Method Validation by Phase of Development

An Acceptable Analytical Practice

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This article provides guidance for reasonable, minimally acceptable method validation practices and a foundation for assessing the risks and benefits associated with method validation programs. It is based on material developed from a PhRMA 2003 workshop about acceptable analytical practices. Additional articles from this workshop will cover dissolution of poorly soluble compounds, analytical method equivalency, and justification of specifications.

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Validation of analytical methods is an essential but time-consuming activity for most analytical development laboratories in the pharmaceutical industry. As such, it is a topic of considerable interest in the literature and at pharmaceutical conferences (1–8). The examination of method validation practices is driven by several factors, including the desire to conduct the right science at the right time with optimal resources while maintaining the ability to rapidly implement change during the development process. Yet, many analytical scientists face a quandary: the more time is invested in a method, the more scientists are pressured to minimize changes to that method. This situation is counterproductive to the desired development process, in which one consciously and continuously adjusts to a growing technical and scientific database about the product being developed. Limiting early method validation to the essential elements helps maintain flexibility during the very fluid stages of early development.

Similar to the way scientists have various analytical methods requirements at different stages of the development lifecycle, method validation needs also adjust throughout the lifecycle.The objective during the late-development stages is to provide substantial information about whether a method can be run accurately and consistently under less-controlled circumstances (*e.g.*, in several laboratories with a potentially wide array of instrumentation and equipment). The methods used in early development, however, generally do not face these challenges.

Requirements for method validation are clear for new drug applications (NDA) and many other worldwide marketing applications. These requirements are specified in documents from the International Conference on Harmonization (ICH) (9–10), regulatory agencies (11–12), and pharmacopeias (13–14). The validation guidelines applicable to early drug development phases, however, are not as specific. This lack of guidance, coupled with a generally conservative, risk-averse environment within the pharmaceutical industry can result in the application of more-stringent late-phase method validation requirements to products in early development.

Recognizing the dilemma many pharmaceutical companies face, the PhRMA Analytical Technical Group selected "method validation by phase of development" as a topic in need of an "acceptable analytical practice" or an industry-led guidance.

Purpose of analytical methods by phase of development

Clinical purpose

Early

- To determine the safe dosing range and key pharmacological data (*e.g.,* bioavailability and metabolism) in Phase I trials involving a few healthy volunteers
- To study efficacy in Phase II trials in patients while continuing to test safety

Late

- To prove efficacy, confirm safety, and obtain desired label through Phase III trials involving a large number of patients

Pharmaceutical purpose

Early

- To deliver the correct bioavailable dose
- To identify a stable, robust formulation for the manufacture of multiple, bioequivalent lots for Phase II and III trials

Late

- To optimize, scale-up, and transfer a robust and controlled manufacturing process for the commercial product

Purpose of methods

Early

- To ensure potency, to understand the impurity and degradation product profile, and to help understand key drug characteristics
- To indicate stability and begin to measure the impact of key manufacturing parameters to help ensure drug substance or product consistency

Late

- To be robust, cost effective, transferable, accurate, and precise for specification setting, stability assessment, and approval of final marketed products

The scope was limited to small-molecule drug substances and drug products in the clinical phase of development. Biopharmaceuticals, raw materials, intermediates, in-process controls, excipients, and bioanalytical and preclinical methods were excluded. A committee (which included the authors of this article) presented starting-point views on the topic at the September 2003 PhRMA workshop and the subject was debated by attendees. The group preferred a phased approach to method validation. Consensus could not be reached, however, regarding the specific details of what should be included, delayed, or eliminated when validating methods in early development.

This article provides guidance on reasonable, minimally acceptable method validation practices that are based on sound scientific principles and the experiences of the authors and workshop attendees. The article also provides some framework and foundation on which analytical scientists can assess the risks and benefits associated with their own method validation programs.

Purpose of analytical methods by phase of development

According to ICH and Food and Drug Administration guidances, the objective of method validation is to demonstrate that analytical procedures "are suitable for their intended purpose" (10–11). Therefore, to understand how a method should be validated at various phases of development, it is important to understand the analytical method's purpose at various developmental stages. The method's purpose should be linked to the clinical studies' purpose and the pharmaceutical purpose of the product being studied (see sidebar, "Purpose of analytical methods by phase of development").

The purposes of initial clinical trials is to determine a safe dosing range and key pharmacological data, typically in healthy human volunteers. As development continues, clinical studies are conducted on increasing numbers of patients to prove efficacy while continuing to study the drug's safety profile.

The purposes of pharmaceutical products in early phases is to deliver a known dose that is bioavailable. As product development continues, increasing emphasis is placed on identifying a stable, robust formulation from which multiple, bioequivalent lots can be manufactured and ultimately scaled-up, transferred, and controlled for commercial manufacture.

The purposes of initial analytical methods are to ensure potency, which can relate directly to the requirement of a known dose; to identify impurities (including degradation products) in the drug substance and product, which can relate to the drug's safety profile; and to help evaluate key drug characteristics such as crystal form, drug release, and drug uniformity because these properties can compromise bioavailability. As development continues, the purposes of the analytical methods mirror those of the pharmaceutical product. The methods should be stability-indicating and capable of measuring the effect of key manufacturing parameters to help ensure that the drug substance and product are consistent. Ultimately, in the subsequent development stages, the methods must be robust, cost effective, transferable, and of sufficient accuracy and precision for specification setting and stability assessment of marketed products.

Method validation plays a key role in ensuring analytical methods are suitable for these intended purposes. Validation studies conducted during early development should ensure that analytical methods are appropriately assessing the product's potency and safety. The number of validation studies required to provide this assurance vary and will be discussed in detail later in this article.

Benefits and risks of phased method validation

Although performing validation in phases has clear benefits, it also must be noted that potential risk is associated with this approach. The risk can be reduced significantly if the analytical scientist has a good understanding of the analytical methodology's limitations and a basic understanding of the chemistry or process used to produce the drug substance or product. With a strong technical base and the use of good method development practices (15), analytical scientists are much more likely to develop a suitable method for its intended purpose and limit the risk of delaying some method validation experiments. Ultimately, analytical scientists are responsible for the scientific defense of their methods; and thus, it is useful to review potential benefits and risks associated with performing method validation in phases.

Reasons for implementing a phased approach to method validation in early development include ongoing method development and optimization, a changing synthetic route for the drug substance, a changing formulation, and a high product-

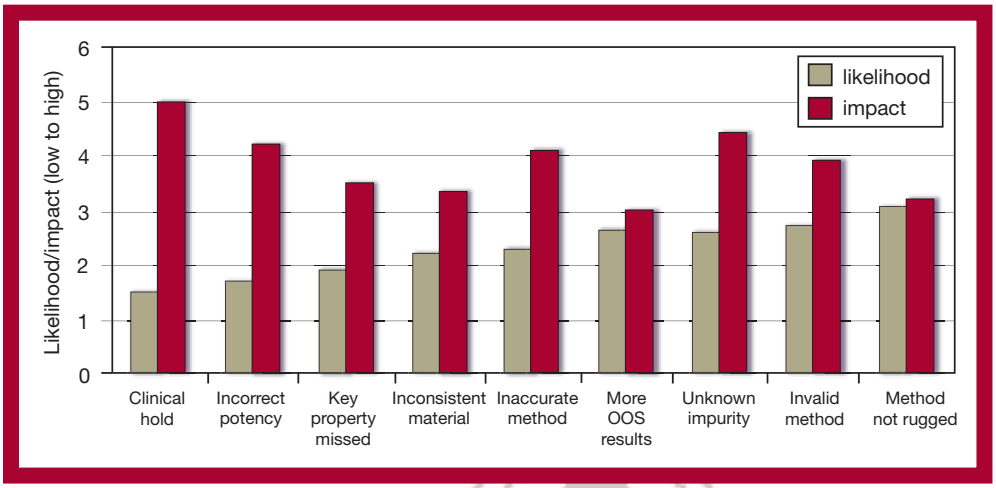

Figure 1: Method validation risk assessment (average values, $n = 38$).

attrition rate. Given the desire to rapidly implement change in early development and the business driver to do more with less, a phased method validation approach can lead to benefits such as:

- fewer resources devoted to method validation;
- lower costs;
- more flexibility in early development;
- more time to focus on analytical science.

These benefits help analytical scientists focus more on the items that truly affect product quality and on the advancement of pharmaceutical medicines in general. It also is important that a phased approach to method validation not compromise product safety or increase other risks associated with the development of new drugs.

To help assess those risks, a two-step approach was taken at the September 2003 workshop. First, attendees were asked to share problems encountered later in development that could have been detected and avoided by more method validation in early development. Second, attendees were asked to numerically assess (on a 1 to 6 scale) the likelihood and the impact of the following predefined risks, using their experiences and their company practices for methods, method validation, specification setting, and/or product quality in general:

- clinical hold (regulatory risk);
- unknown impurity (including degradation product) in a drug substance batch not used in toxicological studies;
- incorrect potency administered;
- key drug substance or product property not discovered;
- inconsistent product not discovered;
- increased out-of-specification (OOS) results;
- invalid/inadequate method not discovered until later in development;
- method not rugged and cannot be run by other laboratories;
- imprecise or inaccurate method leading to poor specification setting or stability assessment.

In response to the first question, the problems shared by workshop attendees were able to be resolved and had little long-term effect on the products in question. The issues mostly related to inadequate method ruggedness (*e.g.,* changing instruments leading to different HPLC gradients and linear ranges, and discovering problems not seen when the method was run by fewer analysts).

In response to the second question, survey results indicated that the likelihood of the predefined risks occurring is low. If they did occur, however, their effect could be relatively high (see Figure 1). Consistent with the feedback from the first question in which method ruggedness issues were raised but were resolved, the response with the highest likelihood (method ruggedness) was rated with one of the lowest impact scores.

At the end of the workshop, after discussing potential reductions in method validation, attendees were asked to take a second look at the survey and determine whether their rating on the likelihood of any predefined risks would change as a consequence of reducing method validation. The consensus was that the likelihood of the risks would not change and remained low.

Recommended approaches to early-phase method validation

At the September 2003 workshop, method validation characteristics for several drug-substance and drug-product methods were discussed. These methods are listed in Table I for drug substance and Table II for drug products. Time constraints prevented discussions about other methods such as those that detect various crystalline forms; therefore, those methods are not discussed in this article.

As stated previously, consensus was not reached on which the specific details of which aspects of method validation studies should be eliminated or delayed during early development. Participants agreed, however, that method validation should be phased.

Experiments that should be considered in a phased method validation program are described. In some cases, the suggested number of tests may not be sufficient to perform formal statistical analyses (*e.g.,* least-squares analysis of linearity data, relative standard deviation of precision data), but the number of tests should be sufficient to determine whether the method validation characteristic is likely to cause a problem. In addition, the suggested experiments assume that one analysis (*e.g.,* one injection for HPLC) will be conducted for each sample preparation leading to one reportable result from the method. If the method requires different replication (*e.g.,* multiple injections from each preparation), then this should be taken into account during method validation.

Phased validation of drug substance methods. Two key drug substance methods required to help ensure the safety and potency of pharmaceutical products are methods for assay and organic impurities. Specificity and quantitation limit are the primary validation characteristics to ensure that these methods meet

Table I: Recommended drug substance method validation during early development.

	Assay	Organic impurities
Accuracy	Inferred from precision, linearity, and specificity	Inferred from precision, linearity, and specificity
Repeatability	Determined from three sample preparations	Determined from three sample preparations
Intermediate precision	Delay*	Delay*
Specificity	Show resolution of drug substance from most likely impurities	Show resolution from most likely impurities
Quantitation limit	N/a	Confirmed to be no greater than the reporting limit
Detection limit	N/a	Delay*
Linearity	Determine from impurity linearity if appropriate or three levels 80–120% of the concentration specified in the method	Determine from three concentrations (*e.g.,* for area % methods, test at sample concentration, at 1% of that level, and at the quantitation limit)
Range	Defined by the linearity work	Defined by the linearity and quantitation limit work
Robustness	Solution stability and information gathering	Solution stability and information gathering

* Experiments can be delayed until later in development.
N/a denotes not applicable per ICH Q2A.

their intended purposes of potency and safety. Experiments that demonstrate specificity, quantitation limit, and other ICH method validation characteristics (including ones that can be delayed) are summarized in Table I. To simplify terms, organic impurity methods will be referred to as *impurity methods.*

Accuracy. Because it is rare to have more than one assay and impurity method available early in development, it is often difficult to determine the accuracy of an assay or impurity method by comparison with a secondary method. Rather, accuracy can be inferred from the precision, linearity, and specificity studies. In addition, it may be useful to assess the overall mass balance of the main component and known impurities to verify the accuracy of these methods.

Repeatability. The repeatability of the assay and impurity methods should be assessed by testing three sample preparations. The results from these studies should give the analytical scientist a sufficient estimate of the assay's and impurity methods' precision.

Intermediate precision. During early stages of development, when methods are operated typically in one laboratory by a few analysts, it is not necessary to determine the intermediate precision of an assay or impurity method.

Specificity. Assay and impurity methods specificity should be evaluated during the early development stages and then regularly reviewed and re-evaluated as changes are made to the drug substance synthetic process. During early development, the assay and impurity methods should separate the most likely impurities (*e.g.,* synthetic impurities, degradation products). In addition, it is important to show separation of the main component and impurities from the raw materials and intermediates, particularly those used in subsequent parts of the synthetic process. Furthermore, it is useful to demonstrate the separation of known and likely side-products in the final synthetic steps. As the synthetic process continues to change, the analytical scientist should evaluate the potential for generating new impurities or side-products and demonstrate the capability of the assay and the impurity methods to separate new intermediates, side-products, and raw materials as appropriate.

Once a method is used to monitor the drug substance's stability attributes, the stability-indicating capabilities of the method must be demonstrated. In general, this task is accomplished by showing that the method can separate major degradation products generated from forced degradation studies (16).

Quantitation limit. Regardless of the phase of development, the quantitation limit for an impurities method should be no greater than its reporting limit. As specified in ICH Q2A, it is not necessary for the quantitation limit to be determined for the assay method.

Detection limit. During early development, it is not critical to have a defined detection limit for an impurities method because verifying that the reporting limit can be quantified is sufficient. Rather, determination of the detection limit can be delayed until later development when the ICH Q3A(R) reporting limits are required (17).

Linearity. When performing full method validation according to ICH guidelines, a minimum of five concentrations normally is used to establish the linear range. With good method development practices (*e.g.,* operating in the linear range of the detector, proper column loading), however, the number of concentrations evaluated can be limited during early development while adding little risk that the methods will later be significantly nonlinear (15).

For early-phase impurity methods, the linear range can be evaluated by ensuring proper quantification at three concentrations. Which concentrations to test will depend on the sample preparation and the type of standardization. For area percentage methods, three concentrations are suggested: at the sample concentration, at 1% of that level, and at the quantitation limit. Data from these three samples ensure that the method's linear range and information on the quantitation limit are acceptable.

If the assay method is the same or similar to the impurity method (which is common practice particularly during early development) and the assay concentration is within the limits tested for the impurity method, no additional linearity data are needed. If the assay method is different, then linearity should be assessed using three concentrations that are 80–120% of the concentration specified by the method.

Range. The working ranges for the two methods are supported by the linearity and quantitation limit experiments described.

Table II: Recommended drug product method validation during early development.

	Assay	Dissolution	Content uniformity	Impurities
Accuracy	Show recovery at 100% for each strength (or bracket)	Show recovery at 50, 75, and 100% (for multiple strengths, 50% of lowest strength to 100% of highest strength)	Show recovery at 70, 100, and 130% (for multiple strengths, 70% of lowest strength to 130% of highest strength)	Show recovery at standard concentration at the highest individual specification and/or at the reporting limit
Repeatability	Determine from three preparations of 100% recovery sample	Determine from three preparations of 100% recovery sample	Determine from three preparations of 100% recovery sample	Determine from three preparations at the standard concentration or the highest individual specification limit
Intermediate precision	Delay*	Delay*	Delay*	Delay*
Specificity	Show assay result is unaffected by impurities and excipients	Show analysis is unaffected by the excipients, and if UV, the media	Show analysis is unaffected by the excipients, and if UV, the excipient solvent	Show resolution of degradation products from synthetic impurities and drug substance
Quantitation limit	N/a	N/a	N/a	Confirmed to be no greater than the reporting limit
Detection limit	N/a	N/a	N/a	Delay*
Linearity	Delay*	Inferred based on accuracy work	Inferred based on accuracy work	Inferred based on accuracy work
Range	Delay*	Defined by accuracy work	Defined by accuracy work	Defined by accuracy work
Robustness	Solution stability and information gathering	Solution stability and information gathering	Solution stability and information gathering	Solution stability and information gathering

* Experiments can be delayed until later in development.
N/a indicates not applicable as per ICH Q2A.

Robustness. During early development, robustness testing can be limited to demonstrating that solutions are adequately stable for their duration of use in the laboratory. During method development and early stages of the project, an analyst should begin to develop an experience base and gather information about which method parameters have the greatest effect on the analytical results and method performance. This experience base can be used in later stages to develop specific robustness experiments and to help establish appropriate system suitability requirements.

Phased validation of drug product methods. During the 2003 workshop, four types of drug product methods were discussed: assay, impurities, dissolution, and content uniformity (CU). The key characteristics for helping ensure product potency and safety are accuracy and specificity. Table II summarizes minimally acceptable method validation studies is provided. The discussions used traditional (immediate release) tablets as a model, and though the principles apply to other dosage forms, the specifics must be interpreted and adapted by an analytical scientist.

Accuracy. ICH requirements state that accuracy may be inferred once precision, linearity, and specificity have been established. With an emphasis on expediting method validation in early development, a minimum number of recovery studies are suggested. These condensed recovery studies are performed in lieu of more extensive linearity and precision studies to demonstrate adequate accuracy and linearity of the methods.

For assay, it is recommended that recovery of the drug substance be determined in the presence of excipients at 100% of the dosage form strength. For dissolution, recovery of the drug substance in the presence of excipients is recommended at 50, 75, and 100% of the dosage form strength. For content uniformity, recovery of the drug substance in the presence of excipients is recommended at 70, 100, and 130% of the dosage form strength. In the case of multiple strengths of similar formulations, further efficiencies may be gained by conducting recovery experiments that bracket the full concentration or strength range.

Early in development, samples of degradation products may be in very short supply, if available at all. Hence, a minimum requirement for demonstrating the accuracy of the impurities method is that recovery is determined using drug substance (in the presence of excipients) at two or three levels (*e.g.,* the standard concentration, the highest individual impurity specification limit, if applicable and if different from the standard concentration, and the reporting limit).

Repeatability. Performing the 100% recovery experiments using three sample preparations for assay, dissolution, and content uniformity should generate a sufficient estimate of the repeatability of the methods. Similarly, conducting the recovery experiment using three sample preparations at the standard concentration or, if applicable, the highest individual specification limit should provide a sufficient estimate of the repeatability of the impurity method. For multiple strengths of similar formulations, bracketing the full concentration or strength range should be sufficient.

Intermediate precision. Similar to the drug-substance recommendations, intermediate precision can be delayed until the meth-

ods are used in multiple laboratories and/or by several different analysts and instruments.

Specificity. As previously discussed, early phase methods must be reliable for determining the potency and safety of the drug product. Therefore, assurance of the assay and impurities method(s) specificity is important even early in development. Demonstrating that the assay result is unaffected by the presence of impurities and formulation excipients is suggested as the minimum for the assay method. For the impurities method at this early stage, the drug product should be appropriately degraded to demonstrate that the degradation products have near-baseline resolution from the main component and synthetic impurities. The analyses for content uniformity and dissolution also should be unaffected by the extracting solvent or media and excipients.

Quantitation and detection limits. Identical to the drug substance recommendations, the quantitation limit for the drug product impurities method should be no greater than its reporting limit. Determination of the detection limit can be delayed until later development when the ICH Q3B(R) reporting limits are required. It is not necessary for the quantitation and detection limits for the assay, CU, and dissolution methods to be determined, as per ICH Q2A (18).

Linearity. When standard concentrations are matched or roughly matched to the expected analyte concentration(s) and good method development practices are used, it is not unreasonable to delay ICH-type linearity studies (15). Rather, adequate linearity for dissolution, CU, and impurities methods can be inferred from the accuracy studies that demonstrate good recovery at various concentrations of key analyte. For the assay method, determining the accuracy at various dosage strengths may help define the linearity. In the absence of a range of strengths, it is reasonable to expect that the results will cover a relatively narrow range, and thus ICH linearity experiments can be delayed.

Range. The working ranges for the dissolution, CU, and impurity methods are supported by the experiments described for accuracy. For assay, determination of range can be delayed for the same reasons described for linearity.

Robustness. Early development is a good time to gather information about the robustness of the methods, but probably too early to begin to conduct designed experiments. Experimentally determined solution stabilities should be established to cover their duration of use in the laboratory.

Other aspects of method validation that can be phased

In addition to optimizing the experiments performed to validate methods during early development, other aspects of method validation can and should be scaled back or delayed to achieve all the benefits of phased method validation. The phasing of documentation, acceptance criteria, and the role of the quality assurance unit were discussed during the workshop. The role of the quality assurance unit is outside the scope of this topic, but recommendations were made for the other aspects.

Regardless of the phase of development, the laboratory raw data used to demonstrate the validity of analytical methods must be properly documented in a notebook or using another

good manufacturing practices-compliant data storage format. A detailed method validation report was not felt to be required by workshop attendees until submission of the final marketing application. Summary reports were recommended to facilitate data retrieval and fulfill potential requests from regulatory agencies for the information (*e.g.,* Phase 2 investigational new drugs [INDs]). This approach also should meet FDA expectations to include "appropriate validation information" in Phase 3 INDs (19).

Concerning acceptance criteria and method validation protocols, attendees felt that internal guidelines or best practice documents are useful for early development. Preapproved protocols and/or rigid acceptance criteria could unnecessarily restrict the scientific evaluation of methods and lead to extensive, unnecessary investigations, however, when the focus in early development should be on the larger issues of potency and safety. During later development when the drug substance and product and corresponding methods/specifications are more established and better understood; when multiple laboratories and stakeholders are involved; and when the method purposes (see sidebar, "Purpose of analytical methods by phase of development") are expanded, acceptance criteria and more-defined method validation standard operating procedures or protocols may be useful.

Conclusions

Regardless of the drug development phase, an analytical scientist must have confidence in the analytical results used to make decisions concerning product progression. Validation of the analytical methods that are used to generate these results requires significant resources, especially to meet the criteria defined in ICH Q2A/B. By phasing these method validation activities, resources can be optimized while maintaining a good scientific approach to pharmaceutical development. It was evident throughout the September 2003 workshop, that the represented pharmaceutical companies use a phased approach to method validation. Each company's phased method validation procedures and processes vary, but the overall philosophy is the same. The extent of and expectations from early-phase method validation are lower than the requirements in the later stages of development. These requirements depend on many factors, including the type of analytical method, the intended use of the method, and the risk associated with delaying full method validation.

The workshop and this article provide a framework on which analytical scientists can evaluate the intended use of their methods and put that in context with what is happening clinically and pharmaceutically by phase of development. We have discussed key risks related to phased method validation and confirmed with workshop attendees that the likelihood of these risks is small and not affected by phasing method validation when put in the context of the whole quality system used by represented pharmaceutical companies. We have suggested some minimally acceptable experiments to ensure the validity of several key drug-substance and drug-product methods and explored phasing other aspects of method validation such as the extent of documentation and the use of acceptance criteria. In the process, we hope these recommendations will provide guidance to analytical scientists in the pharmaceutical industry when dealing with the complexities and challenges associated with method validation during the clinical development phases.

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