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Area under curve method development and validation for estimation of balofloxacin in bulk and tablet dosage form

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ABSTRACT

Simple, precise and economical UV - spectrophotometric method has been developed for the estimation of Balofloxacin in pharmaceutical dosage form. Method applied was area under curve (AUC) in which area under curve was integrated in the wavelength range of 280.40 – 304.0 nm. Calibration curves were plotted for method by using instrumental response at selected wavelengths and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 2-12 µg/ml for the method. Tablet formulation was analyzed and % assay determined was 99.60% – 101.69%. Accuracy and precision studies were carried out and results were satisfactory. The results of the analysis were validated statistically. Limit of detection and limit of quantitaiton were determined for method. The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Balofloxacin in pharmaceutical formulation.

KEYWORDS

Balofloxacin;
UV-spectrophotometry;
Area under curve;
Validation.

INTRODUCTION

Balofloxacin is chemically known as (±)-1-cyclopropyl-6-fluro-1, 4-dihydro-8-methoxy-7-[3-(methyl amino) piperidino]-4-oxo-3-quinolinecarboxylic acid[1]. The molecular formula is $C_{20}H_{24}FN_3O_4$, which corresponds to a molecular weight of 389.4, is a broad spectrum fluorinated quinolone antibacterial. It exhibits excellent antibacterial activity against gram positive bacteria such as multiple-drug-resistant staphylococci and pneumococci. It acts by binding to and inhibiting topoisomerase II (DNA-gyrase) and topoisomerase IV enzymes, which are responsible for the coiling and uncoiling of DNA, which is needed for bacterial cell repair and replication[2,8,9].

A detailed literature survey for Balofloxacin revealed that several analytical methods such as Spectrophotometric methods[3,4] were reported for the quantification of Balofloxacin. There are few RP-HPLC methods were reported for the determination of Balofloxacin in pharmaceutical dosage form[5-7].

The method was validated according to ICH guidelines[10]. Thus the objective of present study was to develop an applicable method for the routine analysis of

Balofloxacin in tablet formulations.

EXPERIMENTAL WORK

Material and method

Balofloxacin working standard was obtained as gift sample from Watson Pharma. The drug was used without further purification. A tablet formulation containing 100 mg of Balofloxacin was purchased from local market. Analytical grade solution was used for the experiment.

Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width ($\Delta\lambda$):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock and working standard solution

The standard stock solution of Balofloxacin was prepared by dissolving accurately weighed 10 mg of the drug in methanol and diluted to 100 mL with same solvent to obtain a final concentration of 100 μg/mL.

Method: area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths 280.4 and 304.0. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The spectrum obtained of zero order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (2-12 μg/mL) versus AUC.

Preparation of sample solution

Ten Balofloxacin tablets (100 mg each) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask and 50 mL methanol was added. After ultrasonic vibration for 10 min, the mixture was diluted to volume with methanol and filtered through Whatman filter paper (No. 41). From the filtrate an appropriate aliquot was taken in such a way that the final concentration in 10 mL is 6.0 μg/mL. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard.

Validation of method

The proposed method was validated as per ICH guidelines[10].

(a) Linearity

For the method, calibration curve was prepared on 3 different days. The calibration curve was constructed by plotting the response (y) versus the theoretical concentrations of standards (x), by using linear regression analysis. Linearity was expressed as a correlation coefficient; the value must be > 0.999.

(b) Precision

The intraday and interday precision of the proposed Spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of Balofloxacin for area under curve 4.0, 6.0, and 8.0 μg/mL and the results are reported in terms of percent relative standard deviation.

(c) Accuracy

The accuracy of the method was determined by calculating recoveries of Balofloxacin by the method of standard additions. The study was performed by spiking three known amount concentration of Balofloxacin (3.2, 4.0, and 4.8 μg/mL; ranging from 80% to 120%) into a prequantified sample solution (4 μg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring the response and by fitting these values to the straight-line equation of calibration curve.

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(d) Specificity

Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the method was said to be specific.

(e) Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot (6.0 µg/mL) in different laboratories by different analysts using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation.

RESULTS AND DISCUSSION

The molecular structure of the Balofloxacin is presented in Figure 1. Methanol was selected as the solvent for Balofloxacin because provides the highest solubility and AUC measurements. Figure 2 shows the absorption spectrum of Balofloxacin (6.0 µg/mL) in Methanol for the method. Optical characteristics of Balofloxacin were calculated by the proposed methods and presented in TABLE 1.

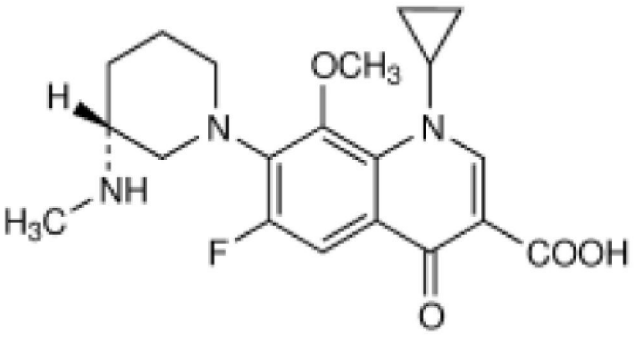

Figure 1 : Chemical structure of Balofloxacin.

TABLE 1 : Optical characteristics of Balofloxacin.

Parameters	Balofloxacin
Beer-Lambert's range(µg/mL)	2-12
λ max(nm)/ wave length range (nm)	293
Slope	0.3804
Intercept	0.2091
Correlation coefficient	0.999
Limit of detection (µg/mL)	0.19
Limit of quantitation (µg/mL)	0.59

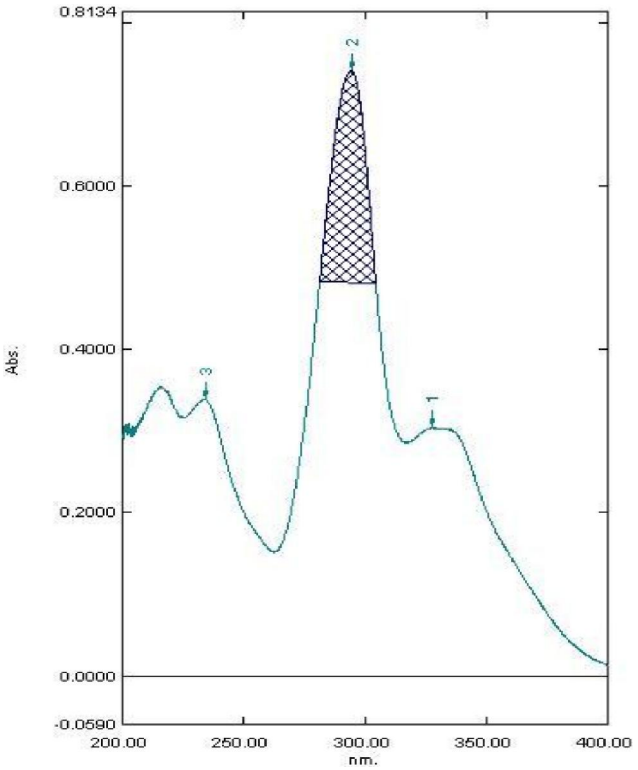

Figure 2 : Area under curve spectrum of Balofloxacin in methanol.

The intra-day and inter-day precision values (%RSD) were calculated (TABLE 3) and lying in the acceptable limits (≤2%) for Balofloxacin. The accuracy of Balofloxacin which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable limits (≤2%) (TABLE 4). This indicates that there was no interference from the excipients present in the dosage form. Ruggedness of proposed method was determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (TABLE 5).

TABLE 2 : Assay results of commercial Balofloxacin tablet.

Balofloxacin marketed formulation	Label claim/Tablet (Balokem)	% Recover*	% RSD
Tablet	100 mg	100.88%	0.78

***Average of three determinations**

TABLE 3 : Precision.

Conc. µg/Ml	Intraday		Interday	
	% Recovery	% RSD	% Recovery	% RSD
4	99.74	1.1226	99.74	0.9806
6	99.81	1.0573	99.64	0.6239
8	99.73	0.7330	99.94	1.0688

TABLE 4 : Accuracy.

Nominal Value	Initial amt.	Added amt.	% Recovery	% RSD
80	4	3.2	100.52	1.06
100	4	4	99.69	1.12
120	4	4.8	100.36	1.19

TABLE 5 : Ruggedness.

Analyst	Amount found of Balofloxacin [%]	%RSD [n=3]
I	99.65	1.07
II	100.20	1.16

n= no. of estimations

CONCLUSION

Method that was developed for the determination of Balofloxacin based on different analytical techniques, UV-Spectrophotometric, AUC method. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Balofloxacin. The proposed Spectrophotometric method will not replace the presently known methods available for the analysis of Balofloxacin. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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