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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR CEFTRIAXONE SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS

ABSTRACT

A simple, sensitive, accurate and precise LC assay method was developed for the quantitative determination of Ceftriaxone Sodium (CTS) in pharmaceutical dosage form. Chromatographic separation was achieved by use of XTerra RP-18 column (250 × 4.0 mm, 5 μm). The described method was linear over a range of 1.0-120 μg mL^{-1} for determination of CTS (r= 0.9992). F-test and t-test at 95% confidence level were used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to be less than critical value. The developed method was found to be simple, specific, robust, linear, precise, and accurate for the determination of CTS in pharmaceutical formulations.

KEYWORDS: Ceftriaxone Sodium, validation, assay, recovery studies.

INTRODUCTION

Ceftriaxone Sodium, Ceftriaxone sodium is chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl 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, (CTS) is a β-lactamase-resistant cephalosporin with an extremely long serum half-life. The bactericidal activity of CTS result from the inhibition of the cell wall synthesis and is mediated through CTS binding to penicillin binding proteins (PBPs). It inhibits the mucopeptide synthesis in the bacterial cell wall. The beta lactam moiety of CTS binds to caboxypeptidase, endopeptidase, transpeptidase, in the bacterial cytoplasmic membrane. These enzymes are involved in cell wall synthesis and cell division. By binding these, CTS results in the formation of defective cell walls and cell death[1], [2].

A literature survey revealed that several methods have been used for determination of CTS which includes Spectrophotometry.[3],[4],[5],[6],[7],[8],[9] High Performance Thin Layer Chromatography[10], High Performance Liquid Chromatography in pharmaceutical formulations[11],[12] and biological fluids[13],[14],[15],[16],[17],[18],[19],[20], Capillary Electrophoresis[21] and differential-pulse adsorptive stripping voltammetry[21].

Although the reported two HPLC methods for estimation of CTS in pharmaceutical formulations present adequate linearity, precision, and recovery, they show a series of limitations including lack of sensitivity, which results in the lower limit of quantification and long chromatographic times (15 min). However, these methods are relatively non-specific, laborious, time consuming and have long retention times. However, the present study achieved satisfactory results in terms of selectivity, linearity, precision and accuracy under simple chromatographic conditions.

EXPERIMENTAL

MATERIALS

CTS reference standard was obtained from Torrent pharma., India. CTS vials (XONE-XP 250mg) were purchased from the local market. HPLC grade Acetonitrile was purchased from Rankem, India, and high pure water was prepared by using Millipore Milli Q plus purification system. Dibasic potassium phosphate, monobasic potassium phosphate, Sodium citrate were purchased from Qualigens Fine chemicals, India.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Quantitative HPLC was performed on Shimadzu HPLC with LC 10 AT VP series pumps besides SPD 10 A VP UV-Visible detector. The chromatographic separations were performed using XTerra, C18, RP column (250 mm × 4mm × 5 µm) maintained at ambient temperature, eluted with mobile phase at a flow rate of 1 mL/min for 10 min. The output signal was monitored and integrated using Shimadzu Class-VP version 6.12 SP1 software. The mobile phase consisted of acetonitrile: phosphate buffer: water (40:4.8:55.2 %v/v/v). Measurements were made with injection volume 20µl and ultraviolet (UV) detection at 270 nm.

Preparation of pH 7.0 buffer

13.6 g of diabasic potassium phosphate and 4.0 g of monobasic potassium phosphate were dissolved in water to obtain 1000 mL solution. This soloution was adjusted with phosphoric acid to yield pH 7.0±0.1

Preparation of pH 5.0 buffer

25.8g of sodium citrate was dissolved in 500 mL of water , pH is adjusted to 5.0± 0.1 with citric acid solution(1g in 5 mL) and then diluted with water to 1000 mL.

Preparation of mobile phase

3.2 g of tetraheptylammonium bromide is dissolved in 400 mL of acetronitrile, then, 44 mL of pH 7.0 buffer and 4 mL pH 5.0 buffer were added to it, the volume was made up to 1000 mL with water, the final solution was filtered through 0.45 membrane filter and degassed.

Preparation of standard and sample solutions

Stock solution of CTS (1mg/mL) was prepared by dissolving 25 mg of CTS in 25 mL of volumetric flask containing 10 mL of mobile phase. The solution was sonicated for about 30 minutes and then made up to volume with mobile phase. Working standard solutions of CTS were prepared by taking suitable aliquots of CTS stock solution and diluted to 10 mL with mobile phase in a 10 mL volumetric flask to yield the drug concentrations in the range of 1-120µg mL^{-1}.

To prepare a sample solution, twenty vials of XONE-XP® 250mg were mixed and an amount of CTS equivalent to 10 mg was diluted with mobile phase and then sonicated for 10 min. The sample solution was filtered and the appropriate aliquot was diluted in the mobile phase to obtain a final solution containing 10 μg mL^{-1} of CTS.

Method validation

The validation procedure for the analysis of CTS by LC method followed the International Conference on Harmonization (ICH) guideline and United States Pharmacopoeia. The performance parameters evaluated in this method were specificity, robustness, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and accuracy.

Robustness

Chromatographic parameters (peak retention time, theoretical plates, tailing factor, retention factor, and repeatability) were evaluated using both samples and reference substance solutions (10 μg mL^{-1}) changing wavelength (265 and 275 nm), column temperature (23 and 27 ºC), flow rate (0.8 and 1.2 mL min^{-1}) and acetonitrile concentration (38 and42%).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves (n=3) were linear over the concentration range of 1–120μg/mL. Peak areas of CTS was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve.

LOD and LOQ

LOD and LOQ were determined by reducing the concentration of a standard solution until the CTS peak response was approximately three or ten times, greater than the noise, respectively.

Precision

The precision of the proposed method was evaluated by carrying out six independent (50µg/mL) assays of test sample. RSD (%) of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument.

Accuracy

The accuracy of the method was determined through the recovery test of the samples, using known amounts of CTS reference standard. For LC method, aliquots of 0.4, 0.5 and 0.6 mL of a CTS standard solution (100 μg mL^{-1}) were added to three sample solutions containing a fixed amount of CTS (50 μg) in mobile phase, respectively. Therefore, this recovery study was performed at a final concentration solution of 80%, 100% and 120% level of CTS. All solutions were prepared in triplicate and analyzed.

System suitability test

System suitability tests were performed to ensure that the LC system and procedure are capable of providing quality data based on USP 31 requirements. The system suitability parameters include CTS retention time, tailing factor and number of theoretical plates, as well as the peak area relative-standard deviation (RSD, n= 6) of reference standard.

RESULTS AND DISCUSSION

Optimization of LC method

To develop a suitable and robust HPLC method for the determination of CTS in different mobile phases - water : methanol : acetonitrile were used in different compositions(50 : 30 : 20), methanol: water :acetonitrile compositions of mobile phases (50: 30: 20) at different flow rates (0.5, 0.75, 0.8, 1.0, 1.2, 1.5, mL/min) with different detection wavelength. The mobile phase acetonitril: buffer: water (40:4.8:55.2%v/v) at a flow rate of 1.0 mL/ min gave peaks with good resolution for CTS are eluted at retention time around 3.155 min and with symmetric peak shape.

Method Validation

Robustness

The robustness of the method was examined by small variations of critical parameters, and percent of CTS, retention time (R_t), number of theoretical plates (*N*) and tailing factor (*T*), were evaluated (table 1).

The robustness study has been proved that in every employed condition, the chromatographic parameters agreed with established values and the assay data remained acceptable. A tailing factor of 1 refers to a symmetric peak. The calculated values for the tailing factor for each chromatographic condition were in the acceptable range of $0.8 \leq T \leq 1.5$. The number of theoretical plates demonstrated the measure the column efficiency in different conditions. Flow rate (0.8 and 1.2 mL min^{-1}) and percent of acetonitrile (38 and 42%) resulted in changes in the retention time in comparison with the proposed normal condition. However, no significant changes were observed regarding quantification of CTS.

Linearity

The standard curves for CTS were constructed and demonstrated to be linear in the concentration range of 1-120 μg mL^{-1}. The representative linear equation was y = 42748x + 12012, where *x* is the concentration (μg mL^{-1}) and *y* is the peak area. The correlation coefficient was *r*= 0.9993. Linearity data were validated by the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($p < 0.05$).

LOD and LOQ

The limit of quantitation (LOQ) of the present method was found to be 1.0 μg/mL with a resultant %RSD of 0.83% ($n = 5$). The limit of detection (LOD) was found to be 0.3 μg/mL. This low values obtained were indicative of the high sensitivity of the method.

Precision

Precision values obtained for the determination of CTS in samples with their RSD are shown in table 2. F-test and t-test was applied to the two sets of data at 95% confidence level, and no statistically significant difference was observed.

Accuracy

Accuracy was evaluated by the simultaneous determination of the analyte in solutions prepared by the standard addition method. Three different concentrations of CTS standard were added to xone-xp® 250mg solution. The mean recovery was shown (table 3) and this value showed that the method was accurate.

System suitability test

The system suitability parameters evaluated, under the experimental conditions, showed a single peak of the drug around 3.15 min, tailing factor (*T*= 1.15) and number of theoretical plates (*N*= 3288), as well as the peak area relative-standard deviation (RSD= 0.7%, n = 6).

Assay

The validated method was applied to the determination of CTS in commercially available xone-xp® 250mg vials. Fig-1and Fig-2 illustrates two typical HPLC chromatograms obtained from CTS standard solution and from the assay of xone-xp® 250mg vials respectively. The results of the assay (*n* = 9) undertaken yielded 99.58% (%RSD = 1.7%) of label claim for CTS. The observed concentration of CTS was found to be 248.9±4.2µg/mL (mean±SD). The mean retention time of CTS was 3.1 min. The results of the assay indicate that the method is selective for the analysis of CTS without interference from the excipients used to formulate and produce these tablets.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of CTS from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of CTS in pure form and its dosage forms and can also be used for dissolution or similar studies.

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