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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CEFPROZIL IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Three visible spectrophotometric methods have been described for the assay of cefprozil either in bulk form or dosage forms. Methods A-C is based on the oxidation of cefprozil with an excess of oxidant [N-bromosuccinimide (NBS) in methods A & B or chloramine-T (CAT) in method C] in acidic medium. The unreacted oxidant is then estimated colorimetrically by using an oxidisable dye [Celistine blue (CB) in method A or Gallocyanine (GC) in method C] or by p-N-methylaminophenol sulphate (PMAP)-sulphanilamide (SA) reagent in method B. Regression analysis of Beer's law plots showed good concentration range 4.0-20.0 μg/mL, 4.0-20.0 μg/mL and 1.0-10.0 μg/mL for methods A, B and C, respectively and gives reproducible results.

Key words: Spectrophotometry, Cefprozil, NBS, CAT.

INTRODUCTION

Cefprozil (CEF) is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral, intravenous administration and chemically known as (6*R*,7*R*)-7-[(*R)*-2-(*p*-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate. A number of methods such as spectrophotometric[1-10] and HPLC[11-21] were reported for the estimation of CEF. Literature survey revealed that only two visible spectrophotometric[6,7] methods were reported for it quantitative determination in bulk drug and pharmaceutical formulations. The present communication describes three visible

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spectrophotometric methods (A-C) for the assay of CEF in bulk form and dosage forms. Methods A-C are indirect procedures, involving the addition of an excess oxidant and determination of the unreacted oxidant by measuring either the decrease in absorbance of the dye (NBS/CB, method A; CAT/GC, method C) or color produced with PMAP-SA reagent (NBS/PMAP-SA, method B).

EXPERIMENTAL

Instruments

A Milton Roy Spectronic 1201 with 1 cm matched quartz cells was used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

Reagents

All the chemicals and reagents were of analytical grade and the solutions were prepared in triply distilled water. Aqueous solutions of NBS (Loba, 5.62 x 10^{-4}M), CB (Chroma, 5.49 x 10^{-4}M) and HCl (E. Merck, 5M) were prepared for method A. Aqueous solutions of NBS (Loba, 5.62 x 10^{-3}M), PMAP (Loba, 8.71 x 10^{-3}M), SA (Sd Fine Chemicals, 1.16 x 10^{-2}M) and acetic acid (Qualigens, 8.75 x 10^{-1}M) were prepared for method B. Aqueous solutions of CAT (Loba, 7.10 × 10^{-4}M) and GC (Chroma, 2.9 × 10^{-4}M) were prepared for method C.

Preparation of standard drug solution

A 1 mg/mL solution was prepared by dissolving 100 mg of pure CEF in 100 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 200 μg/mL for method A, 100 μg/mL for methods B and C, respectively.

Analytical procedures

Method A: Aliquots of standard CEF solution (0.5–2.5 mL, 200 μg/mL), 1.25 mL of 5M HCl and 2.5 mL of NBS (5.62 x 10^{-4}M) were delivered into a series of 25 mL calibrated tubes and the volume in each tube was brought to 20 mL with distilled water. After 10 min, 5 mL of CB solution was added and mixed thoroughly. The absorbances were measured after 5 min at 520 nm against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances were measured against distilled water. The difference in the decrease in

absorbance between test and blank (or test against reagent blank) corresponding to the consumed NBS and in turn, drug concentration was computed from its calibration graph.

Method B: Aliquots of standard CEF solution (0.5–2.5 mL, 200 μg/mL) were transferred into a series of 25 mL calibrated tubes. 0.5 mL of AcOH and 2.0 mL of NBS (5.62×10^{-3}M) solutions were added to the above solutions and volume in each tube was brought to 10 mL with distilled water and kept aside for 20 min at room temp. Then 2.0 mL of PMAP solution was added. After 2 min, 2.0 mL of SA solution was added and the volume was made up to the mark with distilled water. The absorbances were measured after 10 min at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in the absorbance and in turn, the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of GAT was computed from its calibration graph.

Method C: To each of 25 mL calibrated tubes containing standard CEF solution (0.5–2.5 ml, 100 μg/mL), 1.25 mL of 5M HCl and 2.0 mL of CAT were added and the solution was diluted to 20 mL with distilled water. After 10 min, 5 mL of GC solution was added, mixed thoroughly and the absorbances were measured after 15 min at 540 nm against distilled water. A blank was carried out in a similar manner. The decrease in absorbance corresponding to consumed CAT, which in turn to the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye (GC), against amount of the drug. Amount of the drug in any sample was computed from its calibration graph.

For pharmaceutical formulations

The tablet powder equivalent to 100 mg of CEF was accurately weighed and dissolved in methanol and then filtered. For methods A, B and C, the filtrate was evaporated to dryness and the residue was dissolved in 100 mL of distilled water to achieve a drug concentration of 1 mg/mL, from which, suitable dilutions were performed for methods A, B and C as mentioned earlier.

RESULTS AND DISCUSSION

The optimum conditions for the color development of the method were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Method A involves the oxidation of CEF with excess of NBS (first step) and estimating the unreacted NBS with CB (second step). The effect of reagent concentration (acidity, NBS and CB) and time in each step were studied by means of controlled experiments varying one parameter at a time. Studies of variation of acid concentration indicated that constant absorbance was obtained with 1.0-1.5 mL of 5 M HCl, 0.5-1.0 mL of 5 M H_2SO_4, or 1.5-2.5 mL of 5 M AcOH, when 3.0 mL of 5.62 x 10^{-4} M NBS was used. Since the difference in absorbance between the sample and blank was found to be highest with the addition of HCl and hence, subsequent studies were performed with 1.25 mL of 5 M HCl. In order to ascertain the linear relationship between the volume of added NBS and decrease in absorbance of CB, experiments were carried out in 1.25 mL of 5 M HCl with varying volumes of NBS. The decrease in absorbance was found to be linear up to 2.5 mL of 5.62 x 10^{-4}M NBS with 5.0 mL of 5.49 x 10^{-4}M CB. So fixed amounts of HCl (1.25 mL, 5 M), NBS (2.5 mL, 5.618 x 10^{-4}M) and CB (5.0 mL, 5.49 x 10^{-4}M) were taken for further investigations. Time span of 5 to 15 min for the reaction between GAT and NBS in the first step and 3 to 10 min between NBS and CB in the second step resulted in constant and maximum difference in absorbance of test and blank solutions. Hence, reaction periods of 10 and 5 min were maintained in subsequence studies of the first and second step, respectively. The color was found to be stable up to 30 min. The absorption spectra of the colored species in the proposed method show characteristic λ_{max} 520 nm.

Method B involves two stages, namely oxidation with excess of NBS and the determination of unreacted NBS using PMAP-SA reagent. Oxidation of GAT with 1.5-3.0 mL of NBS (5.61 x 10^{-3}M) solution gave maximum and reproducible absorbance values. The effect of time and temperature of oxidation on the absorbance of the colored species was studied by conducting the oxidation at different temperatures for different time intervals. Oxidation times ranging from 10-20 min at room temp (28 ± 5^0C) gave constant and reproducible absorbance values. Prolonging the oxidation time beyond 20 min and increasing the temperature gave erratic results. The pH of the solution at 2.9 ± 0.2 was found to be the best for attaining maximum sensitivity. This was achieved by the addition of 0.5 mL of 8.75 x 10^{-1}M acetic acid. Use of 1.0-2.0 mL of PMAP solution and 1.0-2.5 mL of SA solution afforded maximum absorbance value. A waiting period of 1-3 min was necessary between the addition of PMAP and SA solutions for the generation of p-N-methyl benzoquinone monoimine (PMBQMI) by the action of NBS on PMAP. Prolonging the waiting period beyond 3 min resulted in low absorbance values, owing to the partial hydrolysis of PMBQMI formed *in situ* to the quinone state. Among the water miscible solvents examined, water was found to be the best for final dilution of the solution. Maximum color intensity was attained within 10 min after the final dilution and remained

stable for next 40 min. The absorption spectra of the colored species in the proposed method show characteristic λ_{max} 520 nm.

Method C involves the oxidation of CEF with excess of CAT (first step) and estimating the unreacted CAT with GC (second step). The effect of reagent concentration (acidity, CAT and GC) and time in each step was studied by means of controlled experiments by varying one parameter at a time. Studies of variation of acid concentration indicated that constant absorbance was obtained with 1.0-1.5 mL of 5 M HCl, 0.5-1.0 mL of 5 M H_2SO_4, or 1.5-2.5 mL of 5 M AcOH, when 3.0 mL of CAT was used. Since the difference in absorbance between the sample and blank was found to be highest with the addition of HCl and therefore, subsequent studies were performed with 1.25 mL of 5 M HCl. In order to ascertain the linear relationship between the volume of added CAT and decrease in absorbance of GC, experiments were carried out in 1.25 mL of 5 M HCl with varying volumes of CAT. The decrease in absorbance was found to be linear up to 2.0 mL of CAT with 5.0 mL of GC. So fixed amounts of HCl (1.25 mL, 5 M), CAT (2.0 mL, 7.10×10^{-4}M) and GC (5.0 mL, 2.9×10^{-4}M) were taken for further investigations. Time span of 5 to 15 min for the reaction between GAT and CAT in the first step and 10 to 20 min between CAT and GC in the second step resulted in constant and maximum difference in absorbance of test and bulk solutions. Hence, reaction periods of 10 and 15 min were maintained in subsequence studies of the first and second step, respectively. The color was found to be stable up to 60 min. The absorption spectra of the colored species in the proposed method show characteristic λ_{max} 540 nm.

Analytical data

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table 1. The precision of each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results obtained are incorporated in Table 1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and is presented in Table 1. Commercial formulations containing GAT were successfully analyzed by the proposed methods. The results obtained by the proposed and reference methods (UV) for dosage forms were compared statistically by the t – and F – tests (Table 2). This comparison shows that there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were performed by

adding a fixed amount of the drug to the pre-analyzed formulations. The amount of drug found and the % recovery were calculated in the usual way.

Table1: Optical characteristic, precision and accuracy of the proposed methods for cefprozil

Optical characteristics	Method A	Method B	Method C
	NBS/CB	NBS/PMAP-SA	CAT/GC
λ_{max} (nm)	520	520	540
Beer's Law limits (μg/mL)	4-20	4-22	2-10
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.46 x 10^4	1.32 x 10^4	2.95 x 10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.028	0.031	0.014
Regression Equation y = a + bc (i) Slope (b)	0.0361	0.0322	0.0732
(ii) Intercept (a)	-0.0013	0.0058	-0.0023
Correlation coefficient (r)	0.9999	0.9999	0.9999
% error in bulk sample**	-0.044	0.072	0.065
% range of error 0.05 level	0.151	0.147	0.287
0.01 level	0.223	0.218	0.425
Relative Standard Deviation*	0.181	0.176	0.343

*Average of six determinations considered. **Average of three determinations

Table 2: Assay of CEF in pharmaceutical formulations

Pharmaceutical formulations (Labeled amount)	Amount found by proposed methods*			Reference method #	% Recovery by proposed methods**		
	A	B	C		A	B	C
Tablet I (200 mg)	199.6 ± 0.38 F = 1.35 T = 0.61	199.78 ± 0.38 F = 1.34 T = 0.98	199.55 ± 0.42 F = 1.64 T = 0.6	199.62 ± 0.33	99.79 ± 0.19	99.89 ± 0.20	99.77 ± 0.21

Cont…

Pharmaceutical formulations (Labeled amount)	Amount found by proposed methods*			Reference method #	% Recovery by proposed methods**		
	A	B	C		A	B	C
Tablet II (200 mg)	199.74 ± 0.44 F = 2.66 T = 1.22	199.12 ± 1.0 F = 1.95 T = 1.3	199.6 ± 0.6 F = 1.46 T = 1.4	199.34 ± 0.71	99.87 ± 0.22	99.56 ± 0.5	99.44 ± 0.64
Tablet III (400 mg)	398.42 ± 1.43 F = 1.83 T = 1.99	399.83 ± 2.59 F = 1.00 T = 0.83	399.68 ± 2.86 F = 1.10 T = 0.99	396.55 ± 1.94	99.60 ± 0.35	99.95 ± 0.64	99.92 ± 0.71
Tablet IV (400 mg)	398.56 ± 1.43 F = 1.83 T = -5.00	396.79 ± 2.4 F = 1.15 T = 1.19	398.85 ± 1.94 F = 2.41 T = 0.18	397.33 ± 2.58	99.64 ± 0.35	99.19 ± 0.60	99.72 ± 0.48

#Developed in the laboratory using methanol

*Average ± Standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, t = 2.57, F = 5.05.

**After adding 3 different amounts of the pure labeled to the pharmaceutical formulation, each value is an average of 3 determinations.

Interference studies

The interference studies in the determination of CEF in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, lactose, gelatin, talc, magnesium stearate, aluminum hydroxide, sorbitol, calcium silicate and glycerin do not interfere, even when present in excess than the anticipated amount. However, a preliminary clean up procedure with methanol is necessary to avoid interference due to the presence of reducing sugars like lactose if present, prior to the estimation of GAT in formulations for methods A, B and C, respectively.

CONCLUSION

The proposed methods are applicable for the assay of cefprozil and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and

λ_{max} among the proposed methods are C > A > B and C > A = B respectively. The proposed methods are simple, selective and can be used in the routine determination of CEF in bulk samples and formulations with reasonable precision and accuracy.

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