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Stability Indicating RP-HPLC method for the *Ceftaroline Fosamil acetate*

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ABSTRACT:

Plan: *Stability indicating RP-HPLC method for the ceftaroline fosamil acetate.*
Preface: *The developed method is simple, fast and accurate and can be used for routine analysis of Market formulations.*
Methodology: *Stability indicating reverse phase high performance liquid chromatography method was developed and validated for the analysis of ceftaroline fosamil in bulk form. Chromatographic separation was achieved on a X- Bridge shield RP (4.6×100mm, 5µ) column, maintained at 30^0c with a mobile phase consisting of acetonitrile and ammonium dihydrogen ortho phosphate buffer in the ratio of 10: 90. The mobile phase pumped at a rate of 1 ml/ min. and the detection was carried out at 242.6nm by PDA detector. The retention time was obtained 4.312.The peak area plot was linear over the concentration range of 50µg/ml to 250µg/ml. The different experimental parameters affecting the stability were optimized. The method was validated for accuracy, precision, specificity, robustness, LOD and LOQ in accordance with International Conference on Harmonization (ICH) guidelines.*
Outcome: *The proposed method was successfully applied for the analysis of ceftaroline fosamil in bulk form.*
Keywords: *Ceftaroline Fosamil, RP-HPLC method, Validation.*

1. INTRODUCTION

The cephalosporin class of antimicrobial agents is known for its broad spectrum of activity, proven efficacy and favourable safety profile, making it the most commonly prescribed class of antimicrobials.[1-6] There are four recognized class generations of cephalosporins based on spectra of activity.[7] The CLSI designates ceftaroline as a member of a new subclass of antimicrobials, cephalosporins with anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity.[8]

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Ceftaroline has also been described in the literature as a 'fifth-generation' cephalosporin.[9] Ceftaroline was developed by modifying the structure of the fourth-generation cephalosporin cefozopran[10] . The Prodrug, ceftaroline fosamil, which contains a phosphono group to increase water solubility, is rapidly converted in plasma into the bioactive agent, ceftaroline (Figure 1)[11].

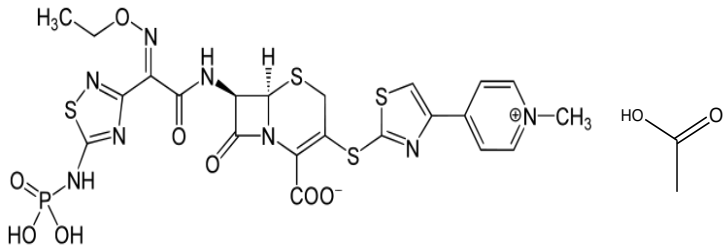


Figure 1. Structure of Ceftaroline Fosamil acetate

2. MATERIALS & METHODS

2.1. Chemicals

Ceftaroline Fosamil (95% purity) was kindly supplied by jubilant chemsys Ltd, Noida. HPLC- grade methanol, ammonium dihydrogen orthophosphate, acetonitrile; Sigma Aldrich Ltd. and high purity water was prepared using Milli-Q water purification system (Millipore, USA)

2.2. Equipment

Waters (e2695 separation module) with 2998 PDA detector was used for HPLC study along with auto sampler, X-Bridge Shield RP 18, (4.6×100mm, 5µm) column and Empower software with computer.

2.3. Chromatographic Condition

The mobile phase was used ammonium dihydrogen ortho phosphate with acetonitrile (90:10), at a flow rate of 1mL/min. The eluate was monitored at 242.6nm. Separation was carried out at temperature of 30°C.

2.4. Preparation of stock solution

10 mg of Ceftaroline Fosamil was accurately weighed and transferred to a 10 ml volumetric flask. 5 ml of diluents was added, sonicated and the volume was made up to 10 ml to yield 1000ppm stock solution. The stock solution was protected from light using aluminium foil.

2.5. Preparation of standard solution:

The standard solution was prepared 1 ml above stock solution was pipette out and make up 5 ml in volumetric flask. The final concentration of standard solution 200µg/ml. This stock solution was subsequently used for preparation of working standards in concentration ranges of 50µg/ml to 250µg/ml

2.6. Preparation of Buffer

575 mg ammonium dihydrogen ortho phosphate was weighed accurately and dissolved in 1000 ml water and pH was adjusted to 6.00 ± 0.05 with orthophosphoric acid (1%v/v). It was filtered through 0.5µ or membrane filter.

2.7. Diluents

Water: Methanol (2:2) as diluents.

3. Method development

In this trail it was found that in this condition ceftaroline peak was eluted well and peak shape is good which finalized column for Ceftaroline Fosamil.

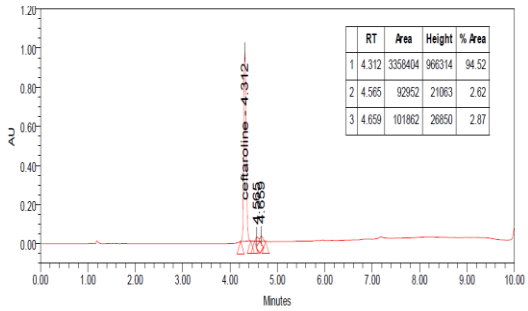


Figure No. 2 Chromatogram of Ceftaroline Fosamil

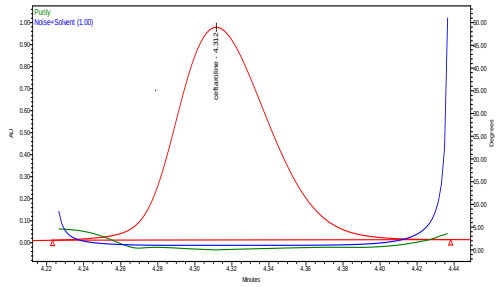


Figure No. 3 purity plot diagram of Ceftaroline Fosamil

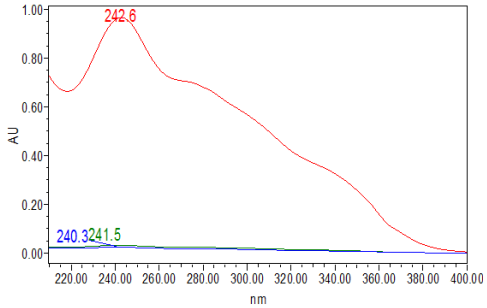


Figure No. 4 UV absorption spectrum of Ceftaroline Fosamil

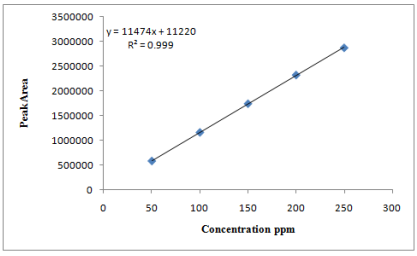
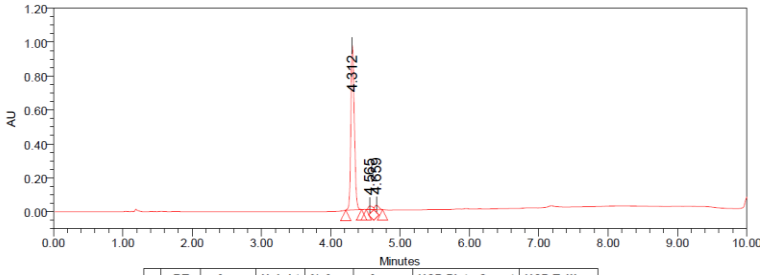


Figure No. 5 Calibration curve of Ceftaroline Fosamil



	RT	Area	Height	% Area	Asym	USP Plate Count	USP Tailing
1	4.312	3367231	967036	94.50	29572.80	35252.97	1.22

Figure No. 6 Chromatogram of System Suitability Parameters of Ceftaroline Fosamil

3.1. Linearity

Various aliquots were prepared from the stock solution (1000µg/ml) ranging from 50µg/ml to 250µg/ml and the resulting solutions were analyzed with the help of RP-HPLC using water: methanol as blank. (Fig.5)

3.2. Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations, i.e 100, 150, 200µg/ml of specification limit in triplicate as per ICH guidelines. For each concentration three sets were prepared and injected and % recovery was found to be 95%-98%.

3.3. Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, 200µg/ml of solution was prepared and the peak area was reported. In the intra-day variation study, 200µg/ml of solution was prepared. The results were indicating by % RSD.

3.4 .Limit of Detection & Limit of Quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily to quantify as an exact value. The LOQ is the concentration that can be quantify reliably with a specified level of accuracy and precision. The LOD & LOQ were calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

$$LOD = Cd \times Syx / b \text{ and } LOQ = Cq \times Syx / b$$

Where Cd and Cq are the coefficients for LOD and LOQ. Syx is the residual Variance of the Regression, and b is the Slope. Calculation was performed by using values of Cd and Cq of 3.3 and 10.

3.5 .Robustness

The robustness of the method was evaluated by assaying standard solutions after slight but deliberate changes in the analytical conditions flow rate (± 0.1 ml/min), and changing the column temperature (25°C,30°C, 35°C).

3.6. System Suitability Parameters-

For system suitability parameters tailing factor, theoretical plate, retention time, asymmetric factor and plate height of the peaks were calculated.

3.7. Stability Studies

The stability study of Ceftaroline Fosamil was carried out in acidic as well as, basic and oxidative conditions at room temperature. For this 0.1N solution of acid, alkali and 1% hydrogen peroxide was prepared and 1ml of working standard was taken. Equivalent quantities of drug and 0.1N solutions of acid, alkali and 1%peroxide were mixed in three different vail for stability studies.

Table No. 1 Stability in strong acidic condition (0.1 N HCL)

S.NO.	***Time (hr.)***	***% Purity***	***% Degradation***
1	0	94	0
2	12	85	10
3	24	83	12
4	36	72	24

Table No. 2 Stability in strong basic condition (0.1 N NAOH)

S.NO.	***Time (hr.)***	***% Purity***	***% Degradation***
1	0	77	0
2	12	41	47
3	24	45	42
4	36	53	32

Table No. 3 Stability in strong oxidative condition (1% H_2O_2)

S.NO.	***Time (hr.)***	***% Purity***	***% Degradation***
1	0	91	0
2	12	85	7
3	24	81	11
4	36	64	32

After studies of 36 hr. in different conditions (basic, acidic and peroxide).it was observed that the drug Ceftaroline Fosamil was degraded in acid and peroxide and basic solutions at room temperature.

4. RESULTS AND DISCUSSION

Based on interrelationship between columns, pH, mobile phase composition and its ratio the optimized parameters were sets and the peak was obtained at the retention time of 4.312 min. Ceftaroline Fosamil shows the absorbance maxima at 242.6 nm (Figure 4). The accuracy of the method was assessed by determination of recovery for three concentration (corresponding to 100, 150, 200 µg/mL of test solution conc.) covering the range of the method. For each concentration three sets were prepared and injected and % recovery 95-98%. Which showed that there no interference from the excipients. The LOD & LOQ was obtained 4.2498 & 12.8782 µg/mL respectively.

Table No. 4 Results of Validation Parameters

S.No.	*Parameters*	*Results*
1	Linearity range (µg/ml)	50-250
2	LOD (µg/ml)	4.2498
3	LOQ (µg/ml)	12.8782
4	Slope	11474
5	Intercept	11220
6	Correlation co-efficient (R^2)	0.999
7	Accuracy	95-98%
8	%RSD	
	Intraday precision	0.444%
	Inter day precision	1.35%

Table No.5 purity data table

Name	*R T (min)*	*Purity angle*	*Purity threshold*	*area*	*% area*	*height*
Ceftaroline	4.312	0.264	0.285	2315865	94.52	966314

5. CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of ceftaroline fosamil in bulk form. The results of analysis revealed that the proposed method was suitable for the analysis with virtually no interference of the usual additives present in pharmaceutical formulations. This method can be adopted for routine quality control of Ceftaroline Fosamil in bulk. All the acceptance criteria's of the parameters selected for validation are satisfied as per ICH guideline.

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