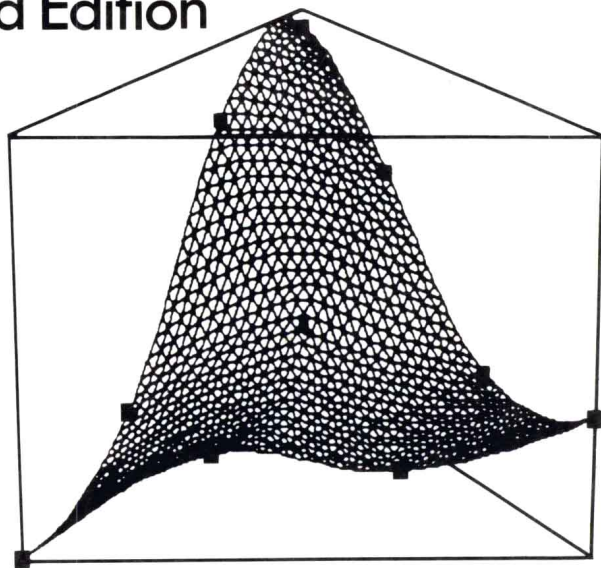




ANALYTICAL CHEMISTRY BY OPEN LEARNING

High Performance Liquid Chromatography

Second Edition



Sandie Lindsay

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Published in 1992 by John Wiley & Sons Ltd
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Library of Congress Cataloging-in-Publication Data:

Lindsay, Sandie.

High performance liquid chromatography / Sandie Lindsay.—2nd ed.
p. cm.—(Analytical Chemistry by Open Learning)

Includes bibliographical references (p.) and index.

ISBN 0 471 93180 2 (cloth) : ISBN 0 471 93115 2

(paper)

1. High performance liquid chromatography—Programmed instruction.

2. Chemistry, Analytic—Programmed instruction. I. ACOL (Project)

II. Title. III. Series: Analytical Chemistry by Open Learning

(Series)

QD79.C454L54 1992

543'.0894—dc20

91-428293

CIP

British Library Cataloguing in Publication Data:

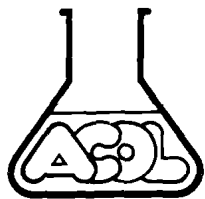
A catalogue record for this book is available from the British Library

ISBN 0 471 93180 2 (cloth)

ISBN 0 471 93115 2 (paper)

Typeset in 11/13pt Times by Text Processing Dept, John Wiley & Sons Ltd, Chichester
Printed and bound in Great Britain by Courier International Ltd, East Kilbride, Scotland

High Performance Liquid Chromatography



Analytical Chemistry by Open Learning

Project Director

BRIAN R CURRELL
Thames Polytechnic

Project Manager

JOHN W JAMES
Consultant

Project Advisors

ANTHONY D ASHMORE
Royal Society of Chemistry

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To achieve the full benefit from an open learning text you need to plan your place and time of study.

- Find the most suitable place to study where you can work without disturbance.
- If you have a tutor supervising your study discuss with him, or her, the date by which you should have completed this text.
- Some people study perfectly well in irregular bursts, however most students find that setting aside a certain number of hours each day is the most satisfactory method. It is for you to decide which pattern of study suits you best.
- If you decide to study for several hours at once, take short breaks of five or ten minutes every half hour or so. You will find that this method maintains a higher overall level of concentration.

Before you begin a detailed reading of the text, familiarize yourself with the general layout of the material. Have a look at the course contents list at

the front of the book and flip through the pages to get a general impression of the way the subject is dealt with. You will find that there is space on the pages to make comments alongside the text as you study—your own notes for highlighting points that you feel are particularly important. Indicate in the margin the points you would like to discuss further with a tutor or fellow student. When you come to revise, these personal study notes will be very useful.

¶ When you find a paragraph in the text marked with a symbol such as is shown here, this is where you get involved. At this point you are directed to do things: draw graphs, answer questions, perform calculations, etc. Do make an attempt at these activities. If necessary cover the succeeding response with a piece of paper until you are ready to read on. This is an opportunity for you to learn by participating in the subject and although the text continues by discussing your response, there is no better way to learn than by working things out for yourself.

We have introduced self assessment questions (SAQs) at appropriate places in the text. These SAQs provide for you a way of finding out if you understand what you have just been studying. There is space on the page for your answer and for any comments you want to add after reading the author's response. You will find the author's response to each SAQ at the end of each Chapter. Compare what you have written with the response provided and read the discussion and advice.

At intervals in the text you will find a Summary and List of Objectives. The Summary will emphasize the important points covered by the material you have just read and the Objectives will give you a checklist of tasks you should then be able to achieve.

You can revise the Unit, perhaps for a formal examination, by re-reading the Summary and the Objectives, and by working through some of the SAQs. This should quickly alert you to areas of the text that need further study.

Study Guide

High performance liquid chromatography (HPLC) is the most powerful of all the chromatographic techniques. It can often easily achieve separations and analyses that would be difficult or impossible using other forms of chromatography. On the other hand, there are very many things that can go wrong with such separations; there are probably more pitfalls in HPLC than in any other form of chromatography. To avoid these, you have to have the sort of experience that is difficult to obtain by reading textbooks. Only by doing a great deal of experimental work (and making many mistakes) can you hope to achieve the necessary practical skills.

This is not to say that the theoretical side of the subject is unimportant. In chromatography, theory has always led experimental work. The great advances that have been made in liquid chromatography in the last 10–15 years have been achieved through a better theoretical understanding of the technique. You will not be able to use HPLC to full advantage unless you have a proper understanding of how it works. Liquid chromatography is a very wide ranging subject and to understand it you will have to have some knowledge of many different areas of physical and analytical chemistry. I will assume you have studied chemistry up to the standard of the BTEC higher certificate and that you have a knowledge of physics and mathematics up to about ‘A’ level standard. I will also assume that you are familiar with *ACOL: Chromatographic Separations*. You will find it helpful to have had some experience with the use of analytical instruments such as spectrophotometers and chart recorders.

As well as dealing with the basic principles of the method, I have attempted to give a brief coverage of one or two more specialized topics, for instance in Sections 6.5, 10.2 and 10.6. If you are new to chromatography and find these too difficult, you can treat them as optional if you wish.

Because HPLC covers such a wide area, you are bound to find that there are some topics that you would like to study in more detail than is given in this text. Suitable books that you could use as a starting point are listed in the bibliography. Because HPLC is a technique that is still developing, textbooks often contain some material that is obsolete. To get up to date information, especially on columns and instrumentation, you have to use the chromatographic literature, or catalogues and applications literature from equipment manufacturers. A selection of applications literature available from manufacturers is given at the end of Chapter 8.

Supporting Practical Work

1. GENERAL CONSIDERATIONS

The experiments below use reverse phase chromatography with bonded silica columns and uv absorbance detection. If more extensive experimental facilities are available, some additional experiments are suggested. These are concerned with the preparation and evaluation of columns, and with the use of other detectors and modes of hplc. It should be possible to complete each experiment within a three hour practical period.

2. AIMS

- (a) To provide practical experience in the use of basic hplc equipment.
- (b) To demonstrate the various parameters that control hplc separations.
- (c) To show the use of the technique for separations and quantitative analysis.
- (d) To illustrate some of the important principles from the theory part of the Unit.

3. SUGGESTED EXPERIMENTS

- (a) The effect of mobile phase flow rate and dead volume on column performance.
- (b) The effect of mobile phase composition on retention and selectivity in a reverse phase separation.

- (c) Determination of 4-hydroxy-3-methoxy benzaldehyde (vanillin) in vanilla essence.
- (d) Determination of aspirin and caffeine in an analgesic tablet.

4. ADDITIONAL EXPERIMENTS

- (a) Preparation and evaluation of an hplc column.
 - (b) Analysis of sugars in fruit juice.
 - (c) The use of extraction techniques in the separation of carotene pigments from fruit.
-

Bibliography

Textbooks on high performance liquid chromatography.

1. J.J. Kirkland and L.R. Snyder, *Introduction to Modern Liquid Chromatography*, 2nd edn., Wiley, 1979.
2. R.J. Hamilton and P.A. Sewell, *Introduction to High Performance Liquid Chromatography*, Chapman and Hall, 1982.
3. C.F. Simpson, Ed. *Techniques in Liquid Chromatography*, Wiley, 1984.
4. J.H. Knox, Ed. *High Performance Liquid Chromatography*, Edinburgh University Press, 1982.
5. Veronika R. Meyer, *Practical High Performance Liquid Chromatography*, Wiley, 1988.

Reference 1 is a very comprehensive treatment. References 2, 4, 5 and parts of 3 are simpler treatments which are more suited to this text.

Useful material on HPLC can also be obtained from manufacturers catalogues and applications notes (see Chapter 8, after the Summary) and from a number of free journals including:

LC-GC International, Chester Business Park, Chester, CH4 9QH.
Chromatography and Analysis, 27 Norwich Road, Halesworth, Suffolk,
IP19 8BX.

Laboratory Equipment Digest, 30 Calderwood Street, London SE18 6QH.

Additional reading for specialised topics is given at the end of chapters.

Acknowledgements

I am grateful for assistance from Alan Curtis and Cecil Lobo (Bush Boake Allen Ltd), John Mills (Varian Associates), Tony Green (Eurocolour Ltd), Ken Evans (ICI Colours and Fine Chemicals), Dave Cook (Dyson Instruments) and, especially, from Tom Donovan (Biotage).

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