Spectrophotometric Determination of Ceftiofur Hydrochloride Using *N*-Bromosuccinimide and *p*-Dimethylaminobenzaldehyde

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Abstract: Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of ceftiofur based on the formation of oxidation and condensation products. Method A involves the oxidation of the ceftiofur (CEFT) with *N*-bromosuccinimide (NBS) and determination of the unconsumed NBS with *p*-*N*-methylaminophenol (PMAP)-sulfanilamide (SA) (PMAP-SA) reagent. Method B includes the determination of unreacted NBS using a known excess of celestine blue (CB) and measuring the remaining dye. Condensation of *p*-dimethylaminobenzaldehyde (PDAB) with the drug was the basis of method C. Determination of CEFT in bulk form and in pharmaceutical formulations was also incorporated.

Keywords: Ceftiofur, *N*-Bromosuccinimide, *p*-Dimethylaminobenzaldehyde, Spectrophotometry.

Introduction

Ceftiofur (Scheme 1) (CAS Number: [80370-57-6]; IUPAC Name: (6R,7R)-7- [[[2z)-(2-Amino-4 thiozyl (methoxyimino) acetyl] amino] -3- [[2-furanyl carbonyl} thio], methyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene -2- carboxylic acid) is a part of a family of powerful antibiotics¹. They are known as the third generation cephalosporins. The non-steroidal anti-inflammatory drugs (NSAIDS), such as flunixin, ketoprofen and carprofen were used in conjunction with ceftiofur, in the treatment of naturally occurring bovine respiratory diseases.

Ceftiofur(CEFT) has worldwide approvals for respiratory disease in swine, ruminants and horses and has also been approved for foot rot and metritis infections in cattle. A very few physicochemical methods have been reported in the literature for the assay of CEFT in

biological fluids and pharmaceutical formulations. Most of them are based on spectrophotometric methods^{2,3}, HPLC⁴⁻⁸, GC^{9,10}, fluorimetry¹¹⁻¹³, LC-MS¹⁴, GC-MS¹⁵⁻¹⁷, TLC¹⁸ and Mass¹⁹. The analytically useful functional groups in CEFT includes 2-amino-4-thiazoyl, β -lactam, carboxyl and double bond in dihydrothiazine have not been fully exploited for designing suitable spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy.

Scheme 1. Structure of ceftiofur.

A reported UV-Visible spectrophotometric method has been adopted for the determination of CEFT in pharmaceutical formulations (Tablets) and used as reference method to compare the results obtained by the proposed methods.

Experimental

An Elico UV-Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

All the chemicals and reagents used were of analytical grade and the aqueous solutions were freshly prepared with triple distilled water. A 1 mg/mL stock solution was prepared by dissolving 100 mg of pure CEFT in 100 mL of distilled water and working standards of required concentration were prepared. NBS (*N*-Bromosuccinimide) (Loba; 0.088%, 4.94×10^{-3} M), PMAP (*p*-*N*-Methylaminophenol sulphate) solution (0.3%, 8.71×10^{-3} M) and SA (Sulphanilamide) solution (Sd-fine; 0.2%, 1.16×10^{-2} M) for method A, NBS solution (Loba; 0.01%, 5.618×10^{-4} M), CB (Celestine blue) solution (Chroma; 0.005%, 5.497×10^{-4} M) and 5 M hydrochloric acid (E.Merck) for method B, PDAB (*p*-Dimethylaminobenzaldehyde) (BDH, 0.4%, 2.63×10^{-3} M) and conc. H_2SO_4 (Merck) for method C were prepared.

Recommended procedures

Method A

Aliquots of standard CEFT solution (1.0-5.0 mL, $200 \mu\text{g mL}^{-1}$) were transferred into a series of 25 mL calibrated tubes. Then 0.5 mL (8.75×10^{-1} M) of acetic acid and 2 mL (4.94×10^{-3} M) of NBS solutions were added and kept aside for 15 min at room temperature. Then 1.5 mL (8.71×10^{-3} M) of PMAP solution was added. After 2 min 2.0 mL (1.16×10^{-2} M) of SA solution was added. The volume was made up to the mark with distilled water as blank. The absorbance was measured after 10 min at 520 nm against distilled water. A blank experiment was also carried out without 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 CEFT was computed from its calibration graph Figure 1.

Method B

Aliquots of standard CEFT solution (0.5-3.0 mL, $20 \mu\text{g mL}^{-1}$) were transferred into a series of 25 mL calibrated tubes. Then 1.25 mL (5.0 M) of HCl and 2.5 mL (5.618×10^{-4} M) of NBS were added. The volume was brought to 15 mL with distilled water. After 10 min, 10 mL (5.50×10^{-4} M) of CB solution was added and mixed thoroughly. The absorbance was measured after 5 min at

540 nm. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbance was measured against distilled water. The decrease in absorbance corresponding to consumed NBS and in turn the drug concentration was obtained by subtracting the decrease in absorbance of the test solution (dye-test) from that of the blank solution (dye-blank). The amount of CEFT was computed from its calibration graph Figure 2.

Figure 1. CEFT - NBS/PMAP – SA system; (a) Absorption spectrum (b) Beer's law plot.

Figure 2. (a) CEFT - NBS/CB system; Absorption spectrum.

Figure 2. (b) CEFT - NBS/CB system; Beer's law plot.

Method C

To each of 10 mL calibrated tubes, aliquots (0.5-2.5 mL, 400 $\mu\text{g/mL}$) of methanolic standard drug solution, 2.0 mL of PDAB and 3.0 mL of conc H_2SO_4 were added successively and the total volume in each flask was brought to 9 mL by adding methanol and placed in hot water bath for 25 min. Then the flasks were cooled and made up to the mark with methanol and the absorbance was measured after 5 min at 540 nm against a reagent blank prepared in a similar way. The concentration of the drug sample was computed from Beer-Lambert's plot Figure 3.

Figure 3. CEFT – PDAB system.

Results and Discussion

Chemical reactions of methods - Method A

The oxidant (NBS) reacts with CEFT and forms an oxidation product (Scheme 2). The unreacted NBS then reacts with metol (PMAp as sulphate) giving rise to *p*-N-methylbenzoquinone monoamine (PMBQMI) which in turn forms charge transfer complex with sulfanilamide (SA).

Method B

As in method A, in this method NBS reacts with CEFT and forms an oxidation product (Scheme 3). The unreacted NBS then quantitatively decolorizes celestine blue, by disrupting the chromophores and auxochromes present in celestine blue.

Scheme 2.

Scheme 3.

Method C

The NH_2 group in thiazolyl moiety of CEFT reacts with PDAB to form a colored species, azomethine.

Validation of methods

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

The optical characteristics such as Beers law limits, molar absorptivity and Sandells sensitivity for the methods (A-C) are given Table 1. The precision of the method to the drug was found by measuring the absorbance of 6 separate 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) and standard error of estimation (Se) for each system.

Table 1. Optical and regression characteristics, precision and accuracy of the proposed methods for CEFT.

Parameter	Method-A	Method-B	Method C
$\lambda_{\max}$, nm	520	540	540
Beer's law limits, $\mu\text{g/mL}$	4.0-24.0	0.4-2.4	20-120
Detection limit, $\mu\text{g/mL}$	0.0107	0.08537	5.994
Molar absorptivity, $\text{L mol}^{-1}\cdot\text{cm}^{-1}$	1.212×10^4	1.401×10^5	2.213×10^3
Sandell's sensitivity, $\mu\text{g cm}^{-2}/0.001$ absorbance unit	0.1254	2.49×10^{-2}	0.4296
Optimum photometric range, $\mu\text{g/mL}$	6-24	1.15-2.14	45-105
Regression equation, $Y=a+bc$ slope (b)	0.02418	0.25	9.209×10^{-3}
Standard deviation in slope (S_b)	6.039×10^{-5}	5.36×10^{-3}	1.026×10^{-3}
Intercept (a)	1.749×10^{-3}	4.68×10^{-12}	5.749×10^{-3}
Standard deviation in intercept (S_a)	8.011×10^{-4}	7.114×10^{-3}	6.806×10^{-2}
Standard error in estimation (S_e)	7.638×10^{-4}	6.78×10^{-3}	6.490×10^{-2}
Correlation coefficient (r)	0.9990	1.0000	0.9993
Relative standard deviation, % *	0.816	0.7077	0.9905
0.05 level	0.938	0.8137	1.1389
0.01 level	1.471	1.276	1.7860
% error in bulk samples **	0.312	0.282	0.348

Table 2. Assay of CEFT in pharmaceutical formulations.

Sample	Amount taken, mg	Amount found by proposed methods			Amount found by proposed methods			Amount found by proposed methods			Reference method	% Recovery by proposed methods		
		Met A	F-test	t-test	Met B	F-test	t-test	Met C	F-test	t-test		Met A	Met B	Met C
Tablet 1	50	49.76	1.75	0.675	49.63	1.55	0.86	49.73	1.61	1.15	50.22	99.94	99.73	99.93
Tablet 2	50	49.66	2.52	0.57	49.78	1.66	0.302	49.76	3.69	1.27	49.93	99.85	99.92	99.92
Tablet 3	50	49.76	1.79	0.49	49.68	1.98	0.70	49.86	1.50	0.91	49.96	99.95	99.84	99.91
Tablet 4	50	49.89	1.89	0.49	49.76	1.43	0.69	49.78	1.86	0.64	50.17	99.85	99.93	99.85

The accuracy of the methods was ascertained by comparing the results by proposed and reference methods (UV) statistically by the *t*- and *F*- tests Table 2. The comparison shows that there is no significant difference between the results of studied methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods the excipients present in pharmaceutical formulations do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were carried out. The recovery of the added amounts of standard drug was studied at 3 different levels. Each level was repeated 6 times. From the amount of drug found, the percentage recovery was calculated from the amount of drug found.

The high $\lambda_{\max}$ values of all the proposed methods have a decisive advantage since the interference from the associated ingredients should be generally less at higher wavelengths than at lower wavelengths. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision, accuracy and constitute better alternatives to the existing ones to the routine determination of CEFT in bulk forms and pharmaceutical formulations.

Conclusions

The proposed methods exploit the various functional groups in CEFT molecule. The decreasing order of sensitivity ($\epsilon_{\max}$) among the proposed methods is Method B > Method A > Method C, respectively. The concomitants which do not contain the functional groups chosen in the present investigation do not interfere in the color development by the proposed methods. Thus the proposed methods are simple, sensitive and selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of CEFT in bulk drugs and pharmaceutical formulations.

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