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RESEARCH ARTICLE

Development and Validation of a Stability-Indicating Liquid Chromatographic Method for the Determination of Cefditoren Pivoxil in Presence of Degradation Products

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Abstract: A novel stability indicating liquid chromatographic method was developed for the determination of Cefditoren pivoxil in presence of degradation products using Zorbax SB C18 (150 mm × 4.6 mm i.d., 3.5 µm particle size) column with a flow rate 1.0 mL/min (UV detection 210 nm). Linearity was observed over a concentration range 0.1–200 µg/mL with regression equation y = 34809 x + 30603 (R^2 = 0.9994). Forced degradation studies were performed and Cefditoren pivoxil is reported to be highly sensitive towards alkaline conditions in comparison to oxidation. The method was validated as per ICH guidelines.

Keywords: Cefditoren pivoxil, Stability-indicating, Liquid chromatography, Validation, ICH

Introduction

Cefditoren pivoxil is a third-generation semi-synthetic cephalosporin antibiotic (Figure 1). It is effective in treating both gram-positive and gram-negative organisms and respiratory tract infections. It is chemically (7*R*) - 7-((*Z*)- 2-(2-aminothiazol- 4-yl)- 2-(methoxyimino) acetamido)- 3-((*Z*)- 2-(4-methylthiazol- 5-yl) vinyl)- 8-oxo- 5-thia-1-azabicyclo[4.2.0] oct-2-ene- 2-carboxylic acid ($C_{25}H_{28}N_6O_7S_3$) with molecular weight 620.73 g/mol. It is a prodrug which is hydrolyzed by esterases during absorption, and the drug is distributed in the circulating blood as active Cefditoren[1].

A thorough literature survey reports that very few analytical methods were reported for the determination of Cefditoren in biological fluids[2-4] and in pharmaceutical dosage forms using HPLC[5-8], UPLC[9] and spectrophotometry[10]. The authors have proposed a stability indicating liquid chromatographic method for Cefditoren pivoxil in presence of its degradation products.

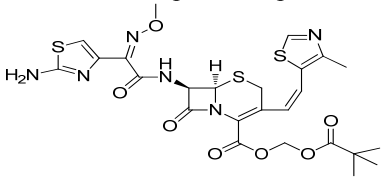

Figure 1. Chemical structure of Cefditoren pivoxil

Experimental

Cefditoren pivoxil standard (purity 99.50%) was obtained from Cipla Limited (India) and was used as it is without further purification. All other chemicals were of analytical grade (Merck). Cefditoren pivoxil is available as tablets (Label claim 200 mg) with brand names CEFTORIN® (Cipla Lmited, India) and ZOSTUM-O® (Zuventus, India).

Instrumentation and chromatographic conditions

Chromatographic separation was achieved by using Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) for HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector, maintained at 25 ºC.

Isocratic elution was performed using tetra butyl ammonium hydrogen sulphate: acetonitrile (50:50, v/v) as mobile phase. The overall run time was 10 min. with flow rate 1.0 mL/min with UV detection at 210 nm. 20 µL of sample was injected into the HPLC system.

Preparation of tetra butyl ammonium hydrogen sulphate buffer solution

3.3954 g of Tetra butyl ammonium hydrogen sulphate (10 mM) was accurately weighed and dissolved in HPLC grade water in a 1000 mL volumetric flask (pH 3.37).

Preparation of stock solution

Stock solution was prepared by accurately transferring about 10 mg of Cefditoren pivoxil in to a 10 mL volumetric flask with mobile phase. Further dilutions were done on daily basis from the stock solution with mobile phase (tetra butyl ammonium hydrogen sulphate solution: acetonitrile, 50:50, v/v). Prior to injection all solutions were filtered through 0.45 µm membrane filter.

Method validation

The method was validated for linearity, limit of quantitation (LOQ), limit of detection (LOD), intra/inter-day precision, accuracy, robustness and specificity[11].

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the assay analyte concentration (0.1-200 µg/mL). 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. A graph was drawn by taking the concentration of the drug on the x-axis and the corresponding peak area on the y-axis.

Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) and limit of detection (LOD) were based on the standard deviation of the response and the slope of the constructed calibration curve as described in International Conference on Harmonization guidelines Q2 (R1).

Precision

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Cefditoren pivoxil at three concentration levels (20, 50 and 100 µg/mL) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated.

The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (20, 50 and 100 µg/mL) and each value is the average of three determinations. The %RSD of three obtained assay values on three different days was calculated.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%) and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Cefditoren pivoxil in the drug product. The study was carried out in triplicate at a total concentration 18, 20 and 22 µg/mL. The percentage recovery in each case was calculated.

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (208 and 212 nm), percentage of acetonitrile in the mobile phase (52 and 48%), flow rate (0.9 and 1.1 mL/min) and pH (3.3 and 3.5). Robustness of the method was studied with 100 µg/mL of Cefditoren pivoxil.

Analysis of commercial formulations (Tablets)

Twenty tablets were procured from the local pharmacy store, weighed and crushed in to fine powder. Powder equivalent to about 10 mg Cefditoren pivoxil was accurately transferred into a 10 mL volumetric flask and made up to volume with acetonitrile. The contents were sonicated for 30 min to enable complete dissolution of Cefditoren pivoxil and then the solution was filtered. The filtrate was further diluted with mobile phase to yield 100 µg/mL.

Forced degradation studies/Specificity

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method[12]. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Cefditoren pivoxil and refluxed for 30 min at 80 ºC in thermostat and then diluted with mobile phase to give a final concentration of 100 µg/mL All the solutions were analysed after 24 h.

The acidic and alkaline degradations were performed using hydrochloric acid (0.1 M) and in sodium hydroxide (0.01 M) at 80 ºC in a thermostat and the stressed samples were instantly cooled with a mixture of ice and water, neutralized and diluted with mobile phase as per the requirement. Oxidation was performed using H_2O_2 solution where as photolysis was performed on exposure of the drug solution to UV light (365 nm) for 6 hours in UV light chamber.

Results and Discussion

The authors have developed a validated stability indicating RP-HPLC method for Cefditoren pivoxil in presence of degradation products. A comparative study was done for the performance characteristics of the present stability indicating liquid chromatographic methods with the reported methods in the literature (Table 1).

HPLC method development and optimization

Initially the drug samples were analyzed using a mobile phase consisting of tetra butyl ammonium hydrogen sulphate buffer solution: acetonitrile (50:50, v/v) with a flow rate of 0.8 mL/min where the drug was eluted at 6.23 min with little tailing. Therefore the flow

rate was modified to 1.0 mL/min for which the retention time of the drug was eluted at 2.876 min (UV detection at 210 nm) as a sharp peak without tailing and therefore the same chromatographic conditions were chosen for the entire study.

Table 1. Comparison of the performance characteristics of the present method with the published HPLC methods

Method /Reagent	λ nm	Linearity µg/mL	Remarks	Ref
Ammonium acetate: methanol: acetonitrile (50:50, v/v)	295	(100-4000)×10^{-3}	Rat plasma	[3]
Methanol: Potassium dihydrogen phosphate (75:25, v/v)	231	40–120	Narrow linearity range	[5]
Water: Methanol (adjusted to pH = 6) (20:80, v/v)	256	3-30	Very narrow linearity range	[6]
Phosphate buffer (pH 8.0): acetonitrile (40:60, v/v)	220	0.1-200	Wide linearity range	[7]
Water-acetonitrile (50:50, v/v)	218	0.1-250	Isocratic mode Stability indicating	[8]
Acetonitrile: Ammonium acetate (pH 6.7)	-	80-120	Gradient mode	[9]
TBHS: ACN (isocratic mode) (50:50, v/v)	210	0.1-200	Stability indicating method with wide linearity range	Present work

Linearity

The typical chromatogram obtained for Cefditoren pivoxil was shown in Figure 2A. Cefditoren pivoxil obeys Beer-Lambert's law over tconcentration range 0.1-200 µg/mL (Table 2) with regression equation y = 34809 x + 30603 (r^2 = 0.9994) (Figure 3).

The LOQ and LOD were determined based on the 10 and 3.3 times the standard deviation of the response, respectively, divided by the slope of the calibration curve. The LOQ is found to be 0.0792 µg/mL and the LOD is found to be 0.0241 µg/mL.

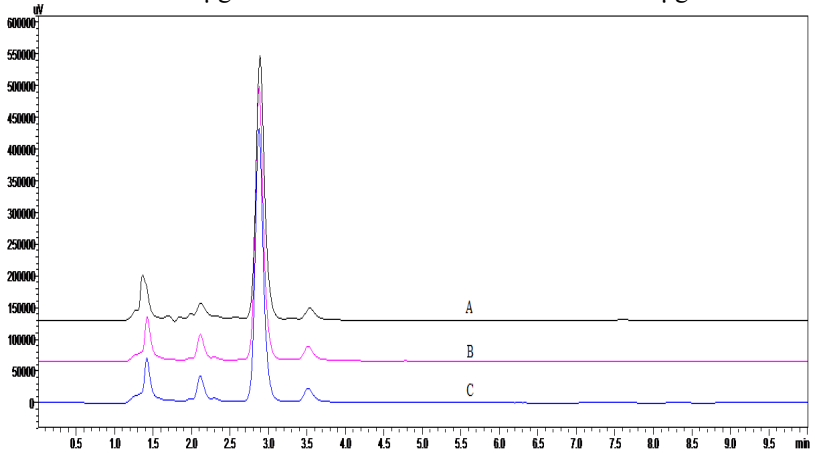

Figure 2. Typical Chromatograms of Cefditoren pivoxil (100 µg/mL) (A), CEFTORIN (B) ZOSTUM-O (C) (Label claim: 200 mg)

Table 2. Linearity of Cefditoren pivoxil

Conc. µg/mL	*Mean peak area ± SD	RSD %
0.1	3852 ± 12.48	0.32
1	36629 ± 103.66	0.28
5	169635 ± 843.09	0.50
10	338542 ± 1100.26	0.33
20	802587 ± 2199.09	0.27
50	1856362 ± 2636.03	0.14
100	3533668 ± 8834.17	0.25
150	5325463 ± 25029.68	0.47
200	6900145 ± 35190.74	0.51

**Mean of three replicates*

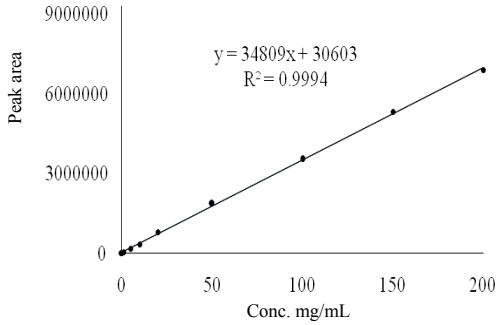

Figure 3. Calibration curve of Cefditoren pivoxil

Precision and accuracy

The % RSD was found to be 0.76-0.99 (intra-day) and 0.49-0.96 (inter-day) in precision studies where as in accuracy studies the percentage recovery was found to be 98.87-98.97% with percentage RSD 0.44-0.46 indicating that the method is precise and accurate (Table 3).

Robustness

The percentage RSD was found to be 98.87-98.98 which is less than 2.0% indicating that the proposed method is robust (Table 4).

Table 3. Precision and accuracy studies of Cefditoren pivoxil

Conc. µg/mL	Intra-day precision	Inter-day precision
	* Mean peak area ± SD %RSD	* Mean peak area ± SD % RSD
20	801561.00±7911.37 (0.99)	797571.33±4587.46 (0.58)
50	1807286.33±14897.66 (0.82)	1836719.67±17559.51 (0.96)
100	3514885.33±26718.79 (0.76)	3513687.67±17372.18 (0.49)

Accuracy				
Spiked conc. µg/mL	Total conc. µg/mL	* Mean peak area ± SD % RSD	Drug found, µg/mL	% Recovery
8 (80 %)	18	650071.67±2824.20 (0.46)	17.80	98.87
10 (100 %)	20	719660.00 ±3021.02 (0.44)	19.80	98.98
12 (120 %)	22	788532.67±3350.70 (0.44)	21.77	98.97

**Mean of three replicates*

Table 4. Robustness study of Cefditoren pivoxil

Parameter	Condition	*Mean peak area	*Mean peak area ± SD % RSD	*Assay %
Flow rate (±0.1 mL/min)	0.9	3493573	3532076.00±37732.20 (1.07)	99.95
	1.0	3533668		
	1.1	3568987		
Detection wavelength (± 2 nm)	208	3535521	3522240.33 ± 21418.09 (0. 16)	99.68
	210	3533668		
	212	3497532		
Mobile phase composition (TBAHS: acetonitrile) (± 2 %, v/v)	48:52	3485243	3528234.33 ± 40548.48 (1.15)	99.85
	50:50	3533668		
	52:48	3565792		
pH (± 0.1 unit)	3.3	3493546	3530997.00 ± 36189.50 (1.02)	99.92
	3.4	3533668		
	3.5	3565777		

**Mean of three replicates*

Analysis of commercial formulations (Tablets)

The proposed method was applied to the determination of Cefditoren Pivoxil tablets and the assays was calculated as 98.70-99.22% (Table 5) and no interference was observed with the excipients Figure 2B and 2C.

Table 5. Analysis of Cefditoren pivoxil commercial formulation (Tablets)

Formulation	Labeled claim, mg	*Amount found, mg	*Recovery, %
CEFTORIN	200	197.40	98.70
ZOSTUM-O	200	198.44	99.22

**Mean of three replicates*

Forced degradation studies/Specificity

Cefditoren pivoxil was eluted at 2.883 min and when exposed to stress conditions the drug peak retains its symmetry as well as its retention time and did not interfere with the degradant peaks indicating the method is specific. The representative chromatograms obtained during the assay of stressed samples were shown in Figure 4a-4e. Cefditoren pivoxil was totally destroyed in alkaline environment. The carboxylic moiety present in the drug structure may be responsible for the total degradation in the alkaline condition. Figure 4b shows the absence of Cefditoren pivoxil peak which has to be appeared at 2.8 min. In alkaline degradation study extra peaks were observed at 1.372 min and at 1.597 min indicating that Cefditoren pivoxil is highly sensitive towards alkaline conditions. During the oxidation, Cefditoren pivoxil has shown 40.32% degradation indicating that the drug is less sensitive towards oxidation in comparison to alkaline environment. In oxidative stress extra peaks were observed at 1.399 min and at 1.973 min indicating that Cefditoren pivoxil is sensitive towards oxidation conditions. Cefditoren pivoxil has undergone 3.51, 5.20 and 4.97% degradation during acidic, thermal and photolytic degradations respectively which is less than 10% (Table 6). In all the studies Cefditoren pivoxil has reported theoretical plates more than 2000 and the tailing factor less than 1.5 indicating that the proposed method is selective.

Table 6. Forced degradation studies of Cefditoren pivoxil

Stress Conditions	*Mean peak area	*Drug recovered %	*Drug decomposed %	Theoretical Plates	Tailing factor
Standard drug (Untreated)	3533668	100	-	2819.132	1.015
Acidic degradation	3409534	96.49	3.51	2800.820	1.006
Alkaline degradation	19166	0.54	99.46	2817.927	1.008
Oxidative degradation	2108888	59.68	40.32	2829.038	1.001
Thermal degradation	3350045	94.80	5.20	2828.947	1.011
Photolytic degradation	3357997	95.03	4.97	2913.039	1.023

**Mean of three replicates*

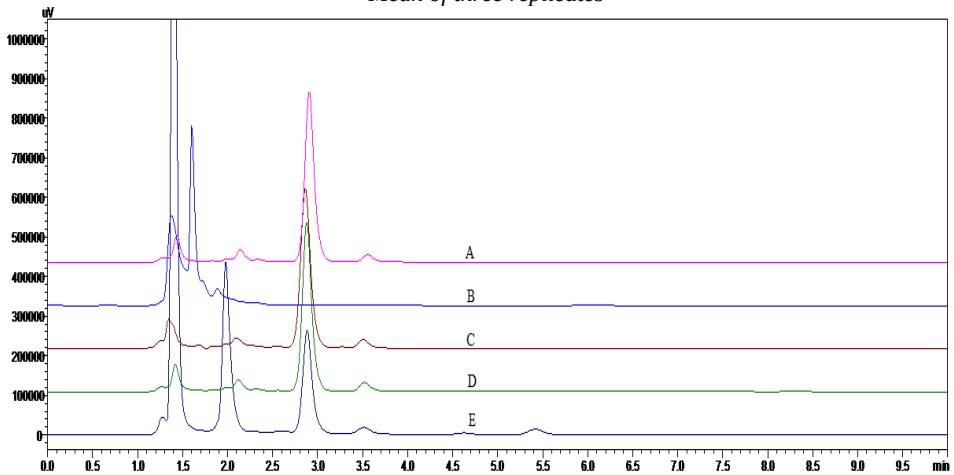

Figure 4. Typical Chromatograms of Cefditoren pivoxil on photolytic [A], alkaline [B], acidic [C], thermal [D] and oxidative [E] degradations

Conclusion

This stability-indicating and validated HPLC method is selective, precise, accurate and can be applied for the determination of Cefditoren pivoxil.

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