

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF CEFTRIAXONE IN STERILE POWDER FOR INJECTION WITHOUT USING ION PAIRING REAGENT

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ABSTRACT

Objective - The objective of the work was to develop a rapid, accurate, precise and specific stability-indicating HPLC method for the determination of Ceftriaxone in sterile powder for injection without using ion pairing reagent.

Methods - The chromatographic separation was achieved on an Acclaim 120 C₁₈(100x4.6)mm, 5µm, stainless steel column with detection wavelength of 240nm using an isocratic mobile phase mixture of phosphate buffer(pH 7), acetonitrile and triethylamine (90:10:0.2 v/v/v) at a flow rate of 1.0 mL/min. The performance of the method was validated according to the present ICH guidelines for specificity, linearity, accuracy, precision and robustness. The specificity of the method was ascertained by acidic, basic, oxidative and photolytic stress study test.

Results - The retention time of Ceftriaxone under these conditions were 3.0 minutes with chromatographic parameters better than the minimum requirements of United States Pharmacopoeia. The calibration curve for the drug was linear in the range of 0.5-50 µg/mL with a R² value of 0.9995 and regression equation $y = 25.235x + 3.1698$. The relative standard deviation for the intra-day and inter-day precision studies was 0.58 and 0.83, respectively and the average recovery of the sample was 100.5%. There was no interference due to peaks obtained for degraded products with the Ceftriaxone peak which ensures the specificity of the method.

Conclusion - The proposed method was successfully employed for quantification of Ceftriaxone in sterile powder for injection dosage form. The method can be applied for the routine quality control analysis of the Ceftriaxone in sterile powder for injection.

Keywords: Ceftriaxone, Sterile powder for injection, ICH guideline, Validation, sTability indicating method, HPLC, Ion pairing reagent.

INTRODUCTION

Ceftriaxone(CTX) is a third-generation cephalosporin with a broad-spectrum of bactericidal activity in vivo and in vitro against aerobic gram-negative and gram-positive micro-organisms, including penicillin resistant pneumococci, and some anaerobic bacteria. It also has high stability against degradation by β-lactamases[1,2]. The bactericidal activity of CTX results from the inhibition of cell wall synthesis and is mediated through its binding to penicillin binding proteins. It inhibits the mucopeptide synthesis in the bacterial cell wall. The beta lactam moiety of CTX binds to carboxypeptidase, endopeptidase, transpeptidase enzymes present in the bacterial cytoplasmic membrane which are involved in cell wall synthesis and cell division. By binding with these enzymes, CTX results in the formation of defective cell walls and cell death[3].It is one of the proposed antibiotic regimens for the treatment of leptospirosis[4]. CTX sodium is chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2methoxyiminoacetyl amido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]-3-cephem-4-carboxylic acid, disodium salt[5,6]. It differs from other third-generation cephalosporins because of its comparatively long half-life of 8-10 hrs which allows once daily administration[7]. This unusual property may be attributed to the presence of highly acidic, heterocyclic system on the 3-thiamethyl group. The chemical structure of CTX is shown in Figure 1. CTX is listed in the United

States Pharmacopoeia (USP)[8], British Pharmacopoeia[9] and Indian Pharmacopoeia[10].

There are various analytical methods available for the analysis of CTX including spectrophotometry[11,12,13,14], liquid chromatography[7,15,16,17], differential-pulse adsorptive stripping voltammetry[18] and TLC[19,20], either alone or in combination with other drugs. An exhaustive review of the analytical methods available for the analysis of CTX has also been referred[21]. The HPLC methods[7,15] used ion pairing reagents which are having the disadvantage of large volumes of mobile phase consumption which may be required to equilibrate the reverse-phase column, tendency to produce tailing peaks, particularly as the column ages and damage to the silica based packing-material of the analytical column by the quaternary ammonium salts used as ion pairing reagent. The other HPLC method[16] has not established the chromatographic parameters properly and the method also uses a large amount of acetonitrile in the mobile phase which is a disadvantage considering its cost and the negative environmental effects. In this article the authors describe a simple, accurate, precise and sensitive HPLC method with properly established chromatographic parameters, which also overcomes the drawbacks of ion pairing reagents, for the estimation of CTX in sterile powder for injection. The parameters developed in this method were also compared with the United States Pharmacopoeia(USP) requirements.

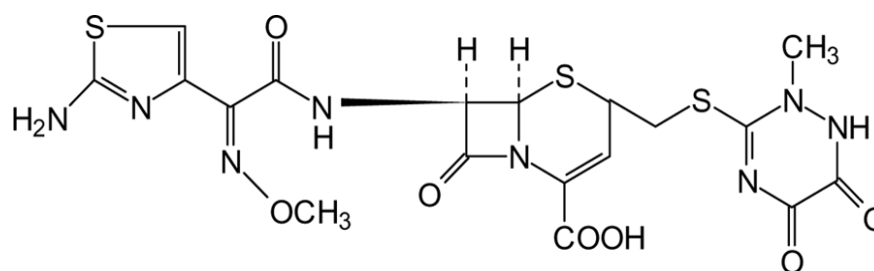


Fig. 1: Chemical Structure of Ceftriaxone

MATERIALS AND METHODS

Chemicals and Reagents

CTX sodium was obtained as gift sample from Alkem Laboratories, Sikkim, India. Acetonitrile and potassium phosphate used were of HPLC grade and purchased from S.D Fine Chem Ltd., Mumbai, India. Triethylamine used was of AR grade and purchased from S.D Fine Chem Ltd., Mumbai, India. Commercially available CTX sterile powder for injection vials Traxol (Cachet Pharmaceuticals, India) and Keftra (IPCA laboratories, India) were procured from local market.

Instrumentation

Shimadzu (Japan) LC-20 AT liquid chromatography equipped with a 20 μ L loop, in isocratic mode with Prominence SPD-20A UV-Visible detector was used for quantitative HPLC determination. The HPLC system was equipped with Spinchrom (Shimadzu) software for data collection and processing. Sartorius (CP225D) electronic balance was used for weighing the materials.

Chromatographic Conditions

The chromatographic separation was performed on Dionex Acclaim 120 C₁₈ (100x4.6 i.d)mm, 5 μ m, stainless steel column. The mobile phase consisting of a mixture of acetonitrile, potassium phosphate buffer and triethylamine in the ratio of 10:90:0.2, (pH 7.0) was delivered at a flow rate of 1.0 mL/min. The mobile phase was filtered through 0.45 μ m membrane filter and degassed by sonication prior to use. Separation was performed at ambient temperature and detection was made at 240nm.

Preparation of Standard Solution

A stock solution of CTX containing 1.0 mg/mL was prepared by dissolving 25mg into a 25mL volumetric flask, added 10mL mobile phase, shaken for 5 minutes, sonicated for 10 minutes and diluted to volume with the mobile phase. Working standard solution of 10 μ g/mL was prepared by suitably diluting the stock solution with the mobile phase.

Preparation of Sample Solution

An appropriate weight of the sample containing 25mg CTX was transferred into a 25mL volumetric flask, added 10mL mobile phase, shaken for 5 minutes, sonicated for 10 minutes and diluted to volume with the mobile phase to furnish a solution containing 1.0 mg/mL. The solution was filtered and the filtrate was suitably diluted with the mobile phase to give a final concentration of 10 μ g/mL of CTX.

RESULTS AND DISCUSSION

Method Development

Conditions were developed for a simple, accurate, precise and sensitive isocratic reverse phase HPLC method for the determination of CTX in sterile powder for injection.

Taking into consideration the instability of CTX sodium in strong alkaline and acidic conditions, the pH value of the mobile phase should be limited within the range of 5 to 8. CTX is highly polar in nature so method development started with 85 volume ammonium acetate buffer (0.05M) and 15 volume acetonitrile but peaks were showing tailing and it was eluting too early to have a good retention factor. Triethylamine was added in the mobile phase to reduce the tailing but it increased the pH of the mobile phase to 9.0 and it was found that when pH was adjusted to lower value the tailing also increased. So the buffer was changed to potassium phosphate and it was observed that in the phosphate buffer the tailing value was reduced to acceptable level. The reason behind this may be the strong cationic action of potassium which neutralizes the negatively charged portions of CTX and improves its retention property. Acetonitrile was selected for the organic phase instead of methanol because of its higher eluting ability but when the retention time came too quickly its amount was decreased to 10 volumes only. This resulted in good separation and symmetrical peak with high plate numbers and acceptable retention factor. There was also another mechanism by which some interactions between polar silanol groups of stationary phase and polar functional groups of CTX takes place resulting in producing a tailing peak. Addition of triethylamine improves the separation by masking polar silanol groups on the stationary phase, thus enabling CTX molecule to move through the column without interference from the stationary phase. The effect of the flow rate was investigated by varying the flow rate of the mobile phase from 0.7 to 1.3 mL/min. However, a flow rate of 1.0 mL/min gave an optimal signal to noise ratio with a reasonable separation time and, hence, permitted good analytical conditions. Under these experimental conditions sharp peak with good theoretical plate numbers were obtained for CTX at the retention time of about 3 minutes.

System Suitability Parameters

These are integral part of chromatographic methods and are used to verify that the reproducibility of the system are adequate for the analysis to be performed. Typical parameters such as tailing factor, number of theoretical plates, retention factor and percentage relative standard deviation (RSD) of five replicate injections were established for the method and it was compared with the USP requirements and presented in the table 1. The table clearly shows that the proposed method meets and exceeds the minimum requirements set forth by USP for the assay of CTX in sterile powder for injection formulation. Representative chromatogram of standard CTX is given below in figure 2.

Method Validation

The validation of the method was performed as per the ICH guidelines [22], USP [8] and other available literatures [23,24,25,26].

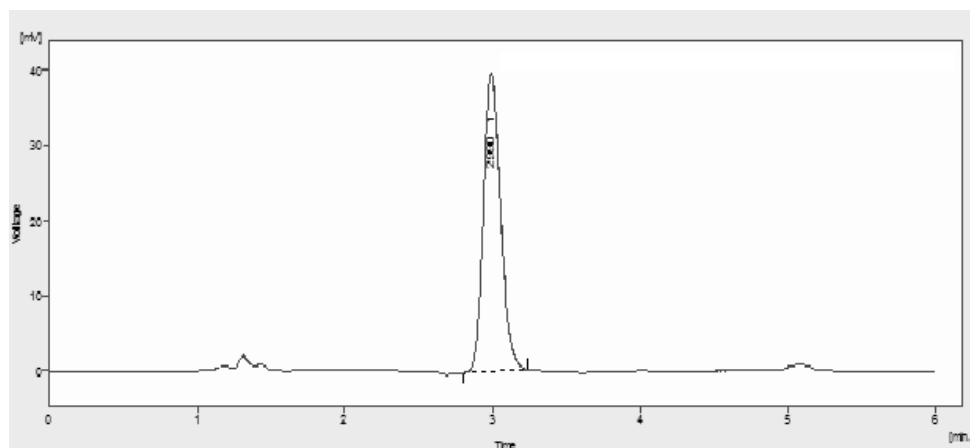


Fig. 2: Representative Chromatogram of Standard Ceftriaxone

Table 1: Comparison of System Suitability Parameters of the Proposed Method with Minimum Requirements of USP

Method	Retention time (min)	Tailing factor	Retention factor	Theoretical Plates	% RSD of five replicate injections
Proposed method	3.0	1.2	2	3300	0.18
USP Method	NA	NMT 2	NA	NLT 1500	NMT 2%

NA - not available, NMT - not more than, NLT - not less than

Linearity

The linearity of the method was checked by analyzing eight different solutions having concentration in the range of 0.5–50 µg/mL of CTX (0.5, 1, 5, 10, 20, 30, 40 and 50 µg/mL). Each solution was prepared in triplicate. The peak areas obtained from different concentrations of the drug were used to calculate linear regression equation. This was $Y = 25.235X + 3.1698$ with correlation coefficient value of 0.999. The high value of the correlation coefficient was indicative of linear relationship between analyte concentrations and peak areas. The linearity data are summarized in table 2.

Precision

The precision of the chromatographic method, reported as percentage RSD, was estimated by measuring intra-day and inter-day assay precision on six separate weights of the sample at 100% test concentration. The percentage RSD values obtained were 0.58 and 0.83 for the intra-day and inter-day precision respectively. The precision data are summarized in table 3.

Accuracy

The accuracy of the method was determined by addition of known amounts of CTX to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution and a standard solution containing 250µg/mL was

prepared separately using mobile phase. Further dilutions were made with the mobile phase to get the final concentrations of 5.0, 10.0 and 15.0 µg/mL of CTX. Mean recovery for CTX from the spiked sample was found to be 100.61%, indicating that the developed method was accurate for the determination of CTX in sterile powder for injection. The accuracy data are summarized in table 4.

Specificity

It is the ability of a method to measure analyte concentration in the presence of impurities or degradation products. Stress testing of the drug substance can create different degradation products and the ability of the method to analyze the required analyte even in this condition can validate the stability-indicating power of the analytical method used.

In the present study, injections of the blank were performed to demonstrate the absence of interference with the elution of the CTX. Stress studies were also performed to evaluate the specificity of CTX under four different stress conditions. Acid hydrolysis (0.1M HCl) for 30 minutes, base hydrolysis (0.1M NaOH) for 10 minutes, light degradation for 24 hrs and oxidative degradation (5.0% H₂O₂) for 30 minutes was carried out. The stress studies data are summarized in table 5. It was evident from the stress chromatograms obtained as shown in figure numbers 3-6 that the degradation peaks eluted were not interfering with the CTX peak. Therefore, it confirms the specificity of the method.

Table 2: Linearity Results of the Method

Name of the drug	Concentration (µg/mL)	Regression equation	R ²
Ceftriaxone	0.5 - 50	$y = 25.235x + 3.1698$	0.9995

Table 3: Precision Study Result

Parameters	Intra-day Precision (% assay)*	Inter-day Precision (% assay)*
1st weight	101.10	102.07
2 nd weight	101.93	102.26
3 rd weight	101.45	101.55
4 th weight	100.56	101.00
5 th weight	101.31	102.13
6 th weight	102.21	100.08
Mean	101.42	101.51
Standard Deviation	0.58	0.84
% RSD	0.58	0.83

*Mean of three replicate studies

Table 4: Recovery Result of the Method

S. No.	Standard drug conc.(µg/mL) (a)	Sample drug conc.(µg/mL) (b)	Total drug conc.(µg/mL) (c)	Total amount found*(µg/mL) (d)	% Recovery of standard (d-b)/a X 100
1	2.5	2.5	5	5.05	102.00
2	7.5	2.5	10	9.89	98.53
3	12.5	2.5	15	15.12	100.96

*Mean of three replicate studies

Table 5: Results from the Forced Degradation Study of the Method

Stress Parameters	Sample treatment	Assay (%)	Degradation (%)
Reference	Fresh solution	98.04	0
Acid hydrolysis	0.1M HCl for 30 mins	91.52	6.65
Base hydrolysis	0.1M NaOH for 10 mins	83.28	15.06
Oxidative	5.0% H ₂ O ₂ for 30 mins	60.78	38.00
Light degradation	UV Light for 24 h	75.87	22.61

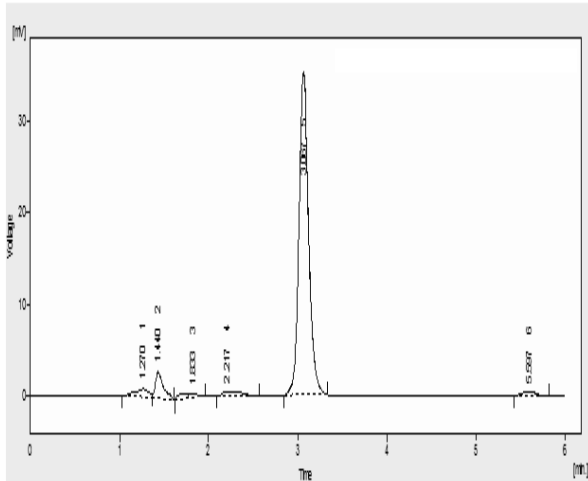


Fig. 3: Acid degradation chromatogram

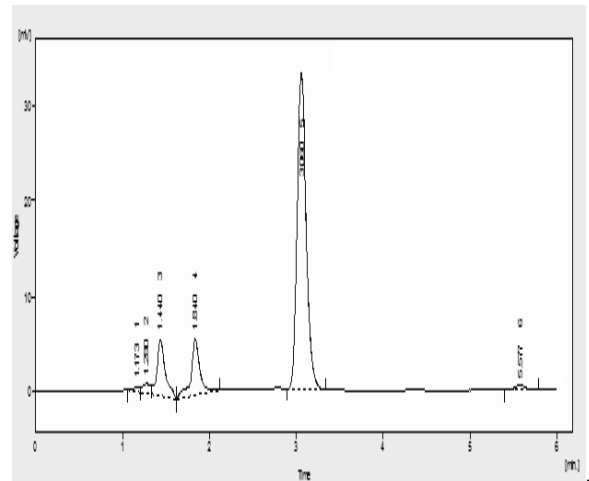


Fig. 4: Base degradation chromatogram

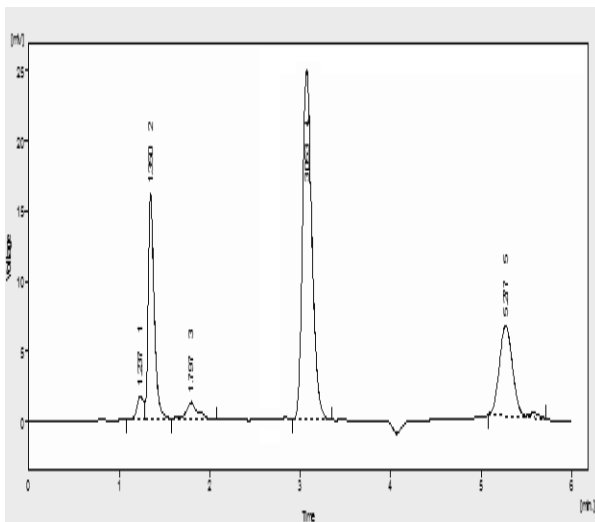
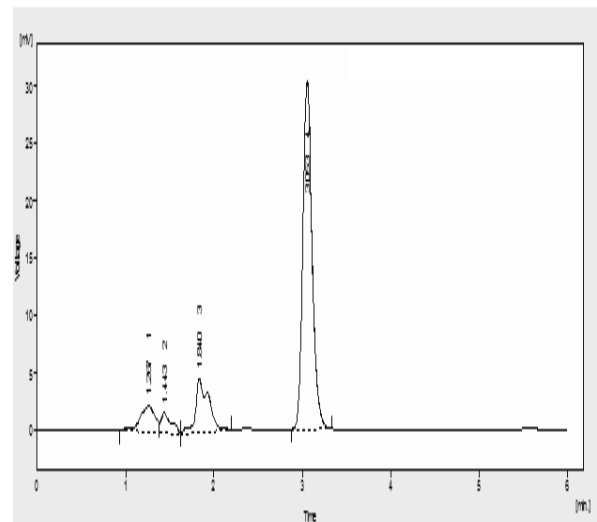


Fig. 5: UV degradation chromatogram

Fig. 6: H₂O₂ degradation chromatogram

Robustness

The ability of a method to remain unaffected by small and deliberate variations in method parameters provides an indication of its reliability for routine analysis and this can be evaluated by performing robustness studies. It was performed by deliberately altering the experimental conditions and evaluating the assay percentage, peak tailing and number of theoretical plates in the changed conditions. The flow rate of the mobile phase was changed by 10% to 0.9 mL/min and 1.1 mL/min and the composition of the acetonitrile in the mobile phase was also changed by 10% to 9 ml and 11 ml and the effect was studied. Similarly, the effect of pH was studied at 6.8 and 7.2 instead of 7.0. For all changes in conditions, the sample was analyzed in triplicate. Only one condition of the experiment was changed at a time keeping the other parameters constant at the optimum value. The assay values for all deliberate changes of conditions were within 98.41-102%.

CONCLUSION

The proposed stability indicating reverse phase HPLC method was found to be specific, precise, accurate, and rapid for the determination of CTX in sterile powder for injection. The method parameters established in the proposed method meets and exceeds the USP requirements. The method is simple and cost effective with a run time of 6 minutes and it can be used in quality control laboratory for the analysis of CTX without interference from commonly used additives.

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