ANALYTICAL METHOD VALIDATION / VERIFICATION PROTOCOL FOR LIVOFLOXACIN TABLETS (FOR ASSAY)

METHOD VERIFICATION

PROTOCOL FOR (ASSAY)

LEVOFLOXACIN TABLETS USP 500 MG

BY

HIGH PREFORMANCE LIQIUD CHROMATOGRAPHY

Protocol No.	
Supersedes	NIL
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1.0 Protocol Approval:

This is a specific Protocol for Method Verification ofLevofloxacin 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 LIVOFLOXACIN TABLETS (FOR ASSAY)

2.0 Objective:

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

4.0 Responsibility:

To conduct the Method Verification for Levofloxacin 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 andReport. 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.

ANALYTICAL METHOD VALIDATION / VERIFICATION PROTOCOL FOR LIVOFLOXACIN TABLETS (FOR ASSAY)

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

8.2Reagent:

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 : 25 cm × 4.6 mm, 5 µm (L1)

Wavelength : 360 nm.

Flow Rate : 0.8 ml /min.

Injection volume : 25 µl.

Temperature : 45 ºC.

Run time: 2 times of Levofloxacin retention time.

9.1.2 **Diluent:** Acetonitrile: Water (20:80).

9.1.3 **Blank:**Mobile Phase.

9.1.4 **Mobile Phase:** Transfer 874 mg of cupric sulphate ,918 mg of L-isoleucine and 5.94 gm of ammonium acetate in to a beaker add 700 ml water and mix for dissolve and add 300 ml methanol shake for mix.

9.1.5 **Placebo Solution:**Weight and transfer 700 mg placebo in to 500 ml volumetric flask disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter and further dilute 2 ml to 50 ml with mobile phase.

9.1.6 **Standard stock solution:**Weight and transfer about Eq. to 100 mg ofLevofloxacinin 50 ml volumetric flask add 20 ml diluent shake and sonicate for dissolve and make up with diluent.

9.1.7 **Standard solution:**Further dilute 5 ml to 50 ml with mobile phase. **(Standard solution 200-ppm).**

9.1.8 **Test solution stock:**Take 5 intact tablet in to 500 ml volumetric flask disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter.

9.1.9 **Test solution:** Further dilute 2 ml Test stock solution to 50 ml with mobile phase.

9.1.10 **Procedure:** Separately inject25 µl one injection of blank,placebo, five injections of standard preparation followed by two injections of sample preparation.

9.1.11 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.1.12 **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 **Standard stock solution:**Weight and transfer about Eq. to 100 mg of Levofloxacinin 50 ml volumetric flask add 20 ml diluent shake and sonicate for dissolve and make up with diluent.

9.2.1.3 **Standard solution:**Further dilute 5 ml to 50 ml with mobile phase. **(Standard solution 200-ppm).**

9.2.1.4 **Procedure:** Separately inject 25µl one injection of Blank, and six injections of standard solution.

9.2.1.5 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.2.1.6 **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 **Standard stock solution:**Weight and transfer about Eq. to 100 mg ofLevofloxacinin 50 ml volumetric flask add 20 ml diluent shake and sonicate for dissolve and make up with diluent.

9.2.2.3 **Standard solution:**Further dilute 5 ml to 50 ml with mobile phase. **(Standard solution 200-ppm).**

9.2.2.4 **Test solution stock:**Take 5 intact tablet in to 500 ml volumetric flask disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter.

9.2.2.5 **Test solution:** Further dilute 2 ml Test stock solution to 50 ml with mobile phase.

9.2.2.6 **Procedure:** Separately inject 25 µl one injection of blank and resolution, five injections of standard preparation followed by two injections of each sample preparation.

9.2.2.7 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

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

9.2.2.9 **Calculation:** Calculate the content of Levofloxacin(in mg): -

ANALYTICAL METHOD VALIDATION / VERIFICATION PROTOCOL FOR LIVOFLOXACIN TABLETS (FOR ASSAY)

$$\frac{At}{As} = \frac{Ws}{50} \times \frac{5}{50} \times \frac{500}{5} \times \frac{50}{2} \times \frac{361.95}{370.40} \times \frac{P}{100}$$

Where

A_T: Area of Levofloxacin peak from one injections of test preparation.

A_S: Average area of Levofloxacin Working Standard USP from five injections of standard Preparation.

W_S : Weight of Levofloxacin Working Standard USP in mg .

P : Potency of Levofloxacin 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 **BlankSolution:** As per specificity test.

9.2.3.2 **Standard stock solution:**Weight and transfer about Eq. to 100 mg ofLevofloxacinin 50 ml volumetric flask add 20 ml diluent shake and sonicate for dissolve and make up with diluent.

9.2.3.3 **Standard solution:**Further dilute 5 ml to 50 ml with mobile phase. **(Standard solution 200-ppm).**

9.2.3.4 **Test solution stock:**Take 5 intact tablet in to 500 ml volumetric flask disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter.

9.2.3.5 **Test solution:** Further dilute 2 ml Test stock solution to 50 ml with mobile phase.

9.2.3.6 **Procedure:** Separately inject 25 µl one injection of blank, five injections of standard preparation followed by two injections of each sample preparation.

9.2.3.7 **System Suitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.2.3.8 **Acceptance criteria:**

- Percentage RSD shall be Not more than 2.0 % for six results.
- Analyst 1 & analyst 2 results cumulativeRSD shall be Not more than 2.0 %.
- Analyst 1 & analyst 2 resultsCoefficient of determinationR^2should be greater than 0.99

9.2.3.9 **Calculation:** Calculate the content of Levofloxacin (in mg): -

$$\frac{At}{As} = \frac{Ws}{50} \times \frac{5}{50} \times \frac{500}{5} \times \frac{50}{2} \times \frac{361.95}{370.40} \times \frac{P}{100}$$

Where

A_T: Area of Levofloxacin peak from one injections of test preparation.

A_S: Average area of Levofloxacin Working Standard USP from five injections of standard Preparation.

W_S : Weight of Levofloxacin Working Standard USP in mg .

P : Potency of Levofloxacin 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 Solution:**As per specificity test.

9.3.2 **Standard stock solution:**Weight and transfer about Eq. to 200 mg ofLevofloxacinin 100 ml volumetric flask add 50 ml diluent shake and sonicate for dissolve and make up with diluent.

9.3.3 **Standard Solution:** Further transfer 10 ml of this solution in to a 100 ml volumetric flak, dilute with mobile phaseto 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 into a100 ml volumetric flak, dilute with mobile phase to volume, and mix.

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

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

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

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

9.3.5 **Procedure:** Separately inject25 µl one injection of blank, five injections of standard preparation followed by one injections of each concentration samplesolution.

9.3.6 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 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 25 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 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 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.

9.5.1.1 **Blank Solution:** As per specificity test.

9.5.1.2 **Standard stock solution:**Weight and transfer about Eq. to 100 mg ofLevofloxacinworking standard USPin 50 ml volumetric flask add 20 ml diluent shake and sonicate for dissolve and make up with diluent.

9.5.1.3 **Standard solution:**Further dilute 5 ml to 50 ml with mobile phase. **(Standard solution 200-ppm).**

9.5.1.4 **Test solution stock** Weight and transfer 700 mg placebo in to 500 ml volumetric flask disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter and further dilute 2 ml to 50 ml with mobile phase.

9.5.1.5 **Test solution:** Further dilute 2 ml Test stock solution to 50 ml with mobile phase.

9.5.2 **Recovery for 80 % level:**Transfer and weight a quantity of the powder about 700 mg of Placebo in to a 500-mL volumetric flask, and spiked Eq. to2.000 g of Levofloxacinworking standard USPadd about disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter and further dilute 2 ml to 50 ml with mobile phase.**(Test solution: 160 PPM).**

9.5.3 **Recovery for 100 % level:** Transfer and weight a quantity of the powder about 700 mg of Placebo in to a 500-mL volumetric flask, and spiked Eq. to2.500 g of Levofloxacinworking standard USPadd about disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter and further dilute 2 ml to 50 ml with mobile phase.**(Test solution: 200 PPM).**

9.5.4 **Recovery for 120 % level:** Transfer and weight a quantity of the powder about 700 mg of Placebo in to a 500-mL volumetric flask, and spiked Eq. to3.000 g of Levofloxacinworking standard USPadd about disperse in 375 ml diluent allow to stand for 15 min shake for 30 min and make up with diluent than filter with 0.45 µ membrane filter and further dilute 2 ml to 50 ml with mobile phase.**(Test solution: 240 PPM).**

9.5.5 **Procedure:** Separately inject 25 µl one injection of blank, five injections of standard preparation followed by triplicate injections of each sample solution.

9.5.6 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

9.5.7 **Acceptance criteria:**

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

9.5.7.2 Percentage RSD shall be Not more than 2.0 %.

9.5.8 **Calculation:**

Recovery in (mg):

$$\text{At} \times \frac{\text{Ws}}{\text{As}} \times \frac{5}{50} \times \frac{500}{1} \times \frac{50}{2} \times \frac{361.95}{370.40} \times \frac{P}{100} \times 1$$

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 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 25 µl one injection of blank, five injections of fresh standard, one injection ofinitial standardand preparation followed by two injections of sample solution.

9.7.6 **SystemSuitability:**The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 %.

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 Levofloxacin (in mg): -

$$\frac{A_T}{A_S} \times \frac{W_S}{50} \times \frac{5}{50} \times \frac{500}{5} \times \frac{50}{2} \times \frac{361.95}{370.40} \times \frac{P}{100}$$

Where

A_T:Average area of LevofloxacinUSPpeak from two injections of test .

A_S: Average area of LevofloxacinUSPpeak from five injections of standard .

W_S : Weight of LevofloxacinUSPworking standard in mg.

P : Potency of LevofloxacinUSPworking 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 SOP No – .....

9.12.2 USP / ICH/ IHS.

9.13 Abbreviations:

QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
Sr.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
%	Percent

9.14 Revision History:

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