HPLC

A Practical User's Guide

SECOND EDITION

Marvin C. McMaster

WILEY-INTERSCIENCE
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PREFACE

High-pressure liquid-solid chromatography (HPLC) is rapidly becoming the method of choice for separations and analysis in many fields. Almost anything that can be dissolved can be separated on some type of HPLC column. However, with this versatility comes the necessity to think about the separation desired and the best way to achieve it. HPLC is not now and probably never will be a turn-key, push-button type of operation. Many dedicated system-in-a-box packages are sold for specific separations, but all of these still offer wide possibilities for separation. Changing the column and the flow rate lets you change the separation and the amount of sample you can inject. This is not the worst thing in the world, for it does create great opportunity for the chromatographer and a great deal of job security for the instrument operator.

Fortunately, controlling separations is not nearly as complicated as much of the literature may make it seem. My aim is to cut through much of the detail and theory to make this a usable technique for you. The separation models I present are those that have proven useful to me in predicting separations. I make no claim for their accuracy, except that they work. There are many excellent texts on the market, in the technical literature, and on the Internet, continuously updated and revised, that present the history and the current theory of chromatography separations.

This book was written to fill a need, hopefully, your need. It was designed to help the beginning as well as the experienced chromatographer in using an HPLC system as a tool. Twenty-five years in HPLC, first as a user, then in field sales and application support for HPLC manufacturers, and finally working as a teacher and consultant has shown me that the average user wants an instrument that will solve problems, not create new ones.

I will be sharing with you my experience gained through using my own instrument, through troubleshooting customer's separations, and from field demos; the tricks of the trade. I hope they will help you do better, more rapid separations and methods development. Many of the suggestions are based on tips and ideas from friends and customers. I apologize for not giving them credit, but the list is long and my memory is short. It has been said that plagiarism is stealing ideas from one person and research is borrowing from many. This book has been heavily researched and I would like to thank the many

who have helped with that research. I hope I have returned more than I borrowed.

I have divided this guide into three parts. The first part should give you enough information to get your system up and running. When you have finished reading it, put the book down and shoot some samples. You know enough now to use the instruments without hurting them or yourself. When you have your feet wet (not literally I hope), come back and we will take another run at the material in the book.

Part II shows you how to make the best use of the common columns and how to keep them up and running. (Chapter 6 on column healing should pay for the book in itself.) It discusses the various pieces of HPLC equipment, how they go together to form systems, and how to systematically troubleshoot system problems. We will take a look at the newest innovations and improvements in column technology and how to put these to work in your research. New detectors are emerging to make possible analysis of compounds and quantities that previously were not detectable.

Finally, in Part III, we will talk about putting the system to work on real-world applications. We will look at systematic methods development, both manual and automated, and the logic behind many of the separations that others have made. We will discuss how to interface the HPLC system to computers and robotic workstations. I will also give you my best guesses as to the direction in which HPLC columns, systems, detectors, and liquid chromatography/mass spectrometer (LC/MS) systems will be going.

It is important to give credit where it is due. Christopher Alan McMaster created many of the illustrations in this text before he died of the ravages of muscular dystrophy six years ago. I supplied hand-drawn sketches of the illustrations I used on boards in my classes. Chris turned them into art on his Macintosh. His collaborative efforts are greatly missed.

A brief note is required about the way I teach. First, I have learned that repetition is a powerful tool, not a sign of incipient senility as many people have hinted. Second, I have found in lecturing that few people can stand more than 45 minutes of technical material at one sitting. However, I have also learned that carefully applied humor can sometimes act as a mental change of pace. Properly applied, it allows us to continue with the work at hand. So, occasionally, I will tiptoe around the lab bench. I do not apologize for it, but I thought you ought to know.

The instrument itself is the most effective teacher. Think logically about the system and the chemistry and physics occurring inside the column. You will be surprised how well you will be able to predict and control your separation.

Remember! HPLC is a versatile, powerful, but basically simple separation tool. It is a time machine that can speed your research and, thereby, allow you to do many things not possible with slower techniques. It is both an analytical and a preparative machine. When I finish, I hope you will have the confidence to run your instrument, make your own mistakes, and be able to find your own solutions.

Your HPLC success depends on three things:

1. The suitability of the equipment you buy,
2. Your ability to keep it up and running (or find someone to service it), and
3. The support you receive, starting out in new directions or in solving problems that come up.

Marvin C. McMaster
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