A Validated Quantitative Estimation and Stability Indicating Reversed-phase-high Performance Liquid Chromatography Method for Balofloxacin in Bulk and its Tablet Formulation

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ABSTRACT

This paper describes the development of a stability-indicating reversed-phase-high performance liquid chromatography (RP-HPLC) method for balofloxacin (BFX) in the presence of its degradation products generated from forced decomposition studies. The drug substance was found to be susceptible to stress conditions of acid, base, oxidation, wet heat, and photo degradation. The drug was found to be stable to the dry heat condition attempted. Successful separation of the drug from the degradation products formed under stress conditions was achieved on a Zorbax C_{18} column (150 mm × 4.6 mm id, 5 microns particle size) using methanol-water pH adjusted to 2.5 by orthophosphoric acid (40:60, v/v) as the mobile phase at a flow rate of 0.5 mL/min at 30°C temperature. Quantification was achieved with photodiode array detection at 293 nm over the concentration range 50-150 µg/ml with mean recovery of 100.0 ± 0.53% for BFX by the RP-HPLC method. Statistical analysis proved the method is repeatable, specific, and accurate for estimation of BFX. Because the method could effectively separate the drug from its degradation products, it can be used as a stability-indicating method.

Keywords: Balofloxacin, reversed-phase-high performance liquid chromatography, orthophosphoric acid, validation

INTRODUCTION

Balofloxacin (BFX) is an orally active of third generation fluoroquinolone antibiotic. The chemical name of BFX[1,2] is 1-cyclopropyl-6-fluoro-1, 4-dihydro-8 methoxy-(methylamino1-piperidinyl)-4-oxo-3-quinoline carboxylic acid and its molecular formula is $C_{20}H_{24}FN_3O_4$. BFX is a fluoroquinolone that has a broader spectrum of activity and reduced toxicity than other fluoroquinolones and structure of BFX shown in Figure 1. BFX is used for the treatment of urinary tractinfection.[3] It exhibits excellent antibacterial activity against Gram-positive bacteria such as multiple drug-resistant, staphylococci, and pneumococci; BFX acts by binding and inhibiting topoisomerase-II (deoxyribonucleic acid [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.[4] Few analytical methods were reported in the literature, such as fluorescent spectroscopy,[5] reversed-phase-high performance liquid chromatography (RP-HPLC) with fluorescence detection,[6] liquid chromatography-electron ionization mass spectrometry,[7] luminescence spectroscopy[8] methods have been developed for the determination of BFX either in bulk or tablet formulations. However, no stability indicating RP-HPLC method is available for estimation of BFX in bulk and its pharmaceutical dosage form.

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DOI: 10.5530/phm.2014.1.1

MATERIALS AND METHODS

Balofloxacin pure drug was obtained as a gift sample from Hetero Labs Pvt. Ltd., Hyderabad Andhra Pradesh, India. Balofox-100 Mg tablets are manufactured by Hetero Pvt. Ltd., Himachal Pradesh, India and marketed by Abbott Health Care Pvt. Ltd., Mumbai, India. Baloflox-100 mg tablets manufactured by Hetero Mumbai, India were procured from the local pharmacy. High performance liquid chromatography (HPLC) grade methanol and water (filtered through 0.2 µ filters) were purchased from

Merck, Mumbai, India. Orthophosphoric acid (OPA) was purchased from Rankem, RFCL Ltd., New Delhi, India.

Preparation of solutions

A volume of 1000 ml of HPLC grade water (filtered through 0.2 µ filters) was degassed and adjusted the pH to 2.5 by 0.1 M OPA.

Preparation of stock solution

A total of 100 mg of BFX pure drug was dissolved in 100 mL of HPLC grade methanol to get a concentration of 1000 µg/ml.

Preparation of working standard solution

A volume of 10 ml of stock solution was taken in 100 ml volumetric flask and diluted up to the mark with HPLC grade water to get a concentration of 100 µg/ml.

Preparation of sample solution

A total of 20 tablets of BFX were powdered and an amount of the powder equivalent to 100 mg of the drug was accurately weighed and transferred to the 100 ml volumetric flask, made up to the volume with methanol. The solution was placed in an ultrasonicator for 20 min and filtered through a 25 mm, 0.45 µm nylon syringe filter. 10 ml of this solution was taken and diluted to 100 ml by using a HPLC grade water to get a final concentration of 100 µg/ml. Five replicate sample solutions were prepared in a similar manner.

Instrumentation

Waters HPLC system consisting of a WATERS 2695 separation module, an inbuilt auto sampler, a column oven and WATERS 2996 photodiode array detector was employed for throughout the analysis. Chromatography was performed on a Zorbax C_{18}- 5 µm, 150 mm × 4.6 mm column. A Digisum DI 707 digital pH meter used for pH adjustment and a band line sonerex sonicator was used for sonication. The data were acquired using the Empower 2 software and other details of the instrumentation are given in Table 1.

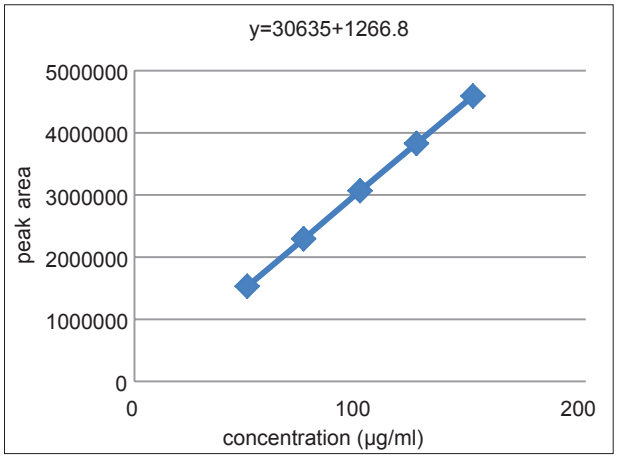

Figure 1. Linearity graph of balofloxacin.

Optimized chromatographic conditions

Chromatography was performed on a Zorbax C_{18}- 5 µm, 150 mm × 4.6 mm column using mobile phase containing a mixture of HPLC water pH adjusted to 2.5 with OPA: Methanol 60:40%v/v. The mobile phase was filtered through the membrane filter (0.45 µm) and vacuum degassed by sonication prior to use. The pump pressure and run time was maintained at 1500-2000 psi and 10 min respectively. Chromatography was performed at ambient temperature (30°C) under isocratic conditions at a flow rate is 0.5 ml/min and detection was done at 293 nm. Instrumentation and optimized chromatographic conditions for proposed method details are shown in Table 1.

Forced degradation sample study/specificity

The study was intended to ensure the separation of BFX and its degradation products. Forced degradation study was performed to evaluate the stability indicating properties and specificity of the method.[9] As per the ICH guideline Q2(R1) [10] provides the stress conditions to be performed for degradation products, which states that the stress samples should be stored under relevant stress conditions such as light, heat, humidity, acid/base hydrolysis, and oxidation. As per the guideline multiple stress studies were performed as indicated below and they were chromatographed along with anunstressed sample.

Table 1 Instrumentation and optimized chromatographic conditions for proposed method

Instrumentation	Optimized chromatographic conditions
HPLC	WATERS 2695 separation module
Detector	WATERS 2996 PDA
Column	Zorbax SD C_{18}
Column temperature	30°C
Flow rate	1.0 ml/min
Injection volume	5 µl
Wavelength	293 nmt
Run time	4.0 min
Diluents	Methanol
Mobile phase composition	Water: methanol 60:40 (water pH adjusted to 2.5 by OPA)

HPLC: High performance liquid chromatography, PDA: Photo diode array, OPA: Ortho phosphoric acid

Acid induced degradation

Solution containing 100 mg of BFX was treated with 10 mL 1N HCl sonicated or reflex for 30 min and then add 10 ml of 1N NaOH to neutralize and make up with methanol (diluent).

Base induced degradation

Solution containing 100 mg of BFX was treated with 10 mL 1N NaOH sonicated or reflex for 30 min and then add 10 ml of 1N HCL to neutralize and make up with methanol (diluent).

Oxidative condition

Hydrogen peroxide induced degradation. Solution containing 100 mg of BFX was treated with 6% w/v H_2O_2 at 40°C for 6 h was cooled and diluted with methanol.

Photolytic degradation study

A total of 100 mg of BFX was exposed to the ultraviolet light/sunlight for 7 days. This drug powder was transferred in 50 ml volumetric flask, diluted to the volume with methanol.

Aqueous degradation study

A total of 100 mg of BFX was added to 25 ml of water and refluxed for 6 h at 60°C after cooling make up the volume to 50 ml with methanol.

RESULTS AND DISCUSSION

Assay of B tablets

The developed method was applied to the assay of BFX tablets. The content was calculated as an average of six determinations, and experimental results were given in Table 2. The results were very close to the labeled value of commercial tablets. There presentative standard and sample chromatograms of BFX were shown in Figures 2 and 3, respectively.

Validation study of balofloxacin

The Method validation was performed as per ICH guidelines[11] for the determination of BFX in bulk and in the pharmaceutical dosage forms. The method was validated with respect to parameters, including specificity, accuracy, precision, linearity, robustness, system suitability, limit of detection (LOD), and limit of quantification.

Table 2 Assay results of BFX formulation

Formulations	Standard peak area	Sample peak area	Labeled amount (mg)	Amount found (mg)	%Assay± RSD*
Balofox (Abbott)	3,064,366	3,060,365	100	99.48	99.48±0.14

*Average of three determinations. RSD: Relative standard deviation, BFX: Balofloxacin

Specificity

The specificity was examined for non-degraded and degraded samples the solutions of after stress conditions of acid hydrolysis at 363 K, oxidation (H_2O_2). The HPLC method for determination of BFX was found selective in the presence of degradation products as shown in Figure 4. Peaks were symmetrical, clearly separated from each other [Figure 4]. Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of BFX peaks. The peak purity values were more than 98.79% for BFX at 273 nm, which proves that degradants were not interfering with the main peak. Forced degradation results are given in Table 3.

Accuracy (Recovery studies)

The accuracy was determined by calculating the recovery of BFX at 50%, 100%, and 150% was added to a pre-quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of BFX at

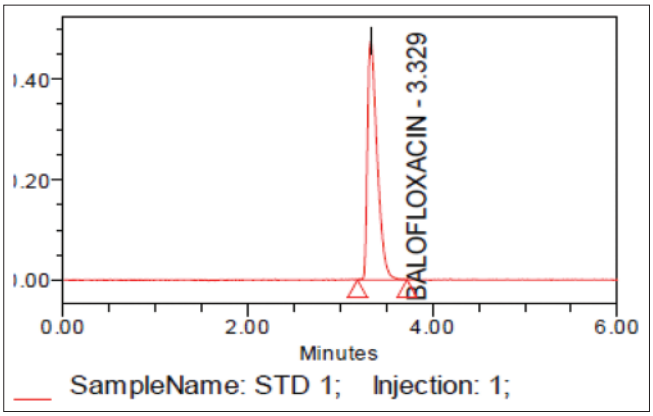

Figure 2. 2-reverse phase-high performance liquid chromatography chromatogram of balofloxacin standard.

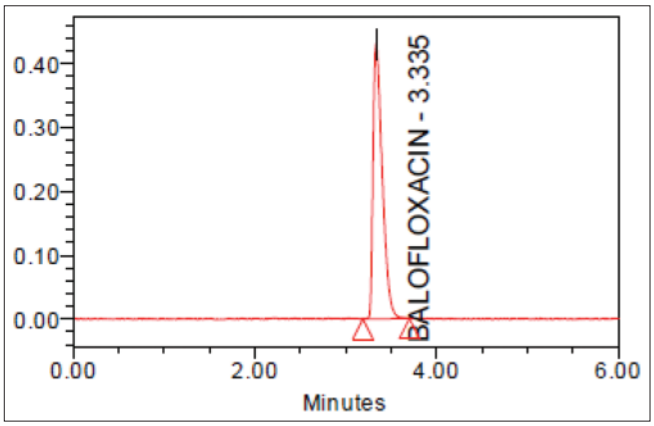

Figure 3. Reverse phase-high performance liquid chromatography chromatogram of balofloxacin formulation (tablets).

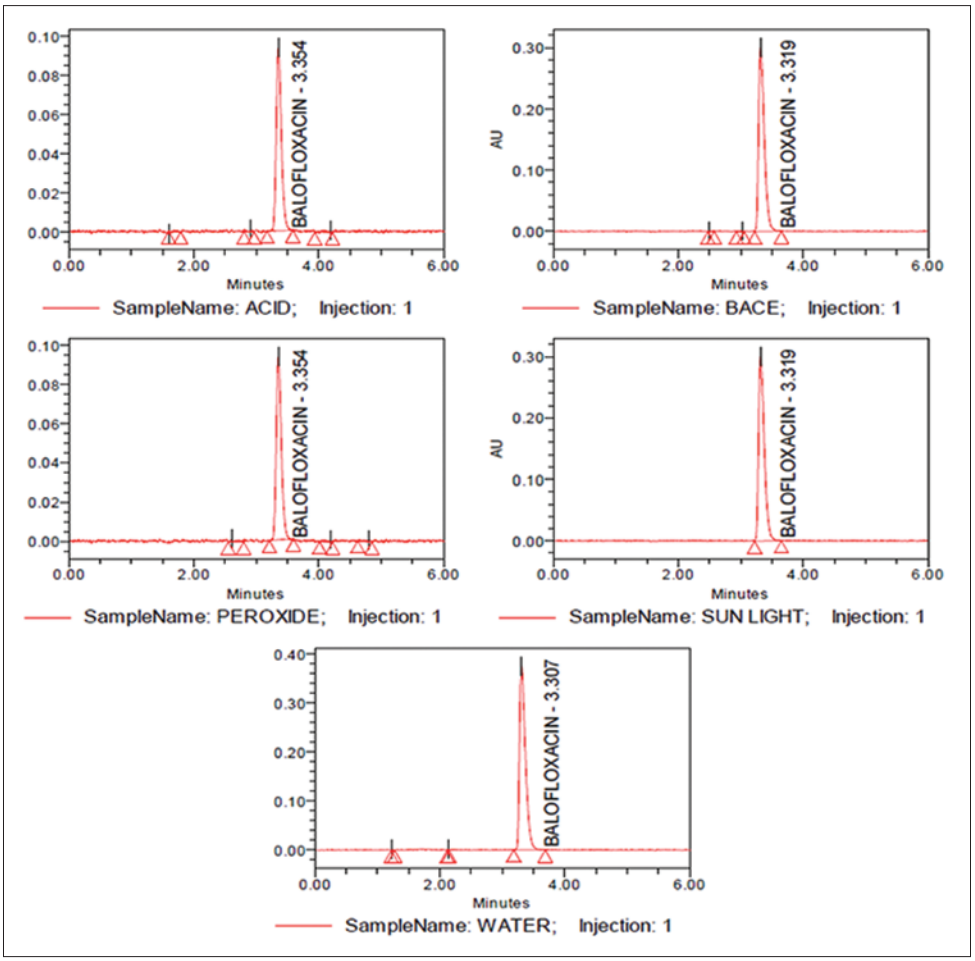

Figure 4. Reverse phase-high performance liquid chromatography chromatograms of degradation peaks in various stress conditions.

Table 3 Forced degradation studies of BFX

Stress conditions	*Drug recovered (%)	*Drug decomposed (%)
Standard drug	100	0
Acidic degradation	91	9
Basic degradation	94	6
Oxidative degradation	90	10
Photolytic degradation	97	3
Aqueous degradation	86	14

BFX: Balofloxacin

Table 4 Recovery data for the proposed RP-HPLC method

Concentration level %	Amount added (µg/ml)	Amount found (µg/ml)	Area obtained	Mean %recovery±SD*
50	50.00	49.95	1,532,382	49.99±0.14
100	100.00	99.76	3,069,777	100.6±0.25
150	150.00	149.69	4,567,421	149.85±0.35

*Average of three determinations. SD: Standard deviation, HPLC: High performance liquid chromatography

each level was not <99% and not more than 101%. The percentage recovery of BFX was found to be in the range of 100-101%. The results are shown in Table 4.

Precision

Precision should be investigated using homogeneous, authentic samples. Precision of the analytical method was expressed as a standard deviation and percentage of relative standard deviation of the series of replicate measurements. Precision of BFX estimation by the proposed method was ascertained by replicate analysis of homogeneous samples of BFX standard solutions in the intraday under the similar conditions. The system precision and method precision results were shown in Table 5.

Linearity

Linearity of the proposed method was established by using series of standard solutions of BFX at concentration levels from 50 to 150%, such as 50%, 75%, 100% 125%, and 150%. This linearity studies are repeated for 3 times with different stock solutions. The curve obtained by concentration on x-axis and peak area on y-axis against showed linearity in the concentration range of 50-150 µg/ml of BFX and linearity graph is shown in Figure 1. The regression equation was found to be y = 30635x

Table 5 Method precision results of the proposed RP-HPLC method

Injections	Rt	Peak area
1	3.291	3,067,665
2	3.287	3,061,482
3	3.288	3,060,365
4	3.290	3,061,218
5	3.290	3,067,460
6	3.291	3,068,851
Mean	3.289	3,064,501
S.D	0.0016	3864.91
%RSD	0.048	0.126

Rt: Retention time, SD: Standard deviation, RP-HPLC: Reverse phase-high performance liquid chromatography, RSD: Relative standard deviation

+1266 with regression coefficient is $r^2 = 0.999$. The linearity and statistical analysis of data are shown in Table 6.

Robustness

The robustness was evaluated by the analysis of BFX under different experimental conditions such as slight changes in chromatographic conditions such as change of flow rate (±0.2 ml/min), temperature (±5°C), and mobile phase composition (±5%). It was observed that there were no marked changes in the chromatograms, and the parameters are within the limit, which indicates that the method has the robustness and suitability for routine use. The complete results are shown in Table 7, and the method is having a good system suitability.

System suitability

The system suitability test was carried out on freshly prepared BFX standard solution (100%) was used for the evaluation of the system suitability parameters such as area, retention time, unique selling proposition peak tailing, and the number of theoretical plates, LOD and limit of quantitation (LOQ). Five replicate injections for a system suitability test were injected into the chromatographic system.

LOD

The LOD has established the minimum concentration at which analyze can be reliably detected. LOD is determined by the signal to noise ratio and signal to noise ratio 3:1 is generally considered acceptable for estimating the detection limit, and it was found to be 1.1 µg/ml.

LOQ

The LOQ has established the minimum concentration at which analyte can be reliably quantified. LOQ is determined by the signal to noise ratio, and a typical signal to noise ratio is 10:1 is acceptable for estimating the quantitation limit and it was found to be 3.7 µg/ml. Finally, the proposed method is having a good system suitability and system suitability parameters as shown in Table 8.

Table 6 Linearity analysis data for BFX

Concentration of BFX (µg/ml)	Percentage of working standard	Peak area
50	50	1,530,187
75	75	2,292,907
100	100	3,066,670
125	125	3,829,065
150	150	4,591,565

BFX: Balofloxacin

Table 7 Robustness results of BFX

Parameter	Plate count	Tailing	Rt	Peak area
Flow rate (0.8 ml/min)	7053	1.6	4.17	183,353
Flow rate (1.2 ml/min)	6280	1.5	2.80	1,197,149
Column temperature (25°C)	6678	1.5	3.32	1,427,503
Column temperature (35°C)	6698	1.5	3.34	1,437,873

BFX: Balofloxacin, Rt: Retention time

Table 8 System suitability parameters proposed for RP-HPLC method

Parameters	Values
Wavelength (λ max)	293 nm
Linearity range (µg/ml)	50-150
Regression equation	Y=30635.6x+1266
Regression coefficient (r^2)	0.99
Retention time (min)	3.34
Theoretical plates	6395
Tailing factor	1.5
Limit of detection (µg/ml)	1.1
Limit of quantitation	3.7

RP-HPLC: Reverse phase-high performance liquid chromatography

CONCLUSION

A stability-indicating RP-HPLC method was developed, validated and applied for the determination of BFX in pharmaceutical dosage forms. The developed method was validated and as per ICH guidelines and was found to be accurate, precise, robust, and specific. The chromatographic elution step is undertaken in a short time (<4 min). No interference from any components of pharmaceutical dosage form or degradation products was observed, and the method has been successfully used to perform long-term and accelerated stability studies of BFX formulations. Therefore, the method is suitable for use the routine quality control analysis of BFX in application programming interface or in pharmaceutical dosage forms.

ACKNOWLEDGMENT

The authors would like to thank the management of Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India for then donation of drug used in this investigation and SSJ College of Pharmacy for providing the facilities to carry out the research work.

REFERENCES

1. O'Neil MJ. The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals. 14th ed. White House Station, New Jersey: Merck & Co. Inc.; 2006. p. 941.
2. Sweetman SC. Martindale: The Complete Drug Reference. 37th ed. London: Pharmaceutical Press; 2011. p. 229.
3. Alksne L. Balofloxacin Choongwae. Curr Opin Investig Drugs 2003;4:224-9.
4. Ross DL, Elkinton S, Riley CM. Physicochemical properties of the fluoroquinolone antimicrobials IV 1-octanol/water partition coefficients and their relationships to structure. Int J Pharm 1992;88:379-89.
5. Marutani K, Matsumoto M, Otabe Y, Nagamuta M, Tanaka K, Miyoshi A, *et al.* Reduced phototoxicity of a fluoroquinolone antibacterial agent with a methoxy group at the 8 position in mice irradiated with long-wavelength UV light. Antimicrob Agents Chemother 1993;37:2217-23.
6. Qi XJ, Wang JX, Liu ZR. Fluorescent spectroscopy of balofloxacin. Chin J Anal Chem 2006;34:1047.
7. Bian Z, Tian Y, Zhang Z, Xu F, Li J, Cao X. High performance liquid chromatography-electrospray ionization mass spectrometric determination of balofloxacin in human plasma and its pharmacokinetics. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850:68-73.
8. Chu Z, Wang L, Guo C, Jiang W. Luminescence enhancement effect for the determination of balofloxacin with balofloxacin europium (III) sodium dodecylbenzene sulfonate system. J Lumin 2009;129:90-4.
9. ICH Stability Testing of New Drug Substances and Products Q1A (R2). In: Proceedings of International Conference on Harmonization; 2003.
10. International Conference on Harmonization (ICH) Q2 (R1): Validation of Analytical Procedures – Test and Methodology. Geneva, Switzerland; 2005.
11. FDA. Reviewer Guidance: Validation of Chromatographic Methods. Washington, USA: Center for Drug Evaluation Research (CDER); 1994.