Research Article

ESTIMATION AND VALIDATION OF LEVOFLOXACIN IN BULK AND EYE DROP FORMULATION BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, rapid, accurate, precise and economical UV spectrophotometric method has been developed for the determination of levofloxacin (LEV) in bulk and eye drop formulation. The method was developed using distilled water as solvent for preparing dilutions. This method obeys Beer's law in the concentration range of 10-30 µg/mL with correlation coefficient of 0.9975 and exhibiting maximum absorption at 288 nm. The method is accurate and precise as validated and there was no interference from any common pharmaceutical additives and excipients. The results of analysis were validated statistically and by recovery studies. Thus this UV method can be used in the routine quantitative analysis of levofloxacin in eye drop solutions.

Keywords: Levofloxacin (LEV), Eye drop, Interference, UV spectrophotometric method.

INTRODUCTION

Levofloxacin hemihydrate (LEV) (Fig. 1) chemically, [(-)(s)-9-fluro-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 an optically L-isomer of ofloxacin.[1] It is a broad spectrum fluoroquinolone class of antibacterial agent and effective against many gram positive and gram negative bacteria.[2,3] It is a potent inhibitor of bacterial DNA gyrase enzyme (topoisomerase II & IV), which is necessary for negative supercoiling of DNA prior to replication.[4]

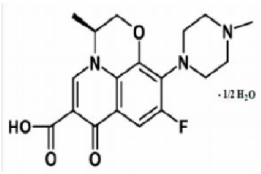

Figure 1: Structure of levofloxacin hemihydrate.

Levofloxacin hemihydrate (LEV) eye drop is available for topical treatment of eye infection by gram negative bacteria. Literature survey reveals that several methods have been developed for the quantitative determination of LEV in formulations (singly or combined) as well as in plasma and urine. These include capillary electrophoresis and UV spectrophotometry,[5-7] HPLC,[8-11] simultaneous HPTLC method with ornidazole[12] and flow injection analysis.[13]

Though LEV has been determined by various techniques in bulk, tablet (singly or combined) as cited above, no method for LEV in eye drop has yet been reported. Since there is lack of method to directly estimate LEV in eye drop formulation, this paper describes simple, rapid, accurate, precise and economical methods for determination of LEV in eye drop by zero order spectrophotometry. This is supported by the fact that after obtaining the spectra no interference was observed by the excipients for quantitative estimation.

MATERIALS AND METHODS

Instruments

UV-visible double beam spectrophotometer, JASCO V-630 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. Shimadzu AY220 balance was used for weighing the samples. All the chemicals used were of AR grade. Distilled water was used throughout the experimental work.

Materials

The commercially available eye drop, Leeflox manufactured by Centaur Pharmaceuticals (Label claim: Levofloxacin Hemihydarte 0.5% w/v) was procured from local market. All the chemicals and reagents were of analytical grade.

Selection of wavelength

The dilutions were obtained and the solutions were scanned in UV range (200-400nm) in 1.0cm cell against solvent blank. The study of spectrum reveals that LEV show a well-defined λ_{max} at 288 nm. This wavelength was selected for development of method (Fig. 1).

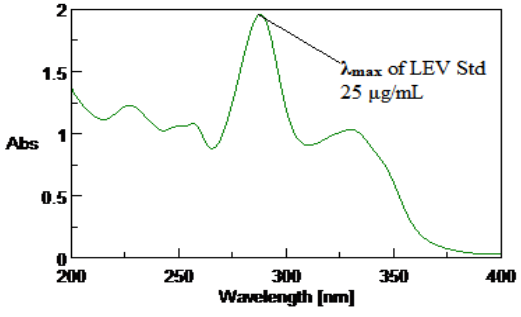

Figure 1: λ_{max} graph for Levofloxacin pure drug

Preparation of standard stock solution

Accurately weighed 10 mg of LEV pure drug taken in separate 100mL volumetric flask and dissolved with 70 mL of distilled water and shaken for 15 min and then diluted with distilled water to get 100 µg/mL standard stock solution.

Construction of calibration curve

Aliquots of standard stock solution were pipetted out and suitably diluted with distilled water to get the final concentration of 10-30 µg/mL (Overlain spectra for dilutions in linearity range is given in fig. 2). The solution was scanned in the spectrum mode from 400 nm - 200 nm wavelength range. Calibration curve was constructed by plotting the absorbance against the concentration and regression equation was computed (Fig. 3).

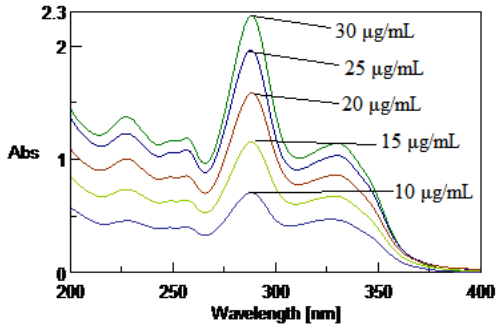

Figure 2: Spectra for various dilutions in linearity range

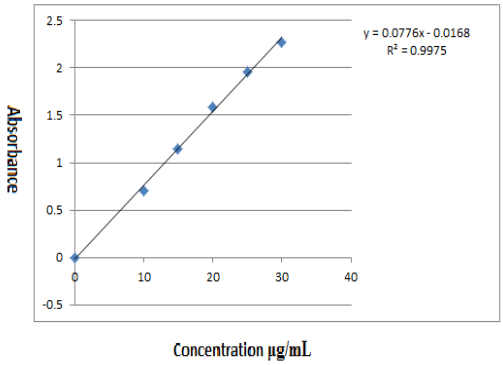

Figure 3: Calibration curve of Levofloxacin [Linearity]

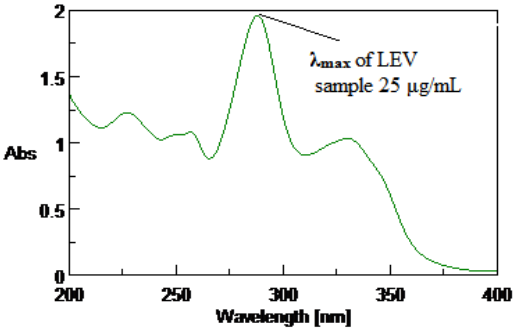

Figure 4: λ_{max} graph for Levofloxacin eye drop preparation.

Analysis of formulation

For the estimation of LEV from eye drop, a portion of the formulation was taken in separate 100 mL volumetric flask and subsequently diluted with distilled water to get the final concentration of 25 µg/mL. Evaluation was performed with double beam spectrophotometer for LEV at 288 nm. The spectrum for sample preparation is given in fig. 4.

Method Validation

Various optical parameters and regression characteristics for levofloxacin are given in table 1.

Table 1: Optical parameters and regression characteristic for LEV

Parameter	Data
Beer-Lambert's Law range (Linearity)	10-30 µg/mL
λ_{max} (nm)	288 nm
Regression equation (Y=mx + c)	Y = 0.0776x-0.0168
Slope (m)	0.0776
Intercept (c)	0.0168
R^2	0.9975

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed six times with tablet formulation. The standard deviation, coefficient of variation and standard error was calculated (Table 2).

Table 2: Analysis of formulation

Drug concentration taken (µg/mL)	Absorbance reading (at 320 nm)	% Amount found
25	1.9591	99.96
25	1.9511	99.56
25	1.9623	100.13
25	1.9498	99.49
25	1.9617	100.10
25	1.9547	99.74
Average		99.83
S.D		0.2743
COV		0.2747
S.E		0.1119

S.D: Standard Deviation, COV: Coefficient of variation, S.E: Standard Error.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level (Table 3).

Table 3: Result of recovery study

% Level of standard drug added	% Recovery	% Mean recovery ± S.D	% RSD
80%	99.69±0.92	99.75±0.92	0.9223
	100.05±1.08		
	99.53±0.76		
100%	99.23±0.69	100.01±0.74	0.799
	101.02±0.56		
	99.77±0.97		
120%	99.18±1.05	99.90±0.91	0.9109
	101.31±0.97		
	99.21±0.73		

S.D: Standard Deviation, % RSD: Relative Standard Deviation

Table No. 4: Validation data for precision study

Drug concentration taken (µg/mL)	Intraday precision		Interday precision	
	% Content*	% RSD	% Content*	% RSD
25	99.79	0.721	99.68	0.825

*Average of six determinations, % RSD: Relative Standard Deviation

Intermediate precision (Interday and Intraday)

The interday and intraday precision by the assay of the sample solution on the same day and on different days at different time intervals was carried out respectively (Table 4).

RESULTS AND DISCUSSION

The proposed method for estimation of LEV in eye drop preparation was found to be simple, rapid, accurate, precise and economical. The λ_{max} of LEV was found at 288.0 nm. Drug follows Beer-Lambert's law over the concentration range of 10-30 µg/mL giving the linear equation as y = 0.0776x - 0.0168 and R^2= 0.9975 for the proposed method. The values of standard deviation were found satisfactory and the recovery studies were close to 100%. Thus being a rapid method it can be applied in routine analysis of LEV in eye drop preparation.

The proposed method is based upon direct estimation of LEV in eye drop preparation at 288 nm. The mean percentage content of drug was found to be within the limit which is determined by taking average of six readings. The developed method was validated as per ICH guidelines for repeatability, intermediate precision and recovery studies. The precision of the method was checked in terms of Inter-day and Intra-day, where methods were repeated on six different days and also repeated on six different time periods in same day. The accuracy of the method was proved by performing recovery studies in the commercially available formulations. Moreover there is no interference from the excipients present in the formulations.

CONCLUSION

In the above developed method, there was no additional extraction or separation procedure to extract the active ingredient from the formulation and this can be explained from the fact that the spectra obtained can be overlain without much interference. The error in quantifications can be decreased by the elimination of this procedure. Hence, the developed method is simple, rapid, accurate, precise and economical for the routine estimation of LEV in its pharmaceutical dosage form (eye drop).

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