

DEVELOPMENT AND VALIDATION OF A DENSITOMETRIC HPTLC METHOD FOR QUANTITATIVE ANALYSIS OF CEPPODOXIME PROXETIL IN HUMAN PLASMA

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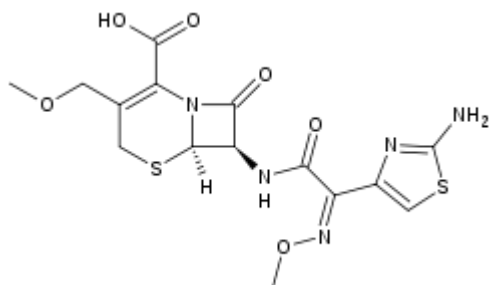
ABSTRACT

A simple, specific, accurate and precise high-performance thin-layer chromatographic method for analysis of Cefpodoxime proxetil (CP) in human plasma has been developed. The method use aluminium plates coated with silica gel 60 F₂₅₄ as stationary phase and chloroform-methanol-toluene 3:3:4 (v/v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 290 nm. The CP was satisfactorily resolved from plasma peaks with R_F values 0.65 ± 0.05. The respective calibration plot was found to be linear over the range 100-600 ng band⁻¹ for Cefpodoxime proxetil, respectively. This method has been successfully validated and applied for the analysis of drug in human plasma. The recovery of Cefpodoxime Proxetil from human plasma using the described precipitation procedure was about 94.75 %. The method was validated for precision, accuracy, specificity, recovery and stability.

Keywords: High-performance thin-layer chromatography, Cefpodoxime proxetil, Densitometry.

INTRODUCTION

Cefpodoxime proxetil (6R, 7R)-7-[[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxy imino-acetyl] amino]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid is a cephalosporin antibiotic used to treat a variety of bacterial infections¹.



Cefpodoxime proxetil is indicated for the treatment of patients with mild to moderate infections caused by susceptible strains of the designated microorganisms in the conditions listed below. Recommended dosages, durations of therapy, and applicable patient populations vary among these infections. Cefpodoxime proxetil is a new oral esterified cephem antibiotic with a broad antibacterial spectrum. The dissolution of cefpodoxime proxetil is pH dependent.

Cefpodoxime exhibits potent, broad-spectrum activity against gram-negative and gram-positive bacteria including antistaphylococcal activity. It is highly stable in the presence of β -lactamase enzymes. As a result, many organisms, which produce β -lactamase and are therefore resistant to penicillin and some cephalosporins, may be susceptible to cefpodoxime. Cefpodoxime is indicated for the treatment of patients infected with susceptible strains of microorganisms which include a wide range of gram-positive & gram-negative bacteria. It is highly stable in presence of β -lactamase enzyme, so it is more effective against gram-positive bacteria than other 3rd generation oral Cephalosporins.

Cefpodoxime is a semisynthetic oral third generation cephalosporin. It has a broad spectrum of activity, efficacy and safety profile which makes it ideal for treatment of many community acquired infections in the era of increasing drug resistance. Since 1989 oral third generation cephalosporins that have been introduced in the market are cefpodoxime, cefixime, cefdinir, and

cefprozil. In vitro susceptibility testing which determines the minimum concentration necessary for a particular antibiotic to inhibit or kill most strains of bacterial species and pharmacodynamic modeling are useful but have limitations. Antibiotics with lower side effect profile, infrequent dosing, good palatability in suspension and short duration of treatment may lead to better outcomes. Cefpodoxime fulfills most of these criteria.

Cefpodoxime is bactericidal and acts by inhibition of bacterial cell wall synthesis. It passes through porin channels in the bacterial cell wall and binds to the penicillin binding proteins (PBP) in the cell membrane. This leads to reduced synthesis of peptidoglycans and results in damage to cell wall².

Literature survey reveals several HPLC methods for the determination of CP in plasma, sinus mucosa and in pharmaceutical dosage form as a single drug or in combination with other drugs³⁻⁶. Specific RP-HPLC method for quantification of cefpodoxime proxetil in rat⁷, Voltametric and HPTLC methods have been reported for the estimation of CP in formulation⁸⁻¹⁰. This paper describes a simple, specific, accurate, precise and sensitive HPTLC method for determination of cefpodoxime proxetil in human plasma. The proposed method was optimized and validated in accordance with US-FDA guidelines¹¹.

MATERIALS AND METHODS

Reagents and Chemicals

Methanol, toluene, chloroform (all AR grade), Acetonitrile HPLC grade were obtained from Thomas Baker (chemicals) Pvt. Limited (Mumbai, India). Standard bulk drug samples of Cefpodoxime proxetil by Cadila Pharmaceuticals Ltd. (Ahmedabad, India).

Instrumentation and Chromatographic Conditions

Chromatography was performed on 10 cm X 10 cm aluminum plates precoated with silica gel 60 F₂₅₄ (E. Merck, Darmstadt, Germany). Before use the plates were prewashed with methanol and activated at 110 °C for 5 min. Samples were applied to the plates as bands 6 mm wide and 6.8 mm apart by means of a Camag (Switzerland) Linomat 5 sample applicator equipped with a 100 μ L syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a 10 cm X 10 cm twin trough glass chamber (Camag) using chloroform-methanol-toluene (3:3:4 v/v/v) as mobile phase after saturation of the chamber with mobile phase vapor for 25 min.

The development distance was 9 cm and the development time approximately 30 min. After chromatography the plates were dried in a current of air by use of a hair dryer. Densitometric scanning was performed with a Camag TLC Scanner 3 at 290 nm for all measurements. The scanner was operated by Wincats software Version 1.4.2. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm X 0.45 mm and the scanning speed was 20 mm s⁻¹.

a) Preparation of Standard Stock Solutions

A Standard stock solution of Cefpodoxime proxetil was prepared by dissolving 5 mg drug in 10 ml methanol to furnish a concentration of 500 µg/ml. To prepare the calibration plot the stock solution of CP was diluted to 100, 200, 300, 400, 500, and 600 ng band⁻¹ with methanol.

b) Preparation of plasma sample solution:

To 0.5ml of human plasma, 0.5 ml Cefpodoxime proxetil calibration solutions, 1 ml ACN was added to a glass tubes. Each sample was vortex mixed for 3 min and centrifuged (2500 rpm for 20 min). After centrifugation, 20-µl aliquots of supernatant of each concentration were spotted on to the TLC plate.

Selection of Detection Wavelength

After chromatographic development, bands were scanned over the range 200–400 nm (spectrum scan speed 100 nm s⁻¹) and the spectra were overlaid. At 290 nm, CP has considerable absorbance so this wavelength was selected as detection wavelength (Fig. 1).

Validation

The method was validated in accordance with FDA guidelines

Calibration Plot

The calibration plot for the HPTLC method was constructed by analysis of six solutions containing different concentrations of Cefpodoxime proxetil (100, 200, 300, 400, 500, and 600 ng band⁻¹). In the range 100–600 ng band⁻¹ the data were best fitted by a linear equation $mx + b = y$, the coefficient of determination (R^2) was 0.998 (Table 1 and Figure 2).

Selectivity

Six blank plasma samples were tested for interferences by of RF values obtained from human plasma samples spiked with CP. Result are given in Figure 3.

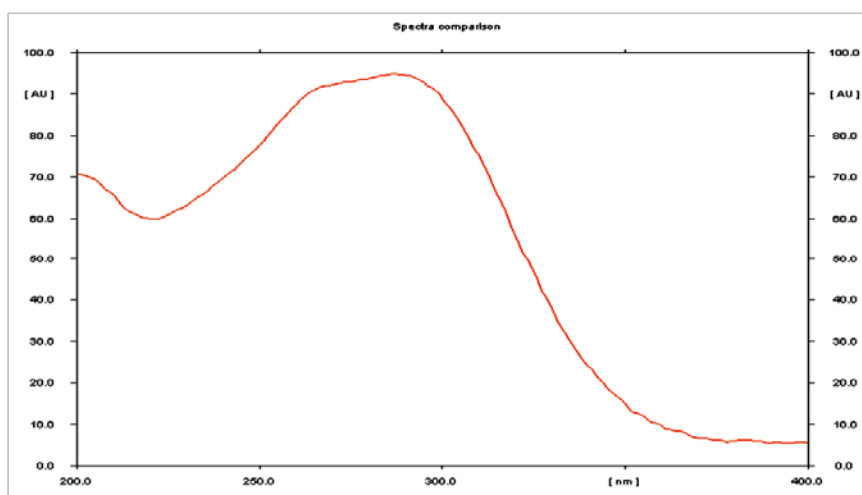


Fig. 1: Representative spectrum of Cefpodoxime proxetil from 200 to 400 nm

Table 1: Method validation data for quantification of Cefpodoxime Proxetil by HPTLC

Method characteristic	Value
Range	100-600 ng band ⁻¹
coefficient of determination (R^2)	0.998
Lower limit of quantification (LLOQ)	100 ng band ⁻¹
System suitability	RSD = 5%

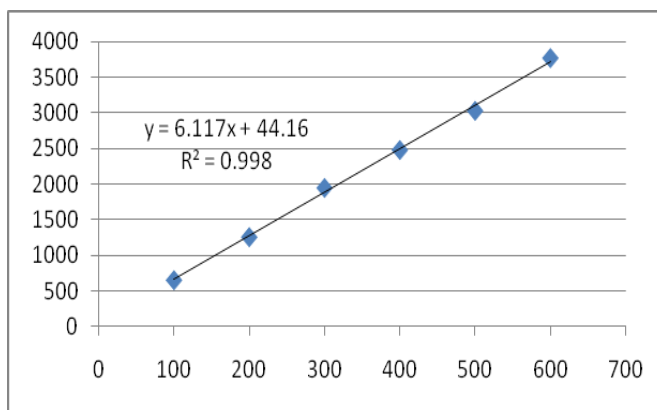


Fig. 2: Calibration curve of CP in plasma sample

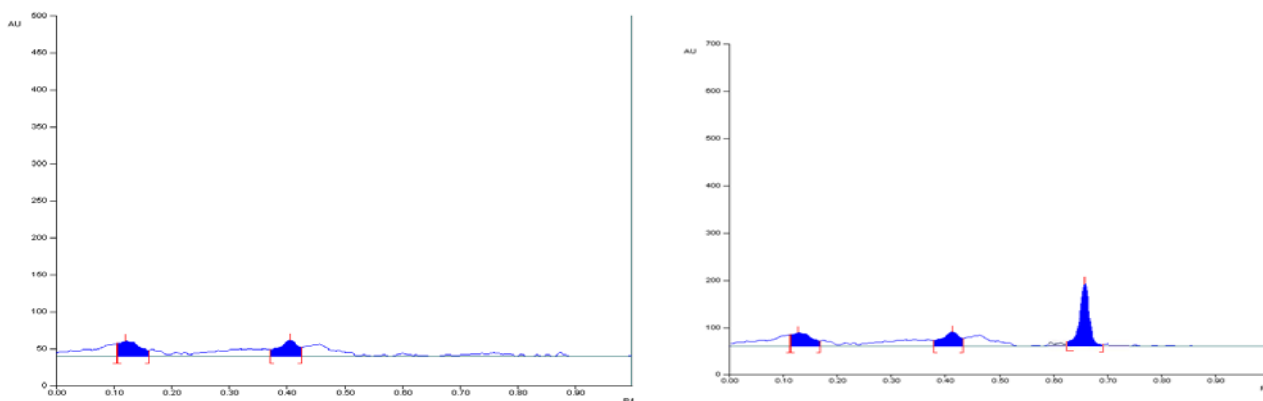


Fig. 3: Typical Chromatogram of (a) Blank human plasma (b) Cefpodoxime proxetil (100 ng/band, Rf- 0.65 ± 0.05) extracted from human plasma

Table 2: Recovery of Cefpodoxime Proxetil

Concentration of Drug [ng band ⁻¹]	Mean amount of Drug found +SD [ng band ⁻¹]	RSD [%]
200	190.49 ± 7.9685	4.18
300	290.94 ± 12.1705	4.29
400	386.36 ± 15.1574	3.92

Recovery

Recovery from human plasma samples was evaluated in triplicate for each of three concentrations of Cefpodoxime proxetil; the response for each level being compared with that from the corresponding standard solution. Recovery was from 94.95 to 05.47 % (Table 2).

Accuracy and Precision

The mean values for accuracy were within 15 % of the actual value (except at the LLOQ where 20 % is allowed) whereas for precision the relative standard deviation (RSD) values did not exceed the limit of 15% (except at the LLOQ where 20% is allowed).

Repeatability

The repeatability (intra-assay precision) of the method was evaluated in triplicate on the same day for three different concentrations performing of Cefpodoxime proxetil. The results, expressed as mean amount of drug found, are shown in Table 3.

Reproducibility

The reproducibility (inter-assay precision) was evaluated in triplicate for three different concentrations of Cefpodoxime proxetil on three consecutive days (fresh samples were prepared every day). The results, expressed as mean amounts of drug found, are shown in Table 4.

Table 3: Repeatability analysis of Cefpodoxime Proxetil

Level	Concentrations [ng band ⁻¹]	Mean amount [ng band ⁻¹]	Mean Recovery [%]	RSD [%]
1	200	Plasma	95.47	1.99
		Solution		
		191.18 ± 3.82		
2	300	281.17 ± 17.44	93.83	6.20
		299.65 ± 7.61		
3	400	366.62 ± 4.30	94.95	1.17
Average Mean Recovery		94.75		

Table 4: Reproducibility of analysis of Cefpodoxime Proxetil

Concentration of Drug [ng band ⁻¹]	Mean amount of Drug found ± SD [ng band ⁻¹]			Mean from 3 days ± SD [ng band ⁻¹]	RSD [%]
	Day 1	Day 2	Day 3		
	200	192.31 ± 9.18	184.17 ± 10.93		
300	293.10 ± 9.60	289.79 ± 13.97	302.39 ± 23.41	295.09 ± 6.53	2.21
400	383.41 ± 20.43	391.99 ± 8.37	379.41 ± 14.98	384.94 ± 6.43	1.67

Stability

Samples of Cefpodoxime proxetil at three concentrations were tested in triplicate through three freeze-thaw cycles and during storage at -5 ± 0 °C. The stability of samples in the bench top and the stability of the stock solution were also tested.

RESULTS AND DISCUSSION

Recovery of Cefpodoxime proxetil from human plasma was from 94.95 to 05.47 % compared with the standard solution, and the variability (RSD) on the same day and on different days was <15%. The range quantified in human plasma using linear regression was from 100 to 600 ng band⁻¹

Cefpodoxime proxetil was shown to be stable through three freeze-thaw cycles, during storage for 3 weeks at -5 ± 0 °C, during storage in bench top for 6 h, and in the stock solution for 5hr. 30 min; the results obtained were precise and accurate.

The mobile phase resolved the drug efficiently. The RF values of Cefpodoxime proxetil was 0.65 ± 0.05 . The advantages of the method are that it uses a small amount of sample (20µL), the volume of mobile phase used is approximately 10 ml per plate, and reading or detection of the plates takes approximately 7 min.

CONCLUSION

This HPTLC method for quantification of Cefpodoxime Proxetil in human plasma is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma.

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