ANALYTICAL METHOD VALIDATION PROTOCOL FOR ASSAY OF CELECOXIB CAPSULES

Name of the Product	**:**	Celecoxib Capsules
Department	**:**	Quality Control
Protocol No.	**:**	
Effective Date	**:**	

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR ASSAY OF CELECOXIB CAPSULES

INDEX

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR ASSAY OF CELECOXIB CAPSULES

1. **OBJECTIVE:** The objective of this protocol is to establish a documented evidence for the assay method validation at site of Celecoxib Capsules.

2. **SCOPE:** This Protocol is applicable for assay test of Celecoxib Capsules for Specificity and Precision for export market in Quality Control Department.

3. **RESPONSIBILITY:** Officer/Executive-QC is responsible for the preparation of the protocol. The protocol shall be reviewed / approved by the Officer/Executive/Manager –QC/QA, approved by Head QA/QC.

4. **REFERENCES:**

   SOP: Operation and calibration of HPLC.

   SOP: Procedure Validation Activities.

   SOP: Operation and calibration of analytical balance.

   SOP: Procedure for handling of reference/working standard.

   SOP: Handling procedure of high performance liquid chromatography column

5. **METHODOLOGY:**

   ASSAY (BY HPLC)

   Equipment /Instrument Required:

- HPLC
- Analytical Balance
- Sonicator
- Calculator

Glass wares required:

- Volumetric Flask
- Beaker
- Bulb Pipette

Reagent Required (HPLC grade or equivalent)

- Methanol
- Hydrochloric acid
- Water

Working/Reference standard:

- Celecoxib BP

Chromatographic Conditions:

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR ASSAY OF CELECOXIB CAPSULES

Column : Use a stainless steel column (10 cm × 4.6 mm) packed with base-deactivated, end-capped octadecylsilyl silica gel for chromatography (3 µm) (Hypersil BDS is suitable).
Flow rate : 1.5 ml/min
Wave length : 280 nm
Injection Volume : 10 µl
Column temperature : Ambient

Equilibrate the column for at least 30 minutes with methanol and equilibrate with the initial mobile phase for at least 5 minutes.

Mobile phase: Mobile phase A: Methanol.

Mobile phase B: 0.5% w/v solution of ammonium acetate.

[Dissolve 5 gm of Ammonium Acetate into 1000 ml of water.]

Use gradient elution and the mobile phases described below.

Time (min)	Mobile phase A (percent (V/V)	Mobile phase B (percent (V/V)
0	30	70
10	100	0
12	100	0

Standard Solution: Weigh accurately to 50 mg of Celecoxib working standard into 100 ml volumetric flas 50 ml of methanol, sonicate for dissolve and make up to mark with methanol. Filter and discard first fe Further dilute 2 ml to 100 ml with methanol.

Sample Preparation: Crush the 20 Capsules. Weigh the powder equivalent to 50 mg of Celecoxib into 1 volumetric flask, add 50 ml of methanol, sonicate to dissolve and make up to mark with methanol. Filt discard first few ml. Further dilute 2 ml to 100 ml with methanol.

Take the absorbance at 255 nm of both solutions and calculate the content of Celecoxib.

Calculation:

$$= \frac{\text{Spl Abs.}}{\text{Std Abs.}} \times \frac{\text{Std Wt.}}{100} \times \frac{2}{100} \times \frac{100}{\text{Spl. Wt.}} \times \frac{P}{2} \times \frac{\text{Avg. Wt}}{100} \times \frac{\text{Claim}}{100}$$

Where,

Spl. Abs. = Absorbance of Sample preparation

Std. Abs. = Absorbance of Standard preparation

Std. Wt = Weight of working standard of Celecoxib

Spl. Wt. = Weigh of Sample

P = Potency of Working Standard

6. PARAMETERS:

The following validation parameter shall be considered for validation.

System Suitability

Specificity

Precision

System Precision

Method Precision

Intermediate Method Precision

6.1 SYSTEM SUITABILITY TEST:

Objective: To demonstrate and verify that the system suitability parameters of the system are adequate for the subjected analysis.

Note: For detailed of procedure, refer methodology as described under section 5.

Procedure: Inject one injection of blank and five injection of standard solution.

Acceptance criteria: %RSD of five replicate standard solution areas should not be more than 2.0%.

6.2 SPECIFICITY

The specificity of an analytical method is its ability to measure unequivocally the analyte in the presence of components that may be expected to be present in the test matrix**.**

Note: For detailed of procedure, refer methodology as described under section 5.

Preparation of placebo: Dissolve 1200 mg powder in 500 ml volumetric flask, add about 350 ml methanol, sonicate for 20 minutes and makeup the volume with methanol. Filter, Further Dilute 25 ml of the filtrate to 50 ml with 0.002 M HCL.]

Procedure: Separately inject 10 µl of followings solution into the chromatograph.
Blank - Single
Placebo – Single
Standard solution - Single
Test solution – Single

Acceptance criteria: There should not be any peak interference at the Retention Time of Celecoxib peak.

6.3 PRECISION:

The measure of how close the data values are to each other for a number of determinations under the same analytical condition

Note: For detailed of procedure, refer methodology as described under section 5.

System precision: Inject five replicate injections of the standard solution.

Method precision: Inject duplicate injections of each six test solution.

Intermediate method precision: Second analyst repeats method precision on different instrument and columns etc.

6.4 SYSTEM PRECISION:

Procedure: Separately inject equal volumes (10µl) of blank in single and standard solution in five replicate into the HPLC and record the chromatograms, measure the responses for the major peaks.

Acceptance criteria: %RSD of five replicate standard solution areas should not be more than 2.0%.

6.5 METHOD PRECISION:

Note: For detailed procedure, refer methodology as described under section 5.

Sample Preparation: Dissolve 1300 mg powder in 500 ml volumetric flask, add about 350 ml methanol, sonicate for 20 minutes and makeup the volume with methanol. Filter, Further Dilute 25 ml of the filtrate to 50 ml with 0.002 M HCL.

Note: Sample to be prepared six times.

Procedure: Separately inject equal volumes (10µl) of blank in single, standard solution in five replicate and six samples solution in duplicate into the HPLC and record the chromatograms and measure the responses for the major peaks.

System suitability: The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the two principal peaks is at least 2. If necessary adjust the concentration of methanol in the mobile phase or adjust the time program for the linear gradient and the relative standard deviation for replicate injections is not more than 2.0 %

Acceptance criteria: %RSD of six test assay result should not be more than 2.00%.

6.6 INTERMEDIATE METHOD PRECISION:

Second analyst will analyze six samples on different instrument and different column and different day.

Mobile Phase, Standard solution, Sample solution, Chromatographic condition, Procedure and Calculation are same as per Method Precision.

Acceptance criteria: %RSD of six test assay result should not be more than 2.00%.

%RSD of 12 test assay results of analyst I and analyst II should not be more than 5.00%.

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR ASSAY OF CELECOXIB CAPSULES

Reports: Prepare the report of analytical Method Validation and compile the data as per below table.

Validation Parameters	Acceptance criteria
System suitability	
% RSD	NMT 2.0%
Specificity	There should not be any peak interference at the retention time of Celecoxib peak.
Precision	
System precision	RSD NMT 2.0%
Method Precision (Analyst I)	RSD of six test assay result should not be more than 2.00%.
Intermediate precision (Analyst II)	RSD of six test assay result should not be more than 2.00%.
Results of Twelve sample of both analyst	%RSD of 12 test assay results of analyst I & analyst II should not be more than 5.00%.

7. ABBREVIATION:

AMV	:	Analytical Method Validation
QC	:	Quality Control
QA	:	Quality Assurance
mL	:	Milli Liter
SST	:	System Suitability Test
i.e.	:	That is
UV	:	Ultra-violet
mcg/ml	:	Microgram/milliliter
µl	:	micro liter
µm	:	Micro meter
nm	:	Nanometer
cm	:	Centimeter
mg	:	Milligram
hrs	:	Hours