Contents

List of Contributors *xiii*
Foreword *xv*
The Structure of "The HPLC-Expert 2" *xvii*

1 When Should I Use My UHPLC as a UHPLC? *1*
Stavros Kromidas
1.1 Introduction *1*
1.2 What Do I Want to Achieve and What Is a UHPLC Capable of? *2*
1.2.1 What Do I Want to Achieve? *2*
1.2.2 What Is a UHPLC Capable of? *2*
1.3 What Is Required from an HPLC Method? *3*
1.3.1 Separate Well *3*
1.3.2 Separate Fast *12*
1.3.3 Improve Mass Sensitivity *13*
1.3.4 Robust Separations in Routine Use *15*
1.4 The UHPLC in Routine Use – A Brief Report *17*
1.5 How Can the Potential of UHPLC Effectively Be Fully Exploited? (See Also Chapters 2, 3, and 9) *20*
1.5.1 Dead Volumes *20*
1.6 Summary and Outlook *22*
1.6.1 Outlook *24*
References *25*

Part I Hardware and Software, Separation Modes, Materials *27*

2 The Modern HPLC/UHPLC Device *29*
2.1 The Modern HPLC/UHPLC System *29*
Steffen Wiese and Terence Hetzel
2.1.1 Today's Demands on the Individual Modules *29*
2.1.1.1 Overview *29*
2.1.2 UHPLC Pump Technology *30*
2.1.2.1 High- and Low-Pressure Pumps *30*
2.1.2.2 Gradient Delay Volume *34*

2.1.3 Autosampler *35*
2.1.3.1 Fixed-Loop Autosamplers *36*
2.1.3.2 Flow-Through Autosamplers *38*
2.1.3.3 Review of the Advantages and Disadvantages of Fixed-Loop and Flow-Through Autosamplers *40*
2.1.4 Column Oven *41*
2.1.5 Detectors *44*
2.1.6 Capillaries and Fittings *47*
Acknowledgment *50*
References *50*
2.2 The Thermostate of Columns – A Minor Matter *52*
Michael Heidorn and Frank Steiner
2.2.1 Thermal Modes of Column Thermostats *54*
2.2.2 Temperature Differences between Column and Mobile Phase *57*
2.2.3 Frictional Heat – Just a Phenomenon in UHPLC? *62*
2.2.4 Thermostatic Control in Method Transfer, Method Speed-Up, and Method Development *68*
Literature *71*

3 The Issue of External Band Broadening in HPLC/UHPLC Devices *73*
Monika Dittmann
3.1 Introduction *73*
3.2 Theoretical Background *74*
3.2.1 Efficiency and Resolution of Modern UHPLC Columns *74*
3.2.2 Estimation of Column Peak Volumes *76*
3.3 Extracolumn Dispersion in (U)HPLC Systems *78*
3.3.1 Sources of External Band Broadening in HPLC/UHPLC Systems *78*
3.3.1.1 Injection Systems *79*
3.3.1.2 Tubing *80*
3.3.1.3 Fittings and Connections *83*
3.3.1.4 Heat Exchangers *84*
3.3.1.5 Detection *85*
3.3.2 Determination of External Band Broadening *88*
3.3.2.1 Analysis of Extracolumn Volume without Column (Short Circuit Method) *88*
3.3.2.2 Analysis of Extracolumn Volume Including a Column *89*
3.4 Impact of External Contributions in Different Application Areas *90*
3.4.1 Impact on Isocratic Separations *90*
3.4.2 Impact on Gradient Separations *92*
3.5 Optimization of HPLC/UHPLC Systems *94*
3.5.1 Testing of Column Performance *95*
3.5.2 Other Isocratic Separations *95*
3.5.3 High-Resolution Gradient Separations *96*
3.5.4 Fast Gradient Separations *96*
3.6 Conclusions *97*
References *98*

4 The Gradient; Requirements, Optimal Use, Hints, and Pitfalls *101*
Frank Steiner
4.1 Instrumental Influences in Gradient Elution – An Overview *101*
4.1.1 The Gradient Delay Effect and the Gradient Dwell Volume of a System *101*
4.1.2 The Role and Function of the Gradient Mixer *103*
4.1.3 Deviations from Ideal Behavior of Gradient Generation Resulting from Fundamental Physicochemical Phenomena *106*
4.1.4 Instrumental Influences on Gradient Elution Outside the Pump *112*
4.1.5 The Stress and Wear on Columns in Gradient Methods *115*
4.2 Gradient Elution Technology and How to Systematically Characterize Gradient Instrumentation *117*
4.2.1 Physicochemical Effects of High Pressure on Liquids *117*
4.2.2 The Need of Solvent Degassing *120*
4.2.3 The Different Types of Pump Technology (Serial, Parallel, Cam Drive, Linear Drive) and Their Specific Properties and Requirements *122*
4.2.4 The Specific Gradient Pump Type and Its Implications for Practical Operation *125*
4.2.5 HPG Pumps and How Discontinuous Pump Cycles Resulting from Pressure Pulsation Impact Retention Time Precision in Practice *127*
4.2.6 LPG Pumps and How Their Immanent Discontinuous Generation of Gradient Composition May Impact Retention Time Precision in Practice *132*
4.2.7 Thermal Effects in Gradient Pumps and How Intelligent Instrument Control Can Minimize the Consequences on Chromatography *134*
4.2.8 Ultrafast Methods with Very Steep or Ballistic Gradients *137*
4.2.9 Fundamental Considerations on the Determination of a Gradient Delay Volume *143*
4.2.10 The Marker Pulse Method as a Quick Way for GDV Determination *145*
4.2.11 The Dolan Test as the Classical Method for GDV Optimization *148*
4.2.12 Designs of Mixers and Their Effectiveness Relative to Their GDV Contribution *150*
4.2.13 Systematic Characterization of the Mixing Efficiency and Gradient Formation of a Pump *155*
4.2.14 Optimizing the Mixing Volume in Dependence of Pump Type and Flow Rate for Demanding Applications Such as TFA Gradients *162*
4.2.15 Exceptional Elution Behavior of Proteins with Mobile-Phase Mixing Ripples *168*
References *169*

5 Requirements of LC-Hardware for the Coupling of Different Mass Spectrometers *171*
Terence Hetzel, Thorsten Teutenberg, Christoph Portner, and Jochen Tuerk
5.1 Introduction *171*
5.2 From Target Analysis to Screening Approaches *171*

5.2.1 Target Analysis *171*
5.2.2 Suspected-Target Screening *172*
5.2.3 Nontarget Screening *172*
5.3 What Should Be Considered for UHPLC/MS Hyphenation? *173*
5.3.1 The Interface and the Optimum Flow Rate *173*
5.3.2 Optimization of MS Parameters *174*
5.3.3 Optimization of the Chromatographic Parameters *174*
5.3.4 Choice of the Suitable Column and Column Dimension *176*
5.4 Target Analysis Using Triple-Quadrupole Mass Spectrometry *178*
5.5 Screening Approaches Using LC-MS *185*
5.6 Miniaturization – LC-MS Quo Vadis? *189*
References *192*

6 2D chromatography – Opportunities and limitations *193*
Thorsten Teutenberg and Juri Leonhardt
6.1 Introduction *193*
6.2 Why Two-Dimensional HPLC? *193*
6.3 Peak Capacity of One- and Two-Dimensional Liquid Chromatography *195*
6.3.1 Peak Capacity of One-Dimensional Liquid Chromatography *195*
6.3.2 Peak Capacity of Two-Dimensional Liquid Chromatography *196*
6.3.2.1 Heart-Cut 2D LC (LC-LC) *196*
6.3.2.2 Comprehensive 2D LC (LC × LC) *197*
6.4 Modulation *200*
6.4.1 Online Heart-Cut 2D LC *200*
6.4.2 Comprehensive Online 2D LC *200*
6.4.3 Stop-Flow and Offline LC × LC *202*
6.5 Practical Problems of Online LC × LC *203*
6.5.1 Compatibility of the Solvent Systems *203*
6.5.2 Dilution *203*
6.5.3 High Flow Rate *203*
6.5.4 Compatibility with Mass Spectrometry *203*
6.6 Development of a Miniaturized LC × LC System *204*
6.6.1 Technical Platform *204*
6.6.2 Selection of the Stationary Phase *204*
6.6.3 Selection of the Mobile Phase and Temperature *205*
6.6.4 Column Dimension and Modulation *205*
6.6.5 Gradient Programming and Overall Analysis Time *206*
6.6.6 Coupling with Mass Spectrometry *206*
6.7 Real Applications *207*
6.7.1 Measurement of a Reference Standard *207*
6.7.2 Measurement of a Real Sample *209*
6.8 Advantages of the MS/MS Functionality *211*
6.9 General Comments to Specific Aspects of an LC × LC System *211*

6.9.1 Offline LC × LC versus Online LC × LC *211*
6.9.2 Stop-Flow LC × LC *214*
6.9.3 Multiple Heart-Cut LC-LC and Selected LC × LC (sLC × LC) *214*
6.10 Method Development and Gradient Programming *215*
6.11 Presentations of the Instrument Manufacturers (in Alphabetical Order) *215*
6.11.1 Commercially Available Solutions for LC × LC *216*
6.11.1.1 Agilent *216*
6.11.1.2 Shimadzu *216*
6.11.1.3 Thermo/Dionex *216*
6.11.2 Further Systems *216*
6.11.2.1 Sciex *216*
6.11.2.2 Waters *216*
6.12 2D LC – Quo Vadis? *217*
6.12.1 Software *217*
6.12.2 System Setup *217*
6.12.3 Peak versus Peak Capacity *218*
References *219*

7 Materials in HPLC and UHPLC – What to Use for Which Purpose *223*
Tobias Fehrenbach and Steffen Wiese
7.1 Introduction *223*
7.2 Requirements for Materials in UHPLC *225*
7.2.1 Mechanical Stability *225*
7.2.2 Chemical Stability *225*
7.2.3 Analyte Compatibility/Biocompatibility *226*
7.3 Flow Paths in UHPLC Systems *227*
7.3.1 Low-Pressure and High-Pressure Flow Path *227*
7.3.2 Mobile-Phase and Sample Flow Path *228*
7.4 Low-Pressure Flow Path *229*
7.5 High-Pressure Flow Path *231*
7.5.1 Pumps *231*
7.5.1.1 Inlet and Outlet Valves *231*
7.5.1.2 Pump Head *233*
7.5.1.3 Pump Pistons and Piston Seals *236*
7.5.1.4 Practical Aspects *237*
7.5.2 Autosamplers *238*
7.5.2.1 Materials *238*
7.5.2.2 Sample Needles, Sample Vials, and Closures *238*
7.5.2.3 Injection Valves *239*
7.5.2.4 Practical Aspects *241*
7.5.3 Tubing and Fitting Systems *242*
7.5.3.1 Outline *242*
7.5.3.2 Materials *243*

7.5.3.3 Tubing *244*
7.5.3.4 Fitting Systems *246*
7.6 When and Why Can an Inert UHPLC System Be Required? *248*
7.6.1 Concept of Inertness *248*
7.6.1.1 General Inertness *248*
7.6.1.2 Analyte-Specific Inertness *249*
7.6.2 Nature of the Passive Layer *249*
7.6.2.1 Passive Layers of Chromium Alloys *251*
7.6.2.2 Passive Layers of Titanium Alloys *252*
7.6.3 Requirements and Interactions *253*
7.6.3.1 Mechanical and Physical Integrity of the UHPLC System *253*
7.6.3.2 Requirement of the Detection Method *254*
7.6.3.3 Interaction of Analyte and UHPLC System *254*
7.6.4 Passivation Strategies and Methods *258*
References *261*

Part II Experience Reports, Trends *269*

8 What a Software has to Possess in Order to Use the Hardware Optimally *271*
Arno Simon
8.1 Functionality and Handling *271*
8.1.1 Integration *272*
8.1.2 Instrument Control *273*
8.1.3 Useability *274*
8.1.4 Ease of Use *274*
8.1.5 User Interface *275*
8.1.6 Multilingual *276*
8.2 Data Exchange *277*
8.2.1 Import and Export of Data *278*
8.3 From PCs Scalability to Global Installation *278*
8.3.1 Software Placement *278*

9 Aspects of the Modern HPLC Device – Experience Report of an Operator *281*
Steffen Wiese and Terence Hetzel
9.1 Introduction *281*
9.2 Determination of the Gradient Delay Volume *281*
9.3 High-Throughput Separations *285*
9.4 Method Transfer between UHPLC Systems of Different Manufacturers *287*
9.5 Application of Elevated Temperatures *290*
9.6 Large-Volume Injection (LVI) *293*
9.7 UHPLC Separation with 1 mm ID Columns *296*
Acknowledgment *299*
References *299*

10 Experiences of an Independent Service Engineer – Hints and Recommendations for an Optimal Operation of Agilent and Waters-Devices *301*
Siegfried Chroustovsky
10.1 Introduction *301*
10.2 The Degasser, Principles *301*
10.2.1 Different Manufacturers, Different Concepts *302*
10.3 The Pump, Principles *303*
10.3.1 Different Manufacturers, Different Concepts *305*
10.4 The Autosampler, Principles *306*
10.4.1 Different Manufacturers, Different Concepts *307*
10.5 The UV Detector, Principles *308*
10.5.1 Different Manufacturers, Different Concepts *309*

11 The Analyte, the Question, and the UHPLC – The Use of UHPLC in Practice *311*
Stefan Lamotte
11.1 Introduction *311*
11.2 When Does It Make Sense to Use UHPLC and When Should I Better Use Conventional HPLC? *311*
11.3 Dissolution Tests in Pharmaceutical Industry *313*
11.4 Method Development and Optimization *314*
11.5 Typical "Classical" Liquid Chromatographic Analysis *314*
11.6 Fast (Most Second) Dimension of Multidimensional Chromatography *315*
11.7 Separation of (Bio)polymers *316*
11.8 Process Analysis (PAT) *316*
11.9 Conclusion *316*
References *316*

12 Report of Device Manufacturers – Article by Agilent, Shimadzu, and ThermoScientific *319*
12.1 Agilent Technologies *319*
Jens Trafkowski
References *328*
12.2 HPLC Current Status and Future Development *328*
Björn-Thoralf Erxleben
12.3 Thermo Fisher Scientific, Germering *334*
Frank Steiner
12.3.1 Total System Requirements and Related Key Experiences *334*
12.3.1.1 NanoLC *335*
12.3.1.2 HPLC and UHPLC on Two Instrumental Platforms (UltiMate 3000, Vanquish) *336*
12.3.1.3 Viper-Based System Tubing *338*
12.3.2 The Contribution of the Individual Components to the Success of a System *338*

12.3.2.1 The Flow Delivery Device – Much More Than a High Pressure Pump *339*
12.3.2.2 The Injector and Liquid Handling Devices for Robust and Ultra-Precise Sample Dosage Even in High-Throughput Workflows *340*
12.3.2.3 New Ways of Column Thermostatting to Combine Highest UHPLC Column Efficiency and Best Method Transfer Capabilities *342*
12.3.2.4 How to Detect Fast and Ultraefficient UHPLC Separations *344*
12.3.3 2D-LC and Alternative Ways to Increase Productivity for Analyzing Complex Samples – and Outlook to Changing Paradigms *346*

About the Authors *349*

Index *355*