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A Validated High performance liquid chromatography (HPLC) Method for the Estimation of Cefuroxime axetil

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ABSTRACT

Cefuroxime axetil (CFA) is the oral prodrug formulation of the injectable antibiotic cefuroxime sodium. It is the second generation cephalosporin with both an intravenous and oral formulation. CFA is the 1-acetoxyethyl ester of cefuroxime. A rapid and reproducible High Performance Liquid Chromatographic method has been developed for the estimation of CFA in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on an ODS C18column (150 x 4.6 mm x 5 µm length), using a mixture of methanol and 0.01M potassium dihydrogen orthophosphate buffer (pH-2.0±0.05) (60:40 v/v) as the mobile phase at a flow rate of 0.8 mL/min and the detection was done at 248 nm was developed and fully validated for the determination of CFA. The retention time of the drug was 3.693 min. The method produced linear responses in the concentration range of 0.45 to 80 µg/mL of CFA. Developed HPLC method was sensitive with LOD= 0.26 μgmL^{-1} and LOQ= 0.58 μgmL^{-1}. The method was successfully validated in accordance to ICH guidelines and was found to be reproducible for analysis of the drug in parental preparations.

Key words: Cefuroxime axetil; HPLC; Validation.

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INTRODUCTION

Cefuroxime axetil (CFA), (1*RS*)-1-[(acetyl)oxy]ethyl (6*R*,7*R*)-3-[(amino carbonyloxy) methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-2-carboxylate, is an orally absorbed pro-drug of cefuroxime that is used in the treatment of common community acquired infections because of its *in vitro* antibacterial activity against several Gram-positive and Gram-negative organisms [1]. It is highly effective against many of the common respiratory pathogens including *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxdla catarrhalis*. It is one of the few oral cephalosporins with some activity against isolates of *S. pneumoniae* that are intermediately resistant to *penicillin* [2]. Cefuroxime axetil is a second-generation cephalosporin that contains the classic β-lactam ring structure. Bactericidal activity *in vivo* is resultant of its binding to essential target proteins, termed the penicillin-binding proteins, which are located in the bacterial cell wall. Inhibition of these proteins leads to bacterial cell wall elongation and leakage, thus the bacteria are unable to divide and mature [3]. Cefuroxime is excreted by glomerular filtration and tubular secretion [4]. Chemical structure of CFA is shown in Fig. 1.

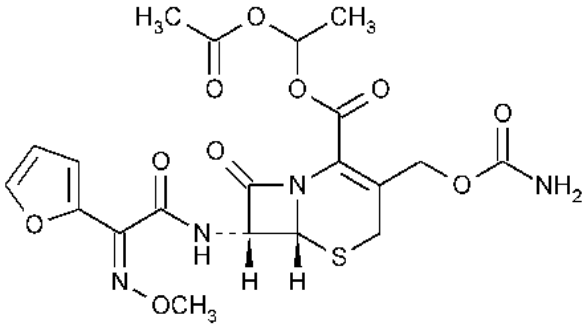

Fig 1: Chemical structure of cefuroxime axetil

In the present study the techniques were used for the determination of CFA are reported to include thin layer chromatography [5], High performance liquid chromatography (HPLC) [6, 7 and 8] infra-red spectrum and UV Spectrophotometry [9]. To develop a rapid, sensitive and validated HPLC method for the estimation of cefuroxime axetil. The method was validated and selectivity, limits of detection and quantification, linearity, precision and assay were determined. Validation process was mainly based on the ICH guideline [12]. To shorten the period of development, the chemical stability of Cefuroxim® tablets was evaluated in accelerated storage conditions at high temperature and high relative humidity (RH) by high-performance liquid chromatographic method. The applicability of this method in determining the drug in commercial dosage forms was also studied.

MATERIALS AND METHODS

Chromatographic conditions

A HPLC system with (LCIDAT VP pumps with gradient mixer assembly, spd –10A VP UV visible Spectrophotometric detector, SIL10ADVP auto injector and SCL-10A vp system controller

[SHIMADZU – VP series]. Data handling system [Class vp software of Shimadzu, version 6.12 or equivalent], Analytical Column : A stainless steel column-18 150mm long , 4.6mm internal diameter filled with octyl silane chemically bonded to porous silica particles of 5μm diameter. Isocratic single pump was employed in the study. HPLC grade methanol and potassium dihydrogen phosphate A.R grade, water for HPLC was prepared from MINI-Q grade were used for preparing the mobile phase. Cefuroxime axetil from Aurobindo labs Hyderabad, India.

Preparation of buffer

Dissolve 4.0g of potassium dihydrogen orthophosphate in 100ml of water. Adjust to pH of the buffer to 2.0±0.05 with orthophosphate acid. Filter through 0.45 μ or fine porosity filter membrane filter.

Standard and Test solutions

Weigh about 5gm of Cefuroxime axetil reference samples into a 2.5ml of clean dry volumetric flask, add 5ml of acetonitrile and sonicate to dissolve. Make up to volume with water. Filter through 0.45μ or finer porosity membrane filter, to the given reaction sample [≅ 0.5ml add 10ml of acetonitrile and sonicate. filtration 0.45μ or finer porosity membrane filter]

Evaluation of suitability

Inject 20μl of system suitability solution before and after deanalysis into the chromatograph and record the chromatograms. The column efficiency as determined from Cefuroxime axetil peak is not less than 4000 theoretical plates and asymmetry for the same peak is not more than 2.0.

Procedure

Separately inject 20μl of diluent sample solution into the chromatograph and record the chromatograms. Examine the diluent chromatograms for any extraneous peaks and disregard. Corresponding peaks observed in the chromatograms of the sample solution, disregard any peak less than 0.05% area in the chromatogram of the sample solution.

Estimation of Cefuroxime axetil in Parenteral Preparations

The commercially available Cefuroxime axetil lyophilized injection of 4mg in 5ml strength of Cefuroxim® was chosen for this purpose. Take all the 5 vials containing Cefuroxime axetil parenteral preparations in each vial 4 mg in 5 ml was reconstituted with water for injection. Transfer them into a 25 ml volumetric flask mix thoroughly. It is diluted with mobile phase to get the concentration of 10μg/mL. High performance liquid chromatography (HPLC), infra red spectrum and UV Spectrophotometry. To develop a rapid, sensitive and validated HPLC method for the estimation of cefuroxime axetil.

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RESULTS AND DISCUSSION

HPLC

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of Cefuroxime axetil in pharmaceutical dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of methanol and 0.01M potassium dihydrogen orthophosphate buffer in different combinations were tested as mobile phase on a C-18 stationary phase. A binary mixture of Methanol and 0.01M potassium dihydrogen orthophosphate buffer in 60:40v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for Cefuroxime axetil acid was 3.693. A model chromatogram is shown in Figure 2. The system suitability parameters including retention time, area, height, area (%) are shown in Table 1.

TABLE 1: System suitability parameters for cefuroxime axetil

Peak	Retention time	Area	Height	Area (%)
1	2.19	3.538	0.653	0.025
2	2.5	4.65	0.59	0.033
3	2.947	1.774	0.316	0.013
4	3.693	14128.255	992.519	99.774
5	4.097	22.044	3.843	0.156

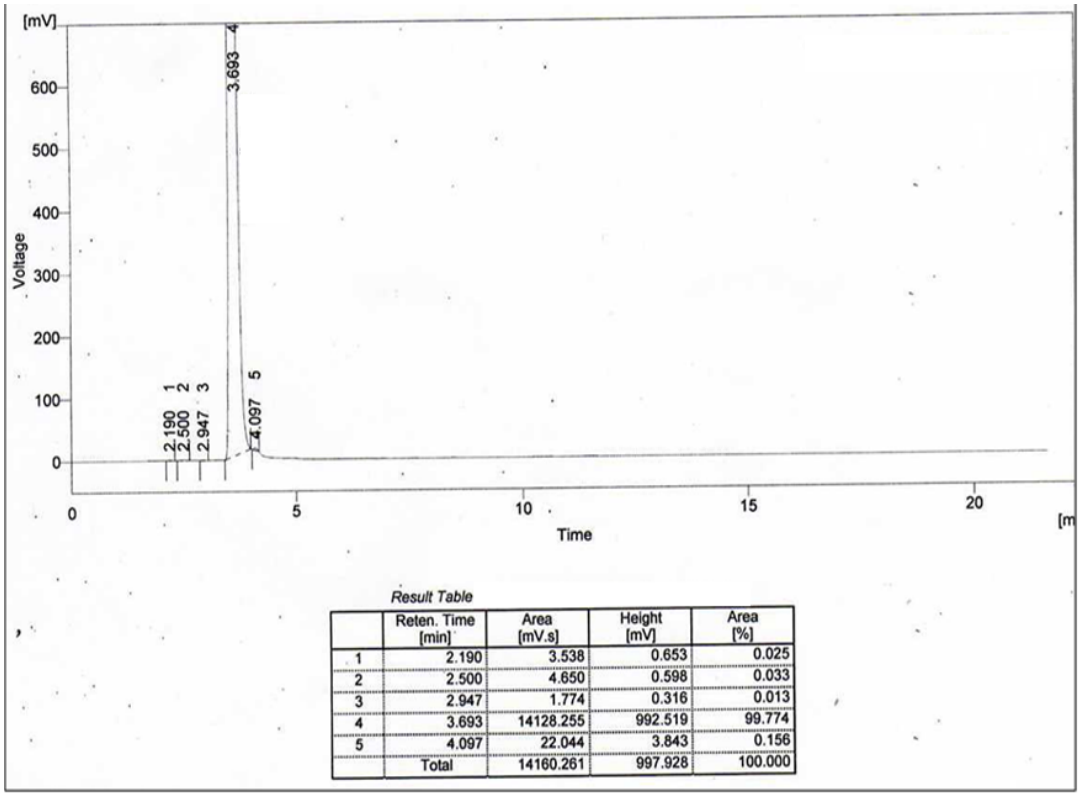

Fig 2: Chromatogram of Cefuroxime axetil

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VALIDATION OF THE METHOD

The method was validated with respect to linearity, accuracy and precision for the Cefuroxime axetil, as summarized here.

Linearity

The linearity was evaluated and established by triplicate injection of the working standard solutions of Cefuroxime axetil. The peak areas were measured and the ratios the peak areas of cefuroxime axetil to that of the internal standard were calculated for each injection. The linear regression was computed by the least square method using Microsoft Excel program to determine the slopes and correlation coefficients for the calibration graphs between the peak area ratios of compounds to internal standard were plotted against the compound concentration to generate calibration curves. Reported statistical data showed that calibration curves generated linear results over the investigated concentration range: for Cefuroxime axetil from 0.100 to 1.000 mg ml−1 ($y = 28.49x + 0.509$; $R^2 = 0.986$), where y is peak area ratio, x is concentration of the compound and R is the correlation coefficient.

Accuracy and precision

When Cefuroxime axetil solutions containing 4 to 10 µg /mL was analyzed by the Cefuroxime axetil for finding out intra and inter-day variations as low coefficient of variation was observed as shown in Table 2. This shows that the present HPLC method is highly precise. The amounts of Cefuroxime axetil obtained from the pre-analyzed samples containing known amounts of added drug are shown in Table 3. About 99.66% of Cefuroxime axetil could be recovered from the pre-analyzed samples indicating the high accuracy of the Cefuroxime axetil. Limits of detection (LOD) were estimated by analyzing serial dilution of working standards of the compounds, corresponding to the concentration at the lower end of each of the calibration curves (assayed in triplicate). Limits of quantification (LOQ) were determined experimentally, by injecting the decreasing concentrations of investigated calibration solutions to obtain the lowest reproducible measurement of peak areas. Reported values are shown in Table 4**.**

TABLE 2: Precision for cefuroxime axetil

Concentration of Cefuroxime axetil (µg/mL)	Observed concentration of Cefuroxime axetil (µg /mL)			
	Intra-day		Inter-day	
	Mean(n=6)	RSD%	mean(n=6)	RSD%
5	4.88 ± 0.10	1.89	4.950±0.046	1.929
10	5.97 ± 0.07	1.68	5.954±0.072	1.723
15	7.39 ± 0.19	2.99	8.940±0.082	3.549

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TABLE 3: Accuracy for Cefuroxime axetil

Labeled amount (mg)	Amount of standard drug added to pre analyzed formulation (mg)	Amount of drug recovered (mg) mean± S.D (n=6)	% recovery mean ± S.D
4	4	5.89±0.78	99.69±0.098
4	8	8.92±0.85	99.72±0.128
4	12	12.82±0.29	99.58±0.027

TABLE 4: Limits of detection (LOD) and quantification (LOQ) of Cefuroxime axetil

LOD & LOQ	Cefuroxime axetil (µg /mL)
Limit of detection (LOD)	0.26
Limit of quantification (LOQ)	0.58

CONCLUSION

The HPLC method developed for quantitative determination of Cefuroxime axetil is precise, accurate, and selective. The method was completely validated and satisfactory results were obtained for all the method validation data tested.

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