Practical Pharmaceutical Chemistry

Fourth Edition – Part Two

Edited by

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Fourth edition,
in two parts

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Part Two

The Athlone Press
London

First published in Great Britain 1988
by The Athlone Press, 44 Bedford Row
London WC1R 4LY

British Library Cataloguing in Publication Data

Beckett, A. H.
Practical pharmaceutical chemistry.—
4th ed.
Pt. 2
1. Drugs—Analysis
I. Title II. Stenlake, J.B.
615'.19015 RS189

ISBN 0-485-11323-8

Library of Congress Cataloging in Publication Data

Beckett, A. H. (Arnold Heyworth)
Includes bibliographies and indexes.
1. Chemistry, Pharmaceutical. I. Stenlake, J.B.
(John Bedford) II. Title [DNLM: 1. Chemistry,
Pharmaceutical. QV 744 B396p]
RS403.B43 1988 615'.1901 87-14470
ISBN 0-485-11323-8 (pt. 2)

Typeset by KEYTEC, Bridport, Dorset
Printed in England at the University Press, Cambridge

Preface to the Fourth Edition

A spate of official publications, including the *British Pharmacopoeia* 1980, and its Addenda (1981, 1982, 1983 and 1986), the *British Pharmacopoeia (Veterinary)* 1985, the combined *United States Pharmacopoeia* XXI and *National Formulary* XVI and the second edition of the *European Pharmacopoeia*, call for yet another revision of *Practical Pharmaceutical Chemistry*. In this, the fourth edition, we have endeavoured to reflect the growing international convergence of policy and practice both in the change of subtitle of Part 1 to Pharmaceutical Analysis and Quality Control, and in the breadth of its content.

The objectives of this revision have been achieved in part by reducing the heavy dependence of earlier editions on the methods of the *British Pharmacopoeia*. Wherever possible, examples are based on drugs and dosage forms that are in widespread and common use in Britain, continental Europe and North America. Additionally, some reference to veterinary pharmaceuticals is made where they provide appropriate examples. As in previous editions, substances that are the subject of monographs in the British Pharmacopoeia are denoted by their British Approved Names (BAN), and are distinguished from United Adopted Names (Usan) where these are given by setting BANs in italics.

The discussion of drug registration has also been broadened to include reference to FDA and EEC procedures, the control of veterinary as well as human medicines, and the need in the United Kingdom for biologically based products to be manufactured in conformity with the requirements of the *Biological Compendium* (1977).

The detailed chapter-by-chapter revision in Part 1 encompasses the changeover in European analytical practice from NORMALITY to MOLARITY, which despite the reservations of some analysts, particularly in relation to oxidation-reduction titrations, is now virtually complete. The expansion of rapid complexometric titration methods, with the consequent almost complete demise of the older much slower gravimetric methods, is reflected in the regrouping of complexometric, argentometric and gravimetric methods into a single chapter. A brief treatment of variables in quantitative analysis appropriate to the application of chemical methods has been included for the first time, and the chapter on the analysis of dosage forms has been updated to better reflect both the range of products and the methods used in their control. In this respect the need for control analysts to embrace an

appreciation of biological methods is reflected in the inclusion of short sections on sterility testing, on microbiological contamination and challenge tests for antimicrobial preservatives, on microbiological assays and on enzymes in pharmaceutical analysis.

There have been few, if any, major innovations in physical methods applicable to pharmaceutical analysis since the publication of the third edition in 1976. However, there have been a number of improvements and changes of emphasis. These are reflected in Part 2 of the present edition, which has been extensively revised in an endeavour to give a broader coverage of the most widely used techniques and a better balance of material that fairly reflects modern practice. In particular, the steady growth in importance of quantitative chromatographic techniques is recognised in the broader coverage and depth accorded to gas chromatography and high performance liquid chromatography, the inclusion of a short section on capillary column gas chromatography, and the transfer from Part 1 of important sections on ion exchange and size exclusion chromatography. The treatment of NMR spectroscopy has also been extended to include a brief introduction to ^{13}C NMR, and the coverage of radiopharmaceuticals increased to include radionuclide generators and quality control of radiopharmaceuticals – a subject of special interest to those engaged in hospital pharmacy. To balance these expansions, coverage of electrochemistry and polarography has been compressed into a single chapter – a degree of emphasis that is more in keeping with the rather modest role that these techniques continue to play in the practice of pharmaceutical analysis.

Two new chapters have been added to improve cohesion in Part 2. The first, by way of introduction, sets out the contribution and role of physical methods of analysis in the various phases of drug development, in quality control within the factory and in independent control laboratories, and in the clinic. The second, by way of conclusion, consists of a series of 'workshop style' exercises, with separate solutions, to illustrate and give practice in the application of spectroscopic techniques in structural elucidation and verification of identity.

Many analytical methods serve a common purpose in their suitability both for the quality control of pharmaceutical products and the study of their absorption, distribution, metabolism and excretion whether in laboratory animals or human subjects. The importance of developing sensitive, cold methods, as opposed to those based on radiolabelled compounds, is widely recognised in clinical pharmacology. Hospital pharmaceutical departments well equipped for essential quality control work are in a unique position with staff, equipment and laboratory facilities to undertake pharmacokinetic and metabolic studies, and even to provide a routine pharmacokinetic monitoring service if this is required. With such developments in mind, we have continued to feature applications of the various separation and spectroscopic methods to drug metabolism and pharmacokinetics together with relevant practical exercises.

J.B.S.

Part 2

Physical Methods of Analysis

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Acknowledgements

Permission to reproduce the following Tables and Figures to gratefully acknowledged:

Tables
3.6, Stanton-Redcroft Ltd; 14.2, *Analytical Chemistry*.

Figures
3.5, HIAC/Royco Instruments Corp,; 3.6, Malvern Instruments; 3.17, Stanton-Redcroft Ltd; 3.12, 3.13, 3.14, 3.15, 3.16, 3.19, 10.3, 11.15 and 11.25, Perkin-Elmer Ltd; 6.14 and 6.15, Pye Unicam; ***, 8.1, and 8.3, Corning-EEL; 9.1, American Instrument Co Inc.; 10.4, N.B.Colthup, American Cyanamide Co; 11.3 and 12.7, Varian Associates; 11.4, *Angew. Chemie*, International Edition and Professor J.D.Roberts; 11.5 and 11.6 *Chemical and Engineering News*; 11.18b and c from *Introduction to Practical High Resolution Nuclear Magnetic Resonance* by D.Chapman and P.D.Magnus, Academic Press; 11.22, 11.28 and 11.31, *Journal of Chemical Education*; 11.26, *Chemical and Engineering News* and Dr.F.A.Bovey; 11.27, adapted from Chan and Hill, *Tetrahedron*, Pergamon Press; 11.29 and 11.30, *Jurnal of Pharmaceutical Sciences*; 11.33, 12.10 and 12.19, Wiley-Interscience; 12.1, adapted from K.Biemann, Mass Spectrometry, McGraw-Hill Publishing Co Ltd; 12.2 John Wiley and Sons and Drs.R.M.Silverstein and G.C.Bassler; 12.3, A.E.I. Ltd and Heyden and Son Ltd; 12.4 and 12.6, Finnegan Corporation; 12.8, *Journal of the American Chemical Society*; 12.9 adapted from *Industrial Research*; 12.11, *Biomedical Mass Spectrometry*; 12.12, Heydon and Son Ltd; 12.13, The Chemical Society and Dr.R.I.Reed; 12.14, McGraw-Hill Publishing Co Ltd and Professor K. Biemann; 12.18, *Arzneim. Forsch.*; 12.20, *Analytical Chemistry*; 13.9, J.R.French and the Radiochemical Centre, Amersham.

Contents

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Instrumental methods in the development and use of medicines

J. B. STENLAKE

Introduction

Quality assurance plays a central role in determining the safety and efficacy of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standardisation and quality control of medicinal products. They are equally important in pharmacokinetics and in drug metabolism studies, both of which are fundamental to the assessment of bioavailability and the duration of clinical response.

Modern physical methods of analysis are extremely sensitive, providing precise and detailed information from small samples of material. They are for the most part rapidly applied, and in general are readily amenable to automation. For these reasons they are now in widespread use in product development, in the control of manufacture and formulation, as a check on stability during storage, and in monitoring the use of drugs and medicines.

Product characterisation for drug development

Once a new drug candidate has been identified for development and clinical trial, physico-chemical methods are called into play at every stage of the development program. In the first instance, they are essential for product characterisation. This is a basic prerequisite to ensure that the animal toxicological studies required to establish safety prior to clinical trial are soundly based. Such studies are time consuming and grossly expensive, and have proved to be one of the prime factors in raising drug development costs to their currently near-prohibitive levels. The product under test must therefore be precisely characterised, and its quality clearly defined to ensure that the maximum amount of useful information is obtained from each test. In particular, product characterisation is necessary to eliminate variations in pharmacological and therapeutic responses and to determine the origin of any toxic effects that may be uncovered. It is also an essential means of ensuring that successive batches of material used in toxicological studies are of like quality, and, equally important, that a reasonable correlation of analaytical profiles is established between material used in animal toxicology and that later produced for clinical trial and marketing.

Such demanding requirements can only be met by employing the full

range and sensitivity of modern physico-chemical separative and analytical techniques. Similarly, sensitive and specific methods for identifying decomposition rates are essential for monitoring stability during toxicological and clinical testing, and in determining the ultimate shelf life of the product. They are equally important in establishing metabolic profiles, for studying release rates from oral solid dosage forms, and in acquiring pharmacokinetic data.

The first step in product characterisation is to establish the precise chemical identity of the product. It is important to determine whether the material is a compound, i.e. a single chemical entity, a mixture of closely related compounds, a mixture of isomers, or merely a loose molecular complex of readily dissociable components. Such information is fundamental to a proper evaluation of the biological properties of the material. Thus *Gentamicin*, for example, consists of a mixture of variable composition dependiong on the source, of the closely related components Gentamicin C_1, C_{1a}, C_2, and C_{2a}, which differ in antibiotic activity. Likewise, only the (*E*)-isomer of *Triprolidine Hydrochloride* is an effective antihistamine, and *Crotamiton*, which is used in the treatment of scabies, consists solely of the (*E*)-isomer. Similarly, the sympathomimetic effect of adrenaline resides almost exlusively in the (*R*)-isomer. Such important physical characteristics could only be precisely, accurately, and rapidly determined by instrumental methods.

For compounds of synthetic origin, identity is usually clearly defined in the great majority of cases by the synthetic route employed. However, it is essential not only that identity be confirmed by alternative means but that the means employed should be capable of providing rapid verification whenever this may be required at any stage of the development program. Modern spectroscopic techniques, such as as ^1H and ^{13}C NMR (Chapter 11) and mass spectrometry (Chapter 12), together with ultraviolet (Chapter 7) and infrared spectroscopy (Chapter 10) are sensitive tools for such purposes. This is shown in the many examples described in the relevant chapters, and in the exercises in their application (Chapter 14). They are invaluable in the resolution of ambiguities where two or more alternative products may result from synthesis, and in the precise characterisation of complex natural products. Thus, the small structural differences that characterise the various components of *Gentamicin* are readily detectable by ^1H NMR, so much so that methods based on these differences have been applied for controlling the composition of the mixture, though superior methods based on high performance liquid chromatography are now available for routine use.

The interpretation of spectroscopic data obtained from compounds, however, is wholely dependent on the knowledge that the material under study is homogeneous. Powerful separative techniques, particularly chromatography in all its many forms (Chapter 4), provide sensitive methods for both purification and analysis of product homogeneity. Gas and high performance liquid chromatography, which make use of electronic recorders, are also eminently suitable and widely used for

quantitative determination of the composition of mixtures of related compounds such as the gentamicins and even mixtures of isomers. The speed and high separative power of capillary gas chromatography (Chapter 4) make it a particularly useful, if highly specialised, technique for the separation of complex mixtures during the research phase of drug development.

Where the product consists of more than one isomer, the isomers must be capable of separate identification and measurement to establish means of ensuring batch to batch consistency of isomer composition. For most optically active compounds, a simple polarimetric measurement of the specific optical rotation at the wavelength of the sodium D-line will suffice (Chapter 2). Occasionally, however, where the measured rotation is small, measurements on a more sensitive instrument at other wavelengths either directly or after derivatisation may be necessary to secure adequate control of the product. Thus, contamination of *Ethambutol Hydrochloride*, which is the *S,S*-isomer, by its inactive *R,S*-isomer is limited in a test that requires the formation of a complex and measurement of its optical rotation using a spectropolarimeter.

In exceptional cases, the identification of optical isomers differing in only one of several chiral centres may call for the use of optical rotatory dispersion or circular dichroism (Chapter 2) to provide a degree of sensitivity that cannot be obtained from simple measurements of optical rotation. For example, the antibiotic *Phenethicillin Potassium*, which is prepared synthetically from 6-aminopenicillanic acid and racemic phenoxypropionyl chloride, is a mixture of the two expected diasterioisomers, but varies in composition depending on the solvent and crystallisation procedures used in its manufacture. The two isomers show differences in their antibacterial spectrum and the product may vary widely in composition. The two isomers give distinct peaks in their circular dichroism spectra, but of opposite sign, from which the composition of the isomer mixtures may be readily determined, and these correlate well with the results of microbiological assay (Stenlake *et al.*, 1972). NMR using chiral lanthanide shift reagents (Chapter 11) also provides a useful alternative method for the examination of diastereoisomeric mixtures.

NMR is particularly valuable in distinguishing geometrical isomers, as for example the *cis*- and *trans*-2,6-dimethyl-1-benzylpiperidines (Chapter 11); also *Crotamiton* (Chapter 14, Compound 16). Similarly, the correct assignment of the C-8 proton signals to the *cis*- and *trans*-isomers of the muscle relaxant *Atracurium Besylate* required the use of a combination of NMR nuclear Overhauser experiments (Chapter 11) and synthesis of a model compound, laudanosine methiodide, with ^{13}C-enriched methyl iodide (Lindon and Ferrige, 1980). Likewise, high resolution ^{1}H NMR and high performance liquid chromatographic characteristics of the *cis-cis, cis-trans*- and *trans-trans*-isomers of atracurium besylate provide a basis for the development of sensitive, accurate and rapid methods capable of establishing the isomer composition of the material (Stenlake *et al.*, 1984).

It is important not only to establish the chemical identity of any new

drug substance but also to identify and quantify potential impurities at an early stage in development. These impurities relate to the source materials (i.e. the substance itself if it is a natural product, or starting materials for synthesis), the manufacturing process and the stability of the product.

Relatively unsophisticated techniques such as those of thin-layer chromatography are now in widespread use for the detection and quantitation of organic impurities, but in the development phase formulation of such tests necessarily rests on the formal identification of the chemical structure of each impurity, and this calls for the same heavy dependence on spectroscopic techniques as is required for the characteristation of the investigational drug itself. Inorganic impurities that might interfere with the assessment of toxicological profiles are confined to residues from toxic elements arising from catalysts and reagents used in synthesis. Trace amounts of toxic metal catalysts, such as nickel and platinum, are readily determined directly at parts per million levels by atomic absorption spectrophotometry (Chapter 8). Traces of toxic non-metals such as boron, derived from the use of borohydride reagents, are not amenable to direct measurement in this way, and require preliminary treatment to destroy the organic matter before determination by some suitable spectroscopic means.

Product development

The design of appropriate dosage forms for any new therapeutic entity is just as important and just as challenging as the other aspects of development. In this, the solid state characters of the active ingredients are especially relevant to the efficacy of oral dosage forms, such as tablets, capsules, oral suspensions, preparations for topical use and aerosols.

Compounds that are water-soluble are usually released readily to solution and hence are available for systemic absorption. Poorly soluble substances, however, may be only marginally effective or may produce erratic clinical responses, unless presented in an appropriate physical form. The definition and control of polymorphism (Chapter 3) can be critical for some compounds, e.g. *Chloramphenicol Palmitate*, which can exist in two polymorphic forms, one of which is biologically inactive. The preparation of dosage forms such as *Chloramphenicol Palmitate Mixture* (Chloramphenicol Palmitate Oral Suspension USP) requires careful control to minimise the formation of the inactive form. Infrared absorption (Chapter 10) and thermal analysis (Chapter 3) provide sensitive methods for the detection and control of such polymorphism. The former is used to control the limit of the inactive polymorph A in *Chloramphenicol Palmitate Mixture*.

The particle size of relatively insoluble compounds is also a critical factor in ensuring effective and consistent response in products for oral administration (e.g. *Digoxin* and *Griseofulvin* in tablets), injectable

suspensions (e.g. *Insulin Zinc Suspension*) and topical preparations (e.g. *Hydrocortisone Acetate Ointment*). Similar control of particle or droplet size for all compounds, irrespective of solubility, is essential for the production of aerosols. The preparation of lipid suspensions for total parenteral nutrition is also dependent on adequate means of controlling droplet size. Particle size, and particle size distribution, can be determined (Chapter 3) by simple manual methods using microscopy and the measurement of sedimentation volumes. Instrumentation methods based on the measurement of electrical conductivity as in the Coulter counter, or requiring the measurement of specific surface area from the permeability to gas flow, offer considerable advantages. Such instrumental methods are particularly useful where large numbers of samples need to be examined, as for example in in-process controls of injectable suspensions and in routine checks for unwanted particulate contamination of injection solutions.

Viscosity (Chapter 2) measurements are used as a means of determining the molecular size of high molecular weight materials to control their properties, as in the various grades of *Methylcellulose* and *Dextran Intravenous Infusions*. When applied by methods suitable for measurements on non-Newtonian systems, viscosity also provides an important way of controlling the flow properties of semi-solid preparations such as ointments and creams.

Production and pharmacopoeial controls

Once established as marketable products, all drug substances and their formulated dosage forms are subject to strict quality assurance procedures. These encompass manufacturing methods, in-process controls, release specifications, and finally check specifications that are applicable throughout the shelf life of the product. In this respect it is important to emphasise the distinction between analytical procedures suitable for use by the manufacturer within the factory in connection with in-process controls and quality control at the time of manufacture (release specifications) on the one hand, and on the other hand procedures suitable for incorporation into a published pharmacopoeial monograph (check specifications) that are capable of independent assessment. Clearly, the latter must necessarily take into account any deterioration in the product during storage, so that the extent to which check specifications differ from release specifications depends on the stability and the intended shelf life of the product. Pharmacopoeial standards must also take account of sample size, which may well be smaller than desirable for analytical accuracy. Tolerances are therefore often wider than in release specifications.

There may also be differences so far as methodology is concerned. The manufacturer, with his complete knowledge of the drug and the additives used in its formulation, is in the best possible position to choose the most specific and sensitive procedures. Sophisticated in-

strumental methods capable of automation to deal with large numbers of samples linked to computerised data recording systems for automatic calculation and recording of results are rapidly becoming the norm. Pharmacopoeial methods, on the other hand, must be such that they are widely available and in common use in control laboratories, whether these be in hospitals, in government departments or independent organisations. Such methods are therefore often simpler.

As a consequence of these latter requirements, the wet chemical methods described in Part 1, whether of classical origin or more recent innovations, have generally been favoured in pharmacopoeial specifications. Even so, such methods have always been supplemented by the measurement of physical constants wherever appropriate, since the facilities for the measurement of, for example, melting and boiling range, refractive index and optical rotation with relatively unsophisticated equipment are readily available in most laboratories. The widespread availability of relatively cheap ultraviolet and infrared spectrometers has brought a degree of precision and simplification into the characterisation and standardisation of drugs, and such techniques are now widely used in both the factory and independent control laboratories. Thin-layer chromatography, likewise, is commonplace, sensitive, quick and easy to apply, and hence is also in widespread use both for verification of identity, and in control tests to limit the degree of contamination by related substances and decomposition products, in both factory and pharmacopoeial specifications.

The much higher instrumental costs of advanced analytical methods, such as nuclear magnetic resonance spectroscopy, mass spectrometry, and gas and high performance liquid chromatography, tend to limit the use of these techniques in pharmacopoeial monographs to the control of complex molecules that cannot be sufficiently closely identified or standardised by simpler methods. Thus NMR is used to identify the antibiotics *Framycetin Sulphate* and *Gentamicin Sulphate*. High performance liquid chromatography is in widespread use because of its versatility in the analysis of complex antibiotics and peptide hormones in addition to its separative power and sensitivity for the analysis of synthetic isomer mixtures; it is increasingly used in the control of related substances in both medicinal agents and their formulated products.

The move towards instrumental methods, particularly in the drive to reduce dependence on biological assays and limit tests requiring animals, has led to the acceptance of a number of less widely used methods. These include, for example, electrophoresis on polyacrylamide gel to control related proteins (arginylinsulin, insulin ethyl ester and proinsulin) in *Insulin*, size exclusion chromatography (Chapter 4) to control higher molecular weight proteins in *Insulin*, and spectrofluorimetry (Chapter 9) to control related substances in the veterinary anthelmintic *Haloxon*. The sensitivity of spectrofluorimetry is also the reason for it being chosen as the most suitable technique for the control of Al levels in *Haemodialysis Solutions*. These are used in large volume and regularly over long periods of time. Hence the need for stringent limits on the

permitted levels of this toxic metal. Classical methods for the analysis of aluminium are not suitable, but fluorescence measurement of its 8-hydroxyquinoline derivative provides a sensitive method for control at levels below 15 ppm of aluminium.

One major disadvantage of many of these methods is the need to compensate for instrument and operator variables, both within and between laboratories. For this reason, many of these techniques require the use of reference compounds or internal standards of known quality and composition. The preparation, storing and distribution on an international basis of large numbers of official reference compounds (British, United States and European Pharmacopeia Reference Substances) is expensive, not just in the materials themselves, but also in distribution costs and in staff time devoted to verification of quality and stability during storage. The distribution of reference samples of controlled drugs (drugs of abuse) is also undesirable, and is subject in some countries to special conditions.

Some progress in overcoming such difficulties has been made in recent years, for example in thin-layer chromatography by use of the parent compound under test at multiple plate loadings (e.g. 1%, 0.01% and 0.05%) to provide a basis for assessing the density of chemically related impurity spots as in *Dextromoramide Tartrate*. However, care is necessary in setting standards when this technique is used, since the sensitivities of the parent and the related substances to the visualising reagent may differ, thereby complicating the problem of setting precise limits for the impurities concerned.

The issue of reference spectra by the British Pharmacopeia Commission for use in the verification of identity in place of reference compounds is a major step forwards in overcoming some of the problems of standardising methods to achieve comparable results on an inter-laboratory basis. In particular it overcomes the need for actual sample distribution of controlled drugs.

Notwithstanding the obvious disadvantages attending the use of complex analytical instrumentation in small control laboratories, their selectivity, sensitivity, and speed of operation ensure their continued use on an expanding scale. As already mentioned, they offer, increasingly, meaningful alternatives to distasteful biological methods. The following chapters describe the more important physico-chemical methods of analysis currently in use in connection with the development, control and use of pharmaceutical products.

Drug metabolism and pharmacokinetics

Investigation of the metabolism and pharmacokinetics form an integral part of all drug development programs. Such studies are conducted initially in laboratory animals, but as development proceeds they must be capable of extension to the clinical level in human volunteers and finally in patients undergoing treatment. The scale of difficulty that