

CONTENTS

| | |
|--------------|------|
| PREFACE | xv |
| CONTRIBUTORS | xvii |
| ACRONYMS | xix |

PART I METHODOLOGY

| | |
|--|----|
| 1. Introduction to Mass Spectrometry | 3 |
| <i>Scott A. Smith, Ruth Waddell Smith, Yu Xia, and Zheng Ouyang</i> | |
| 1.1. History | 3 |
| 1.1.1. Atomic Physics | 4 |
| 1.1.2. Early Applications | 7 |
| 1.1.3. Organic Structural Analysis | 7 |
| 1.1.4. The Biological Mass Spectrometry Revolution | 8 |
| 1.2. Ionization Methods | 9 |
| 1.3. Mass Spectrometer Types | 10 |
| 1.3.1. Magnetic Sector Mass Spectrometers | 10 |
| 1.3.2. Quadrupole Mass Filter and Quadrupole Ion Trap Mass Spectrometers | 14 |
| 1.3.3. Time-of-Flight Mass Spectrometers | 19 |
| 1.3.4. Fourier Transform Ion Cyclotron Resonance Mass Spectrometers | 22 |
| 1.3.5. Orbitrap Mass Spectrometers | 25 |
| 1.4. Tandem Mass Spectrometry | 28 |
| 1.4.1. Ion Isolation | 29 |
| 1.4.2. Ion-Molecule Collisions and Collision-Induced Dissociation | 30 |
| 1.4.3. Electron Capture Dissociation and Electron Transfer Dissociation | 32 |
| 1.5. Separation Techniques Coupled to Mass Spectrometry | 35 |
| 1.5.1. Gas Chromatography–Mass Spectrometry | 35 |
| 1.5.2. Liquid Chromatography–Mass Spectrometry | 37 |
| 1.5.3. Capillary Electrophoresis–Mass Spectrometry | 42 |
| 1.5.4. Ion Mobility Spectrometry–Mass Spectrometry | 45 |

CONTENTS

| | |
|--|------------|
| 1.6. Prospects for Mass Spectrometry | 48 |
| References | 51 |
| 2. LC Method Development and Strategies | 59 |
| <i>Gang Xue and Yining Zhao</i> | |
| 2.1. Introduction | 59 |
| 2.2. Column, pH, and Solvent Screening | 60 |
| 2.2.1. Resolution: Goal of Separation | 60 |
| 2.2.2. Screening: Systematic Approach to Seeking Selectivity | 60 |
| 2.2.3. Screening Instrumentation and Controlling Software | 67 |
| 2.3. Gradient and Temperature Optimization | 69 |
| 2.4. Orthogonal Screening | 70 |
| 2.4.1. Method Orthogonality | 71 |
| 2.4.2. Selection of Orthogonal Methods | 72 |
| 2.4.3. Impurity Orthogonal Screening | 74 |
| 2.5. High-Efficiency Separation | 76 |
| 2.6. Conclusions | 78 |
| References | 78 |
| 3. Rapid Analysis of Drug-Related Substances using Desorption Electrospray Ionization and Direct Analysis in Real Time Ionization Mass Spectrometry | 81 |
| <i>Hao Chen and Jiwen Li</i> | |
| 3.1. Introduction | 81 |
| 3.2. Ionization Apparatus, Mechanisms, and General Performance | 83 |
| 3.2.1. Desorption Electrospray Ionization (DESI) | 83 |
| 3.2.2. Direct Analysis in Real Time (DART) | 85 |
| 3.3. Drug Analysis in Biological Matrices using DESI and DART | 87 |
| 3.3.1. DESI Application | 88 |
| 3.3.2. DART Application | 89 |
| 3.4. High-Throughput Analysis | 92 |
| 3.5. Chemical Imaging and Profiling | 94 |
| 3.6. Future Perspectives | 101 |
| References | 101 |
| 4. Orbitrap High-Resolution Applications | 109 |
| <i>Robert J. Strife</i> | |
| 4.1. Historical Anecdote | 109 |
| 4.2. General Description of Orbitrap Operating Principles | 110 |
| 4.3. The Orbitrap is a “Fourier Transform” Device | 112 |

| | | |
|--------|---|-----|
| 4.4. | Performing Experiments in Trapping Devices | 113 |
| 4.4.1. | “Raw” HPLC Data Look Like Infusion Data | 114 |
| 4.4.2. | How Much Mass Resolution Should Be Used During HPLC | 114 |
| 4.5. | Determining Elemental Compositions of “Unknowns” Using an Orbitrap | 115 |
| 4.6. | Orbitrap Figures of Merit in Mass Measurement | 117 |
| 4.6.1. | Accuracy | 117 |
| 4.6.2. | Precision | 118 |
| 4.6.3. | Discussion | 118 |
| 4.7. | HPLC Orbitrap MS: Accurate Mass Demonstration and Differentiation of Small Molecule Formulas Very Proximate in Mass/Charge Ratio Space | 121 |
| 4.8. | Determination of Trace Contaminant Compositions by Simple Screening HPLC-MS and Infusion Orbitrap MS | 122 |
| 4.9. | Determining Substructures: Orbitrap Tandem Mass Spectrometry (MS ⁿ) | 124 |
| 4.10. | Multianalyzer (Hybridized) System: The Linear Ion Trap/Orbitrap for MS/MS and Higher-Order MS ⁿ , $n > 2$ | 127 |
| 4.11. | Mass Mapping to Discover Impurities | 129 |
| 4.12. | The Current Practice of Orbitrap Mass Spectrometry | 131 |
| 4.13. | Conclusion | 132 |
| | References | 132 |
| 5. | Structural Characterization of Impurities and Degradation Products in Pharmaceuticals Using High-Resolution LC-MS and Online Hydrogen/Deuterium Exchange Mass Spectrometry | 135 |
| | <i>Guodong Chen and Birendra N. Pramanik</i> | |
| 5.1. | Introduction | 135 |
| 5.2. | Characterization of Impurities | 137 |
| 5.2.1. | Mometasone Furoate | 137 |
| 5.2.2. | Enol Tautomer Impurity in Hepatitis C Virus (HCV) Protease Inhibitor | 152 |
| 5.3. | Characterization of Degradation Products | 155 |
| 5.3.1. | Everninomicin | 156 |
| 5.3.2. | Posaconazole | 164 |
| 5.4. | Conclusions | 176 |
| | References | 177 |

CONTENTS

| | |
|---|------------|
| 6. Isotope Pattern Recognition on Molecular Formula Determination for Structural Identification of Impurities | 183 |
| <i>Ming Gu</i> | |
| 6.1. Introduction | 183 |
| 6.2. Three Basic Approaches to Isotope Pattern Recognition | 184 |
| 6.2.1. With Centriod Data | 185 |
| 6.2.2. With Profile Data without Peak Shape Calibration | 187 |
| 6.2.3. With Profile Data with Peak Shape Calibration | 189 |
| 6.3. The Importance of Lineshape Calibration | 190 |
| 6.3.1. Lineshape Calibration Using Standards | 191 |
| 6.3.2. Lineshape Self-Calibration | 193 |
| 6.4. Spectral Accuracy | 194 |
| 6.5. Formula Determination with Quadrupole MS | 194 |
| 6.5.1. Impurity Identification with LC-MS | 195 |
| 6.5.2. Impurity Identification with GC-MS | 200 |
| 6.5.3. Pros and Cons of Determination of Elemental Decomposition (DEC) with Quadrupole MS | 201 |
| 6.6. Formula Determination with High-Resolution MS | 203 |
| 6.7. Conclusions and Future Directions | 208 |
| References | 208 |
| PART II APPLICATION | |
| 7. Practical Application of Very High-Pressure Liquid Chromatography Across the Pharmaceutical Development-Manufacturing Continuum | 215 |
| <i>Brent Kleintop and Qinggang Wang</i> | |
| 7.1. Introduction | 215 |
| 7.2. Theory and Benefits of VHPLC | 217 |
| 7.3. VHPLC Method Development | 220 |
| 7.3.1. Adapting Existing HPLC Methods to VHPLC | 220 |
| 7.3.2. Developing New VHPLC Methods | 224 |
| 7.4. Other Practical Considerations | 226 |
| 7.5. VHPLC Method Validation | 227 |
| 7.6. Summary | 229 |
| References | 229 |
| 8. Impurity Identification for Drug Substances | 231 |
| <i>David W. Berberich, Tao Jiang, Joseph McClurg, Frank Moser, and R. Randy Wilhelm</i> | |
| 8.1. Introduction | 231 |

| | |
|--|------------|
| 8.2. Case Studies | 232 |
| 8.2.1. Identification of Impurities in Each Synthetic Step of Drug Substance during Process Development | 232 |
| 8.2.2. Impurity ID by LC/MS during Exploratory Chemistry: Evaluation of New Raw Materials | 237 |
| 8.2.3. Impurity Identification during Accelerated Stability Studies | 243 |
| 8.3. Conclusions | 249 |
| References | 250 |
| 9. Impurity Identification in Process Chemistry by Mass Spectrometry | 251 |
| <i>David Q. Liu, Mingjiang Sun, and Lianming Wu</i> | |
| 9.1. Introduction | 251 |
| 9.2. Experimentation | 252 |
| 9.2.1. Liquid Chromatography Conditions | 252 |
| 9.2.2. LC-MS Systems | 253 |
| 9.2.3. GC-MS System | 253 |
| 9.2.4. Accurate Mass | 253 |
| 9.2.5. Online H/D Exchange LC-MS | 254 |
| 9.3. Applications | 254 |
| 9.3.1. Identification of Reaction Byproducts by Data-Dependent LC/MS ⁿ | 254 |
| 9.3.2. Online H/D Exchange Aids Structural Elucidation of Process Impurities | 257 |
| 9.3.3. LC-MS for Chemical Reaction Impurity Fate Mapping | 260 |
| 9.3.4. GC-MS for Impurity Profiling of Small-Molecule Starting Materials | 262 |
| 9.3.5. Identification of a Process Impurity that Impacts Downstream Formulation | 265 |
| 9.3.6. Differential Fragmentation between Sodiated and Protonated Molecules as a Means of Structural Elucidation | 267 |
| 9.4. Concluding Remarks | 275 |
| Acknowledgments | 275 |
| References | 276 |
| 10. Structure Elucidation of Pharmaceutical Impurities and Degradants in Drug Formulation Development | 279 |
| <i>Changkang Pan, Frances Liu, and Michael Motto</i> | |
| 10.1. Importance of Drug Degradation Studies in Drug Development | 279 |
| 10.2. Drug Degradation Studies in Formulation Development | 281 |

CONTENTS

| | | |
|----------|--|-----|
| 10.2.1. | Drug Substance–Excipient Interaction | 281 |
| 10.2.2. | Small Unknown Peaks (~0.1%) (Low-Dose Drugs <1 mg per Dose) | 282 |
| 10.2.3. | “Busy” LC Chromatogram with Multiple Peaks (Combination Drug Products) | 282 |
| 10.2.4. | Modification of Non-MS-Compatible LC Methods | 282 |
| 10.2.5. | Uncontrollable Multiple Chemical Reactions in Stability Samples | 283 |
| 10.2.6. | Separation Interference and Contamination Induced by Excipients | 283 |
| 10.2.7. | Peak Isolation and NMR Confirmation for Late-Phase Projects | 284 |
| 10.3. | Complexity of Impurity Identification in Drug Development | 284 |
| 10.3.1. | Drug Substance (DS) Degradation | 284 |
| 10.3.2. | DS–Excipient Interaction | 285 |
| 10.3.3. | DS–Residual Solvent Interaction | 287 |
| 10.3.4. | DS–Solvent Impurity Interaction | 287 |
| 10.3.5. | Metal Ion–Catalyzed Reaction | 289 |
| 10.3.6. | DS–Excipient Impurity Interaction | 289 |
| 10.3.7. | DS–Salt Interaction | 291 |
| 10.3.8. | DS–Preservative Interaction | 291 |
| 10.3.9. | Preservative–Excipient Interaction | 292 |
| 10.3.10. | Excipient Degradation | 292 |
| 10.3.11. | Leachables and Extractables | 293 |
| 10.4. | Strategy for Structure Elucidation of Unknowns | 295 |
| 10.4.1. | Non-MS-Compatible Method versus MS-Compatible Method | 295 |
| 10.4.2. | Selection of Ionization Mode (ESI or APCI, Positive or Negative) | 298 |
| 10.4.3. | Multiple Approaches for Structure Elucidation | 298 |
| 10.4.4. | Structure Confirmation | 299 |
| 10.5. | Hyphenated Analytical Techniques Used in Drug Development | 300 |
| 10.5.1. | LC-MS/MS for Fragmentation Pathways | 302 |
| 10.5.2. | High-Resolution MS for Chemical Formula/Elemental Composition | 302 |
| 10.5.3. | SEC/CLND or HPLC/CLND: Nitrogen-Specific Detection | 304 |
| 10.5.4. | GC-MS with EI-CI Combination | 305 |
| 10.5.5. | Headspace GC-MS: Volatile Compounds | 305 |
| 10.5.6. | NMR and LC-NMR | 306 |
| 10.5.7. | TD-GC/MS: Chemical Reactions Attributing to Weight Loss in TGA | 307 |
| 10.6. | Case Studies | 307 |

| | | |
|---|--|------------|
| 10.6.1. | LC-MS, GC-MS, and LC-NMR Studies of a Drug Degradation Product | 307 |
| <i>10.6.1.1. LC-MS Analysis</i> | 308 | |
| <i>10.6.1.2. GC-MS Analysis</i> | 308 | |
| <i>10.6.1.3. LC-NMR Analysis</i> | 308 | |
| 10.6.2. | Strategy for Identification of Leachables in Packaged Liquid Formulation | 313 |
| 10.6.3. | Characterization of Methionine Oxidation in Parathyroid Hormone Formulation | 316 |
| <i>10.6.3.1. Oxidation, Isolation, and Digestion of PTH1-34</i> | 316 | |
| <i>10.6.3.2. Mass Assignment of PTH1-34 Oxidized Variants</i> | 317 | |
| <i>10.6.3.3. Mass Assignment of CNBr Digested Peptide Fragments</i> | 318 | |
| <i>10.6.3.4. LC-MS/MS Studies of Ion Fragments from Oxidized Peptides</i> | 322 | |
| | Acknowledgment | 326 |
| | References | 326 |
| 11. | Investigation of Degradation Products and Extractables in Developing Topical OTC (Over the Counter) and NCE (New Chemical Entity) Consumer Healthcare Medication Products | 337 |
| | <i>Fa Zhang</i> | |
| 11.1. | Introduction | 337 |
| 11.2. | Oxidatively Induced Coupling of Miconazole Nitrate with Butylated Hydroxytoluene in a Topical Ointment | 338 |
| 11.2.1. | HPLC-MS Screening | 339 |
| 11.2.2. | Organic Synthesis | 341 |
| 11.2.3. | Degradation Mechanism | 344 |
| 11.3. | Extractables from Rubber Closures of a Prefilled Semisolid Drug Applicator | 347 |
| 11.3.1. | Isolation of the Extractables | 348 |
| 11.3.2. | Structural Identification of Extractables 5 and 6 | 348 |
| 11.3.3. | Structural Identification of Extractables 7 and 8 | 349 |
| 11.3.4. | Structural Identification of Extractable 9 | 351 |
| 11.4. | New Degradation Products and Pathways of Vitamin D and Its Analogs | 352 |
| 11.4.1. | Thermal Isomerization of Vitamin D ₃ in DMSO | 355 |
| 11.4.2. | Autoxidation of Isotachysterol | 356 |

CONTENTS

| | |
|---|------------|
| 11.4.2.1. <i>Mechanism of Isotachysterol Autoxidation</i> | 362 |
| 11.4.3. Thermal Degradation of Ecalcidene | 364 |
| 11.4.4. Acid-Induced Degradation of Ecalcidene | 368 |
| 11.4.5. Iodine-Induced Degradation of Ecalcidene | 370 |
| 11.4.5.1. <i>cis/trans-Isomerization of Ecalcidene</i> | 371 |
| 11.4.5.2. <i>cis/trans-Isomerization of Previtamin D₃-Type Isomer 24</i> | 372 |
| 11.5. Reductive Degradation of a 1,2,4-Thiadiazolium Derivative | 376 |
| 11.6. Conclusions | 382 |
| References | 383 |
| 12. Characterization of Impurities and Degradants in Protein Therapeutics by Mass Spectrometry | 391 |
| <i>Li Tao, Michael Ackerman, Wei Wu, Peiran Liu, and Reb Russell</i> | |
| 12.1. Introduction to Therapeutic Proteins | 391 |
| 12.2. Recent Advances in Mass Spectrometry | 392 |
| 12.3. Impurities | 393 |
| 12.3.1. Endotoxin | 394 |
| 12.3.2. Residual DNA | 394 |
| 12.3.3. Residual HCP | 395 |
| 12.4. Degradation Products | 395 |
| 12.4.1. Chemical Degradation | 396 |
| 12.4.1.1. <i>Deamidation/Isomerization</i> | 396 |
| 12.4.1.2. <i>Protein Fragmentation</i> | 400 |
| 12.4.1.3. <i>Oxidation</i> | 401 |
| 12.4.2. Variants Caused by Posttranslational Modification | 404 |
| 12.4.2.1. <i>Case Study: Characterization of S-Thiolation on Secreted Proteins from E. coli</i> | 406 |
| 12.4.2.2. <i>TM307</i> | 408 |
| 12.4.2.3. <i>TM485</i> | 408 |
| 12.4.2.4. <i>TM358 and TM687</i> | 410 |
| 12.5. Conclusions | 413 |
| References | 413 |
| 13. Identification and Quantification of Degradants and Impurities in Antibodies | 427 |
| <i>David M. Hambly and Himanshu S. Gadgil</i> | |
| 13.1. Introduction to Antibodies and Protein Drugs | 427 |

| | | |
|------------|---|-----|
| 13.1.1. | Antibody Classification and Subtypes | 427 |
| 13.1.2. | Antibody Structure | 428 |
| 13.1.3. | Antibody-Domain Structure | 429 |
| 13.1.4. | Recombinant Antibody Production | 429 |
| 13.1.5. | Methods for Characterizing Antibody Degradation and Impurity | 430 |
| 13.2. | Overview of Degradations and Impurities in Protein Drugs and Antibodies | 431 |
| 13.2.1. | Chemical Degradations and Impurities | 431 |
| 13.2.1.1. | <i>Methionine Oxidation</i> | 431 |
| 13.2.1.2. | <i>Disulfide Bonds or Reduced Cysteine</i> | 432 |
| 13.2.1.3. | <i>Deamidation of Asparagine and Glutamine</i> | 432 |
| 13.2.1.4. | <i>Isomerization of Aspartic Acid and Glutamic Acid</i> | 433 |
| 13.2.1.5. | <i>Amide Backbone Hydrolysis Reactions</i> | 433 |
| 13.2.1.6. | <i>Glycation of Lysine Residues</i> | 433 |
| 13.2.1.7. | <i>C-Terminal Lysine Variants</i> | 434 |
| 13.2.1.8. | <i>Carbohydrate Variants</i> | 434 |
| 13.3. | Methods Used to Identify and Quantitate Degradations and Impurities | 435 |
| 13.3.1. | Whole-Protein Mass Analysis Methods | 435 |
| 13.3.1.1. | <i>Carbohydrate Variation</i> | 435 |
| 13.3.1.2. | <i>Detection of Lysine C-terminal Variants and Glycated Lysine</i> | 437 |
| 13.3.1.3. | <i>Detection of Disulfide Bond Variants in IgG2 Antibodies</i> | 437 |
| 13.3.2. | Methods for Evaluating the Mass of Protein Fragments | 438 |
| 13.3.2.1. | <i>Limited Digestion Method for Antibodies</i> | 438 |
| 13.3.2.2. | <i>Limited and Reduced Method for Antibodies</i> | 440 |
| 13.3.2.3. | <i>Reduced Protein Mass Analysis</i> | 441 |
| 13.3.3. | Methods for Evaluating Peptides for Impurities and Degradations | 443 |
| 13.3.3.1. | <i>Reduced and Alkylated Peptide Mapping</i> | 443 |
| 13.4. | Conclusions | 450 |
| Appendix | | 450 |
| References | | 453 |
| INDEX | | 461 |