TROUBLESHOOTING LC SYSTEMS

By

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Troubleshooting LC Systems

A Comprehensive Approach to Troubleshooting LC Equipment and Separations

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Common sense is not so common.

—Voltaire

Preface

Over the last 15 years, high-performance liquid chromatography (LC) has made the transition from an instrument used only by experts in research labs to a tool used for routine applications by relatively unskilled workers. With this transition have come major advances in instrumentation and column technology. In the past, the operator had to be a jack-of-all-trades, with a screwdriver, soldering iron, and various wrenches as constant companions in the LC lab. Today, many instruments contain microprocessors as powerful as those of mainframe computers of earlier days. With this technology has come a variety of self-diagnostic tools that allow the LC system to locate many of its own problems.

Traditionally, well-honed LC troubleshooting skills have been a result of years of work at the bench. Today the LC system itself often can do a better job of troubleshooting than the operator can. Yet many of the problems of the past are still the major problems of today: air bubbles, check valves, detector lamps, and, of course, problems with the separation. An added pressure on the operator of today's LC system is that of productivity—the lab often cannot afford unnecessary downtime. This means that the operator has to be a troubleshooting expert, or has to have that expertise at his or her fingertips. The present book was written to provide this expertise in an easy-to-use format for users at all levels of experience.

As a result of several years of teaching short courses on LC plus writing the LC Troubleshooting column for *LC/GC* magazine, we have become aware of two central themes necessary for successful LC troubleshooting. The first is an understanding of how the instrument operates. If the general principles of operation of each module in the LC system are understood, the operator is more likely to avoid problems that result from poor operational technique. The second area is preventive maintenance. For the

most part, there is nothing magic about reliable LC operation. But like an automobile that needs regular oil changes and brake checks, the LC system will provide much better service when certain preventive maintenance procedures are practiced. These two needs are the reason for the strong emphasis on instrument operation and maintenance in this book.

Finally, a book cannot easily satisfy all the needs of all readers, so we have tried to increase its usefulness through its organization. For readers interested in gaining general knowledge, Chapters 6–13 cover the operation, maintenance, and troubleshooting of various modules. In general we have steered away from references to specific instrument brands and models because this would render the book out of date before it was printed. Instead, we have concentrated on generic LC system modules, so that once a technique is learned, it will be useful for years to come. Instrument-specific procedures are best found in operation and service manuals. Although some readers are interested in general information, others will come to this book with a specific problem that must be solved immediately. Study of the comprehensive index plus mastery of the troubleshooting tree of Table 2.3 should help these users find fast first-aid for the problem at hand.

We would be remiss if we did not express our sincere appreciation for the contribution of our colleagues in the review and criticism of the manuscript. Though their input has helped us identify missing topics and clarify several discussions, we accept any responsibility for inaccuracies herein. Participating in the review process were: Ben Buglio of Hoffmann-LaRoche, Ken Cohen of Boehringer-Ingelheim, Nelson Cooke of Beckman/Altex, Russell Gant of Perkin-Elmer, Joe Glajch of DuPont, Mack Harvey of Valco Instruments, Tom Jupille of LC Resources, Kerry Nugent of Michrom Bioresources, Herb Schwartz of Microphoretic Systems, Paul Upchurch of Upchurch Scientific, and Bob Weinberger of Applied Biosystems/Kratos. They greatly improved our book.

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Chapter 1

INTRODUCTION

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If You Can't Wait

Most readers will be reading this chapter as a casual overview of a new book; if you fall into this category, continue. If, however, you are in a jam and have to get an LC (liquid chroma-

tography) problem solved immediately, try one of these three approaches:

1. If you are not sure what the problem is (i.e., you only know the symptom), go to Chapter 2 and follow the flowchart until you find the cause of the problem.

2. If you have isolated the problem to a specific module, consult the appropriate chapter for that module (Chapters 6–13). There is a table of contents at the beginning of each chapter to guide you to the proper section. At the end of each chapter is a summary table giving solutions to the problems which were discussed.

3. If you know the cause of the problem, and it is not clearly classified as originating in a particular module, use the index at the end of the book to help find the proper information.

If you can't wait, read no further in this chapter.

1.1. An Expert at Your Fingertips

In many labs there is an expert who has the ability to fix an LC system faster and more surely than anyone else. This "guru" may be a PhD chemist with years of experience, or a technician with the proper "feel" for what's going on. It can be very frustrating to have spent several hours (or days) trying to fix an especially troublesome problem, only to have someone look over your shoulder and ask, "have you tried..." which then, of course, solves the problem.

Our goal in writing this book is to condense the expertise of many practical LC workers into a form that is useful for beginning and experienced chromatographers alike. We want this book to become your close companion and helper. There are details here on how to perform some pretty simple procedures, but—simple as they may be—they can be hard to learn unless you have someone to teach you. You should not be afraid to try any of the procedures

discussed here, because there is little chance of causing irreparable or expensive damage to your LC system. We purposely have not included discussions of electronics problems, monochromator adjustments, and the like, which lie beyond the expertise of most workers.

We feel that you will be a better chromatographer and therefore be more fully equipped to solve problems quickly if you have an understanding of how the various LC modules work. For this reason, a discussion of the principles of operation of each module is included in the appropriate chapter. It is also important to have a good understanding of the chromatographic process. This is reviewed in Chapter 3.

As in the case of the expert discussed above, this book contains more details about the operation and troubleshooting of LC systems than most users care to know. When a problem arises, however, any of this information can be useful. Because our book is organized around the various system modules, there is considerable overlap in some places. We have often repeated the most useful information and cross-referenced the rest. For this reason, you may find that the index is the most expedient place to locate a specific topic.

1.2. LC vs HPLC

HPLC, HSLC, LC, high-performance liquid chromatography, high-pressure liquid chromatography, high-priced liquid chromatography, high-speed liquid chromatography, liquid chromatography, modern liquid chromatography.... No doubt you've heard all these terms (and more) to describe the same technique. What was high performance ten years ago is pretty low performance today; pressures are high only if there is a problem with the system. We're all talking about the same technique—separations on columns with small (3–10 μm) particles, in fairly short times (typically < 1 h), and at pressures up to 6000 psi. This is in contrast to traditional open-column or thin-layer (TLC) techniques. You probably have noticed by now that we've chosen to use just liquid chromatography (LC) to describe the technique. Although we (and many others) use it, you should note that the "LC" abbreviation is not used universally. For example, LC is not used in the

description of HPLC-grade solvents (typically brand names) nor in other places where clarification is needed (e.g., to distinguish LC from another liquid chromatographic technique such as sample-cleanup columns).

1.3. How to Use This Book

The goal of any troubleshooting technique is to observe, isolate, and correct a problem with a minimum of time, money, and lost data. In order to quickly solve LC problems, it is necessary to understand how our book is organized and how best to use it. Then the right information can be readily accessed for a particular problem.

This book is divided into three main sections, each of which is written for a specific use. The first section, General Considerations, covers basic material that we feel all chromatographers should know in order to do their job well. Chapter 2 contains a comprehensive set of flow-charts to help you quickly isolate and correct LC problems. This is the chapter that you probably will use more than any other in the book, so you should understand how it is organized. You can read (or at least skim) the material in Chapters 3–5 as a review and/or to fill in those areas in which you need to be competent.

The second section of the book, Individual LC Modules, gives a detailed discussion of each of the major parts of the LC system. These chapters cover not only troubleshooting, but also the principles of operation and maintenance. Each chapter contains a list of spare parts that should be stocked for routine maintenance and emergency use. Most readers will not want to take the time to read all of these chapters at one sitting. You are encouraged to look them over so that you are aware of the kinds of information they contain. In this way, you'll have a headstart on finding information when you do need it.

The third section, Troubleshooting the Separation plus Other Problems, concentrates on problems related to the separation (Chapters 14–17) rather than equipment. If you work with method development or have to troubleshoot separation problems, you'll find reading Chapters 14 and 15 especially worthwhile.

We've taken extra care to make the index as comprehensive and useful as possible. Nothing is more frustrating than trying to find information in a book that has an inadequate index. With the index, Chapter 2, and the contents at the beginning of each chapter, you should be able to quickly find specific topics from a number of starting points.

The remainder of this chapter discusses how to use this book to help identify, isolate, and correct LC problems.

Observing That a Problem Exists

Because it is first necessary to know that a problem exists, we stress a knowledge of the normal operation of the LC system. This includes keeping records of system history plus chromatographic performance under standard conditions, as well as using your five senses (and common sense) to know that the LC system is running properly. Written records of normal system operation can make the identification of problems easier. In order to help you decide whether an observation is normal or represents a problem, the chapters covering specific LC modules include a section describing normal operation.

Isolate the Problem

When a problem exists, the problem must be classified and isolated. Chapter 4 (Principles of Troubleshooting) gives additional detail on how to classify a problem. The logic tree in Chapter 2 (Logical Approaches to Troubleshooting) helps you to quickly determine the problem area by giving Yes/No choices (or a limited number of selections). For example, you will observe the problem either (a) in the chromatogram itself, or (b) elsewhere in the system. Problems elsewhere in the system often are easier to fix, because they usually occur in the malfunctioning module. For example, a leaking fitting is easy to isolate, but problems with the chromatogram can be caused by many parts of the system (e.g., noise spikes in the chromatogram can be caused by air bubbles, a bad detector lamp, a poorly operating recorder, or something else). Further work is required to fully isolate chromatographic problems.

In this vein, it is useful to note that Chapter 2 is organized in a symptom–cause–solution manner, so all that you have to know is the symptom(s) that you see when you find a problem. The remaining chapters, on the other hand, are topical in nature and are organized in a cause–symptom–solution sequence. Thus, these chapters are most useful when you know (or suspect) the cause of the problem.

Several things should be kept in mind during problem isolation. *First,* know when to ask for help (see Chapter 5, Prevention of Problems). You should not be afraid to undertake procedures that you have not done before, but you should also know your limitations. For example, most LC users feel comfortable addressing hardware problems by themselves, but not electronic problems.

Second, keep notes on the effect of changes you make in the system. For example, if you suspect that an elevated system pressure is the result of a blocked injection-valve loop, record the pressure, mark the suspect loop so that you can identify it, replace the loop, and record the new pressure. Much troubleshooting time is wasted because of poor recordkeeping. This can result in (a) not being sure of what action corrected the problem, and (b, even worse) putting a faulty component back into the spare parts supply. Future troubleshooting should build on past experience, so that problems are solved more quickly.

Third, approach each problem in a logical, stepwise manner. This is discussed in more detail in Chapter 4 (Principles of Troubleshooting). Many times, simple Yes/No tests can be made to quickly isolate a problem. For example, when the pressure rises, you should not immediately assume that the column frit is blocked and therefore replace the frit or column. Perhaps the problem is caused by a worn injection valve or a blocked connecting tube. Follow a logical pressure-isolation sequence until the blockage is located.

Once the problem has been isolated to a particular module, you may or may not need further help in isolating the cause of the problem. Consult the "Problems and Solutions" section of the appropriate chapter for more help. Most chapters contain a final table summarizing the problems, symptoms, and solutions for the topics discussed in that chapter.

Correct the Problem

Directions for the correction of many LC-system problems are given in chapters relevant to each module. These can be quickly found in one of three ways. *First,* if you are using the troubleshooting outline (Chapter 2), you will be directed to a specific section for directions. *Second,* you can use the index at the end of the book. *Third,* for added convenience, there is a table of contents at the beginning of each chapter to help you quickly locate information in that chapter. This may be more convenient to use than the index at the end of the book if you are interested in problems with just one module.

Several chapters contain exploded diagrams (Disassembly/Assembly Procedures) for some of the more common variations of the module under discussion. These, along with instructions for solving more general problems (e.g., mobile-phase degassing) will help you correct many LC problems. It should be understood, however, that this book is not a substitute for the User's Manual or Service Manual for the LC module or system. Manufacturer's manuals should always be consulted when detailed instructions are needed for the repair of a particular LC brand or model.

1.4. Other Sources of Troubleshooting Information

By its nature, this book cannot contain complete details on how to troubleshoot every brand and model of LC system. For this reason, you will have to rely on other sources of information, and we have listed some of these resources below.

Operation and Service Manuals

Your primary source of specific details for repairing an LC module or system should be the operation and/or service manuals which came with the instrument when it was new. Though not all of these manuals are of equal quality, most contain exploded diagrams and step-by-step procedures for fixing the majority of hardware failures. If electronic diagnostics are available for your LC system, the manual should contain a discussion of the various error messages that you may encounter.

Manufacturer's Hot Lines

Many LC manufacturers have toll-free (800-number) "hot" lines staffed by technicians ready to help you solve your LC problem. Even if the manufacturer does not publicly list this service, all manufacturers will provide some level of troubleshooting help —just call the company number and ask for "technical support".

LC/GC Troubleshooting Articles

LC/GC magazine publishes a monthly column dealing specifically with LC Troubleshooting. Topics include (a) in-depth discussions of the operation and maintenance of the various LC modules, (b) case studies, and (c) responses to reader questions. This column can be a good source of up-to-date troubleshooting information. If you have a specific question, you can write to the editor for advice. Each year the December issue of LC/GC contains an index of the topics discussed for that year.

Troubleshooting Guides

Several manufacturers (e.g., Phenomenex, Isco) have published short troubleshooting guides that can be obtained at no cost. Though these guides are not as comprehensive as this book, they contain compact troubleshooting trees that can help you to isolate and solve LC problems. An alternative to printed troubleshooting guides is *The HPLC Doctor,*[1] a computer-based expert system for LC troubleshooting; it uses logic trees similar to those in Chapter 2 to help you isolate and correct LC problems.

Troubleshooting Video

A video course, *Troubleshooting HPLC Systems*[2] is also available. In three hours of video instruction, the course covers the highlights of this book. Watching a videotape is a good way to get started in LC troubleshooting; the live demonstrations (e.g., changing pump seals) are especially useful if you do not have someone in the lab to teach you such basic troubleshooting techniques.