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Simultaneous determination of cefepime and L-arginine in injections by second-derivative spectrophotometry

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Abstract

A simple spectrophotometric assay for the determination of cefepime and L-arginine in injections is described. Since zero-order spectra showed considerable overlap, second-derivative spectrophotometry was used to enhance the spectral details. A linear relationship between second-derivative amplitude and concentration of each compound was found. Beer's law was obeyed up to 50 and 22 μg ml^{-1} of cefepime and arginine, respectively, in the second-derivative mode. Detection limits were 0.31 and 0.58 μg ml^{-1} for cefepime and arginine, respectively. The method, which is rapid, simple and does not require any separation step, has been successfully applied to the assay of commercial injections containing cefepime and arginine.

Keywords: Analysis of injections; Arginine; Cefepime; Second-derivative spectrophotometry; Simultaneous determination

1. Introduction

Twelve years ago, an article devoted to biomedical applications of derivative spectroscopy was published by Fell [1]. Since then, derivative spectrophotometry has proved to be very useful, mostly in pharmaceutical and clinical chemistry [2]. In pharmaceutical analysis this technique has been profitably applied to the assay of single-component dosage forms in the presence of interfering excipients [3,4] or degradation products [5] and in the analysis of two-component mixtures [6–8].

Cefepime is a new injectable cephalosporin antibiotic with a broader antimicrobial spectrum than that of third-generation cephalosporins and some other β-lactam antibiotics; for parenteral use cefepime may be formulated with L-arginine in the ratio 1:0.72 (w/w). Analytical procedures based on high-performance liquid chromatography (HPLC) have been described for determining cefepime [9]. However, for successful operation this technique requires specialized apparatus and considerable skill.

The present paper describes a derivative spectrophotometric method that is useful for the simultaneous determination of cefepime and L-arginine in commercial injections.

2. Materials and methods

2.1. Reagents and standard solutions

Cefepime monohydrate (batch 4R142) was donated by Bristol Myers-Squibb (USA). L-

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Arginine was purchased from Sigma (St. Louis, MO, USA). Stock solutions of cefepime and L-arginine (0.5 and 0.36 mg ml^{-1}, respectively) were prepared in distilled water. Series of working standards of cefepime and L-arginine (5–50 and 3.6–36 μg ml^{-1}, respectively) were prepared by dilution and mixing of the stock solutions.

2.2. Samples

Maxipime injection (Bristol Myers-Squibb, USA) was assayed by subjecting to the general procedure aliquots of the injection with a nominal concentration of 20 and 14.4 μg ml^{-1} of cefepime and L-arginine, respectively. The concentrations of the two components were calculated from regression equations relating the derivative amplitude to the concentration of each substance.

2.3. Apparatus

A Shimadzu UV 240 double-beam spectrophotometer with an optional program unit (model OPI-2) and 1-cm quartz cell was used. Suitable settings were: slit width, 2 nm (the response time was automatically adjusted according to the slit width); scan speed, 45 nm min^{-1}; derivative wavelength difference, 4 nm. The recorder scale expansion was optimized to facilitate readings on the recorder trace.

3. Results and discussion

3.1. Spectrophotometric measurements

Fig. 1 shows the zero-order absorbance spectra of cefepime and arginine at similar concentrations in water. The spectra clearly display

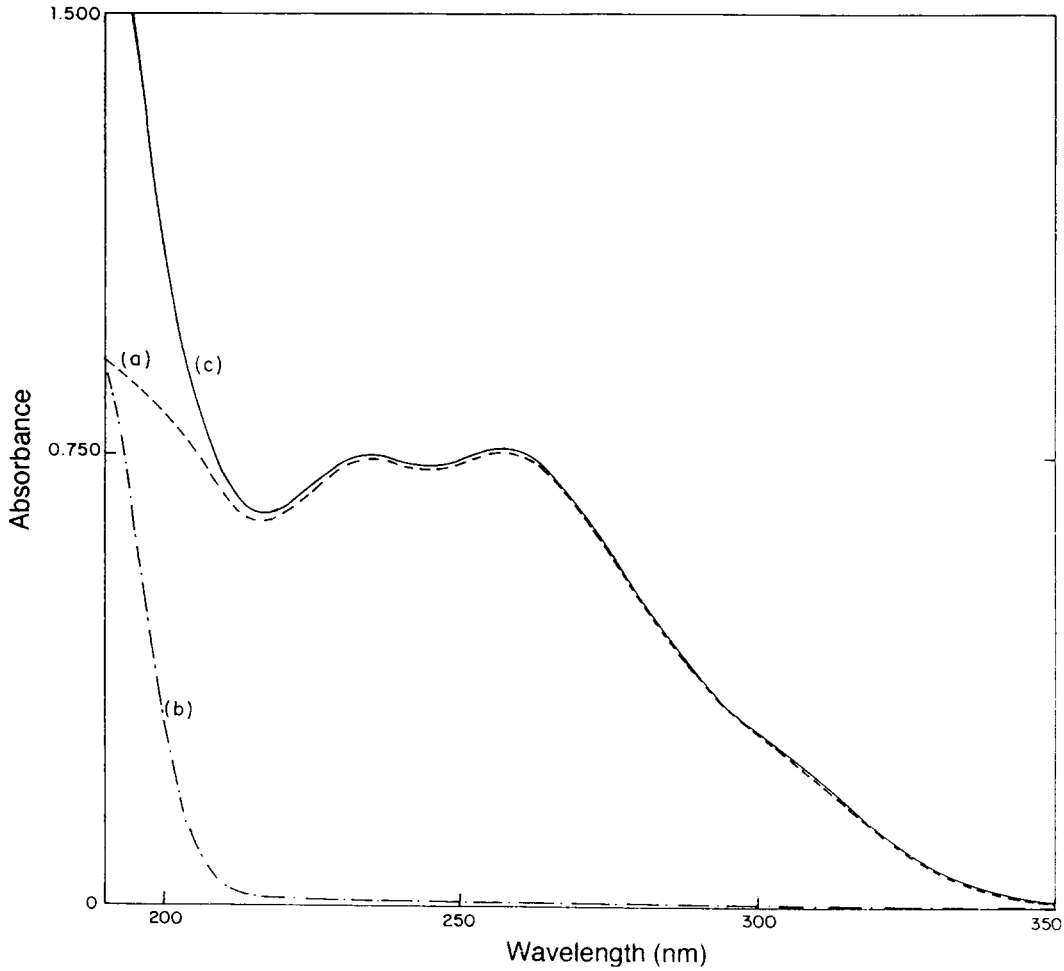

Fig. 1. Zero-order spectra of: (a) cefepime (20 μg ml^{-1}); (b) arginine (14.4 μg ml^{-1}); and (c) cefepime plus arginine (20 and 14.4 μg ml^{-1}, respectively). The reference was water.

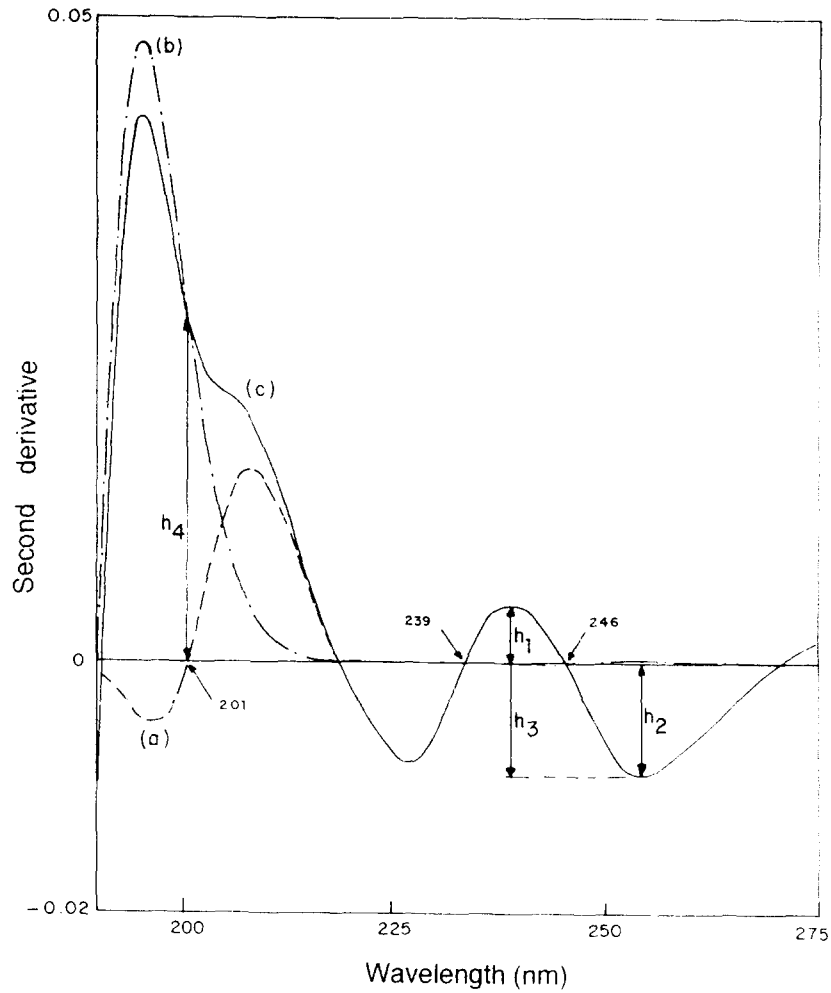

Fig. 2. Second-derivative spectra of: (a) cefepime (20 μg ml^{-1}); (b) arginine (14.4 μg ml^{-1}); (c) a mixture of cefepime (20 μg ml^{-1}) and arginine (14.4 μg ml^{-1}).

considerable overlap. The profiles of the first-derivative spectra are not adequate for the application of this method. However, the second-derivative spectra present spectral features which can be used for the simultaneous determination of the two components (Fig. 2).

The most common procedure for the preparation of an analytical calibration graph involves "peak to peak" and "baseline" measurements (generally called "graphical measurements") and "zero-crossing" measurements. These measurements are made by means of graphical construction on the chart recording of the spectrum [10].

The suitability of different graphical measurements (Fig. 2) was investigated in the sec-

Table 1
Linearity of regression equations in the determination of cefepime and arginine in mixtures by second-derivative spectrophotometry[a]

Compound	Wavelength (nm)	Regression equation	r	Variance (S^2)	Detection limit (μg ml^{-1})
Cefepime	239	$H1 = 2.84 \times 10^{-4}C - 8.00 \times 10^{-6}$	0.9999	1.00×10^{-8}	0.75
Cefepime	254	$H2 = 6.21 \times 10^{-4}C - 4.00 \times 10^{-5}$	0.9999	2.10×10^{-8}	0.49
Cefepime	239/254	$H3 = 9.05 \times 10^{-4}C + 5.00 \times 10^{-5}$	0.9999	1.70×10^{-8}	0.31
L-Arginine	201	$H4 = 2.59 \times 10^{-3}C + 5.05 \times 10^{-4}$	0.9998	5.00×10^{-7}	0.58

[a] Number of samples, $n = 8$; level of significance, $P = 0.05$; C = concentration of the drug (μg ml^{-1}).

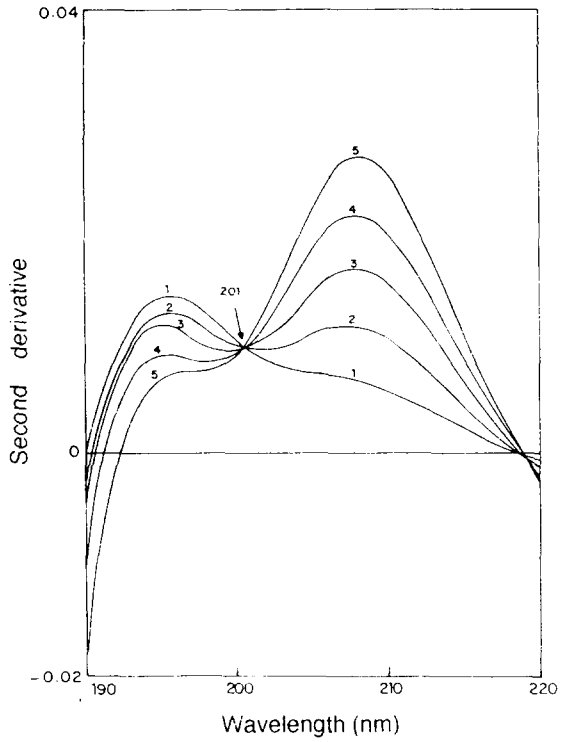

Fig. 3. Second-derivative spectra of mixtures of arginine, 3.6 μg ml^{-1} and cefepime, 5, 10, 15, 20 and 25 μg ml^{-1} (curves 1–5). The reference was water.

ond-derivative mode for both compounds. The heights were not affected by the presence of cefepime and arginine over the full range of concentrations investigated in the second-derivative mode (Fig. 3). The spectral measurement of arginine at 201 nm ($h4$; i.e. on the slope of the derivative spectrum) is reliable [6,8,11,12] as shown by the accuracy and repeatability of the experimental results.

3.2. Statistical analysis of results

Linearity and detection limits

Using the derivative spectra, linear regression equations for mixtures of cefepime and arginine were established. These are given in Table 1 together with correlation coefficients and variances ($P = 0.05$) ($n = 8$). The high values of the correlation coefficients indicate the good linearity of all calibrations. The small degree of scatter of the experimental data points around the line of regression is confirmed by the small values of the variance. Because the intercepts on the y-axis are close to zero, a single-point calibration is justified; for cefepime the largest slope (sensitivity) was obtained for measurement $H3$ so that this was chosen for the assay. The ordinate values, H, of the equations were calculated from the amplitude measurements (mm) and standardized as follows [10]: H = recorder divisions (h mm) x scale expansion/150 mm full scale. Beer's law was followed for concentrations up to 50 and 22 μg ml^{-1} of cefepime and arginine, respectively, in the second derivative mode. The lower detection limit (DL) was calculated by means of the following relationship [13]:

$$\mathrm{DL} = \left(S^2 n - \frac{2}{n} - 1\right)^{1/2} \frac{t}{b}$$

where n = number of samples; b = slope of line of regression; t = Student's t value ($P = 0.05$); and S^2 = variance.

Table 3
Recovery of cefepime and arginine from injections[b]

Cefepime, second derivative			Arginine, second derivative
239 nm	254 nm	239/254 nm	201 nm
99.7 ± 1.3	100.4 ± 1.2	100.1 ± 1.1	98.7 ± 0.8

[a] Mean and standard deviation for ten determinations, given as a percentage of the declared content.
[b] Maxipime injection, 1 g of cefepime and 0.72 g arginine in each vial.

Table 2
Replicate determination of mixtures of cefepime and arginine

Actual content Cefepime/arginine (μg ml^{-1})	Found (μg ml^{-1})			
	Cefepime, second derivative		Arginine, second derivative	
	239 nm[a]	254 nm[a]	239/254 nm[a]	201 nm[a]
20.0/14.4	20.25 ± 0.23	19.99 ± 0.26	20.12 ± 0.24	14.13 ± 0.08
10.0/7.2	9.81 ± 0.15	10.07 ± 0.11	9.94 ± 0.09	7.14 ± 0.06
15.0/10.8	14.82 ± 0.22	15.40 ± 0.17	15.11 ± 0.13	10.62 ± 0.07

[a] Mean and standard deviation (μg ml^{-1}) for eight determinations.

Table 4
Recovery of cefepime and arginine added to injection

Injection	Cefepime/arginine (μg ml^{-1})[a]	Cefepime/arginine		
		Added (μg ml^{-1})	Found (μg ml^{-1})[b]	Recovery (%)
Maxipime	10.00 7.20	5.00:3.60	14.93:10.76	98.6:98.9
		10.00:7.20	20.08:14.47	100.8:101.0
		15.00:10.80	25.15:18.10	101.0:100.9

[a] Obtained by dilution of commercial injection.
[b] Mean of three determinations. Cefepime and arginine were measured at 239 nm 254 nm and 201 nm, respectively.

Accuracy and precision

To test the accuracy and precision of the proposed methods, eight successive determinations of mixtures of cefepime and arginine were carried out. The results reported in Table 2 show that the accuracy and precision were satisfactory.

3.3. Application to a commercial formulation of injection

The method was applied to the determination of cefepime and arginine in injections of Maxi-pime which comprise only this sample binary mixture (with no other added excipients, e.g. buffer salts). Ten replicate determinations were made. Satisfactory results (Table 3) were obtained for the recovery of both compounds and are in good agreement with the label claims. To verify the accuracy of the proposed method, recovery experiments were carried out by the standard addition method. The results obtained (Table 4) showed a satisfactory recovery and confirmed the accuracy of the method.

In summary, the method has been validated with respect to simple binary mixtures of cefepime and arginine and may be applicable only to such simple mixtures in absence of any degradation products. The results confirm that for these mixtures derivative spectrophotometry offers accuracy and precision with the added advantage of speed, simplicity and low detection limits.

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