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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1075-1080, July-Sept 2011

Development and Validation of RP-HPLC Method for Quantitative Estimation of Cefoperazone in Bulk and Pharmaceutical Dosage Forms

A. Sathish Kumar Shetty*, Yashwanth R., Manzoor Ahmed, Sridhar. B.K., Vijaya Kumar.M.L.

Department of Pharmaceutical Analysis, National College of Pharmacy, Balarj Urs Road, Shimoga (District), Karnataka, India – 577 201.

Corres. author: drskshetty@rediffmail.com*
yashusaga@gmail.com
Cell: 09449100007,Fax: 08182 273796.

Abstract: A simple, precise, specific and accurate RP-HPLC method has been developed for the determination of Cefoperazone in bulk and pharmaceutical dosage forms. Chromatography was performed on a supelco RP C-18 Column (25cm x 4.6 mm i.d.,particle size 5 µm) with ammonium acetate buffer of pH 5.5 and methanol in the ratio of 30:70 (v/v) as a mobile phase at a flow rate of 0.5 ml min^{-1}. Detection was performed at 250 nm. The retention time of Cefoperazone was found to be 3.512 min. By adoption of this procedure Cefoperazone is eluted completely. Linear calibration plots were obtained between 20-100µg mL^{-1}. The method of analysis was used for quantification in pharmaceutical preparations with a coefficient of variation <2%. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: Cefoperazone, ammonium acetate, methanol, coefficient of variation.

Introduction

Cefoperazone is a third generation cephalosporin antibiotic indicated for the treatment of patients infected with susceptible strains of microorganisms like Respiratory tract infections (upper and lower), urinary peritonitis, cholangits, septicemia, meningitis, skin and soft tissue infections bone and joint infections[1].

Cefoperazone is chemically 7[R{2-(4-ethyl-2,3-dioxopiperazin-1-yl-carboxamide)-2-4-hydroxylphenyl} acetamide]-3-[1-methyl-1H-tetrazol-5yl-thiomethyl]]-3-cephem-4caboxylate [3], [4].and the structural formula and shown in (Figure I). The molecular formula is $C_{25}H_{27}N_9O_8S_2$ and molecular weight is 667.65 g moL^{-1}. It is freely soluble in water, soluble in methanol and very slightly soluble in alcohol. It is official drug in Indian Pharmacopoeia 2010[3], British Pharmacopoeia 2009[4], and United States

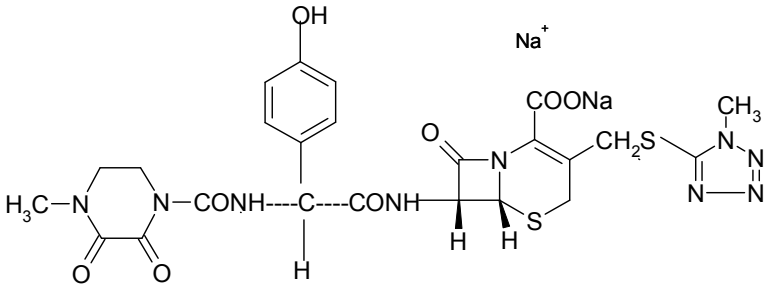

Figure I: Chemical Structure of Cefoperazone

Pharmacopoeia 2004[5]. Literature survey reveals that, stability-indicating HPLC[6], TLC[7], Fluorimetric determination were found and few spectrophotometric methods for the quantitative estimation of Cefoperazone in bulk and pharmaceutical formulations[8-9] have been developed. The proposed method describes a sensitive, simple, precise and accurate RP-HPLC method for the estimation of Cefoperazone in bulk and dosage pharmaceutical formulations form with subsequent validation as per ICH guidelines.

Experimental

Materials and Methods

A gradient HPLC (Shimadzu, class VP-Series) equipped with Supelco Reverse Phase C-18 Column (25cm x 4.6 mm i.d.,particle size 5 µm) was used, LC-10 AT VP pump, UV/VIS detector SPD-10A VP with N-2000 CHROMTECK (Shimadzu) software, o-Phosphoric acid AR grade, Double distilled water and Methanol HPLC grade. The optimized chromatographic conditions are summarized in (**Table I).**

Preparation of mobile phase

300 ml of HPLC grade Methanol was mixed with 128.5 ml of 0.05% of Ammonium acetate which was prepared in double distilled water and its pH was adjusted to 5.5 using ortho-phosphoric acid. Then it was ultrasonicated for 20 minutes and then filtered through 0.4 µm membrane filter paper**.**

Preparation of standard stock solution of Cefoperazone

25 mg of standard Cefoperazone was weighed accurately and transferred to 25 ml volumetric flask and dissolved in 10 ml of mobile phase and then volume was made up to the mark with mobile phase to get 1000 µg mL^{-1} of standard stock solution 'A' of the drug. These stock solutions were filtered through 0.4µm membrane filter paper.

Preparation of marketed formulations

Cefoperazone equivalent to 100 mg was weighed and transferred to 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were ultrasonicated for 20 minutes and the final volume was made up to the mark with mobile phase to get 1000 µg mL^{-1} of standard stock solution 'B' of the drug. Then the above prepared solution was filtered through 0.4 µm membrane filter paper.

Chromatographic condition

The mobile phase containing Methanol and Ammonium acetate buffer in the ratio of (70:30) was selected as the optimum composition of mobile phase, because it was found that this solvent system eluted the drug with good resolution. The flow rate was set to 0.5 ml min^{-1} and UV detection was carried out at 250 nm. The mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.4µm membrane filter paper. All determinations were performed at constant column temperature (25^0C).

Analysis and preparation of calibration curve for Cefoperazone

Appropriate aliquots were pipetted out from the standard stock solution 'A' (1000 µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20-100 µg mL^{-1} of Cefoperazone. 20 µl of each solution were injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The Cefoperazone was eluted at 3.512 min as shown in (**Figure: II),** the calibration curve was constructed by plotting average peak area versus concentration and was presented in (**Figure: III).** The method was extended for determination of Cefoperazone in pharmaceutical dosage form. The linearity range was found to be 20-100 µg mL^{-1}.

Table I: Optimized Chromatographic conditions for the proposed method

Parameters	Optimized condition
Linearity range (µg mL^{-1})	20-100
Detection wavelength (nm)	250
Temperature	25^0C
Retention Time (t) (min)	3.512
Run time (min)	15.0
Limit of Detection (µg mL^{-1})	0.299
Limit of Quantification (µg mL^{-1})	0.908

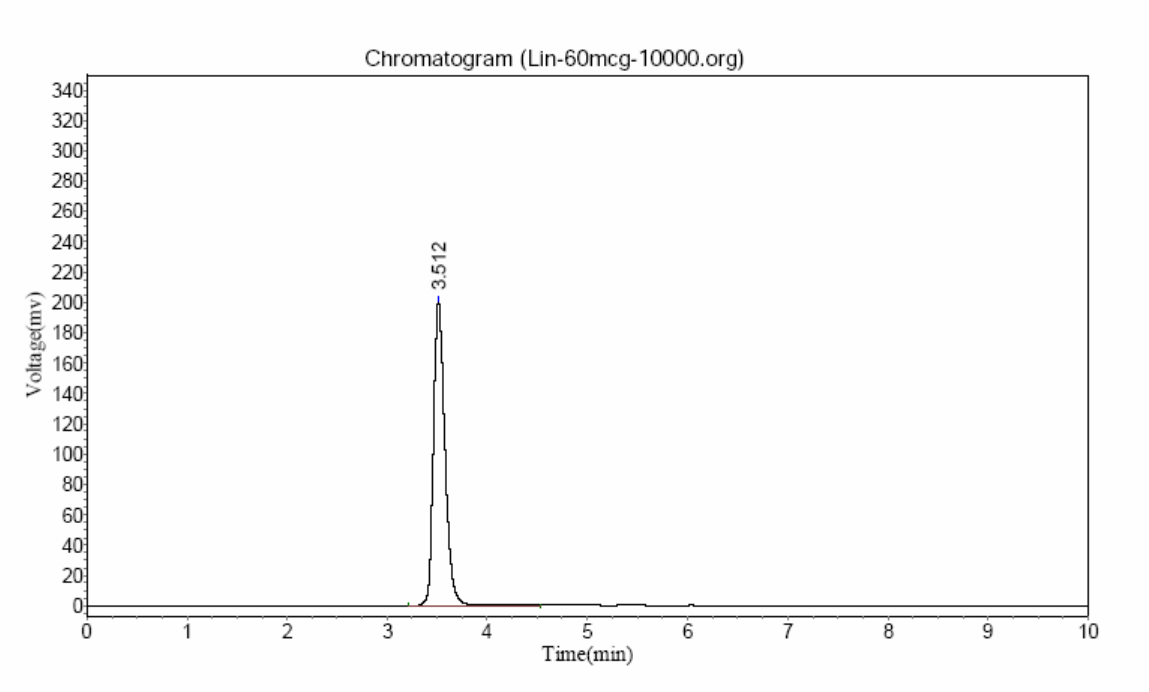

Figure II: Chromatogram of Cefoperazone by RP-HPLC method.

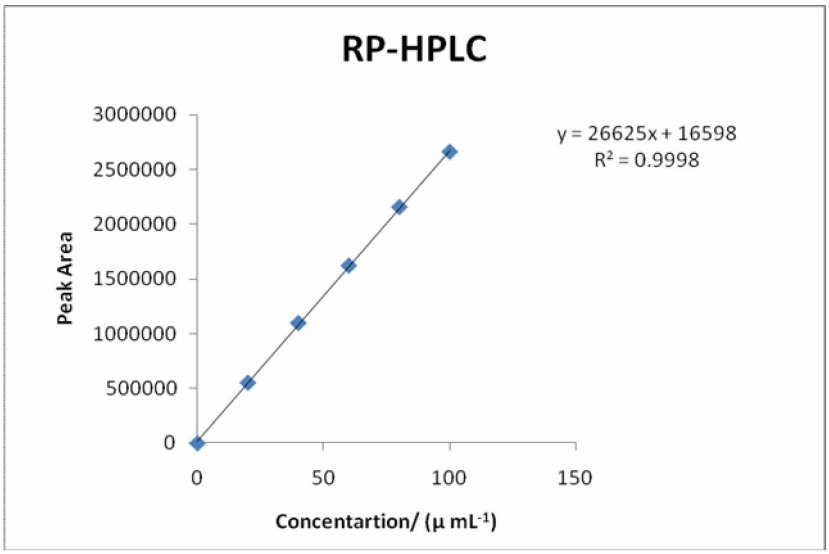

Figure III: Calibration curve of Cefoperazone at 240 nm by RP−HPLC method.

Analysis of Cefoperazone in formulations

From this stock solution 'B', various dilutions of the sample solution were prepared and analyzed. A 20 µl volume of each sample solution was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 250 nm and the amount of drug present in the sample was determined. The proposed methods were validated as per the ICH guidelines.

Method validation [10-13]

Accuracy

The procedure for the preparation of solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the same manner as explained above. The solutions were filtered through 0.4 µm membrane filter paper and then they were subjected to analysis by RP-HPLC method under the same chromatographic conditions as described above. At each level, six determinations were performed. The results obtained were compared with expected results and were statistically validated.

Precision

Intraday and inter-day precision were carried out for the various concentrations of the sample at different time intervals in the same day and at same time on different days. The concentration of the sample solution was determined as per the procedure given for the tablet formulation by determining peak area at selected analytical wavelength 250 nm. The variation of the results within the same day was analyzed and statistically validated.

Linearity and range

Appropriate aliquots were pipetted out from the standard stock solution 'A' in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20-100 µg mL^{-1} of the drug.

The solutions were injected using a 20 µl fixed loop in to the chromatographic system at the flow rate of 0.5 ml min^{-1} and the effluents were monitored at 250 nm, chromatograms were recorded.

Robustness

The evaluation of robustness showed the reliability of analysis with respect to deliberate variations in method parameters. The various concentrations were prepared and injected into sample injector of HPLC six times under different parameters like deliberate variations in flow rate, detection (nm).

Table II: System Suitability Test Parameters for the proposed method

Parameters	Optimized condition
Retention Time (t) (min)	3.512
Theoretical plates (N)	4578.918
Peak asymmetry	1.2914

Table III: Regression analysis of the Calibration curve for the proposed method

Parameters	Optimized condition
Linearity range (µg mL^{-1})	20-100
Regression equation (Y=mx +c)	
Slope (m)	26625
Intercept (c)	16598
Correlation coefficient (r^2)	0.9998
Relative standard deviation (%)	0.8315
Retention time (mins)	3.512

Table IV: Summary of Validation Parameters for the proposed method

Parameters	Values
Limit of detection (µg mL^{-1})	0.299
Limit of quantitation (µg mL^{-1})	0.908
*Accuracy (% RSD)	
80%	0.0320
100%	0.0288
120 %	0.2624
*Precision (% RSD)	
Intra Day	0.0310
Inter Day	0.0147
*Robustness (% RSD)	
Change in Flow rate	
0.4 ml/min	0.1578
0.6 ml/min	0.2673
Change in Detection wavelength	
248nm	0.1489
252nm	0.2880

*Mean of six determinations, RSD indicates relative Standard deviation

Result and Discussion

In this method the conditions were optimized to obtain elution of Cefoperazone. Mobile phase and flow rate selection was based on peak parameters (height, tailing factor, theoretical plates, capacity or asymmetry), run time, resolution. The system with Ammonium acetate: Methanol (30:70 v/v) with pH 5.5 with system suitability parameters as shown in (Table II).

The run time was set at 15 min and R_t for Cefoperazone was found 3.512±0.0155 min with standard deviation less than 1% with a good linear relationship (r^2 = 0.9998) was observed between the concentration of Cefoperazone and the respective peak areas in the range 20-100 µg mL^{-1}. The regression of Cefoperazone was found to be Y = 26625X + 16598, where 'Y' is the peak area and 'X' is the concentration of Cefoperazone (Table III).

The proposed RP-HPLC method was validated for intra and inter-day precision with %RSD, 0.03105 and 0.01478 respectively. A known amount of the pure drug solution (80, 100 and 120 %) was added to the powder sample of the formulation and subjected for the recovery studies. High recovery was obtained indicating that the proposed method is highly accurate. Robustness was determined by changing the parameters like flow rate and detection using similar operational and environmental conditions. (Table IV)

The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations.

Conclusion

Thus, it can be concluded that the method developed in the present investigation was simple, sensitive, accurate, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Cefoperazone in pharmaceutical dosage forms.

Acknowledgement

We would like thank to Karnataka Antibiotics and Pharmaceutical Ltd. Banglore, India for providing reference sample of Cefoperazone to facilitate this work.

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