Contents

Preface XIII
List of Contributors XV
Structure of the Book XVII

Part 1 Evaluation and Estimation of Chromatographic Data 1

1 Evaluating Chromatograms 3
Hans-Joachim Kuss and Daniel Stauffer
1.1 Efficiency 4
1.2 EMG Model 5
1.3 Chromatogram 7
1.4 Selectivity 8
References 8

2 Integration Parameters 9
Daniel Stauffer and Hans-Joachim Kuss
2.1 Peak Recognition Methods 9
2.1.1 The Classical Method 9
2.1.2 Alternative Method 12
2.2 Integration and Integration Parameters 12
2.2.1 Data Acquisition and Integration with Empower 2 12
2.2.2 Data Acquisition and Integration with Chromeleon 14
2.2.3 Data Acquisition and Integration with EZChrom Elite 14
2.2.4 Data Recording and Integration with ChemStation 16

3 Integration Errors 17
Hans-Joachim Kuss
3.1 What Does the Literature Say on Integration Errors? 17
3.2 Integration in Routine Practice 19
Contents
3.2.1 Integration, Simple and Automatically Feasible? 20
3.2.2 Comparison of Integration Systems with a Small Number of Tall Peaks 20
3.2.3 Comparison of Integration Systems for Numerous Small Peaks 25
3.3 Chromatogram Simulation 28
3.3.1 Simulation of a Digital Chromatogram 29
  3.3.1.1 One Peak 30
  3.3.1.2 Several Peaks 32
  3.3.1.3 Noise 32
  3.3.1.4 Drift 33
  3.3.1.5 Peak Area 34
  3.3.1.6 Peaks Merged Together 34
  3.3.1.7 Unclear Baseline 37
  3.3.1.8 Tailing 37
  3.3.1.9 Data Point Interval 37
  3.3.1.10 Other Characteristic Quantities 43
  3.3.1.11 Gas Chromatogram 44
  3.3.1.12 Applications of the Simulation 45
  3.3.2 Simulation of a Standard Curve 47
  3.3.2.1 Tenfold Simulation at the Limit of Quantification 55
  3.3.3 Simulation of an Isocratic Chromatogram 55
  3.3.4 Simulation of a Gradient Chromatogram 63
  3.3.5 By-Product Analysis 68
  3.3.6 Post-Simulation of Real Chromatograms 78
References 82

4 Simulation of Chromatograms 83
  Uwe D. Neue
  4.1 Introduction 83
  4.2 Peak Simulation 84
    4.2.1 Symmetrical Peaks 84
    4.2.2 Peak Tailing 85
  4.3 The Baseline 88
  4.4 The Chromatogram 89
  4.5 Simple Retention Modeling and Real Chromatograms 91
    4.5.1 Isocratic Chromatography 91
    4.5.2 Gradient Chromatography 95
  4.6 Outlook 102
References 103

5 Integration of Asymmetric Peaks 105
  Hans-Joachim Kuss
  5.1 The Valley Between Merged Peaks 108
  5.2 Small Peak Between Larger Ones 110
  5.3 Peak Pairs 112
5.4 Simulation of a Calibration 113
5.5 Exponential Skim 117
5.6 Integration of Merged Peaks 120
5.7 Integration in Daily Practice 121

References 122

6 Deconvolution 123

Mike Hillebrand

6.1 Introduction 123
6.1.1 Software Applications for Deconvolution 126
6.2 Influences on Deconvolution 127
6.2.1 The Baseline Path 128
6.2.2 Peak Homogeneity – Number of Peaks 128
6.2.3 Noise 129
6.2.4 Peak Symmetry 129
6.3 Deconvolution of Gaussian-Shaped Peaks 129
6.3.1 Westerberg Report Simulation 132
6.4 Deconvolution of Peaks with Tailing 134
6.4.1 Peaks with Different Tailing 137
6.5 Deconvolution of Gradient Runs 141
6.5.1 Complex Baseline 141
6.6 Real Chromatogram 147
6.7 Outlook 149

References 150

7 Interpretation of Chromatograms 153

Hans-Joachim Kuss

7.1 Using the Peak Height 153
7.2 Evaluation 154
7.2.1 Evaluation Methods 155
7.3 Calibration 156
7.3.1 Linear Regression 157
7.3.2 Weighted Linear Regression 161

References 165

8 General Interpretation of Analytical Data 167

Joachim Ermer

8.1 (Normal) Distribution of Analytical Data 167
8.2 Problems of Prediction 171
8.3 Analytical Variability 174
8.3.1 Variability Contributions and Precision Levels 174
8.3.2 System or Injection Precision 176
8.3.3 Repeatability 178
8.3.4 Reproducibility 179
8.3.4.1 Number of Series 180
VIII Contents

8.3.4.2 Analysis of Variances 180
8.3.4.3 Benchmarking for Reproducibility 182
8.3.5 Consequences for the Design of an Analytical Procedure 183
8.3.6 Concentration Dependence of the Precision 185
8.3.7 Conclusions 187
8.4 Key Points 188

References 188

9 Metrological Aspects of Chromatographic Data Evaluation 191
Ulrich Panne
9.1 Introduction 191
9.2 Measurement Uncertainty 193
9.3 Traceability of Analytical Measurements 205

References 208

Part 2 Characterization of the Evaluation of Different Chromatographic Modes 211

10 Evaluation and Estimation of Chromatographic Data in GC 213
Werner Engewald (Translator: Mike Hillebrand)
10.1 Introduction 213
10.2 How Does GC Differ from HPLC? 214
10.2.1 What Consequences Result from These Differences? 215
10.2.1.1 Applicability of GC 215
10.2.1.2 Sample Injection 219
10.2.1.3 Column 219
10.2.1.4 Detector 221
10.2.1.5 Fast Gas Chromatography 222
10.3 Qualitative Analysis 223
10.3.1 Introductory Remarks 223
10.3.1.1 Fingerprint Analysis 223
10.3.2 Comparison of Retention Times 225
10.3.3 Relative Retention Times 225
10.3.4 Retention Time Locking (RTL) 226
10.4 GC-MS Coupling 227
10.4.1 MS as an Identifying Detector (Scan Mode) 227
10.4.2 Use of Spectral Libraries 228
10.4.3 Spectrum Deconvolution 231
10.4.4 MS as a Mass-Selective Detector 233
10.4.4.1 Mass Fragmentography (Reconstructed or Extracted Ion Chromatogram) 233
10.4.4.2 SIM (Single Ion Monitoring) or MID (Multiple Ion Recording) 234
10.4.5 Chemical Ionization 235
16 Requirements of (Chromatographic) Data in Pharmaceutical Analysis 321
Joachim Ermer
16.1 System Suitability Tests 321
16.1.1 European Pharmacopoeia (EP) 322
16.1.1.1 Chromatographic Parameters 325
16.1.1.2 Signal-to-Noise Ratio 326
16.1.1.3 System Precision 327
16.1.2 US Pharmacopoeia 328
16.1.3 FDA Reviewer Guidance 329
16.2 Acceptance Limits for the Specification and Precision 330
16.2.1 Assay 330
16.2.1.1 Based on the Method Capability Index 330
16.2.1.2 Based on the 95% Prediction Interval (DPhG-Approach) 331
16.2.2 Impurity Determination 332
16.2.2.1 Acceptance Limits of the Specification 332
16.2.2.2 Quantitation Limit and Variability 332
16.2.3 Key Points 332
16.3 Interpretation and Treatment of Analytical Data 333
16.3.1 Prerequisites 333
16.3.2 Measurement Principles and Variation 333
16.3.3 Outlying Results 334
16.3.3.1 Outlier Test According to Hampel 334
16.3.4 Comparison of Analytical Results 335
16.3.4.1 Precision 335
16.3.4.2 Accuracy 337
16.3.5 Key Points 338
References 338

17 Evaluation and Valuation of Chromatographic Data 341
Stavros Kromidas
17.1 Introduction 341
17.2 The Situation – or Why Does so Little Change? 341
17.3 How Can Something Change and When is it Really Necessary? 343
17.4 Who Can Change Something? 345

Index 347

Contents of the CD 357