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Research Article

SIMPLE AND VALIDATED UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF LEVOFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The purpose of this study was the development of an analytical methodology for the determination of levofloxacin in bulk drug, tablets and infusion using UV spectrophotometer. The simplified methodology consisted of distilled water, the λmax of the drug was found to be 289 nm. The analytical method was successfully validated in order to verify its proper linearity, accuracy, precision, limit of quantitation and limit of detection for the goal intended and its further implementation for the quantification of the active compound in the different pharmaceutical dosage form for routine analysis.

Keywords: Levofloxacin, UV Spectrophotometer, Distilled water

INTRODUCTION

Levofloxacin (Fig. 1), (*S*)-(-)-9-fluro-2,3,-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid[1], a third-generation fluoroquinolone, is the active levo-isomer of ofloxacin and is twice as active as the parent drug. It is active against both Gram-positive and Gram-negative bacteria. Levofloxacin is administrated to patients with urinary, respiratory or cutaneous infections, in 500 mg/day doses. Levofloxacin is mainly excreted in urine (>85%), in unaltered form[2]. No pharmacokinetics differences between oral or intravenous administration have been observed. It functions by inhibiting the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and topoisomerases. In Gram-positive bacteria, the stabilized complexes are between DNA and topoisomerase IV, with the drugs showing a 1000-fold selectivity for the bacterial enzyme over the corresponding enzyme in human cells. In Gram-negative bacteria, the main target for fluoroquinolones is the complex between DNA and a topoisomerase II enzyme called DNA gyrase. It is required when the DNA double helix is being supercoiled after replication and transcription. Thus, the main effects of fluoroquinolones are the inhibition of DNA supercoiling and the damage to DNA, whose synthesis is required for bacterial growth[3-5].

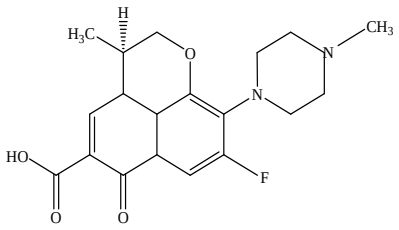

Fig. 1: The chemical structure of levofloxacin.

Some papers have described the analysis of levofloxacin by spectrophotometer[6-7] and by other analytical methods (HPLC, LC/MS, HILIC-MS/MS)[1, 8-11].

The increasing utilization of this fluoroquinolone drug as an antibacterial agent demands the development of new and alternative methods to successfully determine levofloxacin in raw material and pharmaceutical dosage forms. Therefore, an attempt to design a method of estimation, which may be superior in some context to the existing ones, was thought to be worth the effort. Thus, the aim of the study was to develop and validate analytical methods to quantify levofloxacin in bulk drug, tablets and infusion, using UV spectrophotometer, for rapid determination of which should offer simplicity, reproducibility, sensitivity, and accuracy.

MATERIALS AND METHODS

Materials and Apparatus

Levofloxacin was kindly provided as a gift sample from CIPLA, Baddi, Himachal Pradesh (India). LEVODAY (Levofloxacin tablets, 500 mg) (Zydus Cadilla, Ahmedabad, India), L-CIN IV (Levofloxacin Infusion I.P.) (Ahlcon Parenterals (India) Ltd, Rajsthan, India) were purchased from local market. Double distilled water was used as solvent. Stock standard solution of 50 µg/ml was prepared by dissolving levofloxacin in distilled water. Working standard solution of different concentration of 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml and 8 µg/ml was prepared by dilution of stock standard solution with distilled water. Stock standard solution was stable for several weeks at room temperature.

A double beam UV-Visible Spectrophotometer (UV-1800, Shimadzu, Japan) with a pair of 10 mm matched quartz cells and 1 nm bandwidth was used for the absorbance measurements. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. A centrifuge (Remi motors, Mumbai, India) was used for the centrifuge the tablet sample solution.

Standard solution and working solution preparation

Standard stock solution was prepared by dissolving 5 mg of standard drug sample in 10 ml volumetric flask (50 µg/ml) was made up by distilled water. From this stock solution (50 µg/ml) suitable working solution of different concentration of 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml and 8 µg/ml were made. The absorbance of these dilutions was measured at 289 nm (Fig. 2).

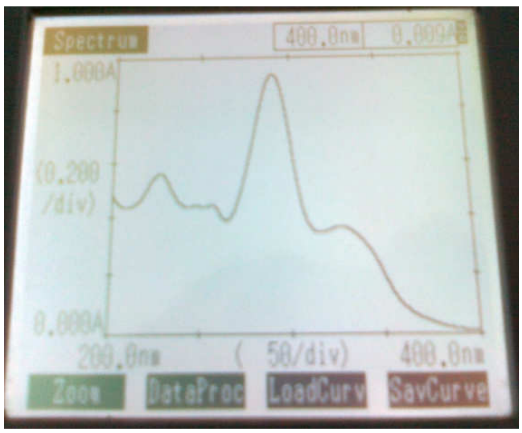

Fig. 2: Spectra of levofloxacin.

Sample Preparation (Tablet treatment)

Twenty tablets of levofloxacin were individually weighed and triturated to obtain homogeneous mixture. Then 7.25 mg of finely powder was accurately weighed using electronic balance and dissolved in 10 ml distilled water (725 µg/ml). The sample was centrifuged for 30 min and filtered through Whatman filter paper no. 40. Further dilution was made from this stock solution to get the absorbance.

Sample Preparation (Infusion treatment)

Accurately measured 1 ml of infusion equivalent to 5 mg of levofloxacin was diluted to 10 ml with distilled water (500 µg/ml). Further dilution of 3.6 µg/ml, 4 µg/ml and 4.4 µg/ml were made to get the absorbance.

Method Validation

The method analytical performance was validated by evaluation of the following parameters: linearity, intra-day and inter-day precision and accuracy, lower limit of quantitation, lower limit of detection ruggedness and recovery according to ICH guidelines[12-14].

Linearity

Standard solution containing 50 µg/ml of Levofloxacin in distilled water was prepared. Aliquots of these solutions were diluted in distilled water, to 6 different concentrations, corresponding to 0.5, 1, 2, 4, 6 and 8 µg/ml of Levofloxacin. Calibration curve with concentration versus absorbance was plotted (Fig. 3); and correlation coefficient (R^2) and regression equation for levofloxacin was 0.9998, y=0.1123x+0.0016, respectively (Table 1).

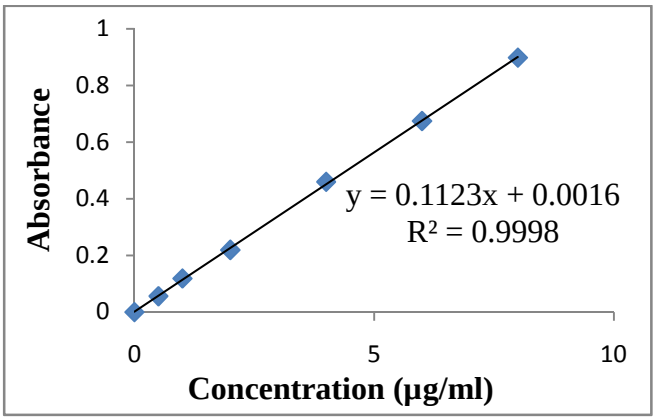

Fig. 3: Calibration curve of levofloxacin.

Table 1: Results of least square regression analysis for the estimation of levofloxacin.

Concentration (µg/ml)	Absorbance at 289 nm [Mean ± SD (n=3)]	C.V. (%)
0.5	0.056 ± 0.0010	1.78
1	0.119 ± 0.0015	1.29
2	0.219 ± 0.0012	0.53
4	0.460 ± 0.0015	0.33
6	0.674 ± 0.0010	0.15
8	0.898 ± 0.0006	0.06

Accuracy

To evaluate the accuracy of the proposed method, recovery tests were carried out with all samples. Recovery tests were performed by adding known amounts of standard solutions to sample followed by analysis using proposed method at 80%, 100% and 120% level. Working standard was added to the fixed concentration (2 µg/ml) of the tablet solution and infusion. Levofloxacin reference standard was accurately weighed and added to a mixture of tablet excipients and infusion at three different concentrations is shown in Table 2.

Precision

The precision of proposed method was evaluated through intra-day and inter-day repeatability of responses of sample solutions. All solutions were prepared fresh and precision is expressed as relative standard deviation (R.S.D.) amongst responses in each case. Inter-day and intra-day variation was taken to determine intermediate precision of the proposed methods. Different levels (low, medium, high) of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation. Percent RSD (% RSD) was found to be lower than 2% in each level (Table 3).

Ruggedness

Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot by different analyst using similar operational and environmental conditions and data is presented in Table 4.

Limit of Detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of levofloxacin by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (Table 5).

Table 2: Recovery study of levofloxacin in different dosage form.

dosage form Pharmaceutical	Levels	Concentration of drug taken (µg/ml)	Concentration of standard added (µg/ml)	Standard recovered (µg/ml)	% Recovery
Tablet	80%	2.0	1.6	3.58	99.0%
	100%	2.0	2.0	4.01	100.5%
	120%	2.0	2.4	4.37	98.5%
Infusion	80%	2.0	1.6	3.57	98.27%
	100%	2.0	2.0	3.98	99.20%
	120%	2.0	2.4	4.39	99.23%

Table 3: Precision data of levofloxacin in different levels (low, medium, high).

Amount of levofloxacin spotted (µg/ml)	Amount detected (µg/ml) (Mean ± SD)	%RSD
Intra-day (n=6)		
0.5	0.4458 ± 0.005138	1.15
4	3.7464 ± 0.005138	0.14
8	7.7508 ± 0.070378	0.91
Inter-day (n=6)		
0.5	0.4338 ± 0.004538	1.04
4	3.6465 ± 0.005138	0.15
8	7.8173 ± 0.048398	0.62

Table 4: Ruggedness data of levofloxacin by different analyst.

Concentration (µg/ml)	Analyst I	Analyst II	Mean ± SD	%RSD
0.5	0.056	0.057	0.0565 ± 0.0007	0.072
1	0.119	0.116	0.118 ± 0.0017	1.41
2	0.219	0.216	0.217 ± 0.0026	1.2
4	0.459	0.467	0.463 ± 0.0042	0.92
6	0.674	0.668	0.671 ± 0.0038	0.56
8	0.897	0.906	0.902 ± 0.0064	0.71

Table 5: Collective performance data for the analysis of levofloxacin by the proposed method.

Analytical parameters	Values
λmax (nm)	289
Beer's law limits (µg/ml)	0.5-8
Correlation coefficient (R^2)	0.9998
Regression equation (y=bx+a)	y=0.1123x+0.0016
Slope (b)	0.1123
Intercept (a)	0.0016
LOD (µg/ml)	0.044
LOQ (µg/ml)	0.134
Precision (% RSD)	>2%
Accuracy (% recovery)	98-101
Ruggedness (% RSD)	>2%

RESULTS AND DISCUSSION

The proposed method was found to be simple, accurate, precise, economical and rapid for the routine analysis of levofloxacin. Levofloxacin follows linearity in the concentration range of 0.5 to 8 µg/ml. The regression equation, y = 0.1123x+ 0.0016 was obtained from calibration curve data. The correlation coefficient (R^2) was 0.9998.The value of intercept is close to zero (0.0016), which shows good linearity of the calibration graph and obey the beer's law (Fig. 2).The accuracy of the proposed method was proved by recovery study in the commercially available formulation (Levofloxacin tablet 500 mg and Levofloxacin infusion I.P 100 ml). Percent recovery results are given in Table 2. It was found in the range of 98 to 101%. The Precision of the proposed method was checked in terms of the inter-day and intra-day time periods. Percent RSD was found to be lower than 2%, results are shown in Table 3. LOD and LOQ were found to be 0.044 µg/ml, 0.134 µg/ml respectively (Table 5). Ruggedness of the proposed method in terms of %RSD was found to lower than 2% (Table 4).

CONCLUSIONS

The method that proposed in this work for the quantitation of levofloxacin was simple, rapid, accurate and precise. The proposed method is also inexpensive due to use of distilled water for the dilution. Therefore, this method can be used for routine analysis of levofloxacin in bulk and pharmaceutical formulations like tablet, infusion.

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