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SAMPLE PREPARATION IN **LC-MS** **BIOANALYSIS**

EDITED BY
WENKUI LI
WENYING JIAN
YUNLIN FU

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Sample Preparation in LC-MS Bioanalysis

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Sample Preparation in LC-MS Bioanalysis

Edited by

Wenkui Li, Wenying Jian, and Yunlin Fu

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Preface

Sample preparation is a pivotal part of the integral LC-MS bioanalysis, which has been heavily employed in the determination of drugs, drug metabolites, biomarkers, and other molecules of interest in various biological matrices (e.g. fluids or tissues) for decades. It has been playing an important role in a variety of human health-care studies, ranging from drug discovery and development, therapeutic drug monitoring, to biomarker analysis. While highly sophisticated LC-MS systems with better sensitivity and higher bioanalytical throughput have been continuously introduced, challenges that remain unchanged are the sample preparation prior to LC-MS quantitation, for which data quality has direct impact on study conclusion.

The purpose of sample preparation is not only to make the analyte(s) of interest available in sample extracts at an appropriate concentration for MS detection but also to remove interfering matrix elements (e.g. phospholipids and salts) that, if not addressed properly, can alter MS response (e.g. signal suppression). In quantitative LC-MS bioanalysis, clean sample extracts means: (i) better chromatography, (ii) lower limit of quantification, (iii) decreased assay variability (due to reduced matrix effects), (iv) less chance of false-positive/negative results, (v) longer column lifetime, (vi) less instrument downtime, and (vii) minimized costs in manpower and equipment maintenance, etc. In practice, the best sample preparation strategies should always be considered, evaluated, and implemented whenever possible in developing a robust quantitative LC-MS bioanalytical method.

As a companion for the previously published *Handbook of LC-MS Bioanalysis: Best Practice, Experimental Protocols and Regulations* (Li, Zhang, and Tse, 2013, Wiley), the current book is to provide a timely and comprehensive update along with representative experimental protocols on all important sample preparation techniques for quantitative LC-MS bioanalysis of small and large molecules. The 26 chapters of the book are divided into three parts. The first part of the book is focused on not only the basic but also the contemporary sample preparation techniques in LC-MS bioanalysis. These include

Protein Precipitation, Liquid-Liquid Extraction, and Solid-Phase Extraction (Chapter 1), Online Extraction and Column Switching (Chapter 2), Equilibrium Dialysis, Ultracentrifugation, and Ultrafiltration (Chapter 3), Phospholipid Depletion (Chapter 4), Salting-out Assisted Liquid-Liquid Extraction (SALLE) (Chapter 5), Supported Liquid Extraction (SLE) (Chapter 6), Immunocapture (Chapter 7), Microextraction (Chapter 8), Microsampling (Chapter 9), Extraction via Nanomaterials (Chapter 10), Extraction via Molecularly Imprinted Polymers (MIP) (Chapter 11), Stir-bar Sorptive Extraction (Chapter 12), Monolithic Spin Column Extraction (Chapter 13), Aptamer-based Sample Preparation (Chapter 14), and Sample Extraction via Electromembranes (Chapter 15).

In Part II, the current sample preparation techniques for LC-MS bioanalysis of biological sample matrices other than common whole blood, plasma, or serum are discussed in detail along with experimental protocols. These matrices include but are not limited to Tissues, Hair, Nail, Skins, and Bones (Chapter 16), Peripheral Blood Mononuclear Cells (Chapter 17), Urine, Cerebrospinal Fluid, Synovial Fluid, Sweat, Tears, and Aqueous Humor (Chapter 18), and Liposomal Samples (Chapter 19).

Part III of the book is focused on sample preparation for LC-MS bioanalysis of challenging molecules. This part starts with some Key Pre-analytical Considerations in Quantitative LC-MS Bioanalysis (Chapter 20), which is followed by Derivatization strategies for enhancing assay sensitivities in quantitative LC-MS bioanalysis of molecules with poor ionization efficiency (Chapter 21). Sample preparation for quantitative LC-MS bioanalysis of Lipids is captured in Chapter 22. In Chapter 23, detailed instructions and associated stepwise protocols are provided for LC-MS bioanalysis of peptides. Expanding from peptides, detailed instructions of sample preparation for LC-MS bioanalysis of Proteins, Oligonucleotides, and Antibody-drug Conjugates (ADCs) are captured in Chapters 24, 25, and 26, respectively.

Our purpose in committing to this project was to provide scientists in industry, academia, and regulatory

agencies with all “practical tricks” in extracting various analyte(s) of interest from biological samples for LC-MS quantification according to the current health authority regulations and industry practices. In this book we are confident that we have accomplished our goal. The book represents a major undertaking which would not have been possible without the contributions of all the authors and the support of their families. We also wish to thank the terrific editorial staff at John Wiley & Sons and give a special acknowledgment to Michael Leventhal, Manag-

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List of Abbreviations

2D	two-dimensional
3NPH	3-nitrophenylhydrazine
5-FU	5-fluorouracil
5-HETE	5-hydroxyeicosatetraenoic acid
AA	acrylamide
AA	alendronic acid
AAC	α 1-antichymotrypsin
ACE	angiotensin I converting enzyme
ACE	automatic cartridge exchange
ACN	acetonitrile
ADA	anti-drug antibody
ADC	antibody–drug conjugate
ADME	absorption, distribution, metabolism, and excretion
ADP	adenosine diphosphate
ADS	alkyl-diol-silica
AFA	adaptive focused acoustics
AFMC	aptamer-functionalized monolithic column
AFMPC	aptamer-functionalized material-packed column
AFM	aptamer-functionalized material
AFOTCC	aptamer-functionalized open tubular capillary column
AFSC	aptamer-functionalized spin column
AG	2-arachidonoylglycerol
AGP	acid glycoprotein
AIBN	azo(bis) isobutyronitrile
AML	acute myeloid leukemia
AMP	adenosine monophosphate
APA	anti-peptide antibody
APCI	atmospheric pressure chemical ionization
Apt-AC	aptamer-based affinity column
Apt-AuNR	aptamer-functionalized gold nanorod
Apt-MM	aptamer-functionalized magnetic material
Apt-MNP	aptamer-functionalized magnetic nanoparticle
Apt-PANCMMA	aptamer-functionalized poly(acrylonitrile-co-maleic acid)
Apt-PP-fiber	aptamer-based-polypropylene fiber
Apt-SA-SPE	aptamer-based surface affinity solid-phase extraction
Apt-SBSE	aptamer-functionalized stir-bar sorptive extraction
Apt-SPE	aptamer-based solid-phase extraction
Apt-SPME	aptamer-based solid-phase microextraction
ATP	adenosine triphosphate
AUC	area under the curve
AuNP	gold nanoparticle

BAL	bronchoalveolar lavage
BEAD	bead extraction and acid dissociation
BEH	bridged ethylene hybrid
BLQ	below limit of quantification
BNP	B-type natriuretic peptide
BP	bisphosphonate
BP-3	benzophenone-3
BPA	bisphenol A
BSA	bovine serum albumin
BSL-2	biosafety level-2
BSTFA	<i>N,O</i> -bis(trimethylsilyl)trifluoroacetamide
CAD	collision-activated dissociation
Cape	capecitabine
CCSHLLE	counter current salting-out homogenous liquid–liquid extraction
CDA	cytidine deaminase
CDI	carbonyl diimidazole
CDR	complementarity-determining region
CE	capillary electrophoresis
CE	cholestryl oleate
CHAPS	3-([3-cholamidopropyl]dimethylammonio)-1-propanesulfonate
CID	collision-induced dissociation
CIP	chiral imprinted polymer
CNBF	4-chloro-3,5-dinitrobenzotrifluoride
CNS	central nervous system
CNT-PDMS	carbon nanotube–poly(dimethylsiloxane)
CNT	carbon nanotube
COXs	cyclooxygenases
CPT	cell preparation tube
CSF	cerebrospinal fluid
CV	coefficient of variation
CZE-C ⁴ D	capillary zone electrophoresis with capacitively coupled contactless conductivity detection
D	distribution ratio
D2EHPA	di-(2-ethylhexyl)phosphoric acid
DAD	diode array detection
DADPA	diaminodipropylamine
DAG	diacylglycerol (1,3-dilinoleoyl- <i>rac</i> -glycerol)
DAR	drug-to-antibody ratio
DBS	dried blood spot
DCM	dichloromethane
DEHP	di-(2-ethylhexyl) phosphate
DEME	dynamic electromembrane extraction
DEX	dextromethorphan
DI	direct immersion
DIC	diclofenac
DIEA	diisopropylethylamine
DI-SDME	direct immersion single-drop microextraction
DLLE	dispersive liquid–liquid extraction
DLLME	dispersive liquid–liquid microextraction
DMBA	dimethylbutylamine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DNS-Cl	dansyl chloride
DOPA	dihydroxyphenylalanine

DOR	dextrorphan
DP IV	dipeptidyl peptidase IV
DPBS	Dulbecco's phosphate-buffered saline
DPX	disposable pipette extraction
DSEA	dansyl sulfonamide ethyl amine
D-SPE	dispersive solid-phase extraction
DTT	dithiothreitol
DVB	divinylbenzene
DXR	doxorubicin
EA	ethyl acetate
EBF	European Bioanalytical Forum
ECAPCI	electro capture atmospheric pressure chemical ionization
ED	equilibrium dialysis
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDC/NHS	<i>N</i> -(3-dimethylaminopropyl)- <i>N</i> -ethylcarbodiimide hydrochloride/ <i>N</i> -hydroxysuccinimide
EDTA	ethylenediaminetetraacetic acid
EG	ethylene glycol
EGDMA	ethylene glycol dimethacrylate
EHS	ethylhexyl salicylate
ELISA	enzyme-linked immunosorbent assay
EME	electromembrane extraction
EME-DLLME	electromembrane extraction dispersive liquid–liquid microextraction
EME-LDS-USAEME	electromembrane extraction low-density solvent-based ultrasound-assisted emulsification electromembrane microextraction
EM-SPME	electromembrane-surrounded solid-phase microextraction
ENB	1-ethyl-2-nitrobenzene
EPR	enhanced permeation and retention
ESI	electrospray ionization
EtOH	ethanol
FA	fatty acid
FA	formic acid
FBAL	α -fluoro- β -alanine
FBS	fetal bovine serum
Fc	fragment crystallizable region/constant region
FcRn	human neonatal Fc receptor
FDA	Food and Drug Administration
FLD	fluorescence detection
FLM	free liquid membrane
Fmoc-Cl	9-fluorenylmethoxycarbonyl chloride
FNME	fiber-packed needle microextraction
GAC	green analytical chemistry
GC	gas chromatography
GC-FID	gas chromatography–flame ionization detection
GC-MS	gas chromatography–mass spectrometry
GIP	glucose-dependent insulintropic peptide
GLP-1	glucagon-like peptide-1
GMA	glycidylmethacrylate
GnRH	gonadotropin-releasing hormone
GPChos	glycerophosphatidylcholines
GPCs	glycerophosphatidylcholines
GPE	gum-phase extraction
GPI	glycosylphosphatidylinositol
HETP	height equivalent to a theoretical plate
HFIP	1,1,1,3,3,3-hexafluoro-isopropanol

HF-LPME	hollow fiber liquid-phase microextraction
HILIC	hydrophilic interaction liquid chromatography
HIV	human immunodeficiency virus
HMS	homosalate
HND-G	high nitrogen-doped graphene
HNE	human neutrophil elastase
HPIM	homemade polymer inclusion membrane
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HS	headspace
HSSBSE	headspace stir-bar sorptive extraction
HS-SDME	headspace single-drop microextraction
HTLC	high-turbulence liquid chromatography
IACUC	Institutional Animal Care and Use Committee
IAE	immunoaffinity extraction
IAM	iodoacetamide
IA-SPE	immunoaffinity solid-phase extraction
IC	immunocapture
iCAT	isotope-coded affinity tag
ICP-MS	inductively coupled plasma mass spectrometry
ID	internal diameter
IGF	insulin-like growth factor
IgG	immunoglobulin G
IL-21	interleukin-21
IMAC	immobilized metal ion affinity chromatography
IPA	isopropanol
IS	internal standard
ISET	integrated selective enrichment target
IS-MRM	in-source multiple reaction monitoring
ISR	incurred sample reanalysis
ISTD	internal standard
ITMS	ion trap mass spectrometry
iTRAQ	isobaric tags for relative and absolute quantification
IT-SPME	in-tube solid-phase microextraction
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous
IVT	<i>in vitro</i> transcription
IX-SPE	ion exchange-solid-phase extraction
Kb/p	blood to plasma ratio
Ke/p	red blood cell partition coefficient
LBA	ligand-binding assay
LC	liquid chromatography
LC-MS	liquid chromatography–mass spectrometry
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LC-UV/FL	liquid chromatography with ultraviolet/fluorescence detection
LD	liquid desorption
LGPCChos	lysoglycerophosphocholines
LLE	liquid–liquid extraction
LLOQ	lower limit of quantification
LOXs	lipxygenases
LPCs	lyso-phosphatidylcholines
LPME	liquid-phase microextraction
LSC	liquid scintillation counting
MA	methacrylate, methyl acrylate

MA	minodronic acid
MAA	methacrylamide
MAA	methacrylic acid
mAb	monoclonal antibody
MADB	poly(methacrylic acid-3-sulfopropyl ester potassium salt-co-divinylbenzene)
MAG	monoacylglycerol (1-stearyl-rac-1 glycerol)
MALDI	matrix-assisted laser desorption ionization
MAX	mixed-mode anion exchange
MCV	mean cell volume
MCX	mixed-mode cation exchange
MDA-LDL	malondialdehyde-modified low-density lipoprotein
MDMA	3,4-methylenedioxy- <i>N</i> -methylamphetamine
MDS	myelodysplastic syndromes
MeOH	methanol
MEPS	microextraction by packed sorbent
MF	matrix factor
MI-MSPE	molecularly imprinted micro-solid-phase extraction
MIPs	molecularly imprinted polymers
MISPE	molecularly imprinted solid-phase extraction
MISPE-DPE	molecularly imprinted solid-phase extraction with differential pulsed elution
MISPE-PE	molecularly imprinted solid-phase extraction with pulsed elution
MIST	metabolites in safety testing
MIT	molecular imprinting technology
MLLE	micro-liquid-liquid extraction
MMA	methylmalonic acid
MMAE	monomethyl auristatin E
MMST	monolithic molecularly imprinted polymer sol-gel packed tip
MNP	magnetic nanoparticle
MPB	2-bromo-3'-methoxyacetophenone
mPGES-1	microsomal prostaglandin E synthase-1
MPS	3-methacryloyloxypropyltrimethoxysilane
MRM	multiple reaction monitoring
mRNA	messenger RNA
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSP	magnetic supraparticle
MSPD	matrix solid-phase dispersion
MSPE	magnetic solid-phase extraction
MTBE	methyl <i>tert</i> -butyl ether
MTBSTFA	<i>N</i> -(<i>tert</i> -butyldimethylsilyl)- <i>N</i> -methyl trifluoroacetamide
MW	molecular weights
MWCNT	multiwall carbon nanotube
MWCO	molecular weight cutoff
NAaPs	nucleic acid associated proteins
NAb	neutralizing antibody
NA	nucleic acid
NCEs	new chemical entities
NEM	<i>N</i> -ethylmaleimide
NHS	<i>N</i> -hydroxysuccinimide
NK	natural killer
NPOE	2-nitrophenyloctyl ether
NPPE	2-nitrophenyl pentyl ether
NPs	nanoparticles
NSB	nonspecific binding

NSE	neuron-specific enolase
NTproBNP	N-terminal pro-B-natriuretic peptide
OC	octocrylene
OD-PABA	ethylhexyl dimethyl <i>p</i> -aminobenzoate
ODS	octadecyl
OH-PAH	monohydroxylated polycyclic aromatic hydrocarbon
OH-PDMS	hydroxyl polydimethylsiloxane
OTT	open tubular trapping
OxLDL	oxidized low-density lipoprotein
P	partition ratio
PA	phosphatidic acid
PA	polyacrylate
PA-EG	poly(methyl methacrylate/ethyleneglycoldimethacrylate)
Pa-EME	parallel electromembrane extraction
PALME	parallel artificial liquid membrane extraction
PANCMMA	poly(acrylonitrile-co-maleic acid)
PAR	peak area ratio
PBD	pyrrolbenzodiazepine
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline with Tween-20
PCA	perchloric acid
PCB	polychlorinated biphenyl
PCI	protein C inhibitor
PCs	phosphatidylcholines
PD	pharmacodynamics
PD	phospholipid depletion
PDMS	polydimethylsiloxane
PE	phosphoethanolamine
PEG	polyethylene glycol
PEME	pulsed electromembrane extraction
PEO	polyethylene oxide
PE	phosphatidylethanolamine
PFB	pentafluorobenzyl
PG	phosphatidylglycerol
PGs	prostaglandins
PHMB	4-(hydroxymercuri)benzoate
PI	phosphatidylinositol
PK	pharmacokinetics
PK/PD	pharmacokinetic/pharmacodynamic
PK/TK	pharmacokinetic/toxicokinetic
PKU	phenylketonuria
PLs	phospholipids
PMMA	pentamethylated minodronic acid
PMMA	poly(methyl methacrylate)
PMSF	phenylmethylsulfonyl fluoride
poly(GMA-co-EDMA)	poly(glycidyl methacrylate-coethylene dimethacrylate)
PP	polypropylene
PPB	plasma protein binding
PPESK	poly(phthalazine ether sulfone ketone)
PP-fiber	porous polymer-coated fiber
PPT	protein precipitation
PPY	polypyrrole
ProGRP	pro-gastrin releasing peptide

PS	phosphatidylserine
PTFE	polytetrafluorethylene
PTV	programmable temperature vaporize
PU	polyurethane foams
PUFA	polyunsaturated fatty acids
QC	quality control
QTOF	quadrupole time-of-flight
QuEChERS	quick, easy, cheap, effective, rugged, safe extraction method
RA	risedronic acid
RAM	restricted access material
RBC	red blood cell
REC	extraction recovery
RED	rapid equilibrium dialysis
rhTRAIL	recombinant human tumor necrosis factor-related apoptosis-inducing ligand
RISC	RNA-induced silencing complex
ROS	reactive oxygen species
RP	reversed phase
RP-SPE	reversed-phase solid-phase extraction
RPV	rilpivirine
SA-EME	surfactant-assisted electromembrane extraction
SALLE	salting-out assisted liquid–liquid extraction
SAX	strong anion exchange
SBSE	stir-bar sorptive extraction
SCAP	sample card and prep
SCIT	(+)-(S)-citalopram
SCX	strong cation exchange
SDCIT	(+)-(S)-desmethylocitalopram
SDDCIT	(+)-(S)-didesmethylocitalopram
SDF	stromal cell-derived factor
SDME	single-drop microextraction
SDS-PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SDU	solvent delivery unit
sEGFR	soluble epidermal growth factor receptor
SELEX	systematic evolution of ligands by exponential enrichment
SF	synovial fluid
SHBG	sex hormone-binding globulin
SIL	stable isotope labeled
SIL-IS	stable isotopically labeled internal standard
SiNWA	silicon nanowire array
SISCAPA	stable isotope standards and capture by anti-peptide antibodies
SLE	supported liquid extraction
SLM	supported liquid membrane
SM	sphingomyelin
SPDE	solid-phase dynamic extraction
SPE	solid-phase extraction
SPME	solid-phase microextraction
SRM	selected reaction monitoring
SRM	single reaction monitoring
SSH	steroid sex hormone
SWCNT	single-wall carbon nanotube
TAG	triacylglycerol (1,3-dipalmitoyl,2oleoyl-glycerol)
TAHS	<i>p</i> - <i>N,N,N</i> -trimethylammonioanilyl <i>N'</i> -hydroxysuccinimidyl carbamate iodide
TBS	tris-buffered saline
TCA	trichloroacetic acid
TCAFMF	thermally controlled aptamer-functionalized microfluid