

Contents

Preface	v
Chapter 1	
Introduction	1
Chapter 2. Theory of high-performance liquid chromatography (Brief summary)	
Fundamental chromatographic relationships	7
Retention parameters	7
Retention volume	8
Column performance parameters	9
Characterization of separation methods based on the interactions of the solutes with the mobile and stationary phases	15
Normal-phase chromatography	15
Separations in reverse-phase systems	19
References	27
Chapter 3. Column selection	
The separation column	29
Stationary phases for high-performance reverse-phase chromatography	30
Silica-based stationary phases	30
Polymer-based reverse-phases	41
Special reverse-phase packings	43
Mixed stationary phases	43
Stationary phases used for hydrophobic interaction chromatography	46
Stationary phases used for affinity chromatography	47
Chiral stationary phases	50
Stationary phases used for ion-exchange chromatography	55
Functional groups and acid-base properties of ion exchanges	55
Properties of matrix	55
Ion-exchange capacity and porosity	56
Stationary phases used for size-exclusion chromatography	57
Packings for size-exclusion chromatography	57
Practical problems in size-exclusion chromatography	58
References	61

Chapter 4. Mobile phase selection

Solvent selectivity—isoelutropic mobile phases	65
Choice of eluents for reverse-phase chromatography	67
Influence of mobile phase selection	67
Influence of solute structure	68
Effect of added organic amines on reverse-phase separations	68
Application of various amines as mobile phase additives for alkaloid separations	69
Silanol masking procedure	69
Regular retention behavior due to silanol masking	72
Silanophilic interactions and selectivity	74
Evaluation of column performance in reverse-phase chromatography	75
Column selectivity of reverse-phase packing	78
Silanol activity	80
Hydrophobicity of bonded phases	84
Column performance assessment—Conclusions	84
How to assess column performance	93
References	95

Chapter 5. Special methods and techniques used in reverse-phase chromatography

Reverse-phase ion-pair chromatography on chemically bonded stationary phases	99
Factors influencing ion-pair separations	99
Adsorption of ion-pair reagent on the surface of chemically bonded reverse-phases	112
Optimization of the RP-IPC procedure	112
Application of reverse-phase ion-pair chromatography for diverse analytical separation purposes	114
Chiral separations	115
Comparison of different chiral separation modes	118
Cyclodextrin inclusion complex formation	124
Strategies for determining drug enantiomers in biological fluids with coupled column liquid chromatography	138
Indirect detection methods	142
Basic principles	142
Design of the detection system	146
Detection sensitivity	146
Application of indirect detection in reverse-phase chromatography	147
Indirect fluorescence and electrochemical detection	147
Multicolumn operation	149
Sample cleanup using on-line multidimensional techniques	149
Multimode HPLC	151

Isolation and characterization of biologically active peptides and proteins (biopolymers) aided by reverse-phase HPLC	160
References	176
Chapter 6. Optimization in reverse-phase chromatography	
Fundamental aspects and principles of phase system optimization in reverse-phase chromatography	181
Most important considerations for initial (trial) separations	182
Optimization of the separation system	183
Peak purity assessment	214
Practical aspects of phase system optimization	216
Prechromatographic considerations	216
Selection of experimental conditions for initial (trial) separations	223
Evaluation of experimental data obtained in the initial experiment	242
Phase system optimization	242
Selection of a suitable optimization method	252
Brief summary of optimization methods with regard to practical applicability	253
System optimization	260
Other practical considerations for phase system optimization in reverse-phase chromatography	260
Mobile phase effects	260
Stationary phase effects—Column deterioration	276
Unsatisfactory reproducibility	276
References	279
Chapter 7. Method validation	
Definitions	281
Validation research	281
Validation routine	288
Analytical performance parameters	288
Practical approaches to select analytical performance parameters for method validation	290
Practical aspects to yield quantitative data	290
Evaluation of experimental data	294
Ruggedness testing	298
Type-A investigations	299
Type-B investigations	299
Checking sample preparation for ruggedness—Type-B-1 investigations	300
Checking chromatographic separation for ruggedness—Type-B-2 investigations	306
Planning and evaluation of ruggedness tests	311
Practice of method validation	314
Preinformation required to start validation experiments	315
Specific Knowledge of the Analyst	320

Evaluation of the validation plans	320
Master sample collection for validation experiments	320
Required level of method validation	320
Validation research	321
Validation of sample preparation	322
Validation routine	334
Quality assurance of the experimental data—Documentation	339
Method validation package	340
Analytical report	340
Standard operating procedures for satisfactorily validated HPLC methods according to GLP requirements	340
HPLC method validation data for registering drugs	344
References	347
Index	349