ISOLATION and IDENTIFICATION of DRUGS

in pharmaceuticals body fluids and post-mortem material

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Preface

During the period which has elapsed since the end of the second World War, the identification of drugs and other potentially poisonous substances has become increasingly urgent and increasingly difficult.

The urgency of the problem can be seen from the figures for the number of deaths due to accidental and suicidal poisoning in England and Wales during the decade 1956 to 1965:

Year	*1956*	*1957*	*1958*	*1959*	*1960*	*1961*	*1962*	*1963*	*1964*	*1965*
Suicide	860	835	877	899	943	1131	1441	1833	1928	1916
Accident	356	353	349	372	448	464	573	672	732	719
Total	1216	1188	1226	1271	1391	1595	2014	2505	2660	2635

Disquieting as these figures are, it must be realised that they take no account of the much larger number of cases of non-fatal poisoning which occur every year nor do they give any idea of the extensive loss of life among domestic animals and wild creatures due to the widespread use of chemicals on the land.

The increasing difficulty of the task is mainly due to the vast increase in the numbers of compounds—drugs, pesticides, solvents, and so on—which have come into everyday use during this period. Apart from the problem arising from the sheer number of the substances for which tests must now be made, a further difficulty stems from the fact that many of these compounds come on the market before any technique for isolating and identifying them has been described. Furthermore, this multiplicity means that many of the classical tests, long regarded as specific for certain substances, are now given by a dozen or more compounds, thus necessitating the carrying out of additional confirmatory reactions.

It is for these reasons that this book has been produced.

We have attempted to make it of value to as wide a readership as possible and have thus included rapid screening techniques for the hospital biochemist working in circumstances where speed may make the difference between life and death, precise methods for the forensic scientist whose findings must stand up to cross examination in a court of law, simple techniques for the chemist working under primitive conditions in the field, and sophisticated procedures for the research worker with full instrumentation at his disposal.

It would not have been possible to produce this book without the unstinting help given me from many sources, and my most grateful thanks are due to Judith Berle for her unfailing help and enthusiasm during the last four years, to my collaborators, D. I. Chapman, A. S. Curry, C. Daglish, R. H. Fox, G. Higgins, J. V. Jackson, H. Leach, M. S. Moss, R. L. Smith, and H. M. Stevens, not only for writing the chapters in Part 1 of this book but also for giving so generously of their time in trying out techniques and checking figures in their own laboratories, to H. V. Street for investigating the behaviour of several hundred compounds by reversed phase paper chromatography, to W. J. Wilson

for providing physical constants, to S. C. Jolly for checking the structural formulae, to Linda Dawe for secretarial assistance, to Sandra Roper and Betty Forman for technical help, to the various individuals and firms mentioned on p. *xxii* whose generous gifts of drugs made possible the experimental work undertaken to produce many of the tests given in this book, and finally to R. G. Todd and the staff of the Extra Pharmacopoeia, especially Edith J. Condon, Maureen A. Dempsey, and B. J. D. Morgan, for their skilled and able assistance in turning an Idea into a Book.

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E. G. C. C.

Introduction

The book is divided into four parts.

Part 1 contains ten chapters which include practical details for carrying out the procedures referred to in the subsequent parts of the book. Seven of these chapters deal with particular analytical techniques—paper, thin-layer, and gas chromatography, ultraviolet and infra-red spectrophotometry, colour tests, and crystal tests. The remaining three chapters are devoted to rapid screening methods, extraction techniques, and the metabolism of drugs.

Part 2 consists of a series of monographs on over 1000 drugs and related compounds, arranged in alphabetical order of their main titles. Each monograph gives the name of the compound with synonyms and proprietary names, its chemical name, structural formula, molecular weight, and physical properties, followed by the relevant analytical data and notes on its metabolism and toxicity.

Part 3 is an arrangement of the more important analytical data of Part 2 in sequential order and tabular form.

Part 4 comprises three appendixes. Appendix 1 describes the reagents and Appendix 2 the tests employed in the analytical procedures referred to in Parts 1 and 2. Appendix 3 is a bibliography of the 900 references cited in the text.

In using the data in Part 2, the following notes may be helpful.

Nomenclature

The main titles of the monographs in Part 2 are usually British Approved Names but for those substances for which such names have not been adopted, preference has been given to International Non-Proprietary Names or United States Adopted Names, where these exist; for pesticides, the British Standard Recommended Common Names have been used. The main title 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 monographs.

Chemical names are in accord with the current practice in Great Britain and generally follow the recommendations of the Chemical Society. The names used for complex radicals or groups are those recommended by the British Pharmacopoeia Commission, namely:

Recommended Name	*Chemical Name*
acetonide	isopropylidene ether of a dihydric alcohol
amsonate	4,4 -diaminostilbene-2,2′-disulphonate
camsylate	camphorsulphonate
closylate	*p*-chlorobenzenesulphonate

Recommended Name	*Chemical Name*
cypionate	β-cyclopentylpropionate
edisylate	ethane-1,2-disulphonate
eglumine	*N*-ethylglucamine
embonate	4,4′-methylenedi-(3-hydroxy-2-naphthoate)
enanthate	heptanoate
esylate	ethanesulphonate
gluceptate	glucoheptonate
isethionate	2-hydroxyethanesulphonate
meglumine	*N*-methylglucamine
mesylate	methanesulphonate
napadisylate	naphthalene-1,5-disulphonate
napsylate	naphthalene-2-sulphonate
pivalate	trimethylacetate
theoclate	8-chlorotheophyllinate
tosylate	toluene-*p*-sulphonate

Classification
At the head of each monograph an indication is given of the classification of the compound according to its therapeutic or commercial use or its pharmacological action.

Physical Properties
Only those physical properties likely to be useful to the analyst have been given. The melting-points recorded in the individual monographs are listed in sequential order on pages 603 to 611. Unless otherwise specified in the text, the stated solubilities are within the temperature range 15° to 25°.
Temperatures are expressed throughout the text in degrees Celsius (centigrade).

Screening Tests
These tests are intended primarily for the use of hospital biochemists and others who need to obtain results in the minimum of time without carrying out detailed analyses—see pages 3 to 15.

Extraction
This entry in the monographs in Part 2 indicates briefly the fraction in which the compound is likely to be encountered; it should be used in conjunction with the extraction scheme outlined on page 26.

Paper Chromatography
Details of the various systems used will be found on pages 34 to 42. The main system for screening for basic drugs is system P1, page 34. Iodoplatinate spray and bromocresol green spray are the best location reagents for this system but, should they fail to react, any of the other reagents listed on page 34 may be used. A list of Rf values for system P1, in sequential order, is given on pages 612 to 627. System P2, page 34, may be used for screening for barbiturates and systems P6 and P7, page 37, for neutral drugs. Other systems are included which deal with particular groups, such as salicylates, corticosteroids, and thiazide diuretics.

Reversed phase chromatography, systems RP1a and RP1b, pages 41 and 42,

is useful in that it may be employed for acidic, basic, or neutral drugs. Rf values, in sequential order, for system RP1a will be found on pages 630 to 633 and for RP1b on pages 633 to 636.

Thin-layer Chromatography

Details of the various systems used are given on pages 46 to 58. The main system for screening for basic drugs is system T1. The best location reagents are acidified iodoplatinate spray and potassium permanganate spray. A list of Rf values for this system, in sequential order, is given on pages 637 to 650. System T10, page 52, may be used for barbiturates. Other systems are included which deal with particular groups, such as corticosteroids, cardiac glycosides, and sulphonamides.

Gas Chromatography

Details of the various systems used are given on pages 82 and 83 and lists of the relative retention times, in sequential order, on pages 656 to 662. The choice of method depends to some extent on the equipment available. System G2/225 is useful for general screening and system G5 for barbiturates.

Ultraviolet Spectrophotometry

See pages 84 to 102. It would, of course, have been desirable to have reproduced the actual curve for each compound but the cost would have been prohibitive. Instead, the wavelengths of the principal peak, of any subsidiary peaks, and of any significant inflexions or shoulders are recorded for the individual compounds in their respective monographs in Part 2.

A list of the principal peaks for compounds in acid solution is given in Table A on pages 670 to 680. Table B on pages 680 to 683 lists the principal peaks for compounds in alkaline solution when values for these compounds in acid solution are not recorded in Part 2 or when the values in alkaline solution differ significantly from those in acid solution, and Table C on pages 683 to 687 lists the principal peaks in neutral solution for compounds for which values in acid or alkaline solution are not recorded in Part 2.

Infra-red Spectrophotometry

See pages 103 to 122. Wherever possible, absorption spectra obtained with potassium bromide disks have been chosen. An index of principal peaks in ascending order of wavenumber will be found on pages 689 to 723 and reproductions of the absorption spectra on pages 727 to 793. Instructions on how to use the former are given on page 688.

Colour Tests

See pages 123 to 134. Where the entries under this heading in the monographs in Part 2 are preceded by the word MICRO, this means that they are carried out by the microtechnique described on page 133. The sensitivity given is that of the smallest quantity that may be expected to give a satisfactory result.

The colours produced by the various compounds in response to the sulphuric acid–formaldehyde test—the Marquis test—are listed on pages 663 to 669.

Crystal Tests
Where the entries under this heading in the monographs in Part 2 are preceded by the word MICRO, this indicates that the tests are carried out by the micro-technique described on page 136. The sensitivity given is that of the most dilute solution from which crystals may be expected to form.

Metabolism
Under this heading in the monographs in Part 2 a brief statement is made on the metabolic fate of each drug where this is known.

Dose
The dose recorded under this heading in the monographs in Part 2 usually indicates the maximum daily adult dose that may be given by mouth for therapeutic purposes. It is intended solely as a guide in deciding whether the amount taken by an individual falls within the therapeutic range.

Toxicity
Lethal doses in animals are given in milligrams per kilogram body-weight (mg/kg), the route of administration being indicated. Lethal doses in man are, of course, rough approximations. Where possible, examples of actual cases recorded in the literature have been cited; in choosing suitable examples, preference has been given to case reports in which the levels of the drug in body fluids and tissues have been recorded.

Use of Italic Type
In the text of Part 2, the names of those reagents that are defined in Appendix 1, page 797, are printed in *italic* type. Italic type is also used for botanical names and names of micro-organisms.

How to use the book

In cases of emergency
Turn to the chapter on rapid screening starting on page 3. This gives simple methods for identifying the common drugs that are responsible for about 90% of the cases of poisoning requiring hospital treatment.

When there is strong presumptive evidence of the identity of the drug under investigation
Turn to Part 2 and consult the monograph on the compound in question, apply the suggested method of extraction, details of which will be found in the chapter starting on page 16, and then submit the isolated material to any of the methods of identification given in the monograph. The choice of method will depend on the nature of the compound, the expertise of the analyst, the equipment available, and the urgency of the problem.

When there is no clue to the identity of the drug
Apply one of the general extraction methods outlined on pages 21 to 28, submit the residues from the various fractions to suitable chromatographic screening procedures, make a provisional identification by means of the analytical indexes in Part 3, and confirm the identification by carrying out the tests suggested in the appropriate monograph in Part 2.

Thus, for example, a residue in the acid ether fraction might be submitted to paper chromatography by system P2, page 34, or thin-layer chromatography by system T10, page 52. An Rf value of 1·40 in the former case or 0·44 in the latter would, by reference to the analytical indexes in Part 3, suggest quinalbarbitone, page 530. This could then be confirmed by gas chromatography (system G5, page 83—retention time relative to barbitone, 3·02) or by infra-red spectrophotometry (principal peaks at 1559, 1648, and 1690 cm^{-1}—see page 761).

Similarly, a residue in the alkaline chloroform fraction submitted to chromatography by the paper system P1, page 34, might give an absorbing spot, positive with iodoplatinate, at Rf 0·31, and by the thin-layer system T1, page 46, a spot at Rf 0·23, also positive with iodoplatinate. If reference is now made to the tables of Rf values for the P1 and T1 systems, pages 612 and 637, and lists are made of all the spots fulfilling the above conditions between 0·29 and 0·33 on the former system and 0·21 and 0·25 on the latter, it will be found that strychnine, page 545, is the only compound common to both lists. Should no instrumentation be available, confirmation may be made (1) by rerunning the test material on paper and thin-layer chromatograms against a known sample of strychnine, (2) by evaporating a microdrop (see page 133) of a solution of the test material in dilute acetic acid with a microdrop of a 0·5 per cent solution of ammonium vanadate, moistening the residue with a microdrop of concentrated sulphuric acid, and observing the colour change—blue → purple → red, and (3) by

mixing a microdrop of a similar solution of the unknown with a microdrop of a 5 per cent solution of platinic chloride by the hanging drop technique described on page 136 and observing the shape of the crystals formed.

Comment se servir de ce livre

Dans les cas d'urgence
Consulter le chapitre sur les méthodes de dépistage rapides commençant à la page 3. Ce chapitre décrit des méthodes simples permettant d'identifier les produits toxiques communs qui sont la cause d'environ 90% des cas d'empoisonnement nécessitant un traitement hospitalier.

Lorsque des preuves suffisamment évidentes permettent de soupçonner l'identité du produit toxique que l'on recherche
Consulter, dans la Deuxième Partie, la monographie du composé en question et appliquer les méthodes d'extraction suggérées dont les détails sont donnés dans le chapitre commençant à la page 16. Soumettre ensuite le produit isolé à n'importe laquelle des méthodes d'identification indiquées dans la monographie. Le choix de la méthode dépendra de la nature du produit, de l'expérience de l'analyste, des appareils disponibles et du degré d'urgence du problème.

Lorsqu'il n'existe aucune indication quant à l'identité du produit toxique
Appliquer une des méthodes générales d'extraction décrites de la page 21 à la page 28 et soumettre les résidus des diverses fractions à des tests appropriés d'identification chromatographique. Faire une identification préliminaire en consultant les tableaux analytiques de la Troisième Partie et confirmer l'identification en procédant aux tests suggérés dans la monographie appropriée de la Deuxième Partie.

Ainsi, par exemple, un résidu dans la fraction éther acide peut être soumis à la chromatographie sur papier suivant le système P2, page 34, ou à la chromatographie en couche mince suivant le système T10, page 52. Une valeur Rf de 1,40 dans le premier cas et de 0,44 dans le second, confrontées aux tableaux analytiques de la Troisième Partie, laisseraient supposer qu'il s'agit de sécobarbital (quinalbarbitone), page 530. Ceci peut ensuite être confirmé par chromatographie gazeuse (système G5, page 83—temps de rétention relatif au barbital, 3,02) ou par spectrophotométrie aux rayons infrarouges (valeurs maximales principales à 1559, 1648 et 1690 cm^{-1}—voir page 761).

De même, un résidu dans la fraction chloroforme alcalin soumis à la chromatographie sur papier selon le système P1, page 34, peut donner un point d'absorption positif avec le platiiodure à Rf 0,31, et selon le système T1 en couche mince, page 46, un point à Rf 0,23, positif également avec le platiiodure. Si l'on consulte alors les tableaux des valeurs Rf relatives aux systèmes P1 et T1, pages 612 et 637, et si l'on dresse les listes de tous les points remplissant les conditions ci-dessus, entre 0,29 et 0,33 pour le premier système, et 0,21 et 0,25 pour le second, on verra que la strychnine, page 545, est le seul produit commun aux deux listes. Si l'on ne dispose d'aucun appareil, il est possible d'obtenir confirmation, (1) en comparant les chromatogrammes sur papier et en couche mince du produit testé

avec ceux d'un échantillon que l'on sait être de la strychnine; (2) en faisant évaporer une microgoutte (voir page 133) d'une solution du produit testé dans de l'acide acétique dilué, avec une microgoutte d'une solution de vanadate d'ammoniaque à 0,5%, en humidifiant ensuite le résidu avec une microgoutte d'acide sulfurique concentré, et en observant le virage—bleu → pourpre → rouge; et (3) en mélangeant une microgoutte d'une solution semblable du produit inconnu avec une microgoutte d'une solution de chlorure de platine à 5%, suivant la technique de la goutte en suspension décrite à la page 136, et en observant la forme des cristaux qui se forment.

Anleitung zum Gebrauch des Buches

Im Notfall
Schlagen Sie das auf Seite 3 beginnende Kapitel auf, das schnelle Prüfungsmethoden beschreibt. Hier finden Sie einfache Verfahren zum Nachweis der gebräuchlichen Arzneimittel, die für ungefähr 90% aller Spitalbehandlung benötigenden Vergiftungen verantwortlich sind.

Wenn deutliche Anzeichen die Identität des Arzneimittels, die untersucht wird, vermuten lassen
Schlagen Sie Teil 2 auf und suchen Sie in der Einzelabhandlung über die in Frage stehende Verbindung Rat, wenden Sie das vorgeschlagene Verfahren zur Absonderung, über welches Sie näheres in dem auf Seite 16 beginnendem Kapitel finden, an und unterziehen Sie dann den abgetrennten Stoff irgendeiner der in der Abhandlung genannten Methoden der Identifizierung. Die Wahl der Methode hängt von der Beschaffenheit der Verbindung, der Erfahrung des Untersuchers, der verfügbaren Apparatur und der Dringlichkeit der Aufgabe ab.

Wenn keine Hinweise auf die Identität des Arzneimittels bestehen
Wenden Sie eines der auf Seite 21–28 kurz beschriebenen allgemeinen Absonderungsverfahren an, unterziehen Sie die Rückstände der verschiedenen Fraktionen den geeigneten chromatographischen Untersuchungen, und unternehmen Sie eine vorläufige Identifizierung mit Hilfe der analytischen Tabellen im Teil 3. Bestätigen Sie den Befund durch die Prüfungen, die in den entsprechenden Abhandlungen in Teil 2 vorgeschlagen werden.

So zum Beispiel kann ein Rückstand im sauren Ätheranteil nach System P2, Seite 34 der Papierchromatographie unterzogen werden, oder der Dünnschichtchromatographie nach System T10, Seite 52. Ein Rf-Wert von 1,40 im ersten Falle oder 0,44 im zweiten weist, wenn man in den analytischen Tabellen im Teil 3 nachsieht, auf Quinalbarbiton, Seite 530. Dieser Befund kann durch Gaschromatographie bestätigt werden (System G5, Seite 83—Retentionszeit bezüglich auf Barbiton, 3,02), oder durch Infrarotspektroskopie (Hauptbanden bei 1559, 1648, und 1690 cm^{-1}—siehe Seite 761).

In ähnlicher Weise kann ein Rückstand des alkalischen Chloroformanteils, wenn er einer chromatographischen Untersuchung nach dem Papiersystem P1, Seite 34 unterzogen wird, einen Absorptionsflecken geben, der bei Rf 0,31 mit Jodoplatinat positiv reagiert. Nach dem Dünnschichtsystem T1, Seite 46, kann er einen Flecken bei Rf 0,23 geben, der gleichfalls mit Jodoplatinat positiv ist. Wenn man sich jetzt auf die Tabellen der Rf-Werte für die P1 und T1 Systeme, Seite 612 und 637, bezieht, und Listen aller Flecken anlegt, die den obigen Bedingungen zwischen 0,29 und 0,33 im ersten System und zwischen 0,21 und 0,25 im zweiten System genügen, dann findet man, dass Strychnin, Seite 545, die einzige Verbindung ist, die beiden Listen gemeinsam ist. Falls keine Instrumente

zur Verfügung stehen, kann eine Bestätigung gefunden werden durch (1) vergleichende Papier- und Dünnschichtchromatogramme des zu untersuchenden Stoffes und einer sicheren Strychninprobe, (2) durch Verdampfen eines Mikrotropfens (siehe Seite 133) einer Lösung des zu untersuchenden Stoffes in verdünnter Essigsäure mit einem Mikrotropfen einer 0,5 prozentigen Lösung von Ammoniumvanadat, Befeuchten des Rückstandes mit einem Mikrotropfen konzentrierter Schwefelsäure und Beobachten des Farbumschlages—blau → purpur → rot, und (3) indem man einen Mikrotropfen einer ähnlichen Lösung des unbekannten Stoffes mit einem Mikrotropfen einer 5 prozentigen Lösung von Platintetrachlorid im Verfahren der hängenden Tropfen mischt (das Verfahren wird auf Seite 136 beschrieben) und die Gestalt der sich bildenden Kristalle beobachtet.

Cómo usar el libro

En las urgencias médicas
Consultar el capítulo sobre cribado rápido que comienza en la página 3. En él se describen métodos sencillos para la identificación de drogas comunes, que son las responsables de un 90% de los casos de envenenamiento que precisan hospitalización.

Cuando existan pruebas bien fundadas acerca de la identidad de la droga que se está investigando
Pásese a la Parte 2 y consúltese la monografía correspondiente al compuesto investigado, aplicando el método de extracción que se sugiera, según los datos que se encontrarán en el capítulo que comienza en la página 16; someter entonces la substancia aislada a cualquiera de los métodos de identificación indicados en la monografía. El método a utilizar dependerá de la naturaleza del compuesto, la pericia del analista, del equipo de que se disponga, y de la urgencia del caso.

Cuando no haya pista alguna identificadora de la droga
Aplíquese uno de los métodos generales de extracción indicados en las páginas 21 a 28, sometiendo los resíduos de las diversas fracciones a procedimientos adecuados de cribado cromatográfico, haciendo una identificación provisional utilizando los índices analíticos de la Parte 3, y confirmando la identificación mediante las pruebas sugeridas en la correspondiente monografía de la Parte 2.

Así, por ejemplo, un resíduo en la fracción ácida de éter podría someterse a cromatografía sobre papel siguiendo el sistema P2, página 34, o a cromatografía de capa delgada según el sistema T10, de la página 52. Un valor Rf = 1,40, en el primer caso, o de 0,44 en el segundo, al hacer comparación con los índices analíticos de la Parte 3, sugeriría la presencia de secobarbital (quinalbarbitone), página 530. Esto podría confirmarse entonces mediante cromatografía en fase gaseosa (sistema G5, página 83—tiempo de retención relativo al barbital, 3,02) o mediante espectrofotometría infrarroja (máximas principales a 1559, 1648, y 1690 cm^{-1}—véase página 761).

De igual modo, un resíduo en la fracción alcalina de cloroformo sometido a cromatografía sobre papel siguiendo el sistema P1, página 34, pudiera dar una mancha de absorción, positiva empleando yodoplatinato, a Rf = 0,31, y mediante el sistema T1 de capa delgada, página 46, una mancha a Rf = 0,23, igualmente positiva con yodoplatinato. Si ahora consultamos las tablas de valores Rf correspondientes a los sistemas P1 y T1, páginas 612 y 637, y se preparan listas a base de todas las manchas que cumplen las anteriores condiciones comprendidas entre 0,29 y 0,33 para el primer sistema, y entre 0,21 y 0,25 para el segundo, encontraremos que la estricnina, página 545, es el único compuesto común a ambas listas. De no disponerse de los instrumentos necesarios, la confirmación puede verificarse (1) volviendo a hacer avanzar a la substancia

problema sobre cromatogramas obtenidos mediante papel y capa delgada de una muestra conocida de estricnina, (2) evaporando una microgota (véase la página 133) de una solución problema en ácido acético diluído, con una microgota de una solución al 0,5 por ciento de vanadato amónico, humedeciendo el resíduo con una microgota de ácido sulfúrico concentrado, y observando el cambio de color—azul → púrpura → rojo, y (3) mediante la mezcla de una microgota de una solución semejante del problema, con uña microgota de una solución al 5 por ciento de cloruro platínico, siguiendo la técnica de la gota suspendida, según se describe en la página 136, y estudiando la forma de los cristales que se hayan formado.