Method Validation in Pharmaceutical Analysis

A Guide to Best Practice

Edited by
Joachim Ermer, John H. McB. Miller

WILEY-VCH Verlag GmbH & Co. KGaA

Method Validation in Pharmaceutical Analysis

Edited by

J. Ermer and J. H. McB. Miller

Related Titles from Wiley-VCH:

M. S. Lee

LC/MS Applications in Drug Development

2002

ISBN: 0-471-40520-5

M. Stoeppler, W. R. Wolf, P. J. Jenks (Eds.)

Reference Materials for Chemical Analysis

Certification, Availability, and Proper Usage

2001

ISBN: 3-527-30162-3

J. M. Miller, J. B. Crowther (Eds.)

Analytical Chemistry in a GMP Environment

A Practical Guide

2000

ISBN: 0-471-31431-5

Method Validation in Pharmaceutical Analysis

A Guide to Best Practice

Edited by
Joachim Ermer, John H. McB. Miller

WILEY-VCH Verlag GmbH & Co. KGaA

Edited by

Dr. Joachim Ermer
sanofi-aventis
Industriepark Höchst
Build. G875
65926 Frankfurt
Germany

Dr. John H. McB. Miller
European Directorate for the Quality
of Medicines (EDQM)
16, Rue Auguste Himly
67000 Strasbourg
France

This book was carefully produced. Nevertheless, editors, authors, and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.
applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Printed in the Federal Republic of Germany.
Printed on acid-free paper.

Typesetting Kühn & Weyh, Satz und Medien, Freiburg
Printing betz-druck GmbH, Darmstadt
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN-13: 978-3-527-31255-9
ISBN-10: 3-527-31255-2

Preface

A number of articles and guidelines already exist dealing with the validation of analytical methods. However, the editors consider that none of the texts completely covers all aspects pertinent to analytical validation for, in particular, methods in pharmaceutical analysis. The editors have attempted, with the authors of the relevant chapters, to bring all these elements together in one book that will be useful to both analysts in the pharmaceutical industry (and beyond) as well as to assessors at the registration authorities for medicines.

Methods used in pharmaceutical analysis must be sufficiently accurate, specific, sensitive and precise to conform to the regulatory requirements as set out in the relevant guidelines of "The International Conference of Technical Requirements for the Registration of Pharmaceutical for Human Use " (ICH), which are applied by the licensing authorities and by some pharmacopoeias. The chapters in Part I deal specifically with the fundamentals of the different validation parameters, giving special emphasis to practical examples and recommendations. It is not intended to replace statistical textbooks but the editors have attempted to provide sufficient background information, illustrated by practical examples to aid the reader in understanding and choosing the relevant parameters and acceptance criteria to be considered for the application of any one analytical procedure to a particular purpose.

Contributions to Part II of this book deal with the life-cycle approach to validation starting with the qualification of equipment employed, the adaptation of ICH guidelines to the early stages of drug development, the relation between analytical variability and specification acceptance criteria, the continual assessment of the performance of the methods when in regular use, the transfer of analytical procedures, and out-of-specification results. There are also chapters dealing with the validation of pharmacopoeial methods and future perspectives for validation.

December 2004

John H. McB. Miller
Joachim Ermer

Contents

Preface *V*

List of Contributors *XIII*

Part I Fundamentals of Validation in Pharmaceutical Analysis *1*

1 Analytical Validation within the Pharmaceutical Environment *3*
Joachim Ermer

1.1 Regulatory Requirements *4*
1.2 Integrated and Continuous Validation *5*
1.3 General Planning and Design of Validation Studies *7*
1.3.1 Always Look on the ''Routine' Side of Validation *8*
1.4 Evaluation and Acceptance Criteria *9*
1.4.1 What does Suitability Mean? *9*
1.4.2 Statistical Tests *12*
1.5 Key Points *14*

2 Performance Parameters, Calculations and Tests *21*
2.1 Precision *21*
Joachim Ermer
2.1.1 Parameters Describing the Distribution of Analytical Data *22*
2.1.2 Precision Levels *30*
2.1.3 Acceptable Ranges for Precisions *35*
2.1.4 Sources to Obtain and Supplement Precisions *49*
2.1.5 Key Points *51*
2.2 Specificity *52*
Joachim Ermer
2.2.1 Demonstration of Specificity by Accuracy *55*
2.2.2 Chromatographic Resolution *55*
2.2.3 Peak Purity (Co-elution) *57*
2.2.4 Key Points *62*

2.3 Accuracy 63
Joachim Ermer
2.3.1 Drug Substance 64
2.3.2 Drug Product 67
2.3.3 Impurities/Degradants and Water 71
2.3.4 Cleaning Validation Methods 74
2.3.5 Acceptance Criteria 77
2.3.6 Key Points 79
2.4 Linearity 80
Joachim Ermer
2.4.1 Unweighted Linear Regression 81
2.4.2 Weighted Linear Regression 94
2.4.3 Non-linear and Other Regression Techniques 97
2.4.4 Key Points 98
2.5 Range 99
Joachim Ermer
2.6 Detection and Quantitation Limit 101
Joachim Ermer and Christopher Burgess
2.6.1 Analytical Detector Responses 102
2.6.2 Requirements for DL/QL in Pharmaceutical Impurity Determination 104
2.6.3 Approaches Based on the Blank 108
2.6.4 Determination of DL/QL from Linearity 110
2.6.5 Precision-based Approaches 117
2.6.6 Comparison of the Various Approaches 118
2.6.7 Key Points 119
2.7 Robustness 120
Gerd Kleinschmidt
2.7.1 Terminology and Definitions 120
2.7.2 Fundamentals of Robustness Testing 122
2.7.3 Examples of Computer-assisted Robustness Studies 126
2.8 System Suitability Tests 170
John H. McB. Miller
2.8.1 Introduction 170
2.8.2 Non-chromatographic Techniques 170
2.8.3 Separation Techniques 171

3 Case Study: Validation of an HPLC-Method for Identity, Assay, and Related Impurities 195
Gerd Kleinschmidt

3.1 Introduction 195
3.2 Experimental 197
3.3 Validation Summary 197
3.3.1 Specificity 200
3.3.2 Linearity 200
3.3.3 Precision 200

3.3.4 Accuracy *200*
3.3.5 Detection and Quantitation Limit *201*
3.3.6 Robustness *201*
3.3.7 Overall Evaluation *201*
3.4 Validation Methodology *201*
3.4.1 Specificity *201*
3.4.2 Linearity *202*
3.4.3 Accuracy *205*
3.4.4 Precision *208*
3.4.5 Range *210*
3.4.6 Detection Limit and Quantitation Limit *210*
3.4.7 Detection Limit and Quantitation Limit of DP1 *212*
3.4.8 Robustness *212*
3.5 Conclusion *212*

References Part I *213*

Part II Life-cycle Approach to Analytical Validation *227*

4 Qualification of Analytical Equipment *229*
David Rudd

4.1 Introduction *229*
4.2 Terminology *230*
4.3 An Overview of the Equipment Qualification Process *231*
4.4 Documentation of the EQ Process *233*
4.5 Phases of Equipment Qualification *234*
4.5.1 Design Qualification (DQ) *234*
4.5.2 Installation Qualification (IQ) *236*
4.5.3 Operational Qualification (OQ) *237*
4.5.4 Performance Qualification (PQ) *237*
4.6 Calibration and Traceability *238*
4.7 Re-qualification *239*
4.8 Accreditation and Certification *241*
4.9 References *241*

5 Validation During Drug Product Development – Considerations as a Function of the Stage of Drug Development *243*
Martin Bloch

5.1 Introduction *243*
5.2 Validation During Early Drug Development *244*
5.2.1 Simplifications During Early Development *246*
5.2.2 Example 1: Assay or Content Uniformity of a Drug Product by HPLC During Early Drug Product Development: Proposal for a Validation Scheme *248*

5.2.3 Variation of Example 1: More than on Strength of Drug Product *250*
5.2.4 Example 2: Degradation Products from a Drug Product by HPLC During Early Drug Product Development: Proposal for a Validation Scheme *251*
5.2.5 Example 3: Residual Solvents of a Drug Product by GC During Early Drug Product Development: Proposal for a Validation Scheme *257*
5.2.6 Example 4: Analytical 'Method Verification' for GLP Toxicology Study *258*
5.2.7 Example 5: Dissolution Rate of a Drug Product During Early Drug Product Development: Proposal for Validation Schemes *259*
5.2.8 Validation of other Tests (Early Development) *263*
5.3 References *264*

6 Acceptance Criteria and Analytical Variability *265*
Hermann Watzig

6.1 Introduction *265*
6.2 Analytical Variability *266*
6.2.1 Uncertainty of the Uncertainty *266*
6.2.2 Estimating the Analytical Uncertainty *269*
6.3 Acceptance Criteria *274*
6.3.1 Assay of Drug Substances *274*
6.3.2 Assay of Active Ingredients in Drug Products *274*
6.3.3 Dissolution Testing *276*
6.3.4 Stability Testing *276*
6.3.5 Impurities *277*
6.4 Conclusions *277*
6.5 References *278*

7 Transfer of Analytical Procedures *281*
Mark Broughton and Joachim Ermer (Section 7.3)

7.1 Overview *281*
7.1.1 Transfer Process *282*
7.2 Process Description *283*
7.2.1 Method Selection *283*
7.2.2 Early Review of the Analytical Procedure *285*
7.2.3 Transfer Strategy *286*
7.2.4 Receiving Laboratory Readiness *287*
7.2.5 Self-qualification *290*
7.2.6 Comparative Studies *290*
7.3 Comparative Studies *291*
7.3.1 General Design and Acceptance Criteria *291*
7.3.2 Assay *293*
7.3.3 Content Uniformity *297*
7.3.4 Dissolution *297*
7.3.5 Minor Components *298*

7.4 Conclusion *299*
7.5 References *300*

8 Validation of Pharmacopoeial Methods *301*
John H. McB. Miller

8.1 Introduction *301*
8.2 Identification *304*
8.3 Purity *307*
8.3.1 Appearance of Solution *308*
8.3.2 pH or Acidity/Alkalinity *308*
8.3.3 Specific Optical Rotation *310*
8.3.4 Ultraviolet Spectrophotometry *310*
8.3.5 Limit test for Anions/Cations *310*
8.3.6 Atomic Absorption Spectrometry *312*
8.3.7 Separation Techniques (Organic Impurities) *313*
8.3.8 Loss on Drying *319*
8.3.9 Determination of Water *319*
8.3.10 Residual Solvents or Organic Volatile Impurities *322*
8.4 Assay *326*
8.4.1 Volumetric Titration *327*
8.4.2 Spectrophotometric Methods *329*
8.5 Conclusions *332*
8.6 References *332*

9 Analytical Procedures in a Quality Control Environment *337*
Raymond A. Cox

9.1 Monitoring the Performance of the Analytical Procedure *337*
9.1.1 Utilization of Blanks *337*
9.1.2 System Suitability Test Parameters and Acceptance Criteria *338*
9.1.3 Use of Check or Control Samples *339*
9.1.4 Analyst Performance *341*
9.1.5 Instrumental Performance *342*
9.1.6 Reagent Stability and Performance *343*
9.1.7 Internal Limits and Specifications *343*
9.2 Use of Control Charts *344*
9.2.1 Examples of Control Charts *344*
9.2.2 Population in Control Charts *347*
9.2.3 Cost of Control Charts *347*
9.3 Change Control *348*
9.3.1 Basic Elements of Test Procedure Change Control *348*
9.3.2 Change Control for Calibration and Preventative Maintenance *349*
9.3.3 Future Calibration and Preventative Maintenance *350*
9.4 When is an Adjustment Really a Change? *350*
9.4.1 Chromatographic Adjustments versus Changes *351*

9.5 Statistical Process Control (SPC) 351
9.5.1 Purpose of Control Charts 352
9.5.2 Advantages of Statistical Process Control 352
9.6 Revalidation 352
9.6.1 Revalidation Summary 354
9.7 References 354

10 Aberrant or Atypical Results 355
Christopher Burgess

10.1 Laboratory Failure Investigation 355
10.2 Basic Concepts of Measurement Performance 357
10.3 Measurements, Results and Reportable Values 359
10.4 Sources of Variability in Analytical Methods and Procedures 361
10.5 Analytical Process Capability 362
10.6 Classification of Atypical or Aberrant Results 366
10.7 Statistical Outlier Tests for Out-of-Expectation Results 371
10.8 Trend Analysis for Quality Control 378
10.9 CuSum Analysis of System Suitability Data 380
10.10 Summary 385
10.11 References 385

11 Future Trends in Analytical Method Validation 387
David Rudd

11.1 Introduction 387
11.2 'Real Time' Analytical Methodologies 389
11.3 Validation Consequences of 'Real Time' Analytical Methodologies 390
11.4 Additional Validation Factors 393
11.4.1 To Calibrate or not to Calibrate? 393
11.5 Validation of Analytically-based Control Systems 394
11.5.1 What is the Basis for the Decision-Making Process? 394
11.5.2 What are the Acceptable Operating Ranges? 395
11.5.3 Robustness of Process Signature 395
11.6 Continuous Validation 395
11.7 Conclusion 396
11.8 References 396

Index 399

List of Contributors

Dr. Martin Bloch
Analytical Research and Development
Novartis
WSJ-360.1104
4002 Basel
Switzerland

Mark Broughton
Head of QC Analytics
Holmes Chapel
Aventis
London Road, Holmes Chapel Crewe,
Cheshire CW4 8BE
UK

Dr. Christopher Burgess
Burgess Consultancy
'Rose Rae', The Lendings, Startforth,
Barnard Castle, Co,
Durham DL12 9AB
United Kingdom

Mr. Ray Cox
Retired from:
Abbott Laboratories
Manager Corporate Compendia and
Reference Standards
1222 Pigeon Creek Rd
Greeneville, TN 37743
USA

Dr. Joachim Ermer
Director of Analytical Processes and
Technology
Global Analytical Development,
QO TSS Aventis
Industriepark Höchst
Build. G875
65926 Frankfurt am Main
Germany

Dr. Gerd Kleinschmidt
Head of Laboratory (New Projects and
Technologies) Global Pharmaceutical
Development Analytical Sciences,
GDPAnSc Aventis
Industriepark Höchst
Build. H790
65926 Frankfurt am Main
Germany

Dr. John H. McB. Miller
Head of the Division III (Laboratory)
European Directorate for the Quality of
Medicines (EDQM)
16, rue Auguste Himly
67000 Strasbourg
France

Dr. David Rudd
Glaxo Smithkline
Building 5
Park Road,
Ware Hertfordshire SG12 0DP
United Kingdom

Prof. Hermann Wätzig
Technical University Braunschweig
Institut für Pharmazeutische Chemie
Beethovenstr. 55
38106 Braunschweig
Germany

Part I:
Fundamentals of Validation in Pharmaceutical Analysis

1 Analytical Validation within the Pharmaceutical Environment

Joachim Ermer

Validation is, of course, a basic requirement to ensure quality and reliability of the results for all analytical applications [8]. However, in comparison with analytical chemistry, in pharmaceutical analysis, some special aspects and conditions exist that need to be taken into consideration. For example, the analytical procedures (apart from pharmacopoeial monographs) are often in-house developments and applications. Therefore, the degree of knowledge and expertise is initially much larger compared with standard methods. The same can be assumed for the samples analysed. The matrix (placebo) in pharmaceutical analysis is usually constant and well known and the ranges where the sample under analysis can be expected are usually well defined and not very large. Evaluation (of batches, stability investigations, etc.) is based on the results of various procedures or control tests, thus their performances can complement each other. Acceptance limits of the specification are fixed values, often based on tradition, as in the case of assay of an active ingredient, or they may be based on specific toxicological studies, which take large safety factors into account, as for impurities. Last, but not least, validation in pharmaceutical analysis has its own regulations. These few – by far from exhaustive – remarks should make it obvious that these special considerations will have an impact on the way validation in pharmaceutical analysis is performed.

The first part of this book focusses on the fundamentals of validation in pharmaceutical analysis, the 'environmental' framework as well as the *implications* for experimental design and suitable calculations. Of course, the basic principles of validation are the same for any analytical procedure, regardless of its field of application. However, the discussions and recommendations focus on pharmaceutical applications, so the reader needs to adjust these to suit his or her purpose, if different. Nevertheless – as validation should never be regarded as simply working through a checklist – this is also required in the case of pharmaceutical analysis, but perhaps to a lesser extent, compared with other areas of application.

1.1 Regulatory Requirements

"The object of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose" [1a], determined by means of well-documented experimental studies. Accuracy and reliability of the analytical results is crucial for ensuring quality, safety and efficacy of pharmaceuticals. For this reason, regulatory requirements have been published for many years [1–7].

The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) was initiated in 1990, as a forum for a constructive dialogue between regulatory authorities and industry, in order to harmonise the submission requirements for new pharmaceuticals between Europe, the United States of America and Japan. One of the first topics within the Quality section was analytical validation and the ICH was very helpful in harmonising terms and definitions [1a] as well as determining the basic requirements [1b]. Of course, due to the nature of the harmonisation process, there are some compromises and inconsistencies. In Table 1-1, the required validation characteristics for the various types of analytical procedures are shown.

Table 1-1: Validation characteristics normally evaluated for the different types of test procedures [1a] and the minimum number of determinations required [1b]

Validation characteristic	Minimum number	Analytical procedure			
		Identity	Impurities		Assay [1]
			Quantitative	Limit	
1. Specificity [2]	Not applicable	Yes	Yes	Yes	Yes
2. Linearity	5	No	Yes	No	Yes
3. Range	Not applicable	No	Yes	No	Yes
4. Accuracy	9 (e.g. 3 × 3)	No	Yes	No	Yes
5. Precision					
Repeatability	6 or 9 (e.g. 3 × 3)	No	Yes	No	Yes
Intermediate precision/ Reproducibility [3]	(2 series) [4]	No	Yes	No	Yes
6. Detection limit	Approach dependent	No	No [5]	Yes	No
7. Quantitation limit		No	Yes	No	No

Yes / No normally evaluated / not evaluated
1 including dissolution, content/potency
2 lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
3 reproducibility not needed for submission
4 no number given in [1b], logical conclusion
5 may be needed in some cases

Two guidelines on validation were issued by the US Food and Drug Administration (FDA), one for the applicant [2], the other for inspectors and reviewers [3]. The first one is also intended to ensure that the analytical procedure can be applied in an FDA laboratory and therefore requires a detailed description of the procedure, reference materials, as well as a discussion of the potential impurities, etc. The second guideline focuses on reversed-phase chromatography and provides a lot of details with regard to critical methodological issues, as well as some indication of acceptability of results. A revised draft of the first guideline was published in 2000 [4]. According to the title *"Analytical procedures and methods validation"*, it also includes the content and format of the analytical procedures, the requirements for reference standards and various types of analytical technique. Therefore, this guidance is more comprehensive than the ICH Guidelines, but is rather too focussed on providing 'instrument output/ raw data'. As this is an inspection and documentation issue, it should be separated from the validation. A very detailed discussion is provided in the Canadian guideline [7] with respect to requirements and particularly acceptance criteria. Although this allows some orientation, the given acceptance criteria were sometimes rather too ambiguous, for example, the intermediate precision / reproducibility of less than 1% for drug substances (see Section 2.1.3.2 and Fig. 2.1-12).

So why is it still important to discuss validation?

First of all, the ICH guidelines should be regarded as the basis and philosophical background to analytical validation, not as a checklist. *"It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product"* [1b]. It will be shown in the next sections that suitability is strongly connected with the requirements and design of the given analytical procedure. As this obviously varies, at least with the type of procedure, it must be reflected in the analytical validation. This includes the identification of the performance parameters relevant for the given procedure, the definition of appropriate acceptance criteria and the appropriate design of the validation studies. In order to achieve this, the analyst must be aware of the fundamental meaning of these performance parameters, as well as the calculations and tests and their relationship to the specific application. The former is discussed in detail in Chapter 2, the latter in the following sections. A lack of knowledge or (perhaps) a wrong understanding of 'efficiency' will lead to validation results that address the real performance of the analytical procedure only partly or insufficiently. This is, at the very least a waste of work, because the results are meaningless. Unfortunately, this can also be found rather too frequently in publications, although to a varying extent for the different validation characteristics. Such common insufficiencies are discussed in the respective sections of Chapter 2.

1.2
Integrated and Continuous Validation

Validation should not be regarded as a singular activity [4], but should always be understood with respect to the life cycle of the analytical procedure. Starting with the method development or optimisation, the performance of the analytical proce-

dure should be matched to the requirements in an *iterative process*. Some validation characteristics, such as specificity (selective separation) or robustness, are more important in this stage (see Section 2.7). However, this depends on the type of procedure. In the case of a complex sample preparation, or cleaning methods (see Section 2.3.4), precision and accuracy may play an important role in the optimisation process. One should also be aware that the validation requested for submission, i. e. a demonstration of the *general* suitability of the respective analytical procedure – can only be considered as a basis. The user of any method has to guarantee that it will stay consistently in a validated status, also referred to as the life-cycle concept of analytical validation [9]. In this process, an increasing amount of information can be compiled.

This does not necessarily mean that additional work always needs to be done. During the actual application of the methods, a lot of data is generated, but often left unused ('data graveyard'). In order to make rational and efficient use of these data, they must be transformed to information (i.e., processed and condensed into performance parameters). When enough reliable information is compiled, it can be further processed to gain knowledge that eventually enables us to achieve a better understanding and control of the analytical procedure (see also Section 2.1.4 and Chapter 9). The whole process is well known as an *'information pyramid'* (Fig. 1-1). This knowledge can also be used to improve analytical procedures, for example, by changing from the traditional 'daily' calibration in an LC assay to a quantitation using 'predetermined' calibration parameters (comparable to a specific absorbance in spectrophotometry), with advantages both in efficiency and reduced analytical variability [10].

Transfers of analytical procedures to another site of the company or to a contract laboratory – quite common nowadays – often result in a challenging robustness test, especially if not appropriately addressed in the validation. Acceptance criteria for a successful transfer may be derived from the validation itself, or from the same principles as for calculations and tests in validation, because here the performance of the analytical procedure is also addressed (see Chapter 7). On the other hand, comparative studies will provide quite reliable performance data of the analytical procedure (see Section 2.1.3.2).

Besides this 'horizontal' integration, analytical validation also needs to be included in the whole system of *Analytical Quality Assurance* (AQA) [8], i.e., 'vertical' integration. This involves all (internal and external) measures which will ensure the quality and reliability of the analytical data, such as an equipment qualification program (see Chapter 4), appropriate system suitability tests (see Section 2.8), good documentation and review practices, operator training, control charts (see Chapter 9), etc.

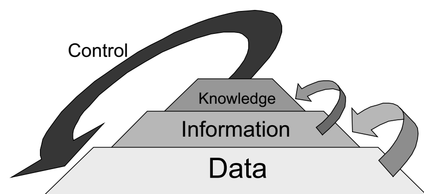

Figure 1-1 Information pyramid.

1.3 General Planning and Design of Validation Studies

Performance is strongly connected with the requirements and design of the given analytical procedure (see Section 1.4.1). As this obviously varies, it must be reflected in the planning and design of the analytical validation. Consequently, a checklist approach is not appropriate. In order to ensure thorough planning, i.e., to identify the relevant performance parameters, to define appropriate acceptance criteria and then to design the studies accordingly, *validation protocols* should be prepared. In addition to this 'good science' reason, protocols can also be regarded as a general GMP requirement and are common practice also the in case of process validation, cleaning validation, equipment qualification, transfer, etc.

The analyst may be faced with the problem of the iterative nature of the method development / validation process. However, here one may distinguish between performance parameters (and the corresponding validation characteristics) of the final analytical procedure and those obtained or derived from different method conditions, such as specificity and robustness. The former can be addressed (before starting the experimental studies, following usual practice) in the protocol, the latter can be referred to in the validation report and/or protocol (see Chapter 5).

Of course, the *extent and depth* of the validation studies, as well as acceptance criteria, should be defined in relation to the required performance ('importance') and the 'environment' of the respective analytical procedure, such as the stages of development (see Chapter 5), or the stages of manufacturing / synthesis. Important or critical procedures (within the context of validation) can be expected to have tighter specification limits. In these cases, such as the assay of active or of critical impurities, it is recommended to address the validation characteristics *separately* (for example, precision with authentic samples and accuracy with spiked samples), in order to increase the power of the results. In other cases, such as the determination of other ingredients or of impurities or water sufficiently below specification limits, several validation characteristics, for example, precision, linearity, and accuracy (quantitation) limit in dependence on the range, see Section 2.6.4) can be investigated simultaneously, using the same spiked samples.

The ICH Guidelines [1a,b] are mainly focused on chromatographic procedures, as can be seen in the methodology guideline [1b]. Therefore, they should be regarded more as a guide to the *philosophy of validation* – i.e., used to identify relevant performance parameters of the given analytical procedure – than as a 'holy grail'. If the special conditions or techniques are not covered in the ICH guideline, the validation approach must then be adapted accordingly (see Chapter 11). The FDA Guidance [4], and the Technical Guide of the European Pharmacopoeia (EP) [11], as well as Chapter 8 also provide details for specific analytical techniques.

1.3.1 Always Look on the 'Routine' Side of Validation

Curiously, one aspect often neglected during validation is its primary objective, i.e., to obtain the real *performance of the routine application* of the analytical procedure. As far as possible, all steps of the procedure should be performed as described in the control test. Of course, this cannot always achieved, but at least the analyst should always be aware of such differences, in order to evaluate the results properly.

What does this mean in practice?

For example, precision should preferably be investigated using *authentic* samples, because only in this case is the sample preparation identical to the routine application. It is also important to apply the intended calibration mode exactly as described in the analytical procedure. Sometimes the latter is not even mentioned in the literature. Precision is reported only from repeated injections of the same solution, ignoring the whole sample preparation. This is certainly not representative for the (routine) variability of the analytical procedure (see Section 2.1.2). Investigating pure solutions is usually of very limited practical use, for example, in the case of cleaning methods (see Section 2.3.4) or quantitation limit (see Section 2.6), or may even lead to wrong conclusions, as the following examples will show.

The minor (impurity) enantiomer of a chiral active ingredient was analysed by chiral LC using an immobilised enzyme column (Chiral-CBH 5 µm, 100 × 4 mm, ChromTech). The quantitation should be carried out by area normalisation (100%-method, 100%-standard), which would require a linear response function and a negligible intercept for both active and impurity enantiomer (see also Section 2.4.1). The experimental linearity investigation of dilutions of the active, revealed a clear deviation from a linear response function (Fig. 1-2). However, when the design was adjusted to simulate the conditions of the routine application, i.e., spiking the impurity enantiomer to the nominal concentration of the active, an acceptable linear relationship was found. Although a slight trend remained in the results, the recoveries between 99 and 105% can be regarded as acceptable for the intended purpose. A possible explanation for such behaviour might be that the interaction between the enantiomers and the binding centres of the immobilised enzyme (cellobiohydrolase, hydrolysing crystalline cellulose) is concentration dependent. Maintaining the nominal test concentration in the case of the spiked samples, the sum of both enantiomers is kept constant and consequently so are the conditions for interactions. In this case, the linearity of the active enantiomer cannot be investigated separately and the validity of the 100% method must be demonstrated by obtaining an acceptable recovery.

Stress samples

Another area where the primary focus of validation is often ignored is the use of stress test samples (see also Section 2.2). At least some of the applied conditions [1g] will result in degradation products without any relevance for the intended storage condition of the drug product. Therefore, such samples should be used with reasonable judgement for method development and validation. It is the primary objective of a suitable (impurity) procedure (and consequently its validation) to address degra-

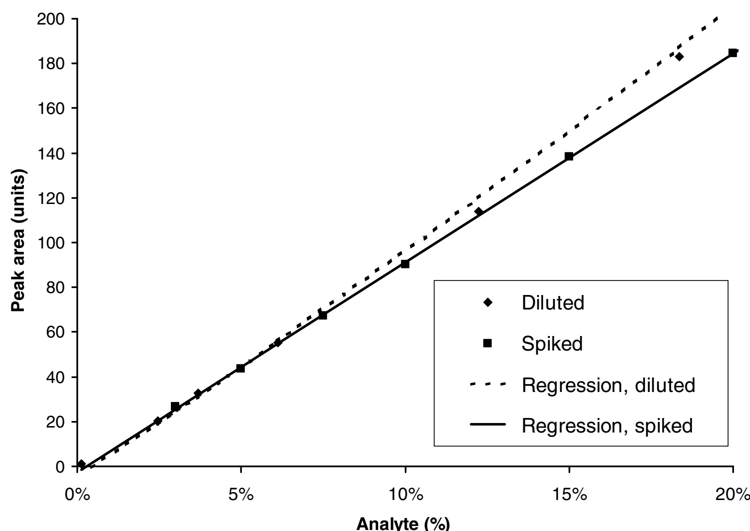

Figure 1-2 Linearity investigation of an enantiomeric LC determination. The diamonds and the squares represent dilutions of the active enantiomer and spikings of the impurity enantiomer to the active enantiomer, respectively. An obvious deviation from a linear function is observed in the case of the dilutions (broken line, polynomial to 3rd order), in contrast to the impurity in the presence of the active enantiomer (solid line, linear regression). The concentration on the x-axis is given with reference to the nominal test concentration of the active enantiomer.

dants "likely to be present" [1b], rather than a 'last resort'. However, it is also reasonable to allow for some 'buffer' [12].

Sometimes, applying artificial conditions cannot be avoided, in order to approach validation parameters, as in recovery investigations (see Section 2.3.2) or in dissolution, where no homogeneous samples are available. In the latter case, the assay part of the analytical procedure may be investigated separately. However, possible influences on the results due to the different application conditions need to be taken into account in the evaluation process as well as in the definition of acceptance criteria.

1.4 Evaluation and Acceptance Criteria

1.4.1 What does Suitability Mean?

The suitability of an analytical procedure is primarily determined by the requirements of the given test item, and secondly by its design (which is normally more flexible). Usually, the (minimum) requirements are defined by the acceptance limits of the specification (often termed traditionally as 'specification limits', but according to ICH [1e], the term 'specification' defines a *"list of tests, references to analytical proce-*

dures, and appropriate acceptance criteria"). For some applications, the requirements are explicitly defined in the ICH Guidelines. For example, the reporting level for unknown degradants in drug products is set to 0.1% and 0.05% for a maximum daily intake of less and more than 1 g active, respectively [1d] (Table 2.6-1). In the case of cleaning validation, the maximum acceptable amount of cross-contamination can be calculated based on the batch sizes and doses of the previous and subsequent product, the toxicological or pharmacological activity and/or the safety factors, and the so called specific residual cleaning limit (SRCL) [13]. Consequently, the corresponding test procedure must be able to quantify impurities or residual substance at this concentration with an appropriate level of precision and accuracy (see Section 2.3.4).

With respect to stability studies, the analytical variability must be appropriate to detect a (not acceptable) change in the tested property of the batch. This is illustrated in Figure 1-3 for determination of the content of active ingredient. The intrinsic degradation of 1.0% within 36 months can be reliably detected by an assay with a true variability of 0.5% (Fig. 1-3A), but not by one with 2.0% variability (Fig. 1-3B). Generally, acceptance limits of the specification (SL) have to enclose (at least) both the analytical and the manufacturing variability (see Chapter 6). Rearranging the equation describing this relationship (Eq. 6-12), the maximum permitted analytical variability can be calculated from the acceptance limits of the specification (Eq.1-1).

$$RSD\mathrm{max}(\%) = \frac{|(BL-SL)| * \sqrt{n_{assay}}}{t(P,df)} \tag{1-1}$$

SL: Acceptance limits of the specification for active (% label claim).

BL: Basic limits, 100% – maximum variation of the manufacturing process (in %). In case of shelf-life limits, the lower basic limit will additionally include the maximum acceptable decrease in the content.

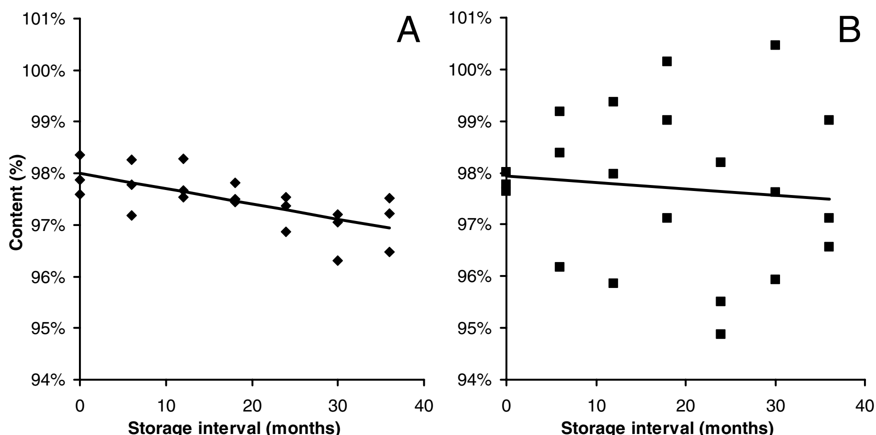

Figure 1-3 Illustration of the requirements for assay of an active ingredient during a stability study. The three individual results per storage interval were simulated based on a 1% decrease of content within 36 months and a normally distributed error of 0.5% (A) and 2.0% (B) using Eq. (2.1-3). The slope of the regression line in B is not significant.

n_{assay}: Number of repeated, independent determinations in routine analyses, insofar as the mean is the reportable result, i.e., is compared to the acceptance limits. If each individual determination is defined as the reportable result, n=1 has to be used.

$t(P,df)$: Student t-factor for the defined level of statistical confidence (usually 95%) and the degrees of freedom in the respective precision study.

The same basic considerations of the relationship between content limits and analytical variability [14] were applied to the system precision (injection repeatability) requirements of the EP [15] (see Section 2.8.3.8). The method capability index (see Section 10.5, Eq. 10-5) is based on similar considerations. However, here the normal distribution is used to describe the range required for the analytical variability (see Section 2.1.1). Consequently, the method capability index must be applied to single determinations (or to means if the standard deviation of means is used) and requires a very reliable standard deviation, whereas Eq.(1-1) can take a variable number of determinations directly into account, as well as the reliability of the experimental standard deviation (by means of the Student t-factor).

Of course, the precision acceptance limit thus obtained will be the minimum requirement. If a tighter control is needed, or if a lower variability is expected for the given type of method (analytical state of the art, see Section 2.1.3), the acceptance limits should be adjusted. A further adjustment may be required if there is a larger difference between repeatability and intermediate precision, i.e., if there is a larger inter-serial contribution (Eq. (2.1-10), Section 2.1.3.2). In such a case, an increased number of determinations in the assay will only reduce the repeatability variance, but not the variance between the series (s^2_g). Therefore, the term $\sqrt{n_{assay}}$ must be transferred to the left-hand side of Eq. (1-1) and $RSD_{max}(\%)$ rearranged to $\sqrt{s_g^2 + \frac{s_r^2}{n_{assay}}}$. This term corresponds to the standard deviation of the means from the routine assay determinations.

Many other performance parameters are linked with the analytical variability. Therefore, once an acceptable precision is defined, it can serve as an orientation for other acceptance criteria (for details, see Table 1-2 and Sections 2.1–2.6). As far as possible, *normalised (percentage) parameters* should be defined as validation acceptance limits, because they can be compared across methods and therefore more easily drawn from previous experience.

As can be seen from Eq. (1-1), the number of determinations also influences the acceptable performance, as well as the intended calibration mode (see Section 2.4). In principle, the analyst is rather flexible in his/her decision, provided that the minimum requirements are fulfilled. Often, the design of the calibration is more influenced by tradition or technical restrictions (for example the capabilities of the acquisition software) than by scientific reasons. Sometimes a 'check standard' is applied, i.e., the standard prepared and used for calibration is verified by a second standard preparation, the response of which needs to be within an acceptable range of the first one (e.g. ± 1.0%). This approach is not optimal. If the 'check standard' is only used for verification, 50% of the available data are ignored. Increasing the number of determi-

nations improves the reliability of the mean (see Fig. 2.1-4A). Therefore, it would be preferable to calculate the mean from all standard preparations (after verification of their agreement), in order to reduce the variability of the standard that will be included in the result for the sample (see discussion on repeatability and intermediate precision, Section 2.1.2). Of course, if the overall variability utilising only the first standard preparation is still acceptable, the procedure will be suitable. However, the analyst must be aware of the inter-relations and their consequences in order to make an appropriate decision and evaluation. This example also highlights the importance of applying the intended calibration, exactly as described in the control test for the intermediate precision study, otherwise the obtained result will not reflect the performance of the routine analytical procedure.

1.4.2 **Statistical Tests**

Significance Tests
Statistical significance tests should very cautiously be (directly) applied as acceptance criteria, because they can only test for a *statistical* significance (and with respect to the actual variability). On one hand, due to the small number of data normally used in pharmaceutical analysis, large confidence intervals (see Section 2.1.1) may obscure unacceptable differences (Fig. 1-4, scenario 3, S). On the other hand, because of sometimes abnormally small variabilities in (one of) the analytical series (that, however, pose no risk for routine application), differences are identified as significant which are of no *practical relevance* (Fig. 1-4, scenario 1, S) [16]. The analyst must decide whether or not detected statistical differences are of practical relevance. In addition, when comparing independent methods for the proof of accuracy, different specificities can be expected which add a systematic bias, thus increasing the risk of the aforementioned danger. Therefore, a statistical significance test should always be applied (as acceptance criteria) in a two-tiered manner, including a measure for practical relevance. For example, in the case of comparison of results with a target value, in addition to the nominal value *t*-test (see Section 2.3.1, Eq. 2.3-2), an upper limit for the precision and a maximum acceptable difference between the mean and the target value should be defined, in order to avoid the scenario 3 illustrated in Figure 1-4 (S).

Equivalence Tests
Such measures of practical relevance are an intrinsic part of the so-called *equivalence tests* [16, 28] (see also Section 7.3.1.3). In contrast to the significance tests, where the confidence intervals of the respective parameter(s) must include the target value (Fig. 1-4, scenario 2 and 3, S), equivalence tests, must be within an *acceptable range*. This measure of practical relevance is defined by the analyst. It is obvious in Figure 1-4, that such equivalence tests are robust with respect to small (scenario 1, E), but sensitive to large (scenario 3, E) variabilities.

Absoute Acceptance Limit
Another alternative is to use *absolute acceptance limits*, derived from experience (see Section 2.1.3) or from statistical considerations, as described in Section 1.4.1 for pre-

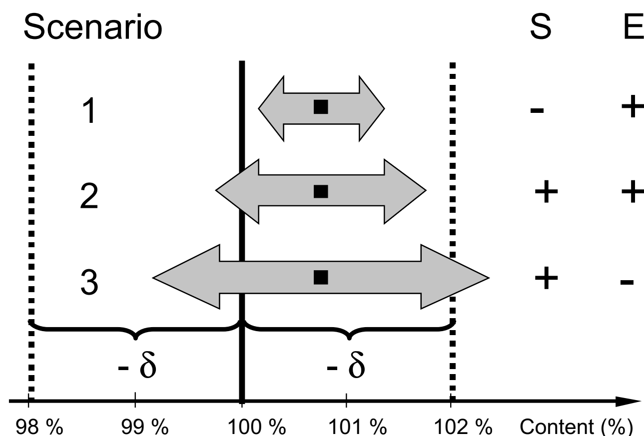

Figure 1-4 Illustration of statistical significance (S) and equivalence (E) tests for the example of a comparison between a mean and a target value of 100% (e.g., a reference or theoretical recovery). The acceptable deviation δ from the target (for the equivalence test) is symbolised by vertical dotted lines, the means, with confidence intervals indicated by double arrows. The outcome of the statistical tests for the three scenarios is indicated by '+' and '–' for 'pass' and 'fail' of the respective (H0) hypothesis, these are 'no statistical significant difference' and 'acceptable difference' for significance and equivalence test, respectively.

cision, and for a maximum acceptable difference in accuracy (see Section 2.3.5). In contrast to the equivalence tests, the actual variability of the data is neglected for the purpose of comparison (if means are used). However, usually the variability will be investigated separately.

If validation software is used, it must be flexible enough to meet these precautions [28].

Of course, statistical significance tests also have their merits, if properly applied. Even if a small variability does not pose a practical risk, when the suitability of a procedure is investigated, it may be assumed that such data are not representative for the usual (routine) application of the analytical procedure. This is an important consideration when the true parameter (standard deviation, mean) is the investigational objective, for example, the true precision of an analytical procedure, or if a reference standard is characterised. In collaborative trials, significance tests such as outlier tests are often defined as intermediary acceptance criteria for checking the quality of the data [17–19]. Deviating (i.e., unrepresentative) results (laboratories) are removed before proceeding to the next step, in which results are combined.

1.5 Key Points

- Validation should address the performance of the analytical procedure under conditions of routine use.
- Suitability is strongly connected with both the requirements and the design of the individual analytical procedure.
- Consequently, the analyst has to identify relevant parameters which reflect the routine performance of the given analytical procedure, to design the experimental studies accordingly and to define acceptance criteria for the results generated.
- Absolute, preferably normalised parameters should be selected as acceptance criteria. These can be defined from (regulatory) requirements, statistical considerations, or experience. Statistical significance tests should be applied with caution, they do not take into consideration the practical relevance.
- Validation must not be regarded as a singular event. The analyst is responsible for the continued maintenance of the validated status of an analytical procedure.

Acknowledgements

Some of the examples presented in my chapters, as well as the experience gained, are based on the work of, and discussion with, many colleagues in Aventis. Their important input is greatfully acknowledged, but I will abstain from an attempt to list them, both because of space as well as the danger of forgetting some of them. I would like to acknowledge in particular, John Landy, Heiko Meier, and Eva Piepenbrock.