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HPLC METHOD DEVELOPMENT OF LEVOFLOXACIN BY RP-HPLC IN ITS BULK DOSAGE FORMS

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ABSTRACT
A fast, simple, sensitive, precise, accurate and reproducible Reverse phase high performance chromatographic method was developed and validated for the analysis of levofloxacin in bulk dosage forms. The separation was conducted by using C-18 RP-HPLC column, which was maintained at ambient temperature. The mobile phase consisting of potassium di-hydrogen ortho phosphate, methanol and acetonitrile in the ratio 70:15:15 v/v was delivered at a rate of 1.5 ml/min. The analysis was detected by using UV detector at the wave length of 295nm.The method is validated for its specificity, precision, accuracy, linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml (r^2 =0.999). The retention time for levofloxacin was found to be 8.80±25min. Limit of quantification of the method is 0.192µg/ml and limit of detection is 0.075µg/ml.
Key words: Levofloxacin, validation, C-18 column, Reverse phase

INTRODUCTION

Levofloxacin is chemically is known as (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0^ {5, 13}] trideca-5, 7, 9(13), 11-tetraene-11-carboxylic acid. Chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe bacterial infections. Like other fluoroquinolones, it acts on bacterial topoisomerase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Levofloxacin has activity against a broad range of Gram-positive and Gram-negative organisms. Levofloxacin also appears to have improved activity against Streptococcus pneumonia compared with ciprofloxacin or ofloxacin[1]. Various methods are used for the determination of drugs in pharmaceutical formulations. These methods include titrimetry, flourimetry, UV spectrophotometry, infra red spectroscopy, differential scanning calorimetric (DSC), chromatographic methods etc. Among these, chromatographic methods are frequently used for the qualitative & quantitative analysis of drug substances, and raw materials.[2]

This paper describes a fast, sensitive, rapid and accurate method for the analysis of levofloxacin in bulk dosage forms by using Reverse phase-High performance liquid chromatography(RP-HPLC).The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Instrumentation

Quaternary isocratic HPLC (Younglin HPLC YL9000 series) with YL 9110 Pump and with autochrom 3000 software and UV-Vis detector YL9120, electronic balance (Shimadzu) was used for the purpose of weighing samples were injected on to HPLC system using Hamilton micro syringe.

Reagents and chemicals

Levofloxacin was obtained from FDC limited Goa and acetonitrile and water employed for the preparation of mobile phases were of HPLC grade (qualigens fine chemicals, Mumbai). All the other chemicals and solvents viz potassium dihydrogen ortho phosphate, methanol, acetonitrile are of ambient grade.

Chromatographic conditions

The mobile phase for the proposed method, potassium di hydrogen ortho phosphate, methanol and acetonitrile (70:15:15) was filtered through a 0.45-µ m membrane filter. It was degassed with a helium spurge for 20 min pumped from the reservoir to the column (c -18, 250 x 4.6mm) at a flow rate at 1.5 ml/min. The run time was set at 10 min the column temperature was maintained at 20^{0}C. prior to injecting the drug solution in to the column; the column was equilibrated for at least 1 hour with the mobile phase flowing through the system. The eluent was monitored at 295nm. The data was stored and analyzed with the soft ware "autochrom-3000" (youngling).

Preparation of standard stock solution

A stock solution of Levofloxacin was prepared by dissolving Levofloxacin (100 mg) in a volumetric flask (100 ml) containing 25 ml of diluents, sonicated for 20 min and then made up to the volume with diluents to 100ml. Working standard solution of Levofloxacin (300µg/ml) was prepared by suitable dilution of stock solution with diluents. Linearity solutions were prepared in diluents containing RS (10-100µg/ml). Each of these drug solutions (20µl) was injected into the column and the peak area and retention times were recorded.

Selection of mobile phase

The solution of Levofloxacin was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, acetonitrile and phosphate buffer in different proportions were tried and finally phosphate buffer and Methanol and acetonitrile (70:15:15v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Levofloxacin.

Preparation of mobile phase

Mobile phase comprised of potassium dihydrogen ortho phosphate (Adjusted to P^H 4.5 ±0.05 with ortho phosphoric acid), Methanol and acetonitrile (70:15:15v/v). Mobile phase was filtered through a 0.45-µ m membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.5 ml/min), which yields a column back pressure of 653-750 psi .Run time was set as 10 min, column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 295 nm and data were acquired, stored and analyzed with the software"Autochro-3000" (Young Lin).

EVALUATION OF ANALYTICAL METHODS

Linearity

Aliquots ranging from 10-100µg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Levofloxacin, the higher concentration range was used to improve signal to noise ratio. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100µg/ml for Levofloxacin[3].

Precision

The precision of the method based on intra- day repeatability was determined by replicate analysis of five sets of samples[4, 5]. The inter day reproducibility of the method was validated using five sets. The coefficient of variation were calculated from the ratio of standard deviation (SD) to the means expressed as percent RSD (%RSD)

Limit of Detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula,

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = standard deviation of the response
S = slope of the calibration curve

Limit of Quantification (LOQ)

The limit quantification is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula,

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = standard deviation of the response
S = slope of calibration.

RESULTS AND DISCUSSION

Checking the resolution of drug and material

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Levofloxacin was injected to get the chromatogram. The retention time for Levofloxacin was found to be 8.80 ±.0.25 min. (Table 1)

Linearity

The data of the Peak area of the Drug Vs drug concentration were evaluated by linear regression analysis as shown in the (Table 2) and calibration curve obtained after plotting drug concentration Vs area is shown in (figure 2). Linear regression analysis demonstrated that the chromatograph response for the drug was highly linear (r^2=0.999) in the studied concentration range of 10-100µg/ml.

Precision

The result depicted in the table 3a,3b indicate that the given method has sufficient precision as indicated by the correspoing values of % RDS ranging 0.25 for intra- day studies and 0.22 for inter-day studies respectively. The values of % RDS for both the studies are well below 1.0% constructing adequate precision.

Limit of Detection and Quantification (LOQ&LOD)

Standard error and slope of linearity data is used to predict LOD and LOQ of levofloxacin and precision was established at the predict concentration. The result was shown in table 4.

CONCLUSION

The developed method was validated in terms of precision, linearity, limit of detection & and limit of quantification. A good linear relationship was observed from levofloxacin in the concentration range of 10-100µg/ml. The co-relation co-efficient for levofloxacin was found to be as 0.999.The intra and inter day precision was good enough to indicate that the developed method is precise and reproducible.

This demonstrated that the current developed RP-HPLC method is simple, linear, and precise.

ACKNOWLEDGMENT

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Table:1 Resolution of drug material

S.No.	Drug	RT (min)	Peak Area	Height	Plates	HETP
1.	Levofloxacin	8.8	3675822	43314	5986	0.0568

Table: 2 Linearity of Levofloxacin

S.No	Concentration (µg/ml)	RetentionTime (min)	Peak Area
1	10	8.805	824576
2	25	8.802	2324568
3	50	8.802	3675822
4	75	8.805	5742139
5	100	8.809	7842369

Table: 3a Intra-Day Precision for Levofloxacin

Concentration(µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3773448	3675822.5	46779.35	0.22
50	3762621			
50	3675822			
50	3793748			
50	3723218			

Table:3b Inter-day precision of Levofloxacin

Concentration (µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3765423	3833523	42900.08	0.25
50	3674569			
50	3675822			
50	3745123			
50	3745698			

Table: 4 Limit of Detection and Quantification (LOQ&LOD)

Limit of Detection	Limit of Quantification
0.075µg/ml	0.192192µg/ml

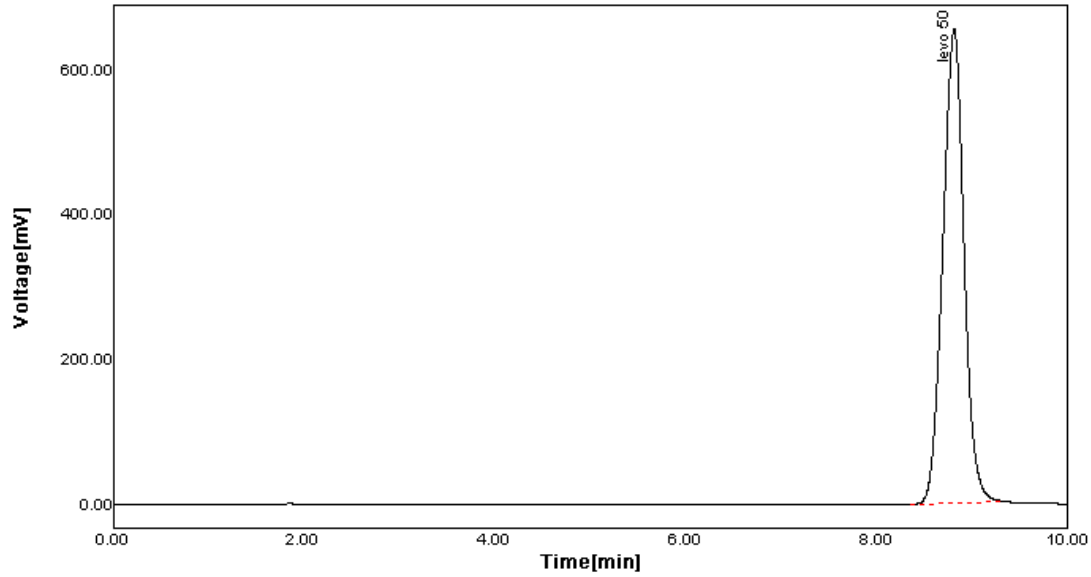

Figure: 1Atypical chromatogram for levofloxacin solution (50µg/ml)

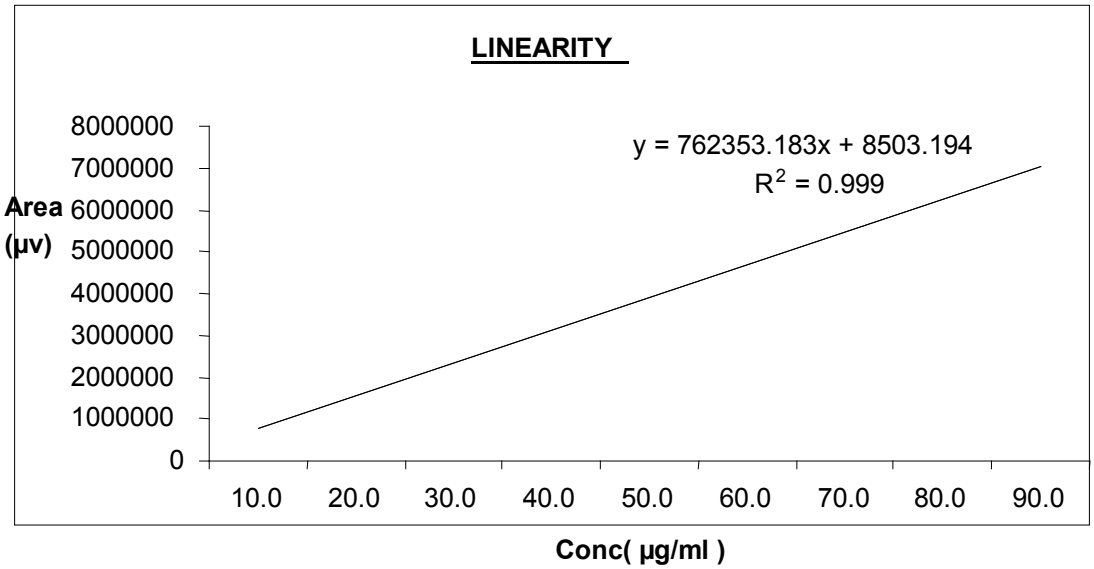

Figure: 2 Calibration curve of levofloxacin

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