CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1214525

Available online at: http://www.iajps.com Research Article

VALIDATED HPLC METHOD FOR ESTIMATION OF BALOFLOXACIN IN BULK AND DOSAGE FORM

Rameshpetchi Rajendran[1], Devikasubramaniyan[2]*, Ramya Sri Sura[3], R. Swathi[4], R. Sainandhan Rao[5], K. Sindhu[6], D. Lavanya[7]

[1,2,4,5,6,7]Bomma Institute of Pharmacy, Behind Eenadu office, Allipuram, Khammam,Telangana, 507318

[3]University of Technology, Osmania University, Hyderabad, Telangana, 500007

Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Balofloxacin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Sunfire C18 (4.6×150mm, 5µm) column using a mixture of Methanol: Water (50:50 v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 245nm. The retention time of the Balofloxacin was 2.6 ±0.02min. The method produce linear responses in the concentration range of 35-175µg/ml of Balofloxacin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Balofloxacin, RP-HPLC, validation.*

Corresponding author:

Devikasubramaniyan,
Bomma Institute of Pharmacy,
Behind Eenadu office,
Allipuram, Khammam,
Telangana, 507318

QR code

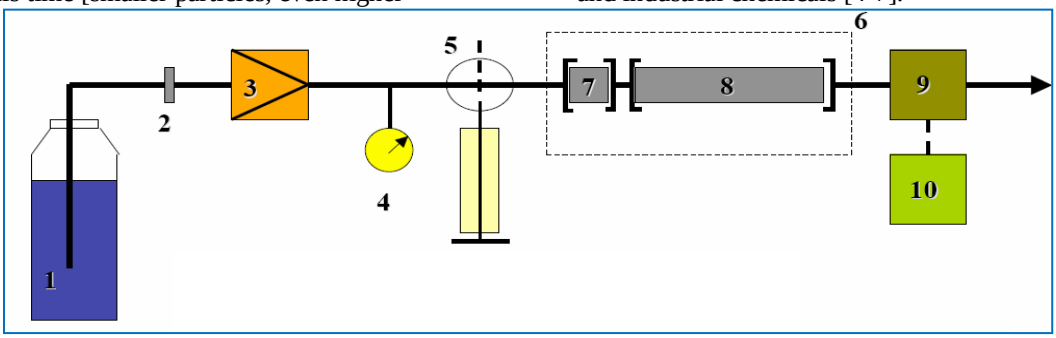
Please cite this article in press Devikasubramaniyan et al., ***Validated Hplc Method for Estimation of Balofloxacin in Bulk and Dosage Form,*** *Indo Am. J. P. Sci, 2018; 05(04).*

INTRODUCTION:

High Performance Liquid Chromatography (HPLC)

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography [1-3].

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals [4-7].



1 = eluent reservoir
2 = filter
3 = high pressure pump with pulse dampener
4 = pressure gauge
5 = sample injection valve with syringe
6 = column oven
7 = guard column
8 = column
9 = detector
10 = recorder (integrator, PC etc.)

Fig.1: High-Performance Liquid Chromatography [HPLC] System

Balofloxacin 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid. s used to treat a variety of bacterial infections of the urinary tract and lungs.

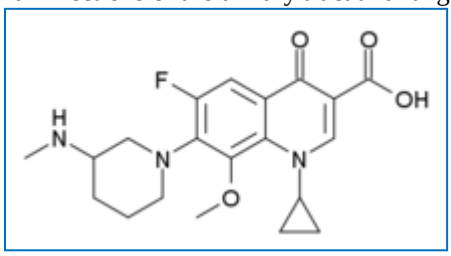


Fig. 2: chemical structure of Balofloxacin

MATERIALS AND METHODS:

Accurately measured 500ml (50%) of HPLC Water and 500ml of Methanol (50%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Instrumentation and Chromatographic conditions

The analysis was performed by using Sunfire C18 column, 4.6×250mm internal diameter with 5 micron particle size column and UV detector set at 290nm nm, in conjunction with a mobile phase of Methanol

in the ratio of 100v/v (pH 5 adjusted with OPA) at a flow rate of 0.9 ml/min. The retention time of Balofloxacin was found to be 2.635 minute. The 10µl of sample solution was injected into the system

Preparation of standard solution [8-10]:
Accurately weigh and transfer 10 mg of Balofloxacin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 1.05ml of the above Balofloxacin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Mobile Phase Optimization [11-16]:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 50:50 v/v respectively.

Optimization of Column:
The method was performed with column like Sunfire C18 (4.6×250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow
(Optimized chromatogram):
Column : Sunfire C18 (4.6×250mm)5µ
Column temperature : 35ºC
Wavelength : 290nm
Mobile phase ratio :Methanol:Water (50:50)V/V
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6min

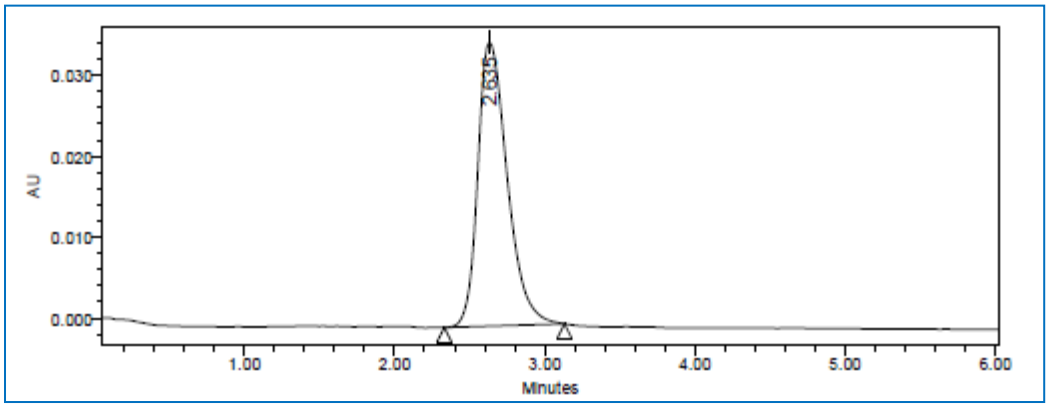


Fig. 3: Typical chromatogram of mixture of Standard solution.

VALIDATION
PREPARATION OF MOBILE PHASE:
Preparation of mobile phase:
Accurately measured 500ml (50%) of HPLC Water and 500ml of Methanol (50%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration
Diluent Preparation:
The Mobile phase was used as the diluent.
Linearity
The linearity of was obtained in the concentration ranges from 35-175µg/ml

Table 1: Linearity data of Balofloxacin

Concentration Level (%)	Concentration µg/ml
60	35
80	70
100	105
120	140
140	175

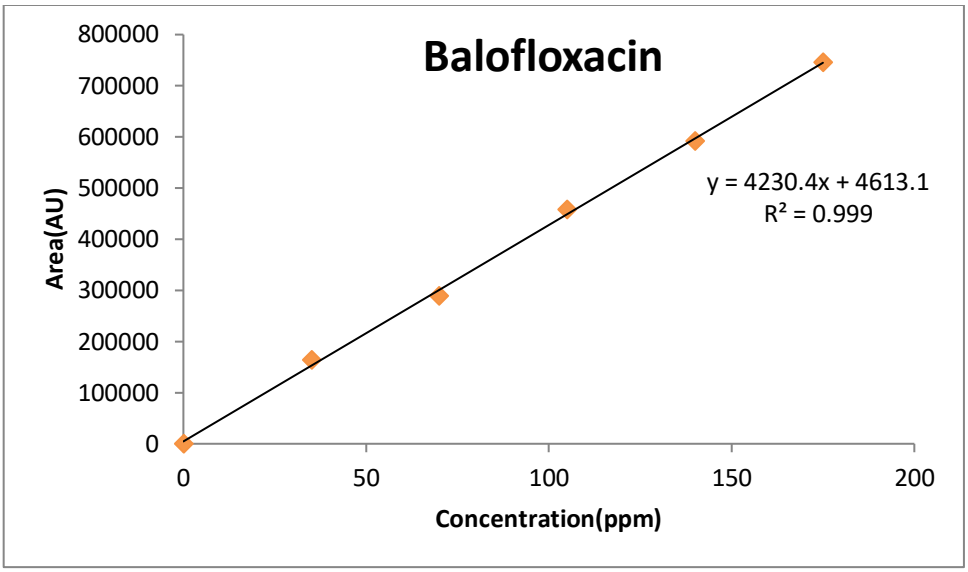


Fig.4. calibration graph of Balofloxacin

LINEARITY PLOT

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 60-140 µg/mL for Balofloxacin. The graph was plotted for concentration versus peak area. The results were shown in Table-1 and fig 4.

Precision

Repeatability [17-20]

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated. The results were shown in Table 2.

Table 2: Results of repeatability for Balofloxacin

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Balofloxacin	2.632	486861	35474	6918	1.32
2	Balofloxacin	2.633	489909	37144	7958	1.32
3	Balofloxacin	2.634	488067	35614	7929	1.28
4	Balofloxacin	2.632	485982	36367	5967	1.29
5	Balofloxacin	2.635	488912	35613	4948	1.28
Mean			487946.2			
Std.dev			1568.095			
%RSD			0.321366			

Accuracy

Balofloxacin tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-4

Table 4: The accuracy results for Balofloxacin

%Concentration (at specification Level)	Peak area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	235213	52.5	52.48	98.7	99.2%
100%	476585	105	104.9	99.3	
150%	774778	157.5	157.4	99.7	

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.The LOD value for Balofloxacin 8.2µg/ml.

Quantitation limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ value for Balofloxacin 24.9 µg/ml

ROBUSTNESS [21-24]

The robustness was performed for the flow rate 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Balofloxacin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of Balofloxacin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 5.

Table 5: Results for Robustness of Balofloxacin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	457254	2.635	9362	1.1
Less Flow rate of 0.8mL/min	458816	3.997	9917	1.12
More Flow rate of 1 mL/min	450018	1.942	7917	1.3
Less organic phase (about 5 % decrease in organic phase)	449175	2.598	8937	1.1
More organic phase (about 5 % Increase in organic phase)	450181	2.634	9472	1.3

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 290nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Sunfire C18 (4.6×150mm, 5µm) because it was giving good peak.40 º C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.9ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Water (50:50 v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Balofloxacin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Balofloxacin was freely soluble in ethanol, methanol and sparingly soluble in water.

Table 6: Summary data for Balofloxacin

Parameters	Balofloxacin
Retention Time (min.)	2.635
Linearity (µg/ml)	35-175
Correlation Coefficient (r^2)	0.999
Slope	4230
Y - intercept	4613
LOD (µg/ml)	8.2
LOQ (µg/ml)	24.9
Repeatability (% RSD) n=6	0.321366
Intraday Precision (% RSD) n=6	1.035617
Interday Precision (% RSD) n=6	1.43223
Accuracy (%)	99.2%

ACKNOWLEDGEMENT:
The authors are thankful to Sura Pharma Labs, Dilshuknagar, Hyderabad for providing necessary facilities for this entire research work.

REFERENCES:

1. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1-8.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1stedition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/columncare.pdf
9. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
14. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
15. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
16. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
17. Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), PP 1-7.
18.
19. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.

20. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
21. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
22. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
23. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. ***Journal of Pharmaceutical Technology*** (2003), 5, PP 110-114.
24. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
25. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).