## Contents

Preface XI Introduction XIII

## Part I Fundamentals 1

- 1.1 Chromatography 2
- 1.2 Chromatographic Figures of Merit 4
- 1.3 The Resolution of Two Peaks 6
- 1.4 Reduced Parameters 8
- 1.5 The Van Deemter Curve 10
- 1.6 Peak Capacity and Number of Possible Peaks 12
- 1.7 Statistical Resolution Probability: Simulation 14
- 1.8 Statistical Resolution Probability: Example 16
- 1.9 Precision and Accuracy of an Analytical Result 18
- 1.10 Standard Deviation 20
- 1.11 Variability of the Standard Deviation 22
- 1.12 Uncertainty Propagation 24
- 1.13 Reproducibility in Trace Analysis 26
- 1.14 Ruggedness 28
- 1.15 Calibration Curves 30
- 1.16 The HPLC Instrument 32
- 1.17 The Detector Response Curve 34
- 1.18 Noise 36
- 1.19 The Playground Presented as an Ishikawa Diagram 38
- 1.20 The Possible and the Impossible 40

## Part II Pitfalls and Sources of Error 43

- 2.1 Mixing of the Mobile Phase 44
- 2.2 Mobile Phase *p*H 46
- 2.3 Adjustment of Mobile Phase *p*H 48
- 2.4 Influence of the Acid Type and Concentration in the Eluent 50
- 2.5 Water as an Unintentional Additive in the Mobile Phase 52

2.7 Inadequate Purity of a Mobile Phase Solvent 56	
2.8 Inadequate Purity of a Mobile Phase Reagent 58	
2.9 Incomplete Degassing 60	
2.10 System Peaks and Quantitative Analysis 62	
2.11 Sample Preparation with Solid Phase Extraction 64	
2.12 Inadequate Stabilization of the Extraction Solvent 66	
2.13 Poor Choice of Sample Solvent: Peak Distortion 68	
2.14 Poor Choice of Sample Solvent: Tailing 70	
2.15 Sample Solvent and Calibration Curve 72	
2.16 Impurities in the Sample 74	
2.17 Formation of a By-Product in the Sample Solution 76	
2.18 Decomposition by the Sample Vial 78	
2.19 Artifact Peaks from the Vial Septum 80	
2.20 Formation of an Associate in the Sample Solution 82	
2.21 Precision and Accuracy with Loop Injection 84	
2.22 Injection Technique 86	
2.23 Injection of Air 88	
2.24 Sample Adsorption in the Loop 90	
2.25 Extra-Column Volumes 92	
2.26 Dwell Volume 94	
2.27 Elution at $t_0$ 96	
2.28 Classification of C <sub>18</sub> Reversed Phases 98	
2.29 Different Selectivity of C <sub>18</sub> Reversed Phases 100	
2.30 Different Batches of Stationary Phase 102	
2.31 Chemical Reaction within the Column 104	
2.32 Tailing of Phosphate Compounds in the Presence of Steel	106
2.33 Recovery and Peak Shape Problems with Proteins 108	
2.34 Double Peaks from Stable Conformers 110	
2.35 Influence of Temperature on the Separation <i>112</i>	
2.36 Thermal Non-Equilibrium within the Column 114	
2.37 Influence of the Flow Rate on the Separation 116	
2.38 Influence of Run Time and Flow Rate on Gradient Separati	ions 118
2.39 UV Spectra and Quantitative Analysis 120	
2.40 UV Detection Wavelength 122	
2.41 Different Detection Properties of Diastereomers 124	
2.42 Fluorescence Quenching by Air 126	
2.43 Detector Overload in UV 128	
2.44 Detector Overload in ELSD 130	
2.45 Influence of the Retention Factor on Peak Height <i>132</i>	
2.46 Influence of the Flow Rate on Peak Area 134	
2.47 Leaks in the HPLC Instrument 136	
2.48 Impairment of Precision as a Result of Noise 138	
2.49 Determination of Peak Area and Height at High Noise 1/	40

- 2.50 Peak Height Ratios 142
- 2.51 Incompletely Resolved Peaks 144
- 2.52 Area Rules for Incompletely Resolved Peaks 146
- 2.53 Areas of a 1 : 10 Peak Pair 148
- 2.54 Heights of a 1 : 10 Peak Pair 150
- 2.55 Quantitative Analysis of a Small Peak 152
- 2.56 Incompletely Resolved Peaks with Tailing 154
- 2.57 Integration Threshold and Number of Detected Peaks 156
- 2.58 Detector Time Constant and Peak Shape 158
- 2.59 Quantitative Analysis in the 99% Range 160
- 2.60 Correlation Coefficient of Calibration Curves 162

## Part III Useful Strategies 165

- 3.1 Column Tests 166
- 3.2 Apparatus Tests 168
- 3.3 Wavelength Accuracy of the UV Detector 170
- 3.4 Internal Standards 172
- 3.5 A Linearity Test 174
- 3.6 Rules for Accurate Quantitative Peak Size Determination 176
- 3.7 High-Low Chromatography 178
- 3.8 Control Charts 180
- 3.9 Verification of the Analytical Result by Use of a Second Method 182
- 3.10 Description of Ruggedness 184
- 3.11 Rules for Passing On an HPLC Method 186
- 3.12 Quality Assurance in the Laboratory 188
- 3.13 Standard Operating Procedures 190
- 3.14 Method Validation 192
- 3.15 Some Elements of Validation 194
- 3.16 A Validation Example 196
- 3.17 System Suitability Test 198
- 3.18 From Repeatability to Reproducibility 200
- 3.19 Measurement Uncertainty 202
- 3.20 Formal Quality Assurance Systems 204

Index 207