Research Article

Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Ceftaroline Fosamil in Bulk and Its Parenteral Dosage Forms

A. Suneetha and K. China Venkanna

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh 522002, India

Correspondence should be addressed to A. Suneetha; drasuneetha@gmail.com

The present method describes the development of a validated RP-HPLC method for determination of ceftaroline fosamil in presence of its degradation products or other pharmaceutical excipients. The drug substance was subjected to stress conditions of acid, alkali, and oxidative and thermal degradation studies. Separation was carried out on a C-18 X-terra column (Waters Corporation, 250 mm × 4.6 mm I.D.; particle size 5 μm) using 40 : 30 : 30 [buffer : acetonitrile : methanol] as mobile phase at a flow rate of 1.0 ml/min. UV detection was performed at 242 nm. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision, and robustness. The assay method was found to be linear in the range of 40 to 120 μg/mL with a correlation coefficient of 0.9999. The percentage recovery of active pharmaceutical ingredient from parenteral dosage form ranged from 99.5 to 100.2%. The method precision for determination of ceftaroline was below 0.85%. The results showed that the developed RP-HPLC method is suitable for determination of ceftaroline fosamil in bulk as well as stability samples of pharmaceutical dosage forms containing various excipients.

1. Introduction

Ceftaroline fosamil [1, 2] is chemically 4-[2-[[(6R, 7R)-2-carboxy-7-[[(2Z)-(ethoxyimino)[5-(phosphonoamino)-1,2,4-thiadiazol-3-yl]acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl]thio]-4-thiazolyl]-1-methyl-pyridinium, inner salt, monoacetate, and monohydrate. It is one of the most widely used drugs for the treatment of community-acquired bacterial pneumonia, skin, and skin structure infection. It is marketed mainly as intravenous solutions and has a metabolic half-life of the order of 2.5 hrs. International Conference on Harmonization (ICH) has made the need of a stability-indicating assay method for every drug candidate mandatory. A stability-indicating assay method helps in establishing the inherent stability of the drug which in turn provides assurance on detection changes in identity, purity, and potency of the product on exposure to various conditions. Therefore, it is necessary to study the degradation

studies of ceftaroline fosamil by exposing to a variety of stress conditions like acidic, alkali, dry heat, and photolytic and oxidative stress. As per the ICH guidelines, stress testing of the drug substance aids in identifying the likely degradation products, which in turn can help in establishing the degradation pathways and the intrinsic stability of the molecule with validation of the analytical procedures used. According to the literature survey, a few methods have been reported on comparative pharmacokinetics of ceftroline fosamil in rats, rabbits and monkeys [3], pathogens [4–7], and UV spectrophotometric method [8]. There is no LC method that has been published so far and thus, the present study was aimed for establishing a simple, accurate, and rapid RP-HPLC method for determination of ceftaroline fosamil in presence of its degradation products or other pharmaceutical excipients. The method was validated following analytical performance parameters suggested by ICH guidelines [9, 10] (see Figure 1).
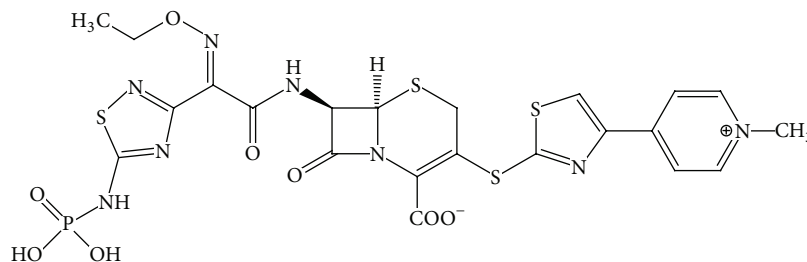
FIGURE 1: Structure of ceftaroline fosamil.

2. Experimental

2.1. Instrumentation. Prominence HPLC system that consisted of isocratic LC 20AT pump, UV-visible, SPD 20A detector, and a DGU-14A degasser model was used for method development and forced degradation studies. The data were acquired and processed by CLASS-VP software (Shimadzu, Kyoto, Japan).

2.2. Chemicals and Solvents. Ceftaroline fosamil pure drug and vials were obtained from Forest Laboratories, Inc. St. Louis, USA. HPLC grade methanol, water, and acetonitrile were procured from Merck (Mumbai, India). Hydrochloric acid, potassium dihydrogen orthophosphate and KOH were obtained from Qualigens Ltd., Mumbai.

2.3. Chromatographic Conditions. The separations were carried out on a C-18 reversed phase column (Phenomenex; Prodigy ODS3V, 250×4.6 mm, 5μ) using mobile phase that consists of buffer : acetonitrile : methanol in $40 : 30 : 30$ ratio at a flow rate of 1.5 mL/min. A wavelength of 242 nm was employed for detection.

2.4. Preparation of Standard Solution. A standard stock solution of ceftaroline fosamil was prepared by dissolving 50 mg drug in methanol in order to make a concentration of 1 mg/mL.

2.5. Analysis of Marketed Formulation. Each vial contains 400 mg of ceftaroline fosamil, and it was dissolved in sufficient quantity of methanol, and the volume was made up to 100 mL with same solvent. The solution was filtered through 0.45μ membrane. It was further diluted to acquire a concentration within the linearity range.

3. Results and Discussion

3.1. Method Development. To develop a suitable and robust LC method for determination of ceftaroline fosamil, different mobile phases were employed to achieve the best separation with good resolution. The method development was started with Phenomenex C-18 (250×4.6 mm, 5μ) column with following mobile phases like methanol : water, water : acetonitrile; and methanol : acetonitrile : water; in all these conditions peak was eluted at void volume, and retention time is more. For next trial buffer : acetonitrile : methanol

TABLE 1: Summary of assay results for ceftaroline fosamil.

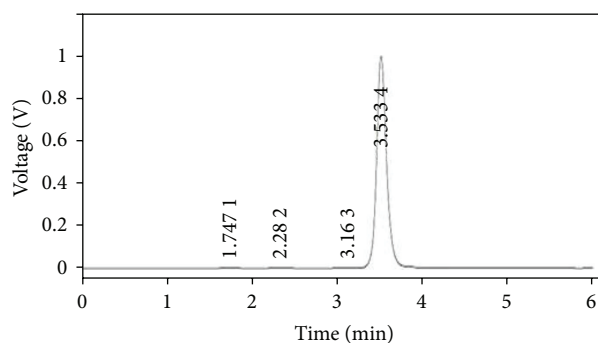| Formulation used	Label claim	Amount found[*]	% Assay[*]
Ceftroline fosamil	400 mg	398.53 mg	99.63 ± 0.58

[*] Average of six determinations.

FIGURE 2: Typical standard chromatogram of ceftaroline fosamil.

in the ratio of $40 : 30 : 30$ was used at a flow rate 1.0 mL/min, and detection was performed at 242 nm, and under this peak shape was good (Figure 2), and retention time was found to be 3.53 min. Ceftaroline shows significant UV absorbance at wavelength 242 nm; hence, this wavelength has been chosen for detection for analysis. The amount of drug present in the marketed formulation was calculated, and the % purity was found to be 99.63%. The results are incorporated in Table 1.

3.2. Analytical Method Validation. The developed chromatographic method was validated for specificity, linearity, precision, accuracy, sensitivity, robustness, and system suitability.

3.2.1. Forced Degradation Studies. Forced degradation studies under acidic, alkaline, and neutral conditions were performed by refluxing using a heating mantle with temperature control, which was carried out in a stability chamber equipped with light sources as defined under option 2 of the ICH guideline Q1B.

   Acid hydrolysis of the drug was carried out in presence of different concentrations of HCl (0.1 M, 1 M, 2 M, and 5 M). The solutions were refluxed for 8 hours in 0.1 M HCl, 12 hours in 1 M HCl, 1 day in 2 M HCl, and 2 days in 5 M HCl then cooled to room temperature and diluted to volume with
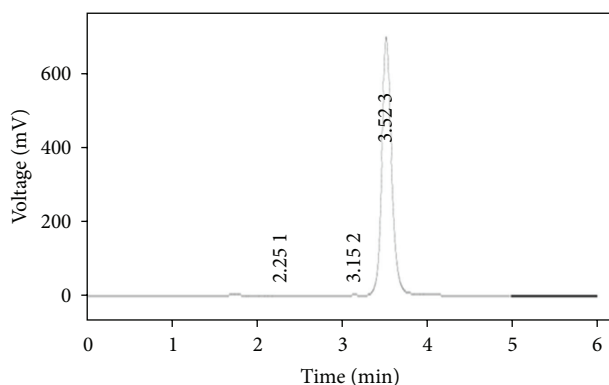
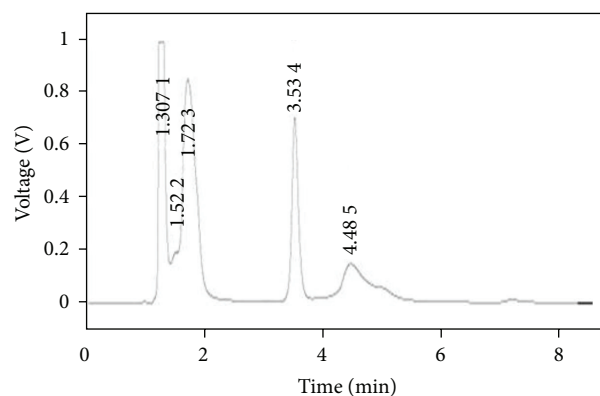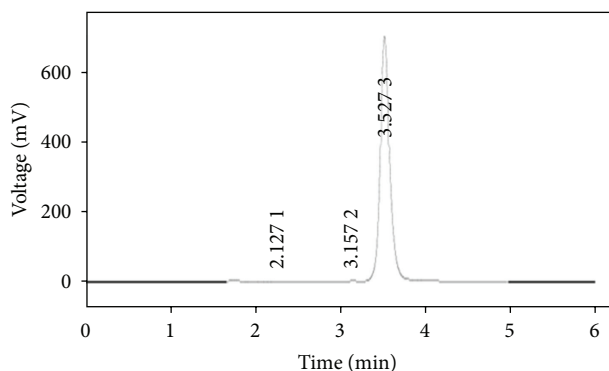FIGURE 3: Typical chromatogram of 0.1 M acid stressed sample.

FIGURE 5: Typical chromatogram of 3% H_2O_2 stressed sample.

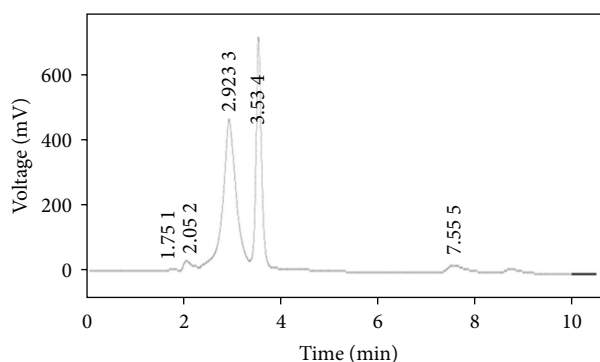FIGURE 4: Typical chromatogram of alkali stressed sample.

FIGURE 6: Chromatogram of direct sunlight stressed sample.

methanol, and then the samples were injected into the HPLC system (Figure 3).

For *Alkaline hydrolysis,* 1 mg/mL of ceftaroline solution and 5 mL of 0.1 M NaOH was transferred into 50 mL flask, and the resulting solution was refluxed for 8 hours. After exposure for the required duration of time the samples, were diluted with mobile phase, and then the samples were injected into the HPLC system after filtration, through 0.45 μ filters (Figure 4).

For *Oxidative stress,* 5 mL of 3% hydrogen peroxide solution was added to sample solution. Initially the studies were carried out in 3% H_2O_2 for 6 hours at room temperature and continued for 1 day at room temperature, and finally the studies were also carried out in 10% and 27% H_2O_2, for 1 day at room temperature. After exposure for the required duration of time, the samples were injected into the HPLC system after filtration (Figure 5).

For *hot air oven degradation products,* 10 mg of drug was stored at 80°C for 1 hour in oven then dissolved in 10 mL of methanol, and further 5 mL of this solution is diluted to 50 mL with mobile phase, and the resulting chromatogram was recorded (Figure 6).

For *photolytic degradation,* some drug molecules undergo degradation upon exposure to sun light, which necessitates special storage conditions and protection from light. Sample solution of the drug was prepared by dissolving 10 mg of drug in 10 mL of mobile phase from which further 5 mL of solution was diluted to 50 mL with mobile phase. It was subjected

to degradation under direct sun light for 6 hours, and the resulting chromatogram was recorded (Figure 7).

Freezer degradation was performed by pipetting 5 mL of sample stock solution into 50 mL volumetric flask, and it was subjected to degradation by treating the sample under 2–8°C for 3 hours, and the chromatogram was recorded (Figure 8).

From the above data of degradation profile, it can be concluded that ceftaroline fosamil has shown significant sensitivity under oxidation, sun light, and hot air oven. The drug was degraded in 3% H_2O_2, sun light, and oven. There is no major degradation products observed in acid, alkali, and freezer conditions, and the results are shown in Table 2.

3.2.2. Linearity. Linearity of the method was established in the concentration range from 40 to 120 μg/mL, and the correlation coefficient obtained was greater than 0.9998 which indicates that the method obeys Beer's law (Figure 9).

3.2.3. LOD and LOQ. The limit of detection and limit of quantitation for ceftaroline fosamil was calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. By the analysis of samples with known concentrations of analyte and establishing the minimum level at which the analyte can be reliably detected and we found 1.64 μg/mL of ceftaroline fosamil. Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and

Table 2: Forced degradation studies by proposed HPLC method.

S. NO	Stressed condition	R_t (min)	% Purity	% of degradation
1	0.1 M HCL	3.520	99.1	Stable
2	0.1 M NaOH	3.527	99.8	Stable
3	3% H_2O_2	3.530	46.0	54
4	Sunlight	3.530	37.0	63
5	Hot air oven	3.527	60.3	39
6	Freezer	3.520	99.4	Stable

Figure 8: Typical chromatogram of freezer stressed sample.

Figure 7: Chromatogram for thermal stressed degradation sample.

Figure 9: Calibration plot for ceftaroline fosamil.

precision. LOQ was found to be 4.85 μg/mL of ceftaroline fosamil.

3.2.4. Precision. To determine the intraday and interday precision of the method, repeatability studies were performed. The intraday and interday precision studies were carried out on three different concentrations (80, 100, and 120 μg/mL). The samples were injected in triplicate on the same day and also on three different days. Concentration was calculated from the areas obtained, and the results were expressed as % RSD. Results are summarized in Table 3.

3.2.5. Accuracy. Accuracy of the method was assessed by three different concentrations of pure drug that were added to a known preanalysed sample, and the total concentration was determined in triplicate, and the percent recovery of the added pure drug was found in the range of 98.76 to 99.98%, thus indicates that the method is found to be more accurate.

3.2.6. Robustness. Robustness of the developed method was demonstrated by purposely altering the experimental conditions. It was carried out with variation of mobile phase composition ±0.2%, flow rate ±0.1 ml/min and detection wavelength ±2 nm and observed that the results were unaffected, hence the developed method is said to be more robust.

3.2.7. System Suitability. System suitability tests were carried out on freshly prepared standard stock solutions of ceftaroline fosamil, and it was calculated by determining the standard deviation of ceftaroline fosamil by injecting standard solutions in five replicates at frequent time intervals, and the values were recorded in Table 3.
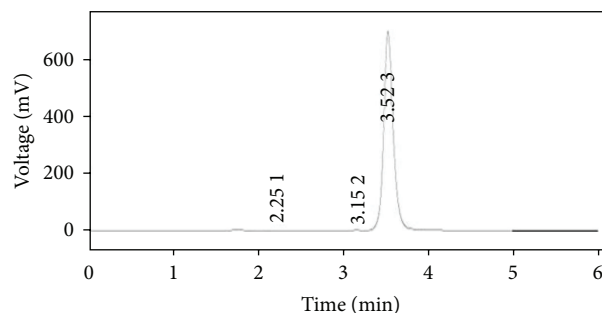
Table 3: Validation parameters.

Parameters	Ceftaroline fosamil
Retention time (min)	3.533
Peak asymmetric factor	1.02
Number of theoretical plates	6803
% RSD of peak area	0.98
Linearity range (μg/mL)	40–120
LOD (μg/mL)	1.64
LOQ (μg/mL)	4.85
Accuracy (%)	98.76–99.98
Precision (% RSD)	
Intraday precision ($n = 3$)	0.45–0.98
Interday precision ($n = 3$)	0.65–1.25

4. Conclusion

The developed stability indicating HPLC method for quantitative estimation of ceftaroline fosamil in bulk and pharmaceutical dosage forms is fast, simple, accurate, and more precise. Validation of this method was accomplished, getting results meeting all requirements. Thus, the developed HPLC method can be used for routine quality control tests.

Acknowledgments

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