Arab Gulf J. Scient. Res. **13**(2), pp. 213-224 (1995)

Spectrophotometric Determination of Cefotaxime and Cefadroxil by Alkaline Degradation to Hydrogen Sulphide and Formation of Violet Colour

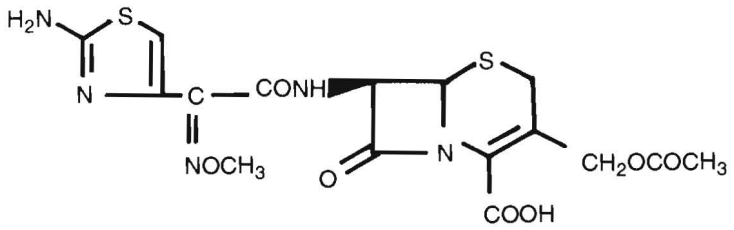
Abdulrahman A. Alwarthan, Fadia H. Metwally and Salma A. Al-Tamimi

Chemistry Department, College of Science, King Saud University, P.O.Box. 2455, Riyadh 11451, Saudi Arabia

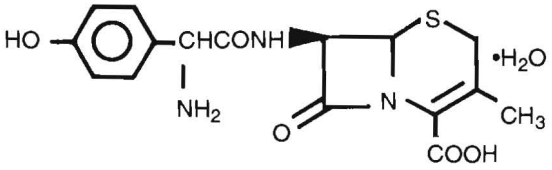
ABSTRACT. A spectrophotometric method is described for the determination of cefotaxime and cefadroxil in various drug formulations. The method is based on the hydrolysis of cefotaxime and cefadroxil in sodium hydroxide that releases hydrogen sulphide, which is then reacted with p-phenylenediamine dihydrochloride in an acidic solution and oxidising the intermediate with Fe (III) ions to give the violet colour. The absorbance of the colour developed is measured at ca. 597 nm for both the drugs. The effect of reagents concentrations and reaction conditions is discussed. The method has been successfully applied to the analysis of some pharmaceutical formulations, particularly of the injection and capsule types.

Cefotaxime (I), one of the third generation cephalosporins, and cefadroxil (II), one of the first generation cephalosporins, are considered as broad-spectrum antibiotics primarily used to treat bacterial infections of the skin, soft tissues and the urinary tract.

Several methods have been reported for the quantitative determination of cefotaxime and cefadroxil. These include spectrophotometric (Alwarthan et al. 1993, Abdel-Khalek and Mahrous 1984, and Issopoulos 1989), high-performance liquid chromatographic (HPLC) (Fabre *et al.* 1986, and Gaitonde and Jayade 1991) and fluorimetric methods (Fabre *et al.* 1985).



I



II

This paper describes the spectrophotometric determination of (I) and (II) after their degradation in 0.5 mol/l sodium hydroxide at room temperature to produce hydrogen sulphide to be utilized for dye formation (violet color). The method is applied to the analysis of various pharmaceutical preparation products of cefotaxime and cefadroxil.

Experimental

Apparatus

A varian Model DMS 100 spectrophotometer interfaced with a Varian Model DS 15 Data station and a Hewlett-Packard Model 82905 B Printer, was used for the absorbance measurements. Mathched sets of 10 mm silica cells were used throughout.

Materials

The procedure was applied on formulations which were obtained from local sources.

a) Claforan, 1g cefotaxime sodium, I.V. injection (Laboratories Roussel), Lot/Batch: 130-189 A.

b) Ultracef, 500 mg of cefadroxil per capsule (Bristol Laboratories Division of Bristol-Myers Company, Syracuse, New York, U.S.A).

c) Ultracef, cefadroxil suspension, 250 mg of cefadroxil per 5 ml (Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York, U. S. A).

Reagents

The cefotaxime sodium and cefadroxil were standards from Sigma Chemical Co. (Dorset, UK) and were used as received. All other chemicals were of analytical reagent grade. Distilled deionized water was used throughout.

Cefotaxime solution. A stock solution of 10^{-2} mol/l was prepared by dissolving 0.2387 g in 0.5 mol/l NaOH and then diluting to 50 ml with 0.5 mol/l NaOH.

Cefadroxil solution. A stock solution of 10^{-2} mol/l was prepared by dissolving 0.1817 g in 0.5 mol/l NaOH and then diluting to 50 ml with 0.5 mol/l NaOH.

p-Phenylenediamine dihydrochloride (PPDD) solution. 0.1 mol/l solution was prepared by dissolving 4.5268 g of PPDD in water and then diluting to 250 ml with the same solvent.

Iron (III) chloride hexahydrate [Fe(III)]. A stock solution of 0.1 mol/l was prepared by dissolving 6.7587 g in 250 ml of distilled water and adding a few milliliters of 0.1 mol/l sulphuric acid for stabilization.

Analysis of authentic samples:

5 ml from the stock solution of each drug was pipetted and transferred into a 50 ml calibrated flask and diluted to volume with 0.5 mol/l sodium hydroxide. The flask was heated in a water bath at a temperature of 70° C for about 30 min. This temperature and period of time was found satisfactory to produce maximum yield of hydrogen sulphide.

An aliquot of cefotaxime (0.5 μg/ml-382 μg/ml) and cefadroxil (0.3 μg/ml-291 μg/ml) were transferred to a 25 ml calibrated flasks containing 2.5 ml of 0.01 mol/l PPDD. The mixture was shaken for few seconds. To this mixture, 2ml of 0.01 mol/l Fe (III) were added. Again the flasks were shaken after being stoppered for few seconds, then diluted to the mark with distilled water and the absorbance was read at λ_{max} = 597.3 nm for both the drugs. The absorbance was measured against a blank treated similarly but containing no drug. The calibration curve for both drugs were obtained by applying the same procedure using standard cefotaxime and cefadroxil solutions.

Analysis of dosage forms

For injection. Accurately known weight of the drug sample, equivalent to 239 mg of the active constituent was transferred into a 50 ml standard flask, dissolved in 0.1 mol/l sodium hydroxide and was then made up to volume with 0.1 mol/l sodium hydroxide. An aliquot of this solution giving an analyte concentration of 191 µg/ml was transferred into a 25 ml calibrated flask and analysed as for authentic samples.

For capsules. The contents of ten capsules of the drug were weighed and powdered. An amount of powder equivalent of 0.1817 g of the active constituent was weighed accurately and transferred into a 50 ml calibrated flask. It was shaken with 25 ml of 0.5 mol/l sodium hydroxide, then the solution was filtered through a whatman No.1 filter paper. The residue was washed with 0.5 mol/l sodium hydroxide and the volume was completed to 50 ml with 0.5 mol/l sodium hydroxide. An aliquot of this solution giving an analyte concentration of 145 µg/ml was transferred into a 25 ml calibrated flask and analysed as for authentic samples.

For suspension. The powder of the bottle was well mixed. An accurately weighed amount, equivalent to 238.7 mg of the drug, was placed in a 50 ml calibrated flask and few milliliters of 0.1 mol/l sodium hydroxide were added to make up to volume. An aliquot of this solution equivalent to 145 µg/ml was pipetted into a 25 ml calibrated flask and analysed as for authentic samples.

Results and Discussion

Different cephalosporins were shown to give different yields of hydrogen sulphide during their hydrolysis in sodium hydroxide solution. The yields were highly reproducible for individual cephalosporins, and can be used for the determination of cephalosporins quantitatively (Fogg *et al.* 1982, Abdalla *et al.* 1982). The proposed method which has been based on the alkaline hydrolysis for cefotaxime and cefadroxil, used for the quantitative estimation of cefotaxime and cefadroxil, used for the quantitative estimation of cefotaxime and cefadroxil in their pure form and real samples. Fig. 1 shows the absorption spectra for the pure drugs and for the reaction products in the range 190-750 nm.

Choice of analytical conditions:

Several analytical conditions such as reagent concentrations, temperature and heating time were optimized to achieve high sensitivity, low blank reading and high stability.

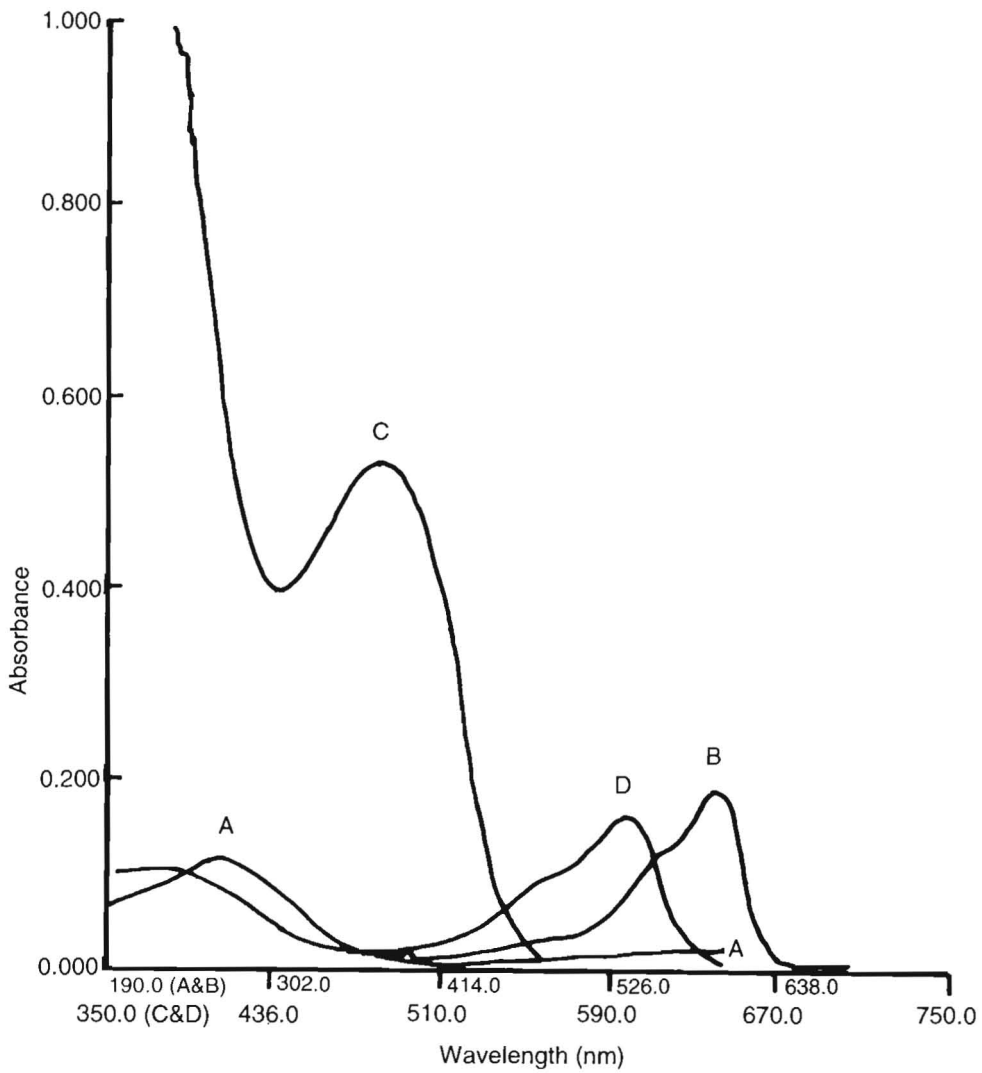


Fig. 1. Absorption spectra of the cephalosporins; A, Pure cefotaxime; B, Products of cefotaxime with reagents; C, Pure cefadroxil; D, Products of cefadroxil with reagents.

Effect of temperature and heating time:

The solution (25 ml), containing 2 ml of cefotaxime or cefadroxil ($4x\ 10^{-4}$ mol/l each drug), 2.5 ml of PPDD ($4x\ 10^{-3}$ mol/l) and 2 ml of Fe (III) ($1x\ 10^{-3}$ mol/l) was heated from 30 to 100° C and for different periods of time from 5 to 60 min. It was found that heating the solutions at 70° C for 35 min and at 80° C for 10 min are the best conditions that resulted in highest absorbance of colour formation for cefotaxime and cefadroxil, respectively.

Effect of Fe (III) concentration:

The effect of Fe (III) concentration colour formation of the violet dye, in presence of $4x10^{-3}$ M PPDD, was studied by varying the concentration of Fe (III) from $4x10^{-4}$ mol/l to $1x10^{-1}$ mol/l. As shown in Table 1, 0.01 mol/l Fe (III) solution was sufficient to give highest absorbance of colour formation for both drugs.

Table 1. Effect of Fe (III) concentration on the colour formation of the violet dye for both drugs, in presence of $4x10^{-3}$ M PPDD

[Fe (III)] (mol/l)	Absorbance*	
	Cefotaxime	Cefadroxil
$4x10^{-4}$	0.101	0.017
$2x10^{-3}$	0.527	0.354
$5x10^{-3}$	0.697	0.759
$8x10^{-3}$	0.737	0.833
0.01	0.838	0.859
0.05	0.810	0.799

* Average of four determinations.

Effect of PPDD concentration:

The effect of PPDD concentration on colour formation of the violet dye, in presence of 10^{-3} M Fe (III), was similarly investigated by taking various concentrations of PPDD. It was observed that 0.01 mol/l PPDD gave maximum absorbance in its reaction with both drugs, as shown in Table 2.

Using the above described optimum parameters, a calibration curve correlating the absorbance versus concentration was found to be linear with slope of 0.20 and 0.11 and correlation coefficient of 0.999 and 0.998 for cefotaxime and cefadroxil, respectively. Beer's law was obeyed over the range 0.5-380 µg/ml and 0.5-300

Table 2. Effect of PPDD concentration on the colour formation of the violet dye for both drugs, in presence of 10^{-3} M Fe (III)

[PPDD] (mol/1)	Absorbance*	
	Cefotaxime	Cefadroxil
$4x10^{-4}$	0.07	0.243
$2x10^{-3}$	0.357	0.734
$5x10^{-3}$	0.633	0.967
0.01	0.743	0.987
0.06	0.702	0.923

* Average of four determinations.

μg/ml for cefotaxime and cefadroxil (Fig. 2), respectively. To obtain the detection limits for both drugs, successive dilutions of 1 μg/ml solution of each drug were carried out for this study. The detection limits for cefotaxime and cefadroxil (defined as the amount of the drug that gave a signal twice the background noise) were as low as 0.2 μg/ml and 0.4 μg/ml, respectively. The relative standard deviation for cefotaxime and cefadroxil were 1.2% and 1.6% for $6x\ 10^{-4}$ mol/1 (for ten replicate determinations), respectively.

In order to study the precision and sensitivity of the proposed method, standard solutions containing three different concentrations of each drug were prepared and four absorbance measurements were made on each standard drug solution according to the recommended procedure. The over-all relative standard deviation (RSD) of twelve determinations was 1.18% and 1.59%, while the mean standard analytical error ($SD/\sqrt{n}$) was 0.38 and 0.35 for cefotaxime and cefadroxil, respectively. The results obtained are shown in Table 3.

Interferences

In order to assess possible analytical applications of the described spectrophotometric procedure, the influence of some excipients used in pharmaceutical preparations was studied. The results are shown in Table 4. A solution of 10^{-4} mol/1 of each potential interferent was prepared which also contained 10^{-5} mol/1 of cefotaxime or cefodroxil. As can be seen from this table, the interferents did not affect the absorbance in the case of cefotaxime, while most of the interferents increase the absorbance in the case of cefadroxil. Talc on the other hand, interfered seriously by decreaing the absorbance.

Table 3. Test on precision of the proposed procedure

Compound	Claimed	Amount, μg/ml		Standard Analytical error (SD/ $\sqrt{n}$)
		Found ± SD*	RSD%	
Cefotaxime	50	50.22 ± 0.45	1.30	0.23
	70	70.15 ± 0.85	1.19	0.43
	90	89.7 ± 0.96	1.05	048
		Mean	1.18	0.38
Cefadroxil	40	39.9 ± 0.55	1.65	0.28
	60	59.8 ± 0.85	1.57	0.43
	80	80.15 ± 0.70	1.55	0.35
		Mean	1.59	0.35

* Average of four determinations.

Table 4. Effect of various additives used as excipients (all in 10^{-4} mol/l) on the absorbance of 10^{-5} mol/l drugs

Additive	Mean recovery (%) (n = 4)	
	Cefotaxime	Cefadroxil
Glucose	100.6	111.5
Fructose	100.6	109.3
Lactose	99.2	111.2
Nicotinamide	100.6	107
Carbowax[a]	100.6	113
Starch	100.3	106.4
Talc	99.7	68.2

[a]polyethylene glycol 4000.

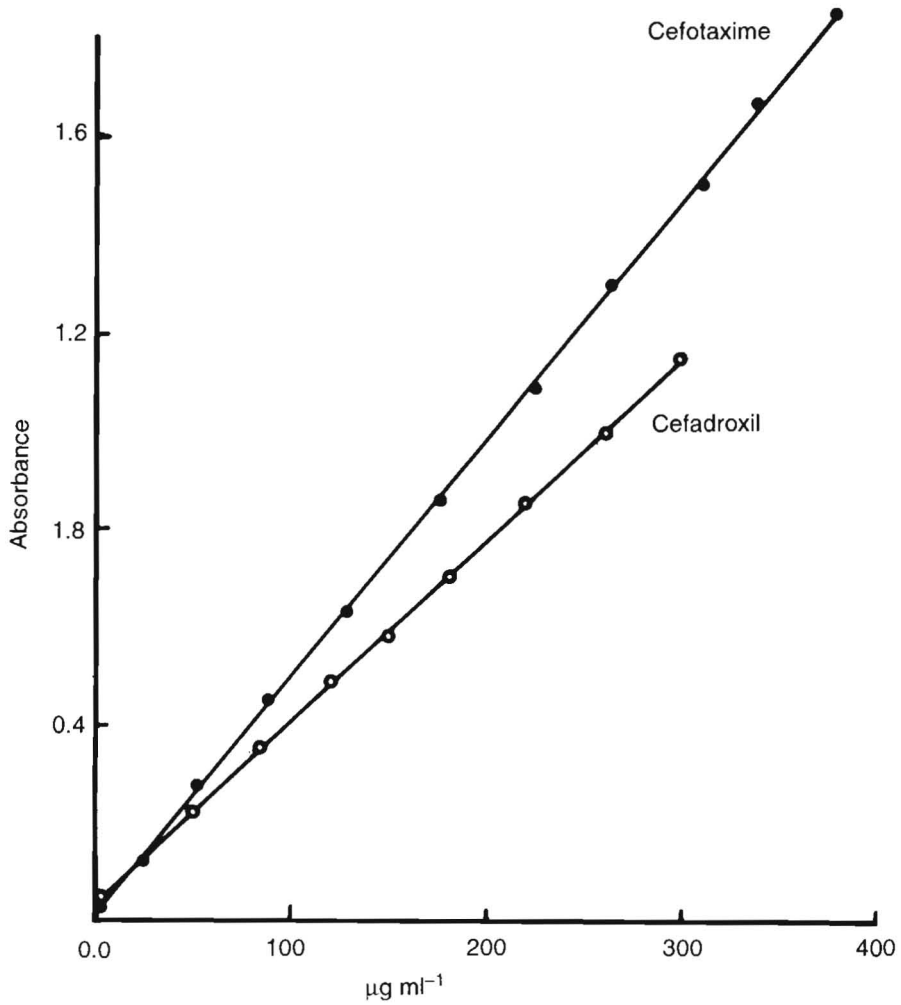


Fig. 2. Calibration curve for cefotaxime and cefadroxil products.

Applications

The proposed method was applied to the determination of cefotaxime and cefadroxil in some drug formulations. The performance of the recommended method was satisfactory as shown in Table 5.

Table 5. Determination of cefotaxime and cefadroxil in drug formulations by the proposed method.

Drug formulations	Amount of drug (mg)		Recovery (%)
	Claimed	Found*	
Cefotaxime Claforan I.V. injection (1g) (Roussel)	500	500.8	100.2
Cefadroxil Ultracef-capsules (500 mg) (Bristol)	500	502.6	100.5
Ultracef-suspension (250 mg) (Bristol)	250	248	99.2

* Average of four determinations.

The proposed method has the advantage of being rapid, simple and applicable over a wider range [compared to the ethylene blue method (Alwarthan *et al.* 1993)] of the drugs. Moreover, the method can be satisfactorily applied to the determination of the drug in both bulk and pharmaceutical formulations without further pretreatment.

References

Abdalla, M. A., Fogg, A. G. and **Burgess, C.** (1982) Selective Spectrophotometric Determination of Cephalosporins by Alkaline Degradation to Hydrogen Sulphide and Formation of Methylene Blue, *Analyst* **107:** 213-216.

Abdel-Khalek, M.M. and **Mahrous, M.S.** (1984) Use of Ammonium Molybdate in the Colorimetric Assay of Cephalosporins, *Talanta* **31**: 635-637.

Alwarthan, A. A., Metwally F. H. and **Al-Tamimi, S. A.** (1993) Spectrophotometric Assay of Certain Cephalosporins Based on Formation of Ethylene Blue, *Anal. Lett.* **26:** 2619-2635.

Fabre, H., Blanchin, M.D. and **Tjaden, U** (1986) High-performance Liquid Chromatography with Anodic Amperometric Detection for the determination of Cefotaxime and Its Metabolites, *Analyst* **111:** 1281-1284.

Fabre, H., Blanchin, M.D., Lerner, D. and **Mandrou, B.** (1985) Determination of Cephalosporins Utilising Thin-layer Chromatography with Fluorescamine detection, *Analyst* **110:** 775-778.

Fogg, A. G., Abdalla, M. A. and **Henriques, H. P.** (1982) Titrimetric Determination of the Yield of Sulphide Formed by Alkaline Degradation of Cephalosporins, Analyst **107:** 449-452.

Gaitonde, C.D. and **Jayade, P.P.** (1991) Liquid Chromatography and Gas Chromatography **9:** 34.

Issopoulos, P. B. (1989) Spectrophotometric Determination of Certain Cephalosporins Using Molybdophosphoric Acid, Part II. Determination of Cefadroxil, Cefapirin, Ceforanide and Cefuroxime, *Analyst* **114:** 237-239.

(Received 16/04/1994;
in revised form 06/02/1995)

التقدير الطيفي الانتقائي للسيفوتاكسيم والسيفودروكسيل بواسطة إنحلالهما إلى كبريتيد الهيدروجين ومن ثم تكوين لون بنفسجي

عبد الرحمن بن عبد الله الورثان و فاديه حسين متولي و سلمى علي التميمي

قسم الكيمياء - كلية العلوم - جامعة الملك سعود

ص .ب (٢٤٥٥) - الرياض ١١٤٥١ - المملكة العربية السعودية

يشتمل هذا البحث على نتائج لطريقة تحليلية طيفية لتقدير كل من السيفوتاكسيم والسيفودروكسيل . وتعتمد هذه الطريقة التحليلية على حلمأة السيفوتاكسيم والسيفودروكسيل في هيدروكسيد الصوديوم مما يؤدي إلى إطلاق كبريتيد الهيدروجين وهذا بدوره يتم مفاعلته مع بارا - فينيلين ثنائي الأمين ثنائي كلوريد الهيدروجين في وسط حمضي . بعد ذلك يتم أكسدة الناتج الوسطي بواسطة الحديد الثلاثي مما يؤدي إلى إعطاء ناتج بلون بنفسجي . ويتم قياس الامتصاص للدوائين السيفوتاكسيم و السيفودروكسيل عند طول موجة مقدارها 597 نانوميتر) .

وقد تمت كذلك مناقشة تأثير تراكيز الكواشف وظروف التفاعل على القياسات الطيفية . وقد تم تطبيق هذه الطريقة التحليلية بنجاح على قياس هذين الدوائين في بعض التركيبات الصيدلانية .