METHOD VERIFICTION

PROTOCOL FOR (ASSAY)

DICLOFENAC SODIUM TABLET IP

BY

HIGH PREFORMANCE LIQIUD CHROMATOGRAPHY

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ANALYTICAL METHOD VERIFICATION PROTOCOL FOR DICLOFENAC SODIUM TABLETS IP

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1.0 Protocol Approval:

This is a specific Protocol for Method Validation of Diclofenac Sodium Tablet IP.

1.1 **Initial Approval:** This Protocol has been approved by the following:

	Name	Designation	Signature	Date
Prepared by (QC)				
Checked by (QC)				
Reviewed by (QA)				

1.2 **Final Approval:** Final approval has been given by the following:

	Name	Designation	Signature	Date
Approved By (Head-Quality Assurance)				

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2.0 Objective:

This protocol shall be providing the documented evidence, that the Method Verification for Diclofenac Sodium Tablet IP in with the specified quality attributes in consistent manner.

3.0 Scope:

This protocol shall be used to provide the procedure for the Method Verification for Diclofenac Sodium Tablet IP.

4.0 Responsibility:

To conduct the Method Verification for Diclofenac Sodium Tablet IP. The Validation team is described through the following responsibility table.

S.No.	Department	Responsibility
1.	Quality Control	1) QC Chemist shall be responsible for conducting the verification carry out the verification analysis. 2) QC Executive or Designee shall be responsible for preparation of Verification Protocol, Reporting, Planning and Monitoring. 3) QC Manager shall be responsible for checking of Verification Protocol and Report. 4) QC Manager or Designee shall be responsible for provide the training for staff.
2.	Quality Assurance	QA Head or Designee shall be responsible for final approval of Testing Protocol.

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5.0 Specification:

S.No.	Parameters	Acceptance Criteria
1.	Specificity	No interference with blank or placebo.
2.	Precision	NMT - RSD < 2%.
	1)System Precision	Standard RSD shall be Not more than 2.0 %.
	2) Method Precision	Results RSD shall be Not more than 2.0 %.
	3) Intermediate Precision	Percentage RSD shall be Not more than 2.0 % for six results. Analyst 1 & Analyst 2 results cumulative RSD shall be Not more than 2.0 %. Analyst 1 & analyst 2 results Coefficient of determination R^2should be greater than 0.99
3.	Linearity	Coefficient of determination R^2should be greater than 0.995
4.	Range	Concentration where data can be reliably determined (98 to 102 % recovery)
5.	Accuracy	98 to 102 % (in range 80 to 120%)
6.	Robustness	Results RSD shall be not more than 2.0 %
7.	Solution stability	Results RSD shall be not more than 2.0 %

6.0 Analytical Method Verification Plan:

i) The experiment may be performed as sequential or parallel operation.

ii) Sample sequence for each experiment may run independently or together with necessary alteration of sample sequence.

iii) Same experiment may be use for more than one parameter.

7.0 Deviation: Any deviation for validation experiments and acceptance criteria (if observed) should be reported and justified**.**

8.0 Methodology for Verification:

8.1 Equipment:

S.No.	Instrument Name	Manufactured By	Model No.	Calibration Date

8.2 Reagent:

S.No.	Name	Manufactured By	Batch/Lot. No.	Mfg Date	Exp Date

8.3 **Working Standard:**

S.No.	Name	Manufactured By	Batch/AR. No.	Mfg. Date	Exp. Date

9.0 Experimental plan & Data evaluation:

9.1 **Specificity:** Specificity is the evidence of suitable separation of all solvents so that each solvent can specified & quantified.

9.1.1 **Chromatographic Conditions:**

Equipment : High Performance Liquid Chromatography

Column : 4.6 mm × 25-cm, 5µm (C18)

Wavelength : 254 nm.

Flow Rate : 1.5 ml /min.

Injection volume : 20 µl.

Column Temperature: 30°C

9.1.2 **Blank:** Mobile Phase

9.1.3 **Buffer:** Dissolve 3.12 g of Sodium Di-hydrogen Orthophosphate in water and dilute to 1000 ml with water, adjusting the pH to 6.5 ± 0.05 with Sodium Hydroxide solution.

9.1.4 **Mobile phase:** A mixture of 25 volumes of buffer solution, 40 volumes of methanol and 35 volumes of acetonitrile,

9.1.5 **Placebo Solution:** Weight and transfer 118 mg placebo in to a 100-m volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-mL volumetric flask, dilute with diluent to volume, and mix than filter with 0.45 µ membrane filter.

9.1.6 **Standard solution:**Transfer about to 40 mg of Diclofenac sodium in to a 100-ml volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-mL volumetric flask, dilute with diluent to volume, and mix. **(Standard Solution 200 ppm).**

9.1.7 **Test Solution:** Use the 20 tablets powder and transfer about to 40 mg of Diclofenac Sodium in to a 100 ml volumetric flask, add 50 ml of diluent dissolve and sonicate for 20 min at 40°C and make up with diluent dissolve by swirling dilute with diluent to volume, and mix. Further transfer 10 ml of this solution in to a 20-mL volumetric flask, dilute with diluent to volume, and mix. Filter with 0.22 µm pore size filter paper. **(Test solution 200 ppm).**

9.1.8 **Procedure:** Separately inject 1 µl one injection of blank, placebo, five injections of standard preparation followed by two injections of sample preparation.

9.1.9 **Gradients program:**

Time (min)	Solution A	Solution B
0.00	70	30
0.50	70	30
8.50	5	95
10.00	5	95
10.01	70	30
14.00	70	30

9.1.10 **System Suitability:** The test is not valid unless the tailing factor is not more than 1.2 and the relative standard deviation for replicate injections is not more than 1.0 %.

9.1.11 **Observation:**

Components	Acceptance Criteria
Diluent	There shall be no interference in Sample response due to blank and placebo.
Placebo	
Standard	
Sample	

9.2 Precision:

9.2.1 **System Precision:** Precision is the agreement between a set of replicate measurements.

9.2.1.1 **Blank & Chromatographic Conditions:** As per specificity test.

9.2.1.2 **Standard solution :**Transfer about to 40 mg of Diclofenac sodium in to a 100-ml volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-ml volumetric flask, dilute with diluent to volume, and mix. **(Standard Solution 200 ppm).**

9.2.1.3 **Procedure:** Separately inject 1µl one injection of Blank, and six injections of standard solution.

9.2.1.4 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.2.1.5 **Acceptance criteria:** Percentage RSD shall be not more than 2.0 % for replicate standard.

9.2.2 **Method Precision:** Repeatability evaluates the variation experienced by a single analyst on a single instrument Repeatability is performed by analyzing multiple replicates of an assay composite sample using the analytical method. The recovery value is calculated and reported for each value.

9.2.2.1 **Blank & Chromatographic Conditions:** As per specificity test.

9.2.2.2 **Standard Solution :** Transfer about to 40 mg of Diclofenac Sodium in to a 100-ml volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-ml volumetric flask, dilute with diluent to volume, and mix. **(Standard Solution 200 ppm).**

9.2.2.3 **Test solution:** Use the 20 tablets powder and transfer about to 40 mg of Diclofenac Sodium in to a 100 ml volumetric flask, add 50 ml of diluent dissolve and sonicate for 20 min at 40°C and make up with diluent dissolve by swirling dilute with diluent to volume, and mix. Further transfer 10 ml of this solution in to a 20-mL volumetric flask, dilute with diluent to volume, and mix. Filter with 0.22 µm pore size filter paper. **(Test solution 200 ppm)** Prepare the six separate samples.

9.2.2.4 **Procedure:** Separately inject 1 µl one injection of blank, five injections of standard preparation followed by two injections of each sample preparation.

9.2.2.5 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.2.2.6 **Acceptance criteria:** Percentage RSD shall be not more than 2.0 % for six results.

9.2.2.7 **Calculation:** Calculate the content of Diclofenac sodium (in mg): -

$$\frac{W_s}{A_s} \times \frac{10}{100} \times \frac{100}{20} \times \frac{20}{W_t} \times \frac{P}{10} \times \frac{}{100} \times \text{Average wt.}$$

Where

A_T: Area of Diclofenac sodium peak from one injections of test preparation.

A_S: Average area of Diclofenac Sodium working standard USP five injections of preparation.

W_S: Weight of Diclofenac Sodium working standard USP in mg.

W_t: Weight of test in mg.

P : Potency of Diclofenac sodium working standard USP used in percent.

9.2.3 **Intermediate Precision:** Intermediate precision was formally known as ruggedness. A second analyst repeats the repeatability analysis on a different day using different conditions and different instruments. The recovery values are calculated and reported. A statistical comparison is made to the first analyst's results.

9.2.3.1 **Blank & Chromatographic Conditions:** As per specificity test.

9.2.3.2 **Standard Solution:** Transfer about to 40 mg of Diclofenac sodium in to a 100 ml volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this

solution in to a 20 ml volumetric flask, dilute with diluent to volume, and mix. **(Standard solution 200 ppm).**

9.2.3.3 **Test solution:** Use the 20 tablets powder and transfer about to 40 mg of Diclofenac sodium in to a 100 ml volumetric flask, add 50 ml of diluent dissolve and sonicate for 20 min at 40°C and make up with diluent dissolve by swirling dilute with diluent to volume, and mix. Further transfer 10 ml of this solution in to a 20-ml volumetric flask, dilute with diluent to volume, and mix. Filter with 0.22 µm pore size filter paper. **(Test solution 200 ppm)** Prepare the six separate samples.

9.2.3.4 **Procedure:** Separately inject 1 µl one injection of blank, five injections of standard preparation followed by two injections of each sample preparation.

9.2.3.5 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.2.3.6 **Acceptance criteria:**

- Percentage RSD shall be Not more than 2.0 % for six results.
- Analyst 1 & analyst 2 results cumulative RSD shall be Not more than 2.0 %.
- Analyst 1 & analyst 2 results Coefficient of determination R^2should be greater than 0.99.

9.2.3.7 **Calculation:** Calculate the content of Diclofenac sodium (in mg):

$$\frac{Ws}{As} \times \frac{10}{100} \times \frac{100}{20} \times \frac{20}{Wt} \times \frac{P}{10} \times \frac{}{100} \times \text{Average wt.}$$

Where

A_T: Area of Diclofenac sodium peak from one injections of test preparation.

A_S: Average area of Diclofenac sodium working standard USP five injections of preparation.

W_S: Weight of Diclofenac sodium working standard USP in mg.

W_t: Weight of test in mg.

P: Potency of Diclofenac sodium working standard USP used in percent.

9.3 Linearity: Linearity evaluates the analytical procedure's ability (within a given range) to obtain a response that is directly proportional to the concentration (amount) of analyte standard.

9.3.1 **Blank & Chromatographic Conditions:** As per specificity test.

9.3.2 **Standard stock solution:** Weight and transfer about Eq. to 40 mg of Diclofenac sodium in 100 ml volumetric flask add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix.

9.3.3 **Standard Solution:** Further transfer 10 ml of this solution in to a 100 ml volumetric flak, dilute with diluent to volume, and mix.

9.3.4 Prepare five standard solutions of the analyte at 80%, 90%, 100%, 110%, and 120% of the method concentration using serial dilutions from a Standard stock solution.

9.3.4.1 **Standard Preparation for 80 %:** To 8 ml of the Standard stock resulting solution in to a100 ml volumetric flask, dilute with diluent to volume, and mix.

9.3.4.2 **Standard Preparation for 90 %:** To 9 ml of the Standard stock resulting solution in to 100 ml volumetric flak, dilute with diluent to volume, and mix.

9.3.4.3 **Standard Preparation for 100%:** To 10 ml of the Standard stock resulting solution in to 100 ml volumetric flak, dilute with diluent to volume, and mix.

9.3.4.4 **Standard Preparation for 110%:** To 11 ml of the Standard stock resulting solution in to 100 ml volumetric flak, dilute with diluent to volume, and mix.

9.3.4.5 **Standard Preparation for 120%:** To 12 ml of the Standard stock resulting solution in to 100 ml volumetric flak, dilute with diluent to volume, and mix.

9.3.5 **Procedure:** Separately inject 1µl one injection of blank, five injections of standard preparation followed by one injections of each concentration sample solution.

9.3.6 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.3.7 **Acceptance criteria:**

9.3.7.1 Coefficient of determination (r2) should be greater than 0.995

9.4 **Range:** Range is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

9.4.1 **Low and High concentration Preparation:** Prepare standard solutions of the analyte at ~80%, and 120% of the method concentration using serial dilutions from a Standard stock solution as per Linearity test.

9.4.2 **Procedure:** Separately inject 1 µl one injection of blank, five injections of standard solution followed by six replicate injections of each lower and higher concentration sample solution.

9.4.3 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.4.4 **Acceptance criteria:**

9.4.4.1 Percentage RSD shall be Not more than 2.0 % for each concentration.

9.4.4.2 Coefficient of determination (r2) should be greater than 0.99

9.5 **Accuracy:** Accuracy expresses the closeness of agreement between the value found and the value that is accepted as either a conventional true value or an accepted reference value. It may often be expressed as the recovery by the assay of known, added amounts of analyte. Samples (spiked placebos) are prepared

normally covering 80%, 100%& 120% of the nominal sample preparation concentration each in triplicate. These samples are analyzed and the recoveries of each are calculated.

9.5.1 **Blank & Chromatographic Conditions:** As per specificity test.

9.5.2 **Standard Solution:**Transfer about to 40 mg of Diclofenac sodium in to a 100-mL volumetric flask, add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20 ml volumetric flask, dilute with diluent to volume, and mix. **(Standard solution 200 ppm).**

9.5.3 **Test solution:**

9.5.3.1 **Recovery for 80 % level:** Transfer and weight a quantity of the powder about 118 mg of Placebo in to a 100-ml volumetric flask, and spiked 32 mg of Diclofenac sodium working standard USP add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-ml volumetric flask, dilute with diluent to volume, and mix than filter with 0.45 µ membrane filter. **(Test solution: 160 PPM).**

9.5.3.2 **Recovery for 100 % level:** Transfer and weight a quantity of the powder about 118 mg of Placebo in to a 100-mL volumetric flask, and spiked 40 mg of Diclofenac sodium working standard USP add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20-mL volumetric flask, dilute with diluent to volume, and mix than filter with 0.45 µ membrane filter.**(Test solution: 200 PPM).**

9.5.3.3 **Recovery for 120 % level:** Transfer and weight a quantity of the powder about 118 mg of Placebo in to a 100 ml volumetric flask, and spiked 48 mg of Diclofenac sodium working standard USP add 50 ml of diluent and dissolve by swirling dilute with diluent to volume, and mix. Transfer 10 ml of this solution in to a 20 ml volumetric flask, dilute with diluent to volume, and mix than filter with 0.45 µ membrane filter. **(Test solution: 240 ppm).**

9.5.4 **Procedure:** Separately inject 1 µl one injection of blank, five injections of standard preparation followed by triplicate injections of each sample solution.

9.5.5 **System Suitability:** The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.5.6 **Acceptance criteria:**

9.5.6.1 Each individual sample recovery should lie within the range of 98% to 102%.

9.5.6.2 Percentage RSD shall be Not more than 2.0 %.

9.5.7 **Calculation:**

Recovery in (mg):

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{10}{20} \times \frac{100}{110} \times \frac{20}{100} \times P \times 1$$

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Recovery in (mg)

Recovery in (%): ----------X 100

Spiked amount in (mg)

9.6 **Robustness:** Robustness is the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters.

9.6.1 **For Wavelength of UV-Visible Detector:** The procedure shall be used to verify that error in the detector wavelength at most, ±2 nm and check the system suitability parameters.

9.6.2 **For Flow rate:** The procedure shall be used to verify that error in the flow rate at most, ± 10% of flow rate and check the system suitability parameters.

9.6.3 **For Column temperature:** The procedure shall be used to valid that error in the Column temperature at most, ± 0.5°C and check the system suitability parameters.

9.7 **Solution stability:** Stability is determined by comparing the response and impurity profile from aged standards or samples to that of a freshly prepared standard and to its own response from earlier time points.

9.7.1 Prepare fresh blank, resolution and standard as per the test method.

9.7.2 Analyze these solutions as per the test method.

9.7.3 Analyze these sample versus fresh standard with initial, 4, 8, 12 and 24 hours.

9.7.4 Calculate the percent recoveries calculated for all solutions.

9.7.5 **Procedure:** Separately inject 1 µl one injection of blank, five injections of fresh standard, one injection of initial standard and preparation followed by two injections of sample solution.

9.7.6 **System Suitability:** The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than2.0 %.

9.7.7 **Acceptance Criteria:**

- For assay level standards, the fresh standard and the verification standard should not differ more that 2.0%.
- For the assay level, the standard and sample solutions are considered sufficiently stable over time if the recovery value does not vary more than 2.0 % from the initial result.

9.7.8 **Calculation:** Calculate the content of Diclofenac sodium (in mg): -

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{10}{20} \times \frac{100}{Wt} \times \frac{20}{10} \times \frac{P}{100} \times \text{Average Wt}$$

Where

A_T : Average area of Diclofenac Sodium USP peak from two injections of test .

A_S : Average area of Diclofenac Sodium USP peak from five injections of standard.

W_S : Weight of Diclofenac Sodium USP working standard in mg.

W_t: Weight of test in mg.

P : Potency of Diclofenac Sodium USP working standard used in percent .

9.8 Deviations: State the impact of the variation or deviation on the ability of the experiment to be suitable to Validation.

9.9 Recommendations: Indicate any changes that need to be made to the Test Method before it should be approved. These changes should be a result of the robustness testing outcome and may include modifying or supplementing the System Suitability section of the Test Method and/or adding caution statements about requirements for analyst control of experimental parameters.

9.10 Attachments: Calibrated equipment list, signature log of executors, copies of pertinent training records, data tables, chromatograms or printouts from equipment, figures as defined by results presentation and appropriate notebook references or pages.

9.11 Conclusion: Summarize the results of the Validation Study and conclude whether or not the Test Method is appropriate for its intended use base on the Validation results given in this report and the acceptance criteria set forth in the Validation Protocol.

9.12 Reference:

9.12.1 SOP No……

9.12.2 USP/ICH/IHS.

9.13 Abbreviations:

QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
S.No.	Serial Number
SPE	Specification
USP	United states pharmacopeia
IHS	In -House
ICH	International conference on Harmonization
RSD	Relative standard deviation
mg	Milligram
nm	Nanometre

9.14 Revision History:

S.No.	Revision No	Details of Changes	Reason for Revision