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International Journal of Health Research, March 2008; 1(1): 11-20

Available online at http://www.ijhr.org

Review Article

Open Access
Online Journal

Method Development and Validations: Characterization of Critical Elements in the Development of Pharmaceuticals

Received: 12-Feb-08 Revision received: 23-Feb-08 Accepted for publication: 28-Feb-08

Abstract

This review discusses the conceptual aspects of method validation, its management, processes and schemes and highlights method validation key performance characteristics. Although a thorough validation cannot rule out all potential problems, the process of method development and validation should address the most common ones. Examples of typical problems that can be minimized or avoided are synthesis impurities that co-elute with the analyte peak in an HPLC assay; a particular type of column that no longer produces the separation needed because the supplier of the column has changed the manufacturing process; an assay method that is transferred to a second laboratory where they are unable to achieve the same detection limit; and a quality assurance audit of a validation report that finds no documentation on how the method was performed during the validation. Problems increase as additional people, laboratories, and equipment are used to perform the method. When the method is used in the developer's laboratory, a small adjustment can usually be made to make the method work, but the flexibility to change it is lost once the method is transferred to other laboratories or used for official product testing. This is especially true in the pharmaceutical industry, where methods are submitted to regulatory agencies and changes may require formal approval before they can be implemented for official testing. The best way to minimize method problems is to perform adequate validation experiments during development.

Keywords*: Method validation, method development, pharmaceutical analysis*

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Introduction

Method validation is the process of proving that an analytical method is acceptable for its intended purpose[1-3]. For pharmaceutical methods, guidelines from the United States Pharmacopeia (USP), International Conference on Harmonisation (ICH), and the Food and Drug Administration (FDA) provide a framework for performing such validations. In general, methods for regulatory submission must include studies on specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness. The literature contains diverse approaches to performing validations[3,13,17]. Although there is general agreement about what type of studies should be done[13], there is great diversity in how they are performed[13, 17]. This report presents an approach to performing validation studies that encompasses much of the current literature and provides practical guidance. This approach should be viewed with the understanding that validation requirements are continually changing and vary widely, depending on the type of drug being tested, the stage of drug development, and the regulatory group that will review the drug application.

In the early stages of drug development, it is usually not necessary to perform all of the various validation studies. Many researchers focus on specificity, linearity, accuracy, and precision studies for drugs in the pre-clinical through Phase II (preliminary efficacy) stages. The remaining studies are performed when the drug reaches the Phase III (efficacy) stage of development and has a higher probability of becoming a marketed product[3,4-6]. The process of validating a method cannot be separated from the actual development of the method conditions, because the developer will not know whether the method conditions are acceptable until validation studies are performed. The development and validation of a new analytical method may therefore be an iterative process. Results of validation studies may indicate that a change in the procedure is necessary, which may then require revalidation.

The steps of methods development and method validation depend upon the type of method being developed however, the following steps are common to most types of projects: they are method development plan definition, background information gathering, laboratory method development, generation of test procedure, methods validation protocol definition, laboratory methods validation, validated test method generation, validation report. A well-developed method should be easy to validate. (Jay Breaux et al (2003).

Why is analytical method validation required?

Method Validation is required for the following reasons:

1. Assuring quality and achieving 3 a levels
2. Achieving acceptance of products by the international agencies.
3. Mandatory requirement purposes for accreditation as per ISO 17025 guidelines
4. Mandatory requirement for registration of any pharmaceutical product or pesticide formulation.

Validated methods are only acceptable for undertaking proficiency testing.

Various parameters for method validation

Breaux et al [13] reported that **v**alidation requirements depend upon the type of test method, including

- Specificity – ability to measure desired analyte in a complex mixture,
- accuracy – agreement between meas-

ured and real value,

- linearity – proportionality of measured value to concentration,
- precision – agreement between series of measurements,
- range – concentration interval where method is precise, accurate, and linear,
- detection limit – lowest amount of analyte that can be detected,
- quantitation limit – lowest amount of analyte that can be measured, and robustness – reproducibility under normal but variable laboratory conditions.

Only specificity is needed for an identification test. However, the full range of specificity, accuracy, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), precision, and robustness testing is needed for more-complex methods such as quantitative impurity methods.

Specificity/Selectivity

According to Green [18], developing a separation for chromatographic methods involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, synthesis intermediates, excipients, degradation products, process impurities, etc.) is compared with the response of a solution containing only the analyte. Other potential sample components are generated by exposing the analyte to stress conditions sufficient to degrade it to 80-90% purity. For bulk pharmaceuticals, stress conditions such as heat (50 |AoC), light (600 FC), interest, and that there is no interference from excipient(s) and/or degradation products and/or impurities. Determination of this can be carried out by assessing the peak identity and purity. Diode array detectors can facilitate the development and validation of HPLC assays. Spectral data obtained from diode array detectors, effectively supplement the retention time data for peak identification, also spectral manipulation often provides information about the peak purity.

The figures below lists several of the techniques available for assessing peak identity and purity. Purity index is a measure of the peak's relative purity, measured using a full comparison of spectral data for the leading and training edge of the peak (Figure 1). A value of 1.5 is commonly accepted to indicate a pure peak but >1.5 would indicate the presence of an impurity [9], as shown in Figure 2[15]. The peak identification [9] is a comparison of the apex spectrum against that of a reference (stored under the same

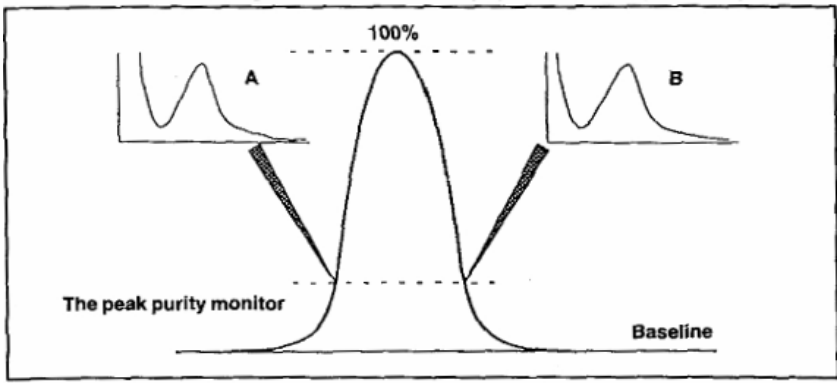

Figure 1: The leading and trailing spectra used for purity analysis

conditions). If an alternate chromatographic column is to be allowed in the final method procedure, it should be identified during these studies. Once acceptable resolution is obtained for the analyte and potential sample components, the chromatographic parameters, such as column type, mobile-phase composition, flow rate, and detection mode, are considered set. An example of specificity criteria for an assay method is that the analyte peak will have baseline chromatographic resolution of at least 1.5 from all other sample components. If this cannot be achieved, the unresolved components at their maximum expected levels will not affect the final assay result by more than 0.5%. An example of specificity criteria for an impurity method is that all impurity peaks that are 0.1% by area will have baseline chromatographic resolution from the main component peak (s) and, where practical, will have resolution from all other impurities.

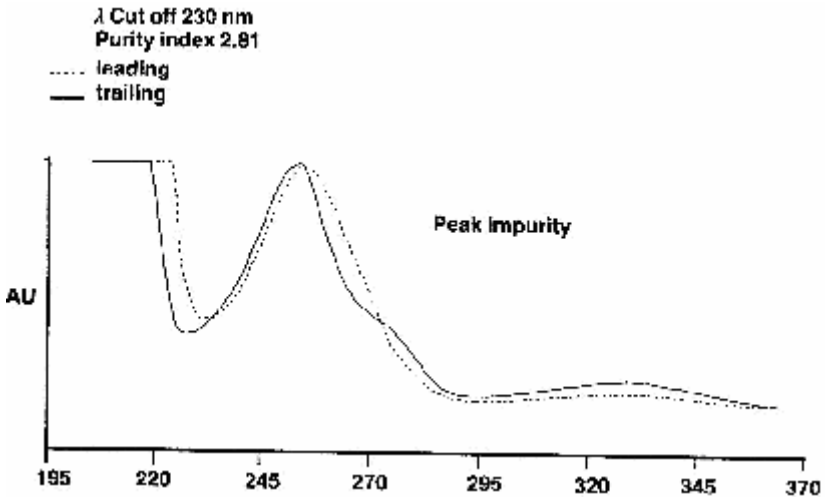

Figure 2: Spectra comparison showing peak purity

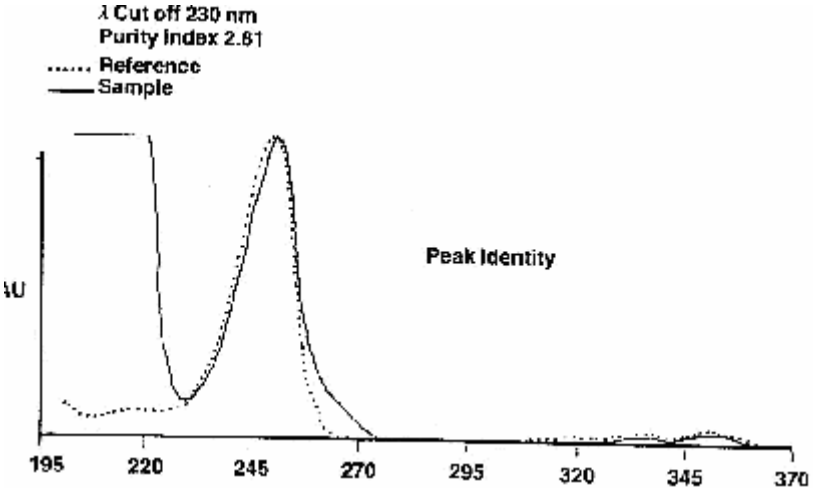

Figure 3: Comparison of reference versus sample apex spectra for peak identity

Selectivity studies should also assess interferences that may be caused by the matrix, e.g., urine, blood, soil, water or food. Optimized sample preparation can eliminate most of the matrix components [17]. The absence of matrix interferences for a quantitative method should be demonstrated by the analysis of at least five independent sources of control matrix.

Precision and Reproducibility

Chitlange et al [19] indicated that the precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The measured standard deviation can be subdivided into three categories: repeatability, intermediate precision and reproducibility [20, 21].

Repeatability is obtained when one operator using one piece of equipment over a relatively short time-span carries out the analysis in one laboratory. At least 5 or 6 determinations of three different matrices at two or three different concentrations should be done and the relative standard deviation calculated.

The AOAC [14] manual for the Peer-Verified Methods program includes a table with estimated precision data as a function of analyte concentration. Intermediate precision is a term that has been defined by ICH [20] as the long-term variability of the measurement process. It is determined by comparing the results of a method run within a single laboratory over a number of weeks. A method's intermediate precision may reflect discrepancies in results obtained by different operators, from different instruments, with standards and reagents from different suppliers, with columns from different batches or a combination of these. The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same results once the development phase is over.

Reproducibility, as defined by ICH [2, 21] represents the precision obtained between laboratories. The objective is to verify that the method will provide the same results in different laboratories [17]. Reproducibility of an analytical method is determined by analyzing aliquots from homogeneous lots in different laboratories with different analysts, and by using operational and environmental conditions that may differ from, but are still within, the specified parameters of the method (interlaboratory tests). Validation of reproducibility is important if the method is to be used in different laboratories. Some typical variations affecting a method's reproducibility are [17]:

- Differences in room temperature and humidity;
- Operators with different experience and thoroughness;
- Equipment with different characteristics, e.g. delay volume of an HPLC system;
- Variations in material and instrument conditions, e.g. in HPLC, mobile phases composition, pH, flow rate of mobile phase;
- Variation in experimental details not specified by the method;
- Equipment and consumables of different ages;
- Columns from different suppliers or different batches; and
- Solvents, reagents and other material with varying quality.

Accuracy and Recovery

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree [1-3]. Accuracy can also be described as the closeness of agreement between the values that is adopted, either as a conventional, true or accepted reference value, and the value found [17].

The true value for accuracy assessment can be obtained in several ways. One approach is to compare the results of the method with results from an established reference method. This assumes that the uncertainty of the reference method is known. Alternatively, accuracy can be assessed by analyzing a sample with known concentrations (e.g., a control sample or certified reference material) and comparing the measured value with the true value as supplied with the material. If certified reference materials or control samples are not available, a blank sample matrix of interest can be spiked with a known concentration by weight or volume. After extraction of the analyte from the matrix and injection into the analytical instrument, its recovery can be determined by comparing the response of the extract with the response of the reference material dissolved in a pure solvent. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. If validated correctly, the recovery factor determined for different concentrations can be used to correct the final results [17]. The concentration should cover the range of concern and should include concentrations close to the quantitation limit, one in the middle of the range and one at the high end of the calibration curve. Another approach is to use the critical decision value as the concentration point that must be the point of greatest accuracy [17]. The expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration. AOAC manual for the Peer-Verified Methods program includes a table with estimated recovery data as a function analyte concentration [17].

The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates each). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value, together with the confidence intervals [17].

Linearity and calibration curve

The linearity of an analytical method is its ability to elicit test results that are (directly or by means of well-defined mathematical transformations) proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighings of synthetic mixtures of the test product components, using the proposed procedure [17].

In chromatographic analyses, linearity may be determined by a series of three to six injections of five or more standards whose concentrations span 80-120 percent of the expected concentration range. The response should be (directly or by means of a well-defined mathematical calculation) proportional to the concentrations of the analytes. A linear regression equation applied to the results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that there is no effect on the accuracy of the method [13, 19].

Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million)

obtained by the analytical method [17]. For assay tests, the ICH requires [2] the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity (whichever is greater to 120 percent of the specification [17].

Limit of Detection

The limit of detection (LOC) is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. LOC is frequently confused with the sensitivity of the method [1-3, 13]. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass [17-18].

In chromatography, the detection limit is the injected amount that results in a peak with a height at least two or three times as high as the baseline noise level. Besides this signal/noise method, the ICH describes three more methods:

- **Visual inspection:** The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected [17-18].
- **Standard deviation of the response based on the slope of the calibration curve:** A specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. The residual standard deviation of a regression line, or the standard deviation of y-intercepts of regression lines, may be used as the standard deviation.
- **Standard deviation of the response based on the standard deviation of the blank:** Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses [17].

Limit of Quantitation

The limit of quantitation is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography, typically requiring peak heights 10 to 20 times higher than the baseline noise [1-3, 17]. If the required precision of the method at the limit of quantitation has been specified, the number of samples with decreasing amounts of the analyte is injected six times. The calculated RSD percent of the precision is plotted against the analyte amount. The amount that corresponds to the previously defined required precision is equal to the limit of quantitation. It is important to use not only pure standards for this test but also spiked matrices that closely represent the unknown samples.

Limit of Detection

For the limit of detection, the ICH [2] recommends, the visual inspection, the standard deviation of the response and the slope of the calibration curve, in addition to the procedures as described above. Any results of limits of detection and quantitation measurements must be verified by experimental tests with samples containing the analytes at levels across the two regions. It is equally important to assess other method validation parameters, such as precision, reproducibility and accuracy, close to the limits of detection and quantitation [17-18].

Ruggedness

Ruggedness is not addressed in the ICH documents Its definition has been replaced by reproducibility, which has the same meaning as ruggedness, defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness [17, 19, 22] is determined by the analysis of aliquots from homogeneous lots in different laboratories.

Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range [17].

Obtaining data on these effects helps to assess whether a method needs to be revalidated when one or more parameters are changed, for example, to compensate for column performance over time. In the ICH document, it is recommended to consider the evaluation of a method's robustness during the development phase, and any results that are critical for the method should be documented. This is not, however, required as part of a registration [17].

Stability

Many solutes readily decompose prior to chromatographic investigations, for example, during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials (in refrigerators or in an automatic sampler). Under these circumstances, method development should investigate the stability of the analytes and standards [17, 19].

The term system stability has been defined as the stability of the samples being analyzed in a sample solution. It is a measure of the bias in assay results generated during a pre-selected time interval, for example, every hour up to 46 hours, using a single solution. System stability should be determined by replicate analysis of the sample solution. System stability is considered appropriate when the RSD, calculated on the assay results obtained at different time intervals, does not exceed more than 20 percent of the corresponding value of the system precision. If, on plotting the assay results as a function of time, the value is higher, the maximum duration of the usability of the sample solution can be calculated.

The effect of long-term storage and freeze-thaw cycles can be investigated by analyzing a spiked sample immediately after preparation and on subsequent days of the anticipated storage period [17]. A minimum of two cycles at two concentrations should be studied in duplicate. If the integrity of the drug is affected by freezing and thawing, spiked samples should be stored in individual containers, and appropriate caution should be employed for the study of samples.

Conclusion

Analytical methods development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The official test methods that re-

sult from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products.

For a method development and validation programme to be successful, an holistic approach is recommended. A common challenge encountered during methods development and validation is that methods are typically developed by the research and development (R&D) department, whereas validation is typically the responsibility of a validation group. It is important that the R&D and validation groups work as one team. Various groups may also be responsible for ensuring the suitability of the methods to support early clinical phases and commercial manufacturing. The transfer of analytical methods from one group to another then becomes an important step for ensuring that the proper validation is in place to justify its intended use [13]. As several groups will run the method during its progression from development to validation, the method must be robust. This means that the method should provide reliable data, both on a wide range of equipment and in the hands of several chemists.

Finally, one of the key requirements for methods validation (which is also one of the key challenges) is that only well-characterised reference materials with well-documented purities should be used during methods validation activities. The challenge stems from the fact that, in some cases, the tools used to characterise reference standard materials are being developed and validated at the same time as the reference standard itself.

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