

ESTIMATION OF A CEFDITOREN FROM A BIOLOGICAL FLUID

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Abstract

A simple, sensitive, and selective high-performance liquid chromatographic method for the determination of cefditoren in human plasma has been developed. In the presented work, a liquid-liquid extraction reversed-phase high-performance liquid chromatographic (LLE-RP-HPLC) method with UV detection has been validated. The internal standard method was used for quantification and experimental design was used to establish this analytical method. On a reverse phase C18 column, separation was accomplished using an isocratic mobile phase of acetonitrile: water (50:50, v/v). The linear range was estimated to be 1-8 µg/ml. Accuracy and precision both intra-day and inter-day (measured by relative standard deviation, and relative error, respectively) were consistently less than 7% and 5%. The stability of cefditoren was good and stable in human plasma under various conditions. It is suitable for both routine analysis and clinical studies and is cost-effective, simple to use, and has excellent reproducibility.

Keywords: Cefditoren, RP-HPLC, Liquid-Liquid Extraction, Validation.

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INTRODUCTION

Cefditoren(7-[(Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamidol-3(Z)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate) is the third-generation oral cephalosporin with a broad spectrum of activity against pathogens, including both gram-positive and gram-negative bacteria, and is stable to hydrolysis by many common β -lactamases.¹ It is approved for use in the treatment of acute exacerbations of chronic bronchitis(AECB), mild-to-moderate community-acquired pneumonia (CAP), acute maxillary sinusitis, acute pharyngitis/tonsillitis, and uncomplicated skin structure infection.²

Cefditoren is formulated as a pivoxil ester. After oral administration, cefditoren pivoxil is rapidly and completely hydrolyzed by intestinal esterases to form cefditoren (the active metabolite) and pivalate. After a single 400 mg dose, the mean maximum plasma concentration (C_{max}) ranges from 3.8 to 4.6 mg/l. Area under the plasma concentration-time curve (AUC) values ranged between 11.4 and 17.4 mg/l on average. The mean apparent volume of distribution following a single intravenous dose of 100 mg is 9.3 L. With a renal clearance of 4.1-5.6 L/h after multiple doses, cefditoren is mostly eliminated as an unchanged drug by the kidneys; its elimination half-life is 1.5 hours.^{1,3}

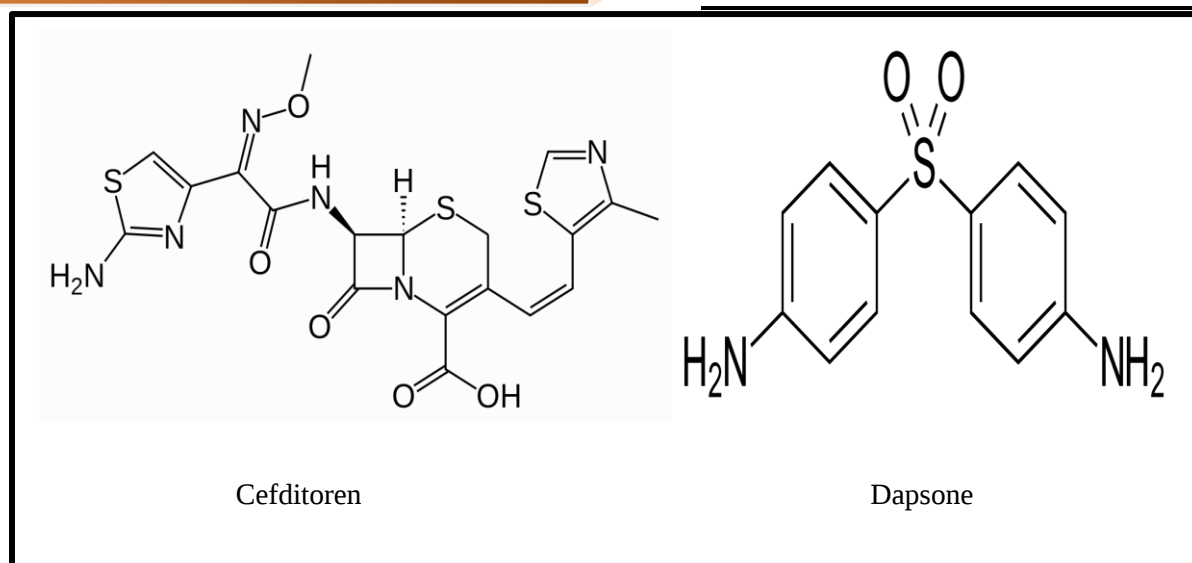


Fig. 1: Structure of Cefditoren and dapsone

A literature survey revealed that there are few chromatographic methods reported for the estimation of cefditoren from human plasma. The method reported includes HPLC-UV involving solid-phase extraction.⁴ Although solid-phase extraction equipment may not be available in most laboratories, this process is time-consuming and expensive. Thus, there is a need to develop a simple, economical, and rapid method for the estimation of cefditoren from human plasma. In this paper, we used an HPLC method with UV detection, and the results were satisfactory. This method used a simple liquid-liquid extraction that was highly reproducible, making it suitable for pharmacokinetic or bioequivalence studies. The method reported in this paper is a simple, rapid, cost-effective HPLC method to quantify the cefditoren with UV detection using liquid-liquid extraction. This method is fully validated as per US FDA guidelines.

EXPERIMENTAL

Materials and Method

Cefditoren's working standard was obtained as a gift sample from Zenvision pharma LLP. The dapsone working standard (internal standard) was obtained as a gift sample from Atul Ltd (Mumbai, India). HPLC grade methanol and acetonitrile (purity 99.9%) were procured from Molychem (Maharashtra, India). The HPLC-grade water was obtained by double distillation and passed through a 0.45 μm filter. All other reagents were of analytical grade (Molychem, Maharashtra).

Chromatographic Conditions

The HPLC system consisted of an Agilent -1200 series with a quaternary pump, UV detector, and Rheodyne manual sample injector fitted with a 20 μL loop. The Chemstation software, version B.02.03 was used for data acquisition. Separation was performed using a guard column ThermoFisher C18 column and analytical column Cosmos C18 (250 $\times$ 4.6 mm, 5 μm). The chromatographic analysis was performed at ambient temperature. The mobile phase consisted of the mixture of solvents acetonitrile: water in the ratio of 50:50 v/v. The prepared mobile phase was filtered through a 0.45 μm membrane filter and ultrasonically degassed prior to use. A UV detector

set at 270 nm was used to monitor the eluent, and the peak area was recorded using a chromatographic data system. The study was carried out at a constant flow rate of 1 ml/min.

Samples Preparation

Preparation of Stock Solution in Dilution Solution

Cefditoren and the internal standard (IS) for dapsone were prepared as separate stock solutions in methanol at 1 mg/ml. Working solutions were prepared by diluting the stock solutions with dilution solution.

Preparation of Stock Solution in the Plasma

For the calibration curve, six different concentrations (0.5, 1, 2, 4, 6, and 8 µg/ml) in fresh plasma were prepared by adding the required volume of working solution of analyte to blank plasma. The final plasma concentration for the internal standard was 0.5 µg/ml.

Extraction of Cefditoren from Plasma

An aliquot quantity of 1 ml human plasma was taken in a 15 ml stoppered glass tube and spiked with 0.5 µg cefditoren and 0.5 µg of dapsone. The spiking was done by the addition of 20 µl of 25 µl methanolic solution of cefditoren followed by vortex mixing for 1 min. The spiked plasma was acidified by the addition of 10 µl of acetic acid. 5 ml of diethyl ether was added. The tube was shaken on a reciprocating shaker at 100 strokes per mins for 45 mins. 3 ml of the organic layer was transferred to a separate tube and evaporated to dryness. The residues obtained upon evaporating to dryness were reconstituted with 250 µl of mobile phase and the same was injected into the HPLC system.

Method Validation

The proposed RP-HPLC method was approved in accordance with USFDA (CDER) guidelines.⁵ The developed method was validated in plasma for selectivity, limits of detection and quantification, linearity, accuracy and precision, recovery, and stability. Quality control samples were used for the assay of inter-day and intra-day accuracy and precision. Plasma calibration curves were generated by using the analyte to internal standard area ratio as the Y-axis and the analyte concentration (µg/ml) as the X-axis. Five replicates of the calibration curve were prepared to take each concentration five times. The linear regression equation ($y=mx+c$) was used to fit the spiked concentration and peak area of cefditoren. Precision is reported as % RSD of the estimated concentrations and accuracy is expressed as $[(\text{Interpolated concentration} - \text{nominal concentration}) / \text{nominal concentration}] \times 100$.

Calibration and Linearity

A calibration curve was constructed by plotting the response ratios with respect to analyte concentrations ranging from 1-8 µg/ml in human plasma. These calibration curves were generated over the course of five days. Using weighted (1/X) least squares regression analysis, linearity was assessed. A standard curve was found to be validated if the coefficient of correlation should be near 0.99 or better. The acceptability criterion for each back-calculate value, with the exception of LLOQ (20%), should not be higher than 15%. At least 75% of standards, including the LLOQ, and 67% of the QC samples (low, medium, and high), should comply with the abovementioned limits for the standard curve to be accepted.

Selectivity

To determine the amount to which endogenous plasma components may interfere with the analyte or the internal standard, five randomly selected blank human plasma samples were passed through the extraction method and chromatographed. The retention time of all analytes in standard blank samples was $\leq 20\%$ of the drug's area in the extracted LLOQ samples, and the peak area at the retention time of IS was $\leq 5\%$ of the IS area in the extracted LLOQ sample, as per the acceptance limit. It was determined that there were no significant interferences in any of the five samples.

Extraction Recovery

To evaluate the extraction recovery of the analyte, the peak areas of the drug from the prepared plasma quality control samples were evaluated. As low-quality control (LQC), and high-quality control (HQC) samples, respectively, $1\mu\text{g/ml}$, and $7\mu\text{g/ml}$ plasma samples were selected. The peak areas of the extracted LQC and HQC were compared to the absolute peak areas of the unextracted samples having the same drug concentration as 100%. Three replicates of each QC sample were used to assess the extraction recovery of cefditoren in order to achieve good extraction efficiency. Additionally, the extraction of IS was determined.

Accuracy and Precision

Accuracy and precision were also determined from LQC ($1\mu\text{g/ml}$), MQC ($4\mu\text{g/ml}$), and HQC (7Mg/ml). Three replicates of each concentration were analyzed on the same day to determine the within-run accuracy and precision of the method. To confirm the between-run accuracy and precision three replicates of each concentration on the first day morning and three replicates of each concentration on the second day and third days were analyzed.

Stability Study

Two studies were carried out to evaluate the stability of cefditoren in plasma: a short-term stability study and a freeze-thaw study. Plasma blank samples were spiked with cefditoren at a concentration of $1\mu\text{g/ml}$ (LQC), and $7\mu\text{g/ml}$ (HQC), and each concentration was carried out three times. A short-term stability test was performed at room temperature. After 12 hours at room temperature with cefditoren spiked plasma samples, the samples were extracted and then analyzed. For freeze-thaw stability, spiking samples were evaluated immediately after preparation and daily after three consecutive days of repeated freeze-thaw cycles at -20°C .

RESULTS AND DISCUSSION

Chromatography

Representative chromatograms of blank plasma and plasma spiked with cefditoren and IS are shown in Fig. 2 and 3, respectively. The retention time for the cefditoren and internal standard were 7.336 mins and 3.729 mins, respectively. Good separation of analyte and IS with low background noise was observed. For the analysis of the plasma sample, the chromatographic run time was 10 minutes. The peak of cefditoren was well resolved without any interference from endogenous material. The sensitivity (limit of quantification and detection) and specificity of the method was tested and no potential metabolites of the drug or any endogenous compounds were detected at the retention time of the drug. The study involved HPLC-UV with liquid-liquid extraction instead of solid-phase extraction reported in the paper earlier. The described chromatographic system was used to analyze both mobile phase and plasma solutions, and in both cases, i.e., mobile phase and plasma solutions, a linear connection between peak area and

concentration were observed. The reproducibility data and recovery data clearly show the accuracy and validity of the method, respectively.

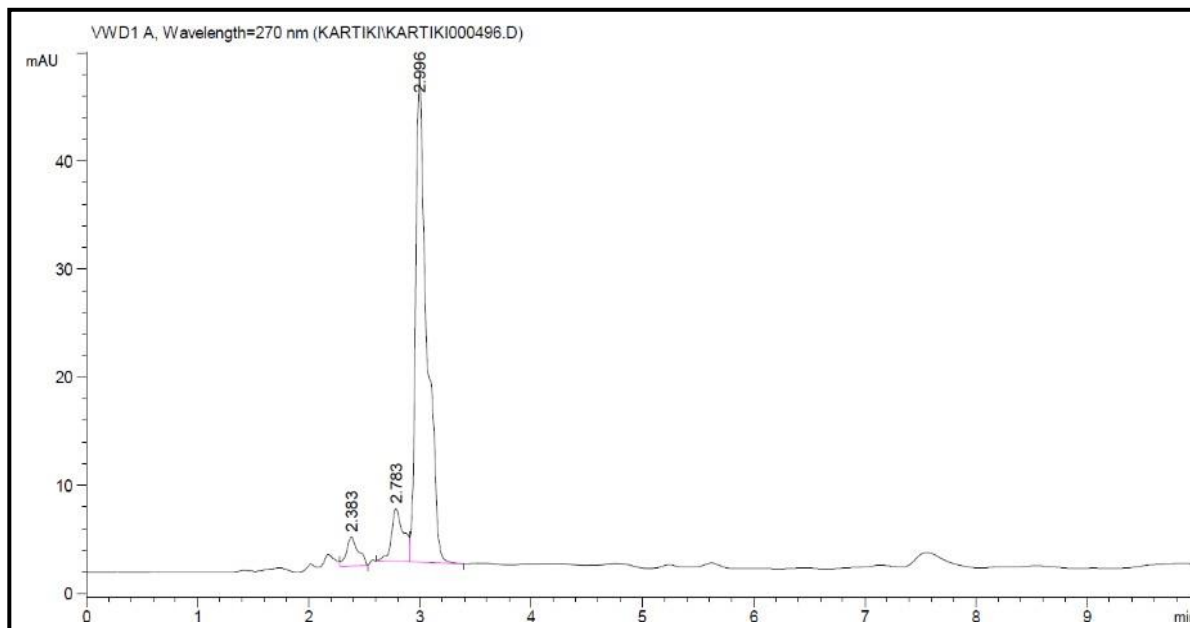


Fig. 2: Representative chromatogram of blank plasma

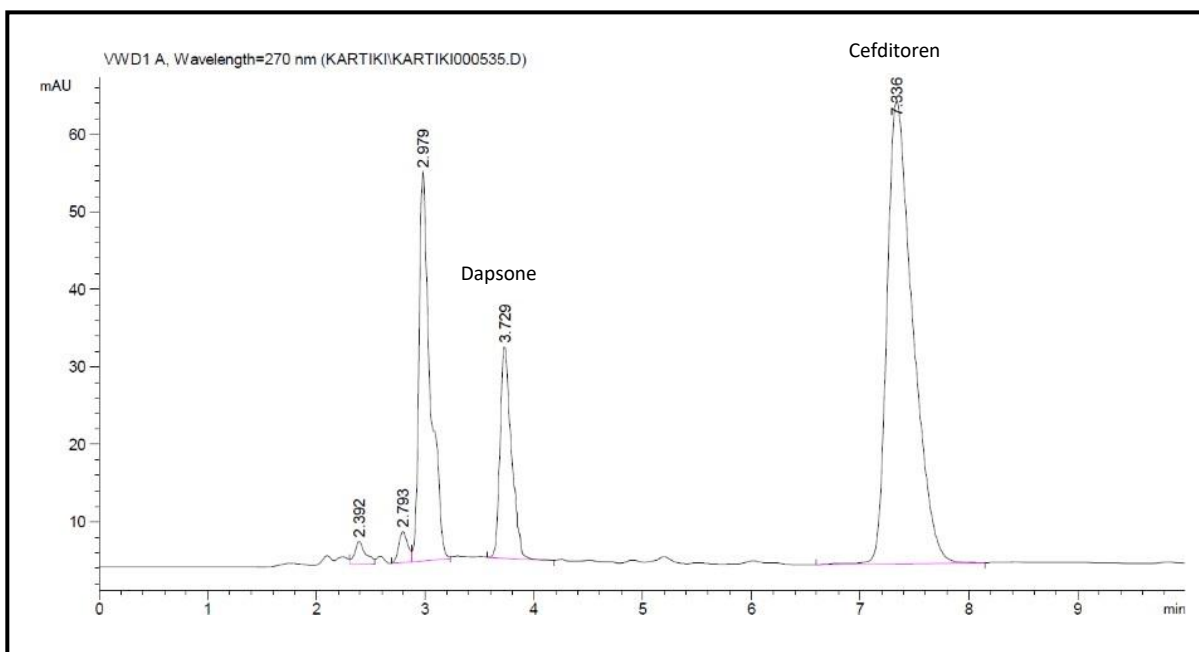


Fig. 3: Representative chromatogram of plasma spiked with cefditoren and internal standard

Linearity

The peak area ratio of cefditoren to IS in human plasma was linear with respect to the analyte concentration over the range of 1-8 µg/ml. Based on the analysis of the data using linear regression with/without intercepts and weighting factors, the calibration model was selected. The linear equation of the form $y = mx + c$ with weighting factor $1/X$ could be used to obtain the best fit for

the calibration curve. The calibration curve's weighted linear regression equation for the analyte was $y = 0.5151x - 0.0647$, where y represented the peak area ratio of the analyte to the IS and x represented its concentration. The correlation coefficients (r^2) for cefditoren were above 0.9867 from each standard curve of five separate runs.

Accuracy and Precision

The accuracy and precision of the method were assessed using spiked plasma standards at three different concentrations (1, 4, and 7 $\mu\text{g/ml}$). The intra-day and inter-day variations for cefditoren were calculated using data accumulated over a period of 3 days. Intra-day accuracy and precision were evaluated by replicate analysis of a spiked pooled plasma containing cefditoren at three different concentrations ($n=3$). Inter-day accuracy and precision were similarly evaluated ($n=3$). Results are presented in Table 1. These indicate the high accuracy and precision of the method.

Table 1: Accuracy and precision of cefditoren

Concentration added ($\mu\text{g/ml}$)	Intra-day ($n=3$)			Inter-day ($n=3$)		
	Concentration found (mean $\pm$ SD, $\mu\text{g/ml}$)	Precision (% RSD)	Accuracy (% RE)	Concentration found (mean $\pm$ SD, $\mu\text{g/ml}$)	Precision (% RSD)	Accuracy (% RE)
1	1.05 ± 0.05	4.78	5	1.02 ± 0.03	3.43	2
4	3.9 ± 0.25	6.4	-2	4.1 ± 0.20	4.88	2.5
7	7.3 ± 0.3	4.17	4.2	7.1 ± 0.12	1.8	1.4

Recovery

The recovery of cefditoren from plasma was analyzed using the method mentioned in the previous section. The recovery of cefditoren was assessed at two different concentrations. The extraction recovery of cefditoren at low and high-quality control samples was 89.52% and 87.22%, respectively. The recovery of internal standard was 81.06%

Stability

According to the procedure described in the previous section, the stability of cefditoren in plasma was assessed under a variety of conditions. Table 2 summarises all stability results. The test performed at room temperature showed that two QC samples were stable for 12 hrs. After exposing samples to three freeze-thaw cycles, no significant decrease in cefditoren concentration in plasma was found. It indicated that cefditoren was stable in human plasma under the conditions.

Table 2: Stability of cefditoren

Stability conditions	Concentration added ($\mu\text{g/ml}$)	Concentration found (mean, $\mu\text{g/ml}$)	Precision (% RSD)	% Nominal
Short-term stability	1	1.05	4.78	105
	7	7.3	4.17	104.2
Three freeze-thaw cycles	1	0.95	11.75	95
	7	7.3	3.43	104.2

CONCLUSION

The HPLC-UV method claims to employ liquid-liquid extraction to prepare samples, is effective for quantifying cefditoren in human plasma samples, and has been completely validated in

accordance with US-FDA guidelines. The validation data demonstrated high accuracy and precision. This paper describes a rapid and precise HPLC method for quantifying cefditoren that could be employed to monitor plasma concentrations during human clinical pharmacokinetic studies.

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REFERENCES

1. Wellington, K., Curran, M. P., Cefditoren pivoxil: A review of its use in the treatment of bacterial infections. *Drugs* 64, 2597 (2004)
2. Wellington, K., & Curran, M. P. (2005). Spotlight on cefditoren pivoxil in bacterial infections. *Treatments in Respiratory Medicine*, 4(2), 149-152.
3. Guay, D. R. P., Review of cefditoren, an advanced-generation, broad-spectrum oral cephalosporin. *Clin. Ther.* 23, 1924 (2001)
4. Nirogi, R. V., Kandikere, V. N., Shrivastava, W., & Mudigonda, K. (2006). Quantification of the cephalosporin antibiotic cefditoren in human plasma by high-performance liquid chromatography. *Arzneimittelforschung*, 56(04), 309-313.
5. US Department of Health and Human Services. (2001). Bioanalytical method validation, guidance for industry, 2001. <http://www.fda.gov/cder/guidance/4252fnl.Htm>.