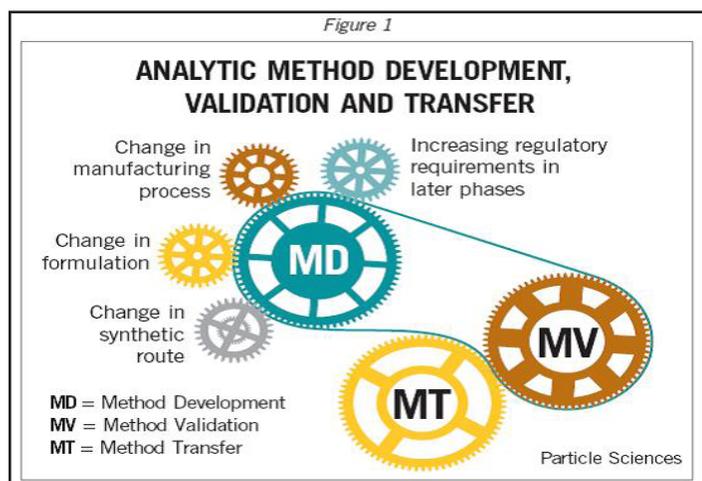# The Central Role Of Analytic Method Development And Validation In Pharmaceutical Development

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Analytic method development, validation, and transfer are key elements of any pharmaceutical development program. This article will focus on development and validation activities as applied to drug products. Often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency. These method-related activities are interrelated. They are iterative, particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes encountered during drug development may require modifications to existing analytic methods. These modifications to the methods, in turn, may require additional validation or transfer activities, as shown below (Figure 1).

Figure 1

ANALYTIC METHOD DEVELOPMENT, VALIDATION AND TRANSFER

Change in manufacturing process
Increasing regulatory requirements in later phases
Change in formulation
Change in synthetic route

MD = Method Development
MV = Method Validation
MT = Method Transfer

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Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use." Method transfer is the formal process of assessing the suitability of methods in another laboratory. Each of these processes contributes to continual improvement of the methods and results in more efficient drug development. Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. According to the International Conference on Harmonization (ICH), the most common types of analytic procedures are:

(i) Identification tests
(ii) Quantitative tests of the active moiety in samples of API or drug product or other selected component(s) in the drug product
(iii) Quantitative tests for impurities' content
(iv) Limits tests for the control of impurities

Method development (Figure 2) is a continuous process that progresses in parallel with the evolution of the drug product. The notion of phase appropriate method development is a critical one if time, cost, and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments. These may include forced degradation studies, which are an integral part of development of a stability-indicating method. API is typically subjected to degradation by acid, base, oxidant, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients. Additional experiments help to define the system suitability criteria that will be applied to future analytic sample sets. System suitability tests are a set of routine checks to assess the functionalities of the instrument, software, reagents, and analysts as a system. Final method system suitability parameters may be determined from evaluations of method robustness performed under statistical design of experiments. The goal is to identify the critical parameters and to establish acceptance criteria for method system suitability.

Figure 2

ANALYTICAL METHOD DEVELOPMENT PROCESS

	Early Development	GLP	GMP (Phase I & II)	GMP (Phase III+)
Method Goal	Solid, well-understood method	Scientifically sound method	Minimally validated method	Fully validated method
Analytic Goals	Build robustness into the method	Establish results for appropriate validation elements	Limited precision and robustness studies	Extensive evaluation of precision and robustness
Validation Requirements	Not a requirement, unless requested by a client	Not a requirement, unless requested by a study director	Required by Phase II	Required
Documentation Requirements	Good documentation practices	Good documentation practices, usually no written protocol or report	Written protocol and report with acceptance criteria by Phase II	Full validation protocol and report

Design Space → Knowledge Base
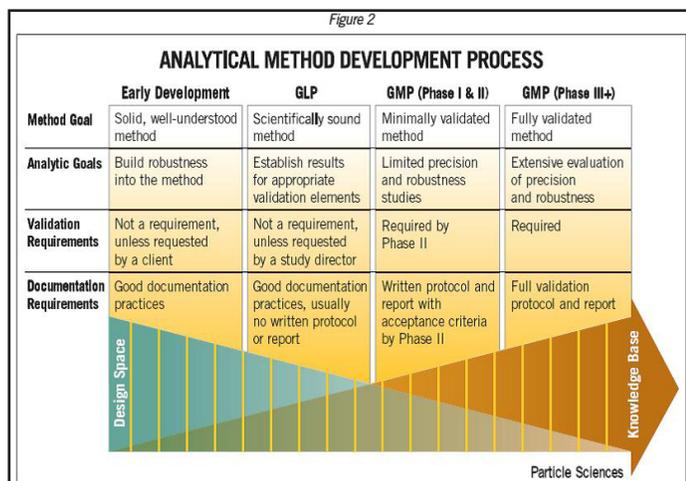
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Table 1

Parameter	Definition
Accuracy	an assessment of the difference between the measured value and the real value
Precision	a measure of the agreement for multiple measurements on the same sample
Specificity	the ability to assess the analyte when in the presence of other components
Limits of detection and quantitation	the lowest amounts of analyte that can be detected / determined accurately, respectively
Linearity and range	the proportionality of the measurement to the concentration of the analyte within a specified range
Robustness	a check of the effect of deliberate small changes to the method on the results

Elements of Validation

The validation of an analytic method demonstrates the scientific soundness of the measurement or characterization. It is required to varying extents throughout the regulatory submission process. The validation practice demonstrates that an analytic method measures the correct substance, in the correct amount, and in the appropriate range for the intended samples. It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. Resources for information and approaches to method validation are listed in the footnotes. In order to perform method validation, the laboratory should be following a written standard operating procedure (SOP) that describes the process of conducting method validation. The laboratory should be using qualified and calibrated instrumentation with a corresponding operating SOP. There should be a well-developed and documented test method in place and an approved protocol should be in place prior to the execution of any validation experiments. The protocol is a plan that describes which method performance parameters will be tested, how the parameters will be assessed, and the acceptance criteria that will be applied. Finally, samples of API or drug product, placebos, and reference standards are needed to perform the validation experiments. The method performance parameters that are applicable to most methods are shown in Table 1.

Approaches to Validation Experiments

Accuracy is established by quantitation of the sample against a reference standard for API, or spiking placebo with API for drug product. It can also be determined by comparison of results from alternate measurement techniques. Precision is determined by multiple measurements on an authentic, homogeneous set of samples. Samples may be analyzed on different days, by different analysts, on different instruments, or in different laboratories. There are three levels of precision validation evaluations – repeatability, intermediate precision, and reproducibility. Repeatability is a measure of precision under the same conditions over a short period of time. Intermediate precision is a measure of precision within the same laboratory by different operators, using different instruments, and making

measurements on different days. Reproducibility assesses precision between two or more laboratories. Specificity can be established by a number of approaches, depending on the intended purpose of the method. The ability of the method to assess the analyte of interest in a drug product is determined by a check for interference by placebo. Specificity can be assessed by measurement of the API in samples that are spiked with impurities or degradants, if available. If API-related compounds are not available, drug can be stressed or force-degraded in order to produce degradation products. In chromatographic separations, apparent separation of degradants may be confirmed by peak purity determinations by photodiode array, mass purity determinations by mass spectroscopy (MS), or by confirming separation efficiency using alternate column chemistry. During forced degradation experiments, degradation is targeted at 5 to 20% degradation of the API, in order to avoid concerns about secondary degradation. The limit of detection and limit of quantitation are based on measurement signal-to-noise ratios of 3 and 10, respectively. Standards or samples at concentrations near the expected limits are measured. Signal-to-noise can be generated by software, manually measured, estimated from standard deviation calculations, or limits may be empirically determined. Linearity is established by measuring response at various concentrations by a regression plot, typically by method of least squares. The response may require mathematical manipulation prior to linearity assessments. A visual inspection of the linearity plot is the best tool for examining proportionality of the response. The range is established by the required limits of the method and the point at which linearity is compromised. Robustness is typically assessed by the effect of small deliberate changes to chromatographic methods on system suitability parameters such as peak retention, resolution, and efficiency. Experimental factors that are typically varied during method robustness evaluations include:

(i)   Age of standards and sample preparations
(ii)   Sample extraction time
(iii)   Variations to pH of mobile phase
(iv)   Variation in mobile phase composition
(v)   Analysis temperature
(vi)   Flow rate

(vii)   Column lot and/or manufacturer
(viii) Type and use of filter against centrifugation

Robustness experiments are an ideal opportunity to utilize statistical design of experiments, providing data-driven method control. The ICH guidance on validation separates types of methods according to the purpose of the method and lists which evaluations are appropriate for each type 2. The ICH guidance also suggests detailed validation schemes relative to the intended purpose of the methods. It lists recommended data to report for each validation parameter. Acceptance criteria for validation elements must be based on the historical performance of the method, the product specifications, and must be appropriate for the phase of drug development.

Timing of Validation

As previously mentioned, the path to validation forms a continuum. It begins in the early phases of drug development as a set of informal experiments that establish the soundness of the method for its intended purpose. It is expanded in intensity and extent throughout the regulatory submission process into a fully documented report that is required by NDA submission at Phase III and in support of commercial production. It is repeated whenever there is a significant change in instrumentation, method, specifications, and process, if applicable.

Conclusion

Analytic method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently contradictory, validated methods produce results within known uncertainties. These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product. The time and effort that are put into developing scientifically-sound, robust, and transferable analytic methods should be aligned with the drug development stage. The resources that are expended on method validation must be constantly balanced with regulatory requirements and the probability for product commercialization.

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About The Authors

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Laurie Goldman joined Particle Sciences in 1997 as Analytic and Quality Manager. Building on a solid quality control and quality assurance framework from previous experience in the electronic chemicals, mining, and inks/coatings industries, she established and grew the state-of-the-art analytical laboratory at Particle Sciences. She has coauthored numerous technical publications. She holds a B.S. in Chemistry from Muhlenberg College and has been certified in statistical process control, quality engineering, and quality auditing.

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Before joining Particle Sciences, Dr. Lee held senior management positions at Novavax, Inc., Lyotropic Therapeutics, Inc., and Imcor Pharmaceutical Corp. Dr. Lee holds Bachelor of Science degrees in Biology and Chemistry from the University of Washington and a PhD in Physical Bioorganic Chemistry from the University of California-Santa Barbara.

Dr. Lee has published articles in numerous peer-reviewed journals and three book chapters plus holds 11 issued patents and 14 provisional or PCT patent applications. Dr. Lee has more than 22 years of experience in pharmaceutical research and development of both therapeutic drugs and diagnostic imaging agents. He maintains strong academic ties, including an appointment as Adjunct Associate Professor of Pharmaceutical Chemistry at the University of Kansas in 1992, and serving as a reviewer for both the International Journal of Pharmaceutics and Journal of Pharmaceutical Sciences.