CRC Handbook of Chromatography

Carbohydrates

Volume II

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CRC Press
Boca Raton Ann Arbor Boston

Library of Congress Cataloging-in-Publication Data
(Revised for volume 2)

Carbohydrates.

(CRC handbook of chromatography) (CRC series in chromatography)
"Editor-in-chief, Joseph Sherma"—Vol. 2 t.p.
Includes bibliographical references.
1. Chromatographic analysis—Handbooks, manuals, etc.
2. Carbohydrates. I. Churms, Shirley C., 1938-
II. Sherma, Joseph. III. Series.
QD117.C5C37 547.7'8046 80-29541
ISBN 0-8493-3061-0 (v. 1)
ISBN 0-8493-3062-9 (v. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-3062-9

Library of Congress Card Number 80-29541
Printed in the United States

CRC HANDBOOK OF CHROMATOGRAPHY

SERIES PREFACE

The *CRC Handbook of Chromatography* series began with two volumes that were published in 1972. These volumes, written by Dr. Gunter Zweig and myself, covered all compound types and chromatographic methods. The first volume on carbohydrates by Shirley C. Churms, published in 1982, was the third volume in the series, and the first in the current format in which each book is devoted to a particular class of compounds or chromatographic method. The first volume by Dr. Churms is among the best selling and most highly acclaimed in the entire series, which now numbers 25 volumes overall with the publication of this second book on carbohydrates. Dr. Churms is an internationally recognized expert on the chromatography of carbohydrates and I am gratified that she was willing and able to update her original coverage of the field at this time.

Future volumes in the *Handbook of Chromatography* series are now being planned or written. These include additional volumes on pesticides, polymers, lipids, steroids, and hydrocarbons, and coverage for the first time of toxins, vitamins, and chiral separations. I would appreciate hearing from readers who have suggestions for topics or authors for subsequent volumes in the series. I would also be interested in receiving comments or corrections on the present volume or any others that have already been published.

Joseph Sherma, Ph.D.
Easton, PA
August 1990

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PREFACE

Since the compilation of *CRC Handbook of Chromatography: Carbohydrates*, Volume I, which was completed early in 1979, there have been many developments in the field, particularly in liquid chromatography, owing to the rapid proliferation of HPLC methods during the past decade. Gas chromatography, too, has been revolutionized by the increasing use of capillary columns and the advent of chiral phases and some novel methods of derivatization, which have greatly improved resolution of related sugars, including enantiomeric pairs. Derivatization has become important in HPLC, also, owing to the greatly enhanced sensitivity of detection possible by use of chromophoric and, especially, fluorescent derivatives; this applies equally to PC and TLC. In addition to pre- and postcolumn derivatization, other methods of detection have been developed for HPLC: in particular, coupling of HPLC and MS is now a reality and electrochemical methods have become important, especially in the relatively new technique of ion chromatography, the application of which to analysis of mono- and oligosaccharides has produced some remarkable results. Another new technique which shows great promise in the analysis of oligosaccharides and glycoconjugates is supercritical fluid chromatography.

This new handbook, which has been compiled almost entirely from literature published during the period 1979 to 1989, has been structured to take cognizance of these developments. Since HPLC has been the main area of growth during the period under review, the section on liquid chromatography data has been greatly expanded, as have those on detection and derivatization methods in liquid chromatography. The vital importance of mass spectrometry in GC analysis is reflected in the inclusion of relevant mass spectral data for various derivatives used, including some of the newer ones. These new methods of derivatization, and improvements in the older methods, are given in detail in Section III. Although application of supercritical fluid chromatography to carbohydrates is not yet widespread, its potential is great enough to justify the inclusion of a new subsection covering the data available at present, and the recent coupling of SFC to MS is discussed in Section II.

To allow some more space for all this new material and a comprehensive list of important literature references relating to the new methods, it has been decided to omit the separate section on chromatographic materials that was included in Volume I. Developments in this area are so rapid at present that the currency of any such directory will inevitably be of short duration, and therefore to attempt to update the list published in Volume I seemed futile. Instead, relevant details of the packings or plates used are given in the footnotes to tables showing data obtained with these chromatographic materials, and the names of manufacturers, from whom further details can be obtained, are also appended in such footnotes.

Shirley C. Churms, Ph.D.
October 1989

THE EDITOR-IN-CHIEF

Joseph Sherma, Ph.D., received a B.S. in chemistry from Upsala College, East Orange, NJ in 1955 and a Ph.D. in analytical chemistry from Rutgers University, New Brunswick, NJ, in 1958 carrying on his thesis research in ion exchange chromatography under the direction of the late William Rieman III. Dr. Sherma joined the faculty of Lafayette College, Easton, PA in September, 1958, and is presently Charles A. Dana Professor and Head of the Chemistry Department. At Lafayette, he teaches three courses in analytical chemistry.

Dr. Sherma, independently and with others, has written or edited about 350 research papers, chapters, books, and reviews involving chromatography and other analytical methodology. In addition to being Editor-in-Chief of the CRC *Handbook of Chromatography* series, he co-edits the series *Analytical Methods for Pesticides and Plant Growth Regulators*, previously published by Academic Press and now by CRC Press. He is co-editor for residues and trace elements of the *Journal of the Association of Official Analytical Chemists* and a member of the editorial board of the *Journal of Planar Chromatography*. He is consultant on analytical methodology for many companies and federal agencies.

Dr. Sherma has received three awards for superior teaching and scholarship at Lafayette College and the E. Emmet Reid Award for excellence in teaching, presented at the Middle Atlantic Regional meeting of the ACS. He is a member of the ACS, AIC, Phi Lambda Upsilon, Sigma Xi, and AOAC. Dr. Sherma's current research interests are in quantitative TLC, mainly applied to clinical analysis, pesticide residues, lipids, and food additives.

DEDICATION

This volume is dedicated to Professor Alistair M. Stephen, in deep gratitude for many happy and productive years under his inspiring leadership, and to colleagues, past and present, in the Carbohydrate Research Group at the University of Cape Town, whose cooperation and camaraderie have helped to ease the high pressure of carbohydrate chromatography

Shirley C. Churms
October 1989

THE EDITOR

Shirley C. Churms (born Macintosh) graduated at the University of Cape Town, South Africa, and then undertook research, at the same University, on cation-exchange processes in aqueous monoethanolamine, for which the degree of Ph.D. was awarded in 1962. She spent her postdoctoral year at the Imperial College of Science and Technology in London, England, as the holder of the Ohio State Fellowship, awarded by the International Federation of University Women; during this period she carried out an extensive survey of the properties of inorganic ion-exchangers and commenced an investigation of cation- and anion-exchange on amphoteric hydrated alumina. In 1964 she returned to the University of Cape Town and during the next 4 years she continued her research on inorganic ion-exchangers, supervised a group of research students working on various aspects of ion-exchange, and lectured in the Department of Chemistry at the University. In 1967 the work of the ion-exchange group was recognized by the South African Chemical Institute in the award of the African Explosives and Chemical Industry medal for one of its publications

A change in research interests occurred in 1968, when Dr. Churms was appointed Research Associate in the newly formed Carbohydrate Chemistry Research Unit, sponsored by the South African Council for Scientific and Industrial Research, which functioned in the Department of Chemistry (from 1974 until 1988 in the separate Department of Organic Chemistry), under the direction of Professor Alistair M. Stephen. The Unit, which became the Carbohydrate Research Group in 1985, used chromatographic methods, particularly gas chromatography, very extensively in analyses of the products of degradative studies of polysaccharides, and Dr. Churms developed a special interest in the application of gel-permeation (now steric-exclusion) chromatography in these structural studies. Publication of a major review on this topic in *Advances in Carbohydrate Chemistry and Biochemistry* in 1970 resulted in an invitation from Dr. Erich Heftmann to contribute a chapter on chromatography of carbohydrates to the Third Edition of the well-known book on chromatography of which he is the Editor. She has subsequently remained a co-author in the Fourth Edition and, recently, the Fifth Edition of this seminal work. She was Volume Editor, and a major contributor, for Volume I of the present Handbook, published by CRC Press in 1982. To date, Dr. Churms has been the author of seven major reviews and, in addition to the chapters for Heftmann's *Chromatography* already mentioned, co-author (with Professor Stephen) of a further three chapters to be published in books dealing with various aspects of polysaccharide chemistry. She has been author or co-author of over 50 published papers and numerous presentations at national and international conferences, and is currently a member of the Editorial Board for the Symposium Volumes of the *Journal of Chromatography*.

ACKNOWLEDGMENTS

The financial support of the University of Cape Town and the Foundation for Research Development of the South African Council for Scientific and Industrial Research is gratefully acknowledged. I thank the University of Cape Town for a grant of study leave during the writing of this book and Professor Alistair M. Stephen for his patience and encouragement, as well as for many helpful discussions of aspects of chromatography. I am indebted also to Mrs. Evelyn Rossmeisl, of the Inter-Library Loans Department in the University of Cape Town Library, for her invaluable assistance in obtaining some of the literature reviewed in this book, and to Dr. Bill Edwards, of the Dionex Corporation, Sunnyvale, California, for kindly sending me some literature on ion chromatography from sources not otherwise accessible to me.

Special thanks are due to Mrs. Patsy Alexander and Mrs. Jean Goode for their excellent processing of the manuscript. The cooperation of the editorial staff of CRC Press is also gratefully acknowledged.

LIST OF ABBREVIATIONS

Ac = acetyl
Ara = arabinose
Asn = asparagine
atm = atmosphere
CI = chemical ionization
d = days
Da = daltons
DEAE = diethylamino ethyl
DP = degree of polymerization
EI = electron impact
Et = ethoxyl
f = furanose
FAB = fast atom bombardment
fmol = femtomole
FI = flame ionization detector
Fru = fructose
Fuc = fucose
Gal = galactose
GalA = galacturonic acid
GalN = 2-amino-2-deoxygalactose (galactosamine)
GalNAc = 2-acetamido-2-deoxygalactose (*N*-acetylgalactosamine)
GalNAc-ol = 2-acetamido-2-deoxygalactitol (*N*-acetylgalactosaminitol)
GC = gas chromatography
GLC = gas liquid chromatography
Glc = glucose
GlcA = glucuronic acid
GlcN = 2-amino-2-deoxyglucose (glucosamine)
GlcNAc = 2-acetamido-2-deoxyglucose (*N*-acetylglucosamine)
GlcNAc-ol = 2-acetamido-2-deoxyglucitol (*N*-acetylglucosaminitol)
Glc-ol = glucitol
Gly = glycine
h = hour(s)
HPLC = high-performance liquid chromatography
I.D. = inner diameter
k′ = capacity factor = $(t_r - t_o) / t_o$
K_{av} = SEC partition coefficient = $(V_e - V_o) / (V_t - V_o)$
K_d = alternative SEC partition coefficient = $(V_e - V_o) / V_i$
kPa = kilopascal
LC = liquid chromatography
M = molecular weight (molar mass); in Table EL I = mobility
$\overline{M}_n$ = number-average molecular weight
$\overline{M}_w$ = weight-average molecular weight
Man = mannose
mCi = millicurie
Me = methyl
meq = milliequivalent
min = minute(s)
m*M* = millimolar
mmol = millimole

MPa = megapascal (S.I. unit of pressure)
ms = millisecond
MS = mass spectrometry
m/z = mass/charge ratio
n.a. = not available
NeuAc = *N*-acetylneuraminic acid (sialic acid)
ng = nanogram
nm = nanometer
nmol = nanomole
p = pyranose
PC = paper chromatography
pg = picogram
pmol = picomole
ppb = parts per billion
ppm = parts per million
r = relative retention time
R_F = migration distance relative to solvent front
R_{Glc} = migration distance relative to glucose
Rha = rhamnose
RI = refractive index detector
s = second
SCOT = support-coated open tubular
SEC = steric-exclusion chromatography
Ser = serine
SFC = supercritical fluid chromatography
SS = stainless steel
Thr = theonine
TLC = thin-layer chromatography
TMS = trimethylsilyl
t_o = retention time of solvent front
t_r = retention time
Tyr = tyrosine
U = units (of enzyme)
UV = ultraviolet absorbance
v = volume
V_e = elution volume
V_i = internal solvent volume in SEC
V_o = void volume
V_t = total volume in SEC
w = weight
WCOT = wall-coated open tubular
Xyl = xylose

Other abbreviations are defined in the test.

TABLE OF CONTENTS

Volume II

Section I

Chromatographic Data

Section I.I.

GAS CHROMATOGRAPHY TABLES

As in Volume I, these tables are grouped according to the type of volatile derivative used in gas chromatography (GC), with the derivatives arranged in alphabetical order for easy reference. In the present volume the emphasis is mainly on capillary GC, although in many cases retention data for chromatography on equivalent packed columns are included for comparison. The new tables do not necessarily supersede those in Volume I; many should be regarded rather as supplementary to the earlier compilations, as indicated in footnotes to the present tables. A few of the derivatives included in Volume I have not been used extensively in recent years and are, therefore, omitted here, while several new derivatives are included for the first time in this volume. Of special interest are those permitting resolution of enantiomers, which has also been achieved by the use of recently developed chiral phases. Tables devoted to this important aspect will be found at the end of Section I.I.

TABLE GC 1
Peracetylated Alditols, Aminodeoxyalditols, and Inositols: GLC on Packed Columns

Packing	P1	P1	P2	P3	P4
Temperature (°C)	T1	T2	T3	195	195
Gas; flow rate (ml/min)	N_2; 40	He; 20	N_2; 20	N_2; 45	N_2; 40
Column					
Length, cm	158	100	183	200	250
Diameter (I.D.), cm	0.4	0.2	0.18	0.3	0.4
Form	Packed	Packed	Packed	Packed	Packed
Material	Glass	Glass	Glass	Glass	SS
Detector	FI	FI	FI	FI	FI
Reference	1	2	3	4	5
Parent compound	**r[a]**	**r[a]**	**r[b]**	**r[c]**	**r[a]**
Rhamnose	—	—	—	0.18	—
Fucose	0.32	—	—	0.20	—
Ribose	—	—	—	0.27	—
Arabinose	0.46	0.52	—	0.30	—
Xylose	0.59	0.64	—	0.39	—
Mannose	0.77	0.79	—	0.78	0.69
Galactose	0.83	0.84	—	0.93	0.79
Glucose	0.91	0.91	—	1.00	0.87
2-Amino-2-deoxyglucose	0.57[d]	—	—	0.46[e]	—
2-Amino-2-deoxymannose	—	—	—	0.48[e]	—
2-Amino-2-deoxygalactose	0.73[d]	—	—	0.54[e]	—
2-Acetamido-2-deoxyglucitol	—	—	1.44[f]	—	—
2-Acetamido-2-deoxygalactitol	—	—	1.55[f]	—	—
2-Acetamido-2-deoxymannitol	—	—	1.55[f]	—	—
myo-Inositol	1.00	1.00	—	—	1.00
chiro-Inositol	—	—	—	—	0.72
neo-Inositol	—	—	—	—	0.81
muco-Inositol	—	—	—	—	1.17
scyllo-Inositol	—	—	—	—	1.53

[a] t_r relative to *myo*-inositol hexaacetate (39.2 min,[1] 8.3 min,[2] 25 min[5]).
[b] t_r relative to peracetylated D-*gluco* heptitol (10.5 min).
[c] t_r relative to glucitol hexaacetate (53.94 min).
[d] As 2,5-anhydrohexitol acetates (2,5-anhydromannitol and -talitol from 2-amino-2-deoxyglucose and -galactose) produced by deamination.
[e] *N*-methylated ($HCHO/NaBH_3CN$) between reduction and acetylation.
[f] For simultaneous analysis of acetamidodeoxyhexoses as *O*-methyloxime acetates see Table GC 7, Reference 3.

Packing
P1 = 3% SP-2340 on Supelcoport® (100—120 mesh).
P2 = 3% diethylene glycol adipate, stabilized, on Chromosorb® W HP (100—120 mesh).
P3 = 2% EGSS-X on Chromosorb® W AW DMCS (60—80 mesh).
P4 = 0.5% QF-1 + 0.5% LAC-2R-446 on Chromosorb® W HP (80—100 mesh).

Temperature
T1 = 150 → 220°C at 2°C/min.
T2 = 190 → 260°C at 5°C/min.
T3 = 210 → 240°C at 2°C/min.

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