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Spectrophotometric Estimation of Cefuroxime and Ceftazidime in Bulk and in Dosage Forms

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Abstract:- Three simple, accurate and sensitive spectrophotometric methods (A, B and C) for the determination of cefuroxime and ceftazidime in bulk samples and in dosage forms are described. They are based on the reaction with nitrous acid forming a nitroso derivatives which can be measured at λ_{max} 350 and 355 nm for cefuroxime (I) and ceftazidime (II), respectively (method A) or by oxidation of drug I or II with an excess of freshly prepared hypobromite and the residual hypobromite was treated with sodium fluorescein at the optimum experimental conditions and measured at λ_{max} at 517 nm (method B). Method C is based on the formation of tris (o-phenanthroline) iron(II) complex (ferroin) upon the oxidation of the studied drug I or II with an iron (III)-o-phenanthroline mixture in acetate buffer solution of pH 3.6 and measuring at λ_{max} 509 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 0.2 – 6.0, 0.2 – 3.2 and 0.1 – 5.6 μg ml^{-1} for methods A, B and C, respectively. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration range was 0.2 – 5.6 μg ml^{-1}. The validity of the proposed methods was tested by analysing dosage forms containing the studied drugs I and II. The relative standard deviations were ≤ 1.25% with recoveries 98.6 – 101.4% .

Key words: Cephalosporins determination, spectrophotometry, ferroin complex, nitroso derivatives, hypobromite.

Introduction

Cephalosporins are a large class of antibiotic agents which are derived from microorganism cephalosrium acremonium. They resemble penicillin in structure with variation in chemical structure next to beta lactam ring. Despite this similarity to penicillin, they offer broader antimicrobial coverage.

Several procedures have been reported in the literature for the analysis of cephalosporins. These methods are spectrophotometry[1-6], polarography[7,8], capillary zone electrophoresis[9], immunoassay analysis[10] and high performance liquid chromatography (HPLC) [11,21]

In the present work, three simple, accurate and sensitive methods for the determination of cefuroxime and ceftazidime in the presence of various excipients and diluents are developed. These methods are also applicable for determination of the two mentioned drugs in some pharmaceutical dosage forms without previous separation.

Experimental

Apparatus

A Shimadzu 260 UV-Visible spectrophotometer with 10 mm matched quartz cells was used for all the absorbance measurements. An Orion research model 601A/digital ionalyzer pH-meter was used for check the pH of all solutions.

Reagents

All chemicals were of analytical or pharmacopoeial grade and double distilled water was used throughout.

Aqueous solutions of sodium nitrite (20%, W/V), sodium hydroxide (20%, W/V), hydrochloric acid (5 and 10%, V/V) , and ammonium chloride (15%,W/V) were prepared for method A.

Aqueous solution of potassium hypobromite was prepared by addition of 12 ml 20% HCl to 6.0 ml of 0.1 M potassium bromate in 5% potassium bromide, then sufficient amount of 0.5M NaOH was added to produce hypobromite and complete the volume to 250 ml with bidistilled water in a 250 ml calibrated flask. Sodium fluorescein was prepared by dissolving 0.3803 g sodium fluorescein (BDH, Pool, UK) in water and completed to the mark in a one liter calibrated fask with the same solvent. Acetate buffer solutions of pH ranges 2.16-5.85 were prepared as recommended previously[22] [for Method B].

1,10-Phenanthroline-iron(III) colour reagent was freshly prepared by mixing 0.198 g of 1,10-phenanthroline (Sigma Chemicals, St. Louis, USA), 2.0 ml of 1.0 mol/L hydrochloric acid and 0.160 g of ferric ammonium sulphate (BDH, Pool, UK) and diluting with water to 100 ml in a 100 ml calibrated flask. This reagent is stable for more than 6.0 weeks if stored in a dark bottle a way from light (for method C).

Preparation of standard stock drug solution.

Standard cefuroxim sodium and cefazidime (Glaxo, Egypt, SAE, Cairo, Under the licence from Glaxo group, England) [1mg / ml] was prepared by dissolving 100 mg in

100 ml distilled water. The working standard solutions were obtained by further dilution of stock solution with water.

Analysis of pure sample

Method A

Aliquots of standard drug solution equivalent to 2.0-60 μg were pipetted into a series of 10 ml calibrated flasks. 1.0 ml of 5.0 % HCl followed by 1.0 ml of 20% Sodium nitrite were added, and heated in a water bath at 60 °C for 10 min. The mixture was cooled, then a dropwise of 5.0 ml of 15% NH_4Cl was added under stirring condition. 2.0 ml of 20 % NaOH was added and the volume was completed to the mark with water. The absorbance was measured at 350 and 355 nm for drug I and II, respectively, against a reagent blank prepared similarly without drug.

For ceftazidime the yellow colour developed spontaneously on cold, whereas heating for 10 min in waterbath at 60 °C is necessary for production of yellow colour for cefuroxime.

Method B

Aliquots of standard solutions containing drugs I and II in the ranges 5.0-80 μg were transferred into 25 ml calibrated flasks, 2.0 ml of potassium hypobromite reagent was added. After five mins, 2.0 ml of acetate buffer solutions of pH 5.5 and 5.7 for I and II, respectively, were added followed by 1.0 ml sodium fluorescein reagent. The volume was completed to the mark with water and the absorbance was measured at 517 nm after 10 min against a reagent blank prepared similarlly.

Method C

Aliquots of the sample solutions containing 0.1-56 μg were pipetted and transferred into a series of 10 ml calibrated flasks. 2.0 ml of 1,10- phenanthroline-iron(III) reagent was added and the mixture was heated at 75 °C for 5.0 min and cooled. The solution was completed to the mark with water and the absorbance was measured at λ_{max} 509 nm against a reagent blank prepared in the same way without drug.

Analysis of phamaceutical dosage forms (Vials)

The content of 10 vials was mixed and weighed. The average weigh of one vail was calculated. 20 mg of the mixed powder was dissolved in 10 ml water and then completed to the mark with water in 50 ml calibrated flask. This solution was used after appropriate dilution with water and continued as mentioned above for methods A, B and C.

Results and discussion

The absorption spectra of the reaction products in methods A, B and C show characteristic λ_{max} values (Fig. 1). The experimental reaction conditions were stablished by variation of one parameter at a time[23].

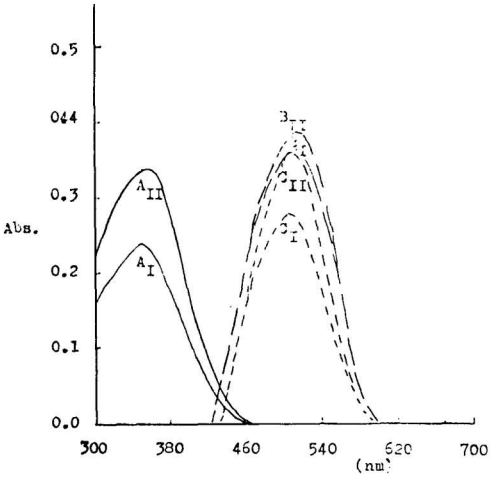

Fig (l) Absorbance spectra for method A (A_I and A_{II}), method B (B_I and B_{II})

And method C (C_I and C_{II}) using drug I and II, respectivily.

In method A, 0.8-1.2 ml of each of 5.0% HCl and $NaNO_2$ were the optimum concentration ranges for complete nitrosation on cold for ceftazidime and after heating in a water bath at 60°C for 7.0-15 min for cafuroxime. Hence, 1.0 ml of both 5.0% HCl and $NaNO_2$ was added and heated for 10 min at 60 °C were chosen for drug I. For maximum absorbance 4.0-7.0 ml of 15% NH_4Cl and 1.5-2.4 ml of 20% NaOH was necessary for completation of nitrosation reaction. 5.0 ml of 15% NH_4Cl and 2.0 ml of 20% NaOH were selected for the procedure. Maximum colour intensity was attained 5.0 min after the addition of NaOH and the coloured species was stable for 4.0 h.

Method B involves two stages: oxidation of drug I or II with excess of freshly prepared hypobromite and the residual hypobromite was treated with sodium fluorescein in buffered medium of pH 5.5 as optimal, where equivalent eosin was produced with λ_{max} at 517 nm. 2.0 ml of both potassium hypobromite and of acetate buffere of pH 5.5 and 5.7 for drug I and II, respectively was the optimum for the first stage. For the second one, 1.0 ml of sodium fluorescein reagent was found optimal, since the results were highly concordant at these conditions.

In Method C, 1.6-2.5 ml of 1,10- phenanthroline-iron(III) reagent with heating at 75 °C for 10 min, was found to give maximum colour intensity. The order of addition was found not to have a significant effect. The coloured species (ferroin) was stable for more than 12 h.

Analytical data

Beer's law limits, molar absorptivities, regression equations and correlation coefficients obtained by linear least squares treatment of the results are given in Table 1. The precision and accuracy were found by analysis of six separate samples containing known amounts of the drug (4.0 μg for method A, 2.0 μg for method B and 3.0 μg for method C) and the results are summarized in the same Table . The relative standard deviation 1.25% and ±1.4% range of error at the 95% confidence level are also given.

The values obtained by the proposed and reference [21,24] methods for pure drugs I and II are compared in view of accuracy (t-test) and precision (F-value)[25]. The results are in good agreement with lower values of t-and F-tests (Table 1) compared with the theoretical values indicating that there is no significant difference between the proposed and reference methods [21,24].

Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods

Parameters	I			II		
	A	B	C	A	B	C
Beer's law limits (μg ml^{-1})	0.2-6.0	0.2-3.2	0.4-4.4	0.2-6	0.8-2.4	0.2-5.6
Ringbom limits (μg ml^{-1})	0.5-5.6	0.3-2.9	0.2-4.0	1.8-5.5	04-2.1	0.5-5.2
Molar absorptivity ($L.mol^{-1}cm^{-1}$)	7.64×10^4	1.15×10^5	7.64×10^4	7.59×10^4	8.48×10^4	8.04×10^4
Sandell's sensitivity (ng cm^{-2})	8.33	5.55	6.96	5.89	5.26	5.56
Detection limits (μg ml^{-1})	0.25	0.21	0.12	0.25	0.11	0.22
Quantification limits (μg ml^{-1})	0.85	0.72	0.39	0.83	0.35	0.78
Regression equation[a]						
Slope (b)	0.12	0.18	0.145	0.17	0.19	0.18
Intercept (a)	0.008	0.010	-0.038	0.010	-0.017	0.017
Correlation coefficient (r)	0.9999	0.9997	0.9994	0.9992	0.9999	0.9990
Standard deviation of slope (S_b)	2.85×10^{-4}	3.11×10^{-4}	4.22×10^{-4}	3.38×10^{-4}	3.88×10^{-4}	4.0×10^{-4}
Standard diviation of intercept (S_a)	3.13×10^{-4}	3.66×10^{-4}	3.98×10^{-4}	3.56×10^{-4}	4.07×10^{-4}	3.7×10^{-4}
Standard error of estimation (S_e)	3.14×10^{-4}	4.04×10^{-4}	4.38×10^{-4}	3.78×10^{-4}	3.58×10^{-4}	4.37×10^{-4}
Relative standard deviation [b] (%)	0.58	0.83	0.64	0.83	0.60	0.76
% Range of error [b] (95% confidence limit)	0.95	0.34	0.75	0.78	0.25	1.10
t-test (2.57)[c]	1.17	1.63	1.24	1.48	1.79	1.55
F-value (5.05)[c]	2.56	3.12	2.77	2.81	3.40	3.09

[a] with respect to A = a + bC were C is concentration in μg ml^{-1} and A is theabsorbance

[b] Six replicate samples (concentrations 4.0, 2.0 and 3.0 μg ml^{-1} of pure drug I or II for methods A, B, and C, respectively).

[c] value in parantheses is the theoretical values for five degree of freedom and 95% confidence levels.

The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from series of 13 blank solutions for each procedures. The limits of detection (K=3) and of quantification (K=10) were established according to IUPAC definitions[26].

Effect of interfering species

The criterion of interference was an error of more than 3.0 % in absorbance. Experiments showed that there was no interference from additives and excipients e.g. lactose, glucose, fractose, calcium hydrogen phosphate, magnesium stearate and starch for the examined methods A, B and C. Also, there was no interference from common degradation products resulted from thermal decomposition of drug I and II indicating the ability to use the proposed methods in routine quality control for phamaceutical dosage forms.

Analytical applications

The proposed methods were applied to determine drug I and II in pharmaceutical dosage forms (commercial products randomly collected from local pharmacies). Table 2 list the results obtained by the proposed and reference methods [21,24] [based on HPLC and A 1 % in case of cefuroxime and ceftazidime, respectively]. The results indicate good agreement with the reference method. The proposed methods can be recommended for routine analysis in the majority of drug quality control laboratories. Another favourable characteristic of the methods is the stability of absorbance of the coloured species formed which are stable for 4.0, 6.0 and 12 h, using methods A, B and C, respectively.

On comparing the obtained results by the proposed methods with those of the reference methods[21,24], the t-test for accuracy and F-values for precision assessment[25] was used. The calculated values did not exceed the corresponding theoretical values, indicating insignificant difference between results. The proposed methods were more accurate, with high recoveries amounting to 99.8±1.2%, 100.3±1.3% and 100.5±1.1% for method A, B and C, respectively, compared with 100.8±1.7% using the reference methods [21, 24].

Table 2: statistical analysis of the results obtained by applying the proposed methods to pharmaceutical preparations.

procedure	Zinnate 1.5 g of cefuroxime	Fortum 500 mg of ceftazidime
Method A		
Recovery	100.52±0.99	100.89±0.93
t-test (2.57)[a]	0.85	1.96
F-Vatue (5.05)[a]	1.06	2.53
Method B		
Recovery	100.7±0.77	99.00±0.83
t-test (2.57)[a]	0.5	1.4
F-Vatue (5.05)[a]	1.79	1.3
Method C		
Recovery	98.93±0.82	100.21±1.03
t-test (2.57)[a]	1.2	0.6
F-Vatue (5.05)[a]	2.03	1.17
Reference method		
Recovery	100.50±0.575	99.81±0.951

[a]-value in paranthesis are the theoretical value of t and F-value for Five degree of freedom and 95% confidence limit.

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