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Development and Validation of Chromatographic method for the determination of Cefoxitin sodium in pharmaceutical dosage forms

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ABSTRACT

A reverse Phase high performance liquid chromatographic method was developed for the determination of Cefoxitin Sodium in Pharmaceutical dosage form. The separation was effected on Zorbax SB Phenyl column (150mm x 4.6 mm, 5.0µ) using a mobile phase mixture of 0.01Msodium dihydrogen orthophosphate and methanol in the ratio of 70:30 v/v at a flow rate of 1.5 ml/min. the detection was made at 254nm. The retention time of Cefoxitin sodium was found to be 8.59 min. Calibration curve was linear over the concentration range of 75 to 225µg/ml. The proposed method was validated as per the ICH guidelines. The method was accurate and precise and found to be suitable for the quantitative analysis of the drug in injection dosage form.

Keywords: cefoxitin sodium, validation, HPLC

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INTRODUCTION

Cefoxitin [Fig. 1] is a cephamycin antibacterial that differs structurally from the cephalosporins by the addition of a 7-α-methoxy group to the 7-β-aminocephalosporanic acid nucleus. It is generally classified with the second-generation cephalosporins and can be used similarly to cefamandole for the treatment of susceptible infections. However, because of its activity against Bacteroides fragilis and other anaerobic bacteria, it is used principally in the treatment and prophylaxis of anaerobic and mixed bacterial infections, especially intra-abdominal and pelvic infections. Indications include endometritis (prophylaxis at caesarean section), pelvic inflammatory disease, and surgical infection (prophylaxis). It may also be used in the treatment of gonorrhoea and urinary-tract infections [1]. Literature survey revealed that only a few methods based on HPLC [2-16], LC/MS [17-20], HPTLC [21] and UPLC-MS/MS [22] were reported for the determination of Cefoxitin sodium from dosage forms and in biological fluids either singly or with its degradation products. The present investigation by the author describes a rapid, accurate and precise RP-HPLC method for the determination of Cefoxitin Sodium from dosage forms. The detector responses were linear in the concentration range of 75-225 µg/ml of the drug. The method was validated as per ICH [23] guidelines.

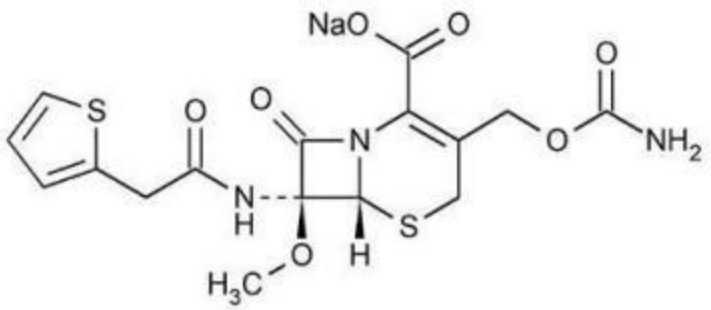

Fig. 1: Molecular structure of Cefoxitin

MATERIALS AND METHODS

Chromatographic condition

A Schimadzu prominence high pressure liquid chromatographic instrument provides with a Zorbax SB Phenyl (150x4.6mm, 5 µ), an LC 20 AD pump and an SPD 20A UV-visible detector was employed in the study. A 20 µL Hamilton injection syringe was used for sample injection. Data acquisition was done by using Spinchrome software.

HPLC grade Methanol and Sodium dihydrogen orthophosphate AR grade were used in the study. Triple distilled water used in the study was prepared in house using Borosil Glass Distillation Unit.

A freshly prepared binary mixture of 0.01M sodium dihydrogen orthophosphate and Methanol in the ratio of 70:30 v/v (pH adjusted to 2.5 with o-phosphoric acid) was used as the

mobile phase and also as diluents for preparing the working solutions of the drug. The mobile phase was filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.5 ml/min. The detection of the drug was carried out at 254 nm.

Drug samples

The reference sample of cefoxitin sodium was supplied by M/s Alkem laboratories, Mumbai. The branded formulation of Cefoxitin sodium (Mefoxin) was purchased from the local market.

Preparation of stock and working standard solutions of Cefoxitin Sodium

Accurately weighed 75 mg of Cefoxitin sodium standard was transferred into a 100 mL volumetric flask containing 30 mL of the diluent. The solution was sonicated for 5 min and then volume was made up with a further quantity of the diluent to get a concentration of 0.75 mg/mL solution. 10 mL of this solution was further diluted to 50 mL with the mobile phase to get a working standard solution of 150 µg/mL of cefoxitin sodium.

Preparation of Sample (injection) solution

Pool the contents of 10 vials. An accurately weighed portion of this powder equivalent to 25 mg of Cefoxitin sodium was transferred to a 50 mL volumetric flask containing 30mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of the diluent. Then this mixture was filtered through whatman No.41filter paper and 10 mL of this filtrate was further diluted to 50 mL with diluent.

Linearity and construction of calibration curve

The quantitative determination of the drug was accomplished by an external standard method. The column was equilibrated with the mobile phase for at least 30 min prior to injection of the drug solution.

Linearity of peak area response was determined by taking measurements at seven concentration points (six replicates at each point). Working dilutions of cefoxitin sodium in the range of 75-225 µg/mL were prepared by taking suitable aliquots of the working standard solution in different 10 ml volumetric flasks and diluting up to the mark with the mobile phase. Ten microlitre quantity of the dilutions was injected each time in to the column at a flow rate of 1.5ml/min. Each dilution was injected six times in to the column. The drug in the eluates was monitored at 254 nm and the corresponding chromatograms were obtained. From these chromatograms the mean peak area were calculated and a plot of concentrations over the peak areas was constructed. The regression of the plot was computed by least square regression

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method. A linear relationship in the range was found to be 75-225 µg/mL of the drug between the concentration of cefoxitin sodium and respective peak area. This regression equation was later used to estimate the amount of cefoxitin sodium in pharmaceutical dosage forms. A representative chromatogram for the separation of Cefoxitin sodium is given in Fig. 2.

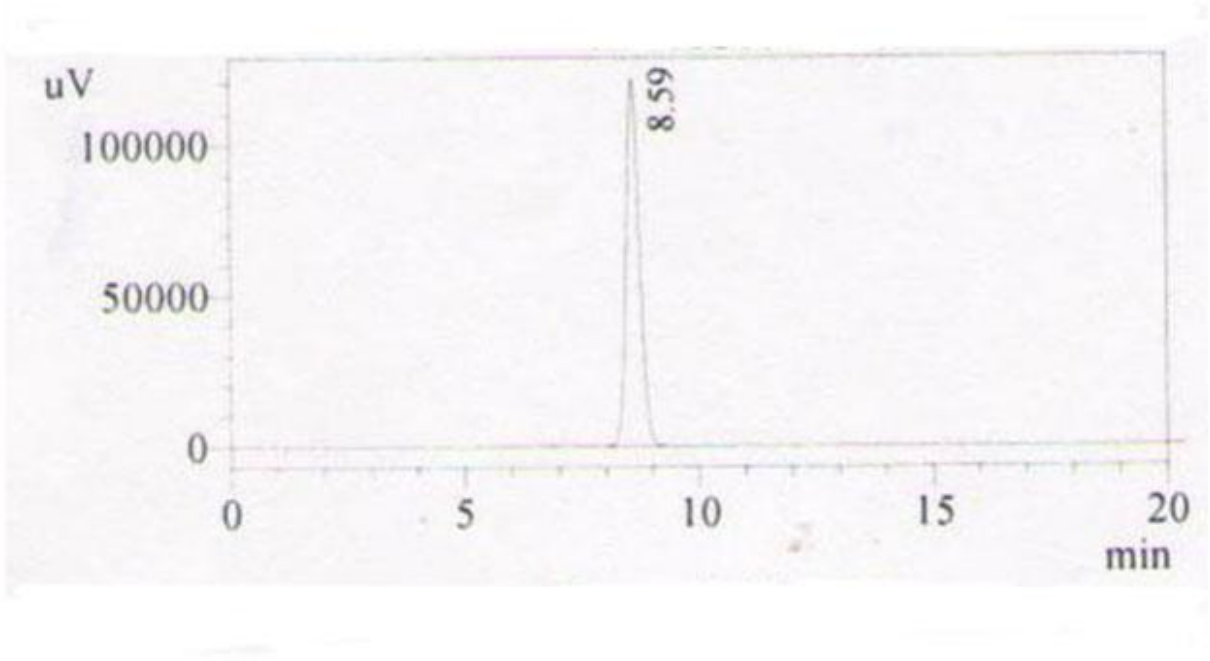

Fig. 2: A typical chromatogram of Cefoxitin sodium standard

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of Cefoxitin Sodium in bulk drug and pharmaceutical dosage forms. In order to achieve optimum separation of the component peaks, a binary mixture of 0.01M Sodium dihydrogen orthophosphate and Methanol (pH- 2.5) in a proportion of 70:30 v/v was selected as the mobile phase. The retention time obtained for cefoxitin sodium was 8.59 min. Each of the samples was injected six times and the same retention times were observed in all cases. The peak areas of Cefoxitin sodium were reproducible as indicated by low coefficient of variation. A good linear relationship (r = 0.9999) was observed between the concentration of Cefoxitin sodium and the respective peak areas. The regression curve was constructed by the linear regression fitting and its mathematical expression was y= 16075x-1334 (where y gives peak area and x is concentration of the drug). The regression characteristics are given in Table 1. When cefoxitin sodium solutions containing 75, 120, 135, 150, 165, 180 and 225 µg/mL were analyzed by the proposed method for finding out intra and inter-day variation, low coefficient of variation was observed in Table 2. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

High recovery values obtained from the different dosage forms by the proposed method indicates the method is accurate. The drug content in injection was quantified using the

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proposed analytical method. The injection vials has showed an average recovery of 100.2 %. The low coefficient of variation indicated the reproducibility of the assay of Cefoxitin sodium in dosage forms. The results are given in Table 3.

Table 1: Calibration data of the proposed method

Level	Concentration of Cefoxitin sodium (µg/mL)	Mean peak area
Level -1	75	1205667
Level -2	120	1929062
Level -3	135	2170196
Level -4	150	2411334
Level -5	165	2652429
Level -6	180	2893611
Level -7	225	3617252
Slope		**16075.46**
Intercept		**-1334.63**
Correlation Coefficient		**0.9999**
Range: 50 to 150 % of target concentration (i.e. 75 to 225 µg/mL)		

Table 2: Precision of the proposed method

Injection number	Area of Cefoxitin sodium	Acceptance criteria
1	2411323	%RSD of peak areas of Cefoxitin sodium should not be more than 2.0
2	2412334	
3	2410296	
4	2411315	
5	2411298	
6	2411362	
%RSD		**0.03**

Table 3: Recovery study

Level	Amount of Cefoxitin sodium spiked (µg)	Amount of Cefoxitin sodium recovered (µg)	% Recovery	%RSD
50%	75	75.18	100.24	0.24
	75	75.44	100.60	
	75	75.50	100.70	
100%	150	149.50	99.67	0.43
	150	150.56	100.41	
	150	150.62	100.41	
150%	225	225.70	100.31	0.26
	225	224.68	99.86	
	225	224.70	99.87	
Mean % recovery				**100.23**
Overall %RSD				**0.93**

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicated the robustness of the method. The

robustness study results are presented in Table 4. The lowest values of LOD and LOQ as obtained by the proposed method indicate the sensitivity of the method. The standard solution of the drug was stable up to 24 hr as the difference in percent assay during the above period is within limit.

Table 4: Robustness study

Condition	Mean area	% assay	% difference
Unaltered	2412332	99.82	-
Flow rate at 1.3 mL/min	2411698	99.05	0.73
Flow rate at 1.7mL/min	2412012	99.02	0.70
Mobile phase:			
(Buffer(72):Methanol(28))	2412253	99.14	0.82
(Buffer(68):Methanol(32))	2412165	98.87	0.55
pH of buffer at 2.3	2411314	99.29	0.97
pH of buffer at 2.7	2411314	99.17	0.85

System suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor, the numbers of theoretical plates are all in the acceptance limits. The system suitability results are shown in Table 5.

Table 5: System suitability

Parameter	Values
Linearity study	75-225 µg/mL
LOD	0.11
LOQ	0.35
Tailing factor	0.90
Theoretical plates	4288

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of cefoxitin sodium in pharmaceutical dosage forms with short analysis time of less than 10 min.

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