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REVERSE PHASE-HPLC METHOD FOR THE ANALYSIS OF CEFPROZIL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of cefprozil in bulk drug sample and pharmaceutical dosage forms using RPC-18 column. The mobile phase consists of mixture of trifluoroacetic acid in water (75 volume), trifluoroacetic acid in acetonitrile (25 volume) and diluent (phosphate buffer) in the ratio of 60 : 40, respectively and was pumped at 1.0 mL/ min at 40ºC. The detection was carried out at 290 nm and the calibration curve was linear in the range of 0.2 µg/ mL to 20 µg/ mL. The method was statistically validated for its linearity, precision and accuracy. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining cefprozil in bulk drug samples or in pharmaceutical formulations.

Key words: Cefprozil, RP-HPLC.

INTRODUCTION

Cefprozil[1-3] is chemically (6R, 7R)-7-[(R)-2-amino-2-(-p-hydroxyphenyl) acetamidol]-8-oxo-3-(1-propenyl)-5-thia-1-azobicyclo [4,2,0] octo-2-ene-2-carboxylic acid monohydrate. Cefprozil is bactericidal and is used in the treatment of susceptible infections, skin and soft tissue infection and should probably be classified as a second generation cephalosporins, beta-lactum and other inhibitors of cell wall synthesis.

Drug structure

Literature survey reveals that few methods are available like LC method for the estimation of cefprozil in human plasma and its pharmacokinetics[4]. Spectrophotometric

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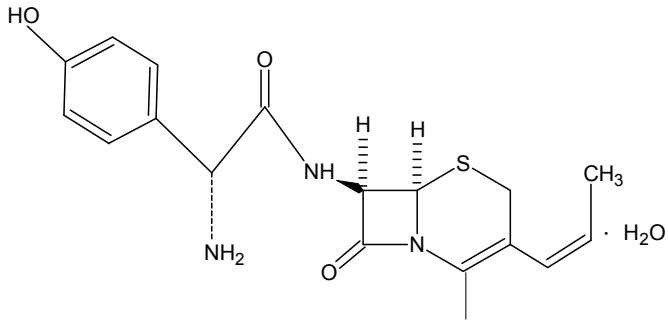
determination of cefprozil in pharmacokinetics dosage forms in urine and in the presence of its alkaline induced degradation products[5]. HPLC method for simultaneous determination of cefprozil diastereomers in human plasma[6], spectroscopic analytical study for the charge-transfer complexation of certain cephalosporin with chloranillic acid[7] and flow injection, chemiluminescent determination of cefprozil using (2,2′-bipyridyl) ruthenium-1,1,-permangnate systems[8] have been reported. The aim of this study is to develop a simple, rapid, precise and accurate reverse-phase HPLC method for the determination of cefprozil in bulk drug samples or in pharmaceutical dosage forms.



EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class-VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard)^{TM}, LC-18, 2 cm, Supelco Inc., Bellefonte, PA and RPC-18 column (150 mm x 4.6 mm ID, particle size 5 μm) was used. The HPLC system was equipped with the software Class-VP series Version 6.01 (Shimadzu).

Chemicals and reagents

Pure samples of cefprozil were obtained as gift sample from Ranbaxy Laboratories Limited (Dewas), India. Acetonitrile (HPLC grade), disodium hydrogen orthophosphate dihydrin (AR grade) and orthophosphoric acid and trifluoroacetic acid (AR grade), water (HPLC grade Qualigens). The commercially available cefprozil tablet claimed to contain 250 mg of drug were procured from local market.

Chromatographic conditions

The contents of the mobile phase were prepared by adding 1.0 mL of trifluoroacetic acid in 1000 mL of water (mobile phase A) and 1 mL of trifluroacetic acid in 1000 mL of acetonitrile (mobile phase-B). The mobile phase A and B were mixed in the ratio of 75 : 25 volume.

Preparation

Phosphate buffer was prepared by dissolving 3.5 g of disodium hydrogen orthophosphate dihydren in 1000 mL of water and pH was adjusted to 8.0 ± 0.05 with orthophosphoric acid. The diluent was prepared by mixing phosphate buffer and acetonitrite in the ratio of 60 : 40. The contents of the mobile phase A and B in the ratio of 75 : 25 were filtered before use through 0.45 μm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/ min, which yielded a column back pressure of 138-140 Kg/cm². The run time was set at 15 min and the column temperature was maintained at 30ºC. Mobile phase-A was prepared by adding 1 mL of trifluoroacetic acid to 1000 mL of water HPLC grade water. Mobile phase-B was prepared by adding 1 mL of trifluoroacetic acid to 1000 mL of acetonitrile. The volume of the injection loop was 20 μL. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The elements were monitored at 290 nm and the data were acquired, stored and analyzed with the software Class-VP series version 6.01 (Shimadzu).

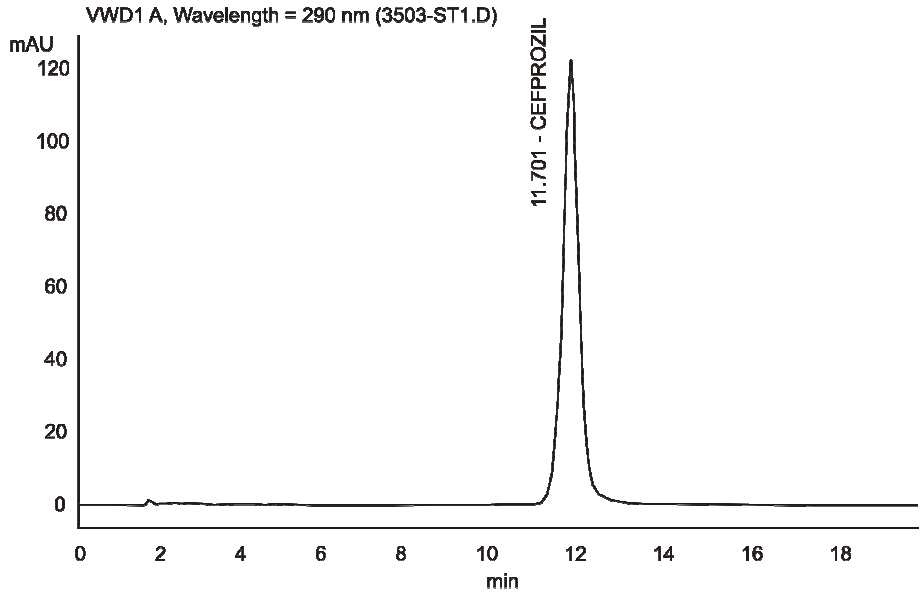
Procedure

About 100 mg of cefprozil was accurately weighed and dissolved in acetonitrile so as to give 1 mg/mL solution. Subsequent dilutions of this solution were made with diluent to get concentrations of 0.2 to 20 μg/ mL of cefprozil. The standard solutions prepared as above were injected six times into the column at a flow rate of 1.0 mL/ min. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of cefprozil in tablet dosage forms.

Cefprozil solutions containing 8 μg/ mL, 12 μg/ mL and 20 μg/ mL were subjected to the proposed HPLC analysis for finding out the intra- and inter-day variations. The recovery studies were carried out by adding known amount of cefprozil to the preanalyzed samples and subjecting them to the proposed HPLC method.

Assay

Twenty tablets each containing 250 mg were weighed and powdered. An accurately weighed portion of the powder equivalent to 100 mg of cefprozil was transferred to a 100 mL volumetric flask containing 50 mL of acetonitrile. The contents of the flask were sonicated for 15 min to dissolve cefprozil and made up to volume with mobile phase and the resulting mixture was filtered through a 0.45 μm filter. One mL of this solution was added to a 100 mL volumetric flask and made up to the volume with mobile phase. This solution (20 μL) was injected six times into the column. The mean values of peak areas of six such determinations were calculated and the drug content in the tablet was quantified using the regression equation obtained above. The same procedure was followed for the estimation of cefprozil in other commercially available tablet dosage forms.



RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of cefprozil in bulk samples or pharmaceutical dosage forms. The column pressure varied from 138 to 140 Kg/cm². The retention time for cefprozil was 11.701 min for a run period of 20 min. Each of the sample was injected 6 times and the same retention time were observed in all cases. The peak area of different

concentrations set up as above were calculated and the average values for 6 such determinations are shown in Table 1.

Table 1. Calibration of the HPLC method

Concentration of Cefprozil (μg/ mL)	Peak area (n = 6)
0.2	136
0.5	338
1.0	679
2.0	1359
3.0	2038
5.0	3395
6.0	4076
10.0	6792
12.0	8148
15.0	10187
20.0	13583

Regression equation (from 0.2 to 20 μg/mL):
$y = -0.001269 + 0.52609$ ($r = 0.9996$)

The peak area for drug solution was reproducible as indicated by low coefficient of variation (1.96%). A good linear relationship ($r = 0.9996$) was observed between the concentrations of cefprozil and the respective peak areas. The calibration graph was found to be $y = -0.001269 + 0.52609\ x$, where y is the peak area and 'x' is the concentration of cefprozil in the range of 0.2 to 20 μg/ mL when cefprozil solution containing 8 μg/ mL, 12 μg/ mL and 20 μg/ mL were analyzed by the proposed reverse phase HPLC method for finding out intra and inter-day variations A low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The amount of cefprozil from the pre-analyzed sample containing known amounts of the drug are shown in Table 3. About 99.97% cefprozil could be recovered from the preanalyzed sample indicating the high accuracy of the proposed HPLC method.

Table 2: Inter and intra-day precision for cefprozil assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration of cefprozil (μg/ mL)	Observed concentration of cefprozil (μg/ mL)			
	Intra-day		Inter-day	
	Mean (n = 6)	% CV	Mean (n = 6)	% CV
8	7.97	0.42	8.04	0.72
12	12.02	0.34	12.05	0.93
20	20.07	0.97	19.95	0.82

Table 3. Experimental values obtained in the recovery test for cefprozil tablets by proposed HPLC method

Amount of drug added (μg) to drug solution/ powdered tablet formulation	Recovery from drug solution		Recovery from powdered tablet formulation	
	Mean (± SD) amount (μg) found (n = 6)	Mean (± SD) % recovery (n = 6)	Mean (± SD) amount (μg) found (n = 6)	Mean (± SD) % recovery (n = 6)
4	3.991 ± 0.35	99.77 ± 0.02	4.023 ± 0.21	100.57 ± 0.01
8	8.014 ± 0.61	100.12 ± 0.06	7.987 ± 0.01	100.23 ± 0.02
12	12.07 ± 0.02	100.84 ± 0.03	12.21 ± 0.03	99.97 ± 0.01

Table 4. Mean (± SD) amount of cefprozil in tablet dosage forms by the proposed HPLC methods

Brand of the tablet	Labelled amount of drug (mg)	Mean (± SD) amount found (mg) by the proposed method (n = 6)	Mean (± SD) % labelled amount (n = 6)
AA	250	250.25 ± 0.03	100.30 ± 0.02
BB	250	249.72 ± 0.05	99.80 ± 0.05

AA = 3 Ceff 250 mg (Alkem, Ulticare), BB = Refzil O Distab 250 mg (Ranbaxy)

The drug content in the tablets was quantized using the proposed analytical method. The mean content of cefprozil in two different brands of tablet dosage form is

shown in Table 4.

The absence of additional peaks indicates no interference of the excipients used in the tablet. The tablets were found to contain 99.8 ± 0.05% to 100.3 ± 0.02% of the labeled amount. The low 1% CV indicates the reproducibility of the assay of cefprozil in the tablet dosage form. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

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