Int J Pharm Bio Sci 2013 Jan; 4(1): (P)102 - 120

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

DEVELOPMENT OF SOME NEW AND SENSITIVE ANALYTICAL METHOD FOR THE ESTIMATION AND VALIDATION OF LEVOFLOXACIN BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

YEDLA NAGARAJ*

Department of Pharmaceutics, MLR Institute of Pharmacy, Hyderabad, India.

ABSTRACT

A Rapid, precise, accurate, specific and simple RP-HPLC method was developed for determination of Levofloxacin in pharmaceutical formulation. The presented method is simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. A RP-HPLC assay utilized. A HPLC assay utilized symmetry C – 18 (4.6× 150mm, 5 μm), with mobile phase composition of Acetonitrile: Potassium dihydrogen orthophosphate [60:40] of pH 3 was used, and flow rate was 0.7 mL min^{-1} with UV detection at 295 nm. The retention time Levofloxacin of was 2.448 min. The total HPLC run time was less than 5 min. Linearity was observed over concentration range of 20-60 µg/ml for Levofloxacin. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability. Commercial tablet formulations and laboratory prepared dilutions were successfully analyzed using the developed methods.

KEYWORDS: **Levofloxacin, Retention time, Linearity and Run time**

YEDLA NAGARAJ
Department of Pharmaceutics, MLR Institute of Pharmacy, Hyderabad, India.
nagarajzerik@gmail.com

Corresponding author

INTRODUCTION

High Performance Liquid Chromatography (HPLC)[1-2]

The modern form of column chromatography called high performance, high pressure, high resolution and high speed liquid chromatography. The essential equipment consists of an eluent, reservoir, a high pressure pump, and an injector for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures. The systems used are often described as belonging to one of four mechanistic types, absorption, partition, ion exchange and size exclusion. Adsorption chromatography arises from interaction between solutes on the surface of the solid stationary phase. Partition chromatography involves a liquid stationary phase, which is immiscible with the eluent and coated on an inert support. Adsorption and partition systems can be normal phase (stationary phase more polar than eluent) or reversed phase (stationary phase less polar than eluent) ion exchange chromatography involves a solid stationary phase with anionic or cationic groups on the surface to which solute molecules of opposite charges are attached. Size exclusion chromatography involves a solid stationary phase with controlled pore size. Solutes are separated according to their molecular size, the large molecules enable to enter the pores eluting first.

Reversed phase chromatography[3]

In 1960s chromatographers started modifying the polar nature of silonol group by chemically reacting silica with organic silanes, the object was to make silica less polar or non-polar so that polar solvents can be used to separate water- soluble polar compounds. Since the ionic nature of the chemically modified silica is now reversed i.e., It is non polar or the nature of the phase is reversed. The chromatographic separation is carried out with silica is referred to as reversed-phase chromatography. A large no of chemically bonded stationary phases based on silica are available commercially table list some of the functional groups bonded in chemically modifies silica. Silica based stationary phases are still most popular in reversed phase chromatography however higher adsorbents based on polymer (styrene-divinyl benzenes polymer) are slowly gaining ground. Simple compounds are better retained by the reversed phase surface, the less water soluble (i.e., the more non polar) they are. The retention decreases in the following order; aliphatic > induced dipoles (eg.CCl4) < permanent dipoles (eg. $CHCl_3$) > weak Lewis bases (eg.ethers, aldehydes, ketones) > strong Lewis bases (amines) > weal Lewis acids (alcohols, phenols)> strong Lewis acids (carboxylic acids) also the retention increases as the no of carbon atoms increases.

In reversed phase systems the strong attractive forces between water molecules arising from the 3-dimensional intermolecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non-polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phaseschemically bonded octadecyl silane (ODS) an alkane with 18 carbon atoms is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations used ODS HPLC columns. The solvent strength in RP HPLC is reversed

from that of adsorption chromatography[4,5] (Silica gel) as stared earlier. Water interacts strongly with silonal group, so that; adsorption of sample molecules becomes highly restricted and rapidly eluted as a result. Exactly oppositely applies in reversed phase systems, water cannot wet the non-polar (hydrophobic) alkali groups such as c-18.of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and it gives slowest elution rate. The elution time (retention time) in reversed phase chromatography increases with increase in the amount of water in the mobile phase.

MATERIALS AND METHODS

Equipments and apparatus

The Waters HPLC system consisting of PDU detector, Column C-18 (2) (4.6 x 150 mm, 5 μm) with UV detection at 295 nm. Analytical weighing balance (AFCOSET, ER-200A series) was used for weighing, Sonicator (Ultra Sonic Cleaner, pH meter (Adwn Series: AD 1020), Millipore filtration kit for solvents and sample filtration were used throughout the experiment. The Empower2 software Quadra channel was used for acquisition, evaluation and storage of chromatographic data

Reagents and Pharmaceutical Preparations

Levofloxacin was kindly gifted by Dr. Reddy Labs, Hyderabad, India certified to contain between 98.5% and 101.00% purity. The drugs are used without further purification.

Collection of solvents

HPLC grade Methanol, Ortho phosphoric acid, Acetonitrile and water was used as solvent throughout the experiment and all of them are of MERCK products.

Chromatographic condition

The following RP-HPLC condition was maintained throughout the experiment.

Buffer

A solution of 7 gm of Potassium Dihydrogen Phosphate is dissolved in 100 ml of HPLC grade water, mix well by using sonicator, and make up the volume to 1000 ml with water. pH of the resulting solution was adjusted to 3.0. The above solution was filtered through a 0.45 μm pore size nylon filter and degassed by ultrasonicator. RP-HPLC experiments were carried out using binary pump. In one solvent reservoir methanol and in another reservoir pH 3.0 was taken.

Recording the Chromatogram

The Empower2 software- Quadra channel was used for acquisition, evaluation and storage of chromatographic data with the following Details.
Retention time (RT), peak area, peak height, percentage area, assigning the name to the Peaks.

Preparation of standard Levofloxacin solution

Accurately weighed 10 mg of pure drug was taken in clean, dry 10 ml volumetric flask and dissolved in 7ml of mobile phase and sonicate to dissolve it completely and made up the volume to 10 ml with mobile phase. This gave 1000 μg/mlof drug concentration. Further 0.4 ml of above solution was pipetted out into 10 ml volumetric flask and volume was made up to 10 ml with mobile phase. Mix well and filter through 0.45μm filter. This solution gave 80 μg/ml concentration.

Pharmaceutical Preparations:

A total of 5 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 10 mg of Levofloxacin was taken and dissolved in 7 ml of mobile phase and sonicate for five minutes. About 2 ml of mobile phase was added and sonicate for further 5 minutes. The mixture was shaken well for 2 minutes and transferred to a 10 ml

volumetric flask through a 0.45 filter The residue was washed thrice with mobile phase and the combined filtrate was made up to the mark with mobile phase. This gave 1000 μg/ml of drug concentration. Further 0.4 ml of above solution was pipetted out into 10 ml volumetric flask and volume was made up to 10 ml with mobile phase. This solution gave 20 μg/ml concentration.

Buffer preparation

Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 3.0 with Orthophosphoric acid

Mobile phase

Acetonitrile + Buffer (60:40)

Determination of Levofloxacin by Reverse Phase High Performance Liquid Chromatography (RP-HPLC)[6,7]:

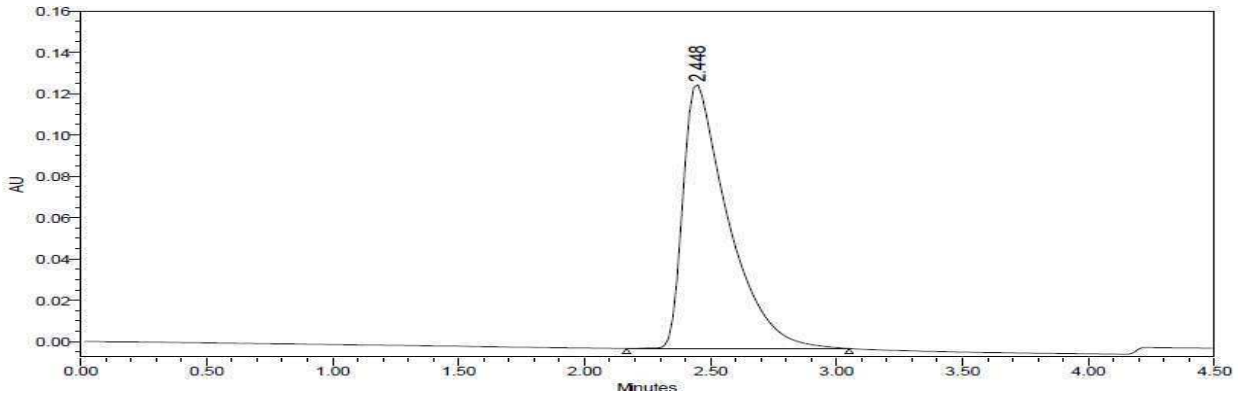
A Rapid, precise, accurate, specific and simple RP-HPLC method was developed for determination of Levofloxacin in pharmaceutical formulation. The presented method is simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. A RP-HPLC assay utilized Symmetry C-18 (4.6 x 150 mm, 5 μm), with mobile phase composition of Acetonitrile: Potassium dihydrogen orthophosphate [60:40] of pH 3 was used, and flow rate was 0.7 ml min^{-1} with UV detection at 295 nm.. The retention time Levofloxacin of was 2.448min. The total RP-HPLC running time was less than 5 min. Linearity was observed over concentration range of 20-60 μg/ml for Levofloxacin. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability. Commercial tablet formulations and laboratory prepared dilutions were successfully analyzed using the developed methods.

CHROMATOGRAPHIC CONDITIONS

Flow rate	**:**	0.7ml/min
Column	**:**	C-18 (4.6 x 150mm, 5μm)
Detector	**:**	Photo Diode Array detector
Detector wave length	**:**	295nm
Column temperature	**:**	Ambient
Injection volume	**:**	20μl
Run time	**:**	5 minutes
Retention Time	**:**	2.448 min
Diluents	**:**	Acetonitrile: pH 3.0 Buffer (60:40)

METHOD VALIDATION [8]

Levofloxacin Method Trails
Trail 1



S. No	Retention time	Area	height	USP plate count	USP tailing
1	2.448	1592117	127768	886.2	2.0

1) SPECIFICITY
Levofloxacin Identification
Solutions of standard and Sample are prepared as per test method and injected into the chromatographic system.

Acceptance criteria
Chromatogram of Standard and sample should be identical with near retention time.

Figure 1
Chromatogram of Levofloxacin standard

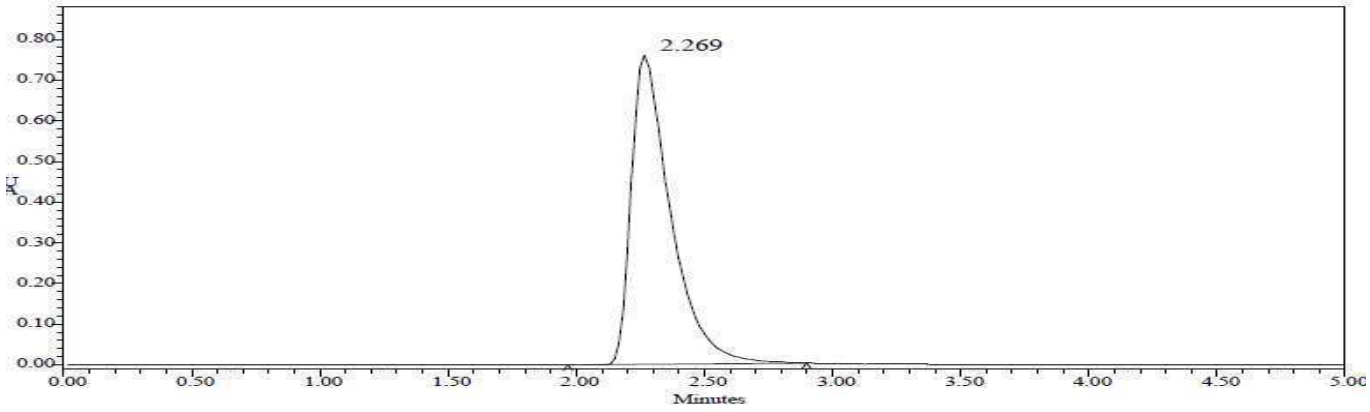
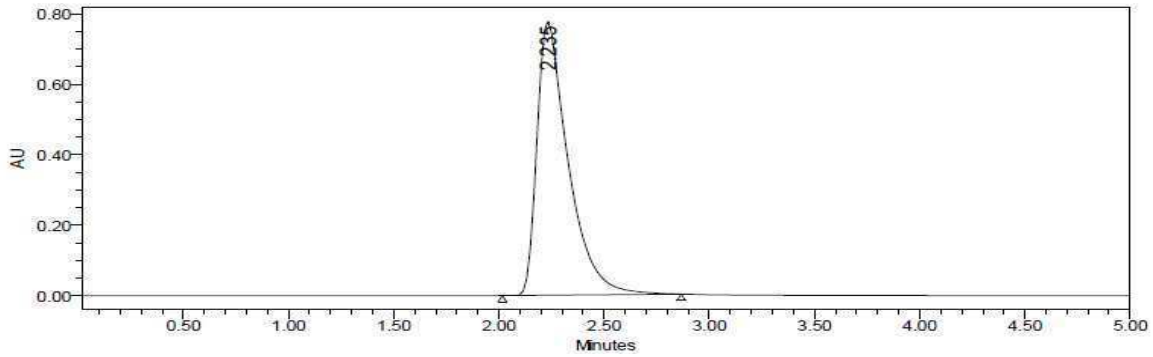


Figure 2
Chromatogram of Levofloxacin sample



2) ACCURACY (RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed as per test method with equivalent amount of into each volumetric flask for each spike level to get the concentration of Levofloxacin equivalent to 80%, 100%, and 120% of the labeled amount as per the test method. The average % recovery of Levofloxacin was calculated. Separately inject the individual concentrations in to the chromatograph.The mean % recovery of the Levofloxacin at each level should be not less than 98.5.0% and not more than 101.0%.

Figure 3
Chromatogram of Levofloxacin for accuracy studies (50 µg/ml)

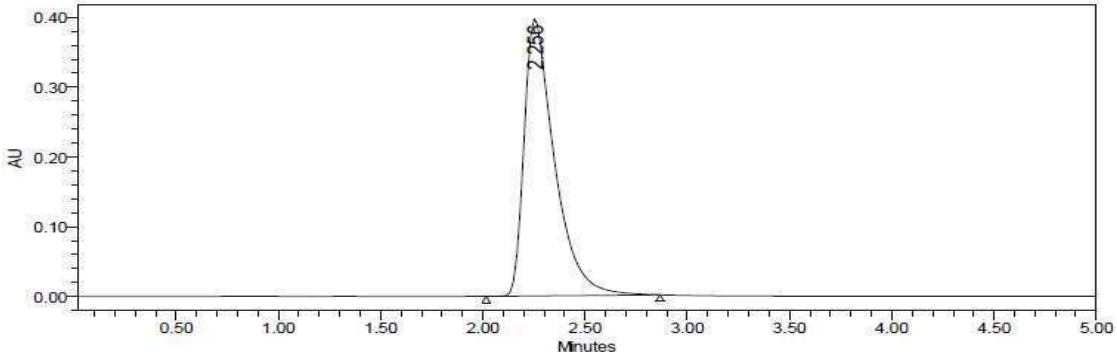


Figure 4
Chromatogram of Levofloxacin for accuracy studies (100 µg/ml)

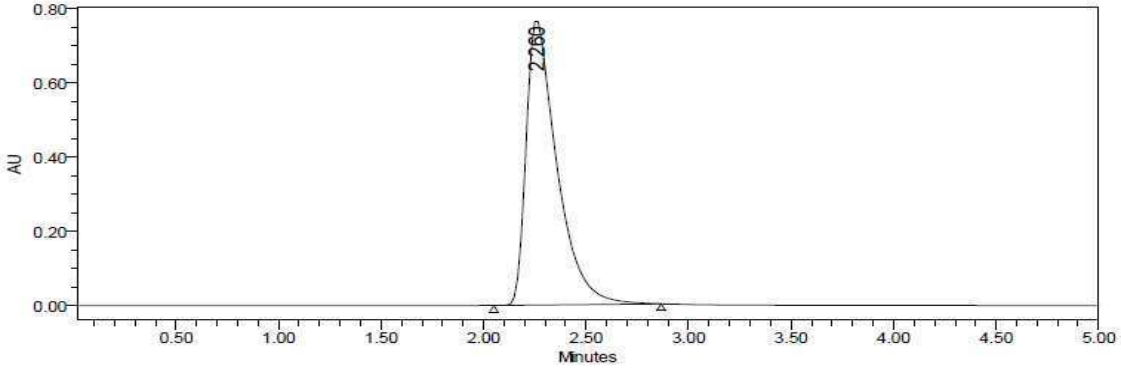
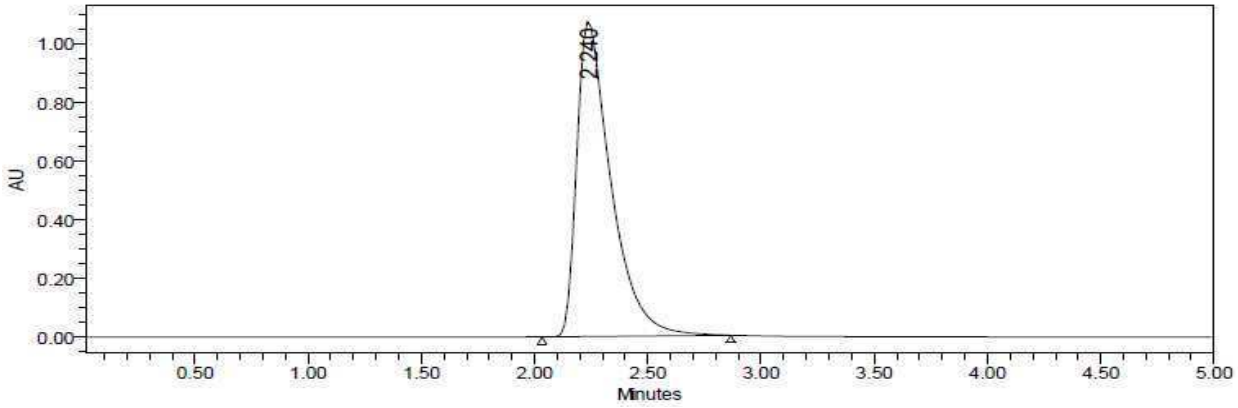


Figure 5
Chromatogram of Levofloxacin for accuracy studies (150 µg/ml)



3. PRECISION

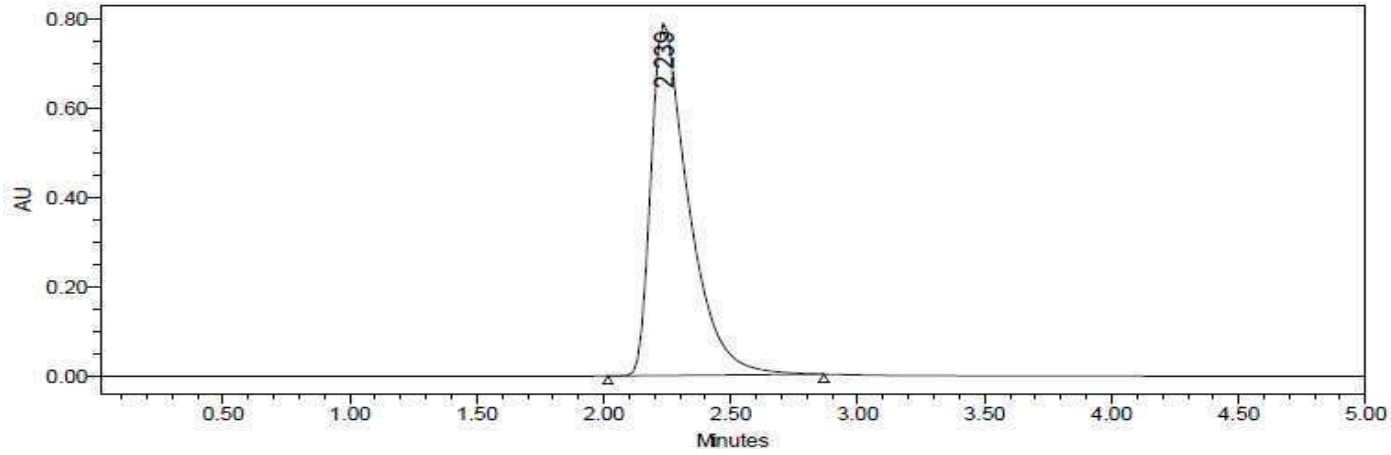
System precision

Standard solution prepared as per test method and injected 5 times.

Method precision

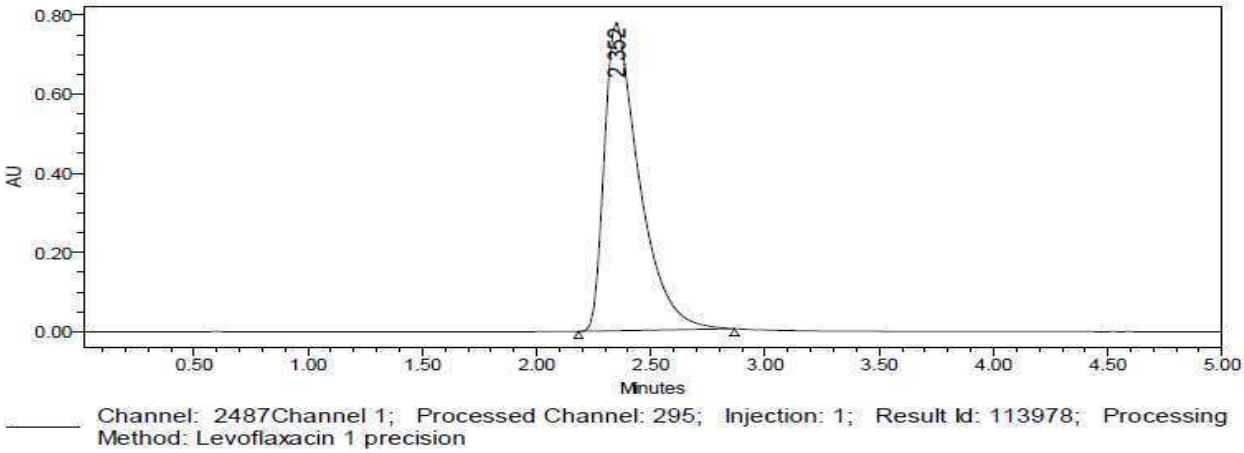
Prepared five sample preparations individually using single batch of tablets (250mg) as per test method and injected each solution.

Figure 6
Chromatogram of Levofloxacin for precision studies



3.1 METHOID PRECISION

Figure 11
Chromatogram of Levofloxacin for precision studies (40 µg/ml)



ASSAY
Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Levofloxacin obtained with test preparation
AS = Peak Area of Levofloxacin obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Figure 16
Assay of Sample

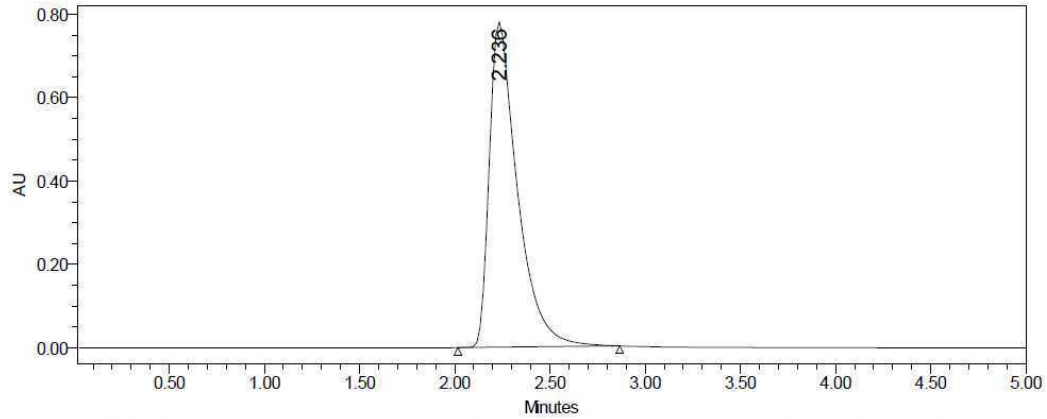
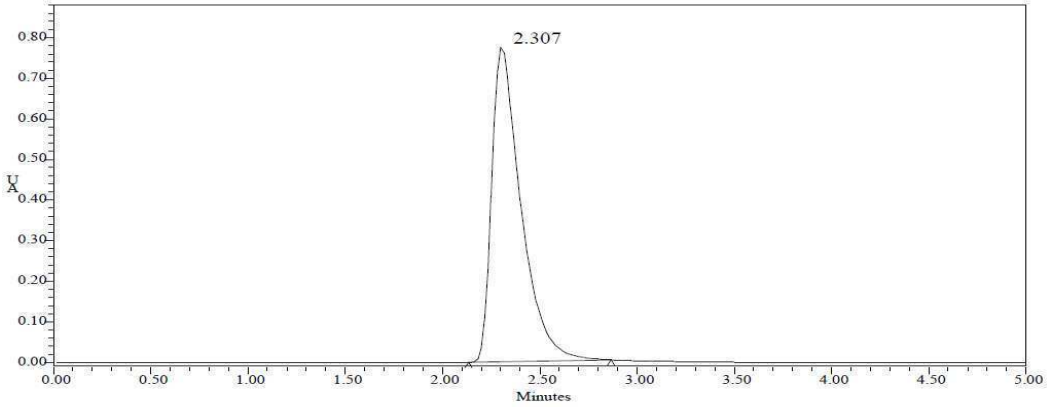
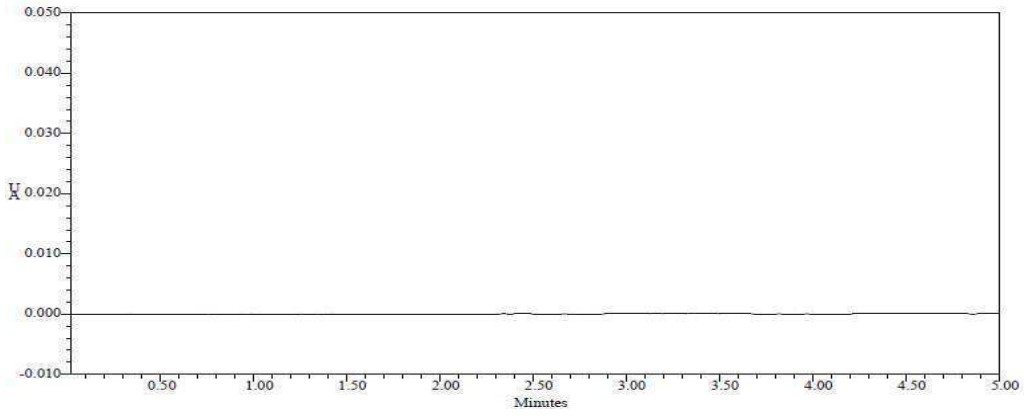


Figure 17
Assay of standard 1



Figure; 19
Assay of Blank



S. No	Baseline noise
1	0.048

4. LINEARITY

Preparation of stock solution

Accurately weigh and transfer 10mg of Levoflaxacin API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20µg/ml) 0.2ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (30µg/ml) 0.3ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (40µg/ml) 0.4ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (50µg/ml) 0.5ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (60µg/ml) 0.6ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

PROCEDURE

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Linearity Results

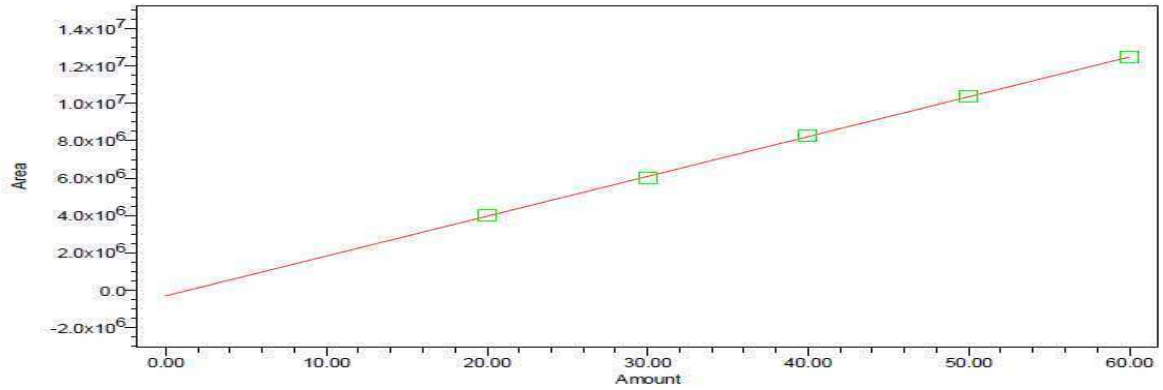
S. No	Linearity Level	Concentration	Area
1	I	20µg/ml	3990640
2	II	30µg/ml	6027684
3	III	40µg/ml	8250070
4	IV	50µg/ml	33247570
5	V	60µg/ml	12472251
Correlation Coefficient			0.999

Acceptance Criteria

Correlation coefficient should be not less than 0.999.

CALIBRATION CURVE

Figure 20
Linearity of Levofloxacin



S.No	Conc in µg/ml	Response
1	20	3990640
2	30	6027684
3	40	8250070
4	50	10380756
5	60	12472251

5 LIMIT OF DETECTION

Preparation of 40µg/ml solution

Accurately weigh and transfer 10mg of Levoflaxacin Working standard into a 10 mL Volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

Preparation of 0.18% solution At Specification level (0.007µg/ml solution)

Acceptance Criteria

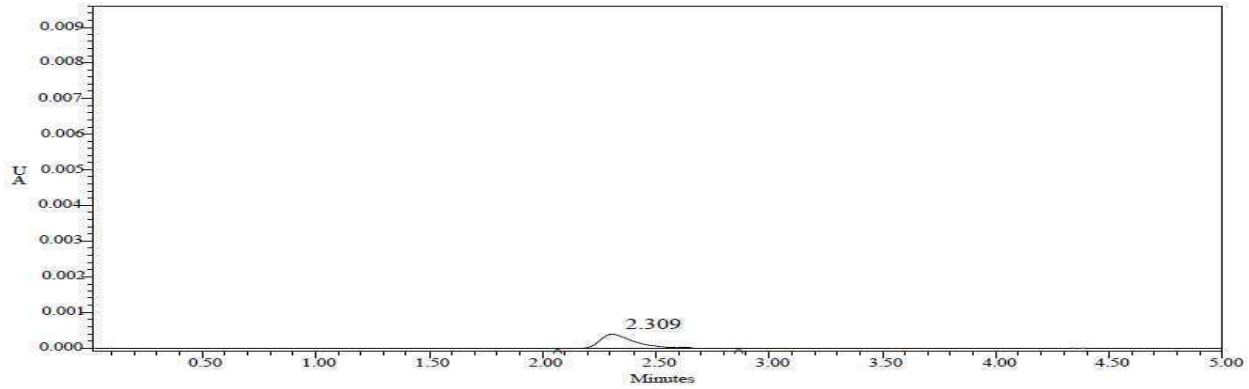
S/N Ratio value shall be 3 for LOD solution.

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.185mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank
Signal Obtained from LOD solution (0.18% of target assay concentration)
S/N = 142/48= 2.95

Figure 25
Chromatogram of LOD



S.NO	Retention Time	Area	Height
1	2.309	1454	142

6. LIMIT OF QUANTIFICATION

Preparation of 40µg/ml solution

Accurately weigh and transfer 10mg of Levoflaxacin Working standard into a 10 ml Volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of 0.5% solution At Specification level (0.02µg/ml solution)

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.Further pipette 0.5mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

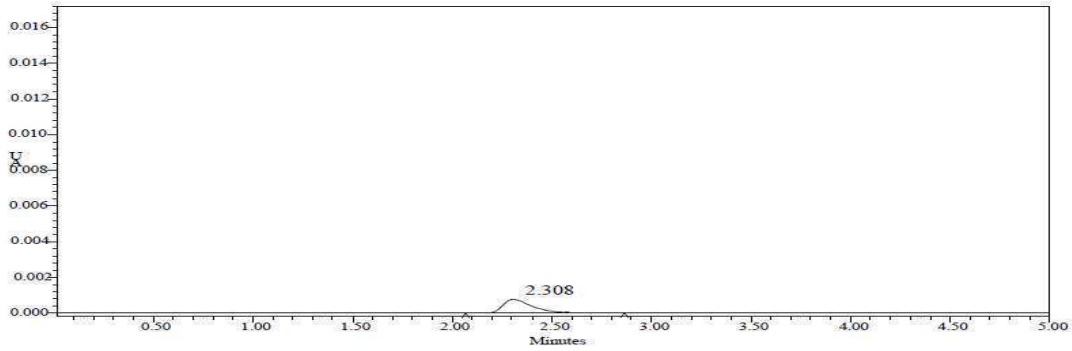
Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 48µV
Signal Obtained from LOD solution (0.5% of target assay concentration) : 492 µV
S/N = 492/48 = 10.25

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ solution.

Figure 26
Chromatogram of LOQ



S.NO	Retention Time	Area	Height
1	2.308	5038	492

7. ROBUSTNESS

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.6 to0.8ml/min.

Standard solution 40 µg/ml was prepared and analysed using the varied flow rates along with method flow rate. The results are summarizedon evaluation of the above results, it can be concluded that the variation in flow rate do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

		System Suitability Results	
S.No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.6	2269.0	1.8
2	0.7	2088.3	1.8

**** Results for actual flow (0.7 ml/min) have been considered from Assay standard.***

Figure 27
Chromatogram of Robustness (flow rate at 0.6ml)

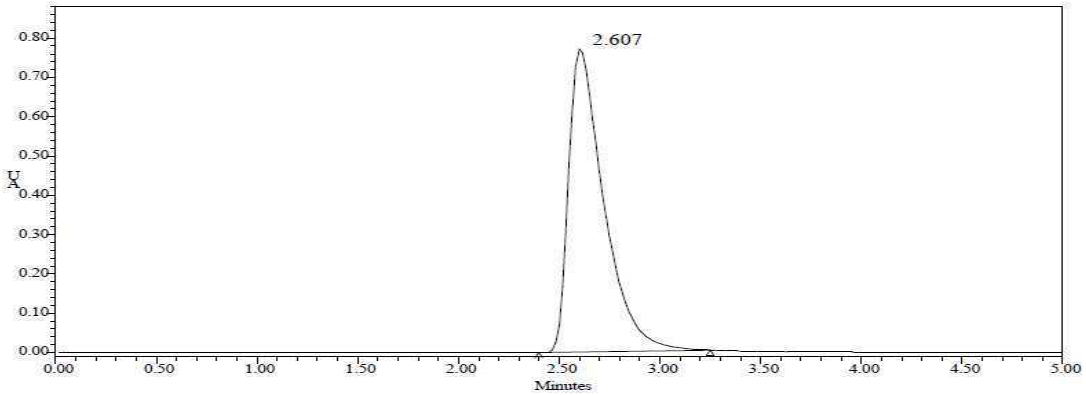
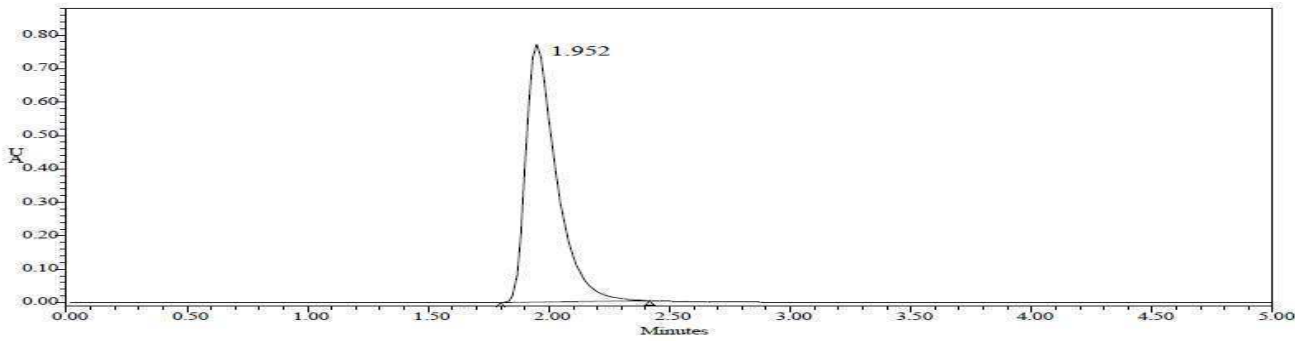


Figure 28
Chromatogram of Robustness flow rate at 0.8 ml



b). The Organic composition in the Mobile phase was varied from 65% to 55%.
Standard solution 40 µg/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. The results are summarized on evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10%.

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2277.8	1.8
2	*Actual	2088.3	1.8
3	10% more	2066.8	1.7

Figure 29
Chromatogram of Robustness less organic

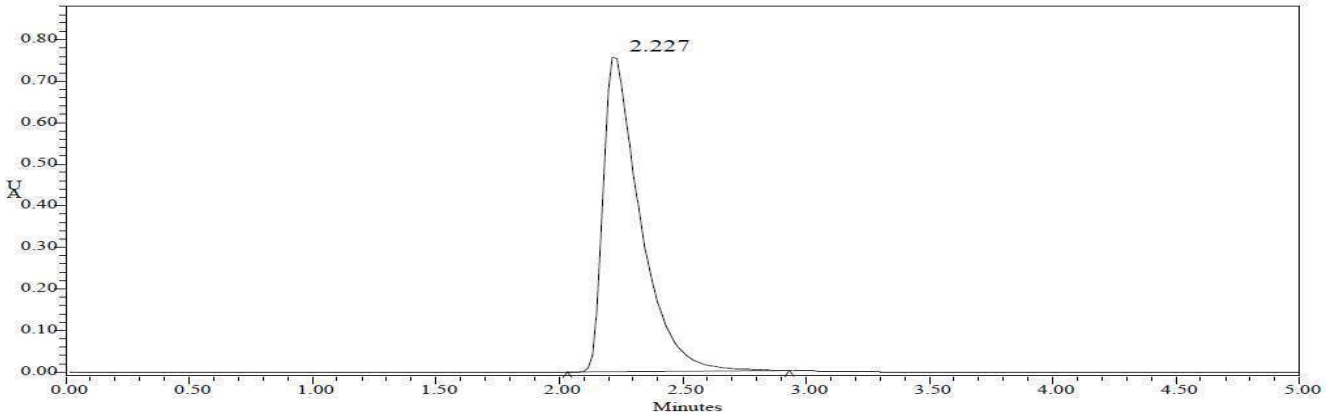
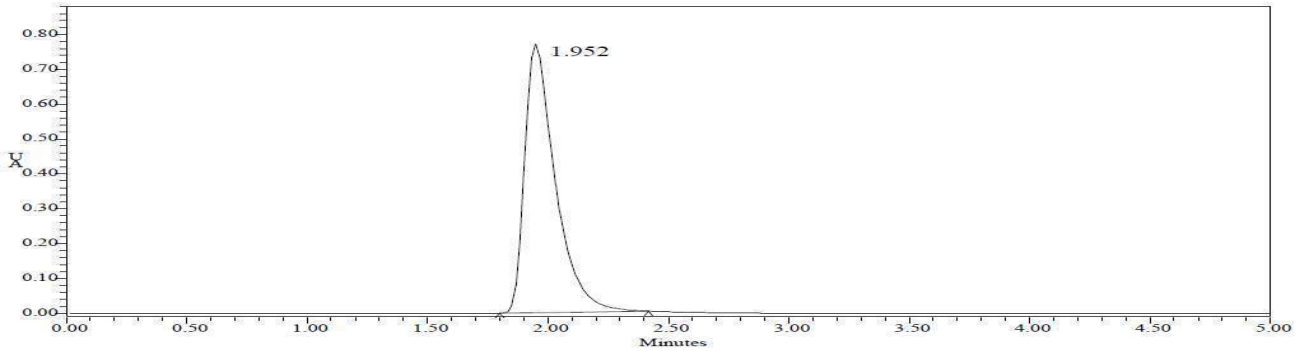


Figure 30
Chromatogram of Robustness more organic



**** Results for actual Mobile phase composition (60:40Acetonitrile: Buffer) have been considered From Assay standard***

RESULTS

TABLE 1
LEVOFLOXACIN METHOD DEVELOPMENT TRAILS

S. No	Retention time	Area	height	USP plate count	USP tailing
1	1.419	7736119	159547	2953.9	0.7
2	1.356	395198	58672	367.3	0.7
3	1.054	1172313	44583	273.9	4.7
4	1.072	1664673	156013	23.5	2.0
5	2.823	1832007	121950	752.7	2.1
6	2.448	1592117	127768	886.2	2.0

TABLE 2
ACCURACY (RECOVERY) STUDIES OF LEVOFLOXACIN IN RP- HPLC

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4102149	5.0	5.03	100.7%	99.5%
100%	8029842	10.0	9.86	98.6%	
150%	11308394	14.0	13.8	99.2%	

TABLE 3
SYSTEM PRECISION STUDIES OF LEVOFLOXACIN IN RP- HPLC

Injection	Area
Injection-1	8243214
Injection-2	8265240
Injection-3	8240898
Injection-4	8251762
Injection-5	8242549
Average	8248733
Standard Deviation	10145.9
%RSD	0.12

TABLE 4
INTERMEDIATE PRECISION STUDIES OF LEVOFLOXACIN

Injection	Area
Injection-1	8156179
Injection-2	8153201
Injection-3	8153251
Injection-4	8158345
Injection-5	8155309
Average	8155257
Standard Deviation	2158.6
%RSD	0.03

System Suitability Results

1). Tailing factor Obtained from the standard injection is 1.8
2). Theoretical Plates Obtained from the standard injection is 2088.3

Acceptance Criteria

The % RSD for the area of Five standard injections results should not be more than 2%.

ROBUSTNESS STUDIES OF LEVOFLOXACIN IN RP- HPLC

TABLE 5
EFFECT OF VARIATION OF FLOW RATE

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	2269.0	1.8
2	0.7	2088.3	1.8
3	0.8	2145.1	1.7

TABLE 6
EFFECT OF VARIATION OF ORGANIC PHASE

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2277.8	1.8
2	*Actual	2088.3	1.8
3	10% more	2066.8	1.7

TABLE 7
LINEARITY RESULTS

S.No	Linearity Level	Concentration	Area
1	I	20µg/ml	3990640
2	II	30µg/ml	6027684
3	III	40µg/ml	8250070
4	IV	50µg/ml	33247570
5	V	60µg/ml	12472251
Correlation Coefficient			0.999

7. ASSAY RESULTS:

Weight of 5 tablets: 4.2090 grams
Average Weight : 0.8398 grams

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

$$\frac{8019412}{} \times \frac{10}{} \times \frac{0.4}{} \times \frac{10}{} \times \frac{10}{} \times \frac{99.8}{} \times \frac{839.8}{} \times 100 = \mathbf{98.7\%}$$

$$\frac{}{8104063} \times \frac{}{10} \times \frac{}{10} \times \frac{}{16.79} \times \frac{}{0.4} \times \frac{}{100} \times \frac{}{500}$$

8. LIMIT OF DETECTION

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 48µV
Signal Obtained from LOD solution (0.18% of target assay concentration) : 142 µV
S/N = 142/48= 2.95

Acceptance Criteria

- S/N Ratio value shall be 3 for LOD solution.

9. LIMIT OF QUANTIFICATION

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 48µV
Signal Obtained from LOD solution (0.5% of target assay concentration) : 492 µV
S/N = 492/48 = 10.25

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ solution.

DISCUSSION

RP-HPLC METHOD

The objective of the proposed work was to develop methods for the determination of Levofloxacin and to validate the methods according to USP and ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of Levofloxacin. The present developed HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of Levofloxacin in commercial dosage forms. In RP- HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, resolution. The average retention time for Levofloxacin was found to be 2.448 min. According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 20-60 µg mL^{-1}. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 98.5 – 101 %.Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using five concentrations analyzed on three trials over a period of three days. These results show the accuracy and reproducibility of the assay.

Ruggedness of the proposed method was determined by analysis of aliquots from different environmental conditions; the % R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

The proposed methods are accurate, simple, rapid and selective for the estimation of Levofloxacin in pharmaceutical formulations

CONCLUSION

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool.

The run time of the HPLC procedure is only 15 minutes. Good agreement was seen in the assay results of pharmaceutical formulation as well as in laboratory prepared mixtures by developed methods. We concluded that all the proposed methods are a good approach for obtaining reliable results and were found to be suitable for the routine estimation of Levofloxacin in pharmaceutical formulation.

REFERENCES

1. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development 2nd ed. New York: John Wiley & sons; 1997. p. 46-51.
2. Sandy L. High performance liquid chromatography. John Wiley & sons; 1991. p. 81-87.
3. Frank S. Hand book of instrumental techniques for analytical chemistry. New Delhi: Pearson education; 2007. p. 481-498.
4. Kaur H. Spectroscopy. Meerut:Pragathi prakashan. 1999; p. 258-317.
5. Kellermann Hurtado, Daniele Rubert Nogueira, Fernanda Bortolini, Luclia Magalh, es da Silva' Estevan Zimmermann ' Marin Jost e. Souza. Determination of Levofloxacin in a Pharmaceutical Injectable Formulation by Using HPLC and UV Spectrophotometric Methods. Journal of Liquid Chromatography & Related Technologies 2007 30(13) :1981 – 1989.
6. Ashuthoshkar. Pharmaceutical drug analysis. Newdelhi: New age Internationals. p. 293-313.
7. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development 2nd ed. New York: John Wiley & sons; 1997. p. 46-51.
8. Satish A. Patel, Arun M. Prajapati, Paresh U. Patel, Natubhai J. Patel,Jayesh B. Vaghmasi. Development and Validation of Column High-Performance Liquid Chromatographic and Derivative Spectrophotometric Methods for Determination of Levofloxacin and Ornidazole in Combined Dosage Forms. International Journal of AOAC 2008;91(4):1060-327