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

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ABSTRACT

A simple, rapid, and sensitive High-Performance Liquid Chromatography (HPLC) method was developed and validated for the estimation of Cefiderocol in bulk drug and injectable dosage forms. The separation was achieved using a mobile phase of 10 mM KH_2PO_4: Methanol (20:80 v/v), pH adjusted to 4.0 with orthophosphoric acid, at a flow rate of 1.0 mL/min, with detection at 258 nm. The method showed good linearity over the concentration range of 5–25 µg/mL (r^2 = 0.9993). System suitability parameters, including retention time, tailing factor, and number of theoretical plates, were within acceptable limits. Accuracy was confirmed through recovery studies (98.28–98.85%), and precision studies demonstrated low %RSD values, indicating excellent repeatability and intermediate precision. The method was robust and sensitive, with LOD and LOQ values of 0.15 µg/mL and 0.45 µg/mL, respectively. Forced degradation studies under acidic, alkaline, oxidative, and photolytic conditions confirmed the stability-indicating nature of the method. The developed HPLC method is reliable, accurate, and suitable for routine quality control and stability analysis of Cefiderocol in pharmaceutical formulations.

Keywords: Cefiderocol, HPLC, Method Development, Validation, Stability-Indicating, Injectable Formulation, Forced Degradation.

INTRODUCTION

Cefiderocol is a novel siderophore cephalosporin antibiotic, designed to combat multidrug-resistant Gram-negative bacterial infections (Ito *et al.*, 2017). Its unique mechanism involves utilizing bacterial iron transport systems to gain entry into cells, thereby enhancing its efficacy against resistant strains, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae*. Given the rising prevalence of antimicrobial resistance, accurate and reliable quantification of Cefiderocol in bulk drug and pharmaceutical formulations is critical for quality control, dosage standardization, and therapeutic monitoring.

High-Performance Liquid Chromatography (HPLC) is a widely accepted analytical technique in pharmaceutical analysis due to its sensitivity, specificity, reproducibility, and ability to separate complex mixtures (Ayre *et al.*, 2013). HPLC-based methods are particularly valuable for the quantification of antibiotics, ensuring precise determination in both bulk drug substances and finished dosage

forms. Developing a robust, validated HPLC method for Cefiderocol enables accurate assessment of drug content, stability studies, and routine quality control in pharmaceutical industries. The present study focuses on the development and validation of a simple, rapid, and sensitive HPLC method for the estimation of Cefiderocol in bulk and marketed formulations in accordance with International Council for Harmonisation (ICH) guidelines (Swathi *et al.,* 2024). Method validation includes evaluation of parameters such as linearity, accuracy, precision, specificity, limit of detection, limit of quantification, and robustness, ensuring reliability and reproducibility of the analytical procedure (Venkanna *et al.,* 2025).

MATERIALS AND METHODS

Selection of Mobile Phase

To estimate Cefiderocol in fixed-dose formulations, several mobile phase compositions in different ratios were initially evaluated. Based on system suitability parameters such as retention time (RT), tailing factor, number of theoretical plates, and HETP, the most suitable mobile phase for analysis was found to be 10 mM KH_2PO_4: Methanol (20:80 v/v), pH adjusted to 4.0 with orthophosphoric acid (OPA) **(**Gandhimathi *et al.,* 2003). Prior to use, the mobile phase was filtered through a 0.45 µm filter to remove particulate matter and degassed by sonication. The flow rate employed for the analysis was 1.0 mL/min**.**

Preparation of Mobile Phase

The mobile phase was prepared by mixing 20 parts of 10 mM KH_2PO_4 buffer with 80 parts methanol. The mixture was filtered through a 0.45 µm filter paper, and the pH was adjusted to 4.0 using OPA.

Selection of Diluent

The diluent used for sample preparation was chosen to be compatible with the mobile phase and to ensure no significant effect on the retention or resolution of the analyte (Ali *et al.,* 2021). After several trials, methanol was selected as the diluent.

Preparation of Stock Solution

Accurately weighed 10 mg of Cefiderocol was transferred into a 50 mL volumetric flask, dissolved in 10 mL of methanol, and sonicated for 10 minutes. The volume was then made up to 50 mL with methanol and mixed thoroughly using a vortex to ensure complete dissolution. The solution was filtered through Whatman filter paper (No. 41) to obtain a stock solution (Stock-A) of 200 µg/mL**.**

Preparation of Sub-Stock Solution

From Stock-A, 5 mL was taken and transferred into a 10 mL volumetric flask, diluted to volume with methanol to prepare Stock-B with a concentration of 100µg/mL**.**

Preparation of Working Solutions

Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL of Stock-B were separately transferred into 10 mL volumetric flasks and diluted to volume with methanol, yielding working solutions of 5, 10, 15, 20, and 25 µg/mL of Cefiderocol.

Linearity and Calibration Graph

To establish the linearity of the method, a series of dilutions ranging from 5–25 µg/mL were prepared. All solutions were filtered through a 0.2 µm membrane filter and injected into the HPLC system (Kumari and Chourasiya, 2025). Chromatograms were recorded at 258 nm**,** and each injection was repeated three times. A calibration graph was plotted between the mean peak area and the

corresponding concentration, and the regression equation was determined.

System Suitability Parameters

Separation parameters were set, and the mobile phase was allowed to saturate the column at a flow rate of 1.0 mL/min. After complete column equilibration, three replicates of the working standard solution of 10 µg/mL Cefiderocol were injected separately. Peak reports and column performance data, including retention time, tailing factor, and number of theoretical plates, were recorded for all chromatograms.

Linearity

Linearity refers to the ability of the analytical method to produce results that are directly proportional to the concentration of analyte within a given range (Bhagwate and Gaikwad, 2013). Calibration solutions of 5, 10, 15, 20, and 25 µg/mL were prepared and injected in triplicate. The mean peak areas were plotted against their respective concentrations to construct a calibration curve. The regression equation and correlation coefficient (r^2) were determined. The response ratio (response factor) was calculated by dividing the area under the curve (AUC) by the corresponding concentration.

Specificity

The specificity of the method was evaluated to confirm that Cefiderocol could be accurately measured in the presence of expected impurities, degradation products, and matrix components.

Accuracy

Accuracy was assessed through recovery studies. Known quantities of standard Cefiderocol were added to pre-analyzed sample solutions at 80%, 100%, and 120% levels, and the percent recovery was determined to validate the method (Ivanova *et al.,* 2005).

Precision

Precision of the method was determined at three levels:

Repeatability – Assessed by analyzing five replicates at each concentration within the linearity range (5–25 µg/mL) under the same conditions over a short interval (Safeer *et al.,* 2010).

Intermediate Precision

Day-to-Day Precision

Analyst-to-Analyst Precision

Reproducibility – Verified under different laboratory conditions to ensure method reliability (Prameela *et al.,* 2013).

Robustness

Robustness was evaluated by introducing deliberate small variations in the mobile phase composition (Chaudhari *et al.,* 2013). The ratio of 10 mM KH_2PO_4: Methanol (20:80 v/v) was altered to 15:85 v/v and 10:90 v/v to assess the method's capacity to remain unaffected.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve, following ICH guidelines (Kharoaf *et al.,* 2012).

Analysis of Drug in Injectable Formulation

An amount equivalent to 10 mg of Cefiderocol was transferred into a 100 mL volumetric flask, dissolved in 25 mL methanol by sonication for 10 minutes, and the volume was made up to 100 mL with methanol. The solution was filtered through Whatman filter paper (No. 41), and working

samples of various concentrations were prepared (Jothieswari *et al.,* 2011).

Forced Degradation Studies

To demonstrate that the method is stability-indicating, forced degradation studies were performed on Cefiderocol powder using HPLC with a UV detector. 20 µL of each degraded sample was injected (Bhavyasri *et al.,* 2020).

Acid Degradation – 50 mg of Cefiderocol was placed in a 50 mL round-bottom flask, mixed with 50 mL of 0.1 N HCl, and stirred at 80°C for 8 hours. Samples were diluted to 10 µg/mL and analyzed by HPLC to determine percent degradation.

Alkaline Hydrolysis – 50 mg of Cefiderocol was mixed with 50 mL of 0.1 M NaOH in a 50 mL flask, stirred at 80°C for 8 hours, diluted to 10 µg/mL, and analyzed.

Oxidative Degradation – 50 mg of Cefiderocol was treated with 50 mL of 3% hydrogen peroxide, stirred at room temperature for 24 hours, diluted to 10 µg/mL, and analyzed.

Thermal Degradation – 50 mg of Cefiderocol was placed in a petri dish and stored in an oven at 50°C for 4 weeks**.** Samples were withdrawn, diluted to 10 µg/mL, and analyzed by HPLC.

RESULTS AND DISCUSSION

The developed HPLC method for the estimation of Cefiderocol in bulk and injectable formulations demonstrated excellent chromatographic performance. The system suitability parameters (Table 1) indicate that the method provides sharp, symmetrical peaks with a tailing factor of 1.19 ± 0.0562, a high number of theoretical plates (3266.667 ± 6.8605), and a consistent retention time (5.224 ± 0.0014 min), confirming the suitability of the selected mobile phase and chromatographic conditions.

Linearity studies over the range of 5–25 µg/mL showed a strong correlation between peak area and concentration (r^2 = 0.9993), with a slope of 128.63 and intercept of 4.428 (Table 2), indicating that the method is capable of producing proportional and reproducible responses across the intended range.

Accuracy, evaluated through recovery studies at 80%, 100%, and 120% levels, yielded recoveries between 98.28% and 98.85% (Table 3), confirming that the method is accurate and free from interference from excipients in the formulation. Precision studies (Table 4) showed low %RSD values for repeatability (0.146) and intermediate precision (0.142), demonstrating that the method is precise and reliable under both intra-day and inter-day conditions.

The robustness of the method was established by minor deliberate variations in mobile phase composition, with the mean % recovery remaining consistent (98.607 ± 0.104, Table 5), indicating that the method is resilient to small experimental changes. The LOD and LOQ values were found to be 0.15 µg/mL and 0.45 µg/mL, respectively (Table 6), reflecting the high sensitivity of the developed method.

Analysis of the injectable formulation showed a % assay of 99.80% with low %RSD (0.132, Table 7), confirming the suitability of the method for routine quality control of marketed products.

Forced degradation studies (Table 8) demonstrated that Cefiderocol undergoes moderate degradation under acidic (13.15%) and alkaline (15.35%) hydrolysis, while

oxidative and photolytic stress resulted in lower degradation (7.54% and 6.48%, respectively). These results confirm that the developed method is stability-indicating, capable of separating the intact drug from its degradation products.

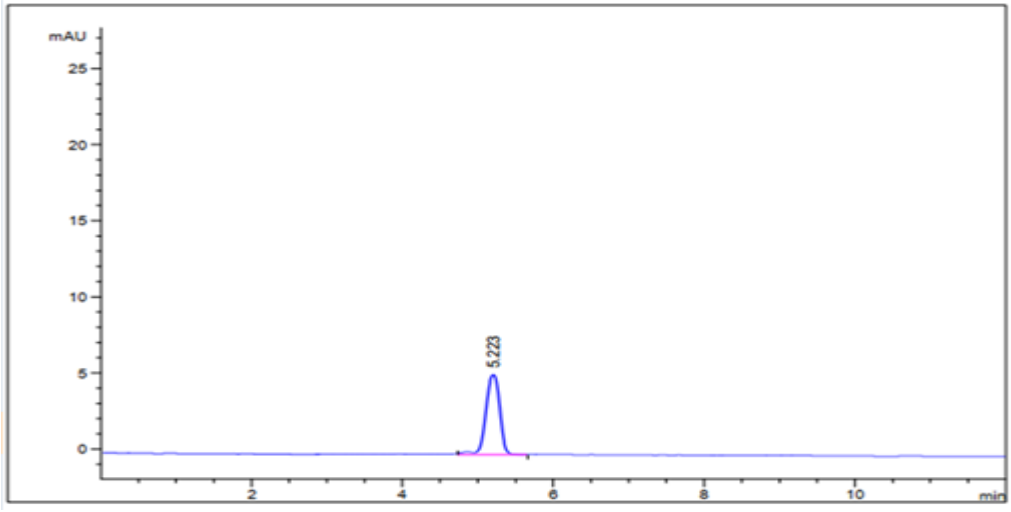

Figure 1: Chromatogram of standard

Table 1: Results of system suitability parameters

Parameters	Cefiderocol
No. of Theoretical Plates	3266.667±6.8605
Tailing Factor	1.19±0.0562
Retention time	5.224±0.0014

Table 2: Results of Linearity of Cefiderocol

Parameter	Cefiderocol
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.9993
Slope (m)*	128.63
Intercept (c)*	4.428

*Value of five replicate

Table 3: Results of recovery study

% Level	% MEAN±SD*
80%	98.28±1.230
100%	98.85±0.712
120%	98.63±0.366

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

Table 4: Results of precision

Parameter	% MEAN±SD*
Repeatability	98.523±0.146
Intermediate precision	
Day to day precision	98.317±0.142

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

Table 5: Results of Robustness

Parameter	% MEAN±SD*
Robustness	98.607±0.104

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

Table 6: Results of LOD and LOQ

S. No.	LOD (µg/ml)	LOQ (µg/ml)
1.	0.15	0.45

Table 7: Analysis of injectable formulation

	Cefiderocol
Label Claim (mg)	1gm
% Found (mg)	0.998 gm
% Assay	99.80
% RSD	0.132

Table 8: Results of Forced degradation studies

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.80	0
Acidic hydrolysis	86.65	13.15
Alkaline hydrolysis	84.45	15.35
Oxidative degradation	92.26	7.54
Photolytic degradation	93.32	6.48

CONCLUSION

A simple, rapid, precise, and accurate HPLC method was successfully developed and validated for the estimation of Cefiderocol in bulk and injectable dosage forms. The method is linear, robust, and stability-indicating, making it suitable for routine quality control and stability studies of Cefiderocol 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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