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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF BALOFLOXACIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of the method was to develop a simple, rapid, efficient, cost effective and reproducible, stability indicating (RP-HPLC) Reverse Phase High Performance Liquid Chromatography method for the estimation of Balofloxacin in bulk and tablet dosage form.

Methods: The RP-HPLC analysis was carried out on an Enable C_{18} G column with a mobile phase of Potassium dihydrogen orthophosphate (pH adjusted to 2.5 with 0.1 M ortho phosphoric acid) and Acetonitrile in the ratio of 75:25% v/v. The analyte was detected at 293 nm using PDA detector. The method was validated in terms of linearity, accuracy, precision, LOD (Limit of Detection), LOQ (Limit of Quantification) and robustness as per ICH guidelines.

Results: The method was found to be linear in the range of 10-120 µg/ml. Limit of detection and limit of quantitation was found to be 0.793 and 2.405 µg/ml respectively. Recovery was found to be in the range 100.268- 101.077 % and precision less than 2%. The developed method was successfully applied for the estimation of Balofloxacin in marketed tablet formulation (Baloforce TAB) and percentage assay was found to be 101.690 %. Forced degradation studies were performed under different conditions. The drug was degraded in acidic, basic, oxidative, UV light and sunlight conditions. The peaks of degraded products were well resolved from the actual drug. The results obtained prove that the developed method is a stability indicating method.

Conclusion: The developed RP-HPLC method was simple, rapid, accurate, precise and stability indicating for the estimation of Balofloxacin in bulk and tablet dosage form.

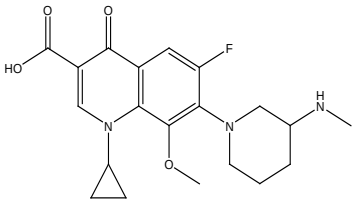
Keywords: Balofloxacin, RP-HPLC, Validation, Stability indicating, Degradation.

INTRODUCTION

Stability indicating method is used to evaluate the ability of analytical method to estimate the analyte and its degradation products without any interference from the degraded products generated by forced degradation studies [1].

According to FDA and ICH guidelines forced degradation studies are conducted to investigate the stability indicating power of the developed analytical method [2, 3].

The developed method is expected to allow the analysis of individual degraded products. Balofloxacin, 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylamino piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid Fig.1: is a Fluoroquinolone antibiotic used as a Broad spectrum antibacterial activity against Gram negative bacterium and anaerobe specially against Gram positive bacterium such as MRSA, Streptococcus pneumonia, Enterococcus faecalis. It inhibits and binds with Topoisomerase II (DNA Gyrase) and Topoisomerase IV enzymes which are responsible for coiling and uncoiling of DNA needed for bacterial cell repair and replication [4,5].



1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline 3-carboxylic acid

Fig. 1: Structure of Balofloxacin

On extensive survey of literature, several analytical methods such as derivative spectroscopy, RP-HPLC, UV-Visible spectrophotometry, Chemiluminescence method, Spectroscopic method using 1% w/v ceric sulphate as chromogenic agent, RP-TLC, Spectrophotometric determination using ion-pair complexation have been reported for estimation of Balofloxacin in bulk and pharmaceutical dosage form [6-16]. Many bio-analytical methods such as determination of Balofloxacin and its marketed formulation from human urine, serum, human plasma, rat plasma, bile, blood and tissue have also been reported in literature [17-23]. However no stability indicating method has been reported for estimation of Balofloxacin from bulk and tablet dosage form. Hence an attempt has been made to develop and validate a stability indicating RP-HPLC method for estimation of Balofloxacin in bulk and tablet dosage form. Forced degradation studies were performed to prove that the developed method is stability indicating.

MATERIALS AND METHODS

Chemicals

Balofloxacin was obtained as gift sample from Zydus Cadila Healthcare Ltd, Ahmedabad. Methanol and acetonitrile of HPLC grade were obtained from Loba Chemie, India. Potassium dihydrogen ortho phosphate and ortho phosphoric acid of HPLC grade were purchased from Loba Chemie, India. Hydrochloric acid, Sodium hydroxide and Hydrogen peroxide of analytical grade was purchased from Loba Chemie, India. Marketed tablet formulation (Baloforce TAB), manufactured by Hetero Labs Ltd, Himachal Pradesh was purchased from local market.

HPLC system

The HPLC system used was LC 20AT Shimadzu system with LC Solutions software. The system was equipped with LC 20AT prominence pump and UV/PDA detector. The chromatographic separation was carried out on Enable C_{18} G column (150×4.6 mm, 5µ). Elution was performed with a mobile phase containing potassium dihydrogen ortho phosphate (pH 2.5) and acetonitrile (75:25 v/v). pH was adjusted to 2.5 by 0.1 M ortho phosphoric acid. Mobile phase was freshly prepared and filtered through 0.45 µm

membrane filter and degassed prior to the analysis.

Preparation of standard stock solution

Standard stock solution was prepared by transferring 100 mg of Balofloxacin to a 100 ml volumetric flask. The volume was made up to 100 ml with methanol-water (80:20).The concentration of the final solution was found to be 1000 µg/ml.

Preparation of working solution

1 ml from the standard stock solution was taken into a 10 ml volumetric flask and the volume was made up to mark with Milli Q water to give a working solution of concentration 100 µg/ml.

Preparation of test sample solution

20 tablets were crushed using a mortar-pestle, powder was weighed and quantity of powder equivalent to 100 mg of Balofloxacin was transferred in a 100 ml volumetric flask, dissolved in solvent (methanol: water 80:20) and sonicated for 30 minutes.

The solution was filtered through Whatman filter paper (No. 41) and residues were washed three times with solvent (5ml). All the filtrates were collected and from this solution 0.5 ml was transferred in 10 ml volumetric flask and volume was adjusted up to mark with Milli Q water to give a concentration of 50 µg/ml of Balofloxacin.

Validation of the method

The developed method was validated according to ICH guidelines in terms of specificity, sensitivity, linearity, LOD, LOQ, accuracy, precision and robustness [3].

Specificity

Specificity was established by complete separation of analyte in the presence of tablet excipients and without interferences at the retention time of Balofloxacin.

Linearity

Linearity was established by least squares linear regression analysis of calibration curve. Linearity was determined in the range of 10-120 µg/ml.

LOD and LOQ

Calibration curve was repeated three times and the standard deviation (SD) of the intercepts was calculated. The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations:

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ = Standard deviation of slope

S = Slope of the Calibration curve

Precision and accuracy

Precision and accuracy of the method was monitored for three days. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. For intraday precision nine determinations of three concentrations were analyzed on the same day. For interday precision nine determinations of three concentrations were analyzed for three consecutive days.

Robustness

Robustness of the method was investigated under a variety of conditions like change in pH of mobile phase (±0.1), flow rate (±0.2 ml/min) and wavelength (±2 nm). In each variation analysis was made in three replicates and %RSD of peak areas were determined.

Forced degradation studies

In order to establish whether the developed method is stability indicating Balofloxacin (API) and formulation were stressed under various conditions (acid, base, oxidation and photolytic) to perform forced degradation studies.

Acid degradation studies

Accurately weighed 100 mg of Balofloxacin was transferred to a 100 ml volumetric flask, dissolved in methanol: water (80:20) and volume was made up to the mark with the same. From this solution 10 ml was taken, transferred to a round bottom flask containing 10 ml 1N HCl and kept in a water-bath at 70^0 C and refluxed for 1 hour. 1 ml sample was withdrawn, transferred to 10 ml volumetric flask every 15 minutes and neutralized with 1 N NaOH. The volume was made up to mark with Milli Q water to give 100 µg/ml of Balofloxacin. The resulting solutions were injected in HPLC and chromatograms were recorded and checked for degradation products.

Base degradation studies

Accurately weighed 100 mg of Balofloxacin was transferred to a 100 ml volumetric flask, dissolved in methanol: water (80:20) and volume was made up to the mark with the same. From this solution 10 ml was taken, transferred to a round bottom flask containing 10 ml 5N NaOH and kept in a water-bath at 70^0 C and refluxed for 2 hours. 1 ml sample was withdrawn, transferred to 10 ml volumetric flask every 15 minutes and neutralized with 5 N HCl. The volume was made up to mark with Milli Q water to give 100 µg/ml of Balofloxacin. The resulting solutions were injected in HPLC and chromatograms were recorded and checked for degradation products.

Oxidation studies

Accurately weighed 100 mg of Balofloxacin was transferred to a 100 ml volumetric flask, dissolved in methanol: water (80:20) and volume was made up to the mark with the same. From this solution 10 ml was taken, transferred to a round bottom flask containing 10 ml 3% H_2O_2 and kept in a water-bath at 70^0 C and refluxed for 2 hours. 1 ml sample was withdrawn, transferred to 10 ml volumetric flask every 15 minutes and the volume was made up to mark with Milli Q water to give 100 µg/ml of Balofloxacin. The resulting solutions were injected in HPLC and chromatograms were recorded and checked for degradation products.

UV degradation

Some amount of Balofloxacin API powder was kept in a petridish and placed under UV light for 4 hours. 1 mg sample was weighed accurately every 15 minutes, transferred to a 10 ml volumetric flask and the volume was made up to the mark with methanol: water (80:20) to give 100 µg/ml of Balofloxacin. The resulting solutions were injected in HPLC and chromatograms were recorded and checked for degradation products.

Sunlight degradation

Some amount of Balofloxacin API powder was kept in a petridish and placed under sun light for 3 hours. 1 mg sample was weighed accurately every 15 minutes, transferred to a 10 ml volumetric flask and the volume was made up to the mark with methanol: water (80:20) to give 100 µg/ml of Balofloxacin. The resulting solutions were injected in HPLC and chromatograms were recorded and checked for degradation products.

RESULTS AND DISCUSSION

Method development

Initially wavelength was selected for the method development and different compositions, pH and flow rate of the mobile phase were tried during method development. The 293 nm was selected for the current method since at this wavelength Balofloxacin can be detected with high sensitivity. In the course of optimizing the composition of mobile phase, acetonitrile in combination with various buffers like phosphate and acetate with varying pH values were tried. After a series of preliminary experiments it was concluded that potassium dihydrogen ortho phosphate buffer resulted in better peak shape. Peak with good shape and symmetry was observed by the mobile phase consisting of phosphate buffer (pH 2.5): acetonitrile (75:25 v/v) set at a flow rate of 1 ml/min. This composition was used for further studies (Fig.2).

Method Validation

Specificity

The specificity of the method is shown in Fig. 3 where Balofloxacin was eluted completely without any interference from tablet excipients at its retention time.

This shows that the excipients of tablets did not interfere with the analyte elution.

Linearity

The calibration curve was constructed between peak area and respective concentrations. The calibration curve was linear over the range of 10-120 µg/ml. Correlation coefficient was found to be 0.997. The regression equation for calibration curve was found to be y= 73288x + 347342. Results of linearity are shown in Table 1 and Fig. 4.

LOD and LOQ

LOD and LOQ were determined by the earlier mentioned equations. LOD was found to be 0.793µg/ml and LOQ was found to be 2.40µg/ml.

Precision and Accuracy

Precision of the method was determined in terms of intraday and interday precision. The % RSD obtained for intraday and interday precision was less than 2%. Accuracy was calculated by recovery studies at three levels viz 80%, 100% and 120% by standard addition method. The percentage recovery was found to be 100.268-101.077%. The values of intra and interday precision are shown in Table 2 and accuracy results are shown in Table 3.

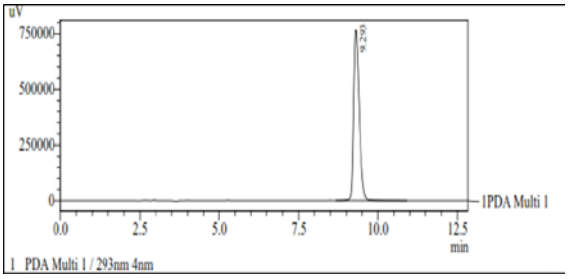


Fig. 2: Chromatogram showing estimation of Balofloxacin (R_t 9.293 min)

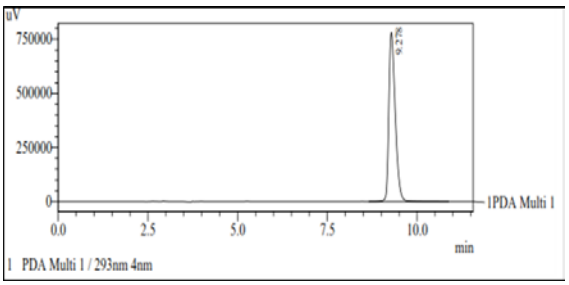


Fig. 3: Chromatogram of test sample solution (marketed formulation)

Table 1: Analytical performance parameters for linearity

Parameters	Balofloxacin
Linearity range	10-120 µg/ml
Regression equation	y =73288x + 347342
Correlation coefficient (r^2)	0.997
Intercept	347342
Slope	73288

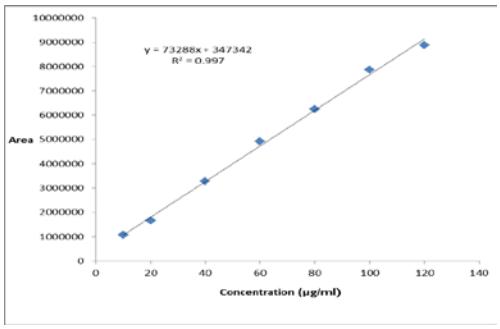


Fig. 4: Linearity of Balofloxacin

Table 2: Intraday and Interday precision results

Concentration (µg/ml)	%RSD	
	Intraday	Interday
10	0.465	1.038
60	0.236	0.531
120	0.084	1.059

Table 3: Accuracy results

S. No.	Spike level	Amount present (µg/ml)	Amount recovered (µg/ml)	% recovery
1.	80	90	90.97	101.077
2.	100	100	100.268	100.268
3.	120	110	110.718	100.652

Table 4: Robustness testing of the method

Factor	Condition	% RSD
pH	2.4	1.314
	2.5	1.595
	2.6	1.545
Flow rate	0.8	1.964
	1.0	1.868
	1.2	1.705
Detection wavelength	291	1.517
	293	1.033
	295	1.898

Robustness

Robustness of the method was determined by deliberately changing parameters like flow rate, pH of mobile phase and wavelength of detection. Samples were analyzed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in Table 4.

Forced degradation studies

All the stressed samples in acid, alkaline, oxidative, UV light and sunlight degradation studies were decomposed to 29.83, 25.22, 18.06, 12.22 and 16.88 % respectively. No degradation peaks were observed in UV and Sunlight degradation but the peak area was decreased. The peaks of degraded products were well separated from the analyte peak with good resolution Fig. 5 (a, b, c, d and e) which indicates that the developed method is stability indicating. The forced degradation studies data are summarized in Table 5.

Assay

The validated method was applied to the determination of Balofloxacin from commercially available Baloforce tablets. Chromatogram obtained by assay of tablets is shown in Fig. 6. The % assay was found to be 101.690 %. The results of assay indicate that the developed method is selective without interference from tablet excipients.

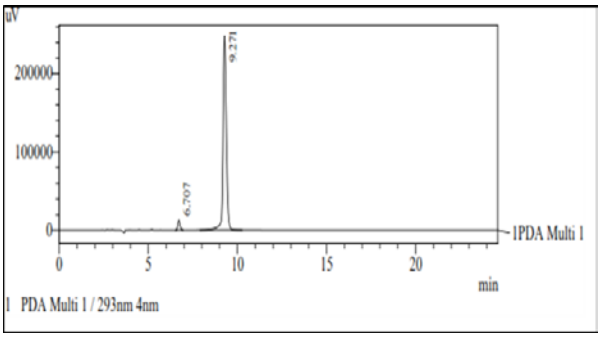


Fig. 5a: Chromatogram showing acid degradation of Balofloxacin (an additional peak is observed at Rt 6.707 which is the peak of degraded product of Balofloxacin)

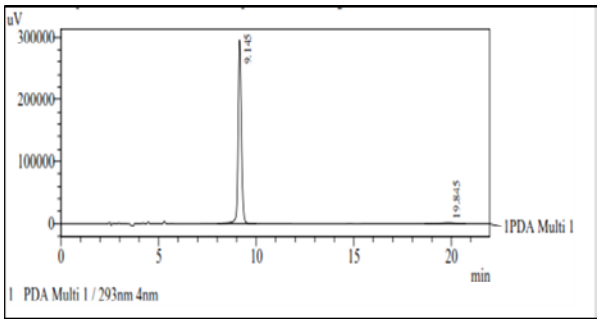


Fig. 5b: Chromatogram showing alkaline degradation of Balofloxacin (an additional peak is observed at Rt 19.845 which is the peak of degraded product of Balofloxacin)

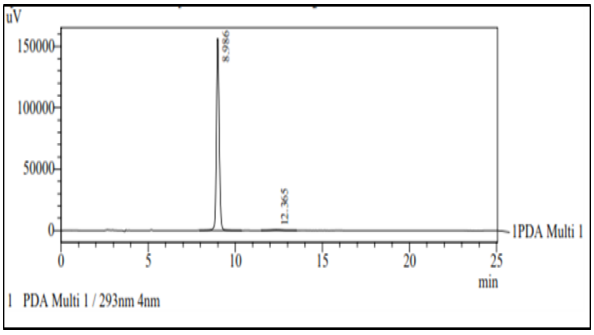


Fig. 5c: Chromatogram showing oxidative degradation of Balofloxacin (an additional peak is observed at Rt 12.365 which is the peak of degraded product of Balofloxacin)

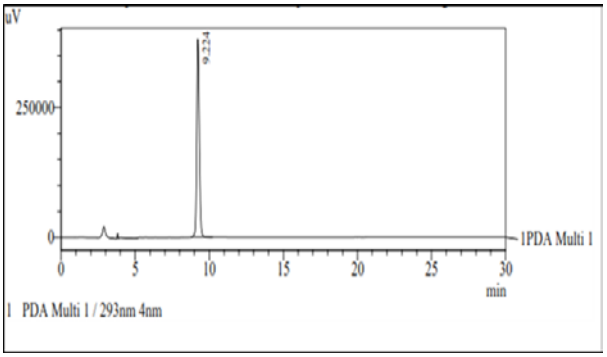


Fig. 5d: Chromatogram showing UV light degradation of Balofloxacin

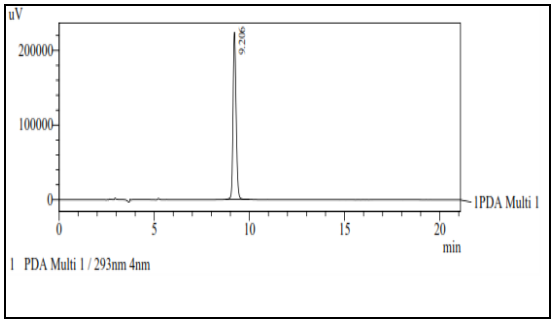


Fig. 5e: Chromatogram showing Sun light degradation of Balofloxacin

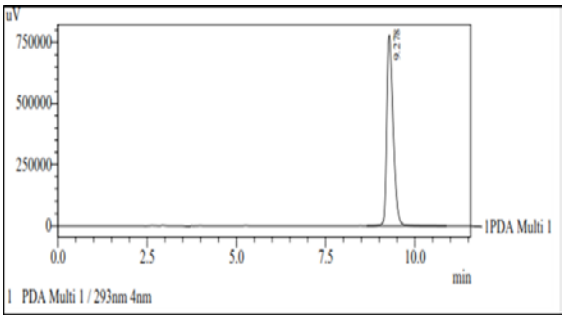


Fig. 6: Chromatogram showing analysis of Baloforce tablets

Table 5: Data of forced degradation studies

S. No.	Stress condition	Time	% Degradation
1.	Acidic hydrolysis (70^{0}C)	1 hour	29.83
2.	Alkaline hydrolysis (70^{0}C)	2 hours	25.22
3.	Oxidative hydrolysis (70^{0}C)	1 hour 30 min	18.06
4.	UV degradation	4 hours	12.22
5.	Sunlight degradation	3 hours	16.88

CONCLUSION

A simple, sensitive, specific, accurate and precise stability indicating RP-HPLC method was developed and validated for the routine analysis of bulk and tablet dosage form of Balofloxacin. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies and excipients found in tablets. The method can be employed for the routine analysis of Balofloxacin.

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CONFLICT OF INTEREST

None declared

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