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Utility of Redox Reactions to Determine Some Cephalosporins Spectrophotometrically

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Abstract

A simple, accurate and sensitive spectrophotometric method for the determination of cefadroxil (CXL), cefaclor (CLR) and cefatoxime (CTXM) in pure, dosage forms, and in the presence of its oxidative degradates is described. The method is based on the oxidation of the studied drug using cerium(IV) ammonium sulphate and subsequent determination of the unreacted Ce(IV) by measuring the decrease in absorbance of rhodamine B (RhB) at a suitable λ_{max}= 540, 541 and 542 nm for CXL, CLR and CTXM, respectively. Regression analysis of Beer/Lambert plots showed good correlation in the concentration ranges: 1.0-10, 1.0-12 and 2.0-8.0 µg ml^{-1} for CXL, CLR and CTXM, respectively. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration ranges were 3.0-8.0, 3.0-9.0 and 4.0-7.0 µg ml^{-1}, respectively. The obtained results are compared statistically using student's t-test and variance ratio F-test with the official methods. Analyzing pure and dosage forms containing CXL, CLR and CTXM tested the validity of the proposed method. The relative standard deviations were ≤ 1.38 with recoveries 98.2-101.2%.

Keyword: Cephalosporins determination; Redox reaction; Spectrophotometry; Dosage forms.
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1. Introduction

Cephalosporins are a class of β-lactam antibiotics (Figure 1), discovered in the 1950s, and produced by various species of the mold cephalosporium and from semi-synthetic processes. Cephalosporin-C which is the prototype of the cephalosporins was isolated in 1956 by Norton and Abraham from Cephalosporium acremorium, and its total synthesis was accomplished by Woodward and co-workers in 1966. Cephalosporins are broad spectrum antibiotics, with high potency against Gram-negative organisms. They are more resistant to inactivation by β-lactamases, particularly those produced by Gram-positive bacteria. They also have relatively low serum protein binding potential and almost all are excreted through the renal route. The cephalosporins are generally used in the treatment of upper respiratory and urinary tract infections. They are also generally useful for the rare patient who is sensitive to penicillin although sensitivity to cephalosporins is sometimes noticed [1]. The basic nucleus of the cephalosporins is 7-amino-cephalosporanic acid (7-ACA), which comprises a dihydrothiazine nucleus and a β-lactam ring. Cephalosporins are traditionally divided into first, second, third, and fourth generation agents, based roughly on the time of their discovery and their antimicrobial properties i.e. spectrum of activity.

The official methods for the analysis of the cephalosporins are HPLC techniques in the reversed phase mode [2,3]. Various other alternative methods have been reported for the analysis of the cephalosporins. The methods include spectrophotometric [4-9], spectrofluorometric [10-13], chemiluminescence [14-16], chromatographic [17-20], and electrochemical methods [21-24]. There are many methods which have been reported based on the hydrolysis of cephalosporins to the residual 7-ACA for the assay of cephalosporins in both bulk samples and pharmaceutical dosage forms. These methods include use of derivatization of the α-aminoacyl functions [25], flow injection analysis following formation of dyes [26], iodine and wool fast blue [27], alkaline hydrolysis [28], flow injection [29], vanadophosphoric acid [30] and more recently 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole [31]. While each of these reported methods has advantages, majority of them are extensively time-consuming and utilizes reagents which are expensive or may in some cases present with certain stability problems.

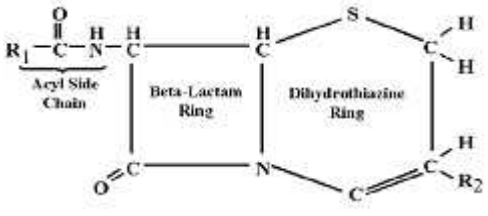

Fig (1): General structure of cephalosporins

Recently, we demonstrated the ability of cerium(IV) ammonium sulphate to oxidize different drugs and we successfully adopted the method for the spectrophotometric determination of famotidine [32] and ranitidine hydrochloride [33] with accuracies comparable to official methods. Generic brands of cephalosporins circulating in the drug market have become too overwhelming in recent times that a simple, rapid and equally accurate method will be required for their quality assessment. In this work, we developed simple approaches for the spectrophotometric determination of three cephalosporins possessing amino groups that can be oxidized with cerium(IV) ammonium sulphate and providing relatively simple and easily adoptable technique for their analysis with the use of inexpensive reagents. The drug market has witnessed the proliferation of many multisource generic brands of cephalosporins in the last decade. The official method for their assay is HPLC which is beyond the reach of the third world economies. A simple spectrophotometric method with readily available reagent will aid in the quality control of these antibacterials.

2. Experimental

Apparatus

All the absorption spectral measurements were made using JASCO V-670 (UV–VIS) spectrophotometer (Japan), with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10 mm matched quartz cells.

Materials and Reagents

All chemicals used were of analytical or pharmacopoeia grade purity, and water was doubly distilled. Pure CXL, CLR and CTXM were obtained from Kahira Pharmaceutical & Chemical industries Co. under license from Smith, Kline & French laboratories, Ltd, Welwyn Grand City, Herts, England. Stock CXL, CLR and CTXM solution (100 µg ml^{-1}) were prepared by dissolving 10 mg in water and adjusted to 100 mL with water. Working solutions of lower concentration were prepared by serial dilutions. Aqueous solutions of rhodamine B (RhB) (BDH, 1.0 mM) were prepared by dissolving an appropriate weight in 100 mL water. A solution of cerium(IV) sulphate (May and Baker, 3.0 mM) was prepared by dissolving known weight of $Ce(SO_4)_2$ in least amount of warm 5.0 M H_2SO_4 in a 250 mL calibrated flask, then adjusted with the same acid to the volume.

General procedure

The proposed method depended on oxidation of CXL, CLR and CTXM performed by adding 10–120 µg ml^{-1} of CXL, CLR and CTXM to an excess volume (1.0 mL) 3.0 mM $Ce(SO_4)_2$ containing 1.0 M H_2SO_4. The solution was boiled in a water bath for 5.0 min. The mixture was cooled and 0.30 mL of 1.0 mM Rh6G was mixed to warm solution and then cooled. The volume was completed to 10 mL with water. The decrease in colour intensities of Rh6G was measured spectrophotometrically at their corresponding maximum wavelength 526 nm, respectively. The concentration range was determined in each case by plotting the concentration of CXL, CLR and CTXM against absorbance at the corresponding λ_{max}.

Preparation of degradation products

A suitable amount (0.1 g) of CXL, CLR and CTXM was dissolved in 10 mL 0.1 M HCl, and then 1.0 mL of 12 % H_2O_2 was added. The solution was boiled in water bath for 45 min, and then diluted in 100 mL volumetric flask to the mark with water. The stock solution was diluted quantitatively to obtain degraded sample of the required concentrations.

Determination of CXL, CLR and CTXM

Procedure for dosage

At least 10 tablets of the CXL, CLR and CTXM drugs were weighed into a small dish, powdered and mixed well. A portion equivalent to 20 mg was weighed and dissolved in 100 mL water, shaken well and filtered through a sintered glass crucible G4. A 10 mL aliquot of the test solution (200 µg ml^{-1} of CXL, CLR and CTXM) was diluted to 100 mL in volumetric flask. One milliliter of this solution was then treated as described above in procedure A, B and C.

3. Results and discussion

This study used to establish simple a spectrophotometric method for the determination of CXL, CLR and CTXM containing sulfur atom, which is liable to atmospheric oxidation forming the S-oxide derivative. The structural activity relationship shows that this oxidative form (S-oxide) is inactive as antibiotic. Hence, for this reason, the establishment of a method that can quantitatively determine the drug in presence of its oxidized form is of great pharmaceutical value. The absorption spectra of the reaction products for CXL, CLR and CTXM drugs show characteristic λ_{max} value (Fig. 2).

The proposed method involved two-stage oxidation of the studied drugs with excess prepared Ce(IV) in acid medium under the effect of heating, and subsequent determination of the unreacted oxidant by measuring the decrease in absorbance of RhB at a suitable λ_{max} around 540 nm. The influence of each of the following variable on the reaction was tested.

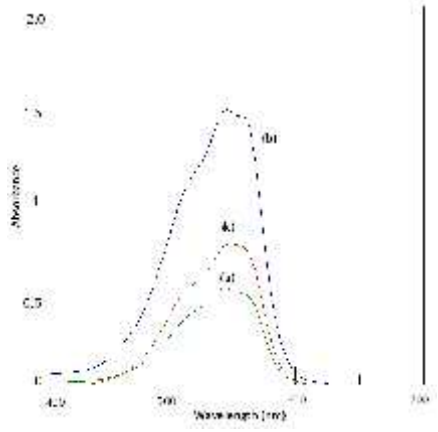

Fig (2): The absorption spectra of the reaction products for CXL, CLR and CTXM drugs show characteristic λ_{max} value

Effect of acid concentration

The most suitable acid to be used with Ce(IV) was found to be sulfuric acid of 1.0 M if present as 0.8, 0.8

and 0.3 mL for CXL, CLR and CTXM, respectively, in the total volume of reaction mixture 10 mL (Fig 3).

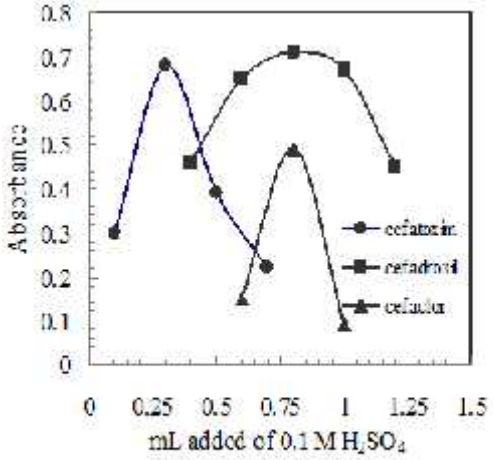

Fig (3): Effect of acidity on the redox reaction between the studied drugs and excess Xe(IV) using RhB

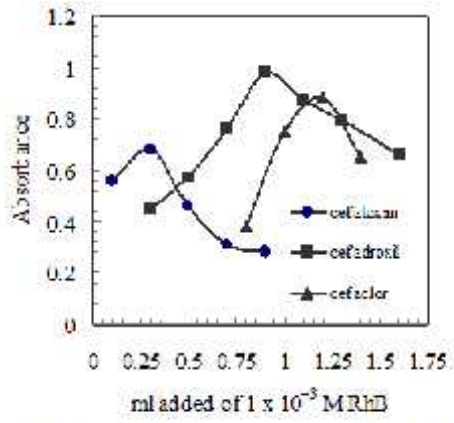

Fig (4): Effect of RhB concentration on the redox reaction condition for the studied drugs using Ce(IV) ion

Effect of temperature and time

Sample solutions containing the studied drug, Ce(IV) and H_2SO_4 were heated at different temperatures ranging from 30 to 60 ºC. The results obtained indicated that the reaction is catalyzed by heat, and the optimum absorbance reached its maximum at 55 ºC. The time required to complete the reaction is 5.0 min.

Effect of cooling

In the proposed method, the addition of RhB to the hot solution gives maximum absorbance, so there is no need to cool the solution before addition of dye.

Effect of dye concentration

To establish the optimum concentration of the reagent, different volumes of 1.0 x 10^{-3} M RhB solution were used (Fig 4). The optimum volume used for the production of maximum and reproducible color intensity is 0.9, 1.2 and 0.3 mL for CXL, CLR and CTXM, respectively.

Quantification

Beer-Lambert law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients obtained by linear squares treatment of the results are given in Table 1. The detection and quantitation limits were calculated from the standard deviation (SD) of the absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K=3) and of quantitation (K=10) were established according to IUPAC definitions [34].

Table (1): regression characteristics using Ce(IV) and Rh.B dye.

Parameters	Drugs		
	cefadroxil	cefatoxim	cefaclor
Maximum wave length λ_{Max} (nm)	540	542	541
Beer's law limits (µg ml^{-1})	0.1– 1.0	0.2– 0.8	0.1 – 1.2
Ringbom limits (µg ml^{-1})	0.3 – 0.8	0.4 – 0.7	0.3 – 0.9
Detection limits LOD (µg ml^{-1})	0.0132	0.0869	0.0406
Quantitation limits LOQ (µg ml^{-1})	0.0402	0.263	0.123
- Regression equation *			
Slope (b)	0.572	0.721	0.430
Standard deviation of slope (S_b)	0.304	0.244	0.360
Intercept (a)	1.2 x10^{-3}	8.1x10^{-2}	1.9 x10^{-3}
Standard deviation of Intercept (S_a)	7.1 x10^{-4}	3.1 x10^{-2}	6.3x10^{-4}
Correlation coefficient (r)	0.990	0.981	0.991

* With respect to A= a + bC were C is concentration of drug in µg mL^{-1} and A is absorbance.

In order to determine the accuracy and precision of the methods, solutions containing three different concentrations of the studied drugs were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2. The RSD % was found to be ≤ 1.38 and the percentage range of error at 95% confidence level (≤ ± 1.44) can be considered to be very satisfactory.

Table (2): Evaluation of accuracy and precision of proposed procedures using Ce(IV)and Rh. B dye

	Taken	Recovery	RSD[a]	RE	Confidence limit [b]
cefadroxil	0.4	101.2	0.99	1.04	0.406 ± 0.0052
	0.6	99.4	0.47	0.49	0.591 ± 0.0073
	0.8	99.6	0.32	0.34	0.789 ± 0.0063
cefatoxim	0.4	100.8	1.19	1.25	0.404 ± 0.0063
	0.6	99.4	0.67	0.70	0.591 ± 0.0105
	0.8	98.9	0.81	0.85	0.773 ± 0.0210
Cefaclor	0.4	101.4	1.00	1.05	0.407 ± 0.0052
	0.6	100.3	0.60	0.63	0.603 ± 0.0063
	0.8	99.4	0.40	0.42	0.788 ± 0.0084

[a] Relative standard deviation for six determinations .
[b] 95 % confidence limits and five degrees of freedom .

Interferences

The criterion of interference was an error of not more than ± 5.0% in absorbance. Experiments showed that there was no interference from additives and excipients, e.g. lactose, glucose, fructose, calcium hydrogen phosphate, magnesium stearate and starch for the examined method. Also, there was no interference from common degradation products results from the oxidation of CXL, CLR and CTXM, which are likely to occur at normal storage condition.

Analytical applications

The proposed method was successfully applied to determine CXL, CLR and CTXM in its dosage forms. The results obtained were compared statistically by Student's t -test (for accuracy) and variance ratio F-test (for precision) with the official methods [35] (based on HPLC method) at 95% confidence level with five degrees of freedom as shown in Table 3. The results showed that the t - and F- values were less than the critical value indicating that there was no significant difference between the proposed and official methods. The proposed method was more accurate with high recoveries than the official methods, so the proposed methods can be recommended for routine analysis in the majority of drug quality control laboratories.

Table (3): Evaluation of accuracy and precision of the three studied drugs (CXL, CTXM, CLR) by the proposed method and official methods in pharmaceutical preparation

Official method			Proposed methods Using RH.B dye								
			CXL (Libodroxl)			CTXM (Rametax)			CLR (Cloracef)		
Taken µg/ml	Found µg/ml	*Recovery (%)*	Taken µg/ml	Found µg/ml	*Recovery (%)*	Taken µg/ml	Found µg/ml	*Recovery (%)*	Taken µg/ml	Found µg/ml	*Recovery (%)*
0.4	0.401	100.2	0.4	0.406	101.5	0.4	0.405	101.2	0.4	0.404	100.8
0.6	0.572	95.3	0.6	0.605	100.8	0.6	0.582	97.0	0.6	0.608	101.3
0.8	0.781	97.6	0.8	0.780	97.5	0.8	0.805	100.6	0.8	0.783	97.8
Mean ± RSD			99.9 ± 1.13			99.7 ± 0.8			99.9 ± 1.24		
N			6			6			6		
t-test			1.43			1.08			1.29		
F-test			3.61			3.17			3.41		

4. Conclusion

The proposed method was advantageous over other reported visible spectrophotometric methods with respect to their higher sensitivity which permits the determination of up to 1.0 µg mL^{-1}, simplicity, reproducibility, precision, accuracy and stability of colored species. The proposed method can be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drug in raw materials, in pharmaceutical formulations and in the presence of their oxidative degradates.

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