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A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF BALOFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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A novel, simple, rapid and precise reverse phase isocratic high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of Balofloxacin in marketed formulations. Estimation of drug in the formulation was done with a C18 column [Agilent ODS UG column. 250 mm × 4.5 mm] using mobile phase of composition Acetonitrile and Phosphate buffer pH 3.2 in the ratio of (60 : 40v/v) and flow rate was 1 ml/min and effluent was monitored at 293 nm. The retention time of Balofloxacin was observed at 2min. The method was found to be linear over a range of 2-10µg/ml for Balofloxacin. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully applied in the estimation of commercial formulations.

KEYWORDS : Balofloxacin, RP-HPLC, Method validation.

INTRODUCTION

Balofloxacin is a fluoroquinolone derivative, has an antibiotic action. Chemically, Balofloxacin is described as 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl amino piperidin-1-yl)-4-oxo quinoline-3-carboxylic acid [1-2].

Balofloxacin is not official in any pharmacopeia. Extensive Literature survey reveals that only few analytical methods have been reported for the estimation of Balofloxacin in bulk and pharmaceutical dosage forms [3-17]. So, an attempt has been made to develop a rapid, precise, accurate, simple, specific and reliable RP-HPLC method for routine analysis of Balofloxacin in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Equipment used

The chromatographic separation was performed on Agilent 1120 Compact Liquid Chromatographic system integrated with a variable wavelength programmable UV detector

and a Rheodyne injector equipped with 20 µl fixed loop. A reverse phase kromasil 100-5C_{18} Column (250 mm × 4.5 mm) was used.

Reagents and chemicals

Pharmaceutical grade pure Balofloxacin was obtained as gift sample from Hetero Laboratories Pvt. Ltd, Hyderabad. Marketed formulation Balox with dose of 100 mg was procured from local market. HPLC grade Acetonitrile, Double distilled water and Laboratory grade Triethylamine, Orthophosphoric acid and Potassium dihydrogen orthophosphate were procured from Merck specialities private limited, Mumbai.

Chromatographic conditions

Kromasil 100-5C_{18} Column (250 mm × 4.5 mm) was used for the chromatographic separation at a detection wavelength of 293 nm. Mobile phase composition of Acetonitrile and Phosphate buffer pH 3.2 in the ratio of (60 : 40 v/v) was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1 ml/min and the injection volume was 20 µl.

Preparation of mobile phase

Mobile phase was prepared by mixing Acetonitrile and Phosphate Buffer pH 3.2 in the ratio of (60 : 40 v/v) and was initially filtered through 0.45 µm Millipore membrane filter and sonicated for 15 min.

Phosphate Buffer pH 3.2 :

Dissolve 0.136 gm of potassium dihydrogen phosphate and 2 ml of triethylamine in 80 ml 0f water, the pH is adjusted to 3.2 with orthophosphoric acid and sufficient quantity of water was added to produce 100ml.

Preparation of Standard Stock Solution

25 mg of pure drug was weighed accurately and transferred into 25 ml volumetric flask and dissolved in 10ml of mobile phase and made up to the volume with mobile phase to get a final concentration of 1000 µg/ml (Working stock solution A).

Preparation of sample stock solution

Marketed tablet formulation (Balox) containing 100mg of Balofloxacin was analyzed by this method. Twenty tablets were accurately weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 25 mg was taken in 25 ml volumetric flask and dissolved in 10 ml of mobile phase. The solution was kept for sonication for 5min. The solution was made up to the mark with the mobile phase and filtered through 0.45 µ membrane filter to get the concentration of 1000 µg/ml (Working stock solution B).

Optimization of HPLC method

The HPLC method was optimized with an aim to develop an accurate and precise method for the estimation of Balofloxacin in pharmaceutical dosage forms. For the method optimization, different mobile phases were tried but acceptable retention times, theoretical plates and good resolution were observed with acetonitrile and Phosphate Buffer pH 3.2 (60 : 40 v/v) using Kromasil 100-5C_{18} Column (250mm × 4.5mm).

VALIDATION OF RP-HPLC METHOD

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with six injections of solutions of 100% concentration having 10 µg/ml of Balofloxacin into the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T), were reported in table 1.

Table 1. System suitability parameters for Balofloxacin

S. No	Balofloxacin		
	Conc. (µg/mL)	R_t (min)	Peak Area
1	10	2.003	138776896
2	10	2.007	138666445
3	10	2.000	138467323
4	10	2.010	138455231
5	10	2.110	138200465
6	10	2.002	138964362
Mean		0.043261	270501.8878
SD		2.357	138588453.7
% RSD		1.835	0.19

Linearity

To establish the linearity of proposed method, appropriate aliquots were pipette out from working stock solution 'A' were to a series of 10 ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain final concentrations ranging from 2-10 µg/ml of Balofloxacin. Three replicates per each concentration were injected and Peak areas of the above solutions were reported. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curve was shown in Figure 3 and their corresponding linearity parameters were given in table 2.

Table 2. Results for linearity of Balofloxacin

Concentration (µg/mL)	Area	Rt
2	35672893	2.030
4	70358147	2.032
6	100625277	2.030
8	140003524	2.032
10	169875321	2.030
Statistical validation data of linearity		
Slope (m)	20637	
Intercept (c)	90093	
Correlation coefficient	0.999	

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample

and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in table 3.

Table 3. Results for accuracy of Balofloxacin

Recovery level	Amount Added (mg)		Amount Found (mg)	% Recovery
	Std.	Test		
50%	1	1	1.98	99.0
100%	3	1	3.99	99.75
150%	5	1	5.98	99.66
Mean % Recovery	99.0-99.66%w/w			

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (10 µg/ml) on the same day. The results were given in table 4.

Table 4. Results for Precision of Balofloxacin Precision for Balofloxacin

Concentration (µg/ml)	Peak area	Retention Time
10	138776896	2.030
10	138609366	2.030
10	143498406	2.007
10	139469972	2.000
10	144525816	2.007
10	140012834	2.007
Statistical validation of precision		
Standard deviation	2567662.59	
Mean	140815548.3	
% RSD	1.823	

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were calculated from the slope (s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10σ/s. The results were given in table 5.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wavelength, etc and the % RSD should be reported.

Table 5. Results for limit of detection and limit of quantification

Parameter	Balofloxacin
Linearity Range (µg/ml)	2-10
Regression Equation	Y = 20637x + 90093
Slope (m)	20637
Intercept (c)	90093
Regression Coefficient (r^2)	0.999
Limit of Detection (µg/mL)	0.055
Limit of Quantitation (µg/mL)	0.167

Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wavelength and ±0.1 ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in table 6 and 7.

Table 6. Results for Robustness of Balofloxacin

Parameter	Flow Rate(ml/min)	
	0.8	**1.2**
Peak area	168836935	115911635
	168535436	115834543
	168357625	115711326
S.D	242300.25	54512.27
Mean	117909998.7	115819168.0
%RSD	0.205	0.047

Table 7. Robustness Data showing Variation in Detection Wave Length

Parameter	Wave Length (nm)	
	291	**295**
	132800198	13876646
	132800178	13876642
Peak area	132800168	13876640
S.D	14.1421	2.8284
Mean	132800181.3	13876642.67
%RSD	1.06	1.89

Assay of marketed formulation

20 µl of sample solution of concentration 10 µg/ml was injected into the chromatographic system and the peak response was measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of standards with the test sample. A typical chromatogram of test solution containing 10 µg/ml of Balofloxacin was shown in figure 4. The results were shown in table 8.

Table 8. Results for assay of marketed formulation

S. No	Label claim (mg/tab)	Amount Obtained in (mg/tab)	Percentage purity (%w/w)
	Balox	Balox	Balox
1	100	97.65	97.65

Results and discussion

After a number of trials with mobile phases of different composition, acetonitrile and phosphate buffer pH 3.2, in the ratio of 60 : 40 v/v was selected as mobile phase because of better resolution and symmetrical peaks. Balofloxacin showed appreciable absorbance at 293 nm when determined spectrophotometrically and hence it was selected as the detection wavelength. The optimized chromatogram of Balofloxacin was shown in Figure 2.

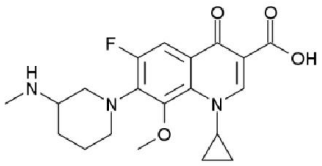

Fig. 1. Chemical Structure of Balofloxacin

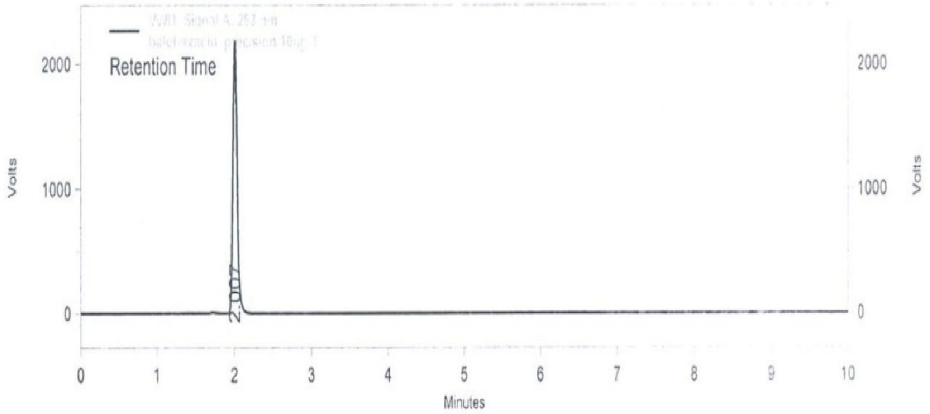

Fig. 2. Optimized chromatogram of Balofloxacin

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatogram confirms the presence of Balofloxacin at 2.0min without any interferences. The parameters were given in table 1.

Concentration range of 2-10 µg/ml was found to be linear with correlation coefficient of 0.999. The results were given in table 2.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 99.0-99.66% w/w. The values obtained were given in table 3.

The proposed method was found to be precise and reproducible with %RSD of 1.823 and it was reported in table 4.

The limit of detection was found to be 0.055 µg/ml and limit of quantitation was found to be 0.167 µg/ml. The values were represented in table 5.

The method was found to be robust after changing the conditions like detection wavelength (± 2 nm) and flow rate (± 0.1 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 6 and 7.

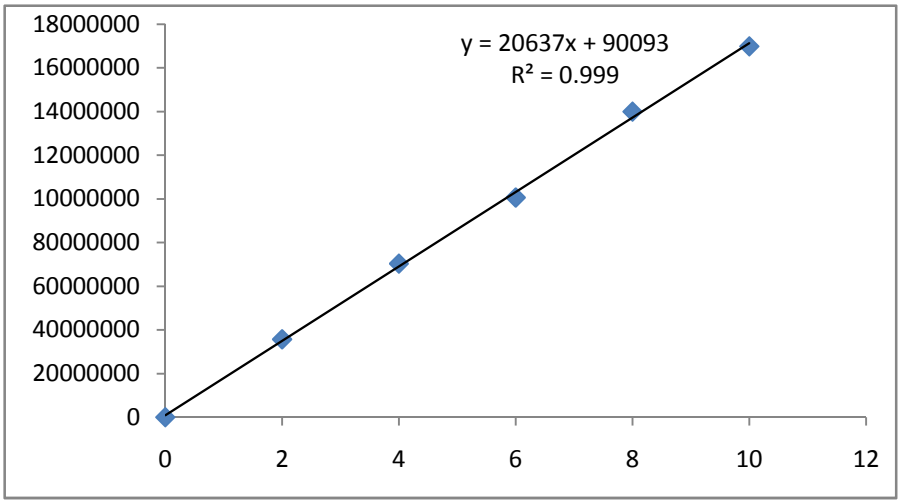

Fig. 3. Calibration curve of Balofloxacin

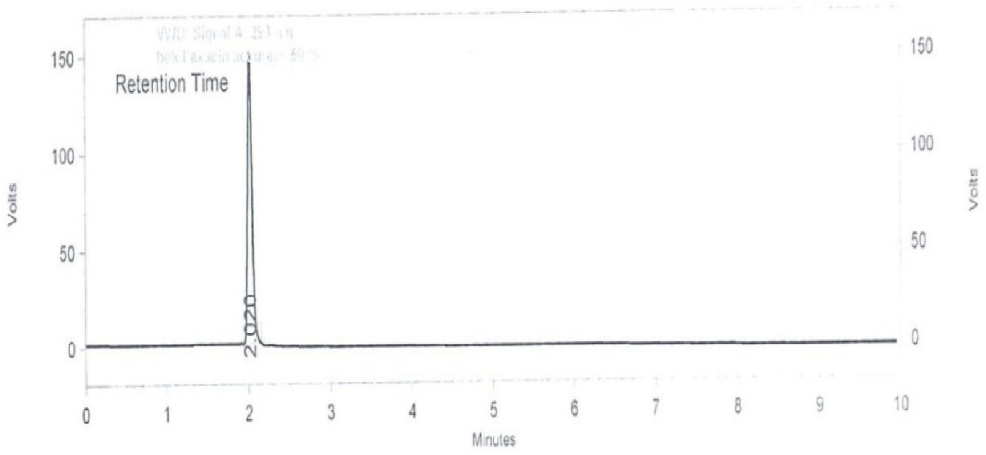

Fig. 4. Chromatogram for assay of marketed formulation containing 100 mg of Balofloxacin

The method was found to be specific after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of commercial formulation. Values obtained were given in table 8.

Conclusion

The proposed RP-HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Balofloxacin by RP-HPLC using UV detector in pharmaceutical dosage forms. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Balofloxacin with no interference from other formulation excipients. The proposed method was highly sensitive, reproducible, reliable, rapid, robust and specific. Therefore, this method can be employed in quality control to estimate the amount of Balofloxacin in bulk and in pharmaceutical dosage forms.

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