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RP-HPLC Estimation of Cefpodoxime Proxetil in Rat Plasma

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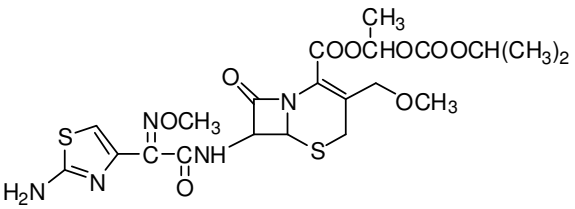
A validated reverse phase HPLC method was developed for the estimation of cefpodoxime proxetil in rat plasma to determine pharmacokinetic parameters such as peak plasma concentration (C_{max}), peak time (t_{max}), area under the curve (AUC_{0-24}), absorption rate constant K_a and biological half-life ($t_{½}$). The samples were chromatographed on a reverse phase column, Luna C18 (250 × 4.6 mm, 5 µm). Detection of cefpodoxime proxetil was carried out at 259 nm using SPDMP 10A photodiode array detector. Mixture of acetonitrile and phosphate buffer (pH 3) (70:30, v/v) was used as mobile phase. Aspirin was used internal standard. The drug was extracted from rat plasma samples by liquid-liquid extraction using methanol as extraction solvent. Calibration curve was linear over the range of 0.05-2 µg/mL of cefpodoxime proxetil. After oral administration of 1.4 mg/kg of rat weight of cefpodoxime proxetil, the plasma concentration- time curve was best confirmed to two-compartment open model. The maximum concentration (C_{max}) 3.24 ± 0.037 µg/mL was obtained at time (t_{max}) of 2.0 ± 0.11 h. The mean area under the curve at 24 h, $AUC_{0\rightarrow24}$ was 39.77 ± 0.03 µg h/mL and at infinity time, $AUC_{24\rightarrow\infty}$ was 43.48 ± 0.06 µg h/mL. The biological half-life, $t_{½}$ was 3.96 ± 0.034 h. The absorption rate constant, K_a was 0.135 ± 0.001 h^{-1} and the elimination rate constant, K_e was 0.175 ± 0.002 h^{-1}.

Key Words: Cefpodoxime proxetil, Reverse phase HPLC, Internal standard, Aspirin, Rat plasma, Accuracy, Precision.

INTRODUCTION

Cefpodoxime proxetil[1-4] (CFDP) is a new oral esterified cephem antibiotic with a broad antibacterial spectrum. Chemically it is 1-[(isopropoxy carbonyl)ethyl ester of (Z)-7-(2-(2-amino-1,3-thiazol-4-yl)-2-methoxy iminoacetamido]-3-methoxy methyl-3-cephem-4-carboxylic acid.



Structure of cefpodoxime proxetil

There were few methods for the quantitative determination of cefpodoxime proxetil in pharmaceutical dosage forms such as suspensions containing cefpodoxime proxetil in combination with clavulanic acid.

Cefpodoxime proxetil is usually preferred in the form of dispersible tablets and there were no reports on validated HPLC technique for estimation of cefpodoxime proxetil in plasma samples useful in evaluation of pharmacokinetic parameters of formulations of cefpodoxime proxetil. Hence the objective of the present research work is to develop high pressure liquid chromatographic technique for estimation of cefpodoxime proxetil in rat plasma to estimate pharmacokinetic parameters of dispersible tablets of cefpodoxime proxetil. As the dispersible tablets are administered in solution form, the rat as an animal model is the right choice in the present study for estimation of pharmacokinetic parameters of dispersible tablets of cefpodoxime proxetil.

EXPERIMENTAL

Pure and certified samples of cefpodoxime proxetil and aspirin were gifted by M/s Karnataka Antibiotics and Pharmaceuticals Ltd., Bangalore, India. Acetonitrile (HPLC grade) and water (HPLC grade) were purchased from Merck specialties Pvt. Limited (Mumbai). All other chemicals and reagents were of analytical grade

A gradient high pressure liquid chromatograph (Shimadzu HPLC with Sphinchrome Software series, Japan) with two CC-10 AT VP pumps was used for the analysis. The samples were chromatographed on a reverse phase Luna C18 (250 × 4.6 mm, 5 µm) column. Detection of Cefpodoxime proxetil

was carried out at 259 nm using SPDMP 10A photodiode array detector. Different mobile phases were tested in order to find the sensitive conditions for the sharp peaks characteristic of the selected internal standard such as aspirin and the drug, cefpodoxime proxetil. Mixture of 0.5 % phosphate buffer (pH 3.5) and acetonitrile (60:40, v/v) was selected as optimum composition for mobile phase. The flow rate was maintained at 1 mL/min. The injector having the capacity of 0-250 µL, Rheodyne Hamilton type was used to inject the samples. The mobile phase was vacuum filtered through 0.45 µm millipore filter paper before use. Samples that are filtered through 0.45 µm millipore filter paper using sample filtration syringe system were used to inject in the system.

Preparation of standard solution of cefpodoxime proxetil: The standard stock solution was prepared with mobile phase to obtain 10 mg/mL solution of cefpodoxime proxetil. Working standard solutions equivalent to 2, 4, 6, 8, 10 and 20 µg/mL of cefpodoxime proxetil were prepared from stock solution.

Preparation of internal standard solution: Aspirin (IS), 10 mg was accurately weighed and dissolved in 100 mL of mobile phase. This solution was suitably diluted to obtain 2 µg/mL of aspirin.

Extraction procedure: 1 mL of blood samples of each rat were collected from retro orbital plexus of rats into eppendorf tubes containing dipotassium ethylenediamine tetra acetic acid (K_2EDTA). The collected blood samples were coagulated by centrifugation at 3000 rpm for 20 min and plasma was separated. 100 µL of clear plasma was spiked with 100 µL of aspirin (IS, 2 µg/mL in mobile phase) and methanol was added to make the volume up to 1 mL (for denaturation and precipitation of plasma proteins). The tubes were tightly capped, vortexed for 10 min and centrifuged for 20 min at 3000 rpm. Then the supernatant clear sample was filtered through 0.45 µm Millipore filter paper and then 100 µL of sample was injected into the system.

Calibration curve: Standard solutions containing 2, 4, 6, 8, 10 and 20 µg/mL of cefpodoxime proxetil were prepared in mobile phase. An aliquot of drug free plasma 100 µL was accurately measured into a stoppered centrifuge vials followed by the addition of 100 µL of 200 ng/mL solution of aspirin (I.S) along with the addition of 100 µL of each cefpodoxime proxetil standard solution. The samples were processed as describe above. The quantification of chromatogram was performed using peak area ratios of the drug to internal standard and average value for 5 such determinations were calculated. The relevant chromatogram is presented in Fig. 1. A standard graph was plotted between the plasma concentrations of cefpodoxime proxetil and the peak area ratio of cefpodoxime proxetil to aspirin (I.S) is shown in Fig. 2.

Accuracy: Accuracy of the present method was determined by recovery studies. The recovery studies were conducted by adding 0.5, 1.0, 2.0 µg of cefpodoxime proxetil to the preanalyzed plasma drug samples containing 500 ng of cefpodoxime proxetil per 500 µL of plasma and subjecting them to present HPLC method of estimation. The accuracy was expressed in terms of present recovery of cefpodoxime proxetil from the preanalyzed samples. The results are given in the Table-1.

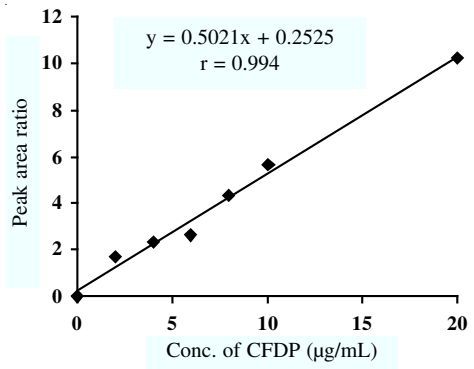


Fig. 1. Calibration curve for the estimation of cefpodoxime proxetil in rat plasma

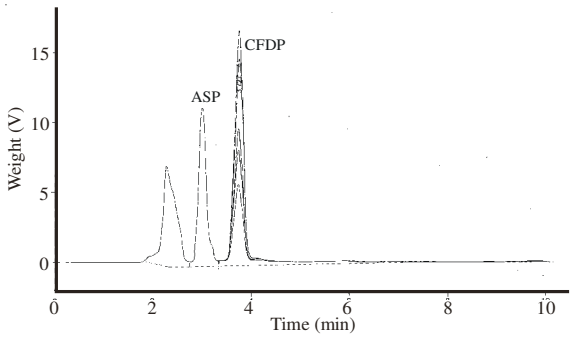


Fig. 2. HPLC chromatogram of blank plasma spiked with aspirin and cefpodoxime proxetil

TABLE-1
RECOVERY OF CEFPODOXIME PROXETIL FROM PREANALYZED PLASMA SAMPLE CONTAINING 500 ng OF CEFPODOXIME PROXETIL PER 500 µL PLASMA (n = 6)

Amount of cefpodoxime proxetil added (µg)	Mean per cent recovery (± SD)
0.5	99.92 ± 2.94
1.0	99.87 ± 2.79
2.0	99.93 ± 3.22

Method validation: The intra-day and inter-day variation of the present HPLC method was estimated by subjecting plasma drug samples (100, 200, 300 ng/mL) prepared on different 5 days of HPLC analysis for five different times. In each case the coefficient of variation in mean peak area ratios of the drug was calculated to find the precision of the present HPLC method. The results are given in the Table-2.

TABLE-2
PRECISION OF THE HPLC METHOD USED FOR THE ESTIMATION OF CEFPODOXIME PROXETIL IN RAT PLASMA (n = 6)

Conc. of cefpodoxime proxetil (µg/mL)	Intra-day		Inter-day	
	Mean peak area ratio	CV (%)	Mean peak area ratio	CV (%)
2	1.76	1.91	1.71	0.98
4	2.31	0.97	2.30	1.19
6	2.71	1.16	2.69	1.08

Pharmacokinetic study: Male wistar albino rats weighing 170-250 g were used in the study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animals were given

standard rat pellets (Gold Mohor Ltd.) and drinking water *ad libitum*.

A group of rats (containing four animals) were administered with commercial cefpodoxime proxetil dispersible tablet solution[5] equivalent to 1.4 mg/kg of rat weight of cefpodoxime. At predetermined time intervals, blood samples (0.2-0.3 mL) were collected from retro orbital plexus of rats into eppendorf tubes containing dipotassium ethylenediamine tetra acetic acid (K_2EDTA). The collected blood samples were coagulated by centrifugation at 3000 rpm for 20 min, plasma was separated and stored in deep freezer until used. Plasma samples of cefpodoxime proxetil were thawed after withdrawal from deep freezer. The plasma concentration of cefpodoxime proxetil was estimated by present HPLC method.

RESULTS AND DISCUSSION

Linearity: As shown in Fig. 1 good linear relationship was observed between the concentrations of cefpodoxime proxetil and the peak area ratios of cefpodoxime proxetil to that of internal standard with a high correlation coefficient (r = 0.994) in the range of 2-20 µg/mL of plasma. The regression of cefpodoxime proxetil concentration over its peak area ratio was found to be y = 0.5021x + 0.2525 where 'y' is the peak area ratio and 'x' is the concentration of cefpodoxime proxetil. The low coefficient of variation values in the peak area ratios indicated the reproducibility of the method.

Accuracy: The high recovery values closer to 100 % with less standard deviation as shown in Table-1 indicated accuracy of the present HPLC technique for estimation of CFDP in rat plasma.

Method validation: The low per cent coefficient of variation values shown in the Table-2 of inter and intra day (precision) studies indicated that the present method is reproducible and valid. Hence, this method was used for the estimation of cefpodoxime proxetil *in vivo* studies.

Pharmacokinetic study: Following oral administration of commercial cefpodoxime proxetil dispersible tablet solution (equivalent to 1.4 mg/kg of cefpodoxime), plasma concentration of CFDP was estimated by present developed HPLC technique and the data is given in Table-3. Plasma concentration-time curve was plotted and given in Fig. 3. The mean area under the curve after 24 h, $AUC_{0\rightarrow24}$ was 39.77 ± 0.03 µg h/mL and at infinity $AUC_{24\rightarrow8}$ was 43.48 ± 0.06 µg h/mL. Peak plasma concentration, C_{max} was 3.24 ± 0.037 µg/mL which appeared at peak time tmax of 2 ± 0.11 h. The half-life, $t_{½}$ of the drug was 3.96 ± 0.034 h. The absorption rate constant K_a was 0.135 ± 0.001 h^{-1} and the elimination rate constant K_e was 0.175 ± 0.002 h^{-1}.

TABLE-3
PLASMA CONCENTRATION-TIME DATA AFTER ORAL ADMINISTRATION OF MARKETED FAST DISPERSIBLE TABLET SOLUTION (n = 4)

Time (h)	Rat 1	Rat 2	Rat 3	Rat 4	Average plasma conc. (µg/mL)	CV (%)
0.25	0.70	0.78	0.65	0.83	0.74	0.09
0.50	1.50	1.62	1.47	1.64	1.56	0.14
0.75	2.09	2.17	2.00	2.25	2.13	0.21
1.00	2.90	3.04	3.11	2.83	2.97	0.27
2.00	3.19	3.08	3.29	3.40	3.24	0.36
4.00	2.75	2.68	2.89	2.96	2.82	0.25
6.00	2.34	2.29	2.59	2.54	2.44	0.29
8.00	1.91	2.19	1.86	2.15	2.03	0.22
12.00	1.37	1.52	1.32	1.49	1.43	0.16
24.00	0.56	0.52	0.78	0.74	0.65	0.07

*Coefficient of variance.
No significant variation between the rat groups ($p < 0.05$).

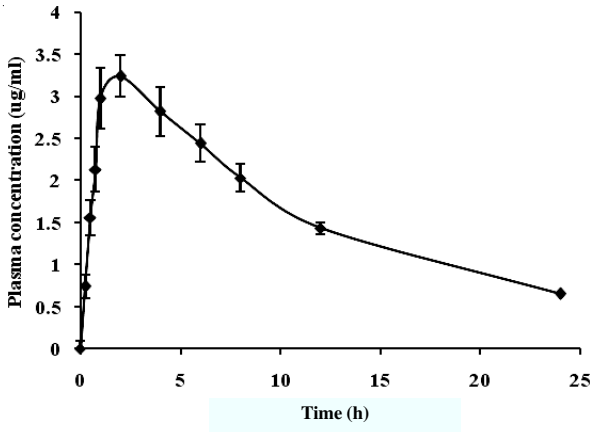


Fig. 3. Mean plasma concentration-time curve

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REFERENCES

1. Physicians Desk References, edn. 60, pp. 2650-2651 (2006).
2. K.D. Tripathi, Essential of Medical Pharmacology, edn. 5, pp. 661-665 (2003).
3. J.G. Hardman and L.E. Limbird, Goodman and Gilman's: The Pharmacological Basis of Therapeutics, edn. 10, pp. 1206-1211 (2001).
4. R.C. Rowe, P.J. Sheskey and P.J. Weller, Hand Book of Pharmaceutical Excipients, edn. 10, pp. 581-584 (2003).
5. M.T. Borin, *Drugs*, **42**, 13 (1991).