View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Universal Journal of Pharmaceutical Research

ORIGINAL RESEARCH ARTICLE

AN OVERVIEW OF ANALYTICAL METHOD VALIDATION

ABSTRACT

In line with the developments in the pharmaceutical field, the product range is constantly being renewed and diversified. There is a need to develop and validate new analytical techniques for new pharmaceutical products. The validation of an improved method is an internationally recognized scientific requirement, as these validation practices are also indicative of the competence of the analytical laboratory.The method development process ensures the applicability and reliability of the data. The result is a more detailed understanding of the standard test methods and an additional insight into the relationship between test methodology and product performance. It is important to validate an advanced method. Because if the method can not be reproduced, the method is meaningless. Validation is always a balance between costs, risks and technical possibilities.

Keywords: Medicines, Quality, Test Method, Validation.

INTRODUCTION

In line with the developments in the pharmaceutical field, the product range is constantly being renewed and diversified. There is a need to develop and validate new analytical techniques for new pharmaceutical products [1]. The validation of an improved method is an internationally recognized scientific requirement, as these validation practices are also indicative of the competence of the analytical laboratory [2].

It is important to assess the applicability and safety of each new pharmaceutical construction/product and to determine whether the method is suitable for the intended target under suitable conditions. Otherwise, the data produced by the method may not shed light on the application, may not evaluate the quality, or may not help solve a particular problem. When applying standard methods to new pharmaceutical productions / products, it should optimize and characterize a standard test method for user-specific applications and verify its suitability. To characterize the product, it is important to well characterize the test method used in the analysis before relying on the data obtained.

Analytical instruments play an important role in the process of obtaining high quality and reliable analytical data; therefore, the quality assurance of analytical equipment in the laboratory must be questioned. Analytical method can be spectroscopic, chromatographic, electrochemical or a combination of other techniques. Analytical method development is generally the process of determining the correct test procedure; concurrent validation is the process of proving that an analytical method for measuring the concentration of subsequent samples is suitable for use in the laboratory. Analytical methods should be used in Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) environments, anddeveloped using the protocols and acceptance criteria specified in Q2 (R1) inthe International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The basic prerequisites for method development are as follows [3-6]:

1. Qualified and calibrated devices;
2. Documented methods;
3. Reliable reference standards;
4. Competent analysts;
5. Sample selection and integrity;
6. Change control.

An analytical procedure is developed to measure a defined property of the target component according to the acceptance criteria specified for that property. In the development of a new analytical procedure, the choice of analytical device and methodology should be based on the intended purpose and scope of the analytical method. Specific parameters that can be evaluated during method development are specificity, linearity, limit of dedection (LOD) and limit of quantification (LOQ), range, accuracy and precision; specificity is always the first parameter to be evaluated as the most

Reviewer's Copy

important feature of analytical methods. However, in the early stages of method development, among other parameters, the robustness of the methods must be assessed; this feature helps to decide which method will ultimately be approved. The development of analytical procedures is based primarily on the basic knowledge and the sum of previous experience. Experiences and experimental data from previous procedures can be used to guide further developments.

Method development commonly includes following steps:

1. Method development plan definition
2. Basic information collection
3. Standard analyte characterization
4. Determination of method requirements
5. Scientific article research
6. Method selection
7. Device installation and preliminary studies
8. Optimization of parameters
9. Documentation of the analytical picture
10. Evaluation of method development with sample application
11. Determination of percentage recovery of the sample
12. Demonstration of quantitative sample analysis
13. Establishing the test procedure
14. Define method validation protocol
15. Validation of laboratory methods
16. Creation of validated test method
17. Validation report

An analytical method details the steps and approaches needed to perform an analysis. These include the preparation of samples, standards and reagents, the use of instruments, the creation of a calibration curve, and the use of formulas for calculation. Analytical method development is required in the following sample types and situations:

1. Herbal products and their activities
2. New processes and reactions
3. Development of new molecules
4. Active components (Macro analysis)
5. Residues (Micro analysis)
6. Determination of impurity
7. Components of the substance in different proportions
8. Decay studies

Reviewer's Copy

In addition to the general principles listed above, the sample preparation section should be seen as an integral step in the analytical method / method development. The most important step of sample preparation is to recognize the sample. The detection or knowledge of relatively rough information such as structure of sample medium, analyte concentration, number of target component in the sample to be analyzed, as well as more detailed physicochemical parameters, such as pKa value, molecular size and weight, electrical charge, solubility, volatility, stability, toxicity, polarity, chemical reactivity and absorptivityof the target compounds provides the analyzer significant advantages in recognizing the sample and analytes.In the light of the information obtained; A sample suitable for starting method development is obtained using the conventional sample preparation or purification techniques.

After the sample preparation, the type of analysis appropriate to the structure of the sample is determined and the method development step is started.

The need for validation of the analytical method has arisen due to international competition and ethical reasons that keep the standard of products high in terms of commercial and market value. Various international organizations have adopted a standard and fixed protocol in accordance with the reference for authorization and licensing. The main rules and guidelines governing quality standards are as follows:

1. Good Manufacturing Practice (GMP) regulations
2. Good Laboratory Practices (GLP) regulations
3. Pharmaceutical Inspection Co-operation Scheme-PIC / S

4th International Conference on Harmonization (ICH)
5. ISO / IEC 17025 quality guide published by International Organization for Standardization
6. World Health Organization (WHO)

If changes are made to non-standard but proven methods, the effect of this change must be documented and validation re-established. If there are standard methods for testing a particular sample, then the most current version must be used. Validation includes determination of requirements, determination of method characteristics, control of the fulfillment of requirements using the method, and a statement of validity [7,8]. To fully understand the impact of changes in method parameters on the analytical procedure, a systematic approach is adopted for initial risk assessment and multivariate method robustness study (experimental design with method parameters); such approaches allow us to understand the effects of parameters on method performance. Evaluation of the performance of a method may include analysis of samples obtained from a range of in-process production steps to the finished product. The information obtained about the sources of the method diversity during these studies can help to evaluate the performance of the method.
A well-developed method is considered to be fully validated [9]. When using any analytical technique for pharmaceutical product analysis, there is a need for a well-developed, detailed, and in-depth method development practice.
Various types of problems are also encountered in method development. For example, stored samples are initially correct, but may slowly become wrong with a low systematic error. On the other hand, it is a known fact that a series dilute curve is concave. Reaction factors decrease with decreasing concentration and increased exposure due to dilution number, surface area contact, and time may cause this problem. In general, detailed problems specific to the analytical approach, such as those known in the art or those listed above, may also be encountered. In addition, the main elements of successful method development and implementation are summarized in Table 1.

Reviewer's Copy

Table1. Considerations for the development and application of a successful analytical method [12].

1	The intended use of the test is clearly defined depending on the desired product property (eg definition, purity, impurities, efficacy, concentration, stability) and requirements of acceptance specifications.
2	It is understood how the method technology functions to generate information about the parameter of interest. System compliance solutions are developed to evaluate method performance regardless of the performance of the test sample.
3	Potential sources of methods and operational variability that may affect reproducibility of the test procedure are found and controlled. System compliance solutions are included to ensure the validity of each test and monitor method performance over time.
4	The suitability of the method for scientifically intended use is confirmed by showing performance parameters such as accuracy, precision (within and between experiments), linearity, range, detection limit (LOD) / quantitative determination limit (LOQ), and specificity (including sample degradation products if they are stability indicator).
5	It is verified that the test is sufficiently robust under the expected conditions of use to statistically support the specification requirements in each phase of product development and commercially.
6	As time passes, it is ensured that the documentation and data obtained from each lifecycle status of the method is preserved in completed, traceable and accessible archive files for use of the product and method to support information management.

METHOD VALIDATION

Method validation can be defined as the process of demonstration that a particular improved analytical method is acceptable for its intended use [9]. Validation is an important requirement in the implementation of an analytical process. The method can be evaluated as a process of validating, defining an analytical requirement, and confirming that the method being examined has consistent performance capabilities as required by the application. With respect to the biotechnological synthesis of pharmaceutical products, validated methods for measuring the amount of both the product and the substrate at different time intervals are essential for accurate calculation of rate coefficients [11,12]. Validation of the method is a continuous process and the latest purpose of validating the analytical method is to make sure that every future measurement in routine analysis is close enough to the actual

unknown value for the analyte content in the sample [13]. Due to the continuous developments in analytical measurement technologies, analytical methods are being updated over time; therefore, the validity and cross-validity of the methods gain importance for the accurate interpretation of the data collected over the years [14,15].

Validation is carried out by the official, approved and signed validation protocol in the Quality Assurance (QA) unit. Validation is complete when the following steps are performed:

i) When all acceptance criteria are shown to be fulfilled,

ii) When results are clearly documented in accordance with Current Good Manufacturing Practices (Current GMP-cGMP),

iii) When the final method validation report, including references to raw data which demonstrate how the acceptance criteria have been met are fully reviewed and approved by related personnel, including employees, management and quality assurance unit.

Validation Guides

1. ICH Q2R (1) Evaluation of Analytical Procedures: Text and Methodology
2. FDA guidance for industry: Analytical procedures and method validation
3. Various validation guidelines of ISO
4. European, US and Turkish Pharmacopoeias

Requirement of Validation of Analytical Method

Method validation is required due to the following reasons [11];

1. Ensuring the quality of pharmaceutical product.
2. Ensuring the acceptance of pharmaceutical products by international organizations.
3. Request for the accreditation of test parameter according to ISO 17025 guidelines.
4. Registration of any pharmaceutical product or formulation (validated methods are only acceptable to participate in the proficiency test).

Validation not only improves processes, but also confirms that the process has been developed appropriately. Validation of the manufacturer's method is important for:

• Deepening understanding of processes and reduce the risk of preventing problems;
• Reducing risk of error cost;
• Reducing the risk of regulatory non-compliance;
• A fully validated process may require less in-process control and end-product testing [16].

Reviewer's Copy

Types of Analytical Procedures to Validate

The discussion of the validation of analytical procedures addresses four most common types of analytical procedures:

1. Identification tests for active or related subtances;
2. Quantitative tests for impurity content;
3. Limit tests for the control of impurities;
4. Quantitative tests of the active moiety in a pharmaceutical sample.

Qualitative or identification tests are intended to provide the identity of the analyte in a sample. This is achieved by comparing a property of the sample (spectrum, chromatographic behavior, chemical reactivity, etc.) to the same property of the reference standard. The impurity test can be a quantitative test or limit test for impurity in a sample. The test is intended to show the purity characteristics of the sample. Analytical method development and its validation place at the heart of impurity investigations that provide information contributing to reproducible production of high quality product. For all significant process and degradation-related impurities, occuring in manufacturing process or storage conditions, to be detected and quantified, specifying how many test should be done is important. The main aspect of this approach is to use as many techniques as possible to search for impurities including various types of analytical techniques with different detection options. The initial method would be severely affected by the characteristics of active substances and their molecular changes during the process and possible by-products or degradation products. Impurity investigation provides specification tests and acceptance limits set up at various control points in the development and manufacturing processes and quality control [20]. A quantitative test requires validation characteristics different from a limit test. Assay procedures are designed to measure the analyte present in a given sample. Here; The assay represents a quantitative measurement of the major component(s) in the pharmaceutical product.

Criteria to be Fulfilled by Validation

Validation of an analytical method demonstrates the scientific soundness of measurement or characterization. Various scopes need to be changed during the application process to the regulatory body. Validation demonstrates that an analytical method measures the correct substance, the right amount, and the appropriate range for the samples. It allows the analyst to figure out the behavior of the method and specify the performance limits of the method [17,18]. To validate the method, it is obligatory to follow the written standard operating instructions that describe the validation process of the method. The laboratory should use qualified and calibrated instruments. A well-developed and documented test method and an approved protocol must be available before validation. The protocol is a systematic plan explaining which method performance parameters should be tested and how to evaluate the parameters with acceptance criteria. As with drugs, validation experiments require an active pharmaceutical ingredient or pharmaceutical product, placebo and reference standards. The criteria that the validation process must fulfill can be listed as follows:

1. The whole method must be validated. At this point it is quite usual to focus on the fixation technique or instrumental measurement, which usually means that only the stage in question is validated. However, the previous steps of the sample pretreatment, extraction or pre-concentrating steps also belong to the analysis method and are very important. Therefore, all of them need to be verified.
2. The whole concentration range must be validated. This is difficult to follow, because one method may work very well in a certain concentration range, but not in others.
3. The entire matrix range must be validated. It is well known that the matrix may have a decisive effect on the analysis. Therefore, and for representation, several matrices must be submitted to validate the method.

As mentioned in the above, validation of the analytical method is the process of proving that the method is suitable for the specified purpose. The results from the method validity study can be used to assess the quality, reliability, and consistency of the method. To validate the method, selectivity, stability, linearity and range, detection limit, quantitative determination limit, precision, accuracy and robustness parameters should be analyzed and shown to be appropriate. Which parameters need to be studied is stated by standards or pharmacopoeias (Table 2).

Reviewer's Copy

Table 2. Basic method validity parameters according to some sources [21].

Parameter	ICH	USP	EP	ISO 17025
Specificity	+	+	+	+
Linearity	+	+	Qn	Qn
Range	+	+	Qn	Qn
Limit of detection	+	L	L	Ql ve L
Limit of quantification	+	Qn	L	L
Precision				
Repeatability	+	+	Qn	Qn
Intraday reproducibility	+	-		
Interday reproducibility	+	-		
Accuracy	+	+	Qn	Qn
Robustness	R	+	-	+
Ruggedness	+	R	+	-

R: recommended, Qn: only in quantitative studies, Ql: only in qualitative studies, L: only in limit tests

Which validation parameters to be studied are mainly related to the purpose of the analysis. The ICH guideline states which parameters should be applied in which case as presented in Table 3 [17].

Table 3. Method-dependent necessity of method validity parameters [17].

Parameter	Analytical method type		
	Identification	Impurity Tests	DetectionDissolution

		Quantification	Limit	Content/Potency
Specificity	+	+	+	+
Linearity	-	+	-	+
Range	-	+	-	+
Limit of detection	-	- (1)	+	-
Limit of quantification	-	+	-	-
Precision				
Repeatability	-	+	-	+
Intraday reproducibility	-	+(2)	-	+(2)
Interday reproducibility	-	+	-	+
Accuracy	-	+	-	+

(1) May be necessary in some cases.

(2) .Where repeatability is performed, intermediate precision is not required

When an analytical procedure (including complementary conditions) is validated (or verified) and applied; this procedure is followed to ensure that it remains consistently appropriate for the intended purpose, throughout its lifetime. Assessment analysis for method performance should be regularly done to assess the need to optimize the analytical procedure, or to revalidate the whole or some part ofanalytical procedure. Ifan analytical procedure only meets the system compliance requirements provided along with repeated changes made under the working conditions stated in this procedure, the analytical procedure has to be suitably reassessed, re-validated or corrected. Choosing new information and risk assessment (a better understanding of current, product critical quality characteristics or awareness of a new impurity etc.) over the course of a warranty lifetime is a guarantee for a new or alternative analytical guidance. New technologies can allow greater understanding and / or confidence in ensuring product quality. The applicant should periodically assess the appropriateness of analytical methods at a glance and consider new or alternative. Where life cycle changes in analytics need to be foreseen, appropriate stored samplesshould be kept to allow comparative studies.The number should be based on scientific principles and risk assessment. For complex products that are sensitive to production changes, replacement samples can be an important tool for making these comparisons. Stored samples used in comparative studies should include samples representing the marketed product and, where possible, important clinical trial materials. When a risk-based assessment or other impact leads to changes in the analytical procedure or to replacement with a new method, or when transfering of the procedure to a new test site; revalidation, a new validation study, analytical method comparability study, or a combination of these studies should be taken into consideration.In some cases, changes to the active substance or pharmaceutical product manufacturing process may also ensure that the analytical procedure is re-validated.In terms of future trends in analytical method validation “real time” analytical methodologies and continious validation issues are taken into consideration [22].

Reviewer's Copy

CONCLUSION

The uniqueness of pharmaceutical products and their applications determine the suitability of a standard test method. By applying the method development process to standard test methods, the generated data is expected to be more meaningful. As a result, improved data quality enables decisions to be made with increased confidence. Not only can problems be detected quickly when reliable data is available, but solutions are also made easier to develop. The method development process ensures the applicability and reliability of the data. The result is a more detailed understanding of the standard test methods and an additional insight into the relationship between test methodology and product performance. It is important to validate an advanced method. Because if the method cannot be reproduced, the method is meaningless. Validation is always a balance between costs, risks and technical possibilities. Therefore, strong in-service training will ensure successful method development and validation.

REFERENCES

[1] Rashmin. An introduction to analytical method development for pharmaceutical formulations. Pharm Rev, 2008;6:1-10.

[2] Taverniers I, Loose MD, Bockstaele EV. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Analyt Chem, 2004;23:535.

[3] ICH, International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q7:Good Manufacturing Practices for Pharmaceutical Ingredients 2000.

[4] USP, Current Good Manufacturing Practices for finished Pharmaceuticals, 21 CFR, Parts 210 and 211, US Food and Drug Administration.

[5] EC, European Commission Final Version of Annex 15 to the EU Guide toGood Manufacturing Practice: Qualification and validation 2001;4:1-10.

[6] McDowall RD. Effective and Practical risk management options for computerized system validation. Quality Assurance Journal 2005;9(3):196-227.

[7] Bansal KS, Layloff T, Bush ED, Hamilton M, Hankinson EA. Qualification of Analytical Instruments for Use in the Pharmaceutical Industry: a Scientific Approach. AAPS Pharm Sci Tech 2004;5:1-8.

[8] FDA, Guidance for Industry: Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation. U.S. Department of Health and Human Services 2000.

[9] Breaux J, Jones K., Boulas P. Understanding and implementing efficient analytical methods development and validation. Pharm Technol Anal Chem Test 2003;5:6-13.

[10] Shintani H. Development of test method for pharmaceutical and biopharmaceutical products. Pharmaceutica Analytica Acta 2013;4(7):258.

[11] Araujo P. Key aspects of analytical method validation and linearity evaluation. J Chromatogr B Analyt Technol Biomed Life Sci, 2009;877:2224-34.

[12] Putheti RR, Okigbo RN, Patil SC, Advanapu MS, Leburu R. Method development and validations: Characterization of critical elements in the development of pharmaceuticals. Int J Health Res 2008;1:11-20.

[13] Lindholma J, Johanssonb M, Fornstedt T. Guidelines for analytical method development and validation of biotechnological synthesis of drugs production of a hydroxyprogesterone as model. J Chromatogr B 2003;791:323-36.

[14] Gonzalez G, Herrador M. A practical guide to analytical method validation including measurement uncertainty and accuracy profiles. Trends Anal Chemical Engineering Journal 2007;26:227-38.

[15] Silva MJ, Preau JL, Needham LL, Calafat AM. Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci 2008;873:180-6.

[16] Jatto E, Okhamafe AO. An overview of pharmaceutical validation and process controls in drug development. Trop J Pharm Res, 2002;1:115-22.

[17] ICH, International Conference on Harmonization Validation of analytical procedures: text and methodology Q2 (R1) 1994.

[18] Chan CC, Lam H, Lee YC, Zhang XM. Analytical Method Validation and Instrument Performance Verification, Hoboken, John Wiley & Sons (Wiley Inter science), New Jersey 2004.

[19] Feinberg M, Boulanger B., Dewe W, Hubert P. New advances in method validation and measurement uncertainty aimed at improving the quality of chemical data. Anal Bioanal Chem 2004;380:502-14.

[20] Ahuja S, Alsante KM. Handbook of Isolation and Characterization of Impurities in Pharmaceuticals, 2004;5:1-414.

[21] ICH, International Conference on Harmonization; Draft Guidance on specifications, Test procedures and acceptance criteria for new drug substances and products: Chemical Substances. Fed Regist. 2000;65:83041-63.

[22] Ermer, J., & Miller, J. H. M. (Eds.). Method validation in pharmaceutical analysis: A guide to best practice. John Wiley & Sons, 2006, 389-395.

Reviewer's Copy