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Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 257–267
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Derivative spectrophotometry in the analysis of mixtures of cefotaxime sodium and cefadroxil monohydrate

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Received 22 April 2002; received in revised form 25 October 2002; accepted 27 October 2002

Abstract

Derivative spectrophotometry (ratio-spectra 1st- and 2nd-derivative and zero-crossing 2nd-derivative techniques) was applied for the determination of some cephalosporins in two component mixtures. Cefotaxime sodium salt ($C_{16}H_{16}O_7N_5S_2Na$) and cefadroxil monohydrate ($C_{16}H_{17}N_3O_5S \cdot H_2O$) were examined. In all procedures, the calibration plots are linear up to 43 μg/ml of each antibiotic, with r ranging from 0.9997 to 0.9999. In the ratio-spectra method, the measurements were taken at 239.5 and 291.5 nm (cefotaxime, 1st-derivative), 238 and 283 nm (cefadroxil, 1st-derivative), 284 and 303 nm (cefotaxime, 2nd-derivative), and 229.5 and 245.5 nm (cefadroxil, 2nd-derivative). Detection limits at $P = 0.05$ level of significance, calculated by a statistical treatment of calibration data, ranged from 0.15 to 0.58 μg/ml. LOD and LOQ ranged, respectively, from 0.19 to 0.51 and from 0.63 to 1.70 μg/ml. By the zero-crossing 2nd-derivative method, lines of regression are linear at 257 and 279 nm (cefotaxime) and 242 and 296 nm (cefadroxil). Detection limits from 0.28 to 0.51 μg/ml. LOD and LOQ from 0.27 to 0.41 and from 0.90 to 1.37 μg/ml, respectively. All the samples were tested for stability in solution and in the course of actual analysis, up to 80 h from their preparation. The developed derivative spectrophotometric methods were applied to synthetic mixtures and the RSD values ranged between 0.05 and 1.35% (ratio-spectra technique) and 0.01 and 1.07% (zero-crossing technique). The methods were also applied to vials and tablets for these drugs. The recoveries obtained were between 100.9 and 102.4% (ratio-spectra) and between 99.8 and 102.0% (zero-crossing). The procedures are simple, rapid, and did not require any preliminary separation or treatment of the samples. Instrumentation commonly available was utilised. The cephalosporins analysed are frequently used antibiotics of relevant clinical and pharmacological importance; hence this work would be of interest for the readers of journals devoted to pharmaceutical and biomedical analysis.

Keywords: Cefotaxime; Cefadroxil; Derivative spectrophotometry

1. Introduction

Cefotaxime and cefadroxil are the members of the cephalosporin antibiotic class of drugs that have a rather wide spectrum of activity and are useful as pre-surgery antibiotics. They are also useful for serious infections caused by susceptible strains of micro-organisms in lower respiratory infections, genito-urinary infections, gynaecologic infections, skin infections, and central nervous system infections. These antibiotics work by inhibiting bacterial cell wall biosynthesis and are

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(02)00690-8

active against a wide range of both gram-positive and gram-negative bacteria. A positive feature of these drugs is that they display a resistance to penicillinases and are useful to treat infections that are resistant to penicillin derivatives. Side effects of cefotaxime include diarrhoea and when mixed with alcohol, cefotaxime can cause stomach cramps, nausea, vomiting, headache, fainting, and difficulty in breathing. Analogously, adverse effects of cefadroxil include nausea, epigastric distress, diarrhoea, vomiting, skin rash, urticaria, genital pruritus, pseudomembranous colitis, and genital moniliasis. People who are allergic to penicillin may have equally serious allergic reactions to cephalosporin antibiotics such as cefatoxime and cefadroxil.

In conclusion, cefatoxime and cefadroxil are cephalosporins of clinical and, hence, analytical interests. For their pharmacological importance, coupled with the risk of side effects sometimes developed in the course of the therapy, we deemed useful to propose novel methods for the simultaneous determination of these antibiotics in admixture and for routine quality control of pharmaceutical dosage forms for these drugs. The procedures are rapid, simple and non-destructive, and require instrumentation commonly available in all laboratories.

For the above considerations, and for lack of other methods or methods easier than the present for the simultaneous determination of cefatoxime and cefadroxil, we consider the present work of interest for the readers of journals devoted to biomedical and pharmaceutical analyses.

Cefotaxime is the sodium salt of 7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-7^2(Z)-(*o*-methyloxime), acetate (ester). Molecular formula: $C_{16}H_{16}O_7N_5S_2Na$. Cefadroxil is chemically designated as 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,7-[[amino(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-, monohydrate, [6R[6a, 7b(R*)]]. It has the formula $C_{16}H_{17}N_3O_5S \cdot H_2O$.

Cefotaxime sodium and cefadroxil monohydrate (A and B, respectively, from this point for brevity) have closely overlapping spectra, which prevents the use of zero-order UV–Vis spectrophotometry for their determination. Derivative spectrophotometry is a suitable tool for overcoming this problem. This technique has been successfully applied in pharmaceutical and environmental analyses for the determination of drugs in multi-component systems (see recent examples [1–10]). The present work is a continuation of the author's research on the possibility of the application of derivative spectrophotometry in the analysis of multi-component formulations [11–18], allowing an increase in the selectivity of spectrophotometric determinations. The aim of this work was to develop reliable spectrophotometric procedures for the determination of A and B, either in laboratory samples, or in dosage forms for these drugs.

Various orders of derivative and different kinds of measurements were assayed, i.e., ratio-spectra 1st- and 2nd-derivative and zero-crossing 2nd-derivative. Satisfactory results were obtained with all methods. A brief comparison between the usefulness of different procedures was attempted.

2. Experimental

2.1. Apparatus and instrumental conditions

Absorption and derivative spectra were recorded with a Perkin–Elmer Lambda 3b spectrophotometer running spectrophotometric software Pecss. Suitable settings: 1 nm slit width, 60 nm/min scan speed, and 0.5 nm wavelength interval.

2.2. Reagents

Stock solutions, 0.2 mg/ml in water, of pure samples of cefotaxime sodium (Calbiochem-Behring Corp., USA) and cefadroxil monohydrate (Sigma Chem. Co., USA) were freshly prepared.

Pharmaceuticals. Mixtures of vials of Claforan (Hoechst Pharma, S.p.A., Italy) and Zaviriz (Aventis Pharma, S.p.A., Italy) and tablets of Oradroxil (Lampugnani Farmaceutici, S.p.A., Italy) and Foxil (I. Bir. N-Ist. Bioterapico Nazionale s.r.l.), which are labelled to contain 250 mg of cefotaxime sodium and 1 g of cefadroxil mono-

hydrate per pharmaceutical, respectively. Excipients per tablet: 30 mg starch, 130 mg cellulose microcrystalline, 120 mg idroxypropilcellulose, 10 mg saccharin, and 10 mg magnesium stearate.

2.3. Procedure

Suitable volumes of stock solutions, expected to contain up to 43 μg/ml of each drug, were mixed in a 5 ml calibrated flask and diluted to volume with distilled water.

All reagents were tested for stability in solution and during the actual analysis. The behaviour of the analytes remained unchanged up to about 80 h from their preparation. Further tests of stability (i.e., over 80 h) were found unnecessary and were not made. Before use, the samples were placed in stoppered vessels and stored in a refrigerator (not into freeze). All measurements were made at room temperature.

2.3.1. Ratio-spectra method

According to the theory of the ratio-spectra derivative method [19–23], the absorption spectrum of the mixture was divided, wavelength-by-wavelength, by a standard spectrum of B (B°, 5 μg/ml) for determining A and by a standard spectrum of A (A°, 5 μg/ml) for determining B. Then, the 1st- and 2nd-derivatives of the above ratio-spectra were recorded and the values of the derivatives were measured at suitably selected wavelengths. In particular, the concentration of A was proportional to the value of the 1st-derivative of the ratio-spectra at 239.5 and 291.5 nm and to the value of 2nd-derivative at 284 and 303 nm. The concentration of B was proportional to the value of 1st-derivative of the ratio-spectra at 238 and 283 nm and to the value of 2nd-derivative at 229.5 and 245.5 nm. The concentration of A and B in the mixture was computed from the corresponding calibration graph for each cephalosporin.

2.3.2. Zero-crossing method

The fundamental principles and convention of derivative spectrophotometry have been enounced in the works of O'Haver and Green [24] and Fell and Smith [25]. Initially, the commonest used procedure to obtain calibration graphs was "graphical measurements". The "zero-crossing" technique has found practical applications more recently [11,26,27]. Then it has become the most used procedure to resolve binary mixtures by spectrophotometry.

The 2nd-derivative spectra of mixtures of A and B were recorded against water and the values of derivative were measured at 257 and 279 nm (zero-crossing wavelengths of 2nd-derivative of B) for the determination of A and at 242 and 286 nm (zero-crossing wavelengths of 2nd-derivative of A) to determine B. Then, the concentration of A and B was calculated from the calibration graphs.

2.4. Procedure for pharmaceutical preparations

The method was tested by mixtures of commercial injections and tablets for these drugs (see under Section 2.2). To minimise the variation in the composition of the tablets, five tablets were finely ground and a portion of the powder equivalent to the stated content of one tablet was weighed. Extraction of cephalosporin from powdered tablets with methanol (as described in a previous paper [28]) is preferable to eliminate little interference from the excipients. Samples were prepared by dissolving appropriate amounts of the pharmaceuticals in water. The assays were completed as previously described and the concentration of A and B in the mixture was computed from the corresponding calibration graphs for each drug.

3. Results and discussion

In Fig. 1 are shown the zero-order spectra of A and B, which closely overlap.

3.1. Ratio-spectra method

An accurate choice of either standard divisors or working wavelengths is fundamental [19,21,22]. In particular, by increasing or decreasing the concentration of divisor, the resulting derivative values and, hence, the slope of lines of regression are proportionately decreased or increased, with consequent variation of both sensitivity and line-

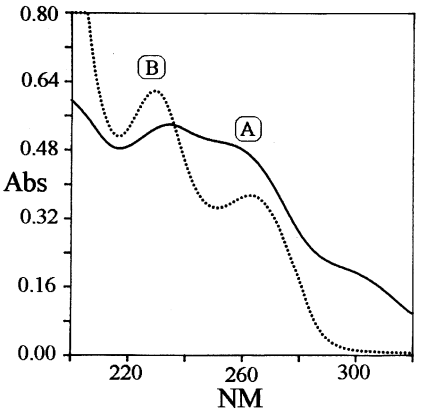

Fig. 1. Absorption spectra of cefotaxime sodium (15 μg/ml, continuous line) and cefadroxil monohydrate (20 μg/ml, dotted line). Reference, water.

arity range. Several tests were made in a preliminary investigation by using standard divisors of A and B in the concentration range from 3 to 10 μg/ml. The best results in terms of signal-to-noise ratio, sensitivity, repeatability, and range of validity of Beer's law were found by using as divisors standard spectra of 5 μg/ml of A (A°) and 5 μg/ml of B (B°). First- and 2nd-derivative of the ratio-spectra were used and the results compared. Higher derivative orders gave less satisfactory results.

In Fig. 2 are shown two series of ratio spectra of A/B° (continuous lines, left ordinate) and B/A° (dotted lines, right ordinate) from 3 to 40 μg/ml.

In Fig. 3 are shown examples of 1st- derivatives of the ratio-spectra A/B° and B/A° (continuous and dotted curves, respectively). In Fig. 4 is reported a series of 2nd-derivatives of the ratio-spectra of A/B° and B/A° (continuous and dotted lines, respectively). For calibration graphs, we selected the wavelengths which exhibited the best linear response to the analyte concentration, i.e., in the 1st-derivative mode 239.5 and 291.5 nm to determine A and 238 and 283 nm to determine B. In the 2nd-derivative mode, 284 and 303 nm for A and 229.5 and 245.5 nm for B. The working wavelengths are reported in the figures. All the above wavelengths correspond to maxima or minima of derivative spectra.

The calibration graphs for each drug were obtained by plotting the values of 1st- and 2nd-

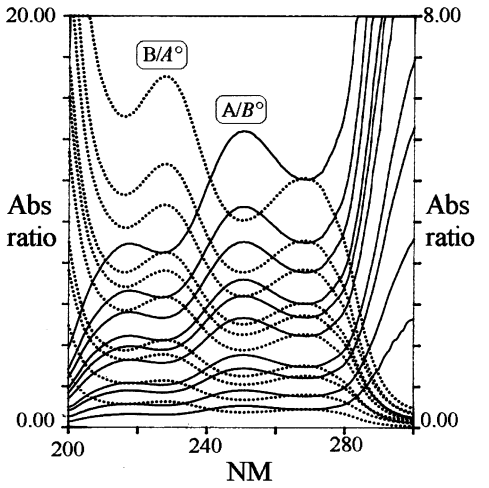

Fig. 2. Ratio-spectra for different concentrations of cefotaxime sodium (divisor B° 5 μg/ml, continuous curves, left ordinate scale) and cefadroxil monohydrate (divisor A° 5 μg/ml, dotted curves, right ordinate scale). Concentration of the drugs: 3, 5, 8, 10, 15, 18, 20, 25, 30, and 40 μg/ml, from the bottom.

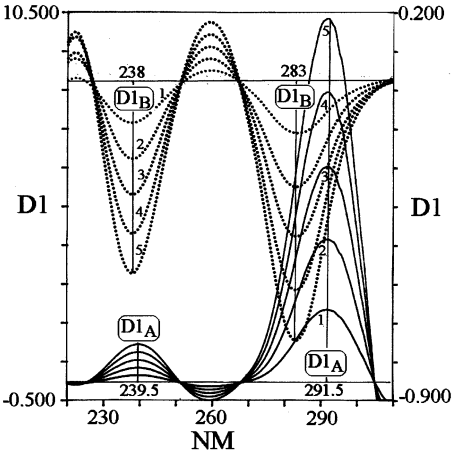

Fig. 3. First-derivative spectra of the ratio spectra of cefotaxime sodium (5, 10, 15, 20, and 25 μg/ml, continuous curves 1–5, left ordinate scale) and cefadroxil monohydrate (5, 10, 15, 20, and 25 μg/ml, dotted curves 1–5, right ordinate scale). The standard divisors are, respectively, B° and A°, both 5 μg/ml. The working wavelengths are indicated.

derivatives of the ratio-spectra A/B° and B/A°, with variable concentrations of A and B, at the above working wavelengths, against the concentration of A and B in the standards, respectively. The equations for calibration curves are compiled in Table 1 under "ratio-spectra", together with

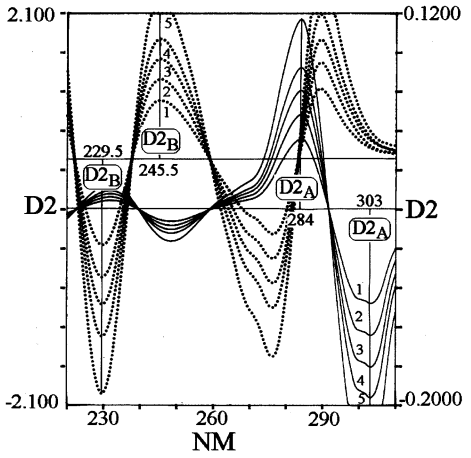

Fig. 4. Second-derivative spectra of the ratio-spectra of cefotaxime sodium (15, 20, 25, 30, and 40 μg/ml, continuous curves 1–5, left ordinate scale) and cefadroxil monohydrate (15, 20, 25, 30, and 40 μg/ml, dotted curves 1–5, right ordinate scale). The standard divisors are, respectively, B° and A°, both 5 μg/ml. The working wavelengths are indicated.

statistical data, including the standard deviations for the slope and the intercept and the detection limits calculated by a statistical treatment of calibration data [11,29,30] at $P = 0.05$ level of significance. Correlation coefficients, r, evidence for both linearity of regression lines and negligible scatter of experimental points. Calibration plots are linear up to 43 μg/ml of each antibiotic. LOD and LOQ values are also reported. LOD is the lowest concentration of analyte that the analytical process can reliably detect, defined as the analyte concentration leading to a signal three times the standard deviation σ of the blank in 10 successive scans. Hence, the limit of detection, LOD, is located 3σ above the measured average blank and the limit of quantitation, LOQ, is 10σ above the blank [31].

In order to verify if the developed method was free from procedural errors depending on the simultaneous presence of more components, the experimental intercepts, a, of regression lines were tested for the significance of the deviation from the expected value, i.e., zero [11,29,30]. The values calculated for t, showed in the table, never exceeded the 95% criterion 2.31, which denotes that the intercepts of all regression lines are not significantly different from zero.

As expected [19–23], the slope of calibration graphs are inversely proportional to the concentration of the divisors. Various divisor concentrations were tested, i.e., 3, 5, 7, and 10 μg/ml and in all cases, the graphs, Slope vs 1/divisor concentration, not shown for brevity, were straight lines with correlation coefficients higher than 0.9997. These results confirm the reliability of the ratio-spectra method in the present instance.

3.2. Zero-crossing method

The more convenient order of derivative and working wavelengths were selected by preliminary tests. The basic requirements of the present procedure were that neither shape of derivative spectra nor location of the zero-crossing wavelengths were dependent on the composition of binary mixtures. Second-derivative proved to be optimal.

The good resolution of the 2nd-derivative spectra allowed to select the optimal working wavelengths from several zero-crossing wavelengths, i.e., those which exhibited the best linear response to analyte concentration and/or higher sensitivity.

In Fig. 5 are shown the 2nd-derivative spectra of A (continuous lines) and B (dotted lines) with the zero-crossing points. Among these, 257 and 279 nm for determination of A and 242 and 296 nm for B were selected as working wavelengths (shown in the figure), in that measurements of the absolute value of the total derivative spectra taken at these wavelengths gave the best response to the analyte concentration. Note that two spectra at different concentrations for each one of the two drugs are reported, in order to put in evidence the repeatability of the values of the zero-points.

In Fig. 6 are presented typical sets of (a) 2nd-derivative spectra of mixtures of 5 μg/ml of B and increasing concentrations of A and (b) 5 μg/ml of A and increasing concentrations of B. It is important to observe that all curves converge, as expected, to distinct isosbestic points, corresponding to the zero-crossing wavelengths of A and B, i.e., irrespective of the variable concentrations of A and B in the mixtures, respectively.

The statistical data for mixtures of A and B are assembled in Table 1 under "zero-crossing". Both,

Table 1
Statistical data for the calibration graphs of cefatoxime and cefadroxil by ratio-spectra and zero-crossing derivative spectrophotometry

Drug	Regression equations	λ (nm)	r	Standard deviations		Detection limit (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)	$t = a/s_a$ [a]
				Intercept, s_a	Slope, s_b				
Ratio-spectra, 1st-derivative									
A	$D1_A = -1.42E-03 + 4.39E-02C_A$	239.5	0.9999	2.48E−03	1.20E−04	0.21	0.25	0.83	0.57
A	$D1_A = -1.14E-02 + 4.12E-01C_A$	291.5	0.9999	1.96E−02	9.48E−04	0.18	0.22	0.73	0.58
B	$D1_B = -1.77E-03 - 2.13E-02C_B$	238	0.9999	2.46E−03	1.19E−04	0.43	0.38	1.27	0.72
B	$D1_B = 3.94E-03 - 2.94E-02C_B$	283	0.9999	1.89E−03	8.20E−05	0.21	0.30	1.00	2.08
Ratio-spectra, 2nd-derivative									
A	$D2_A = -1.10E-03 + 5.10E-02C_A$	284	0.9999	2.29E−03	1.11E−04	0.17	0.19	0.63	0.48
A	$D2_A = 6.21E-04 - 6.73E-02C_A$	303	0.9999	2.63E−03	2.27E−04	0.15	0.22	0.73	0.23
B	$D2_B = 1.41E-03 - 4.79E-03C_B$	229.5	0.9997	7.54E−04	3.65E−05	0.58	0.51	1.70	1.87
B	$D2_B = 7.11E-06 + 3.30E-03C_B$	245.5	0.9999	2.79E−04	1.35E−05	0.31	0.33	1.10	0.03
Zero-crossing, 2nd-derivative									
A	$D2'_A = 2.26E-05 - 2.05E-04C_A$	257	0.9999	1.57E−05	7.62E−07	0.28	0.32	1.07	1.43
A	$D2'_A = -2.65E-06 + 2.34E-04C_A$	279	0.9999	1.76E−05	8.52E−07	0.28	0.27	0.90	0.15
B	$D2'_B = 5.90E-05 + 5.32E-04C_B$	242	0.9999	5.95E−05	2.88E−06	0.42	0.39	1.30	0.99
B	$D2'_B = -1.42E-05 + 1.71E-04C_B$	296	0.9997	2.82E−05	1.36E−06	0.51	0.41	1.37	0.50

A, cefotaxime, B, cefadroxil, C_A and C_B, concentration of drugs (μg/ml). Number of samples, $n = 10$.
[a] Theoretical value of t at $P = 0.05$ level of significance is 2.31.

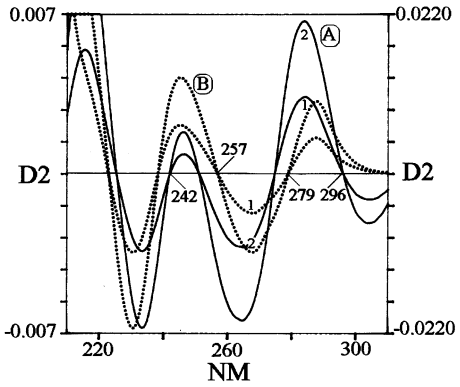

Fig. 5. Second-derivative of cefotaxime sodium: (1) 10 μg/ml and (2) 20 μg/ml (continuous lines, left ordinate), and cefadroxil monohydrate: (1) 10 μg/ml and (2) 20 μg/ml (dotted lines, right ordinate). The zero-crossing working wavelengths are marked.

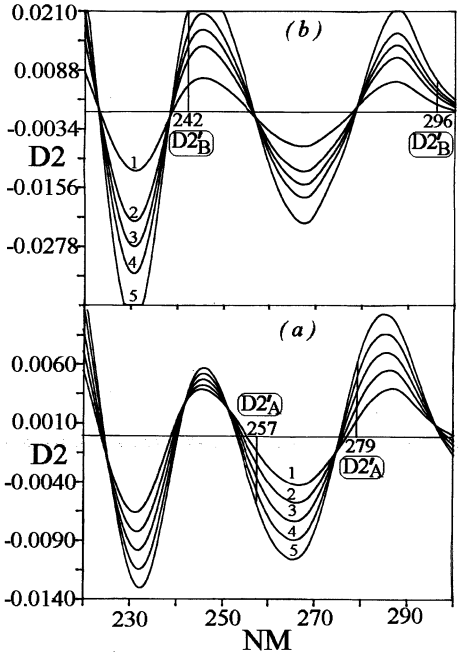

Fig. 6. (a) Second-derivative spectra of mixtures with constant concentration of cefadroxil monohydrate (5 μg/ml) and increasing concentration of cefotaxime sodium (5, 10, 15, 20, and 25 μg/ml, curves 1–5) and (b) 2nd-derivative spectra of mixture with constant concentration of cefotaxime sodium (5 μg/ml) and increasing concentration of cefadroxil monohydrate (10, 20, 25, 30, and 40 μg/ml, curves 1–5). The working wavelengths are indicated.

linearity of regression lines and negligible scatter of experimental points, are evidenced by correlation coefficients. Lines of regression are linear up to 43 μg/ml. Detection limits at $P = 0.05$ level of significance [11,29,30] and LOD and LOQ [31] are reported. Also in the present instance the values calculated for t, shown in the table, never exceed the 95% criterion, 2.31.

3.3. Accuracy and precision

In Table 2 are reported the results obtained by five replicate determinations of A and B in synthetic mixtures. Good accuracy and precision resulted by both ratio-spectra and zero-crossing methods.

The histograms in Fig. 7 represent the confidence limits [29] for the determinations of cefatoxime and cefadroxil in mixtures by the different procedures at $P = 0.05$ level of significance (for the sake of brevity, not all curves calculated were reported in the figure). The histograms are plotted from calibration data in a particular way (11), i.e., as uncertainty (%) on the concentration, (relative error) $t_p s_c/c$ (%) against the concentration of A and B, respectively. s_c is the absolute error in the determination of a given concentration, defined by the expression (29) $s_c = s_0/b[(1 + 1/n)(y_i - \bar{y})^2/b^2(\Sigma c_i^2 - n\bar{c}^2)]^{1/2}$, where s_0 is the variance characterising the scatter of experimental points with respect to the line of regression, t_p is Student's coefficient at the selected level of significance, c_i and y_i are the concentration and experimental ordinate values, respectively, $\bar{c}$ and $\bar{y}$ are the average values for $n = 10$ samples, b the slope of lines of regression.

Obviously s_c and, hence, $t_p s_c/c$ (%), are not experimental values, but they follow from theoretical calculations based on the calibration graphs used in determining the regression lines reported in Table 1; the concentrations utilised were 3, 5, 8, 10, 15, 18, 20, 25, 30, and 40 μg/ml of both cephalosporins. Clearly, if the concentrations were changed, the corresponding histograms would have to be re-examined; however, they give useful indications about the level of precision that may be expected in the range of concentrations examined.

Table 2
Replicate determinations on mixtures of cefatoxime and cefadroxil, by ratio-spectra and zero-crossing derivative spectrophotometry

Nominal value (μg/ml)		Found[a]			
A	B	A (239.5 nm)	A (291.5 nm)	B (238 nm)	B (283 nm)
Ratio-spectra, 1st-derivative					
5.0	10.0	4.98±0.02 (0.45)	4.93±0.07 (1.35)	10.01±0.02 (0.21)	10.13±0.13 (1.32)
10.0	15.0	9.90±0.11 (1.10)	9.91±0.09 (0.95)	14.85±0.14 (0.98)	14.87±0.13 (0.91)
15.0	5.0	14.89±0.12 (0.80)	14.93±0.11 (0.71)	5.07±0.06 (1.20)	4.93±0.05 (1.10)
5.0	30.0	4.95±0.06 (1.31)	4.95±0.06 (1.30)	30.02±0.24 (0.80)	30.07±0.09 (0.31)
10.0	25.0	9.90±0.11 (1.10)	9.92±0.09 (0.95)	25.10±0.13 (0.51)	24.87±0.15 (0.61)
30.0	5.0	29.96±0.05 (0.18)	29.97±0.06 (0.22)	4.96±0.05 (0.95)	4.94±0.06 (1.20)
8.0	25.0	7.94±0.07 (0.91)	7.93±0.07 (0.91)	25.18±0.20 (0.81)	24.88±0.13 (0.53)
20.0	15.0	19.91±0.10 (0.52)	19.93±0.09 (0.45)	14.85±0.13 (0.88)	14.86±0.17 (1.20)
		A (284 nm)	A (303 nm)	B (229.5 nm)	B (245.5 nm)
Ratio-spectra, 2nd-derivative					
5.0	10.0	4.91±0.64 (1.31)	4.93±0.06 (1.28)	10.09±0.11 (1.10)	10.11±0.11 (1.12)
10.0	15.0	9.92±0.08 (0.78)	9.94±0.07 (0.72)	15.09±0.11 (0.71)	14.85±0.16 (1.10)
15.0	5.0	14.92±0.09 (0.61)	14.91±0.11 (0.71)	5.06±0.06 (1.12)	4.94±0.05 (1.11)
5.0	30.0	4.96±0.05 (1.10)	4.96±0.06 (1.11)	30.27±0.25 (0.82)	30.12±0.20 (0.65)
10.0	25.0	9.91±0.08 (0.77)	9.90±0.11 (1.10)	25.22±0.22 (0.86)	24.94±0.08 (0.31)
30.0	5.0	29.94±0.11 (0.36)	29.98±0.08 (0.28)	5.05±0.05 (0.91)	4.96±0.05 (0.93)
8.0	25.0	7.95±0.09 (1.08)	7.94±0.08 (1.04)	24.97±0.05 (0.20)	25.09±0.10 (0.41)
20.0	15.0	19.82±0.17 (0.88)	19.78±0.18 (0.92)	15.20±0.14 (0.95)	14.86±0.13 (0.89)
		A (257 nm)	A (279 nm)	B (242 nm)	B (296 nm)
Zero-crossing, 2nd-derivative					
5.0	30.0	4.99±0.01 (0.28)	5.08±0.03 (0.61)	30.15±0.17 (0.58)	29.82±0.25 (0.71)
10.0	25.0	9.89±0.11 (1.07)	10.03±0.04 (0.38)	25.07±0.08 (0.33)	25.15±0.17 (0.68)
20.0	15.0	20.11±0.12 (0.61)	20.05±0.04 (0.22)	14.93±0.08 (0.55)	14.95±0.06 (0.38)
30.0	5.0	29.87±0.05 (0.17)	29.85±0.17 (0.58)	4.96±0.05 (0.95)	5.04±0.04 (0.88)
8.0	25.0	7.93±0.14 (0.89)	8.11±0.08 (1.04)	25.07±0.08 (0.33)	25.15±0.16 (0.65)
5.0	10.0	4.99±0.02 (0.33)	5.04±0.04 (0.89)	10.04±0.05 (0.51)	9.99±0.02 (0.19)
10.0	15.0	9.90±0.03 (0.28)	9.90±0.03 (0.33)	14.93±0.09 (0.60)	14.96±0.06 (0.43)
15.0	5.0	14.99±0.12 (0.78)	14.93±0.08 (0.52)	4.97±0.04 (0.82)	4.95±0.04 (0.87)

A, cefotaxime, B, cefadroxil.
[a] Mean±standard deviation (μg/ml) for five determinations with RSD% in parentheses.

3.4. Assay of pharmaceutical formulations

The assay was carried out as previously described under Section 2.4. The results of five replicate determinations are shown in Table 3. The level of precision and accuracy is adequate for the quality control analysis of pharmaceutical preparations. Either ratio-spectra or zero-crossing procedures yield good recoveries with a little prevalence of the zero-crossing method. However, the minor differences observed may be considered acceptable.

4. Conclusions

All procedures presented in this paper enable the quantitation of mixtures of cefatoxime sodium and cefadroxil monohydrate with good accuracy and precision, either in pure form or in formulations. Potential little interferences from the excipients of tablets were avoided as described under Section 2.4. The vials did not contain excipients and other kinds of interferences were not found.

One of the purposes of this work was to investigate the capability of two different applica-

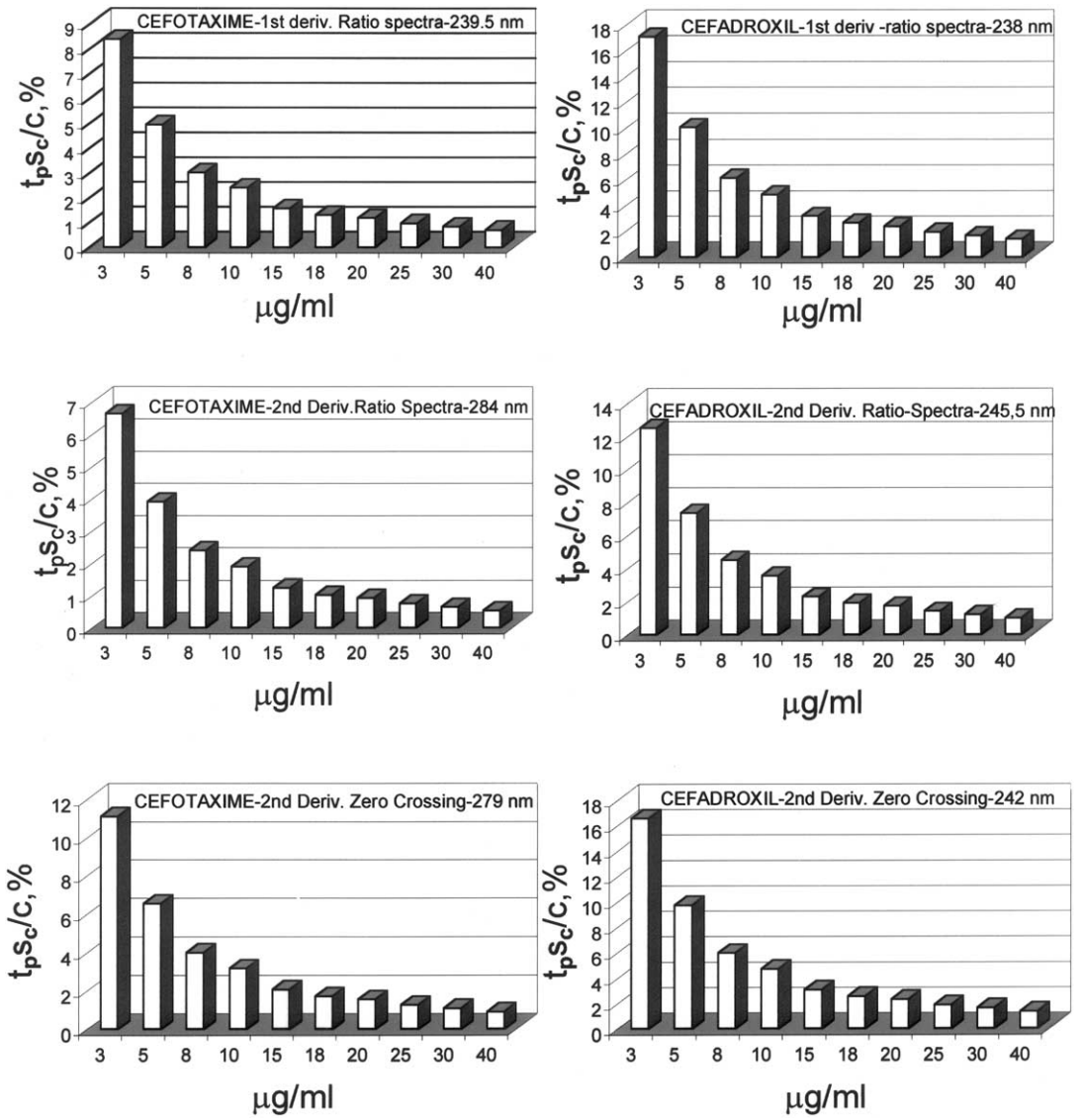

Fig. 7. Histograms of the variation of confidence limits at $P = 0.05$ level of significance, in the form of uncertainty percent on the concentration.

tions of derivative spectrophotometry, ratio-spectra, and zero-crossing and to remark possible differences in the experimental findings. From a comparison of the results obtained with the two methods, they did not show significant differences. For this reason, the zero-crossing method seems to be, in this case, more recommendable than the ratio-spectra method which is tedious and needs a rigid control of various experimental parameters and a greater number of measures. On the other hand, the ratio-spectra method offers the chance of doing measurements in correspondence of peaks, hence a potential greater sensitivity, as demonstrated by the slopes of lines of regression in Table 1.

As concerns the choice of cefatoxime and cefadroxil, we emphasise that these have been selected for their importance in therapeutic field

Table 3
Recovery of cefotaxime and cefadroxil from injections and tablets, by ratio-spectra and zero-crossing derivative spectrophotometry

Mixture	Recovery %[a]			
	A (239.5 nm)	A (291.5 nm)	B (238 nm)	B (283 nm)
Ratio-spectra, 1st-derivative				
Claforan vials	101.7±0.8	100.9.0±0.5		
Foxil tablets			102.0±0.7	101.2±0.4
Zariviz vials	100.9±0.9	100.9±0.7		
Oradroxil tablets			101.3±0.8	99.9±0.8
	A (284 nm)	A (303 nm)	B (229.5 nm)	B (245.5 nm)
Ratio-spectra, 2nd-derivative				
Claforan vials	101.9±0.8	101.0±0.4		
Foxil tablets			101.8±0.6	101.4±06
Zariviz vials	102.0±0.6	102.4±0.8		
Oradroxil tablets			101.9±0.4	102.0±0.3
	A (257 nm)	A (279 nm)	B (242 nm)	B (296 nm)
Zero-crossing, 2nd-derivative				
Claforan vials	100.6±0.5	99.9±0.7		
Foxil tablets			101.8±0.3	100.9±0.8
Zariviz vials	99.8±0.8	99.9±0.8		
Oradroxil tablets			101.8±0.5	102.0±0.4

A, cefatoxime, B, cefadroxil.
[a] Mean±standard deviation for five determinations, given as percentage of the nominal content (the labelled content of pharmaceuticals are given under reagents section).

and the potential toxic effects. Unfortunately, the mixture under study was not easily available in the local markets, i.e., with the two drugs formulated together; hence, the analysis was performed by using mixtures of pharmaceuticals. However, we emphasise that these drugs may be administered simultaneously in some diseases and moreover this is not the only case of literature of mixtures of drugs formulated separately [11,19–21,32–35].

The methods described allow the simultaneous determination of the two antibiotics in admixture and this represents a considerable advantage over existing methods which enable the determination of cephalosporins in question one at time. A comparison of the present data with a reference method was not made because of the absence of an official method for this binary mixture of cephalosporins.

It cannot be excluded that other techniques, such as HPLC, electrophoresis, etc., would also give good results. The technology and applications of HPLC, e.g., have been developed at a fast rate during the past years (although the detector in some instance remains the factor that sometimes limits the ultimate sensibility and flexibility). Unfortunately, the above techniques generally need sophisticated and more expensive instrumentation in respect to spectrophotometry, which offers the chance of using instrumentation commonly available in all research and analysis laboratories.

The procedures described are sensitive and work without solving equations or complicated pretreatment of the samples. Satisfactory recoveries were found from injections and tablets.

For the above reasons, coupled with analysis time, ease of operation, and widespread availability of commercial instruments with derivative capability, the described procedures offer a distinct advantage over other techniques and confirm their suitability for routine analysis of mixtures of cefotaxime and cefadroxil and for control purposes of pharmaceutical dosage forms for these drugs.

The above considerations added to the importance of the two cephalosporins in pharmaceutical and clinical field (as remarked under Section 1), and the need of analytical procedures for their determination, would make the present work of interest for the readers of journals like JPBA.

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