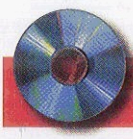
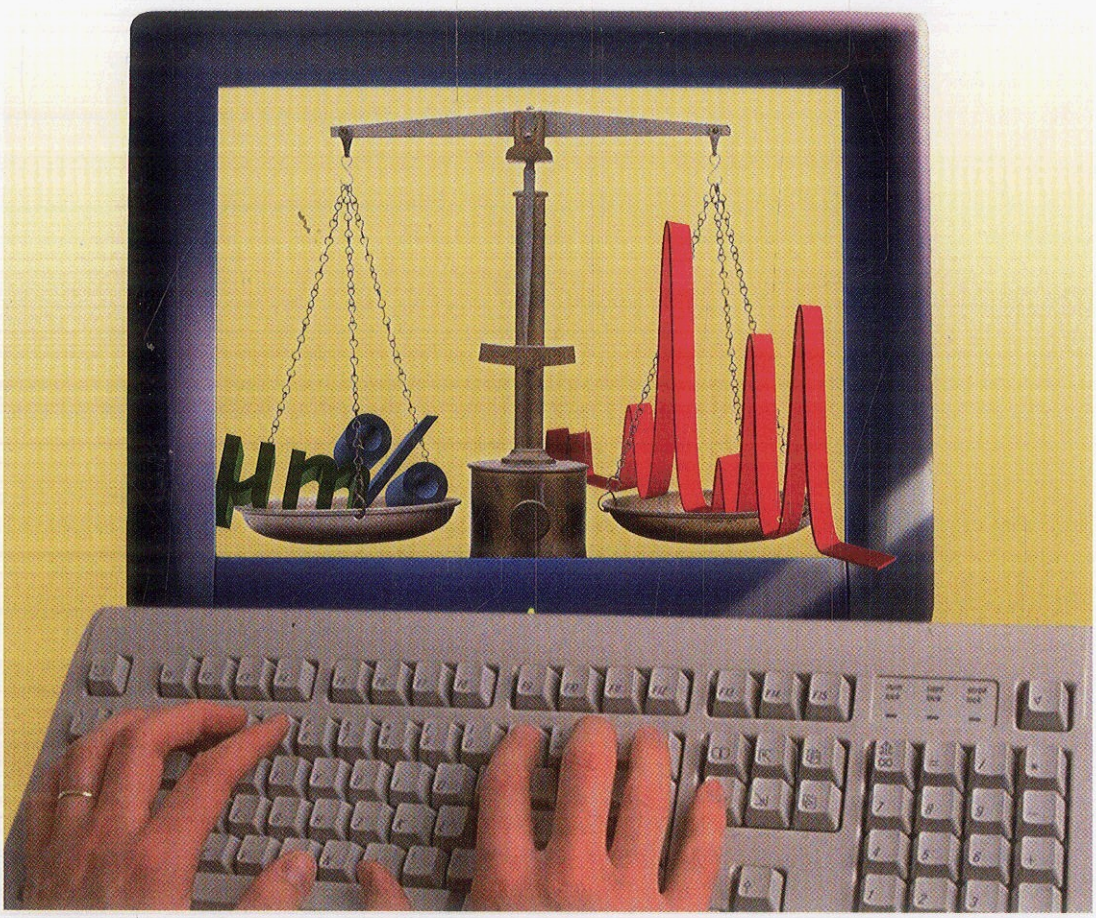
Edited by Hans-Joachim Kuss
and Stavros Kromidas

WILEY-VCH

Quantification in LC and GC

A Practical Guide to
Good Chromatographic Data





Contents

Preface *XIII*

List of Contributors *XV*

Structure of the Book *XVII*

Part 1 Evaluation and Estimation of Chromatographic Data *1*

1 Evaluating Chromatograms *3*
Hans-Joachim Kuss and Daniel Stauffer
1.1 Efficiency *4*
1.2 EMG Model *5*
1.3 Chromatogram *7*
1.4 Selectivity *8*
References *8*

2 Integration Parameters *9*
Daniel Stauffer and Hans-Joachim Kuss
2.1 Peak Recognition Methods *9*
2.1.1 The Classical Method *9*
2.1.2 Alternative Method *12*
2.2 Integration and Integration Parameters *12*
2.2.1 Data Acquisition and Integration with Empower 2 *12*
2.2.2 Data Acquisition and Integration with Chromeleon *14*
2.2.3 Data Acquisition and Integration with EZChrom Elite *14*
2.2.4 Data Recording and Integration with ChemStation *16*

3 Integration Errors *17*
Hans-Joachim Kuss
3.1 What Does the Literature Say on Integration Errors? *17*
3.2 Integration in Routine Practice *19*

Quantification in LC and GC: A Practical Guide to Good Chromatographic Data
Edited by Hans-Joachim Kuss and Stavros Kromidas

ISBN: 978-3-527-32301-2

3.2.1 Integration, Simple and Automatically Feasible? *20*
3.2.2 Comparison of Integration Systems with a Small Number of Tall Peaks *20*
3.2.3 Comparison of Integration Systems for Numerous Small Peaks *25*
3.3 Chromatogram Simulation *28*
3.3.1 Simulation of a Digital Chromatogram *29*
3.3.1.1 One Peak *30*
3.3.1.2 Several Peaks *32*
3.3.1.3 Noise *32*
3.3.1.4 Drift *33*
3.3.1.5 Peak Area *34*
3.3.1.6 Peaks Merged Together *34*
3.3.1.7 Unclear Baseline *37*
3.3.1.8 Tailing *37*
3.3.1.9 Data Point Interval *37*
3.3.1.10 Other Characteristic Quantities *43*
3.3.1.11 Gas Chromatogram *44*
3.3.1.12 Applications of the Simulation *45*
3.3.2 Simulation of a Standard Curve *47*
3.3.2.1 Tenfold Simulation at the Limit of Quantification *55*
3.3.3 Simulation of an Isocratic Chromatogram *55*
3.3.4 Simulation of a Gradient Chromatogram *63*
3.3.5 By-Product Analysis *68*
3.3.6 Post-Simulation of Real Chromatograms *78*
References *82*

4 Simulation of Chromatograms *83*
Uwe D. Neue
4.1 Introduction *83*
4.2 Peak Simulation *84*
4.2.1 Symmetrical Peaks *84*
4.2.2 Peak Tailing *85*
4.3 The Baseline *88*
4.4 The Chromatogram *89*
4.5 Simple Retention Modeling and Real Chromatograms *91*
4.5.1 Isocratic Chromatography *91*
4.5.2 Gradient Chromatography *95*
4.6 Outlook *102*
References *103*

5 Integration of Asymmetric Peaks *105*
Hans-Joachim Kuss
5.1 The Valley Between Merged Peaks *108*
5.2 Small Peak Between Larger Ones *110*
5.3 Peak Pairs *112*

5.4 Simulation of a Calibration *113*
5.5 Exponential Skim *117*
5.6 Integration of Merged Peaks *120*
5.7 Integration in Daily Practice *121*
References *122*

6 Deconvolution *123*
Mike Hillebrand
6.1 Introduction *123*
6.1.1 *Software Applications for Deconvolution* *126*
6.2 Influences on Deconvolution *127*
6.2.1 The Baseline Path *128*
6.2.2 Peak Homogeneity – Number of Peaks *128*
6.2.3 Noise *129*
6.2.4 Peak Symmetry *129*
6.3 Deconvolution of Gaussian-Shaped Peaks *129*
6.3.1 Westerberg Report Simulation *132*
6.4 Deconvolution of Peaks with Tailing *134*
6.4.1 Peaks with Different Tailing *137*
6.5 Deconvolution of Gradient Runs *141*
6.5.1 Complex Baseline *141*
6.6 Real Chromatogram *147*
6.7 Outlook *149*
References *150*

7 Interpretation of Chromatograms *153*
Hans-Joachim Kuss
7.1 Using the Peak Height *153*
7.2 Evaluation *154*
7.2.1 Evaluation Methods *155*
7.3 Calibration *156*
7.3.1 Linear Regression *157*
7.3.2 Weighted Linear Regression *161*
References *165*

8 General Interpretation of Analytical Data *167*
Joachim Ermer
8.1 (Normal) Distribution of Analytical Data *167*
8.2 Problems of Prediction *171*
8.3 Analytical Variability *174*
8.3.1 Variability Contributions and Precision Levels *174*
8.3.2 System or Injection Precision *176*
8.3.3 Repeatability *178*
8.3.4 Reproducibility *179*
8.3.4.1 Number of Series *180*

8.3.4.2 Analysis of Variances *180*
8.3.4.3 Benchmarking for Reproducibility *182*
8.3.5 Consequences for the Design of an Analytical Procedure *183*
8.3.6 Concentration Dependence of the Precision *185*
8.3.7 Conclusions *187*
8.4 Key Points *188*
References *188*

9 Metrological Aspects of Chromatographic Data Evaluation *191*
Ulrich Panne
9.1 Introduction *191*
9.2 Measurement Uncertainty *193*
9.3 Traceability of Analytical Measurements *205*
References *208*

Part 2 Characterization of the Evaluation of Different Chromatographic Modes *211*

10 Evaluation and Estimation of Chromatographic Data in GC *213*
Werner Engewald (Translator: Mike Hillebrand)
10.1 Introduction *213*
10.2 How Does GC Differ from HPLC? *214*
10.2.1 What Consequences Result from These Differences? *215*
10.2.1.1 *Applicability of GC* *215*
10.2.1.2 Sample Injection *219*
10.2.1.3 Column *219*
10.2.1.4 Detector *221*
10.2.1.5 Fast Gas Chromatography *222*
10.3 Qualitative Analysis *223*
10.3.1 Introductory Remarks *223*
10.3.1.1 Fingerprint Analysis *223*
10.3.1.2 Peak Purity *224*
10.3.2 Comparison of Retention Times *225*
10.3.3 Relative Retention Times *225*
10.3.4 Retention Time Locking (RTL) 226
10.4 GC-MS Coupling *227*
10.4.1 *MS as an Identifying Detector (Scan Mode)* *227*
10.4.2 Use of Spectral Libraries *228*
10.4.3 Spectrum Deconvolution *231*
10.4.4 MS as a Mass-Selective Detector *233*
10.4.4.1 Mass Fragmentography (Reconstructed or Extracted Ion Chromatogram) *233*
10.4.4.2 SIM (Single Ion Monitoring) or MID (Multiple Ion Recording) *234*
10.4.5 Chemical Ionization *235*

10.5 Quantitative Analysis *236*
10.5.1 Setting up an Analysis Sequence *236*
10.6 Isotope Dilution Analysis (IDA) or Stable Isotope Dilution Analysis (SIDA) *237*
10.7 Matrix Effects in Trace Analysis *237*
10.8 Headspace-GC *238*
10.9 Estimation of the Correction Factor with the FID *239*
References *241*

11 Data Evaluation in LC-MS *243*
Hartmut Kirchherr
11.1 Introduction *243*
11.2 Influence of the Matrix in Chromatography *244*
11.3 Internal Standards *252*
11.4 Adjustments to the Mass Spectrometer *255*
11.5 Evaluation Software *258*
References *259*

12 Evaluation of Chromatographic Data in Ion Chromatography *261*
Heiko Herrmann and Detlef Jensen
12.1 Introduction *261*
12.2 Eluents *262*
12.2.1 Purity *262*
12.3 The Water Dip – the Solvent Peak in Ion Chromatography *263*
12.4 Contaminants *266*
12.5 Calibration Functions *268*
References *268*

13 Qualification of GPC/GFC/SEC Data and Results *271*
Daniela Held and Peter Kilz
13.1 Introduction *271*
13.2 Principles of GPC/SEC Data Processing *272*
13.2.1 Calculation of Molar Mass Averages *274*
13.3 Guidelines, Standards and Requirements for GPC/SEC Data Processing *277*
13.4 Validation and Tests for GPC/SEC Data Evaluation *279*
13.4.1 Description of a General Verification Procedure for GPC/SEC Software *279*
13.4.2 Validation of the Molar Mass Distribution Calculation *280*
13.5 Influence of Data Processing, Calibration Methods and Signal Quality on Accuracy and Precision of GPC/SEC Results *284*
13.5.1 Validation of GPC/SEC Evaluation with External Molar Mass Calibration *284*
13.5.2 Validation of GPC/SEC Evaluation with Viscometry Set-ups *285*

13.5.3 Influence of Detector Noise on the Accuracy of GPC/SEC Results *285*
13.5.4 Influence of the Detector Drift on the Accuracy of GPC/SEC Results *287*
13.5.5 Influence of the Number of Data Points on the Accuracy of GPC/SEC Results *288*
13.6 Influence of GPC/SEC Specific Parameters on the Precision and Accuracy of GPC/SEC Results *290*
13.6.1 Influence of Parameters for GPC/SEC with External Molar Mass Calibration *290*
13.6.1.1 Influence of the Calibration Curve *290*
13.6.1.2 Influence of Instrument Parameters (Instrument Performance) *294*
13.6.1.3 Influence of Evaluation Parameters *295*
13.6.2 Influence of Parameters for GPC/SEC with Light Scattering Detection *297*
13.6.2.1 Influence of Instrument Calibration *299*
13.6.2.2 Influence of Instrument Performance *299*
13.6.2.3 Influence of Evaluation Parameters *299*
13.6.3 Repeatability and Reproducibility of GPC/SEC Analyses *299*
13.7 Summary *301*
References *302*

Part 3 Requirements for Chromatographic Data Analysis from the Viewpoint of Organisations and Public Authorities *303*

14 The Science Behind the Pharmaceutical Regulatory Chromatographic Procedures *305*
Linda Ng
14.1 Introduction *305*
14.2 Instrument Qualification *306*
14.3 Chromatographic Procedures *307*
14.4 Method Design, Development and Validation *309*
14.5 Compendial Procedures *311*
14.6 Conclusions *311*
References *312*

15 Interpretation of Chromatographic Data According to the Pharmacopoeias – Control of Impurities *313*
Ulrich Rose
15.1 Outline *313*
15.2 Interpretation of Qualitative Data *313*
15.2.1 System Suitability Test *316*
15.3 Interpretation of Quantitative Data *318*
References *320*

16 ***Requirements of (Chromatographic) Data in Pharmaceutical Analysis*** *321*
Joachim Ermer
16.1 System Suitability Tests *321*
16.1.1 European Pharmacopoeia (EP) *322*
16.1.1.1 Chromatographic Parameters *325*
16.1.1.2 Signal-to-Noise Ratio *326*
16.1.1.3 System Precision *327*
16.1.2 US Pharmacopoeia *328*
16.1.3 FDA Reviewer Guidance *329*
16.2 Acceptance Limits for the Specification and Precision *330*
16.2.1 Assay *330*
16.2.1.1 Based on the Method Capability Index *330*
16.2.1.2 Based on the 95% Prediction Interval (DPhG-Approach) *331*
16.2.2 Impurity Determination *332*
16.2.2.1 Acceptance Limits of the Specification *332*
16.2.2.2 Quantitation Limit and Variability *332*
16.2.3 Key Points *332*
16.3 Interpretation and Treatment of Analytical Data *333*
16.3.1 Prerequisites *333*
16.3.2 Measurement Principles and Variation *333*
16.3.3 Outlying Results *334*
16.3.3.1 Outlier Test According to Hampel *334*
16.3.4 Comparison of Analytical Results *335*
16.3.4.1 Precision *335*
16.3.4.2 Accuracy *337*
16.3.5 Key Points *338*
References *338*

17 **Evaluation and Valuation of Chromatographic Data** *341*
Stavros Kromidas
17.1 Introduction *341*
17.2 The Situation – or Why Does so Little Change? *341*
17.3 How Can Something Change and When is it Really Necessary? *343*
17.4 Who Can Change Something? *345*

Index *347*

Contents of the CD *357*