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Research Article

Determination of Balofloxacin in Pharmaceutical formulation by Zero, First and Second order Derivative Spectrophotometric methods

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ABSTRACT

In this study, zero, first and second order derivative spectrophotometric methods were developed for quantitative determination of Balofloxacin in pharmaceutical preparation. In zero order spectrophotometry, absorbance values were measured at 286 nm. In first derivative spectrophotometry, amplitude values were measured at 295 nm. In second derivative spectrophotometry, amplitude values were measured at 266 nm. Parameters such as linearity, precision, accuracy were studied according to the International Conference on Harmonization Guidelines. Calibration curves were linear between the concentration range of 10-50 µg/ml, 2-10 µg/ml and 10-50 µg/ml for zero, first and second order derivative spectrophotometric methods. The RSD values of system and method precision for Balofloxacin were less than 2%, the mean recovery value of Balofloxacin was in between 98-102% for pharmaceutical preparation. All the methods developed were successfully applied to a tablet formulation and the results were found to be within the limits.

Keywords: Anti bacterial; Balofloxacin; First and second derivative spectroscopy; Fluoroquinolone; UV spectroscopy.

INTRODUCTION

Balofloxacin (Figure-1) [1-Cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy- 7-(3-methylamino- piperidin -1-yl)-4-oxoquinoline-3-carboxylic acid; Balofloxacin (BLX) is the fourth generation of a new class of synthetic antibacterial fluoroquinolone agents. BLX was first commercialized in Korea in 2002 (Alksne.L, 2003). It has a broad antibacterial spectrum, ranging from gram-positive bacteria to gram-negative bacteria. BLX exhibited excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneumococci (Ito T. *et al.,* 1992; Gohara.Y *et al*., 1993). The methoxy group at the 8-position reduces photoallergic responses (Matsumoto.M *et al*., 1992; Marutani.K *et al*., 1998). BLX was largely (70–80% of the dose) excreted via urine as the unchanged chemical constitution (Nakagawa.T *et al*., 1995; Kozawa.O *et al*., 1996). Several analytical techniques have been utilized for the determination of BLX including high-performance liquid chromatography with spectrofluorimetric detection (HPLC–FLD) (Nakashima.M *et al*., 1995), High-performance liquid chromatography – electrospray ionization–mass spectrometry (HPLC–ESI–MS) (Bian.Z.W *et al*., 2007;Qi.X.J *et al*., 2006) , and fluoro spectrophotometry (Qi X.J *et al*., 2006). The present study reports newly developed zero, first and second order derivative spectroscopy for estimation of BLX in bulk and pharmaceutical formulations. The developed UV- spectrophotometric methods is easy to handle and requires less time for the analysis. It is also a simple, highly rapid and economic friendly method.

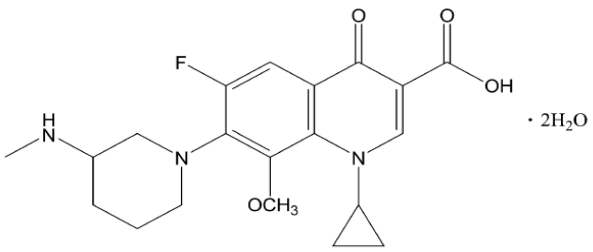

Figure 1: Chemical structure of Balofloxacin

MATERIALS AND METHODS

Instruments

A Perkin elmer double beam spectrophotometer (model lamda 25) with 1 cm matched quartz cell used for all spectral measurements with λ25 software

Chemicals

Balofloxacin reference standard (Abott India Limited, Goa) having a potency of 99.8%w/w.

Solutions

Stock solution 1mg/ml is prepared by dissolving pure drug in distilled water.

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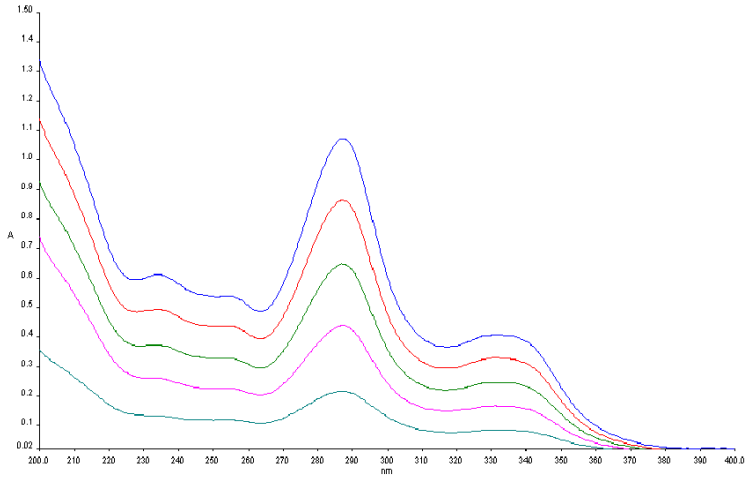

Figure 2: Zero order UV spectrum of Balofloxacin

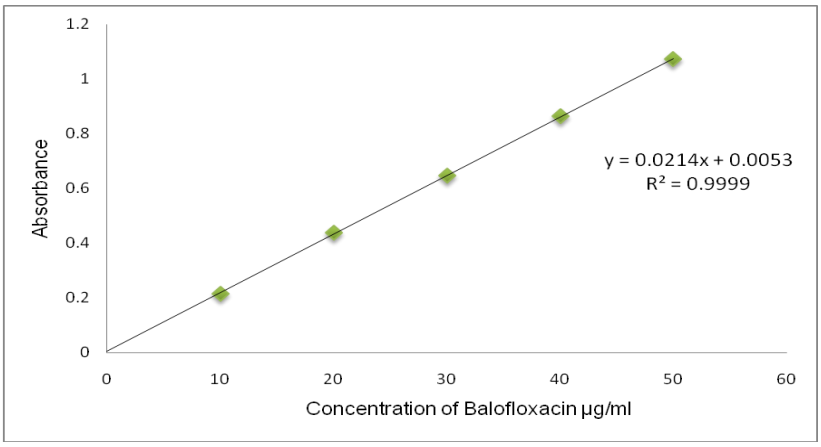

Figure 3: Zero order Calibration curve

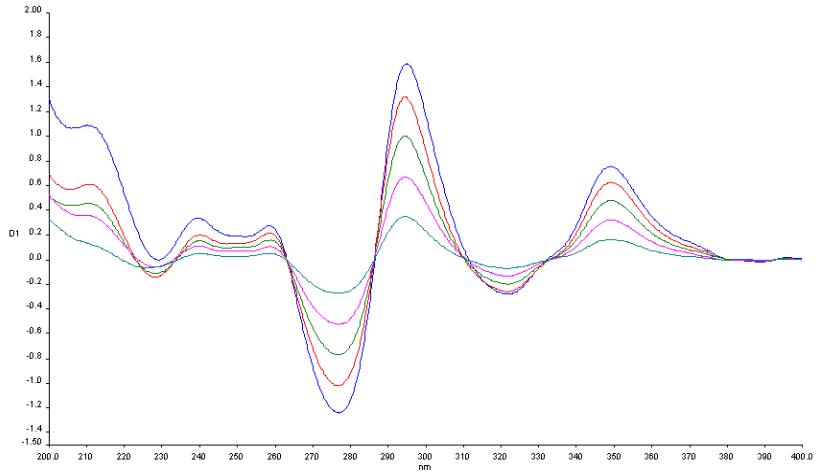

Figure 4: First order UV spectrum of Balofloxacin

Procedure

Determination of BLX by simple, first and second derivative spectrophotometry:

The zero order absorption spectrum of pure BLX was recorded between 200nm-400nmfor spectrophotometric determination and calibration graph was also obtained. The λmax was obtained at 286nm.The first and second derivative spectra were plotted with delta (amplitude) values against concentration. Calibration graphs were obtained at the selected wave length of the first and second derivative spectra with the aim of best linearity and maximum absorption.

Validation

Linearity and range

Linearity of the concentrations was taken in the range of 10-50µg/ml, 2-4µg/ml, and 10-50µg/ml for zero, first and second derivative spectroscopy respectively.

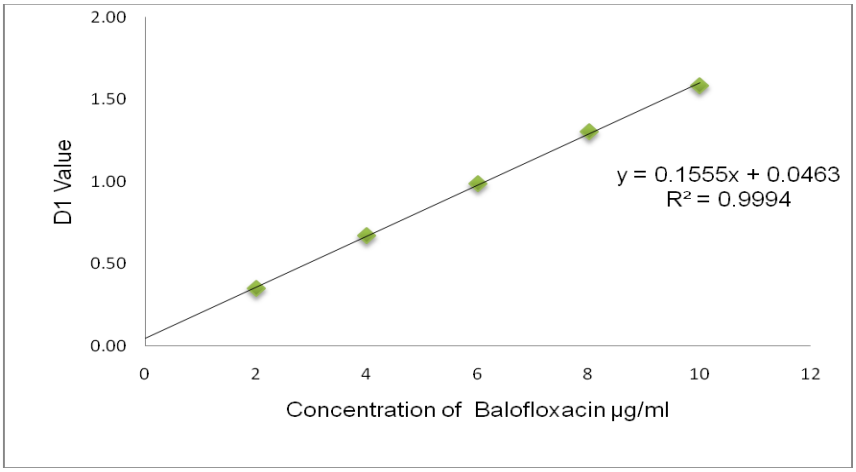

Figure 5: First order Calibration curve

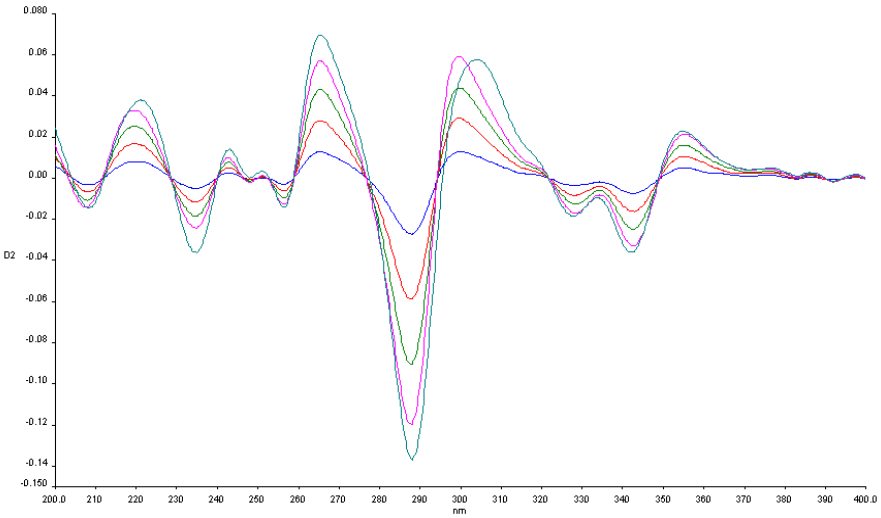

Figure 6: Second order UV spectrum of Balofloxacin

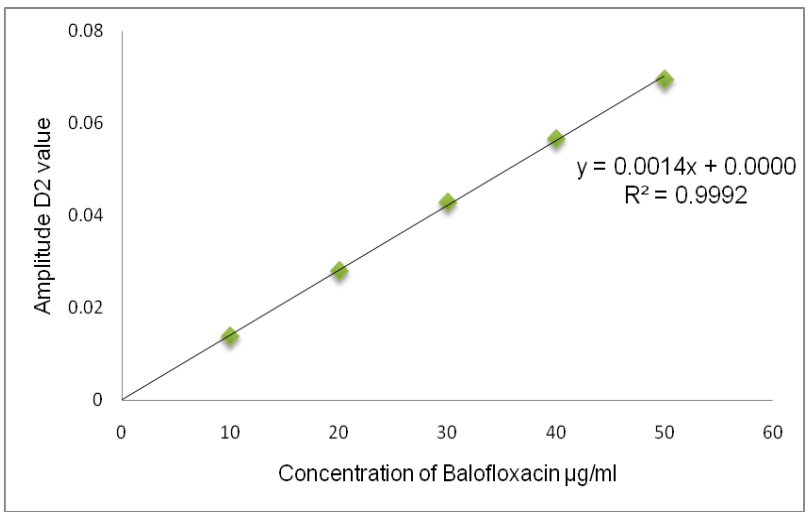

Figure 7: Second order Calibration curve

Accuracy

Accuracy of proposed method from excipients was determined by recovery experiments. Recovery experiments were carried out in three levels of concentration. The amounts of standard recovered were calculated in the terms % recovery.

Precision

It is expressed as the percentage relative standard deviation (%RSD) which is calculated as per the following expression:

$$\%\ \text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Average}}\ \text{X}\ 100$$

Table 1: System suitability parameters

Parameters	Zero order mode	First order derivative (D1) mode	Second order derivative (D2) mode
Wave length measured (nm)	286	295	266
Linearity range (µg/ml)	10- 50	2 - 4	10- 50
Correlation Coefficient	0.9999	0.9994	0.9992
LOD	1.0200	0.3050	1.6827
LOQ	3.0908	0.9242	5.0989

Table 2: Recovery Studies

Parameters	Zero order mode			First order derivative (D1) mode			Second order derivative (D2) mode		
Accuracy level (%)	80	100	120	80	100	120	80	100	120
Amount added (µg/ml)	10	10	10	3	3	3	10	10	10
Amount present (µg/ml)	14	20	26	1.8	3	1.2	14	20	26
Amount recovered (µg/ml)	23.88	30.04	35.96	4.8	5.99	7.28	24.19	30.06	36.55
% Recovery	99.16	100.19	99.85	99.94	99.78	101.9	101.32	100.32	102.13

Table 3: System Precision studies

Parameters	Zero order mode	First order derivative (D1) mode	Second order derivative (D2) mode
Mean (Absorbance and Amplitude value)	0.6485	1.0007	0.04325
SD	0.0057	0.0023	0.0001
% RSD	0.88	0.2336	0.1981
N	6	6	6

System precision

The system precision is performed on standard solutions by measuring the absorbance, amplitude values of first and second derivative spectroscopy at 30µgml, 6µg/ml and 30µg/ml respectively for six times. The system precision was determined by calculating the percentage Relative Standard Deviation (% RSD).

Method Precision

The method precision is performed by measuring the absorbance and amplitude values for D1 & D2 of six assay sample solutions. The method precision was determined by calculating the percentage Relative Standard Deviation (% RSD).

Assay sample preparation

The average tablet mass was calculated from the mass of 10 tablets of Baloforce (100 mg BLX tablet, which was composed of BLX and some common excipients). They were then finely ground, homogenized and portion of the powder equivalent to 100mg was weighed accurately, transferred into a 100 ml measuring flask, 50ml of distilled water was added, sonicated for five minutes and diluted to scale with distilled water, mixed well and then filtered through a Whatman No 42 paper. Approximate dilutions were made at concentrations of 30µg/ml, 6µg/ml and 30µg/ml with distilled water. Zero, first, and second and order derivative spectra were recorded against distilled water.

RESULTS AND DISCUSSION

Linearity

By following the linearity Zero, first and second derivative spectroscopy were determined. From this R^2 values are obtained as 0.9999, 0.9994, and 0.9992 for zero, first and second derivative and the values are given in Table 1 and in Fig 2-7.

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ of BLX by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (Table 1).

Accuracy

The mean absolute recovery of balofloxacin is between 98-102%, the values were given in Table 2.

Table 4: Method Precision studies

Parameters	Zero order mode	First order derivative (D1) mode	Second order derivative (D2) mode
Mean (% Assay)	99.90	101.09	100.82
SD	0.2842	0.2824	0.4003
% RSD	0.2845	0.2793	0.3971
N	6	6	6

Table 5: Assay of Balofloxacin

Parameters	Zero order mode	First order derivative (D1) mode	Second order derivative (D2) mode
Mean (% Assay)	99.80	100.86	100.68
SD	0.1740	0.1740	0.1740
% RSD	0.1743	0.1743	0.1743
N	3	3	3

Table 6: Comparison of method precision results by F-test

Methods	Calculated F value	T Table value
Zero with first order derivative	1.02	5.05
Zero with second order derivative	0.49	5.05
First order derivative with Second order derivative	0.50	5.05

Precision

By the precision studies (system precision and method precision) the relative standard deviation values were obtained as less than 2%, the values were given in Table 3 and 4.

Assay

By performing assay the amount of BLX present in tablets was estimated and the results are shown in Table 5

Comparison of the methods

Zero, first and second order derivative spectrophotometric methods were applied for the determination of the commercial tablet (Table 4). The results show the high reliability and reproducibility of three methods. The best results obtained at 286 nm, 295 nm, and 266nm for zero, first and second order derivative spectrophotometric methods were statistically compared using the F-test. At 95% confidence level, the calculated F-values do not exceed the theoretical values (Table 6). Therefore, there is no significant difference between zero, first and second order derivative spectrophometric methods.

CONCLUSION

Finally with the above results it is concluded that the developed method is simple, rapid and accurate which can be applied to the estimation of BLX in bulk and pharmaceutical formulations with minimum errors. Also, no significant difference was found between the proposed spectrophotometric methods by F-test. Therefore, developed methods can be recommended for routine and quality control analysis of BLX.

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