

CONTENTS

Preface	xv
Acknowledgment	xvii
Editors	xix
Contributors	xxi
1 Antibiotics: Groups and Properties	1
<i>Philip Thomas Reeves</i>	
1.1 Introduction, 1	
1.1.1 Identification, 1	
1.1.2 Chemical Structure, 2	
1.1.3 Molecular Formula, 2	
1.1.4 Composition of the Substance, 2	
1.1.5 pK_a , 2	
1.1.6 UV Absorbance, 3	
1.1.7 Solubility, 3	
1.1.8 Stability, 3	
1.2 Antibiotic Groups and Properties, 3	
1.2.1 Terminology, 3	
1.2.2 Fundamental Concepts, 4	
1.2.3 Pharmacokinetics of Antimicrobial Drugs, 4	
1.2.4 Pharmacodynamics of Antimicrobial Drugs, 5	
1.2.4.1 Spectrum of Activity, 5	
1.2.4.2 Bactericidal and Bacteriostatic Activity, 6	
1.2.4.3 Type of Killing Action, 6	
1.2.4.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration, 7	
1.2.4.5 Mechanisms of Action, 7	
1.2.5 Antimicrobial Drug Combinations, 7	
1.2.6 Clinical Toxicities, 7	
1.2.7 Dosage Forms, 8	
1.2.8 Occupational Health and Safety Issues, 8	
1.2.9 Environmental Issues, 8	

CONTENTS

- 1.3 Major Groups of Antibiotics, 8
 - 1.3.1 Aminoglycosides, 8
 - 1.3.2 β -Lactams, 10
 - 1.3.3 Quinoxalines, 18
 - 1.3.4 Lincosamides, 20
 - 1.3.5 Macrolides and Pleuromutilins, 21
 - 1.3.6 Nitrofurans, 27
 - 1.3.7 Nitroimidazoles, 28
 - 1.3.8 Phenicol, 30
 - 1.3.9 Polyether Antibiotics (Ionophores), 31
 - 1.3.10 Polypeptides, Glycopeptides, and Streptogramins, 35
 - 1.3.11 Phosphoglycolipids, 36
 - 1.3.12 Quinolones, 36
 - 1.3.13 Sulfonamides, 44
 - 1.3.14 Tetracyclines, 45
- 1.4 Restricted and Prohibited Uses of Antimicrobial Agents in Food Animals, 52
- 1.5 Conclusions, 52
- Acknowledgments, 53
- References, 53

2 Pharmacokinetics, Distribution, Bioavailability, and Relationship to Antibiotic Residues

61

Peter Lees and Pierre-Louis Toutain

- 2.1 Introduction, 61
- 2.2 Principles of Pharmacokinetics, 61
 - 2.2.1 Pharmacokinetic Parameters, 61
 - 2.2.2 Regulatory Guidelines on Dosage Selection for Efficacy, 64
 - 2.2.3 Residue Concentrations in Relation to Administered Dose, 64
 - 2.2.4 Dosage and Residue Concentrations in Relation to Target Clinical Populations, 66
 - 2.2.5 Single-Animal versus Herd Treatment and Establishment of Withholding Time (WhT), 66
 - 2.2.6 Influence of Antimicrobial Drug (AMD) Physicochemical Properties on Residues and WhT, 67
- 2.3 Administration, Distribution, and Metabolism of Drug Classes, 67
 - 2.3.1 Aminoglycosides and Aminocyclitols, 67
 - 2.3.2 β -Lactams: Penicillins and Cephalosporins, 69
 - 2.3.3 Quinoxalines: Carbadox and Olaquinox, 71
 - 2.3.4 Lincosamides and Pleuromutilins, 71
 - 2.3.5 Macrolides, Triamilides, and Azalides, 72
 - 2.3.6 Nitrofurans, 73
 - 2.3.7 Nitroimidazoles, 73
 - 2.3.8 Phenicol, 73
 - 2.3.9 Polyether Antibiotic Ionophores, 74
 - 2.3.10 Polypeptides, 75
 - 2.3.11 Quinolones, 75
 - 2.3.12 Sulfonamides and Diaminopyrimidines, 77
 - 2.3.13 Polymyxins, 79
 - 2.3.14 Tetracyclines, 79
- 2.4 Setting Guidelines for Residues by Regulatory Authorities, 81
- 2.5 Definition, Assessment, Characterization, Management, and Communication of Risk, 82

- 2.5.1 Introduction and Summary of Regulatory Requirements, 82
- 2.5.2 Risk Assessment, 84
 - 2.5.2.1 Hazard Assessment, 88
 - 2.5.2.2 Exposure Assessment, 89
- 2.5.3 Risk Characterization, 90
- 2.5.4 Risk Management, 91
 - 2.5.4.1 Withholding Times, 91
 - 2.5.4.2 Prediction of Withholding Times from Plasma Pharmacokinetic Data, 93
 - 2.5.4.3 International Trade, 93
- 2.5.5 Risk Communication, 94
- 2.6 Residue Violations: Their Significance and Prevention, 94
 - 2.6.1 Roles of Regulatory and Non-regulatory Bodies, 94
 - 2.6.2 Residue Detection Programs, 95
 - 2.6.2.1 Monitoring Program, 96
 - 2.6.2.2 Enforcement Programs, 96
 - 2.6.2.3 Surveillance Programs, 97
 - 2.6.2.4 Exploratory Programs, 97
 - 2.6.2.5 Imported Food Animal Products, 97
 - 2.6.2.6 Residue Testing in Milk, 97
- 2.7 Further Considerations, 98
 - 2.7.1 Injection Site Residues and Flip-Flop Pharmacokinetics, 98
 - 2.7.2 Bioequivalence and Residue Depletion Profiles, 100
 - 2.7.3 Sales and Usage Data, 101
 - 2.7.3.1 Sales of AMDs in the United Kingdom, 2003–2008, 101
 - 2.7.3.2 Comparison of AMD Usage in Human and Veterinary Medicine in France, 1999–2005, 102
 - 2.7.3.3 Global Animal Health Sales and Sales of AMDs for Bovine Respiratory Disease, 103
- References, 104

3 Antibiotic Residues in Food and Drinking Water, and Food Safety Regulations

111

Kevin J. Greenlees, Lynn G. Friedlander, and Alistair Boxall

- 3.1 Introduction, 111
- 3.2 Residues in Food—Where is the Smoking Gun?, 111
- 3.3 How Allowable Residue Concentrations Are Determined, 113
 - 3.3.1 Toxicology—Setting Concentrations Allowed in the Human Diet, 113
 - 3.3.2 Setting Residue Concentrations for Substances Not Allowed in Food, 114
 - 3.3.3 Setting Residue Concentrations Allowed in Food, 114
 - 3.3.3.1 Tolerances, 115
 - 3.3.3.2 Maximum Residue Limits, 116
 - 3.3.4 International Harmonization, 117
- 3.4 Indirect Consumer Exposure to Antibiotics in the Natural Environment, 117
 - 3.4.1 Transport to and Occurrence in Surface Waters and Groundwaters, 119
 - 3.4.2 Uptake of Antibiotics into Crops, 119
 - 3.4.3 Risks of Antibiotics in the Environment to Human Health, 120
- 3.5 Summary, 120
- References, 121

4 Sample Preparation: Extraction and Clean-up **125***Alida A. M. (Linda) Stolker and Martin Danaher*

- 4.1 Introduction, 125
- 4.2 Sample Selection and Pre-treatment, 126
- 4.3 Sample Extraction, 127
 - 4.3.1 Target Marker Residue, 127
 - 4.3.2 Stability of Biological Samples, 127
- 4.4 Extraction Techniques, 128
 - 4.4.1 Liquid–Liquid Extraction, 128
 - 4.4.2 Dilute and Shoot, 128
 - 4.4.3 Liquid–Liquid Based Extraction Procedures, 129
 - 4.4.3.1 QuEChERS, 129
 - 4.4.3.2 Bipolarity Extraction, 129
 - 4.4.4 Pressurized Liquid Extraction (Including Supercritical Fluid Extraction), 130
 - 4.4.5 Solid Phase Extraction (SPE), 131
 - 4.4.5.1 Conventional SPE, 131
 - 4.4.5.2 Automated SPE, 132
 - 4.4.6 Solid Phase Extraction-Based Techniques, 133
 - 4.4.6.1 Dispersive SPE, 133
 - 4.4.6.2 Matrix Solid Phase Dispersion, 134
 - 4.4.6.3 Solid Phase Micro-extraction, 135
 - 4.4.6.4 Micro-extraction by Packed Sorbent, 137
 - 4.4.6.5 Stir-bar Sorptive Extraction, 137
 - 4.4.6.6 Restricted-Access Materials, 138
 - 4.4.7 Solid Phase Extraction-Based Selective Approaches, 138
 - 4.4.7.1 Immunoaffinity Chromatography, 138
 - 4.4.7.2 Molecularly Imprinted Polymers, 139
 - 4.4.7.3 Aptamers, 140
 - 4.4.8 Turbulent-Flow Chromatography, 140
 - 4.4.9 Miscellaneous, 142
 - 4.4.9.1 Ultrafiltration, 142
 - 4.4.9.2 Microwave-Assisted Extraction, 142
 - 4.4.9.3 Ultrasound-Assisted Extraction, 144
- 4.5 Final Remarks and Conclusions, 144
- References, 146

5 Bioanalytical Screening Methods **153***Sara Stead and Jacques Stark*

- 5.1 Introduction, 153
- 5.2 Microbial Inhibition Assays, 154
 - 5.2.1 The History and Basic Principles of Microbial Inhibition Assays, 154
 - 5.2.2 The Four-Plate Test and the New Dutch Kidney Test, 156
 - 5.2.3 Commercial Microbial Inhibition Assays for Milk, 156
 - 5.2.4 Commercial Microbial Inhibition Assays for Meat-, Egg-, and Honey-Based Foods, 159
 - 5.2.5 Further Developments of Microbial Inhibition Assays and Future Prospects, 160
 - 5.2.5.1 Sensitivity, 160
 - 5.2.5.2 Test Duration, 161
 - 5.2.5.3 Ease of Use, 161

5.2.5.4	Automation, 161	
5.2.5.5	Pre-treatment of Samples, 162	
5.2.5.6	Confirmation/Class-Specific Identification, 163	
5.2.6	Conclusions Regarding Microbial Inhibition Assays, 164	
5.3	Rapid Test Kits, 164	
5.3.1	Basic Principles of Immunoassay Format Rapid Tests, 164	
5.3.2	Lateral-Flow Immunoassays, 165	
5.3.2.1	Sandwich Format, 166	
5.3.2.2	Competitive Format, 166	
5.3.3	Commercial Lateral-Flow Immunoassays for Milk, Animal Tissues, and Honey, 168	
5.3.4	Receptor-Based Radioimmunoassay: Charm II System, 170	
5.3.5	Basic Principles of Enzymatic Tests, 171	
5.3.5.1	The Penzyme Milk Test, 171	
5.3.5.2	The Delvo-X-PRESS, 172	
5.3.6	Conclusions Regarding Rapid Test Kits, 174	
5.4	Surface Plasmon Resonance (SPR) Biosensor Technology, 174	
5.4.1	Basic Principles of SPR Biosensor, 174	
5.4.2	Commercially Available SPR Biosensor Applications for Milk, Animal Tissues, Feed, and Honey, 175	
5.4.3	Conclusions Regarding Surface Plasmon Resonance (SPR) Technology, 176	
5.5	Enzyme-Linked Immunosorbent Assay (ELISA), 178	
5.5.1	Basic Principles of ELISA, 178	
5.5.2	Automated ELISA Systems, 178	
5.5.3	Alternative Immunoassay Formats, 179	
5.5.4	Commercially Available ELISA Kits for Antibiotic Residues, 179	
5.5.5	Conclusions Regarding ELISA, 180	
5.6	General Considerations Concerning the Performance Criteria for Screening Assays, 181	
5.7	Overall Conclusions on Bioanalytical Screening Assays, 181	
	Abbreviations, 182	
	References, 182	
6	Chemical Analysis: Quantitative and Confirmatory Methods	187
	<i>Jian Wang and Sherri B. Turnipseed</i>	
6.1	Introduction, 187	
6.2	Single-Class and Multi-class Methods, 187	
6.3	Chromatographic Separation, 195	
6.3.1	Chromatographic Parameters, 195	
6.3.2	Mobile Phase, 195	
6.3.3	Conventional Liquid Chromatography, 196	
6.3.3.1	Reversed Phase Chromatography, 196	
6.3.3.2	Ion-Pairing Chromatography, 196	
6.3.3.3	Hydrophilic Interaction Liquid Chromatography, 197	
6.3.4	Ultra-High-Performance or Ultra-High-Pressure Liquid Chromatography, 198	
6.4	Mass Spectrometry, 200	
6.4.1	Ionization and Interfaces, 200	
6.4.2	Matrix Effects, 202	
6.4.3	Mass Spectrometers, 205	
6.4.3.1	Single Quadrupole, 205	
6.4.3.2	Triple Quadrupole, 206	

CONTENTS

- 6.4.3.3 Quadrupole Ion Trap, 208
- 6.4.3.4 Linear Ion Trap, 209
- 6.4.3.5 Time-of-Flight, 210
- 6.4.3.6 Orbitrap, 212
- 6.4.4 Other Advanced Mass Spectrometric Techniques, 214
 - 6.4.4.1 Ion Mobility Spectrometry, 214
 - 6.4.4.2 Ambient Mass Spectrometry, 214
 - 6.4.4.3 Other Recently Developed Desorption Ionization Techniques, 216
- 6.4.5 Fragmentation, 216
- 6.4.6 Mass Spectral Library, 216
- Acknowledgment, 219
- Abbreviations, 220
- References, 220

7 Single-Residue Quantitative and Confirmatory Methods **227**

Jonathan A. Tarbin, Ross A. Potter, Alida A. M. (Linda) Stolker, and Bjorn Berendsen

- 7.1 Introduction, 227
- 7.2 Carbadox and Olaquinox, 227
 - 7.2.1 Background, 227
 - 7.2.2 Analysis, 229
 - 7.2.3 Conclusions, 230
- 7.3 Cefotiofur and Desfuroylcefotiofur, 230
 - 7.3.1 Background, 230
 - 7.3.2 Analysis Using Deconjugation, 231
 - 7.3.3 Analysis of Individual Metabolites, 232
 - 7.3.4 Analysis after Alkaline Hydrolysis, 232
 - 7.3.5 Conclusions, 233
- 7.4 Chloramphenicol, 233
 - 7.4.1 Background, 233
 - 7.4.2 Analysis by GC-MS and LC-MS, 233
 - 7.4.3 An Investigation into the Possible Natural Occurrence of CAP, 235
 - 7.4.4 Analysis of CAP in Herbs and Grass (Feed) Using LC-MS, 236
 - 7.4.5 Conclusions, 236
- 7.5 Nitrofurans, 236
 - 7.5.1 Background, 236
 - 7.5.2 Analysis of Nitrofurans, 236
 - 7.5.3 Identification of Nitrofuran Metabolites, 237
 - 7.5.4 Conclusions, 239
- 7.6 Nitroimidazoles and Their Metabolites, 239
 - 7.6.1 Background, 239
 - 7.6.2 Analysis, 240
 - 7.6.3 Conclusions, 241
- 7.7 Sulfonamides and Their N^4 -Acetyl Metabolites, 241
 - 7.7.1 Background, 241
 - 7.7.2 N^4 -Acetyl Metabolites, 242
 - 7.7.3 Analysis, 243
 - 7.7.4 Conclusions, 244
- 7.8 Tetracyclines and Their 4-Epimers, 244
 - 7.8.1 Background, 244
 - 7.8.2 Analysis, 245
 - 7.8.3 Conclusions, 246
- 7.9 Miscellaneous, 246

- 7.9.1 Aminoglycosides, 246
- 7.9.2 Compounds with Marker Residues Requiring Chemical Conversion, 247
 - 7.9.2.1 Florfenicol, 247
- 7.9.3 Miscellaneous Analytical Issues, 250
 - 7.9.3.1 Lincosamides, 250
 - 7.9.3.2 Enrofloxacin, 251
- 7.9.4 Gaps in Analytical Coverage, 251
- 7.10 Summary, 252
- Abbreviations, 253
- References, 254

8 Method Development and Method Validation

263

Jack F. Kay and James D. MacNeil

- 8.1 Introduction, 263
- 8.2 Sources of Guidance on Method Validation, 263
 - 8.2.1 Organizations that Are Sources of Guidance on Method Validation, 264
 - 8.2.1.1 International Union of Pure and Applied Chemistry (IUPAC), 264
 - 8.2.1.2 AOAC International, 264
 - 8.2.1.3 International Standards Organization (ISO), 264
 - 8.2.1.4 Eurachem, 265
 - 8.2.1.5 VICH, 265
 - 8.2.1.6 Codex Alimentarius Commission (CAC), 265
 - 8.2.1.7 Joint FAO/WHO Expert Committee on Food Additives (JECFA), 265
 - 8.2.1.8 European Commission, 266
 - 8.2.1.9 US Food and Drug Administration (USFDA), 266
- 8.3 The Evolution of Approaches to Method Validation for Veterinary Drug Residues in Foods, 266
 - 8.3.1 Evolution of “Single-Laboratory Validation” and the “Criteria Approach,” 266
 - 8.3.2 The Vienna Consultation, 267
 - 8.3.3 The Budapest Workshop and the Miskolc Consultation, 267
 - 8.3.4 Codex Alimentarius Commission Guidelines, 267
- 8.4 Method Performance Characteristics, 268
- 8.5 Components of Method Development, 268
 - 8.5.1 Identification of “Fitness for Purpose” of an Analytical Method, 269
 - 8.5.2 Screening versus Confirmation, 270
 - 8.5.3 Purity of Analytical Standards, 270
 - 8.5.4 Analyte Stability in Solution, 271
 - 8.5.5 Planning the Method Development, 271
 - 8.5.6 Analyte Stability during Sample Processing (Analysis), 272
 - 8.5.7 Analyte Stability during Sample Storage, 272
 - 8.5.8 Ruggedness Testing (Robustness), 273
 - 8.5.9 Critical Control Points, 274
- 8.6 Components of Method Validation, 274
 - 8.6.1 Understanding the Requirements, 274
 - 8.6.2 Management of the Method Validation Process, 274
 - 8.6.3 Experimental Design, 275

CONTENTS

- 8.7 Performance Characteristics Assessed during Method Development and Confirmed during Method Validation for Quantitative Methods, 275
 - 8.7.1 Calibration Curve and Analytical Range, 275
 - 8.7.2 Sensitivity, 277
 - 8.7.3 Selectivity, 277
 - 8.7.3.1 Definitions, 277
 - 8.7.3.2 Suggested Selectivity Experiments, 278
 - 8.7.3.3 Additional Selectivity Considerations for Mass Spectral Detection, 279
 - 8.7.4 Accuracy, 281
 - 8.7.5 Recovery, 282
 - 8.7.6 Precision, 283
 - 8.7.7 Experimental Determination of Recovery and Precision, 283
 - 8.7.7.1 Choice of Experimental Design, 283
 - 8.7.7.2 Matrix Issues in Calibration, 286
 - 8.7.8 Measurement Uncertainty (MU), 287
 - 8.7.9 Limits of Detection and Limits of Quantification, 287
 - 8.7.10 Decision Limit ($CC\alpha$) and Detection Capability ($CC\beta$), 289
- 8.8 Significant Figures, 289
- 8.9 Final Thoughts, 289
- References, 289

9 Measurement Uncertainty

295

Jian Wang, Andrew Cannavan, Leslie Dickson, and Rick Fedeniuk

- 9.1 Introduction, 295
- 9.2 General Principles and Approaches, 295
- 9.3 Worked Examples, 297
 - 9.3.1 EURACHEM/CITAC Approach, 297
 - 9.3.2 Measurement Uncertainty Based on the Barwick–Ellison Approach Using In-House Validation Data, 302
 - 9.3.3 Measurement Uncertainty Based on Nested Experimental Design Using In-House Validation Data, 305
 - 9.3.3.1 Recovery (R) and Its Uncertainty [$u(R)$], 306
 - 9.3.3.2 Precision and Its Uncertainty [$u(P)$], 312
 - 9.3.3.3 Combined Standard Uncertainty and Expanded Uncertainty, 312
 - 9.3.4 Measurement Uncertainty Based on Inter-laboratory Study Data, 312
 - 9.3.5 Measurement Uncertainty Based on Proficiency Test Data, 317
 - 9.3.6 Measurement Uncertainty Based on Quality Control Data and Certified Reference Materials, 319
 - 9.3.6.1 Scenario A: Use of Certified Reference Material for Estimation of Uncertainty, 320
 - 9.3.6.2 Scenario B: Use of Incurred Residue Samples and Fortified Blank Samples for Estimation of Uncertainty, 324
- References, 325

10 Quality Assurance and Quality Control

327

Andrew Cannavan, Jack F. Kay, and Bruno Le Bizec

- 10.1 Introduction, 327
 - 10.1.1 Quality—What Is It?, 327

- 10.1.2 Why Implement a Quality System?, 328
- 10.1.3 Quality System Requirements for the Laboratory, 328
- 10.2 Quality Management, 329
 - 10.2.1 Total Quality Management, 329
 - 10.2.2 Organizational Elements of a Quality System, 330
 - 10.2.2.1 Process Management, 330
 - 10.2.2.2 The Quality Manual, 330
 - 10.2.2.3 Documentation, 330
 - 10.2.3 Technical Elements of a Quality System, 331
- 10.3 Conformity Assessment, 331
 - 10.3.1 Audits and Inspections, 331
 - 10.3.2 Certification and Accreditation, 332
 - 10.3.3 Advantages of Accreditation, 332
 - 10.3.4 Requirements under Codex Guidelines and EU Legislation, 332
- 10.4 Guidelines and Standards, 333
 - 10.4.1 Codex Alimentarius, 333
 - 10.4.2 Guidelines for the Design and Implementation of a National Regulatory Food Safety Assurance Program Associated with the Use of Veterinary Drugs in Food-Producing Animals, 334
 - 10.4.3 ISO/IEC 17025:2005, 334
 - 10.4.4 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed (Document SANCO/10684/2009), 335
 - 10.4.5 EURACHEM/CITAC Guide to Quality in Analytical Chemistry, 335
 - 10.4.6 OECD Good Laboratory Practice, 336
- 10.5 Quality Control in the Laboratory, 336
 - 10.5.1 Sample Reception, Storage, and Traceability throughout the Analytical Process, 336
 - 10.5.1.1 Sample Reception, 336
 - 10.5.1.2 Sample Acceptance, 337
 - 10.5.1.3 Sample Identification, 337
 - 10.5.1.4 Sample Storage (Pre-analysis), 337
 - 10.5.1.5 Reporting, 338
 - 10.5.1.6 Sample Documentation, 338
 - 10.5.1.7 Sample Storage (Post-reporting), 338
 - 10.5.2 Analytical Method Requirements, 338
 - 10.5.2.1 Introduction, 338
 - 10.5.2.2 Screening Methods, 338
 - 10.5.2.3 Confirmatory Methods, 339
 - 10.5.2.4 Decision Limit, Detection Capability, Performance Limit, and Sample Compliance, 339
 - 10.5.3 Analytical Standards and Certified Reference Materials, 339
 - 10.5.3.1 Introduction, 339
 - 10.5.3.2 Certified Reference Materials (CRMs), 340
 - 10.5.3.3 Blank Samples, 341
 - 10.5.3.4 Utilization of CRMs and Control Samples, 341
 - 10.5.4 Proficiency Testing (PT), 341
 - 10.5.5 Control of Instruments and Methods in the Laboratory, 342
- 10.6 Conclusion, 344
- References, 344