



DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC-METHOD FOR ESTIMATION OF CEFOTAXIME SODIUM IN INJECTION

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Abstract

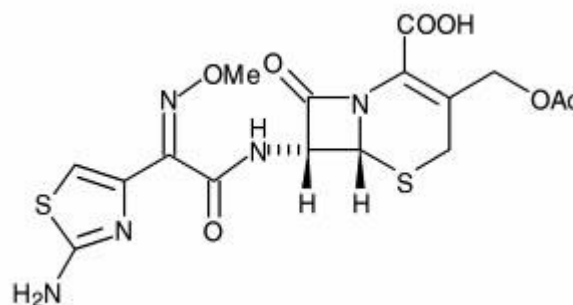
A simple, stability-indicating, reversed-phase liquid chromatographic method was developed for the determination of Cefotaxime sodium in the presence of its degradation products. The analysis was carried out by using ODS (150 x 4.6mm) 5 μ m column. Mobile phase used was a mixture of buffer, methanol and acetonitrile in the ratio of 80:15:05, at a flow rate of 1.3 mL per minute and injection volume of 20 μ L with UV-detection at 254 nm with a run time of 10 minutes. The stability indicating capability of the method was proven by subjecting the drugs to stress conditions such as alkaline and acid hydrolysis, oxidation, photolysis, thermal degradation and resolution of the degradation products formed therein. This method showed good linearity in the range of 51.348 to 359.438 μ g/mL. The suggested method was successfully applied for the analysis of Cefotaxime sodium from injection formulation and found the average assay data of 98.86% with a relative standard deviation of 0.48% for 6 samples. The Cefotaxime sodium was found to be stable in solution up to 14 hours at room temperature. The method validation data showed excellent results for precision, linearity and specificity. The present method can be successfully used for routine quality control and stability studies.

Key words: Cefotaxime sodium, HPLC-Method, Stability Indicating.

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) with molecular formula C₁₆H₁₆N₅NaO₇S₂¹. Cefotaxime is a third-generation parenteral cephalosporin antibacterial used in the treatment of infections due to susceptible Gram-positive and Gram-negative bacteria, including infections of the abdomen, bones and joints, CNS, skin and skin structure, genito-urinary tract (including gonorrhoea) and respiratory tract, in gynaecological infections, and in early Lyme disease^{2, 3}. Cefotaxime has a pKa of 3.4. A 10% solution in water has a pH of between 4.5 and 6.5. Cefotaxime Sodium is freely soluble in water and slightly soluble in alcohol⁴.

This antibiotic displays a high antimicrobial potency, a broad antibacterial spectrum, high resistance against the action of beta-lactamases, as well as a low index of side effects and lowered toxicity profile compared to the predecessors⁵.



Structure of Cefotaxime

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Literature survey revealed that several HPLC⁶⁻¹⁴ HPTLC^{15,16} and spectrophotometric methods¹⁷⁻²² have been used for determination of Cefotaxime Sodium. Spectrophotometric methods are absolutely non-stability indicating and the published HPLC methods did not talk about the stability indicating nature and no common method is seen for the estimation of potency

and impurities in a single method. Since Cefotaxime is unstable in solution, its estimation method should be stability indicating in the assay method in order to estimate the actual Cefotaxime content. In the present study rapid, precise, accurate, simple and stability indicating method was developed for the estimation of Cefotaxime Sodium from injections and also verified the separation of its impurities in the same method and found that all the impurities are eluted within 10 minutes of HPLC run time.

Materials and Methods

Instrumentation

The high pressure liquid chromatographic (HPLC) system was used bearing the configuration by equipped with pump (Waters 2695 separators module), auto sampler, thermo stated column compartment and detector (Waters 2487 dual wavelength absorbance detector) controlled by Empower software. A column Symmetry ODS, 150mm X 4.6mm, 5 μ , Make: Waters, was used as a stationary phase.

Materials

Working standard and impurities of Cefotaxime sodium were received as a gift sample from Kreszent Pharma. Disodium hydrogen orthophosphate anhydrous, Potassium dihydrogen orthophosphate anhydrous and Orthophosphoric acid used were of analytical grade and methanol and acetonitrile were procured from commercial source and HPLC grade water was obtained using Millipore water purification system. All other chemicals were of analytical reagent grade unless specified.

Methods

Chromatographic conditions:

The analysis was carried out on binary HPLC system using the column Symmetry ODS, 150mm X 4.6mm, 5 μ m with UV- detection of 254 nm at 25°C. The injection volume was 20 μ l with a flow rate of 1.3 ml per minute and a run time of 10 minutes.

Mobile phase and solutions

Prepared a mixture of buffer (2.8 gm of disodium hydrogen orthophosphate anhydrous + 1.4gm potassium dihydrogen orthophosphate anhydrous in 1000 ml of water), methanol and acetonitrile in the ratio of 80:15:05. Adjusted the pH between 5.9 to 6.1 with diluted ortho phosphoric acid. Degassed and filtered through 0.45 μ membrane filter

Standard & Resolution solution

Preparation of standard solution

Weighed and transferred accurately 26.3mg of Cefotaxime sodium working standard into 100 ml volumetric flask; dissolved and diluted up to the volume with mobile phase and mixed well.

Preparation of resolution solution

Weighed and transferred accurately 1.2mg of each Cefotaxime sodium working standard and impurity-B into 10 ml of volumetric flask; dissolved the material in 1 ml of 1N hydrochloric acid (diluted 8.5 ml of concentrated hydrochloric acid to 100 ml with HPLC grade water) and 2 ml of acetonitrile. Diluted the solution to the volume with mobile phase and mixed well.

Calibration

Seven different concentrations (51.348-359.43 μ g/ml) of Cefotaxime sodium solutions were prepared for linearity studies. The responses were measured as peak areas and plotted against concentration.

Estimation of Cefotaxime sodium from injectable formulation

Accurately weighed and transferred about 24.9 mg of Cefotaxime sodium for injection in to 100 mL volumetric flask, shaken well to dissolve and diluted to volume with mobile phase.

Forced degradation sample solutions – Forced degradation samples are neutralized after treatment of the sample in acidic and basic conditions.

Stressed sample solutions are further diluted with mobile phase to get a final concentration of 0.25mg/ml.

Validation Parameters

Various method validation parameters ascertained are as follows:

Precision

Injection repeatability (System precision):

The injection repeatability was established by six replicate injections of the standard solution containing the analyte of interest.

Sample repeatability (Method precision):

The sample repeatability was established by carrying out the analysis of the analytes six times.

Specificity /selectivity

Specificity of the method was evaluated by injecting the blank, impurities spiked in sample and the sample solution to check the interference if any at the retention time of Cefotaxime peak.

Stress testing (Forced degradation)

The Cefotaxime for injection sample was subjected to forced degradation under the following stress conditions.

i) Hydrolytic and Oxidative degradation

The Cefotaxime for injection sample was separately treated with 5ml of 0.5 N Hydrochloric acid, 5ml of 1N Sodium Hydroxide and 5ml of 10%v/v solution of hydrogen peroxide for 2 minutes.

ii) Thermal Degradation

The sample was subjected to thermal degradation by keeping the sample at 80°C for 18 hours and analyzed as per the method.

iii) Photolytic Degradation

The sample was exposed to sun light for about 24 hours and analyzed as per the developed method.

The spectral purity of the main peaks were evaluated using photodiode array detector and Empower software used to verify that the degradation peaks are well resolved from the main peaks.

Stability of the solution

A sample solution of Cefotaxime for Injection was prepared as per the test method, analyzed initially and also analyzed at different time intervals by keeping the sample solution at room temperature.

System suitability tests

The resolution solution was prepared and injected as per the test method during the course of validation study. The resolution between Cefotaxime and Impurity-B peaks was calculated using the chromatographic software. The standard solution was prepared and injected as per the test method. The tailing factor, theoretical plates of Cefotaxime peak and the percentage relative standard deviation of five replicate injections of standard preparation were calculated.

Results and Discussion

Precision

The values of the relative standard deviation of six replicate injections of the standard solution containing were lie well within the limits ($\% \text{RSD} \leq 2$), indicating the injection repeatability of the method. Specimen chromatogram of standard is given in figure 1.

Specificity /selectivity

Specificity of the method was evaluated by injecting the blank to check the interference if any at the retention time of Cefotaxime peak from the blank solution. There was no peak eluted at the retention time Cefotaxime peak from the blank solution shows the method is specific. Also five impurities (namely impurity A, B, C, D and E) of Cefotaxime spiked in the sample were eluted separately and not interfered with the principle peak. Spiked impurity chromatogram and UV spectrum of Cefotaxime are given in figure 2 and 3 respectively.

Stress testing (Forced degradation)

The results of forced degradation studies conducted on Cefotaxime for injection sample are summarized in Table 1. At every stage of forced degradation study peak purity of the Cefotaxime peak was checked using photodiode array detector (PDA). The peak purity data was found passed and it revealed that there are no co eluting peaks at the retention time of Cefotaxime peak and it is homogeneous. This illustrates the test method to separate degradation products from Cefotaxime peak.

Linearity

Calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a

correlation coefficient of 0.999 over the calibration ranges tested. The linearity of response for Cefotaxime was determined in the range of 51.348 to 359.438 mcg/ml. Linearity graph is given in figure 4.

Table 1 : Forced Degradation

Mode of Degradation	% Assay	% Degradation w.r.t. untreated sample	Peak Purity
Untreated sample	98.86	-	Passes (No flag)
Acid	81.54	17.32	Passes (No flag)
Alkali	75.67	23.19	Passes (No flag)
Hydrogen Peroxide	80.25	18.61	Passes (No flag)
Thermal	97.07	1.79	Passes (No flag)
Photolytic	98.14	0.72	Passes (No flag)

Accuracy

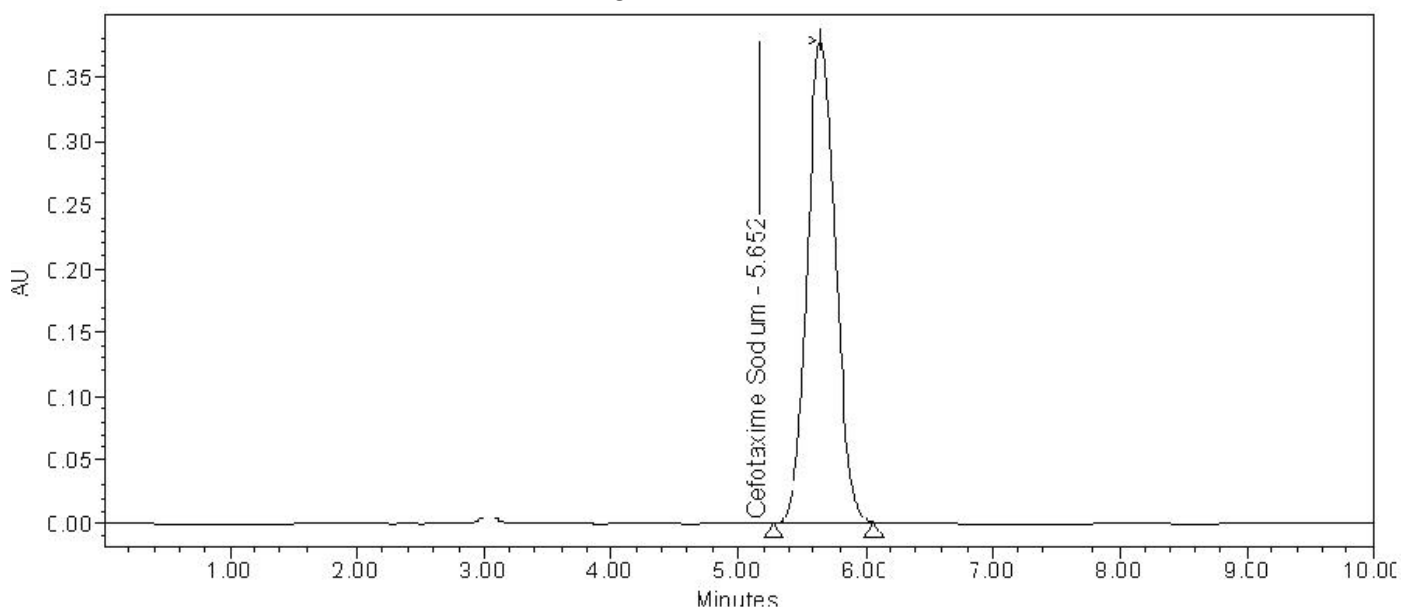
Accuracy of the method is inferred from the data of specificity, linearity and precision data

System suitability and stability of analytical solution

The tailing of Cefotaxime sodium was found in the range of 1.4 to 1.6. The % RSD for five replicate injection of standard was found in the range of 0.29 – 0.65. Resolution between the peaks was found in the range of 14.1 to 15.1.

A sample solution of Cefotaxime for injection was analysed at different time intervals by keeping the sample solution at room temperature. The cumulative % RSD of area of Cefotaxime was found to be 1.36 indicates that solution is stable up to at least 14 hours.

Figure 1.
Chromatogram of Pure Cefotaxime



Conclusion

The analytical method is simple, specific and precise. The method is stability indicating and all the five potential impurities are eluted in the short HPLC run time of 10 minutes, where no published method is

available for the estimation of potency and impurities in a common method and in a very short time. Hence it can be used for routine quality control analysis as well as stability studies of injections, bulk and also for other dosage forms like solids (Tablet/Capsule).

Figure 2.
Chromatogram of test solution spiked with impurities

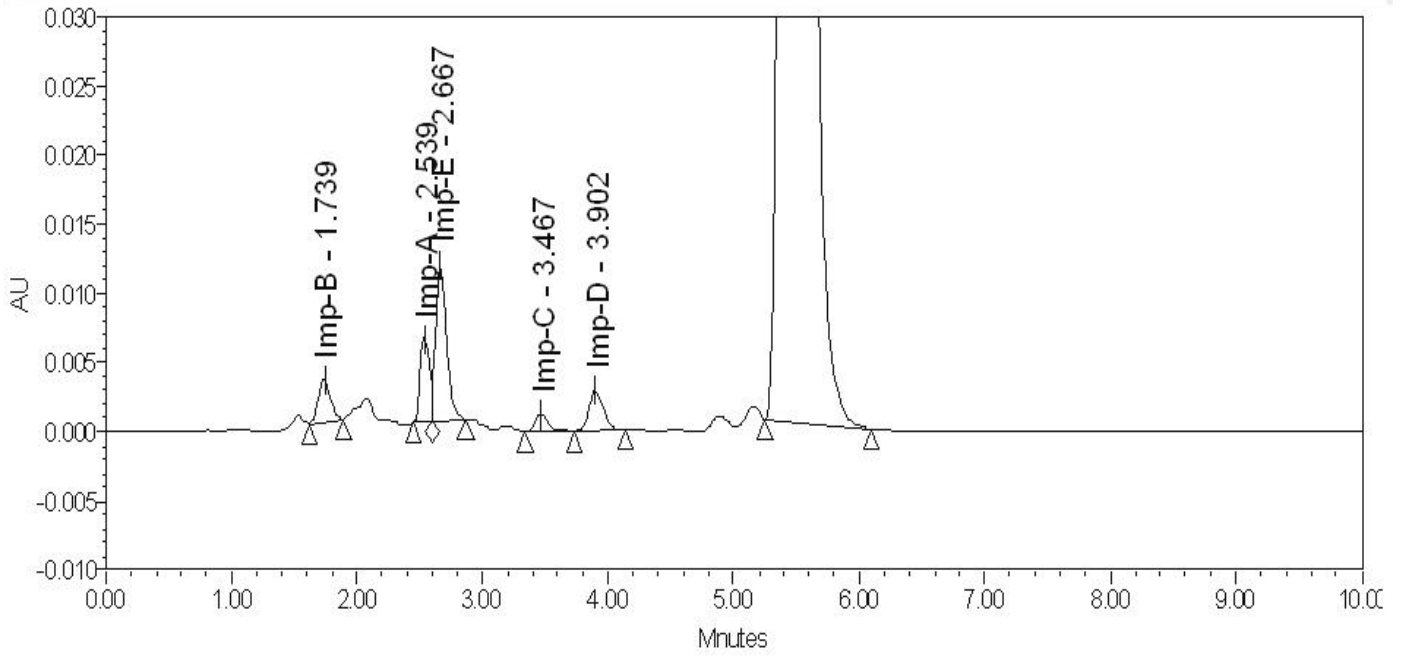


Figure 3.
UV spectrum of Cefotaxime

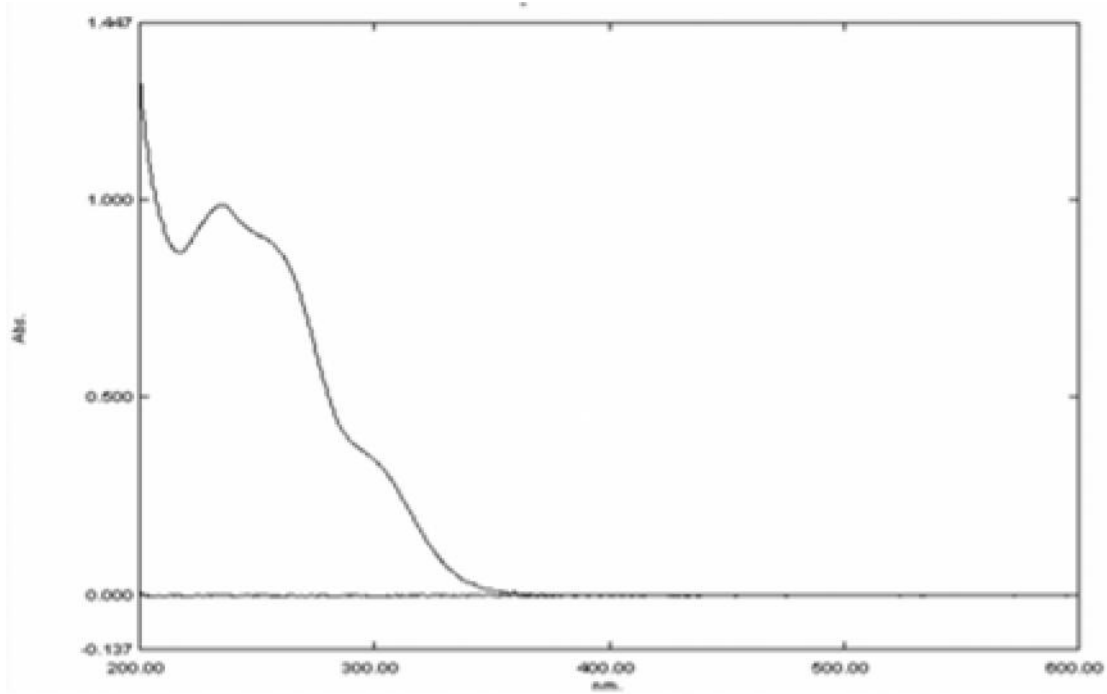
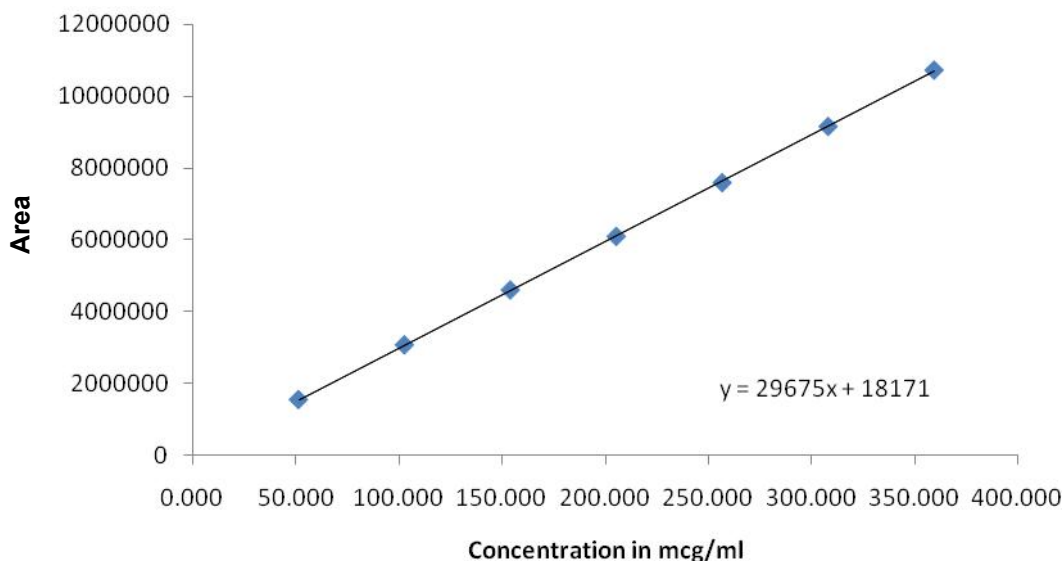


Figure 4.
Linearity Graph



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