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First and second derivative spectrophotometric determination of cefoperazone and sulbactam in injections

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Abstract: A simple, spectrophotometric assay to measure the concentrations of cefoperazone and sulbactam in injectable formulations is described. Since zero-order spectra are subject to interference, derivative spectrophotometry was used to enhance the spectral details. A linear relationship between derivative amplitudes and the concentrations of the compounds was found. Beer's law is obeyed up to 75 and 80 μg ml^{-1} of cefoperazone in the first and second derivative modes, respectively, and up to 75 μg ml^{-1} of sulbactam in the second derivative mode. Detection limits were 0.64 and 0.88 μg ml^{-1}, respectively for cefoperazone in the first and second derivative modes and 0.30 μg ml^{-1} for sulbactam in the second derivative mode. The method is rapid, simple, does not require a separation step and has successfully been applied to the assay of commercial injections containing cefoperazone and sulbactam.

Keywords: *Cefoperazone determination; sulbactam determination; derivative spectrophotometry; simultaneous determination; analysis of injections.*

Introduction

Derivative UV spectrophotometry has proved to be a valuable technique for the identification and quantitation of several organic compounds including drugs [1, 2]. In pharmaceutical analysis this technique is particularly useful in 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]. Cefoperazone–sulbactam is a (2:1, w/w) combination of cefoperazone, an extended-spectrum cephalosporin active against Gram-positive and Gram-negative bacteria, and sulbactam, a potent β-lactamase inhibitor. The concomitant administration of sulbactam enhances the activity of cefoperazone against microorganisms.

Analytical procedures based on high-performance liquid chromatography (HPLC) have been described for determining cefoperazone and sulbactam [9–12]. However, this technique requires special apparatus and considerable skill to obtain reliable results. The present paper describes a derivative spectrophotometric method for the simultaneous determination of both drugs in commercial injections.

Materials and Methods

Reagents and standard solutions

Cefoperazone sodium (batch 31824) and sulbactam sodium (batch 906-391056) were donated by Pfizer, S.A. (Spain). Stock solutions of cefoperazone and sulbactam (0.5 and 0.25 mg ml^{-1}, respectively) were prepared in distilled water. Series of working standards of cefoperazone and sulbactam (5–100 and 2.5–40 μg ml^{-1}, respectively) were obtained by dilution and mixing of the stock solutions.

Samples

Sulperazone injection (Pfizer GmbH, Germany) was assayed as follows. Aliquots of the injection with a nominal concentration of 20 and 10 μg ml^{-1} (cefoperazone and sulbactam, respectively) were subjected to the general procedure. The concentration of the two components was calculated from the regression equations relating the derivative amplitudes to the concentrations of the drugs.

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Apparatus

A Shimadzu UV 240 double-beam spectrophotometer with an optional program unit model OPI-2 and 1-cm quartz cells 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.

Results and Discussion

Spectrophotometric measurements

Since cefoperazone and sulbactam have ionizable groups, the effect of pH on the derivative spectra was studied. Over the pH range 4–8 the spectra were unchanged.

Figure 1 shows the zero-order absorption spectra of cefoperazone and sulbactam at similar concentrations in water. The spectra clearly display considerable overlap. However, the first and second derivative spectra have spectral features that can be used for the simultaneous determination of the two components (Figs 2 and 3).

The commonest derivative measurements for the construction of analytical calibration graphs are "peak to peak" and "peak to baseline" measurements (generally called graphical measurements) and "zero-crossing" measurements. These are obtained by

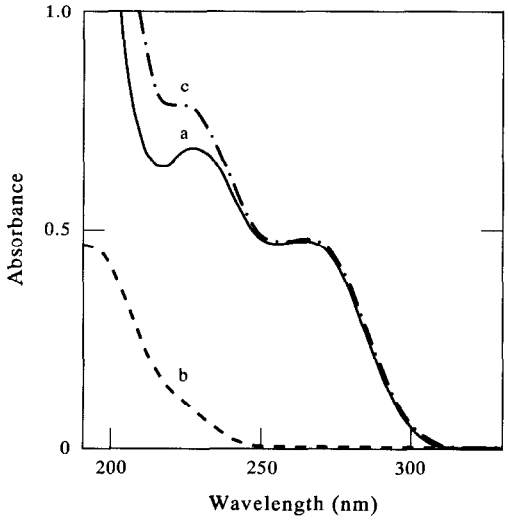

Figure 1
Zero-order spectra of (a) cefoperazone (20 μg ml^{-1}); (b) sulbactam (10 μg ml^{-1}); and (c) cefoperazone plus sulbactam (20 and 10 μg ml^{-1}, respectively). The reference was water.

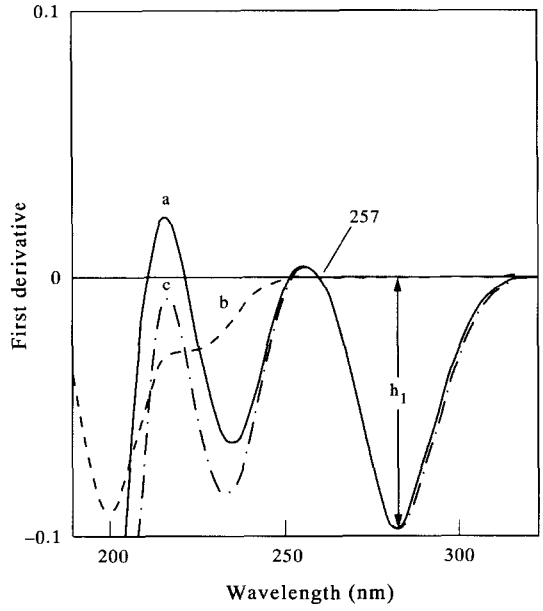

Figure 2
First derivative spectrum of (a) cefoperazone (20 μg ml^{-1}); (b) sulbactam (10 μg ml^{-1}) and (c) a mixture of cefoperazone (20 μg ml^{-1}) and sulbactam (10 μg ml^{-1}). The arrow indicates the zero-crossing wavelength of cefoperazone.

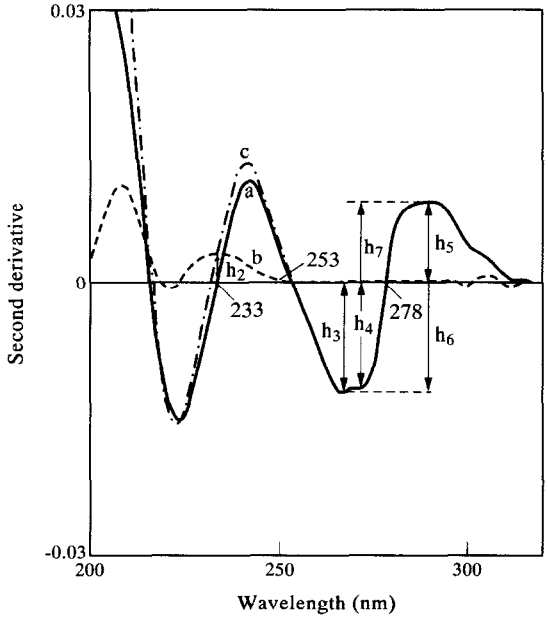

Figure 3
Second derivative spectrum of (a) cefoperazone (20 μg ml^{-1}); (b) sulbactam (10 μg ml^{-1}) and (c) a mixture of cefoperazone (20 μg ml^{-1}) and sulbactam (10 μg ml^{-1}). The arrows indicate the zero-crossing wavelengths of cefoperazone and sulbactam.

measurements on the chart recording of the spectrum [15].

The suitability of different graphical and zero-crossing measurements (Figs 2 and 3) was investigated in two derivative modes for both compounds. The quantitation of sulbactam was found to be not possible by the first derivative mode since poor results were obtained: the scatter of experimental points was unacceptable and the calibration curves were insufficiently linear.

The measured values were not affected by the presence of the other component over the full range of concentrations investigated in the first and second derivative mode (Fig. 4). The spectral measurement of sulbactam at 233 nm (h2; i.e. on the slope of the derivative spectrum) is reliable [6, 8, 13, 14] as shown by the accuracy and repeatability of the experimental results shown below.

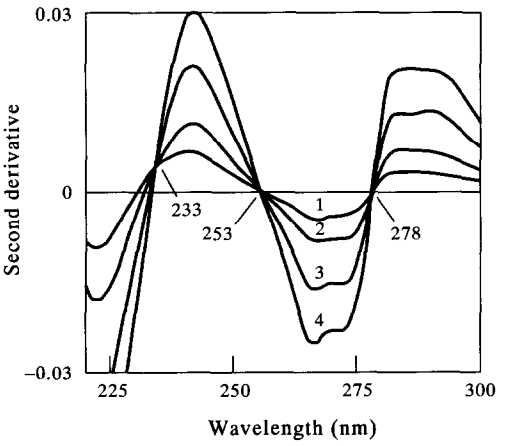

Figure 4
Second derivative spectra of mixtures of sulbactam, 20 μg ml^{-1} and cefoperazone, 10, 20, 40 and 60 μg ml^{-1} (curves 1–4). The reference was water.

Statistical analysis of results

Linearity and detection limits. By using the derivative spectra, linear regression equations for mixtures of cefoperazone and sulbactam were established. These are given in Table 1, together with correlation coefficients, and variances at the $P = 0.05$ level of significance ($n = 8$). 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 variance. Because the intercepts on the y-axis are close to zero, a single-point calibration was justified. The ordinate values, H, of the equations were calculated from the amplitude measurements (mm) standardized as follows [15]: H = recorder divisions (hmm) × scale expansion/100 mm full scale. Beer's law was obeyed for concentrations up to 75 and 80 μg ml^{-1} of cefoperazone in the first and second derivative modes, respectively and up to 70 μg ml^{-1} of sulbactam in the second derivative mode. For cefoperazone, the largest amplitude was $H1$ and so this amplitude was used for the assay.

The detection limit (DL) was calculated by means of the following relationship [16]:

$$\sqrt{(S^2n - 2/n - 1)}\cdot t/b,$$

where n is the number of samples, b is the slope of line of regression, t is the Student's t-value at the $P = 0.05$ level of significance and S^2 is the variance.

Accuracy and precision. To test accuracy and precision of all the methods proposed, eight successive determinations of mixtures of cefoperazone and sulbactam were carried out. The

Table 1
Linearity of the determination of cefoperazone and sulbactam in mixtures by first and second derivative spectrophotometry

Compound	(nm)	Regression equation	r	Variance (S^2)	Detection limit (μg ml^{-1})
Cefoperazone	277.5	$H1 = 1.80 \times 10^{-3} + 4.71 \times 10^{-3} C$	0.9999	2.00×10^{-6}	0.64
Sulbactam	233	$H2 = 1.57 \times 10^{-4} + 3.20 \times 10^{-4} C$	0.9999	2.03×10^{-9}	0.30
Cefoperazone	267.5	$H3 = 2.92 \times 10^{-4} + 4.39 \times 10^{-4} C$	0.9979	5.43×10^{-8}	1.20
Cefoperazone	272.5	$H4 = 4.45 \times 10^{-5} + 4.47 \times 10^{-4} C$	0.9994	4.80×10^{-8}	1.05
Cefoperazone	290	$H5 = 4.64 \times 10^{-5} + 3.20 \times 10^{-4} C$	0.9998	1.80×10^{-8}	0.95
Cefoperazone	267.5/290	$H6 = 3.54 \times 10^{-4} + 7.59 \times 10^{-4} C$	0.9999	1.10×10^{-7}	0.99
Cefoperazone	272.5/290	$H7 = 4.44 \times 10^{-4} + 7.67 \times 10^{-4} C$	0.9999	9.00×10^{-7}	0.88

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

Table 2
Replicate determinations of mixtures of cefoperazone and sulbactam (all values are in μg ml^{-1})

Actual content C/S†	Found						
	Cefoperazone, first derivative			Cetoperazone, second derivative			Sulbactam, second derivative
	277.5 nm*	267.5 nm*	272.5 nm*	290 nm*	267.5/290 nm*	272.5/290 nm*	233 nm*
20:10	20.10 ± 0.011	20.07 ± 0.011	19.89 ± 0.015	19.91 ± 0.010	19.78 ± 0.013	19.92 ± 0.007	10.05 ± 0.009
10:15	10.04 ± 0.007	10.02 ± 0.09	9.81 ± 0.006	9.85 ± 0.011	9.87 ± 0.008	9.97 ± 0.013	15.04 + 0.007
15:10	15.05 ± 0.011	15.08 ± 0.006	14.84 ± 0.015	14.89 ± 0.007	14.91 ± 0.010	14.90 ± 0.018	9.94 ± 0.011

*Mean and standard deviation (μg ml^{-1}) for eight determinations.
†C = cefoperazone and S = sulbactam.

Table 3
Recovery* of cefoperazone and sulbactam from injections†

Cefoperazone, first derivative	Cetoperazone, second derivative					Sulbactam, second derivative
277.5 nm	267.8 nm	272.5 nm	290 nm	267.5/290 nm	272.5/290 nm	233 nm
101.2 ± 48	98.7 ± 0.85	102.1 ± 63	99.3 ± 0.60	102.4 ± 0.56	99.5 ± 0.37	101.1 ± 0.52

*Mean and standard deviation for eight determinations, given as a percentage of the declared content.
†Sulperazone injection, 1 g of cefoperazone and 0.5 g sulbactam/vial.

Table 4
Recovery of cefoperazone and sulbactam added to injections

Injection	Cefoperazone–Sulbactam (μg ml^{-1})*	Cefoperazone–Sulbactam		
		Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)
Sulperazone	20.00:10.00	10.00:5.00	30.09:15.02	100.3:100.1
		20.00:10.00	40.12:20.07	100.3:100.4
		30.00:15.00	50.27:25.04	100.5:100.2

*Obtained by dilution of commercial injections.
†Average of three determinations. Cefoperazone and sulbactam were measured at 277.5 and 233 nm, respectively.

results reported in Table 2 show that the accuracy and precision were satisfactory.

Application to a commercial formulation of injection

The method was applied to the determination of cefoperazone and sulbactam in injections of Sulperazone, which is a simple binary mixture (with no added excipients, e.g. buffering salts). Ten replicate determinations were made. The results (Table 3) for both compounds were in good agreement with the label claims. In order to verify the accuracy of the described method, recovery experiments by the standard addition method were carried out. 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, and may be applicable only to, simple binary mixtures of cefoperazone and sulbactam, confirming that derivative spectrophotometry offers accuracy and precision with the added advantage of speed, simplicity and low detection limits.

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