

CRC

HANDBOOK
of
CHROMATOGRAPHY

Gunter Zweig
Joseph Sherma
Editors-in-Chief

Stanley Blackburn
Peptides — Volume I

CRC

PRESS

CRC Handbook of Chromatography: Peptides

Volume I

Author

Stanley Blackburn, Ph.D., C. Chem., F.R.S.C.

Leeds, England

CRC Series in Chromatography
Editors-in-Chief

Gunter Zweig, Ph.D.

President
Zweig Associates
Washington, D.C.

Joseph Sherma, Ph.D.

Professor of Chemistry
Lafayette College
Easton, Pennsylvania



CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data

Blackburn, S. (Stanley)
Peptides.

(CRC series of chromatography)

Bibliography: v. 1,
Includes index.

1. Peptides--Analysis. 2. Chromatographic analysis.

I. Title. II. Series.

QP552.P4B55 1986 547.7'56 86-6133

ISBN-0-8493-3065-3

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

All rights reserved. This book, or any parts thereof, may not be reproduced in any form without written consent from the publisher.

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

© 1986 by CRC Press, Inc.

International Standard Book Number 0-8493-3065-3 (v. 1)

Library of Congress Card Number 86-6133

Printed in the United States

CRC Series in Chromatography

Editors-in-Chief

Gunter Zweig, Ph.D. and Joseph Sherma, Ph.D.

General Data and Principles

Gunter Zweig, Ph.D. and
Joseph Sherma, Ph.D.

Lipids

Helmut K. Mangold, Dr. rer. nat.

Hydrocarbons

Walter L. Zielinski, Jr., Ph.D.

Carbohydrates

Shirley C. Churms, Ph.D.

Inorganics

M. Qureshi, Ph.D.

Drugs

Ram Gupta, Ph.D.

Phenols and Organic Acids

Toshihiko Hanai, Ph.D.

Terpenoids

Carmine J. Coscia, Ph.D.

Amino Acids and Amines

S. Blackburn, Ph.D.

Steroids

Joseph C. Touchstone, Ph.D.

Polymers

Charles G. Smith,
Norman E. Skelly, Ph.D.,
Carl D. Chow, and Richard A. Solomon

Pesticides and Related Organic Chemicals

Joseph Sherma, Ph.D. and
Joanne Follweiler, Ph.D.

Plant Pigments

Hans-Peter Köst, Ph.D.

**Nucleic Acids and
Related Compounds**
Ante M. Krstulovic, Ph.D.

SERIES PREFACE

The present volume of the Handbook of Chromatography series on the subject of peptides by Dr. Stanley Blackburn is a continuation and extension of Dr. Blackburn's earlier book on amino acids and amines (CRC, 1983). We agree with Dr. Blackburn that the topic of peptide separation and identification has grown exponentially during the past 10 years since Volumes I and II of this series first appeared. There has been an actual revolution in the thinking and practice of protein characterization and amino acid sequence determination. Peptide identification and resolution have played a major role in these endeavors. We, as series editors, therefore, are grateful to the immense task that Dr. Blackburn has undertaken single-handedly to produce a most valuable and important volume in this series.

Other volumes of the Handbook of Chromatography that have previously appeared cover topics such as phenols and organic acids, drugs, terpenoids, pesticides, and lipids and fatty acids. Volumes that will appear in the near future will cover plant pigments, hydrocarbons, nucleic acid and derivatives, and inorganic ions and compounds.

We welcome suggestions and ideas from our faithful readers for other topics that should be included in the Handbook of Chromatography and prospective authors. Specific suggestions and corrections pertaining to this volume should be directed to the volume editor, Dr. Stanley Blackburn.

Gunter Zweig
Joseph Sherma

PREFACE

The past few years have seen a tremendous increase in the number of publications describing the chromatography of peptides. This is due in part to the overall increase in chromatographic literature, reflected in a greater number of special journals, but other factors have also played a part. One such factor is the discovery of the opioid peptides and enkephalins, together with the need for efficient methods for their separation and estimation. Another is the introduction of greatly improved methods of peptide fractionation, such as the use of alkyl-bonded stationary phases for liquid chromatography. A new volume of the Handbook of Chromatography dealing with peptides is therefore timely.

The present book represents a logical extension of the *Handbook of Chromatography: Amino Acids and Amines* (Blackburn, 1983) to the topic of peptides. It supplements data given in Volume I of the Handbook of Chromatography and covers the literature since 1970. In addition to the tables of chromatographic data, sections of the volume describe methods of sample preparation and derivatization and detection reagents. A number of articles describing techniques applicable to peptide separation, including chromatographic procedures for peptide separation, fluorescence methods of peptide detection, methods of peptide mapping, the prediction of retention times and gel permeation chromatography supplement the information on methods and techniques presented in Volume II of the Handbook of Chromatography. A book directory gives information on sources for further detailed reading, while chromatographic materials listed in another section will direct the reader to commercial sources of supply.

S. Blackburn

THE EDITORS-IN-CHIEF

Gunter Zweig, Ph.D., received his undergraduate training at the University of Maryland, College Park, where he was awarded the Ph.D. in biochemistry in 1952. Two years following 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 series. *Paper Chromatography* (1952) was also translated into Russian.

From 1953 to 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 to 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, New York (research on environmental pollution), and in 1973 he became Chief, Environmental Fate Branch, Environmental Protection Agency (EPA) in Washington, D.C. From 1980 to 1984 Dr. Zweig was Visiting Research Chemist in the School of Public Health, University of California, Berkeley, where he was doing research on farmworker safety as related to pesticide exposure.

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* (published by Academic Press); the pesticide book 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 to 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 80 scientific papers on diverse subjects in chromatography and biochemistry, besides being the holder of three U.S. patents. In 1985, Dr. Zweig became president of Zweig Associates, Consultants in Arlington, Va.

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 Charles A. Dana Professor and Head of the Chemistry Department. He is in charge of two courses in analytical chemistry, quantitative analysis and instrumental analysis. At Lafayette he has continued research in chromatography and had additionally worked a total of 14 summers in the field with Harold Strain at the Argonne National 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. and Advanced Separation Technologies, Inc.

Dr. Sherma and Dr. Zweig co-authored or co-edited the original Volumes I and II of the *CRC Handbook of Chromatography*, a book on paper chromatography, seven volumes of the series *Analytical Methods for Pesticides and Plant Growth Regulators*, and the Handbooks of Chromatography of drugs, carbohydrates, polymers, phenols and organic acids, amino acids and amines, pesticides, terpenoids, and lipids. Other books in the pesticide series and further volumes of the *CRC Handbook of Chromatography* are being edited with Dr. Zweig,

and Dr. Sherma has co-authored the handbook on pesticide chromatography. Books on quantitative TLC and advances in TLC were edited jointly with Dr. Touchstone. A general book on TLC was written with Dr. Bernard Fried, the second edition of which is now being written. Dr. Sherma has been co-author of nine biennial reviews of column and thin layer chromatography (1968—1984) and the 1981, 1983, and 1985 reviews of pesticide analysis for the ACS journal *Analytical Chemistry*.

Dr. Sherma has written major invited chapters and review papers on chromatography and pesticides in *Chromatographic Reviews* (analysis of fungicides), *Advances in Chromatography* (analysis of nonpesticide 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), *CRC Critical Reviews in Analytical Chemistry* (pesticide residue analysis), *Comprehensive Biochemistry* (flat bed techniques), *Inorganic Chromatographic Analysis* (thin layer chromatography), *Journal of Liquid Chromatography* (advances in quantitative pesticide TLC), and *Preparative Liquid Chromatography* (strategy of preparative TLC). Dr. Sherma is editor for residues and elements of the AOAC and is scientific coordinator of the AOAC.

Dr. Sherma spent 6 months in 1972 on sabbatical leave at the EPA Perrine Primate Laboratory, Perrine, Fla., with Dr. T. M. Shafik, two summers (1975, 1976) at the USDA in Beltsville, Md. with Melvin Getz doing research on pesticide residue analysis methods development, and one summer (1984) in the food safety research laboratory of the Eastern Regional Research Center, Philadelphia, with Daniel Schwartz doing research on the isolation and analysis of mutagens in cooked meat. He spent three months in 1979 on sabbatical leave with Dr. Touchstone developing clinical analytical methods. A total of more than 270 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 and food additive determinations.

Dr. Sherma has written an analytical quality control manual for pesticide analysis under contract with the USEPA and has revised this and the EPA Pesticide Analytical Methods Manual under a 4-year contract 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 a manual on the analysis of food additives for the FDA, both of these projects as technical editor for the AOAC. He is preparing three additional FDA manuals on animal drug and food additives analysis, and analytical field operations.

Dr. Sherma taught the first prototype short course on pesticide analysis, with Henry Enos of the EPA, for the Center for Professional Advancement. He was editor of the Kontes TLC quarterly newsletter for 6 years and also has taught short courses on TLC for Knotes and the Center for Professional Advancement. He is a consultant for numerous industrial companies and federal agencies on chemical analysis and chromatography and regularly referees papers for analytical journals and research proposals for government agencies. At Lafayette, Dr. Sherma, in addition to analytical chemistry, teaches general chemistry and a course in thin layer chromatography.

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, AIC, and AOAC.

THE AUTHOR

Dr. Stanley Blackburn gained both his Honours B.Sc. degree in Chemistry and his Ph.D. degree in Organic Chemistry at the University of Leeds, England. He is a former research scientist at the Wool Industries Research Association, Leeds, where his research interests included the development of chromatographic techniques, the structure and amino acid sequence of the proteins of wool keratin, and the end group determination of peptides and proteins.

Dr. Blackburn is a Chartered Chemist, a Fellow of the Royal Society of Chemistry, and a member of the Biochemical Society and the American Chemical Society. His current work is centered on scientific writing and documentation. He has written more than 30 scientific papers dealing with protein analysis and structure and is the author of several texts, including *Amino Acid Determination: Methods and Techniques*, *Protein Sequence Determination: Methods and Techniques*, and *Enzyme Structure and Function*, all published by Marcel Dekker. He is also the author of the recently published *CRC Handbook of Chromatography: Amino Acids and Amines*.

TABLE OF CONTENTS

Section I: Tables

I.I.	Gas Chromatography Tables	3
I.II.	Liquid Chromatography Tables	11
I.III.	Paper Chromatography Tables	131
I.IV.	Thin Layer Chromatography Tables.....	135

Section II: Techniques

II.I.	High Performance Liquid Chromatography of Peptides	199
II.II.	Gel Permeation Chromatography of Peptides.....	215
II.III.	Techniques of Peptide Mapping	225
II.IV.	The Prediction of Peptide Retention Times in High Performance Liquid Chromatography (HPLC)	231
II.V.	Fluorescence Techniques for the Detection of Peptides	237
II.VI.	The Electrochemical Detection of Peptides.....	247

Section III:	Detection Reagents.....	263
--------------	-------------------------	-----

Section IV:	Methods of Sample Preparation Including Derivatization.....	281
-------------	---	-----

Section V:	Products and Sources of Chromatographic Materials	333
------------	---	-----

Section VI:	Chromatography Book Directory	341
-------------	-------------------------------------	-----

Section VII:	Reviews of Chromatographic Methods and Equipment.....	347
--------------	---	-----

Section VIII:	Indexes.....	347
---------------	--------------	-----

Section I *Tables*

- I.I. Gas Chromatography Tables
- I.II. Liquid Chromatography Tables
- I.III. Paper Chromatography Tables
- I.IV. Thin Layer Chromatography Tables

Section I.I.

GAS CHROMATOGRAPHY TABLES

Wherever possible, tables are arranged according to classes of chemical compounds. This was not always possible when different chemical compounds were chromatographed under the same experimental conditions. The reader is referred to the compound index for specific compounds that may appear in different tables.

Table GC 1
TRIMETHYLSILYL DERIVATIVES OF
PEPTIDES

Packing	P1
Temperature	T1
Gas	He
Column	
Length (m)	12
Diameter (mm, I.D.)	0.2
Form	Capillary
Material	Fused silica
Detector	FID
Sequence peak no.	Dipeptide
1	Gly-Gly
2	L-Val-Gly
3	Gly-L-Leu
	L-Leu-Gly
4	L-Ala-L-Ala
5	Gly-L-Ser
6	Gly-L-Thr
7	L-Ala-L-Val
8	L-Ala-L-Leu
9	L-Ala-L-Ile
	L-Leu-L-Ala
10	α -L-Asp-Gly
11	Gly-L-Met
	L-Met-Gly
12	Gly-L-Asp
13	Gly-L-Ile
14	L-Val-L-Val
	L-Ser-L-Ala
15	DL-Ala-DL-Ser
16	DL-Ala-DL-Ser
17	L-Ala-L-Thr
18	Gly-L-Ser
19	L-Ala-L-Asn
20	Gly-L-Thr
21	Gly-L-Phe
	L-Phe-Gly
22	Gly-L-Glu
23	Gly-L-Val
	L-Leu-L-Leu
24	Gly-L-Leu
25	L-Ala-L-Asp
26	Gly-L-Ile
	L-Ala-L-Asn
27	L-Leu-L-Ser
28	L-Ala-L-Met
29	L-Met-L-Ala
30	L-Ala-L-Glu
31	L-Phe-L-Ala
32	L-Ala-L-Phe
33	γ -L-Glu-L-Leu
34	Gly-L-Asp
	L-His-Gly
35	L-Ser-L-Met
36	Gly-L-Met

Table GC 1 (continued)
TRIMETHYLSILYL DERIVATIVES OF
PEPTIDES

Sequence peak no.	Dipeptide
37	L-Phe-L-Val
38	L-Val-L-Phe
39	DL-Leu-DL-Phe
40	Gly-L-Glu
41	DL-Leu-DL-Phe
42	L-Ser-L-Phe
43	Gly-L-Phe
44	L-His-L-Ala
45	L-Ala-L-His
46	L-Met-L-Met
47	L-Ala-L-Tyr
48	L-Tyr-L-Ala
49	L-Tyr-Gly
50	L-Trp-Gly
51	L-Met-L-Phe
52	L-His-L-Ser
53	Gly-L-His
54	L-Phe-L-Phe
55	L-Trp-L-Ala

Packing: PI = SP-2100 (methyl silicone liquid phase) coating of capillary column.

Temperature: T1 = Programed at 4°C/min from 100 to 160°C, 6°C/min from 160 to 230°C and maintained at 230°C for 15 min.

REFERENCE

1. Dizdaroğlu, M. and Simic, M. G., *Anal. Biochem.*, 108, 269, 1980.

Reproduced by permission of Academic Press, Inc.

Table GC 2
TRIMETHYLSILYL DERIVATIVES OF
DIPEPTIDE DIASTEREOMERS

Packing	PI
Temperature	T1
Gas	He
Column	
Length (m)	12
Diameter (mm, I.D.)	0.2
Form	Capillary
Material	Fused silica
Detector	FID

Sequence peak no. (see Figure GC 1)	Dipeptide
1	L-Ala-L-Ala
	D-Ala-D-Ala
2	L-Ala-D-Ala

Table GC 2 (continued)
TRIMETHYLSILYL DERIVATIVES OF
DIPEPTIDE DIASTEREOMERS

Sequence peak no. (see Figure GC 1)	Dipeptide
	D-Ala-L-Ala
3	L-Ala-L-Val
	D-Ala-D-Val
4	L-Ala-D-Val ^a
	D-Ala-L-Val ^a
5	L-Ala-L-Leu
	D-Ala-D-Leu ^a
6	L-Ala-D-Leu ^a
	D-Ala-L-Leu ^a
7	L-Ala-L-Ser
	D-Ala-D-Ser ^a
8	L-Ala-D-Ser ^a
	D-Ala-L-Ser ^a
9	L-Leu-L-Val
	D-Leu-D-Val ^a
10	L-Leu-D-Val ^a
	D-Leu-L-Val ^a
11	L-Leu-L-Leu
	D-Leu-D-Leu
12	L-Leu-D-Leu
	D-Leu-L-Leu
13	L-Ala-L-Phe
	D-Ala-D-Phe
14	D-Ala-L-Phe
15	L-Leu-L-Phe
	D-Leu-D-Phe ^a
16	L-Leu-D-Phe ^a
	D-Leu-L-Phe ^a
17	L-Leu-L-Tyr
18	D-Leu-L-Tyr

^a The assignment of the peaks corresponding to these compounds was based on the order of elution of the other dipeptides.

Packing: P1 = SE-54 (5% phenyl, 1% vinylmethylsilicone gum, siloxane deactivated) coating a capillary column.

Temperature: T1 = Programed at 2°C/min from 70 to 150°C, then at 3°C/min from 150 to 250°C.

REFERENCE

1. Dizdaroglu, M. and Simic, M. G., *J. Chromatogr.*, 244, 293, 1982.

Reproduced by permission of Elsevier Science Publishers B.V.

Table GC 3
RETENTION INDEX INCREMENTS OF AMINO ACID RESIDUES IN
OLIGOPEPTIDE-DERIVED O-TRIMETHYLSILYLATED PERFLUORO-
DIDEUTEROALKYL POLYAMINO ALCOHOLS

The amino acid sequencing of peptides with up to 30 amino acid residues can be carried out by gas chromatography/mass spectrometry (GC/MS). Key steps in the procedure are a partial hydrolysis to small peptides followed by derivatization, separation of the derivatives by GC, and finally identification by MS. The retention indexes of particular peptide derivatives can be related to the amino acid composition of the original oligopeptide and thus can be used as a reliable parameter for identification. The retention indexes of derivatives can be approximately calculated by adding increments which are assigned to each amino acid residue. The retention index increments are derived from the retention indexes of known oligopeptides.

Amino acid residue	Retention index increment
Ala	335
Gly	340
Val	425
Leu	475
Ile	475
Pro	495
Pro*	610
< Glu	610
Hyp	650
Ser	540
Thr	535
Asn	640
Asp	640
Gln	740
Glu	740
Met	825
Arg	705
Arg*	805
Lys	805
Phe	960
AEtCys	995
CMCys	1020
BzlCys	1285
Tyr	1225
His	1340
Trp	1660

Abbreviations: Pro*, derivative containing a pyrrolidine ring; Arg*, ω -trideuteromethyl ornithine derivative; < Glu, 2-pyrrolidone-5-carboxylic acid; AEtCys, S-aminoethyl cysteine; CMCys, S-carboxymethyl cysteine; BzlCys, S-benzyl cysteine.

REFERENCE

1. Nau, H. and Biemann, K., *Anal. Biochem.*, 73, 139, 1976.

Reproduced by permission of Academic Press, Inc.