ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Development and Validation of Analytical Method for Estimation of Balofloxacin in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT:
The objective of the present study is to develop simple, accurate, sensitive and economic method for effective quantitative determination of Balofloxacin in active pharmaceutical ingredient. as well as in Pharmaceutical dosage forms by using HPLC. The newly developed method is validated in accordance with the analytical parameters for quantitative estimation of Balofloxacin in pharmaceutical dosage forms as per ICH guidelines. The method was validated for parameters like accuracy, linearity, precision, specificity, ruggedness, robustness, and system suitability. The detection was carried out using UV detector at 249 nm. The solutions were injected at a constant flow rate of 1 ml/min. the linearity range of Balofloxacin was found to be 10-60 µg/ml. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Balofloxacin. LOD were found to be 0.210 µg/ml and LOQ found to be 0.637µg/ml for Balofloxacin. The results obtained on the validation parameters met ICH guidelines; it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: High Performance Liquid Chromatography, Active Pharmaceutical Ingredient, Validation, Dosage Form.

INTRODUCTION:
Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air, generally used for drug analysis are spectral methods, chromatographic methods, electro analytical techniques, and miscellaneous techniques like conventional titrimetric, gravimetric and Polari metric methods[1,2].

Received on 27.03.2021 Modified on 26.05.2021
Accepted on 03.07.2021
Research J. Pharm. and Tech. 2022; 15(7):2992-2996.
DOI: 10.52711/0974-360X.2022.00499

Analytical method development:
The number of drugs introduced into the market is increasing every year. These Drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopeia. It will happen when the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities. Development of patient resistance and introduction of better drugs by competitors, under these conditions, standards and analytical procedures for these drugs may not be available in the Pharmacopeia, it becomes necessary, there is a need for to develop newer analytical methods for such drugs[3,4].

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not

be available in the literature due to patent regulations; Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipient.

- Analytical methods for the quantization of the drug in biological fluids may not be available, Analytical methods for a drug in combination with other drugs may not be available the existing analytical procedures may require expensive reagents and solvents.
- It may also involve cumbersome extraction and separation procedures and these may not be reliable[4,5,6].

MATERIALS AND METHODS:

Balofloxacin (API) was obtained as a gift sample from Bio Leo Analytical Labs India Pvt.Ltd. HPLC Grade Methanol; HPLC Grade Acetonitrile from E.Merck, Potassium dihydrogen phosphate was obtained as a gift sample from SD Fine Chem.

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Balofloxacin and working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)[7]

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Balofloxacin working standard into a 10 mL clean dry volumetric flask add about 7 mL of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)[7]

Preparation of Level – I to VI (100 ppm- 600 ppm Balofloxacin):

Required volume (1.0 to 6.0 ml) of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Method development and optimization of chromatographic parameters:

1. Selection of wavelength:

The sensitivity of the HPLC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. A UV spectrum of Balofloxacin was recorded between 200-400nm[8].

2. Selection of chromatographic methods:

The proper selection of chromatographic parameters depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic, so reversed phase chromatography can be used because of its simplicity and suitability[9,10].

Estimation of drugs in formulation by developed RP-HPLC Method:

The objective of this study was to develop and validate RP-HPLC method used for determination of Balofloxacin in bulk dosage form.

3. Validation of Developed Rp-Hplc Method:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristics of the method meet the requirements for intended analytical application. Performance characteristics are expressed in terms of analytical parameters. After development of RP-HPLC method for estimation of Balofloxacin. Validation of the method was carried out according to ICH guidelines[10,11,12].

a. Accuracy:

The accuracy of measurement is defined as the degree of closeness of the measured value to the true value. Typically, accuracy is represented and determined by recovery studies. This study was performed by spiking analyse matrices. For assay methods, spiked placebo samples are prepared at three concentration levels of 50%, 100% and 150%[11].

b. Precision:

The precision of the method was performed by intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated[12]**.**

c. Linearity:

Different concentrations of the pure drugs were injected into the chromatographic system**.** Calibration curve of Balofloxacin was constructed by plotting peak area vs. applied concentrations**.** The obtained results have shown an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area at each level vs. concentration of analyse was calculated[13]**.**

d. Limit of detection (LOD):

From standard solution pipette out 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents Pipette 0.2mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. The solution was injected and chromatogram the Limit of detection was found to be 0.210 µg/ml for Balofloxacin[14].

e. Limit of Quantification (LOQ):

From standard solution pipette out 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.65mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to

the mark with diluent. The solution was injected and chromatogram the Limit of Quantification was found to be 0.637µg/ml for Balofloxacin[15]

f. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the Flow rate, Mobile Phase composition and Temperature Variation was made to evaluate the impact on the method[13,14,15].

RESULTS AND DISCUSSION:

1. Selection of wavelength:

A UV spectrum of Balofloxacin was recorded between 200-400nm. Drug showing maximum absorption at a wavelength of 293 nm.

2. Selection of chromatographic methods:

Optimized Chromatographic Parameters:

Equipment: High performance liquid chromatography equipped with Auto Sampler UV detector

Column: Symmetry C_{18} (3.9 x 150mm, 5µm, Make: waters)

Mobile phase: P^H 5.0: ACN (50:50)

Flow rate: 1 ml / min

Wavelength: 293 nm

Injection volume: 20 µl

Column oven: Ambient

Run time: 8min

3. Validation of developed RP-HPLC method:

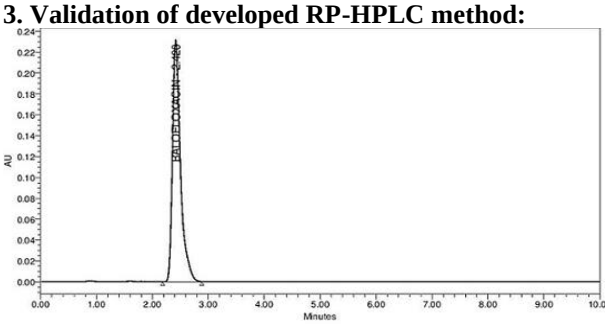

Fig.1: Chromatogram for accuracy 50%

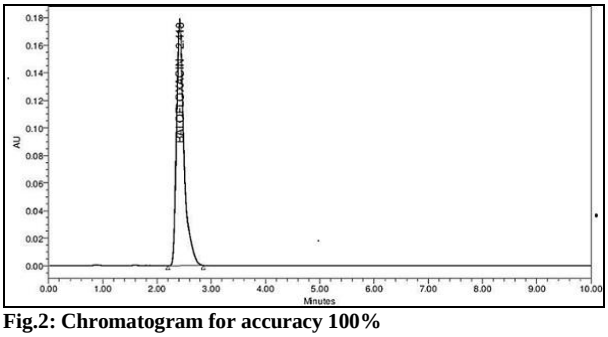

Fig.2: Chromatogram for accuracy 100%

a. Accuracy:

Table: 1 Accuracy results for Balofloxacin

Drug	% Level	Amount Added (mg)	Amount found (mg)± SD	% Recovery	Mean Recovery**
Balofloxacin	50	5	4.950± 1.12	98.79	98.86
	100	10	9.897±1.24	98.97	
	150	15	14.824±1.45	98.82	

**Average of three determinations

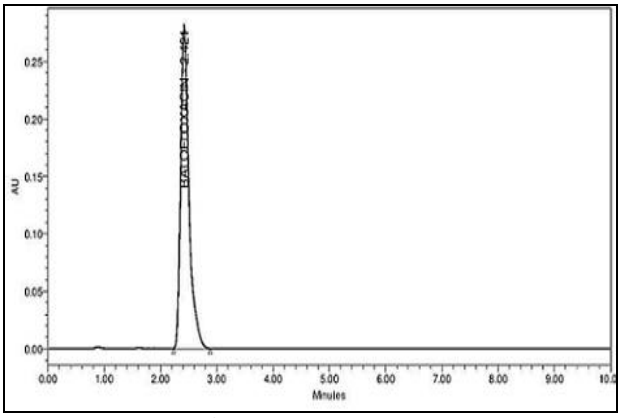

Fig.3: Chromatogram for accuracy 150%

Acceptance Criteria:

- The % recovery for each level should be between 98.79 to 98.97%. Accuracy results of Balofloxacin represented in Table 1 and Figure 1,2, and 3

b. Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or Relative Standard deviation. Results represented in table 2.

Table 2: System precision results

S. No.	Linearity Level	Balofloxacin	
		Conc. (µg/ml)	Area
1	I	10	286910
2	II	20	579740
3	III	30	865836
4	IV	40	1151386
5	V	50	1440983
6	IV	60	1727507
Correlation coefficient		0.999	

Table 3: Linearity results for Balofloxacin

Injection	Rt (min)	Area of Balofloxacin
Injection-1	2.516	1169233
Injection-2	2.5	1166925
Injection-3	2.486	1162653
Injection-4	2.475	1160155
Injection-5	2.469	1160674
Average	2.4892	1163928
Standard Deviation	**0.020579**	**3660.103**
%RSD	**0.83**	**0.31**

c. Linearity:

Acceptance Criteria:

The calibration curves were linear in the range 10-60 µg/ml for Balofloxacin. The correlation coefficient ('r') value was found to be 0.9999 for Balofloxacin. Results are represented in table 3.

d. LOD results

Table 4: LOD results for Balofloxacin

S. No	Drug	LOQ	
1	Balofloxacin	Concentration (µg/ml)	40
		Retention time (t_R)	2.4000
		Height (µv)	124058
		Area	3336

Table 5: LOQ results for Balofloxacin

S.No	DRUG	LOD	
1	Balofloxacin	Concentration (µg/ml)	40
		Retention time (t_R)	2.4000 min
		Height	124058
		Area	1141103

Acceptance Criteria:

Signal to noise ratio should be equal to 3. Results are represented in table 4.

e. LOQ results:

Acceptance Criteria:

Signal to noise ratio should be equal to 10. Results are represented in table 5.

f. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Results represented in table 6.

Table 6: Study of Robustness (Effect of Mobile Phase)

Factor	Retention Time	Tailing Factor	USP Plate Count
Mobile Phase (v/v)	Balofloxacin	Balofloxacin	Balofloxacin
45:55	3.206	1.67	3371
50:50	2.516	1.58	2490.7
55:45	1.910	1.48	3739

Table 7: System Suitability Parameters

S. No.	Parameters	Balofloxacin
1	Area	1134371
2	Retention time (Rt)	2.394
3	Resolution (Rs)	10.7
4	Tailing factor (T)	1.58
5	No. of theoretical plates (N)	3739

Table 8: Validation parameter of Balofloxacin

S. no	Parameter	Balofloxacin	ICH acceptance limit
1	Accuracy	98.86%	98-102%
2	Precision	0.31	%RSD < 2
3	Correlation coefficient	0.9999	Not less than 0.999
4	LOD	S/N =3	S/N =3
5	LOQ	S/N =10	S/N =10
6	USP Resolution	....	Not less than 2
7	USP Tailing	1.58	Less than 2
8	USP Plate count	3739	Not less than 2000

System Suitability Parameters

Acceptance Criteria: TF <2, Plate Count > 2000, Rs >2. System suitability parameters represented in table 7 and validation parameter of Balofloxacin represented in table 8.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Balofloxacin was done by RP-HPLC. The Phosphate buffer of p^H 5.0 and the mobile phase was optimized with consists of Acetonitrile: Phosphate buffer mixed in the ratio of 50:50 % v/ v. A C_{18} column C18 (4.6 x 150mm, 4.5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 249 nm. The solutions were chromatographed at a constant flow rate of 1 ml/min. the linearity range of Balofloxacin was found to be from 10-60 µg/ml. linear regression coefficient was shown 0.9999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Balofloxacin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH guidelines. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

ACKNOWLEDGEMENT:

The authors are thankful to Vignan's Foundation for Science Technology and Research (VFSTR) Management for providing the entire necessary facilities and infrastructure.

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