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REVIEW ARTICLE

AN OVERVIEW OF ANALYTICAL METHOD VALIDATIONUmut BESKAN¹ , Sümeýra TUNA YILDIRIM² , Evren ALGIN YAPAR^{1*} ¹Department of Analysis and Control Laboratories, Turkish Medicines and Medical Devices Agency, Sıhhiye, Ankara, Turkey.²Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Yalınzbağ, Erzincan, Turkey.**ABSTRACT**

In line with the developments in the pharmaceutical field, the product range is constantly being renewed and diversified. New analytical techniques need to be developed and validated 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 is a process that ensures the applicability and reliability of the data. The result is a more comprehensive understanding of the standard test methods and a further insight into the connection between test methodology and product quality. It is important to validate an advanced method. Because if the method can not be reproduced, the method is meaningless. Validation is a continuous balance among costs, risks and technical possibilities.

Keywords: Medicines, quality, test method, validation.

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INTRODUCTION

In line with the developments in the pharmaceutical field, the product range is constantly being renewed and diversified. New analytical techniques need to be developed and validated for new pharmaceutical products¹. 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 laboratory².

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

The process of obtaining accurate and reliable analytical data gives an important role to analytical instruments; therefore, the quality assurance of analytical equipment in the laboratory must be questioned. Analytical method can be based on

spectroscopy, chromatography, electrochemistry or a combination of those techniques. Analytical method development refer generally to the process of determining the correct test procedure; concurrent validation is the process of showing that the developed analytical method for measuring the concentration of subsequent samples is suitable for usage in the laboratory. Analytical methods should be used in Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) environments, and developed using the protocols and quality control criteria specified in Q2 (R1) in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The following are the basic prerequisites for analytical method development³:

1. The devices that are routinely qualified and calibrated;
2. Well documented methods;
3. Reliable reference standards;
4. Competent and experienced analysts;
5. Proper sample selection and batch integrity;
6. Checking for any changes.

An intended property of the component of attention is measured by developing the analytical procedure that has met the quality control criteria specified for that property⁴. In the development process of a new analytical procedure, the selection of analytical instrument and methodology should rest on the

intended purpose and coverage of the analytical method⁵. The important parameters that can be assessed throughout the method development process are specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ), range, accuracy and precision; specificity is always the first parameter to be evaluated as the most important feature of analytical methods. However, in the initial stages of method development process, along with other parameters, robustness must be evaluated so that; this parameter helps to decide which method will be verified finally⁶. The development of analytical procedures is based mainly on the basic knowledge and the sum of previous experience. Experiences and experimental data from previous procedures can be used to contribute to 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 initial studies
8. Parameter optimization
9. Documentation of the analytical picture
10. Assessment of method development with sample implementation
11. Determination of percentage recovery of the sample
12. Demonstration of quantitative analysis for samples
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 specifies the steps and approaches needed to perform an analysis. These include the preparation of selected samples, reference or primary standards and chemical reagents, the usage of instruments, the plotting of a calibration curve, and the usage of formulas for calculation. Analytical method development is felt necessity for the following sample types and situations:

1. Herbal products and their activities
2. New processes and reactions
3. New molecule development
4. Active components (Macro analysis)
5. Residues (Micro analysis)
6. Impurity investigation
7. Components of the substance in different proportions
8. Decay studies

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 recognizing 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 absorptivity of 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 increased due to international competition and ethical reasons that keep the quality of products high in terms of economic 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
 1. 4th International Conference on Harmonization (ICH)
 4. ISO/IEC 17025 quality guide published by International Organization for Standardization
 5. World Health Organization (WHO)

If non-standard but proven methods are changed, 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 comprises determination of requirements and method characteristics, checking of the fulfillment of requirements using the method, and a statement of validity^{7,8}. To fully recognize 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); this approach allow us to figure out the effects of parameters on method performance. Assessment of the performance of a method may include analysis of samples selected from a range of in-process production steps to the final product. The information obtained about the sources of the method diversity during these studies can contribute to evaluation for the method performance.

A well-developed method is considered to be fully validated⁹. 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.

Table 1: Considerations for a successful analytical method development and its application¹².

1	The intended use of the test is clearly defined depending on the desired product properties (eg definition, purity, impurities, efficacy, quantity, stability) and requirements of quality specifications. It is figured out how the method technique functions to generate information about the interested parameter.
2	System compliance solutions are developed to evaluate method performance regardless of the performance of the test sample.
3	Potential sources for methods and operational variability that may influence on reproducibility of the test procedure are found and handled. System compliance solutions are taken into account to ensure the validity of each test and monitor the performance of method over time.
4	The method suitability for scientifically intended use is confirmed by showing performance parameters like accuracy, precision (within and between experiments), linearity, range, detection limit (LOD)/quantitative determination limit (LOQ), and specificity (including sample degradation products if they indicate stability).
5	It is verified that the test is sufficiently robust under the expected conditions of usage to statistically support the specification requirements in each phase of product development.
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 described as the process of demonstration that the developed analytical method is acceptable for its intended use⁹. The implementation of an analytical process importantly requires validation. The method can be evaluated as a process of validating, specifying an analytical requirement, and confirming that the method being examined has consistent performance efficiencies as required by the application¹⁰. Relating to the biotechnological synthesis of pharmaceutical products, validated methods for measuring the amount of both the product and the substrate at different timeframes are fundamental to accurate calculation of rate coefficients^{11,12}. Method validation is a continuous process and the last purpose of validating the analytical method is to make sure that every subsequent measurement in routine analysis is close enough to the actual unknown value for the analyte concentration in the sample¹³. Due to the continuous developments in the technologies of analytical measurement, analytical methods are restored over time; therefore, the validation and cross-validation of the methods gain momentum for the accurate judgment of the data collected over the years^{14,15}.

Validation is carried out according to the official, approved and signed validation protocol in the Quality

Assurance (QA) unit. Validation process is completed by performing the following steps:

- i. When all acceptance criteria are shown to be fulfilled,
- ii. When results are clearly documented corresponding to Current Good Manufacturing Practices (Current GMP-cGMP),
- iii. When the final report for method validation, including references to raw data which demonstrate how the acceptance criteria have been met are entirely checked 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 imperative due to the following reasons¹¹;

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

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

- Extending the understanding of processes and reducing the risk of preventing problems;
- Reducing risk of error cost;
- Reducing the risk of regulatory non-compliance;
- An entirely validated process may require less in-process control and end-product testing¹⁶.

Types of analytical procedures to validate

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

1. Identification tests for active or related substances;
2. Quantitative tests for impurity content;
3. Limit tests for the control of impurities;
4. Quantitative tests for 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 purity characteristics of the sample is understood by impurity investigations. 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, occurring 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 control²⁰. 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

an analytical method validation exhibits the scientific strength of measurement or characterization. Various scopes need to be changed during the application process to the regulatory body. Validation proves that an analytical method measures the intended substance with the right amount, at the suitable range for the samples. It helps 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 method validation process. The laboratory should use there should be routinely qualified and calibrated instruments in the laboratory. A well-developed and documented test method and an approved protocol must be available before validation. The protocol is a standardized plan stating which method performance parameters will be tested and how to evaluate the parameters according to 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 method must be validated thoroughly. At this point it is quite conventional to concentrate on the fixation technique or instrumental measurement, which usually means that only the part in question is validated. On the other hand, the previous stages of the sample pretreatment, extraction or pre-concentrating steps also belong to the analysis method and have great importance. Thus, all of them need to be verified.
2. The whole concentration range must be validated. This is difficult to follow, because one method may show good performance in a certain concentration interval, but not in others.
3. The validation for entire matrix range must be performed. It is well known that the matrix may have a definitive 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).

Table 2: Basic method validity parameters according to some sources²¹.

Parameter	ICH	USP	EP	ISO 17025
Specificity	+	+	+	+
Linearity	+	+	Qn	Qn
Range	+	+	Qn	Qn
Limit of detection	+	L	L	Q1 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, Q1: 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 3¹⁷.

When an analytical procedure (along with complementary methods) 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 of analytical procedure. If an 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. Over the course of the lifetime of a product, New information and risk assessment (a better realizing of product the critical quality characteristics of product or awareness of a new impurity etc.) may be a guarantee for a new or alternative analytical method development and its validation²⁰. New technologies and innovations can allow better understanding and/or confidence in ensuring product quality. The applicant should frequently assess the suitability of analytical methods for a product and consider new or alternative methods. Where life cycle changes in analytics need to be foreseen, appropriate stored samples should be kept to allow comparative studies. The number of the stored samples should stand on scientific principles and risk assessment. For complex products that are affected easily from the adjustment made on production,

replacement samples can be an important tool for making these comparisons²¹. The samples representing the marketed product and, where possible, important clinical trial materials should be among the stored samples used in comparative studies. When a risk-based assessment or other impact leads to some changes in the analytical procedure or to replacement with a new method, or when transferring of the procedure to a new test laboratory; method revalidation, a new method validation study, analytical

method comparability study, or a combination of these studies should be taken into consideration. In some cases, modifications 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 continuous validation issues are taken into consideration²².

Table 3: Method-dependent necessity of method validity parameters¹⁷

Parameter	Analytical method type			
	Identification	Impurity Tests		Detection Dissolution Content/ Potency
		Quantification	Limit	
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

CONCLUSION

The suitability of a standard test method is determined by the singularity of pharmaceutical products and their applications. The method development application to standard test methods is expected to result in the more meaningful generated data. As a conclusion, enhanced data quality enables decisions to be made with increased confidence. Not only can troubles be observed quickly when reliable data is available, but solutions become also easier to find. The method development process along with validation 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.

AUTHOR'S CONTRIBUTION

All authors have worked equally for this work.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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