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RESEARCH ARTICLE

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE QUNTIFICATION OF CEFOTAXIME SODIUM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, fast & precise Reverse Phase High Performance Liquid Chromatographic method was developed for the determination of Cefotaxime Sodium in pharmaceutical dosage form. The RP-HPLC separation was achieved on hypersil C_{18} column (250 mm, 4.6 mm, 5µm) using mobile phase consisting of buffer solution (sodium dihydrogen phosphate) : acetonitrile [37: 63 v/v, pH=2.75 adjusted with phosphoric acid] at a flow rate of 1ml/min and the retention time was about 6 minutes. The method is selective to Cefotaxime Sodium and able to resolve the drug peak from formulation excipients. The system suitability with retention time was (Mean + %CV) 8.600 + 0.186.The calibration curve was linear over the concentration range of 1-20µg/ml (r2 = 0.999). The proposed method is accurate and precise (Intra day and Inter day variation, RSD were 0.55-1.67) and linear within the desired range. The LOD and LOQ was detected as 0.0187µg/ml and 0.043µg/ml respectively with r2 = 0.9997. The accuracy result of seventy percent drug (80%) was 99.87%, hundred percent (100%) was 99.93%, and one thirty percent (120%) was 100.18%. Therefore, this method could be used as a more convenient and efficient option for the analysis of Cefotaxime Sodium in raw material and Parentral dosage form.

Keywords: Third generation cephalosporin; Cefotaxime Sodium; Method validation; HPLC method determination; Quantitative analysis

INTRODUCTION

Cefotaxime Sodium is parentral third generation cephalosporin [1, 2]. Several investigations on Cefotaxime Sodium have indicated that it is an parentally active antibiotic with similar antibacterial spectrum and resistance to β-lactamase as for other parenteral third generation cephalosporins [3, 4]. The injection of cefotaxime is useful in emergency cases and used BD in a day. Cefotaxime Sodium has potent antibacterial activity against a wide range of bacteria, highly stable towards β-lactamases and long duration of action [3]. Memon et al. reported Cefotaxime Sodium as a safe, effective, and cheaper oral option for the treatment of multidrug-resistance [5]. The chemical structure of Cefotaxime Sodium consists of the cefem nucleus that is bicyclic and formed with fusion of , a β-lactam ring & 6-membered dihydrothiazine ring. The cephem nucleus incorporates two important groups: the vinyl group at the 3-position, which is responsible for the intestinal absorption of the intact molecule. Other groups are the aminothiazole ring and the acetic acid oxy-imine group on the side chain at the 7-position, which are associated with the antibacterial activity[4]. So it was thought of interest to develop a simple, specific and sensitive RP-HPLC method for determination of Cefotaxime Sodium in Injection[5].

Chemical formula:

$C_{16}H_{16}N_5NaO_7S_2$

A number of methods has been used for the analysis of Cefotaxime Sodium in raw material, in pharmaceutical dosage forms as well as in biological fluids such as Rathinavel et al., who used RP-HPLC method for the estimation of Cefotaxime Sodium in tablets[6], Meng et al., who used liquid chromatographic-tandem mass spectrometric method to determine Cefotaxime Sodium in human plasma[7], also, Al-Momani used Spectrophotometric method for the determination of drug in formulations[8], whereas Eric-Jovanovic et al. used HPTLC method for the determination of Cefotaxime Sodium in dosage forms[9], and finally Liu et al., used HPLC method for the analysis of Cefotaxime Sodium in human plasma and urine[10]

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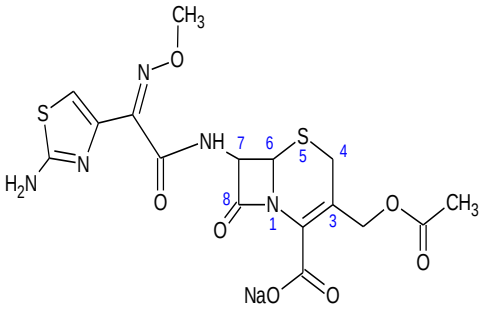

sodium
3-[(acetyloxy)methyl]-7-{[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino) acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

MATERIALS AND METHODS

All the reagents used were of HPLC grade and analytical grade. Reference standard of Cefotaxime Sodium was supplied as gift sample from Sun Pharmaceutical Laboratories Limited, Jammu with purity of 99.987% Injection of three different companies (TAXIMO-500 mg from Alchem Pharma Ltd., BIOTAX-500 mg from Unichem Pharma Ltd. and CLAFORAN-200 mg from M/s MNC Pharma Ltd) were procured from the local pharmacy in the market. A standard stock solution of Cefotaxime Sodium (1 mg/ml) was prepared by dissolving 25 mg of drug powder in 25 ml of acetonitrile[9-12]. Working standard solution (100 µm/ml) was prepared from stock solution by proper dilution with acetonitrile and water mixture.

A Labtronic Model 3201 (LC -2010TH –Liquid Chromatography) equipped with PDA detector. Promesil C_{18} ((250 mm, 4.6 mm, 5µm) column and LC software were used. The mobile phase used was Phosphate buffer : acetonitrile (37:63 v/v) which was filtered through nylon 0.45 µm membrane filter and degassed by ultrasonication for 10 min.

RESULT AND DISCUSSION

Linearity of the method was investigated by serially diluting the working standard to give a concentration range of 1-10 µm/ml and 20 µl from this was injected. The flow rate was maintained at 1 ml/min. temperature of column was kept ambient and the effluent was monitored at 254 nm. Calibration curve was constructed by plotting concentration against peak area. The method was validated for linearity, precision, accuracy, specificity, limit of detection and limit of quantification as per ICH guidelines.

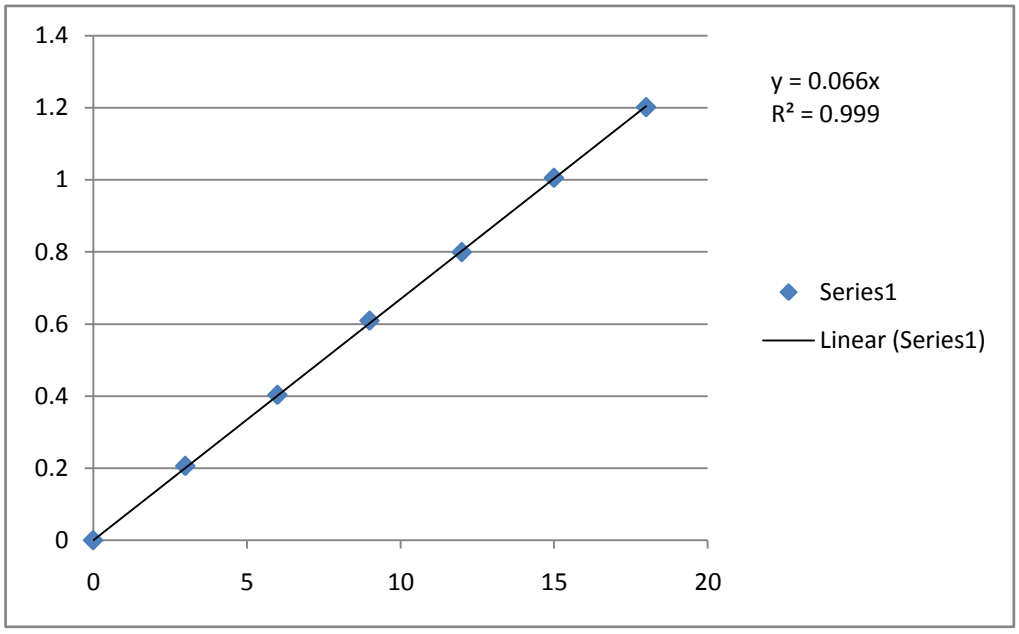

Figure 1: Standard Curve of Cefotaxime Sodium

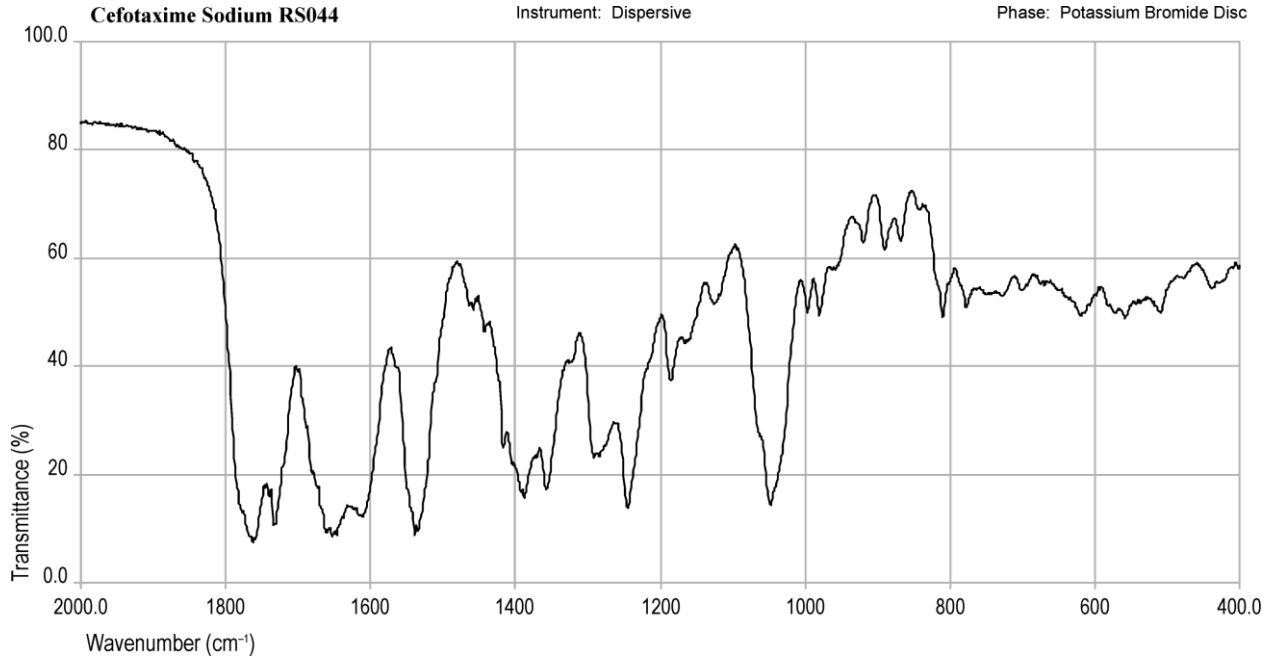

Figure 2: Infra Red Spectrum of Cefotaxime Sodium

Assay of Injection of Cefotaxime Sodium were perfomed. Ten Injection of each company of strength 250 mg, 500 mg and 2 g were weighed and ground to a fine powder. A quantity of powder equivalent to 10 mg of Cefotaxime Sodium was transferred to 10 ml volumetric flask, dissolved and diluted with acetonitrile and water mixture to obtain 1 mg/ml. The solution was sonicated for 15 minute and filtered through 0.45 µm membrane filter. The solution was further diluted to obtain concentration 10 µm/ml. Peak area of the above prepared solutions of Cefotaxime Sodium were measured by using above mentioned chromatographic conditions and the amount of Cefotaxime Sodium were found from regression equation.

To optimize the HPLC parameters, several mobile phase compositions were tried. Various mobile phases having different ratios of methanol, water and acetonitrile were tried. Drug was retained in mobile phase consisting of Phosphate buffer : acetonitrile (40 : 60 v/v) and methanol : acetonitrile (45 : 55 v/v). In methanol : water (60 : 40 v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of Phosphate buffer : acetonitrile (37:63 v/v). Quantification was achieved with PDA detection at 277 nm based on peak area. The retention time of Cefotaxime Sodium obtained was 6.60±0.132 (fig. 3). The system suitability tests for HPLC were carried out on freshly prepared solution of Cefotaxime Sodium (10 µg/ml) and parameters were studied. The results were summarized in Table 1.

Table 1: HPLC instrumentation & chromatographic conditions for Cefotaxime Sodium

Parameters	**Description**
Instrument	A HPLC instrument Labtronic with Model 3201
Column	Hypersil C-18, (250 mm, 4.6 mm, 5µm)
	Different mobile phase used for Trial 1 to 6
Flow Rate	1.0 mL/minute
Detection wavelength	277 nm
Injection Volume	10µL
Run Time	25 minutes

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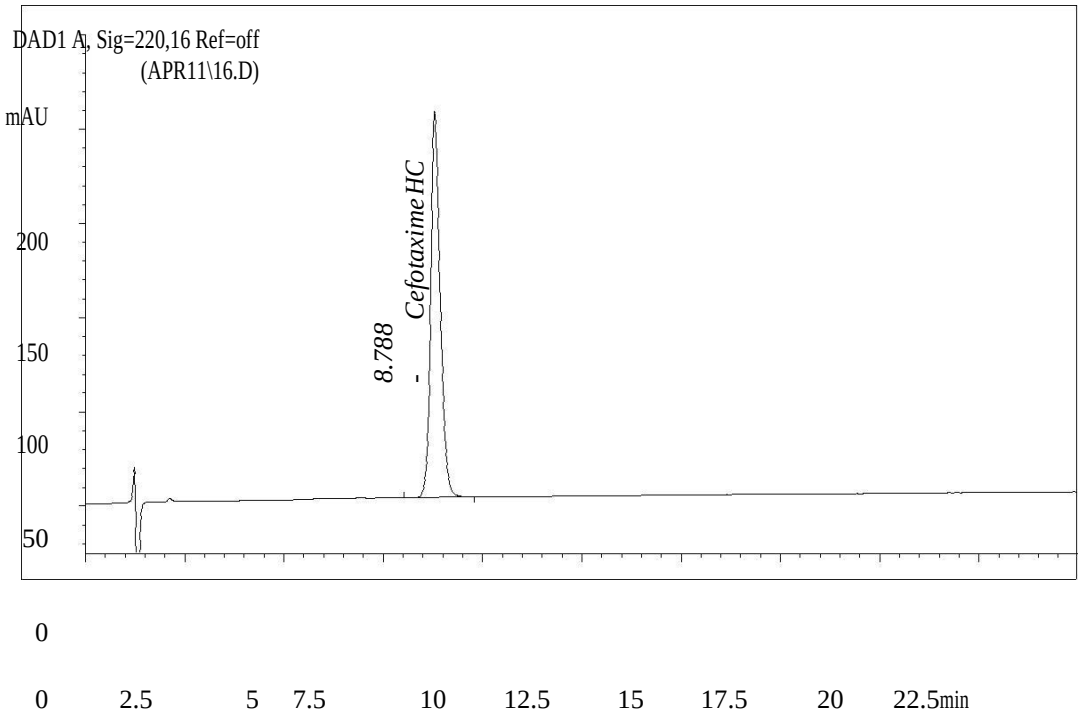

Figure 3: Overlay chromatogram of standard Cefotaxime Sodium (10 µg/ml)

Table 2: System Suitability Test Parameters

Parameters	RP-HPLC method
Retention time, min	8.600±0.186
Tailing factor	1.034±0.328
Asymmetry factor	1.142±0.358
Theoretical plates	6753±0.648
Resolution	2.754±0.329

The linear regression date showed a good linear relationship over the concentration range of 1-10 µg/ml as summarized in Table 3. The limit of detection (LOD) and the limit of quantification(LOQ) of the drug were found by scanning the solution of Cefotaxime Sodium having different lower concentrations and the LOD and LOQ were found to be 0.5 and 1 µg/ml indicates that method is sensitive (Table 3). The intraday and interday precision were determined by analyzing standard solution of Cefotaxime Sodium at three different concentration levels (6, 8, 10 µg/ml). The % RSD for intraday and interday precision was found to be 0.235 – 0.752% and 0.534-1.070% respectively which indicate that method is precise (Table 3). Repeatability of the method was studied by injecting 10 µg/ml solution of Cefotaxime Sodium for six times and peak area was measured and % RSD was calculated which was found to be 0.189 shows repeatability of the method (Table 3). Accuracy of the method was evaluated by standard addition method in which appropriate portion of stock solutions of Cefotaxime Sodium were spiked into blank placebo matrix to produce concentrations of 80 100 and 120% of theoretical concentration. The mean recovery of spiked samples obtained was in range of 99.87 to 100.18 reveals no interference of excipients and shows that method is accurate. The proposed validated method was successfully applied to determine Cefotaxime Sodium in tablet form. The results obtained for tablets of Cefotaxime Sodium were comparable with the corresponding labeled amounts (0.5 mg/tab) (table 3). Robustness of the method was estimated by changing the mobile phase composition (3±3), wavelength ±1 nm, injection volume (20±2µl), column temperature (40 ± 3^0) and RSD values for all these changes calculated were less than 2 indicate that proposed method is robust. The proposed RP-HPLC method was accurate, precise, sensitive and rapid. The method also can be extended for the routine analysis of Cefotaxime Sodium in tablet dosage form.

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Table 3: Regression characteristics and Validation Parameters

Sr. No.	Parameter	Value
1.	λ_{max} (nm)	254
2.	Linearity range	1– 20
3.	Correlation coefficient (r^2)	0.999
4.	Regression equation	Y=0.66X +0.003
5.	Intercept (a)	.0026
6.	Slope (b)	0.9967
7.	Limit of detection (LOD µg/ml)	0.325
8.	Limit of quantification(LOQ µg/ml)	0.956
9.	Accuracy (%)	99.87-100.18
10.	Repeatability (RSD, %, n=6)	0.189
11.	Precision (RSD %), Interday (n=3)	0.534-1.070%
12.	Intraday (n=3)	0.235-0.752

Table 4: Results of analysis of commercial Dosage form of Cefotaxime Sodium

Tablet Formulation	Label claim(mg)	Drug added (mg)	% Label claim estimated*(Mean ± S.D.)	% Coeff. of variation	Standard error
I(TAXIMO)	250	100	99.876 ± 1.446	1.325	0.853
II(BIOTAX)	500	300	99.934 ± 1.278	1.268	0.624
III (CLAFORAN)	2000	1000	100.183 ±0.785	0.683	0.513

*Average of six determinations

CONCLUSION

It is thus concluded that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of this drug in pharmaceutical tablet dosage forms. The proposed method shall prove equally effective to analyze Cefotaxime Sodium in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis.

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