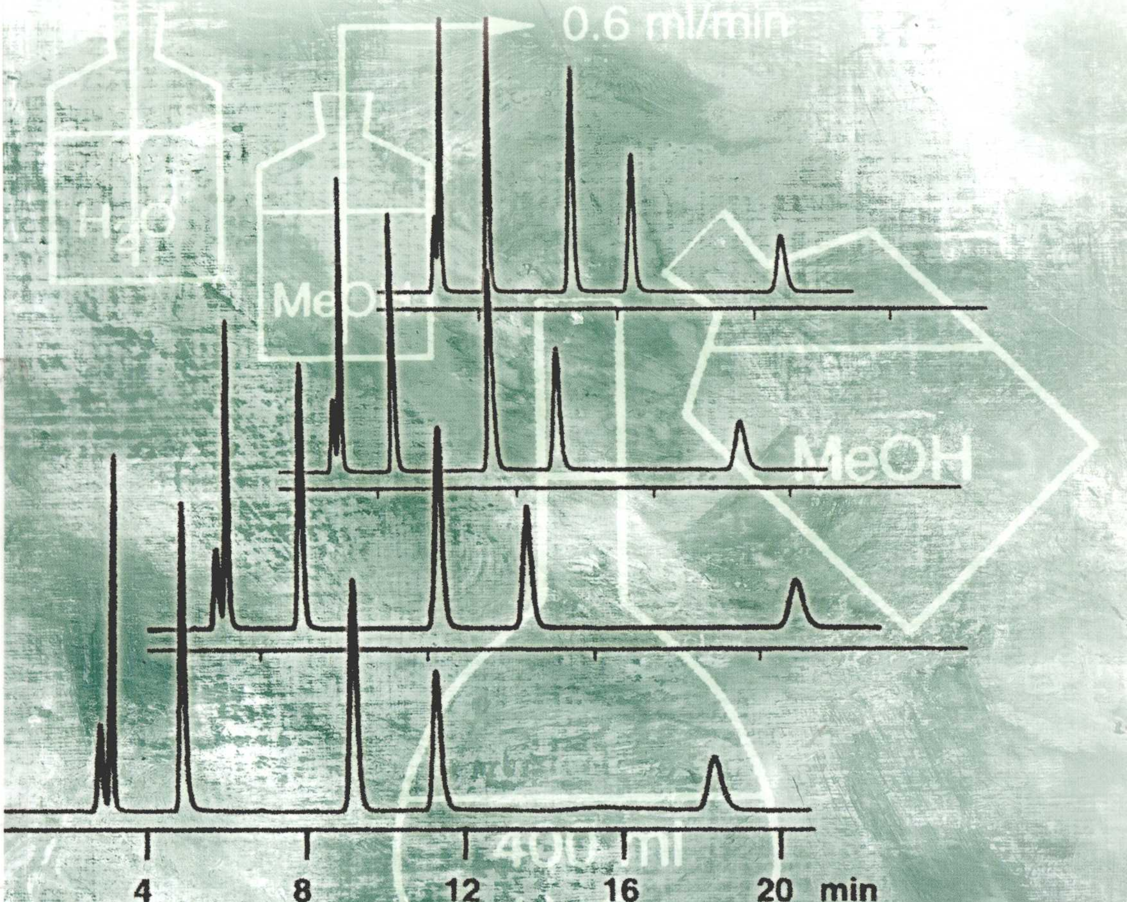
Veronika R. Meyer

WILEY-VCH

Pitfalls and Errors of HPLC in Pictures

Third, Revised and Enlarged Edition



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The Author

Dr. Veronika R. Meyer
EMPA St. Gallen
Lerchenfeldstrasse 5
9014 St. Gallen
Switzerland
e-mail: veronika.meyer@empa.ch

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1 wort = 1 millibild
(A picture says more than a thousand words)

Graffiti on the Baltzerstrasse in Bern

Preface

Errors are a common companion of all human activity, including work in the laboratory. Yet it is a great pity if erroneous results are produced with great effort and by using expensive instruments and demanding procedures. Therefore a book about sources of errors in high performance liquid chromatography, one of today's most widely used analytical methods, is not superfluous. Maybe the topic is not welcomed enthusiastically but I hope I have found a design which encourages reading and thinking.

In conception, at least, possible problems can be divided into two categories. 'Errors' are troublesome opponents of accurate and precise analytical results which, however, can be understood; we need to remember and to anticipate them. In contrast, 'pitfalls' are totally unexpected intruders and the secret behind them is difficult to discover. The worst are those which are not detected but which affect the result anyway. Nevertheless, the book does not distinguish between the two types. The readers decide how they classify them. With increasing experience in HPLC it should become easier to avoid the pitfalls.

The third edition could be expanded with new examples and proposals. Many people helped me with examples, hints or ideas on how to improve the text and figures. I want to thank all of them. Special thanks to the publisher who supports the idea of a picture book, not for children but for novices and experts in the analytical laboratory. I hope that the book will be a useful aid in daily laboratory work thanks to intelligible explanations and lucid illustrations.

St. Gallen, August 2012 *Veronika R. Meyer*

Introduction

This book is not an introductory text to HPLC and also not a troubleshooting guide of the kind "what shall I do if my instrument does not work?". It does not replace such books but is intended to complement them. Some texts which, according to my personal opinion, are very useful and should therefore be present in the HPLC laboratory are listed on the next page.

Now this book on your desk is a picture book. The figures are at least as important as the texts; sometimes more information can be found in them than could be given in the short descriptions. It is possible, and in principle recommended, to study all the pages in sequence from beginning to end. This method guarantees that one learns about errors which are uncommon and unexpected. On the other hand each pair of pages is limited to one topic, linked to other pages by arrows only, and can therefore be studied in isolation. The index at the end of the book can help you find the right pages when a problem occurs, although it must be stated once again that quick troubleshooting advice is not usually provided.

The book is divided into three parts:

Part I briefly presents some basic facts about HPLC. Many topics may be absent because this is not a textbook, but the matter presented is of utmost relevance in HPLC. Thus the topics discussed should act as reminders and be used for revision. Whoever understands Part I knows a lot about HPLC – more than it seems at first glance.

Part II lists the pitfalls and sources of error. They are in a logical sequence, as far as this is possible, following the flow path in an HPLC instrument, from the preparation of the mobile phase to data evaluation. The list is somewhat arbitrary, and not all errors are of equal importance with regard to their possible consequences. It would, however, be dangerous to distinguish between grave and harmless errors. A minute error can cause much damage under special circumstances.

Part III gives some hints on what can be done to avoid errors. Again this synopsis is very heterogeneous in character. This does not diminish its value, of course.

Incompleteness is an inevitable feature of this book. I am grateful for all hints on other pitfalls and sources of error or on how to avoid them.

Recommended Texts

Veronika R. Meyer
Practical High Performance Liquid Chromatography
Wiley, Chichester
5th edition 2010

John W. Dolan and Lloyd R. Snyder
Troubleshooting LC Systems
Humana Press, New Jersey
1989

Paul C. Sadek
Troubleshooting HPLC Systems: A Bench Manual
Wiley-Interscience, New York
2000

Stavros Kromidas
Practical Problem Solving in HPLC
Wiley-VCH, Weinheim
2000

Stavros Kromidas
More Practical Problem Solving in HPLC
Wiley-VCH, Weinheim
2004

Lloyd R. Snyder, Joseph J. Kirkland and Joseph L. Glajch
Practical HPLC Method Development
Wiley-Interscience, New York
2nd edition 1997

Norman Dyson
Chromatographic Integration Methods
Royal Society of Chemistry, London
2nd edition 1998

Contents

Part I
Fundamentals

Pitfalls and Errors of HPLC in Pictures, Third Edition. Veronika R. Meyer.

1.1 Chromatography

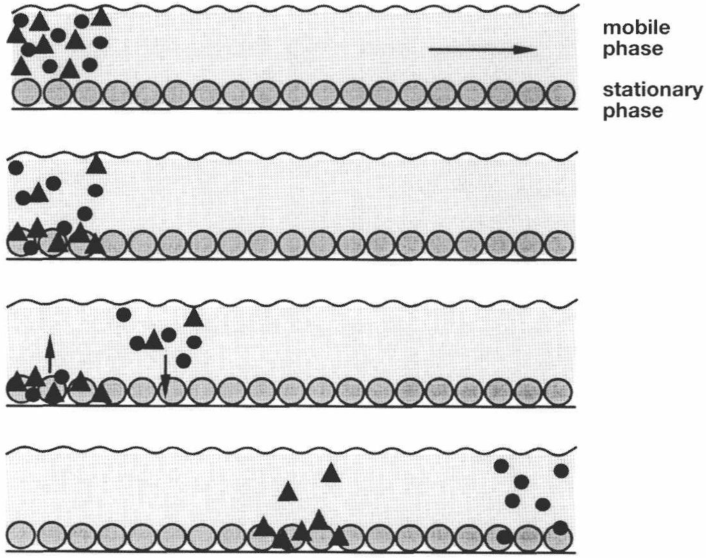
In chromatography, a physical separation method, the components of a mixture are partitioned between two phases. One of the phases stays in its place and is called the stationary phase, whereas the other moves in a definite direction and is called the mobile phase.

According to the type of mobile phase we distinguish between gas chromatography, supercritical fluid chromatography, and liquid chromatography.

The separation is based upon the different partition coefficients of the sample components between the two phases. It is helpful to divide the chromatographic column into small hypothetical units, the socalled theoretical plates. Within each plate a new partition equilibrium is established. The narrower a theoretical plate, the more equilibrium processes can take place within a column of given length and the more demanding the separation problems which can be solved.

The figure shows the separation of two compounds. One of these prefers the mobile phase but also enters the stationary phase. For the other compound the preference is the other way round. Thanks to this large difference in their properties the two types of molecule can easily be separated. They are transported through the column by the flow of the mobile phase and thereby reach zones where new equilibria are formed again and again.

In the drawing, such a theoretical plate has a height of approximately 3 1/2 stationary phase particle diameters. This height depends on the packing quality of the column, on the mass transfer properties of the phases, and on the analytes involved. Plate height is a function of the particle diameter of the stationary phase. For good columns, plate heights are equal to ca. 3 particle diameters irrespective of the particle size. A fine packing, e.g. with a 3-μm phase, gives four times as many theoretical plates as does a 10-μm packing if identical column lengths are compared. The column with the fine packing can therefore be used for more difficult separation problems.


mobile
phase
stationary
phase

1.2
Chromatographic Figures of Merit

To judge a chromatogram it is necessary to calculate some data which can be easily obtained. The integrator or data system yields the retention times, t_R, and peak widths, w; perhaps it is advisable to determine the peak width at half height, $w_{1/2}$. In addition the breakthrough time or 'dead time', t_0, must be known although it can be a problem to measure it unambiguously. In principle, the first baseline deviation after injection marks t_0. Then the following data can be calculated:

1) **Retention factor**, k (formerly capacity factor, k'):

$$k = \frac{t_R - t_0}{t_0}$$

The retention factor is a measure of the retention of a peak. It depends only on the phase system (the types of mobile and stationary phase) and on the temperature.

2) **Separation factor**, α:

$$\alpha = \frac{k_2}{k_1}$$

Two compounds can be separated only if α is higher than 1.0 in the selected phase system. For HPLC separations α should be 1.05 or higher (→ 1.3).

3) **Theroetical plate number, *N***:

$$N = 16\left(\frac{t_R}{w}\right)^2 = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2 = 2\pi\left(\frac{h_P t_R}{A_P}\right)^2$$

where h_P = peak height and A_P peak area. The plate number is a measure of the separation performance of a column. (The equations given here are in principle only valid for symmetrical peaks.)

From the plate number it is possible to calculate the height, H, of a theoretical plate (e.g., in μm):

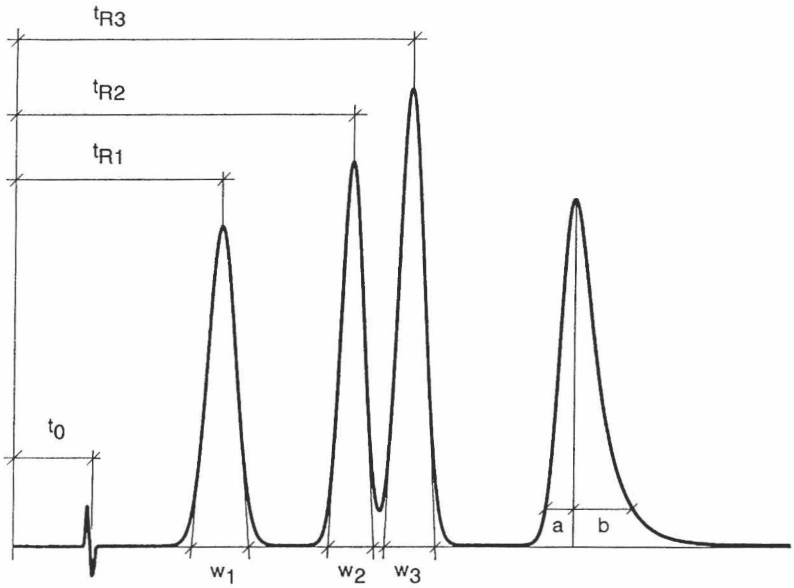
$$H = \frac{L_c}{N}$$

where L_c = column length.

4) **Tailing *T*** (for asymmetric peaks):

$$T = \frac{b}{a}$$

where a and b are determined at 10% of peak height.



t_{R3}
t_{R2}
t_{R1}
t_0
a
b
w_1
w_2
w_3