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Development and validation of RP-HPLC method for estimation of Cefotaxime sodium in marketed formulations.

Lalitha N*[1], Sanjay Pai PN[1]

1) Department of Quality Assurance, Al-Ameen College of Pharmacy, Bangalore, India.

ABSTRACT: A RP-HPLC assay method has been developed and validated for cefotaxime. An isocratic RP-HPLC was developed on a SS Wakosil II- C_8 column (250 mm 4.6 mm i.d., 5 µm) utilizing a mobile phase of ammonium acetate buffer (pH 6.8) and acetonitrile (85:15 v/v) with UV detection at wavelength 252 nm at the flow rate 0 .8 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 10-70 µg/ml. Limit of detection and Limit of quantification was found to be 0.3 µg/ml and 0.6 µg/ml respectively. The % recovery ranged within 97-102 %. Method, system, interday and intraday precision was found to be within the limits of acceptance criteria. Method was found to be rugged when analysis was carried out by different analyst. The method was found to be sensitive and efficient with 2216 theoretical plates, 0.1128 mm HETP and tailing factor 1. The method was suitable for the quality control of cefotaxime in injection formulations.

KEY WORDS:
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INTRODUCTION

Cefotaxime sodium is sodium 7-[2-(2-amino-4-thiazolyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate 72 (Z)-(o-methyloxime), acetate (ester). (Fig1) with molecular formula $C_{16}H_{16}N_5NaO_7S_2$.[1] It is a 1st generation cephalosporin, inhibits cell wall biosynthesis. Literature survey revealed that ESR[2], various spectrophotometric and HPLC methods are available for estimation of cefotaxime sodium.[3-17] In the present study a simple rapid precise and accurate RP-HPLC method was developed for the estimation of cefotaxime sodium.

MATERIALS AND METHODS:

Pure Cefotaxime was obtained as gift sample from Karnataka Antibiotics Pharmaceuticals Ltd, Bangalore, India. Cefotaxime Injection was purchased from local market. HPLC grade methanol was purchased from Qualigens fine chemicals, Mumbai, India. Millipore water was obtained from the Millipak 0.22µm filter. Buffer materials and all other chemicals were of analytical-reagent grade. An HPLC system consisted model 10AT Shimadzu- SPD10A detector, the column used was a SS Wakosil II- C_8 (250 mm × 4.6 mm i.d., 5 µm). The separation was carried out under isocratic elution of the mobile phase, ammonium acetate buffer (pH 6.8) and acetonitrile in the ratio of (85:15 v/v) at the flow rate 0 .8 ml/min. The column temperature was ambient, the wavelength was monitored at 252 nm and the injection volume was 100 µl. Standard stock solution of 1 mg/ml of cefotaxime 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 0.1–100 µg/ml of standard cefotaxime. 100 µl of each working standard solution was injected into the chromatograph and chromatogram was recorded. The

*Corresponding Author:
E-mail: lallubalu@rediffmail.com

standard calibration curve was constructed in the concentration range of 5-100 µg/ml, with concentration on X- axis, peak area on Y- axis and regression equation was calculated.

Accurately weighed 10 mg of cefotaxime sodium 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 chromatograph was recorded. The concentration of cefotaxime in working sample solution was calculated from regression equation using average peak area of three replicates of working sample solution. 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 cefotaxime in mobile phase.

RESULTS AND DISCUSSION

The developed analytical method was validated as per ICH method validation guidelines (Table 1). The validation parameters addressed were LOD, LOQ, linearity, accuracy, precision (system, method, inter-day and intra-day), robustness, ruggedness, specificity and stability of cefotaxime in mobile phase. A simple and rapid isocratic RP-HPLC method for estimation of cefotaxime was developed on a SS Wakosil II- C_8 column (250 mm × 4.6 mm i.d., 5 µm) utilizing a mobile phase of ammonium acetate buffer (pH 6.8) and acetonitrile (85:15v/v) with UV detection at wavelength 252 nm at the flow rate 0.8 ml/min. Cefotaxime was eluted at retention time 5.47 min (Fig. 1). Three independent determinations were performed at each concentration and the response for the drug was found linear in the concentration range of 10 to 70 µg/ml. The standard calibration curve was plotted and regression equation was calculated as y = 49692x+87045 with R^2 = 0.9976 (Fig. 2), percentage recovery ranges from 97–102% (Table 1). The method was found to be specific for the estimation of cefotaxime in marketed injection formulations. The method was found to be sensitive and efficient with 2216 theoretical plates, 0.1128 mm HETP and tailing factor 1. The developed method was successfully applied for the assay of marketed formulation, the working sample solution showed 97.33 % w/w of cefotaxime sodium.

Conclusion

The study presents a reverse phase HPLC estimation of cefotaxime 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 cefotaxime injection formulation, as no interference was observed due to excipient or other components present.

FIGURE 1: STRUCTURE OF CEFOTAXIME SODIUM

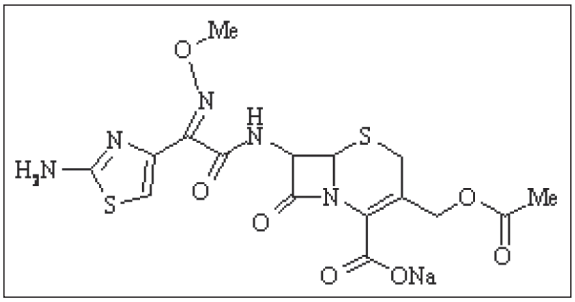

FIGURE 2: CHROMATOGRAM SHOWING SAMPLE SOLUTION OF CEFOTAXIME AT CONCENTRATION OF 10µg/ml.

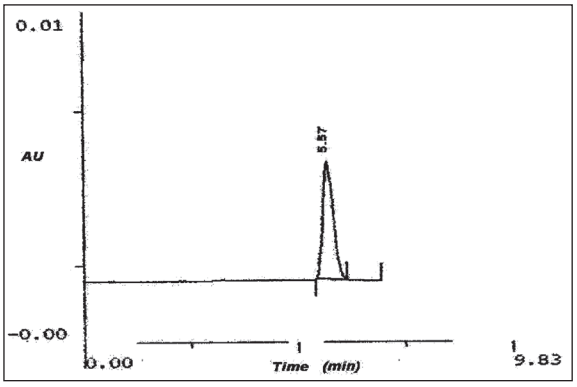

FIGURE 3: LINEARITY GRAPH OF CEFOTAXIME SODIUM

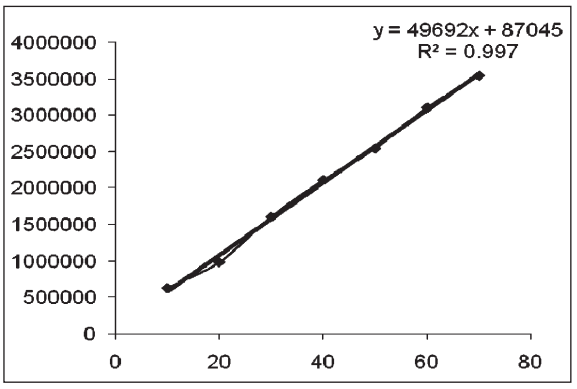

TABLE 1: RECOVERY STUDY DATA OF CEFOTAXIME

Sl. No.	Std. cefotaxime conc. mcg/ml (a)	Sample cefotaxime conc. mcg/ml (b)	Total conc. mcg/ml (c)	Peak area	Total amount of cefotaxime from Std graph mcg/ml (d)	Recovery of standard drug mcg/ml (d-b) = (e)	% Recovery of standard (e x 100 / c)
1	10	10	20	979221	19.70	9.70	97.05
2	30	10	40	2006242	41.63	31.63	101.23
3	50	10	60	3034843	61.06	51.06	102.12

TABLE 2: REGRESSION ANALYSIS OF THE CALIBRATION CURVES FOR PROPOSED METHOD

Parameters	Values
Calibration ranges (mcg/ml)	10–70
Slope	49692
Intercept	87045
Correlation coefficient (R^2)	0.9976
Regression	49692x+87045

TABLE 3: SUMMARY OF VALIDATION PARAMETERS

Parameters	Values
Detection limit (mcg/ml)	0.3
Quantitation limit (mcg/ml)	0.6
Accuracy (%)	97 to 102%
Precision (RSD[a])	
Intraday (n = 3)	1.2917
Interday (n = 3)	0.9050
Repeatability (RSD[a] , n = 3)	0.1659
Ruggedness (% Assay)	
Analyst 1	99.51
Analyst 2	99.34

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