CRC

HANDBOOK of CHROMATOGRAPHY

Gunter Zweig
Joseph Sherma
Editors-in-Chief

Ram N. Gupta
Drugs
Volume II

CRC PRESS

CRC Handbook of Chromatography Drugs

Volume II

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CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data
Main entry under title:

Drugs.

CRC handbook of chromatography.
Bibliography: p.
Includes index.
1. Drugs--Analysis. 2. Chromatographic analysis. I. Gupta, Ram N. II. Sunshine, Irving. III. Series.
RS189.D79 615'.19015 81-10157
ISBN 0-8493-3030-0 (set) AACR2

Direct all inquiries to CRC Press, Inc., 2000 N.W. 24th Street, Boca Raton, Florida 33431.

International Standard Book Number 0-8493-3030-0 (Complete Set)
International Standard Book Number 0-8493-3031-9 (Volume I)
International Standard Book Number 0-8493-3032-7 (Volume II)
Library of Congress Card Number 81-10157
Printed in the United States

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CRC HANDBOOK OF CHROMATOGRAPHY

Series Preface

This Handbook of Chromatography, Drugs by Ram N. Gupta, is one in a series of separate volumes devoted to a single class of chemical compounds or to compounds with a similar use pattern, like the prospective volumes on pesticides and terpenoids. When Volumes I and II of the Handbook of Chromatography were first published in 1972, the editors made an attempt to select the data so as to accomplish the coverage of most organic and inorganic compounds in a volume of about one thousand pages. However, during the ensuring ten years, the literature of chromatography, especially high-performance liquid chromatograph (HPLC), has grown to such an extent that, after an initial intent to update Volumes I and II, it was decided to publish separate volumes devoted to specific subjects. The present volume on the Chromatography of Drugs is an example of the expanded Handbook Series. In selecting Volume Editors, the Editors-in-Chief endeavored to select scientists with extensive knowledge and expertise in the chromatography of specific compounds. The Editor of this Volume, Dr. Ram N. Gupta is renowned in the field of chromatography of drugs, which is evident from the comprehensive and authoritative treatment of the subject found in this Volume. We have given each Volume Editor wide latitude in designing a format that would be most useful to the reader and do justice to the particular subject being covered. Subsequent volumes of this series will include the chromatography of pesticides, steroids, lipids and fatty acids, terpenoids, plant pigments, hydrocarbons, amino acids, inorganic compounds, polymers, and nucleic acids and associated compounds.

We invite readers to communicate with the Volume Editor for comments and corrections and to the Editors-in-Chief for suggestions for future volumes. The Editors-in-Chief want to thank Dr. Gupta for his outstanding effort and the cooperation of his associates.

Gunter Zweig, Ph.D.
Joseph Sherma, Ph.D.
Spring, 1981

PREFACE

In the last decade the most noted application of chromatography has been in the field of drug analysis. Demands for high sensitivity and selectivity in the analysis of drugs have been partly responsible for the development of sensitive and selective detectors and highly efficient columns for both gas (GC) and high-pressure liquid chromatography (HPLC).

As soon as a new drug is developed, the analytical laboratory of the pharmaceutical company proceeds to develop a sensitive analytical procedure to study the pharmacokinetics of the drug. In some cases, alternative analytical procedures are developed simultaneously in a number of centers where the drug is undergoing clinical trials. With rare exceptions, chromatographic procedures are used for the analysis of new drugs. In some instances, when the drug concentration per unit volume of the specimen is very low, immunoassays are also attempted.

In the last decade the role of the clinical chemistry laboratory has been augmented. In addition to providing analyses for different constituents both for diagnosing disease and for demonstrating symptomatic drug overdose, analyses are now being performed to monitor drug treatment. It is believed that the control of epilepsy has improved significantly with the measurement of therapeutic concentrations of antiepileptic drugs. This demand for therapeutic drug monitoring is being extended to more drug classes, e.g., antiarrhythmic drugs, antidepressants, neuroleptics, etc.

The American Society of Clinical Pathologists, the American Society of Clinical Chemists, and a number of European societies have made available voluntary quality control schemes whereby analysts can compare their performance in drug monitoring and detection with other analysts working in the field.

The spectrophotometric or colorimetric procedures used for routine quality control in the production of pharmaceuticals are being replaced by chromatographic procedures. These procedures have the capacity of detecting potentially harmful trace impurities in drugs. However, spectrophotometric or colorimetric procedures are still preferred in clinical laboratories for the rapid detection of some drugs in emergency situations, e.g., salicylates and acetaminophen.

In the last few years, there has been a phenomenal increase in publications describing the use of HPLC; however, GC is still popular and many improvements in instrumentation have been achieved. Thus, it is now quite convenient to use nitrogen-selective detectors in which the salt bead is heated electrically. With the introduction of flexible break-resistant capillary columns, GC has offered a new potential for ultratrace analysis in complex matrices. Another factor in favor of GC is that the mobile phase does not present a disposal problem.

In North America, quantitative thin-layer chromatography (TLC) is not as popular as it is in Europe. One advantage of TLC is the ability to separate a number of samples simultaneously, and the separated spots can be quantitated relatively rapidly by *in situ* densitometry. The spots can be made colored or fluorescent by spraying or dipping the plate in suitable reagents. Postcolumn reactions in HPLC to increase the detection sensitivity are still not very popular. TLC remains the method of choice all over the world for the qualitative detection of drugs of abuse. There have been advances in the manufacture of precoated TLC plates. High-performance TLC plates and chemically bonded reverse-phase TLC plates are now commercially available.

The purpose of this handbook is to provide a reference source of different chromatographic techniques available for the analysis of drugs.

I am grateful to Mrs. Elaine Moore for her skillful assistance in preparing the manuscript. Mrs. Diane Lewis helped to organize the filing system of reprints. Ms. Pamela Woodcock of CRC Press provided all the required editorial assistance.

Ram N. Gupta

THE EDITORS-IN-CHIEF

Gunter Zweig, Ph.D., received his undergraduate and graduate training at the University of Maryland, where he was awarded the Ph.D. in biochemistry in 1952. For two years after his graduation, Dr. Zweig was affiliated with the late R. J. Block, pioneer in paper chromatography of amino acids. Zweig, Block and Le Strange wrote one of the first books on paper chromatography which was published in 1952 by Academic Press and went into three editions, the last one authored by Gunter Zweig and Dr. Joe Sherma, the co-Editor-in-Chief of this Handbook. Paper Chromatography (1952) was also translated into Russian.

From 1953 till 1957, Dr. Zweig was research biochemist at the C. F. Kettering Foundation, Antioch College, Yellow Springs, Ohio, where he pursued research on the path of carbon and sulfur in plants using the then newly developed techniques of autoradiography and paper chromatography. From 1957 till 1965, Dr. Zweig served as lecturer and chemist, University of California, Davis and worked on analytical methods for pesticide residues, mainly by chromatographic techniques. In 1965, Dr. Zweig became Director of Life Sciences, Syracuse University Research Corporation (research on environmental pollution), and in 1973 he became Chief, Environmental Fate Branch, Environmental Protection Agency in Washington, D.C. In 1980, he was appointed Senior Science Advisor in the same agency. During his government career, Dr. Zweig continued his scientific writing and editing. Among his works are (many in collaboration with Dr. Sherma) the now 11-volume series on Analytical Methods for Pesticides and Plant Growth Regulators (Academic Press); the Pesticide Chemistry series for CRC Press; co-editor of Journal of Toxicology and Environmental Health; co-author of basic review on paper and thin-layer chromatography for Analytical Chemistry from 1968-1980; co-author of applied chromatography review on pesticide analysis for Analytical Chemistry, beginning in 1981. Among the scientific honors awarded to Dr. Zweig during his distinguished career are the Wiley Award in 1977, Rothschild Fellowship to the Weizmann Institute in 1963/64; the Bronze Medal by the EPA in 1980. Dr. Zweig has authored or co-authored over 75 scientific papers on diverse subjects in chromatography and biochemistry, besides being the holder of three U.S. patents. At the present time (1980/82), Dr. Zweig is Visiting Scholar in the School of Public Health, University of California, Berkeley, where he is doing research on farmworker safety as related to pesticide exposure.

Joseph Sherma, Ph.D., received a B.S. in Chemistry from Upsala College, East Orange, N.J., in 1955 and a Ph.D. in Analytical Chemistry from Rutgers University in 1958. His thesis research in ion exchange chromatography was under the direction of the late William Rieman III. Dr. Sherma joined the faculty of Lafayette College in September, 1958, and is presently full professor there in charge of two courses in analytical chemistry. At Lafayette he has continued research in chromatography and has additionally worked a total of 12 summers in the field with Harold Strain at the Argonne Nationa Laboratory, James Fritz at Iowa State University, Gunter Zweig at Syracuse University Research Corporation, Joseph Touchstone at the Hospital of the University of Pennsylvania, Brian Bidlingmeyer at Waters Associates, and Thomas Beesley at Whatman, Inc. Dr. Sherma and Dr. Zweig (who is now with U.S. EPA) co-authored Volumes I and II of the *CRC Handbook of Chromatography*, a book on paper chromatography, and 6 volumes of the series *Analytical Methods for Pesticides and Plant Growth Regulators*. Other books in the pesticide series and further volumes of the *CRC Handbook of Chromatography* are being edited with Dr. Zweig, and Dr. Sherma will co-author the Handbook on Pesticide Chromatography. A book on quantitative TLC (Wiley-Interscience) was edited jointly with Dr. Touchstone. Dr. Sherma has been co-author of seven biennial reviews of liquid chromatography (1968-1980) and the 1981 review of pesticide analysis for the journal *Analytical Chemistry*. Dr. Sherma has authored major invited chapters and review papers on chromatography and pesticides in *Chromatographic Reviews* (analysis of fungicides), *Advances in Chromatography* (analysis of non-pesticide pollutants), Heftmann's *Chromatography* (chromatography of pesticides), Race's *Laboratory Medicine* (chromatography in clinical analysis), *Food Analysis: Principles and Techniques* (TLC for food analysis), *Treatise on Analytical Chemistry* (paper and thin layer chromatography), and *CRC Critical Reviews in Analytical Chemistry* (pesticide residue analysis). A general book on thin layer chromatography co-authored by Dr. Sherma is now in press

at Marcel Dekker. Dr. Sherma spent six months in 1972 on sabbatical leave at the EPA Perrine Primate Laboratory, Perrine, Florida, with Dr. T. M. Shafik, and two additional summers (1975, 1976) at the USDA in Beltsville, Maryland, with Melvin Getz doing research on pesticide residue analysis methods development. He spent three months in 1979 on sabbatical leave with Dr. Touchstone developing clinical analytical methods. A total of more than 200 papers, books, book chapters, and oral presentations concerned with column, paper, and thin layer chromatography of metal ions, plant pigments, and other organic and biological compounds; the chromatographic analysis of pesticides; and the history of chromatography have been authored by Dr. Sherma, many in collaboration with various co-workers and students. His major research area at Lafayette is currently quantitative TLC (densitometry), applied mainly to clinical analysis and pesticide residue determinations. Dr. Sherma has written an analytical quality control manual for pesticide analysis under contract with the U.S. EPA and has revised this and the EPA Pesticide Analytical Methods Manual under a four-year contract (EPA) jointly with Dr. M. Beroza of the AOAC. Dr. Sherma has also written an instrumental analysis quality assurance manual and other analytical reports for the U.S. Consumer Product Safety Commission, and is currently preparing a manual on the analysis of food additives for the U.S. FDA, both of these projects also in collaboration with Dr. Beroza of the AOAC. Dr. Sherma taught the first, prototype short course on pesticide analysis, with Henry Enos of the EPA, for the Center for Professional Advancement. He is editor of the Kontes TLC quarterly newsletter and also teaches short courses on TLC for Kontes and the Center for Professional Advancement. He is a consultant for several industrial companies and federal agencies on chemical analysis and chromatography and regularly referees papers for analytical journals and research proposals for government agencies. Dr. Sherma has received two awards for superior teaching at Lafayette College and the 1979 Distinguished Alumnus Award from Upsala College for outstanding achievements as an educator, researcher, author, and editor. He is a member of the ACS, Sigma Xi, Phi Lambda Upsilon, SAS, and AIC.

THE EDITOR

Ram N. Gupta, Ph.D., is Assistant Clinical Chemist at the St. Joseph's Hospital in Hamilton and Associate Professor in the Department of Pathology at McMaster University, in Hamilton.

Dr. Gupta received his M.Sc. degree in 1962 and Ph.D. degree in 1963 in Organic Chemistry from McMaster University. He continued working in the Chemistry Department of McMaster University as a research associate until 1971 when he moved to the Department of Pathology at the same university.

Dr. Gupta has been elected as a fellow of the Chemical Institute of Canada. He is a member of the American Chemical Society, American Association of Clinical Chemists, Canadian Society of Clinical Chemists and the Association of Clinical Biochemists (U.K.). He is the author of more than 40 scientific publications.

His present research interests are the development of chromatographic procedures for the assay of drugs and other biochemicals in biological fluids.

THE CONSULTING EDITOR

Dr. Irving Sunshine is Chief Toxicologist at the Cuyahoga County (Cleveland), Ohio Coroner's Office; Professor of Toxicology in the Department of Pathology and Professor of Clinical Pharmacology in the Department of Medicine at the School of Medicine, Case Western Reserve University; Chief Toxicologist for the University Hospitals in Cleveland, Ohio; Director of the Cleveland Poison Information Center; and Editor-In-Chief for Biosciences for CRC Press, Inc. He is a Diplomate of both the American Board of Clinical Chemistry and The American Board of Forensic Toxicology and is on the Board of Directors of both these organizations.

Born in New York City, he obtained all his formal education in various Colleges of New York University, earning the B.Sc., M.A., and Ph.D. degrees. While earning his Ph.D., he taught chemistry in various colleges in the New York area and during the war, he worked during the "grave yard" shift on a pilot plant for the separation of uranium isotopes as a part of the "The Manhattan Project". His development in toxicology was encouraged by two memorable mentors, Dr. Alexander O. Gettler and Dr. Bernard Brodie.

To my teacher

Professor Ian D. Spenser

TABLE OF CONTENTS

ORGANIZATION OF TABLES AND EXPLANATION OF ABBREVIATIONS

The drugs have been arranged alphabetically according to their generic names. Some of the commonly used synonyms and proprietary names for them are listed in the Appendix and included in the Index. Drugs for which a suitable chromatographic procedure could not be found in the current literature are not included in the handbook. Steroidal drugs will be included in another volume of this series of handbooks.

Gas Chromatography (GC)

Specimen — B = whole blood, plasma, or serum; U = urine; D = dosage form (pharmaceutical preparation, plant material, etc.); NA = not available; CSF = cerebrospinal fluid. Other kinds of specimens have been described without using abbreviations.

S — Sensitivity of the procedure. We have categorized a procedure as 1. if the authors have used that procedure for a single-dose pharmacokinetic study; as 2. if the procedure has been used to measure concentration after multiple dosage. In many instances, a procedure categorized as 2. may be adequate for pharmacokinetic study. Category 3. has been assigned to those procedures which have been used to analyze dosage forms or to obtain a semiquantitative or a qualitative result.

Column — Columns are made of glass unless noted otherwise. Length is given in meters and inner diameter in millimeters.

Packing — In a few cases, the chemical names of liquid phases have been changed to commonly used abbreviations; support size if given in other units has been changed to mesh sizes.

Oven temp. — Oven temperature is given for isothermal operation only. It is merely indicated that the procedure uses temperature programming.

Gas — Gas flow if given in units other than mℓ/min has been indicated by a footnote.

Det. — Detector. FID = Flame Ionization Detector; NPD = Nitrogen Phosphorus Detector, Alkali Flame Ionization Detector, Thermionic Sensitive Detector, or Nitrogen Specific Detector; ECD = Electron Capture Detector; MS-EI = Electron-Impact Mass Spectrometer, and MS-CI = Chemical Ionization Mass Spectrometer. Any other detector used has been indicated without using abbreviations.

RT (min) — Retention time in minutes. This column gives the retention time of the title drug as it appears in the chromatogram of the procedure. It may be the retention time of the parent drug or its derivative if formed during the treatment of derivatization process. A dash (—) indicates that the title drug is not analyzed in the procedure under review, whereas NA indicates that the retention time is not available.

Internal Standard — The names of the compounds used as internal standards are given in full. Any abbreviation used is explained by a footnote. A dash (—) indicates that no internal standard was used in the procedure. The retention time in minutes is given in parentheses as it appears in the chromatogram. It may be of the parent compound or its derivative if formed during the derivatization process.

Deriv. — Derivative. This column indicates that the specimen or its extract at some stage has been subjected to derivatization. A footnote indicates the derivatizing agent when a number of alternative reagents are available to prepare a particular derivative.

Other Compounds (RT) — Metabolites of the parent drug or other similar or unrelated drugs, when analyzed simultaneously with the title drug are listed in this column. Their retention times in minutes are given in parentheses.

Ref. — Reference.

High-Pressure Liquid Chromatography (HPLC)* (See under GC for the explanation of common columns)

Column — Columns are made of steel unless noted otherwise. Length is in centimeters and inner diameter in millimeters. Temperature other than ambient is indicated by a footnote.

Packing — Packing is described by the trade names as used by the authors.

Elution — Unless noted otherwise the procedure uses isocratic elution. In this column, the eluting solvent is given a number and the corresponding solvent is described at the end of the table.

Flow Rate — Flow rate given in other units has been changed to mℓ/min; a footnote indicates that only the pump pressure is given.

Det. — Detection. Wavelength (nm) for ultraviolet (UV) or visible absorption is given. Fl = fluorescence; λ_{ex} = excitation wavelength, λ_{fl} = emission wavelength. Other modes of detection are indicated without the use of abbreviations.

Thin-Layer Chromatography (TLC) (See under GC and HPLC for the explanation of common columns)

Plate — Unless otherwise noted, plates are made of glass. "Laboratory" indicates that the plates have been coated by the authors in their laboratory. A few cases of paper chromatography have also been included under TLC. The manufacturer of paper and the kind of paper used are indicated under plate.

Solvent — Developing solvent is given a number, and the corresponding solvent is described at the end of the table.

Post-Separation Treatment — Sp: The plate is sprayed with the described reagent. D: The plate is dipped in the described reagent. E: The plate is exposed to the vapors of the described reagent.

Det. — Qualitative detection is indicated as Visual. Wavelength (nm) for short or long wave UV lamp is given when fluorescence or quenching of fluorescence is observed under a UV light. When the plate is scanned with a densitometer for quantitative analysis, the mode of scanning is indicated as reflectance, transmission or reflectance/transmission for simultaneous mode. Wavelength (nm) for scanning mode is given and for fluorescence scanning both excitation wavelength (λex) and emission wavelength (λfl) are given.

* Also known as High Performance Liquid Chromatography.

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Gas Chromatography

Specimen (mℓ)	S	Column m × mm	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
B (1)	2	1.8 × 2	5% FFAP Gas Chrom® W (80/100)	220	Nitrogen (35)	FID	4.3	3-Methyl-3-phenylbutyric acid (2.9)	—	—	1
B (0.1)	1	1.8 × 3	10% 3-Cyanopropyl-silicone Gas Chrom® Q (100/120)	190	Argon 95 + Methane (55)	ECD	9.1	4-Isobutyl-phenyl acetic acid (13.3)	Pentafluorobenzyl	—	2
B (2)	2	1.8 × 4	10% SP-216-PS Supelcoport (100/120)	200	Nitrogen (80)	FID	8.9	Myristic acid (3.6)	—	—	3

High-Pressure Liquid Chromatography

Specimen (mℓ)	S	Column cm × mm	Packing (μm)	Elution	Flow rate (mℓ/min)	Det. (nm)	RT (min)	Internal standard (RT)	Other compounds (RT)	Ref.
B (1)	2	25 × 4.6	LiChrosorb® RP-18 (10)	E-1	2	UV (220)	6.5	Cinnamic acid (2.7)	—	4

Note: E-1 = 0.01 *M* Orthophosphoric acid + methanol.
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Gas Chromatography

Specimen (mℓ)	S	Column m × mm	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
B (5)	2	2 × 4	6% Dexsil® 300 GC Chromosorb® W (NA)	200	Nitrogen (60)	FID	NA	—	Methylsilyl	Ibuprofen (NA)	1[a]

[a] Analysis has also been carried out by thin-layer chromatography.

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