

SPECTROPHOTOMETRIC DETERMINATION OF BALOFLOXACIN BY ION-PAIR COMPLEXATION REACTION IN THE BULK AND TABLET DOSAGE FORMS

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Received: 19 July 2013, Revised and Accepted: 2 September 2013

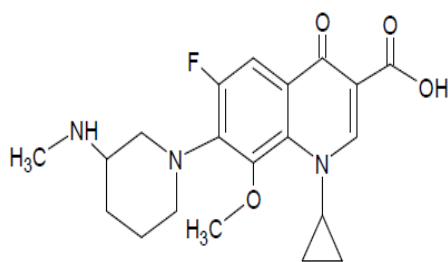
ABSTRACT

A rapid, sensitive and accurate spectrophotometric method for the determination of balofloxacin, an antibacterial fluoroquinolone has been developed and validated for its analysis in tablet formulation. In this method, balofloxacin is allowed to react with the bromocresol green (BCG) dye in aqueous acidic buffer. The yellow colored complex species was formed that was extracted into organic solvent, dichloromethane. The formed complex is quantified spectrophotometrically at the absorption maxima at 412 nm. Linearity was observed over the concentration range of 4-30 µg/ml, with the calibration curve having a good coefficient of correlation of 0.9940. The validated method was accurate as found from recovery of 96.65%-101.26% when applied for marketed tablet formulation of balofloxacin, and thus can be used in quality control and its routine analysis.

Keywords: Balofloxacin, Spectrophotometric, Analytical method validation, Citrate buffer, Bromocresol green, Anhydrous sodium sulphate

INTRODUCTION

Balofloxacin (BLFX), 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin 1-yl)-4-oxoquinoline-3-carboxylic acid [1], 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 [2]. Several analytical methods such as UV spectrophotometric method [1, 3], HPLC method in biological fluids [4], HPLC method in human plasma with solid extraction [5], RP-HPLC [6], RP-HPLC with fluorescence detection [7], HPLC-electrospray ionization mass spectroscopy [8] 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

MATERIALS AND METHODS

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

Preparation of standard drug solution

A 1 mg/ml of stock solution was prepared in a 10 ml volumetric

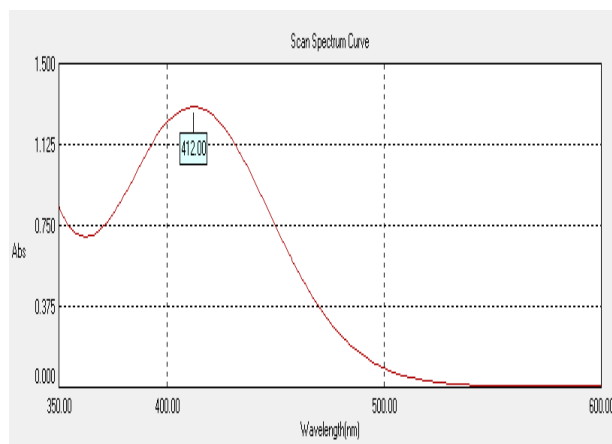
flask by dissolving 0.01 g of balofloxacin in 0.1N HCl (hydrochloric acid), diluting to the mark with the same acid. Then it was appropriately diluted to obtain a concentration of 100 µg/ml.

Preparation of 0.4M citrate buffer

0.4M citrate buffer was prepared by mixing various volumes of 0.4M citric acid and 0.4M sodium citrate solution to the required pH 3.

Bromocresol green solution**Standard Calibration Plot**

Aliquots of the stock solution ranging from 0.4 ml to 5 ml were taken in separate 100 ml separating funnels and 1 ml each of 0.4M citrate buffer pH 3 was added, followed by 2 ml of bromocresol green indicator solution which was used as a reagent was added, mixed well, further volume brought to 10 ml with distilled water. The funnels were shaken vigorously with 10 ml dichloromethane for 2 min and allowed to stand for clear separation of the two phases. The yellow coloured organic phase was collected by passing it over the anhydrous sodium sulphate powder. The absorbance of the organic phase was measured at 412 nm against a reagent blank simultaneously.

**Fig.1: Absorption spectra of ion-pair complex**

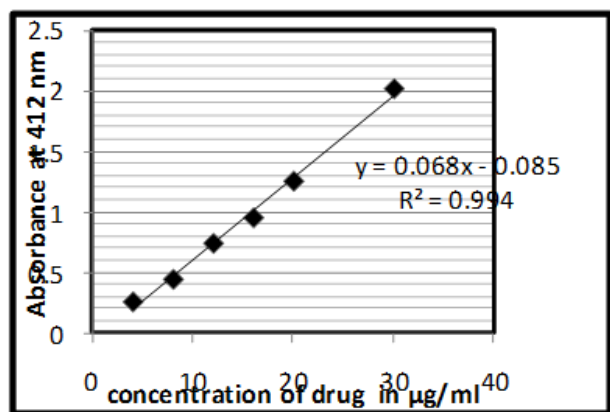


Fig. 2: Standard Calibration Plot

Further, with optimized conditions, the proposed method was validated for linearity, accuracy, precision, sensitivity, reproducibility and stability of colour. Recovery studies were carried out by mixing standard solutions of the drug at 5 different levels (66% to 133%) 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.

Table 1: Validation parameters

Parameters	Observations
Linearity	4-30 µg/ml
Precision	0.36% RSD
Sandell's sensitivity	$15.91 \times 10^{-3} \mu\text{g}/\text{cm}^2$
Equation of linearity graph	$0.0682X + 0.0854$
Slope:	0.0682
Intercept:	0.0854
Stability of Colour	24 hr

Table 2: Results of recovery study

Standard Conc. (in µg/ml)	Sample Conc. (in µg/ml)	Absorbance at 412 nm	Total concentration as calculated from linearity graph (in µg/ml)	% Std Recovery
8	12	1.190	19.33	96.65%
10	12	1.350	21.66	98.45%
12	12	1.512	23.66	98.58%
14	12	1.766	26.33	101.26%
16	12	1.825	28.00	100.00%

(*Average of three readings)

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. Different aliquot volumes of this solution was taken in 100 ml separating funnel to which 1 ml each of 0.4 M citrate buffer pH 3 was added, followed by 2 ml of bromocresol green indicator solution was added, mixed well, further brought to volume of 10 ml with distilled water. The funnels were shaken vigorously with 10 ml dichloromethane for 2 min and allow to stand for clear separation of the two phases. The yellow coloured organic phase was collected by passing it over the anhydrous sodium sulphate powder. The absorbance of the organic phase was measured at 412 nm against a reagent blank simultaneously.

RESULTS AND DISCUSSION

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH. The ion-pair extractive spectrophotometry has been applied to the estimation of numerous compounds; possessing basic moieties (secondary or tertiary amino group) by using an anionic dye as a reagent and organic solvent as an extractant. [9].

The charge-transfer (CT) reactions are quantitative and produce coloured solutions for some drugs and therefore can be explored for colorimetric techniques. Few drugs can be determined by colorimetric method based on formation of colored CT complexes with electron acceptors. BCG is an acid sulphonaphthalein dyestuff. The color of such dyes is due to opening of lactoid ring and subsequent formation of quinoid group. It behaves as a strong electron acceptor due to the presence of the strong electron withdrawing sulphonic acid group conjugated with the aromatic ring system [10]. The organic layer after extraction obtained was crystal yellow in colour. The developed method was found to show linearity over the concentration range of 4 – 30 µg/ml. Accuracy was determined from recovery study at 5 different levels of 66 %, 82%, 100%, 118% and 133% by adding standard solution of balofloxacin

to previously analysed tablet samples of balofloxacin. Average recovery of 96.65% to 101.26% indicated accuracy of the method. Sandell's sensitivity was determined and found to be $15.91 \times 10^{-3} \mu\text{g}/\text{cm}^2$. The colour was stable up to 24 hr. The results of assay showed that the amount of drug determined by new method was in good agreement with the label claim of formulation.

CONCLUSION

From the above results it can be concluded that the new visible spectrophotometric method for balofloxacin is simple, rapid, accurate, precise and economical. Hence the method can be applied for quantitative analysis of balofloxacin in bulk and pharmaceutical tablet dosage forms.

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