Edited by Hans-Joachim Kuss
and Stavros Kromidas

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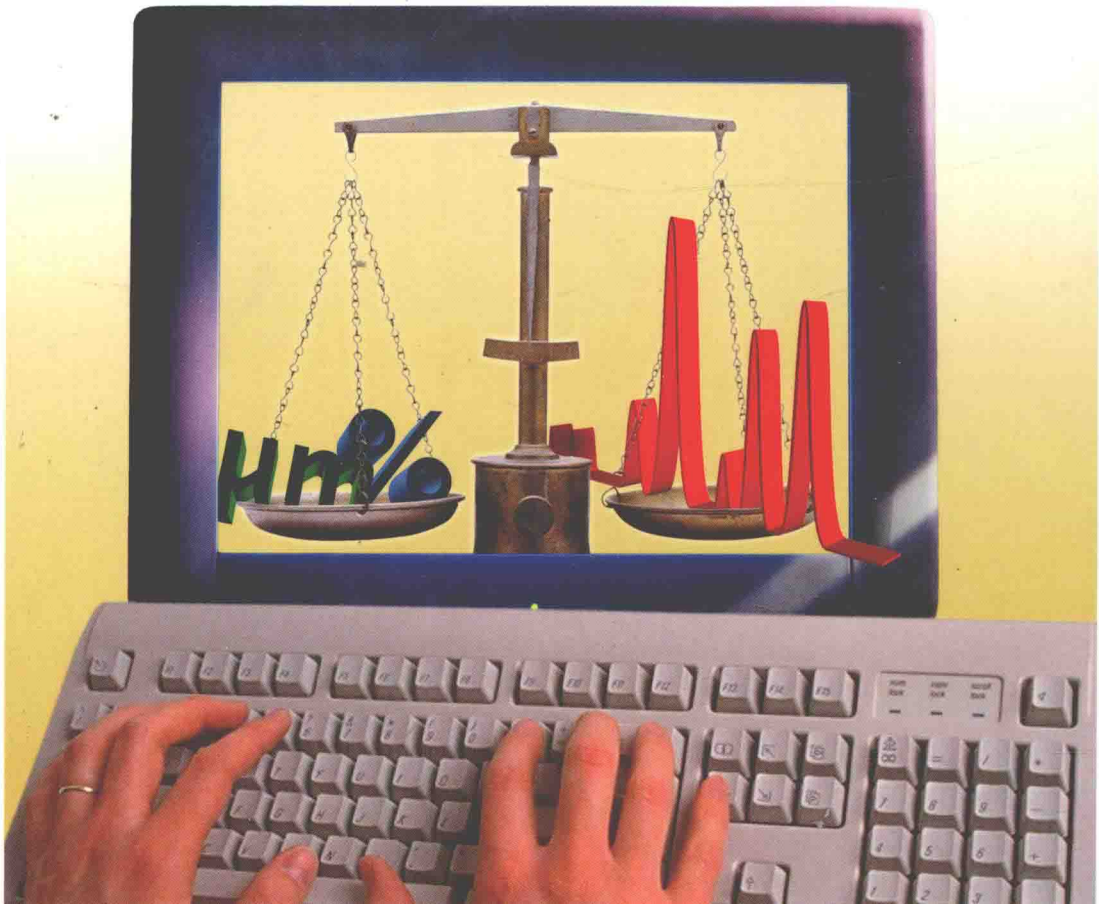
Quantification in LC and GC

A Practical Guide to
Good Chromatographic Data



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A Practical Guide to Good Chromatographic Data

Edited by
Hans-Joachim Kuss and Stavros Kromidas

WILEY-VCH Verlag GmbH & Co. KGaA

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Preface

With chromatography as many substances as possible should be separated from each other as well as possible. A poor separation is not "repairable" later. However, we have the impression that the following evaluation and assessment of the results are not always treated with due caution under everyday stress. One likes to rely on the computer programs – finally the integration software is validated and this is "covered" by the manufacturer's certificate. It is hardly registered as a problem that the integration algorithms have changed only insignificantly for 30 years and that the area of peaks merged together is always determined simply by perpendicular drop. The assessment of the results is often based on consideration of the questions: "Does the c.v. match?" and "Is my value in spec?" Sometimes this may suffice, sometimes not.

We would like to deal with the following questions in this book: How accurate are the integrated areas or heights of commercial systems? Can one check this at all? How important are the integration parameters, what effect do they have on results? However, we also wanted to have a look at the questions: "How can I really judge a result?" How correct can a result be in reality?" and finally: "What do the authorities have to say on this subject, what must I do, what am I free to do?" To this end we have invited experienced colleagues who have made their knowledge available in various areas. Our many thanks are due to these colleagues.

On the one hand, it is the aim of the book to show that one perhaps should not always blindly trust integration systems and that it is worthwhile developing a deeper appreciation of integrating. On the other hand, we want to make a contribution to the critical analysis of chromatographic results, primarily for quantitative statements used as a basis for decisions. We hope to give the reader many suggestions and ideas for finding his own "right" evaluation and assessment practice. We thank the WILEY-VCH publishing house, and particularly Lesley Belfit and Frank Weinreich, for very close cooperation.

Saarbrücken and München,
August 2009

Hans-Joachim Kuss
Stavros Kromidas

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Structure of the Book

Part 1: Evaluation and Estimation of Chromatographic Data

In Part 1 it is shown, how chromatographic data are obtained and how the results are evaluated.

In Chapter 1 (Hans-Joachim Kuss and Daniel Stauffer) it is described how a chromatogram arises and which factors influence the shape, the height and the area of the peaks. The most important equations for Gaussian or exponential modified Gaussian (EMG) peaks are given.

In Chapter 2 (Daniel Stauffer and Hans-Joachim Kuss) the integration parameters are explained: What do they mean and what are they called in different integration systems.

Chapter 3 (Hans-Joachim Kuss) deals mainly with the integration and integration errors. The question considered is the accuracy of commercial integration systems. Furthermore, the possibility of checking this using a chromatogram simulation is described with Gaussian peaks to verify the results.

Chapter 4 (Uwe Neue) treats the simulation of EMG-peaks and gives the theoretical background for peaks in gradient hplc.

Chapter 5 (Hans-Joachim Kuss) describes the special difficulties of the integration of asymmetric (EMG)-peaks.

Chapter 6 (Mike Hillebrand) treats the possibility of "recalculating" merged peaks with the deconvolution method.

Chapter 7 (Hans-Joachim Kuss) treats the calculations for evaluation and calibration, including weighted calibration.

The general criteria for the judgement of analytical data are introduced systematically and analyzed critically in Chapter 8 (Joachim Ermer).

Most of the chromatograms and/or tables in Chapters 3 to 8 can be found on the supplementary CD. The examples are ordered following the book's table of contents. Each time a remark such as "Chromatogram.cdf" or "Table.xls" is found in the text, the corresponding file can be found on the CD.

Part 1 concludes with Chapter 9 (Ulrich Panne) which considers the metrological aspects of analytical data together with a detailed discussion of the measuring uncertainty.

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Part 2: Characterization of the Evaluation of Different Chromatographic Modes

The evaluation of chromatographic results depends on the chromatographic techniques used. An overview is given of some special conditions for: Gas chromatography in Chapter 10 (Werner Engewald), LCMS coupling in Chapter 11 (Hartmut Kirchherr), ion chromatography in Chapter 12 (Heiko Herrmann and Detlef Jensen) and gel permeation chromatography in Chapter 13 (Daniela Held and Peter Kilz).

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

The area regulated most strongly is certainly the pharmaceutical one. The requirements are explained from three different viewpoints: the US guidelines in Chapter 14 (Linda Ng), the EU guidelines in Chapter 15 (Ulrich Rose) and the view of the pharmaceutical industry in Chapter 16 (Joachim Ermer). Instead of an epilogue, the everyday dealing with analysis results is discussed in the final Chapter 17 (Stavros Kromidas).

The individual chapters were written to represent closed modules, but "jumping" between them is possible at any time. It is the aim of the editors to introduce a book of ideas and to give a lot of suggestions so that one can possibly find one's own solutions. Therefore, the divergent opinions of the authors on topics were accepted. Some repetitions were also accepted so as not to impair the harmony in the textual context. The reader may profit from the different representation of the topics and the individual weighting of the authors.

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
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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*

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