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Novel spectrophotometric methods for the determination of phenolic β-lactam antibiotic (Cefprozil) in bulk and dosage forms

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ABSTRACT

Three simple, accurate and economical spectrophotometric methods in ultraviolet and visible region were developed for the determination of cefprozil in bulk and dosage forms have been described. The method A is based on the reduction of ferric ion into ferrous ion by the mentioned drug in the presence of 1,10-phenanthroline to form a highly stable orange red colored ferrion complex measured at 500 nm.The method B is based on the oxidation of MBTH with Fe(III) followed by coupling with mentioned drug to form a highly stable violet colored chromogen measured at 620 nm.The method C is based on the reduction of Folin Ciocalteau (FC) reagent in alkaline medium by cefprozil leading to the formation of intense blue colored chromogen measured at 740 nm. Common execipients used as additives in pharmaceutical preparations do not interfere in the proposed methods. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed methods.

Key words: Cefprozil, ferrion complex, MBTH and F.C.reagent.

INTRODUCTION

Cefprozil,5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid,7-[[amino(4-hydroxyphenyl)acetyl]amio]-8-oxo-3-(1-propenyl)-,(6*R*,7*R*)-7-[(*R*)-2-amino-2-(*p*-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0.] Oct-2-ene-2-carboxylic acid, is a semi synthetic broad-spectrum cephalosporin antibiotic[1,2], which is currently available in a oral dosage form (i.e., tablet and suspension) for the treatment of respiratory tract and skin or skin structure infections in both adults and children. The bactericidal action of Cefprozil results from inhibition of cell wall synthesis. Few analytical techniques have been reported for the determination of cefprozil in bulk and dosage forms including HPLC[3-5], LC-ESI-MS[6], and UV spectophotometry[7,8]. Existing analytical methods reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the analytically useful functional groups in Cefprozil. Hence there is a need to develop sensitive and selective, and economical validated visible spectrophotometric methods, which prompted the author to carry out in this accord. The present paper describes three methods have been developed based on different chemical reactions and is extended to dosage forms as well.

EXPERIMENTAL

All the spectral measurements were made on ELICO SL-159 Double beam spectrophotometer. All chemicals were of analytical grade.

1,10-phenanthroline(Merck), MBTH (sd. fine),Folin-Ciocalteau reagent (sd. fine), Fe(III)Cl_3 (A.R.), o-Phosphoric acid (A.R.), Sodium Hydroxide (A.R.), Sodium carbonate (A.R.)and Hydrochloric acid (sd. fine) were used. Distilled water was used to prepare all solutions and in all experiments.

Reagents

Method A

Fe(III)Cl_3 Solution (sd.fine;0.054 %, 3.32×10^{-3} M)

Prepared by dissolving 54 mg of anhydrous Fe (III) Cl_3 in 100 ml of distilled water.

o-Phenanthroline Solution (loba;0.2%,1.10×10^{-2}M)

Prepared by dissolving 200 mg of o-Phen in 100 ml distilled water with warming.

o-Phosphoric acid: (sd.fine; 2.0×10^{-2} M)

Prepared by dilution 1.27ml of 10 ml of o-Phosphoric acid to 100 ml distilled water.10 ml of this stock solution was diluted to 100 ml with distilled water.

Method B

MBTH (3-methyl-2-Benzothialinone hydrazone hydrochloride) solution: (loba; 0.5%, 2.14×10^{-2} M)

Prepared by dissolving 500 mg of MBTH in 100 ml distilled water.

Fe(III)Cl_3 Solution(sd.fine; 0.25 %, 1.53×10^{-3} M)

Prepared by dissolving 250 mg of anhydrous Fe (III) Cl_3 in 100 ml of distilled water.

NaoH solution: (loba; 0.1M)

Prepared by dissolving 400 mg of NaoH in 100 ml of distilled water and standardized.

Hcl solution :(sd.fine; 1 M)

Prepared by dissolving 8.6 ml of Conc. Hcl in 100 ml of distilled water and standardized.

Method C

Folin-Ciocalteau reagent (FC); Loba, 2N)

Used as it is.

Sodium carbonate (Na_2Co_3) solution (10%, 9.43×10^{-1} M)

Prepared by dissolving 10 mg of Sodium carbonate in 100 ml of distilled water.

Preparation of standard drug solution

Cefprozil (pure or formulation) (100mg) was accurately weighed, dissolved in 0.1N HCl (20 ml) and transferred to standard 100 ml volumetric flask. The final volume was made up to the mark with 0.1N HCl .The final concentration was brought up to 200 $\mu g.mL^{-1}$ (method A) ,40 $\mu g.mL^{-1}$ method B) and 400 $\mu g.mL^{-1}$ (method C) respectively.

Procedure for the assay of cefprozil in pharmaceutical dosage forms

Twenty tablets of the cefprozil drug were weighed and powdered, and a quantity of the powder equivalent to 100 mg was transferred into a 100 ml volumetric flask, dissolved in 5 ml of methanol, stirred well for 2 minutes. The solution was mixed well by shaking for 10 minutes, and then make up to the mark with acetonitrile. The solution was filtered. The filterate was quantitatively diluted with methanol to yield concentrations in the linear range of the assay of cefprozil.

Recommended procedures for the determination of cefprozil

Aliquots (0.5-2.5 ml, 200$\mu g.mL^{-1}$) of standard cefprozil were transferred into a series of 25 ml calibrated flasks and then solutions of Fe (III) Cl_3 (1.5 ml) and o-Phen (2.0 ml)was added successively .The total volume in each flask was brought to 10.0 ml with distilled water and heated for 30 minutes in a boiling water bath at 90 ^oC .After cooling to room temperature,2.0 ml of o-Phosphoric acid was added, the volume in each flask was made up to the mark with distilled water. The absorbance of colored complex solution was measured after 5 minutes at 500nm against reagent blank prepared similarly.

Method B

Aliquots (0.5-2.5 ml, 40 $\mu g.mL^{-1}$) of standard cefprozil were transferred into a series of 10 ml calibrated tubes and then 1.0 ml of water, 0.5 ml of 0.5% MBTH and 0.5 ml of 0.1N NaoH were added to each tube. The contents were heated for 10 minutes in a water bath at 100 ^oC and cooled for 5 minutes in a water bath at 15 ^oC. Then 0.5 ml of 1N Hcl and 2.0 ml of Fe (III) Cl_3 solution were added successively and kept aside for 1hour .The absorbance was measured at 620 nm against reagent blank.

Method C

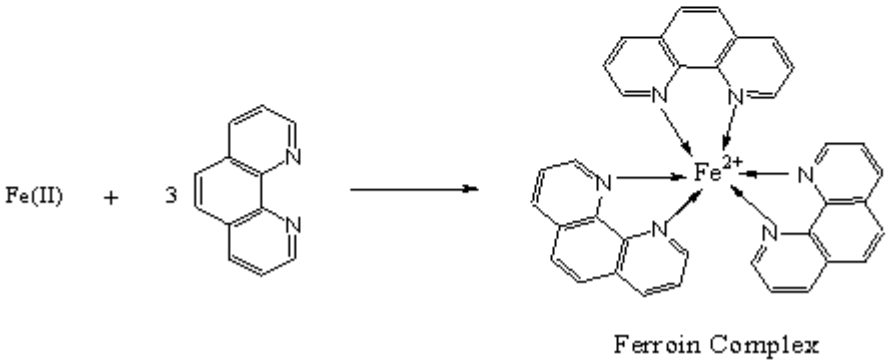
Delivered aliquots of standard cefprozil solution (0.5-2.5 ml, 400 $\mu g.mL^{-1}$) into a series of 25 ml calibrated tubes and the volume was adjusted to 3.0 ml with distilled water. To each of the test

Step - I

Cefprozil + Fe(III) ⟶ Oxidation products of Cef + Fe(II) + Fe(III) (Unreacted)

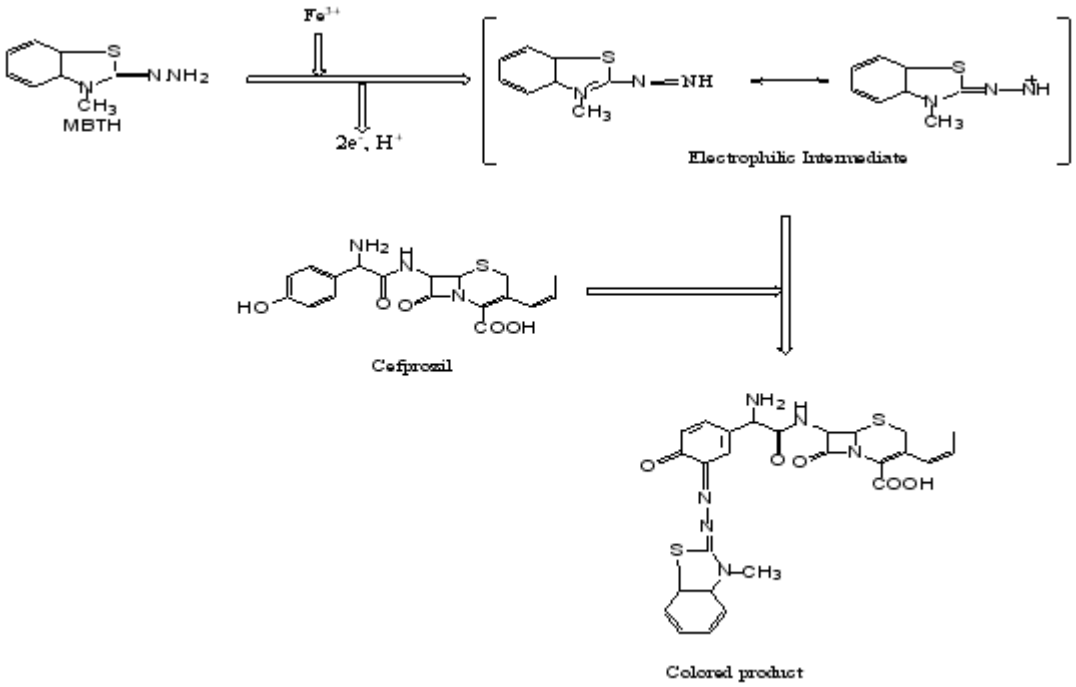
Fe(III) + o-Phosphoric acid ⟶ Complex (unreactive)

Step - II



Ferroin Complex

Scheme 1



Scheme 2

Table 1: Optical characteristics and precision

Optical Characteristics	Method A	Method B	Method C
λmax (run)	500	620	740
Beer's law limits(μg.mL^{-1})(C)	4 - 20	2 - 10	8 - 40
Molar absorptivity (lit.mol^{-1}cm^{-1})	7.27×10^3	1.44×10^4	4.67×10^3
Sandell's sensitivity (μg/cm^2)-0.001 abs units	0.0171	0.0126	0.0229
Regression equation(Y = a + bc)*			
Slope (b)	0.0174	0.0350	0.0113
Intercept (a)	0.0035	0.0019	0.0035
Correlation coefficient (r)	0.9999	0.9999	0.9999
% RSD	0.8191	0.8181	0.7555
Range of errors**			
Confidence limits with 0.05 level	0.8599	0.8589	0.7931
Confidence limits with 0.01 level	1.3485	1.3469	1.2438

´Y is the absorbance and C is the concentration μg.mL^{-1}

´For six measurements

Table 2: Estimation of cefprozil in pharmaceutical dosage forms

Sample	Labeled amount (mg)	Amount obtained (mg) Proposed methods*			UV method	%Recovery of Proposed methods**		
		Method A	Method B	Method C		Method A	Method B	Method C
Tablet-1	250	248.02	247.04	246.35	249	99.61	99.21	98.94
Tablet- 2	500	497.08	496.03	495.01	498	99.82	99.60	99.39

*Average of six determinations

** Mean and standard deviation of six determinations

tubes, 5.0 ml of Na_2CO_3 and 1.5 ml of F.C. reagent were added and kept aside for 15 minutes. The volume was adjusted to the mark with distilled water. The absorbance was measured at 740 nm against a reagent blank prepared under identical conditions. In all the above methods, a calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve, or deduced using a regression equation, calculated from Beers law data.

RESULTS AND DISCUSSION

In method A, the ferric ion was reduced by phenolic OH group of the drug to ferrous ion, which reacts with 1, 10-phenanthroline and forms ferrion complex (Scheme 1) which exhibiting λ_{max} at 500 nm. In method B, MBTH looses two electrons and one proton on oxidation, forming the eletrophilic

intermediate which has been postulated to be the active coupling species. The intermediate reacts with cefprozil to form colored species(Scheme 2) exhibiting λ_{max} at 620 nm. In method C, the reduction of heteropolyacid, phospho- molybdotungstic acid, the well known Folin-Ciocalteau reagent (FC), by the cefprozil in the presence of alkali thereby producing one or more possible reduced species which have a characteristic intense blue colored chromogen with λ_{max} at 740 nm.

Beer's law was obeyed over the concentration range of 4-20 $\mu g.mL^{-1}$ for method A, 2-10 $\mu g.mL^{-1}$ for method B and 8-40 $\mu g.mL^{-1}$ for method C respectively. The proposed procedures are validated by determining various optical parameters, which are listed in Table 1. The linearity, intercepts and the slope have been calculated using regression equation Y= a+bC, where Y represents optical density, 'C', the concentration of the drug in $\mu g.mL^{-1}$ and 'a' and 'b' represents intercepts and slope respectively. Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and dosage samples of the drug, whose concentration lie within Beer's law range.

The values of standard deviation (% R.S.D.) and percent range of error (0.05 level and 0.01 level confidence limits) were calculated for the above three methods are presented in Table 1. The values obtained for the determination of Cefprozil in different brands of Tablet samples 1and 2 by the proposed and U.V methods are compared in Table-2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table 2.

For method A and B, the reaction of colored species formation were slow at room temperature 25 °C and requires longer time for completion. Hence, efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum color intensity was obtained by heating the reaction mixture at 90°C on a boiling water bath for 30 minutes, for method A and at 100°C on boiling water bath for 5 minutes, for method B. For method C, room temperature 25°C is sufficient for the development of colored species .The absorbences remained constant at room temperature for more than 10 and 6 hours for method A and B respectively. In method C, the color was found to stable for more than 4 hours at room temperatures.

CONCLUSIONS

The methods reported here are found to be simple, sensitive, accurate and precise. Further, spectrophotometric methods involve simple instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories cannot afford to have. The present methods involve the formation of highly stable colored species which makes it easier for the determination of cefprozil from pharmaceutical dosage forms in a routine manner. Further statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the methods.

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