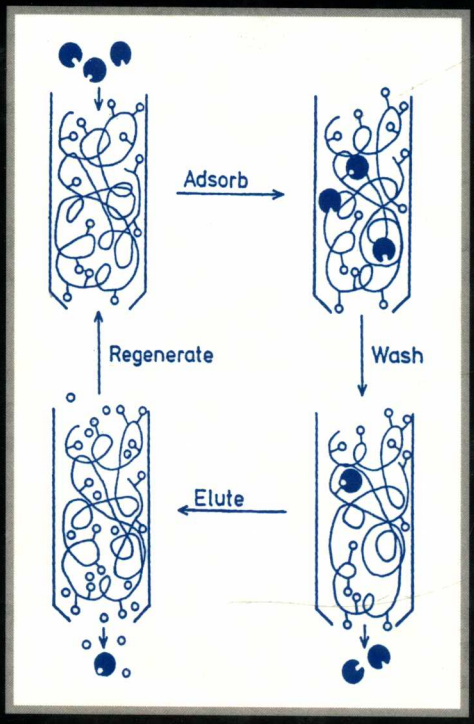
CHROMATOGRAPHIC SCIENCE SERIES VOLUME 78

Handbook of HPLC



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Handbook of HPLC

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MARCEL DEKKER, INC. NEW YORK · BASEL

Library of Congress Cataloging-in-Publication Data

Handbook of HPLC / edited by Elena Katz . . . [et al.].
p. cm.—(Chromatographic science series; v. 78)
Includes bibliographical references and index.
ISBN 0-8247-9444-3 (acid-free paper)
1. High performance liquid chromatography. I. Katz, Elena. II. Series:
Chromatographic science; v. 78.
QD79.C454H36 1998
543'.0894—dc21 98-35549
CIP

This book is printed on acid-free paper.

Headquarters
Marcel Dekker, Inc.
270 Madison Avenue, New York, NY 10016
tel: 212-696-9000; fax: 212-685-4540

Eastern Hemisphere Distribution
Marcel Dekker AG
Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
tel: 44-61-261-8482; fax: 44-61-261-8896

World Wide Web
http://www.dekker.com

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

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

PRINTED IN THE UNITED STATES OF AMERICA

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the first time. Moreover, the text is detailed enough to provide the latest innovations and refresh one's memory about those techniques that have now become standard.

Elena Katz
Roy Eksteen
Peter Schoenmakers
Neil Miller

Preface

During the last decade, high-performance liquid chromatography (HPLC) has become the analytical method of choice for product separation, purification, and detection in many areas such as pharmaceuticals, biotechnology, environmental monitoring, and applied research.

The development and availability of HPLC columns of different geometries and various stationary phases have been accompanied by the development of rugged and reliable HPLC instrumentation, the performance of which is now compatible with that of the modern, highly efficient columns.

Many excellent, but specialized, books devoted to both HPLC theory and applications have been published. This handbook encompasses all the aspects of the method, presented in four parts: fundamentals, HPLC techniques, instrumentation, and applications. Part I, fundamentals, covers the HPLC theory that is necessary to understand in order to successfully practice the method. Part II, HPLC techniques, discusses different HPLC modes—reversed-phase HPLC and ion-exchange, size exclusion HPLC among others—and critically addresses their applicability and limitations, with the emphasis on how to select an appropriate technique to achieve desirable solutions. Part III, instrumentation, describes the principles and practical operations of modern HPLC equipment: pumps, detectors, injection devices, and data handling systems. Part IV, applications, covers a comprehensive array of the HPLC applications in different areas of industry and applied research. All the chapters contain extensive lists of tables and references.

This book has been written to satisfy the need for detailed information about one of the most widely employed methods of laboratory analysis. It is eminently suitable as a textbook for undergraduate college students and new practitioners who look for a better understanding of the HPLC concepts that are usually only briefly discussed in primers on analytical chemistry. On the next level, the book provides a thorough overview of the HPLC literature by experts in their respective fields, while avoiding excessive details in the text by using appropriate referencing of the relevant literature. We hope that this approach makes the handbook an invaluable starting point for graduate students who look for the basic ideas behind HPLC techniques and applications. For those already experienced in the art and practice of HPLC, the handbook is designed to become an often used source of information by including many tables and figures that contain practical data, most of which have been brought together for

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Handbook of HPLC

Contents

1
Retention and Selectivity

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I. PRINCIPLES OF DISTRIBUTION AND ADSORPTION

Chromatographic separation can be attained for compounds that migrate at different speeds through a "chromatographic bed." The phenomenon of different migration velocities is based on different *retentions* of the migrating compounds ("separands" or "analytes") caused by the elementary process of different distributions of the separands between two phases: a mobile phase and a stationary phase.

In liquid chromatography (LC), the mobile phase is a liquid. The corresponding stationary phases consist predominantly of solid surfaces. These surfaces can be the original support materials themselves, or they may consist of various chemical structures attached to the support materials. (Such modifications are discussed in detail later.) This type of chromatography is addressed as *liquid–solid chromatography* (LSC). [In the physicochemical treatment, the total stationary phase, beyond the surface of the adsorbent, is considered to also include the first adsorbed layers of the mobile phase.]

In a different type of chromatography, which nowadays is used only in very few practical applications of classic chromatography [1,2], the stationary phase is also a liquid, which is adsorbed as a bulky film onto a solid support. Stationary liquid-phase and mobile liquid–phase are immiscible; their equilibrium composition is interrelated by the tie-lines (connodes) of the miscibility gap. Mobile and stationary phases employed are usually at least ternary mixtures, occasionally including polymeric components [1]. This type of chromatography is addressed as *liquid–liquid chromatography* (LLC). In "ideal" LLC the influence of the surface on the partition equilibrium is negligible. Partition between two liquid phases is also the basis of countercurrent chromatography [3]. In this technique, the liquid stationary phase is not adsorbed onto any support material, but the liquid mobile phase moves through the stationary liquid (e.g., in the form of upstreaming liquid bubbles or by motion in a helical coil). All commonly used experimental techniques use density differences between the two liquid phases as driving forces for phase movement.

To distinguish between the LSC and LLC distribution equilibria, one can call the first one an adsorption equilibrium, the second one a partition equilibrium.