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Original Research Article

SIMPLE COST EFFECTIVE STABILITY INDICATING HPLC METHOD FOR THE ESTIMATION OF BESIFLOXACIN

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ABSTRACT

A simple, rapid, accurate, precise, cost-effective, and stability-indicating RP-HPLC method was developed and validated for the estimation of Besifloxacin in pharmaceutical dosage form. Chromatographic separation was achieved using a mobile phase consisting of 10 mM potassium dihydrogen phosphate and methanol in the ratio of 20:80 v/v at a flow rate of 1.0 ml/min. Detection was carried out at 247 nm using a UV detector. The developed method showed good linearity in the concentration range of 5–25 µg/ml with a correlation coefficient (r^2) of 0.999. The method was validated according to ICH guidelines for parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The percentage recovery values were found to be within acceptable limits, indicating good accuracy of the method. Precision studies demonstrated low %RSD values, confirming the reproducibility of the developed method. The LOD and LOQ values were found to be 0.65 µg/ml and 1.85 µg/ml, respectively, indicating adequate sensitivity. Forced degradation studies under acidic, alkaline, oxidative, and photolytic conditions confirmed the stability-indicating capability of the developed method. The proposed RP-HPLC method was found to be simple, reliable, economical, and suitable for routine quantitative analysis of besifloxacin in pharmaceutical formulations.

Keywords: Besifloxacin, RP-HPLC, Stability-indicating method, Method validation, Forced degradation studies, ICH guidelines; Pharmaceutical analysis, Eye drop formulation.

INTRODUCTION

Besifloxacin is a fourth-generation fluoroquinolone antibiotic widely used in ophthalmology for the treatment of bacterial conjunctivitis and other ocular infections. It exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative microorganisms by inhibiting bacterial DNA gyrase and topoisomerase IV enzymes, thereby preventing bacterial DNA replication and cell division (Mah *et al.*, 2016).

Due to its potent antimicrobial efficacy, excellent ocular penetration, and reduced bacterial resistance, besifloxacin has gained considerable importance in ophthalmic therapy (Totoli and Salgado, 2018).

Analytical method development plays a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical formulations. Among the various analytical techniques available, High Performance Liquid Chromatography (HPLC) is considered one of the most reliable and widely accepted

methods for the quantitative estimation of drugs in bulk and dosage forms. HPLC methods provide high accuracy, precision, specificity, and reproducibility, making them suitable for routine quality control analysis (Pawar, 2024). Stability-indicating analytical methods are essential for determining the stability profile of pharmaceutical compounds under different stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation (Vajir and Patil, 2026). These methods help in identifying degradation products and ensure that the analytical procedure can accurately measure the active pharmaceutical ingredient without interference from impurities or degradation products. According to International Council for Harmonisation (ICH) guidelines, stability-indicating methods are necessary for the validation and regulatory approval of pharmaceutical products (Qiu and Norwood, 2007).

Several analytical methods have been reported for the estimation of fluoroquinolone antibiotics; however, only limited methods are available for the determination of besifloxacin. Many of the reported methods involve complex mobile phase compositions, expensive solvents, longer retention times, or sophisticated instrumentation, which increase the overall cost and analysis time. Therefore, there is a need to develop a simple, rapid, economical, and stability-indicating HPLC method for the estimation of besifloxacin in pharmaceutical dosage forms (Sousa *et al.*, 2012; Czyrski *et al.*, 2017; Speltini *et al.*, 2010).

The present study aims to develop and validate a simple and cost-effective stability-indicating HPLC method for the estimation of besifloxacin. The developed method is intended to provide accurate, precise, robust, and reproducible results with shorter analysis time and minimal solvent consumption. The method will be validated according to ICH guidelines with respect to parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection, and limit of quantification. Additionally, forced degradation studies will be performed under various stress conditions to establish the stability-indicating capability of the proposed method.

MATERIALS AND METHODS

Materials

Besifloxacin was obtained as a gift sample and used as the working standard. HPLC grade methanol and acetonitrile were used as solvents for chromatographic analysis. Potassium dihydrogen phosphate (KH_2PO_4), hydrochloric acid, sodium hydroxide, and hydrogen peroxide were of analytical reagent grade and used for preparation of mobile phase and forced degradation studies. Double distilled water was used throughout the study. Commercially available besifloxacin eye drop formulation was procured from the local market and used for assay analysis. Whatman filter paper No. 41 and membrane filters of 0.45 µm and 0.2 µm pore sizes were used for filtration of solutions before chromatographic analysis.

Methods

Selection of Mobile Phase

Different mobile phase compositions were initially evaluated for the estimation of Besifloxacin in pharmaceutical dosage form as reported by Sravya and Kuber, (2023). Various chromatographic conditions were optimized by considering system suitability

parameters such as retention time (RT), tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP). Among the different combinations tested, the mobile phase consisting of 10 mM potassium dihydrogen phosphate (KH_2PO_4) and methanol in the ratio of 20:80 v/v was found to be most suitable for the analysis. The mobile phase was filtered through a 0.45 µm membrane filter to remove particulate matter and degassed by sonication prior to use. The chromatographic analysis was carried out at a flow rate of 1.0 ml/min.

Selection of Diluent

The diluent selected for sample preparation was compatible with the mobile phase and did not produce any significant interference with the retention and resolution of the analyte. After several experimental trials, methanol was selected as the suitable diluent for the study.

Preparation of Stock Solution

An accurately weighed quantity of 10 mg of besifloxacin was transferred into a 50 ml volumetric flask and dissolved in 10 ml of methanol. The solution was sonicated for 10 minutes to ensure complete dissolution, and the volume was made up to the mark with methanol. The resulting solution was vortex mixed and filtered through Whatman filter paper No. 41. This solution containing 200 µg/ml of besifloxacin was designated as Stock Solution A.

Preparation of Sub-Stock Solution

From Stock Solution A, 5 ml was pipetted into a 10 ml volumetric flask and diluted up to the mark with methanol to obtain a concentration of 100µg/ml. This solution was designated as Stock Solution B.

Preparation of Standard Working Solutions

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml were withdrawn separately from Stock Solution B and transferred into individual 10 ml volumetric flasks. The volume was adjusted up to the mark with methanol to obtain final concentrations of 5, 10, 15, 20, and 25 µg/ml of besifloxacin, respectively.

Linearity and Calibration Curve

To establish the linearity of the developed analytical method, a series of standard solutions in the concentration range of 5–25 µg/ml were prepared. All solutions were filtered through a 0.2 µm membrane filter before injection into the HPLC system. The chromatograms were recorded at 247 nm, and each concentration was analyzed in triplicate. A calibration curve was constructed by plotting mean peak area against corresponding drug concentration, and the regression equation was determined as described by Jain *et al.,* (2018).

System Suitability Parameters

Prior to analysis, the chromatographic system was equilibrated with the optimized mobile phase at a flow rate of 1.0 ml/min until complete column saturation was achieved. Thereafter, three replicate injections of standard besifloxacin solution were made, and chromatograms were recorded. System suitability parameters including retention time, theoretical plates, tailing factor, and HETP were evaluated to ensure adequate performance of the chromatographic system as reported by Sharma *et al.*, (2012).

Validation of Developed Method

Linearity

Linearity of the developed method was evaluated by analyzing five different

concentrations ranging from 5–25 µg/ml. Each concentration was injected in triplicate, and the corresponding peak areas were recorded. The mean peak area for each concentration was calculated, and the calibration curve was plotted. The regression equation and correlation coefficient were determined to assess the linear relationship between concentration and peak area as described by Jain *et al.,* (2010).

Specificity

Specificity of the method was evaluated to determine the ability of the analytical procedure to measure the analyte accurately in the presence of impurities, degradation products, and formulation excipients without any interference.

Accuracy

The accuracy of the developed method was assessed by recovery studies using the standard addition method. Pre-analyzed sample solutions were spiked with known amounts of standard drug at 80%, 100%, and 120% concentration levels, and the percentage recovery was determined according to the method described by Jain *et al.,* (2019).

Precision

The precision of the developed method was evaluated in terms of repeatability and intermediate precision.

Repeatability

Repeatability studies were carried out by analyzing five replicate injections of besifloxacin at concentrations of 5, 10, 15, 20, and 25µg/ml under the same operating conditions within a short time interval. The results demonstrated the precision of the analytical method as described by Pandya and Rajput, (2018).

Intermediate Precision

Intermediate precision was evaluated by performing the analysis on different days using five replicate injections at five concentration levels within the linearity range. The obtained results indicated satisfactory day-to-day precision of the developed method.

Robustness

Robustness of the method was evaluated according to ICH guidelines by introducing small deliberate changes in chromatographic conditions, particularly in the composition of the mobile phase. The effect of these changes on chromatographic performance was studied to determine the reliability of the method under varied conditions.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve as described by Athavia and Dedania, (2017).

Analysis of Drug in Eye Drop Formulation

An accurately measured quantity of eye drop formulation equivalent to 2 mg of besifloxacin was transferred into a 100 ml volumetric flask. About 25 ml of methanol was added, and the solution was sonicated for 10 minutes to ensure complete dissolution of the drug. The volume was then made up to the mark with methanol and filtered through Whatman filter paper No. 41. Appropriate dilutions were prepared from the filtrate and analyzed by the developed HPLC method. The assay results of the formulation were recorded as described by Rao, (2021).

Forced Degradation Studies

Forced degradation studies were performed to establish the stability-indicating capability of the developed HPLC method. The degradation studies were conducted under acidic, alkaline, oxidative, and thermal stress conditions, and the samples were analyzed using HPLC coupled with a UV detector. A volume of 20 µl of each degraded sample solution was injected into the chromatographic system as described by Rajakumari and Rao, (2016).

Acidic Degradation

Fifty milligrams of besifloxacin was transferred into a 50 ml round-bottom flask containing 50 ml of 0.1 N hydrochloric acid solution. The mixture was continuously stirred for 8 hours at 80°C. The resulting solution was diluted appropriately to obtain a concentration of 10 µg/ml and analyzed by HPLC to determine the percentage degradation.

Alkaline Degradation

Fifty milligrams of besifloxacin was treated with 50 ml of 0.1 M sodium hydroxide solution in a round-bottom flask and subjected to constant stirring for 8 hours at 80°C. The degraded sample was diluted to obtain a concentration of 10 µg/ml and analyzed by HPLC.

Oxidative Degradation

For oxidative degradation studies, 50 mg of besifloxacin was treated with 50 ml of 3% hydrogen peroxide solution and kept under constant stirring at room temperature for 24 hours. The resulting solution was diluted to obtain a final concentration of 10 µg/ml and analyzed chromatographically.

Thermal Degradation

Thermal degradation studies were carried out by placing 50 mg of besifloxacin in a petri dish and storing it in a hot air oven maintained at 50°C for four weeks. The degraded sample was suitably diluted to obtain a concentration of 10 µg/ml and analyzed using the developed HPLC method.

RESULTS AND DISCUSSION

A simple, rapid, economical, and stability-indicating RP-HPLC method was successfully developed and validated for the estimation of Besifloxacin in pharmaceutical dosage form. The optimized chromatographic conditions produced a sharp and symmetric peak with satisfactory retention time, as shown in Figure 1. The developed method demonstrated good chromatographic performance with acceptable system suitability parameters including retention time, theoretical plates, and tailing factor.

The validation results summarized in Table 1 indicated that the developed method complied with ICH guidelines for analytical method validation. The system suitability study showed low %RSD values, confirming the reliability and reproducibility of the chromatographic system. The response ratio data also demonstrated consistent detector response over the selected concentration range.

The linearity study presented in Table 2 revealed that the method exhibited excellent linearity within the concentration range of 5–25 µg/ml with a correlation coefficient (r^2) of 0.999, indicating a strong correlation between concentration and peak area. The regression equation further confirmed the proportional relationship between analyte concentration and detector response.

Accuracy studies performed at 80%, 100%, and 120% levels showed percentage recoveries close to 100% with low standard deviation and %RSD values, as presented in Table 1. These findings indicated that the method was accurate and free from interference by formulation excipients.

The precision of the developed method was evaluated by repeatability and intermediate precision studies. The low %RSD values obtained for repeatability and day-to-day precision studies confirmed that the method was precise and reproducible under normal analytical conditions. Robustness studies also demonstrated that small deliberate variations in chromatographic conditions did not significantly affect the analytical performance of the method.

The sensitivity of the developed method was evaluated in terms of limit of detection (LOD) and limit of quantification (LOQ). The obtained LOD and LOQ values, shown in Table 3, demonstrated that the method was sufficiently sensitive for routine analysis of besifloxacin in pharmaceutical formulations.

Assay analysis of the marketed formulation showed satisfactory drug content with acceptable %RSD values, indicating suitability of the developed method for routine quality control applications.

Forced degradation studies were carried out under acidic, alkaline, oxidative, and photolytic stress conditions to establish the stability-indicating capability of the method. The results presented in Table 4 showed significant degradation under acidic and alkaline conditions, whereas comparatively lower degradation was observed under oxidative and photolytic conditions. The developed method successfully separated the degradation products from the drug peak, confirming its specificity and stability-indicating nature.

Overall, the developed RP-HPLC method was found to be simple, accurate, precise, robust, sensitive, and cost-effective for the quantitative estimation of besifloxacin in pharmaceutical dosage forms and suitable for routine quality control as well as stability studies.

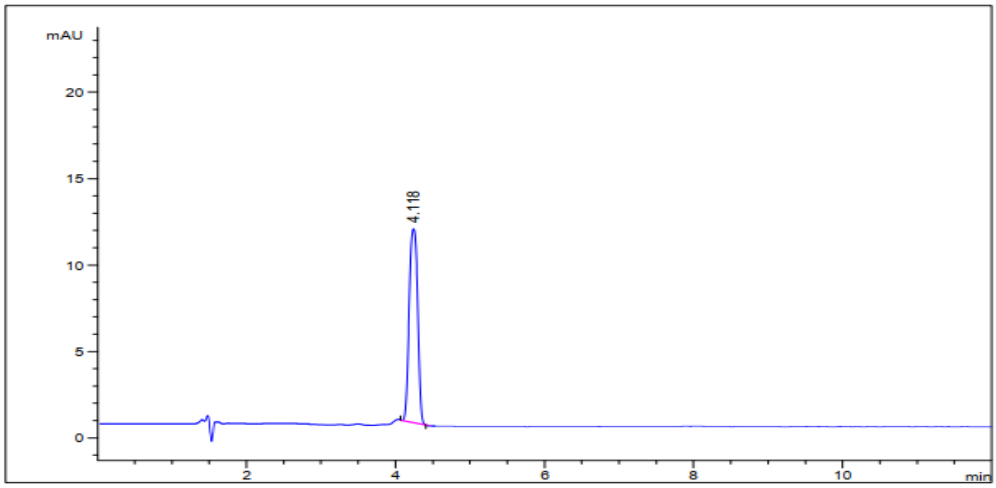

Figure 1: Standard Chromatogram of Besifloxacin

Table 1: Validation Parameters of HPLC Method for Besifloxacin

Validation Parameter	Mean	SD	% RSD
System Suitability	261.880	9.252	3.533
Response Ratio	26.494	0.363	1.137
Accuracy (80% Level)	99.42	0.723	0.727
Accuracy (100% Level)	99.87	0.098	0.098
Accuracy (120% Level)	99.38	0.977	0.983
Repeatability	98.516	0.104	0.106
Intermediate Precision (Day-to-Day)	98.859	0.106	0.108
Robustness	98.607	0.104	0.105
Assay of Formulation	96.66	—	0.145

Table 2: Linearity Data of Besifloxacin

Parameter	Result
Linearity Range	5–25 µg/ml
Regression Equation	y = 26.64x – 1.535
Correlation Coefficient (r^2)	0.999

Table 3: LOD and LOQ of Besifloxacin

Parameter	Value (µg/ml)
LOD	0.65
LOQ	1.85

Table 4: Summary of Forced Degradation Studies of Besifloxacin

Stress Condition	Drug Recovered (%)	Drug Decomposed (%)
Standard Drug	99.80	0.20
Acidic Hydrolysis	80.45	19.55
Alkaline Hydrolysis	88.20	11.80
Oxidative Degradation	95.10	4.90
Photolytic Degradation	89.60	10.40

CONCLUSION

A simple, rapid, accurate, precise, economical, and stability-indicating RP-HPLC method was successfully developed and validated for the estimation of Besifloxacin in pharmaceutical dosage form. The developed method showed excellent linearity within the concentration range of 5–25 µg/ml with satisfactory accuracy, precision, robustness, and specificity in accordance with ICH guidelines. The low values of LOD and LOQ indicated good sensitivity of the method for routine analytical applications. Forced degradation studies demonstrated that the developed method was capable of effectively separating the drug from its degradation products under various stress conditions, confirming its stability-indicating nature. The assay results of the marketed formulation were found to be within acceptable limits, indicating suitability of the method for routine quality control analysis.

The proposed RP-HPLC method is simple, cost-effective, reliable, and suitable for routine quantitative estimation and stability studies of besifloxacin in pharmaceutical formulations.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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