

# Standard Operating Procedure

## Title: HPLC Method Development & Validation Procedure

### 1.3. USP Tailing

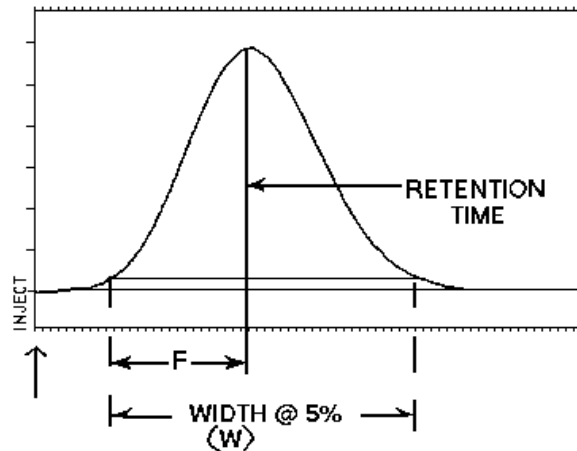
1.3.1. The Tailing factor, T is an indicator of peak skewness and is calculated using the equation:

$$T = \frac{W}{2F}$$

where

W = Peak width at 5% of peak height

F = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height.



## 2. Specificity (or Selectivity)

- 2.1. The Specificity of an analytical method is its ability to accurately measure the analyte in the presence of components that may be present in the sample matrix, (e.g. excipients, solvents, breakdown products (BDP) and impurities).
- 2.2. The method often also needs to be able to measure the level of any detectable BDP or impurities present. Stress Testing (refer 4.) enables the BDP to be characterised by their retention times.
- 2.3. The analyte peak must be well separated from any peaks due to excipients, breakdown products, impurities and void volume. Coelution between any of the peaks of interest should not occur.
- 2.4. For stability indicating chromatographic methods, peak purity (homogeneity) should be demonstrated. This can be done by peak slicing using diode-array UV detection (PDA) and comparing the results obtained with reference standard and drug degraded under stress conditions.
- 2.5. The specificity of a test method is determined by comparing test results from the analysis of samples containing impurities, degradation products, or placebo ingredients with those obtained from the analysis of samples without impurities, degradation products, or placebo ingredients. The bias of the assay is a measure of the specificity.

## 3. System Suitability Requirements

- 3.1. During method development, the following system suitability conditions need to be met for the analyte and breakdown product/impurity peaks:

# Standard Operating Procedure

## Title: HPLC Method Development & Validation Procedure

### 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  $\leq 2\%$  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  $\pm 2\%$  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.
- $$\% \text{ Accuracy} = \frac{\text{Experimental Assay} - \text{Theoretical Assay}}{\text{Theoretical Assay}} \times 100$$
- 7.4. Typical accuracy acceptance criteria are  $\geq 98\%$  and  $\leq 102\%$ .
- Typical % RSD acceptance criteria (over all concentration levels) is  $\leq 2\%$ .
- 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).