

Contents

List of Contributors XIII

The structure of "The HPLC-Expert" XV

Preface XVII

1	LC/MS Coupling	1
1.1	State of the Art in LC/MS	1
	<i>Oliver Schmitz</i>	
1.1.1	Introduction	1
1.1.2	Ionization Methods at Atmospheric Pressure	3
1.1.2.1	Overview about API Methods	4
1.1.2.2	ESI	4
1.1.2.3	APCI	6
1.1.2.4	APPI	7
1.1.2.5	APLI	7
1.1.2.6	Determination of Ion Suppression	8
1.1.2.7	Best Ionization for Each Question	9
1.1.3	Mass Analyzer	9
1.1.4	Future Developments	11
1.1.5	What Should You Look for When Buying a Mass Spectrometer?	11
1.2	Technical Aspects and Pitfalls of LC/MS Hyphenation	12
	<i>Markus M. Martin</i>	
1.2.1	Instrumental Considerations	13
1.2.1.1	Does Your Mass Spectrometer Fit Your Purpose?	13
1.2.1.2	(U)HPLC and Mass Spectrometry	17
1.2.2	When LC Methods and MS Conditions Meet Each Other	35
1.2.2.1	Flow Rate and Principle of Ion Formation	35
1.2.2.2	Mobile Phase Composition	37
1.2.3	Quality of Your Mass Spectra and LC/MS Chromatograms	39
1.2.3.1	No Signal at All	40
1.2.3.2	Inappropriate Ion Source Settings and their Impact on the Chromatogram	41
1.2.3.3	Ion Suppression	43
1.2.3.4	Unknown Mass Signals in the Mass Spectrum	44

1.2.3.5	Instrumental Reasons for the Misinterpretation of Mass Spectra	49
1.2.4	Conclusion	51
1.2.5	Abbreviations	52
1.3	LC Coupled to MS – A User Report	53
	<i>Alban Muller and Andreas Hofmann</i>	
1.3.1	Conditions of the Ion Chromatography	56
1.3.2	Gradient Generator	56
1.3.3	Transitions	56
	References	58
2	Optimization Strategies in RP-HPLC	61
	<i>Frank Steiner, Stefan Lamotte, and Stavros Kromidas</i>	
2.1	Introduction	61
2.1.1	Speed of Analysis	61
2.1.2	Peak Resolution	62
2.1.3	Limit of Detection and Limit of Quantification	62
2.1.4	Costs of Analysis	63
2.2	LC Fundamentals	64
2.2.1	Peak Resolution	64
2.2.2	Optimization of Efficiency (The Kinetic Approach)	69
2.2.2.1	The Term Describing the Eddy Dispersion (A-Term)	71
2.2.2.2	The Term Describing the Longitudinal Diffusion of Analyte Molecules (B-Term)	72
2.2.2.3	The Term Describing the Hindrance of Analyte Mass Transfer (C-Term)	72
2.2.3	The Influence of the Column Dimension	73
2.3	Methodology of Optimization	76
2.3.1	How to Optimize Selectivity	76
2.3.1.1	The Role of Selectivity in Practical Method Optimization	77
2.3.1.2	How to Control Selectivity in HPLC?	78
2.3.2	The Role of Temperature in HPLC	85
2.3.2.1	Retention and Selectivity Control via Temperature: Possibilities and Limitations	86
2.3.2.2	Separation Acceleration through Temperature Increase	90
2.3.3	The Value of Mobile Phase Composition versus Temperature in the Strive for Optimization in HPLC	98
2.3.4	Accelerating Separations through Efficiency Improvement of Stationary Phases	104
2.3.4.1	Systematic Speed-Up by Optimization of Particle Diameter and Column Length	104
2.3.4.2	Monoliths and Solid Core versus Fully Porous Phase Materials	113
2.3.5	Optimizing Resolution by Particle Size and/or Column Length	116
2.3.6	High-Resolution 1D-LC and 2D-LC to Fully Exploit the Potential	120

2.3.6.1	The 2D-LC Approach to Increase Peak Capacities Beyond These Limits	121
2.3.6.2	Boosting Peak Capacity in 1D-LC Further and How This Translates into Analytical Value	127
2.3.7	Optimization of Limits of Detection and Quantification	131
2.3.7.1	Absolute Detection Limit (Related to Analyte Mass on Column)	132
2.3.7.2	Concentration Detection Limit	134
2.3.8	Practical Guide for Optimization	135
2.3.8.1	General Optimization Workflow and Important Considerations and Precautions	135
2.3.8.2	Overview on Valuable Rules and Formula	136
2.4	Outlook	137
	References	148
3	The Gradient in RP-Chromatography	151
3.1	Aspects of Gradient Optimization	151
	<i>Stavros Kromidas, Frank Steiner, and Stefan Lamotte</i>	
3.1.1	Introduction	151
3.1.2	Special Features of the Gradient	151
3.1.3	Some Chromatographic Definitions and Formulas	153
3.1.4	Detection Limit, Peak Capacity, Resolution: Possibilities for Gradient Optimization	156
3.1.4.1	Detection Limit	156
3.1.4.2	Peak Capacity and Resolution	158
3.1.5	Gradient "Myths"	162
3.1.6	Examples for the Optimization of Gradient Runs: Sufficient Resolution in an Adequate Time	164
3.1.6.1	About Irregular Components	164
3.1.6.2	Preliminary Remarks, General Conditions	164
3.1.7	Gradient Aphorisms	173
3.2	Prediction of Gradients	177
	<i>Hans-Joachim Kuss</i>	
3.2.1	Linear Model: Prediction from Two Chromatograms	177
3.2.1.1	What Does the Retention Factor k Tell Us?	178
3.2.1.2	What Do the Two Retention Factors k_g and k_e Mean?	181
3.2.1.3	How Does the Integration- and Control System See the Gradient?	182
3.2.1.4	How Does the HPLC-Column See the Gradient?	182
3.2.1.5	How Do the Substances to Be Analyzed See the Gradient?	183
3.2.1.6	Interpretation of the $\ln(k)$ to %B Graph	183
3.2.1.7	The Instrumental Gradient Delay (Dwell Time)	185
3.2.1.8	Extension of the Gradient Downwards by Constant Slope	189
3.2.2	Curvilinear Model: More than Two Input Chromatograms	189
3.2.2.1	The $\ln(k)$ -Straight Lines Are Often not Straight at All	189

3.2.2.2	The $\ln(k)$ to %B Fit According to Neue	190
3.2.2.3	Predictions with Excel	193
3.2.2.4	The Interaction Is Temperature Dependant	194
3.2.2.5	Optimization Parameters	195
3.2.2.6	Commercial Optimization Programs	195
3.2.2.7	How Accurate Must the Prediction of k_g and k_e Be?	197
3.2.3	How to Act Systematically?	198
3.2.4	List of Abbreviations	199
	References	200
4	Comparison and Selection of Modern HPLC Columns	203
	<i>Stefan Lamotte, Stavros Kromidas, and Frank Steiner</i>	
4.1	Supports	203
4.1.1	Why Silica Gel?	204
4.2	Stationary Phases for the HPLC: The Historical Development	205
4.3	pH Stability and Restrictions in the Use of Silica	208
4.4	The Key Properties of Reversed Phases	209
4.4.1	The Hydrophobicity of Reversed Phases	209
4.4.2	The Hydrophobic Selectivity	210
4.4.3	The Silanophilic Activity	210
4.4.4	Shape Selectivity (Molecular Shape Recognition)	211
4.4.4.1	Why Is This So?	211
4.4.5	The Polar Selectivity	211
4.4.6	The Metal Content	212
4.5	Characterization and Classification of Reversed Phases	212
4.5.1	The Significance of Retention and Selectivity Factors in Column Tests	216
4.5.1.1	Preliminary Remark	216
4.5.1.2	Criteria for the Comparison of Columns	216
4.5.2	Column Comparison, Comparison Criteria: Similarity of Selectivities	220
4.5.3	Two Simple Tests for the Characterization of RP Phases	223
4.5.3.1	Test 1	224
4.5.3.2	Test 2	224
4.6	Procedure for Practical Method Development	224
4.6.1	The Interaction between Mobile and Stationary Phase	224
4.6.1.1	Why Is This?	225
4.6.2	Which Columns Should Be Used, and How Do I Use Them?	226
4.6.3	What to Do, When the Analytes Are Very Polar and Are not Retained on the above-Mentioned Columns?	229
4.6.3.1	AQ Columns, Polar RP Columns, and Ion-Pair Chromatography	229
4.6.3.2	Mixed-Mode Columns	231
4.6.3.3	Ion-Exchange Columns/Ligand-Exchange Chromatography	232
4.6.3.4	HILIC (Hydrophilic Interaction Liquid Chromatography)	232

4.6.3.5	Porous Carbon	233
4.7	Column Screening	234
4.8	Column Databases	239
	References	240
5	Introduction to Biochromatography	243
	<i>Jürgen Maier-Rosenkranz</i>	
5.1	Introduction	243
5.2	Overview of the Stationary Phases	245
5.2.1	Base Materials	246
5.2.2	Characterization of Stationary Phases	246
5.2.2.1	Particle Form	247
5.2.2.2	Particle Size	247
5.2.2.3	Pore Size and Surface	249
5.2.2.4	Loading Density	250
5.2.2.5	Purity	251
5.2.2.6	Functional Group	252
5.3	Reversed-Phase Chromatography of Peptides and Proteins	252
5.3.1	Retention Behavior of Peptides and Proteins	252
5.3.2	Gradient Design	252
5.3.3	Organic Modifier	254
5.3.4	Ion Pair Reagent	255
5.3.5	Influence of the pH Value	255
5.3.6	Pore Size	256
5.3.7	Bonding Chemistry	257
5.4	IEC Chromatography of Peptides and Proteins	257
5.4.1	IEC Parameters	259
5.4.1.1	Ionic Strength of the Sample	259
5.4.1.2	Buffer Concentration of the Eluent	259
5.4.1.3	pH Value	259
5.4.1.4	Organic Modifier	259
5.4.1.5	Temperature	259
5.4.1.6	Flow Rate	260
5.4.1.7	Pore Size	260
5.4.1.8	Loading and Injection Volume	260
5.5	Size-Exclusion Chromatography of Peptides and Proteins	261
5.5.1	SEC Parameters	263
5.5.1.1	Particle Size	263
5.5.1.2	Pore Size Distribution	263
5.5.1.3	Pore Volume	264
5.5.1.4	Flow Rate	264
5.5.1.5	Temperature	264
5.5.1.6	Viscosity	264
5.5.1.7	Loading and Injection Volume	264
5.6	Further Types of Chromatography – Brief Descriptions	264

5.6.1	Hydrophobic Interaction Chromatography	264
5.6.2	Hydrophilic Interaction Chromatography	264
5.6.3	Affinity Chromatography (AC)	265
5.7	Summary	266
6	Comparison of Modern Chromatographic Data Systems	267
	<i>Arno Simon</i>	
6.1	Introduction	267
6.2	The Forerunners for CDS	267
6.3	CDS Today	268
6.4	Advantages and Disadvantages of File-Based CDS	268
6.5	Advantages and Disadvantages of Database-Supported CDS	269
6.6	CDS in a Network Environment	270
6.7	Instrument Control	271
6.8	Documentation and Compliance	272
6.9	Brief Overview of Current Systems	273
6.9.1	Atlas	273
6.9.2	ChemStation	273
6.9.3	Agilent OpenLAB CDS	274
6.9.4	Chromeleon	274
6.9.5	Empower	274
6.9.6	EZchrom	274
6.9.7	Tabular Comparison of Empower and Chromeleon	275
6.10	The CDS of Tomorrow	277
6.10.1	MS Integration	277
6.10.2	Large Installation	278
6.10.3	Easy and Intuitive Usability	279
6.11	Special Extensions	279
6.11.1	Support of Peak Integration	279
6.11.2	Column Administration	280
6.11.3	Instrument Usage	280
6.11.4	Connection of Balances	281
6.12	Open Interfaces	282
6.12.1	Instrument Integration	282
6.13	The CDS in 20 Years	283
	Acknowledgment	283
7	Possibilities of Integration Today	285
	<i>Mike Hillebrand</i>	
7.1	Peak Overlay - Effect on the Chromatogram	285
7.2	Separation Techniques for Higher-Level Peaks	286
7.2.1	Lot Method	286
7.2.2	Error by the Vertical Skim Overlapping Peaks (Area Rules to V.R. Meyer)	287

7.2.3	Tangential and Valley-to-Valley Separation Method	288
7.2.4	Gaussian and Exponential Separation Method	288
7.3	Application of Separation Methods	288
7.4	Chromatogramsimulation	289
7.5	Deconvolution	290
7.6	Evaluation of Separation Methods	292
7.7	Practical Application of Deconvolution	294
	References	299
8	Smart Documentation Strategies	301
	<i>Stefan Schmitz</i>	
8.1	Introduction	301
8.2	Objectives of Documentation	303
8.2.1	Documentation from the Organizational Point of View	304
8.2.2	Documentation from the Process Point of View	305
8.2.3	Documentation from the Communication Point of View	307
8.2.4	Documentation from the Information Point of View	309
8.2.5	Documentation from the Knowledge Storage Point of View	310
8.2.6	Regulatory Requirements for Laboratory Documentation	314
8.3	The Life Cycle Model for Regulated Documents in Practice	315
8.4	Dealing with Hybrid Systems Comprising Paper and Electronic Records	317
8.4.1	Advantages and Disadvantages of Paper Versus Electronic Documents	317
8.4.2	Implementation Strategy	319
8.5	Preview	320
	References	321
9	Tips for a Successful FDA Inspection	323
	<i>Stefan Schmitz and Iris Retzko</i>	
9.1	Introduction	323
9.2	Preparation with the Inspection Model	324
9.2.1	Materials, Reagents, and Reference Standards	325
9.2.2	Facilities and Equipment	326
9.2.3	Laboratory Controls	328
9.2.4	Personnel	329
9.2.5	Quality Management	330
9.2.6	Documents and Records	332
9.3	Typical Course of an FDA Inspection	333
9.4	During the Inspection	335
9.4.1	Behavior in Inspections	336
9.4.2	Lab Walkthrough	338
9.4.3	The Inspection in the Audit Room (Front Office)	338
9.4.4	Dealing with Obviously Serious Observations	339
9.4.5	Documentation of Observations on Form FDA 483	340

9.5	Post-Processing of the Inspection	341
	Further Readings	341
10	HPLC – Link List	343
	<i>Torsten Beyer</i>	
10.1	Chemical Data	343
10.2	Applications/Methods	344
10.2.1	Authorities and Institutions	344
10.2.2	Manufacturers of Analytical Instruments and Columns	344
10.2.3	Journals and Web Portals	345
10.3	Troubleshooting	345
10.4	Background Information and Theory	346
10.5	Literature	347
10.5.1	Publishing Companies for Journals, Books, and Databases	347
10.5.2	Scientific Journals (Full Access with Costs)	347
10.5.3	OpenAccess Journals	349
10.5.4	Free Commercial Journals and Web Pages with Focus on Chromatography	349
10.5.5	Literature Search Engines	350
10.6	Databases with Costs	350
10.6.1	STN Databases	350
10.6.2	Data on Chemical Media	350
10.6.3	Literature	350
10.7	Apps	350
10.8	Social Media	351
10.9	Twitter Pages (Examples)	351
10.10	Facebook Pages (Examples)	351
	Index	353