Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

A NOVEL RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF BALOFLOXACIN IN TABLETS

M. SINDHUSRI, T. SWETHA, A. RAMADEVI, A. ASHOK KUMAR*

Department of Pharmaceutical analysis and Quality Assurance, Vijaya college of pharmacy, Munaganur (village), Hayathnagar (mandal), Hyderabad 501511, India.
Email: ashok576@gmail.com

Received: 12 Oct 2014 Revised and Accepted: 08 Nov 2014

ABSTRACT

Objective: To develop a rapid, accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Balofloxacin in tablets.

Methods: The optimized method uses a reverse phase column, Enable Make C18G (250 X 4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 2.3): acetonitrile in the proportion of 20:80 v/v, flow rate of 1.0 ml/min and a detection wavelength of 280 nm using a UV detector.

Results: The developed method resulted in Balofloxacin eluting at 2.6 min. Balofloxacin exhibited linearity in the range 2.5-7.5µg/ml. The precision is exemplified by relative standard deviation of 1.08%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies.

Conclusion: A rapid, accurate, precise and linear isocratic RP-HPLC method was developed and validated for the quantitative estimation of Balofloxacin in tablets as per ICH guidelines and hence it can be used for routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Balofloxacin, Method development, Validation.

INTRODUCTION

Balofloxacin,(fig. 1) is a fourth generation Fluoroquinolone antibiotic used as a broad spectrum antibacterial drug exhibiting activity against Gram negative bacterium and anaerobe specially against Gram positive bacterium such as MRSA, Streptococcus pneumonia, Enterococcus faecalis. Balofloxacin chemically is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylamino piperidin-1-yl)-4-oxoquinoline -3-carboxylic acid. Balofloxacin has an empirical formula of $C_{23}H_{26}N_2O_2$. $C_4H_6O_4$ and a molecular weight of 480.55. Mechanism of action includes inhibiting and binding 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 [1-6]. Balofloxacin is prescribed for various infectious diseases such as opthalmitis, sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, urinary tract infections [7].

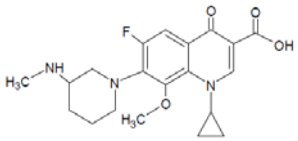

Fig. 1: Structure of Balofloxacin

Literature survey reveals chromatographic methods [3,8-10] and spectrophotometric methods [6,11-13] for the analysis of Balofloxacin in pharmaceutical dosage forms and few bioanalytical methods for the analysis of Balofloxacin in human plasma [14-16] and in urine [17]. In this article, we report a totally new, simple, accurate and precise RP-HPLC isocratic method for the determination of assay of Balofloxacin in tablets and validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Balofloxacin with purities greater than 99% was obtained as the gift sample from Chandra labs, Hyderabad, India and tablet formulation [BALOFORCE] was procured from MEDPLUS Pharmacy, Hyderabad, India with labelled amount 100mg of Balofloxacin. Acetonitrile (HPLC grade), water (HPLC grade), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Labsolutions lite" software. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Method

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Balofloxacin. Suitable wavelength selected was 280 nm (fig. 2).

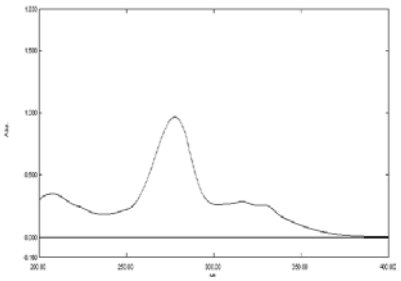

Fig. 2: UV spectrum of Balofloxacin

Chromatographic conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X4.6 mm; 5µ), mobile phase consisting of triethylammonium phosphate buffer (adjusted using 30% v/v of ortho phosphoric acid pH 2.3): acetonitrile in the proportion of 20:80 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 280 nm.

Buffer preparation

The buffer solution was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 2.3 using 30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

Mobile phase preparation

The mobile phase was prepared by mixing acetonitrile and buffer in the ratio of 80:20 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Diluent

100% pure acetonitrile is used as a diluent.

Preparation of stock and working standard solution

10mg of Balofloxacin was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluents and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as stock standard solution (100µg/ml). From this stock solution, 0.5 ml was pipetted out and made up to 10 ml using the diluent to get a concentration of 5µg/ml, treated as 100% target concentration.

Preparation of stock and working sample solution

Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 100 ml diluent and then stirred for 25 minutes followed by filtration through 0.45µ nylon membrane filter to get primary sample stock solution of 1000µg/ml.1 ml of the above stock solution was pipetted out and made up to 10 ml to get secondary stock solution of 100 µg/ml. Pipette out 0.5 ml from the above secondary stock solution and make up to 10 ml to get a concentration of 5µg/ml, equivalent to a concentration of working standard of 5 µg/ml.

RESULTS AND DISCUSSION

Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i. e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Balofloxacin at 2.6 min. Fig. **3** and **4** represent chromatograms of blank solution and the standard solution (5µg/ml) respectively. The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor of the standard at the working concentration are given in table 1**.**

Fig. 3: Typical Chromatogram of Blank solution

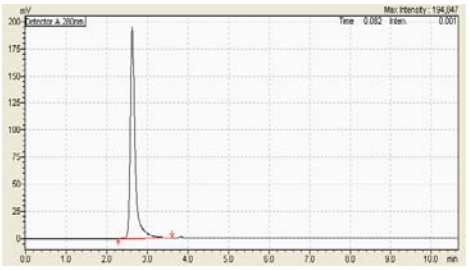

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitability studies results

Parameters	Balofloxacin
Retention time (min)	2.6
Number Of Theoretical plates (N)	3453
Tailing factor (T)	1.6

In order to test the applicability of the developed method to a commercial formulation, BALOFORCE was chromatographed at working concentration (5µg/ml) and it is shown in fig. 5. The sample peak was identified by comparing the retention time with the standard drug fig. 4. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

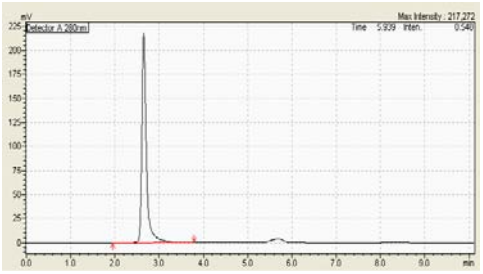

Fig. 5: Typical chromatogram for the tablet formulation

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [18] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy and precision.

Specificity

Fig. 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and the sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of Balofloxacin. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solution at the working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 2.

Method precision

Method precision was determined by performing assay of sample under the tests of repeatability (Intraday precision) at working concentration.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 3).

Table 2: System precision results of Balofloxacin

S. No.	Peak area
1	2195914
2	2215798
3	2166082
4	2141393
5	2234526
6	2234835
Average	2198091.3
SD	38039.84
%RSD	1.73

Table 3: Intraday precision results

S. No.	Balofloxacin % Assay
1	99.56
2	99.2
3	101.05
4	101.16
5	99.09
6	101.5
Average	100.26
S. D.	1.09
% R. S. D.	1.08

Linearity

Standard solutions of Balofloxacin at different concentrations level (50%, 75%, 100%, 125%, and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration level of drug within the concentration range (50-150µg/ml) for the drug and the results are given in Tables 4-5 and fig. 6. The correlation coefficient of Balofloxacin is greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear.

Table 4: Linearity of the chromatography system

Drug	Linearity range (µg/ml)	R^2	Slope	Intercept
Balofloxacin	2.5-7.5	0.995	10892.68	986768.8

Table 5: Calibration data for Balofloxacin

% Level	Concentration (µg/ml)	Peak Area
50	2.5	1512669
75	3.75	1804618
100	5	2095914
125	6.25	2380837
150	7.5	2586144

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 6**.** The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

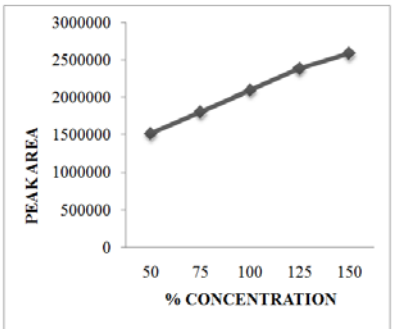

Fig. 6: Linearity curve for Balofloxacin

Table 6: Results of Accuracy studies for Balofloxacin

% Level	% Mean recovery
50	100.86
100	100.59
150	99.84

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision and linearity for the quantitative estimation of Balofloxacin in tablets. The precision is exemplified by relative standard deviation of 1.08%. A good linear relationship was observed for the drug between concentration ranges of 2.5 and 7.5µg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise and linear and therefore the method can be used for the routine analysis of Balofloxacin in tablets.

ACKNOWLEDGEMENT

The authors would like to thank the management of Vijaya college of pharmacy (VJYH), Hyderabad, for providing the necessary facilities to carry out of this research work. The authors are grateful to Chandra labs, Hyderabad for providing gift sample of Balofloxacin.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. 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.
2. Ashok Reddy S, Chandra Sekhar KB. Development and validation of analytical method for estimation of balofloxacin in bulk and Pharmaceutical dosage form. J Global Trends Pharm Sci 2012;3(2):647-55.
3. Ravisankar P, Devalarao G, Devadasu Ch, Gopal reddy P, Srinivasa babu P, Afzal basha SK, *et al.* A novel validated RP-HPLC method for the determination of Balofloxacin in bulk and pharmaceutical dosage forms. Int J Pharm Ind Res 2013;8(2):127-36.
4. Ito T, Otsuki M, Nishino T. *In vitro* antibacterial activity of Q-35, a new fluoroquinolone. Antimicrob Agents Chemother 1992;36:1708-14.
5. Gohara Y, Arai S, Akashi A, Kuwano K, Tseng CC, Matsubara S, Matumoto M, *et al*. *In vitro* and *In vivo* activities of Q-35, a new

fluoroquinolone, agaist Mycoplasma pneumonia. Antimicrob Agents Chemother 1993;37:1826-30.

6. Seetharaman R, Lakshmi KS, Dinesh kumar B, Hariprasad G, Ravi KR. Determination of Balofloxacin in pharmaceutical formulation by Zero, First and Second order derivative spectrophotometric methods. Int J Res Pharm Sci 2011;2(3):438-43.
7. Alksne L. Balofloxacin choongwae. Curr Opin Investig Drugs 2003;4(2):224-9.
8. Nyola NK, Govindasamy J. Estimation of Balofloxacin in active pharmaceutical ingredients and pharmaceutical formulations by different methods. Novel Sci Int J Pharm Sci 2012;1(7):425-9.
9. Naga Raju P, Devala Rao G, Rajendra Prasad Y. Development and validation of a Reverse Phase-HPLC Method for the determination of Balofloxacin in bulk and pharmaceutical dosage forms. Drug Invention Today 2012;4(12):655-8.
10. Thumar PM, Patel VB. Development and validation of analytical method for estimation of Balofloxacin in bulk and pharmaceutical dosage Form. Int J Pharm Tech Res 2011;3(4):1938-41.
11. Pai S, Nayak S. Development of a new spectroscopic method for determination of Balofloxacin in tablet dosage form. Int J Sci Res 2013;2(7):76-7.
12. Pai SP. Spectrophotometric determination of Balofloxacin by ion-pair complexation reaction in the bulk and tablet dosage forms. Asian J Pharm Clin Res 2013;1(1):22-4.
13. Reddy SA, Sekhar KC. Development and validation of analytical method for estimation of Balofloxacin in bulk and pharmaceutical dosage form. J Global Trends Pharm Sci 2012;3(2):647-55.
14. 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(1):68-73.
15. Wang D, Xie L, Wang J, Liu X. Determination of Balofloxacin in plasma by HPLC-Fluorescence and its pharmacokinetics in rats. J Chin Pharm University 2004;35(2):160-3.
16. Chu ZJ, Dong YB, Yang FH, Wang L. Determination of the content of Balofloxacin in human plasma by RP-HPLC method and its application in therapeutic drug monitoring. J Pharm Care Res 2008;8(3):223-5.
17. Sui Y, Guo T, Zhang J, Shi G, Tang Y, Zhao L. Determination of Balofloxacin in human urine by RP-HPLC with fluorescence detection. Journal of Shenyang Pharm University 2007;11:691-4.
18. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures: Text and methodology ICH Q2 (R1); 2005.