ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

REVERSED-PHASE TLC/ DENSITOMETRY METHOD FOR ESTIMATION OF BALOFLOXACIN IN BULK AND IN TABLET DOSAGE FORM

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Manuscript Info

Manuscript History:

Received: 12 August 2013
Final Accepted: 24 August 2013
Published Online: September 2013

Key words:
Balofloxacin,
RP-HPTLC,
Accuracy, Precision,
Recovery, Validation.

Abstract

A simple, selective, precise, sensitive and accurate high performance thin layer chromatographic method of analysis for the determination of Balofloxacin in both bulk drug and in formulation was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The solvent system consists of methanol: water: triethylamine (6:4:0.5 v/v/v). This system was found to give compact spots for Balofloxacin (R_f value of 0.48 ± 0.02). Dentiometric analysis of Balofloxacin was carried out in the absorbance mode at 266 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.999 with respect to peak height and peak area, in the concentration range of 500-3000 ng per band. The method was validated for accuracy, precision and recovery. The method is simple and accurate, separation is good, right Balofloxacin quality control has obvious practical value.

Introduction

Balofloxacin (BLEX or BALO), 1-Cyclopropyl -6-fluro-8-methoxy-7- (3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid is a broad spectrum fluorinated quinolone antibacterial (Figure I). BLX is fourth generation of new class of synthetic antibacterial fluroquinolone agent. BLX was first commercialized in Korea in 2002. It has broad antibacterial spectrum ranging from gram positive to negative. BLX exhibite excellent antibacterial activity against gram positive bacteria using multiple drug resistant staphylococci and pneumococci. It acts by binding to and inhibiting topoisomerase II (DNA - gyrase) and topoisomerase IV esnzyme which are responsive for coiling and uncoiling of DNA which is needed for bacterial cell repair and replication. In literature, various analytical methods, such as RP-HPLC (Nakagawa T, et al. 1995(2), CHU Zhi-jie et al. 2008 (3), Mi Yaxian, et al. 2010(4)), RP-HPLC with fluorescence detection (Yin S., et al. 2007(5)), HPLC-Electrospray ionization mass spectroscopy (Bian Z, et al. 2007(6)) have been developed for determination of Balofloxacin. However, no UV spectrophotometric method is available for estimation of balofloxacin either in bulk or in dosage form.

Experimental

Materials and Reagents

Balofloxacin was provided as a gift sample by Alkem Ltd. Mumbai. Drug was used without any further purification. All other reagents required for experimentation were of analytical reagent (AR) grade. Chemicals used for this experiment were Methanol (AR grade), water (Distilled), Triethylamine (AR grade) these chemicals were purchased from Merck Chemicals.

Instrumentation and chromatographic condition

Chromatography was performed on 20 cm ×10 cm coated with 200-µm layers of silica gel 60 RP-18 F254S(Merck, Darmstadt, Germany, supplied by Merck India, Mumbai, India) was used. Before chromatography the plates were prewashed with methanol and activated at 105^0C for 5 min. The samples were applied as 6 mm wide bands with the help of Linomat 5 sample applicator (Muttenz, Switzerland) fitted with a 100-µL sample syringe

(Hamilton, Bonaduz, Switzerland). The plate was developed in a pre-saturated Camag twin trough glass chamber (20 cm × 10 cm). In this method Methanol: Water: Triethylamine (6:4:0.5 *v/v*), was used as mobile phase and optimized chamber saturation time was 30 min. The plate was developed to a distance of 8.0 cm and scanned densitometrically using Camag TLC Scanner 3 equipped with win CATS software version 1.3.0 at 266 nm for this method. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200- 400 nm. Evaluation was performed using peak area with linear regression (Figure II).

.**Preparation of standard and sample solutions**

A sample of pure Balofloxacin was accurately weighed (10 mg), transferred into a 10 ml volumetric flask. It is dissolved in 5ml of methanol and then diluted up to the mark with methanol to give standard stock solution having concentration of 1000ng/µl.

Calibration Curve

A stock solution of Balofloxacin (1000ng/µl) was prepared in methanol. Different volumes of stock solution 0.5, 1, 1.5, 2, 2.5, 3 µl were spotted in duplicate on TLC plate to obtain concentrations at 500-3000 µg of Balofloxacin respectively. The data of peak height/ area were treated by linear least square regression method (Table I).

Table I: Linearity of BALO for proposed method (n=6)

Parameters	BALO
Linearity range (ng/band)	500 - 3000
Slope	5.514
Intercept	7172
Correlation Coefficient (r^2)	0.999

Precision

Repeatability of the sample application and measurement of peak area were carried out using 1000, 1500, 2000 ng/ mL three replicates of each spot. The intra and inter day variation for the determination of Balofloxacin was carried out at 500, 1000, 1500 ng/band (Table II).

Recovery

The analyzed samples were spiked with extra 80, 100 and 120 ng of the standard balofloxacin and the mixture was reanalyzed by the proposed method. The experiment was conducted in triplicates. This was done to check for the recovery of the drug at different levels in the formulation (Table III).

Application of the proposed method for simultaneous estimation of the drugs in tablets

Twenty tablets (Baloforce) (contained 100 mg of BALO) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 100 mg BALO was transferred to a 100 mL volumetric flask and methanol (35 mL) was added followed by ultrasonication for 10 min, volume was adjusted to mark and filtered using 0.45 µm filter (Mill filter, Milford, MA) and 10 mL of filtrate was further diluted to 10 mL with methanol. Appropriate volume 10 µL, was spotted for assay of BALO. The plate was developed and scanned as described in above chromatographic condition (Table IV).

Ruggedness and Robustness

Ruggedness of the method was performed for BALO by different analysts maintaining similar experimental and environmental conditions.

Robustness of the method was performed by introducing various changes in the previous chromatographic conditions; effects on the results were examined for method (Table V).

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently. The peak purity of BALO extracted from tablet and standard BALO was tested at the peak - start (S), peak - apex (A) and at the peak - end (E) position (Figure III).

Sensitivity

The sensitivity of measurements of BALO by the use of the proposed method was estimated in terms of the Limit of Quantitation (LOQ) and the lowest concentration detected under the chromatographic conditions as the Limit of Detection (LOD).

LOQ and LOD were calculated by the use equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The results were recorded for the methods. Different validation parameters for the methods for determining BALO content were summarized in (Table VI).

Table II: Accuracy of BALO for proposed method (n=3)

Sr. No.	Initial amount	Level of recovery	Amount of drug added	% Recovery	% RSD
1	1000	80%	800	98.52	1.04
2	1000	100%	1000	100.92	1.08
3	1000	120%	1200	101.24	0.99

Table III: Precision of BALO

Drug	Conc. [ng/band]	Intra-day Amount Found [µg/mL]		Inter-day Amount Found [µg/mL]	
		Mean	% RSD [n= 3]	Mean	% RSD [n= 3]
BALO	1000	12596	0.58	12596	0.58
	1500	15569	0.91	15569	0.91
	2000	18179.67	0.78	18179.67	0.78

Table IV: Tablet assay of BALO

Component	Amount taken in [µg/mL]	Amount found [µg/mL] Mean ± SD;[n = 6]	% Amount Found	% RSD
BALO	6	12662.67± 118.47	99.09 ± 1.2	1.36

Table No V: Optimized Chromatographic Conditions

Parameters	SD of peak area	%RSD
Mobile phase composition	-	-
Methanol : water : triethylamine (6 :4 :0.5, v/v/v)	180.35	1.42
Methanol : water : triethylamine (7 :3 :0.5, v/v/v)	137.68	1.09
Mobile phase volume		
5.0 ml	31.79	0.49
10.0 ml	69.53	1.08
Development distance		
7 cm	54.41	0.85
7.5 cm	49.54	0.77
8 cm	42.40	0.66

Relative humidity		
55	62.23	0.97
65	50.09	0.78
Duration of saturation		
10 min	58.36	0.91
15 min	58.98	0.92
20 min	57.60	0.89
Activation of prewashed TLC plates		
8 min	61.07	0.95
10 min	60.33	0.94
12 min	70.11	1.09
Time from spotting to chromatography	54.41	0.85
Time from chromatography to scanning	51.36	0.80

Table VI: Summary of validation data

Parameter	**BALO**
Linear range (ng/band) [n=6]	500 – 3000
Correlation coefficient (r^2)	0.999
Limit of detection (µg/mL)	0.48
Limit of quantification (µg/mL)	1.42
% Recovery [n=3]	98.52 -100.24
Precision [%RSD]	
Repeatability [n=6]	0.92
Intra-day [n=3]	0.75
Inter-day [n=3]	0.63
Robustness	Robust

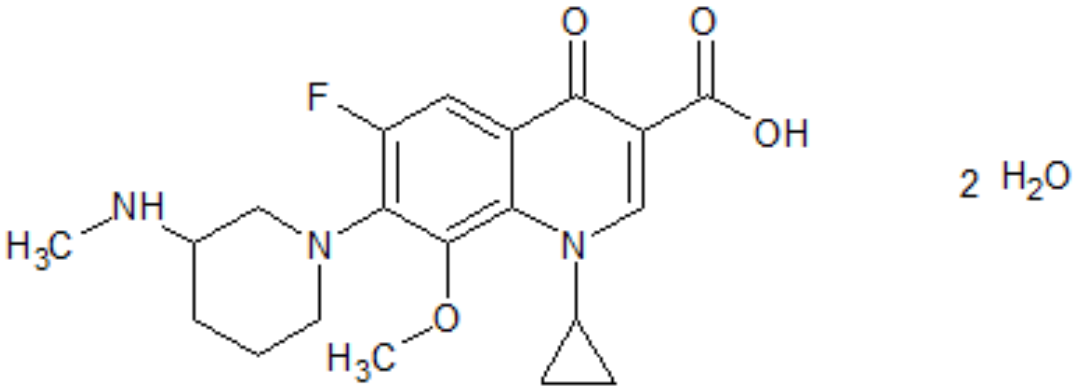

Figure I: Chemical structure of Balofloxacin

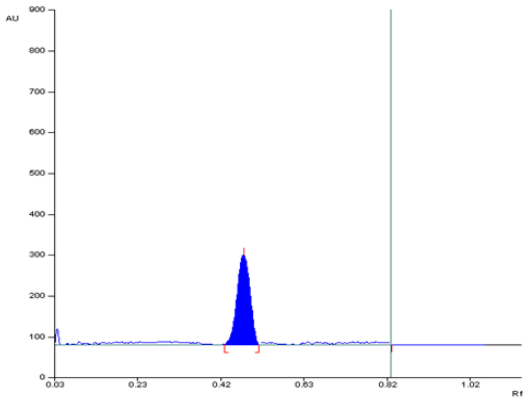

Figure II: Densitogram of Standard Balofloxacin (R_f 0.48 ± 0.02) measured at 266 nm.

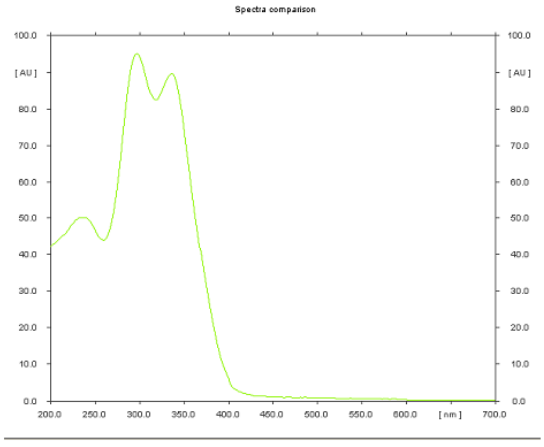

Figure No: III: peak purity of BALO extracted from tablet and standard BALO was tested at the peak - start (S), peak - apex (A) and at the peak - end (E) position.

Result and Discussion

An RP-HPTLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Optimization of method was done by altering almost all the chromatographic conditions and the effect on R_f and peak shape was monitored for BALO. The final chromatographic conditions was performed on 20 cm × 10 cm aluminum-backed RP-HPTLC plates coated layers of silica gel $60F_{254}$ S (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 105 – 110°C for 15 min prior to chromatography. The samples were applied on the plates as bands, under continuous flow of nitrogen, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with a 100-µL syringe. Finally Methanol: Water: Triethylamine in the ratio (6:4:0.5 *v/v*). The chamber saturation time was 30 min. The R_f of BALO was found to be 0.48 ± 0.02.

Conclusion

The modalities adopted in experiment were successfully validated as per ICH guidelines. The proposed Method was validated by preliminary analysis of standard sample and by recovery studies for the determination of BALO in bulk and in tablet dosage form and the percentage of average recoveries of BALO was obtained 100.57 and 98.22 respectively. The proposed Method provide simple, rapid, accurate, precise and specific. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its

good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Balofloxacin in tablet formulation.

Acknowledgement

The authors are thankful to Alkem Pharmaceutical Ltd. Mumbai for providing drug sample and also Dr. S. J. Surana Principal of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing essential facilities to carry out research work.

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