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SHORT COMMUNICATION

Development and validation of the stability-indicating LC–UV method for the determination of Cefditoren pivoxil

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KEYWORDS

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Abstract An isocratic RP-HPLC method was developed for the determination of Cefditoren pivoxil in pharmaceutical formulations using a C-18 column with water–acetonitrile (50:50, v/v) as mobile phase and flow rate 1.2 mL/min (UV detection at 218 nm). Linearity was observed in the concentration range 1.0–250 μg/mL (R^2=0.999) with regression equation y=24194x+10749. The forced degradation studies were performed by using HCl, NaOH, and H_2O_2, and thermal and UV radiation. Cefditoren pivoxil is more sensitive towards oxidation and alkaline conditions and resistant towards acidic and photolytic degradations. The method was validated as per ICH guidelines.

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1. Introduction

Cefditoren pivoxil (Fig. 1) is a third-generation semi-synthetic cephalosporin antibiotic for oral administration. The therapeutic potential of Cefditoren has been well documented to include broad-spectrum effective coverage against both gram-positive and gram-negative organisms to treat respiratory tract infections [1]. Chemically, Cefditoren pivoxil is (-)-(6R, 7R)-2,2-dimethylpropionyloxymethyl7-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(Z)-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate with empirical formula $C_{25}H_{28}N_6O_7S_3$ and molecular weight 620.73. There are various methods in literature for the determination of Cefditoren pivoxil in human plasma [2–4] and in pharmaceutical dosage forms using HPLC, UPLC and spectrophotometry [5–8] but this method is selective and specific. HPLC is the analytical technique that still dominates the pharmaceutical quality control in the industrial environment. The aim of this work was to develop a validated stability indicating HPLC method for the determination of Cefditoren pivoxil in pharmaceutical dosage forms.

2. Experimental

Cefditoren pivoxil standard (purity 99.50%) was obtained from Cipla Ltd., India. All other chemicals and solvents were

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obtained from Merck (India) and were of analytical grade. HPLC grade water was purchased from Qualigens (India).

Cefditoren pivoxil is available as tablets (Label claim: 200 mg) with brand names SPECTRACEF® (Aristo Pharmaceuticals, India), CEFTORIN® (Cipla Ltd., India) and ZOSTUM-O® (Zuventus, India). All chemicals were of an analytical grade and used as received.

Chromatographic separation was achieved by using a C-18 (250 mm × 4.6 mm i.d., 5 µm particle size) column of Shimadzu Model CBM-20 A/20 Alite, equipped with SPD M20A prominence photodiode array detector, maintained at 25 °C. Isocratic elution was performed using acetonitrile and water (50:50, v/v). The overall run time was 10 min and the flow rate of the mobile phase was 1.2 mL/min. The wavelength of the PDA detector was set at 218 nm. 20 µL of sample was injected into the HPLC system.

Stock solution was prepared by accurately weighing 25 mg of Cefditoren pivoxil in a 25 mL amber volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in a solvent mixture of acetonitrile and water (50:50, v/v; mobile phase). Solutions were filtered through a 0.45 µm membrane filter prior to injection.

Twenty tablets were purchased from the local market, weighed and crushed to a fine powder. Powder equivalent of 25 mg Cefditoren pivoxil was accurately weighed into a 25 mL volumetric flask, made up to volume with mobile phase, sonicated for 30 min and filtered. The filtrate was diluted with mobile phase as per requirement. The solutions were filtered through a 0.45 µm nylon filter before injections.

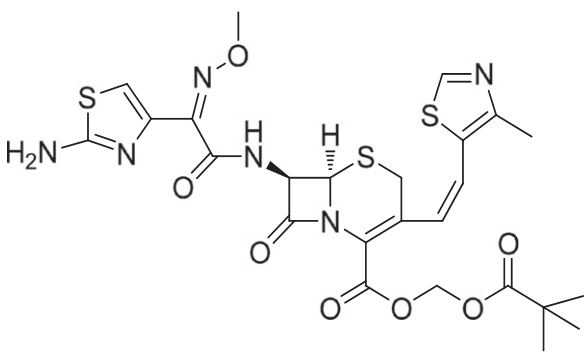

Figure 1 Chemical structure of Cefditoren pivoxil.

2.1. Forced degradation studies/specificity

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method [9]. 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 °C in a thermostat and then diluted with mobile phase to give a final concentration of 100 µg/mL.

The acidic and alkaline degradations of Cefditoren pivoxil in aqueous solutions were studied in hydrochloric acid (0.1 M) and in sodium hydroxide (0.01 M) at 80 °C and the stressed samples were instantly cooled with a mixture of ice and water, neutralized and diluted with mobile phase. Oxidation was conducted by using 2.0 mL of 30% H_2O_2 solution whereas photolysis was performed by exposing the drug solution to UV (365 nm) for 6 h in a UV light chamber.

2.2. Method validation

The method was validated for the following parameters: system suitability, linearity, limit of quantification (LOQ) and limit of detection (LOD), precision, accuracy, selectivity and robustness [10].

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the assay analyte (1, 5, 10, 20, 50, 100, 150, 200 and 250 µg/mL). 20 µL of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted.

Table 1 Linearity of Cefditoren pivoxil.

Conc. (µg/mL)	[a]Mean area ± SD (n = 3)	[a]RSD (%)
1	24224.00 ± 51.35	0.21
5	128880.00 ± 417.57	0.32
10	240569.00 ± 680.81	0.28
20	484027.00 ± 2405.61	0.49
50	1252384.00 ± 4070.24	0.33
100	2443214.00 ± 6694.41	0.27
150	3672705.00 ± 5215.24	0.14
200	4844477.00 ± 15889.88	0.33
250	6033216.00 ± 13695.40	0.23

[a]Mean of three replicates.

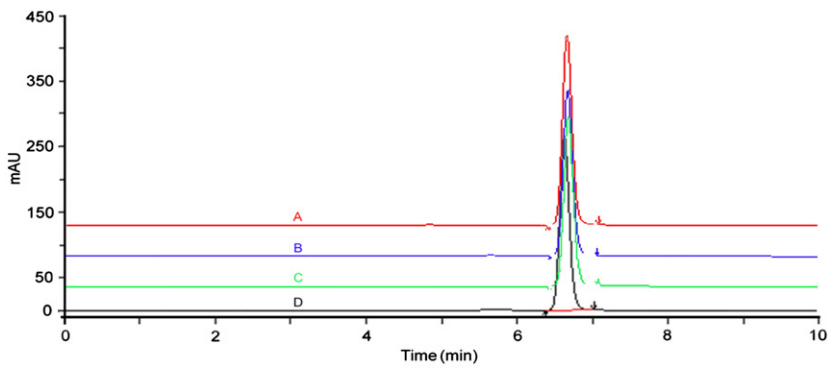

Figure 2 Representative chromatograms of Cefditoren pivoxil (100 µg/mL) (A), SPECTRACEF® (200 mg) (B), CEFTORIN® (200 mg) (C) and ZOSTUM-O® (200 mg) (D).

The intra-day and inter-day precision of the assay method was evaluated at three concentration levels (10, 20 and 50 μg/mL; $n=3$) and the % RSD of three obtained assay values on three different days was calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method and the study was carried out in triplicate at 36, 40 and 44 μg/mL.

The robustness of the assay method was established by introducing small changes [9] in the HPLC conditions which included wavelength (216 and 220 nm), percentage of acetonitrile in the mobile phase (48% and 52%) and flow rate (1.1 and 1.3 mL/min). Robustness of the method was studied using six replicates at a concentration level of 20 μg/mL of Cefditoren pivoxil.

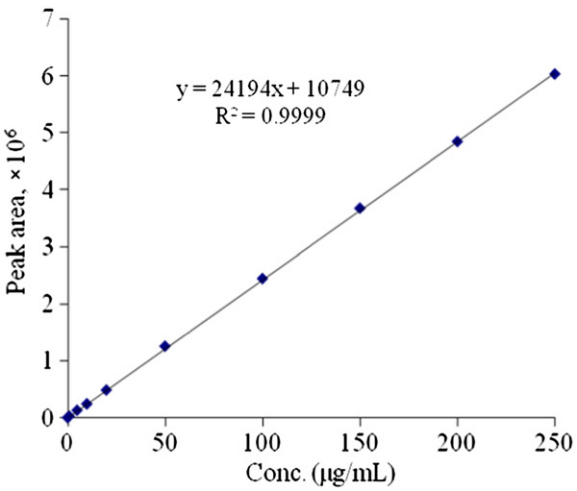

Figure 3 Calibration curve of Cefditoren pivoxil.

3. Results and discussion

The present method is a stability indicating RP-HPLC method which was not reported earlier and also specific because the drug peak was well separated even in the presence of degradation products. The representative chromatogram obtained for the drug is shown in Fig. 2A. The representative

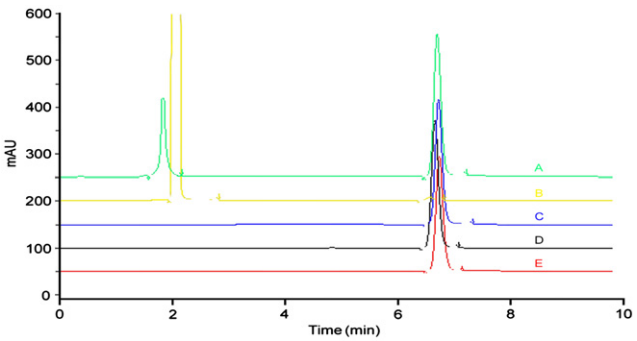

Figure 4 Representative chromatograms of Cefditoren pivoxil (100 μg/mL) on alkaline (A), oxidative (B), acidic (C), thermal (D) and photolytic (E) degradations.

Table 2 Intra-day and inter-day precision studies of Cefditoren pivoxil.

Sample no.	Conc. (μg/mL)	Intra-day precision		Inter-day precision	
		Mean[a] ± SD	RSD (%)	Mean[a] ± SD	[a]RSD (%)
1	10	240562.00 ± 575.53	0.24	240160.30 ± 1210.88	0.50
2	20	483649.33 ± 488.24	0.10	484082.30 ± 2122.54	0.44
3	50	2407203.00 ± 7674.96	0.32	2398627.00 ± 13779.59	0.58

[a]Mean of three replicates.

Table 3 Accuracy–recovery study of Cefditoren pivoxil by standard-addition method.

Sample no.	Spiked concentration (μg/mL)	[a]Measured concentration (μg/mL)	Recovery[a] (%)	[a]RSD (%)
1	16 (80%)	15.93	99.56	0.1424
2	20 (100%)	19.87	99.35	
3	24 (120%)	23.91	99.62	

[a]Mean of three replicates.

Table 4 Analysis of Cefditoren pivoxil commercial formulation (tablets).

Sample no.	Formulation	Labeled claim (mg)	[a]Amount found (mg)	[a]Recovery (%)
1	CEFTORIN®	200	198.96	99.48
2	SPECTRACEF®	200	199.23	99.62
3	ZOSTUM-O®	200	199.64	99.82

[a]Mean of three replicates.

Table 5 Forced degradation studies of Cefditoren pivoxil.

Stress conditions	[a]Drug recovered (%)	[a]Drug decomposed (%)
Standard drug	100.00	–
Acidic hydrolysis	91.46	8.54
Alkaline hydrolysis	68.17	31.83
Oxidative degradation	36.28	63.72
Thermal degradation	99.91	0.09
Photolytic degradation	99.95	0.05

[a]Mean of three replicates.

chromatograms obtained for Cefditoren pivoxil from the extracted marketed formulations are shown in Fig. 2B–D.

Linearity was evaluated in the concentration range 1.0–250 μg/mL (Table 1). The calibration curve (Fig. 3) was described by the equation, $y=24194x+10749$ with correlation coefficient 0.999 and RSD less than 0.5% (0.142–0.497). The % RSD in precision (Table 2), accuracy (Table 3) and robustness studies was found to be less than 2.0%, indicating that the method is precise, accurate and robust. The LOD and LOQ were found to be 0.2093 and 0.6351 μg/mL respectively. The assay results obtained from the marketed formulations are given in Table 4. The capacity factor was more than 2, theoretical plates were 8476 (more than 2000) and tailing factor was 1.12 (less than 2) for the Cefditoren pivoxil peak.

Typical chromatograms obtained following the assay of stressed samples are shown in Fig. 4A–E. Cefditoren pivoxil shows significant degradation in alkaline hydrolysis and oxidative stress conditions. 31.83% of Cefditoren pivoxil was degraded in alkaline conditions with an extra peak at 1.816 min and this may be due to the presence of the aminothiazol-4-yl-2-methoxyiminoacetamido moiety present in the chemical structure. Cefditoren pivoxil was also very much sensitive to oxidative conditions as 63.72% was decomposed (Table 5).

4. Conclusion

This stability-indicating and validated HPLC method is precise, accurate and robust, can be applied for the determination of Cefditoren pivoxil in pharmaceutical dosage forms.

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