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UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF CEFPIROME SULPHATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, and precise UPLC method is developed for the quantitative determination of Cefpirome sulphate in pharmaceutical dosage form. The chromatographic separation of Cefpirome sulphate was achieved with an phenomnex c18 (2.5 X 100 mm, 3.0 µm) analytical column using 0.01M phosphate buffer and acetonitrile taken in 50:50%v/v and the response was detected at 265 nm by using PDA detector. The retention time was found to be 0.652 min. The described method shows excellent linearity over a range of 7.5-75 µg/mL. The correlation coefficient for Cefpirome sulphate was found to be 0.999. The relative standard deviation for six measurements in two sets of Cefpirome sulphate in injection is always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination of Cefpirome sulphate in pharmaceutical preparations.

Key words: UPLC, Cefpirome sulphate, Assay, Validation parameters.

INTRODUCTION

Cephalosporins are bactericidal antibiotics that inhibit cell wall synthesis of bacteria. These antibiotics are derived from cephalosporin C which was for the first time isolated from the cultures of Cephalosporium acremonium in 1948 by an Italian scientist, Giuseppe Brotzu. The first agent was cephalothin, discovered in 1964. Now new generation cephalosporins are available along with four generations of cephalosporins in pharmaceutical dosage forms.

Cefpirome sulphate is belongs to fourth generation cephalosporin drug and its molecular formula is C22H24N6O9S3. Route of administration for cefpirome sulphate is orally. And it is having anti microbial and anti bacterial characters.

The literature survey reveals that there is no UPLC analytical method available for estimation of Cefpirome sulphate in pharmaceutical dosage form. The reported methods available for the estimation of Cefpirome sulphate individually are spectro photometric method and HPLC methods. Since the lack of official high performance liquid chromatographic methods for the simultaneous estimation of Cefpirome sulphate, we have planned to develop a simple, precise, economic and accurate Stability indicating UPLC method development and validation for the estimation of Cefpirome sulphate in pharmaceutical dosage form.

MATERIAL AND METHODS

Instrumentation:

Waters-Acquity UPLC system equipped with auto sampler, and DAD Detector was used for the separation. An analytical column; Phenomnex C_{18} (2.5 x 100mm, 3.0µm) was used in the analysis with

the flow rate is 0.3 ml per minute and wave length is 265 nm and injection volume is 2 µl and column oven temperature is 40^0C with the run time of 3 minutes. For data collection and processing, Chromatographic software Empower -2 was used.

Reagents and chemicals:
The standard cefpirome sulphate drug was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile and ammonium acetate and all other chemicals were purchased from Merck chemical division, Mumbai. HPLC grade water was used throughout the study. Formulation was purchased from the pharmacy.

Preparation of Phosphate buffer:
To prepare Phosphate buffer solution, By adding 6.8gms of potassium di hydrogen ortho phosphate in a 1000ml water. Adjust this solution to pH 3.5

Preparation of mobile phase:
Mix a mixture of above buffer 500 mL (50%) and 500 ml Acetonitrile HPLC (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

Diluents Preparation:
Water : Acetonitrile(50:50) ratio

Standard Stock Solution Preparation:
Accurately weigh and transfer 10 mg of Cefpirome sulphate 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)

Sample Solution Preparation:
Accurately weigh and transfer equivalent to 10 mg of Cefpirome sulphate 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).
Further pipette 1.5 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3.0 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

METHOD VALIDATION:

System suitability:
System suitability parameters are determined in the different view of parameters like area**, t**ailing factor and theoretical plates.

Precision:
Repeatability:
Pipette out 1.5 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3.0 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.This standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision:
To evaluate the intermediate precision of the method, Precision was performed on different day by using different make column of same dimensions. Pipette out 1.5 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
Further pipette 3.0 ml of Cefpirome sulphate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy:
The recovery experiments were performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 50, 100 and 150% of labeled amount of the Cefpirome sulphate. Three replicate samples of each concentration level were prepared and the %recovery at each level (n = 3), and mean% recovery (n=9) were determined.

Linearity:
From the stock solution pipette out 0.5, 1, 2, 3.0, 4 & 5 ml was taken into the clean and dry 10 ml volumetric flask and make up the solution with diluents to prepare the concentration of 7.5-75ppm of Cefpirome. Inject each level into the chromatographic system three times and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

LOD and LOQ:
The limit of detection (LOD) and limit of quantitation (LOQ) for DCZ was determined by using calibration curve method.

Solution stability:
The stability of the standard solution was tested at intervals of 24 hours by injecting six times of

standard solution.

Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.27 ml/min to 0.33ml/min, mobile phase is varied ±5% and the temperature is varied from 35 to 45 degrees Celsius.

Assay:
The Content of Cefpirome sulphate in the pharmaceutical dosage form was found by using the developed method. The percentage purity of Cefpirome sulphate was found to be 99.68%, and %RSD values for Cefpirome sulphate was within limit of ≤2.

RESULTS & DISCUSSION

The chromatographic method was optimized by using different stationary phases like C18, C8, CN and different mobile phases containing buffers like phosphate, ammonium acetate, and triethylamine with different pH (2–5), and organic modifier (acetonitrile) were used. Finally, the chromatographic separation was achieved using Phenomnex C_{18} (2.5 x 100mm, 3.0μm) column. Changing the composition of mobile phase optimized the chromatographic method. To develop a stability-indicating method assessing the effect of change of proportion, the pH of mobile phase was maintained at 3.5 and the drug was well-resolved at mobile phase composition of buffer–acetonitrile (50:50, v/v) and the flow rate of mobile phase 0.3mL/min, and column temperature 40°C was optimal. The analyte had adequate retention, peak shape, less tailing and the chromatographic analysis time was less than 3 min.

The developed method was validated for system suitability, Linearity, accuracy, precision, LOD, LOQ and robustness. All the results were found to be within the limits and were summarized in the table 1.

CONCLUSION

A new, simple, rapid and precise ultra performance liquid chromatographic method was developed for the estimation of Cefpirome sulphate in pharmaceutical dosage form. Hence this method can be applied for the estimation of Cefpirome sulphate in drug testing laboratories and pharmaceutical industries.

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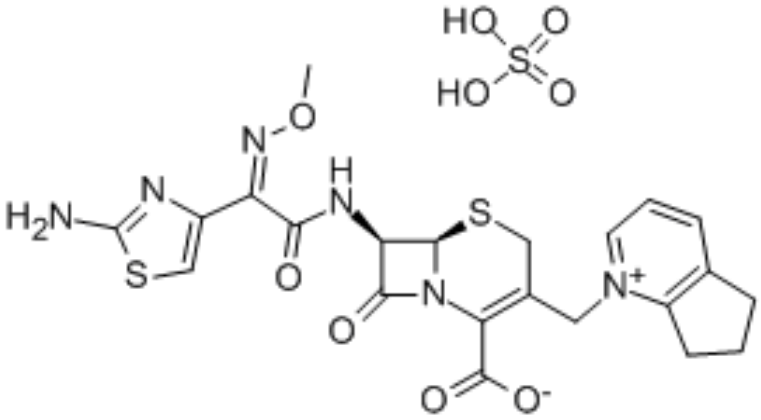

Fig 1. Structure of cefpirome sulphate

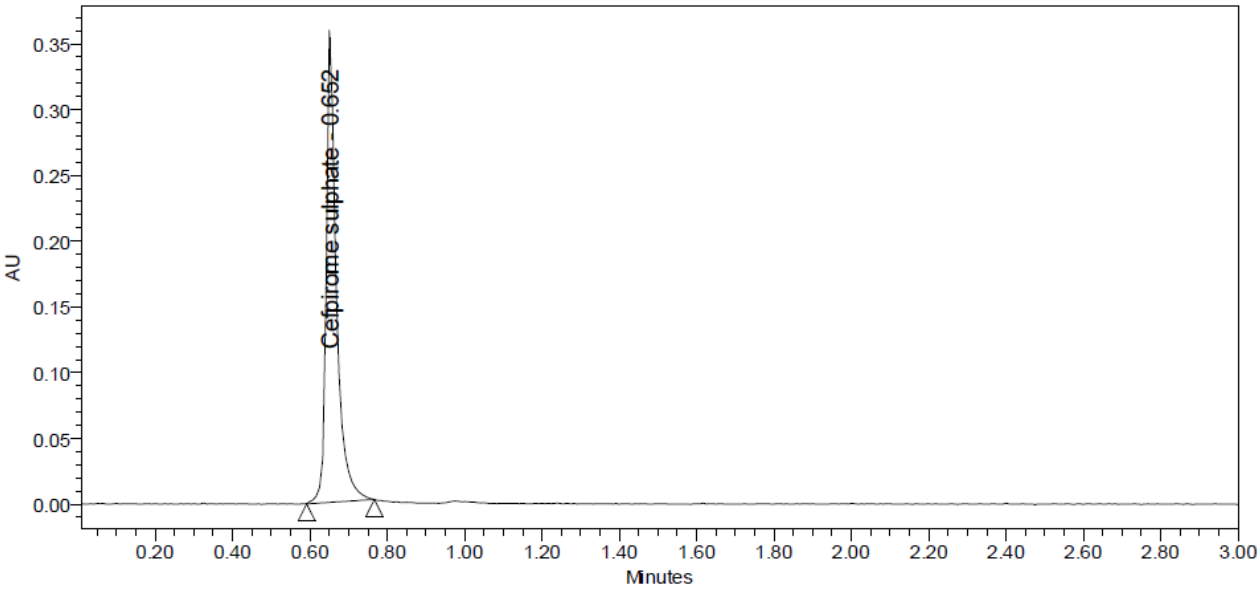

Fig. 2. Standard chromatogram of cefpirome sulphate

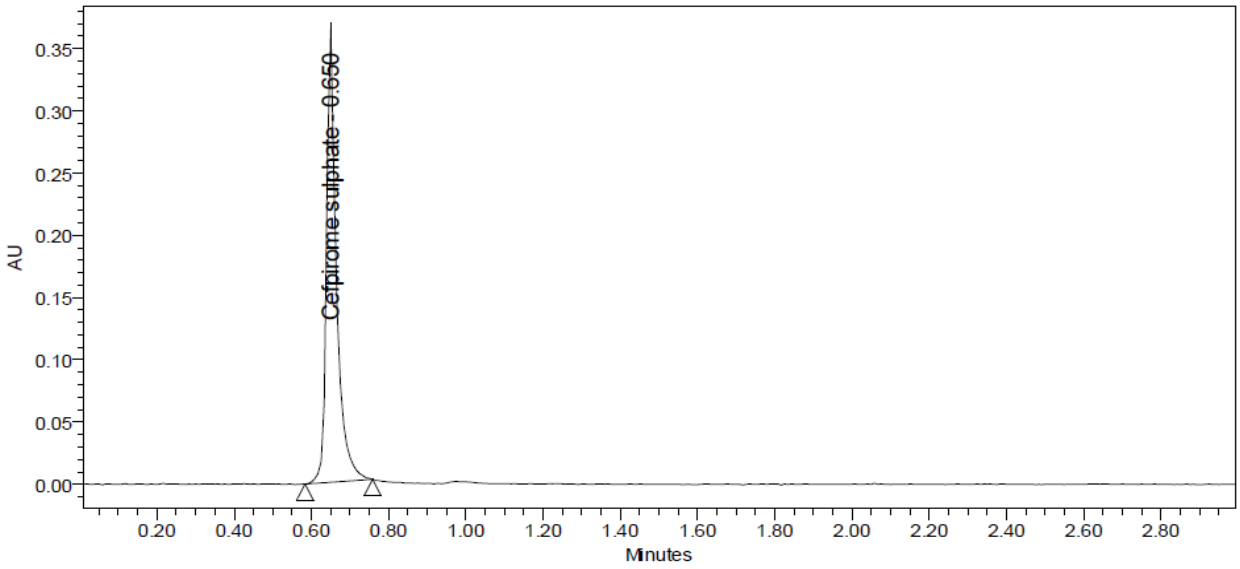

Fig. 3. Assay chromatogram of cefpirome sulphate

Table 1: Results of validation parameters

S. No.	Validation Parameter	Result	ICH guidelines
1	System suitability: No of theoretical Plates Tailing factor	 2842 1.52	 >2000 <2
2	Specificity	No other peaks at drug peak	No interference of excipients with drug peak

3	Linearity	R^2= 0.9995	R^2= 0.999
4	Intermediate Precision Repeatability	%RSD = 1.37 %RSD = 0.81	%RSD = <2 %RSD = <2
5	Accuracy	%RSD = 1.67	%RSD = <2
6	Lod and Loq	0.35 & 1.08µg/ml	Sensitive
7	Solution Stability	SD= 0.11	SD<2
8	Robustness	%RSD<2	%RSD = <2

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