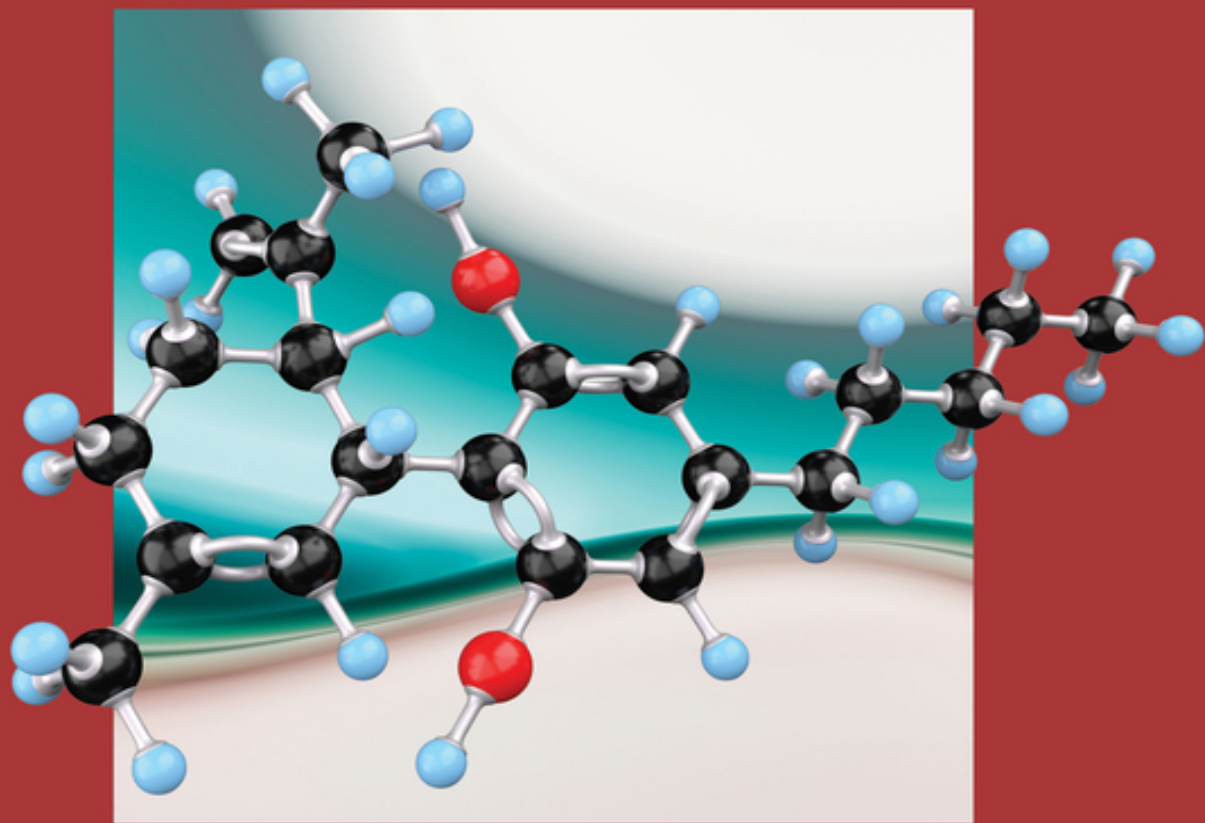


SECOND EDITION



FUNDAMENTALS OF ANALYTICAL TOXICOLOGY

CLINICAL AND FORENSIC

ROBERT J. FLANAGAN
EVA CUYPERS
HANS H. MAURER
ROBIN WHELPTON

WILEY

FUNDAMENTALS OF ANALYTICAL TOXICOLOGY

FUNDAMENTALS OF ANALYTICAL TOXICOLOGY

Clinical and Forensic

Second Edition

Robert J. Flanagan

*King's College Hospital NHS Foundation Trust
UK*

Eva Cuypers

*Maastricht Multimodal Molecular Imaging Institute
Maastricht University
The Netherlands*

Hans H. Maurer

*Department of Experimental and Clinical Toxicology
Saarland University
Germany*

Robin Whelpton

*Formerly School of Biological and Chemical Sciences
Queen Mary University of London
UK*

WILEY

This edition first published 2020
© 2020 John Wiley & Sons, Ltd

Edition History

John Wiley & Sons, Ltd (1e, 2007)

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at <http://www.wiley.com/go/permissions>.

The right of Robert J. Flanagan, Eva Cuypers, Hans H. Maurer and Robin Whelpton to be identified as the authors of this work has been asserted in accordance with law.

Registered Offices

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

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

Editorial Office

The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty

In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. While the publisher and authors have used their best efforts in preparing this work, they make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives, written sales materials or promotional statements for this work. The fact that an organization, website, or product is referred to in this work as a citation and/or potential source of further information does not mean that the publisher and authors endorse the information or services the organization, website, or product may provide or recommendations it may make. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for your situation. You should consult with a specialist where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Cataloging-in-Publication Data

Names: Flanagan, Robert J., author.

Title: Fundamentals of analytical toxicology : clinical and forensic /

Robert J. Flanagan, King's College Hospital NHS Found Trust, UK,

Eva Cuypers, Maastricht University, The Netherlands, Hans H. Maurer,

Department of Experimental and Clinical Toxicology, Saarland University,

Germany, Robin Whelpton, Formerly School of Biological and Chemical

Sciences, Queen Mary University of London, UK.

Description: Second edition. | Hoboken, NJ : Wiley, 2020. | Includes bibliographical references and index.

Identifiers: LCCN 2020007512 (print) | LCCN 2020007513 (ebook) | ISBN

9781119122340 (cloth) | ISBN 9781119122364 (adobe pdf) | ISBN

9781119122371 (epub)

Subjects: LCSH: Analytical toxicology.

Classification: LCC RA1221 .F86 2020 (print) | LCC RA1221 (ebook) | DDC

615.9/07-dc23

LC record available at <https://lccn.loc.gov/2020007512>

LC ebook record available at <https://lccn.loc.gov/2020007513>

Cover Design: Wiley

Cover Images: © Xuanyu Han/Getty Images,

© Alexander Limbach/Shutterstock

Set in 10/12pt, TimesLTStd by SPi Global, Chennai, India.

Contents

Preface	xxiii
Health and Safety	xxv
Nomenclature, Symbols, and Conventions	xxvii
Uniform Resource Locators	xxix
Amount Concentration and Mass Concentration	xxxii
Acknowledgements	xxxiii
List of Abbreviations	xxxv
SECTION A THE BASICS	1
1 Analytical Toxicology: Overview	3
1.1 Introduction	3
1.2 Modern analytical toxicology	4
1.2.1 Analytical methods	5
1.2.2 Systematic toxicological analysis	6
1.2.3 Ethanol and other volatile substances	8
1.2.4 Trace elements and toxic metals	8
1.3 Provision of analytical toxicology services	10
1.3.1 Samples and sampling	10
1.3.2 Choice of analytical method	10
1.3.3 Method validation and implementation	11
1.3.4 Quality control and quality assessment	13
1.4 Applications of analytical toxicology	15
1.4.1 Clinical toxicology	15
1.4.2 Forensic toxicology	17
1.4.3 Testing for substance misuse	18
1.4.4 Therapeutic drug monitoring	19
1.4.5 Occupational and environmental toxicology	20
1.5 Summary	21
References	21
2 Sample Collection, Transport, and Storage	23
2.1 Introduction	23
2.2 Clinical samples and sampling	23
2.2.1 Health and safety	23
2.2.2 Clinical sample types	25
2.2.3 Blood and blood fractions	25
2.2.3.1 Arterial blood	25
2.2.3.2 Venous blood	25

2.2.3.3	Serum	28
2.2.3.4	Plasma	28
2.2.3.5	Blood cells	29
2.2.3.6	Dried blood spots	30
2.2.3.7	Volumetric microsampling	30
2.2.4	Urine	31
2.2.5	Stomach contents	31
2.2.6	Faeces	32
2.2.7	Tissues	32
2.3	Guidelines for sample collection for analytical toxicology	32
2.3.1	Sample collection and preservation	35
2.3.2	Blood	36
2.3.2.1	Collection of blood post-mortem	37
2.3.3	Urine	37
2.3.4	Stomach contents	39
2.3.5	Oral fluid	40
2.3.6	Sweat	40
2.3.7	Exhaled air	40
2.3.8	Cerebrospinal fluid	41
2.3.9	Vitreous humour	41
2.3.10	Synovial fluid	41
2.3.11	Pericardial fluid	41
2.3.12	Intraosseous fluid	41
2.3.13	Liver	42
2.3.14	Bile	42
2.3.15	Other tissues	42
2.3.16	Insect larvae	42
2.3.17	Keratinaceous tissues (hair and nail)	43
2.3.18	Bone	44
2.3.19	Injection sites	44
2.3.20	Scene residues	45
2.4	Sample transport, storage, and disposal	45
2.5	Common interferences	47
2.6	Summary	48
	References	48
3	Basic Laboratory Operations	52
3.1	Introduction	52
3.1.1	Reagents and standard solutions	53
3.1.2	Reference compounds	55
3.1.3	Preparation and storage of calibration solutions	56
3.2	Aspects of quantitative analysis	58
3.2.1	Analytical error	58
3.2.1.1	Confidence intervals	59
3.2.2	Minimizing random errors	60
3.2.2.1	Preparation of a solution of known concentration	60
3.2.3	Accuracy and precision	61
3.2.3.1	Assessing precision and accuracy	61

3.2.3.2	Detecting systematic error (fixed bias)	62
3.2.3.3	Identifying sources of variation: analysis of variance	62
3.2.3.4	Measurement uncertainty	64
3.2.4	Calibration graphs	64
3.2.4.1	Linear regression	65
3.2.4.2	Testing for linearity	66
3.2.4.3	Weighted linear regression	67
3.2.4.4	Non-linear calibration	68
3.2.4.5	Residuals and standardized residuals	69
3.2.4.6	Blank samples and the intercept	70
3.2.4.7	Method of standard additions	70
3.2.4.8	Limits of detection and quantitation	71
3.2.4.9	Curve fitting and choice of equation	71
3.2.4.10	Single-point calibration	72
3.2.5	Batch analyses	72
3.2.6	Random access analysis	73
3.3	Use of internal standards	74
3.3.1	Advantages of internal standardization	74
3.3.1.1	Reproducibility of injection volume	74
3.3.1.2	Instability of the detection system	75
3.3.1.3	Pipetting errors and evaporation of extraction solvent	75
3.3.1.4	Extraction efficiency	75
3.3.1.5	Derivatization and non-stoichiometric reactions	76
3.3.2	Internal standard availability	76
3.3.3	External calibration	76
3.3.4	Potential disadvantages of internal standardization	77
3.3.5	Quantity of internal standard added	78
3.4	Method comparison	78
3.4.1	Bland–Altman plots	79
3.5	Non-parametric statistics	80
3.5.1	Sign tests	81
3.5.1.1	Wilcoxon signed rank test	81
3.5.2	Runs test	82
3.5.3	Mann–Whitney U-test	82
3.5.4	Spearman rank correlation	83
3.5.5	Non-parametric regression	83
3.6	Quality control and quality assessment	84
3.6.1	Quality control charts	85
3.6.1.1	Shewhart charts	85
3.6.1.2	Cusum charts	85
3.6.1.3	J-chart	86
3.6.2	External quality assessment	86
3.6.3	Toxicology external quality assessment schemes	87
3.7	Operational considerations	89
3.7.1	Staff training	89
3.7.2	Recording and reporting results	90
3.8	Summary	91
	References	91

4	Aspects of Sample Preparation	94
4.1	Introduction	94
4.2	Modes of sample preparation	97
4.2.1	Protein precipitation	97
4.2.2	Liquid–liquid extraction	98
4.2.2.1	pH-Controlled liquid–liquid extraction	102
4.2.2.2	Ion-pair extraction	103
4.2.2.3	Supported liquid extraction	103
4.2.2.4	Immobilized coating extraction	104
4.2.3	Solid phase extraction	104
4.2.3.1	Types of stationary phase	106
4.2.4	Solid phase microextraction	108
4.2.5	Liquid phase microextraction	110
4.2.6	Supercritical fluid extraction	111
4.2.7	Accelerated solvent extraction	112
4.3	Plasma protein binding	112
4.3.1	Ultrafiltration	113
4.3.2	Equilibrium dialysis	114
4.4	Hydrolysis of conjugated metabolites	115
4.5	Extraction of drugs from tissues	117
4.6	Summary	117
	References	118
5	Colour Tests, and Spectrophotometric and Luminescence Techniques	120
5.1	Introduction	120
5.2	Colour tests in toxicology	120
5.3	Colour tests for pharmaceuticals and illicit drugs	122
5.4	UV/Visible spectrophotometry	123
5.4.1	The Beer–Lambert law	123
5.4.2	Instrumentation	126
5.4.2.1	Derivative spectrophotometry	129
5.4.3	Spectrophotometric assays	130
5.4.3.1	Salicylates in plasma or urine	131
5.4.3.2	Carboxyhaemoglobin in whole blood	131
5.4.3.3	Cyanide in whole blood by microdiffusion	132
5.5	Fluorescence and phosphorescence	134
5.5.1	Intensity of fluorescence and quantum yield	135
5.5.2	Instrumentation	136
5.5.3	Fluorescence assays	137
5.5.3.1	Fluorescence measurement of quinine	137
5.6	Chemiluminescence	138
5.6.1	Instrumentation	140
5.6.2	Chemiluminescence assays	140
5.7	Infrared and Raman spectroscopy	141
5.7.1	Instrumentation	141
5.7.2	Applications	142
5.8	Summary	143
	References	143

6	Immunoassays and Related Assays	145
6.1	Introduction	145
6.2	Basic principles of competitive binding assays	145
6.2.1	Antibody formation	146
6.2.2	Selectivity	147
6.2.3	Performing the assay	148
6.2.3.1	Classical radioimmunoassay	148
6.2.3.2	Modern radioimmunoassay	149
6.2.4	Non-isotopic immunoassay	150
6.2.5	Assay sensitivity and selectivity	150
6.2.6	Immunoassay development	151
6.3	Heterogeneous immunoassays	151
6.3.1	Tetramethylbenzidine reporter system	151
6.3.2	Antigen-labelled competitive ELISA	152
6.3.3	Antibody-labelled competitive ELISA	152
6.3.4	Sandwich ELISA	153
6.3.5	Lateral flow competitive ELISA	154
6.3.6	Chemiluminescent immunoassay	154
6.4	Homogenous immunoassays	155
6.4.1	Enzyme-multiplied immunoassay technique	155
6.4.2	Fluorescence polarization immunoassay (FPIA)	156
6.4.3	Cloned enzyme donor immunoassay	158
6.5	Microparticulate and turbidimetric immunoassays	159
6.5.1	Microparticle enzyme immunoassay (MEIA)	159
6.5.2	Chemiluminescent magnetic immunoassay (CMIA)	160
6.6	Assay calibration, quality control, and quality assessment	160
6.6.1	Immunoassay calibration	160
6.6.2	Drug screening	161
6.7	Interferences and assay failures	162
6.7.1	Measurement of plasma digoxin after F _{ab} antibody fragment administration	163
6.8	Aptamer-based assays	163
6.9	Enzyme-based assays	163
6.9.1	Paracetamol	163
6.9.2	Ethanol	164
6.9.3	Anticholinesterases	165
6.10	Summary	165
	References	166
SECTION B	SEPARATION SCIENCE	167
7	Separation Science: Theoretical Aspects	169
7.1	General introduction	169
7.2	Theoretical aspects of chromatography	170
7.2.1	Analyte phase distribution	170
7.2.2	Column efficiency	171
7.2.3	Zone broadening	173
7.2.3.1	Multiple path and eddy diffusion	173

7.2.3.2	Longitudinal diffusion	173
7.2.3.3	Resistance to mass transfer	173
7.2.4	Kinetic plots	175
7.2.5	Extra-column contributions to zone broadening	176
7.2.6	Temperature programming and gradient elution	176
7.2.7	Selectivity	177
7.2.8	Peak asymmetry	178
7.3	Measurement of analyte retention	179
7.3.1	Planar chromatography	179
7.3.2	Elution chromatography	180
7.4	Summary	181
	References	181
8	Planar Chromatography	182
8.1	Introduction	182
8.2	Qualitative thin-layer chromatography	183
8.2.1	Thin-layer plates	183
8.2.2	Sample application	184
8.2.3	Developing the chromatogram	185
8.2.4	Visualizing the chromatogram	186
8.2.5	Interpretation of thin-layer chromatograms	189
8.2.6	TIAFT-DFG R_f data compilation	190
8.2.7	Toxi-Lab	190
8.3	Quantitative thin-layer chromatography	190
8.3.1	Forced-flow planar chromatography	190
8.3.2	Quantitative high-performance thin-layer chromatography	191
8.4	Summary	192
	References	192
9	Gas Chromatography	193
9.1	Introduction	193
9.2	Instrumentation	194
9.2.1	Injectors and injection technique	195
9.2.1.1	Cryofocusing/thermal desorption	196
9.2.2	Detectors for gas chromatography	198
9.2.2.1	Thermal conductivity detection	198
9.2.2.2	Flame ionization detection	198
9.2.2.3	Nitrogen/phosphorus detection	199
9.2.2.4	Electron capture detection	200
9.2.2.5	Pulsed discharge detection	200
9.2.2.6	Flame photometric detection	202
9.2.2.7	Atomic emission detection	202
9.2.2.8	Chemiluminescent nitrogen detection	202
9.2.2.9	Fourier transform infra-red detection	202
9.2.2.10	Vacuum UV detection	203
9.3	Columns and column packings	203
9.3.1	Stationary phases	204

9.3.2	Packed columns	206
9.3.3	Capillary columns	206
9.3.4	Multidimensional gas chromatography	209
9.4	Headspace and 'purge and trap' analysis	210
9.5	Formation of artefacts in gas chromatography	213
9.6	Derivatization for gas chromatography	213
9.7	Chiral separations	217
9.8	Summary	219
	References	220
10	Liquid Chromatography	223
10.1	Introduction	223
10.2	General considerations	224
10.2.1	The column	225
10.2.2	Column configuration	226
10.2.3	Column oven	227
10.2.4	The eluent	227
10.2.5	The pump	228
10.2.6	Sample introduction	230
10.2.7	System operation	231
10.3	Detection in liquid chromatography	232
10.3.1	UV/Visible absorption detection	232
10.3.2	Fluorescence detection	233
10.3.3	Chemiluminescence detection	234
10.3.4	Electrochemical detection	235
10.3.5	Chemiluminescent nitrogen detection	235
10.3.6	Aerosol-based detectors	236
10.3.6.1	Evaporative light scattering detection	236
10.3.6.2	Condensation nucleation light scattering detection	237
10.3.6.3	Charged aerosol detection	237
10.3.7	Radioactivity detection	237
10.3.8	Chiral detection	238
10.3.9	Post-column modification	239
10.3.10	Immunoassay detection	239
10.4	Columns and column packings	240
10.4.1	Column packings	240
10.4.1.1	Chemical modification of silica	241
10.4.1.2	Bonded-phase selection	242
10.4.1.3	Stability of silica packings	243
10.4.1.4	Monolithic columns	243
10.4.1.5	Surface porous particles	244
10.4.1.6	Hybrid particle columns	244
10.4.1.7	Restricted access media	244
10.5	Modes of liquid chromatography	245
10.5.1	Normal-phase chromatography	245
10.5.2	Hydrophilic interaction liquid chromatography	245
10.5.3	Reverse-phase chromatography	246

10.5.4	Ion-exchange chromatography	246
10.5.5	Ion-pair chromatography	247
10.5.6	Affinity chromatography	247
10.5.7	Size exclusion chromatography	248
10.5.8	Semi-preparative and preparative chromatography	249
10.6	Chiral separations	250
10.6.1	Chiral stationary phases	251
10.6.1.1	Amylose and cellulose polymers	251
10.6.1.2	Crown ethers	251
10.6.1.3	Cyclodextrins	251
10.6.1.4	Ligand exchange chromatography	252
10.6.1.5	Macrocyclic glycopeptides	252
10.6.1.6	Pirkle brush-type phases	252
10.6.1.7	Protein-based phases	254
10.6.2	Chiral eluent additives	254
10.7	Derivatives for liquid chromatography	255
10.7.1	Fluorescent derivatives	255
10.7.2	Chiral derivatives	255
10.8	Use of liquid chromatography in analytical toxicology	256
10.8.1	Acidic and neutral compounds	258
10.8.2	Basic drugs and quaternary ammonium compounds	259
10.8.2.1	Non-aqueous ionic eluent systems	260
10.8.3	Chiral analysis	260
10.9	Summary	261
	References	262
11	Supercritical Fluid Chromatography	264
11.1	Introduction	264
11.2	General considerations	267
11.2.1	The pump	268
11.2.2	The eluent	269
11.3	Detection in supercritical fluid chromatography	269
11.4	Columns and column packings	269
11.5	Chiral separations	270
11.6	Toxicological and forensic applications	272
11.7	Summary	273
	References	273
12	Capillary Electrophoretic Techniques	275
12.1	Introduction	275
12.2	Theoretical aspects	276
12.2.1	Electrophoretic mobility	276
12.2.2	Efficiency and zone broadening	278
12.2.3	Joule heating	278
12.2.4	Electrodispersion	279
12.2.5	Adsorption of analyte onto the capillary wall	279
12.2.6	Reproducibility of migration time	279

12.3	Sample injection in capillary electrophoresis	280
12.3.1	Hydrodynamic injection	280
12.3.2	Electrokinetic injection	281
12.3.3	Sample 'stacking'	281
12.4	Detection in capillary electrophoresis	281
12.5	Other capillary electrokinetic modes	282
12.5.1	Micellar electrokinetic capillary chromatography	283
12.5.2	Capillary electrochromatography	283
12.6	Capillary electrophoretic techniques in analytical toxicology	285
12.6.1	Chiral separations	285
12.7	Summary	287
	References	287
13	Mass Spectrometry	289
13.1	Introduction	289
13.2	Instrumentation	291
13.2.1	Sector instruments	292
13.2.2	Quadrupole instruments	293
13.2.3	Ion trap quadrupole instruments	294
13.2.4	Controlled fragmentation	295
13.2.5	Quadrupole ion trap	296
13.2.6	Time-of-flight instruments	296
13.2.7	Ion cyclotron resonance	298
13.2.8	Orbitrap mass analyzer	298
13.3	Gas chromatography-mass spectrometry	299
13.3.1	Analyte ionization in gas chromatography-mass spectrometry	299
13.3.1.1	Electron ionization	299
13.3.1.2	Chemical ionization	301
13.3.2	Gas chromatography-combustion-isotope ratio mass spectrometry	303
13.4	Liquid chromatography-mass spectrometry	304
13.4.1	Analyte ionization in liquid chromatography-mass spectrometry	304
13.4.1.1	Electrospray and ionspray ionization	306
13.4.1.2	Atmospheric pressure chemical ionization	307
13.4.1.3	Atmospheric pressure photoionization	308
13.5	Supercritical fluid chromatography-mass spectrometry	308
13.6	Capillary electrophoresis-mass spectrometry	309
13.7	Direct introduction mass spectrometry	310
13.7.1	Flow injection analysis-mass spectrometry	310
13.7.2	High-performance thin-layer chromatography-mass spectrometry	311
13.7.2.1	Elution-based approaches	312
13.7.2.2	Desorption-based approaches	312
13.7.3	Desorption electrospray ionization mass spectrometry	313
13.7.4	Paperspray ionization-mass spectrometry	313
13.7.5	Laser diode thermal desorption mass spectrometry	314
13.7.6	Matrix assisted laser desorption ionization mass spectrometry	314
13.8	Presentation of mass spectral data	315
13.9	Interpretation of mass spectra	317

13.10	Quantitative mass spectrometry	320
13.10.1	Stable isotope-labelled internal standards	321
13.10.2	Assay calibration	322
13.10.3	Isotopic internal calibration	323
13.11	Mass spectrometry imaging	324
13.12	Summary	325
	References	325
14	Ion Mobility Spectrometry	329
14.1	Introduction	329
14.1.1	Interactions with buffer gas	330
14.2	Theoretical aspects	331
14.3	Types of ion mobility spectrometry	332
14.3.1	Drift time ion mobility spectroscopy	333
14.3.2	High field asymmetric waveform ion mobility spectroscopy	333
14.3.3	Travelling wave ion mobility spectrometry	334
14.3.4	Trapped ion mobility spectrometry	335
14.4	Resolving power	336
14.5	Interfacing ion mobility spectrometry	336
14.5.1	Selected ion flow tube mass spectrometry	338
14.6	Applications of ion mobility spectrometry in analytical toxicology	339
14.6.1	Direct analysis	339
14.6.2	Interfaced techniques	340
14.6.3	Chiral separations	341
14.7	Summary	342
	References	342
SECTION C	ESSENTIAL PHARMACOKINETICS	345
15	Absorption, Distribution, Metabolism, and Excretion of Xenobiotics	347
15.1	Introduction	347
15.2	Movement of drugs and other xenobiotics around the body	347
15.2.1	Passive diffusion	348
15.2.1.1	pH-Partition relationship	348
15.2.1.2	Other physiochemical properties	349
15.2.2	Carrier-mediated transport	349
15.3	Routes of administration	351
15.3.1	Oral dosage	351
15.3.1.1	Pre-systemic metabolism	353
15.3.2	Intravenous injection	354
15.3.3	Intramuscular and subcutaneous injection	354
15.3.4	Sublingual and rectal administration	354
15.3.5	Intranasal administration	354
15.3.6	Transdermal administration	354
15.3.7	Inhalation	355
15.3.8	Other routes of administration	355
15.4	Distribution	355
15.4.1	Ion-trapping	356

15.4.2	Binding to macromolecules	356
15.4.2.1	Plasma protein binding	356
15.4.3	Carrier-mediated transport	357
15.5	Metabolism	357
15.5.1	Phase 1 metabolism	358
15.5.1.1	The cytochrome P450 family	358
15.5.1.2	Other phase 1 oxidases	359
15.5.1.3	Enzymatic reductions	360
15.5.1.4	Hydrolysis	361
15.5.2	Phase 2 reactions	361
15.5.2.1	D-Glucuronidation	361
15.5.2.2	O-Sulfation and N-acetylation	362
15.5.2.3	O-, N- and S-Methylation	363
15.5.2.4	Conjugation with glutathione	363
15.5.2.5	Amino acid conjugation	364
15.5.3	Stereoselective metabolism	364
15.5.4	Metabolic reactions of toxicological importance	365
15.5.4.1	Oxidative dealkylation	365
15.5.4.2	Hydroxylation	366
15.5.4.3	S- and N-oxidation	367
15.5.4.4	Oxidative dehalogenation	368
15.5.4.5	Desulfuration	369
15.5.4.6	Trans-sulfuration and trans-esterification	370
15.5.5	Enzyme induction and inhibition	370
15.5.5.1	Enzyme induction	370
15.5.5.2	Enzyme inhibition	371
15.6	Excretion	371
15.6.1	The kidney	372
15.6.1.1	Tubular secretion	372
15.6.1.2	Renal excretion of metabolites	372
15.6.2	Biliary excretion	373
15.6.2.1	Recycling of xenobiotics	373
15.7	Pharmacogenetics and pharmacogenomics	373
15.7.1	Cytochrome P450	374
15.7.2	Atypical pseudocholinesterase	375
15.7.3	Alcohol dehydrogenase and aldehyde dehydrogenase	375
15.7.4	Thiopurine methyltransferase	376
15.7.5	N-Acetyltransferase	376
15.7.6	UDP-Glucuronosyltransferases	376
15.8	Summary	376
	References	377
16	Pharmacokinetics	379
16.1	Introduction	379
16.2	Fundamental concepts	379
16.2.1	Rates, rate constants, and orders of reaction	379
16.2.1.1	First-order elimination	380
16.2.1.2	Zero-order elimination	380

16.2.2	Dependence of plasma half-life on volume of distribution and clearance	381
16.2.2.1	Apparent volume of distribution	381
16.2.2.2	Clearance	381
16.3	Absorption and elimination	382
16.3.1	First-order absorption	382
16.3.2	Quantification of F	383
16.3.3	Maximum concentration (C_{\max})	383
16.4	Drug accumulation	384
16.4.1	Intravenous infusion	384
16.4.1.1	Loading doses	384
16.4.2	Multiple dosage	385
16.5	Sustained-release preparations	386
16.6	Non-linear pharmacokinetics	387
16.6.1	Example of non-linear kinetics in overdose	389
16.7	Multi-compartment models	390
16.7.1	Calculation of rate constants and volumes of distribution	391
16.7.2	Multiple-compartment models in analytical toxicology	391
16.8	Non-compartmental methods	392
16.9	Factors affecting pharmacokinetic parameters	393
16.9.1	Gastrointestinal contents and gastrointestinal motility	393
16.9.2	Age	394
16.9.2.1	Effect of age on renal function	395
16.9.3	Sex	395
16.10	Disease	396
16.11	Pharmacokinetics and the interpretation of results	397
16.11.1	Back-calculation of dose or time of dose	397
16.11.1.1	How much substance was administered?	398
16.11.1.2	When was the substance administered?	399
16.11.1.3	Prediction of ethanol concentrations	399
16.12	Summary	402
	References	402
SECTION D ANALYTICAL TOXICOLOGY		405
17	Toxicology Testing at the Point of Contact	407
17.1	Introduction	407
17.2	Use of point of contact testing	408
17.2.1	Samples and sample collection	409
17.3	Toxicology testing at the point of contact	412
17.3.1	Ethanol	412
17.3.1.1	Breath ethanol	412
17.3.1.2	Oral fluid ethanol	412
17.3.2	Substance misuse	413
17.3.2.1	Oral fluid testing	414
17.3.2.2	Autopsy specimens	417

17.4	Interferences and adulterants	418
17.5	Quality assessment	419
17.6	Summary	419
	References	419
18	Laboratory Testing for Substance Misuse	422
18.1	Introduction	422
18.1.1	Matrix and sampling	423
18.1.1.1	Urine	423
18.1.1.2	Oral fluid	424
18.1.1.3	Hair	424
18.1.1.4	Blood	424
18.1.1.5	Exhaled air	424
18.1.1.6	Sweat	424
18.1.2	'Cut-off' concentrations	425
18.2	Urine testing	425
18.2.1	Sample adulteration	426
18.2.2	Analytical methods	428
18.2.2.1	Immunoassay	428
18.2.2.2	Chromatographic methods	430
18.2.2.3	Assay calibration and acceptance criteria	430
18.2.3	'Cut-off' concentrations	431
18.3	Oral fluid testing	433
18.3.1	Sample collection and storage	433
18.3.1.1	Oral fluid collection devices	433
18.3.2	Road-side testing procedures and 'cut-off' concentrations	434
18.3.3	Analytical methods	435
18.3.4	'Cut-off' concentrations	436
18.4	Blood testing	437
18.4.1	Legislative limits drugs and driving	437
18.4.2	Analytical methods	438
18.5	Hair testing	438
18.5.1	Surface contamination	438
18.5.2	Analytical methods	440
18.5.3	Assay calibration and quality assessment	441
18.5.4	'Cut-off' concentrations	442
18.5.5	Ethanol markers	443
18.5.5.1	Ethyl glucuronide	444
18.5.5.2	Fatty acid ethyl esters	444
18.5.6	Children	444
18.6	Breath testing	445
18.6.1	Collection devices	446
18.7	Sweat testing	446
18.8	Summary	446
	References	447

19	General Analytical Toxicology	452
19.1	Introduction	452
19.2	Gas chromatography	453
19.2.1	Qualitative analyses	453
19.2.2	Quantitative analyses	454
19.2.2.1	Ethanol and other volatiles	454
19.2.2.2	Carbon monoxide and cyanide	455
19.3	Gas chromatography-mass spectrometry	456
19.3.1	Qualitative analysis	457
19.3.1.1	Targeted analysis	457
19.3.1.2	Systematic toxicological analysis	459
19.3.2	Quantitative analysis	462
19.4	Liquid chromatography	464
19.4.1	Qualitative analysis	464
19.5	Liquid chromatography-mass spectrometry	464
19.5.1	Qualitative analysis	468
19.5.1.1	Targeted analysis	468
19.5.1.2	Systematic toxicological analysis	468
19.5.2	Quantitative analysis	468
19.5.2.1	Batch analysis	468
19.5.2.2	Emergency toxicology	469
19.6	Liquid chromatography-high resolution mass spectrometry	469
19.6.1	Qualitative analysis	471
19.6.1.1	Targeted analysis	471
19.6.1.2	Systematic toxicological analysis	471
19.6.2	Quantitative analysis	473
19.7	Summary	473
	References	475
20	Therapeutic Drug Monitoring	479
20.1	Introduction	479
20.2	Sample collection	480
20.3	Sample types	481
20.3.1	Blood and blood fractions	481
20.3.1.1	Dried blood spots	482
20.3.1.2	Volumetric microsampling devices	482
20.3.2	Urine	482
20.3.3	Oral fluid	483
20.3.4	Keratinaceous samples	483
20.3.5	Other alternative matrices	483
20.4	Analytical methods	483
20.5	Factors affecting interpretation of results	485
20.6	Gazetteer	486
20.6.1	Antiasthmatics	486
20.6.2	Anticoagulants	487
20.6.3	Antiepileptic drugs	488

20.6.4	Anti-infectives	488
	20.6.4.1 Antibiotics	488
	20.6.4.2 Antifungal drugs	490
	20.6.4.3 Antimalarials	490
	20.6.4.4 Antiretroviral drugs	492
20.6.5	Anti-inflammatory drugs	492
	20.6.5.1 Therapeutic antibodies	493
20.6.6	Antineoplastic drugs	493
	20.6.6.1 Chemotherapeutic agents	493
	20.6.6.2 Protein kinase inhibitors	493
	20.6.6.3 Therapeutic antibodies	495
20.6.7	Cardioactive drugs	495
	20.6.7.1 Digoxin	495
	20.6.7.2 Other cardioactive drugs	495
20.6.8	Immunosuppressants	495
20.6.9	Psychoactive drugs	497
	20.6.9.1 Lithium	497
	20.6.9.2 Antidepressants	497
	20.6.9.3 Antipsychotics	499
20.7	Summary	499
	References	499
21	Trace Elements and Toxic Metals	505
21.1	Introduction	505
21.2	Sample collection and storage	505
21.3	Sample preparation	507
	21.3.1 Analysis of tissues	508
	21.3.2 Analyte enrichment	509
21.4	Atomic spectrometry	509
	21.4.1 General principles of optical emission spectroscopy	509
	21.4.2 Atomic absorption spectrometry	510
	21.4.2.1 Flame atomization	511
	21.4.2.2 Electrothermal atomization	512
	21.4.2.3 Sources of error	512
	21.4.3 Atomic emission and atomic fluorescence spectrometry	514
	21.4.3.1 Optical emission spectrometry	514
	21.4.3.2 Atomic fluorescence spectrometry	515
	21.4.4 Inductively coupled plasma-mass spectrometry	516
	21.4.4.1 Ion sources	516
	21.4.4.2 Mass analyzers	516
	21.4.4.3 Interferences	516
	21.4.5 Vapour generation approaches	517
	21.4.5.1 Hydride generation	517
	21.4.5.2 Mercury vapour generation	518
	21.4.6 X-Ray fluorescence	519
21.5	Colorimetry and fluorimetry	520

21.6	Electrochemical methods	521
21.6.1	Anodic stripping voltammetry	521
21.6.2	Ion-selective electrodes	522
21.7	Catalytic methods	523
21.8	Neutron activation analysis	523
21.9	Chromatographic methods	524
21.9.1	Chromatography	524
21.9.2	Speciation	524
21.10	Quality assessment	525
21.11	Summary	525
	References	525
22	Clinical Interpretation of Analytical Results	527
22.1	Introduction	527
22.2	Clinical toxicology	529
22.2.1	Pharmacokinetics and the interpretation of results	531
22.3	Forensic toxicology	533
22.3.1	Drug-facilitated assault	533
22.3.2	Fabricated illness	534
22.3.3	Post-mortem toxicology	534
22.4	Gazetteer	539
22.4.1	Alcohols	540
22.4.1.1	Ethanol	540
22.4.1.2	Ethylene glycol and methanol	541
22.4.2	Anabolic steroids	542
22.4.3	Antidepressants	544
22.4.4	Antidiabetic drugs	544
22.4.4.1	Insulin and C-peptide	544
22.4.4.2	Insulin analogues	546
22.4.4.3	LC-MS of insulin and insulin analogues	546
22.4.5	Antiepileptics	547
22.4.6	Antipsychotics	547
22.4.7	Barbiturates	547
22.4.8	Benzodiazepines	548
22.4.9	Carbon monoxide	548
22.4.10	Cannabinoids	549
22.4.10.1	Synthetic cannabinoids	550
22.4.11	Cardioactive drugs	553
22.4.12	Diuretics and laxatives	553
22.4.13	Hallucinogens	554
22.4.13.1	<i>N</i> -Benzylphenethylamines	554
22.4.13.2	2,5-Dimethoxy-substituted phenethylamines	555
22.4.13.3	Mescaline and psilocybin	555
22.4.14	γ -Hydroxybutyrate and γ -butyrolactone	555

22.4.15	Inorganic anions	556
22.4.15.1	Azide	556
22.4.15.2	Cyanide and thiocyanate	556
22.4.15.3	Nitrite	557
22.4.15.4	Phosphide	557
22.4.15.5	Sulfide	557
22.4.16	Ketamine	557
22.4.17	Non-opioid analgesics	558
22.4.18	Opioids	559
22.4.18.1	Buprenorphine	560
22.4.18.2	Codeine/codeine analogues	560
22.4.18.3	Diamorphine/morphine	560
22.4.18.4	Fentanyl	562
22.4.18.5	Methadone	562
22.4.18.6	Novel synthetic opioids	562
22.4.18.7	Tramadol	567
22.4.19	Organophosphorus compounds	568
22.4.20	Phosphodiesterase 5 inhibitors	569
22.4.21	Stimulants and related compounds	569
22.4.21.1	Amphetamine and metamphetamine	569
22.4.21.2	Cocaine	570
22.4.21.3	MDMA and related compounds	571
22.4.22	Trace elements/toxic metals	572
22.4.23	Volatile substances	572
22.5	Sources of further information	574
22.6	Summary	576
	References	576
	Index	587

Preface

The analytical toxicologist may be required to detect, identify, and in many cases measure a wide variety of compounds in samples from almost any component of the body or in related materials such as residues in syringes or in soil. Many difficulties may be encountered. The analytes may include gases such as carbon monoxide, drugs, solvents, pesticides, metal salts, and naturally-occurring toxins. Some poisons may be individual chemicals and others complex mixtures. New drugs, pesticides, and other substances continually present novel challenges in analysis and in the interpretation of the results of the analysis. The analyte might be an endogenous compound such as acetone, or an exogenous compound such as a drug and/or metabolite(s) of the drug, whilst the sample matrix may range from urine to bone.

Many biological samples contain muscle, connective tissue, and so forth, which may have to be separated or degraded prior to an analysis, as well as a multitude of small and large molecular weight compounds. The concentration of the analyte to be measured can range from g L^{-1} (parts per thousand) in the case of blood ethanol to $\mu\text{g L}^{-1}$ (parts per thousand million) in the case of plasma digoxin, and even ng L^{-1} (parts per million million) in the case of the potent opioid carfentanil. The stability of the analytes in biological samples also varies considerably, ranging from a few minutes for protease sensitive peptides and esters such as aspirin and diamorphine, to several years for some other drugs and pesticides.

This book aims to give principles and practical information on the analysis of drugs, poisons and other relevant analytes in biological and related specimens, particularly clinical and forensic specimens, i.e. it is a 'toolkit' in modern parlance. As such, this volume extends the scope of the World Health Organization (WHO) basic analytical toxicology manual¹ and builds on the success of the first edition of this work that appeared in 2007.² Moreover, it is intended to complement Dr Randall Baselt's *Disposition of Toxic Drugs and Chemicals in Man* (Edition 12. Seal Beach: Biomedical Publications, 2020), which remains the seminal reference work as regards the interpretation of analytical toxicology data.

A major difficulty in writing any textbook is deciding on the order of presentation. Having taken account not only of reviewer comments on the first edition, but also of the advances in analytical methods on the one hand, and the range of analyses that may now be required on the other, the material has been updated, expanded and presented in a new order. However, much of the discussion of the historical development of analytical toxicology present in the first edition has been removed to save space. On the other hand, some discussion of more traditional methods such as thin-layer chromatography has been retained for the simple reason that such methods are still used in many parts of the world.

After providing some background information, Section A outlines basic laboratory operations (aspects of sample collection, transport, storage, and disposal, use of internal standards, method implementation/validation, quality control and quality assessment, staff training, laboratory

¹Flanagan RJ, Braithwaite R, Brown SS, Widdop B, De Wolff FA. *Basic Analytical Toxicology*. Geneva: WHO, 1995; available at www.who.int/ipcs/publications/training_poisons/analytical_toxicology.pdf

²Flanagan RJ, Taylor A, Watson ID, Whelpton R. *Fundamentals of Analytical Toxicology*. Chichester: Wiley, 2007

accreditation, etc.) and basic methodology ranging from simple colour tests through spectrophotometry to immunoassay and enzyme-based assays. Section B discusses separation science in detail (chromatography and electrophoresis, mass spectrometry, and ion mobility spectrometry). Section C reviews xenobiotic absorption, distribution and metabolism, and pharmacokinetics. Section D aims to unify this material and discusses point-of-contact testing, laboratory-based substance misuse and general toxicology screening, therapeutic drug monitoring, and trace elements and toxic metals analysis. The section concludes with a general discussion on the interpretation of analytical toxicology results.

Health and Safety

This book is intended for use by scientists trained appropriately in laboratory work. Care should be taken to ensure the safe handling of all chemical and biological materials, and particular attention should be given to the possible occurrence of allergy, infection, fire, explosion, or poisoning (including transdermal absorption or inhalation of toxic vapours). Readers are expected to consult current local health and safety regulations and to adhere to them.

Nomenclature, Symbols, and Conventions

We have followed IUPAC nomenclature for chemical names except when Chemical Abstracts nomenclature or trivial names are more readily understood. With regard to symbols, we have adopted the convention that variables and constants are italicized, but labels and mathematical operators are not. Thus, for example, the acid dissociation constant is written K_a , K being the variable, a being a label to denote that it is an acid dissociation constant. The notation for the negative logarithm of K_a is pK_a – p is a mathematical symbol and is not italicized. Where the subscript is a variable then it is italicized, so the concentration at time t , is C_t , but the concentration at time 0 is C_0 . Note especially that relative molecular mass (molecular weight, relative molar mass), the ratio of the mass of an atom or molecule to the unified atomic mass unit (u), is referred to throughout as M_r . The unified atomic mass unit, sometimes referred to as the dalton (Da), is defined as one twelfth of the mass of one atom of ^{12}C . The symbol amu for atomic mass unit can sometimes be found, particularly in older works. The unified atomic mass unit is not a Système International (SI) unit of mass, although it is (only by that name, and only with the symbol u) accepted for use with SI.

As to drugs and pesticides, we have used recommended International Non-proprietary Name (rINN) or proposed International Non-proprietary Name (pINN) whenever possible. For mis-used drugs, the most common chemical names or abbreviations have been used. It is worth noting that for rINNs and chemical nomenclature, it is now general policy to use ‘f’ for ‘ph’ (e.g. sulfate not sulphate), ‘t’ for ‘th’ (e.g. chlortalidone not chlorthalidone) and ‘i’ for ‘y’ (mesilate not mesylate for methanesulfonate, for example). However, so many subtle changes have been introduced that it is difficult to ensure compliance with all such changes. Names that may be encountered include the British Approved Name (BAN), the British Pharmacopoeia (BP) name, the United States Adopted Name (USAN), the United States National Formulary (USNF) name, and the United States Pharmacopoeia (USP) name. Where the rINN is markedly different from common US usage, for example acetaminophen rather than paracetamol, meperidine instead of pethidine, the alternative is given in parentheses at first use and in the index.

Isotopically-labelled compounds are indicated using the usual convention of square brackets to denote the substituted atoms, and site of substitution where known. For example, $[^2\text{H}_3\text{-}N\text{-methyl}]$ -hyoscine indicates that the hydrogen atoms in the N -methyl group have been substituted by deuterium – this should not be confused with N -methylhyoscine (methscopolamine).

A useful source of information on drug and poison nomenclature is the *Merck Index Online* (www.rsc.org/Merck-Index/). Chemical Abstracts Service (CAS) Registry Numbers (RN) provide a unique identifier for individual compounds, but it is important to note that salts, hydrates, racemates, etc., each have their own RNs. Similarly, when discussing dosages we have tried to be clear when referring to salts, and when to free acids, bases, or quaternary ammonium compounds.

The oxidation number of metal ions is given by, for example, iron(II), but older terminology such as ferrous and ferric iron for Fe^{2+} and Fe^{3+} , respectively, will be encountered in the literature.

We emphasize that cross-referral to an appropriate local or national formulary is mandatory before any patient treatment is initiated or altered. Proprietary names must be approached with caution – the same name is sometimes used for different products in different countries.