SOP for Analytical Method Validation By HPLC

1.0 OBJECTIVE:

1.1 To describe the procedure for the Analytical Validation of HPLC method.

2.0 SCOPE:

2.1 This procedure is applicable for validating the method by High Performance Liquid Chromatography in the Quality Control Laboratory .

3.0 RESPONSIBILITY:

3.1 Officer - Quality Control
3.2 Executive - Quality Control

4.0 ACCOUNTABILITY:

4.1 Incharge - Quality Control

5.0 REFERENCE(S):

5.1 In- House.

6.0 PROCEDURE:

6.1 HPLC method either used for the Assay or for the Content Uniformity determination will be validated for the following parameters.

6.1.1 Specificity.
6.1.2 Precision. (System precision and Method precision).
6.1.3 Accuracy.
6.1.4 Linearity.
6.1.5 Ruggedness.
6.1.6 Robustness.

6.2 The Following factors will be also determined as described in the Pharmacopoeia i.e. BP or USP for system suitability.

6.2.1 Resolution factor.
6.2.2 Column efficiency.
6.2.3 Tailing factor.
6.2.4 Relative standard deviation of replicate injections.
6.2.5 Relative retention time.

6.3 In a HPLC, analysis will be performed through replicate injections. The minimum number of replicate injection is five.

6.4 **Parameter :** Specificity

6.4.1 To ensure the satisfactory resolution of the principle peak from the nearest secondary peak resolution factor and tailing factor will be calculated. The minimum requirement of resolution factor will be decided for each sample.

6.6 **Parameter :** Precision

6.6.1 Five standard solutions to be prepared and analyzed as per described in procedure and data to be record as follows

Sample No.	Weight in mg	Peak Area	Peak Area / mg

Arithmetic Mean
Relative Std. Deviation

6.7 **Parameter :** Linearity

6.7.1 Five solutions of the standard preparation to be prepared as described in the test procedure. So that concentration described for the test solution lies at the mid-point on the straight line of the linearity curve

Sample No.	Concentration	Area

6.8 **Parameter :** Accuracy

6.8.1 The true peak area for the standard preparation and deviation will be determined on five replicate injections from the calculated value. The data will be recorded as follows

Calculated Value.	Sample Value	Percent

Arithmetic Mean
Relative Std. Deviation

6.9 **Parameter :** Ruggedness

6.9.1 The test procedure will be reproduced with another HPLC column in the same laboratory and employing the same method in different pharmaceuticals so as to determine intra-lab and inter-lab variation. Similar exercise to be done on different days and on same day by different analyst to determine inter-day and inter-analyst variation.

6.9.2 All the various chromatographic factors as described in BP, USP will be determined for all HPLC test procedures as per methodology given in the pharmacopoeia. The factors to be determined are given below for ready reference.

6.9.2.1 Resolution factor.
6.9.2.2 Column efficiency / number of theoretical plates.
6.9.2.3 Tailing factor.
6.9.2.4 Relative standard deviation of replicate injection.
6.9.2.5 Relative retention time.

7.0 HISTORY:

7.1 Details are given below.

SOP No.	REASON FOR CHANGE	EFFECTIVE DATE

8.0 ABBREVIATIONS : The abbreviations used in the SOP are :

- SOP- Standard Operating Procedure
- No. - Number
- QA - Quality Assurance
- QC - Quality Control