

Quantitative Applications of Mass Spectrometry

Irma Lavagnini

University of Padova, Padova, Italy

Franco Magno

University of Padova, Padova, Italy

Roberta Seraglia

National Council of Research, Padova, Italy

Pietro Traldi

National Council of Research, Padova, Italy



JOHN WILEY & SONS, LTD

Quantitative Applications of Mass Spectrometry

Quantitative Applications of Mass Spectrometry

Irma Lavagnini

University of Padova, Padova, Italy

Franco Magno

University of Padova, Padova, Italy

Roberta Seraglia

National Council of Research, Padova, Italy

Pietro Traldi

National Council of Research, Padova, Italy



JOHN WILEY & SONS, LTD

Copyright © 2006

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester,
West Sussex PO19 8SQ, England

Telephone (+44) 1243 779777

Email (for orders and customer service enquiries): cs-books@wiley.co.uk

Visit our Home Page on www.wileyeurope.com or www.wiley.com

All Rights Reserved. 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 under the terms of the Copyright, Designs and Patents Act 1988 or under the terms of a licence issued by the Copyright Licensing Agency Ltd, 90 Tottenham Court Road, London W1T 4LP, UK, without the permission in writing of the Publisher.

Requests to the Publisher should be addressed to the Permissions Department, John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England, or emailed to permreq@wiley.co.uk, or faxed to (+44) 1243 770620.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The Publisher is not associated with any product or vendor mentioned in this book.

This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the Publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Other Wiley Editorial Offices

John Wiley & Sons Inc., 111 River Street, Hoboken, NJ 07030, USA

Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 42 McDougall Street, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

John Wiley & Sons Canada Ltd, 22 Worcester Road, Etobicoke, Ontario, Canada M9W 1L1

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Library of Congress Cataloging-in-Publication Data

Quantitative applications of mass spectrometry/Irma Lavagnini ... [et al.].

p. cm.

Includes bibliographical references and index.

ISBN-13: 978-0-470-02516-1 (pbk. : acid-free paper)

ISBN-10: 0-470-02516-6 (pbk. : acid-free paper)

1. Mass spectrometry. 2. Chemistry, Analytic—Quantitative.

I. Lavagnini, Irma.

QD96.M3Q83 2006

543'.65—dc22

2005036663

British Library Cataloguing in Publication Data

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

ISBN-13 978-0-470-02516-1 (Paperback)

ISBN-10 0-470-02516-6 (Paperback)

Typeset in 10/12 pt Times by Thomson Press (India) Ltd, New Delhi, India

Printed and bound in Great Britain by TJ International Ltd, Padstow, Cornwall

This book is printed on acid-free paper responsibly manufactured from sustainable forestry in which at least two trees are planted for each one used for paper production.

*To our past, present and future students who stimulate our interest
in the research and who, hopefully, have learnt or will learn
something from our efforts*

Contents

<i>Preface</i>	ix
<i>Acknowledgements</i>	xi
<i>Introduction</i>	xiii
1 What Instrumental Approaches are Available	1
1.1 Ion Sources	1
1.1.1 Electron Ionization	3
1.1.2 Chemical Ionization	4
1.1.3 Atmospheric Pressure Chemical Ionization	6
1.1.4 Electrospray Ionization	8
1.1.5 Atmospheric Pressure Photoionization	11
1.1.6 Matrix-assisted Laser Desorption/Ionization	12
1.2 Mass Analysers	14
1.2.1 Mass Resolution	14
1.2.2 Sector Analysers	15
1.2.3 Quadrupole Analysers	19
1.2.4 Time-of-flight	25
1.3 GC/MS	27
1.3.1 Total Ion Current (TIC) Chromatogram	27
1.3.2 Reconstructed Ion Chromatogram (RIC)	28
1.3.3 Multiple Ion Detection (MID)	29
1.4 LC/MS	29
1.5 MS/MS	30
1.5.1 MS/MS by Double Focusing Instruments	30
1.5.2 MS/MS by Triple Quadrupoles	31
1.5.3 MS/MS by Ion Traps	32
1.5.4 MS/MS by Q-TOF	34
References	34
2 How to Design a Quantitative Analysis	37
2.1 General Strategy	38

2.1.1	Project	41
2.1.2	Sampling	41
2.1.3	Sample Treatment	42
2.1.4	Instrumental Analysis	43
2.1.5	Method Validation	53
	References	53
3	How to Improve Specificity	55
3.1	Choice of a Suitable Chromatographic Procedure	56
3.1.1	GC/MS Measurements in Low and High Resolution Conditions	56
3.1.2	LC/ESI/MS and LC/APCI/MS Measurements	61
3.2	Choice of a Suitable Ionization Method	79
3.3	An Example of High Specificity and Selectivity Methods: The Dioxin Analysis	85
3.3.1	Use of High Resolution MID Analysis	85
3.3.2	NICI in the Analysis of Dioxins, Furans and PCBs	93
3.3.3	MS/MS in the Detection of Dioxins, Furans and PCBs	95
3.4	An Example of MALDI/MS in Quantitative Analysis of Polypeptides: Substance P	101
	References	106
4	Some Thoughts on Calibration and Data Analysis	107
4.1	Calibration Designs	108
4.2	Homoscedastic and Heteroscedastic Data	108
4.2.1	Variance Model	109
4.3	Calibration Models	109
4.3.1	Unweighted Regression	109
4.3.2	Weighted Regression	119
4.3.3	A Practical Example	126
4.4	Different Approaches to Estimate Detection and Quantification Limits	130
	References	132
	<i>Index</i>	135

Preface

This book has been born from the long-term collaboration (and friendship!) existing between two research groups operating in the Padova area. The first has operated for more than 30 years in the field of analytical chemistry, the second for nearly the same amount of time in organic mass spectrometry. The exchange of specific knowledge and experiences between the two groups has been very fruitful, in particular in the development phase of quantitative analyses by mass spectrometry. Both operative and theoretical aspects have been the objects of many discussions and this was fundamental to clarify those doubts that have arisen for those working in the research and analytical fields.

In the last two decades mass spectrometry has shown a phenomenal growth and nowadays it is an essential tool in environmental and biomedical fields. The problem that can arise from this wide expansion is that mass spectrometry is often mainly considered as a 'magic box' in which on one side a sample is introduced and on the other side the analytical data come out. The software (and the marketing!) has removed all the doubts and critical analysis of the data.

With this book we wish to present some very basic information to the scientists and technicians working in the field of quantitative organic mass spectrometry.

Our efforts have been devoted to authoring a book that is easy to read for researchers who are not necessarily physicists or chemists, but mainly for those who, for the first time, face all the problems arising from the development and use of a quantitative procedure. The last chapter presents a description of the theoretical aspects related to calibration and data analysis and is devoted to those who wish to learn more about these aspects.

The picture we have chosen for the cover is a Dolomite peak. Leaving aside the banal shape similarity with a chromatographic peak, this choice was made because the mountain is a good teacher of life: if you want to reach the top of the peak directly you must exert a lot of energy or, alternatively, you must study its structure and choose the right way to reach the summit with less effort. In other words each

mountain climbed requires both a general strategy and many tactical choices to be performed along the way. Thus, the same approach must be used in the development of a quantitative analysis by mass spectrometry: be sure of each step you are doing, otherwise the peak will remain out of reach!

Acknowledgements

The authors wish to thank sincerely Dr. Roberta Zaugrando (Venice University) for the dioxine analysis data and Profs. Gloriano Moneti and Giuseppe Pieraccini (Florence University) for the data related to testosterone analysis.

Introduction

Nowadays, mass spectrometry (MS) is one of the most frequently employed techniques in performing quantitative analysis. Its specificity, selectivity and typical limit of detection are more than enough to deal with most analytical problems.

This is the result of significant effort, either from the scientists working in the field or from the manufacturing industry, devoted to the development of new ionization methods, expanding the application fields of the technique, and new analysers capable of increasing the specificity mainly by collisional experiments (MS/MS or “tandem mass spectrometry”) or by high mass accuracy measurements.

Thus, the MS panorama is made up of many instrumental configurations, each of which have specific positive and negative aspects and different cost/benefit ratios.

Of course, these mass spectrometric approaches are usually employed when linked to suitable chromatographic (C) systems. The synergism obtained allows C-MS to be used worldwide and is of considerable interest to researchers involved in basic chemistry, environmental and food controls, biochemistry, biology and medicine.

It is to be expected that this diffusion will grow in the future, due to the relevance of the information that quantitative MS can provide, in particular in the field of public health. For this reason, some basic information on the phenomena which form the basis of different instrumental approaches, the general strategy to be employed for the development of a quantitative analysis, the role of the specificity in this context and some theoretical aspects on calibration and data analysis, are of interest and this book aims to cover, as simply as possible, all these aspects.

