

LC/MS

A Practical User's Guide

MARVIN C. McMASTER



A JOHN WILEY & SONS, INC., PUBLICATION

LC/MS

LC/MS

A Practical User's Guide

MARVIN C. McMASTER



A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2005 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data:

McMaster, Marvin C.

LC/MS: a practical user's guide / Marvin C. McMaster.

p. cm.

Includes bibliographical references and index.

ISBN-13 978-0-471-65531-2 (cloth)

ISBN-10 0-471-65531-7 (cloth)

1. Liquid chromatography—Handbooks, manuals, etc. 2. High performance liquid chromatography—Handbooks, manuals, etc. 3. Mass spectrometry—Handbooks, manuals, etc. I. Title.

QD79.C454M363 2005

543'.84—dc22

2004063820

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

*To the memory of my son, Chris McMaster, my writing partner and the artist
on the first two books in this series. Chris has passed on to bigger and better
things painting sunrises and rainbows.*

CONTENTS

Preface	xi
1 Introduction to LC/MS	1
1.1 Why LC/MS?, 1	
1.2 Molecular Weights and Structure Studies, 4	
1.3 LC/MS Systems, 4	
1.4 System Costs, 7	
1.5 Competitive Systems, 7	
2 The HPLC System	9
2.1 HPLC System Components, 9	
2.2 Gradient versus Isocratic Systems, 14	
2.3 Micro HPLC Systems, 16	
2.4 HPLC Tubing and Fittings, 18	
3 The HPLC Column and Separation Modes	21
3.1 Column Construction, 21	
3.2 Column Packing Materials, 23	
3.3 Normal-Phase Columns, 25	
3.4 Other Bonded-Phase Silica Columns, 26	
3.5 Optimizing Reverse-Phase Column Use, 28	
3.6 Silica Ion-Exchange Columns, 30	
3.7 Silica Size-Separation Columns, 31	

3.8	Zirconium Bonded-Phase Columns, 31	
3.9	Polymer Reverse-Phase Columns, 32	
4	HPLC and Column Maintenance	33
4.1	HPLC Maintenance, 33	
4.2	Column Maintenance, 37	
5	Sample Preparation and Separations Development	41
5.1	Mobile-Phase Preparation, 41	
5.2	Mobile-Phase pH Control Using Buffers, 42	
5.3	Sample Preparation, 44	
5.4	Cartridge Column Cleanup, 44	
5.5	On-Column Sample Concentration, 45	
5.6	Isocratic and Gradient Methods Development, 46	
5.7	Automated Methods Development, 49	
6	LC/MS Interfaces	51
6.1	Solvent Removal and Ionization, 51	
6.2	Atmospheric-Pressure Interfaces, 52	
6.3	Electrospray Interface, 53	
6.4	Ion Spray Interface, 54	
6.5	Secondary Detectors, 55	
7	LC/MS Overview	59
7.1	HPLC and the Ionization Source, 60	
7.2	Vacuum Pumps, 61	
7.3	Analyzer and Ion Detector Designs, 61	
7.4	Data and Control Systems, 66	
7.5	Peak Detection, ID, and Quantitation, 69	
8	Mass Analyzers	71
8.1	Quadrupole Analyzer, 71	
8.2	Ion Trap Analyzer, 74	
8.3	Linear Ion Trap Analyzer, 78	
8.4	Time-of-Flight Analyzer, 79	
8.5	Fourier Transform Analyzer Design, 81	
8.6	Magnetic Sector Analyzers, 83	
9	Mass Spectrometer Maintenance	85
9.1	High-Vacuum Operation, 85	
9.2	MS Hardware Maintenance, 88	
9.3	System Electrical Grounding, 92	

10	Application Areas for LC/MS	95
10.1	Compound Discovery,	95
10.2	Identification of Complex Biological Compounds,	96
10.3	Analysis of Trace Impurities and Metabolites,	97
10.4	Arson Residue Investigation,	98
10.5	Industrial Water and Pesticide Analysis,	98
10.6	Toxicology and Drugs of Abuse,	98
10.7	Clinical Therapeutic Drug Screening,	99
10.8	Pesticide Manufacturing,	101
11	Trace Analysis and LC/MS/MS	103
11.1	LC/MS/MS Triple-Quadrupole System,	103
11.2	MS/MS Operating Modes,	104
11.3	Ion Trap MS/MS Operation,	106
11.4	Hybrid LC/MS/MS Systems,	108
12	Drug Discovery and Benchtop LC/MS	111
12.1	Activity Screening,	111
12.2	Standardized LC/MS Screening,	113
12.3	Molecular Fragmentation for Structural Determination,	115
12.4	Process Monitoring,	116
13	Proteomics: LC/MALDI/TOF and MS/MS Libraries	119
13.1	Protein Molecular-Weight Determination by LC/MS,	120
13.2	De Novo Protein Purification,	121
13.3	Protein Analysis by Two-Dimensional GEP and LC/TOFMS,	122
13.4	LC/MS/MS Identification of Peptide Structures,	122
13.5	Tracer Labeling for Peptide ID,	124
13.6	Posttranslational Modified Protein,	124
13.7	Transient Peptides and Accumulation Proteins,	124
14	The Future of LC/MS	127
14.1	Instrumentation Improvements,	127
14.2	Affordable Benchtop LC/LITMS,	129
14.3	User-Customized Data Libraries,	129
14.4	Nucleomics and Restriction Fragment Analysis,	130
Appendix A	LC/MS Frequently Asked Questions	131
Appendix B	Solvents and Volatile Buffers for LC/MS	139
Appendix C	Guide to Structure Interpretation	143

Appendix D	Glossary of LC/MS Terms	149
Appendix E	LC/MS Selective Reading List	155
Index		157

PREFACE

I consult and teach extension courses on laboratory instrumentation and computers at the University of Missouri–St. Louis. I taught a course called *Practical HPLC* for a number of years while working as a sales representative and technical support specialist for a variety of instrument companies. The first book in this series, *HPLC: A Practical User's Guide*, arose out of a need for a textbook for my course. At the end of that book I wrote a chapter on a rising research technique that I felt would eventually transform the life of the average laboratory chemist and provide a tool for definitive identification of the compounds that he or she was producing.

I next had an opportunity to work with a manufacturer of control and data systems for GC/MS equipment. I added consulting and teaching in this specialty to my portfolio and designed a book, *GC/MS: A Practical User's Guide*, to provide a teaching tool. Again, I added a final chapter on the growing art of LC/MS. I feel another book and course are needed now that commercial sales of LC/MS systems has nearly equaled those of GC/MS systems. This tool combines my expertise and interests in several separations areas.

I do not attempt to write the definitive book for a new instrumentation specialty. I want to put together a useful tool for introducing the technique and providing practical information on how to use it. I try to look at complicated material, internalize it, and present it in a way that is understandable and useful for solving laboratory problems. When inexpensive, easy-to-use LC/MS systems appear on the end of every laboratory bench, I would like to have a copy of this book setting next to them to lay the groundwork for getting the most out of the system.

When I teach practical courses, I use an overhead projector and a PowerPoint slide set to provide the theme and illustrations for the course. I realize that

if I were buying this book to use as a teaching text book, it would be very useful to have the slide set on a CD-ROM disk. In the back of this book I have included such a disk with my slide set, searchable files on LC/MS Frequently Asked Questions, a glossary of terms, and useful LC/MS tables. For the LC/MS students, this provides a series of self-study guides for learning or honing their LC/MS skills. I hope the readers of this book will find these additional tools useful. I plan to add similar tools to later editions of my other books.

I wish to thank the following companies for permission to use drawings and illustrations from their brochures and Web sites: Agilent Technologies, Applied Biosystems, ESA, Varian, and Waters Corporation. I have found in teaching that pictures truly are worth a thousand words. Their kind assistance has helped me keep this book down to a reasonable size. I never have cared for “rat killer” manuals.

MARVIN C. MCMASTER

Florissant, Missouri

1

INTRODUCTION TO LC/MS

Liquid chromatography (LC) combined with mass spectrometry (MS) creates an ideal analytical tool for the laboratory. The high-performance liquid chromatograph (HPLC) has been the laboratory tool of choice for separating, analyzing, and purifying mixtures of organic compounds since the 1970s.

An HPLC column can separate almost any mixture that can be dissolved. A mass spectrometer can ionize the separated peak solution and provide a molecular weight for each peak component. An LC/MS/MS system can fragment the parent ion into a distinctive fragmentation pattern and can separate the daughter ions for identification and quantitation. The characteristic fragmentation pattern from each parent ion can be identified by comparison to fragmentation patterns produced by standard computerized databases. The output of the HPLC system can be divided for analysis by other HPLC detectors or for preparative sample recovery, since only a small portion of the column effluent is required for mass spectral analysis.

1.1 WHY LC/MS?

The preferred tool until the turn of the millennium for separating a mixture and providing definitive identification of its components was the gas chromatograph/mass spectrometer (GC/MS). However, this technique was limited by three main factors:

1. Sample volatility.
2. The fact that aqueous samples require extraction.
3. Thermal degradation of samples in the GC oven.

Not all compounds are volatile enough to be introduced or eluted off a GC column. Aqueous mixtures have to be extracted and/or derivatized before injection, adding to analysis cost and bringing sample handling errors into peak quantitation. The columns available were not able to resolve all mixtures of compounds. This problem has been eliminated somewhat with new varieties of columns. Oven-temperature programming remains the principal variable available for separating compounds in a mixture. The final oven temperature necessary to remove a large compound from a column can degrade many thermally labile compounds.

In the last two years, LC/MS sales have nearly equaled GC/MS sales because of the additional compounds that can be analyzed by LC/MS and the greater range of separation variables that can be utilized in HPLC separation. The editors of *Analytical Instrumentation Industry Reports* say that in 2000 the global GC/MS market was \$300 million and that LC/MS sales reached \$250 million. This does not indicate parity, but it does show that the gap is closing. One industry analyst predicted that LC/MS sales should top \$1 billion by 2005. The difference in cost of a HPLC system and its interface compared to a gas chromatograph must be factored into these numbers when comparing unit costs. An isocratic HPLC system costs 50% more than a basic GC module. The cost difference nearly doubles when you add in the cost of an atmospheric-pressure interface (API). Gradient HPLC configuration increases the cost to triple that of a GC module. However, all of these costs are overshadowed by the price of a mass spectrometer.

For LC/MS to be a major player in the analytical laboratory, there are factors limiting performance that must be overcome:

- Analyzer signal swamping by the elution solvent.
- Solvent composition changing in gradient elution.
- Buffer use for pH control.
- Ionization of neutral peak components.

By far the most important of these is the volume of eluting solvent necessary to displace the compounds separated from the HPLC column. The mass analyzer is quickly overwhelmed by the signal from the solvent if the HPLC output is introduced directly into the mass spectrometer. The analyte signal is buried beneath this solvent signal avalanche. The solvent signal saturation effect occurs even if a low-molecular-weight solvent such as methanol or water is chosen and a low analyzer mass cutoff range is selected to exclude the solvent's peak signal. A method for in-stream solvent removal with concurrent sample concentration must be provided to connect the column effluent to a high-vacuum mass spectrometer. The HPLC solvent gradient used to resolve closely eluting HPLC peaks and decrease HPLC run times also produces solvent composition changes that further complicate the solvent-masking effect of analyte signal.

Many compounds resolved by the HPLC column require pH control to adhere to the column long enough to be eluted. Removal of nonvolatile buffer and ion-pairing reagents commonly used in HPLC separations from the effluent is the next

problem that must be handled. Direct introduction of inorganic compounds into a high-vacuum system will cause mass spectrometer inlet fouling and loss of signal. Organic buffers used instead of inorganic buffers exhibit the same problems as those found with organic solvents: They overwhelm the analyzer and detector. Replacing nonvolatile buffers and reagents with volatile equivalents allows them to be removed like solvent. The final hurdle is that neutral compounds separating off the HPLC column must be converted to charged molecular ions or fragmented into charged ions that can be separated by the analyzer.

API using ion spray and electrospray interfaces provides many of the answers to these problems. At least part of the stream from the HPLC is sprayed over a high-voltage coronal discharge needle in a heated chamber, vaporizing the solvent and charging the suspended molecule, creating a molecular ion. A neutral flowing curtain gas sweeps much of the solvent and volatile additives out of the interface before the ionized analyte is pulled into the pinhole entrance to the high-vacuum environment of the analyzer. One of Jack Henion's papers produced at Cornell University reports that he operated an ion spray interface at effluent flow rates of 2 mL/min of methanol/water containing phosphate buffer to feed sample into a Hewlett-Packard MSD mass spectrometer with its vacuum provided by a tiny turbo pump, but this should be looked on as an exception to the rule of using volatile components.

Liquid chromatography provides a wide variety of operating variables that can be used to control and optimize a separation:

- Column-bonded phase selection with rapid column switching.
- Major solvent change with rapid reequilibration.
- Mobile-phase polarity adjustment and gradient operation.
- Packing support selection for pH and temperature stability.
- Temperature programming.

Most HPLC separations have been carried out using reverse-phase silica columns, with non-polar-bonded phases eluting compounds with polar solvents. A wide variety of bonded phases are available to achieve these separations. Various nonpolar mobile-phase solvents can be selected to shift elution orders of compounds on the same type of column. Mixing nonpolar solvents with water can change solvent polarity, increasing or decreasing partitioning with bonded-phase packing.

Traditional HPLC column supports have had nonpolar bonded phase bound to a silica matrix. These bonded phases are unstable under strongly acidic conditions, and the silica matrix dissolves rapidly at mildly basic pH. Newer polymeric and zirconium matrixes provide reverse-phase columns that are both pH and temperature stable. These packing materials allow operation at high or low pH without using buffers. Zirconium packing allows use of temperature as a separations variable using a temperature-controlled column jacket. Thermally labile compounds would have some of the same problems as those seen in a GC oven, but the temperature control range is much lower in HPLC, due to solvent volatility.