

# APPLICATIONS OF MODERN MASS SPECTROMETRY



Editors:

**Atta-ur-Rahman, FRS**

**M. Iqbal Choudhary**

**Syed Ghulam Musharraf**

**Bentham Books**

# **Applications of Modern Mass Spectrometry**

***(Volume 2)***

Edited by

**Atta-ur-Rahman, *FRS***

*Kings College  
University of Cambridge  
Cambridge  
UK*

**M. Iqbal Choudhary  
Mustafa (PBUH) Prize Laureate**

&

**Syed Ghulam Musharraf**

*International Center for Chemical and Biological Sciences,  
(HEJ Research Institute of Chemistry and Dr. Panjwani  
Center for Molecular Medicine and Drug Research),  
University of Karachi, Karachi-75270, Pakistan*

## **Applications of Modern Mass Spectrometry**

*(Volume 2)*

Editors: Atta-ur-Rahman, M. Iqbal Choudhary & Syed Ghulam Musharraf

ISSN (Online): 2717-6037

ISSN (Print): 2717-6029

ISBN (Online): 978-981-5050-05-9

ISBN (Print): 978-981-5050-06-6

ISBN (Paperback): 978-981-5050-07-3

©2024, Bentham Books imprint.

Published by Bentham Science Publishers Pte. Ltd. Singapore. All Rights Reserved.

First published in 2024.

## **BENTHAM SCIENCE PUBLISHERS LTD.**

### **End User License Agreement (for non-institutional, personal use)**

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the book/echapter/ejournal (“**Work**”). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: [permission@benthamscience.net](mailto:permission@benthamscience.net).

### **Usage Rules:**

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

### ***Disclaimer:***

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction, advertisements or ideas contained in the Work.

### ***Limitation of Liability:***

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

### **General:**

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of Singapore. Each party agrees that the courts of the state of Singapore shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

**Bentham Science Publishers Pte. Ltd.**

80 Robinson Road #02-00

Singapore 068898

Singapore

Email: [subscriptions@benthamscience.net](mailto:subscriptions@benthamscience.net)



## CONTENTS

<b>PREFACE</b> .....	i
<b>LIST OF CONTRIBUTORS</b> .....	iii
<b>CHAPTER 1 ION MOBILITY-MASS SPECTROMETRY FOR MACRO- MOLECULE ANALYSIS</b> .....	1
<i>Mehmet Atakay, Hacı Mehmet Kayılı, Ülkü Güler and Bekir Salih</i>	
<b>INTRODUCTION</b> .....	1
IM-MS Analysis in Proteomics .....	4
IM-MS Analysis of Glycoconjugates .....	8
Main Strategies for the Analysis of Glycoconjugates by Mass Spectrometry .....	9
IM-MS Analysis of Intact Glycopeptides .....	10
IM-MS Analysis of Intact Glycans .....	11
Evaluation of IM-MS Platforms in Isomeric Glycan Analysis .....	12
Polymer Characterization using IM-MS .....	14
<b>CONCLUDING REMARKS</b> .....	20
<b>ACKNOWLEDGEMENT</b> .....	21
<b>REFERENCES</b> .....	22
<b>CHAPTER 2 RECENT ADVANCEMENTS IN THE DETECTION OF ORGANIC CONTAMINANTS IN WASTEWATER USING ADVANCED MASS SPECTROMETRY</b> .....	36
<i>Senuri Kumara, Laksiri Weerasinghe and Imalka Munaweera</i>	
<b>INTRODUCTION</b> .....	37
Basic Components of the Mass Spectrometer .....	38
Sample Analysis and Preparation .....	39
<b>MAJOR EMERGING CONTAMINANTS, THEIR ABUNDANCE AND THEIR TOXICITY</b> .....	40
Pharmaceuticals .....	40
Pesticides .....	41
Per-fluorinated Compounds .....	42
<b>EVOLUTION OF MASS SPECTROMETRY</b> .....	43
3.1. History of Mass Spectrometry in brief .....	43
Sample Extraction Techniques .....	44
Sample Separation Techniques .....	45
Sample Ionization Sources .....	47
Mass Analyzers .....	48
Miniaturizing and Automation of Mass Spectrometry .....	49
<b>DETECTION OF ORGANIC CONTAMINANTS IN WASTEWATER USING ADVANCED MASS SPECTROMETRY</b> .....	51
Detection of Pharmaceuticals, Hormones and Illicit Drugs in Wastewater Using Advanced Mass Spectrometry .....	51
Detection of Pesticides in Wastewater Using Advanced Mass Spectrometry .....	59
Detection of PFASs in Wastewater Using Advanced Mass Spectrometry .....	62
Detection of other Emerging Contaminants in Wastewater Using Advanced Mass Spectrometry .....	65
<b>CONCLUSION AND FUTURE PROSPECTS</b> .....	68
<b>REFERENCES</b> .....	73
<b>CHAPTER 3 POISONOUS SUBSTANCES IN TROPICAL MEDICINAL AND EDIBLE PLANTS: TRADITIONAL USES, TOXICOLOGY, AND CHARACTERIZATION BY HYPHENATED MASS SPECTROMETRY TECHNIQUES</b> .....	90

*Amanda E. de Athayde, Monalisa A. Moreira, Gabriella B. Souza, Tiago Tizziani,  
Maique W. Biavatti and Louis P. Sandjo*

<b>INTRODUCTION</b>	91
<b>EXAMPLE OF TROPICAL EDIBLE PLANTS USED IN TRADITIONAL PHARMACOPEIA AS MEDICINE</b>	92
Plants Rich In Methylxanthine Alkaloids	92
Plants Rich In Steroidal Alkaloids	93
Plants Rich In Pyrrolizidine Alkaloids	93
Plants Rich In Tropane Alkaloids	93
Plants Rich In Piperidine Alkaloids	94
<b>ALKALOIDS IN FOODS AND THEIR TANDEM MASS BEHAVIOR</b>	94
Alkaloids In Foods, their Pharmacological Effect and their Mass Spectrometry Identification	94
<i>Methylxanthine Alkaloids</i>	94
<i>Steroidal Glycoalkaloids</i>	97
<i>Pyrrolizidine Alkaloids</i>	100
<i>Tropane Alkaloids (TAs)</i>	103
<i>Piperidine Alkaloids (PAs)</i>	106
<i>Nicotinic Acid Alkaloids (NAAs)</i>	107
<i>Other Poisoning Nitrogen-Containing Metabolites</i>	110
<b>EXTRACTION OF POISONING ALKALOIDS FROM CONTAMINATED FOOD</b>	116
<b>COMPARISON OF THE MAIN ANALYTICAL METHODOLOGIES FOR THE DETECTION OF ALKALOIDS</b>	116
<b>CONCLUSION</b>	119
<b>ACKNOWLEDGEMENTS</b>	119
<b>REFERENCES</b>	119
<b>CHAPTER 4 LC-MS ANALYSIS OF ENDOGENOUS NEUROPEPTIDES FROM TISSUES OF CENTRAL NERVOUS SYSTEM: AN OVERVIEW</b>	127
<i>Neva Alasağ and Erol Şener</i>	
<b>INTRODUCTION</b>	127
Neuropeptides	128
Mass Spectrometric Analysis of Neuropeptide Families	130
Sample Preparation from Brain Tissues	134
Homogenization	135
Microdialysis	137
Ultrafiltration (UF)	139
Solid-phase Microextraction (SPME)	139
Solid-phase Extraction (SPE)	140
Protein Precipitation (PP)	141
Liquid-liquid Extraction (LLE)	142
Recent Advances in Sample Preparation Techniques	143
Supported Liquid Extraction (SLE)	144
Phospholipid Removal Plates	144
Magnetic Beads	145
Turbo Flow	146
Monolithic Spin Column Extraction	146
Microextraction by Packed Sorbent (MEPS)	147
Carbon Nanotubes (CNTs)	148
Restricted Access Materials (RAM)	149
Immunosorbents	149

Molecularly Imprinted Polymers (MIPs) .....	150
Aptamers .....	151
Guide to Sample Preparation Prescriptions of Brain Tissues .....	152
<i>Sample Preparation of Primate and Rat Brain</i> .....	152
<i>Sample Preparation of Rat Brain</i> .....	153
<i>Sample Preparation of Brain Tissue</i> .....	153
<i>Sample Preparation of Brain Tissue</i> .....	154
<i>Sample Preparation of Mouse Spinal Cords</i> .....	154
<i>Sample Preparation of Rat Brain</i> .....	155
<i>Sample Preparation of the Human Brain</i> .....	155
<i>Sample Preparation of Rat Brain</i> .....	155
<i>Sample Preparation of Rodent Spinal Cord</i> .....	156
<i>Sample Preparation of Rat Brain</i> .....	156
LC-MS/MS Analysis .....	157
Ionization Sources for Neuropeptides .....	157
Electrospray Ionization (ESI) .....	158
Matrix-Assisted Laser Desorption Ionization (MALDI) .....	158
Secondary Ion Mass Spectrometry (SIMS) .....	159
Mass Analyzers for Neuropeptides .....	159
Quadrupole Mass Analyzers .....	159
Triple Quadrupole (QQQ) Mass Analyzers .....	160
Ion Trap (IT) Mass Analyzers .....	161
Orbitrap Mass Analyzers .....	161
Time of Flight (TOF) Mass Analyzers .....	162
Fragmentation Techniques for Neuropeptides .....	162
Quantitative Analysis of Neuropeptides .....	166
<b>CONCLUDING REMARKS</b> .....	168
<b>ACKNOWLEDGEMENTS</b> .....	169
<b>REFERENCES</b> .....	169

## **CHAPTER 5 ADVANCES IN STRUCTURAL PROTEOMICS USING MASS**

<b>SPECTROMETRY</b> .....	188
<i>Sarah Otun, Tshele Mokhantso and Ikechukwu Achilonu</i>	
<b>INTRODUCTION</b> .....	188
<b>IDENTIFICATION OF PROTEIN STRUCTURES BY MASS SPECTROMETRY</b> .....	189
Conventional Protein MS Techniques .....	190
<i>Electrospray Ionisation (ESI)</i> .....	190
<i>Matrix-assisted Laser Desorption/Ionisation (MALDI)</i> .....	192
Tandem Protein Mass Spectrometry Methods .....	193
<i>Limited Proteolysis in Tandem with Mass Spectrometry</i> .....	193
<i>Exchange of Hydrogen and Deuterium in Tandem with MS (HDX-MS)</i> .....	194
<i>Oxidative Footprinting in Tandem with Mass Spectrometry (OFP-MS)</i> .....	195
<i>Affinity-purification Mass Spectrometry (AP-MS)</i> .....	196
<i>Chemical Crosslinking and Chemical Labelling in Tandem with MS (CXL-MS)</i> .....	196
Mass Spectrometry of Multiprotein Complexes .....	197
<i>Instruments for Fragmentation in Conjunction with Mass Spectrometry</i> .....	197
<i>Protein Map Generation</i> .....	198
<i>Protein-ligand, Small-molecule, and Drug Interactions</i> .....	199
<i>Ion Mobility-mass Spectrometry of Intact Protein Complexes</i> .....	199
<i>Integrating Mass Spectrometry and Molecular Modelling</i> .....	200



<b>EMERGING MASS SPECTROMETRY TECHNOLOGIES FOR STRUCTURAL PROTEOMICS</b>	201
<b>CONCLUDING REMARKS</b>	202
<b>ACKNOWLEDGEMENT</b>	202
<b>REFERENCES</b>	202
<b>CHAPTER 6 RECENT TRENDS OF MODERN MASS SPECTROMETRY: APPLICATION TOWARDS DRUG DISCOVERY AND DEVELOPMENT PROCESS</b>	209
<i>Shweta Sharma</i>	
<b>INTRODUCTION</b>	209
<b>PRINCIPLE</b>	210
<b>DRUG DISCOVERY METHOD USING MASS SPECTROMETRY</b>	210
Sample Preparation and Introduction	210
Ionization	210
Mass Analysis	211
<i>Time-of-Flight (TOF)</i>	211
<i>Quadrupole</i>	211
<i>Ion Trap</i>	211
Detection and Data Analysis	211
<i>Identification</i>	211
<i>Characterization</i>	211
Quantification	211
Metabolite Identification	212
Screening	212
<b>RECENT TRENDS IN MODERN MASS SPECTROMETRY</b>	213
High-Resolution Mass Spectrometry (HRMS)	213
Ambient Ionization Mass Spectrometry	215
Data- Independent Acquisition Mass Spectroscopy	215
Tandem Mass Spectrometry (LC-MS/MS)	216
<b>APPLICATION OF MODERN MASS SPECTROMETRY IN DRUG DISCOVERY AND DEVELOPMENT</b>	217
Drug Metabolism Studies	217
Bioanalytical Analysis of Drugs and their Metabolites	217
Lead Compound Identification and Validation	218
Pharmacokinetic and Pharmacodynamics Studies	218
High Throughput Screening (HTS)	218
<b>ADVANTAGES</b>	219
Sensitivity	219
Specificity	219
Speed	219
<b>CHALLENGES</b>	219
Complexity of Data	219
Sample Preparation	219
Instrumentation Costs	219
<b>CONCLUSION</b>	219
<b>REFERENCES</b>	220
<b>SUBJECT INDEX</b>	447

## PREFACE

Mass spectrometry is a unique analytical tool that offers unmatched sensitivity and selectivity levels for a wide range of analyses. The most recent applications of mass spectrometry are mostly oriented toward biochemical problems, such as proteomes, metabolomes, high-throughput drug discovery, and metabolism. Other analytical applications are routinely applied in pollution control, food control, forensic science, natural products or process monitoring, and many others.

The present volume of “*Application of Mass Spectrometry*” provides a useful insight into some of these developments. The present 2nd volume of this book series comprises 6 comprehensive reviews written by the leading practitioners of mass spectrometry. These articles present diverse applications of mass spectrometry in fields such as proteomics, peptidomics, drug development and discovery, toxicology, and environmental analysis. Moreover, the use of advanced ionization techniques, i.e., ion mobility, particularly in the analyses of macromolecules, is also discussed in this volume.

Mehmet Atakay *et al.* have discussed the use of an advanced mass spectrometry approach, ion mobility spectrometry (IM-MS), in the field of macromolecule analysis, such as proteomics, glycoproteomics, and polymer characterization. Sarah Otun *et al.* have described the use of different mass spectrometric approaches and tools in the understanding of structural proteomics. Neva Alasağ *et al.* have elaborated on the use of liquid chromatography-mass spectrometry (LC-MS) as a powerful analytical technique for separating and quantifying endogenous neuropeptides in the central nervous system (CNS) and organisms. Louis P. Sandjo *et al.* have focused on the separation and detection of toxic plant-based metabolites in tropical medicinal and edible plants. Shweta Sharma has reviewed the use of mass spectrometry in various stages of the drug discovery and development process, including target identification, hit identification, lead optimization, and drug metabolism and pharmacokinetic studies. Imalka Munaweera *et al.* highlighted the qualitative and quantitative detection of a diverse range of organic contaminants in environmental samples utilizing advanced mass spectrometry.

We are grateful to all the authors for their excellent scholarly contributions and for the timely submissions of their review articles. We would also like to express our gratitude to Mrs. Fariya Zulfiqar (Manager Publications) and Mr. Mahmood Alam (Director Publications) of Bentham Science Publishers for the timely completion of the volume in hand. We sincerely hope that the efforts of the authors and the production team will help readers better understand and appreciate the versatility and robustness of mass spectrometry and motivate them to conduct good-quality research and development work in this exciting area.

**Atta-ur-Rahman, FRS**  
Kings College  
University of Cambridge  
Cambridge  
UK

**M. Iqbal Choudhary**  
**Mustafa (PBUH) Prize Laureate**

**&**

**Syed Ghulam Musharraf**  
International Center for Chemical and Biological Sciences  
(HEJ Research Institute of Chemistry and Dr. Panjwani  
Center for Molecular Medicine and Drug Research)  
University of Karachi, Karachi-75270, Pakistan

## List of Contributors

<b>Amanda E. de Athayde</b>	Programa de Pós-Graduação em Farmácia, CCS, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Bekir Salih</b>	Department of Chemistry, Hacettepe University, Ankara, Turkey
<b>Erol Şener</b>	Pharmacy Faculty, Department of Analytical Chemistry, Anadolu University, Eskişehir, Turkey
<b>Gabriella B. Souza</b>	Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Hacı Mehmet Kayılı</b>	Department of Biomedical Engineering, Karabük University, Karabük, Turkey
<b>Imalka Munaweera</b>	Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka Instrument Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
<b>Ikechukwu Achilonu</b>	Department of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa
<b>Laksiri Weerasinghe</b>	Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
<b>Louis P. Sandjo</b>	Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Mehmet Atakay</b>	Department of Chemistry, Hacettepe University, Ankara, Turkey
<b>Monalisa A. Moreira</b>	Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Maique W. Biavatti</b>	Programa de Pós-Graduação em Farmácia, CCS, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Neva Alasağ</b>	Department of Analytical Chemistry, Anadolu University, Health Science Institute, Eskişehir, Turkey
<b>Senuri Kumarage</b>	Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
<b>Sarah Otun</b>	Department of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa
<b>Shweta Sharma</b>	Department of Chemistry, Career College, Barkatullah University, Bhopal-462023, India
<b>Tiago Tizziani</b>	Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
<b>Tshele Mokhantso</b>	Department of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa
<b>Ülkü Güler</b>	Department of Chemistry, Hacettepe University, Ankara, Turkey

## CHAPTER 1

# Ion Mobility-Mass Spectrometry for Macromolecule Analysis

Mehmet Atakay<sup>1</sup>, Hacı Mehmet Kayılı<sup>2</sup>, Ülkü Güler<sup>1</sup> and Bekir Salih<sup>1,\*</sup>

<sup>1</sup> Department of Chemistry, Hacettepe University, Ankara, Turkey

<sup>2</sup> Department of Biomedical Engineering, Karabük University, Karabük, Turkey

**Abstract:** The need for conformational information is increasing by the time in studies on macromolecules. For example, proteins may have various functions and properties depending on their folding states that make their conformational analyses very important. Mass spectrometry is one of the most effective analytical techniques that separate ions in the gas phase by their mass-to-charge ratio. It provides useful data on molecular characterization in many areas of research with high precision, accuracy, and sensitivity. Although mass spectrometry is a very powerful analytical technique, it cannot distinguish different species having identical mass-to-charge ratio. The analytical technique combining mass spectrometry with ion mobility spectrometry (IM-MS), which provides information about the three-dimensional structure of an ion, solves this problem by separating them according to their collision cross sections (CCS) in the gas phase. This analytical method also provides the advantages of higher precision and better resolution in the rapid analysis of different types of complex samples. The separation of isomers with the same molecular weight, increasing the dynamic range and distinguishing ions from chemical noise are the most important features that this technique contributes to mass spectrometry. As improvements have been made in IM-MS technology, the number and quality of publications in the areas where this technique is used increases rapidly. In this chapter, the use of IM-MS techniques in the fields such as proteomics, glycoproteomics and polymer characterization are explained by presenting their various applications in the literature.

**Keywords:** Conformational Characterization, Glycoproteomics, Ion Mobility-Mass Spectrometry, Proteomics, Polymer Characterization.

## INTRODUCTION

The status and use of ion mobility-mass spectrometry (IM-MS) in various research areas have been increasing rapidly in recent years [1]. The interest in this analytical method is increasing in parallel with the improvements in the parameters such as high sensitivity, resolution power and low amount of sample

\* Corresponding Author Bekir Salih: Department of Chemistry, Hacettepe University, Ankara, Turkey; Tel: +90 312 2977975; Fax: +90 312 2992163; E-mail: bekir@hacettepe.edu.tr

in the analysis with the developing technology. The basics of ion mobility spectrometry are similar to the principles of mass spectrometric techniques. Thus, these two techniques can be easily used together in a combined system. Modern IM-MS techniques having high ion mobility resolution, sensitivity and applicability for a wide variety of samples started to be developed in the 1990s. These developments coincide with the period when ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) were first used in mass spectrometric analysis [2 - 4]. In 2006, a company manufacturing mass spectrometers combined ion mobility and mass spectrometry techniques in an instrument and launched it on the market. Since then, the use of ion mobility technology has become increasingly common in various research areas for differentiation, identification, and structural analysis of species [5]. Today, commercially available IM-MS instruments having different ion mobility technology provided by different manufacturers are used in studies [1].

Ion mobility spectrometry is still widely used alone in defense, security and environmental analysis applications [6 - 8]. Over the past two decades, very rapid and significant advances have been made in the development of systems in which ion mobility and mass spectrometry are combined. These advances are particularly concerned with the ability to trap, transmit and focus ions between regions under different vacuum values using electrodynamic fields. Considering the very important analytical advantages provided by IM-MS instruments, ion mobility technique has become a preferable option especially in studies carried out in the “omics” fields [9]. IM-MS technique, which has an important place in various research fields, has become an analytical system that is also sought after and interested in several applications performed on the industrial scale.

Ion mobility spectrometers simply measure the time spent by ions as they travel through an electrical field with the help of a buffer gas. Ion mobility ( $K$ ), collision cross section ( $\Omega$ ), the Boltzmann constant ( $k_b$ ), neutral number density ( $N$ ), mass of ion ( $m_i$ ), mass of buffer gas ( $m_N$ ), charge of ion ( $z$ ), and electronic charge ( $e$ ,  $1.602 \times 10^{-19}$  C) is defined by the Mason-Champ equation [10, 11].

$$K = \frac{\sqrt{18\pi}}{16} \frac{ze}{\sqrt{k_B T}} \sqrt{\left(\frac{1}{m_i} + \frac{1}{m_N}\right)} \frac{1}{N} \cdot \frac{1}{\Omega} \quad (1)$$

The collision cross section (CCS) value of an ion can provide detailed information about its size and shape. When two different molecules having identical mass-to-charge ratios are analyzed using IM-MS, they can be separated from each other

according to their mobility in the ion mobility cell depending on their shape and size characteristics. The arrival time data obtained from the acquisition with an IM-MS device should be converted to the CCS values of the analyzed species according to the performed calibration calculations using standard molecules with known CCS values. There are free software and databases that can be helpful in such CCS calibration and estimation processes. A list of common CCS databases and software used in CCS calibration, prediction, and estimation with their URLs are given in Table 1.

The collision cross section of an ion depending on its mobility and the type of buffer gas used can also be calculated by appropriate computational methods. Thus, the theoretical CCS values can be compared with the experimental values obtained from IM-MS analyses. The theoretical average CCS values are determined by taking into account the collisions of ions with the buffer gas in the ion mobility cell by the computational simulation of analysis conditions [12]. In structural biology studies, the conformations of biomolecules or their complexes are investigated by comparing the experimental CCS values with data obtained from theoretical calculation [13].

**Table 1. List of common CCS databases and software.**

CCS Database / Software	Description	URL	Refs.
MOBCAL	calculating CCS values implementing the Projection Approximation, Exact Hard Spheres Scattering model, and MD Calculations	<a href="https://nano.lab.indiana.edu/software/">https://nano.lab.indiana.edu/software/</a>	[14, 15]
GlycoMob	CCS database for glycomics	<a href="http://www.glycomob.org/">http://www.glycomob.org/</a>	[16]
ISICLE	simulating CCS values, NMR chemical shifts conformers	<a href="https://github.com/pnnl/isicle">https://github.com/pnnl/isicle</a>	[17, 18]
MetCCS	predicting CCS values of metabolites	<a href="http://www.metabolomics-shanghai.org/MetCCS/">http://www.metabolomics-shanghai.org/MetCCS/</a>	[19]
LipidCCS	predicting of CCS values for lipids	<a href="http://www.metabolomics-shanghai.org/LipidCCS/">http://www.metabolomics-shanghai.org/LipidCCS/</a>	[20]
Bush Lab Collision Cross Section database	presenting CCS of small molecules, peptides, denatured proteins, native-like proteins, and native-like protein complexes	<a href="http://depts.washington.edu/bushlab/ccsdatabase/">http://depts.washington.edu/bushlab/ccsdatabase/</a>	[21 - 28]
Clemmer Group Cross Section Database	presenting CCS of peptides, proteins, and oligonucleotides	<a href="https://clemlab.siteshost.iu.edu/Research/CrossSection%20Database/cs_database.php">https://clemlab.siteshost.iu.edu/Research/CrossSection%20Database/cs_database.php</a>	[29 - 38]
Unified CCS Compendium	interactive repository of experimentally acquired CCS values of molecular standards and classes	<a href="https://mcleanresearchgroup.shinyapps.io/CCS-Compendium/">https://mcleanresearchgroup.shinyapps.io/CCS-Compendium/</a>	[39]
CCSbase	presenting CCS values of lipids, water-soluble metabolites, small molecules, drugs, etc.	<a href="https://ccsbase.net/">https://ccsbase.net/</a>	[40]
PIXIE	extracting arrival times by drift tube ion mobility spectrometry and calculating the associated CCSs	<a href="https://github.com/PNNL-Comp-Mass-Spec/PIXIE">https://github.com/PNNL-Comp-Mass-Spec/PIXIE</a>	[41]
IMPACT	calculating CCSs of proteins in structural biology and proteomics applications	<a href="https://process.innovation.ox.ac.uk/software">https://process.innovation.ox.ac.uk/software</a>	[42]
EM <sup>3</sup> IM	estimation of CCS from electron microscopy density maps	<a href="http://emn3im.chem.ox.ac.uk/">http://emn3im.chem.ox.ac.uk/</a>	[43]
PNNL Collision Cross Section Database	CCS database for metabolites	<a href="https://metabolomics.pnnl.gov/">https://metabolomics.pnnl.gov/</a>	[44]

## CHAPTER 2

## Recent Advancements in the Detection of Organic Contaminants in Wastewater Using Advanced Mass Spectrometry

Senuri Kumarage<sup>1</sup>, Laksiri Weerasinghe<sup>1</sup> and Imalka Munaweera<sup>1, 2,\*</sup>

<sup>1</sup> Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

<sup>2</sup> Instrument Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

**Abstract:** With the increase of industrialization and urbanization, pollution of clean water has become a critical issue in the contemporary world. Despite organic pollutants such as pharmaceuticals, pesticides, industrial chemicals, poly- and per-fluoroalkyl substances (PFASs) and hormones, contaminants originating from the industrial effluents, urban run-offs, agricultural run-offs and domestic sewage have become a greater threat to the aquatic eco-systems. The availability of some of these highly potent contaminants at low concentrations and the simultaneous analysis of multiple samples have been identified as the major concerns in current analytical methods in water pollution analysis. In this regard, modern mass spectrometric methods have emerged as suitable techniques for the analysis of smallest concentrations even at a level of nanograms or femtograms while allowing the detection of hundreds of analytes in a single analysis within a short duration of time.

Recently, combinational mass spectrometric analysis has become the state of the art in several qualitative and quantitative analyses of organic pollutants in water. The sensitivity of the detection has been enhanced by coupling with various sample extraction methods, chromatographic techniques and different mass analyzers in mass spectrometry. Utilization of modern sample extraction methods coupled with mass analyzers has facilitated the accuracy of the detection of organic pollutants in water samples. Sample extraction methods involve sophisticated solid-phase extraction, solid-phase microextraction, and liquid-liquid extraction methods, whereas mass analyzers include time-of-flight, orbitrap, ion-trap and triple quadrupole, *etc.* The hallmark of these hyphenated techniques is the ability of allowing the screening of targeted analytes, non-targeted analytes and suspect analytes without the need of authentic standards. This chapter will focus on the recent advancement of mass spectrometry in qualitative and quantitative analysis of several organic contaminants in wastewater samples.

\* Corresponding author Imalka Munaweera: Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka; E-mail: imalka@sjp.ac.lk



**Keywords:** Emerging Contaminants, PFAs, Hyphenated Techniques, Industrial Chemicals, Mass Spectrometry, Non-target Analysis, Organic Pollutants, Pesticides, Pharmaceuticals, Recent Advancements, Sample Extraction, Target Analysis, Wastewater Analysis.

## INTRODUCTION

Contamination of water resources is one of the most serious issues that must be addressed in order to preserve the ecosystem and ensure its long-term viability. Currently, the use of enormous numbers of different chemicals all over the world, has a huge impact on the ecosystems due to their unavoidable intrusion. Any chemical that could cause adverse effects to the ecological systems or to human health and also frequently detecting in increasing concentration in the environment but yet not being monitored by the established environmental monitoring programs is referred to as an emerging contaminant (EC) [1, 2] and the existence of such compounds in the environment could even be permanent due their constant input from various unregulated sources. Since the acquisition, consumption, and usage of such contaminants are not governed by any legislation, the elimination of these substances in natural sources *via* treatment plants has not been prioritized. In this regard, pharmaceuticals, personal care products, hormones, per-fluorinated compounds, flame retardants, endocrine disruptors, pesticide, plasticizers, impurities from commercial formulations and surfactants, are all considered ECs that could pose a threat to environmental ecosystems [3].

To acquire early identification and exact quantification of every component capable of compromising ecosystems, and global health integrity, a comprehensive examination of environmental contamination necessitates persistent innovation in technology and analytical methodologies [4]. In this sense, mass spectrometry (MS) stands to be the most effective approach, since it has demonstrated constant and massive growth in concept, apparatus, and implementations since its invention [5 - 7]. MS is distinguished by its high sensitivity, high resolution, quantitative capabilities, and robust repeatable fast analysis. High sensitivity allows for the detection of molecules at trace amounts or in limited samples, while high resolution allows for molecular identification by comparing their mass/charge ( $m/z$ ) or fragmentation patterns [8 - 10]. With its quantitative capabilities, the concentrations of analytes in real samples could be reported, while high speed analysis enables rapid process monitoring or high analytical performance [7, 11].

## Basic Components of the Mass Spectrometer

Multiple improvements have been made to increase the performance of MS since its inception in analytical chemistry. To ionize, scan, focus, fragment, and identify chemical structures, a variety of methods have been devised. The main components of a mass spectrometer include an ion source, a mass analyzer and a detector. Only ions of compounds or molecules can be detected by a mass spectrometer and differentiation between radicals and neutral molecules cannot be done. Ionization of materials is hence a prerequisite for MS analysis. To ionize the samples, sample molecules are first exposed to an ion source. The most prevalent ionization techniques are electron ionization (EI), chemical ionization (CI), electrospray ionization (ESI), atmospheric-pressure chemical ionization (APCI) and matrix-assisted laser desorption/ionization (MALDI) [12, 13]. After ionization, the newly formed ions of molecules are sent to the mass analyzer to be sorted as per their  $m/z$  ratio. Different types of mass analyzers are used alone or in combinations and they have been classified into two major types as ‘beam analyzers’ and ‘trapping analyzers’. In beam analyzers, ions escape the ion source in a beam and pass through the analyzing field to the detector, whereas in trapping analyzers, ions are trapped in the analyzing field after being produced in the analyzer or injected from an external ion source [13]. Quadrupole (Q), quadrupole ion trap ( $Q_{IT}$ ), time of flight (TOF), Fourier-transform ion-cyclotron resonance (FT-ICR), and orbitrap are the widely used mass analyzers up to date. Q and TOF are examples of beam analyzers while  $Q_{IT}$ , FT-ICR and orbitrap are trapping analyzers. In addition, combinations of the previously mentioned mass analyzers, such as triple quadrupole (QQQ), time-of-flight/time-of-flight (TOF/TOF), and quadrupole/time-of-flight (Q/TOF) are used and are termed as Tandem Mass analyzers (MS/MS). These MS/MS are again classified as tandem-in-space or tandem-in-time. Each step of MS/MS requires a separate analyzer in tandem-in-space devices. In tandem-in-space instruments, beam-type analyzers are utilized. Instruments used in trapping are usually tandem-in-time. The different steps of MS/MS are carried out in the same analyzer but at different times [13]. A multitude of factors impact the choice of a mass analyzer, including the intended  $m/z$  range to be studied, the mass of the analytes, the required resolving power, the ability to interface with the mass spectrometer's ion source, and the required limit of detection [12, 14]. With the exception of FT-ICR, after passing through the above mentioned mass analyzers, the ions are identified and converted into an electrical signal by the detectors, which is generally proportionate to their abundance in the ion beams. Some detectors, known as point ion detectors, can only detect a single ion at a time, but others, known as array detectors, can scan many masses at once [6]. MS detectors include the photo plate detector, Faraday cup detector, electron multiplier detectors such as the Discrete Dynode Electron Multiplier and Continuous Dynode Electron Multiplier, Micro channel Plate

## CHAPTER 3

## Poisonous Substances in Tropical Medicinal and Edible Plants: Traditional Uses, Toxicology, and Characterization by Hyphenated Mass Spectrometry Techniques

Amanda E. de Athayde<sup>1</sup>, Monalisa A. Moreira<sup>2</sup>, Gabriella B. Souza<sup>2</sup>, Tiago Tizziani<sup>2</sup>, Maique W. Biavatti<sup>1</sup> and Louis P. Sandjo<sup>2,\*</sup>

<sup>1</sup> Programa de Pós-Graduação em Farmácia, CCS, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

<sup>2</sup> Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

**Abstract:** Alkaloids are natural metabolites containing nitrogen atoms, produced for different biological functions by plants, animals, and microorganisms. In most cases, its production is related to the defense mechanism of an organism through allelopathic effects. Because of this allelopathic property, some of these alkaloids are used as pesticides and can somehow be found in food and beverages as exogenous contaminants. Other contaminations by alkaloids come from industrial processing; so, ingestion of contaminated food or drinks can cause poisoning or death. Many of these plants, although composed of toxic substances, are also used as traditional medicines. Therefore, the compilation of these plants, their chemical constituents, and their pharmacological effects remain important. This paper aims to report traditional preparations and the use of edible plants containing toxic components, their toxicological records of a part of these poisonous metabolites, some regulations on their tolerable dose, and appropriate hyphenated techniques related to mass spectrometric for their separation, detection, quantification, and characterization. In addition, a particular emphasis will be placed on the properties of the stationary and mobile phases used for these studies. The fragmentation mechanism pathways based on mass spectrometry data for these substances will be widely described, and the diagnostic peak will be highlighted.

**Keywords:** Alkaloids, Edible plants, Mass spectrometry, Toxicology, Traditional remedy.

\* Corresponding author Louis P. Sandjo: Programa de Pós-Graduação em Química, Departamento de Química, CFM, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil; Tel: +554837213624; E-mail: p.l.sandjo@ufsc.br

## INTRODUCTION

Many communities in tropical areas have a dynamic relationship with plants in nature including plants-food, plant-remedy, and plant-spiritual thinking. Sometimes, different parts of the same plant can serve simultaneously as food, traditional remedy, or be endowed with spiritual power as believed by certain local communities. In any case, the use of plants as food and as remedy mostly relies on their nutritional value and their chemical constitution. However, the chemical constituents of these plants are not always beneficial for humans and sometimes can present toxic effects. The danger associated with plants used as food and traditional medicines depends mostly on their preparations or manipulation. For instance, the preparation can require cooking or fermentation before consumption or ingestion as a fresh vegetable. So, the thermal exposure and the fermentation step can be crucial factors to avoid intoxication. Therefore, this review will particularly present alkaloids in medicinal and edible plants. Alkaloids are nitrogen-containing natural products whose biosynthetic precursors are proteinogenic (the twenty amino acids found in proteins) and non-proteinogenic amino acids (those which are not incorporated in protein biosynthesis: anthranilic acid, L-dopamic acid, hydroxyproline). Different alkaloid nuclei containing aromatic systems, saturated ring, and acyclic atoms sequences are found among the skeletal structure of these metabolites. Nitrogen-containing metabolites can be separated into five groups, including true alkaloids, protoalkaloids, polyamine alkaloids, amides alkaloids, and pseudo alkaloids. The predominant skeletons in food are pyrrolizidine, tropane, piperidine, quinolizidine, isoquinoline,  $\beta$ -carboline, quinoline, ergot alkaloids, pyrrolidinylpyridine, steroidal alkaloids, pyrrolopyridine, and purine [1]. These metabolites are produced by plants, animals, and microorganisms and can present beneficial effects or can be harmful to humans [1 - 3]. Despite the adverse effects associated with alkaloids, some of them are found in trace or are highly concentrated in the daily human diet. Consequently, their existence in beverages, feeds, and foods can cause damages such as hepatotoxicity, cirrhosis, carcinogenicity, mutagenicity, teratogenicity, immunotoxicity, neurotoxicity, and estrogenicity [4, 5]. For this reason, governmental agencies and authorities have set food control strategies and regulations by defining thresholds for toxic alkaloids concentration in food products [6]. The effective concentration of these compounds depends on the amount consumed, and beyond this limit, possible food poisoning can be observed.

Import and conservation of food products always present a high risk of contamination, especially when the contaminant is not traceable and unknown or when the product comes from countries that lack adequate quality of monitoring infrastructure. Therefore, from an economic point of view, the standard of safety

and quality of food products remains a priority for food manufacturers and industries. Quality control, food ingredient, functional ingredients, and so on relied on traditional analytical techniques, such as Nuclear Magnetic Resonance, NMR; Infrared, IR; and Ultraviolet, UV, mass spectrometry (EI, MALDI, ESI, APCI *etc.*) and separation techniques (high-performance liquid chromatography, HPLC; gas chromatography, GC; capillary electrophoresis, CE and supercritical fluid chromatography, SFC). Two of these techniques can be hyphenated, such as liquid chromatographic coupled to mass spectrometry (LC-MS), gas chromatography associated with mass spectrometry (GCMS), and liquid chromatography coupled to nuclear magnetic resonance (LC-NMR). These hyphenated techniques allow simultaneous characterization of the components of a mixture, although the choice of analytical techniques depends entirely on the physical and chemical properties of the sample to be analyzed.

Hereby, we will report on toxic alkaloids found in plants and used as food and as medicinal remedies, their pharmacological effect, and how to use the mass spectrometry device for their detection and identification

## **EXAMPLE OF TROPICAL EDIBLE PLANTS USED IN TRADITIONAL PHARMACOPEIA AS MEDICINE**

### **Plants Rich In Methylxanthine Alkaloids**

#### ***Coffee Tree***

The infusion of leaves and roasted seeds of *Coffea arabica* is used in Haiti to treat anemia, asthenia, and edema. In western India, asthma symptoms are alleviated with the use of an aqueous extract from dried seeds [7]. However, a side effect manifested by excessive bleeding during menstruation was observed in Nepal after consuming *Coffea benghalensis* flowers [8]. Disorders related to coffee intake have been previously demonstrated in several studies [9, 10].

#### ***Cocoa Tree***

The whole grains of *Theobroma cacao* are used in the traditional Ghanaian pharmacopoeia for the treatment of diabetes, digestive and thoracic problems. In addition, seed powder after fermentation is used to prevent heart disease [11]. Its beans are also used in Mexico as a traditional remedy to cure mouth ulcers and toothache [12].

---

**CHAPTER 4**

---

# LC-MS Analysis of Endogenous Neuropeptides from Tissues of Central Nervous System: An Overview

Neva Alasağ<sup>1,\*</sup> and Erol Şener<sup>2</sup>

<sup>1</sup> Department of Analytical Chemistry, Anadolu University, Health Science Institute, Eskişehir, Turkey

<sup>2</sup> Pharmacy Faculty, Department of Analytical Chemistry, Anadolu University, Eskişehir, Turkey

**Abstract:** In recent years, various methods and technological advances demonstrated that neurochemical measurements have contributed to significant improvements in our understanding of the relationship between chemistry in the central nervous system (CNS) and the organism. Techniques based on Liquid Chromatography-Mass Spectrometry (LC-MS) are potent approaches for separating and quantifying endogenous neuropeptides in CNS. The separation ability and reliability of LC with sensitivity and selectivity of MS have become a valuable combination for peptide analysis either qualitatively or quantitatively. Thus, new peptides have been identified using this technique. When applied to disease models, pathophysiological mechanisms can be identified and used as drug targets or biomarkers. Due to the low concentrations of neuropeptides in the biological samples, they restrict developing analysis methods and the understanding of their biological function. This book chapter focuses on novel developments of LC-MS/MS for endogenous neuropeptides. It has also emphasized the applications that cite preparation techniques used for brain tissue analysis, published in recent years.

**Keywords:** LC-MS/MS, Endogenous neuropeptide, Sample preparation, Brain tissues, Microdialysis, Central nervous system, Neuropeptide families, Mass spectrometry, Mass analyzers, Ionization sources, Fragmentation techniques, Neuropeptide analysis.

## INTRODUCTION

Neuropeptides are the largest class of neuromessengers and have gained significant attention from researchers due to the diversity of their chemical structure, mechanisms of intercellular communication, localization within cells,

---

\* **Corresponding author Neva Alasağ:** Department of Analytical Chemistry, Anadolu University, Health Science Institute, Eskişehir, Turkey; E-mail: [nalasag@anadolu.edu.tr](mailto:nalasag@anadolu.edu.tr)

and regulation of their functions in many biological and physiological processes.

Neuropeptide analysis in biological samples has significantly contributed to neuroscience research for identifying new peptides and biomarkers, having a better understanding of the disease, and developing new strategies for treatment. However, there are common challenges related to neuropeptide studies owing to vastly variable molecular weights and structural diversity, present at low endogenous concentrations in biological samples, products of proteolytic processing and posttranslational modifications (PTMs). Despite many difficulties that make neuropeptides study challenging, much advancement has been made on new technological developments, applications, and perspectives in this field [1 - 3].

Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA) methods are widely used for neuropeptide analysis. Although these methods are sensitive, most antisera are not unique to a single peptide and can cross-react with other peptides. Also, only a certain number of neuropeptides can be analyzed with these methods. Using highly selective, sensitive, robust, and accurate liquid chromatography-mass spectrometry (LC-MS), procedures have been carried out to separate and quantify peptides from biological samples. Simultaneous identification and determination of many neuropeptides in a sample are possible using mass spectrometry [4 - 6].

This book chapter demonstrates the use of mass spectrometric analysis of neuropeptide families in mammals to prepare a guide for researchers in this area. It also focuses on preparation and pre-treatment techniques for endogenous neuropeptides.

### **Neuropeptides**

In complex neural networks, the chemical language of nerve cell communication compounds (called neuromessengers), also known as neurotransmitters, plays a crucial role in signal transduction and affects neural activity in the brain. Previously, only a few substances appeared as signal molecules; nowadays, more categories of molecules, including amino acids, biogenic amines, gaseous messengers, lipid derivatives, purines, pyrimidines, appear to have a signaling function in the nervous system [7, 8]. Neuropeptides have only been investigated as neurotransmitters in recent years, and their wide range has made them different from the amino acid and biogenic amine neurotransmitters [9].

The term “neuropeptides” was first launched by David de Wied in 1971. They were firstly found in the nervous system, as indicated by their name. They consist of 3 to 100 amino acid residues, which are much larger and synthesized

differently than classical neurotransmitters. They are released in a smaller amount than small-molecule transmitters. Neuropeptides are thousands of times more active than small-molecule transmitters. Another feature of neuropeptides is that they cause long-lasting effects. Some of these long-term effects include changes in the cell's metabolic function, changes in the activation or deactivation of specific genes in the cell nucleus, and long-term changes in the number of excitatory or inhibitory receptors. These effects can sometimes take days, months, or even years [7, 10].

Neuropeptides contain many different endogenous peptides, which act as neurotransmitters or neuromodulators in the central and peripheral nervous systems and neurohormones in the endocrine system. They influence the regulation of many biological and physiological functions, including sleep, pain, fear, reproduction, metabolism, depression, learning, homeostatic mechanisms, water retention, *etc* [1, 3, 4, 11 - 19]. According to the research of PubMed, some endogenous neuropeptides and their physiological functions are given, as shown in Table 1.

**Table 1. Some Neuropeptides and Physiological Functions.**

Neuropeptides	Physiological functions	Refs.
Pyroglutamylated arginine- phenylalanineamide peptide (QRFP- 26)	Food intake	[20]
Cortistatin	Inflammation, pain, stress	[21]
Neuropeptide B (NPB)	Feeding, energy metabolism, hormone secretion	[22]
PACAP	Reflex	[23]
Corticotropin-releasing factor (CRF)	Stress	[24]
Orexin B	Passive avoidance learning	[25]
VIP	Feeding behavior, metabolic hormone release, body mass composition and energy balance	[26]
Nociceptin (N/OFQ)	Emotional memory, aversive learning	[27]
Dynorphin-A	Hibernation, climatic modulation	[28]



## CHAPTER 5

## Advances in Structural Proteomics using Mass Spectrometry

Sarah Otun<sup>1,\*</sup>, Tshele Mokhantso<sup>1</sup> and Ikechukwu Achilonu<sup>1</sup>

<sup>1</sup> Department of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa

**Abstract:** Structural proteomic techniques have recently evolved because of advances in mass spectrometry (MS). Several MS techniques, such as, Hydrogen-deuterium exchange, oxidative footprinting or radical probe mass spectrometry, chemical crosslinking, affinity purification, and ion mobility separation, can now be used to analyse protein interaction networks, conformational changes, protein structures, and other downstream applications. This article examines proteomic MS techniques' progression from conventional to advanced techniques, tandem MS techniques, MS of multiprotein complexes, and emerging MS techniques for structural proteomics. Also, the applications that were gleaned from these techniques were reviewed. Lastly, the future of this rapidly emerging field was highlighted.

**Keywords:** Affinity purification, Chemical crosslinking, Hydrogen-deuterium exchange, Ion mobility, Mass spectrometry, Oxidative footprinting, Radical probe mass spectrometry (RP-MS), Structural proteomics.

### INTRODUCTION

The characterisation of protein structures, protein assemblies and systematic evaluation of protein-protein interactions are instances of structural proteomics, that is, the application of protein chemistry and modern mass spectrometry techniques to these and other proteomic analyses [1]. Several MS-based techniques (such as, hydrogen-deuterium exchange, oxidative footprinting, chemical crosslinking, and affinity purification, to name a few) can now determine the atomic-level structure of the complex, including its shape, cavity size, protein subunit distance, inter-subunit angles, protein connectivity, monomer topology, subcomplex topology, protein-protein interface structure, and more [2, 3]. Nonetheless, the vast diversity, transient nature, and low relative quantities of biomolecules in biological samples make it difficult to determine the essential

<sup>1</sup> **Corresponding author Sarah Otun:** Department of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa; E-mail: Oluwatobi.otun@wits.ac.za

structural characteristics of these protein complexes [3]. Consequently, designing more sensitive MS techniques capable of capturing the dynamic behaviour of protein complexes is crucial to resolving this problem. Nevertheless, there have been actual barriers to implementing such a thorough approach.

Furthermore, the sensitivity, speed, and precision have reinforced MS's status as a crucial structural proteomics tool, enabling researchers to understand several cellular processes' interconnectivity better. The structure and dynamics of multiprotein complexes at any concentration and in any solution using mass spectrometry can be investigated [2]. MS has been combined with other techniques, such as hydrogen-deuterium exchange (HDX), chemical crosslinking (CXL), oxidative footprinting (OFP), limited proteolysis (LP), affinity purification (AP), and ion mobility (IM) separation, to reveal the three-dimensional structure of multiprotein complexes [4].

Structural proteomics uses two primary kinds of MS techniques: 1). those that derive structural information from measurements of protein ions in the gas phase, and 2). those that offer spatial constraints from measurements of proteins in solution [3]. The structural integrity of complex proteins must be preserved during the transition to the gas phase for downstream applications [5]. To do this, MS instruments have been constructed or modified to raise their ion guide pressures, include low-frequency quadrupole mass analysers, and use their more considerable acceleration potentials [3, 5]. Although each MS technique may have a wide variety of physical issues, combining them can yield additional knowledge that can be utilised to solve previously unsolvable structural biology problems. MS may be used, for instance, to discover chemical changes in proteins in a solution intended to affect their structure or dynamics [6].

This article reviews the most recent advancements in the structural characterisation of proteins using MS-based techniques. Although MS measurement of entire protein complexes is a relatively new method for examining protein structure, it is already a solid research tool, and we concentrate on its role in structural proteomics. This review highlights that by combining MS technologies, a range of techniques for understanding protein network composition and three-dimensional structure may be accessible.

## **IDENTIFICATION OF PROTEIN STRUCTURES BY MASS SPECTROMETRY**

Protein sequences are abundant, with approximately 141,621,564 entries in the UniprotKB, but protein structures are scarce (158,271 in the UniprotKB as of February 2023, [www.pdb.org](http://www.pdb.org)). Alternative, high-throughput methods for studying protein structure are necessary to reduce this disparity. Despite their frequent

inability to provide complete, high-detail structural information, the value of these higher-throughput methods lies in their ability to offer either a localised, highly detailed view of a small portion of the structure or a global, high-sensitivity view of the structure's dynamical behaviour [7]. Many MS-based methods are utilised to determine protein structure, highlighting the divergence from the conventional focus on identifying and measuring these macromolecules. To assess the stoichiometry of a protein or protein complex, the conventional approach of mass spectrometry entails an examination of the protein or protein complex [8].

### **Conventional Protein MS Techniques**

Mass spectrometry is essential for precise sequencing and characterisation of proteins [9]. Whole proteins may be ionised using electrospray ionisation (ESI) or matrix-assisted laser desorption/ionisation (MALDI). Both approaches characterise proteins in ways compatible with modern mass spectrometers' capabilities and mass range. The initial stages involve ionising proteins in one of two ways and passing them into a mass analyser in their natural form. In protein research, this method is known as “top-down” analysis (Fig. 1c). For low-throughput single-protein research, the top-down technique is often utilised [10]. Proteases such as trypsin and pepsin are used in electrophoretically separated proteins to enzymatically degrade the separated proteins into smaller peptides in solution or gel. Protein analysis from the “bottom-up” (Fig. 1a) has also been used to investigate the distribution and position of post-translational changes, such as phosphorylation, on proteins [11]. Another possible paradigm gaining support is the “middle-down” paradigm (Fig. 1b). This research focuses on proteolytic peptides larger than the average tryptic peptide [12]. Both ESI and MALDI employ these models, and they are further discussed.

#### ***Electrospray Ionisation (ESI)***

Electrospray ionisation (ESI) is used in mass spectrometry to generate ions by delivering a high voltage to a liquid to create an aerosol (Fig. 2a) [13]. It works against the inherent propensity of macromolecules to break apart when ionised, making it an extremely successful ionisation approach [14]. Unlike traditional ionisation methods, ESI may generate multiple-charged ions, expanding the analyser's mass range and allowing it to handle the kDa-MDa orders of magnitude often seen in proteins and similar polypeptide fragments [15]. Due to the low degree of fragmentation during ESI, it is also known as a “soft ionisation” technique [16]. Although the molecular ion (or, more precisely, a pseudo-molecular ion) is usually always identified, the simple mass spectrum obtained offers very little structural information. This disadvantage may be overcome by employing a tandem mass spectrometer (ESI-MS/MS) in combination with ESI

---

**CHAPTER 6**

---

## **Recent Trends of Modern Mass Spectrometry: Application towards Drug Discovery and Development Process**

**Shweta Sharma<sup>1,\*</sup>**

<sup>1</sup> *Department of Chemistry, Career College, Barkatullah University, Bhopal-462023, India*

**Abstract:** Mass spectrometry has evolved significantly in recent years and has become a powerful analytical tool in the field of drug discovery and development. It allows for the identification and characterization of small molecules, peptides, and proteins in complex biological samples with high sensitivity and accuracy. This chapter provides an overview of the recent trends in modern mass spectrometry and its application towards the drug discovery and development process. It discusses the advancements in mass spectrometry technology, such as high-resolution mass spectrometry (HRMS), ambient ionization mass spectrometry (AIMS), data-independent acquisition (DIA) mass spectrometry, tandem mass spectrometry (LC-MS/MS), and how they have enabled the analysis of complex biological samples. The chapter also highlights the use of mass spectrometry in various stages of the drug discovery and development process, including target identification, hit identification, lead optimization, and drug metabolism and pharmacokinetic studies. Additionally, it discusses the challenges and future prospects of mass spectrometry in drug discovery and development. Overall, mass spectrometry has revolutionized the drug discovery and development process and will continue to play a crucial role in the future.

**Keywords:** Ambient ionization mass spectrometry (AIMS), Drug discovery, Drug development, Data-independent acquisition (DIA) mass spectrometry, High-resolution mass spectrometry (HRMS), Mass spectrometry, Tandem mass spectrometry (LC-MS/MS).

### **INTRODUCTION**

Mass Spectrometry (MS) has long been recognized as a powerful analytical technique with widespread applications in various fields, including chemistry, physics, and biology. In recent years, mass spectrometry has emerged as a key technology in drug discovery and development, playing a crucial role in the iden-

---

\* **Corresponding author Shweta Sharma:** Department of Chemistry, Career College, Barkatullah University, Bhopal-462023, India; E-mail: shwetasharma2703@gmail.com

tification, characterization, and quantification of small molecules and large biomolecules [1, 2]. With the growing complexity of drug molecules and the demand for faster and more efficient drug discovery and development processes, there has been a significant increase in the adoption of modern mass spectrometry techniques [3].

In this chapter, we will discuss the recent trends of modern mass spectrometry in the context of drug discovery and development. We will explore how mass spectrometry has evolved over the years, driven by advancements in instrumentation and methodologies, and how it has been successfully applied in various stages of the drug development process. We will also discuss the recent and important application of mass Spectrometry in the field of drug discovery.

## **PRINCIPLE**

To understand the current state of mass spectrometry in drug discovery and development, it is important to first highlight its fundamental principles. It is an analytical technique that measures the mass-to-charge ratio ( $m/z$ ) of ions in a sample [4]. It involves the ionization of a sample, typically through techniques such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI), followed by separation and detection of these ions based on their  $m/z$  values. The resulting mass spectrum provides information on the identity, quantity, and structure of the sample components, making it a valuable tool for the identification and quantification of drug molecules [5, 6].

## **DRUG DISCOVERY METHOD USING MASS SPECTROMETRY**

Mass spectrometry (MS) plays a crucial role in modern drug discovery, particularly in the identification and characterization of potential drug candidates. Here's a detailed overview of how mass spectrometry is utilized in the process [7 - 10] (Fig. 1).

### **Sample Preparation and Introduction**

The process begins with the preparation of samples containing the compounds of interest, which could be from natural sources, synthetic chemicals, or biological extracts. These samples are then introduced into the mass spectrometer for analysis.

### **Ionization**

In the mass spectrometer, the sample is ionized to create charged particles. This step is critical as it allows the molecules to be analyzed based on their mass-to-charge ratio ( $m/z$ ). Common ionization techniques include electrospray

ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), each suitable for different types of compounds.

### **Mass Analysis**

Once ionized, the ions are accelerated and separated based on their mass-to-charge ratio in the mass analyzer. There are several types of mass analyzers used in drug discovery MS, including:

#### ***Time-of-Flight (TOF)***

Measures the time it takes for ions of different masses to reach a detector.

#### ***Quadrupole***

Uses radiofrequency voltages to selectively transmit ions based on their  $m/z$  ratio.

#### ***Ion Trap***

Captures and releases ions based on their mass-to-charge ratio using electromagnetic fields.

### **Detection and Data Analysis**

The ions reaching the detector are converted into electronic signals, which are then processed by a computer. This data provides information about the mass-to-charge ratios of the ions present in the sample.

#### ***Identification***

MS can identify unknown compounds by comparing their mass spectra to databases of known compounds.

#### ***Characterization***

MS can provide detailed structural information about the molecular composition of the compounds, including fragmentation patterns.

### **Quantification**

MS is also used for quantifying the amount of specific compounds present in a sample. This quantitative data is crucial in assessing the potency and efficacy of drug candidates.

## SUBJECT INDEX

### A

Abdominal cramps 115  
 Acid(s) 104, 107, 109, 111, 117, 116, 118,  
     119, 142, 153, 155, 156, 158, 165, 191  
     acetic 142, 153, 155, 156, 158, 191  
     formic 104, 109, 111, 117, 118, 119, 158  
     nicotinic 107, 109, 116  
     perchloric 142  
     phosphoric 165  
 Affinity-purification mass spectrometry 196  
 Agents 4, 19, 52, 53, 141, 149  
     antihyperglycaemic 53  
     antimicrobial 149  
     cardiovascular 52  
     cationizing 19  
     signaling 4  
 Ageratum conyzoides 93  
 Amino acid(s) 6, 8, 91, 110, 113, 114, 128,  
     162  
     nonproteinogenic 91  
     sequence analyses 6, 8  
 Angiotensin analogues 163  
 Anti-leukemic agent 151  
 Antipyretic remedy 93  
 Applications, downstream 188, 189  
 Aromatic nitro compounds 67  
 ARVD metabolites 58  
 Atmospheric 18, 38, 48, 63, 71, 92, 95, 98,  
     117, 134  
     -pressure chemical ionization (APCI) 38,  
     48, 63, 71, 92, 95, 98, 117, 134  
     pressure photo ionization (APPI) 48, 63,  
     71, 95, 98, 117  
     solid analysis probe (ASAP) 18  
 Automated operations 51  
 Azolic antimycotics 56

### B

Back-pressure regulator (BPR) 46  
 Bacteria, antibiotic-resistant 41

Based LC-MS techniques 145  
 Bioanalytical assays 216  
 Biochemical 41, 193, 214  
     pathways 214  
     processes 41, 193  
 Biogenic amines 128  
 Biological processes 165  
 Biopharmaceutical analysis 149  
 Biosynthetic pathway 107, 112  
     of cyanogenic glycosides 112  
 Boltzmann constant 2  
 Brain 127, 128, 135, 138, 141, 152, 153, 155  
     homogenate 141  
     microdialysis 138  
     tissue analysis 127  
 Brominated triazine-based flame retardants 65

### C

Calcitonin-gene related peptide 134  
 Calyculin, protein phosphatase inhibitor 194  
 Camellia sinensis 109  
 Cannabinoid 57  
 Capillary electrophoresis 14, 92, 134, 158,  
     195  
     microchip 195  
 Capillary zone electrophoresis 12  
 Carbon nanotubes (CNTs) 148  
 Cellular stress response 197  
 Chains, fluorinated aliphatic 42  
 Chemical(s) 36, 37, 38, 39, 42, 46, 48, 56, 59,  
     63, 67, 68, 70, 71, 134, 135, 148, 158,  
     196  
     anthropogenic 42  
     fingerprinting 48  
     industrial 36, 37  
     ionization, atmospheric-pressure 38, 63, 134  
     linkers 196  
     vaporizes deposition (CVD) 148  
 Chemical derivatization 9, 10  
     methods 9  
     technique 10

Chromatogram 54  
Chromatographic 4, 10, 20, 36, 40, 43, 46, 48, 100, 118, 134, 146, 147  
    analysis 118  
    conditions 134  
    methods 40  
    separation 4, 100, 146, 167  
    techniques 10, 20, 36, 43, 46  
Chromatography 10, 20, 40, 51, 56, 68, 92, 217, 219  
    gas 40, 92, 217  
    liquid adsorption 20  
    porous graphitic carbon 10  
    supercritical fluid 40, 56, 92  
Cisplatin binding 201  
Clarithromycin 53  
Coated glass capillaries (CGC) 48  
Collision(s) 1, 2, 3, 10, 12, 20, 47, 96, 164, 198  
    cell 164  
    energies 96, 164  
    high-energy 198  
Collisional energy 164  
Column 39, 45, 46, 54, 100, 103, 104, 106, 109, 111, 113, 117, 118, 119, 145, 146, 150  
    analytical 117  
    chromatographic 117  
    chromatography 39  
    methylpolysiloxane 104  
    monolithic 146  
    reversed-phase 106, 118  
Compositions 6, 15, 21, 60, 61, 63, 134, 139, 166, 197  
    amino acid 6  
    enantiomer 60  
    enantiomeric 61  
    isotopic 166  
Conformational analyses 1, 5, 7  
Conformations 18, 19, 200  
    cyclic 19  
    dynamic 18  
    functional 200  
Confusion, mental 110  
Contaminants 36, 37, 40, 42, 48, 65, 66, 91, 113, 119, 149  
    emerging trace 65  
    environmental 42, 48  
Copolymers 15, 16, 17  
    amphiphilic block 15

    cyclic olefin 16  
    polymer-peptide 16  
Copper-binding peptide methanobactin 201  
Coronavirus 151  
Corticotropin-releasing 129, 133  
    factor (CRF) 129  
    hormone 133

## D

Data-independent acquisition mass spectroscopy 215  
Depression, respiratory 106  
Deprotonated peptides 164, 165  
Detection 59, 116, 118  
    mass spectrometric 118  
    methodologies 116  
    of pesticides in wastewater 59  
Detector, fluorescence 11  
Devices, centrifugal filter 153  
Differential mobility 7, 10, 13, 16, 17, 47  
    analyzers (DMA) 47  
    spectrometry (DMS) 7, 10, 13, 16, 17, 47  
Digestion 9, 131, 135, 191, 193  
    enzymatic 131  
    proteolytic 9, 191  
Direct analysis in real time (DART) 48, 71, 215  
Discovery, biomarker 136  
Discrete dynode electron multiplier 38  
Diseases 4, 7, 41, 42, 93, 94, 128, 130, 131, 138, 157, 168, 200  
    amyloidosis-type 200  
    cardiovascular 94  
    heart-related 41  
    neurodegenerative 7, 94, 157  
    thyroid 42  
Dispersive magnetic solid-phase extraction (DMSPE) 48  
Dissociation 6, 19, 96, 163, 164, 165, 166, 197  
    collision-activated 96  
    collision-induced 6, 96, 163, 164  
    electron transfer 6, 19, 163, 165, 166  
Drug(s) 41, 127, 131, 216, 217  
    anti-inflammatory 41  
    targets 127, 131  
    treatment 216, 217  
Drug discovery 209, 210, 212, 213, 214, 215, 216, 217, 218, 220  
    applications 216



## ***Subject Index***

method 210  
process 212, 213, 215, 218  
Drug metabolism 209, 214, 216, 217  
pathways 217  
Dyschromatopsia 115

## **E**

Ecosystems 37  
environmental 37  
Effects 90, 93, 94, 104, 106, 115  
allelopathic 90  
anti-inflammatory 93  
anticholinergic 94, 104  
antiepileptic 106  
neurotoxicity 115  
Electron(s) 6, 11, 14, 19, 157, 159, 163, 165,  
166, 198, 201  
activated dissociation 14  
-based dissociation method 165  
capture dissociation (ECD) 6, 163, 165,  
198, 201  
low energy 165  
thermal 165  
transfer dissociation (ETD) 6, 11, 19, 163,  
165, 166  
Electrophoretic techniques 13  
Electrospray 118, 191, 210  
Electrospray ionization (ESI) 2, 38, 43, 47, 48,  
58, 59, 71, 95, 111, 113, 117, 118, 134,  
157, 158, 162, 190, 210, 214, 215  
desorption 215  
technique 157  
Emerging mass spectrometry technologies 201  
Energy balance 129  
Environmental 40, 43, 44, 45, 46, 48, 49, 55,  
56  
analysis 43, 44, 46, 48, 49, 56  
applications 45  
biodegradability 55  
pollutants 40  
Enzyme-Linked Immunosorbent Assay  
(ELISA) 128, 166  
ESI techniques 48, 197  
ETD techniques 20

## **F**

Ferrite-polymer combinations 145

## ***Applications of Modern Mass Spectrometry, Vol. 2 227***

Field asymmetric waveform IMS (FAIMS)  
14, 47  
Fingerprint mass spectra of organic  
compounds 71  
Food 90, 91, 92, 94, 100, 103, 104, 106, 109,  
116, 119  
cereal-based 104  
contaminants 94, 100  
contaminated 90  
Footprinting, oxidative 188, 189, 193, 200  
Fourier-transform ion cyclotron resonance  
(FTICR) 6, 165  
Fragmentation 103, 108, 119, 127, 162, 163,  
164, 165  
mechanism 103, 119  
method 164  
pathway 108  
techniques 127, 163, 164, 165  
techniques for neuropeptides 162  
Fungicides 41, 59

## **G**

GABAA receptors 115  
Gas chromatography (GC) 40, 43, 45, 46, 47,  
54, 65, 66, 67, 70, 92, 217  
Gaseous messengers 128  
Gastrointestinal 94, 113, 131  
disorders 94  
tract 131  
Genes 41, 129, 131, 151  
encoding 131  
resistance 41  
viral 151  
Genetic mutation 41  
Genomic problems 4  
Genotoxicity 100  
Glycan(s) 8, 9, 10, 11, 12, 13  
analysis 9, 13  
fucosylated 12  
isomeric 12, 13  
isomerization 9  
Glycine activity 116  
Glycoalkaloids, steroidal 97, 117  
Glycoproteins 9, 13, 14  
Glycosidic bonds 9, 97  
Gonadotropin-releasing hormone 133  
Growth hormone releasing peptides (GHRP)  
134

**H**

Heart-cutting LC technique 54  
Heat inactivation, microwave-induced 152  
Heating ramp 45  
Hemorrhagic necrosis 100  
Hepatic problems 113  
Hepatomegaly 100  
Hepatoprotective 93  
Hepatotoxic effect 115  
Herbicidal activity 60  
High 39, 40, 45, 46, 48, 49, 56, 59, 62, 65, 66,  
92, 106, 118, 119, 150, 157, 158, 209,  
213, 214, 218, 219  
performance liquid chromatography  
(HPLC) 40, 45, 46, 56, 92, 106, 118,  
119, 150, 157, 158  
-resolution mass spectrometry (HRMS) 39,  
46, 48, 49, 59, 62, 65, 66, 209, 213, 214,  
219  
throughput screening (HTS) 218  
Human immunodeficiency virus (HIV) 14, 51  
Hydrazinolysis 14  
Hyphenated 36, 37, 40, 54, 57, 61, 90, 92  
chromatography-MS techniques 57  
techniques 36, 37, 40, 54, 61, 90, 92  
Hyphenated mass 40, 65, 72, 73  
spectrometric methods 40  
spectrometry methods 65

**I**

Immobilized antibody 149  
IMMS technique 21  
Immunometric 166  
assays 166  
immunoassays 166  
IMS technique 47  
Inert gas 47, 157  
Ion 168  
cyclotron resonance 168  
Ion mobility 1, 2, 3, 4, 5, 7, 8, 13, 14, 16, 17,  
20, 46, 47, 58, 64  
-mass spectrometers 8  
spectrometry (IMS) 1, 2, 3, 4, 7, 8, 13, 14,  
17, 46, 47, 58, 64  
spectrometry technique 4  
technique 2, 5, 16, 17, 20  
Isomeric separation 64  
Isomers, sialic-acid 10

**K**

Kinetic energy 164

**L**

Liquid chromatography (LC) 40, 45, 47, 52,  
54, 59, 70, 92, 118, 127, 130, 133, 134,  
147, 151, 153, 157, 158, 191, 217  
high-performance 92, 130, 157, 158  
-mass spectrometry 127  
Liver dysfunction 100  
Low-pressure gas chromatography (LPGC) 45

**M**

Mass spectrometric analysis 2, 7, 44, 118,  
131, 138, 166  
Mass spectrometry 2, 40, 92, 209  
detection 40  
device 92  
techniques 2  
technology 209  
Mass spectroscopic methods 43  
Microextraction techniques 44  
Molecularly imprinted polymers (MIPs) 147,  
150, 151  
MS analysis 6, 9, 10, 38, 141, 151, 212, 219  
for neuropeptides 141  
MS-based 131, 135, 188, 189, 196, 201, 212  
neuropeptide analysis 131, 135  
based proteomics techniques 212  
techniques 188, 189  
technologies 196, 201  
MS techniques 188, 189  
emerging 188  
proteomic 188  
sensitive 189  
Mucin, gastric 13  
Multiple reaction monitoring (MRM) 48

**N**

Neurochemical analysis 168  
Neurological disorders 97  
Neuropeptides and physiological functions  
129  
Nitrogen-containing 91, 113  
metabolites 91

## **Subject Index**

phytotoxins 113  
Nitrogen gas 158  
Nuclear magnetic resonance spectroscopy  
157, 219

## **O**

Oxidation-footprinting protein mass  
spectrometry (OFPMS) 195  
Oxytocin vasopressin 138

## **P**

Parkinson's disease 104, 130  
Peptide fragmentation 131, 157, 164  
Phosphatases 194, 196  
Phosphoproteomic analysis 164  
Plant(s) 41, 90, 91, 92, 93, 100, 103, 104, 106,  
110, 114, 115, 116  
diseases 41  
medicinal 93, 114, 115  
Poisoning nitrogen-containing metabolites  
110  
Polycyclic aromatic hydrocarbons (PAHs) 65,  
66, 67  
Polymers 141, 150  
neutral 141  
organic 150  
Products, enzymatic digestion 5  
Protease 155, 190, 193  
inhibitors 155  
Protein 91, 188, 198  
biosynthesis 91  
chemistry 188  
-protein network 198  
Proteinase 194  
Proteolysis 193  
Proton(s) 53, 164  
ionizing 164  
-pump inhibitors 53  
Psychiatric disorders 41, 130, 131  
Pulmonary dysfunction 100  
Pyrolysis products 16

## **R**

Radioimmunoassay 128, 130  
Reaction, polymerization 150  
Release 13, 14, 46, 115, 129, 198  
enzymatic glycan 13, 14

## **Applications of Modern Mass Spectrometry, Vol. 2 229**

metabolic hormone 129  
Resonance, nuclear magnetic 92  
Restricted-access materials (RAMs) 140, 149  
Retrodialysis 137  
RNA oligonucleotides 151

## **S**

Secondary ion mass spectrometry (SIMS) 158,  
159, 162  
Sewage treatment plant 60  
Signals 4, 38, 39, 128, 130, 131, 151, 211  
transduction 128  
transmission 130  
electrical 38, 151  
electronic 211  
Soft ionization technique 71, 95, 130, 158,  
168  
SPE and reversed 51, 58  
phase ion-exchange 58  
liquid chromatography 51  
Spectrometry 4, 39, 47, 52, 117, 128, 166,  
195, 209, 213, 214, 219  
accurate liquid chromatography-mass 128  
gas chromatography-mass 47  
high-resolution mass 39, 209, 213, 214, 219  
liquid chromatography-electrospray  
ionization-mass 117  
liquid chromatography-tandem mass 4, 52  
oxidation-footprinting protein mass 195  
Structure-activity relationship (SAR) 214, 215  
Supercritical fluid 39, 40, 46, 56, 70, 92, 116  
chromatography (SFC) 40, 46, 56, 70, 92  
extraction (SFE) 39, 116  
Symptoms, asthma 92

## **T**

Tachycardia 106  
Tandem mass 4, 19, 96, 104, 109, 209, 216,  
219  
analysis 96  
spectrometry 4, 19, 109, 209, 216, 219  
spectrum 104  
Target 59, 61, 193, 194, 215  
potential therapeutic 215  
protein therapy 194  
pesticides 59, 61  
protein 193  
Teas, herbal 104

Techniques 1, 44, 68, 69, 70, 71, 127, 137,  
138, 144, 145, 150, 151, 157, 189, 193,  
196, 217  
affinity-purification 196  
complementary 71  
microdialysis 138  
sensitive 70  
Technology 37, 43, 44, 46, 59, 61, 189, 197,  
212, 217, 219  
combining MS 189  
sewage disposal 61  
Teratogenicity 91  
Thoracic pain dyspnea 115  
Tonsillitis 93  
Transformation products (TPs) 42, 59, 63, 67  
Transient burning sensation 115  
Tumors, painful 93

## U

Ultra-fast liquid chromatograph 138  
Ultraviolet light photodissociation 6

## V

Vasoactive intestinal peptide (VIP) 129, 134

## W

Waals forces 151  
Wastewater-based epidemiology (WBE) 42  
Water 36, 37, 42, 46, 51, 53, 68, 70, 111, 117,  
118, 119, 129, 150  
pollution analysis 36  
resources 37  
retention 129  
treatment 51



## ATTA-UR-RAHMAN, FRS

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has over 1559 international publications in several fields of organic chemistry (h index 76, citations 38,200) including 86 international patents, 70 chapters in books, 875 research publications, and 391 books (11 authored and 380 edited). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.



## M. IQBAL CHOUDHARY

M. Iqbal Choudhary is a distinguished national professor of organic/bioorganic chemistry and honorary advisor at the International Center for Chemical and Biological Sciences (H. E. J. Research Institute of Chemistry, and Dr. Panjwani Center for Molecular Medicine and Drug Research), and coordinator general of COMSTECH (OIC Ministerial Committee). He is among the most prominent scientists of Pakistan, recognized for his original contributions in the fields of natural products and bioorganic chemistry. He has written and edited 27 books and he is the author of over 1275 research papers and chapters in top international science journals as well as 64 US patents with an H-index: 86 and citations: 43,716. He is the volume editor of many international book series and journals. He has served as a visiting faculty in many prestigious universities of the world. He is the fellow of major science academies of world (TWAS, IWAS, PAS), and received prestigious awards and honors, including Chinese Government Friendship Award (2022), Mustafa (PBUH) award (2021), ECO Excellence Award in Education (2016) and MRC Team Impact Prize, UK (2024).



## SYED GHULAM MUSHARRAF

Prof. Dr. Syed Ghulam Musharraf is amongst the most notable young scientists based on his seminal contributions to mass spectrometry and its applications. He obtained post-doctoral training from Austria and USA. On his return, he established a world-class mass spectrometry research laboratory in Pakistan. He has effectively used several mass spectrometric tools for the high-throughput analyses of bioactive compounds and the standardization of botanicals. He is pioneered in biomarker-based metabolomics research in Pakistan. He worked extensively on different cancers, as well as on thalassemia for molecular understanding of disease. He is the author of over 250 research publications (journal cumulative impact factor over 1200, h-index = 38, Google Scholar). Based on his scientific contributions, he has received several international/national awards and honors, including civil award and a D. Sc. Degree.