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Spectrophotometric Methods for the Determination of Cefprozil Using Methyl Orange

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Abstract: The simple, rapid, accurate, precise, cost effective and reproducible UV spectroscopic methods have been developed for the simultaneous estimation of Cefprozil in bulk and combined tablet dosage form. The first method is based upon the simultaneous equation and second upon the determination of Q value. Cefprozil have absorption maxima at 373 nm. Beer's law obeyed in concentration range of 5-35 µg/ml Cefprozil. The recovery studies from tablet are indicative of accuracy of method and are found in between 99.97-100.66 % at three different levels of standard additions.

Key words: Cefprozil · UV Spectroscopy · Simultaneous equation

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) oct-2-ene-2-carboxylic acid.Cefprzil is an orally active cephalosporin which is used in clinical practice. It belongs to the β- lactam group of antibiotics. Its antibacterial activity is dependent on the presence of the β- lactam functionality which is not stable in aqueous conditions. This instability leads to chemical degradation and the formation of 2,5 - dione derivative via intra molecular nucleophilic attack of the primary amine from the side chain of the lactam moiety at neutral or slightly alkaline medium [1, 2]. Literature survey reveals HPLC method for the simultaneous determination of cefprozil diasteriomers, Spectrophotometric determinations, spectrofluorimetric HPTLC [3, 4]. Author of the article and his research team has developed a UV Method development in different pharmaceutical dosage form [5-19] using Methyl orange [20]. The aim of this work is to develop and validate an analytical method by using UV Spectrophotometry for the estimation of Cefprozil in bulk and pharmaceutical dosage forms and also perform degradation studies on the drug as per ICH guidelines using the proposed method [21, 22].

MATERIALS AND METHODS

Instruments: The instrument used for the present study was a UV-Vis double beam spectrophotometer with 1cm matched pair quartz cell. The solvent used was methanol and distilled water (50:50) which was of AR grade, Methyl orange purchased from SD Fine Chemicals Limited, India. Solubility test for the drug Cefprozil was performed by using various solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, Ethyl acetate, ether and Chloroform.

Reagent Preparation: Methyl orange (10 µg mL^{-1}): A 100 µg mL^{-1} dye solution was first prepared by dissolving accurately weighed 58.8 mg of dye (S.D. Fine Chem., Mumbai, India, 80 % dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was further diluted to obtain a working concentration of 10 µg mL^{-1}.

Preparation of Stock Solution: Standard stock solution of Cefprozil was prepared by dissolving 10mg of Cefprozil in 10ml of methyl orange and distilled water (50:50) which gives 100µg/ml. One ml of this stock solution was taken and was diluted up to 10 ml by using methyl orange and distilled water (50:50) to produce a concentration of 100 µg/ml solution.

Preparation of Working Solution: From the above methyl orange 2ml was transferred into 10ml volumetric flask and volume was made up to the mark with methanol to make 100µg/ml. Then the sample was scanned with UV-Vis Spectrophotometer in the range 200-400nm against methyl orange and distilled water (50:50) as blank and the wavelength corresponding to maximum absorbance was noted which is its λ max i.e. at 373 nm.

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Simultaneous Estimation Method [23]: Varying aliquots (3.0 mL) of standard 20 μg mL^{-1} Cefprozil solutions were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 5.0 mL with ether. To each flask were added 1 mL of 0.5 M acetic acid and 1.0 mL of bromate-bromide mixture (20 μg mL^{-1} in KBrO3) by means of micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 20 μg mL^{-1} indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 373.0 nm against a reagent blank after 10 min.

The two equations were constructed based upon the fact that at λ1 and λ2 the absorbance of the mixture is the sum of individual absorbances of Cefprozil.

$$\text{At } \lambda 1,\ A1 = ax1\,bcx + ay1\,bcy \ldots\ldots$$

Where, A1 are absorbances of mixture at 373 nm.

Application of the Proposed Method: An amount of finely ground tablet powder equivalent to 250 mg of Cefprozil was accurately weighed into a beaker, 100 mL of Methyl orange was added and stirred for 20 min and warmed. Then, the content was transferred to a 100 mL calibrated flask, the beaker was washed with water and the washings were also transferred to the flask and the volume was diluted with water to the mark, mixed well and filtered using a Whatmann No 41 filter paper.

Validation of the Developed Methods: The developed methods for simultaneous estimation of Cefprozil were validated as per ICH guidelines.

Effects of Reagent Concentration: The effect of methyl orange concentration on the reaction was checked out at room temperature and away from direct sunlight. The reaction of Cefprozil was dependent on the concentration of dye used. A concentration of 1.5% (w/v) was selected as the optimum reagent concentration. The absorbance of the solution was measured after 20 minutes after adding reagent and up to 6.0 hrs., the reactions was slow and the formed colour was stable up to 10 hrs. To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. From that total amount of drug found and percentage recovery was calculated. To ascertain accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively.

Method Validation

Selectivity: A method is said to be specific when it produces a response only for a single analyte. Selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ) were calculated according to the 3:1 (S/N) and 10:1 (S/N) criterions respectively, where S is the signal of the sample and N is the noise of the corresponding curve.

Linearity and Range: Linearity of the concentrations was taken in the range of 10-50μg/ml for chloroform and 20-100μg/ml for tetra hydro furan respectively.

Accuracy: Accuracy of proposed method from excipients was determined by recovery experiments. Recovery experiments were carried out in three levels of concentration. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of % relative standard deviation. Precision.

It is expressed as the percentage coefficient of variation (%CV) which is calculated as per the following expression:

$$\%CV = (\text{standard deviation} / \text{mean}) * 100$$

Intraday Precision: It was determined by calculating the %coefficient of variation (%CV) of the results obtained in the same day.

Inter Day Precision: It was determined by calculating the percentage coefficient of variation (%CV) of the results obtained over at least three days.

RESULTS AND DISCUSSION

Proposed Spectrophotometric methods have been developed and compared for estimation of Cefprozil. The methods selected for simultaneous estimation analysis were given the satisfactory results. This method utilizes the active analogue principle that lies at the spectroscopic method [5-19]. A novel approach to use 0.366 % SLS as solvent proven beneficial with many respects such as reduction in cost, no use of organic solvent and stable pharmaceutical solvent for analysis. The spectra of Cefprozil exhibit ë max of 373 nm. Additionally one isorptive point was observed at 298 nm. These wavelengths were selected for simultaneous estimation of Cefprozil and are assumed to be sensitive wavelengths. Standard calibration curves for Cefprozil were linear with correlation coefficient of 0.9986 at all selected wavelengths. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of standard additions, recovery in the range of 99.87-101.43 % justifies the accuracy of method. Precision was studied to find out intra and inter-day variations in the test method of Cefprozil. Calibration curves prepared in medium were run in triplicate in the same day and for three consecutive days. % Relative standard deviation (RSD) were calculated which should be less than 2%. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κ S.D.a/b, where κ = 3 for LOD and 10 for LOQ, S.D.a is the standard deviation of the intercept and b is the slope. The LOD and LOQ were 0.93 and 0.53 µg ml^{-1}, respectively. The detection and quantitation limits determined were 0.88 and 0.30 µg ml^{-1}, respectively. These low values indicated the high sensitivity of the purposed method. System was evaluated for reproducibility by injecting six replicates of Cefprozil (1mg/ml) dilution. The coefficient of variation obtained was. The results obtained are given in Table 1. System was suitable for the determination of Cefprozil because the results were reproducible for the analyte. By following the linearity Simultaneous estimation method were determined. From this values are obtained as 0.9986 and the values are given in Table 1.

Table 1: Analytical and regression parameters of the proposed methods

Parameter	Methyl orange
Beer's law limits	5-35
□max, nm	373 nm
Molar absorptivity	5.3×10^3
Sandell sensitivity	0.06865
Limit of detection	0.93
Limit of quantification	0.53
Regression equation *	
(a) Intercept	6.764
(b) Slope	1.06
Correlation coefficient, (r)	0.9986

CONCLUSION

The Simultaneous estimation method of analysis though expensive, can also be used in the routine analysis of Cefprozil in formulations, because multiple samples can be analysed simultaneously. The results obtained by these methods including recovery studies were comparable which proves the repeatability and suitability of the method. The developed methods were found to be simple, sensitive, accurate, precise, cost effective, reproducible and can be used for routine quality control analysis in tablet dosage form.

Table 2: Accuracy of the proposed method

Sample	Label Claim	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added	Amount of Drug recovered	% Recovery	RSD (% n=5)
Method I	250	249.95	80	10	250.21	100.17	0.09
			100	15	249.87	99.90	0.22
			120	20	249.98	99.98	0.10

Table 3: Intraday, Interdays, data of tablet formulation

		Interday precision % COV		
Sample	Intra day precision % COV (n=6)	Day 1[a]	Day 2[a]	Day 3[a]
Method I	0.938	0.671	0.540	0.336

COV: Coefficient of variance

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