Spectrophotometric Determination of Ceftibuten in Bulk and in Pharmaceutical Formulations

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Abstract: A simple and cost effective spectrophotometric method is described for the determination of ceftibuten in pure form and in pharmaceutical formulations. The method is based on the formation of blue colored chromogen, when the drug reacts with Folin-Ciocalteu (F-C) reagent in alkaline medium. The colored species has an absorption maximum at 798 nm and obeys Beer's law in the concentration range 10-50 mcg/mL. The apparent molar absorptivity and sandell's sensitivity were 0.4583×10^4 and 0.3×10^{-4} respectively. The slope and intercept of the equation of the regression line are 4.5×10^{-3} and 1.3×10^{-5} respectively. The optimum experimental parameters for the reaction have been studied and the validity of the described procedure was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed method was successfully applied for the determination of ceftibuten in pharmaceutical formulations.

Keywords: Ceftibuten, F-C reagent, Spectrophotometry.

Introduction

Ceftibuten¹(CTB) is chemically [6R-6 α -7 β (R*)]-7-[α -amino-4-hydroxy phenyl)-acetyl]amino]-8-oxo-3-(1-propenyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate. It is a third generation cephalosporin effective against gram-positive and gram-negative bacteria, including both aerobes and anaerobes. It is indicated for complicated upper and lower urinary tract infections, respiratory tract infections, skin and soft tissue infections and bacteraemia. It is official in Martindale-The Extra Pharmacopoeia². Literature survey reveals that the drug was determined by using HPLC³⁻⁵ and some spectrophotometric methods⁶. According to literature survey there is no method reported for ceftibuten by visible spectrophotometry. Hence, there was an attempt made to develop a simple and sensitive spectrophotometric method for the estimation of ceftibuten in pure drug and in pharmaceutical formulations. The method uses the well known reduction reaction involving Folin-Ciocalteu (F-C) reagent and ceftibuten resulting in the formation of a blue chromogen that could be measured at 798 nm.

Experimental

Apparatus

All spectral characteristics and absorbance measurements were made on ELICO (SL 164) double beam spectrophotometer with 10 mm matched quartz cells. All chemicals used were of analytical reagent grade and double distilled water was used throughout. F-C reagent 2N supplied by S.D. Fine chem. India, Ltd., was used by diluting 50 mL to 100 mL with distilled water. Sodium hydroxide solution (1%) was prepared by dissolving 1 g of sodium hydroxide in 100 mL double distilled water. 10 mcg/mL stock reference solution was freshly prepared from pure sample of ceftibuten by dissolving 100 mg in 100 mL of double distilled water and made necessary dilutions to obtain 10 mcg /mL.

General procedure

Into 10 mL volumetric flask, different aliquots of working standard solution (1-5 mL) were transferred to provide final concentration range of 1-5 mcg/mL. To each flask, 2 mL of F-C reagent and 1 mL of sodium hydroxide were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 798 nm against the reagent blank. The calibration graph was then prepared by plotting the absorbance versus the concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Procedure for tablets

Twenty tablets of Ceftibuten (cedax 400mg, Shionogi USA INC) were weighed accurately and ground into a fine powder. An amount of powder equivalent to 100 mg of ceftibuten was weighed into a 100 mL volumetric flask, 50 mL of distilled water was added and shaken thoroughly for about 10 minutes, then the volume was made up to the mark with the distilled water, mixed well and filtered. Further dilutions were made to make 10 mcg/mL. The assay of the tablets was completed according to general procedure

Results and Discussion

The F-C reagent was used in the determination of many phenolic compounds and a large number of substances of pharmaceutical interest. The proposed method is based on the formation of a blue colored chromogen, following the reduction of phospho-molybdotungstic mixed acids of the F-C reagent by ceftibuten, in the presence of sodium hydroxide, which could be measured at 798 nm. The mixed acids in the F-C reagent were the final chromogen and involve the following chemical species:

Ceftibuten probably effects reduction of 1, 2 or 3 oxygen atoms from tungstate and / or molybdate in the F-C reagent, there by producing one or more possible reduced species which have characteristic intense blue color. The effect of different variables such as nature and strength of alkali, optimum volumes of NaOH and F-C reagent, reaction time and order of addition of reactants were studied and optimized for attainment of maximum color and stability of colored species.

Optimization of conditions and absorption spectrum of the reaction product

Condition under which reaction of ceftibuten with F-C reagent fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32 ± 2 °C).

Selection of reaction medium

To find a suitable medium for the reaction, different aqueous bases were used, such as borax, sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate and sodium hydrogen phosphate. The best results were obtained when sodium hydroxide was used. In order to determine the optimum concentration of sodium hydroxide, different volumes of 1% sodium hydroxide solution (0.5, 1, 1.5, 2.5 mL) were used to a constant concentration of ceftibuten (30 mcg/mL) and the results of the observation were plotted. From the figure it is evident that 1 mL of 1% sodium hydroxide solution was found optimum. Larger volumes had no effect on the absorbance of the colored species.

Effect of F-C reagent concentration

Several experiments were carried out to study the influence of F-C reagent concentration on the color development. It was apparent that 2 mL of reagent gave maximum color and 2 mL of reagent in a total volume of 10 mL was used throughout the work.

Reaction time and stability of the colored species

The color reaction was not instantaneous. Maximum color was developed within 5 minutes of mixing the reactants and was stable for 60 minutes thereafter.

Effect of order of addition of reactants

After fixing all other experimental variables, a few further experiments were performed to ascertain the influence of order of addition of reactants on the color development and the results are presented in Table 1. The order of addition of serial number 1 is recommended.

Table 1. Effect of order of addition of reactants on color development.

S. No.	Order Of Addition	Absorbance ^a
1	CTB+F-C reagent + NaOH	0.18
2	CTB+NaOH + F-C reagent	0.16
3	F-C reagent + CTB + NaOH	0.05
4	F-C reagent + NaOH + CTB	0.16
5	NaOH + F-C reagent + CTB	0.16
6	NaOH + CTB + F-C reagent	0.02

^aFor 20 mcg/mL of CTB.

Absorption spectrum and calibration graph

Absorption spectrum of the colored complex was scanned at 400-820 nm against a reagent blank. The reaction product shows absorption maximum at 798 nm. Calibration graph was obtained according to the above general procedure. The linearity (eight replicates for five different concentrations) was checked by a linear least – squares treatment. All the spectral characteristics and the measured or calculated factors and parameters are summarized in Table 2.

Sensitivity, accuracy and precision

Sandell's sensitivity, molar absorptivity, precision and accuracy were found by performing eight replicate determinations containing $\frac{3}{4}$ th of the amount of upper Beer's law limits. The measured standard deviation (S.D.), relative standard deviation(RSD), and confidence limits (Table 2) can be considered satisfactory.

Interference

Reducing ions, tryptophan, hydroxyproline, 2-and 3-hydroxypyridines, ascorbic acid, uric acid also reduce F-C reagent to molybdenum blue. However these substances are seldom present in the reagents and used in the pharmaceutical formulations. Hence, the method is devoid of error due to above substances.

Table 2. Optical and regression characteristics, precision and accuracy of the proposed method for ceftibuten.

Parameters	Values
$\lambda_{\max}$, nm	798
Beer's law limits, mcg/mL	10-50
Molar absorptivity, L/mol.cm	0.4583 $\times 10^4$
Sandell's sensitivity, mcg/cm ² /0.001 absorbance unit	0.3 $\times 10^{-4}$
Regression equation ($Y = a + bc$)	
Slope(b)	4.5 $\times 10^{-3}$
Standard deviation of slope (S _b)	0.18 $\times 10^{-4}$
Intercept(a)	1.3 $\times 10^{-5}$
Standard deviation of intercept (S _a)	0.18 $\times 10^{-3}$
Standard error of estimation (S _e)	0.16 $\times 10^{-2}$
Correlation coefficient (r)	0.9731
Relative standard deviation % ^a	0.221
%range of error (confidence limits) ^a	
0.05 level	0.178
0.01 level	0.267
%error in bulk samples ^b	0.169

^aAverage of eight determinations; ^bAverage of 3 determinations, In $Y = a + bc$, Y is absorbance and c is concentration.

Application to formulation

The proposed procedure was applied for the determination of ceftibuten in commercially available tablets. The same samples were analysed, simultaneously by the UV reference method and the proposed method. The results obtained from the two sets of analyses were compared statistically. Table 3 summarizes the results.

Table 3. Results of analysis of tablet formulations containing ceftibuten.

Formulation	Labelled amount, mg	Recovery _a $\pm$ S.D., % proposed reference	T _b	F _c
A	40	99.85 $\pm$ 0.092	0.98	1.32
		98.00 $\pm$ 0.102		
B	20	100.12 $\pm$ 0.065	1.26	2.23
		100.05 $\pm$ 0.098		

^a Average of six determinations; ^b calculated t - value ; tabulated t -value for 5 degrees of freedom; and $p=0.05$ is 2.57; ^c Calculated F -value; tabulated F -value for 5 degrees of freedom; and 95% confidence limits is 5.05.

Conclusion

The proposed method was found to be simple, rapid, and inexpensive, and hence can be used for routine analysis of ceftibuten in bulk and in tablet formulations.

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