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Short communication

Spectrophotometric determination of certain cephalosporins through oxidation with cerium(IV) and 1-chlorobenzotriazole

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1. Introduction

Cephalosporins are penicillinase-resistant antibiotics with significant activity against both gram-negative and gram-positive bacteria. Several procedures have been reported in the literature for the analysis of cephalosporins. These methods are spectrophotometry [1–3], high performance liquid chromatography [4,5], capillary electrophoresis [6], fluorimetry [7], polarography [8] and titrimetry [9]. The U.S.P. XXIII [10] specify high performance liquid chromatography for determination of cefotaxime sodium and cefuroxime sodium. Three spectrophotometric methods based on the oxidation of cefadroxil and other cephalosporins have been described. The first method involves oxidation of cefadroxil and cephalexin with ammonium vanadate in concentrated sulphuric acid media followed by subsequent monitoring of the absorbance at 760 nm [11]. The second method involves the oxidative spectrophotometric determination of cefadroxil, cefapirin, ceforanide, and cefuroxime after reaction with molybdophosphoric acid [12]. The third method involves oxidation of cefaclor and cefadroxil with chlorobenzotriazole reagent in strong alkaline medium pH 11.5 followed by subsequent monitoring of the absorbance at 333 nm [13].

On the other hand, 1-chlorobenzotriazole which is N–halogen compound containing three nitrogen ring may undergo certain chemical reactions which prove its usefulness in organic synthesis, thus, 1-CBT oxidizes alcohols to aldehydes and ketones, hydrazo to azo compounds and 1-amino-4,5-diphenyl triazole to diphenylacetylene, and 1-CBT is converted to benzotriazole hydrochloride [14].

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1-CBT reagent was used to assay of some sulphur compounds as thiourea, allylthiourea, phenylthiourea, tolylthiourea, thioacetamide, thiobenzamide, diethylthiocarbamate, ethylphenyldithiocarbamate, diisopropyldithiocarbamate, and methionine [15]. Recently, 1-CBT was used for determination some of phenothiazine derivative [16] and certain sulphur containing drugs, cefaclor and cefadroxil [13].

Cerium (IV) sulphate is a versatile oxidimetric reagent, Its high oxidation potential and excellent solution stability promoted us to use this reagent for the quantitative determination of cefotaxime sodium and cefuroxime sodium.

2. Experimental

2.1. Apparatus

Shimadzu 260 UV recording spectrophotometer equipped with 1 cm matched pair optical quartz cells was used.

2.2. Materials and reagents

All the reagents were of analytical grade. Double distilled water was used.

1. Cefotaxime sodium, Hoechst orients Egypt. Cairo, under the licence from Hoechst AG Frankfurt (Main), Germany.
2. Cefuroxime sodium, Glaxo Egypt S.A.E. Cairo, under the licence from Glaxo Group Ltd., England.
3. Cerium (IV) sulphate (May and Baker, Dagenham, England), 2.8×10^{-3} M solution, was prepared by dissolving 93 mg $Ce(SO_4)_2$ in 100 ml of 4 M perchloric acid.
4. *p*-Dimethylaminobenzaldehyde (Riedel-de-Haën AG, Seelze. Hanover, Germany), 0.2% w/v was prepared by dissolving 200 mg in 1 ml 70% perchloric acid and completing to 100 ml with distilled water.
5. 1-Chlorobenzotriazole, was prepared by method of Johnson et al. [17] and recrystallized from dichloromethane, m.p. (105–106°C). It is purity was checked by iodometric method (99.89%), 0.02 M ethanolic solution was prepared by dissolving 307.2 mg per 100 ml ethanol.

2.3. Standard solutions

Solutions of 0.25 mg ml^{-1} were prepared by dissolving 25 mg of cefotaxime sodium or cefuroxime sodium in distilled water in a 100-ml volumetric flask and diluting to volume. The solutions were stable for at least 48 h if they had been stored in a cool (< 25°C) and dark place.

2.4. Formulations

The following commercial formulations were subjected to the analytical procedures: Claforan vials (Hoechst Orient Egypt, Cairo) containing 524 mg cefotaxime sodium equivalent to 500 mg cefotaxime per vial.and Zinnat vials (Glaxo S.A.E. Egypt, Cairo) containing cefuroxime sodium equivalent to 250 mg cefuroxime per vial..

2.5. General procedures

2.5.1. Procedure using Ce(IV)

Appropriate volumes of solutions prepared from the standard drug solution, in the concentration range stated in Table 1, were placed in 10 ml volumetric flasks, followed by 1 ml of 2.8×10^{-3}M cerium (IV) sulphate, mixed well, and allowed to stand for 20 min at room temperature. 1 ml of 0.2% w/v *p*-DMAB solution was added. The volume was completed with distilled water and the decrease in absorbance (ΔA) at 464 nm against blank in which the drug is omitted.

2.5.2. Procedure using 1-CBT

Appropriate volumes of standard solution in the concentration range stated in Table 1 were placed in 10 ml volumetric flasks, followed by 1 ml 0.02 M 1-CBT ethanolic solution and allowed to stand for 20 min at room temperature. The volume was completed with distilled water and absorbance was measured at 298 nm against blank in which the drug is omitted.

2.5.3. Procedure for pharmaceutical preparations

The contents of two vial bottles was mixed well. An accurately weighted amount of each preparation equivalent to 25 mg of cefotaxime sodium or cefuroxime sodium was transferred into a 100-ml volumetric flask. Enough water was added, with stirring for 5 min, to dissolve the powder. The volume was completed with distilled water and the methods were continued as mentioned under Sections 2.5.1 and 2.5.2.

3. Results and discussion

3.1. For procedure using Ce(IV)

Cerium(IV) sulphate as strong oxidizing agent was utilized extensively for the determination of organic compounds. The proposed procedure involves two steps, the first is concerned with the treatment of the investigated drugs with a known excess amount of Ce(IV) sulphate in 4 M perchloric acid for certain time. The second step involves the determination of the excess unreacted Ce(IV) via its reaction with *p*-DMAB.

Oxidation of the investigated drugs with Ce(IV) can be monitored through measuring the excess of unreacted Ce(IV) at different time intervals since it forms a chromogenic product with *p*-DMAB. Addition of *p*-DMAB solution to a solution of Ce(IV) in 4 M perchloric acid gave a yellow colour with λ max. of 464 nm. The investigated drugs, *p*-DMAB and cerium(IV) sulphate has no absorption above 400 nm (Fig. 1).The decrease in absorption intensity at 464 nm-caused by the presence of the investigated drugs-was found to be proportional to the amount of the drug that has reacted.

The reaction between the investigated drugs and Ce(IV) is complete within 20 min and the colour produced from the reaction between the remaining unreacted Ce(IV) and *p*-DMAB develops immediately and remains stable for at least 20 min at 25 ± 5°C.

Acid medium is needed to prevent precipitation of hydrated ceric oxide, $CeO_2 \cdot xH_2O$. The reaction of the studied compounds with Ce(IV) proceeds quantitatively only in the presence of 4 M perchloric acid. The reaction of Ce (IV) in 3–4 M sulphuric acid ($E^o = 1.44$ V) or nitric acid ($E^o = 1.61$ V) as oxidant for organic compounds proved to be extremely slow and failed to obey any simple stoichiometric relationship. The greater strength of Ce(IV) in perchloric acid medium ($E^o = 1.75$ V) overcomes both the slowness of the oxidation process and the inexact stoichiometry encountered in sulphuric acid.

Dilution of the developed coloured product by different solvents brings about slight bathochromic shifts with methanol, ethanol, acetone, propanol and dioxane relative to water

Table 1
Optical characteristics and statistical data of the regression equations for oxidation of cefotaxime sodium and cefuroxime sodium with Ce(IV) sulphate and 1-CBT

Parameters	Cefotaxime sodium		Cefuroxime sodium	
	Ce(IV)	1-CBT	Ce(IV)	1-CBT
Beer's law limits/μg ml^{-1}	0.5–4.5	10.0–70.0	0.5–4.5	10.0–70.0
Molar absorptivity/mol^{-1} cm^{-1}	8.59×10^4	6.39×10^3	8.36×10^4	6.26×10^3
Sandell's sensitivity[a]	1.79×10^{-2}	1.26×10^{-3}	1.87×10^{-2}	1.43×10^{-3}
Regression equation				
Slope (b)	0.1752	0.0126	0.1773	0.0137
Intercept(a)	0.0113	0.0392	0.0246	0.0161
Correlation coefficient (r)	0.9997	0.9989	0.9998	0.9969

[a] Units, μg cm^{-2} per 0.001 A.

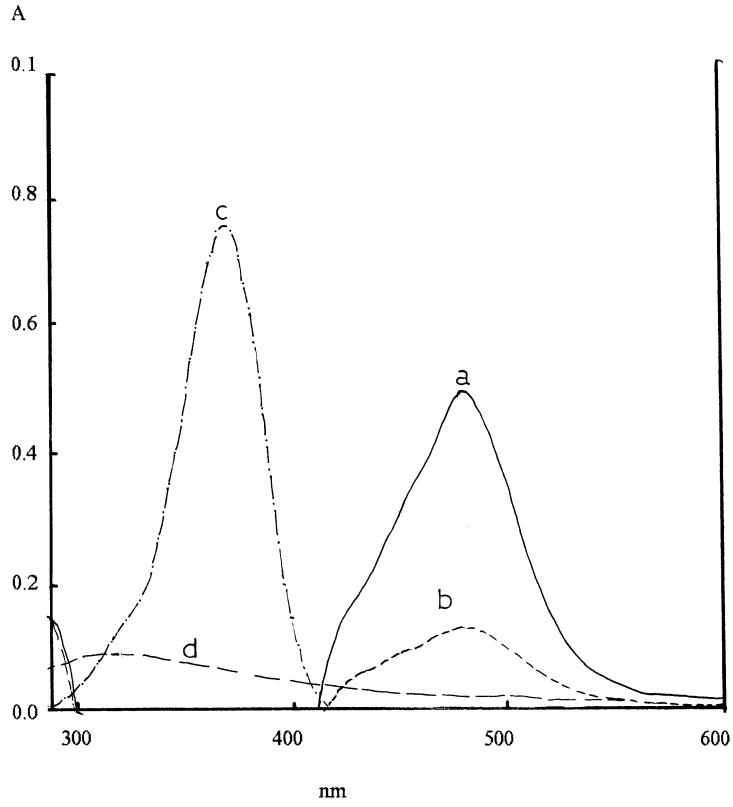


Fig. 1. Absorption spectra of 2.8×10^{-4} M ceric (IV) sulphate (d), 0.02% w/v *p*-DMAB (c) and their reaction product in absence and presence of 2 μg ml^{-1} cefotaxime sodium (a and b, respectively).

which afforded maximum stability and intensity.

The reaction between Ce(IV) and *p*-DMAB was linear at 40–110 μg ml^{-1} concentration range with the following regression equation:

$$A_{464} = 0.0011(\pm 0.09) + 0.0072(\pm 0.11)C$$

$$(r = 0.9989,\ n = 6)$$

C = μg ml^{-1} Ce(IV)

Under the specified reaction conditions, linear correlations were found between A at 464 nm and concentrations of the studied drugs. Beer's law limits, Sandell's sensitivity, regression parameters obtained by least squares treatments of the results are given in Table 1.

The reaction of the excess unreacted Ce(IV) with *p*-DMAB to give the coloured product could be explained as an oxidation process to give *p*-dimethylaminoquinone. It was reported that *p*-DMAB underwent a Dakin-type reaction when treated with hydrogen peroxide at 35°C for 24 hours to give p-dimethylaminophenol and formic acid, furthermore, *p*-dimethyl-aminophenol underwent oxidation to the corresponding quinone [18].

3.2. For procedure using 1-chlorobenzotriazole

The organic positive halogen compounds have been used as oxidizing agent for the oxidation of a variety of organic compounds. The oxidation reactions generally involve the loss of the hydrogen from –C–H, O–H, –N–H, or –S–H bonds. Though the reactions involving addition of oxygen have also been reported. These reactions have

found extensive application in the determination of organic compounds [19].

1-CBT was used to oxidise some sulphur containing compounds as cefotaxime sodium and cefuroxime sodium with the production of yellow colour reaction product with λ max. at 298 nm Fig. 2.

For optimization the reaction condition of 1-CBT with the studied drugs, several factors have been carefully studied. Concerning the effect of pH, it was found that the maximum colour intensity was produced in neutral medium, addition of NaOH cause high decrease in the intensity of the colour (differ from that in [13], in which the colour produced only in strong alkaline medium pH 11.5 using NaOH, in the determination of cefaclor and cefadroxil). Concerning the amount of the reagent solution and effect of the time, 1 ml of 0.02 M 1-CBT ethanolic solution and 20 min were found to be sufficient to obtain complete reaction, where maximum absorbances were obtained and the colour developed was stable for at least 30 min.

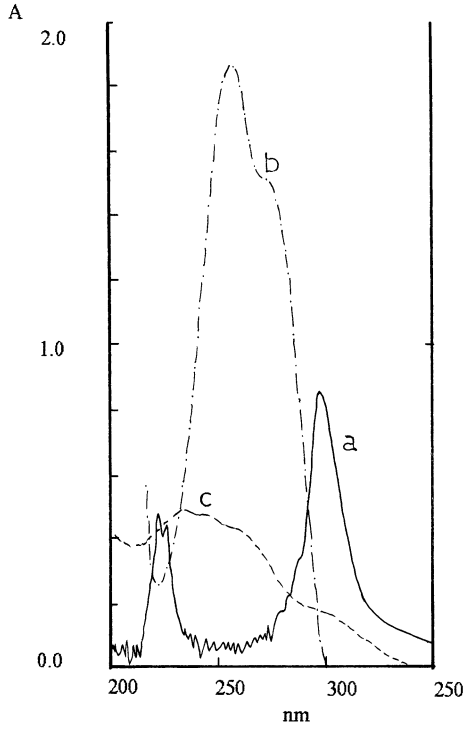


Fig. 2. Absorption spectra of 65 μg ml^{-1} cefotaxime sodium (c), 2×10^{-3} M 1-CBT (b) and their reaction product (a).

The suggested possible reaction bath ways are postulated in Scheme 1.

Under the specified reaction conditions, linear correlations were found between A at 298 nm and concentrations of the studied drugs. Beer's law limits, Sandell's sensitivity, regression parameters obtained by linear squares treatments of the results were given in Table 1

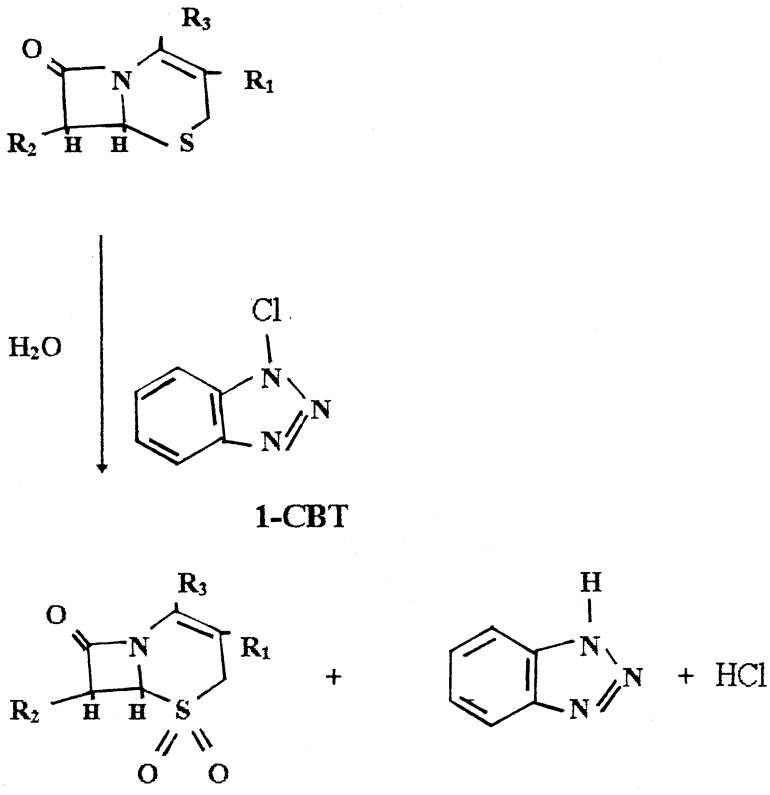
3.3. Accuracy and precision of the methods

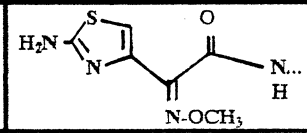
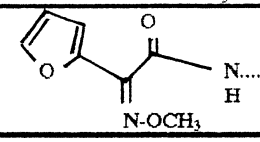
In order to determine the accuracy and precision of the methods, solutions containing three different concentrations of cefotaxime sodium and cefuroxime sodium were prepared and analysed in five replicates. The analytical results obtained from this investigation are summarized in Table 2. The percentage standard deviation and the percentage range of error at 95% confidence level can be considered to be very satisfactory.

The proposed methods for the determination of cefotaxime sodium and cefuroxime sodium were applied to commercial vials together with the official USPXXIII methods [10]. These determinations were carried out on the same batch of samples. The results obtained were compared statistically by Student's t-test and variance ratio F-test (Table 3), which indicates that there was no significant difference between the methods compared.

4. Conclusion

The proposed methods are advantageous when compared to many of the reported spectrophotometric and titrimetric methods because of their higher sensitivity which permits the determination of up to 0.5 μg ml^{-1}. They can be applied for the quality control analysis of cephalosporins containing dosage forms without interference. Although the methods cannot differentiate between



	R1	R2	R3
Cefotaxime sodium	$-CH_2-O-COCH_3$		-COONa
Cefuroxime sodium	$-CH_2-O-CONH_2$		-COONa

Scheme 1.

the two cephalosporins, this shortcoming does not affect their usefulness in routine analysis and content uniformity determination of these drugs as they are singly prescribed.

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

Compared method	Added/μg ml^{-1}	Found/ ± S.D.[a]	RSD%	Confidence limits[b]
Cefotaxime sodium				
Using Ce(IV)	0.5	0.51 ± 0.01	0.96	0.51 ± 0.0124
	1.5	1.53 ± 0.03	1.96	1.53 ± 0.0373
	2.5	2.55 ± 0.03	1.17	2.55 ± 0.0373
Mean			1.70	
Using 1-CBT	15	15.2 ± 0.10	0.66	15.2 ± 0.1243
	30	31.0 ± 0.08	0.26	31.0 ± 0.0995
	45	44.7 ± 0.22	0.49	44.7 ± 0.2735
Mean			0.47	
Cefuroxime sodium				
Using Ce(IV)	0.5	0.47 ± 0.01	2.13	0.47 ± 0.0124
	1.5	1.52 ± 0.03	1.97	1.52 ± 0.0373
	2.5	2.44 ± 0.03	1.23	2.44 ± 0.0373
Mean			1.78	
Using 1-CBT	15	14.2 ± 0.11	0.77	14.2 ± 0.1368
	30	30.7 ± 0.07	0.23	30.7 ± 0.0870
	45	45.9 ± 0.05	0.11	45.9 ± 0.0621
Mean			0.37	

[a] Mean ± standard deviation for five determination.
[b] Confidence limits at $P = 0.95$ and four degrees of freedom.

Table 3
Determination of cefotaxime sodium and cefuroxime sodium in commercial preparation using the proposed methods compared statistically with an official methods [10]

Compound formulation	Recovery ± S.D.%[b]		
	Proposed procedure		Official method[a]
	Using Ce (IV)	Using 1-CBT	
Cefotaxime sodium			
Claforan vials	97.37 ± 0.59	97.55 ± 0.46	97.60 ± 0.44
t[c]	0.70	0.17	
F[d]	1.80	1.09	
Cefuroxime sodium			
Zinnat vials	98.93 ± 0.18	99.16 ± 0.39	99.01 ± 0.19
t	0.68	0.77	
F	1.11	4.21	

[a] USP XXIII [10].
[b] Mean ± standard deviation of five determination.
[c] Tabulated t-value for $P = 0.05$ and eight degrees of freedom is 2.306.
[d] Tabulated F-value for $P = 0.05$ and $f_1 = f_2 = 4$ is 6.39.

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