Analytical Method Validation Report

Levofloxacin Tablets 500 mg

This document is an exercise on Analytical Method Validation of the various analytical Methods used in determination of active ingredients in Quality Control Laboratory

CONTRIBUTIONS:
This protocol is a team effort of Quality control Laboratory chemists to achieve the objective of validating the analytical methods carried out to estimate the contents of pharmaceutical products manufactured.

Analytical Method Validation Protocol Number			
Validation Frequency	Analytical Methods should be validated during and at the end of development process and after any significant change in analytical method.		
	Designation	**Name of the Person**	**Sign /Date**
Prepared By	Officer QC		
Checked By	Manager QC		
Reviewed By	Manager QA		
Approved By	Operation Head		

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

What is Validation?

Validation is the evaluating of processes, products or analytical methods to ensure compliance with product or method requirements. One of the most popular definitions of Validation came from the 'US FDA' General Principle of Validation **"Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes."**

The term Validation & Qualification are often mixed up and there is also some overlap. Equipment Qualification means checking an instrument for compliance with previously defined functional and performance specifications. For Operational Qualification generic standards and analytical conditions are used rather than real sample conditions. Validation relates more to the entire but sample specific process including sample preparation, analysis, and data evaluation.

Validation efforts in the analytical laboratory should be broken down into separate components addressing the equipment and the analytical methods run on that equipment. After these have been verified separately they should be checked together to confirm expected performance limits **(System-Suitability Testing),** and finally the sample analysis data collected on such a system should be authenticated with suitable validation checkouts. All methods / equipment that are used to create, modify, maintain, archive or distribute critical data for cGMP/GLP.

Analytical method should be validated prior to routine use and after changing method parameters.
Peoples involved in Validation exercise should be qualified for their jobs. This includes education, training and/or experience.
Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.
Typical analytical performances characteristics that should be considered in the validation of the types of methods are as follows.

- **Accuracy**
- **Precision**
- **Specificity**
- **Detection Limit**
- **Quantitation Limit**
- **Linearity**
- **Range**
- **Robustness**

USP 30 in "(1225) Validation of compendial procedures" says Category I (Analytical methods for Quantization of major components of bulk drug substances or active ingredients including preservative in finished pharmaceutical products) should comply with **Accuracy, Precision, Specificity, Linearity, Robustness, & Range.**

However after discussions with many experts & referring some of the IDAM – APA magazines, we have decided to at least comply with **Accuracy, Linearity, Precision, and Robustness.**

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Validation Report

Once the method has been validated, a validation report should be prepared that includes.

- Objective & scope of the method (applicability, type).
- Summary of the methodology.
- Type of compound & matrix.
- All chemical, reagents, reference standards, detailed instruction on their preparation.
- Method parameters.
- Detailed condition on how the experiments were conducted including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.
- Statistical procedures & representative calculations.
- Representative plots
- Performance data for acceptance limit
- Criteria for revalidation
- Summary & conclusions
- Approval with name, designations, date & signatures of those responsible for the review & approval of the analytical test procedure.

Validation Report For Levofloxacin Tablets

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE: The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

Product Name	Levofloxacin Tablet**.**
Ingredient	Levofloxacin Hemihydrate eq. to Levofloxacin
Label Claim	Each film coated tablet contains Levofloxacin Hemihydrate eq. to Levofloxacin 500 mg
Test Method	Liquid Chromatography.

Specificity (**Diluents Interference**)

Placebo Preparation: A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the mobile phase is used as the placebo solution. Area at 274 nm, Observation Result: Nil

Conclusion for Specificity: We observed that at wavelength 274 nm there is no significant area for placebo (Diluents) for Levofloxacin tablets assay method. Therefore specificity of the method considered acceptable.

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System Accuracy

The system precision of the above method was carried out by taking area for six times of the sample preparation of exact weight.

Serial No.	Area of Levofloxacin
1.	5902564.8
2.	5914567.8
3.	5934550.5
4.	5924560.2
5.	5922568.7
6.	5917893.4
Mean	5919762.4
RSD	**0.20%**

Acceptance Criteria: RSD is not more than 2.0%.

Linearity/ Accuracy:

Definition: The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition: The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

Limit: Levofloxacin Tablet.

Label Claim 500 mg/tablet
(Limit: 90.0 % to 110.0 % of the labeled amount).

Chromatographic System

Note: Use freshly prepared solutions and carry out the test protected from light.

- a stainless steel column 25cm x 4mm packed with octadecylsilane bonded to porous silica (5µm).
- Mobile phase: a mixture of 85 volume of buffer solution prepared by dissolving 84 volumes of 0.05M citric acid monohydrate and 1 volume of 1M ammonium acetate, filter and 15 volumes of acetonitrile
- Flow rate: 1ml per minute
- Spectrophotometer set at 293nm

- 10 µl loop injector

Standard Solution: Take 100mg of Levofloxacin hemihydrate RS and dissolve in 100ml of 0.1M hydrochloric acid. Dilute 5ml of the solution to 10ml with water.

Test Solution: Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100mg of Levofloxacin. Disperse in 100ml of 0.1M hydrochloric acid and filter. Dilute 5ml of the solution to 10ml with water.

Chromatographic system: Separately inject equal volumes of the standard solution and sample solution and measure the responses of the major peaks and calculate the content of Levofloxacin.

Procedure: weigh accurately powdered tablet .and prepare the dilution as above mentioned separately of WS and sample. Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Levofloxacin by the formula given below

$$\frac{\text{Sample area} \times \text{WS weight} \times \text{potency of WS} \times \text{Average Weight} \times 100}{\text{Standard area} \times \text{Sample weight} \times 100 \times \text{claim}} = \quad \%$$

S. no.	Standards	Area
	Standard-1	5856795.6
	Standard-2	5867586.4
	Standard-3	5857452.6
	Standard-4	5847921.5
	Standard-5	5854235.1
	Standard-6	5846541.3
	Mean	5856798.2
	RSD	**0.12%**

Samples	Sample Area	Mean
Sample-A-01 70 mcg	821564.2	821861.9
Sample-A-02 70 mcg	822564.8	
Sample-A-03 70 mcg	821456.7	
Sample-B-01 80 mcg	940561.5	941134.1
Sample-B-02 80 mcg	941567.2	
Sample-B-03 80 mcg	941273.5	
Sample-C-01 90 mcg	1094562.2	1095598.1
Sample-C-02 90 mcg	1102475.8	
Sample-C-03 90 mcg	1089756.2	
Sample-D-01 100 mcg	1204578.9	1202323.7
Sample-D-02 100 mcg	1197824.6	
Sample-D-03 100 mcg	1204567.8	

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

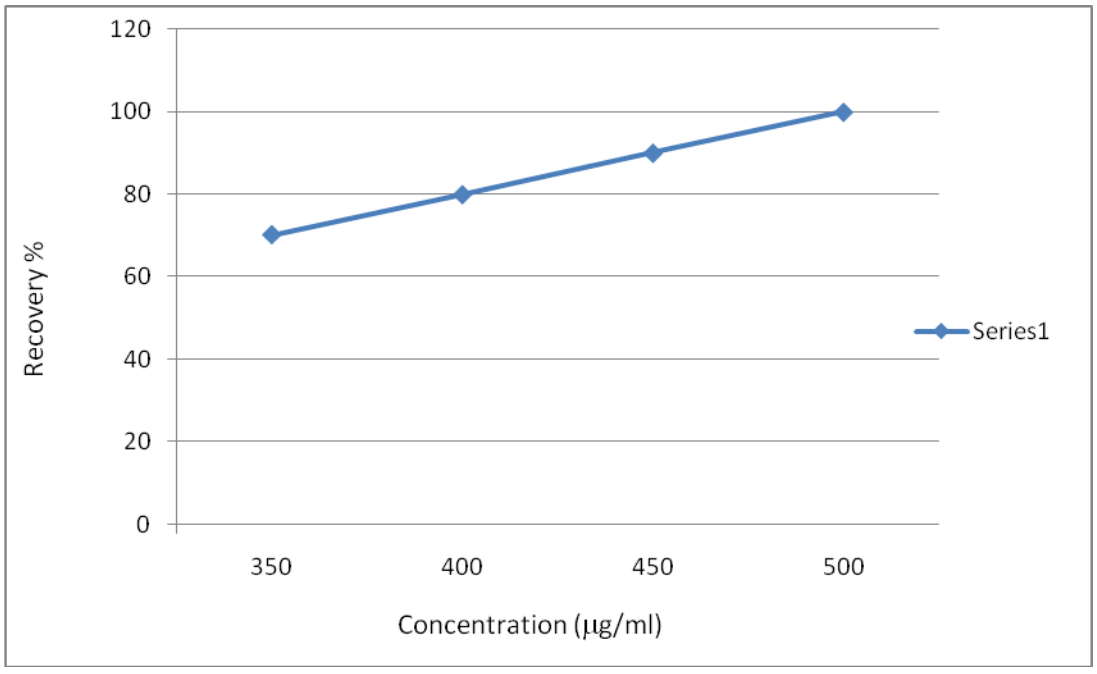
Data Collection:

Concentration (µg/ml)	Concentration in %	Sample Mean Area	Recovery%
350	70%	821861.9	70.10%
400	80%	941134.1	79.85%
450	90%	1095598.1	89.95%
500	100%	1202323.7	99.90%

From the above results, draw a curve.

Linearity plot for Levofloxacin tablets

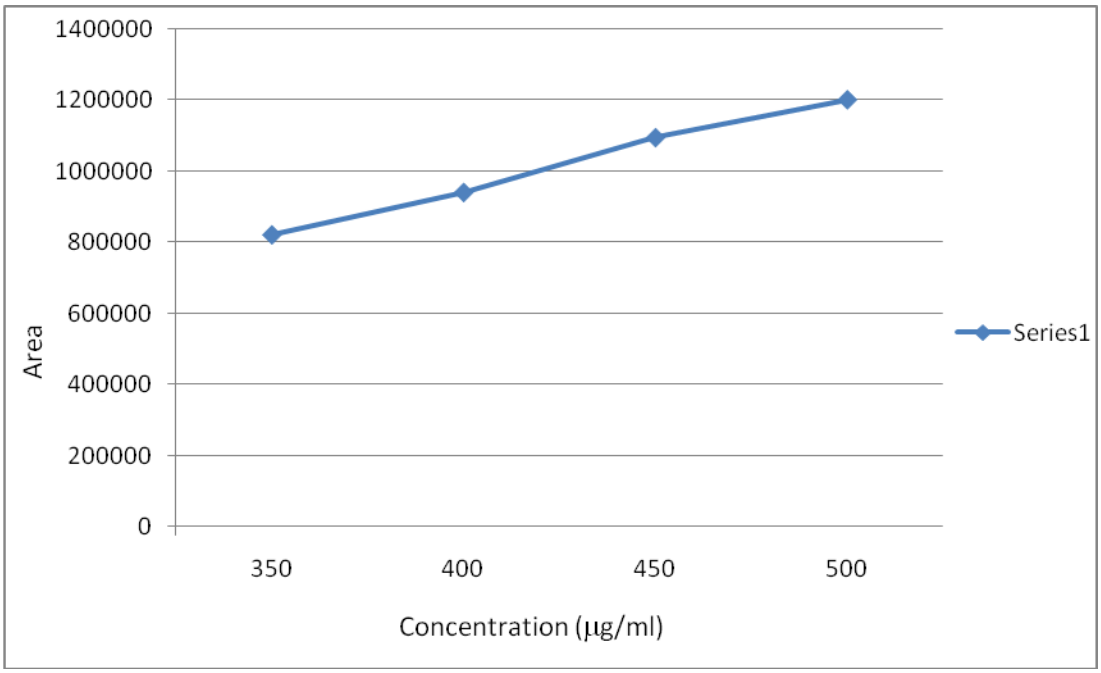
Concentration (µg/ml)	Recovery %
350	70.10
400	79.85
450	89.95
500	99.90



Concentration (µg/ml) Area

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

350	821861.9
400	941134.1
450	1095598.1
500	1202323.7



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R-squared value ($R^{2)}$

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

$y = m x + b$

Where m is the slope and b is the intercept.

x = concentration

y = Area Value

Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x

b ⟶ 0.163

We can arrive sample concentration from the above equation is 100 mcg

$$\frac{\text{Sample area X WS weight X potency of WS X Average Weight X 100}}{\text{Standard area X Sample weight X 100 X claim}}$$

= %

Conclusion for Linearity: The graphical representation & data collected during this exercise proves Levofloxacin Tablet for demonstrate linearity in the range of 70% to 100% when determined by Liquid Chromatographic method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision

Levofloxacin Tablet**.**

Label Claim 500 mg/tablet (Limit: 90.0 % to 110.0 % of the labeled amount).

Chromatographic System

Note: Use freshly prepared solutions and carry out the test protected from light.

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ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

- Mobile phase: a mixture of 85 volume of buffer solution prepared by dissolving 84 volumes of 0.05M citric acid monohydrate and 1 volume of 1M ammonium acetate, filter and 15 volumes of acetonitrile
- Flow rate: 1ml per minute
- Spectrophotometer set at 293nm
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Procedure: weigh accurately powdered tablet .and prepare the dilution as above mentioned separately of WS and sample. Inject in HPLC and collect the DATA from received chromatogram from HPLC and calculate quantity of Levofloxacin by the formula given below

$$\frac{\text{Sample area} \times \text{WS weight} \times \text{potency of WS} \times \text{Average Weight} \times 100}{\text{Standard area} \times \text{Sample weight} \times 100 \times \text{claim}} = \quad \%$$

Standard Wt. taken: Take 100mg of Levofloxacin hemihydrate RS and dissolve in 100ml of 0.1M hydrochloric acid. Dilute 5ml of the solution to 10ml with water.

Sample Dilutions: By "……"

Sample A 152.1 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample B 152.2 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample C 152.3 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample D 152.2 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

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Sample E 152.4 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample F 152.2 mg of sample, disperse in 100 ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Test Data Collection

S.no.	Standards	Area
1.	Standard-1	5856795.6
2.	Standard-2	5867586.4
3.	Standard-3	5857452.6
4.	Standard-4	5847921.5
5.	Standard-5	5854235.1
6.	Standard-6	5846541.3
	Mean	5856798.2
	RSD	**0.12%**

Samples		Sample Area	Mean
Sample A	T1	5914578.6	5909426.6
	T2	5904274.6	
Sample B	T1	5920456.7	5917565.5
	T2	5914674.3	
Sample C	T1	5923456.1	5919065.3
	T2	5914674.5	
Sample D	T1	5914456.2	5916167.0
	T2	5917897.8	
Sample E	T1	5907892.4	5910174.5
	T2	5912456.7	
Sample F	T1	5924562.4	5926227.5
	T2	5927892.6	

Estimated Amount

- Assay on % of Theory for sample A---- 99.91%
- Assay on % of Theory for sample B---- 100.08%
- Assay on % of Theory for sample C-----100.25%
- Assay on % of Theory for sample D-----100.02%
- Assay on % of Theory for sample E-----99.88%
- Assay on % of Theory for sample F-----100.35%

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Table for Six Replicate Assays:

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.91%	100.02%	0.15%
Sample B	100.08%		
Sample C	100.25%		
Sample D	100.02%		
Sample E	99.88%		
Sample F	100.35%		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Conclusion for precision: The overall % Relative standard deviation for Levofloxacin tablets there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision – (Within laboratory variations such as different days, analyst & equipments):

Analyst: "……."

Standard Wt. taken: Take 100mg of Levofloxacin hemihydrate RS and dissolve in 100ml of 0.1M hydrochloric acid. Dilute 5ml of the solution to 10ml with water.

Sample A 152.1 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample B 152.0 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample C 152.1 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample D 152.0 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample E 152.1 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

Sample F 152.0 mg of sample, disperse in 100ml of 0.1M hydrochloric acid, sonicate to dissolve and make up to 100 ml and filter. Dilute 5ml of the solution to 10ml with water.

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

Standards	Area
Standard 1	5866794.2
Standard 2	5864231.2
Standard 3	5854781.4
Standard 4	5845672.5
Standard 5	5854758.5
Standard 6	5844674.2
Mean	5857247.5
RSD	**0.14%**

Samples		Sample Area	Mean
Sample A	T1	5904567.2	5908457.3
	T2	5912347.5	
Sample B	T1	5920412.5	5918018.0
	T2	5915623.5	
Sample C	T1	5927894.5	5921326.6
	T2	5914758.8	
Sample D	T1	5916645.2	5915611.0
	T2	5914576.8	
Sample E	T1	5907892.4	5911228.5
	T2	5914564.5	
Sample F	T1	5924567.8	5926166.3
	T2	5927764.8	

Calculation:
Levofloxacin Content

$$\frac{\text{Sample area X WS weight X potency of WS X Average Weight X 100}}{\text{Standard area X Sample weight X 100 X claim}} = \%$$

Estimated Amount analyst by "……."

- Assay on % of Theory for sample A ----99.99%
- Assay on % of Theory for sample B ----100.15%
- Assay on % of Theory for sample C ----100.45%
- Assay on % of Theory for sample D ----100.05%
- Assay on % of Theory for sample E ----100.12%
- Assay on % of Theory for sample F -----100.56%

Test Data analyst by "……"

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.99%	100.15%	0.17%
Sample B	100.15%		
Sample C	100.45%		
Sample D	100.05%		
Sample E	100.12%		
Sample F	100.56%		

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

Test Data analyst by "……"

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.91%	100.02%	0.15%
Sample B	100.08%		
Sample C	100.25%		
Sample D	100.02%		
Sample E	99.88%		
Sample F	100.35%		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.17% & 0.15% Levofloxacin tablets there is no significant difference between two analysts Within laboratory variations such as different days, analyst & equipments.
Therefore reproducibility of the method considered to be acceptable.

2 Robustness:
To demonstrate the analytical method is capable to yield reproducibility results under;
Small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.
- Change in Flow rate of mobile phase. Record the observation in below observation table.

OBSERVATION TABLE:

Change ratio in the mobile phase					
Mobile phase		Flow rate ml/min	System suitability		
Water	Methanol		Retention time	Theoretical plate	Tailing Factor
848 ml	152 ml	1.0 ml/min.	7.27	2568	1.5
850 ml	150 ml	1.0 ml/min.	7.29	2530	1.5
852 ml	148 ml	1.0 ml/min.	7.31	2598	1.4

Change in flow rate

Change in flow rate at 274 nm				
Mobile phase		System Suitability		
Ratio of Mobile Phase (Water: Methanol)	Change in flow rate	Retention Time	Theoretical Plates	Tailing Factor
850:150	0.8 ml/min.	8.95	2610	1.5
850:150	1.0 ml/min.	7.29	2540	1.5
850:150	1.2 ml/min.	6.15	2605	1.4

ANALYTICAL METHOD VALIDATION REPORT FOR LEVOFLOXACIN TABLETS 500 mg

Acceptance criteria:
Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 1000 & tailing factor is not more than 2.0).

Conclusion for Robustness:
There is no significant difference for Levofloxacin tablets for different conditions**,** such as composition & Flow rate of mobile phase.
Therefore Robustness of the method considered acceptable.

CONCLUSION:
All the analytical parameter are checked as per the approved validation process and found well within specified acceptance criteria.Hence,It is concluded that ,this method is suitable for accurate & precise results for routine analysis.