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Spectrophotometric determination of certain cephalosporins in pure form and in pharmaceutical formulations

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Abstract

A simple and reproducible spectrophotometeric method for the assay of cefotaxime sodium, cefuroxime sodium, and ceftriaxone disodium with metol-chromium(VI) reagent has been developed. The procedure is based on direct oxidation of metol by potassium dichromate in presence of drug in acidic medium and subsequent formation of ternary complex. Beer's law is obeyed in the range 0.2–28 $\mu g\,ml^{-1}$ at λ_{max} 520 nm. For more accurate analysis, Ringbom optimum concentration range is found to be 0.8–26.5 $\mu g\,ml^{-1}$. The molar absorptivity and Sandell sensitivity were calculated. Six replicate analysis of solutions containing seven different concentrations of the examined drugs were carried out and gave a mean correlation coefficient $\leq$0.9996; the factors of the regression line equation for the three cephalosporins were calculated. The proposed method was applied to the determination of the examined drugs in pharmaceutical formulations and the results demonstrated that the method is equally accurate, precise, and reproducible as the official methods.

Keywords: Cefotaxime sodium; Cefuroxime sodium; Ceftriaxone disodium; Metol; Potassium dichromate; Spectrophotometry; Dosage forms

1. Introduction

Cefotaxime sodium, cefuroxime sodium, and ceftriaxone disodium are some of the third-generation cephalosporin antibiotics characterized by a broad antibacterial spectrum and a resistance to beta-lactamase-producing organisms in addition to its antimicrobial activity (streptococci, staphylococci, pneumococci, etc. [1]). Cephalosporins are distributed widely into tissues and body fluids, including pleural, pericardial, and synovial fluids. However, while the earlier cephalosporins failed to penetrate the central nervous system and were unsuccessful in the treatment of meningitis, the third-generation cephalosporins enter the central nervous system and reach therapeutic concentrations, there sufficient for treatment of meningitis caused by aerobic gram-negative bacteria [2]. These characteristics are of considerable clinical and hence, analytical interest [3].

Several analytical procedures are available in the literature for the analysis of cephalosporins, via spectrophotometric [4–10], polarographic [11], stripping voltammetric [11–13], fluorimetric [14,15], and high performance liquid chromatographic [16–18] methods.

The aim of this work was to develop a simple and reproducible spectrophotometric procedure for the determination of cefotaxime sodium, cefuroxime sodium, and ceftriaxone disodium in acidic medium by reaction with metol and potassium dichromate.

2. Experimental

2.1. Material and reagents

All chemicals and reagents used were of analytical grade and all solutions were prepared in doubly distilled water.

A freshly prepared 5×10^{-3} M aqueous solution of metol was prepared by dissolving appropriate weight in water. A 10^{-2} M potassium dichromate was also prepared. Buffer solution, a mixture of 250 ml of 0.2 M potassium hydrogen phthalate and different volume of 0.1 M HCl was diluted to 1 l with water to produce different pH values.

Cefotaxime sodium (I) was obtained from Hoechst Orients Egypt, Cairo, under the license from Hoechst AG,

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Frankfurt/Main, Germany, whereas cefuroxime sodium (II) was obtained from Glaxo Wellcome, Egypt, S.A.E., Cairo, under the license from Glaxo Wellcome group Ltd., England. Ceftriaxone disodium (III) was obtained from the Egyptian International Pharmaceutical Industries Company (EIPICO) under the license from Roche (Switzerland). Stock solutions were prepared by accurately weighing 100 mg of the examined drug into a 100 ml calibrated flask, dissolved in water and kept in the dark to avoid any degradation of the drugs.

2.2. Instrumentation

Spectral and absorbance measurements were made with a JASCO 530 UV-Vis and Perkin-Elmer Lambda 3B spectrophotometer with 10 mm quartz cells. The pH of solutions was checked using an Orion Research Model 601A/digital ionlyser.

2.3. General procedure

Pipette a 1.5 ml aliquot of the examined drug solution (concentration range as indicated in Table 1) in a 10 ml calibrated flask. Add 6.0 ml of buffer solution of pH 2.7, 3.2, and 2.9 using drug I, II, and III, respectively, 1.0 ml of 5×10^{-3} M metol solution (freshly prepared) and 1.2 ml of 0.01 M $K_2Cr_2O_7$. Allow the mixture to stand at $50 \pm 2\,^{\circ}C$ for 5.0 min and then dilute to volume with water. Measure the absorbance at 520 nm against a reagent blank prepared in a similar manner. The examined drug concentration was read from a standard calibration curve prepared with the same manner under identical conditions without the examined drug.

2.4. Formulations

The following commercial formulations were subjected to the analytical procedure, claforan vials (Hoechst Orient Egypt, Cairo) containing 524 mg cefotaxime sodium equivalent to 500 mg cefotaxime per vial, zinnat vials (Glaxo Wellcome, Egypt) containing 263 mg, equivalent to 250 mg cefuroxime and rocephen vials (EIPICO, Egypt) containing ceftriaxone disodium equivalent to 1.0 g ceftriaxone per vial were used.

2.5. Procedure for vials

The contents of each vial was transferred into separate 500 ml calibrated flask and made up to volume with water. Suitable aliquots of the standard drug solutions were mixed with 0.5 ml of the solution prepared above in 50 ml calibrated flask and diluted to the mark with water. The assay was completed as described above under general procedure. The recovery of the drug was computed from the corresponding regression equation.

3. Results and discussion

The spectrum of the oxidation product of metol with potassium dichromate in the presence of drug I, II, or III using 6.0 ml of pH 2.7, 3.2, or 2.9 were found to have an absorption maximum at 520 nm. The maximum color development is observed after heating in a water bath at $50 \pm 2\,^{\circ}C$ for 5 min, which is stable for at least 75 min. The absorption spectra is identical with that of the product obtained by direct oxidation of metol by Cr(VI) at higher pH values. The redox behavior of metol was studied [19] by mixing it with potassium molybdocyanide and deduced rather reasonably that the oxidized species was the *p*-*N*-methylquinoneimine. Cihalik and Vavrejnova [20], who estimated metol potentiometrically by titration with iodine monochloride in weakly acidic or neutral media, also assumed the oxidation product of metol to be *p*-*N*-methylquinoneimine, on the basis of their mole-ratio study.

The absorption spectra of the oxidation product of metol obtained under the optimum experimental conditions and

Table 1
Quantitative parameters for determination of cefotaxime, cefuroxime, and ceftriaxone using metol and $K_2Cr_2O_7$

Parameter	I	II	III
pH	2.7	3.2	2.9
λ_{max} (nm)	520	520	520
Beer's law limits ($\mu g\,ml^{-1}$)	0.2–22.0	0.1–25.0	0.2
Ringbom concentration range ($\mu g\,ml^{-1}$)	0.6–20.5	0.4–23.5	0.8–26.5
Detection limit ($\mu g\,ml^{-1}$)	0.04	0.03	0.05
Quantitation limit ($\mu g\,ml^{-1}$)	0.15	0.10	0.17
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	1.22×10^4	9.91×10^3	1.05×10^4
Sandell sensitivity ($\mu g\,cm^{-2}$)	0.038	0.045	0.055
Regression equation[a]			
Intercept (a)	+0.018	+0.033	−0.027
Slope (b)	0.026	0.022	0.018
Correlation coefficient (r)	0.9996	0.9992	0.9996
Standard deviation (%)	0.49	0.93	0.76
Range of error (%)	±1.1	±1.5	±1.3

[a] $A = a + bC$ (where C is the concentration in $\mu g\,ml^{-1}$).

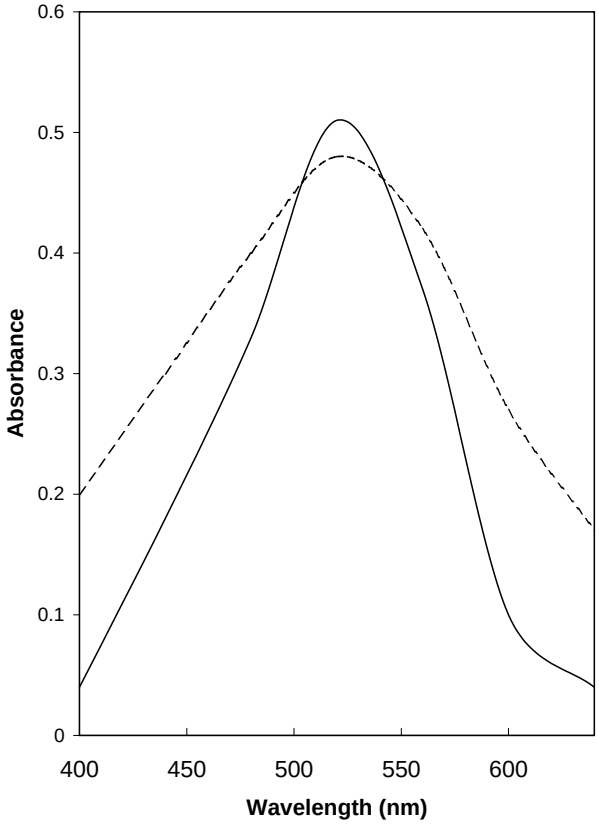

Fig. 1. Absorption spectra of the oxidation product of metol with: (—) Cr(VI) in the presence of ceftriaxone against a reagent blank; (- - -) ICl against water as blank.

that obtained with iodine monochloride oxidation have been found to give the same maximum, indicating their identity (Fig. 1). At 520 nm, the oxidation product has a reasonably high absorbance in comparison with that of Cr(VI).

Before the effect of the examined drugs on the metol-chromium(VI) reaction was investigated, the reactions between Cr(VI) and drug I, II, or III and between Cr(VI) and metol were studied separately at pH 2.7, 3.2, or 2.9, respectively. It was found that metol or the examined drugs do not react separately with Cr(VI) to a detectable extent. However, the color of the $K_2Cr_2O_7$ is gradually replaced by the purple-red color of the metol oxidation product, probably *p-N*-methylquinoneimine, in the presence of the examined drugs. Since the presence of primary aromatic amine in the drug causes an acceleration of the metol oxidation, it is believed that the primary amine must be involved in the rate determining step, and that both Cr(VI) and metol are essential as oxidant and reductant. It is tentatively suggested that the reaction mechanism involves some form of ternary intermediate involving primary aromatic amine, Cr(VI) and metol in which the electron transfer between Cr(VI) and metol is facilitated by the presence of amine. Such ternary intermediate with Cr(VI), EDTA and hydrazine have been reported by Beck and Durham [21] who also observed a limiting rate as the EDTA concentration is increased. These authors believe that EDTA facilitates the reaction between hydrazine and Cr(VI). If Cr(III) resulted from the redox reaction forms a complex with the aromatic primary amine, this could increase the Cr(VI)/Cr(III) potential. Thus, the reaction can proceed as long as there is free amine and hence the absorbance of the oxidation product of metol is proportional to the concentration of amine. Such Cr(III) complexes with primary aromatic amine have already been reported. These complexes are reportedly stable and easily formed only at higher pH values; decrease in pH reduces the stability. In the proposed method, the rate of reaction increase with decreasing pH and this parallelism supports our conclusions about the complex formation with Cr(III).

3.1. Optimization of reaction variables

Investigations were carried out to establish the most favorable conditions for the oxidation process to achieve maximum color development. The influence of some variables on the reaction has been tested. The results of these studies are summarized in the general procedure.

3.2. Analytical validity

A calibration graph was plotted from data of absorbance versus drug concentration of the reference standard and Beer's law was found to be valid for 0.2–28 $\mu g\,ml^{-1}$. For more accurate results, Ringbom optimum concentration range was found to be 0.8–26.5 $\mu g\,ml^{-1}$ (Table 1). The correlation coefficients, intercepts and slopes for the calibration data of drug I, II, and III are calculated using the least squares method.

The reproducibility of the procedure was determined by running six replicate samples, each containing 18 $\mu g\,ml^{-1}$ of drug in the final assay solution. At this concentration, the relative standard deviation was 0.49, 0.93, and 0.74% for drug I, II, and III, respectively.

The performance of the proposed method was assessed by calculation of the *t*- and *F*- values compared with the official method [22]. Mean values were obtained in as Student's *t*- and *F*-test at 95% confidence limits for five degrees of freedom [23]. The results showed that the calculated *t*- (1.54, 1.91, and 1.17) and *F*- (2.94, 3.56, and 2.43) values for I, II, and III, respectively did not exceed the theoretical values 2.57 and 5.05, respectively.

3.3. Sensitivity, accuracy, and precision

The mean molar absorptivity (ε) and Sandell sensitivity (*S*) as calculated from Beer's law are presented in Table 1. In order to determine the accuracy and precision of the method, solutions containing seven different concentrations of drug were prepared and analyzed in quintuplicate. The measured standard deviation (S.D.), relative standard deviation (R.S.D.), the standard analytical errors (SAE) and confi-

Table 2
Evaluation of accuracy and precision of the proposed method compared with that of the official one [22]

Drug	Taken ($\mu g\,ml^{-1}$)	Found[a] ($\mu g\,ml^{-1}$)					
		Official	Proposed	S.D.	R.S.D. (%)	SAE	Confidence limits
I	2.5	2.55	2.52	0.04	0.58	0.016	2.52 ± 0.050
	5.0	4.90	5.05	0.07	0.19	0.029	5.05 ± 0.080
	7.5	7.65	7.45	0.09	1.10	0.037	7.45 ± 0.110
	10.0	10.20	9.90	0.05	0.76	0.020	9.90 ± 0.060
	12.5	12.70	12.40	0.08	0.99	0.033	12.40 ± 0.095
	15.0	15.25	15.15	0.10	1.31	0.042	15.15 ± 0.120
	17.5	17.20	17.60	0.11	1.40	0.046	17.60 ± 0.135
II	3.0	2.95	3.03	0.05	0.64	0.020	3.03 ± 0.060
	6.0	6.10	5.95	0.03	0.41	0.012	5.95 ± 0.035
	9.0	9.15	8.90	0.08	0.96	0.033	8.90 ± 0.095
	12.0	11.80	12.10	0.06	0.75	0.024	12.10 ± 0.020
	15.0	14.80	15.10	0.09	1.15	0.037	15.10 ± 0.110
	18.0	18.20	17.85	0.07	0.97	0.029	17.85 ± 0.080
	20.0	20.30	19.85	0.12	1.45	0.140	19.85 ± 0.145
III	4.0	4.10	4.03	0.03	0.46	0.012	4.03 ± 0.035
	8.0	7.85	8.05	0.07	0.83	0.027	8.05 ± 0.080
	12.0	12.15	11.90	0.05	0.64	0.20	11.90 ± 0.060
	16.0	15.80	15.90	0.08	0.98	0.033	15.90 ± 0.095
	20.0	19.75	20.15	0.06	0.75	0.024	20.15 ± 0.070
	24.0	23.70	24.20	0.09	1.07	0.037	24.20 ± 0.110
	28.0	28.35	27.85	0.04	0.59	0.016	27.85 ± 0.050
Mean					0.76	0.024	

[a] Average of six determinations.

dence limit (Table 2) can be considered satisfactory, at least for the levels of concentrations examined.

The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The detection ($k = 3$) and quantitation ($k = 10$) limits of the method were established according to the IUPAC definitions ($C_1 = KS_0/s$) where C_1 is the detection limit, S_0 is the standard error of blank determination, s is the slope of the standard curve, and K is the constant related to the confidence interval [24]. The calculated values for detection and quantitation limits were recorded in Table 1.

Comparison of the recovery obtained with the proposed method with the purity of the examined drugs as determined according to the official method [22] showed that a high

Table 3
Determination of ceftriaxone in pharmaceutical preparations applying the standard addition technique

Pharmaceutical formulation	Taken ($\mu g\,ml^{-1}$)	Added ($\mu g\,ml^{-}$)[1]	Examined drug, Found[a] ($\mu g\,ml^{-1}$)	
			Proposed ± S.D.	Official ± S.D.
Zinnat (250 mg of II)	10	0	10.05 ± 0.53	9.90 ± 0.75
		2	12.04 ± 0.40	11.95 ± 0.99
		4	13.97 ± 0.53	14.14 ± 0.81
		6	16.10 ± 0.64	16.15 ± 1.03
		8	17.92 ± 0.88	18.22 ± 1.17
		10	19.90 ± 0.58	20.25 ± 1.34
Claforan (500 mg of I)	5	0	4.98 ± 0.34	5.05 ± 0.64
		4	8.95 ± 0.41	9.10 ± 0.41
		8	13.10 ± 0.37	13.15 ± 0.88
		12	17.15 ± 0.81	17.25 ± 1.07
		16	20.83 ± 0.93	20.70 ± 1.13
		20	25.25 ± 0.66	24.60 ± 0.78
Rocephen (1.0 g of III)	20	0.0	19.95 ± 0.55	20.25 ± 1.25
		1.5	21.50 ± 0.77	21.80 ± 1.42
		3.0	22.90 ± 0.82	23.35 ± 0.97
		4.5	24.60 ± 0.68	24.90 ± 1.63
		6.0	25.85 ± 0.56	26.50 ± 1.37
		7.5	27.30 ± 0.74	27.95 ± 1.71

[a] Average of six determinations.

accuracy of the present method. The proposed method is simpler, rapid, and more sensitive than the official method [22]. Moreover, the proposed method could be used for the routine determination of drug I, II, and III in pure form or in pharmaceutical formulations.

3.4. Analytical applications

Vials containing 250, 500, and 1000 mg of I, II, and III, respectively, were analyzed by the proposed method and the accuracy was assessed by the standard addition method in which variable amounts of pure drug was added to the previously analyzed portion of pharmaceutical formulations. Results are recorded in Table 3 and confirmed that the proposed method is not liable to interfere by fillers usually formulated with the standard drugs. The proposed method is highly sensitive, therefore it could be used easily for the routine analysis of pure drugs and their vial formulations.

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