



EXCERPT FROM:

VALIDATION OF ANALYTICAL METHODS FOR PHARMACEUTICAL ANALYSIS

BY OONA MCPOLIN

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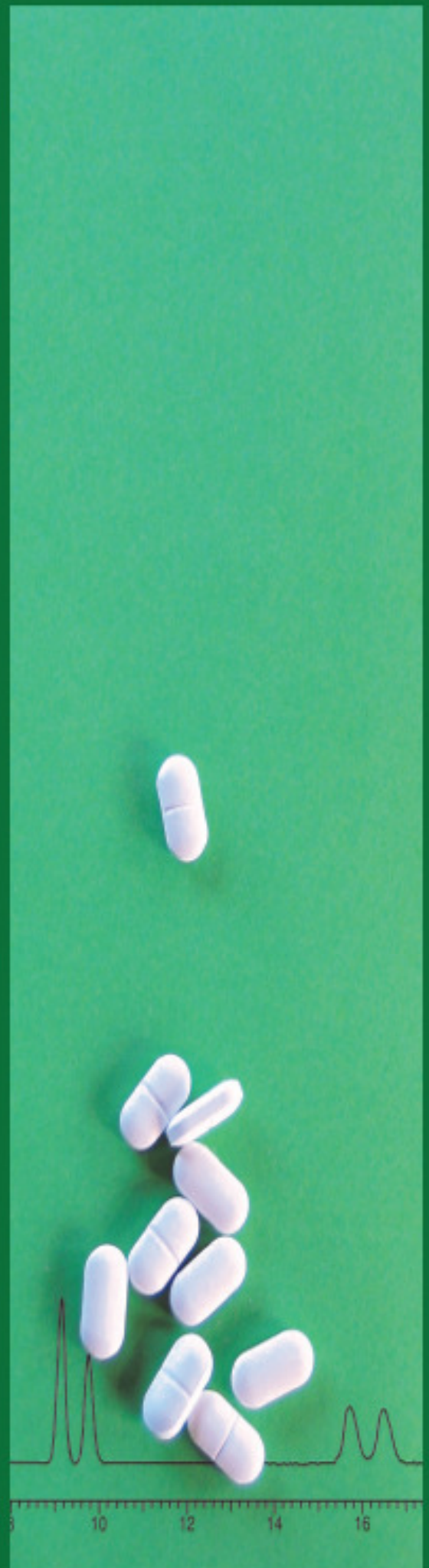
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**Validation
of
Analytical
Methods
for
Pharmaceutical
Analysis**

OONA McPOLIN



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Preface

This book provides guidance on how to perform validation for the analytical methods which are used in pharmaceutical analysis. Validation of the analytical methods which are used during drug development and drug manufacturing is required to demonstrate that the methods are fit for their intended purpose. Additionally, the pharmaceutical industry around the world is subject to extensive regulations due to the nature of its products. Validation is a regulatory requirement and the data generated during an analytical method validation study is included in submissions to regulatory authorities for clinical trial and marketing applications.

The definitive reference for this topic is the guideline produced by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Q2(R1) 'Validation of Analytical Procedures: Text and Methodology'. Sections of the guideline have been reproduced in this book with the kind permission of the ICH Secretariat. All ICH guidelines are available via the website, www.ich.org, and thus the validation guideline may be easily checked for revisions.

The guidance in this book is primarily aimed at analytical methods for small molecules. Reference is made to validation of methods for pharmaceuticals of biological origin, such as proteins and peptides. In principle the ICH guidelines should be applied to these types of compounds, however it is acknowledged they present particular challenges during validation, in particular relating to biological assays. The references provided in Chapter 1 provide more information on the validation of analytical methods for biopharmaceuticals/ biotechnology derived products.

At the back of the book there is a glossary to help the reader become familiar with the terminology used in analytical method validation. When a new term is introduced it is shown in **bold** to indicate to the reader that a definition is available in the glossary. In the appendix there is a list of abbreviations and also a question section so that the reader can test their understanding of the content.

Oona McPolin

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Introduction

Validation is defined as ‘finding or testing the truth of something’. When analytical methods are used to generate results about the characteristics of drug related samples it is vital that the results are trustworthy: they may be used as the basis for decisions relating to administering the drug to patients. A validation study is performed on an analytical method to ensure that reliable results are always obtained.

Validation in the Pharmaceutical Industry

Analytical method validation is just one type of validation required during drug development and manufacturing. To comply with the requirements of current Good Manufacturing Practices (GMP)¹⁻³ pharmaceutical companies should have an overall validation policy which documents how validation will be performed. This will include the validation of: production processes, cleaning procedures, analytical methods, in-process control test procedures, and computerised systems. The purpose of this validation is to show that processes involved in the development and manufacture of drugs, such as production, cleaning and analytical testing, can be performed in an effective and reproducible manner.

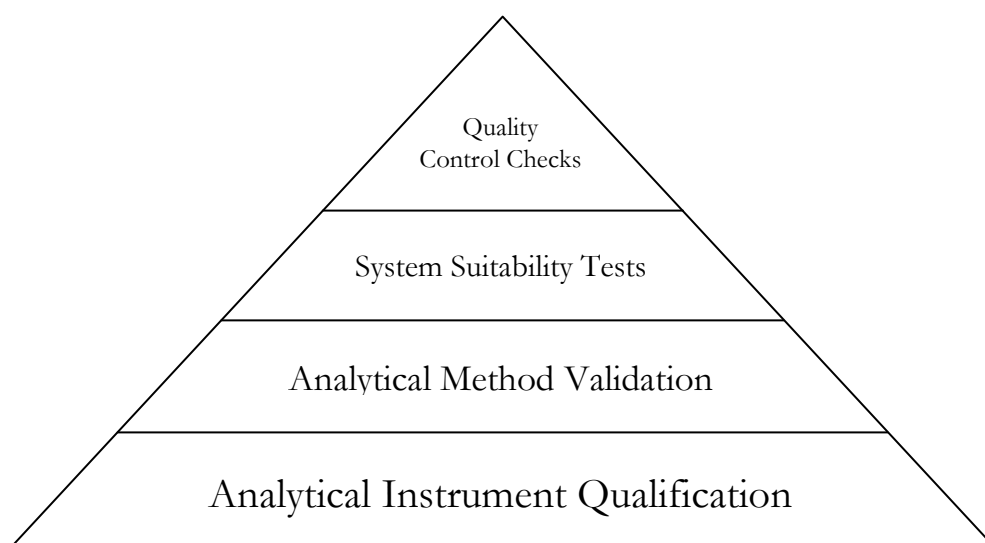
The reason that validation is included in cGMP in this way is to ensure that quality is built in at every step, and not just tested for at the end. ‘Validation is intended to provide assurance of the quality of a system or process through a quality methodology for the design, manufacture and use of that system or process, that cannot be found by simple testing alone.’⁴

Data Quality

The quality of analytical data is assured by the combination of a number of critical components as shown in Figure 1. In the data quality triangle⁵ the components are layered, each layer adds to the overall quality of the data. The base of the triangle is **Analytical Instrument Qualification (AIQ)**, this is documented evidence that an instrument performs suitably for its intended purpose and that it is properly maintained and calibrated. The next layer is analytical method validation, the subject of this book. This is documented evidence that demonstrates that the analytical method is suitable for its intended use. The top layers of the triangle are **system**

suitability tests and **quality control checks**, these are used to demonstrate that the combination of system and method performed as expected at the time of the analysis. System suitability tests are commonly used for chemical analyses, which are usually subject to GMP regulations, and quality control checks are commonly used for bioanalytical analyses, which are usually subject to GLP regulations. Overall, AIQ and analytical method validation assure the quality before the analysis is performed and system suitability tests and quality control checks assure the quality immediately before or during the analysis.

Figure 1 Data quality triangle, from Basal et al in *AAPS PharmSciTech*⁵



Analytical Instrument Qualification (AIQ)

The equipment which is used for all types of validation related to drug development and manufacture needs to be qualified. In the case of analytical methods this refers to the analytical instrumentation required to perform the test. Analytical method validation must be performed on appropriately qualified instruments.

The qualification procedure is usually carried out in four stages during which all actions are documented^{5,6}. These are:

Design Qualification (DQ):

This covers all procedures prior to the installation of the system in the selected environment. The DQ defines the functional and operational specifications of the instrument and details the conscious decisions in the selection of the supplier. For commercial off the shelf (COTS) analytical instrumentation users generally have very little input into the design of the instrument and thus the DQ will detail the user requirements and the rationale for the selection of a particular supplier. For custom designed analytical instrumentation the DQ details the key features of the design and how they address the user requirements.

Installation Qualification (IQ):

This covers all the procedures relating to the installation of the instrument in the selected environment. The IQ establishes that the instrument is received as designed and specified, that it is properly installed in the selected environment and that this environment is suitable for the operation and use of the instrument. The IQ may be carried out by the supplier and/or the user. For some complex instrumentation it may have to be performed by the supplier.

Operational Qualification (OQ):

This is the process of demonstrating that an instrument will function according to its operational specification in the selected environment. The OQ usually takes place after the IQ of a new instrument or after a change to the instrument, such as repair or change of location. As with the IQ, the OQ may be carried out by the supplier and/or the user. IQ/OQ is now offered by most suppliers of analytical instrumentation when a new instrument is purchased.

Performance Qualification (PQ):

This is defined as the process of demonstrating that an instrument consistently performs according to a specification appropriate for its routine use. PQ may be offered as part of a service contract for the routine maintenance of analytical instruments and/or may be carried out by the user. Calibration is part of performance qualification.

This summary gives an overview of equipment qualification, more information can be found in the GMP guidelines¹⁻³, also many articles and books are available on this topic⁵⁻⁷.

The Purpose of an Analytical Method

An analytical method details the steps necessary to perform an analysis. This may include: preparation of samples, standards and reagents; use of the apparatus; generation of the calibration curve, use of the formulae for the calculation, etc. The objective of validation of an analytical method is to demonstrate that the method is suitable for the intended use.

The use of analytical methods during drug development and manufacturing provides information on:

- Potency, which can relate directly to the requirement of a known dose.
- Impurities, which can relate to the safety profile of the drug.
- Evaluation of key drug characteristics such as crystal form, drug release, and drug uniformity, properties which can compromise **bioavailability**.
- **Degradation products**, methods need to be **stability indicating**.

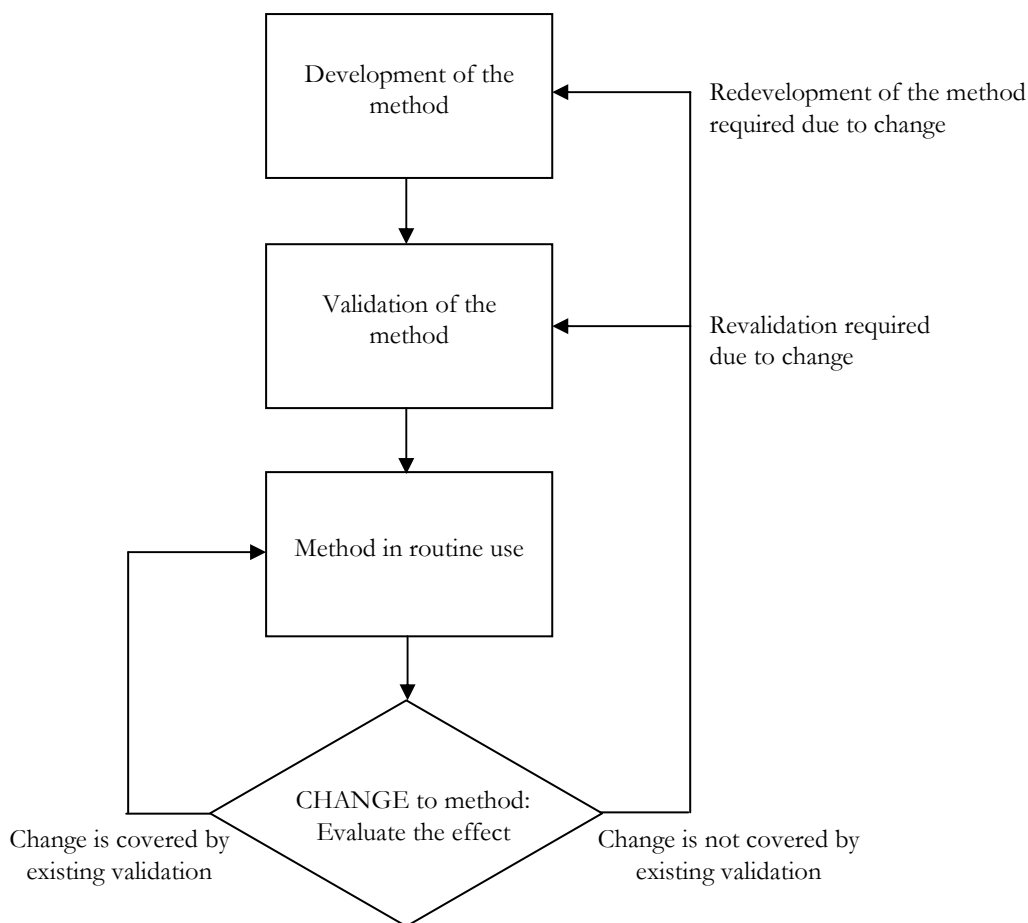
- Effect of key manufacturing parameters, to ensure that the production of drug substance and drug product is consistent.

The validation which is performed on the methods which generate this data needs to demonstrate that they can do so reliably and consistently.

The life cycle of an analytical method

Once a method has been developed and validated it may then be used for routine analysis, as shown in Figure 2. However, changes may occur which make it necessary to evaluate whether the method is still suitable for its intended use. The change may be covered by the existing validation, in which case no further validation is required or the change may result in revalidation, and in some cases, redevelopment of the method followed by validation of the new method.

Figure 2 The life cycle of an analytical method



Examples of potential changes and the effect are shown in Table 1.

Summary

1. Validation is an important procedure in the pharmaceutical industry and is used to ensure that quality is built into the processes supporting drug development and manufacture.
2. Data quality is assured by the combination of four components: Analytical Instrument Qualification (AIQ); analytical method validation; system suitability tests and quality control checks.
3. Validation of an analytical method is intended to demonstrate that it is suitable for its intended use.
4. Guidelines for analytical method validation as applied to pharmaceuticals are available from the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.
5. The type of method and analytical technique used will determine the nature and extent of the validation studies required. The most common methods for validation are identification, assay and impurities determination.
6. The characteristics of validation are: Specificity, linearity, range, accuracy, precision, detection limit, quantitation limit and robustness.

References

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Validation Characteristics

Validation of an analytical method is performed by consideration of a number of characteristics as introduced in Chapter 1. The nature of the analytical method will determine which characteristics should be investigated. In this chapter each characteristic is considered separately in the order in which they are listed in the ICH guidelines¹. The guidance for each as written in the ICH guidelines is provided together with a discussion of the interpretation of the guidance and of the associated practical considerations.

Validation during Method Development

When validation is performed for analytical methods which are used to analyse drug related samples the nature of the investigation is usually confirmatory. During the development of the method many of the validation characteristics have already been considered although probably not in a formal way. For example:

- The concentration of the samples used for the analysis will have been selected so as to achieve a suitable response, e.g. in the case of a HPLC method to determine impurities using a UV detector, the concentration will be selected to fall within the linear range of the detector and to have a suitable response for the low concentrations required.
- The specificity will have been considered during the initial method development, e.g. in any chromatographic method the separation of the components of interest is the basis of the method development process and thus the retention time of components of interest and potential interferences are evaluated during the development of the method.
- An evaluation of the robustness of the method is usually commenced as soon as the method is developed. This will include identification of the critical parameters of the method and an evaluation of the stability of test solutions.

The result of this information gained during method development is that the validation experiments are expected to comply with the selected acceptance criteria and major problems are not usually anticipated. However, it is always possible that the validation study may identify problems that could result in changes to the method.

Statistics in Analytical Method Validation

Statistical analysis of the data produced during a method validation study is required to demonstrate the validity of the method. The statistical procedures which are commonly used are described during the discussion of each validation characteristic in this chapter and also in Chapter 4. In this preceding section the use of the mean, standard deviation and confidence intervals are considered.

Mean

The mean of a dataset is the total sum of all the values in the dataset divided by the number of values in the dataset. It is denoted by \bar{x} (x bar) and is calculated using the following equation:

$$\bar{x} = \frac{1}{n} \sum x_i$$

Consider as an example the analysis of a pharmaceutical sample to determine the amount of active pharmaceutical ingredient present. A single determination or measurement will provide an estimation of the true value. If another determination is performed then the result is likely to be different from the first determination but which is the best estimate of the true value? The mean of the results obtained for the two determinations is normally calculated to provide a better estimate for the true value. As the number of determinations increases the calculated sample mean will be nearer to the true value. In this situation the true value is the population mean, denoted by μ .

Standard deviation

The standard deviation of a dataset is a measure of the spread of the values in the dataset. It is calculated by measuring the difference between the mean and the individual values in the dataset. The standard deviation for a sample is determined using the following equation:

$$\text{Standard deviation, } s = \sqrt{s^2}$$

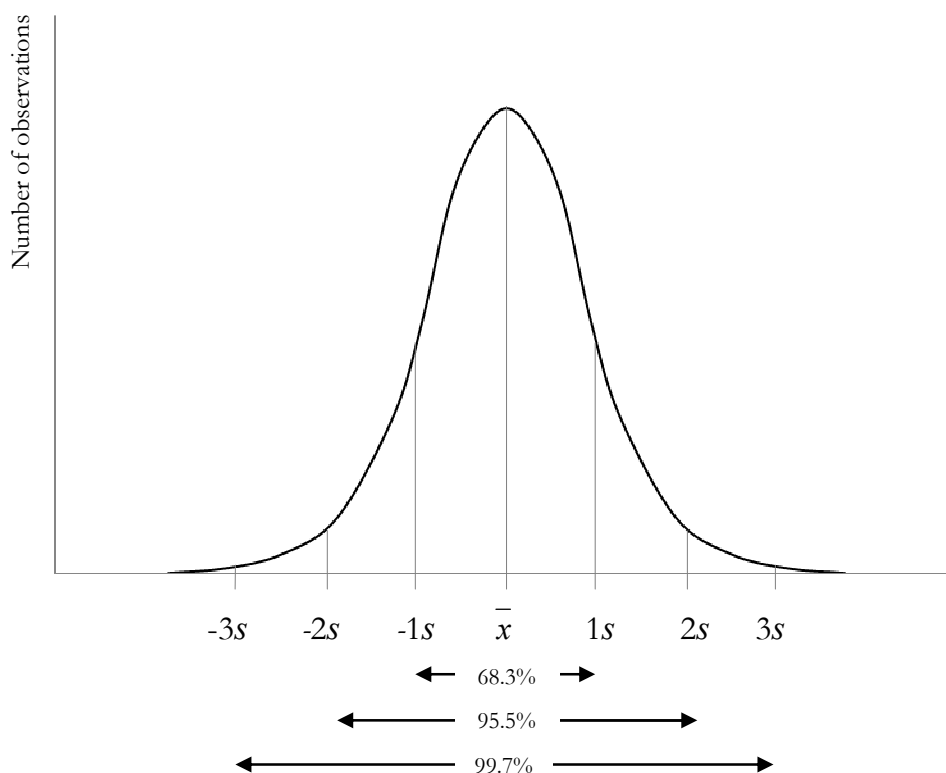
Where:

$$s^2 = \frac{1}{n-1} \sum_i (x_i - \bar{x})^2$$

The calculation for standard deviation assumes a normal distribution of the data. This distribution is shown in Figure 3 and is typical for the results of most physico-chemical analysis. The data is centred about the mean with the majority of the observations near to the mean value. Fewer and fewer observations occur the further they are away from the mean. 68.3 % of the observations lie within 1 standard deviation of the mean, 95.5 % of the observations lie within two standard deviations and 99.7 % lie within 3 standard deviations. When using small data sets, typical in

pharmaceutical analysis, a normal distribution may not be apparent on inspection of the data but this distribution can usually be assumed.

Figure 3 Normal distribution curve



If the population standard deviation, σ , is defined as the standard deviation calculated for a large data set, and the sample standard deviation, s , is defined as the standard deviation of a small subset of this large data set, then it is found that the value calculated for the sample standard deviation on a number of subsets may vary considerably due to random variability in the data. The smaller the number of data, the higher is the variability of the sample standard deviation. As a result of this, it is recommended that the standard deviation should only be calculated for data sets where $n > 3$.

Confidence intervals

Confidence intervals are used to indicate the reliability of an estimate. In the example quoted previously, where the amount of active pharmaceutical ingredient present in a pharmaceutical sample is analysed, the mean result obtained is an estimate of the actual amount present. A confidence interval provides limits around the experimentally determined value of the mean within which the true value (or population mean, μ) lies with a given degree of probability, usually 95%.

The size of the confidence interval depends on the value of the standard deviation, s . The confidence interval determined for a sample where the standard deviation

Linearity

The section on linearity in the ICH guidelines¹ is as follows:

ICH

2. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

A linear relationship should be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included.

In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample.

For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

Verification of the calibration model

If an analytical method is going to be used to generate quantitative results then a method of calibration is required. The purpose of the validation characteristic referred to as 'linearity' is to verify the calibration model. The term 'linearity' is slightly misleading because it implies that the relationship between the test results and the concentration of the analyte should be linear. This is not always the case as is acknowledged in the penultimate paragraph of section 2 in the guidelines, **immunoassays** being quoted as an example. In the FDA guidelines⁴ on bioanalytical method validation the term 'Calibration/Standard Curve' is used instead of linearity.

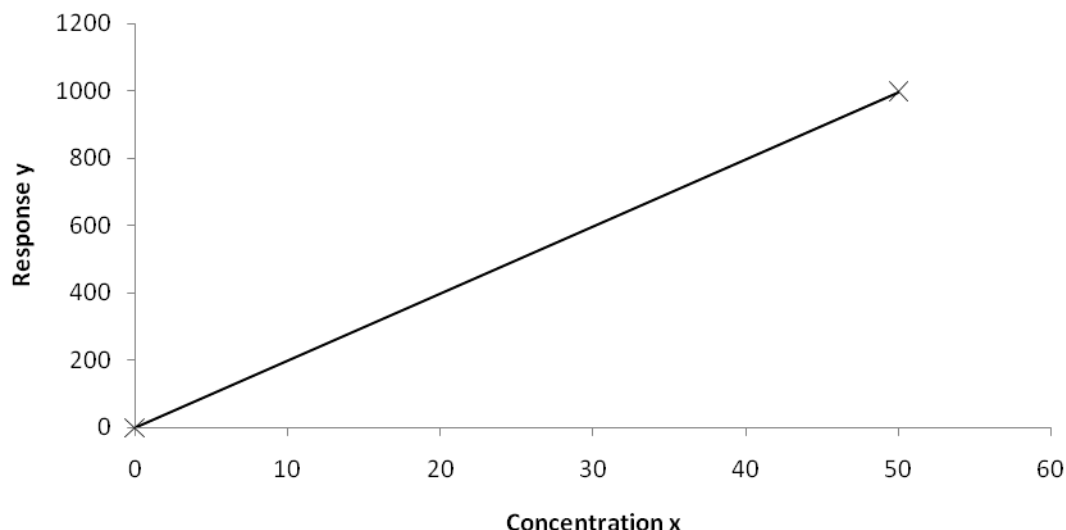
In reality a large number of pharmaceutical analytical methods are based on linear relationships and the terminology is appropriate in these situations. Therefore the term ‘linearity’ is used in the remainder of this book, but it is assumed that the reader understands that a linear calibration may not always apply.

Single point calibration

The most common type of calibration model encountered in the analysis of drug substances and drug products is known as a single point calibration, where the standard is prepared at one concentration level only. In fact this model defines a two point calibration line where one point equals zero and the other the standard concentration. A single point calibration line is shown in Figure 5. The unknown concentration of an analyte in a sample is calculated by entering the response obtained for the analyte when analysed by the method into the equation of the calibration line, $y = mx + c$. In order to force the line through zero, and thus use a single point calibration, the value of ‘c’ must be negligible.

To verify the single point model it is necessary to demonstrate that the value of the ‘c’ (the intercept) is negligible. This is achieved by measuring the response due to a number of different concentration levels across the range of the method and calculating the intercept for the best fit straight line. Since the aim of the investigation is to show that the line goes through zero it is not appropriate to include zero as a point in the investigation unless it is a measured value. For example, the absence of a peak in a chromatographic analysis cannot be interpreted as a zero result. However the measurement of the blank in a UV assay is a measured value and may be included in an investigation of linearity. This applies to all calibration models, not just single point.

Figure 5 Example of a single point calibration



Robustness

The section on robustness in the ICH guidelines¹ is as follows:

ICH

8. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- stability of analytical solutions;
- extraction time.

In the case of liquid chromatography, examples of typical variations are:

- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of gas-chromatography, examples of typical variations are:

- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

Robustness is not included in the tabular summary of required validation characteristics to be tested in the ICH guidelines, however it is expected that it is 'considered at an appropriate stage in the development of the analytical procedure'.¹ Robustness is included in the summary table provided in the draft FDA guidelines²⁴ (see Table 14). Evaluation of the robustness of an analytical method is usually

performed in two phases. Initial robustness testing is part of the method development process. A more formal study is then performed during validation of the method.

The factors which are investigated in robustness studies are method related, they are parameters which are defined in the method, e.g. temperature. Altering the value of these factors is a deviation from the method. In contrast, the factors which are investigated in intermediate precision (sometimes referred to as ruggedness) are non-method related factors, e.g. the analyst performing the analysis. When investigating these factors the method is followed in full without any deviations. Robustness factors are sometimes referred to as internal factors and those relating to intermediate precision as external factors. When an analytical method has been shown to have acceptable intermediate precision and robustness through the validation study, confidence is gained that the method can be used successfully in routine analysis. Also included in robustness testing is the evaluation of the stability of test solutions which are used in the analysis.

Some examples of robustness factors from available literature^{1,25,26} for a variety of analytical techniques are listed below:

High Performance liquid Chromatography (HPLC)

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate
- Buffer concentration (ionic strength)
- Additive concentration
- Gradient slope
- Initial mobile phase composition
- Final mobile phase composition
- Injection volume
- Sample preparation (pH of solutions, reagent concentrations, etc.)
- Equilibration time
- Column age

The Validation Protocol

The planning and design of a validation study involves a number of steps. First, the appropriate validation characteristics, as described in Chapter 2, are selected for the analytical method being studied. Next, the experiments which are required for each characteristic are defined including the concentration levels and the number of replicates to be tested. Then the required results and **acceptance criteria** are defined for each test.

Contents of the Validation Protocol

The validation protocol details the design of the validation study. Its purpose is to provide information on which characteristics will be tested during the study, how the experiments will be performed, and what results will be calculated. Typical information in a validation protocol may include:

- Details regarding the analytical method (or methods) to be validated.
- The validation characteristics which will be investigated for the method(s).
- Details on how the experiments will be performed including: the type and number of solutions to be prepared; how the solutions should be prepared; how measurements should be performed.
- Details of the results which will be calculated (with directions if appropriate).
- The acceptance criteria which will be applied to the results.
- Details of the reference materials which will be used in the validation study.
- Details of batches of the material used during the validation study. In the case of precision studies, particular representative batches may be selected.
- Details of the equipment which will be used in the study.
- Details of responsibilities and required signatures.

Validation Characteristics to be Studied

A tabular summary which details the validation characteristics that should be applied for different types of methods is included in the ICH guidelines¹. This table is extended in the draft FDA guidelines² on ‘Analytical Procedures and Method Validation’ to include specific tests and robustness, and is reproduced below, see Table 14.

Table 14 Recommended validation characteristics of the various types of tests²

Type of analytical procedure	Identification	Testing for impurities		Assay Dissolution (measurement only), Content/potency	Specific Tests
		Quantitative	Limit		
Accuracy	-	+	-	+	+ ⁴
Precision					
Repeatability	-	+	-	+	+ ⁴
Intermediate precision	-	+ ¹	-	+ ¹	+ ⁴
Specificity	+ ²	+	+	+ ⁵	+ ⁴
Detection Limit	-	- ³	+	-	-
Quantitation Limit	-	+	-	-	-
Linearity	-	+	-	+	-
Range	-	+	-	+	-
Robustness	-	+	- ³	+	+ ⁴

NOTE:

- Signifies that this characteristic is not normally evaluated.
- + Signifies that this characteristic is normally evaluated.
- 1 In cases where reproducibility has been performed, intermediate precision is not needed.
- 2 Lack of specificity for an analytical procedure may be compensated for by the addition of a second analytical procedure.
- 3 May be needed in some cases.
- 4 May not be needed in some cases.
- 5 Lack of specificity for an assay for release may be compensated for by impurities testing.

The ‘Type of analytical procedure’ in the table, i.e. identification, assay, testing for impurities and specific tests, was defined previously in Chapter 1. The table lists the

validation characteristics which are regarded as most important for the different types of methods. The ICH guidelines¹ state that ‘the list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis’. Laboratories which perform analytical method validation typically have in-house guidelines or standard operating procedures to describe the approach which should be taken for validation studies. These documents are usually based on the ICH guidelines and provide additional information on the organisational policy regarding which validation characteristics should be investigated for particular types of analytical methods.

Experimental Procedure and Acceptance Criteria

When the relevant validation characteristics have been identified, the experimental procedure which will be used to investigate those characteristics needs to be defined. Minimum requirements for the number of determinations and replicates which should be used are provided in the ICH guidelines. Also required is the rationale for assessing the results which are generated from the study. The acceptance criteria selected may be based on an absolute value of practical relevance or a statistical test may be used. The experimental procedure for performing a validation study and the relevant acceptance criteria are discussed for each validation characteristic below. The values quoted are suggestions provided for the purposes of orientation. Therefore they should be used with caution and may not be appropriate for a particular method.

Specificity

The aim of the specificity investigation is different for identification methods when compared to the aim for assay and impurity methods, due to the difference in the nature of the methods. For identification, specificity demonstrates that the method does not give a positive response for samples other than the one of interest. However, for assay and impurity methods, specificity demonstrates that the response due to the analyte of interest in the sample is not affected by potential interferences which may also be present in the sample. The result of this is that the approach used for the experimental part of the investigation and the acceptance criteria applied for each type of method differs.

Experimental

For identification methods samples will be selected which could be mistaken for the sample of interest. This selection requires knowledge of the analytical method, the manufacturing process for the material in question and knowledge regarding other processes using the same plant. The samples may include materials which are structurally similar or closely related to the analyte, e.g. isolated intermediates for the drug substance. Samples should be prepared in a similar way to the sample of interest and analysed as per method.

For assay and impurity methods the samples tested will contain materials which are potentially present during routine analysis and may interfere with the result, e.g.

Table 17 Suggestions for inclusion in a validation protocol for a quantitative impurities method

Validation characteristic	Experimental details	Acceptance criteria
Specificity	<p>Analyse samples containing potential interferences, e.g. impurities, excipients.</p> <p>Prepare samples by:</p> <ul style="list-style-type: none"> Spiking drug substance/drug product with the potentially interfering material. Prepare samples of the potentially interfering materials. Perform stress studies on drug substance/drug product. <p>Chromatographic:</p> <ul style="list-style-type: none"> Investigate peak purity of analyte peak. 	<p>No interference observed for response due to analyte or impurities of interest.</p> <p>Chromatographic:</p> <ul style="list-style-type: none"> No peaks interfering with the analyte peak or the peaks due to the impurities of interest observed. Minimum resolution between peaks of interest and neighbouring peaks = 1.5. No co-elution detected from peak purity investigation.
Linearity	<p>A least 5 concentrations over the range QL-120% of the specification limit.</p> <p>e.g. 25, 50, 75, 100 and 120% specification limit of the impurity, the values will depend on the values of the specification limit and the quantitation limit for a particular method.</p>	<p>Calibration model valid.</p> <p>Residual plot shows random scatter and no systematic trends.</p> <p>95% confidence interval of intercept includes zero, or intercept is less than 2% of nominal response.</p>
Range	<p>The range is defined by the results obtained for linearity, accuracy and precision.</p>	<p>Linearity, accuracy and precision demonstrated over the range.</p>
Accuracy	<p>At least 9 determinations over 3 concentration levels.</p> <p>e.g. 3 at quantitation limit, 3 at 50% of specification limit, and 3 at 120% of specification limit.</p>	<p>Mean recovery within 90-110%.</p> <p>Individual recoveries within 70-130%.</p> <p>Regression analysis of known vs. estimated:</p> <ul style="list-style-type: none"> Slope within 0.9-1.1 95% confidence interval of slope includes 1
Precision	<p>System precision assessed by 6 replicate measurements/injections.</p>	<p>%RSD \leq 2%</p>

The Validation Report

When the experiments detailed in the validation protocol have been performed the next stage of the validation study is the interpretation of the results. The calculations and statistics associated with each validation characteristic are carried out and the results obtained are assessed against the acceptance criteria to decide if the method meets the validation requirements.

Contents of the Validation Report

The validation report details the results of the validation study. Its purpose is to provide information on which characteristics were tested during the study, the results obtained, and the interpretation of those results. Typical information in a validation report may include:

- Details of the validation protocol.
- Details regarding the analytical method (or methods) validated.
- The validation characteristics which were investigated for the method(s).
- The results which were calculated for each validation characteristic.
- A discussion of the interpretation of the results and how they compare to the acceptance criteria.
- Any relevant validation information which was obtained during method development, e.g. solution stability, robustness, stress studies, etc.
- Details of the reference materials used in the validation study.
- Details relating to batch numbers, etc. of the materials used in the validation study.
- Details of the equipment used in the study, e.g. identifiers and qualification details.
- References to the laboratory notebooks, or equivalent used to record the raw data obtained during the validation study.

- Details of responsibilities and required signatures.

Statistics in Analytical Method Validation

The statistics required for the interpretation of validation results include: the calculation of the mean, standard deviation, confidence intervals and relative standard deviation for data sets obtained; regression analysis for evaluation of linearity and accuracy (these were discussed in Chapter 2); comparative studies; and assessment of the significance of outliers. Validated statistical software packages^{2,3} are normally used for the calculation of all statistics associated with analytical method validation.

Statistical significance

A statistically significant difference means that there is statistical evidence that a difference exists which is unlikely to have occurred by chance. It does not mean that the difference is necessarily large or important and thus may not be of practical relevance. Statistics are very useful to support analytical method validation but the results should be used with caution. Sound scientific judgement is required for interpretation of statistical results.

Comparative studies

Examples of comparative studies which could be performed during method validation are:

- Specificity and accuracy, to compare the results obtained using the method being validated against a second well characterised method.
- Precision, to compare the results obtained during intermediate precision and reproducibility studies.
- Robustness, to compare the effects due to the factors under evaluation.
- Stability of solutions, to compare the results obtained from solution which have been stored against the original results or results obtained for a freshly prepared sample.

Student's t-test

Student's t-tests are one of the most commonly used statistical significance tests applied to small data sets. A t-test may be used to compare two sets of data which are each characterised by their mean, standard deviation and number of data points, provided that the distribution of the data can be assumed to be normal. The outcome of a t-test is that the null hypothesis is accepted or rejected. The null hypothesis is that any differences between the data sets are due to random and not systematic errors, i.e. both methods give the same results, or both samples contain the same amount of analyte.

Case Study

A validation protocol for the analytical method presented in Chapter 3, 'Analytical Method for Determination of the Assay and Degradation Products of 'MiracleCure' 25 mg Tablets by HPLC' (Figure 12), was designed previously (refer to Table 19). The results from this study are presented in a tabular summary format in Table 25.

Table 25 Summary validation report for the analytical method and validation protocol detailed in Chapter 3 (refer to Figure 12 and Table 19)

Validation characteristic	Results	Acceptance criteria										
Specificity	<p>Chromatograms obtained for the specificity solutions showed that there are no interfering peaks.</p> <p>Retention times:</p> <table border="0"> <tr> <td>MiracleCure</td> <td>10.3 minutes</td> </tr> <tr> <td>Impurity X</td> <td>12.4 minutes</td> </tr> <tr> <td>DP 1</td> <td>15.0 minutes</td> </tr> <tr> <td>DP 2</td> <td>37.2 minutes</td> </tr> <tr> <td>Excipients</td> <td>no peak detected</td> </tr> </table> <p>Critical pair resolution = 3.2 (MiracleCure and Impurity X)</p>	MiracleCure	10.3 minutes	Impurity X	12.4 minutes	DP 1	15.0 minutes	DP 2	37.2 minutes	Excipients	no peak detected	<p>No peaks interfering with the peaks due to MiracleCure, Impurity X, DP 1 or DP 2.</p> <p>Minimum resolution between peaks of interest and neighbouring peaks = 2.</p>
MiracleCure	10.3 minutes											
Impurity X	12.4 minutes											
DP 1	15.0 minutes											
DP 2	37.2 minutes											
Excipients	no peak detected											
Linearity – Assay	<p>Calibration model is valid</p> <p>Equation of line, $y = 1219x + 691$</p> <p>Correlation coefficient = 1.000</p> <p>Residual sum of squares = 150186</p> <p>95% confidence interval for the intercept includes zero, -105 to 1487, intercept is 0.56% of nominal response.</p> <p>The plot of residuals shows random scatter and no systematic trends.</p>	<p>Calibration model valid.</p> <p>Residual plot shows random scatter and no systematic trends.</p> <p>95% confidence interval of intercept includes zero, or intercept is less than 2% of nominal response.</p>										
Linearity – DP 1	<p>Calibration model is valid</p> <p>Equation of line, $y = 1303x - 3.17$</p> <p>Correlation coefficient = 0.998</p> <p>Residual sum of squares = 4318</p> <p>95% confidence interval for the intercept includes zero, -18.47 to 12.12, intercept is 0.95% of response at 50% SL.</p> <p>The plot of residuals shows random scatter and no systematic trends.</p>	<p>Calibration model valid.</p> <p>Residual plot shows random scatter and no systematic trends.</p> <p>95% confidence interval of intercept includes zero, or intercept is less than 2% of response at 50% SL (0.25% nominal).</p>										

Glossary

A

Acceptance criteria	The criteria which are applied to the results obtained from a validation study, if the results comply with the criteria then it is concluded that the analytical method is fit for the intended purpose.
Accuracy	The closeness of agreement between a test result and the accepted reference value.
Active Pharmaceutical Ingredient (API)	The 'active' or the 'active pharmaceutical ingredient' is the substance in a drug preparation that is pharmaceutically active.
Analyte	The compound of interest to be analysed.
Analytical Instrument Qualification (AIQ)	Documented evidence that an analytical instrument performs suitably for its intended purpose and that it is properly maintained and calibrated.
Assay	An analytical method to analyse or quantify a substance in a sample.

B

Bioanalysis	The chemical analysis of biological samples, e.g. plasma, urine etc.
Bioassay	A biological test, measurement or analysis to determine whether compounds have the desired effect either in a living organism, outside an organism, or in an artificial environment.
Bioavailability	The amount of drug absorbed into the body.
Bioequivalence	The comparison of the expected in vivo biological equivalence of two proprietary preparations of a drug. If two products are said to be bioequivalent it means that they would be expected to be, for all intents and purposes, the same.
Biopharmaceutical	A drug produced by biotechnology.
Biotechnology	The application of scientific and engineering principles to the processing of materials by biological agents.

List of Abbreviations

AIQ	Analytical Instrument Qualification
API	Active Pharmaceutical Ingredient
CDS	Chromatography Data System
COTS	Commercial Off The Shelf
DAD	Diode Array Detector
DL	Detection Limit
DOE	Design of Experiments
DQ	Design Qualification
EMEA	European Medicines Agency
EP	European Pharmacopoeia
FDA	Food and Drug Administration (US)
GC	Gas Chromatography
GFC	Gel Filtration Chromatography
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation
IMPD	Investigational Medicinal Product Dossier
IND	Investigational New Drug
IQ	Installation Qualification

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