

Contents

Preface to the Fifth Edition	xiii
Important and Useful Equations for HPLC.	1
1 Introduction	5
1.1 HPLC: A powerful separation method	5
1.2 A first HPLC experiment	5
1.3 Liquid chromatographic separation modes	8
1.4 The HPLC instrument.	9
1.5 Safety in the HPLC laboratory	10
1.6 Comparison between high-performance liquid chromatography and gas chromatography	11
1.7 Comparison between high-performance liquid chromatography and capillary electrophoresis	12
1.8 Units for pressure, length and viscosity	13
1.9 Scientific journals	14
1.10 Recommended books	15
2 Theoretical Principles	17
2.1 The chromatographic process	17
2.2 Band broadening	19
2.3 The chromatogram and its purport	23
2.4 Graphical representation of peak pairs with different degree of resolution	30
2.5 Factors affecting resolution	35
2.6 Extra-column volumes (dead volumes)	40
2.7 Tailing	41
2.8 Peak capacity and statistical resolution probability	46
2.9 Effects of temperature in HPLC	49
2.10 The limits of HPLC	51
2.11 How to obtain peak capacity.	55

3	Pumps	59
3.1	General requirements	59
3.2	The short-stroke piston pump	59
3.3	Maintenance and repair	62
3.4	Other pump designs	63
4	Preparation of Equipment up to Sample Injection	65
4.1	Selection of the mobile phase	65
4.2	Preparation of the mobile phase	67
4.3	Gradient systems	68
4.4	Capillary tubing	70
4.5	Fittings	72
4.6	Sample injectors	74
4.7	Sample solution and sample volume	78
5	Solvent Properties	81
5.1	Table of organic solvents	81
5.2	Solvent selectivity	83
5.3	Miscibility	83
5.4	Buffers	84
5.5	Shelf life of mobile phases	87
5.6	The mixing cross	88
6	Detectors	91
6.1	General	91
6.2	UV detectors	96
6.3	Refractive index detectors	99
6.4	Fluorescence detectors	101
6.5	Electrochemical (amperometric) detectors	103
6.6	Light-scattering detectors	104
6.7	Other detectors	106
6.8	Multiple detection	107
6.9	Indirect detection	108
6.10	Coupling with spectroscopy	109
7	Columns and Stationary Phases	117
7.1	Columns for HPLC	117
7.2	Precolumns	119
7.3	General properties of stationary phases	120
7.4	Silica	125
7.5	Chemically modified silica	126
7.6	Styrene-divinylbenzene	129

Contents	ix
7.7	Some other stationary phases 133
7.8	Column care and regeneration. 136
8	HPLC Column Tests 141
8.1	Simple tests for HPLC columns 141
8.2	Determination of particle size 143
8.3	Determination of breakthrough time. 144
8.4	The test mixture 146
8.5	Dimensionless parameters for HPLC column characterization 148
8.6	The van Deemter equation from reduced parameters and its use in column diagnosis 152
8.7	van Deemter curves and other coherences 153
8.8	Diffusion coefficients. 155
9	Adsorption Chromatography: Normal-Phase Chromatography 159
9.1	What is adsorption? 159
9.2	The elutropic series. 162
9.3	Selectivity properties of the mobile phase. 165
9.4	Choice and optimization of the mobile phase 166
9.5	Applications 168
10	Reversed-Phase Chromatography 173
10.1	Principle 173
10.2	Mobile phases in reversed-phase chromatography 174
10.3	Solvent selectivity and strength 177
10.4	Stationary phases 181
10.5	Method development in reversed-phase chromatography 185
10.6	Applications 188
10.7	Hydrophobic interaction chromatography. 191
11	Chromatography with Chemically Bonded Phases 195
11.1	Introduction 195
11.2	Properties of some stationary phases 195
11.3	Hydrophilic interaction chromatography 200
12	Ion-Exchange Chromatography 203
12.1	Introduction 203
12.2	Principle 203
12.3	Properties of ion exchangers. 204

12.4	Influence of the mobile phase	207
12.5	Special possibilities of ion exchange	208
12.6	Practical hints	210
12.7	Applications	213
13	Ion-Pair Chromatography	217
13.1	Introduction	217
13.2	Ion-pair chromatography in practice.	218
13.3	Applications	220
13.4	Appendix: UV detection using ion-pair reagents	221
14	Ion Chromatography	225
14.1	Principle	225
14.2	Suppression techniques	226
14.3	Phase systems	226
14.4	Applications	230
15	Size-Exclusion Chromatography	231
15.1	Principle	231
15.2	The calibration chromatogram.	234
15.3	Molecular mass determination by means of size-exclusion chromatography.	238
15.4	Coupled size-exclusion columns.	241
15.5	Phase systems	243
15.6	Applications	244
16	Affinity Chromatography.	249
16.1	Principle	249
16.2	Affinity chromatography as a special case of HPLC	251
16.3	Applications	252
17	Choice of Method	255
17.1	The various possibilities	255
17.2	Method transfer	260
18	Solving the Elution Problem	263
18.1	The elution problem	263
18.2	Solvent gradients	264
18.3	Column switching	270
18.4	Comprehensive two-dimensional HPLC	272

Contents	xi
18.5 Optimization of an isocratic chromatogram using four solvents	273
18.6 Optimization of the other parameters	276
18.7 Mixed stationary phases	284
19 Analytical HPLC	285
19.1 Qualitative analysis	285
19.2 Trace analysis	287
19.3 Quantitative analysis	291
19.4 Recovery	296
19.5 Peak-height and peak-area determination for quantitative analysis	299
19.6 Integration errors.	303
19.7 The detection wavelength	304
19.8 Derivatization.	306
19.9 Unexpected peaks: Ghost and system peaks	308
20 Quality Assurance	311
20.1 Is it worth the effort?	311
20.2 Verification with a second method	312
20.3 Method validation	312
20.4 Standard operating procedures	314
20.5 Measurement uncertainty	315
20.6 Qualifications, instrument test and system suitability test	317
20.7 The quest for quality	318
21 Preparative HPLC	321
21.1 Problem	321
21.2 Preparative HPLC in practice.	322
21.3 Overloading effects	325
21.4 Fraction collection	328
21.5 Recycling	330
21.6 Displacement chromatography	331
22 Separation of Enantiomers	333
22.1 Introduction	333
22.2 Chiral mobile phases	335
22.3 Chiral liquid stationary phases	336
22.4 Chiral solid stationary phases	337
22.5 Indirect separation of enantiomers	345

23	Special Possibilities.	349
23.1	Micro, capillary and chip HPLC	349
23.2	High-speed and super-speed HPLC	352
23.3	Fast separations at 1000 bar: UHPLC	353
23.4	HPLC with supercritical mobile phases	355
23.5	HPLC with superheated water	359
23.6	Electrochromatography	361
24	Appendix 1: Applied HPLC Theory	363
25	Appendix 2: How to Perform the Instrument Test	373
25.1	Introduction	373
25.2	Test sequence	373
25.3	Preparations	374
25.4	Pump test.	377
25.5	UV detector test	379
25.6	Autosampler test.	383
25.7	Column oven test	383
25.8	Equations and calculations.	384
25.9	Documentation.	385
26	Appendix 3: Troubleshooting	387
26.1	Pressure problems	387
26.2	Leak in the pump system	389
26.3	Deviating retention times	389
26.4	Injection problems	390
26.5	Baseline problems	390
26.6	Peak shape problems	392
26.7	Problems with light-scattering detectors	393
26.8	Other causes	394
26.9	Instrument test	395
27	Appendix 4: Column Packing	397
	Index of Separations	401
	Subject Index	403