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Research Paper

Analytical Methods for Determination of Balofloxacin in Tablets

Chemistry

KEYWORDS : Balofloxacin, Spectrophotometric, Analytical method validation, Ceric sulphate, Methyl orange, Indigo carmine

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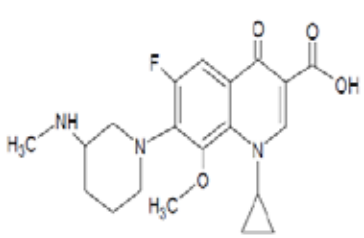
ABSTRACT

Two new, simple, accurate and sensitive spectrophotometric methods has been developed and validated for the analysis of balofloxacin in tablet dosage formulations. The drug undergoes oxidation with a known excess of cerium (IV) sulphate in acidic medium and the residual oxidant is determined by reaction with fixed concentration of methyl orange (λ max 511 nm) in first method and with indigo carmine (λ max 613 nm) in the second method. The two methods have been validated separately. The Beer's law was obeyed in the range of 0.1-2.5 µg/ml with first method and 1-7µg/ml for the second method, with good correlation coefficient of 0.9970 and 0.9980 respectively. The method was validated in terms of accuracy and precision and successfully applied to the determination of the drug in its pharmaceutical formulations. The amount of balofloxacin determined by the proposed method conformed with the label claim of the formulations.

Introduction

Balofloxacin (BLFX), 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin 1-yl)-4-oxoquinoline-3-carboxylic acid (Punam & Vandana, 2011), is a broad spectrum fourth generation fluoroquinolone 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 (Ross, Elkinton, and Riley, 1992). Several analytical methods such as UV spectrophotometric method (Punam & Vandana, 2011), (Ashok Reddy & Chandra Sekhar, 2012), HPLC method in biological fluids (Nakagawa, Ishigai, Hiramatsu, Kinoshita, Ishitani, Ohkubo, and Okazaki, 1995), HPLC method in human plasma with solid extraction (Deng, Xiao, Zhang, Zhang, and Tang, 2007),RP-HPLC (Nyola & Jeyabalan 2012), RP-HPLC with fluorescence detection(Yin, Tao, Jing-wang, Yun-biao & Long-shan, 2007) HPLC-electrospray ionization mass spectroscopy (Bian, Tian, Zhang, Xu, Li, and Cao, 2007) have been developed for determination of balofloxacin. In the present study a simple, rapid and reliable visible spectrophotometric method has been developed and validated for linearity, accuracy, precision and specificity. The method was extended for determination of balofloxacin in the marketed tablet formulation (Baloforce™ Tablet manufactured by Mankind Pharma, New Delhi, India).



Balofloxacin

Instrumentation:

Perkin Elmer Lambda 25 uv/vis spectrophotometer was used for recording the absorbance(Method A) and Labindia Analytical UV 3000 uv/vis spectrophotometer (Method B). Reference standard of Balofloxacin was kindly received from Cirex Pharmaceuticals (P) Ltd, Hyderabad, India. All the solvents used were of analytical grade.

Method:

Preparation of standard balofloxacin solutions (10 µg/ml and 20 µg/ml)

A 1 mg/ml of stock solution was prepared in a 10 ml volumetric flask by dissolving 10 mg of balofloxacin in methanol , diluting to the mark with the same solvent. This was further diluted with the same solvent to yield 10 µg/ml and 20 µg/ml of working standard solution.

Cerium (IV) Sulphate (250 µg/ml):

A 0.01 g/ml cerium (IV) sulphate stock solution was prepared by dissolving 0.5 g of the chemical in 1 M sulphuric acid and transferred into 50 mL volumetric flask and diluting to the mark with the same acid. Stock solution was diluted appropriately with 1 M sulphuric acid to yield 250 µg/ml cerium (IV) sulphate solution.

Methyl Orange (50 µg/ml):

Methyl orange, 50 mg was dissolved in water in a 100 ml volumetric flask, diluted to the mark with the same solvent and filtered to obtain a stock solution of concentration 500 µg/ml. This was then diluted 10-fold to obtain a working concentration of 50 µg/ml.

Indigo carmine (200µg /ml):

A 1000 µg /ml solution was first prepared by dissolving 100 mg of indigo carmine in water and diluting to the mark in a 100 ml volumetric flask with the same solvent. This was further diluted to get 200 µg /ml solution with water.

Sulphuric acid (5 M):

This was prepared by adding 274 ml of concentrated sulphuric acid to 726 ml water with cooling.

Standard Calibration Plot:

Method 1 (Methyl orange):

Aliquot solutions ranging from 0.1-2.5 ml of standard 10 µg/ml balofloxacin solution were transferred into a series of 10 ml volumetric flasks and the total volume was adjusted to 5 ml by adding water. Into all the flasks, 1 ml each of 5 M sulphuric acid and 250 mg/ml cerium (IV) sulphate solutions were added, mixed well and kept aside for 10 min with occasional swirling. Then, 1 ml of methyl orange solution was added and the volume made up to the mark with water and mixed. The absorbance of each solution was measured at 511 nm against a water blank after 5 min.

Method 2 (Indigo Carmine):

Aliquot solutions ranging from 0.5-3.5 ml of standard 20 µg/ml balofloxacin solution were transferred into a series of 10 ml volumetric flasks and the total volume was adjusted to 5 ml by adding water. To each flask, 1 ml of 5 M sulphuric acid and 1 ml of 500 mg/ml cerium (IV) sulphate solutions were added in that order, mixed well and set-aside for 10 min. Then, 1 ml of indigo carmine solution was added and the volume made up with wa-

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ter. The absorbance of each solution was measured at 613 nm against a water blank after 5 min.

Estimation of Balofloxacin from tablets

10 tablets of (*Baloforce*) containing 100 mg of the active ingredient in each tablet was weighed and powdered. Tablet powder, equivalent to 25 mg of balofloxacin was transferred to 25 ml of volumetric flask and sonicated using 5 ml of 0.1N HCl at ambient temperature for 15 min. The resulting solution was filtered using whatman filter paper no. 42 and volume of solution diluted up to the mark with 0.1N HCl. It was further diluted to get the concentration of 10 µg/ml and 20 µg/ml.

Method 1: Different aliquots (0.1-2.5 ml) of a sample 10 µg/ml tablet solution were transferred into a series of 10 ml volumetric flasks and the total volume was adjusted to 5 ml by adding water. Into all the flasks, 1 ml each of 5 M sulphuric acid and 250 mg/ml cerium (IV) sulphate solutions were added, mixed well and kept aside for 10 min with occasional swirling. Then, 1 ml of methyl orange solution was added and the volume made up to the mark with water and mixed. The absorbance of each solution was measured at 511 nm against a water blank after 5 min.

Method 2: Different aliquots (0.5-3.5 ml) of a 20 µg/ml sample solution were accurately measured into a series of 10 ml volumetric flasks and the volume was adjusted to 5 ml by adding water. To each flask, 1 ml of 5 M sulphuric acid and 1 ml of 500 mg/ml cerium (IV) sulphate solutions were added in that order, mixed well and set-aside for 10 min. Then, 1 ml of indigo carmine solution was added and the volume made up with water. The absorbance of each solution was measured at 613 nm against a water blank after 5 min.

Results and Discussion:

The property of cerium (IV) sulphate to cause oxidation of balofloxacin and bleach the colour of methyl orange and indigo carmine dyes have been used for the indirect spectrophotometric assay of balofloxacin. In both methods, the drug was reacted with a measured excess of cerium (IV) sulphate in acid medium and the unreacted oxidant was determined by reacting with either methyl orange or indigo carmine followed by absorbance measurement at 511 or 613 nm. In either method, the absorbance increased linearly with increasing concentration. BLFX, when added in increasing amounts to a fixed amount of cerium (IV) sulphate, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of either dye was added to decreasing amounts of oxidant, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λmax with increasing concentration.

Optimization of Reagents concentrations:

Validation studies were carried out to fix the maximum concentrations of the dyes that could be be used for optimum performance, and these were found to be 1 ml each of 50 and 200 µg/ml for methyl orange and indigo carmine in method 1 and method 2 respectively. A cerium (IV) sulphate concentration of 250 µg/ml was found to destroy the pink colour due to 50 µg/ml of methyl orange, whereas in the case of 200 µg/ml indigo carmine, 500 µg/ml cerium (IV) sulphate was sufficient to bleach the blue colour in acid conditions. Hence, different amounts of BLFX were reacted with 1 ml of 250 µg ml-1 oxidant in Method 1 and 1 ml of 500 µg ml-1 oxidant in Method 2 before determining the residual cerium (IV) sulphate as described under the respective procedures. The reaction was carried out in sulphuric acid medium. The highest intensity was obtained with 5M sulphuric acid. Hence 1 ml of 5 M acid was used to perform the assay procedures.

For quantitative reaction between balofloxacin and cerium (IV) sulphate reaction time of 10 min was validated in both methods. The standing time of 5 min was necessary for the bleaching of dye colour by the residual oxidant. The colour developed was stable for 3 hr in Method 1 and 2 hr in Method 2.

Further, with optimized conditions, both the methods were validated for linearity, accuracy, precision, sensitivity, reproducibility and stability of colour. Recovery studies were carried out by mixing standard solutions of the drug at 3 different levels for Method 1 and Method 2, with previously analyzed tablet samples of balofloxacin. The results of the validation study and recovery study is presented in Table 1 and Table 2 respectively.

Both the methods, one with methyl orange and the other with indigo carmine produced good results when applied to the formulation analysis and conformed to the label claim of the formulation. Hence they can be applied for routine analysis of balofloxacin in tablet formulations.

Table 1: Validation Parameters

Parameters	Observations	
	Method 1	Method 2
Linearity	0.1-2.5 µg/ml	1-7 µg/ml
Precision	0.0722 %RSD	1.744 % RSD
Sandell's sensitivity	2 X 10^{-3}µg/cm^2	5 X 10^{-1} µg/cm^2
Equation of linearity graph Slope: Intercept:	0.266x+0.101 0.266 0.101	0.006x +0.005 0.006 0.005
Stability of Colour	3 hr	2 hr

Table 2: Results of Recovery Study

Methods	Std. Conc (in µg/ml)	Sample Conc (in µg/ml)	Absorbance at 511 nm (method A) 613nm (method B)	Concentration as per standard graph	% of Std Recovery (in %)
1	0.75	1	0.5724	1.77	101.14%
	1	1	0.6411	2.03	101.5%
	1.25	1	0.6947	2.23	99.11%
2	0.75	1	0.0170	1.72	98.28%
	1	1	0.0192	2.05	102.50%
	1.25	1	0.0204	2.23	99.11%

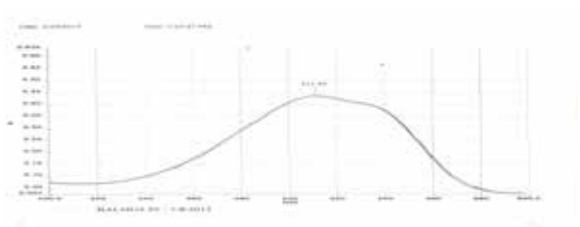

Fig 1: Absorption maxima of the Colour (Method 1)

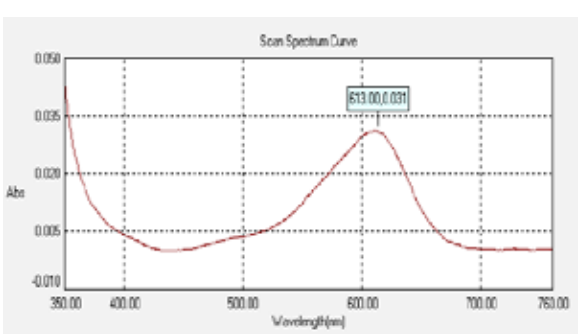

Fig 2: Absorption maxima of the Colour (Method 2)

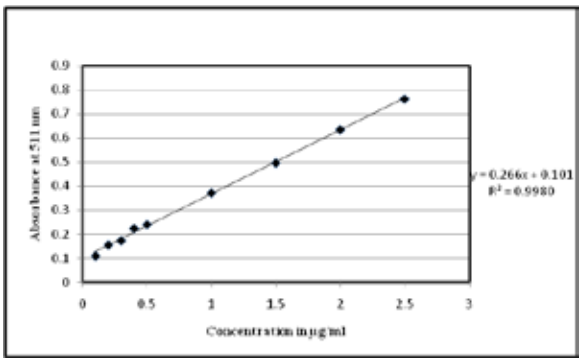

Fig 3: Standard Calibration Plot (Method 1)

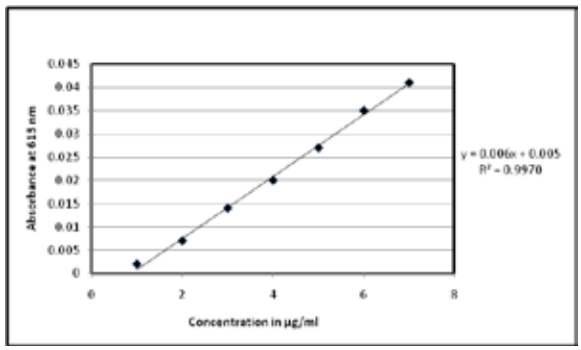

Fig 4: Standard Calibration Plot (Method 2)

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