Procedure

General

The validation of an analytical method is the process by which it is established that the performance characteristics of the method, such as Precision, Accuracy, Specificity, Linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ) and Robustness meet the requirements for the intended applications.

This SOP refers specifically to HPLC. However, the same principles may be applied to validations of other types of analytical procedures.

Well-characterised reference materials with documented purity should be used to perform the validation.

The optimum wavelength for a method can be found by acquiring the chromatographic data on a PDA detector over a large wavelength range, (e.g. 200-400nm). The optimum wavelength is the wavelength, which maximises the response for all the components of interest, but is outside the absorbance for the mobile phase. Before validating an HPLC method, its Specificity must be determined. If the method does not comply with the Specificity requirements, the method must be modified until the acceptance criteria are met. Hence it is essential that the Specificity be adequate, before Precision, Linearity and Accuracy, etc. are performed.

1. Definitions used in determining Specificity

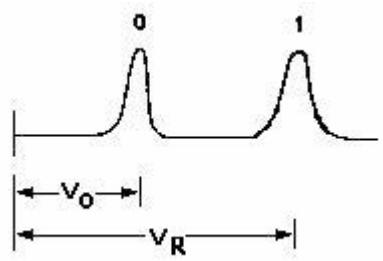
1.1. Capacity Factor (k') is defined by the equation

$$k' = \frac{V_R - V_O}{V_O}$$

where:

V_R = the distance along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak of interest.

V_O the distance along the baseline between the point of injection and a perpendicular dropped from the maximum of an unretained peak.



1.2. Resolution, R

Determine the resolution between adjacent peaks, A and B, using the following equation:

$$R = \frac{V_B - V_A}{1/2\,(W_A + W_B)}$$

where

V = retention time of the peaks (expressed in mm)

W = peak widths at the baseline of tangents drawn on the peak (mm).

6. Assay Precision

6.1. The **Precision** of an analytical method refers to the degree of agreement among individual test results obtained from multiple sampling of the same homogeneous sample. Precision may be considered at 3 levels: Repeatability, Intermediate Precision and Reproducibility. The precision of a method is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

6.2. **Repeatability** may be obtained by:

1) Repeatedly applying the analytical method to multiple samplings (at least 6) of a homogeneous sample at 100% of test concentration,
or;
2) at least 9 determinations covering the specified range for the procedure,
(e.g. 3 concentrations, 3 replicates each). The standard deviation of the results must be ≤ 2% of the mean for the method to meet precision requirements.

6.3. **Intermediate Precision**. This establishes the extent to which random events influence the precision of the method, within the one laboratory. Typical variations to be studied are: different days, different analysts, or different pieces of equipment.

6.4. **Reproducibility** refers to inter-laboratory trials. As a general rule the reproducibility should be within +/- 2% between laboratories for active drugs and 10% for degradation products.

7. Accuracy

7.1. Accuracy may be defined as the closeness of an individual test result to the true test result value. Thus, accuracy is a measure of the exactness of the analytical method. The results of a given method may be high in accuracy but low in precision, and vice versa. Accuracy may often be expressed as percent recovery by the assay of known, added amounts of analyte to the inert matrix.

7.2. Accuracy can be determined by preparing a matrix of the ingredients of the product with the exception of the active component. The active component is then added or 'spiked' in known amounts usually ranging from 75% to 125% of the dosage strength on at least 5 levels (25% - 125% for dissolution studies). The recovery of the known amount is then calculated.

7.3. A minimum of 3 concentrations should be studied, 3 injections per concentration. The accuracy of an analytical method is the closeness of test results obtained by that method to the true or theoretical value.

$$\% \text{ Accuracy} = \text{Experimental Assay} - \frac{\text{Theoretical Assay} \times 100}{\text{Theoretical Assay}}$$

7.4. Typical accuracy acceptance criteria are ≥ 98% and ≤ 102%.

Typical % RSD acceptance criteria (over all concentration levels) is ≤ 2%.

7.5. To validate the accuracy of a method, the analyst must have a standard material of characterised purity in order to know what response to expect in the test method.

8. Limit of Detection / Limit of Quantitation

8.1. For the quantitation of impurities and degradation products, linearity studies should be carried out in the presence of the drug substance. Such studies should be extended to low concentrations to experimentally define actual Limits of Detection (LOD) and Limits of Quantitation (LOQ).

