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Validated RP-HPLC Method for Estimation of Cefprozil in Tablet Dosage Form

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Abstract : A simple, precise, specific and accurate Reverse Phase HPLC method has been developed for the determination of Cefprozil in bulk drug and pharmaceutical formulation. Chromatography was performed on a hypersilthermo C-18 Column (250mm x 4.6 mm i.d., particle size 5 µm), with mixture of acetonitrile and monobasic Ammonium phosphate buffer (50:50v/v) as mobile phase at a flow rate of 1 ml/ min. Detection was performed at 280 nm. The retention time of Cefprozil was found to be 4.55 min. By adoption of this procedure Cefprozil is eluted completely. Linear calibration plots were obtained between 20-100 µg/ml. The method of analysis was used for quantification for Cefprozil in pharmaceutical preparations with a coefficient of variation <2%. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, ruggedness and robustness.

Keywords: Cefprozil, Monobasic ammonium phosphate buffer, Acetonitrile, Coefficient of variation.

Introduction

Cefprozil is second generation cephalosporin antibiotic. It 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 and the structural formula is shown in (Fig: 1). The molecular formula is $C_{18}H_{19}N_3O_5S$ and molecular weight is 407.45 g/mol. It is a White to yellowish crystalline powder, stable at room temperature and freely soluble in water, soluble in methanol and very slightly soluble in alcohol. Cefprozil is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefprozil interferes with an autolysin inhibitor. It is official drug in United States Pharmacopoeia[1-3]. From the literature survey, it was found that Cefprozil was estimated by analytical methods such as few UV-Visible methods[4-5], High-Performance Thin Layer Chromatography (HPTLC) method[6]. The present developed method was simple, precise, specific and accurate.

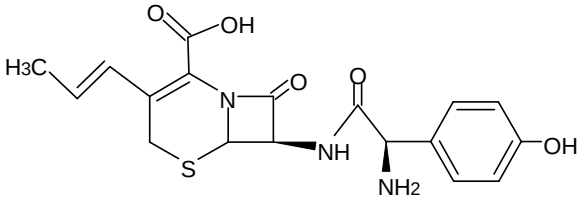

Fig: 1. Chemical Structure of Cefprozil

Experimental

Materials and Methods

In present investigation Merk Hitachi 7110-7400 with one lanchrom hitachi pump, with UV/VIS detector, lanchrom system controller. All the chemicals used in the investigation were of HPLC grade. The chromatographic analysis was performed on a hypersilthermo C-18 Column (250mm x 4.6 mm i.d., particle size 5 µm). The mobile phase consists of a mixture of acetonitrile and monobasic Ammonium phosphate buffer (pH 4.4) in the ratio of 50:50. The optimized chromatographic conditions are summarized in Table 1.

Preparation of mobile phase

500 ml of HPLC grade acetonitrile was mixed with 500 ml of monobasic ammonium phosphate buffer prepared in double distilled water and its pH was adjusted to 4.4 using o-phosphoric acid. Then it was ultrasonicated for 20 minutes and then filtered through 0.45µm membrane filter paper.

Preparation of standard stock solution of Cefprozil

10 mg of standard drug was weighed accurately and transferred to 100 ml volumetric flask. The drug was dissolved in 50 ml of mobile phase with shaking and then volume was made up to the mark with mobile phase to get 100 µg/ml (stock A) of standard stock solution of the drug. This stock solution was filtered through 0.2 µm membrane filter paper.

Preparation of marketed formulations

Twenty tablets of Cefprozil were weighed and the average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 10 mg was weighed and transferred to 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were ultrasonicated for 20 minutes and the final volume was made up to the mark with mobile phase to obtain 100 µg/ml (stock A' solution). This stock solution was then filtered through 0.2 µm membrane filter paper and was used as standard stock solution.

Chromatographic condition

The mobile phase containing Acetonitrile and Monobasic ammonium phosphate (pH 4.4) in the ratio of 50:50 was selected as the optimum composition of mobile phase, as this solvent system resolved for the component ideally. The flow rate was set to 1.0 ml/min and UV detection was carried out at 280 nm. The mobile phase and sample was degassed by sonication for 20 min and filtered through 0.45 µm membrane filter paper. All determinations were performed at room temperature.

Preparation of Calibration Curve and Analysis of Cefprozil

Appropriate aliquots were pipetted out from the standard stock solution (100µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range of 20 - 100 µg/ml of Cefprozil.

Triplicate dilutions of each of the above mentioned concentrations were prepared and 10 µl of each concentration of the drug were injected in to the chromatographic system at the flow rate of 1.0 ml / min and the effluents were monitored at 280 nm, chromatograms were recorded. The Cefprozil was eluted at 4.55 min as shown in Fig: 2.

The calibration curve was constructed by plotting average peak area versus concentration and was presented in Fig: 3.

Analysis of Cefprozil in Pharmaceutical formulations

From stock A' solution, various dilutions of the sample solution were prepared and analysed. A 10 µl volume of each sample solution was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 280 nm and the amount of drug present in the sample was determined.

Method validation [7-9]

The developed analytical method was subjected to validation with respect to various parameters such as accuracy, precision, linearity and range, robustness, LOD and LOQ of the proposed method and it was validated as per the ICH guidelines.

Table 1: Optimized Chromatographic conditions for the proposed method

Parameters	Optimized condition
Linear range (µg/ml)	20-100
Detection wavelength (nm)	280
Temperature	Ambient
Retention Time (t) (min)	4.55
Run time (min)	10
Limit of Detection (µg/ml)	0.02181
Limit of Quantification (µg/ml)	0.06611

Table 2: System Suitability Test Parameters for the proposed method

Parameters	Optimized condition
Retention Time (t) (min)	4.55
Theoretical plates (N)	5986

Table 3: Regression analysis of the Calibration curve for the proposed method

Parameters	Optimized condition
Linearity range (µg/ml)	20-100
Regression equation (Y=mx +c)	
Slope (m)	40061
Standard deviation of slope (S_m)	32.90
Intercept (c)	10572
Standard deviation of Intercept (S_c)	264.88
Correlation coefficient (r^2)	0.999
Relative standard deviation (%)	0.0893
Retention time	4.55

Table 4: Summary of Validation Parameters for the proposed method

Parameters	Values
Limit of detection (µg/ml)	0.021
Limit of quantitation (µg/ml)	0.066
Accuracy (% RSD)	
80%	0.0084
100%	0.0076
120 %	0.0429
Precision (% RSD)	
Intra Day precision	0.1554
Inter Day precision	0.6569
Robustness (% RSD)	
Change in Flow rate	
0.9 ml/min	0.1879
1.1 ml/min	0.2958

Mean of six determinations, RSD indicates relative Standard deviation

Table 5: Assay Results of Cefprozil using proposed method

Brand name	Labeled amount (mg)	Amount found (mg)	%Recovery ± SD**
Brand I	250	249.96	99.92±0.010

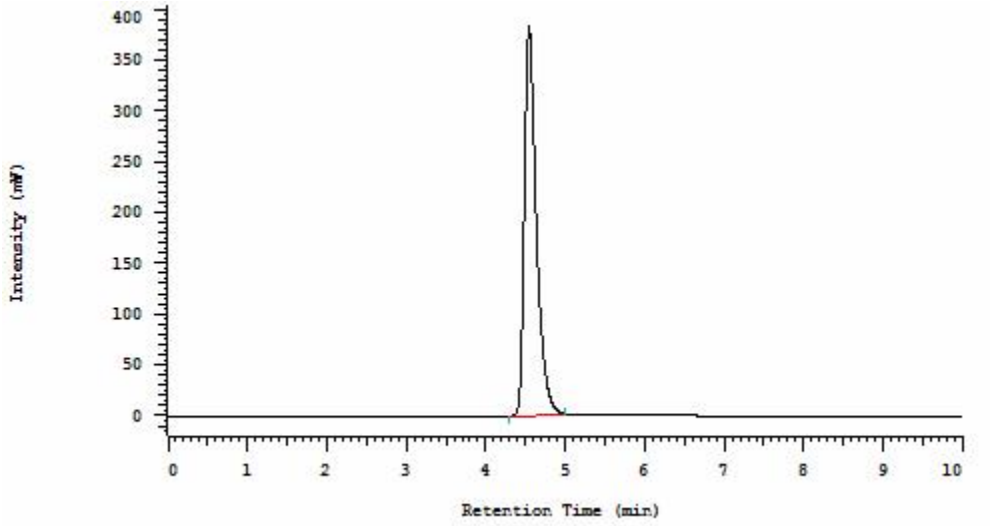

Fig: 2. Chromatogram of Cefprozil by RP-HPLC method.

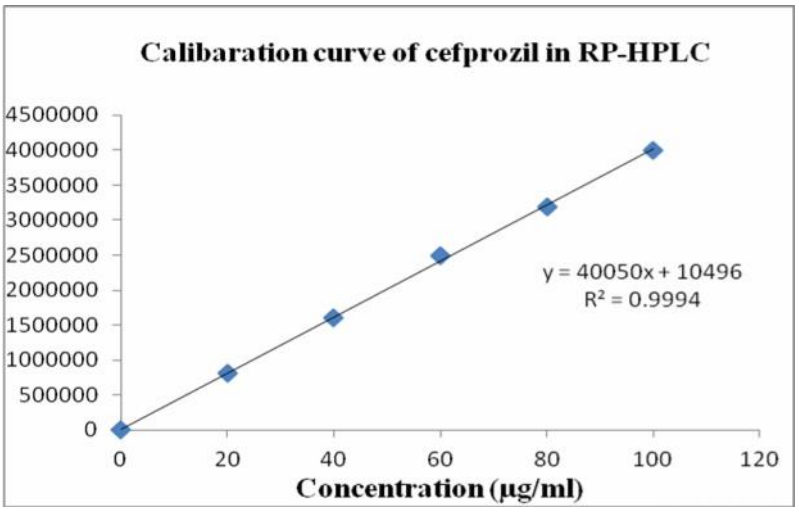

Fig: 3. Calibration curve of Cefprozil at 280 nm by RP−HPLC method.

Results and Discussion

In this method the conditions were optimized to obtain complete elution of Cefprozil. Mobile phase and flow rate selection was based on peak parameters (height, tailing factor and theoretical plates), run time and resolution. The system with a mixture of acetonitrile and monobasic Ammonium phosphate buffer (pH 4.4) in the ratio of 50:50. The system suitability parameters as shown in (Table 2).

The run time was set at 10 min and the retention time for Cefprozil was found 4.55 min as shown in Fig: 2.The sample solution was injected 6 times and the retention times were found to be same. When the concentrations of Cefprozil and its respective peak areas were subjected to regression analysis, a good linear relationship (r^2 = 0.999) was observed between the concentration of Cefprozil and the respective peak areas in the range 20-100 µg/ml. The regression of Cefprozil was found to be Y =

40061X+10572, where 'Y' is the peak area and 'X' is the concentration of Cefprozil. (Table 3)

The proposed RP-HPLC method was validated for intra and inter-day precision with coefficient of variance (%CV), 0.1554 and 0.6569, respectively. (Table 4)

A known amount of the drug solution (80%, 100% and 120 %) was added to the sample of the formulation and subjected to the estimation of the drug for the recovery studies. There was a high recovery of Cefprozil indicating that the proposed procedure for the determination of Cefprozil in the formulation is highly accurate. (Table 4)

The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations.

Conclusion

Thus, it can be concluded that the method developed in the present investigation was economical, simple, sensitive, accurate, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Cefprozil in pharmaceutical dosage forms..

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