METHOD VERIFICATION PROTOCOL FOR (ASSAY) CIPROFLOXACIN TABLETS USP 500 MG BY HIGH PREFORMANCE LIQIUD CHROMATOGRAPHY

Protocol No.	
Supersedes	NIL
Effective Date	
Document contains pages	13

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR CIPROFLOXACIN TABLETS USP 500 MG

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR CIPROFLOXACIN TABLETS USP 500 MG

1.0 Protocol Approval:

This is a specific Protocol for Method Verification of Ciprofloxacin Tablets USP 500 mg.

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)				

ANALYTICAL METHOD VALIDATION/VERIFICATION PROTOCOL FOR CIPROFLOXACIN TABLETS USP 500 MG

2.0 Objective:

This protocol shall be providing the documented evidence, that the Method Verification for Ciprofloxacin Tablets USP 500 mg 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 Ciprofloxacin Tablets USP 500 mg at .....

4.0 Responsibility:

To conduct the Method Verification for Ciprofloxacin Tablets USP 500 mg. The Verification 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	R^2 0.99, similar response ratio
3.	Linearity	R^2 0.99, similar response ratio
4.	Range	Concentration where data can be reliably determined (98 to 102 % recovery)
5.	Accuracy	98 to 102 % (in range 50 to 150%)
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

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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 , L1

Wavelength : 278 nm.

Flow Rate : 1.5 ml /min.

Injection volume : 10 µl.

Temperature : 30 ± 1°C.

9.1.2 **Reagent required:**

- 0.025 M Ortho phosphoric acid. (2.9 ml OPA dilute to 1000 ml)
- Triethylamine
- Acetonitrile
- Water.

9.1.3 **Solution A:** Prepare a mixture of 0.025 M phosphoric acid, previously adjusted (with triethylamine) to a pH of 2.0 ± 0.1

9.1.4 **Blank, Solution B or Diluent:** Solution A and acetonitrile (87:13).

9.1.5 **Solution C:** Prepare a mixture of 0.025 M phosphoric acid, previously adjusted (with triethylamine) to a pH of 3.0 ± 0.1

9.1.6 **Mobile Phase:** Prepare a mixture of Solution C and acetonitrile (87:13).

9.1.7 **Placebo Solution:** Transfer and weight a quantity of the powder about 2000 mg placebo in to a 500-mL volumetric flask, add about 400 mL of diluent, and sonicate for about 20 minutes. Dilute with diluent to volume, and mix. Further dilute 2 mL to 25 mL withdiluent to volume, and mix.

9.1.8 **System suitability solution:**Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.1.9 **Standard preparation:** Weight and transfer equivalent to 50 mg of Ciprofloxacin working standard USP into a 50-mL volumetric flask, add 20 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

Transfer 5 mL of the Standard stock preparation into a 25-mL volumetric flask, dilute withdiluent to volume, and mix. **(200 ppm).**

9.1.10 **Test preparation:** Transfer 5 Tablets in to a 500-mL volumetric flask, add about 400 mL of diluent, and sonicate for about 20 minutesdilute with diluent to volume, and mix.

Transfer 1 mL of the Test stock solution in to a 25mL volumetric flask, dilute withdiluent to volume, and mix. **(200 ppm).**

9.1.11 **Procedure:** Separately inject10 µl one injection of blank,placeboand resolution, five injections of standard preparation followed by two injections of sample preparation.

9.1.12 **SystemSuitability:**

- The retention time for ciprofloxacin is between 6.4 and 10.8 minutes; the relative retention times are about 0.7 for ciprofloxacin ethylene diamine analog and 1.0 for ciprofloxacin.
- The resolution, between the ciprofloxacin ethylene diamine analog peak and the ciprofloxacin peak is not less than 6.
- The column efficiency, determined from the ciprofloxacin peak, is not less than 2500 theoretical plates.
- The tailing factor for the ciprofloxacin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

9.1.13 **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:**As per specificity test.

9.2.1.2 **System suitability solution**— Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.2.1.3 **Standard solution:** Weight and transfer equivalent to 50 mg of Ciprofloxacin working standard USP into a 50-mL volumetric flask, add 20 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

Transfer 5 mL of the Standard stock preparation into a 25-mL volumetric flask, dilute withdiluent to volume, and mix. **(200 ppm).**

9.2.1.4 **Procedure:** Separately inject 10 µl one injection of Blank, System suitability solution and six injections of standard solution.

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:**As per specificity test.

9.2.2.2 **System suitability solution:**Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.2.2.3 **Standard solution:** Weight and transfer equivalent to 50 mg of Ciprofloxacin working standard USP into a 50-mL volumetric flask, add 20 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

Transfer 5 mL of the Standard stock preparation into a 25-mL volumetric flask, dilute withdiluent to volume, and mix. **(Standard solution 200 ppm).**

9.2.3 **Test solution:**Transfer 5 Tablets in to a 500-mL volumetric flask, add about 400 mL of diluent, and sonicate for about 20 minutesdilute with diluent to volume, and mix.

Transfer 1 mL of the Test stock solution in to a 25mL volumetric flask, dilute withdiluent to volume, and mix. Prepare the six separate sample preparation.**(Sample solution 200 ppm).**

9.2.3.1 **Procedure:** Separately inject 10 µl one injection of blank and resolution, five injections of standard preparation followed by two injections of each sample preparation.

9.2.3.2 **System Suitability & Acceptance criteria:**

- The retention time for ciprofloxacin is between 6.4 and 10.8 minutes; the relative retention times are about 0.7 for ciprofloxacin ethylene diamine analog and 1.0 for ciprofloxacin.
- The resolution, between the ciprofloxacin ethylene diamine analog peak and the ciprofloxacin peak is not less than 6.
- The column efficiency, determined from the ciprofloxacin peak, is not less than 2500 theoretical plates.
- The tailing factor for the ciprofloxacin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.
- Percentage RSD shall be not more than 2.0 % for six results.

9.2.3.3 **Calculation:** Calculate the content of Ciprofloxacin(in mg): -

At	Ws5	500	25	331.34	P	
----------X	---------X	--------X	---------X	--------X	----------X	--------- X Average wt
As	50	25	5	1385.82	100	

Where

A_T : Average area of Ciprofloxacin peak from two injections of test preparation.

A_S : Average area of Ciprofloxacin peak from five injections of standard Preparation.

W_S Weight of Ciprofloxacin working standard in mg .

W_t : Weight of sample in mg.

P : Potency of working standard used in percent.

9.2.4 **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.

9.2.4.1 **BlankSolution:** As per specificity test.

9.2.4.2 **System suitability solution**— Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.2.4.3 **Standard solution:** Weight and transfer equivalent to 50 mg of Ciprofloxacin working standard USP into a 50-mL volumetric flask, add 20 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

Transfer 5 mL of the Standard stock preparation into a 25-mL volumetric flask, dilute withdiluent to volume, and mix. **(Standard solution 200 ppm).**

9.2.5 **Test solution:**Transfer 5 Tablets in to a 500-mL volumetric flask, add about 400 mL of diluent, and sonicate for about 20 minutesdilute with diluent to volume, and mix.

Transfer 1 mL of the Test stock solution in to a 25mL volumetric flask, dilute withdiluent to volume, and mix. Prepare the six separate sample preparation.**(Sample solution 200 ppm).**

9.2.5.1 **Procedure:** Separately inject 10 µl one injection of blank and resolution, five injections of standard preparation followed by two injections of each sample preparation.

9.2.5.2 **System Suitability:**

- The retention time for ciprofloxacin is between 6.4 and 10.8 minutes; the relative retention times are about 0.7 for ciprofloxacin ethylene diamine analog and 1.0 for ciprofloxacin.
- The resolution, between the ciprofloxacin ethylene diamine analog peak and the ciprofloxacin peak is not less than 6.
- The column efficiency, determined from the ciprofloxacin peak, is not less than 2500 theoretical plates.

- The tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5%.

9.2.5.3 **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 %.
- Coefficient of determination (r2) should be greater than 0.99

9.2.5.4 **Calculation:** Calculate the content of Ciprofloxacin(in mg): -

$$\frac{At}{As} \times \frac{Ws5}{50} \times \frac{500}{25} \times \frac{25}{5} \times \frac{331.34}{1385.82} \times \frac{P}{100} \times \text{Average wt}$$

Where

A_T : Average area of Ciprofloxacin peak from two injections of test preparation.

A_S : Average area of Ciprofloxacin peak from five injections of standard Preparation.

W_S : Weight of Ciprofloxacin working standard in mg .

W_t : Weight of sample in mg.

P : Potency of working standard 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 Solution:** As per specificity test.

9.3.1.1 **System suitability solution**— Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.3.1.2 **Standard solution stock:** Weight and transfer equivalent to 100 mg of Ciprofloxacin working standard USP into a 100-mL volumetric flask, add 50 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

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

9.3.2 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.

9.3.2.1 **Standard Preparation for 80 %:** To 8 ml of the Standard stock resulting solution ina 50 ml volumetric flak, dilute with a mixture of diluent to volume, and mix.

9.3.2.2 **Standard Preparation for 90 %:** To 9 ml of the Standard stock resulting solution ina 50 ml volumetric flak, dilute with a mixture of diluent to volume, and mix.

9.3.2.3 **Standard Preparation for 100 %:** To 10 ml of the Standard stock resulting solution ina 50 ml volumetric flak, dilute with a diluent to volume, and mix.

9.3.2.4 **Standard Preparation for 110 %:** To 11 ml of the Standard stock resulting solution ina 50 ml volumetric flak, dilute with a mixture diluent to volume, and mix.

9.3.2.5 **Standard Preparation for 120 %:** To 12 ml of the Standard stock resulting solution in a 50 ml volumetric flak, dilute with a diluent to volume, and mix.

9.3.3 **Procedure:** Separately inject10 µl one injection of blank and resolution, five injections of standard preparation followed by one injections of each concentration samplesolution.

9.3.4 **SystemSuitability:**

- The retention time for ciprofloxacin is between 6.4 and 10.8 minutes; the relative retention times are about 0.7 for ciprofloxacin ethylene diamine analog and 1.0 for ciprofloxacin.
- The resolution, between the ciprofloxacin ethylene diamine analog peak and the ciprofloxacin peak is not less than 6.
- The column efficiency, determined from the ciprofloxacin peak, is not less than 2500 theoretical plates.
- The tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5%.

9.3.5 **Acceptance criteria:**

9.3.5.1 Percentage RSD shall be Not more than 2.0 %.

9.3.5.2 Coefficient of determination (r2) should be greater than 0.999

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 10 µl one injection of blank and resolution, five injections of standard solution followed by six replicate injections of each lower and higher concentration sample solution.

9.4.3 **Acceptance criteria:**

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

9.4.3.2 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 Preparation:**As per specificity test.

9.5.2 **System suitability solution**— Transfer about 10 mg of Ciprofloxacin Ethylene diamine analog USP in to a 10-mL volumetric flask add 5 mL of Standard solution dissolve and dilute with Standard solution further dilute 0.5 ml to 10 ml with Standard solution .

9.5.3 **Standard solution:** Weight and transfer equivalent to 50 mg of Ciprofloxacin working standard USP into a 50-mL volumetric flask, add 20 mL of diluent and dissolve by swirling and with the aid of brief sonication dilute with diluent to volume, and mix.

Transfer 5 mL of the Standard stock preparation into a 25-mL volumetric flask, dilute withdiluent to volume, and mix. **(Standard solution 200 ppm).**

9.5.4 **Test preparation:**

9.5.4.1 **Recovery for 80 % level:**Transfer and weight a quantity of the powder about 2.0 g of Placebo in to a 500-mL volumetric flask, and spiked about 1.000 g of Ciprofloxacin working standard USPadd about 400 mL of diluent, and sonicate for about 20 minutes. dilute with diluent to volume, and mix. Further dilute 2 mL to 25 mL withdiluent to volume, and mix. **(Test solution: 160 PPM).**

9.5.4.2 **Recovery for 100 % level:** Transfer and weight a quantity of the powder about 2.0 g of Placebo in to a 500-mL volumetric flask, and spiked about 1.250 g of Ciprofloxacin working standard USPadd about 400 mL of diluent, and sonicate for about 20 minutes. dilute with diluent to volume, and mix. Further dilute 2 mL to 25 mL withdiluent to volume, and mix. **(Test solution: 200 PPM).**

9.5.4.3 **Recovery for 120 % level:** Transfer and weight a quantity of the powder about 2.0 g of Placebo in to a 500-mL volumetric flask, and spiked about 1.500 g of Ciprofloxacin working standard USPadd about 400 mL of diluent, and sonicate for about 20 minutes. dilute with diluent to volume, and mix. Further dilute 2 mL to 25 mL withdiluent to volume, and mix. **(Test solution: 240 PPM).**

9.5.5 **Procedure:** Separately inject 10 µl one injection of blank and placebo, five injections of standard preparation followed by triplicate injections of each sample solution.

9.5.6 **Calculation:**

Recovery in (mg):

$$\frac{\text{At} \quad \text{Wt.}\ 2 \quad 500 \quad 25 \quad P}{\text{As} \quad 500 \quad 25 \quad 51 \quad 100} \times 1$$

Recovery in (%): $\frac{\text{Recovery in (mg)}}{\text{Spiked amount in(mg)}} \times 100$

9.5.7 **Acceptance criteria:**

9.5.7.1 The percent recovery of the spikedstandard should be within 100 ± 2 % for the average of each set of three weights.

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

9.5.7.3 Percentage RSD shall be Not more than 2.0

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, ± 0.2 ml/min and check the system suitability parameters.

9.6.3 **For Column temperature:**The procedure shall be used to verify that error in the Column temperature at most, ± 0.5°Cand 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, resolutionand 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 10 µl one injection of blank and resolution, five injections of fresh standard, one injection ofinitial standardand preparation followed by two injections of sample solution.

9.7.6 **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.

9.7.7 **Calculation:** Calculate the content of Ciprofloxacin USP (in mg): -

$$\frac{A_t}{A_s} \times \frac{Ws5}{50} \times \frac{500}{25} \times \frac{25}{5} \times \frac{331.34}{1385.82} \times \frac{P}{100} \times \text{Average wt}$$

Where

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

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

W_S : Weight of Ciprofloxacin USP working standard in mg.

W_t: Weight of sample in mg.

P : Potency of Ciprofloxacin 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 Verification.

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

9.12 Reference:

9.12.1 USP / ICH/ IHS.

9.13 Abbreviations:

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

9.14 Revision History:

S.No	Revision No	Details of Changes	Reason for Revision
1.	00	NA	New