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IN THE LAB | Method Validation

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Perspectives on Method Validation: Importance of adequate method validation

Editor's Note: This article is the first in a three-part series on method validation. Part two will focus on the application of validation to small-molecule formulations and part three will focus on large-molecule applications.

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The appropriate validation of analytical methods has become an essential part of successful drug development and characterization. Validation of a method involves using experimental design to prove that the method can produce accurate and precise results within the scope of its intended use. Understanding the application and limitations of the test method will allow for accurate assessment of sample information, ranging from process outputs to commercial release testing and many steps in between. If the method validation has not been performed or has been performed in an inadequate manner, the method is not proven to provide reliable data.

The key aspect of method validation is determination of the scope of the method being validated and how that scope will dictate what is to be assessed during validation. This challenge presents itself in the manufacturing environment, where the same analytical method may be used to test samples in a variety of matrices. In this case, validation of the method would be tailored to generate accurate information on several different types of samples, each with unique challenges.

In some cases, appropriate and comprehensive method validation is not being performed, a fact supported by the U.S. Food and Drug Administration's recent citations for inadequate method validation. A majority of audit findings fell into three main categories: the use of a non-validated method for critical decision making; inadequate method validation that did not provide the necessary information; or method validation that lacked appropriate controls to maintain the integrity of the validation. An example from each category is presented below.

Cases Reviewed

In the first case, a pharmaceutical manufacturer was cited for not validating its product quality method. The reason given for the lack of validation was the complexity of the sample matrix being tested. In this case, researchers could have no confidence in the data generated, because if the matrix was in fact complex, significant unseen issues might exist with the accuracy of test data. Regardless of the complexity of the sample matrix, an adequate level of method validation is required to ensure that test results are reliable.

In another case, a firm was cited for using an inadequately validated method, and the issues became more involved. In this case, an in-process product concentration assay was being used to determine low-level impurities in the sample. A properly validated in-process assay could achieve this scope, but according to the citation, the executed validation did not include detection and quantitation limit testing of process impurities; therefore, the method was inappropriate for the determination of low-level impurities. If an impurity was enriched due to a process deviation, the method in place might not be capable of ascertaining the failure. This example highlights the importance of ensuring that the entire scope of the method is considered when designing the validation experiments.

In the third case cited by auditors, the method validation was performed, but the predetermined validation criteria were not properly adhered to or were not specific enough to prohibit reanalysis without due cause. In this case, chromatography results generated in the validation were reanalyzed multiple times without using a standardized analysis mechanism. This led to passing results for data that may have failed the original intention of the validation.

The lack of proper controls and adherence to reprocessing restrictions produced a situation in which the true output of the validation was now in question. This could have been avoided if the data analysis had been consistently performed and the outcome critically evaluated. This example stresses the importance of validations following current good manufacturing process requirements and the importance of the validation protocol itself containing directions on how to handle protocol acceptance criteria failures.

In all the reviewed scenarios, proper validation designed to match the intended use of the method was shown to be critical in the implementation of an accurate and reliable analytical assay.

Review of Validation Definitions

In order to be in compliance with U.S. Food and Drug Administration (FDA) regulations, laboratories regulated by the FDA must validate their analytical procedures. As stated in the Code of Federal Regulations, data must exist that demonstrate that the methods used in testing meet proper standards of accuracy and reliability.[1] The International Conference on Harmonisation (ICH) recognized and addressed the need for universal terminology and understanding of required elements in ICH Guideline Q2(R1), "Validation of Analytical Procedures: Text and Methodology."[2] Within the ICH document, the required validation elements are defined, and recommendations are provided, with respect to the following types of procedures:

- identification tests;
- quantitative tests for impurities;
- limit tests for the control of impurities; and
- assay tests for the active component in a drug substance, drug product, or other selected component(s) in the drug product.

ICH Guideline Q2(R1) also states that the information is not intended to provide direction on how to execute the validations but rather to bridge the differences that may exist between the various compendia and regulators of the European Union, Japan, and the United States. The United States Pharmacopoeia (USP) has adopted, to the fullest extent possible, the ICH guidelines from Q2(R1) into its general chapter <1225>, "Validation of Compendial Procedures."[3] The validation parameters required by the ICH are specificity, precision, linearity, accuracy, range, detection limit, quantitation limit, robustness, and system suitability. Each element is defined, and any differences from USP <1225> have been identified below.[2-3]

Specificity: The ability to assess unequivocally the analyte in the presence of components that may be expected to be present. This may include impurities, degradation products, and matrix components.

Precision: This expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. Repeatability reflects precision under the same operating conditions over a short interval of time, while intermediate precision expresses within-laboratory variation, such as different days, different analyst, different equipment, and reproducibility is an indication of precision between laboratories.

Accuracy: This expresses the closeness of agreement between the value accepted either as the conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure. Both the ICH and the USP provide details regarding acceptable approaches for determining accuracy for drug substance and drug product assay methods and impurity methods. According to the ICH, a minimum of nine measurements over a minimum of three concentration levels should be used to assess accuracy over the range of the method.

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Linearity: This is the ability within a given range to obtain test results directly proportional to the concentration (amount) of analyte in the sample. According to the ICH, linearity should be established using a minimum of five concentrations. The USP addresses linearity and range together, whereas the ICH addresses each separately.

Range: The interval between the upper and lower concentration (amounts) of analyte in the sample—including those concentrations—for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. According to the ICH, the suggested minimum ranges to be evaluated are as follows:

- Assay method (drug product and drug substances): 80% to 120% of the sample concentration.
- Impurity method: Reporting level to 120% of the specification.
- Assay and impurity method combined: 100% level standard is used for determination, reporting level of impurity to 120% of assay specification.
- Content uniformity method: 70% to 130% of the sample concentration, unless a wider, more appropriate range is justified based on the nature of the dosage form (e.g., metered dose inhalers).
- Dissolution method: +/- 20% of the specified range.

Detection Limit: Lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value.

Quantitation Limit: Lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy, typically a trait of assays for low level compounds in the sample matrix (e.g., impurities in drug product or drug substance). Several approaches for determination of both the detection limit and quantitation limit described by the ICH include visual evaluation, signal-to-noise ratio, and standard deviation of the response and slope. The ICH does acknowledge, however, that alternate approaches may be acceptable.

Robustness: Demonstration of the reliability of the analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitability controlled, or a precautionary statement should be included in the procedure. Provided examples of typical variation include:

- analyte stability in solution;
- sample extraction time;
- for high-pressure liquid chromatography instrument methods: variations of pH in mobile phase,

variation of mobile phase composition, different columns (lots/suppliers), temperature, and flow rate; and
- for gas chromatography instrument methods: different columns (lots/suppliers), temperature, and flow rate.

Both the ICH and the USP indicate that robustness may be considered during the method development phase.

System Suitability: This is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. The system suitability parameters for a particular procedure should be established based upon the type of procedure being validated. The ICH indicates that pharmacopoeias should be consulted for additional information.

Both the ICH and the USP recognize the fact that not all analytical procedures require evaluation of all validation elements.

Which Parameters Apply?

The extent of validation testing should be based on the type of analytical method, its intended use, and the stage of development of the product for which the method will be utilized. For example, a quantitative related substances method would require an evaluation of all validation parameters. A method that provides a quantitative determination of only the potency of the active pharmaceutical ingredient (API) would require evaluation of all validation parameters except for detection limit and quantitation limit. An identification test must be able to demonstrate specificity, while specificity must be evaluated along with the determination of the detection limit of the method for a limit test.[2] Table 1 displays the required validation elements for each type of method as presented in USP <1225>.[3]

Validation of analytical test methods is important for every stage of the drug development life cycle. Methods must be shown to be acceptable for their intended use and must provide accurate and reliable results at every stage of development, from pre-clinical trials through approved and marketed product.[2] At every stage, analytical methods must assure product safety, be relevant to future commercial manufacturing, and provide a strong foundation for full ICH validation in anticipation of the marketing application.[4]

Table 1. Data Elements Required for Validation

ANALYTICAL PERFORMANCE CHARACTERISTICS	CATEGORY I	CATEGORY II		CATEGORY III	CATEGORY IV
		Quantitative Assays	Limit Tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

*MAY BE REQUIRED, DEPENDING ON THE NATURE OF THE SPECIFIC TEST.

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Preliminary tests of the method—method feasibility—should be executed during method development to determine the performance characteristics of the method. Feasibility is important during method development to ensure that the analytical test method will be able to meet proposed validation criteria, and to evaluate and establish those criteria based upon the level of validation required at a given stage of the drug's life cycle.[5]

So is full ICH validation necessary at all phases of drug development, or is it acceptable to scale down the extent of validation activities performed for a method depending on the phase of development? The current trend in validation is to take a risk-based approach to validation by considering the phase of drug development and determining the level of validation required to prove suitability of the method for its stated objective. The terms qualification, validation, and verification are commonly used to describe the different levels of validation testing performed according to the phase of drug development.

Because the requirements for validation change throughout the life cycle of drug development and full ICH validation is costly and time-consuming, qualification is often a more practical approach to validation during early development.[6]

Method qualification is an acceptable level of method performance evaluation applied to analytical methods used during preclinical, Phase 1, and early Phase 2 clinical trials. Qualification requires fewer resources, costs less, and affords increased flexibility, an important feature given that analytical methods change throughout the drug development life cycle. During the early phases of drug development, the purposes of analytical methods are to accurately determine the potency of the API and to indicate the stability of the active by determining the degradation profile of the API through forced degradation studies.[6]

Table 2. Drug Development Life Cycle

Pre-IND	PHASE I	PHASE II	PHASE III	PHASE IV
Animal Toxicity Studies	Safety in Healthy Volunteers	Safety in Patients and Preliminary Efficacy	Clinical Efficacy Studies	Product is Marketed
Purpose of Analytical Method – Pre Phase II			Purpose of Analytical Method – Post Phase II	
To ensure potency, to understand the impurity and degradation product profile, and to help understand key drug characteristics. To indicate stability and to begin to measure the impact of key manufacturing parameters to help ensure drug substance or drug product consistency.			To be robust, cost-effective, transferrable, accurate and precise for specification setting. Stability assessment and approval of final marketed products.[7]	

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During method qualification, no predetermined method acceptance criteria may be necessary, and only minimal method performance capabilities must be demonstrated. A method cannot fail a qualification. Rather, the information gained through qualification is used to optimize the method until acceptable performance is demonstrated. [4]

Full ICH validation is required for Phase 3 clinical trials and the release of finished product.[5] During the late phases of drug development, the purposes of analytical methods are to be accurate and precise, robust, specific and indicative of stability, transferable to other analytical facilities, and cost effective.&sup6; Full validation is required for lot release assays; raw material, in-process, and excipient testing; methods that are used to determine expiration dates of finished products; and good laboratory practice studies.[4] The validation parameters defined in the ICH Q2(R1) guidance document must be evaluated as applicable for each method.

During method validation, acceptance criteria for the method are established prior to executing the study. Each test must meet the predetermined acceptance criteria in order for the method to be considered acceptable for its intended use. If the acceptance criteria for a given validation test (e.g., repeatability) are not met, the failure must be investigated to determine assignable cause, such as laboratory error.&sup4; If an unacceptable result is observed during validation and the cause is not determined, the data may indicate that a predetermined specification was not appropriate or that certain aspects of the method, such as system suitability criteria, need to be modified based upon the data generated through validation.

Method verification is performed most often in compendial methodology, which has been previously

validated. Verification shows that the test performs according to specifications when executed for the first time using the personnel, equipment, and reagents available.[3,5]; Method verification is defined in USP <1226>, which indicates that specificity is a critical component for verification and should be evaluated for a given test article.[3]

The intent of method verification is not to repeat method validation of the monograph, unless the compendial method is found to be unsuitable for use with the given test article. If critical method parameters are changed or optimized to accommodate a test article, the method must be revalidated to demonstrate acceptable performance characteristics with the modified parameters.

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