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HPLC method for Pharmacokinetics of cis and trans isomer of cefprozil diastereomers in human plasma

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Abstract

Using an high-performance liquid chromatography (HPLC) system and UV detection, a simple and precise analytical procedure was developed to quantify levels of *cis* and *trans* isomer of cefprozil diastereomers in human plasma. Cefprozil was extracted from plasma samples into 10% perchloric acid. The HPLC system included an acetonitrile-0.05M monopotassium phosphate water (adjust pH to 3.05 with glacial acetic acid) (12:88, v/v), flow rate of 1 mL/min, Ultimate™ XB-C18 column,(5μm，4.6×150mm), and UV detection at 280 nm. The regression of the spiked calibrator curves were linear from 0.02–10 μg/mL and 0.02–1 μg/mL (r^2 =0.9951, 0.9950), respectively. The lower limits of quantification were 0.02 μg/mL, the inter- and intra-day precisions (RSD) were lower than 9.0%, and the extraction recoveries were all more than 90%. The *cis* and *trans* isomers of cefprozil were stable under a variety of storage and process conditions. To demonstrate utility, the pharmacokinetic parameters of *cis* and *trans* isomer of cefprozil were measured in twenty human after oral administration of 500mg cefprozil. The assay was sensitive, accurate and convenient, and can be used for the determination of cis and trans isomer of cefprozil in human plasma.

Key words: cefprozil; *cis* and *trans* isomer; pharmacokinetics; HPLC

1. Introduction

Cefprozil, is considered a second-Generation cephalosporin. It is an oral cephaloporin with a broad in vitro spectrum of resistant against both Gram-positive (GP) and Gram–negative bacteria (GN) by inhibiting bacterial peptidoglycan cell wall synthesis and penicillinase [1-3].

Cefprozilisa mixture of *cis* and *trans* isomers in an approximately 9:1 ratio. The major isomer has the 3-(1-propenyl) substituent in the *cis* configuration. The chemical stability and the pharmacological activity of resistant GP, streptococcus and staphylococcus activity of *cis* isomer is equal to *trans* isomer, but the pharmacological activity of resistant GN of *cis* isomer is 6-8 times as same as *trans* isomer [2].

The principal aim of this study is to develop and validate a method for quantifying cefprozil diastereomers in human plasma. Because both isomers exhibit antimicrobial activities, it is necessary to determine the pharmacokinetics of each isomer respectively. To date, there have been few published methods for the measurement of cefprozi levels in biological tissue [4,5].

Some studies had been developed for the determination and quantification of cefprozil diastereomers separately [4,5]. Although these assays suggested a highly specific and precise bioanalytical method, there were some limitations such as run times were longer than 15 min, and the extract method was complex. Moreover, the low limit of quantification was established to be 100 ng/mL and 20 ng/mL for *cis* and *trans* isomers. However, the MICs of cefprozil against upper

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respiratory infections were in the range of 0.016-4 mg/L which was important considerations for therapy [6], a suitable quantification method was needed to be developed to lower the limit of quantification and improve the sensitivity of cefprozil.

In this study, a simple, facility, and efficient HPLC method was developed, validated, and applied to the pharmacokinetics of *cis* and *trans* isomers of cefprozil in human plasma using fluconazol as an internal standard. The pharmacokinetic parameters would provide the basis for clinical rational drug use.

2. Experimental

2.1 Chemicals and reagents

The cis (LOT: H0E054) and trans (LOT: G0D341) isomers of cefprozil standards (Figure 1) were provided by USP ROCKVILLE. The internal standard, fluconazol (LOT: 100314-200503) were provided by the national institutes for food and drug control (Figure 1). Acetonitrile (HPLC grade, LOT: H31825) was purchased from J.T.BAKER (USA). Glacial acetic acid（HPLC grade, LOT: 605041) was purchased from TEDIA company. Potassium dihydrogen phosphate (analytical grade, LOT: 20080313) was purchased from Laiyang fine chemical industry of Shandong Province. Perchloric acid (analytical grade, LOT: 20020814) was purchased from east chemical industry factory of Tianjin. Blank plasma was purchased from Qilu Hospital of Shandong University.

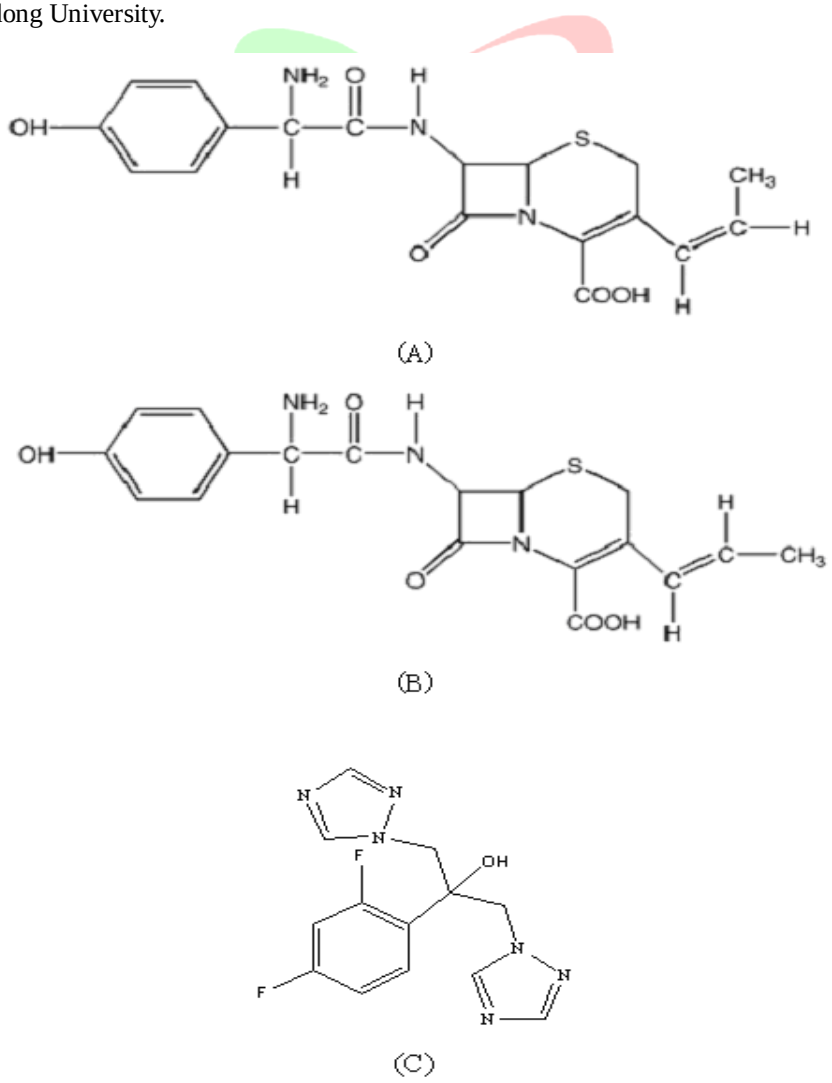

Figure 1 Chemical structures of (A) *cis* isomer of cefprozil, (B) *trans* isomer of cefprozil, and (C) fluconazol

2.2 Preparation of standard solutions

Stock solutions of *cis* and *trans* isomers of cefprozil and IS were prepared in methanol at the concentration levels of 1.0 mg/mL. A series of standard solutions of *cis* and *trans* isomer of cefprozil at the concentration of 1, 10, 100 μg/mL were obtained by step-wise dilution of the stock solution with mobile phase. Standard solution of IS at the concentration of 200 μg/mL were obtained by dilute the stock solution with mobile phase. All the solutions were stored at 4℃, and were brought to room temperature before use.

The standard calibration samples of cefprozil were prepared by adding an appropriate volume of the standard solutions to 0.5mL human plasma, giving the final concentrations of 0.02, 0.05, 0.1,0.5,1, 5, 10 μg/mL of *cis* isomers , and 0.02, 0.05, 0.1, 0.2, 0.5, 1 μg/mL of *trans* isomers.

Quality control (QC) samples at the concentrations of 0.05, 1, 8 μg/mL of cis isomers and 0.05, 0.2, 0.8 μg/mL of trans isomers were prepared in the same way as the calibration standard and stored at 4℃.

2.3 Chromatographic conditions

The essebtial part of the HPLC system consisted of a Model Waters 515 pump, a Model Waters 717 autosampler, and a Model Waters 2487 Dual Wavelength Absorbance Detector.

The analtytic column was Ultimate TM XB-C18 column (5μm，4.6×150mm). The separation was carried out with the mobile phase consisting of acetonitrile-0.05M monopotassium phosphate water (adjust to pH 3.05 with glacial acetic acid) (12:88, v/v) at a flow rate of 1.0 mL/min. The chromatograms were monitored at 280 nm, and the column temperature was room temperature.

2.4 Preparation of the samples

To 0.5 mL plasma sample, 10 μL IS and 100 μL 10% perchloric acid were added. The solution was thoroughly vortex-mixed for 2min, and centrifugated at 10800 rpm for 5 min. 20 μL supernatant were injected into the HPLC system for analysis.

2.5 Preparation of biological samples

The study was approved by the human ethics committee of Qilu Hospital of Shandong University.

Ten healthy male volunteers (aged 18-28 years) participated in the study. All subjects gave written informed consent. The studied men had no history of drug hypersensitivity, and they didn't take any medication in two weeks before entering and during the study. They were determined to be healthy by medical history, physical examination, and blood chemistry test.

After an overnight fast, the subjects received an oral dose of 500 mg cefprozil with 200 mL water at 7 a.m. Blood samples were collected into heparinized tubes at 0, 0.25，0.5，0.75，1.0，1.5，2.0，3.0，4.0，5.0, 6.0，8.0，10.0 and 12.0 h after dosing. Blood samples were centrifuged, and the plasma was separated. The plasma samples were kept frozen at -20℃ until assayed.

3. Results

3.1 Method validation

3.1.1. Specificity The specificity of the method was validated by comparing chromatograms of *cis* and *trans* isomers cefprozil and IS, blank plasma, blank plasma spiked with *cis* and *trans* isomers cefprozil and IS, and plasma from the healthy male volunteers (9-4) after oral 500 mg cefprozil capsules. Retention times for *cis* and *trans* isomer of cefprozil diastereomers and fluconazol (internal standard) were 5.11, 7.38 and 10.36 min, respectively. Typical chromatograms were shown in Figure 2.

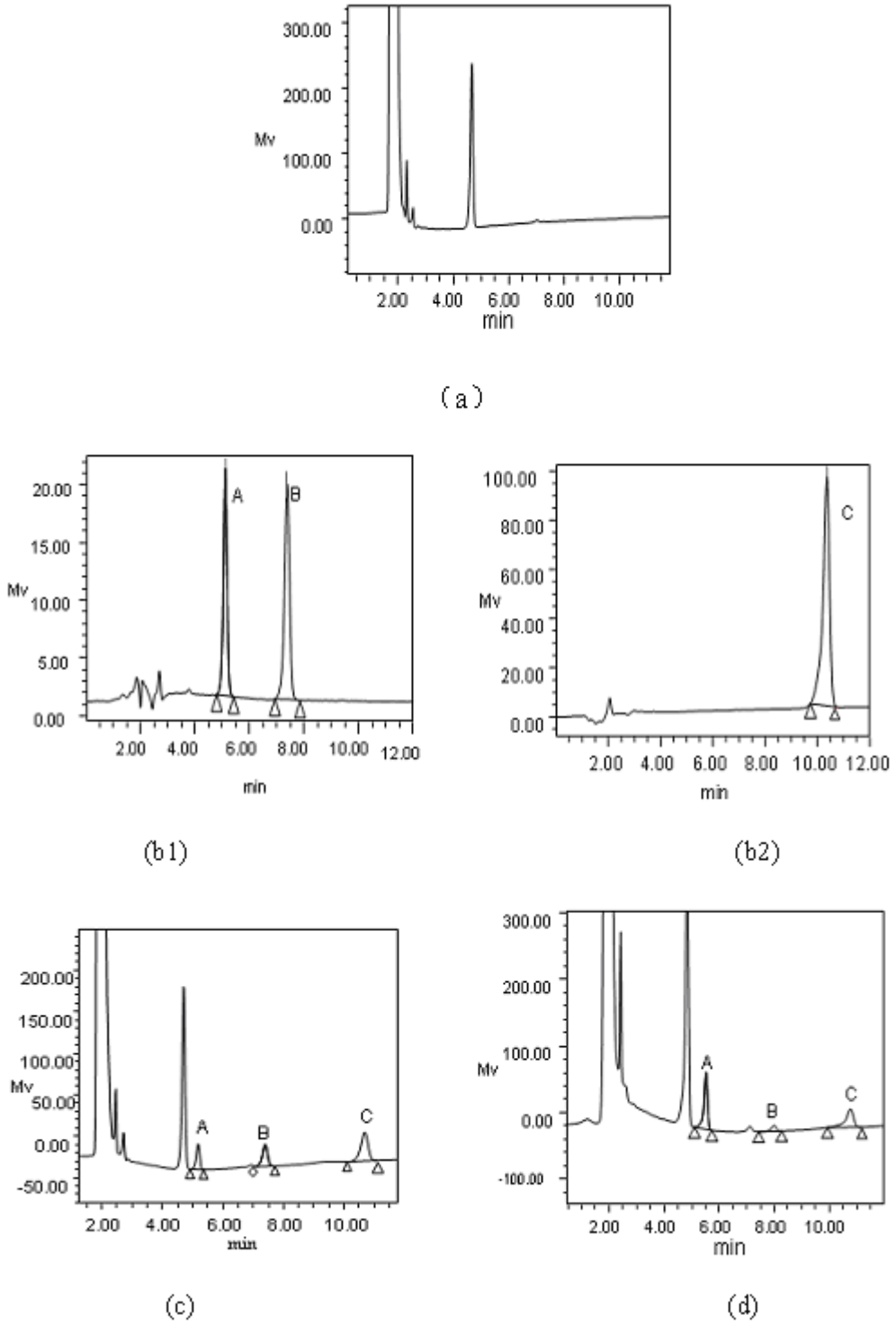

Figure 2 Chromatograms of blank plasma (a), *cis* and *trans* isomer of cefprozil (b1) and IS (b2), blank dog plasma spiked with *cis* and *trans* isomer of cefprozil and IS (c), plasma from a healthy male volunteer (9-4) at 1h after oral cafprozil capsules with IS (d); A: *cis* isomer of cefprozil, B: *trans* isomer of cefprozil, C: IS

3.1.2. Low limit of quantification The lower limit of quantification (LLOQ) was defined as the lowest concentration of the calibration curve at which both precision and accuracy were less than 20%. The current assay offered an LLOQ of 0.02

μg/mL in plasma of *cis* and *trans* isomers of cefprozil which were sufficient for the study of pharmacokinetics following a single oral administration of cefprozil.

3.1.3. Extraction recovery The extraction recoveries of *cis* and *trans* isomers of cefprozil were determined at three QC levels by comparing the peak areas of extracted plasma samples with those of extracted blank plasma spiked with corresponding concentrations. The extraction recovery of IS was also evaluated in the same way. The results showed the mean extraction recoveries of *cis* and *trans* isomers of cefprozil and IS were more than 90%.

3.1.4. Linearity of calibration curve The calibration curves of cefprozil were produced by daily analysis of the spiked calibration samples at seven concentrations in the range of 0.02 to 10 μg/mL of *cis* isomer and 0.02 to 1 μg/mL of *trans* isomer. The linearity of calibration curves was assessed by linear regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$). Therefore, the calibration curves were expressed by the equation of $Y = 1.71X + 7.69\times10^{-3}$ (R^2=0.9951) and $Y = 2.12X - 7.38\times10^{-3}$ (R^2=0.9950), in which y represented the peak-area ratio and x represented the concentration ratio of *cis* or *trans* isomer of cefprozil to IS.

3.1.5. Accuracy and precision Accuracy, intra- and inter-day precisions were assessed with 5 replicates at concentrations of 0.05, 1, 8 μg/mL of *cis* isomer and 0.05, 0.2, 0.8 μg/mL of *trans* isomer on three constant days. The intra- and inter-day precisions were expressed by relative standard deviation (RSD). The accuracy of the method was determined by calculating the percentage deviation observed in the analysis of QC samples. The results were shown in Table 1. It was suggested that the method was accurate and reproducible for the determination of cefprozil in human plasma.

Table 1 Inter- and intra-day precision and accuracy of *cis* and *trans* isomer of cefprozil (n=5)

	Concentration	Precsion (%)		Acurracy (%)	
	(μg/mL)	Intra-day	Inter-day	Intra-day	Inter-day
Cis-cefprozil	0.02	5.90		90.00	
	0.05	2.71	5.51	107.63	103.62
	1	5.46	5.21	109.06	104.42
	8	4.31	3.01	93.58	94.70
Trans-cefprozil	0.02	9.48		95.00	
	0.05	8.55	8.05	97.01	102.73
	0.2	7.73	8.89	107.97	99.32
	0.8	3.74	5.88	106.09	103.53

3.1.6. Stability The stability of *cis* and *trans* isomer of cefprozil was investigated under a variety of storage and process conditions. The analytes were found to be stable after two freeze-thawing cycles (-20 ˚C to room teperature), and in the final plasma extract at room teperature for 8h. No signs of degradation were found under the freeze condition (-20 ˚C) for 30 days. The results were shown in Table 2.

Table 2 stability of the samples

	Concentration (μg/mL)	Fresh sample	−20℃, 30 days	Two freeze-thawing	Stored at room temperature for 8h
Cis-cefprozil	0.05	106.23	95.95	103.50	106.35
	1	107.67	106.32	102.90	105.90
	8	98.43	104.64	101.44	101.40
Trans-cefprozil	0.05	90.39	104.79	87.80	101.25
	0.2	107.29	97.40	93.62	94.36
	0.8	106.63	110.90	100.55	98.54

3.2. Pharmacokinetic parameters

The established HPLC method was successfully used to investigate the pharmacokinetics of the *cis* and *trans* isomer of cefprozil in human plasma, and Drug And Statistic Version 2.0 (DAS, 2.0, P.R. of China) was used to calculate the main pharmacokinetic parameters as shown in Table 3. The mean plasma concentration–time curves of *cis* and *trans* isomers of cefprozil were shown in Figure 3 and 4.

Tables 3 Main pharmacokinetic parameters of *cis* and *trans* isomer of cefprozil in human plasma (mean±SD, *n*=10)

Parameter	*Cis*-cefprozi	*Trans*-cefprozi
V (L/kg)	41.60 ±11.59	41.66±11.73
$t_{1/2}$(h)	1.39 ±0.38	1.11 ±0.38
CL(L/h/kg)	21.02 ±4.17	26.67 ±6.05
Tmax(h)	1.83 ±0.52	1.75 ±0.30
AUC_{0-t}(mg/L*h)	22.13 ±4.57	1.85 ±0.38
$AUC_{0-\infty}$(mg/L*h)	22.25 ±4.60	1.97 ±0.45
MRT_{0-t}(h)	2.82 ±0.42	2.41 ±0.31
$MRT_{0-\infty}$(h)	2.89 ±0.46	2.62 ±0.50
Cmax(mg/L)	7.20 ±1.24	0.74 ±0.24

*T*1/2: terminal elimination half-life; *V*: apparent volume of distribution; CL: clearance; AUC: area under the concentration–time curve; MRT: mean residence time; *C*max: peak concentration; *T*max: time of peak concentration.

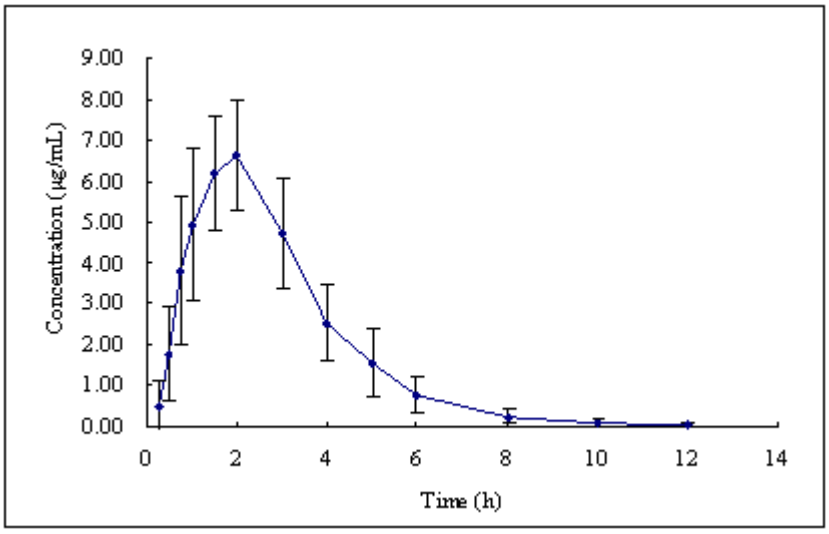

Figure 3 Mean plasma concentration–time curves of *cis*-cefprozi (mean±SD, *n* = 10)

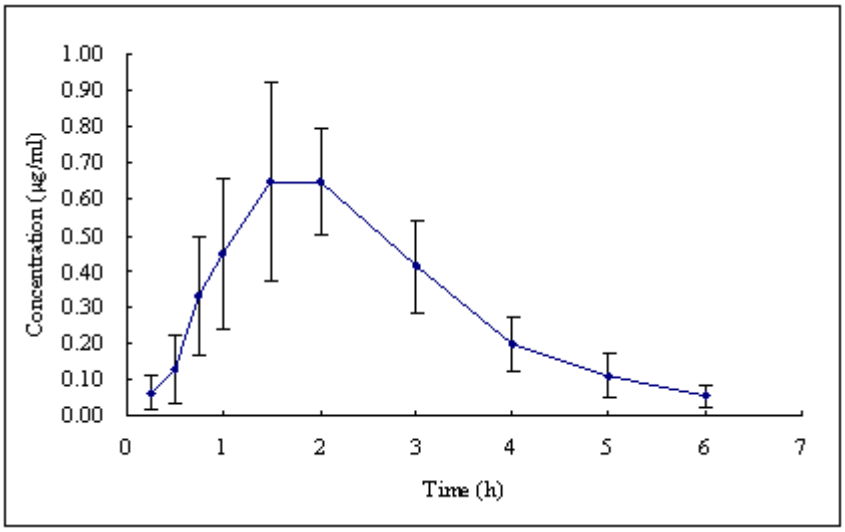

Figure 4 Mean plasma concentration–time curves of *trans* -cefprozi (mean±SD, *n* = 10)

4. Safety evaluation

In the study, only one subject had nausea which continued for 1.5 hours but no affect to the trial. The medical results of 10 subjects after the study were normal.

5. Discussion and conclusions

A simple, facility and shortcut HPLC method was developed, validated, and applied to the pharmacokinetics of cis and trans-isomer of cefprozil diastereomers in human plasma after oral administration at dose of 500 mg.

Ultimate TM XB-C18 column (5μm，4.6×150mm) was selected due to its excellent separation ability for cis and trans isomers, longer service life, and low expenses. Various mobile phase combinations of methanol and acetonitrile with different monopotassium phosphate and PH value were investigated to optimize sensitivity, speed and peak shape. Acetonitrile gave a better response than methanol, and 0.05M monopotassium phosphate water (adjust to pH 3.05 with glacial acetic acid) improved peak shapes and the stability of cis and trans isomers of cefprozil.

In the study, liquid-liquid extraction and protein deposition were tested to extract *cis* and *trans* isomers of cefprozil. Liquid-liquid extraction was discarded because of impurities interference, low extraction recovery, and instability of cefprozil.

Protein deposition method which was simple and facility, was used to dispose the plasma samples. Methanol, acetonitrile, and perchloric acid were tested as the precipitator. Finally, methanol and acetonitrile were discarded because of impurities interference, and 10% perchloric acid was chosen as the precipitator. The extraction procedure was simpler, more rapid, and higher recovery than the reported method [5].

Cefprozil consists of *cis* and *trans* isomers in an approximately 90:10 ratio. Since both isomers exhibit antimicrobial activities, the study analyzed the pharmacokinetic parameters of both isomers. The results showed that the pharmacokinetic parameters were no differences between *cis* and *trans* isomers except AUC and Cmax. The AUC values of *cis* isomer in plasma were about twelfth of that of *trans* isomer, and the Cmax values of *cis* isomer were about tenth of that of *trans* isomer. The result suggested that the metabolic processes of *cis* and *trans* isomer were approximately similar, and there were no transformation between *cis* and *trans* isomer in body.

Cefprozil was a second-generation cephalosporin frequently used in the treatment of community-acquired respiratorytract infections. The MICs of cefprozil against upper respiratory infections were in the range of 0.016-4 mg/L which was important considerations for therapy [6]. In this study, the plasma concentration of *cis* isomer susutained higher than 0.02 μg/mL for 12h, and of *trans* isomer susutained for 6h, which showed that the cefprozil had strong inhibition effect on sensitive bacteria. The results can be used to guide clinical medication for different upper respiratory infections.

The assay was sensitive, accurate and convenient, and can be used for the determination of cis and trans isomer of cefprozil in human plasma. The major advantages of the assay were lower LLOQ, simple sample preparation and a short run time. The metabolic processes of *cis* and *trans* isomer were approximately similar, and there were no transformation between the metabolism of *cis* and *trans* isomer. The results can be used to guide clinical medication for different upper respiratory infections.

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