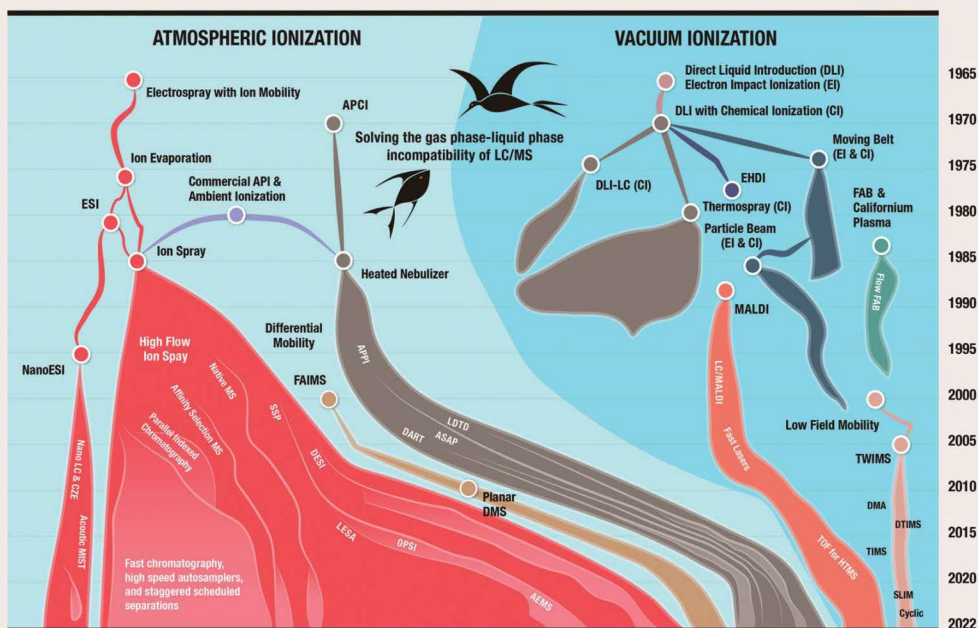


High-Throughput Mass Spectrometry in Drug Discovery

Edited By **Chang Liu** and **Hui Zhang**



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Contents

List of Contributors	<i>xv</i>
Preface	<i>xix</i>
List of Abbreviations	<i>xxi</i>

Section 1 Introduction 1

1	Forty-Year Evolution of High-Throughput Mass Spectrometry: A Perspective	3
	<i>Thomas R. Covey</i>	
1.1	Introduction	3
1.2	Ionization Foundations of High-Throughput Mass Spectrometry	5
1.2.1	Historical Context of the Development of LC/ MS. Ionization in Vacuum or at Atmospheric Pressure?	7
1.2.2	Ambient Sample Introduction Methods (Ambient Ionization) into an API Ion Source Without LC and Their HT-MS Potential	13
1.2.3	Direct and Indirect Affinity Measurements with ESI/MS for HTS	16
1.3	High-Speed Serial Chromatographic Sample Introduction	18
1.3.1	High Flow Rate Ion Sources	19
1.3.2	Fast Serial Scheduled, Staggered Chromatographic Separations with Fast Autosamplers	22
1.3.3	High-Speed Column Stationary Phases	24
1.4	Parallel Chromatographic Sample Introduction	26
1.4.1	Overview of Multichannel Indexed Ion Sources	26
1.4.2	Fluid Indexing	27
1.4.3	Spray Aerosol Indexing	28
1.4.4	Ion Beam Indexing	28
1.4.5	Ionization Indexing	29
1.4.6	Multichannel Autosampler and Pumps	30

- 1.5 High Repetition Rate Lasers 32
- 1.6 Ion Mobility for High-Speed Gas-Phase Separations 35
 - 1.6.1 Motivation and Commercial Options 35
 - 1.6.2 Origins of DMS 36
 - 1.6.3 Chemically Based Selectivity with DMS to Mimic Chromatography 37
- 1.7 Mass Spectrometer Sensitivity 40
 - 1.7.1 Historical Gains and Motivation for Sensitivity Improvements 40
- 1.8 High-Speed Sub-Microliter Volume Sampling 42
 - 1.8.1 Small Sample Size and Low Volume Dispensing HT-MS Technologies 42
 - 1.8.2 Shoot N' Dilute Nanoliter Droplets 44
- 1.9 Conclusions and Future Prospects 53
- References 56

Section 2 LC-MS 75

2 The LeadSampler (LS-1) Sample Delivery System: Integrated Design and Features for High-Efficiency Bioanalysis 77

Brendon Kapinos and John Janiszewski

- 2.1 Introduction 77
- 2.2 Hardware and System Design 80
- 2.3 Software Integration 84
- 2.4 Enabling Emerging Techniques 90
- 2.5 Concluding Remarks 96
- References 97

3 Evolution of Multiplexing Technology for High-Throughput LC/MS Analyses 103

Adam Latawiec

- 3.1 Introduction and Historical Developments 103
- 3.2 Developments Toward Fully Integrated Multiplexing Systems 105
- 3.3 Broadening Customer Options 108
- 3.4 Workflow and End-User Considerations 113
- 3.5 Conclusion 115
- References 116

Section 3 ESI-MS Without Chromatographic Separation 121**4 Direct Online SPE-MS for High-Throughput Analysis in Drug Discovery 123***Andrew D. Wagner and Wilson Z. Shou*

- 4.1 Introduction 123
- 4.2 History of the Development of Direct Online SPE-MS 124
- 4.3 Hardware Details and Data Processing 126
- 4.4 Instrument Performance Highlights 132
- 4.5 Applications 133
- 4.6 Others 134
- 4.7 Future Perspectives 135
- References 135

5 Acoustic Sampling for Mass Spectrometry: Fundamentals and Applications in High-Throughput Drug Discovery 143*Chang Liu, Lucien Ghislain, Jonathan Wingfield, Sammy Datwani, and Hui Zhang*

- 5.1 Introduction 143
- 5.2 Technology Overview 145
 - 5.2.1 AMI-MS 145
 - 5.2.2 ADE-OPI-MS 151
 - 5.2.2.1 System Description 151
 - 5.2.2.2 System Tuning and Assay Development 152
 - 5.2.2.3 ADE-OPI-MS Automated Data Processing and Automation Integration 154
 - 5.3 System Performance 154
 - 5.3.1 AMI-MS Performance 154
 - 5.3.2 ADE-OPI-MS Performance 160
 - 5.4 Applications 162
 - 5.4.1 High-Throughput Screening 162
 - 5.4.1.1 AMI-MS for HTS 162
 - 5.4.1.2 ADE-OPI-MS for HTS 166
 - 5.4.2 High-Throughput ADME 168
 - 5.4.3 In Situ Reaction Kinetics Monitoring 168
 - 5.4.4 Bioanalysis 170

- 5.4.5 Compound QC 171
- 5.4.6 Parallel Medicinal Chemistry 172
- 5.4.7 High-Content Screening 173
- 5.5 Challenges and Limitations 175
- 5.6 Conclusion 176
- References 177

6 Ion Mobility Spectrometry-Mass Spectrometry for High-Throughput Analysis 183

Dylan H. Ross, Aivett Bilbao, Richard D. Smith, and Xueyun Zheng

- 6.1 Introduction of Ion Mobility Spectrometry 183
- 6.2 IMS Fundamental and Experiment 184
 - 6.2.1 Ion Mobility Theory 184
 - 6.2.2 Collision Cross Section Measurement 186
 - 6.2.3 A Typical IMS Experiment 186
- 6.3 IMS Analysis and Applications 187
 - 6.3.1 Separation of Isomeric and Isobaric Species by IMS 187
 - 6.3.2 High-Throughput IMS Measurements and Building a CCS Library 188
 - 6.3.2.1 CCS Measurement of Small Molecules Using DTIMS 190
 - 6.3.2.2 CCS Measurements of Drug Compounds Using TWIMS 193
 - 6.3.2.3 Large-Scale CCS Databases From Prediction Approaches 195
 - 6.3.3 LC-IMS-MS Analysis 195
 - 6.3.4 High-Throughput Analysis Using Rapidfire SPE-IMS-MS 196
 - 6.3.5 Software Tools for IMS Data Analysis 199
- 6.4 High-Resolution SLIM-IMS Developments 200
- 6.5 Conclusions 204
- References 205

7 Differential Mobility Spectrometry and Its Application to High-Throughput Analysis 215

Bradley B. Schneider, Leigh Bedford, Chang Liu, Eva Duchoslav, Yang Kang, Subhasish Purkayastha, Aaron Stella, and Thomas R. Covey

- 7.1 Introduction 215
- 7.2 Separation Speed 216
 - 7.2.1 Classical Low Field Ion Mobility 216
 - 7.2.2 Differential Mobility Spectrometry 217
 - 7.2.2.1 FAIMS 218
 - 7.2.2.2 DMS 219
- 7.3 Separation Selectivity 220
 - 7.3.1 Classical Low Field Ion Mobility 220

- 7.3.2 Differential Mobility Spectrometry 220
- 7.3.2.1 FAIMS 220
- 7.3.2.2 DMS 221
- 7.4 Ultrahigh-Throughput System with DMS 226
- 7.4.1 AEMS Data 231
- 7.4.2 DMS Sensitivity (Ion Transmission) 237
- 7.4.3 Examples of AEMS Analyses with DMS 240
- 7.4.3.1 Example 1. DMS to Eliminate Interferences from Isobaric Species 240
- 7.4.3.2 Example 2. DMS to Eliminate Interferences for Species that are Not Nominally Isobaric 244
- 7.4.3.3 Example 3. DMS to Eliminate Unknown Interferences from Species Endogenous to the Solvent Matrix 250
- 7.4.4 DMS Tuning as a Component of the High-Throughput Workflow 252
- 7.4.5 Automation of the Tuning Process 253
- 7.5 Conclusions 258
- 7.A Chemical Structures 259
- References 262

Section 4 Special Sample Arrangement 267

8 Off-Line Affinity Selection Mass Spectrometry and Its Application in Lead Discovery 269

Christopher F. Stratton, Lawrence M. Szewczuk, and Juncai Meng

- 8.1 Introduction to Off-Line Affinity Selection Mass Spectrometry 269
- 8.2 Selected Off-Line Affinity Selection Technologies and Its Application in Lead Discovery 270
- 8.2.1 Membrane Ultrafiltration-Based Affinity Selection 270
- 8.2.1.1 Introduction of Membrane Ultrafiltration-Based ASMS 270
- 8.2.1.2 Application of Membrane Ultrafiltration-Based ASMS in Lead Discovery 271
- 8.2.1.3 Pulse Ultrafiltration-Based ASMS Technology 273
- 8.2.1.4 Affinity Rank-Ordering Using Pulse Ultrafiltration-Based ASMS 273
- 8.2.1.5 Advantages and Disadvantages of Membrane Ultrafiltration-Based ASMS 275
- 8.2.2 Plate-Based Size Exclusion Chromatography 275
- 8.2.2.1 Introduction of SpeedScreen: A Plate-Based SEC ASMS Technology 275

8.2.2.2	Application of SpeedScreen in Lead Discovery	277
8.2.2.3	Advantages and Considerations of SpeedScreen	278
8.2.3	Bead-Based Affinity Selection	281
8.2.3.1	Introduction to Bead-Based Affinity Selection	281
8.2.3.2	Application and Discussion of Bead-Based Affinity Selection in Lead Discovery	282
8.2.4	Self-Assembled Monolayers and Matrix-Assisted Laser Desorption Ionization (SAMDI)	283
8.2.4.1	Introduction to SAMDI Technology	283
8.2.4.2	Discussion and Proof-of-Concept of SAMDI Technology for Off-Line ASMS	286
8.2.5	Ultracentrifugation Affinity Selection	286
8.2.5.1	Introduction to Ultracentrifugation Affinity Selection	286
8.2.5.2	Discussion and Proof-of-Concept of Ultracentrifugation Affinity Selection for Off-line ASMS	288
8.3	Future Perspectives	291
	References	292
9	Online Affinity Selection Mass Spectrometry	297
	<i>Hui Zhang and Juncai Meng</i>	
9.1	Introduction of Online Affinity Selection-Mass Spectrometry	297
9.2	Online ASMS Fundamental	299
9.3	Instrument Hardware and Software Consideration	300
9.3.1	SEC Selection, Fast Separation, and Temperature	300
9.3.2	MS: Low Resolution and High Resolution	302
9.3.3	Software: Key Features, False Positives, and False Negatives	303
9.3.4	Compound Libraries and Compression Level	305
9.4	Type of Assays Using ASMS	306
9.4.1	Target Identification and Validation	306
9.4.2	Hits ID from Combinatorial Libraries or Compound Collections	308
9.4.3	Hits Characterization and Leads Optimization	308
9.5	Applications Examples and New Modalities of ASMS for Drug Discovery	311
9.6	Future Perspectives	312
	References	313
10	Native Mass Spectrometry in Drug Discovery and Development	317
	<i>Mengxuan Jia, Jianzhong Wen, Olivier Mozziconacci, and Elizabeth Pierson</i>	
10.1	Introduction	317
10.1.1	The Significance of Non-Covalent Protein Complexes in Biology	317

- 10.1.2 Advantages and Disadvantages of Conventional Structural Analytical Techniques 318
- 10.2 Fundamentals of Native MS 320
 - 10.2.1 Principles of Native Electrospray Ionization 320
 - 10.2.2 Specific Sample Preparation to Preserve Non-Covalent Interactions and Be Compatible with ESI-MS Analysis 321
- 10.3 Instrumentation 323
 - 10.3.1 Nano-ESI and ESI 323
 - 10.3.2 Inline Desalting and Separations Coupled to Native Mass Spectrometry 323
 - 10.3.2.1 Inline SEC and Desalting 324
 - 10.3.2.2 Inline IEX 325
 - 10.3.2.3 Inline HIC 325
 - 10.3.2.4 Inline 2D LC 326
 - 10.3.2.5 Compatibility with nESI 326
 - 10.3.3 High-Throughput Native Mass Spectrometry 327
 - 10.3.4 Mass Analyzers 329
 - 10.3.5 Data Processing 329
 - 10.3.5.1 Contrasts Between Non-Native and Native MS Data Processing and Interpretation 329
 - 10.3.5.2 Software for Native MS 330
- 10.4 Application Highlights 330
 - 10.4.1 Using Native MS to Develop Stable Protein Formulations 332
 - 10.4.2 Native MS to Understand Drug/Target Interaction 334
 - 10.4.3 Native Mass Spectrometry and Tractable Protein–Protein Interactions for Drug Discovery 335
 - 10.4.4 Structural Stability Using Collision-Induced Unfolding 336
 - 10.4.5 Vaccines and Virus Proteins Using CDMS 336
- 10.5 Conclusions and Future Directions 337
 - References 337

Section 5 Other Ambient Ionization Other than ESI 347

- 11 Laser Diode Thermal Desorption-Mass Spectrometry (LDTD-MS): Fundamentals and Applications of Sub-Second Analysis in Drug Discovery Environment 349**
Pierre Picard, Sylvain Letarte, Jonathan Rochon, and Réal E. Paquin
 - 11.1 A Historical Perspective of the LDTD 349
 - 11.2 Instrumentation 351
 - 11.2.1 LDTD Process 351
 - 11.2.2 Sample Holder Design 352

11.2.3	Vapor Extraction Nozzle	353
11.3	Theoretical Background	354
11.3.1	Thermal Process	354
11.3.2	Gas Dynamics	358
11.3.3	Ionization	359
11.4	Sample Preparation	362
11.4.1	Motivations	362
11.4.2	General Guidelines	362
11.4.2.1	Compound Detection Background	363
11.4.2.2	Details on Ionic Saturation	364
11.4.2.3	Consideration for Biological Matrices	367
11.5	Applications	370
11.5.1	CYP Inhibition Analysis	371
11.5.2	Permeability	373
11.5.3	Protein Binding	378
11.5.4	Pharmacokinetic	378
11.5.5	Preparation Tips	382
11.6	Conclusion	384
11.6.1	Use and Merits of the Technology	384
11.6.2	Limitations	385
11.6.3	Perspectives	386
	References	387
12	Accelerating Drug Discovery with Ultrahigh-Throughput MALDI-TOF MS	393
	<i>Sergei Dikler</i>	
12.1	Introduction	393
12.2	uHT-MALDI MS of Assays and Chemical Reactions	396
12.2.1	HT-MALDI of Enzymatic Assays	396
12.2.2	Screening Chemical Reactions Using uHT-MALDI	401
12.2.3	uHT-MALDI of Cell-Based Assays	404
12.2.4	uHT-MALDI of Other Types of Assays and Libraries	406
12.3	Bead-Based Workflows	408
12.4	Using Functionalized, Modified, and Microarrayed MALDI Plates for HT-MALDI	411
12.5	Summary and Future Trends	413
	Acknowledgment	414
	References	414

13	Development and Applications of DESI-MS in Drug Discovery	423
	<i>Wenpeng Zhang</i>	
13.1	Introduction	423
13.2	Development of DESI and Related Ambient Ionization Methods	424
13.3	Applications in Drug Discovery	427
13.3.1	Pharmaceutical Analysis and Therapeutic Drug Monitoring	427
13.3.2	Analysis of Drugs in Natural Products	428
13.3.3	DESI-Based Mass Spectrometry Imaging	430
13.3.4	Detection of Drug-Protein Interactions	435
13.3.5	High-Throughput Experimentation	438
13.3.6	High-Throughput Screening	439
13.4	Conclusions and Future Outlook	440
	References	442
	Section 6 Conclusion	453
14	The Impact of HT-MS to Date and Its Potential to Shape the Future of Metrics-Based Experimentation and Analysis	455
	<i>Matthew D. Troutman</i>	
14.1	Defining High-Throughput Mass Spectrometry (HT-MS)	456
14.2	HT-MS: Impact to Date	457
14.3	Considering How HT-MS Will Shape the Future of Drug Discovery	458
	References	462
	Index	467

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Preface

The automated and integrated high-throughput sample analysis is critical to the drug discovery process. Traditional high-throughput bioanalytical technologies such as colorimetric microplate-based readers are often constrained by linear dynamic range. In addition, they need label attachment schemes with the propensity to modify equilibrium and kinetic analysis. On the other hand, mass spectrometry (MS) based methods can achieve label-free, universal mass detection of a wide arrange of analytes with exceptional sensitivity, selectivity, and specificity. However, these techniques are limited by the speed of sample introduction. In recent decades, there have been a lot of efforts to improve the throughput of MS-based analysis for drug discovery. Along with those developments, a dedicated book would be helpful to introduce the fundamentals, experimental details, and applications of a wide variety of technologies that enabled high-throughput mass spectrometry-based screens in supporting broad drug discovery applications. The key research areas include hit discovery by label-free screen, synthetic reaction optimization, lead optimization and SAR support, ADME (absorption, distribution, metabolism, and excretion), toxicology screening, etc.

This book starts with an overview of the 40 years of efforts to improve the analytical throughput of MS-based approaches (Chapter 1). Then, technologies with high-speed sequential and parallel chromatographic sample introduction, high repetition rate lasers, ion mobility, and low-volume MS samplings were summarized.

Due to its high specificity and high sensitivity, the LC-MS technology has been widely used in various steps of the drug discovery workflow. In Part 2 (Chapter 2–3), the efforts to improve the LC-MS analytical throughput are introduced. The development of the high-speed sample introduction for LC-MS and its application on ADME and HTS applications is described in Chapter 2. Another approach for throughput improvement utilizing paralleled multiplexing LC is described in Chapter 3.

Following the conventional LC-MS-based technologies, other electrospray ionization (ESI)MS-based high-throughput platforms without chromatographic

separation are summarized in Part 3 (Chapter 4–7). Direct online solid-phase extraction (SPE) MS and its application in ADME and HTS workflows are described in Chapter 4. The utilization of the acoustic energy for non-contact transfer samples from microplates to MS for high-throughput analysis, including the acoustic mist ionization (AMI) and through the open-port interface (OPI), is summarized in Chapter 5. By skipping the chromatographic separation process, these approaches demonstrated higher analytical throughput than the conventional LC-MS approach. However, there would be the risk of potential isomeric/isobaric interference. Ion mobility spectrometry (IMS) and differential mobility spectrometry (DMS), described in Chapters 6 and 7, respectively, provide the additional dimension of the selectively, potentially solving the specificity issues of these high-throughput technologies for some drug discovery assays.

Part 4 (Chapters 8–10) summarized the MS-based high-throughput hit identification technologies based on the drug-target interaction. Affinity-selection mass spectrometry (ASMS) is a rapidly developing technology for high-throughput hit identification. The off-line and in-line ASMS approaches are introduced in Chapters 8 and 9. In addition, as a direct confirmation tool for the protein-drug binding, native MS has been rapidly developed in the past decade, which is described in Chapter 10.

Part 5 (Chapter 11–13) introduces developments of ambient ionization technologies other than the conventional ESI and their applications in the high-throughput drug discovery workflows, such as Laser Diode Thermal Desorption (LDTD, Chapter 11), Matrix-Assisted Laser Desorption/Ionization (MALDI, Chapter 12), and Desorption Electrospray Ionization (DESI, Chapter 13).

The last chapter (Chapter 14) provides perspectives for future development opportunities after a brief reflection of the realized impacts of high-throughput MS on drug discovery and the pharmaceutical industry.

We believe our goal in this book is accomplished through the extensive coverage of fundamentals, experimental details, and applications of state-of-art technologies that enable high-throughput MS-based screens in supporting drug discovery. We hope it could benefit scientists in pharmaceutical/biopharmaceutical companies and CROs who design and perform the studies and provide analytical support throughout drug discovery processes. We would like to acknowledge the commitment and contributions of all authors of the book chapters and the support and valuable discussions with colleagues and collaborators in the SCIEX research team and Pfizer Discovery Science department. In addition, we sincerely thank the editorial team at John Wiley & Sons, especially Adalfin Jayasingh, Stacey Woods, Jonathan Rose, Andreas Sendtko, and Sabeen Aziz, for their generous support of this book. Finally, we are grateful to our family members for their understanding and support for our editing work in the evening and on weekends.

List of Abbreviations

%-RBA	relative binding affinity percentage
μ FLC	microflow liquid chromatography
2d	two-dimensional
2-HG	2-hydroxyglutarate
3CLpro	3-chymotrypsin-like cysteine protease
4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1
A	pre-exponential factor constant
ACE50	affinity competition experiment 50% inhibitory concentration
AChE	acetylcholinesterase
ADC	antibody–drug conjugate
ADE-OPI-MS	acoustic droplet ejection-open port interface-mass spectrometry
ADME	adsorption, distribution, metabolism, and excretion
AEMS	acoustic ejection mass spectrometry
AMI-MS	acoustic mist ionization-mass spectrometry
AMS	affinity mass spectrometry
ANSI	American National Standards Institute
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
APIs	active pharmaceutical ingredients
APPI	atmospheric pressure photo ionization
ASAP	atmospheric solids analysis probe
ASMS	affinity selection mass spectrometry
ASMS	American society mass spectrometry
Asp	aspartic acid
ATD	arrival time distribution
ATP	adenosine triphosphate

AUC	analytical ultracentrifugation
AUC	area under the curve
BACC	bacterial acetyl coenzyme-A carboxylase
BACE	beta-site APP cleaving enzyme
BAMS	bead assisted mass spectrometry
Bcl-xL	B-cell lymphoma-extra large protein
bdf	batch data file
BE	buffer exchange
Bead-GPS	bead-based global proteomic screening
BFA	bound fraction analysis
BKM120	Buparlisib
BSA	bovine serum albumin
BTE	Boltzmann transport equation
C18	octadecyl stationary phase
C8	octyl stationary phase
CCS	collision cross section
CD	circular dichroism
CDMS	charge detection mass spectrometry
CEM	chain ejection model
cGAMP	cyclic GMP-ATP
cGAS	cyclic GMP-AMP synthase
CHCA	α -cyano-4-hydroxycinnamic acid
CHK1	checkpoint kinase
CID	collision induced dissociation
CIU	collision induced unfolding
CN	cyano stationary phase
CoV	compensation voltage
CPATI	cytosolic proteome and affinity-based target identification
CRIMP	Compression Ratio Ion Mobility Programming
CRM	charged residue model
CV	coefficient of variation
CYP	cytochrome P450
Da	Dalton, measurement unit used in mass spectrometry
DAR	drug-to-antibody ratio
DART	direct analysis in real time
DDI	drug-drug interaction
DEC	desorption enhancing coating
DEL	DNA-encoded library
DESI	desorption electrospray ionization
DHAP	2,5-dihydroxyacetophenone
DHFR	dihydrofolate reductase

diCQA	dicafeoylquinic acid
DI-GCE/MS/MS	direct injection/on-line guard cartridge extraction/tandem mass spectrometry
DIMS	differential IMS
DIOS	desorption ionization on silicon
DLS	dynamic light scattering
DMA	differential mobility analyzer
DMS	differential mobility spectrometry
DP	declustering potential
DQ	DiscoveryQuant
DSF	differential scanning fluorimetry
DT	drift time
DTIMS	drift tube IMS
DUB	deubiquitinase
E_a	energy of activation
ebox	electronics box
E_d	bound dissociation energy
EDTA	ethylenediaminetetraacetic acid
EHDI	electrohydrodynamic ionization
EI	electron impact
EM	electron microscopy
ERK1/ERK2	extracellular signal-regulated kinase 1 and 2
ESI	electrospray ionization
ESI-MS	electrospray ionization mass spectrometry
E_λ	energy associated with the vibrational wavelength
FAB	fast atom bombardment
FAIMS	high field asymmetric waveform ion mobility spectrometry
FAK	focal adhesion kinase
FASN	fatty acid synthase
FIA	flow injection analysis
FLD	fluorescence detector
FP	fluorescence polarization
FTE	full-time equivalent
FTICR	Fourier-transform ion cyclotron resonance
FWHM	full width at half maximum
GABA	γ -aminobutyric
GC	gas chromatography
GLP	good laboratory practice
GPC	gel permeation chromatography
GST	glutathione S-transferase
GWAS	genome-wide association studies

HBSS	Hank's buffered salt solution
HCV	hepatitis C virus
HDMA	high-density micropatterned array
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIC	hydrophobic interaction chromatography
HLM	human liver microsomes
HMW	high molecular weight species
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HT-ADME	high-throughput absorption, distribution, metabolism, excretion
HTE	high-throughput experimentation
HT-LC/MS/MS	high-throughput mass spectrometry
HT-MALDI	high-throughput matrix-assisted laser desorption/ionization
HT-MS	high-throughput mass spectrometry
HTRF	homogenous time-resolved fluorescence
HTS	high-throughput screening
IC ₅₀	half maximal inhibitory concentration
ID	internal diameter
IDH1	isocitrate dehydrogenase 1
IEX	ion exchange chromatography
IM	ion mobility
IMAC	immobilized metal ion affinity chromatography
iMALDI	immuno-matrix-assisted laser desorption/ionization
IMS	ion mobility spectrometry
IR-MALDESI	infrared matrix-assisted desorption electrospray ionization
IS	internal standards
isoAsp	isoaspartic acid
ITC	isothermal titration calorimetry
ITO	indium tin oxide
IVIVC	in vitro to in vivo correlations
<i>k</i>	rate constant
LC	liquid chromatography
LC/MS/MS	liquid chromatography tandem mass spectrometry
LC-MALDI	liquid chromatography-matrix-assisted laser desorption/ionization
LC-MS	liquid chromatography mass spectrometry
LDLR	low-density lipoprotein receptor
LDTD	laser diode thermal desorption
LESA	liquid extraction surface analysis
LLE	liquid-liquid extraction

LOD	limits of detection
LogD	distribution coefficient
LOQ	limit of quantitation
LPS	lipopolysaccharides
M3	microfabricated monolithic multinozzle
mAbs	monoclonal antibodies
MagMASS	magnetic microbead affinity selection screen
MALDI	matrix-assisted laser desorption ionization
MALDI-2	laser-induced postionization
MALDI-FTICR MS	matrix-assisted laser desorption/ionization Fourier-transform ion cyclotron resonance mass spectrometry
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MetAP2	methionyl aminopeptidase 2
MnESI	microflow-nanospray electrospray ionization
MPS	mesoporous silica
MRM	multiple reaction monitoring
MRO	medical review officer
MS	mass spectrometer
MS/MS	tandem mass spectrometry
MSI	mass spectrometry imaging
MTBE	methyl tert-butyl ether
MTP	microtiter plate
MuRF	muscle RING-finger protein
NADPH	nicotinamide adenine dinucleotide phosphate
NALDI	nanostructure-assisted laser desorption/ionization
Nano-DESI	nanospray desorption electrospray ionization
NAPA-LDI	nanopost array-laser desorption/ionization
NDM-1	New Delhi metallo-lactamase1
NDX	native-denatured exchange
nESI	nano electrospray ionization
NHS	<i>N</i> -hydroxysuccinimide
NIMS	nanostructure-initiator mass spectrometry
nL	nanoliter
NMR	nuclear magnetic resonance
nMS	native mass spectrometry
NSAID	nonsteroidal anti-inflammatory drugs
NSP14	nonstructural protein 14
OATP2B1	organic anion transporting polypeptide 2B1
OIMS	overtone IMS
OPSI	open port sampling interface

PAH	polycyclic aromatic hydrocarbon
PBED	polybrominated diphenyl ether
PBS	phosphate-buffered saline, buffer solution about pH 7.4
PCB	polychlorinated biphenyl
PCB	printed circuit board
PC-mass-tags	photocleavable mass-tags
PFAS	per- and polyfluoroalkyl substances
PK	pharmacokinetic
pK_a	acid dissociation constant
PKC α	protein kinase C- α
PMF	peptide mass fingerprinting
PoC	percentage of control
POE	percent of enrichment
PPT	protein precipitation technique
PROTAC	proteolysis targeting chimera
PTP1B	tyrosine phosphatase 1B
PUF-MS	pulsed ultrafiltration-mass spectrometry
PVDF	polyvinylidene difluoride
QA/QC	quality assurance and quality control
qPCR	quantitative polymerase chain reaction
qTOF	quadrupole time-of-flight
QuEChERS	quick easy cheap effective rugged and safe
R	universal gas constant
R^2	coefficient of determination
RAM	restricted access media, usually a type of filtering or extraction media
RAM	restricted access medium
RF-MS	RapidFire – mass spectrometry
ROI	return on investment
RXR α	retinoid X receptor- α
S/N	signal-to-noise ratio
SALLE	salt assisted liquid-liquid extraction
SAM	S-adenosyl-L-methionine
SAMDI	self-assembled monolayers and matrix-assisted laser desorption ionization
SAR	structure-activity relationship
SEC	size-exclusion chromatography
SEC-TID	size-exclusion chromatography for target identification
SEM	scanning electron microscope
SESI	secondary electrospray ionization

SEZ	staggered elution zone chromatography
SIK2	salt-inducible kinase 2
SIMS	secondary ion mass spectrometry
Sirt3	Sirtuin 3
SISCAPA	stable isotope standards and capture by anti-peptide antibodies
SLIM	structures for lossless ion manipulations
SLS	static light scattering
SME	small molecular entity
SmyD3	SmyD3 histone methyltransferase
SNP	single-nucleotide polymorphism
SPE	solid phase extraction
SPE-MS	solid-phase extraction mass spectrometry
SPME	solid-phase microextraction
SPR	surface plasmon resonance
SRM	selected reaction monitoring
SSP	surface sampling probe
SUPER	Serpentine Ultralong Path with Extended Routing
SV	separation voltage
SWATH	sequential window acquisition of all theoretical mass spectra
<i>T</i>	absolute temperature in Kelvin
TCP	tumor cell percentage
THC	tetrahydrocannabinol
TIMS	trapped ion mobility
TLC	layer chromatography
TMA-lyase	trimethylamine-lyase
TM-DESI	transmission mode DESI
TM-IMS	transversal modulation IMS
TOF	time-of-flight
TR-FRET	time-resolved fluorescence energy transfer
TRIS	Tris (hydroxymethyl) aminomethane
TWIMS	traveling wave ion mobility
UFA	unbound fraction analysis
UHPLC	ultrahigh-performance (or pressure) liquid chromatography
UHPLC/MS	ultrahigh-performance liquid chromatography-mass spectrometry
uHT-MALDI	ultrahigh-throughput matrix-assisted laser desorption/ionization
uHTS	ultrahigh-throughput screening
UPLC	ultra performance liquid chromatography

UV	ultraviolet, usually meant to describe absorbances between 190 and 400 nm
UVPD	ultraviolet photodissociation
WBA	whole-body autoradiography
XRD	X-ray diffraction
Δ^9 -THCC	Carboxylic Δ^9 -tetrahydrocannabinol
λ	phonon wavelength