

**BIOANALYTICAL METHOD DEVELOPMENT AND ESTIMATION OF
CEFOTAXIME USING RP-HPLC****Ankush Pushptode* Ankit Diwan, Kuldeep Ganju****Sagar Institute of Pharmacy and Technology, Bhopal (M.P), India***Corresponding Author's E mail: anku1193@gmail.com

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ABSTRACT

A Bioanalytical RP-HPLC assay method has been developed and validated for cefotaxime. An isocratic RP-HPLC method was developed with C18 column (250 mm ×4.6 mm i.d., 5 µm) utilizing a mobile phase of 20mM KH₂P₄ and acetonitrile (80:20 v/v) with UV detection at wavelength 260 nm at the flow rate 1.0ml/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 5-25µg/ml. Limit of detection and Limit of quantification was found to be 0.57µg/ml and 1.54µg/ml respectively. The % recovery ranged within 99.466±0.313 - 99.825±0.269%. 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 suitable for the quality control of cefotaxime in injection formulations.

Keywords: Cefotaxime, Bioanalytical, Method development, Validation.

INTRODUCTION

Bioanalysis is a sub-discipline of analytical chemistry covering the quantitative measurement of xenobiotics (drugs and their metabolites, and biological molecules in unnatural locations or concentrations) and biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems¹. 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 C₁₆H₁₆N₅NaO₇S₂. It is a 1st generation cephalosporin, inhibits cell wall biosynthesis. Literature survey revealed that ESR²⁻³, various spectrophotometric and HPLC methods are available for estimation of cefotaxime sodium⁴⁻¹¹. The pharmaceutical dosage forms are widely present with multiple active components i.e. in combined dosage forms. This has opened new task for analyst for simultaneous estimation of different drugs in such combined dosage forms.

Therefore, in proposed work an attempt to develop simple, accurate and precise method for analysis of Cefotaxime in the plasma and validate them.

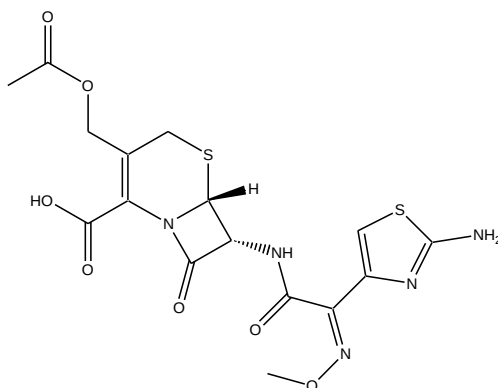


Figure 1: Structure of cefotaxime

MATERIALS AND METHODS:

Method development using RP-HPLC

Selection of precipitating agent

Selection of precipitation agent is based on the solubility of drug and good protein precipitation property. Cefotaxime soluble in acetonitrile and having good precipitating property so the acetonitrile was selected as the protein precipitation and extraction agent.

Selection of Mobile Phase

Initially to estimate Cefotaxime number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20mM KH_2PO_4 : Acetonitrile (pH 4.5) in the ratio of 20:80 v/v. The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Extraction of drug sample Accurately weighed 10 mg of Cefotaxime was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of plasma, then volume was made up to 50 ml with acetonitrile and vortex it to get complete precipitation of plasma protein. Stand it aside for few minute, precipitate of protein settled down then collect the supernatant layer and add 10 ml of acetonitrile in precipitate to complete removal of drug. Centrifuge the collected supernatant layer at 6000 rpm for 7 min at 4°C and then filtered by whatmann filter paper (no.41). Concentration of Cefotaxime in acetonitrile was 200 $\mu\text{g/ml}$. (stock- A)

Preparation of Sub Stock Solution 5 ml of solution was taken from stock-A of Cefotaxime and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml (Stock-B).

Preparation of Different Solution

0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 260 nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of Developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration was recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared and the precision are established in three differences:

1. Repeatability
2. Intermediate precision
 - a) Day to Day
 - b) Analyst to Analyst
3. Reproducibility

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for Cefotaxime indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day to Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations.

Analyst- To- Analyst Precision

Analyst to analyst variation was performed by different analyst in five replicate at five concentrations.

Reproducibility

The reproducibility was performed by chemical to chemical (use of rankem chemicals in place of merck chemicals) variation in five replicate at five concentrations.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Buffer: Acetonitrile (20:80 % v/v), to (15:85 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of Tablet Sample

Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of Cefotaxime was taken in 100-ml volumetric flask. This was than dissolve in 13 ml of plasma by sonication for about 10 minutes. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

RESULTS AND DISCUSSION

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of Cefotaxime were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table 1.

Table 1: Results of system suitability parameters

Parameters	Cefotaxime
No. of Theoretical Plates	3745.500±78.582
Tailing Factor	1.182±0.039
Retention time	7.924±0.001

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 2.

Table 2: Results of linearity of Cefotaxime

Parameter	Cefotaxime
Concentration(µg/ml)	5-25
Correlation Coefficient (r^2)*	0.99
Slope (m)*	98.09
Intercept (c)*	5.474

*value of five replicate

Specificity

Specificity of the method was determined and the peaks of plasma, diluent, mobile phase and excipient of tablets did not interfere with Cefotaxime peaks Figure 2 & 3.

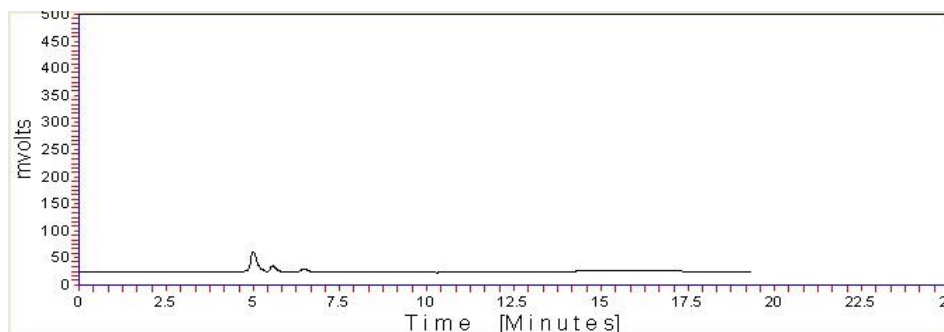


Figure 6.4: Chromatogram of blank with plasma

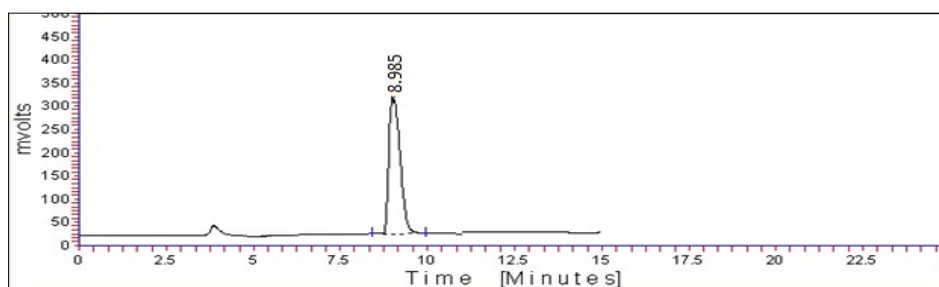


Figure 6.5: Chromatogram of Cefotaxime

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 3.

Table 3: Results of recovery study of Cefotaxime

% Level	% Mean $\pm$ SD*	% RSD*
80%	99.466 $\pm$ 0.313	0.315
100%	99.471 $\pm$ 0.385	0.387
120%	99.825 $\pm$ 0.269	0.269

* Value of three replicate and three concentrations.

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and % RSD are less than 2 indicate the precision of method. Result of precision shown in table 4. The robustness of developed method was checked by changing in the deliberate variation in solvent. Result of robustness shown in table 4.

Table 4: Results of Precision

Parameter	% Mean $\pm$ SD*	% RSD*
Repeatability	99.276 $\pm$ 0.096	0.097
Intermediate precision		
Day to day precision	99.091 $\pm$ 0.127	0.128
Analyst to Analyst	99.219 $\pm$ 0.105	0.106
Reproducibility	98.608 $\pm$ 0.109	0.111
Robustness	98.892 $\pm$ 0.121	0.122

* Value of five replicate and five concentrations

LOD and LOQ

Detection limit and Quantitation limit of described method were observed as 0.570 µg/ml and 1.54 µg/ml, based on the SD of response and slope, which meet the requirement of new method.

Assay of tablet formulation

The results of the analysis of tablet formulation were reported. The assay value of Cefotaxime was close to 100, SD and %RSD are less than 2 indicate the no interference of excipient in the estimation of Cefotaxime table 5.

Table 5: Result of tablet formulation

Mean	I	II
S.D.	98.85	99.12
% RSD	1.012	0.351

CONCLUSION

The result obtained shows the developed methods to be New (no method available for combination this drugs), Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

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