Int. J. Chem. Sci.: 11(1), 2013, 553-568
ISSN 0972-768X
www.sadgurupublications.com

A VALIDATED RP-HPLC METHOD FOR THE ASSAY OF BALOFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

P. RAVISANKAR[*a], Ch. DEVADASU, G. DEVALA RAO[b], P. SRINIVASA BABU, G. SUDHAKAR SAIBABU[b] and P. VENKATESWAR REDDY[c]

Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, GUNTUR – 522213 (A.P.) INDIA
[a]Faculty of Science, Sri Chandrasekharendra Saraswathi Viswa Mahavidyalaya (SCSVMV University), Enathur, KANCHIPURAM – 631561 (T.N.) INDIA
[b]Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, VIJAYAWADA (A.P.) INDIA
[c]Hetero Labs, Jeedimetla, HYDERABAD – 500 055 (A.P.) INDIA

ABSTRACT

A simple, accurate, precise, specific isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of balofloxacin pharmaceutical formulations. RP-HPLC method was developed by using Welchrom C_{18} Column (4.6 x 250 mm, 5 µm), Shimadzu LC-20AT prominence liquid chromatograph. The mobile phase used is phosphate buffer (pH-3.2): acetonitrile (60 : 40% v/v) with a flow rate of 1 mL/min. The responses are measured at 295 nm using Shimadzu SPD-20A prominence UV-Vis detector. The retention times of balofloxacin found to be 5.713 min. The method posses linearity in the range of 2-10 µg/mL and correlation coefficient is 0.9999. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision and specificity according to ICH guidelines. The proposed method provides an accurate and precise quality control tool for routine analysis of balofloxacin in tablet dosage forms.

Key words: Balofloxacin, RP-HPLC, UV-Vis detector, Method validation, Isocratic.

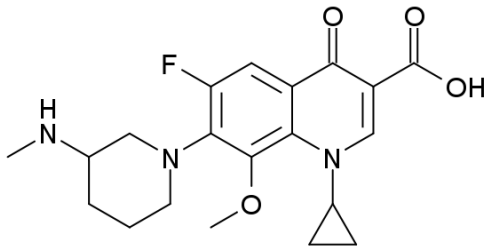
INTRODUCTION

The new fluoroquinolone balofloxacin[1-5] (BLFX) is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid, is a broad

[*]Author for correspondence; E-mail: banuman35@gmail.com

spectrum fluorinated a quinolone antibiotic, prescribed for infective ophthalmitis and sinusitis, chronic bronchitis, acute exacerbation, community-acquired pneumonia, skin infections, urinary tract infection[6]. It exhibits excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneumococci. Balofloxacin acts by binding to and inhibiting topoisomerase II (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[7-9]. Literature survey revealed that very few methods have been reported for the analysis of balofloxacin which include luminescence spectroscopy[7], reverse phase high pressure liquid chromatography[10], LC-MS, HPLC with fluorescent spectroscopy[11], RP-HPLC with fluorescence detection[12], HPLC-Electro spray ionization mass spectroscopy[13], and few UVspectrophotometric methods[14]. The present study illustrates development and validation of simple, sensitive, precise and accurate RP-HPLC method for the determination of new antibacterial fluoroquinolone balofloxacin[15,16] in bulk samples and pharmaceutical tablet dosage forms as per ICH guideline[17].

Chemical name and structure of Balofloxacin



1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl amino piperidin-1-yl)-4-oxo quinoline-3-carboxylic acid

EXPERIMENTAL

Materials and methods

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu LC-20AT prominence liquid chromatograph) with two LC-20AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A prominence UV-Vis detector and Welchrom C_{18} Column (4.6 x 250 mm, 5 µm). The HPLC system was equipped with "Spincotech" software.

Standards and chemicals used

Balofloxacin was provided by Hetero Drugs Limited, Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from S.D Fine-Chem. Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Limited (Mumbai, India). Commercial tablets of balofloxacin were purchased from local market. Balowin-100 (Intra Lab), Bazucin-100 mg tablets manufactured by Lupin Ltd., B-Cin-100 mg tablets are manufactured by Hetero Labs Ltd., and marketed by Lupin Ltd., Mumbai, India.

Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this 55 mL of 0.1 M phosphoric acid was added and pH was adjusted to 3.2 with triethylamime. The above prepared buffer and acetonitrile were mixed in the proportion of 60 : 40 v/v and was filtered through 0.22 µm nylon membrane filter and degassed by sonication.

Preparation of calibration standards

About 100 mg of pure Balofloxacin was accurately weighed and dissolved in 100 mL of mobile phase to get 1 mg/mL stock solution. Working standard solution of balofloxacin was prepared with mobile phase. To a series of 10 mL volumetric flasks, standard solutions of balofloxacin in the concentration range of 2, 4, 6, 8, 10 µg/mL were transferred. The final volume was made with the mobile phase.

RESULTS AND DISCUSSION

Validation study of Balofloxacin

An integral part of analytical method development is validation. Once the method has been devised, it is necessary to evaluate under the conditions expected for real samples before being used for a specific purpose. The method validation was performed as per ICH guidelines for the determination of balofloxacin in bulk and in the pharmaceutical dosage forms. The method was validated with respect to parameters including specificity, Precision, accuracy linearity, robustness, system suitability, limit of detection (LOD) and limit of quantification (LOQ). The goal of this study is to develop rapid HPLC methods for the analysis of balofloxacin in bulk drug samples and tablet formulations using the most commonly employed column (C_{18}) with UV detection at appropriate wavelength. The representative chromatograms indicating the balofloxacin were shown in Fig. 1 to 7. The linearity of the method lies between 1-10 µg/mL (Fig. 8).

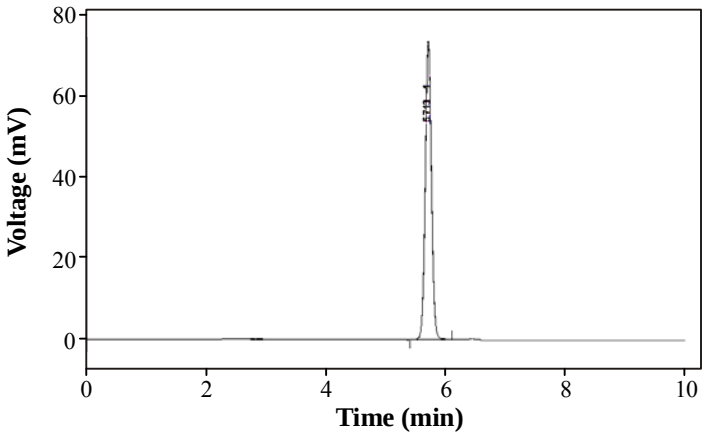


Fig. 1: Standard chromatogram of balofloxacin (10 µg/mL)

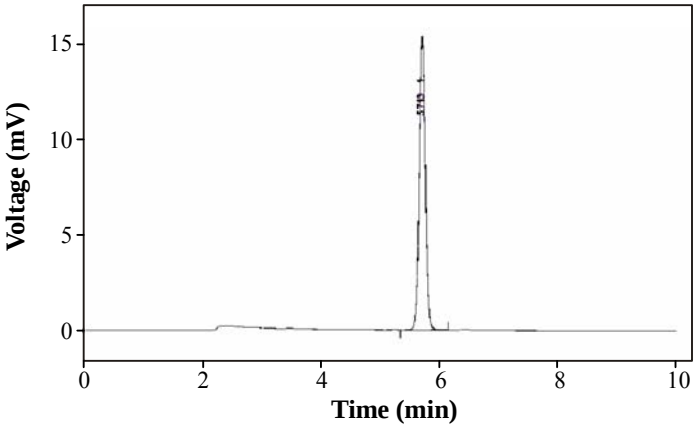


Fig. 2: Standard chromatogram of balofloxacin (2 µg/mL)

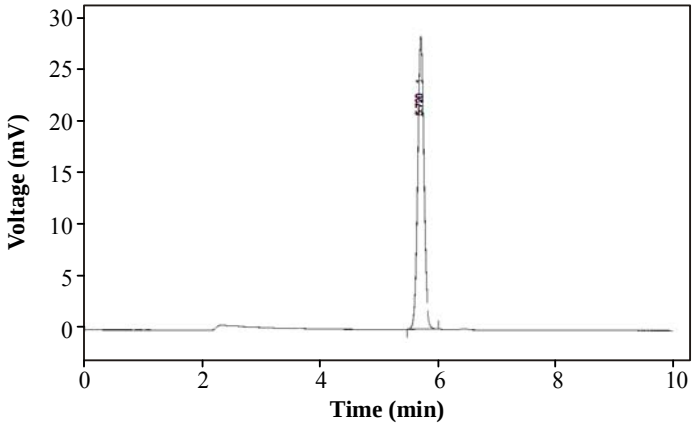


Fig. 3: Standard chromatogram of balofloxacin (4 µg/mL)

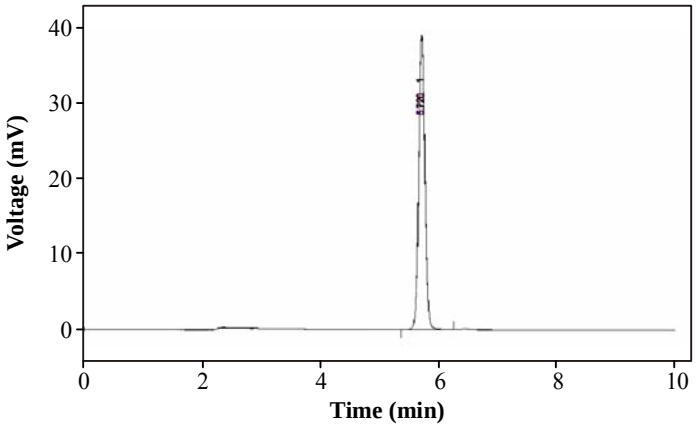


Fig. 4: Standard chromatogram of balofloxacin (6 µg/mL)

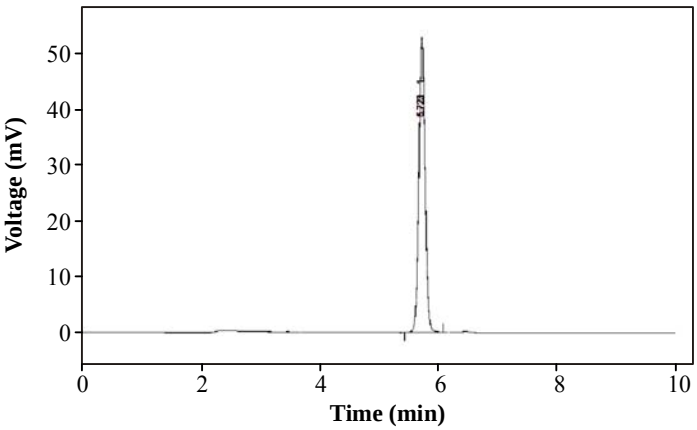


Fig. 5: Standard chromatogram of balofloxacin (8 µg/mL)

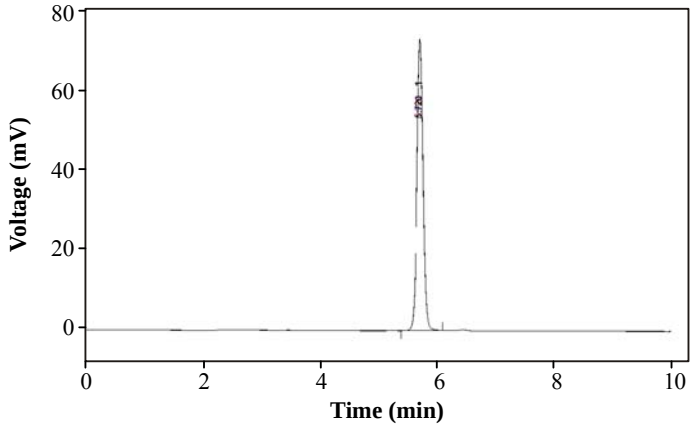


Fig. 6: Standard chromatogram of balofloxacin (10 µg/mL)

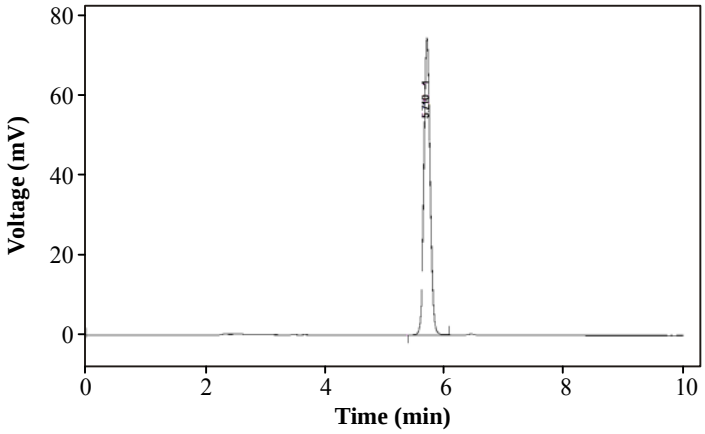


Fig. 7: Sample Chromatogram of balofloxacin

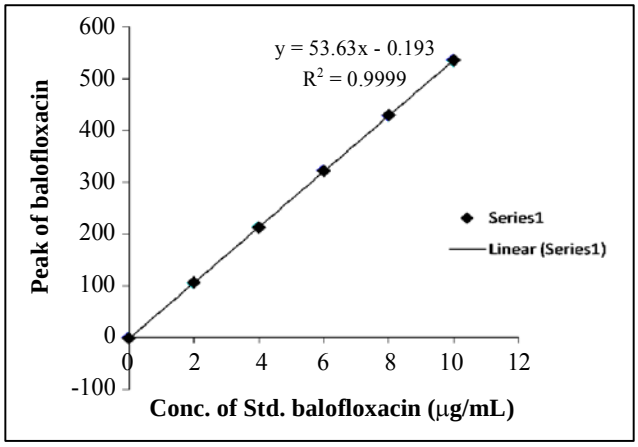


Fig. 8: Calibration plot of balofloxacin

Optical characteristics, regression data, Precision of the proposed method of balofloxacin is shown in Table 9.

System suitability

The HPLC system was stabilized for forty min. One blank followed by six replicates of a single calibration standard solution of baloflaxacin was injected to check the system suitability. To ascertain the systems suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in Table 1.

Table 1: Instrumentation, optimized chromatographic conditions and system suitability parameters for proposed method balofloxacin

Parameter	Chromatographic conditions
Instrument	Shimadzu LC-20AT prominence liquid chromatograph
Column	Welchrom C_{18} Column (4.6 x 250 mm, 5 µm)
Detector	Shimadzu SPD-20A prominence UV-Vis detector
Diluent	Buffer: Acetonitrile (60 : 40 v/v)
Mobile phase	Buffer: ACN (60 : 40 v/v)
Flow rate	1 mL/min.
Detection wave length	By UV at 295 nm.
Run time	10 minutes
Column back pressure	119-125 (Kg/cm^2)
Temperature	Ambient temperature (25^oC)
Volume of injection loop	20 (µL)
Retention time	5.713 min
Theoretical plates[th.pl] (Efficiency)	14,079
Theoretical plates per meter [t.p/m]	282239
Peak asymmetry	1.035

Recommended procedure

Construction of calibration curve

Replicates of each calibration standard solutions were (2,4,6,8,10 µg/mL) were injected in to the chromatogram, the retention times and average peak areas were recorded. Calibration graph was plotted by taking concentration of balofloxacin on X-axis and ratio of peak areas of standard balofloxacin on Y-axis.

Assay of balofloxacin

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 100 mg of BLFX was taken and the drug was extracted in 100 mL of mobile phase. The resulting solution was filtered through 0.22 µm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µL fixed volume loop manual injector. The chromatographic run time of 10 min. was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 295 nm. The amount of drug present in sample was computed from the calibration graph. The results were presented in Table 2.

Table 2: Assay results of balofloxacin formulations

S. No.	Forumlations	Standard peak area	Sample peak area	Labeled amount	Amount found	% Assay ± RSD*
1	Balowin (intra lab)	535.780	535.634	100	99.97	99.97 ± 0.12
2	B-Cin (Lupin)	535.690	535.630	100	99.98	99.98 ± 0.11

*Average of six determinations

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of BLFX in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the sample solution and injected. While the comparison of chromatograms there was no interference from placebo with sample peak. They do not disturb the elution or quantification of balofloxacin. Furthermore, the well-shaped peaks also indicates the specificity of the method. Therefore, it was concluded that the method is specific. The specificity results are shown in Table 3.

Table 3: Specificity study

Name of the solution	Retention time in min.
Blank	No peaks
Balofloxacin	5.710

Precision

Precision of the method was performed as intraday precision and interday precision. To study the intraday precision, six-replicate standard solution of balofloxacin was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.072, which is within the acceptable criteria of not more than 2.0. For interday precision six-replicate standard solution of balofloxacin was injected on the third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.130, which is within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 4.

Table 4: Results of Intraday and interday precision study

Sample	Concentration (PPM)	Injection number	Intraday precision	Interday precision
			Peak area	Peak area
Balofloxacin	10	1	535.630	536.750
	10	2	535.000	535.860
	10	3	536.235	534.950
	10	4	535.600	536.700
	10	5	535.590	535.670
	10	6	535.612	535.550
		Mean	535.612	535.913
		Standard deviation	0.390816453	0.698704
		% RSD acceptance criteria 2.0)	0.072966268	0.130376

Linearity

The linearity graphs for the proposed assay methods were obtained over the concentration range of 2-10 µg/mL BLFX. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented in Table 5. Regression statistics and Anova data is shown in Table 8. The linearity graph of balofloxacin was shown in Fig. 8.

Table 5: Linearity and statistical analysis data for balofloxacin

S. No.	Linearity level (µg/mL)	Concentration (ppm)	Peak area	Slope	Y-intercept	Correlation coefficient(r^2)
1	2	2	107.1268			
2	4	4	213.2400			
3	6	6	322.3804	53.63	-0.193	0.9999
4	8	8	429.5072			
5	10	10	535.6340			

Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of balofloxacin by the method of addition. Known amount of balofloxacin at 25%, 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 balofloxacin at each level was not less than 99% and not more than 101%. The average recovery was found to be 100.05%.

The accuracy results are shown in Table 6.

Table 6: Recovery data of the proposed balofloxacin RP-HPLC method

S. No.	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean % Recovery ± SD*	% RSD #
		5	4.99	267.275		
1	50%	5	5.00	266.300	100 ± 0.01	0.01
		5	5.01	266.265		
		10	9.99	535.634		
2	100%	10	9.98	532.531	99.91 ± 0.025	0.025
		10	10.01	535.630		
		15	15.10	802.900		
3	150%	15	15.02	803.121	100.26 ± 0.05	0.05
		15	15.00	801.890		

* SD Standard deviation

%RSD is percentage of relative standard deviation

Robustness

Robustness of the proposed methods was evaluated by making small changes in flow rate (± 0.2 mL/min), temperature (± 5°C), Mobile phase composition (± 5%), and pH of the buffer solution. The results were found to be not affected by these small alterations. The parameters are within the limit, which indicates that the method has robust and suitability for routine use.

Robustness results are shown in Table 7.

Table 7: Robustness results of balofloxacin

S. No.	Parameters	Optimized	Used	Peak area	Retention time (Rt)	Plate count	Peak asymmetry
1	Flow rate (± 0.2)	1 mL/min	0.8	568.874	6.410	14200	1.190
			1	535.634	5.710	14079	1.035
			1.2	528.784	4.812	13850	1.068
2	Temperature (± 5°C)	30°C	25	548.987	5.120	14028	1.068
			30	535.634	5.710	14079	1.035
			35	578.987	4.980	13902	1.069
3	Mobile phase composition (± 5)	60 : 40	55 : 45	528.200	6.980	13950	1.100
			60 : 40	535.634	5.710	14079	1.035
			65 : 35	528.786	7.620	14220	1.065

Ruggedness

Ruggedness of the method was evaluated by comparing the results of assay of balofloxacin obtained from two analysts, systems and two columns. RSD was always found to be < 2%, which indicates the method is rugged.

Table 8: Regression statistics

Regression Statistics						
Multiple R	0.999993777					
R Square	0.999987555					
Adjusted R Square	0.999984443					
Standard Error	0.791538446					
Observations	6					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	201370.0974	201370.0974	321403.7597	5.80818E-11	
Residual	4	2.506132443	0.626533111			
Total	5	201372.6035				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	-0.19371429	0.572873468	-0.33814498	0.752256607	-1.784266022	1.396837451
X Variable	53.63502286	0.09460694	566.9248272	5.80818E-11	53.37235188	53.89769383

Table 9: Optical characteristics, regression data, precision of the proposed method of balofloxacin

Parameter	Method
Detection wavelength (λ_{max})	By UV at 295 nm
Linearity range (µg/mL)	2-10 µg/mL
Regression equation (Y = a + bc)	Y = 53.63 x -0.193
Slope (b)	53.63
Intercept (a)	-0.193
Standard deviation of slope (S_b)	0.09460694
Standard deviation of intercept (S_a)	0.5728734
Standard error of estimation (Se)	0.7915
Correlation coefficient (r)	0.9999
% Relative standard deviation* i.e., Coefficient of variation (CV)	0.072966268
Limit of detection (µg/mL)	0.055
Limit of quantitation (µg/mL)	0.167
Percentage range of errors* (Confidence limits)	
0.005 significance level	0.076586
0.001 significance level	0.120107

*Average of six determinations

Limit of detection (LOD)

The limit of detection (LOD) is defined as the lowest concentration of the analyte that can be readily detected but not necessarily quantified. LOD is calculated by using the

formula LOD = 3.3(SD)/S. The Limit of detection of balofloxacin was found to be 0.055 µg/mL.

Limit of quantitation (LOQ)

The limit of quantitation (LOQ) is defined as the lowest concentration of the analyte that can be readily quantified with acceptable precision and accuracy. LOQ is calculated by using the formula LOQ = 10(SD)/S. The limit of quantitation was found to be 0.167 µg/mL.

CONCLUSION

Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. This method is simple, reliable, accurate, linear, sensitive, economical and reproducible. Hence this method can be suitable for routine quality control analysis of balofloxacin in active pharmaceutical ingredient (API) and pharmaceutical preparations.

ACKNWOLEDGEMENT

The authors are thankful to Hetero Labs Limited Unit-III, Jeedimetla, Hyderabad for providing the gift sample of pure balofloxacin and to Vignan Pharmacy College for providing the necessary facilities for this research work.

REFERENCES

1. Drug Today Medical Journal, Lorina Publication (India) Inc., Delhi-91, DT 78, **Vol. 1**, (2012) pp. 553-554.
2. http://en.wikipedia.org/wiki/Balofloxacin.
3. Merck and Co. Inc., The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edn., White House Station, Newjeresy (2006) p. 941.
4. Sean C. Sweetman, Martindale, The Complete Drug Reference, 37th Edn., Pharmaceutical Press, London (2011) p. 229.

5. L. George. HPLC Methods for Recently Approved Pharmaceuticals Published by John Wiley & Sons, Inc., Hoboken, New Jersey, Published Simultaneously in Canada, **67** (2005).

6. Alksne L. Balofloxacin Choongwae, Curr Opin Investing Drugs, **4(2)**, 224-229 (2003).

7. D. L. Ross, S. Elkinton and C. M. Riley, Physicochemical Properties of the Fluoroquinolone Antimicrobials IV 1-Octanol/Water Partition Coefficients and Their Relationships to Structure, Int. J. Pharm., **88**, 379-389 (1992).

8. Z. Chu, L. Wang, C. Guo and W. Jiang, Luminescence Enhancement Effect for the Determination of Balofloxacin with Balofloxacin Europium (III) Sodium Dodecyl Benzenesulfonate System, J. Luminescence, **129**, 90-94 (2009).

9. T. Nakagawa, M. Ishigai, Y. Hiramatsu, H. Kinoshita, Y. Ishitani, K. Ohkubo and A. Okazaki, Determination of the New Fluoroquinolone Balofloxacin and its Metabolites in Biological Fluids by High Performance Liquid Chromatography, Arzneimittel-forschung, **45**, 716-718 (1995).

10. Mi Yaxian, Wu Yan, Li Hualong and Li Lijian, Study on Determination of Related Substances in Balofloxacin by RP-HPLC, Tianjin Pharmacy, **05**, 927 (2005).

11. K. Marutani, M. Matsumoto, Y. Otabe, M. Nagamuta, K. Tanaka, A. Miyoshi et al., Reduced Photo Toxicity of a Fluoroquinolone Antibacterial Agent with a Methoxy Group at the 8 Position in Mice Irradiated with Long-Wavelength UV Light. Antimicrobial Agents Chemother., **37**, 2217-2223 (1993).

12. X. J. Qi, J. X. Wang and Z. R. Liu, Fluorescent Spectroscopy of Balofloxacin, Chinease J. Analytical Chem., **34**, 1047 (2006).

13. Z. Bian, Y. Tian, Z. Zhang, F. Xu, J. Li and X. Cao, High Performance Liquid Chromatography Electro Spray Ionization Mass Spectrometric Determination of Balofloxacin in Human Plasma and its Pharmacokinetics, J. Chromatogr B Analyt. Technol. Biomed. Life Sci., **850(1-2)**, 68-73 (2007).

14. M. P. Tumar, B. Vandana, Patel Int. J. Pharm. Tech. Res., **3(4)**, 1938-1941 (2011).

15. O. Kozawa, T. Uematsu, H. Matsuno, M. Niwa, S. Nagashima and M. Kanamaru, Comparative Study of Pharmacokinetics of Two New Fluoroquinolones, Balofloxacin and Grepafloxacin, in Elderly Subjects, Antimicrob. Agents Chemother., **40**, 2824-2828 (1996).

16. T. Uematsu, Y. Ohsawa, A. Mizuno and M. Nakashima, Analysis of a New Fluoroquinolone Derivative (Q-35) in Human Scalp Hair as an Index of Drug Exposure and as a Time Marker in Hair, Int. J. Legal Med., **106**, 237-243 (1994).

17. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 1-13 (2005).

Revised : 08.02.2013 *Accepted : 10.02.2013*