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HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF DELAFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Nischhala Kumari, Ravindra Kumar Chourasiya*
SVN Institute of Pharmaceutical Sciences, Swami Vivekanand University, Sagar (M.P.)-470228, India

***Correspondence Info:**
Ravindra Kumar Chourasiya
SVN Institute of Pharmaceutical Sciences, Swami Vivekanand University, Sagar (M.P.)- 470228, India
Email: ravindracps@gmail.com

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ABSTRACT

A simple, rapid, and precise reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitative determination of Delafloxacin in bulk drug and pharmaceutical tablet dosage forms. Chromatographic separation was achieved on a suitable C18 column using an optimized mobile phase under isocratic conditions, with UV detection at an appropriate wavelength. Delafloxacin showed a well-resolved and symmetrical peak with a retention time of 6.861 min. System suitability parameters confirmed adequate chromatographic performance, with a theoretical plate count of 3200.83 and a tailing factor of 1.1467. The method was linear over the concentration range of 1–5 µg/mL with a correlation coefficient (r^2) of 0.9992. Accuracy studies demonstrated mean recoveries between 99.20% and 99.77%, indicating absence of interference from excipients. Precision studies showed low variability with %RSD values well within acceptable limits for both repeatability and intermediate precision. The method was found to be robust against small deliberate variations in chromatographic conditions. Assay of the marketed tablet formulation revealed a drug content of 99.96% with %RSD of 0.246. The validated RP-HPLC method is accurate, precise, robust, and suitable for routine quality control analysis of Delafloxacin in bulk and pharmaceutical dosage forms.

Keywords: Delafloxacin, RP-HPLC, Method development, Method validation, Pharmaceutical dosage form, Quality control.

INTRODUCTION

Delafloxacin (DLX) is a novel anionic fluoroquinolone antibacterial with broad-spectrum activity against Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Bambeke, 2015). It is indicated for acute bacterial skin and skin-structure infections and other systemic infections, and its unique chemical profile (a weak acid with increased activity at acidic pH) differentiates it from many older fluoroquinolones.

Accurate, precise and stability-indicating analytical methods are essential for quality control of active pharmaceutical ingredient (API) in bulk material and finished dosage forms (Manzoor *et al.*, 2011). Chromatographic methods particularly reversed-phase HPLC remain the workhorse for assay and related-substance testing

because of their high selectivity, robustness and adaptability to forced-degradation studies that generate potential impurities and degradation products. Regulatory guidance (ICH Q2 (R1)) requires demonstration of specificity, linearity, accuracy, precision, detection/quantitation limits, range and robustness for assay methods, and mandates forced-degradation studies (acid/base hydrolysis, oxidation, photolysis and thermal stress) when establishing a method as stability-indicating.

A literature survey shows several analytical approaches for delafloxacin quantification: bioanalytical methods (UPLC–MS/MS) for biological matrices and a small number of HPTLC/UV and fluorescence HPLC reports for tablets and plasma. However, relatively few peer-reviewed, fully validated reversed-phase HPLC methods tailored specifically for routine assay and stability testing in bulk drug and commercial tablet formulations are available; many published procedures either target biological samples (requiring MS detection) or use thin-layer techniques that are less suited for comprehensive forced-degradation and related-substance separation. This gap justifies development of a robust, economical RP-HPLC assay that (1) provides baseline resolution of delafloxacin from its degradation products and common excipients, (2) uses widely available instrumentation and UV detection, and (3) complies with ICH validation requirements so it can be used in quality control environments (Asati and Chourasiya, 2025).

This study therefore aims to develop and validate a simple, selective, and stability-indicating RP-HPLC method for the assay of Delafloxacin in bulk and pharmaceutical dosage forms. The method development includes optimization of stationary phase, mobile-phase composition and pH, flow rate and detection wavelength; forced-degradation experiments will be performed to confirm method specificity and to characterize major degradation pathways (Zubair *et al.,* 2014). The validated method will be demonstrated on commercial tablet formulations and used to report assay and related-substance data in accordance with ICH Q2 (R1).

MATERIALS AND METHODS

Materials

Delafloxacin pure drug was obtained from a certified pharmaceutical supplier. Marketed tablet formulations containing delafloxacin were procured from a local pharmacy for assay studies. HPLC-grade solvents, including methanol, acetonitrile, and water, were used for mobile-phase preparation, along with analytical-grade buffers and reagents. All solutions were freshly prepared, filtered through a 0.45 µm membrane filter, and degassed prior to HPLC analysis. A C18 reversed-phase column was employed for chromatographic separation. All chemicals and reagents used were of analytical or HPLC grade to ensure accuracy and reproducibility of results.

Methods

Initially to estimate of Delafloxacin in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50 v/v (Subramanian and Rajinikanth, 2016). The mobile phase was filtered through 0.45µ filter

paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of standard stock solution Accurately weighed 10 mg of Delafloxacin was transferred into 50 ml volumetric flasks and dissolved in 10 ml of methanol, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of Delafloxacin was 200µg/ml. (Stock- A)

Preparation of Sub Stock Solution 5 ml of solution was taken from stock-A of Delafloxacin transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (methanol) to give concentration of 100µg/ml (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg/ml for drug.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml was prepared (Varshini *et al.*, 2012). All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 254nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System suitability parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Delafloxacin10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample (Dipti *et al.*, 2010). The calibration plot was contracted after analysis of five different (from 1 to 5µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components (Dipti *et al.*, 2010).

Accuracy

Recovery studies were performed to validate the accuracy of developed method (ICH, 2005). To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity

range 1, 2, 3, 4 and 5 µg/ml for Delafloxacin indicates the precision under the same operating condition over short interval time (ICH, 2003).

Intermidiate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations (Priyadarshani *et al.,* 2015). Results of day to day intermediate precision for Delafloxacin reported in table 4.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: acetonitrile (50:50 % v/v), to (45:55 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve (Ramakrishna *et al.,* 2004).

Analysis of marketed formulation

Amount equal to 1 mg of Delafloxacin Inhalation Solution was taken in 10ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of 1µg/ml. This solution is injection 20µl and records the peak area and calculate percentage using calibration curve method.

RESULTS AND DISCUSSION

The present study successfully developed and validated a simple, precise, and robust RP-HPLC method for the determination of Delafloxacin in bulk drug and pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. The chromatogram of the pure drug (Figure 1) demonstrated a sharp, well-resolved peak for Delafloxacin with a retention time of 6.861 min, indicating adequate interaction of the analyte with the stationary phase and suitability of the selected chromatographic conditions for routine analysis.

System suitability parameters (Table 1) confirmed acceptable chromatographic performance. The number of theoretical plates (3200.83) indicated good column efficiency, while the tailing factor (1.1467) was within acceptable limits (<2), demonstrating symmetrical peak shape. The consistent retention time further confirms the reproducibility of the chromatographic system.

Linearity studies (Table 2) showed that the method exhibited excellent linearity over the concentration range of 1–5 µg/mL, with a high correlation coefficient ($r^2 = 0.9992$). The slope and intercept values indicate a strong linear relationship between concentration and peak area, confirming that the method is suitable for quantitative estimation of Delafloxacin at low concentrations.

Accuracy of the method was evaluated through recovery studies at 80%, 100%, and 120% levels (Table 3). The mean recovery values ranged from 99.20% to 99.77%, with low standard deviation, demonstrating that the method is accurate and free from interference by formulation excipients. These results comply with ICH acceptance criteria for assay methods (98–102%).

Precision studies (Table 4) revealed that the method is highly precise. Repeatability and intermediate precision (day-to-day) studies showed % mean values close to 100% with

very low standard deviation, confirming excellent reproducibility of the method under normal operating conditions.

Robustness testing (Table 5) demonstrated that small deliberate variations in chromatographic parameters did not significantly affect the assay results, as evidenced by minimal variation in mean percentage values. This confirms the reliability of the method during routine quality control analysis.

Assay of the marketed tablet formulation (Table 6) showed a mean drug content of 99.96% with a %RSD of 0.246, indicating uniformity of content and suitability of the method for routine analysis of Delafloxacin in pharmaceutical dosage forms. The low %RSD value further confirms the precision and applicability of the method for quality control purposes.

The validated RP-HPLC method satisfies all regulatory requirements for analytical method validation and can be effectively employed for routine assay, quality control, and stability studies of Delafloxacin in bulk drug and tablet dosage forms.

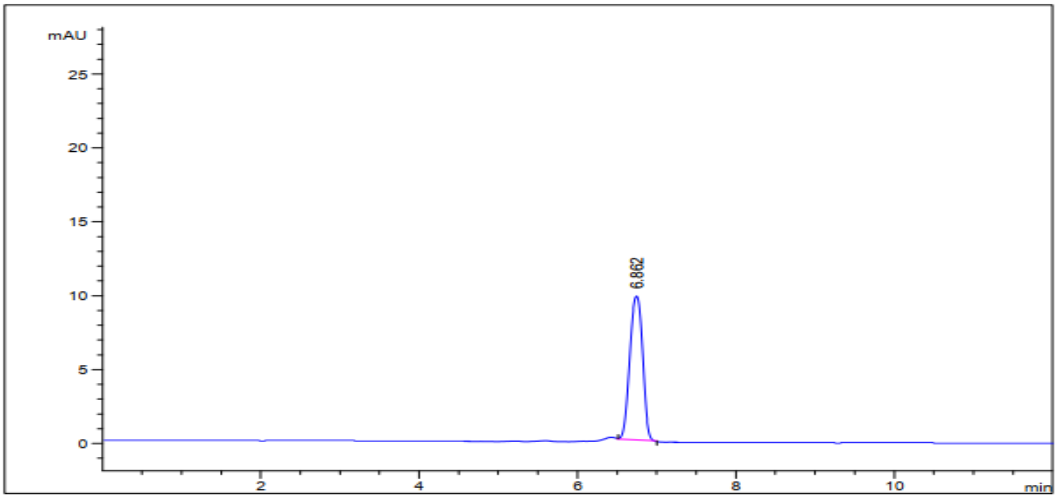

Figure 1: Chromatogram of Delafloxacin

Table 1: Results of system suitability parameters

Parameters	Delafloxacin
No. of Theoretical Plates	3200.83
Tailing Factor	1.1467
Retention time	6.861

Table 2: Results of linearity of Delafloxacin

Parameter	Delafloxacin
Concentration (µg/ml)	1-5
Correlation Coefficient (r^2)*	0.9992
Slope (m)*	798.86
Intercept (c)*	1.498

Table 3: Results of recovery study

% Level	% Mean±SD*
80%	99.48±0.480
100%	99.20±0.230
120%	99.77±0.040

***** Value of three replicate and three concentrations

Table 4: Results of Precision

Parameter	% MEAN±SD*
Repeatability	97.89±0.05
Intermediate precision	
Day to day precision	97.53±0.05

***** Value of five replicate and five concentrations

Table 5: Results of robustness

Parameter	% MEAN±SD*
Robustness	97.64±0.03

*Value of five replicate and five concentrations

Table 6: Assay of tablet formulation

S. No.	Parameter	Delafloxacin
1.	Mean	99.96
2.	S. D.	0.225
3.	% RSD	0.246

CONCLUSION

A simple, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the estimation of Delafloxacin in bulk drug and tablet dosage forms. The method demonstrated excellent linearity, precision, accuracy, and robustness, and is suitable for routine quality control and stability studies of Delafloxacin 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.

REFERENCES

- Van Bambeke, F. (2015). Delafloxacin, a non-zwitterionic fluoroquinolone in phase III of clinical development: Evaluation of its pharmacology, pharmacokinetics, pharmacodynamics, and clinical efficacy. *Future Microbiology*, *10*(7), 1111–1123.
- Ahmed, M., Babu, G. S., & Shetty, A. S. K. (2011). Development and validation of amoxicillin by RP-HPLC method in bulk drug and pharmaceutical dosage forms. *International Journal of ChemTech Research, 3*(3), 1037–1041.
- Asati, S., & Chourasiya, R. K. (2025). UV spectrophotometric method for simultaneous estimation of formoterol fumarate dihydrate and fluticasone

propionate in combined pharmaceutical formulation. *International Journal of Medical, Pharmaceutical and Health Sciences*, *2*(2), 286–293.

- Gajula, R. G., Zubair, M., & Balaram, V. M. (2014). RP-HPLC method development and validation of linagliptin in bulk drug and pharmaceutical dosage form. *Der Pharmacia Sinica*, *5*(5), 123–130.
- Subramanian, P., & Rajinikanth, P. S. (2016). Development and validation of a new RP-HPLC method for the estimation of dutasteride in bulk and pharmaceutical formulations. *Journal of Applied Pharmaceutical Science*, *6*(12), 47–55.
- Varshini, C. A., Kumari, K. S., Sushma, S., & Prakash, K. (2012). Development and validation of RP-HPLC method for simultaneous estimation of alfuzosin hydrochloride and dutasteride in bulk and pharmaceutical dosage form. *Inventi Rapid: Pharm Analysis and Quality Assurance, 4*, 1–4.
- Patel, D. B., Patel, N. J., Prajapati, A. M., & Patel, S. A. (2010). RP-HPLC method for estimation of dutasteride in tablet dosage form. *Indian Journal of Pharmaceutical Sciences*, *72*, 113–116.
- Patel, D. B., & Patel, N. J. (2010). Validated reverse phase high performance liquid chromatographic and high performance thin layer chromatographic method for simultaneous analysis of tamsulosin hydrochloride and dutasteride in pharmaceutical dosage forms. *Acta Chromatographia, 22*, 419–431.
- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2005). *Validation of analytical procedures: Text and methodology Q2(R1).* ICH.
- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2003). *Stability testing of new drug substances and products Q1A(R2).* ICH.
- Ishaq, B. M., Prakash, K. V., & Mohan, G. K. (2014). Simultaneous determination of dutasteride and tamsulosin in pharmaceutical dosage forms by RP-HPLC. *Der Pharma Chemica, 6*, 103–109.
- Priyadarshani, S. B., Chetan, S. C., Preeti, G., Chatrapal, S., Ravindra, K., Mahendra, S. R., & Sujata, S. B. (2015). Analytical method development and validation of dutasteride with tamsulosin in pharmaceutical capsule dosage form by RP-HPLC method. *International Journal of Pharmacy, 5*, 493–499.
- Ramakrishna, N. V. S., Vishwottam, K. N., Puran, S., Koteshawara, M., Manoj, S., & Santosh, M. (2004). Selective and rapid liquid chromatography–tandem mass spectrometry assay of dutasteride in human plasma. *Journal of Chromatography B, 809*, 117–124.