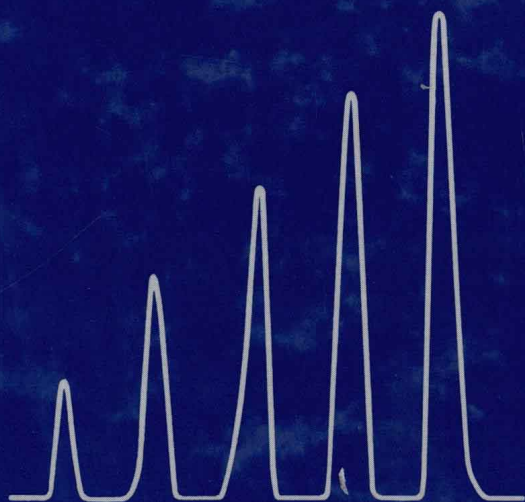


CHROMATOGRAPHIC SCIENCE SERIES VOLUME 66

Thin-Layer Chromatography

Techniques and Applications

Third Edition, Revised and Expanded



Bernard Fried
Joseph Sherma

Library of Congress Cataloging-in-Publication Data

Fried, Bernard.

Thin-layer chromatography : techniques and applications / Bernard
Fried, Joseph Sherma. — 3rd ed., rev. and expanded.

p. cm. — (Chromatographic science series ; v. 66)

ISBN 0-8247-9171-1 (acid-free)

1. Thin-layer chromatography. I. Sherma, Joseph. II. Title.
III. Series: Chromatographic science ; v. 66.

QD79.C8F74 1994

543'.08956—dc20

93-43297

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.

Copyright © 1994 by Marcel Dekker, Inc. All Rights Reserved.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

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

Thin-Layer Chromatography

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 Tater*
54. HPLC in Clinical Chemistry, *J. 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 to the Third Edition

This new edition of our book has been written to update and expand coverage of the topics in the second edition. It provides extensive coverage of qualitative and quantitative thin-layer chromatography (TLC) and high-performance TLC, and compares modern TLC with other separation methods, especially gas and column liquid chromatography. The third edition should appeal to a wide range of scientists, including biologists, chemists, biochemists, biotechnologists, and medical technologists.

We have maintained the overall organization of the second edition, namely, a series of initial chapters on theory and practice, and a second section of chapters concerned with applications to important compound types. As in the previous two editions, practical rather than theoretical aspects of TLC have been stressed. Coverage of certain modern principles, techniques, and instrumentations (such as optimization of separations and densitometry, overpressurized and multiple development, and preparative layer chromatography) has been expanded.

This book is still intended to be an introductory primer. For more detailed coverage of all aspects of TLC the reader is referred to the 1991 *Handbook of Thin-Layer Chromatography*, edited by J. Sherma and B. Fried and published by Marcel Dekker, Inc.

This edition updates and extends references to the literature. The TLC literature is voluminous and references in this book are selective. Citations are mainly to the 1980–1993 literature.

The second part of this book is concerned with the applications of TLC to various classes and compounds. It presents information and various detailed qualitative and quantitative densitometric TLC experiments on organic dyes, lipids, amino acids, carbohydrates, natural pigments, vitamins, nucleic acid derivatives, steroids, pharmaceuticals, organic acids, antibiotics, and insecticides. This section of the book should be useful to students and faculty at academic institutions and should also provide useful experimental procedures for the practicing chromatographer.

The third edition also provides extensive coverage of sample preparation. We feel that this coverage is unique and differs from that found in most other available treatises on TLC.

The suggestions of reviewers have been incorporated into this revision. Comments on this edition as well as notification of errors and suggestions for improvements in future editions will be appreciated.

We thank Mr. Richard L. Everett, Reference and Interlibrary Loan Librarian, Skillman Library, Lafayette College, Easton, Pennsylvania, for his help with literature retrieval.

Bernard Fried
Joseph Sherma

Preface to the First Edition

The purpose of this book is to acquaint the reader with the principles, practices, and applications of thin-layer chromatography (TLC). The book is divided into two parts, the first of which is concerned mainly with general practices of TLC (Chapters 1 to 13) and the second (Chapters 14 to 18) with applications based on compound types. Although we feel this book is a valuable source of practical information for any scientist currently using or contemplating the use of TLC, it was written with special consideration for those without extensive training in analytical chemistry. This includes the majority of biology students and graduate biologists, for whom we feel this book will be especially valuable. After reading our book these people should be better equipped to examine critically the more comprehensive texts already published on TLC.

The authors of this book have had considerable experience in both qualitative and quantitative TLC. One author (J.S.), an analytical chemist, has had widespread experience in the separation sciences. The other author (B.F.), an experimental parasitologist, has recently become involved in TLC analyses of hydrophilic and lipophilic excretions of parasitic worms. Both authors have had considerable experience teaching TLC at Lafayette College and at short courses sponsored by Kontes Co., Inc., Vineland, New Jersey, and the Center for Professional Advancement, East Brunswick, New Jersey. Additionally, the authors have advised scientists from academia, government, and industrial research on principles, practices, and applications of TLC.

At Lafayette, which is an undergraduate teaching institution, we have advised colleagues on TLC. For instance, we helped botanists with TLC procedures for the separation of plant pigments and zoologists with separation problems related to hydrophilic and lipophilic substances from animal tissues and fluids. Several of our colleagues have recently introduced one or more TLC experiments in their courses. The aim of this book, in part, is to aid teachers in introducing TLC experiments in courses. The second part presents various detailed TLC experiments that can usually be performed in one or several 3-hr lab periods. Most experiments utilize inexpensive materials that are readily available.

Our experiments are based on TLC separations on silica and cellulose sorbents. Chapter 3, however, discusses less widely used sorbents such as alumina, Sephadex, ion-exchange materials, polyamides, and others. Since TLC is used mainly as a separation tool for small-molecular-weight, nonionized, lipophilic and hydrophilic substances, our detailed experiments emphasize TLC on silica and cellulose.

We have referred to numerous commercial products in this book. These references are based mainly on our combined experiences with these products and are not meant to imply that any product is better than others not mentioned.

Throughout this book the following conventions have been adopted: the terms "solvent" and "mobile phase" are used more or less interchangeably; "spot" and "zone" are used interchangeably with no designation of a certain shape; mobile phases are designated in volume proportions unless otherwise stated.

Bernard Fried
Joseph Sherma

Contents

<i>Preface to the Third Edition</i>	<i>iii</i>
<i>Preface to the First Edition</i>	<i>v</i>

PART I: GENERAL PRACTICES OF TLC

1. Introduction and History	3
2. Mechanism and Theory	11
3. Sorbents, Layers, and Precoated Plates	25
4. Obtaining Material for TLC and Sample Preparation	51
5. Application of Samples	73
6. Solvent Systems	87
7. Development Techniques	101
8. Detection and Visualization	133
9. Qualitative Evaluation and Documentation	157
10. Quantification	173
	vii

11. Reproducibility of Results	201
12. Preparative Layer Chromatography	209
13. Radiochemical Techniques	221

PART II: APPLICATIONS OF TLC TO DIFFERENT COMPOUND TYPES

14. Basic TLC Design and TLC of Organic Dyes	237
15. Lipids	245
16. Amino Acids	287
17. Carbohydrates	303
18. Natural Pigments	321
19. Vitamins	337
20. Nucleic Acid Derivatives	361
21. Steroids and Terpinoids	373
22. Pharmaceuticals	391
23. Miscellaneous Applications	405
Directory of Manufacturers and Sources of Standards, Sample Preparation Supplies, and TLC Instruments, Plates, and Reagents	421
Glossary	429
<i>Index</i>	439

Thin-Layer Chromatography

Introduction and History

I. INTRODUCTION TO THIN-LAYER CHROMATOGRAPHY

Chromatography encompasses a diverse but related group of methods that permit the separation, isolation, identification, and quantification of components in a mixture. All of the methods involve application of the sample in a narrow (small) initial zone to a stationary phase. Components of the mixture are carried through the stationary phase by the flow of a mobile phase. Separations are based on differences in migration rates among the sample components.

Thin-layer chromatography (TLC) is a mode of liquid chromatography in which the sample is applied as a small spot or streak to the origin of a thin sorbent layer supported on a glass, plastic, or metal plate. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or pressure. TLC separations take place in the “open” layer (or “open bed”), with each component having the same total migration time but different migration distances. The mobile phase in TLC consists of a single solvent or a mixture of organic and/or aqueous solvents. Numerous fixed sorbents have been used, including silica gel, cellulose, alumina, polyamides, ion exchangers, and chemically bonded silica gel. The wide variety of available mobile and stationary phases provides considerable versatility in choosing a system for resolution of virtually all types of compounds.

The basic procedure for classical TLC consists of the following steps:

1. The sample solution is applied to the plate origin as a spot or zone.
2. The sample solvent is allowed to evaporate from the plate.
3. The plate is placed in a closed chamber containing a shallow pool of mobile phase on the bottom.
4. The mobile phase rises by capillary action through the applied spot.
5. Development is continued until the solvent front is about 10–15 cm beyond the origin.
6. The plate is removed from the chamber and the solvent front is marked.
7. Mobile phase is removed from the plate by drying in air or applying heat.
8. If the compounds are not naturally colored or fluorescent, a detection reagent is applied to visualize the zones.
9. The positions of the zones are used for qualitative identification of compounds and the size and/or intensity of the zones for quantification.

TLC can be used both as an analytical and a preparative technique. Although most of this book is devoted to TLC as an analytical procedure, Chapter 12 discusses preparative-scale TLC. TLC provides for separations in the milligram (mg, 10^{-3} g), microgram (μg , 10^{-6} g), nanogram (ng, 10^{-9} g), and picogram (pg, 10^{-12} g) range. Separated substances that are tentatively identified by TLC can be eluted for further characterization by other microanalytical techniques, such as gas chromatography (GC); high-performance liquid chromatography (HPLC); visible, ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry; and electroanalysis. Eluted substances can also be quantified by procedures such as these, but in situ densitometry is the most convenient, accurate, and precise approach for quantitative TLC (Chapter 10). Although many scientists will not have access to a scanner suitable for in situ quantification, several detailed experiments using densitometry have been included in Part II.

II. HISTORY OF TLC

The history of TLC has been reviewed by Stahl (1969), Heftmann (1975), Kirchner (1975, 1978, 1980), Pelick et al. (1966), Jork and Wimmer (1986), and Wintermeyer (1989). TLC is a relatively new discipline, and most chromatography historians date the advent of modern TLC from 1958. The Pelick et al. (1966) review contains a tabulation of significant early developments in TLC and provides translations of the classical studies of Izmailov and Shraiber and of Stahl. In 1938, Izmailov and Shraiber separated certain medicinal compounds on unbound alumina spread on glass plates. Because the solvent was dropped on a glass plate containing the sample and sorbent, their procedure was called drop chromatography. In 1948, Meinhard and Hall used a binder to adhere alumina to

microscope slides; these plates were used for the separation of certain inorganic ions using drop chromatography (Kirchner, 1978). From 1950 to 1954, Kirchner and colleagues at the U.S. Department of Agriculture developed TLC essentially as we know it today. These workers used sorbents bound to glass plates with the aid of a binder, and plates were developed according to conventional ascending development procedures used in paper chromatography. Kirchner used the term "chromatostrips" for his discovery.

The term "thin-layer chromatography" was coined by Egon Stahl in Germany in the late 1950s. Stahl's greatest contribution to the field was the standardization of materials, procedures, and nomenclature and the description of selective solvent systems for the resolution of many important compound classes. His first TLC laboratory manual (Stahl, 1962) popularized TLC, and he elicited the aid of various chemical manufacturers in offering standard materials for TLC. Stahl (1979, 1983) has provided an introspective view of his contributions to the field over a period of 25 years.

Early workers in TLC had no choice but to make their own plates, by procedures that were often messy and arduous and resulted in many rejects. A major breakthrough in this field came in the early 1960s with the commercial availability of convenient precoated plates. This innovation was discussed by Przybylowicz et al. (1965). Today, few workers prepare their own plates in the laboratory unless that require a special type of layer that is not commercially available.

III. LITERATURE OF TLC

Numerous chromatographic, analytical, and biological journals regularly publish articles on TLC, including most notably: *Journal of Planar Chromatography—Modern TLC*, *Journal of Liquid Chromatography*, *Journal of Chromatography*, *Journal of Chromatographic Science*, *Chromatographia*, *Analytical Chemistry*, *Fresenius' Journal of Analytical Chemistry*, *Journal of AOAC International*, *Analytical Biochemistry*, *Journal of Analytical Toxicology*, *Journal of Pharmaceutical and Biomedical Analysis*, *Biomedical Chromatography*, *Analyst (London)*, *Analytical Letters*, *Analisis*, *Clinical Chemistry (Winston Salem, NC)*, *GIT Fachz. Lab. (Glas- und Instrumenten Technik Fachzeitschrift fuer das Laboratorium)*, *GIT—Supplement*, *Comparative Biochemistry and Physiology*, and *Journal of Parasitology*. The *CRC Handbook of Chromatography* series, introduced in 1972 under the joint editorship of Gunter Zweig and Joseph Sherma and continued since 1991 by the latter, began with the publication of two general volumes and now includes 26 additional volumes on carbohydrates, drugs, amino acids and amines, polymers, phenols and organic acids, terpenoids, pesticides, lipids, steroids, peptides, hydrocarbons, pigments, and inorganics. Included in each volume are R_F data, detection reagents, and sample preparation