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RESEARCH ARTICLE

Spectrophotometric Methods for the Assay of Ceftazidime in Bulk and its Pharmaceutical Formulations

L. MOHAN KRISHNA[a*], P. JAYACHANDRA REDDY[a],
V. JAYA SANKAR REDDY[a] and K.V. S. PRASADA RAO[b]

[a]Krishna Teja Pharmacy College, Tirupati, India
[b]Rahul Institute of Pharmaceutical Sciences, Chirla, India
mohanlokireddy@gmail.com

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Abstract: Three visible spectrophotometric methods have been described for the assay of ceftazidime (CTZ) either in bulk form or dosage forms. Methods A-C are based on the oxidation of CTZ with an excess of oxidant (*N*-bromosuccinimide (NBS) in methods A and B or chloramine-T (CAT) in method C) in acidic medium. The unreacted oxidant was then estimated colorimetrically by using an oxidisable dye (Celistine blue (CB) in method A or gallocyanine (GC) in method C) or by *p*-*N*-methyl aminophenol sulphate (PMAP)- sulphanilamide (SA) reagent in method B. Regression analysis of Beer's law plots showed good concentration range 2.0-10.0 μg/mL, 4.0-16.0 μg/mL and 1.0-8.0 μg/mL for methods A, B and C respectively and gives reproducible results.

Keywords: Spectrophotometry, NBS, Ceftazidime , Gallocyanine

Introduction

Ceftazidime (CTZ) is a thrid-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against gram-positive and garm-negative bacteria. Unlike most third-generation agents, it is active against pseudomonas aeruginosa, however it has weaker activity against gram-positive microorganisms and it is not used for such infections. It is also used in the empirical therapy of febrile neutropenia, in combination with other antibiotics, chemically known as (6R,7R,a)-7-(2-aminothiazol-4-yl)- 2-(2-carboxypropan-2-yloxyimino) acetamindo)-8-oxo-3-(pyridinium-1-ylmethyl)-5thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylate. A number of methods such as Spectrophotometic[1-12] and HPLC[13-29], were reported for the estimation of CTZ. Literature survey revealed that only two visible spectrophotometric methods were reported for it quantitative determination in bulk drug and pharmaceutical formulations. The present communication describes three visible spectrophotometric methods (A-C) for the assay of CTZ 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[9], method A; CAT/GC[11], method C) or color produced with PMAP-SA reagent (NBS/PMAP-SA[10], method B).

Experimental

A Milton Roy Spectronic 1201 with 1 cm matched quartz cells were 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, 5.62×10^{-4}M), CB (Chroma, 5.49×10^{-4} M) and HCl (E-merck, 5M) were prepared for method A. Aqueous solutions of NBS (Loba, 5.62×10^{-3} M), PMAP (Loba, 8.71×10^{-3} M), SA (Sd fine chemicals, 1.16×10^{-2} M) and acetic acid (Qualigens, 8.75×10^{-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

One mg/mL solution was prepared by dissolving 100 mg of pure CTZ in 100 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 100 μg/mL for method A, 200 μg/mL for method B and 50 μg/mL for methods C respectively.

Analytical procedures

Method A

Aliquots of standard CTZ solution (1.0–3.0 mL, 50 μg/mL), 1.25 mL of 5 M HCl and 2.5 mL of NBS (5.62×10^{-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 CTZ solution (1.0–3.0 mL, 100 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 CTZ was computed from its calibration graph.

Method C

To each of 25 mL calibrated tubes containing standard CTZ solution (1.0–3.0 mL, 50 μg/mL), 1.25 mL of 5 M 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 injection powder equivalent to 100 mg of CTZ was accurately weighed and 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 above.

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 CTZ 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 perameter 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 $5.62x10^{-4}$ 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 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 $5.62x10^{-4}$M NBS with 5.0 mL of $5.49x10^{-4}$M CB. So fixed amounts of HCl (1.25 mL, 5 M), NBS (2.5 mL, $5.618x10^{-4}$M), CB (5.0 mL, $5.49x10^{-4}$ M) were taken for further investigation. 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 NBS and the determination of unreacted NBS using PMAP-SA reagent. Oxidation of CTZ with 1.5-3.0 mL of NBS ($5.61x10^{-3}$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 ^{0}C) gave constant and reproducible absorbance values. Prolonging the oxidation time beyond 20 min and increasing the temperature gave erratic results. Maintaining 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 8.75×10^{-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 CTZ 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 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), GC (5.0 mL, 2.9×10^{-4} M) were taken for further investigation. Time span of 5 to 15 min for the reaction between CTZ 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 are 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-analysed formulations. The amount of drug found, the % recovery was calculated in the usual way.

Table 1. Optical characteristic, precision and accuracy of the proposed methods for CFT

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	2.0-10.0	4.0-20.0	1.0-6.0
Molar absorptivity, L $mol^{-1}cm^{-1}$	$4.431x10^4$	$3.227x10^4$	$4.787x10^4$
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.014	0.020	0.013
Regression Equation y = a + bc			
(i) Slope (b)	0.0692	0.0632	0.0753
(ii) Intercept (a)	0.0014	-0.127	0.0012
Correlation coefficient (r)	0.9998	0.9999	0.9998
% Error in bulk sample**	0.359	0.074	-0.133
% Range of error 0.05 level	0.362	0.317	0.336
0.01 Level	0.531	0.469	0.497
Relative standard deviation*	0.4291	0.3795	0.4016

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

Table 2. Assay of CFT in pharmaceutical formulations

Pharmaceutical formulations	Amount found by proposed methods*			Reference	% Recovery by proposed methods**		
(Labelled amount)	A	B	C	method #	A	B	C
Inj.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
Inj.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
Inj.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
Inj. 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 CTZ 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 CTZ in formulations for methods A, B and C respectively.

Chemistry of colored species

Methods A and B

These methods are based on the oxidation of CTZ by NBS to form oxidation products (probably mixtures, but reproducible under proposed experimental conditions) besides unreacted NBS, followed by the estimation of unreacted NBS either by CB (method A) or PMAP-SA (method B). In method A, the unreacted NBS decreases the color intensity of CB. NBS is involved in bromination reaction with the dye to form a brominated dye which is colorless. The probable sequences of reactions through analogy are presented in scheme A. In method B, the unreacted NBS develops color when treated with PMAP-SA. The PMBQMI formed *in situ* from PMAP and NBS involves charge-transfer color complex formation with SA.

Method C

In method C, CAT undergoes hydrolysis in aqueous acid medium to give sodium hypochlorite followed by hypochlorous acid. This reacts with CTZ to form the relevant oxidation products, probably a mixture, which appears to be reproducible under the specified experimental conditions. The remaining hypochlorous acid may be responsible for bleaching of the color GC through destruction of the extended chromophoric system.

Conclusion

The proposed methods are applicable for the assay of CTZ 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 CTZ in bulk samples and formulations with reasonable precision and accuracy.

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