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Determination of Cefuroxime Axetil and Cefixime Trihydrate in Pharmaceutical Dosage Forms by RP-HPLC Method

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Abstract

Determination of two generation Cephalosporins (Cefuroxime Axetil and Cefixime Trihydrate) in pharmaceutical dosage forms was carried out employing High Perfeormance Liquid Chromatographic using isocratic separation. Separation was performed on an Enable C_{18} column (250 mm × 4.6 mm, 5.0 µm) using Triethylamine: Methanol: Acetonitrile: Ultra-Pure Water (2:10:20:68 v/v%) as the mobile phase at a flow rate of 1.0 ml/min. The PDA detection wavelength was set at 265 nm. The linearity was observed over a concentration range of 0.1-80 µg/ml for HPLC method (correlation coefficient=0.999). The developed methods were validated according to ICH guidelines. The relative standard deviation values for the method precision studies were <2%, and the accuracy was >99%. The developed method was used successfully for the determination of Cefuroxime Axetil, Cefixime Trihydrate, in Capsule, Tablet and dry syrup formulations.

Keywords: Cefuroxime axetil (CA); Cefixime trihydrate (CT); HPLC

Introduction

Cefuroxime Axetil (CA): 1-Hydroxyethyl (6R,7R)-7-[2-(2-furyl) glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate (Figure 1) is a second generation cephalosporin antibiotic [1].

Cefixime Trihydrate (CT): (6R,7R)-7-{[(2R)-2-amino-2-phenylacetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid hydrate (Figure 2) is a third generation cephalosporin antibiotic [1].

It is used in the treatment of susceptible organsims of the respiratory tract, urinary tract, and skin [2]. According to literature surveys, there are different analytical methods reported for the determination of CA and CT. It includes UV-Visible spectroscopy [3-12], chemiluminescence [13], near infrared spectroscopy [14], potentiometry [15], polarography [16,17], HPLC [18-26], gel filtration chromatography [27], HPLC [28], capillary zone electrophoresis [29], LC-MS [30,31], and MS [32] methods. But no analytical methods are reported for the determination of CA and CT in dry syrup formulation using the mobile phase Triethylamine: Methanol: Acetonitrile: Ultra-Pure Water (2:10:20:68 v/v%) by HPLC (High Performance liquid chromatography). So a successful attempt was made to develop and validate a fast, simple, precise, and accurate HPLC method for the determination of CA and CT in Capsule, Tablet and Syrup formulation. Specificity and stability parameters for the drug were assessed according to ICH [33].

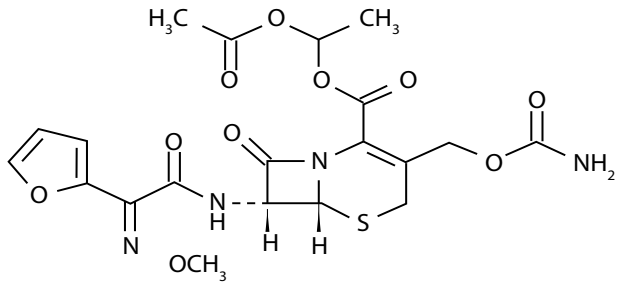

Figure 1: Structure of Cefuroxime axetil (CA).

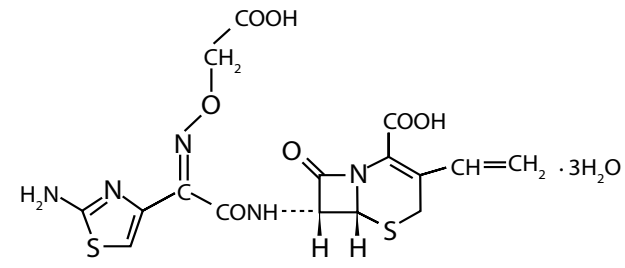

Figure 2: Structure of Cefixime trihydrate (CT).

Experimental and chemical reagents

Cefuroxime Axetil (CA) and Cefixime Trihydrate (CT); (purity of all >99.9%) was obtained as a gift sample from Parabolic Pharmaceuticals Ltd., India. Methanol (Merck Ltd., Mumbai, India) was of HPLC grade. Analytical grade Methanol, Acetonitrile, Triethylamine (Merck GmbH). The Ultra-Pure water for HPLC was obtained by using the TKA Water Purification System, Germany. The Capsule formulation (CT) containing 400 mg/Cap. The tablet formulation (CA) containing 500 mg/Tab. The dry Syrup formulation (CA) and (CT) containing 125 mg/5 mL, 250 mg/5 mL, 100 mg/5 mL, 200 mg/5 mL was bought from the local market.

Instrumentation

Quantitative HPLC was performed on a binary gradient with MERCK-HITACHI Prominence La Chrom pumps, with a 20 µl

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sample injection loop (Auto) and L-2455 PDA detector. The signal was recorded and integrated using EZ Chrom Software. An Enable C_{18}, (250 mm × 4.6 mm i.d. particle size 5 µm) was used for separation. Chromatographic analysis was carried out at ambient temperature on the column using the Triethylamine:Methanol:Acetonitrile:Ultra-Pure Water (2:10:20:68 v/v%) as the mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The PDA detection was carried out at 265 nm.

Preparation of standard and sample solution

Standard stock solutions of CA and CT were prepared by transferring 100 mg of the drug into two separate 100 ml volumetric flasks having 10 ml of diluents and were ultrasonicated for 5 minutes. Finally the volume was made up with suitable diluents, which gave 1000 µg/mL solutions. Powder (Capsule, Tablet and dry Syrup) equivalent to 100 mg of CA and CT was accurately measured and transferred into two separate 100 ml volumetric flasks, containing 10 ml of diluents and ultrasonicated for 20 minutes; the volume was made up and mixed well. Solutions were filtered by a 0.2 µm filter to remove particulate matter, if any. The filtered solutions were properly diluted for analysis as already described. The drug present in the sample solutions was calculated by using the calibration curves. All the solutions were stored at 2-8°C for future use.

Method validation

Specificity: The specificity of the HPLC method was determined by checking the interference of any of the possible degradation products and Absorbances produced during study of CA and CT. The study of the drug was carried out with Methanol, Triethylamine, Ultra-Pure Water for discovering the stability nature of the drug. The degraded samples were prepared by taking suitable aliquots of the drug solution, and then undertaking the respective stress testing procedures for each solution. After the fixed time period, the stressed test solutions were diluted with the mobile phase. For every stress condition, a solution of concentration 50 µg/mL of CA and CT was prepared. The specific stress conditions are described as follows.

Linearity: A twelve-point (0.1, 0.5, 1.0, 5.0, 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL) calibration curves were prepared for the HPLC methods. The peak area for the HPLC was obtained by injecting 20 µl of the drug solution into the column. Calibration curves were plotted by taking the peak area curve on the Y-axis and the concentration (µg/mL) on the X-axis.

Precision: The intraday and interday precision study was carried out to check the reproducibility of the results. A concentration of (15, 20, 25) µg/mL and 30 µg/mL of CA and CT (n=3) were analyzed to find out relative standard deviation (RSD) for HPLC methods.

Accuracy: To check the accuracy of the proposed method, recovery studies were carried out at 80, 100, and 120% of the test concentration. The recovery study was performed three times at each level. The amount of CA and CT present in the sample was calculated using the calibration curves.

Robustness: The robustness of the HPLC method was studied by deliberately changing the method parameters like flow rate of the mobile phase, detection wavelength, and organic phase composition. A series of system suitability parameters like retention time, theoretical plates, and tailing factor were determined for each changed condition according to ICH [33].

Limit of detection and limit of quantitation: The LOD and LOQ were determined separately according to the ICH guidelines. For the HPLC method, concentrations providing a signal-to-noise ratio 3:1 and 10:1 were considered as the LOD and LOQ, respectively.

Results and Discussion

Optimization of the mobile phase was carried out based on the tailing factor and theoretical plates obtained for CA and CT. During the trial runs, the drug was tested with different mobile phase compositions (Triethylamine:Ultra-pure water:Acetonitirle:Methanol v/v%) at various compositions (25:25:25:25 v/v%), (10:20:65:5 v/v%) and (10:20:68:2 v/v%) and flow rates (0.5, 1.0 and 1.2 mL/min). The mobile phase consisting of Triethylamine:Methanol:Acetonitrile:Ultra-Pure Water (2:10:20:68 v/v%) at a flow rate of 1.0 ml/min was selected which gave a sharp, symmetric peak for CA and CT. The retention time for CA and CT was found to be (3.98, 3.67 and 3.16 min) respectively. The run time was 5 min. The tailing factor for CA and CT was found to be (1.48, 1.43, 1.26). PDA detection was carried out at 265 nm. The separation was carried out at room temperature (Figure 3A and 3B).

Specificity

To evaluate the specificity, a PDA detector was applied to find out the peak purity of the chromatographic peaks obtained for the stress-treated drug solution. Peak purity results are indicative for the peak homogeneity. The specificity of the HPLC method was determined by checking the interference of any of the possible degradation products and Absorbances produced during study of CA, CT. The study of the drug was carried out with Methanol, Triethylamine, Ultra-Pure Water for discovering the stability nature of the drug. The degraded samples were prepared by taking suitable aliquots of the drug solution, and then undertaking the respective stress testing procedures for each solution. The UV spectrums (Figure 4) obtained for the blank and placebo show no interference due to the solvent used and presence of the commonly used excipients suggesting the specificity of the two methods.

Linearity

The calibration curves were found to be linear over a concentration range of 0.1-80 µg/mL for methods (correlation coefficient 0.999 for all the methods). The method parameters (overlay chromatograms) and regression data (Linearity series) are shown in Figure 5. Linearity of CA (Figure 6), Linearity of CT (Figure 7), Table 1 for regression data.

Precision

The methods were found to be precise as the RSD (%) values for the precision studies were well below 2% (n=3). The results are shown in Table 2.

Parameter	Cefuroxime Axetil, (CA)	Cefixime Trihydrate, (CT)
Slope	0.199×10^6	0.185×10^6
Intercept	0.205×10^6	0.065×10^6
Correlation Coefficient	0.9995	0.9997
Detection Wavelength, nm	265	
Linear rang, µg/mL	0.1-80	

Table 1: Analysis of method parameters and regression data.

Parameter	Accuracy(recover), %	*Precision(RSD), %	LOD, µg/ml	LOQ, µg/ml
Cefuroxime Axetil, (CA)	100.96%	0.28	0.87	2.66
Cefixime Trihydrate, (CT)	100.75%	0.98	0.67	2.05

Table 2: Summary of validation parameters. *Average of three determination at each level.

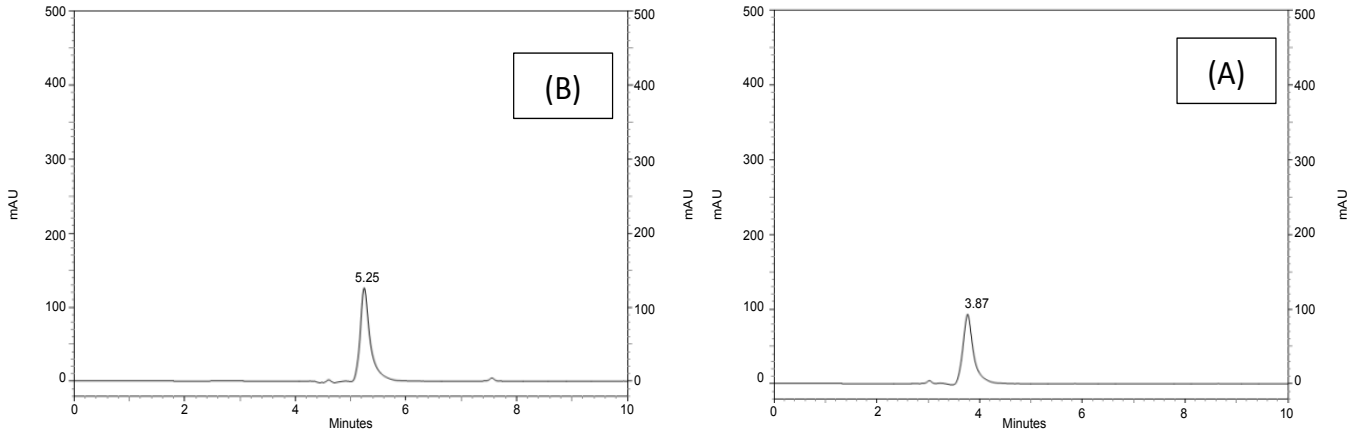

Figure 3: Chromatograms of CA and CT for Method (A) and (B) Respectively.

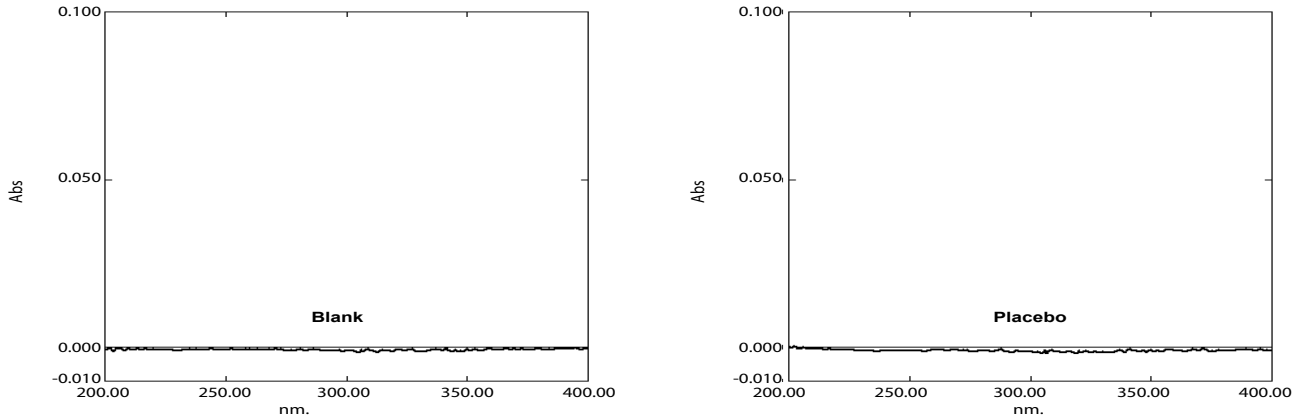

Figure 4: UV Spectrum of Blank Solution (left) and of Placebo Solution (right).

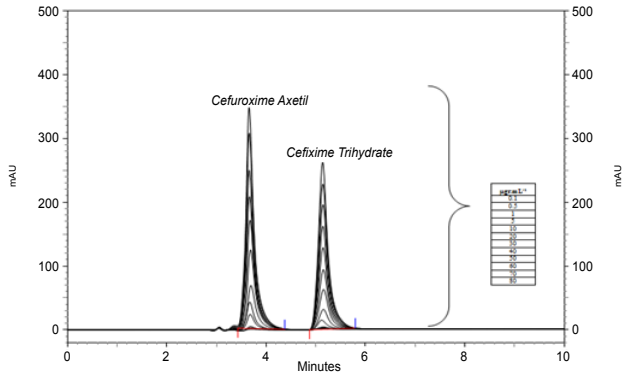

Figure 5: Overlay Chromatograms of CA, CT and linearity rang (0.1-80) µg/mL.

Accuracy

The accuracy of the developed methods was found out by the standard addition method. High recovery values suggest that all three methods are accurate. The results are shown in Table 2.

Limit of detection and limit of quantitation

The LOD and LOQ values shown in Table 2 suggest that the developed methods are sensitive to determine CA and CT.

Robustness

The HPLC method was found to be robust under deliberate changes in the mobile phase flow rate (± 0.1 mL/min), detection wavelength (± 5 nm), and organic phase composition (± 2%). The results of system suitability for the robustness study are shown in Table 2. For the UV spectroscopic methods, changing the slit width shows no significant effect on absorbance, indicating the robustness of the developed methods. No significant changes were obtained in the content of CA and CT during the solution stability studies by the developed methods. The recoveries for the solution stability by the method was found to be 100.96%, 100.75%, respectively.

Analysis of commercial dry syrup formulation

The developed methods were successfully applied for the determination of CA and CT in the Tablet, Capsule and dry Syrup formulation. The result for the assay of CA and CT is shown in Table 3. The assay results

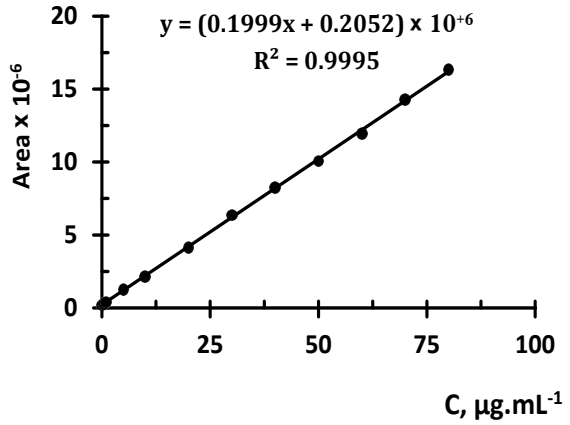

Figure 6: Linearity of CA (0.1-80) µg/mL.

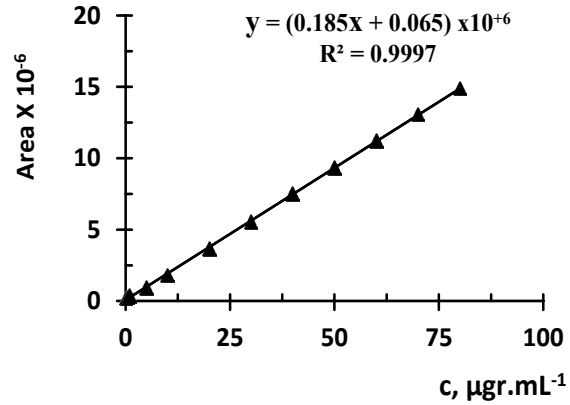

Figure 7: Linearity of CT (0.1-80) µg/mL.

Formulation Label Claim	*RSD%	Recovery(%) ± SD
Zinamed (125 mg/5 mL)	0.01	105.40 ± 0.05
Zednad (250 mg/5 mL)	0.38	105.13 ± 0.19
Zenamed (500 mg/tab)	0.04	104.80 ± 0.02
Sopraxymed (100 mg/5 mL)	1.76	106.60 ± 0.87
Sopraxymed (200 mg/5 mL)	1.01	106.00 ± 0.86
Sopraxymed (400 mg/Cap)	0.93	100.80 ± 0.46

Table 3: Assay of syrup formulation. *Average of three determination at each level.

obtained for CA and CT in the syrup formulation using the HPLC and UV spectroscopic methods.

Conclusion

Three novel analytical methods were developed for the determination of (CA and CT). The validation study shows the methods are specific, linear, precise, accurate, and sensitive in the proposed working range. The methods were found to be fast, simple, accurate, precise, and sensitive. The excipients present in the commercial formulation were found to be non-interfering in the assay results. The methods were successfully applied for the determination of the drug in dry syrup formulation. Furthermore, the developed methods may be applied for the routine analysis of the drug in API, formulations, and dissolution medium.

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