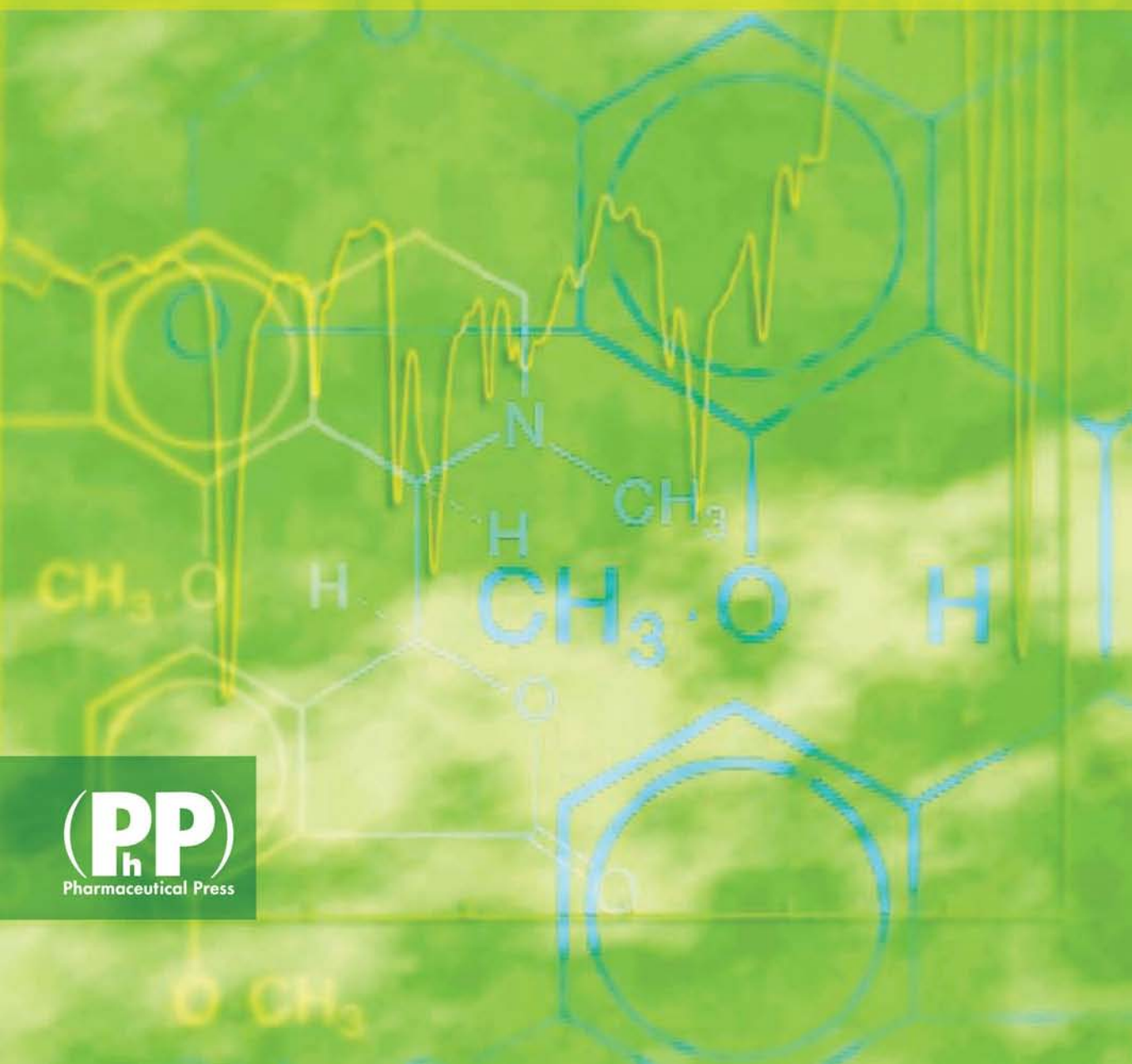
Clarke's Analysis of Drugs and Poisons

Fourth edition

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



Pharmaceutical Press

Clarke's Analysis of Drugs and Poisons

Clarke's Analysis of Drugs and Poisons

in pharmaceuticals, body fluids and postmortem material

FOURTH EDITION

Consulting Editors
Anthony C Moffat
M David Osselton
Brian Widdop

Executive Development Editor
Jo Watts

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Contributors

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Dr Phil Kemp
Analytical Research Laboratories, Oklahoma City, USA

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

Dr Pascal Kintz
Laboratoire Chemtox, Illkirch-Graffenstaden, France

Dr Thomas Kupiec
Analytical Research Laboratories, Oklahoma City, USA

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

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

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

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

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

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

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

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

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

Professor M David Osselton
University of Bournemouth, Dorset, UK

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

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

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

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

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

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

Dr Dave Rudd
GlaxoSmithKline Manufacturing, Ware, Hertfordshire, UK

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

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

Dr Thomas Stimpfl
Department of Forensic Medicine, Wien, Austria

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

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

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

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

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

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

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

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

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

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

About the Editors

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

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

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

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

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

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

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

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

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

Dr Jo Watts
BSc, PhD

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

Foreword

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

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

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

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

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

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

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

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

Robert Wennig, PhD
Past President of TIAFT

Preface

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

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

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

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

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

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

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

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

Volume 1

Part 1: methodology and analytical chapters

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

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

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

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

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

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

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

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

Subject index

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

Volume 2

Part 2: analytical and toxicological data

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

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

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

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

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

Part three: indexes to analytical data

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

Subject index

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

Preparation of this edition

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

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

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

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

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

General Notices

Health and Safety

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

Classification

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

Nomenclature

Monograph Titles

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

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

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

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

IUPAC Names

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

Proprietary Names and Synonyms

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

CAS Registry Numbers

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

Molecular Weights

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

Physical Characteristics

Dissociation Constants

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

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

Melting Points

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

Partition Coefficients

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

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

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

Solubility

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

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

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

Temperature

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

Analytical Data

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

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

Extraction

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

Colour Tests

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

Thin-layer Chromatography

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

Gas Chromatography

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

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

High Performance Liquid Chromatography

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

Ultraviolet Absorption

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

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

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

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

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

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

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

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

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

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

Infrared Absorption

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

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

Mass Spectrum

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

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

Quantification

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

Reagents

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

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

Disposition in the Body

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

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

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

Therapeutic Concentration

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

Toxicity

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

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

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

Volume of Distribution

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

Clearance

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

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

Dose

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

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

Comments

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

Deletions

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

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

Abbreviations

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

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

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

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

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

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

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

PART ONE

Chapters

Methodology and analytical techniques

CHAPTER

1 Hospital Toxicology

DRA Uges

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

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

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

Causes of hospital admissions for poisoning

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

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

Qualitative screening or quantitative analysis?

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

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

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

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

Applications

Confirmation of diagnosis

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

Differential diagnosis of coma

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

Diagnosis of brain death

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

Influence on active therapy

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

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

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

Medicolegal aspects of hospital toxicology

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

Clinical manifestations and biomedical tests

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

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

Other indicative features

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

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

Assays required on an emergency basis

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

Table 1.2 Disturbance of clinical features and indications of possible causes

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

Quality management

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

Table 1.3 Odours associated with poisoned patients

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

Table 1.4 Typical colours of the skin with poisoning

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

Table 1.5 Urine colours associated with various poisons

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

Table 1.6 Biochemical and haematological abnormalities in poisoning

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

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

Request forms

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

Collection and choice of samples

Blood, serum or plasma

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

Table 1.7 Emergency toxicological assays

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

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

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

Urine

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

Stomach contents

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

Request form for toxicological analysis

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

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

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

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

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

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

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

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

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

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

Reference ranges (mg/L):
therapeutic toxic

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

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

Financial information:

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

Figure 1.1 Request form for toxicological analysis.

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

Saliva, sweat and/or oral fluids

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

Toxicological screening

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

Fast limited screening

Immunoassays

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

Alcohol dehydrogenase test for ethanol

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

Direct (colour tests) in serum or urine samples

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

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

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

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

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

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

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

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

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

Confirmation of methanol

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

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

Organophosphorus compounds: cholinesterase inhibitors in serum

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

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

Trichloro-compounds: Fujiwara test in serum or urine

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

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

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

Paraquat and diquat – dithionite test in urine

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

A blue colour indicates the presence of paraquat.

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

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

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

Paracetamol: cresol–ammonia test in urine

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

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

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

Cyanide in blood

Dräger test-tube method

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

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

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

Thallium in urine

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

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

Toxicological screening by thin-layer chromatography

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

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

Solvent extraction procedure for acidic, neutral and basic drugs

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

Back-extraction procedure for stomach contents extracts

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

Drying and concentration

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

Chromatography of acidic and neutral drugs

Location reagents

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

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

Method

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

Visualisation

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

Table 1.8 Sequence of application of acidic extract to TLC plate

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

Table 1.9 TLC data for some acidic and neutral drugs

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

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

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

Chromatography of basic drugs

Solvent extraction from urine

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

Location reagents/spray reagents

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

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

Method

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

Visualisation

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

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

Table 1.10 Sequence of application of basic extract to TLC plate

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

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

Table 1.11 TLC data for some common basic drugs

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

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

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

Chromatography

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

Visualisation procedure

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

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

Gas–liquid chromatography screening for alcohols and other volatile substances

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

Standard solutions in water

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

Internal standard solution in water

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

Apparatus

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

Table 1.12 Thin-layer chromatography of some neuroleptic drugs

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

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

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

Gas chromatographic screening for drugs

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

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

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

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

HPLC screening using the systematic toxicological identification procedure

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

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

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

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

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

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

Screening and quantification using LC-MS(-MS)

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

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

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

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

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

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

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

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

Table 1.13 Chromatographic gradient

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

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

Assay

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

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

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

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

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

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

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

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

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

table continued

Table 1.14 continued

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

Table 1.14 continued

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

table continued

Table 1.14 continued

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

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

Tests for specific compounds and groups of compounds

Alcohols, acetone, acetaldehyde and glycols

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

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

Gas chromatography of alcohols

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

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

Identification assay

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

Quantification

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

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

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

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

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

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

Assay

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

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

Ethylene glycol and diethylene glycol in serum by HPLC

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

Assay

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

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

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

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

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

Screening for abuse of solvents

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

Antidepressants and antipsychotics

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

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

Extraction procedure

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

Chromatography

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

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

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

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

table continued

Table 1.16 continued

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

Table 1.17 Exceptions to UV maxima at 254 nm

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

Table 1.18 Fluorescence detector in series with UV detector

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

Analysis of antidepressants by HPLC

Extraction procedure

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

Chromatography

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

Benzodiazepines, zolpidem and zopiclone

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

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

Benzodiazepines by reversed-phase HPLC

Reagents

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

Extraction procedure

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

Table 1.19 gives the retention times.

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

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

Table 1.19 Retention times and LOQ data for benzodiazepines

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

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

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

Cholinesterase inhibitors (organophosphate and carbamate pesticides)

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

Paraquat and diquat

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

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

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

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

Method

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

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

Chlorophenoxyacetic acid herbicides

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

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

Chlorophenoxyacid herbicides in serum or urine by GLC

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

Chromatography

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

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

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

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

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

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

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

Colorimetric assay for salicylates

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

Assay

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

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

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

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

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

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

Valproic acid determination by GC

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

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

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

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

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

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

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

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

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

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

Internal standard Use 2 mg hexobarbital/L of dichloromethane.

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

Retention times for antiepileptic drugs are given in Table 1.20.

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

Table 1.20 Retention times for antiepileptic drugs

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

Carbon monoxide

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

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

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

Quantification of carboxyhaemoglobin in blood by spectrophotometry

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

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

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

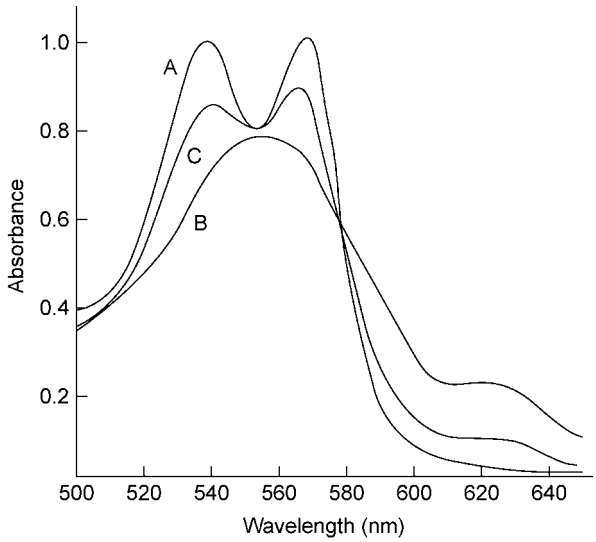

Figure 1.2 Ultraviolet spectra of (A) carboxyhaemoglobin, (B) reduced haemoglobin and (C) a blood sample from a patient poisoned with carbon monoxide.

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

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

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

Metals

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

Theophylline and caffeine

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

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

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

Measurement of xanthines in serum by normal-phase HPLC

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

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

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

Assay

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

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

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

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

Column

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

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

Assay

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

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

Cardioactive drugs

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

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

Measurement of common antiarrhythmic drugs in serum or plasma by HPLC

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

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

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

Assay

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

Retention times for antiarrhythmic drugs are given in Table 1.21.

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

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

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

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

Assay

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

Table 1.21 Retention time and LOQ data for antiarrhythmic drugs

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

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

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

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

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

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

Assay

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

Drugs of abuse

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

Analytical methods

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

Chromatographic analysis of drugs of abuse

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

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

Acid hydrolysis

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

Enzyme hydrolysis

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

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

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

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

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

Assay

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

Plate A, general drugs (mobile phase: ECMA)

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

Plate B, opiates (mobile phase: EMA)

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

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

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

Table 1.22 R_f values and colour reactions for drugs of abuse

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

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

Notes on specific compounds and groups

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

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

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

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

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

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

Table 1.23 HPLC gradient mobile phase at 1 mL/min

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

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

Mobile phases

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

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

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

Assay

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

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

Detection of diuretics in urine by thin-layer chromatography

Reagents

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

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

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

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

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

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

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

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

Method

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

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

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

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

Elution (for all four TLC plates)

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

Detection A

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

Detection B

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

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

Detection of laxatives in urine by thin-layer chromatography

Reference solutions

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

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

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

Assay

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

System A

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

System B

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

Table 1.25 Thin-layer chromatographic data for diuretic drugs

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

table continued

Table 1.25 continued

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

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