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Derivative spectrophotometric determination of the triethylammonium salt of cefotaxime in presence of related compounds from the synthesis

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Abstract

Cefotaxime sodium is a broad spectrum third generation antibiotic. It is obtained by reaction of 7-aminocephalosporanic acid (7-ACA) and *S*-(2-benzothiazolyl)2-amino-α-(methoxyimino)-4-thiazoleethanethioate. 2-Mercaptobenzothiazole is a by-product of this reaction. A derivative spectrophotometric determination of cefotaxime is proposed for its determination in a reaction mixture in the presence of the related compounds from synthesis. With this method Beer's law is obeyed over a concentration range from 0.005 to 0.080 mg ml^{-1} at 276.8 nm ($r = 0.9995$). This technique is accurate, precise (RSD = 0.4%), and has a sensitivity of 1.2% (differences in analytical response of 0.74 μg ml^{-1} could be detected). Recovery experiments of cefotaxime from reaction mixtures include 100% for all assayed concentrations. For these reasons, this technique is found valid for the intended purposes.

Keywords: Cefotaxime; Assay; Derivative spectrophotometry

1. Introduction

Cefotaxime sodium, a semisynthetic β-lactam antibiotic of the cephalosporin group, is a broad spectrum third generation antibiotic. Cefotaxime is not only an active ingredient against a great variety of both Gram negative and Gram positive bacteria, but also a potent β-lactamase inhibitor.

Cefotaxime is synthesized by reaction of 7-ACA and 2-mercaptobenzothiazolyl 2-(2-aminothiazol-

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4-yl)-2-methoxyimino acetate [1]. 2-Mercaptobenzothiazole is a by-product of this reaction. In order to establish the rate of this reaction, an analytical method is necessary to determine cefotaxime in presence of the related compounds from the reaction medium. In this medium, cefotaxime is obtained as its triethylammonium salt (**I**).

An analytical procedure based on HPLC has been described for determination of cefotaxime in bulk drugs [2]. However, this technique requires special apparatus and considerable amounts of solvents and time. Usually, spectrophotometry is not a suitable method for the determination of substances in mixtures, but this condition can be considerably improved by means of derivative spectrophotometry [3]. The present paper describes a new derivative spectrophotometric technique for the determination of **I** in the presence of the related compounds from synthesis.

2. Experimental

2.1. Apparatus

All spectral measurements and treatment of data were carried out with a Pharmacia LKB-Instrument Biochrom 4060 coupled to an IBM-PC computer with Biochrom 4060 Software [4]. A 80 μl flowcell with 10 mm pathway was used.

2.2. Reagents

The triethylammonium salts of cefotaxime (**I**) (99.1%), 7-ACA (**II**) (99.2%) and 2-mercaptobenzothiazole (**III**) (99.3%) were synthesized and purified in the laboratory of Organic Synthesis of the Center of Pharmaceutical Chemistry (Havana, Cuba). The *S*-(2-benzothiazolyl)2-amino-α-(methoxyimino)-4-thiazoleethanethioate (**IV**) was supplied by Alchymars (Milan, Italy). The purity was determined by acid–base titration in non aqueous medium.

2.3. Preparation of solutions

Stock solutions of **I**, **II**, and **III** were separately prepared by weighing 50.0 mg in 50 ml volumetric flasks and using water as the diluent (1 mg ml^{-1}). Since **IV** is slightly soluble in water, a satured solution (3 μg ml^{-1}) was prepared at 25°C and immediately used.

Two sets of five standard solutions of **I** were prepared by transferring 8.0, 4.0, 2.0, 1.0, and 0.5 ml of the stock solution of **I** to 100 ml volumetric flasks. To these sets, 4.0 or 2.0 ml of stock solution of **II** and **III**, and 10.0 ml of stock solution of **IV** were added: in this way one set contained 40 μg ml^{-1} of **II** and **III** and the other set 20 μg ml^{-1}, while the concentration of **IV** was constant in all the resulting solutions (0.03 μg ml^{-1}). The concentrations of cefotaxime were equal to 80, 40, 20, 10 or 5 μg ml^{-1}. Water was always used as the diluent.

2.4. General procedure

The spectrophotometric conditions were as follows: maximum wavelength = 300 nm; minimum wavelength = 200 nm; scan rate = 125 nm min^{-1}; wavelength interval 0.1 nm.

The absorption spectra of the samples were recorded against water and derivative spectra were calculated after subtracting the blank spectrum.

2.5. Validation parameters

Linearity, sensitivity, accuracy, and precision were determined according to reported procedures [5] and ICH Guidelines for Validation of Analytical Procedures [6].

3. Results and discussion

3.1. Spectrophotometric measurements

The zero-order absorbance spectra of 60 μg ml^{-1} solutions of **I**, **II** and **III**, and of a satured solution of **IV**, in the 200–300 nm wavelength region are shown in Fig. 1, whereas Fig. 2 shows the spectrum of the mixture. From these figures it is noted that the absorption spectra of **I**, **II**, and **III** are very similar, and that neither spectrum shows prominent peaks that can be used for reliable direct absorbance measurements.

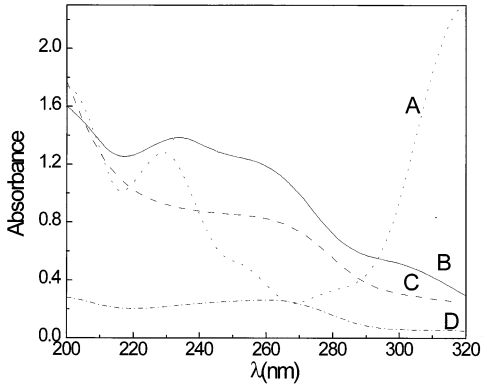

Fig. 1. Zero order absorption spectra of: (A) **III** (60 μg ml^{-1}); (B) **I** (60 μg ml^{-1}); (C) **II** (60 μg ml^{-1}); and (D) **IV** (3 μg ml^{-1}) in distilled water.

Fig. 3 shows the first derivative spectrum (curve A) of a mixture of **II** (40 μg ml^{-1}), **III** (40 μg ml^{-1}), and **IV** (3 μg ml^{-1}), the spectrum (curve C) of the same mixture spiked with of **I** (40 μg ml^{-1}), and the spectrum (curve B) of **I** (40 μg ml^{-1}).

The 'zero-crossing method' was selected for the determination of cefotaxime. This method involves the measurement of the difference in $\Delta A/\Delta\lambda$ between curve A and curve C at a zero-crossing wavelength. The zero-crossing wavelengths in curve A were found at 255.2, 257.0, 266.5, 276.8, and 281.4 nm. The wavelength selected for the intended purpose was 276.8 nm (curve C Fig. 3) [7].

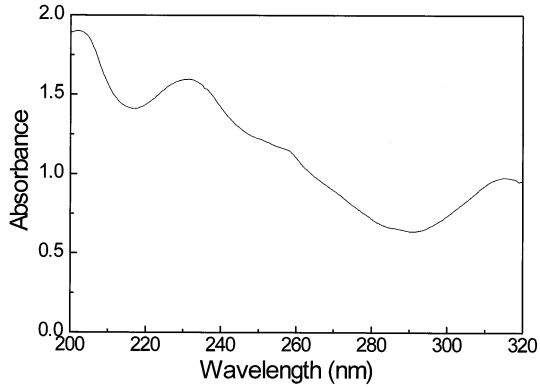

Fig. 2. Zero order absorption spectrum of the mixture of **I** (60 μg ml^{-1}), **II** (60 μg ml^{-1}), **III** (60 μg ml^{-1}) and **IV** (0.03 μg ml^{-1}) in distilled water.

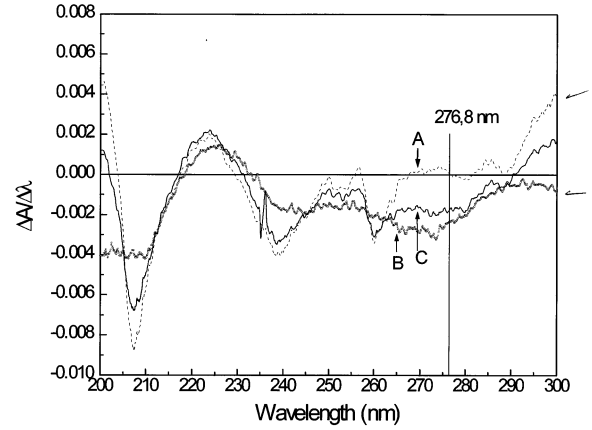

Fig. 3. First derivative spectrum of (A) mixture of **II** (40 μg ml^{-1}), **III** (40 μg ml^{-1}) and **IV** (0.03 μg ml^{-1}); (B) **I** (40 μg ml^{-1}) and (C) mixture of **II** (40 μg ml^{-1}), **III** (40 μg ml^{-1}), **IV** (0.03 μg ml^{-1}) and **I** (40 μg ml^{-1}).

3.2. Validation

3.2.1. Linearity

The estimated values of the linear regression equation for cefotaxime in the mixture were calculated by the method of least squares, and results are given in Table 1. The high value of correlation coefficient ($r = 0.9995$), indicates a positive correlation with a probability higher than 99.9% (r table $= 0.872$ for $\alpha = 0.001$ and $f = 8$) [8]. The calculated determination coefficient (r^2) indicates that the selected concentration range explains 99.9% of the total variance of analytical response (first derivative amplitude). The estimated value

Table 1
Regression analysis of the determination of cefotaxime by first derivative spectrophotometry[a]

Parameters	Values
Linear range (mg ml^{-1})	0.005–0.080
Number of solutions	10
Intercept (a)	0.00011
SD of a	0.00002
Slope (b)	0.03740
SD of b	0.00045
Calibration curve	$y = 0.0374x + 0.00011$
Correlation coefficient (r)	0.9995

[a] y, Height of signal $\Delta A/\Delta\lambda$; x, concentration (mg ml^{-1}); SD, standard deviation.

Table 2
Accuracy and precision of the first derivative spectrophotometric determination of cefotaxime

Amount of cefotaxime added to the mixture (μg ml^{-1})	Amount of cefotaxime found[a] (μg ml^{-1})
15	14.84 ± 0.33
25	24.99 ± 0.24
60	59.85 ± 0.22

[a] Mean and standard deviation for five determinations.

of intercept in ordinate, which is close to 'zero', verifies that the method is proportional and that the Lambert Beer's law is followed within the specified range. The estimated value of the slope is a significant variable since the experimental t value found (t exp = 83.111) is higher than the table value (t table = 2.262 for $\alpha = 0.05$ and $f =$ 9) [8]. This value is sufficiently high to conclude that the probability that it is not equal to zero is also very high, even higher than 99.9% (t table = 4.781 for $\alpha = 0.001$ and $f = 9$) [8]. The relative standard deviation of the slope (RSD = 1.2%) is lower than the accepted 2% to declare a method linear [9].

3.2.2. Sensitivity

The slope of the calibration curve obtained in the determination of the linearity is an indication for the sensitivity of the method. As it is established, both analytical sensitivity, and discriminator capacity increase when the concentration of cefotaxime increases. Thus, the smallest difference of cefotaxime concentration, which can be recorded by this technique, with 95% probability, is equal to 1.2% (0.74 μg ml^{-1}).

3.2.3. Accuracy and precision

Table 2 summarizes experimental results obtained in the determination of the accuracy and precision of the first derivative spectrophotometric determination of cefotaxime. The experimental value of G (G exp = 0.506), which is calculated following a Cochran test and is lower than the tabled value (G table = 0.8709 for three groups and three determinations) [10], lets us assume that the concentration does not have any influence on the variance of results. No significant differences are observed between the amount of cefotaxime added and the amount found (Table 2). For this reason, it is assured that the technique is able to measure the real quantities of cefotaxime in the studied medium. On the other hand, the intermediate precision (RSD = 0.4%), determined during three days and with two analysts using the same apparatus, is significantly lower than the accepted 3% for this analytical parameter [11].

4. Conclusion

The above described technique is appropriate for the quantitative determination of cefotaxime in presence of related compounds from the synthesis. This evidence is demonstrated by the obtained results, which corroborated that the method is linear, accurate and precise. Both sensitivity and accuracy are sufficiently good to use this method in a kinetic study. The procedure is practicable since it involves a minimum of reagents and equipment.

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