



Development and Validation of a Stability Indicating RP-HPLC Method for Balofloxacin

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<http://dx.doi.org/10.13005/ojc/300453>

(Received: September 26, 2014; Accepted: November 20, 2014)

ABSTRACT

The present work describes the development of a stability indicating high performance liquid chromatographic (HPLC) method for the determination of Balofloxacin as bulk drug and as pharmaceutical formulation. The drug was separated using Phenomenex (250 x 4.6mm, 5 μ m particle size) ODS column at a flow rate of 1 mLmin⁻¹ and a SPD 20 A UV detector to monitor the eluate at 298 nm. The isocratic method used a mobile phase consisting of methanol and phosphate buffer of pH 6.8 in the ratio 70:30. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.9998$ in the working concentration range of 5–100 μ g mL⁻¹. The LOD and LOQ were 90 and 273 ng mL⁻¹ respectively. The drug was subjected to stress degradation using acid, alkali, hydrogen peroxide, dry heat, wet heat and uv light. The standard drug peaks were well resolved from the degradation products' peaks with significantly different retention time (Rt).

Key words: HPLC, degradation, stability-indicating, Balofloxacin (BLFX).

INTRODUCTION

Balofloxacin (BLFX), chemically named 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylamino-piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid, is the fourth generation of a new class of synthetic antibacterial fluoroquinolone agents. It has broad antibacterial spectrum, ranging from gram-positive bacteria to gram-negative bacteria. BLFX exhibited excellent antibacterial activity against gram-positive bacteria such as

multiple-drug-resistant staphylococci and pneumococci¹⁻³. The methoxy group at the 8-position reduces photoallergic responses^{4,5} [8,9]. It is prescribed for infective ophthalmitis, sinusitis, chronic bronchitis, pneumonia, skin infections etc. Like other new fluoroquinolones, the bactericidal action of BLFX results from inhibition of the enzymes topoisomerase (II) (DNA gyrase) and topoisomerase (IV)⁶. It was largely excreted via urine as the unchanged chemical constitution.⁷ Different methods have been reported in the

literature for the analysis of BLFX, based on high performance liquid chromatography,⁸⁻¹⁰ HPLC-electro spray ionization - mass spectrometry¹¹, fluorospectrophotometry¹² and UV spectrophotometry^{13,14}. Although these analytical techniques have been successfully applied to analysis of BLFX in pharmaceutical and biological samples, no stability indicating HPLC method is reported so far. As the International Conference on Harmonization (ICH) guidelines require the development of stability-indicating methods (SIAMs) for drug assays in stability tests¹⁵ in the present investigation a simple stability indicating analytical methodology is developed.

EXPERIMENTAL

Materials and Reagents

Balofloxacin API was available as Gift sample from Cirex Pharmaceuticals Ltd, Hyderabad. Methanol, disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from S.D Fine Chemicals (Mumbai, India) and were of HPLC grade. Hydrochloric acid, sodium hydroxide pellets, hydrogen peroxide and orthophosphoric acid were purchased from S.D Fine Chemicals (Mumbai, India) and were of A.R grade. HPLC grade water was prepared in house by distillation of water in triplicate followed by filtration through filter paper of 0.45 μm pore size and 47 mm diameter.

HPLC Instrumentation and Chromatographic Conditions

The HPLC system consists of a Shimadzu LC 20 AD binary pump and SPD 20 A UV detector. The column consists of Phenomenex (250mm length, 4.6 mm internal diameter, 5 μm particle size) ODS. LC Solution software was used for data acquisition. Sample injection was done by Rheodyne manual Injector. Chromatographic separation was achieved using Phenomenex ODS column. The LC system was operated isocratically using a mobile phase consisting of a mixture of methanol and phosphate buffer of pH 6.8 (prepared with 5.04 g of disodium hydrogen phosphate and 3.01 g potassium dihydrogen phosphate dissolved in 1000 mL of triple distilled water) in the ratio of 70:30 as the mobile phase at a flow rate of 1.0 mL/min at room temp. The injection volume was 20 μL for both standard and sample. The UV detector was set at 298 nm and the peak area integration was

done using LC solution software.

Preparation of Balofloxacin standard

The stock solution of BLFX was prepared by weighing 10 mg of the reference substance and transferring to a 10 mL volumetric flask, diluting to 10 mL with methanol (1000 $\mu\text{g}/\text{mL}$). The working standard solution (100 $\mu\text{g}/\text{mL}$) was obtained by dilution of the stock solution using mobile phase.

Preparation of sample solution

Tablets containing 100 mg of BLFX were accurately weighed and crushed to fine powder. A quantity of powder equivalent to 25 mg of BLFX was transferred to a 25 mL volumetric flask and added 10 mL of methanol, kept in an ultrasonic bath for 10 min and made upto the volume with methanol and filtered.

Validation study

The developed method was validated as per ICH guidelines using BLFX with respect to the following parameters: accuracy, precision, LOD, LOQ, specificity, robustness, stability and system suitability.

Linearity

For testing linearity seven calibration standards were prepared in the range of 5 to 100 $\mu\text{g}/\text{mL}$ (5, 10, 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$). Standard curve was obtained by plotting peak area against concentration and the evaluation of linearity was done by linear regression analysis using least square method.

Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were estimated at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of dilute solutions of known concentration.

Specificity

Specificity is the ability of a method to measure analytical response in presence of its potential impurities. Specificity of the method was carried out by the deliberate degradation of the drug by oxidation, heat, hydrolysis (acidic, alkaline, neutral) and photolysis, followed by its analysis using the developed method.

Accuracy

Accuracy of the developed method was assessed in triplicate at three concentration (40, 60 and 80 µg/mL). The percentage recovery was calculated from the linear regression equation obtained in the linearity studies.

Precision

The precision of the analytical method was evaluated by the determination of the repeatability of the method (intra day precision) and intermediate precision (inter day precision) of the sample solutions. Repeatability was calculated by assaying six samples prepared on the same day. Intermediate precision was calculated by assaying 3 days. The relative standard deviation of the area of peaks was calculated.

Robustness

Experimental conditions were deliberately altered, in order to determine the robustness. From the different experimental conditions such as flow rate (1.0 mL/min), lambda max (298 nm) and percentage of methanol (70), each selected factor was changed at three levels (-1, 0, +1). One factor was changed at a time to study the impact of the

change in the experimental conditions on the assay results. Change in the peak area and the retention time were noted at each change in the analytical parameters.

Stability of sample solution

Sample solution was prepared and analysed by the HPLC instrument using fresh mobile phase at different time intervals (0 h, 8 h and 24 h).

RESULTS AND DISCUSSION**Optimization of chromatographic condition**

The chromatographic conditions were optimized with a view to obtain symmetrical peak of BLFX. At the same time the peak should be well separated from the degradation peaks so that it can be quantified even in presence of degradants. Separation was achieved using a mobile phase consisting of methanol and phosphate buffer of pH 6.8 in the ratio of 70:30 by an isocratic profile, at a flow rate of 1 mL min⁻¹. UV detector wavelength was set at 298 nm. The drug was getting eluted at 3.67min and the corresponding chromatogram is given in figure 1.

Table 2: Intra- and inter-day precision

Con.(µg mL ⁻¹)	Intraday precision ^a			Interday precision ^b		
	Mean con.	SD	%RSD	Mean con.	SD	%RSD
20.0	20.73	0.301	1.453	20.624	0.235	1.139
40.0	39.991	0.233	0.582	39.757	0.455	1.144
80.0	81.048	0.182	0.224	80.795	0.314	0.388

^aMean concentration of six trials^bMean concentration of nine trials

Table 3: Data for Recovery studies

Brand	Spiking level (%)	Theoretical content (mg)	Amount found ^a (mg)	Recovery (%) ^b	%RSD ^c
Baloforce	80	16	16.509	101.414	1.631
	100	20	19.702	99.254	0.917
	120	24	24.527	101.197	1.283

^a Mean of three determinations (n=3)

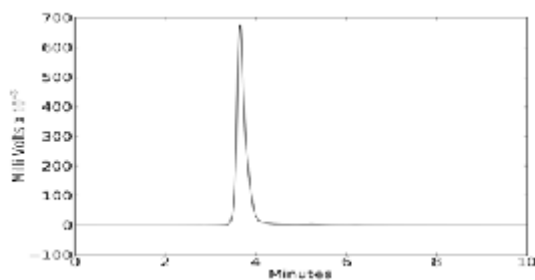
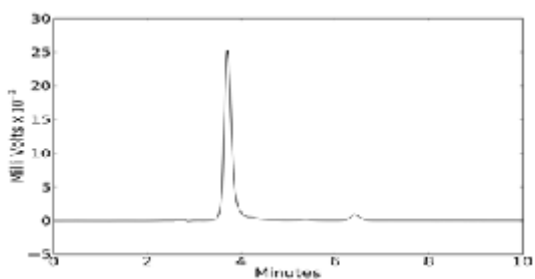


Fig. 1: The chromatogram of BLFX standard



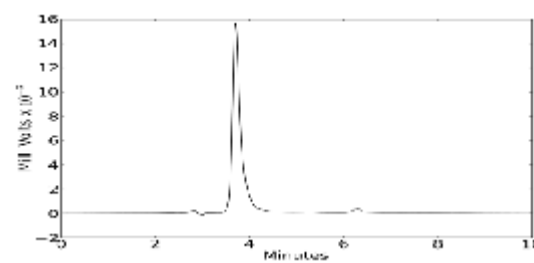
i. Chromatogram for thermal degradation

Limit of detection and limit of quantitation

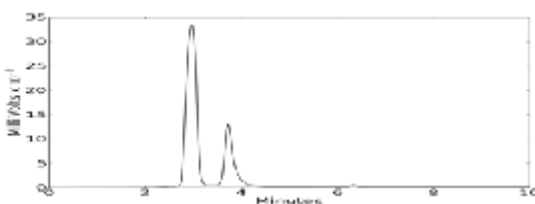
Concentrations of LOD and LOQ were found to be 90 and 273 ng/mL respectively.

Linearity

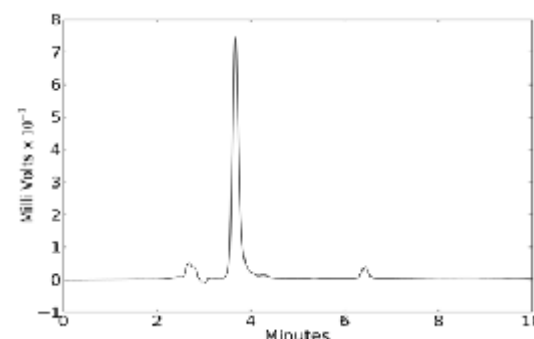
The regression equation for the graph is $y = 91057x + 43149$ and the correlation coefficient, R^2 is 0.9998 showing excellent correlation between the area and the concentration.



ii. Chromatogram for alkaline degradation



iii. Chromatogram for oxidative degradation



iv. Chromatogram for acidic degradation

Fig. 2: Chromatogram of BLFX along with the degradation products under different stress conditions

Precision

The percentage relative standard deviation (%RSD) of the area of BLFX during intra day study was found to be less than 1.5 and for inter day study was found to be less than 1.2 which indicated a good precision of the method (Table II).

Accuracy

The quantitative recovery of BLFX achieved was ranged from 99.280 - 101.524 % with a low % RSD value. The results of the recovery

experiments done at 3 concentration levels and the % RSD values are given in Table III.

Analysis of the marketed formulation

The validated method was applied for the assay of BLFX in 2 brands of Balofloxacin tablets (Baloforce – 100mg, Balotero – 100mg) (Table IV). Due to the absence of extra peaks in the chromatogram, it can be concluded that there was no interference from the excipients in the formulations. The percentage assay values were

found to be 100.181 and 100.850 for Baloforce and Balotero respectively.

Robustness

The results in the robustness study, as shown in table V indicated that the results are not much affected by the small variations in the selected parameters.

Specificity

In order to check the specificity of the proposed method, degradation studies were carried out by using acidic, basic, photolytic, oxidative and thermal conditions. The drug was stressed under various conditions to conduct forced degradation studies. Intentional degradation was performed by subjecting it to various stress conditions of acidic (1 N HCl), basic (0.1 N NaOH), neutral (water), oxidative (3% H₂O₂), thermal (heated

at 105°C) and photolytic degradation (exposed to UV radiations of 254 nm for a period of 48 hrs) to evaluate the ability of the proposed method to separate BLFX from its degradation products. The drug was found to be stable to photolytic and neutral degradation. The results of stress degradation studies are given in Fig.2.

CONCLUSION

A simple and fast stability indicating HPLC method has been developed and validated for the determination of BLFX in API and different pharmaceutical formulations. The method is accurate, precise, and specific and also has the ability to analyse the drug in presence of its degradation products and it can be employed as a stability-indicating assay. Since the drug peak was well separated from degradation products' peaks it can be employed as a stability indicating method.

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