High Performance Thin Layer Chromatographic Method for Estimation of Cefprozil in Tablet Dosage Form

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Abstract: A simple, fast, specific and precise HPTLC method has been developed for the estimation of cefprozil in pure and in tablet dosage forms. Aluminium plates precoated with silica gel G60F₂₅₄ were used as stationary phase and a mixture of chloroform: methanol: toluene: diethyl amine: water in the ratio 4: 4.4: 3.2: 3: 0.8 v/v as mobile phase. Quantification was carried out by the use of a densitometer in absorbance mode at 286 nm. The R_f value of cefprozil was found to be between 0.37 and 0.40. The results of the analysis have been validated statistically and by recovery studies. Linearity was observed in the concentration range of 400-2000 ng/spot.

Keywords: Cefprozil, Estimation in tablets, HPTLC.

Introduction

Cefprozil is chemically (6R, 7R)-7-((R)-2-amino-2-(*p*-hydroxy-phenyl) acetamido) -8-oxo-3-propenyl-5-thia-1-azabicyclo (4.2.0) oct-2-ene-2-carboxylic acid. It is a semi synthetic oral second generation cephalosporin consisting of 9:1 *Z/E* isomeric mixture with a wide antibacterial spectrum of activity. Literature survey reveals HPLC method for the simultaneous determination of cefprozil diastereomers³, flow injection chemiluminescent determination of cefprozil², spectrophotometric determinations^{4,5} and spectrofluorimetric determinations^{6,7}. As there is no HPTLC method at present the author's propose a precise and accurate HPTLC method for the estimation of Cefprozil in pure and in tablet dosage forms.

Experimental

Instrumentation

A Camag HPTLC system comprising of a Linomat-5 applicator and Camag TLC scanner-3 with Wincats 4 software for interpretation of data along with a single pan balance of Mettler-toledo model was used, for the present study. Dissolution of the compounds was enhanced by sonication on a Bandelin sonerex sonicator.

Drugs and chemicals

The authenticity and purity of cefprozil USP was certified by Aurobindo pharma Ltd as 98.5% w/w. Refzil-O tablets (Ranbaxy pharma) containing 250 mg of cefprozil USP were procured from the local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. chloroform, methanol, toluene and diethyl amine procured from Merck Ltd. (Mumbai, India) were used in the study.

Chromatographic conditions

Stationary phase used was aluminium plates precoated with silica gel G 60F₂₅₄ (20 cm x 10 cm). Mobile phase used in the study was a mixture of chloroform: methanol: toluene: diethyl amine: water in the ratio 4: 4.4: 3.2: 3: 0.8 v/v. Development chamber used was a Camag twin trough glass chamber (20 cm x 10 cm) saturated with filter paper for 10 mins. Separation was carried out by ascending development. The size of the plate used was 20 x 10 cm. Sample application position on plate was at 10 mm distance from the base and solvent front was developed to 80 mm. Band of length 8 mm was applied. Deuterium lamp was used and UV detection was carried out at 286 nm. The scanning speed used was 20 mm/sec.

Preparation of standard solution

It is prepared by accurately weighing and transferring 100 mg of cefprozil into a 100 mL volumetric flask and making to volume using 10 mL water and 90 mL methanol. The resulting solution A has a concentration of 1 mg/mL. 2 mL of solution A is made up to 10 mL with methanol to give standard stock solution of concentration 0.2 mg/mL or 0.2 µg/µL.

Results and Discussion

Linearity and calibration

Standard stock solutions of 2, 4, 6, 8 and 10 µL were applied on a TLC plate with the help of a Camag Linomat-5 sample applicator to give concentrations of 400, 800, 1200, 1600 and 2000 ng/spot. The developing chamber was allowed to saturate with mobile phase using a filter paper. The mobile phase was then used for separation of components by ascending development method. After development the plate was removed from the chamber and dried using hot air. The bands were evaluated at 286 nm. A typical HPTLC densitogram of cefprozil standard is shown in Figure 1. The values obtained for the calibration curve are shown in Table 2.

Estimation of cefprozil in tablets

Twenty tablets were weighed and finely powdered. Accurately weighed quantity of tablet powder equivalent to about 10 mg of cefprozil was transferred to a 10 mL volumetric flask, added 1 mL of H₂O and shaken for 5 minutes. The volume was then made up to mark with methanol. The solution was filtered through Whatmann filter paper and the filtrate was appropriately diluted with methanol to get a final concentration of 600 ng/spot of Cefprozil. The resulting solution was used for analysis. Chromatograms were obtained by maintaining the above said chromatographic conditions and evaluation was performed using densitogram area. The results are shown in Table 1.

Table 1. Estimation of cefprozil in tablets

Label claim, mg/tab	Amount found (area), mg/tab
250	245
	249
	251
	255
	252
Mean	250.4
RSD	1.48

Table 2. Calibration of the HPTLC method for the estimation of cefprozil

Conc, ng/spot	Densitogram area
400	1954.5
800	3639.6
1200	5079.5
1600	6010.5
2000	7018.5

Regression equation from 400-2000 ng/spot, $Y=3.234X+880$ ($r^2=0.9997$)

Figure 1. A typical HPTLC densitogram of cefprozil standard

The R_f values were determined and found to be in the range of 0.37-0.40.

Recovery studies

To ensure the reliability of method recovery studies were carried out by mixing a known quantity of standard drug with preanalysed sample and contents were reanalyzed by proposed method. The values are given in Table 3.

Table 3. Results of recovery study

Drug	Amount of standard drug added, $\mu\text{g/mL}$	Amount of drug recovered, $\mu\text{g/mL}$	%Recovery	Mean recovery
Cefprozil	400	415.6	103.9	102.7
Cefprozil	800	812.0	101.5	

Specificity

The specificity was also confirmed by overlaying the spectra of standard Cefprozil with the spectra of sample recorded on TLC scanner in UV range as shown in Figure 2. The various statistical parameters and the method validation parameters were shown in Table 4 & 5.

Table 4. Statistical parameters.

Parameter	Value
Correlation coefficient	0.9997
Coefficient of variation (C.V), %	0.515
Slope (a)	3.234
Intercept(b)	880
Regression equation ($Y=aX+b$)	$Y=3.234X + 880$

$Y=Y$ axis (densitogram area). $X=X$ axis (Concentration of drug in ng/spot).

Figure 2. Overlay absorption spectra of Standard cefprozil & cefprozil from tablets

Table 5. Method validation parameters

Parameter	Value
Accuracy, %, (Area)	101.04±1.78
Linearity range, ng/spot	400-2000, ng/spot
Limit of detection	133, ng/spot
Limit of quantification	400, ng/spot

Conclusion

The proposed HPTLC method was precise, accurate and selective. The method was rapid, sensitive, reproducible and economical. It does not suffer any positive or negative interference due to common excipients present in formulations and can be conveniently used for routine quality control analysis.

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