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NEW HPLC TECHNIQUE ADVANCEMENT AND APPROVAL OF CEIPIME – A FOURTH ERA ANTIBIOTIC

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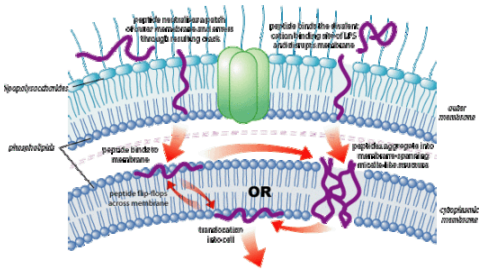
ABSTRACT

A simple and sensitive method was developed for estimation of Cefepime in its bulk and Pharmaceutical formulations and the method is validated according to ICH guidelines for routine practice in the Pharmaceutical industry and academic research and other fields associated with it. The method development was done with RP-HPLC with an equivalent pressure is maintained around 30 MPa, A mobile phase P^H buffer 3.5 and acetonitrile in 50:50 (v/v) ratio. The column X terra C18 (4.6*150, 5µ) with a flow rate 0.8 ml/min a suitable retention peak was obtained on 4.266 min. this method was validated according to ICH guidelines. The system suitability, Linearity, precision, LOD, LOQ, and other stability studies were conducted and all are satisfied the guidelines and the method was suitable or regular practice in Pharmaceutical Analysis of Cefepime.

KEYWORD: Cefepime, RP-HPLC, Validation, Cephalosporin's.

INTRODUCTION

Cefepime is a fourth-generation cephalosporin antibiotic. Cefepime has an extended spectrum of activity against Gram-positive and Gram-negative bacteria, with greater activity against both types of organism than third-generation agents. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms.



Cefipime is chemically (6R, 7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(N-hydroxyimino) acetamido] -3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid". The drug is freely soluble in organic solvents and sparingly soluble in water. It is a white or off white color powder having characteristic odor. In the present study, a rapid, sensitive HPLC method was developed to determine Cefepime. Compared with the previous assay methods single run time of 8 min, this method provided shorter analysis time. The lower limit of quantification (LLOQ) of Cefepime was 8.4 µg /ml, which was sensitive enough to detect relatively low concentration of Cefepime. The linearity, precision and all other parameters are established for regular use in the field of pharmaceutical industry academics.

MATERIALS AND METHODS

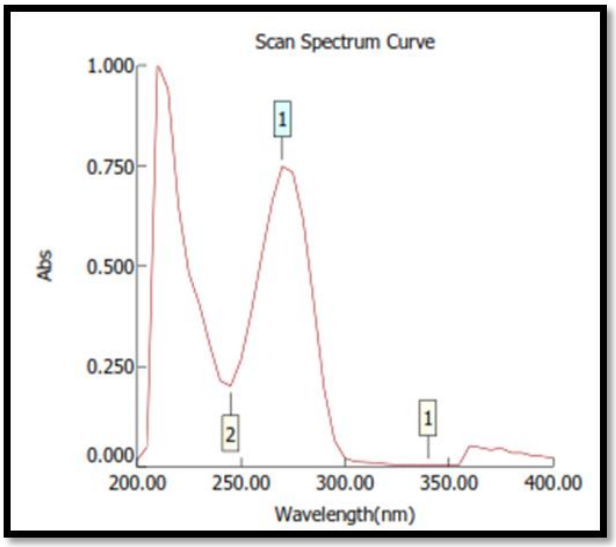
Cefepime Pure drug (99.8%) was procured as a gift sample from HiQ Laboratory, Hyderabad and the solvents used in this are HPLC grade procured from Merck Laboratories acetonitrile, Triethanolamine, water and orthophosphoric acid. The Instrument was utilized for the Method development was HPLC (water 2487 with dual lambda Max detector) which were previously calibrated with caffeine before commencing the Method development. The X Terra Column was Used with a dimensions of 4.6*150 mm and 5µ particle size. All the glass ware used for this is Borocilicated and Class A.

Solvent Preparation: Initially 0.1% Triethanolamine acetate was prepared by adding 1 ml of TEA in 1000 ml of Water HPLC grade and P^H adjusted to 3.5 with ortho Phosphoric acid or Disodium Hydrogen Phosphate, take 500 ml of this solution now this solution was treated as buffer and 500 ml of acetonitrile HPLC grade and mixed with magnetic stirrer and degassed by using vacuum filtration 25µ grade.

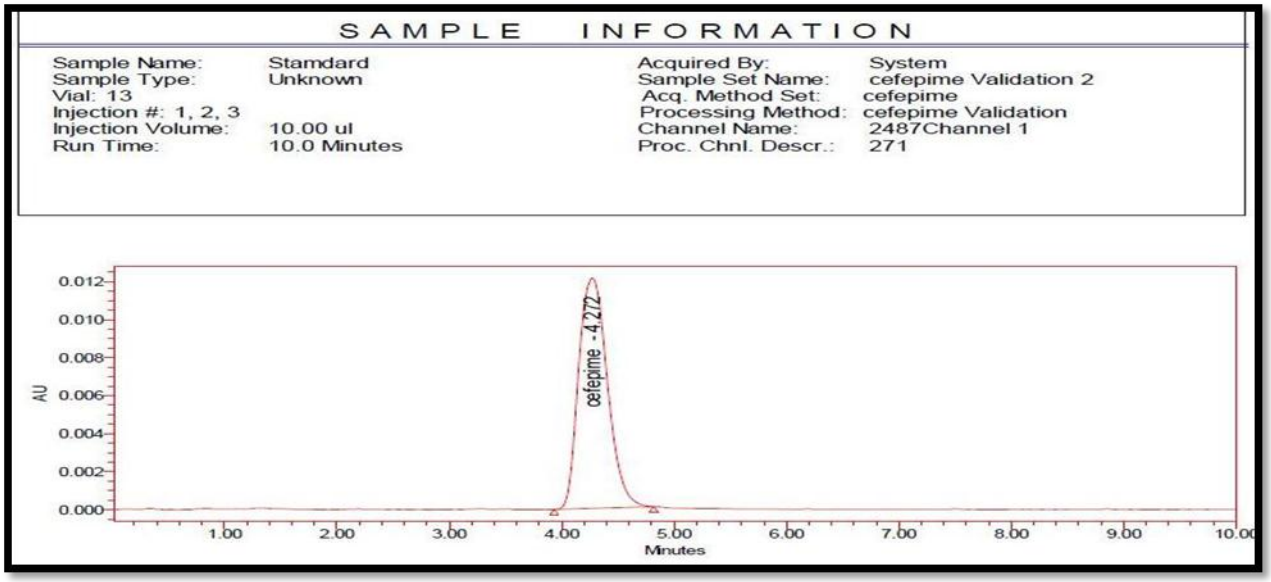
Sample preparation: Accurately weigh and transfer equivalent to 50 mg of **Ultipime O (500mg)** containing Cefepime Hcl sample into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution). Make further dilutions to make Final aliquot was approximately 150 µg/ml.

Preparation of Standard Solution: Accurately weigh and transfer 50 mg of Cefepime Hcl working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Make further dilutions that Final Aliquot Becomes 150 µg/ml.
Selection of wavelength:

The standard Cefepime was dissolved in Mobile Phase having concentration of 50 ppm and observed the lambda max in the UV Instrument. The lambda max was observed in 271 nm. And this will be used in the method development by HPLC.



Method Development: the various mobile phases and columns were used for the analysis of Cefepime in the bulk and Tablet dosage forms. But finally the method was optimized by the conditions using mobile Phase PH 3.5 buffer and acetonitrile in a ratio of 50:50 (v/v), the lambda max adjusted at 271 nm, X terra column 4.6*150 mm with 5µ particle size. Temperature 25^0 C, with flow rate 0.8 ml/min and run time was achieved with in 8 min. finally the retention time was achieved at 4.22 min.



Validation of method: the validation can be according to ICH Q2 R1 guidelines to meet the needs of Pharmaceutical Industry. Validation is very importance in quality control of elements, helps in assuring the materials and also for regulatory requirement to file the product. Whenever method is changed and the change is outside the scope of the original method the validation is required. As per ICH the validation parameters are precision, accuracy, Limit of Detection, Limit of Quantification, Specificity, Linearity and Range, Ruggedness and Robustness.

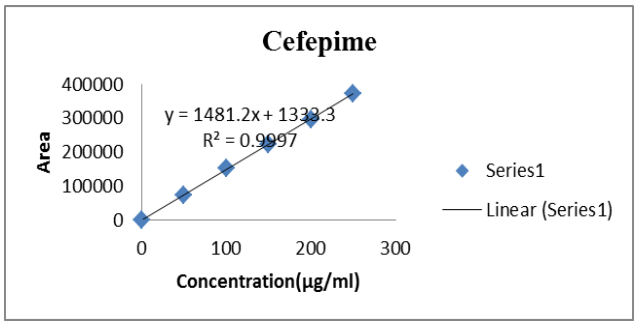
System Suitability: System suitability was used to verify reproducibility of the chromatographic system. It is checked by repetitive injection of the drug solution at the concentration level 150µg/ml for Cefepime to check the reproducibility of the system. At first the HPLC system was stabilized for 40 min. One blank followed by six replicates of a single concentration standard solution of Cefepime was injected to check the system suitability. The parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in table 2.

	Peak name	RT	Area	USP Plates	Tailing
1	Cefipime	4.266	215952	2273.47	1.35
2	Cefipime	4.272	215159	2408.89	1.21
3	Cefipime	4.278	221276	2259.98	1.29
4	Cefipime	4.276	221515	2348.65	1.22
5	Cefipime	4.271	215653	2564.66	1.56
Mean		4.2726	217911	2371.13	1.326
SD		0.004669	3194.597	123.7708	0.142583
%RSD		0.109279	1.46601	5.219907	10.75289

Linearity

The linearity graphs were obtained over the concentration range of 50-250 µg/ml of Cefepime. The various cementations are overlaid and were shown in Fig.3. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve is shown in fig.4.

Sr No.	Concentration	Peak area
01	0 PPM	0
02	50 PPM	74461
03	100 PPM	153992
04	150 PPM	223383
05	200 PPM	294844
06	250 PPM	372305



Precision

Intra-day and inter-day precision study of Cefepime was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 150µg. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results for intra-day and inter-day precision were presented in Table 5 and Table 6 respectively.

conc. in ppm	Rt	Peak area	recovery
50%	4.272	112165.3	25.00
100%	4.272	217462.3	99.63
150%	4.274	335256.7	149.64

Accuracy

The accuracy of the method was determined by calculating recovery of Cefepime by the method of addition. Known amount of Cefepime at 50%, 100% and 150% was added to a pre quantified sample solution.

Robustness: it is done whether the method developed is quality or condition being strong and good condition. Robustness is the ability of a method to cope with errors during the execution and cope with erroneous conditions. It is performed by changing the flow rate, organic phase concentration PH, columns and temperature and in every case the system suitability was measured and compared with ICH guidelines.

LOD and LOQ: Limit of Detection and Limit of Quantitation are used to measure the least concentration of drug by using Method. Cefepime LOD and LOQ are measured by giving a blank Injection (LOB- Limit of Blank) and using the standard deviation of the response and the slope and were found to be 1.86 PPM and 8.66 PPM respectively.

Assay: the sample and standard solutions were injected into the HPLC and the peak areas are measured and found the percentage purity and quantity of tablet. Inject 2 µL of the standard, sample into the chromatographic system and measure the areas for the CEFEPIME Hcl peaks and calculate the % Assay by using the formula. And it was found that 104.49%

CONCLUSION

The developed method was simple and precise for the regular analysis of cefipime in bulk and pharmaceutical dosage forms this was achieved by ICH guidelines. The validation of method followed system suitability, Linearity, accuracy, precession, LOD & LOQs. The method also validated for robustness and ruggedness. This method is also stable under stress conditions have followed the ICH guidelines.

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