METHOD VALIDATION

Introduction

Getting an acceptable separation and detection of compounds is only the first step in a completed method that may be performed for long periods in other laboratories. If the method is used with a product or process, it may be submitted for both internal and official regulatory approval. This could involve agencies such as Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), or their counter-parts around the world.

The transfer of a method is best accomplished by a systematic method validation process. Many workers view validation only as a test of the acceptability of the method using the conditions (e.g., flow rate, sample size, column type) prescribed. However, the real goal of the validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method. It is important to have a well-conceived validation plan for testing the method and acceptance criteria before starting the validation process. Included in this plan should be detailed procedure describing the entire method (including calibration standard and sample preparation, separation, data handling, and calculations) that can conveniently be executed by others. Many official groups have established guidelines or standard procedures for method validation, and some other recommendations exist in published references\textsuperscript{1-7}. However, these guidelines are generally not specific or apply only to certain applications. In this chapter define each of the major items that should be in a good method validation.
Preferred approaches for each phase of a validation process are also given.

**General Approach to Method Validation**

Just as method development will vary with sample and separation goals, so will method validation. An assay for a major component requires a different approach and acceptance criteria than a method for a trace impurity. The frequency with which a method will be used (many times a day, once a day only for a short study, once a month, etc.) also influences the type of validation studies that are needed. An iterative approach to overall method validation often is appropriate. The use of a method early in its development may require only limited validation. For example, for initial R&D studies on a new drug candidate, the analyses may performed in a single laboratory, perhaps by one operator on a single instrument. Preliminary toxicology studies on a new pesticide can also be performed under controlled conditions, which minimizes the need for complete validation studies. An HPLC method for an active drug substance used in initial formulation studies may not require a study of detection limit or ruggedness. Therefore, it is best to prioritize the components of validation studies. In a good validation plan the important studies will be done early and anticipate future needs. Typically, specificity, linearity, accuracy, and precision studies are needed first; complete studies of stability and ruggedness often can come later.
A final method may be performed at different sites. Differences in HPLC instrumentation, laboratory equipment, and reagent sources, and variation in the skills and background of personnel may require specific features in the HPLC method. In addition, the development of different formulations of the same drug with varying strengths or physical forms may require flexibility in method procedures. A method developed for the assay of the main component in a tablet may have to be adapted to function in a lotion, cream, or aerosol. The analysis of residual drug in manufacturing equipment (often needed for cleaning - validation studies) also require method modifications. While these types of applications involve method development study. Requirements for validation at a later stages of product development or commercialization may be more stringent, requiring additional studies.

A preferred approach to method validation is to define and carry out the critical studies needed for each step in a manner that allows use of the new and existing information in a subsequent method improvements or validations. In addition, the routine use of a method outside the originating laboratory can provide valuable information on ruggedness( use of different columns, reagents, instruments, etc.). This information from different laboratories should be accumulated during routine use. These later results may indicate that the method should be modified to improve certain characteristics This iterative process continues until a formal, complete validation is performed and documented (usually prior to submission of a drug application, transfer of the final method to a new site, etc. ).
The individual components of a method validation study are as follows.

- ACCURACY
- PRECISION
- LINEARITY
- RANGE
- LIMIT OF DETECTION
- LIMIT OF QUANTITATION
- SPECIFICITY
- RUGGEDNESS
- ROBUSTNESS
- STABILITY OF SAMPLES
- REAGENTS
- INSTRUMENTS
- SYSTEM SUITABILITY CRITERIA

For the each component of the study, an important consideration is the need to determine (before the validation starts) what constitutes an acceptable result for that study. These acceptance criteria will vary depending on the type of method and its intended use. For example, good precision is more important for an assay of the major component than for a single trace-level impurity.

**Accuracy**: The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose “true value” is known) is analyzed and the measured value should ideally be identical to the true value.
Typically, accuracy is represented and determined by recovery studies. Accuracy determination for an HPLC method should be carried out with a minimum of nine measurements using at least three concentrations. This approach minimizes any variability and/or bias in sample preparation technique and analysis for one sample at only one concentration. An example would be three replicate measurements each of three different concentration preparations.

**Precision**: Precision can be defined as “the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample”\(^2\). A more comprehensive definition proposed by the International Conference on Harmonization (ICH)\(^6\) divides precision into three types namely:

- Repeatability
- Intermediate precision, and
- Reproducibility

**Repeatability**: Is the precision of a method under the same operating condition over a short period of time. This is measured by the sequential, repetitive injection of the same homogeneous sample, followed by the averaging of the peak area value and determination of the relative standard deviation (RSD) of all injections.

**Intermediate precision**: Is the agreement of complete measurements (including standards) when the same method is
applied many times within the same laboratory. This can include full analysis on different days, instruments, or analysts, but would involve multiple preparation of samples and standards.

**Reproducibility**: This examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments. Precision is often expressed by the standard deviation (SD) or relative standard deviation (RSD) of a data set. If a set of \( n \) measurements is performed on a sample, the average value obtained from those \( n \) measurements is defined as

\[
X = \frac{\sum_{i=1}^{n} x_i}{n}
\]

Where \( x_i \) are the individual measurements on the sample. The standard deviation of these data is then

\[
SD = \sqrt{\frac{\sum_{i=1}^{N} (x_i - X)^2}{N - 1}}
\]

And the relative standard deviation (RSD) or coefficient of variation (CV) is

\[
RSD (\%) = \frac{100 \ SD}{X}
\]

\[\text{Image} \]
**Liniearity**: The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data are then processed using a linear least-squares regression. The resulting plot slope, intercept, and correlation coefficient provide the desired information on linearity. A linearity correlation coefficient above 0.999 is acceptable for most method, especially for major components in assay method.

**Range**: The range of a method can be defined as the lower and upper concentrations for which the analytical method has adequate accuracy, precision, and linearity. While a desired concentration range is often known before starting the validation of a method, the actual working range results from data generated during validation studies. For a major component assay, concentrations of standards should be measured at or near the expected target measurement level. The concentration range should encompass values expected in samples to be measured. A good strategy is to perform studies at 50, 100, and 150% of target level.

**Limit Of Detection**: The limit of detection can be defined as the smallest level of an analyte that gives measurable response under stated experimental condition. The LOD is often based on a certain signal-to-noise (S/N) ratio, typically 2 or 3.
**Limit Of Quantitation**: It can be defined as the smallest concentration of an analyte which gives a response that can be accurately and precisely quantified under stated experimental condition.

**Specificity**: This can be defined as the ability to measure accurately the concentration of an analyte in the presence of all other sample materials. Method specificity can be achieved by checking the resolution of all potential interfering compound from the peak of interest.

**Robustness**: The concept of robustness of an analytical procedure has been defined by the ICH\(^6\) as “a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters.” It consists of following parameters.

- Influence of variations in different columns.
- Influence of variations at different column oven temperature.
- Influence of variations at different flow rate.
- Influence of variations at different buffer strength.
- Influence of variations at different pH in mobile phase.
- Influence of variations at different mobile phase composition.

**Degradation Study**: Degradation of an analyte checked under following stressed conditions.

- Acidic condition
- Alkaline condition
- Oxidative condition
- Thermal condition
- Photochemical condition
Correlate the spectrum of an analyte peak initially and after the experiment, the spectrum correlation value more than 99% indicates that no other peak merge with an analyte peak.

**Stability**: During the earlier validation studies, the method developer gained some information on the stability of reagents, mobile phase, standards, and sample solution. For routine testing in which many samples are prepared and analyzed each day, it is often essential that solutions be stable enough to allow for delays such as instrument breakdowns or overnight analyses using autosamplers. At this point, the limits of stability should be tested. Samples should be tested over at least a 48 hrs. period, and quantitation of components should be determined by comparison to freshly prepared standards. If the solutions are not stable over 48 hrs., storage conditions or additives should be identified that can improve stability.
REFERENCES


