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Research Article

Development and Validation of a Stability-Indicating RP-HPLC Method for the Quantitative Estimation of Balofloxacin in Bulk Drug and Pharmaceutical Tablet Dosage Forms

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Balofloxacin is a third-generation fluoroquinolone antibiotic widely used for the treatment of urinary tract and respiratory tract infections caused by susceptible bacterial pathogens. The development of reliable, rapid, and stability-indicating analytical methods is essential to ensure the quality, safety, and efficacy of pharmaceutical formulations during manufacturing and storage. Although several analytical methods have been reported for the estimation of balofloxacin, there remains a need for a simple, accurate, precise, and validated RP-HPLC method suitable for routine quality control analysis. Therefore, the present study was undertaken to develop and validate a stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative estimation of balofloxacin in bulk drug and tablet dosage forms in accordance with International Council for Harmonisation (ICH) guidelines. Chromatographic separation was achieved using a Qualisil BDS C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and phosphate buffer (75:25, v/v; pH 3.0) at a flow rate of 1.0 mL/min, and detection was carried out at 293 nm using a photodiode array detector. The developed method was validated for system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection, and limit of quantification. The calibration curve exhibited excellent linearity over the concentration range of 2–12 µg/mL with a correlation coefficient (r^2) of 0.999. Recovery studies demonstrated excellent accuracy with recoveries ranging from 99.56% to 100.09%, while precision studies showed percentage relative standard deviation values below 2%, confirming the repeatability and reliability of the method. The limits of detection and quantification were found to be 0.21 µg/mL and 0.66 µg/mL, respectively. Forced degradation studies performed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed that the method effectively separated balofloxacin from its degradation products, demonstrating its stability-indicating capability. The validated RP-HPLC method was found to be simple, rapid, accurate, precise, robust, and suitable for routine quality control analysis, stability studies, and assay of balofloxacin in bulk drug and pharmaceutical dosage forms.

Keywords: Balofloxacin; Reverse-phase high-performance liquid chromatography (RP-HPLC); Stability-indicating method; Analytical method validation; ICH guidelines; Pharmaceutical analysis; Forced degradation; Quality control.

INTRODUCTION

The pharmaceutical industry places significant emphasis on ensuring the quality, safety, and efficacy of medicinal products throughout their shelf life. Reliable analytical methods are indispensable for the identification, quantification, and quality assessment of active pharmaceutical ingredients (APIs),

impurities, and degradation products during drug development, manufacturing, and post-marketing surveillance. Among the various analytical techniques available, reverse-phase high-performance liquid chromatography (RP-HPLC) has become one of the most widely employed methods owing to its excellent selectivity, sensitivity, reproducibility, and suitability for routine pharmaceutical quality control [1, 9, 16, 20, 21]. Fluoroquinolones constitute an important class of broad-spectrum antibacterial agents extensively used in the treatment of bacterial infections. Balofloxacin, a third-generation fluoroquinolone antibiotic, exhibits potent antimicrobial activity against both Gram-positive and Gram-negative microorganisms by inhibiting bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, thereby preventing bacterial DNA replication and cell division. Owing to its favorable pharmacokinetic profile, broad antibacterial spectrum, and therapeutic effectiveness, balofloxacin has gained considerable clinical importance in the treatment of respiratory tract infections, urinary tract infections, skin infections, and other susceptible bacterial diseases [6, 22–24, 40]. The therapeutic efficacy of pharmaceutical products largely depends on maintaining the chemical integrity and stability of the active pharmaceutical ingredient throughout manufacturing, transportation, storage, and distribution. Exposure to environmental factors such as heat, light, moisture, acidic or alkaline conditions, and oxidative agents may result in chemical degradation, leading to reduced drug potency, altered pharmacological activity, or the formation of potentially toxic degradation products. Consequently, regulatory agencies recommend the development of stability-indicating analytical methods capable of accurately quantifying the intact drug while effectively resolving degradation products generated under various stress conditions [2–5, 18, 19, 39]. Reverse-phase high-performance liquid chromatography has become the preferred analytical technique for pharmaceutical quality control because it provides rapid analysis, high resolution, excellent precision, and superior sensitivity for the simultaneous separation and quantification of pharmaceutical compounds and their related impurities. Furthermore, RP-HPLC methods are readily adaptable for routine industrial quality control and regulatory compliance because they can be validated according to internationally accepted guidelines. The flexibility of RP-HPLC in terms of column chemistry, mobile phase composition, detector selection, and optimization parameters makes it highly suitable for the analysis of complex pharmaceutical formulations [1, 9, 17, 20, 27, 36].

Several analytical techniques, including ultraviolet (UV) spectrophotometry, high-performance thin-layer chromatography (HPTLC), liquid chromatography–mass spectrometry (LC–MS), and RP-HPLC, have been reported for the estimation of balofloxacin in pharmaceutical dosage forms and biological matrices. Although these methods have demonstrated acceptable analytical performance, several reported procedures require lengthy chromatographic run times, complex mobile phase compositions, expensive instrumentation, or extensive sample preparation. In addition, many published methods lack comprehensive forced degradation studies and complete validation according to the International Council for Harmonisation (ICH) recommendations, thereby limiting their applicability as true stability-indicating methods for routine quality control analysis [6, 7, 22–26, 33]. According to the International Council for Harmonisation (ICH), analytical procedures intended for pharmaceutical quality control should be validated for specificity, system suitability, linearity, accuracy, precision, robustness, ruggedness, limit of detection, limit of quantification, and analytical range before they can be considered suitable for regulatory applications. Furthermore, forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and neutral stress conditions are recommended to establish the stability-indicating capability of the developed analytical method. Such validation ensures that the analytical procedure consistently produces reliable, reproducible, and scientifically acceptable results suitable for routine pharmaceutical analysis and regulatory submissions [4, 5, 18, 19, 39]. Recent advancements in chromatographic science have focused on developing analytical methods that are not only accurate and precise but also rapid, economical, environmentally sustainable, and suitable for routine laboratory applications. Modern pharmaceutical industries increasingly require analytical procedures that reduce solvent consumption, shorten analysis time, improve chromatographic resolution, and

provide enhanced reproducibility without compromising analytical performance. Consequently, considerable research efforts have been directed toward optimizing chromatographic parameters, including mobile phase composition, pH, stationary phase selection, flow rate, and detection wavelength, to achieve efficient and reliable analytical methods for pharmaceutical compounds [10, 11, 20, 21, 28, 30, 34, 35]. Although several analytical procedures for balofloxacin have been published, there remains a need for a simple, rapid, economical, robust, and fully validated RP-HPLC method capable of accurately estimating balofloxacin in bulk drug and tablet dosage forms while effectively separating degradation products generated under various stress conditions. Such a method would provide a practical analytical tool for routine quality control, stability testing, and regulatory evaluation in pharmaceutical industries and research laboratories [6, 7, 22–26, 40]. Therefore, the present investigation was undertaken to develop and validate a simple, rapid, accurate, precise, economical, and stability-indicating RP-HPLC method for the quantitative estimation of balofloxacin in bulk drug and pharmaceutical tablet dosage forms. The developed method was optimized by evaluating various chromatographic parameters, including mobile phase composition, pH, flow rate, and detection wavelength, and subsequently validated in accordance with ICH guidelines. Furthermore, forced degradation studies were performed under acidic, alkaline, oxidative, thermal, photolytic, and neutral stress conditions to establish the stability-indicating capability of the proposed analytical method and demonstrate its suitability for routine pharmaceutical quality control and stability assessment [4, 5, 18, 19, 39].

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Table 1. Chemicals and Reagents Used in the Study

Sr. No.	Chemical/Reagent	Grade	Manufacturer/Supplier	Purpose
1	Balofloxacin Reference Standard	Reference Standard	Alkem Laboratories Ltd., Mumbai, India	Standard drug
2	Balofloxacin Tablets	Commercial Formulation	Procured from Local Market	Sample analysis
3	Acetonitrile	HPLC Grade	Merck Chemicals Ltd., Mumbai, India	Mobile phase preparation
4	Potassium Dihydrogen Phosphate	AR Grade	Standard Laboratory Reagent	Buffer preparation
5	Orthophosphoric Acid	AR Grade	Standard Laboratory Reagent	pH adjustment
6	Double-Distilled Water	Laboratory Grade	In-house	Mobile phase preparation
7	Membrane Filter (0.45 µm)	HPLC Compatible	Millipore/Equivalent	Filtration of mobile phase and samples

2.2 Instrumentation

Table 2. Instruments Used in the Study

Sr. No.	Instrument	Specification/Description	Purpose
1	HPLC System	Shimadzu LC-20 HPLC System	Chromatographic analysis
2	Solvent Delivery System	Quaternary Pump	Delivery of mobile phase
3	Detector	Photodiode Array (PDA) Detector	Detection of analyte
4	HPLC Column	Qualisil BDS C18 (250 mm × 4.6 mm i.d., 5 µm)	Separation of balofloxacin
5	Analytical Balance	Sensitivity ±0.1 mg	Accurate weighing of chemicals and samples
6	Digital pH Meter	Calibrated Digital pH Meter	Adjustment of buffer pH

7	Ultrasonic Bath	Laboratory Ultrasonic Cleaner	Sonication of standard and sample solutions
8	Vacuum Filtration Assembly	0.45 µm Membrane Filtration Unit	Filtration of mobile phase and samples
9	Volumetric Glassware	Class A Volumetric Flasks, Pipettes and Measuring Cylinders	Preparation of analytical solutions

2.3 Chromatographic Conditions

Table 3. Optimized RP-HPLC Chromatographic Conditions for the Determination of Balofloxacin

Parameter	Optimized Condition
Chromatographic system	Shimadzu LC-20 Binary Gradient HPLC
Detector	PDA Detector
Column	Qualisil BDS C18 (250 × 4.6 mm, 5 µm)
Mobile phase	Acetonitrile : 0.02 M Sodium Dihydrogen Phosphate Buffer (75:25, v/v)
Buffer pH	3.0 (adjusted with Orthophosphoric acid)
Flow rate	1.0 mL/min
Detection wavelength	293 nm
Injection volume	20 µL
Run time	10 min *(or actual run time used)*
Retention time	3.20 min
Column temperature	Ambient
Diluent	Methanol

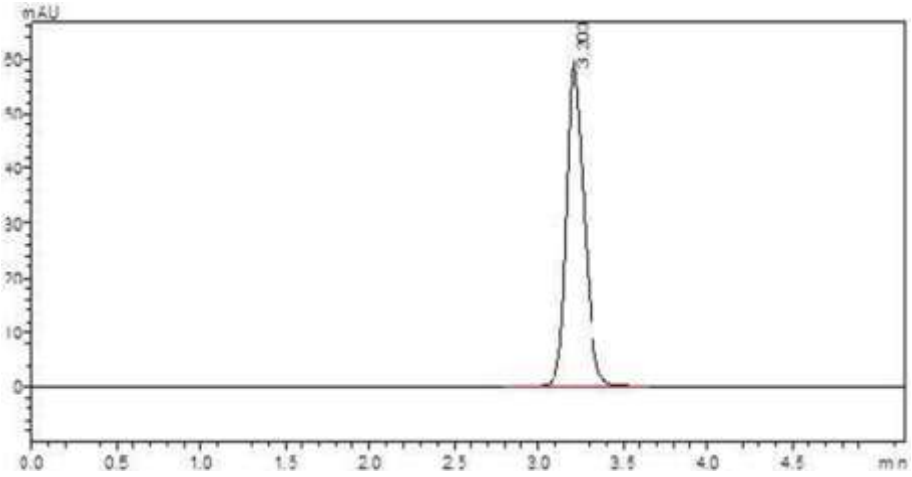

Figure 1. Representative RP-HPLC chromatogram of balofloxacin standard obtained using acetonitrile: phosphate buffer (75:25, v/v; pH 3.0) as the mobile phase, showing a retention time (tR) of 3.20 min.

2.4 Preparation of Standard Solution

An accurately weighed quantity of 25 mg of balofloxacin reference standard was transferred into a 25 mL volumetric flask. The drug was dissolved in a small volume of acetonitrile with sonication, and the volume was adjusted to the mark using the same solvent to obtain a standard stock solution containing 1000 µg/mL of balofloxacin. A suitable aliquot of the stock solution was further diluted with the mobile phase to prepare the working standard solution. Serial dilutions were prepared using the mobile phase to obtain calibration standards in the concentration range of 2–12 µg/mL. All prepared solutions were filtered through a 0.45 µm membrane filter prior to chromatographic analysis.

2.5 Preparation of Sample Solution

Twenty commercially available balofloxacin tablets were accurately weighed individually, and the average tablet weight was determined. The tablets were finely powdered using a clean mortar and pestle. An accurately weighed quantity of tablet powder equivalent to 25 mg of balofloxacin was transferred into a 25 mL volumetric flask. Approximately 15 mL of acetonitrile was added, and the mixture was sonicated for 10–15 min to ensure complete extraction of the drug. After cooling to room temperature, the volume was made up to the mark with acetonitrile to obtain the sample stock solution. The resulting solution was filtered through a 0.45 µm membrane filter, and an appropriate aliquot was further diluted with the mobile phase to obtain the required working concentration for chromatographic analysis. The prepared sample solution was injected into the RP-HPLC system, and the assay was performed by comparing the peak area of the sample with that of the corresponding standard solution.

2.6 Method Development and Optimization

The RP-HPLC method was systematically developed and optimized to obtain satisfactory chromatographic performance for the quantitative estimation of balofloxacin. Various chromatographic parameters, including the stationary phase, mobile phase composition, buffer pH, flow rate, and detection wavelength, were investigated to achieve optimum separation, acceptable retention time, symmetrical peak shape, and adequate resolution. The final chromatographic conditions were selected based on their ability to produce sharp, well-resolved peaks with excellent reproducibility and minimal analysis time, making the method suitable for routine pharmaceutical quality control.

Table 4. Parameters Evaluated During RP-HPLC Method Development and Optimization

Sr. No.	Parameter Evaluated	Purpose
1	Selection of chromatographic column (Qualisil BDS C18, 250 × 4.6 mm, 5 µm)	To achieve efficient separation and good peak shape
2	Optimization of mobile phase composition	To obtain adequate peak separation and acceptable resolution
3	Optimization of buffer pH	To improve peak symmetry, retention, and analyte stability
4	Optimization of flow rate	To obtain an appropriate retention time with satisfactory chromatographic performance
5	Selection of detection wavelength (293 nm)	To achieve maximum detector response and sensitivity
6	Optimization of injection volume	To obtain reproducible peak areas without peak distortion
7	Evaluation of retention time	To ensure consistent elution of balofloxacin
8	Assessment of peak symmetry and peak shape	To obtain symmetr

2.7 Method Validation

The developed RP-HPLC method was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines to establish its suitability for the quantitative estimation of balofloxacin in pharmaceutical dosage forms. The validation parameters evaluated included system suitability, specificity, linearity, accuracy, precision, intermediate precision, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ). The validation studies demonstrated that the developed method was reliable, accurate, precise, reproducible, and suitable for routine quality control analysis.

Table 5. Validation Parameters Evaluated According to ICH Q2(R1) Guidelines

Validation Parameter	Acceptance Criteria
Specificity	No interference at analyte retention time/Rf
Linearity	Correlation coefficient ($R^2 \geq 0.999$)
Accuracy	Recovery 98–102%
Precision	%RSD ≤ 2%
Repeatability	%RSD ≤ 2%
Intermediate precision	%RSD ≤ 2%
Robustness	No significant variation
Ruggedness	Comparable results between analysts/instruments
Limit of Detection (LOD)	As calculated
Limit of Quantification (LOQ)	As calculated
System suitability	Meets predefined acceptance criteria

2.8 Forced Degradation study

Table 6. Forced Degradation Conditions Used for Stability-Indicating Method Development

Stress Condition	Reagent/Condition	Experimental Condition	Purpose
Acid hydrolysis	0.1 N HCl	Reflux for specified time	Acid degradation
Alkaline hydrolysis	0.1 N NaOH	Reflux for specified time	Base degradation
Neutral hydrolysis	Water	Reflux	Hydrolytic degradation
Oxidative degradation	Hydrogen peroxide	3% H_2O_2	Oxidative degradation
Thermal degradation	Dry heat	80–105°C	Thermal stability
Photolytic degradation	UV/Visible light	ICH recommended exposure	Photostability

RESULTS AND DISCUSSION

3.1 Selection and Optimization of the mobile Phase

Different ratios of n-butanol and methanol were tried as mobile phase was tried but, tailing of spot, less persistent spots were observed in most of the attempts. In order to overcome the problems, n-butanol: methanol: ammonia (2.5:1:1.5 v/v/v) was tried and results is good resolution, sharp and symmetrical peak with Rf value of 0.53 for BLF. Table 3.2.1 and chromatogram shown in Figure 3.2.1.

Table 7: Optimization of Mobile Phase

Sr. No.	Solvent System	Composition (*v/v/v*)	R_f BLF
1	chloroform: methanol	2.5:2.5	0.87 tailing
2	n-butanol: methanol	4:1	0.65 tailing
3	n-butanol: methanol: ammonia	2.5:1.5:1	0.75 tailing
4	n-butanol : methanol: ammonia	2.5:1:1.5	0.53

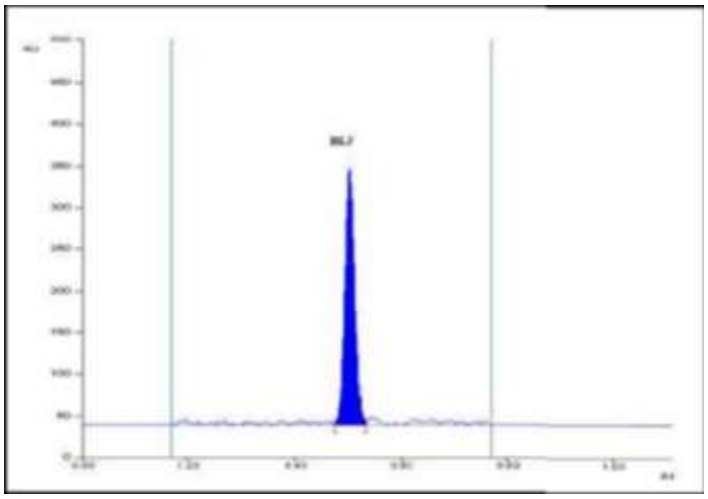

Figure3.2.1: Densitogram of standard BLF(Rf0.53±0.03), measuredat293nm, mobile phase n-butanol: methanol: ammonia (2.5: 1: 1.5 v/v/v).

3.2 Finalized chromatographic conditions

After examining the results from initial experimental conditions, the final working chromatographic conditions are summarized in Table 3.2.2.

Table 8: finalized chromatographic conditions

Parameters	Specifications
Stationary phase	Aluminumbackedsilicagel60F-254 TLC plates, (10cm×10cm, layer thickness0.2mm, E-Merck, Darmstadt, Germany) prewashed with methanol
Mobile phase	n- butanol: methanol: ammonia (2.5:1:1.5*v/v/v*)
Chamber saturation	20 minutes
Migration distance	80 mm
Activation of prewashed plate	10 min
Bandwidth	6 mm
Slit dimensions	6.00 x0.45 mm
Radiation source	Deuterium lamp
Scanning wavelength	293 nm
Distance between bands	15.0 mm

3.3 Linearity Study of BLF

Table 9: Linearity Study of BLF

Sr. No	Concentration in [ng/spot]	Peak area mean ±S.D.	% R.S.D.
1	100	3928.0±66.47	1.16
2	200	5541.8±62.25	1.12
3	300	6900.6±75.92	1.1
4	400	8249.7±59.14	0.71
5	500	9596.6±83.65	0.87
6	600	11349±85.23	0.75

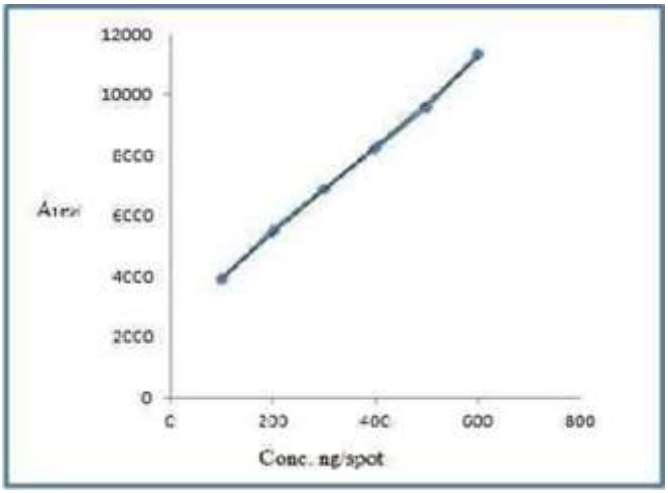

Figure3.2.2: Calibration Curve of BLF; Y=14.462X+2532.5; Where, Correlation coefficient = 0.998, Slope = 14.462, Intercept=2532.5

3.4 Analytical study

Table3.2.4: Analysis of bulk material

Drug	Amount Taken (ng/band)	Amount Found	(ng)	Amount Found %
	400	399.36		99.84
	400	398.71		99.67
BLF	400	399.58		99.89
	400	401.44		100.36
	400	399.92		99.98
	400	400.95		100.23
	Mean ±SD	398.77□3.71		99.69
	%RSD	0.93		0.93

Brand Name: BALOKEM **Batch No.:** BKT1004HB

Mfg. By: Alkem Lab. Ltd. Mumbai. **Average weight:** 257.12mg

Table3.2.5: Analysis of Tablet Formulation

Drug	Amount Taken (ng/band)	Amount Found	(ng)	Amount Found %
	400	405.23		101.30
	400	404.17		101.04
BLF	400	395.52		98.88
	400	408.81		102.20
	400	402.16		100.54
	400	404.23		101.05
	Mean ± SD	403.35□4.41		100.84
	%RSD	1.09		0.99

3.5 Accuracy

Table3.2.6: Recovery studies

Drug	Initial Amount [ng/band]	Amount added (%)	Amount recovered ±S.D. [ng/band] [n=3]	% Recovered	% RSD
	200	80	362.62□1.29	100.73	0.35
BLF	200	100	399.58□1.38	99.89	0.34
	200	120	440.34□3.82	100.07	0.86

Table3.2.7: Precision Studies (Intra-day and Inter-day)

Drug	Conc.[ng/band]	Intra day Amount found[ng]	Inter day Amount found[ng]
		Mean± SD % [n=3] RSD	Mean± SD %RSD [n=3]
	200	201.47 ± 1.97 0.98	201.29 ± 3.10 1.54
BLF	300	299.95 ± 0.97 0.32	300.53 ± 5.13 1.71
	400	400.24 ± 4.33 1.08	399.45 ± 4.52 1.13

3.6 Repeatability Studies

Table3.2.8: Results of Repeatability Studies

Drug	Amount Taken (ng/band)	Amount Found(ng)	Amount Found %
	400	401.65	100.41
	400	400.72	100.18
BLF	400	400.28	100.07
	400	399.29	99.82
	400	403.82	100.95
	400	400.22	100.05
	Mean ±SD	401.001□1.52	100.25
	%RSD	0.39	0.39

3.7 Specificity

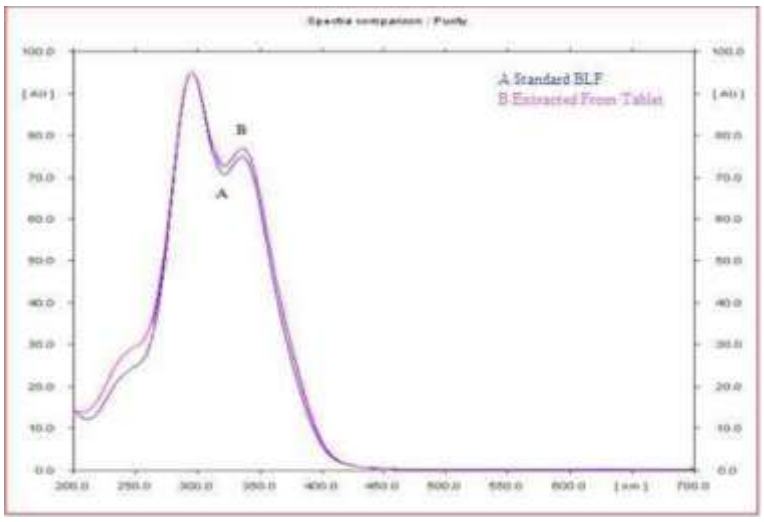

Fig 3.2.3: Peak purity spectra of standard BLF (A), sample (B) extracted from a BLF tablet, scanned at the peak-start, peak-apex, and peak-end positions of the spot (correlation > 0.99)

3.8 Summary of developed method

Table4.1: Summary of developed methods I and II

Parameter	Method I	Method II
Linearity range	2-12 µg/mL	100-600 ng/spot
Correlation coefficient	0.999	0.998
Linearity[equation]	y=96956x+101883	y=14.462x+2532.5
LOD	0.21 µg	8.99 ng
LOQ	0.66 µg	27.25 ng
%Recovery[%RSD][n=3]	0.56– 0.96	0.34– 0.86
Ruggedness[%RSD] Analyst I[n=6]	0.91	0.37
Analyst II[n=6]	0.73	0.69
Precision[%RSD] Inter-Day[n =6]	0.32– 0.48	1.13– 1.71
Intra-Day[n =6]	0.20– 0.40	0.32– 1.08
Repeatability[n =6]	0.68	0.39

3.9 Linearity

Table 3.1.2: Calibration Data for Linearity of Balofloxacin

Sr. No.	Concentration of BLF (µg/mL)	Peak Area (Mean±SD;n=6)	%RSD
1	2	306891 ±1061.33	0.34
2	4	482414 ±996.67	0.20
3	6	670048 ±2373.12	0.35
4	8	886579 ±1234.08	0.13
5	10	1067745 ±1810.76	0.16
6	12	1269769± 3491.43	0.27

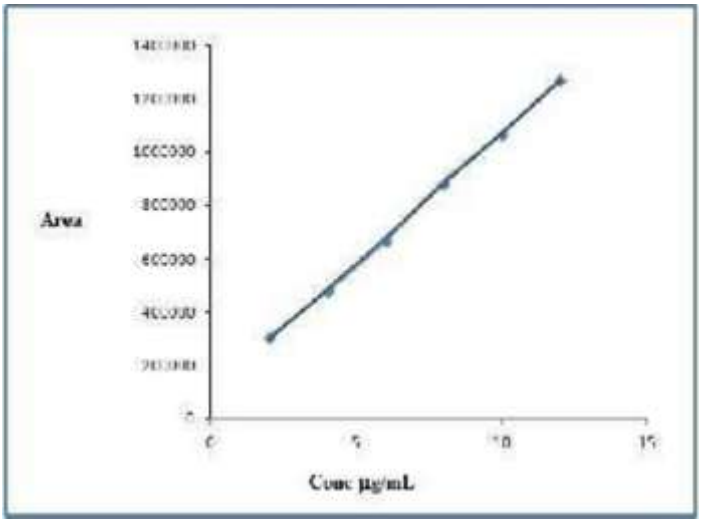

Figure3.1.2: Calibration Curve of Balofloxacin

CONCLUSION

A simple, rapid, accurate, precise, economical, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was

successfully developed and validated for the quantitative estimation of balofloxacin in bulk drug and pharmaceutical tablet dosage forms. The chromatographic conditions were systematically optimized to achieve satisfactory peak resolution, acceptable retention time, excellent peak symmetry, and reproducible chromatographic performance. The developed analytical method was comprehensively validated according to the International Council for Harmonisation (ICH) guidelines for system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantification (LOQ), and all validation parameters complied with the recommended acceptance criteria. The proposed RP-HPLC method exhibited excellent linearity over the selected concentration range with a high correlation coefficient, while recovery studies confirmed the accuracy of the method and precision studies demonstrated excellent repeatability and intermediate precision with percentage relative standard deviation values well within acceptable limits. Robustness and ruggedness studies further established the reliability of the developed method under small deliberate variations in chromatographic conditions and routine laboratory environments. Forced degradation studies performed under acidic, alkaline, oxidative, thermal, photolytic, and neutral stress conditions demonstrated that the developed method effectively separated balofloxacin from its degradation products without interference, thereby confirming its stability-indicating capability. These findings indicate that the method is suitable for monitoring the stability of balofloxacin during pharmaceutical development, manufacturing, storage, and quality assurance studies. Overall, the validated RP-HPLC method is reliable, sensitive, cost-effective, and convenient for routine quantitative analysis of balofloxacin in bulk drug and pharmaceutical tablet dosage forms. Owing to its simplicity, short analysis time, excellent reproducibility, and compliance with ICH validation requirements, the proposed analytical method can be successfully employed for routine quality control testing, stability studies, regulatory submissions, and pharmaceutical research laboratories. Furthermore, the developed method may serve as a useful analytical tool for future formulation development and stability assessment of balofloxacin-containing pharmaceutical products.

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