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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF LEVOFLAXACIN IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple specific and accurate RP-HPLC method has been developed and validated of Levoflaxacin in bulk drug and pharmaceutical dosage forms. The chromatographic conditions were viably created for the unit of Levoflaxacin by using Inertial - ODS C18(250 x 4.6 mm,5µ), stream is 1.0 to 1.2 ml/min, convenient stage extent was Methanol:Water(75:25v/v),recognizable proof wave length was 250nm. Acetonitrile was used in experiment. The results of the tablet analysis were validated with respect to accuracy (recovery), linearity, limit of detection (LOD) and Limit of quantification (LOQ) were found to be satisfactory.

KEY WORDS: Levoflaxacin, RP-HPLC, Acetonitrile, Accuracy, linearity, limit of detection (LOD) and Limit of quantification (LOQ)

1. INTRODUCTION

Levofloxacin is a one of the isomer of ofloxacin.Ofloxacin and Norfloxacin were developed and synthesized by scientists at Daiichi seiyaki[1].From these two isomers Levo form was successfully developed and synthesized in 1985[2, 3]first time it was marketed in Japan in the year1993 and brand name was Cravit[4].Ofloxacin isomer was FDA (food and drug administration) approved in1996 and brand name changed as Levaquin[3].

Levoflaxacin developed and marketed for the use of antibacterial medication[5,6].It is also used for treatment several health issues like

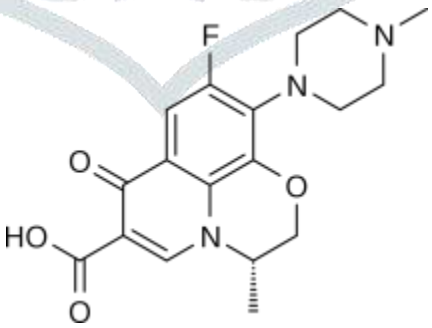
- bacterial infections
- sinusitis
- tuberculosis
- urinary tract infections
- skin diseases
- pregnancy category medicine
- also used as eye drop[7]
- pneumonia

As of 2016rules USA FDA Approved and recommended for the main importance of antibacterial infections[8].Levoflaxacin was shown positive results to control pneumonia[9].In 2007 America recommended for treatment of urinary tract infections and approve by infections disease society of America(IDSA) and recommended by American Thoracic Society. And also recommended to treat different diseases like liver,heart,and lung problems[10].

In 2010 IDSA recommended to treat in adults for urinary tract infections[11]In 2013 many countries have been recommended for treatment of anti bacterial infections[12,13]As per FDA guidelines Levoflaxacine is a pregnancy category medicine it means it can be used for pregnancy and breastfeeding.

GENERAL INFORMATION OF LEVOFLAXACIN

- Synonym: (-)-Ofloxacin
- Drug Bank Code : DB01137
- Molecular formula : $C_{18}H_{20}FN_3O_4$
- IUPAC:(2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10 oxo-4-oxa-1-azatricyclo[7.3.1.0^{5},^{13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid
- Molecular weight : 361.3675
- Chemical Structure



- Appearance : White crystals or fine white powder.
- Solubility : Practically soluble in water.
- Melting Point : 150 °C
- Boiling point : 571.5±50.0 °C at 760 mmHg.
- pKa : Strongest Acidic -5.45 Strongest Basic-6.2
- logP : 2.1

PLAN OF WORK

Present work is to develop and validate a new simple, rapid, and sensitive and for method development and validation Levofloxacin by RP-HPLC in bulk drug and pharmaceutical dosage form.Folowing parameters have been done.

- Importance of the drug(Drug profile)
- Preparations' of the solutions
- Selection of the wavelength

- Selection of stationary phase / column
- Optimization of the method
- Study of the system suitability parameters
- Validation of the proposed method

AIM OF THIS WORK

- To Estimate Levofloxacin simultaneously in dosage forms by RP-HPLC method.
- To validate the method according to ICH guidelines.

Various publications are available for simultaneous estimation, method development and validation of Levoflaxacin by RP-HPLC done single drug in pharmaceutical dosage form.

Hence,there is a need for suitable RP-HPLC Method for routine analysis of Levoflaxacin

The work was an attempt to develop simple, rapid, and sensitive analytical methods for the simultaneous estimation of Levoflaxacin formulation in accordance with ICH(international council on Hormonisation) Q2B guidelines and to extend the method for routine analysis.

2.EXPERIMENTS

2.1.MATERIALS AND METHODS

2.1.1. Chemicals and reagents

Table No.1. List of Chemicals and reagents

1	Levoflaxacin	Lara Pvt, Ltd. Hyderabad, Telangana state, India.
2	Orthophosphoric acid	Merck
3	Acetonitrile	Merck
4	Methanol	Merck
5	Water	Qualigens

2.2. Instruments

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator(FAST CLEAN)

2.3.Selection of wave length (λ max)

Weighed 100 µg/ml of Levoflaxacin in 100ml volumetric flask and prepared in Qualigens water. The resulting solutions were scanned individually on HPLC PDA detector from 200nm to 800 nm and also in UV-Visible spectrophotometer. The optimal response for both of them was obtained at 250 nm. Hence the complete method was processed at the wavelength of 250 nm.

2.4. Preparation of standard solution

100mg of Levoflaxacin was accurately weighed and transferred into a 100 ml clean dry volumetric flask and added methanol sonicated (30 minutes) to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000ppm (Stock solution). Further 4 ml of Levoflaxacin was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent to give a concentration of 40ppm.

2.5. Preparation of sample solution

10ml of Levoflaxacin was pipetted out from the above stock solution into a 100 ml clean dry volumetric flask and diluents was added it and was shaken by mechanical stirrer and sonicated for about 10 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent to give a concentration of 1000ppm and allowed to stand until the the residue settles before taking an aliquot for further dilution (stock solution). 4ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluents up to the mark to give the required concentration.

2.6. pH 3.4 Phosphate Buffer Preparation

2.7218g of KH_2PO_4 was weighed and placed into a 1000ml beaker; it was then dissolved and diluted to 1000ml with HPLC water, with the pH corrected to 3.4 using orthophosphoric acid.

2.7. Mobile Phase

Methanol and Buffer degassed in a 75:25, V/V ratio of Levoflaxacin. Filtered this mobile phase through 0.45micron filter paper.2

2.8.Reversed phase High Performance Liquid Chromatography(RP-HPLC) [14-16]

Most of the drugs are in bulk drug and pharmaceutical dosage form can be analyzed by HPLC method because of the many advantages like rapidity, specificity, accuracy, precision and ease of automation of this method. Some of the advantages are:

- Speed (analysis can be complete in 30 minutes or less)
- More sensitivity (various detectors can be employed)
- Developed resolution (more variety of stationary phases)
- Reusable columns (costly columns but can be used for many analysis)
- Ideal for the substances of low volatility
- Easy sample recovery, handling and maintenance
- Instruments automised (less time taker and less labour)

Nowadays HPLC is widely used in pharmaceutical research and development in following areas:

1) To purification of synthetic or natural compounds.
2) To separation and characterisation of metabolites.
3) To calculate impurities, degradation methods and dissolution studies.
4) Widely used for pharmacokinetic and pharmacodynamic studies.

2.9. Development of the method by RP-HPLC

1. Selection of the solvent:

Solvent to be used as diluents and mobile phase it should be suitable for drug soluble and stable. Solvent must be easily available, low cost of the HPLC grade.

2. Selection of Mobile phase:

For the mobile phase, first it can be decided whether it is an organic or aqueous material should be used. With the RP-HPLC method analysis aqueous or very polar solvents such methanol or acetonitrile should be fixed. If the K' values are too large with anaqueous solvent, organic solvent should be tried. If the K' value are too low with organic solvent the separation should be attempted using a mixture of two solvents with various properties.

- K'-capacity factor is a measurement of the degree where the peak of the interest is located with respect to void volume, i.e. Elution time of non- retained components. Generally the value of K' is > 2.
- If a buffer is used, the p^H as well as ionic strength of the buffer can be tried

In order to select the wavelength to carry out the analysis, critical examination of the Ultraviolet absorbance spectra of the drug should be done. A perfect study of the structure of drug and its physicochemical properties; to select the chromatographic parameters. Selection of method for quantitative chromatographic analysis. Determination of workingconcentration range. Validation of the developed method by following ICH guidelines.

3.RESSULTS AND DISCUSSION

3.1.1.HPLC Method Development for Levoflaxacin

The goal of this study was to improve the assay technique for simultaneous quantification of on literature surveys. As a result, the trials detailed below show how the optimization was accomplished

Trial: 1

Mobile Phase: 100% pure degaussed methanol.

Preparation of Standard Solution:

Weighed 10mg of Levofloxacin drug and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase

Chromatographic Conditions:

Flow rate : 1.0ml/min

Column : Inertsil - C18 ODS columnDetector wavelength : 250nm

Column temp : Ambient

Injection volume : 20µl

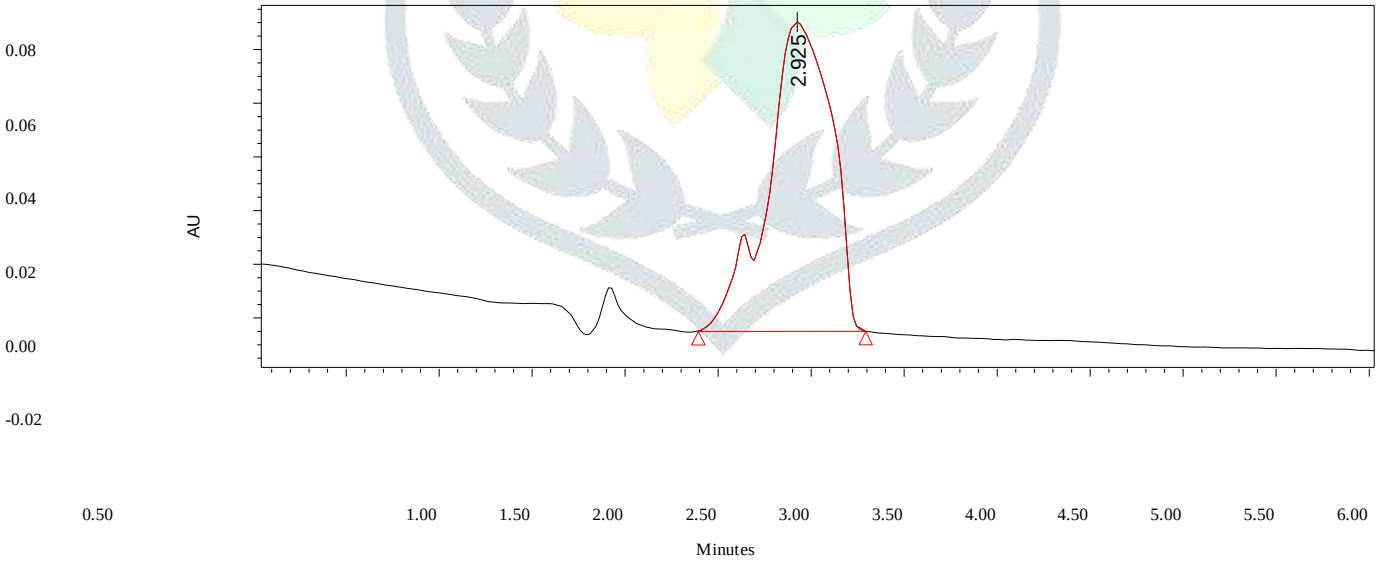
Run time : 6min

Retention time : 2.925

Observation: Peak spliting.The trial 1 chromatogram result was shown in Fig:1

Method development:

Fig1: Chromatogram of Trial 1



Inference : Peak spliting.

S.NO	Name of the peak	Retention time(min)
1.	LEVOFLOXACIN	2.925

Mobile Phase: methanol and Acetonitrile were mixed in the ratio of 90:10V/V and sonicatedto degas.

Preparation of Standard Solution:

Weighed 10mg of Levofloxacin drug and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:

Flow rate : 1ml/min

Column : Inertsil -C18 ODS columnDetector wavelength : 250nm

Column temp : Ambient

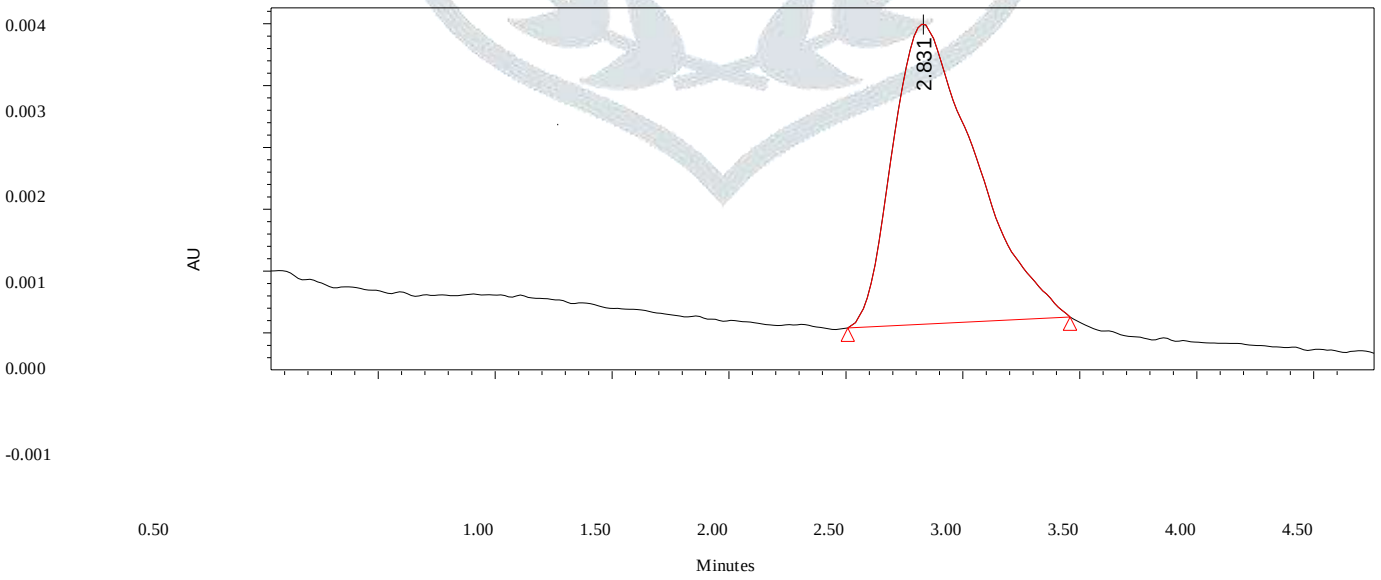
Injection volume : 20µl

Run time : 5min

Retention time : 2.831

Observation: Got more assymetry. The trial 2 chromatogram result was shown in Fig:

Fig 2: Chromatogram of Trial 2:



Mobile Phase: Methanol and Acetonitrile were mixed in the ratio of 80:20 V/V and sonicated to degas.

Preparation of Standard Solution:

Weighed 10mg of Levofloxacin drug and and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:

Flow rate : 1.0ml/min

Column : Inertsil - C18 ODS columnDetector wavelength : 250nm

Column temp : Ambient

Injection volume : 20µl

Run time : 5min

Retention time : 2.808

Observation:

Got Bad peak.. The trial 3chromatogram result was shown in Fig:3

3.2.Optimized method develoment

Mobile Phase: Methanol and Water were taken and sonicated to degas in the ratio of 75:25.

Preparation of stock solution:

Weighed 10mg of Levofloxacin drug and and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 2 ml. was taken from this and diluted to 10ml.with mobile phase.

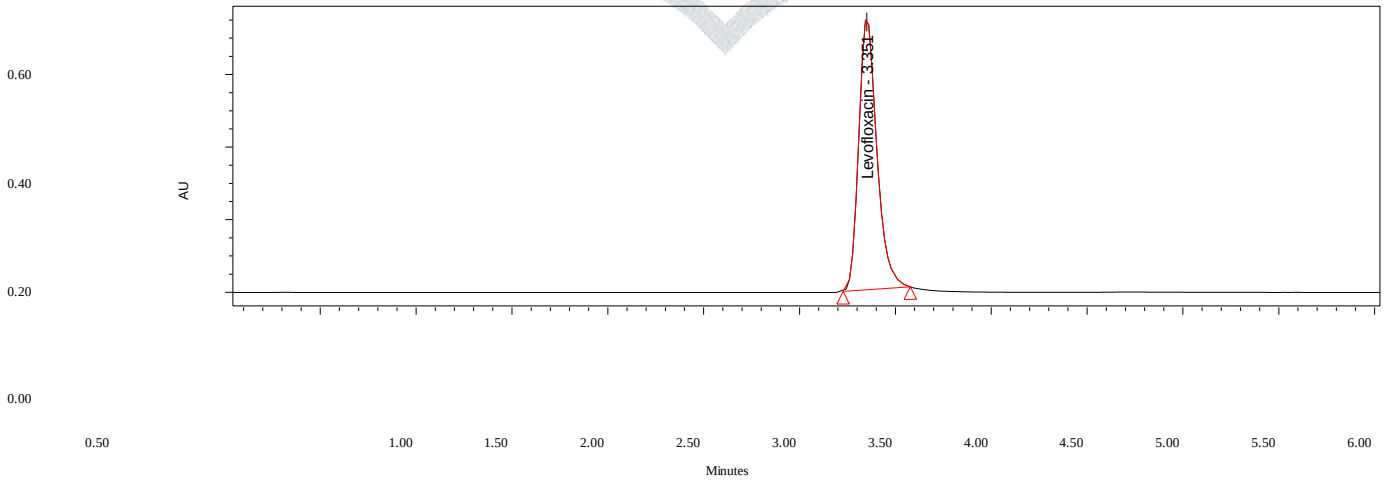
Preparation of working standard solution:

The stock solution equivalent to 20ppm to 80ppm were prepared , sonicated and filtered through 0.45µ membrane.

Optimized chromatographic conditions:

Parameters	**Method**
Stationary phase (column)	Inertsil -ODS C_{18} (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Methanol and Water (75:25)
Flow rate (ml/min)	1.0 ml
Run time (minutes)	6
Column temperature (°C)	Ambient
Volume of injection loop (l)	20
Detection wavelength (nm)	250nm
Drug RT (min)	3.351

Fig 3: Chromatogram of standard



Inference: Got chromatogram at an Rt of 3.351 for standard

S.NO	Name of the peak	Retention time(min)
1	**LEVOFLOXACIN**	3.351

3.2.1. SYSTEM SUITABILITY:

A Standard solution was prepared by using Levofloxacin Working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Levofloxacin **,** retention times and peak areas.

ACCEPTANCE CRITERIA:

1. The % RSD for the retention times of principal peak from 5 replicate injections of eachStandard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections ofeach standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Levofloxacin peaks is NLT 3000.
4. The Tailing factor (T) for the Levofloxacin peaks is NMT 2.0

OBSERVATION:

The %RSD for retention times and peak areas were found to be within the limit.refer table: 1 As sown in fig 6 – 10.

SYSTEM SUITABILITY:

TABLE-1: Data of System suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.353	3348846	19023.845	1.14721
2	3.354	3348675	19010.547	1.13384
3	3.352	3349834	19036.874	1.18742
4	3.352	3348978	19027.254	1.16547
5	3.353	3348226	19084.658	1.17485

Mean	3.3528	3348801	19036.82547	1.1852313
SD	0.000837	591.3275	-------	-------
% RSD	0.024954	0.0176	-------	-------

TABLE-2 Data of Repeatability (System precision)

	Injection	**Peak Areas of Levofloxacin**	**%Assay**
Concentration40ppm	1	3348798	100.18
	2	3349568	100.20
	3	3348271	100.16
	4	3348683	100.18
	5	3348711	100.18
Statistical Analysis	**Mean**	3348962	100.18
	SD	505.97	601.10
	% RSD	0.015	0.015

TABLE-3: Data of Repeatability (Method precision)

	Injection	**Peak Areas of Levofloxacin**	**%Assay**
Concentration40ppm	1	3348900	100.18
	2	3348201	100.16
	3	3348578	100.17
	4	3348907	100.18
	5	3348577	100.17
	6	3348008	100.16
StatisticalAnalysis	**Mean**	3348528	100.17
	SD	364.48	0.0109

Table-4: Data of Intermediate precision (Analyst 2)

	Injection	Peak Areas of Levofloxacin	%Assay
Concentration 40ppm	1	3347897	100.15
	2	3348915	100.18
	3	3347684	100.15
	4	3348555	100.17
	5	3349564	100.18
	6	3348652	100.20
Statistical Analysis	**Mean**	3348544	100.17
	SD	685.27	0.0204
	% RSD	0.0204	0.0204

3.2.3.ACCURACY (RECOVERY):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of F Levofloxacin into each volumetric flask for each spike level to get the concentration of F Levofloxacin equivalent to 50%, 100%, and 150% ofthe labeled amount as per the test method. The average % recovery of Levofloxacin was calculated.

ACCEPTANCE CRITERIA:

The mean % recovery of the Levofloxacin at each spike level should be not less than 98.0% and not more than 102.0%.

TABLE-5: Data of Accuracy

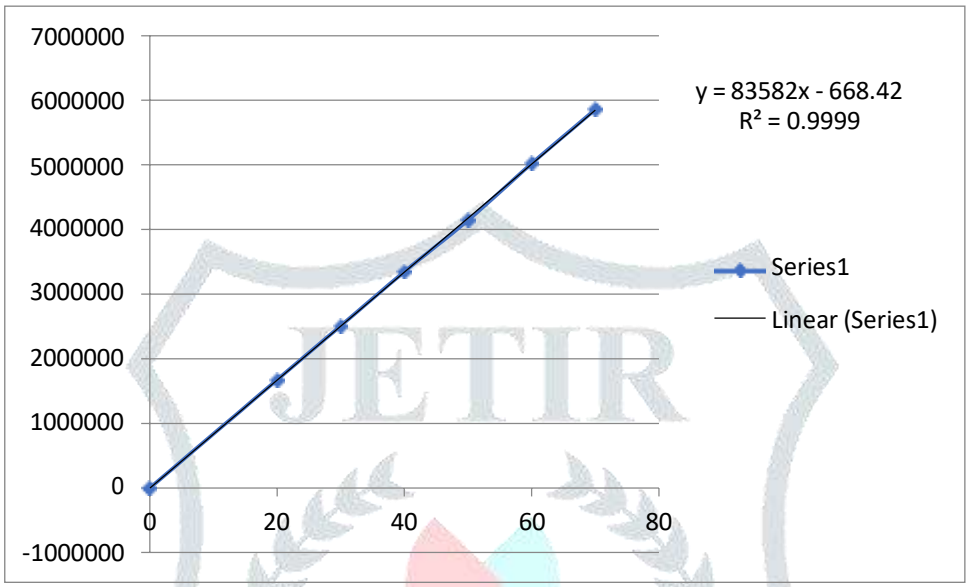
Concentration % of spiked level	Amount added(ppm)	**Amount found (ppm)**	**% Recovery**	**Statistical Analysisof % Recovery**	
50% Sample 1	20	20.04	100.20	**MEAN**	100.19
50% Sample 2	20	20.03	100.18		
50%	20	20.03	100.19	**%RSD**	0.009

Sample 3					
100 % Sample 1	40	40.07	100.17	**MEAN**	100.17
100 % Sample 2	40	40.06	100.15		
100% Sample 3	40	40.07	100.17	**%RSD**	0.013
150% Sample 1	60	60.10	100.17	**MEAN**	100.17
150% Sample 2	60	60.10	100.17		
150% Sample 3	60	60.10	100.17	**%RSD**	0.0013

TABLE-6: Data of Linearity

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	83582
20	1674311	y-Intercept	-668.4
30	2511466	Correlation Coefficient	0.999
40	3348621		
50	4145002		
60	5022932		
70	5860087		

Fig: 4.1inearity Plot (Concentration Vs Response)



4.CONCLUSION

The proposed RP-HPLC, method was suitable methods for the estimation of Levofloxacin in drug substances and drug products. All the validation of parameters results of this method met the criteria of ICH guidelines. The developed and validated method was successfully applied for routine analysis of Levofloxacin in drug substances and drug product.

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