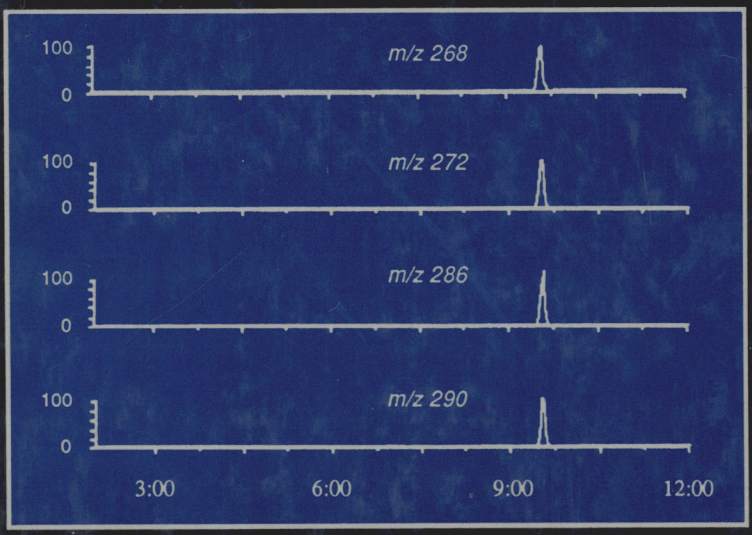
Lipid Chromatographic Analysis



edited by
Takayuki Shibamoto

Lipid Chromatographic Analysis

edited by
Takayuki Shibamoto

Department of Environmental Toxicology
University of California, Davis
Davis, California

Marcel Dekker, Inc. New York • Basel • Hong Kong

Library of Congress Cataloging-in-Publication Data

Lipid chromatographic analysis / edited by Takayuki Shibamoto.
p. cm. -- (Chromatographic science series : v. 65)
Includes bibliographical references and index.
ISBN 0-8247-8941-5 (acid-free paper)
1. Lipids--Analysis. 2. Chromatographic analysis. I. Shibamoto, Takayuki. II. Series: Chromatographic science : v. 65.
QP751.L5463 1994
574.19'247046--dc20 93-37222
CIP

The publisher offers discounts on this book when ordered in bulk quantities. For more information, write to Special Sales/Professional Marketing at the address below.

This book is printed on acid-free paper.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Lipid Chromatographic Analysis

CHROMATOGRAPHIC SCIENCE SERIES

A Series of Monographs

Editor: JACK CAZES
Sanki Laboratories, Inc.
Mount Laurel, New Jersey

1. Dynamics of Chromatography, *J. Calvin Giddings*
2. Gas Chromatographic Analysis of Drugs and Pesticides, *Benjamin J. Gudzinowicz*
3. Principles of Adsorption Chromatography: The Separation of Nonionic Organic Compounds, *Lloyd R. Snyder*
4. Multicomponent Chromatography: Theory of Interference, *Friedrich Helfferich and Gerhard Klein*
5. Quantitative Analysis by Gas Chromatography, *Josef Novák*
6. High-Speed Liquid Chromatography, *Peter M. Rajcsanyi and Elisabeth Rajcsanyi*
7. Fundamentals of Integrated GC-MS (in three parts), *Benjamin J. Gudzinowicz, Michael J. Gudzinowicz, and Horace F. Martin*
8. Liquid Chromatography of Polymers and Related Materials, *Jack Cazes*
9. GLC and HPLC Determination of Therapeutic Agents (in three parts), *Part 1 edited by Kiyoshi Tsuji and Walter Morozowich, Parts 2 and 3 edited by Kiyoshi Tsuji*
10. Biological/Biomedical Applications of Liquid Chromatography, *edited by Gerald L. Hawk*
11. Chromatography in Petroleum Analysis, *edited by Klaus H. Altgelt and T. H. Gouw*
12. Biological/Biomedical Applications of Liquid Chromatography II, *edited by Gerald L. Hawk*
13. Liquid Chromatography of Polymers and Related Materials II, *edited by Jack Cazes and Xavier Delamare*
14. Introduction to Analytical Gas Chromatography: History, Principles, and Practice, *John A. Perry*
15. Applications of Glass Capillary Gas Chromatography, *edited by Walter G. Jennings*
16. Steroid Analysis by HPLC: Recent Applications, *edited by Marie P. Kautsky*
17. Thin-Layer Chromatography: Techniques and Applications, *Bernard Fried and Joseph Sherma*
18. Biological/Biomedical Applications of Liquid Chromatography III, *edited by Gerald L. Hawk*
19. Liquid Chromatography of Polymers and Related Materials III, *edited by Jack Cazes*
20. Biological/Biomedical Applications of Liquid Chromatography, *edited by Gerald L. Hawk*
21. Chromatographic Separation and Extraction with Foamed Plastics and Rubbers, *G. J. Moody and J. D. R. Thomas*

22. Analytical Pyrolysis: A Comprehensive Guide, *William J. Irwin*
23. Liquid Chromatography Detectors, *edited by Thomas M. Vickrey*
24. High-Performance Liquid Chromatography in Forensic Chemistry, *edited by Ira S. Lurie and John D. Wittwer, Jr.*
25. Steric Exclusion Liquid Chromatography of Polymers, *edited by Josef Janča*
26. HPLC Analysis of Biological Compounds: A Laboratory Guide, *William S. Hancock and James T. Sparrow*
27. Affinity Chromatography: Template Chromatography of Nucleic Acids and Proteins, *Herbert Schott*
28. HPLC in Nucleic Acid Research: Methods and Applications, *edited by Phyllis R. Brown*
29. Pyrolysis and GC in Polymer Analysis, *edited by S. A. Liebman and E. J. Levy*
30. Modern Chromatographic Analysis of the Vitamins, *edited by André P. De Leenheer, Willy E. Lambert, and Marcel G. M. De Ruyter*
31. Ion-Pair Chromatography, *edited by Milton T. W. Hearn*
32. Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography, *edited by Steven H. Y. Wong*
33. Affinity Chromatography: Practical and Theoretical Aspects, *Peter Mohr and Klaus Pommerening*
34. Reaction Detection in Liquid Chromatography, *edited by Ira S. Krull*
35. Thin-Layer Chromatography: Techniques and Applications. Second Edition, Revised and Expanded, *Bernard Fried and Joseph Sherma*
36. Quantitative Thin-Layer Chromatography and Its Industrial Applications, *edited by Laszlo R. Treiber*
37. Ion Chromatography, *edited by James G. Tarter*
38. Chromatographic Theory and Basic Principles, *edited by Jan Åke Jönsson*
39. Field-Flow Fractionation: Analysis of Macromolecules and Particles, *Josef Janča*
40. Chromatographic Chiral Separations, *edited by Morris Zief and Laura J. Crane*
41. Quantitative Analysis by Gas Chromatography, Second Edition, Revised and Expanded, *Josef Novák*
42. Flow Perturbation Gas Chromatography, *N. A. Katsanos*
43. Ion-Exchange Chromatography of Proteins, *Shuichi Yamamoto, Kazuhiro Nakanishi, and Ryuichi Matsuno*
44. Countercurrent Chromatography: Theory and Practice, *edited by N. Bhushan Mandava and Yoichiro Ito*
45. Microbore Column Chromatography: A Unified Approach to Chromatography, *edited by Frank J. Yang*
46. Preparative-Scale Chromatography, *edited by Eli Grushka*
47. Packings and Stationary Phases in Chromatographic Techniques, *edited by Klaus K. Unger*
48. Detection-Oriented Derivatization Techniques in Liquid Chromatography, *edited by Henk Lingeman and Willy J. M. Underberg*
49. Chromatographic Analysis of Pharmaceuticals, *edited by John A. Adamovics*
50. Multidimensional Chromatography: Techniques and Applications, *edited by Hernan Cortes*
51. HPLC of Biological Macromolecules: Methods and Applications, *edited by Karen M. Gooding and Fred E. Regnier*

52. Modern Thin-Layer Chromatography, *edited by Nelu Grinberg*
53. Chromatographic Analysis of Alkaloids, *Milan Popl, Jan Fähnrich, and Vlastimil Tatar*
54. HPLC in Clinical Chemistry, *I. N. Papadoyannis*
55. Handbook of Thin-Layer Chromatography, *edited by Joseph Sherma and Bernard Fried*
56. Gas–Liquid–Solid Chromatography, *V. G. Berezkin*
57. Complexation Chromatography, *edited by D. Cagniant*
58. Liquid Chromatography–Mass Spectrometry, *W. M. A. Niessen and Jan van der Greef*
59. Trace Analysis with Microcolumn Liquid Chromatography, *Miloš Krejčí*
60. Modern Chromatographic Analysis of Vitamins: Second Edition, *edited by André P. De Leenheer, Willy E. Lambert, and Hans J. Nelis*
61. Preparative and Production Scale Chromatography, *edited by G. Ganetsos and P. E. Barker*
62. Diode Array Detection in HPLC, *edited by Ludwig Huber and Stephan A. George*
63. Handbook of Affinity Chromatography, *edited by Toni Kline*
64. Capillary Electrophoresis Technology, *edited by Norberto A. Guzman*
65. Lipid Chromatographic Analysis, *edited by Takayuki Shibamoto*
66. Thin-Layer Chromatography: Techniques and Applications, Third Edition, Revised and Expanded, *Bernard Fried and Joseph Sherma*
67. Liquid Chromatography for the Analyst, *Raymond P. W. Scott*

ADDITIONAL VOLUMES IN PREPARATION

Preface

It is well known that chromatography was introduced by Tswett in 1906. However, the technique was not truly utilized until Kuhn, Winterstein, and Lederer applied it in 1931; it has steadily improved since then. The introduction of gas chromatography (GC) by James and Martin in 1952 was one of the most revolutionary events to occur in the field of analytical chemistry. This invention, followed closely by the commercial development of the GC, has allowed us to isolate and identify tremendous numbers of unknown compounds. Today, the most advanced column possesses over 100,000 theoretical plates and can separate more than 1,000 compounds in one run. GC fails, however, in the analysis of nonvolatile compounds. In the 1970s, the high performance liquid chromatograph (HPLC) was developed and marketed to address this need; samples such as lipids, amino acids, and proteins can often be separated using this technique. Recently, supercritical fluid chromatography (SFC) using liquid carbon dioxide as a mobile phase has been developed to analyze some materials not separated by either GC or HPLC.

Analysis of lipids has been one of the most difficult processes in both chemical and biological research. It is particularly difficult to separate a lipid from a mixture because it is soluble in most organic solvents. Lipids are one of the essential components of living matter; they are associated with almost all biological activities including growth, aging, diseases, and protection from harmful agents. Even though recent chromatographic instruments including GC and HPLC can separate most fatty samples, conventional column chromatogra-

phy and thin layer chromatography are important and powerful methods of preparation of samples for analysis. Derivatization techniques are often required to analyze lipids by GC, whereas most lipids can be determined without derivatization by HPLC. On the other hand, GC has higher sensitivity and higher resolution than does HPLC. Obviously, it is extremely important to choose the best chromatographic method to obtain optimal results. This book is prepared with the hope that researchers in this field may be better assisted in choosing their analytical techniques.

Takayuki Shibamoto

Contributors

Debasis Bagchi *Creighton University, Omaha, Nebraska*

Merle L. Blank *Oak Ridge Associated Universities, Oak Ridge, Tennessee*

Gerald A. Cordis *University of Connecticut School of Medicine, Farmington, Connecticut*

Dipak K. Das *University of Connecticut School of Medicine, Farmington, Connecticut*

Susan E. Ebeler *University of California, Davis, California*

Hiranmoy Gangopadhyay *University of Connecticut School of Medicine, Farmington, Connecticut*

Mika Hayakawa *University of Nagoya, Nagoya, Japan*

A. Daniel Jones *Facility of Advanced Instrumentation, University of California, Davis, California*

Randall M. Jones *University of Connecticut School of Medicine, Farmington, Connecticut*

Arnis Kuksis *University of Toronto, Toronto, Canada*

Premysl Mareš *BIO-REGENA Laboratory, Prague, Czech Republic (deceased)*

Kozo Matsumoto *University of Nagoya, Nagoya, Japan*

Nilanjana Maulik *University of Connecticut School of Medicine, Farmington, Connecticut*

Robert A. Moreau *Eastern Regional Research Center, ARS, USDA, Philadelphia, Pennsylvania*

Toshihiko Osawa *University of Nagoya, Nagoya, Japan*

Takayuki Ozawa *University of Nagoya, Nagoya, Japan*

Takayuki Shibamoto *University of California, Davis, California*

Fred L. Snyder *Oak Ridge Associated Medical Universities, Oak Ridge, Tennessee*

Satoru Sugiyama *University of Nagoya, Nagoya, Japan*

Masashi Taguchi *Rigaku International Corporation, Tokyo, Japan*

Eva Tvrzická *Charles University, Prague, Czech Republic*

Miloš Votruba *BIO-REGENA Laboratory, Prague, Czech Republic*

Contents

1

Overview and Recent Developments in Solid-Phase Extraction for Separation of Lipid Classes

Susan E. Ebeler and Takayuki Shibamoto University of California, Davis, California

I. INTRODUCTION

The analysis of lipids frequently involves initial separation of the sample into the various component lipid classes. The separation can either be simple, yielding nonpolar and polar fractions, or complex, resulting in separation and isolation of triglycerides (TG), free fatty acids (FFA), sterols (e.g., cholesterol), sterylesters (SE), glycolipids (GL), acidic phospholipids, neutral phospholipids, and so on. In addition, this preliminary preparation often removes matrix interferences and frequently results in concentration of the analyte(s).

Traditionally, fractionation, cleanup, and concentration of lipid extracts have been achieved through the use of liquid–liquid extraction, thin-layer chromatography (TLC), or liquid–solid column chromatography. Application of TLC for lipid analysis is the subject of Chapter 2 of this volume. Other methods, such as batch adsorption, centrifugation, dialysis, distillation, filtration, lyophilization, precipitation, and Soxhlet extraction, have also been employed (Majors, 1986).

Liquid–solid column chromatography, one of the more commonly used sample preparation methods, relies primarily on partitioning and/or adsorption of the lipid components between the solid and liquid (mobile) phases. Elution of the desired lipid classes is achieved by varying the polarity and strength of the mobile phase. Common stationary phases for column chromatography include silica, alumina, and ion-exchange resins. Application of liquid–solid column

chromatography to the fractionation of lipids has been reviewed extensively (Stein and Lawson, 1966; Litchfield, 1972; Aitzetmüller, 1975; Kuksis, 1977; Christie, 1982; Mangold, 1984).

In the late 1970s small, commercial columns prepacked with a variety of solid stationary phases were introduced. These solid-phase extraction (SPE) cartridges offered several advantages over other sample preparation methods, including traditional column chromatography. Due to their smaller sizes and volumes, SPE columns generally require less eluting solvent. Compared to other methods, reduced solvent use and disposal costs can result in a direct cost savings. According to Tippins (1987), SPE methods can be as much as five times less costly than liquid–liquid extractions. Sample capacities and solvent elution volumes may be appropriate for direct injection onto a gas or liquid chromatograph without further sample preparation, reducing contamination and sample losses incurred during transfer steps. When sample concentration is required, the small solvent volumes are evaporated easily and rapidly. Problems associated with emulsion formation, common with liquid–liquid extractions, are eliminated with SPE.

SPE is a simple, rapid technique and can be up to 12 times faster than liquid–liquid extractions (Tippins, 1987). Packing traditional liquid–solid columns is a time-consuming task and the need for homogeneous packing requires extensive operator skill and training. However, these problems are virtually eliminated with commercial prepacked SPE columns. In addition, the current availability of a wide variety of sorbent types has increased SPE flexibility and selectivity, allowing them to be applied to almost any separation problem. These advantages have allowed SPE to become an increasingly popular tool and often the method of choice in the modern analytical lab for a wide variety of applications, including fractionation of lipid classes.

The purpose of this chapter is to review the principles of SPE and the development of methods for the isolation and fractionation of lipids in a variety of biological, environmental, and food matrices. Traditional column chromatographic methods will be discussed only as they relate to the historical development of SPE methodologies. The SPE separation of oxygenated lipid metabolites (i.e., prostaglandins and leukotrienes), and steroid profiling, although important in a complete lipid analysis, is beyond the scope of this review.

II. PRINCIPLES OF SPE

In general, the principles of traditional liquid–solid column chromatography and high-performance liquid chromatography (HPLC) apply to SPE (i.e., retention and elution depend on the interaction of the analyte with the liquid and solid phases). The relatively large particle sizes and pore sizes of SPE sorbents (generally, 30 to 60 μm, 60 Å) result in low back pressures so that analyte

elution is achieved simply via application of a vacuum (15 in. Hg or less) or a slight positive pressure with a hand-held syringe. These pressure requirements are significantly less than those required for HPLC.

SPE cartridges are generally packed into syringelike polyethylene or polypropylene tubes with 100 to 1000 mg of stationary phase sandwiched between two porous metal or plastic frits. Cartridge sizes vary with the manufacturer, generally ranging from 1 to 7 mL. Several samples can be handled simultaneously by inserting the columns into a pressure or vacuum manifold. Automated and on-line, precolumn SPE sample handling techniques for GC and HPLC are also available (Dimson et al., 1986; Liska et al., 1989; McDowall et al., 1989; Brinkman, 1990).

A number of solid phases are commercially available (Fig. 1). Choice of sorbent and elution solvent will depend on the sample matrix and analyte of interest. The following is a brief discussion of sorbent chemistry and solvent considerations. More detailed reviews are available (Harkey, 1989; Liska et al., 1989; Zief and Kiser, 1990) and several manufacturers provide excellent references and other resources for SPE information (Anon., 1984, 1992; McDonald, 1991).

A. SPE Sorbents

1. Silica

Silica-based stationary phases are widely used for lipid analysis. The polar sites adsorb polar compounds, and analyte retention and elution are related directly to solvent polarity. Ion-exchange effects, due to the mildly acidic nature of the silica particles, also influence separations on silica-based sorbents. Therefore, neutral and basic compounds are more strongly retained than are acids. For example, acidic phospholipids, such as phosphatidic acid (PA), phosphoinositol (PI), and phosphatidylethanolamine (PE), are not retained as strongly as uncharged phospholipids, such as phosphatidylcholine (PC) and sphingomyelin (SM). Highly polar, water-soluble organics adhere tightly and may be difficult to elute.

Silica adsorbs varying amounts of water to the highly active surface silanols. Therefore, the moisture content of the SPE column may affect separation in a given method and may require either drying the columns before use or using solvents with controlled moisture contents (Blunk and Steinhart, 1990).

2. Bonded Phases

Reaction of the surface silanols on silica with a variety of siloxane derivatives results in the formation of bonded silica phases (Fig. 2). As noted previously, a variety of bonded phases are available (Fig. 1) and are normally classified as nonpolar (reverse phase), polar (normal phase), or ion exchange. These classifications are based according to the functional group of the stationary phase. In

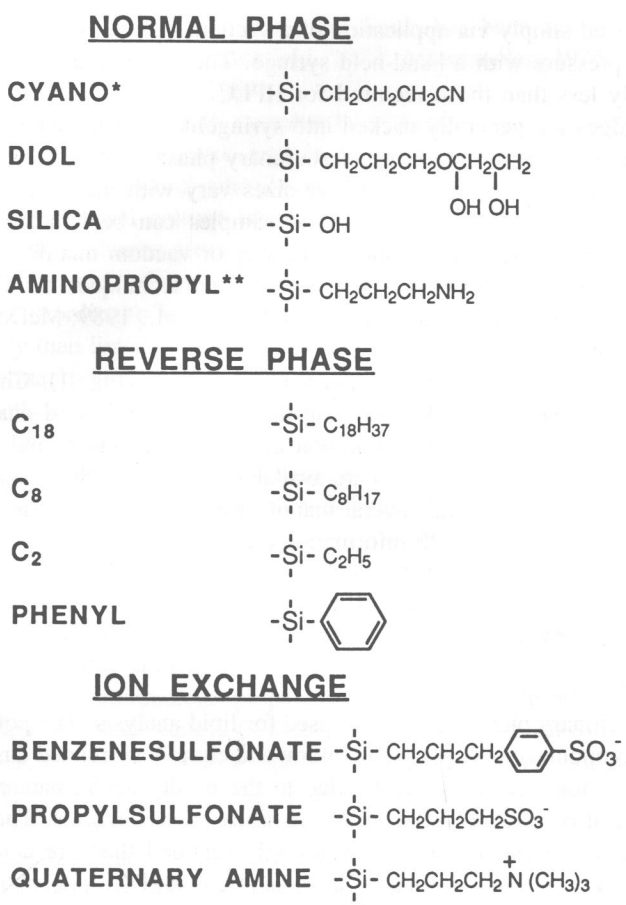


Figure 1 Some commercially available SPE solid phases. (Adapted from Anon., 1992, and Tippens, 1987.)

general, nonpolar phases will be used to retain nonpolar analytes and polar phases to retain polar analytes. Ion-exchange phases depend on ionic interactions as the primary retention mechanism.

B. SPE Elution Solvents

Analyte elution is achieved by selectively desorbing the compound of interest by changing the solvent polarity and/or eluotropic strength. ''Strong'' solvents match the chemical nature and polarity of the sorbent, whereas ''weak'' solvents