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RP-UFLC Method Development and Validation for Simultaneous Estimation of Levofloxacin in Bulk and Tablet

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Abstract
A simple, novel, accurate, precise, linear, rapid, and economical UFLC method was developed to estimate Levofloxacin. The chromatographic separation was achieved using a Phenomenex Luna 5µ C18 (2) 100A (250 x 4.60mm 5 µ) column and binary gradient elution, a mobile phase comprising of Methanol: 0.3% Orthophosphoric acid (70: 30) at P^H 1.66 was adjusted with water. The flow rate was 1.0 ml/min with detection at 294 nm using a UV detector and drug eluted with a retention time of 2.29 min. The calibration curves were linear (r^2=0.999) in the concentration range of 0.2-1.0 µg/ml. The limit of detection and limit of quantitation was0.5931 and 1.7974µg/ml,respectively. Thus the simple, novel, economical, accurate, precise, and rapid UFLC method was developed to estimateLevofloxacin and validated as per ICH guidelines. Hence the method holds good for routine analysis of Levofloxacin in the pure and pharmaceutical dosage form.

Keywords - *Levofloxacin, ICH guidelines, UFLC, Validation*.

I. INTRODUCTION

Levofloxacin (LVFX) is a synthetic fluoroquinolone antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication. It is used to treat a number of bacterial infections, including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis [1,2].

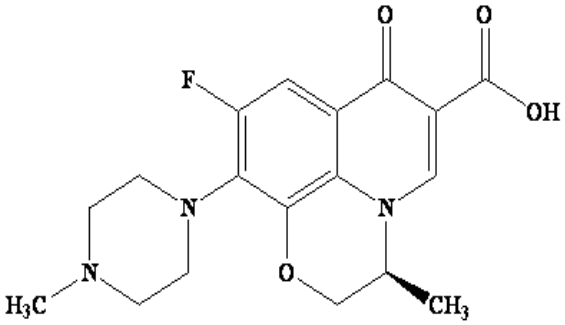

Figure.1: Chemical structure of Levofloxacin.

Levofloxacin is chemically (S)-9-Fluoro-2,3-dihydro-3-methyl- 10-(4-methyl-1-piperazinyl)-7 oxo-7H-pyrido(1,2,3-de) -1,4 benzoxazine-6-carboxylic acid, is a new quinolone antimicrobial agent which exhibits broad-spectrum in-vitro bactericidal activities against gram-positive and gram-negative aerobes[3]. It has a molecular formula of $C_{18}H_{20}FN_3O_4$ and a molecular weight of 361.368g/mol. It has the structural formula (Fig.1). Levofloxacin is a yellowish-white powder, freely soluble in Glacial acetic acid, chloroform, sparingly soluble in water. The brand name of Levofloxacin is Levaquin.

Literature Survey revealed that the drug had been estimated by UV[3-22] spectrophotometric, RP-HPLC[23-34]and HPTLC [35]methodhas been reported so far.

Present work aimed to develop and validate a novel, rapid, simple, precise, and specific UFLC method to estimateLevofloxacin in its bulk and tablet dosage form.

II. MATERIAL AND METHODS

A. Instrumentation

Chromatographic separation was performed on a Shimadzu LC-20AD UFLC system comprising a PDA detector, Shimadzu LC-20AD pump, andPhenomenex Luna 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. A manually operating Prominent autosampler SIL-20 ACHT injector 20µl (20 µl injection valve) was used for injecting sample and standard solution. Data was compiled using the Shimadzu LC Solution software.

B. Chemicals and reagents

- Levofloxacin pure form was obtained as a gifted sample from the pharma industry. Its pharmaceutical dosage form Levoflox labeled claim 500mg(manufactured by Cipla Private limited) was purchased from the local pharmacy, Mandya.
- Methanol, Orthophosphoric acid, and water are available in the Laboratory of JSS College of Pharmacy, Mysore.
- All the chemicals used in this investigation are HPLC grade.

C. Selection of mobile phase

Based on sample solubility, stability, and suitability, various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases,Methanol:0.3%Orthophosphoric acid at PH 1.66 (70:30 v/v) was chosen with detection wavelength 294nm since it gave a sharp peak with good symmetry within limits.
Of Levofloxacin represented in Table 1.

D. Preparation of mobile phase

The mobile phase was prepared by mixing Methanol: 0.3% Orthophosphoric acid (70:30) of PH 1.66. This solution wassonicated for 10mins and filtered using a 0.2µ membrane filter.

E. Chromatographic conditions

The optimized parameters used as a final method for Levofloxacin's estimation are represented in Table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	Methanol: 0.3% Orthophosphoric acid at P^H 1.66 with water (70:30v/v)
Stationary phase	Phenomenex Luna 5µ C18 (2) 100A (250 x 4.60mm, 5 µ)
Wavelength	294nm
Run time	10min
P^H of mobile phase	1.66
Injector	(250 x 4.60mm, 5 µ)
Flow rate	1.0 ml per min
Injection volume	20 µl
Temperature	Ambient
Mode of operation	Binary gradient elution

F. Preparation of standard stock solution

Weigh accurately about 100 mg of Levofloxacin pure drug and then transferred into a 100ml volumetric flask. A portion of diluent is added and sonicated for 10 min to dissolve it completely. The volume is made up to the mark with diluent (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-2), From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-3), from this solution pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml into 10 ml individual volumetric flask and add diluent up to the mark, this gives 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml concentrations.

G. Preparation of sample solution

Ten tablets of Levofloxacin 500 mg were weighed and powdered. The primary stock solution was prepared by dissolving a weight equivalent to 100 mg of Levofloxacin in a 100ml volumetric flask using HPLC grade methanol,and the solution was sonicated for 10 minutes. The concentration of this solution gives (1000mg/ml). This solution was filtered using a 0.2µ membrane filter. The above solution 10ml was pipetted out into a 100ml volumetric flask. The volume was made up to the mark with Methanol and used for the UFLC method analysis.Methanol makes all the dilutions.

H. Flow rate selection

Different flow rates between 0.98 to 1.02 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time.

III. RESULTS AND DISCUSSION

A. System suitability

20 µl of the standard solution was injected under optimized chromatographic conditions to evaluate the system suitability. Parameters such as the number of theoretical plates (N), tailing factor (T), retention time (tR), asymmetry, and area were determined. The obtained values indicate the good performance of the system shown in Fig-2. The values of system suitability parameters were shown in Table- 2.

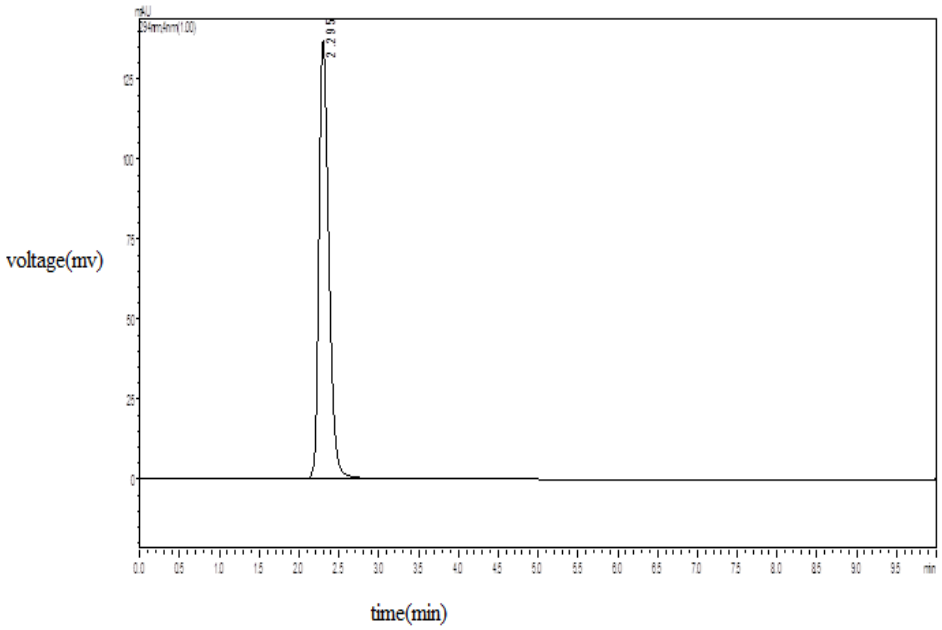

Fig 2: Chromatogram of Levofloxacin

Table 2: Results of System suitability studies

System suitability parameters	Acceptance criteria	Results
Retention time(tR)	-	2.29
Number of theoretical plates(N)	N=≥ 2000	2939
Asymmetry	K=≤ 2.0	0.99
Area	-	94300
Tailing factor(T)	T= ≤ 2.0	1.19

B. Method validation

The method is validated according to the ICH guidelines36-38.

C. Specificity

The UFLC method's specificity was checked for the interference of impurities, degradants, or excipients in the sample solution analysis. It was determined by injecting a volume of 20µl sample solution, and the chromatogram was recorded. There is no impurities interference, excipient on Levofloxacin's peak, indicating the high specificity of the method.

D. Linearity and Range

The calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3. It showed linearity over a concentration range of 0.2-1.0 µg/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected six times. The calibration curve was performed in six replicates.

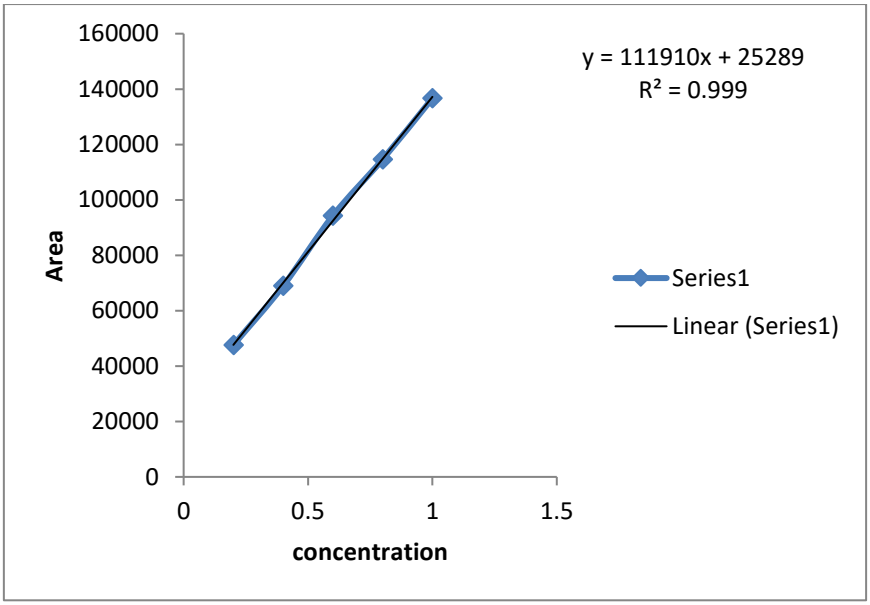

Fig.3: Linearity graph of Levofloxacin

Table 3: Linearity data

Sl. No.	Concentration (µg/ml)	Retention time (min)	Peak area (mv)
1	0.2	2.295	47607
2	0.4	2.294	68955
3	0.6	2.299	94300
4	0.8	2.295	114630
5	1.0	2.295	136679

*indicates an average of six determinations,

Table 4: Regression parameters

Regression parameters	Levofloxacin
Regression Equation	Y=111910x+25289
Slope (b)	111910
Intercept (a)	25289
Correlation Coefficient (r^2)	0.999

E. Precision

The precision of the analytical method was determined by intraday and interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times on the same day. They were injecting six solutions of the same concentration for six different days in a week in interday precision. The results of precision were tabulated in table-5. The mean area's average and standard deviation were taken, and %RSD was calculated and reported. %RSD values were within limits, and the method was found to be precise.

Table 5: Results of Precision studies

Sl.no	Concentration (µg/ml)	Intraday precision (area)	Inter-day precision (area)
1.	0.6	94883	92685
2.	0.6	95686	91842
3.	0.6	97713	91254
4.	0.6	95983	92863
5.	0.6	94956	92857
6.	0.6	95689	92989
Mean		95818.33	92415
Std. dev.*		1026.604	703.5189
%RSD		1.071407	0.76126

*indicates an average of six determinations, RSD indicates the relative standard deviation

F. Accuracy

Recovery studies determined the method's accuracy by determining thedrug's mean recovery at three different levels (50%, 100%, and 150%). At each level, three determinations were performed. A known amount of standard pure drug was added to pre-analyzed tablet powder, and the sample was then analyzed by the developed method. Results of recovery studies were reported in table-6. The observed data were within the range, which indicates good recovery values.

Table 6: Results of Accuracy studies

Level of recovery	Amount of formulation	Amount of Pure drug	The total amount of drug	Peak area	Difference	%recovery	Mean
50	0.4	0.2	0.6	112713	65106	94.41	
	0.4	0.2	0.6	113842	66235	96.05	98.46
	0.4	0.2	0.6	119963	72356	104.93	
100	0.4	0.4	0.8	131213	62258	90.28	
	0.4	0.4	0.8	139948	70993	102.955551	98.42893
	0.4	0.4	0.8	139319	70364	102.043362	
150	0.4	0.6	1	161076	66193	95.9944892	
	0.4	0.6	1	162091	67208	97.4664636	98.0016
	0.4	0.6	1	164213	69330	100.543833	

G. Robustness

The robustness of the analytical method was carried by varying the parameters deliberately from the optimized chromatographic conditions like a PH of mobile phase (variation in ± 0.1 units), flow rate (variation in ± 0.02ml/min.), wavelength (variation in ± 2 nm).The observed results were within the limit. The results were shown in table-7.

Table 7: Results of Robustness studies

Concentration (0.6µg/ml)				
Parameters	Factors	Level	Mean area* ± Standard deviation	%RSD
P^H	1.56	-1	95818±1026.604	1.071
	1.66	0	92415±703.5189	0.761
	1.76	+1	96094±1458.449	1.517
Wavelength (nm)	292	-2	93466±45.50824101	0.048
	294	0	94266.33±39.71565602	0.04213
	296	+2	90571±33.77869151	0.03729
Flow rate (ml/min)	0.98	-2	95542.67±528.9067	0.55358
	1.00	0	91927±719.2767	0.78244
	1.02	+2	92903±74.53858	0.080232

*indicates an average of six determinations,

H. Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was <2, which showed the Ruggedness of the developed analytical method. The values were shown in Table-8.

Table 8: Results of Ruggedness studies

Analysts	Mean area* ± Standard deviation	%RSD
Analyst 1	95818±1026.604	1.07140
Analyst 2	96094±1458.449	1.51773

*indicates an average of six determinations,

I. Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on the standard deviation of the linearity curve's response and slope. LOD and LOQ values of Levofloxacin were shown in Table-9.

Table 9: Results of LOD and LOQ

Parameters	Results
LOD (µg/ml)	0.593147
LOQ (µg/ml)	1.797416

IV. CONCLUSION

From the above, it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ, and Ruggedness) met the predetermined acceptance criteria, as mentioned in ICH guidelines. The developed UFLC method is simple, rapid, accurate, precise, and shown good linearity. Hence it

can be applied for routine analysis of Levofloxacin in bulk and its dosage forms.

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