Oriental Journal of Chemistry Vol. **24**(2), 737-740 (2008)

Estimation of Cefoperazone in marketed formulation by RP-HPLC

N. LALITHA*, VARUN PAWAR, S.B. PURANIK, P.N. SANJAY PAI and G.K. RAO

Department of Chemistry, Al-Ameen College of Pharmacy, Bangalore - 560 027 (India)

(Received: March 03, 2008; Accepted: April 15, 2008)

ABSTRACT

A RP-HPLC assay method has been developed and validated for cefoperazone. An isocratic RP-HPLC was developed on a SS Wakosil II- C_{18} column (250 mm × 4.6 mm i.d., 5 µm) using a mobile phase of phosphate buffer (pH 6.8) and methanol (3:1 v/v) with UV detection at wavelength 254 nm at the flow rate 1 ml/min. The proposed method was validated for sensitivity, selectivity, linearity, accuracy, precision, ruggedness, robustness and solution stability. The response of the drug was linear in the concentration range of 0.4 -100 µg/ml. Limit of detection and Limit of quantification was found to be 0.2 µg/ml and 0.4 µg/ml respectively. The % recovery ranged within 95-106 %. Method, system, interday and intraday precision was found to be within the limits of acceptance criteria. Method was found to be rugged when different analyst carried out analysis. The method was found to be sensitive and efficient with 3370.78 theoretical plates, 0.2966 mm HETP and tailing factor 1. The method was suitable for the quality control of cefoperazone in injection formulations.

Key words: Cefoperazone; RP-HPLC.

INTRODUCTION

Cefoperazone sodium is 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxyphenyl)acetyl]amino}-3-[[(1-methyl-1*H*-terazol-5-yl)thio]methyl]-8-oxo-, monosodium salt with molecular formula $C_{25}H_{26}N_9NaO_8S_2$. It is a 3rd generation cephalosporin, active against gram-negative bacteria but less active against gram positive bacteria. In the present study a simple rapid precise and accurate RP-HPLC method was developed for the estimation of cefoperazone.

EXPERIMENTAL

Materials

Pure cefoperazone was obtained as gift sample from Karnataka Antibiotics Pharmaceuticals Ltd, Bangalore, India. HPLC grade methanol was purchased from Qualigens fine chemicals, Mumbai, India. Millipore water was obtained from the Millipak 0.22µm filter.

Instrumentation and chromatographic conditions

A HPLC system consisted model 10AT Shimadzu- SPD10A detector, the column used was a SS Wakosil II- C_{18} column (250 mm × 4.6 mm i.d., 5 µm). The separation was carried out under isocratic elution of phosphate buffer (pH 6.8) and methanol in the ratio of 3:1 (v/v). The flow rate was 1 ml/min, the column temperature was ambient, the wavelength was monitored at 254 nm and the injection volume was 100 µl.

RESULTS AND DISCUSSION

Standard preparation

Standard stock solution of 1 mg/ml of cefoperazone in mobile phase was prepared in 10

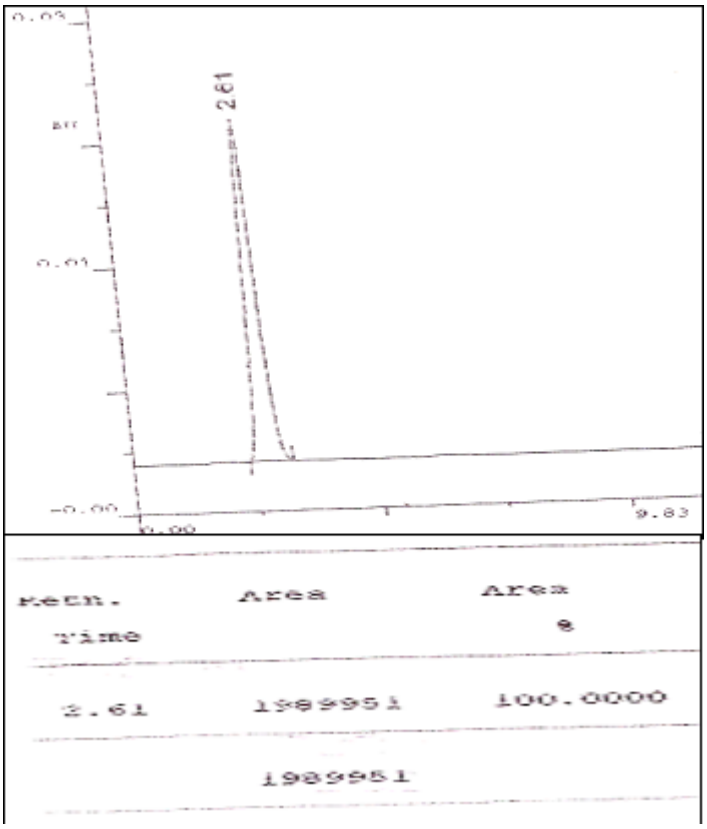
ml volumetric flask. Working solutions were prepared by diluting the aliquots of stock solutions with the mobile phase to contain 0.02–100 µg/ml of standard cefotaxime. 100 µl of each working standard solution was injected into the chromatograph and chromatogram was recorded. The standard calibration curve was constructed in the concentration range of 0.04-100 µg/ml, with concentration on X- axis, peak area on Y- axis and regression equation was calculated.

Sample preparation

Accurately weighed 10 mg of cefoperazone injection dissolved in 10 ml mobile phase, from this solution working sample solution of 10 µg/ml was prepared with the mobile phase in 10 ml volumetric flask and filtered through a 0.22 µm nylon syringe filter. 100µl of working sample solution was injected into the chromatograph and chromatogram was recorded. The concentration of cefoperazone in working sample solution was

Table 1: Validation data of Cefoperazone

S.No.	Parameters		Observations	Acceptance Criteria
1.	LOD (mcg/ml)	0.2	-	
2	LOQ (mcg/ml)	0.4	-	
3	Linearity	Range (mcg/ml)	0.4 - 100	-
		Regression eq^{n}	138510× + 21001	
		R^2	0.9997	-
4	Accuracy(%Recovery)	Level I (80%)	95	% Recovery within 90 to 120%.
		Level II (100%)	97.5	
		Level III (120%)	106	
5	Precision(%RSD)	Method	0.2652	% RSD should be less than 2%.
		System	0.2796	
		Interday	0.905	
		Intraday	1.2917	
6	Ruggedness(%Assay)	Analyst 1	99.51	% Assay within 95-102%.
		Analyst 2	99.34	
7	Robustness			% Assay should be within 95-102%.
	Flow rate (ml/min)	0.8	96.56	
		1.2	98.53	
	PO_4^{2-} buffer : Methanol	3:0.8	97.58	
		3:1.2	98.54	
	Wavelength	252	95.02	
		256	94.85	
	Columns	Accuracil	102.58	
		Wakosil	95.78	



calculated from regression equation using average peak area of three replicates of working sample solution.

Development of the method

A simple and rapid isocratic RP-HPLC method for estimation of cefoperazone was developed on a SS Wakosil II- C_{18} column (250 mm × 4.6 mm i.d., 5 µm) using a mobile phase of phosphate buffer (pH 6.8) and methanol (3:1 v/v) with UV detection at wavelength 254 nm at the flow rate 1 ml/min. Cefoperazone was eluted at retention time 2.6 min (Fig. 1). The method was found to be specific for the estimation of cefoperazone in marketed injection formulations. The method was found to be sensitive and efficient with 3370.78 theoretical plates, 0.2966 mm HETP and tailing factor 1. The developed method was successfully applied for the assay of marketed formulation, the working sample solution showed 101.02 % w/w of cefoperazone.

Validation of the method

The developed analytical method was validated as per ICH method validation guidelines. The validation parameters addressed were LOD, LOQ, linearity, accuracy, precision (system, method, inter-day and intra-day), robustness, ruggedness, specificity and stability of cefoperazone in mobile phase.

CONCLUSION

The study presents a reverse phase HPLC estimation of cefoperazone with UV-detector. Method is simple, economical and less time consuming that the other prescribed methods. The method was validated and found specific, accurate, precise, rugged and robust. The method could be applied with success even to the analysis of marketed products cefoperazone injection formulation, as no interference was observed due to excipient or other components present.

REFERENCES

1. Beckette A. H., Stenlake J.B., *Practical Pharmaceutical Chemistry*. The Anthlone Press; London, 4th edition, **157**: 281-307 (1988).
2. Snyder L.R., Kirkland J.J., Glach J.L., *Practical HPLC Method Development*. A Wiley Interscience Publication; London, 5th edition, 402-420 (2005).
3. Juniper I.R., Method Validation: An Essential Element in Quality Assurance In: Parkany M. Quality assurance and TQM for Analytical Laboratories. The Royal society of chemistry; Thomas Graham House, Cambridge. 114- 120 (1995).
4. USP-27-NF, The United States Pharmacopoeial Convection Inc. Rockville, MD, 350-352 (2004).