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Short Communication

Determination of cephradine and L-arginine in injections by second derivative ultraviolet spectrophotometry

ALBERTO PARRA,*† M. DOLORES GÓMEZ,‡ VINCENTE RÓDENAS,§ JAVIER GARCIA-VILLANOVA§ and MARIA LUISA LÓPEZ§

†*Servicio de Farmacia*/§*Servicio de Análisis Clínicos, Hospital Virgen del Castillo, Avda. de la Feria s/n, 30.510 Yecla, Murcia, Spain*
‡*Servicio de Microbiologia, Hospital Dr. Moliner, Porta-Coeli s/n, 46.118 Serra, Valencia, Spain*

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Introduction

The basis of derivative spectrophotometry [1–5] and its applications have been objects of several reviews [3, 6, 7]. In pharmaceutical analysis it has proven particularly useful in the assay of single components in the presence of excipients [8–12] or degradation products [13] and in the analysis of two-component mixtures [14–19].

Cephradine is a semisynthetic cephalosporin which exhibits a good activity against Gram-positive and Gram-negative bacteria with exception of *Pseudomonas* and *Citrobacter* and which may be formulated for parenteral use with L-arginine in the proportion (2:1, w/w). Methods based on spectrophotometry [20] and HPLC [21] have been described, however many of these assay methods are limited in their applications or considerable skill is required to operate them successfully.

The present paper describes a method based on derivative spectrophotometry for the simultaneous determination of cephradine and L-arginine in commercial injections.

Material and Methods

Reagents and standard solutions

Cephradine was donated by Squibb, S.A. (Spain). A 0.5 mg ml^{-1} cephradine solution was prepared in distilled water. L-arginine was purchased from Sigma Chemical Company (St Louis, MO, USA). A 0.25 mg ml^{-1} solution was prepared in distilled water. Series of working standard solutions of cephradine and L-arginine (1–40 and 0.5–20 μg ml^{-1}, respectively) were obtained by dilution and mixing of the stock solutions. Injectable dosage forms of Velocef (Squibb, S.A., Spain) and Septacef (Septa, S.A., Spain) were utilized. Aliquots of these solutions with a nominal concentration of 20 and 10 μg ml^{-1} (cephradine and L-arginine, respectively) were subjected to the general procedure. The percentage recovery of the two components was computed from the regression equations.

Apparatus

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

Results and Discussion

Spectrophotometric measurements

Figure 1 shows the absorption (zero-order) spectra of (a) cephradine (20 μg ml^{-1}); (b) L-

* Author to whom correspondence should be addressed.

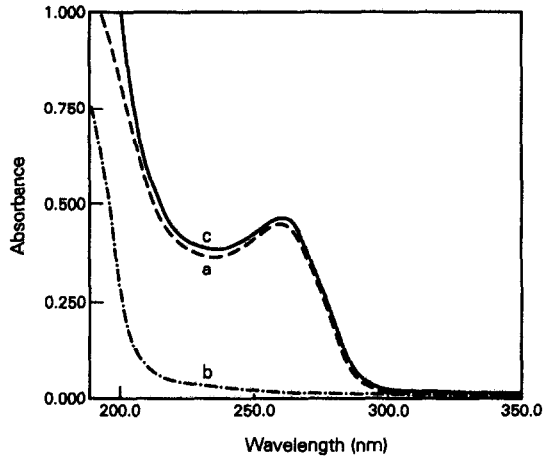

Figure 1
Zero-order spectra of (a) cephradine (20 μg ml^{-1}); (b) L-arginine (10 μg ml^{-1}); and (c) cephradine plus L-arginine (20 and 10 μg ml^{-1}, respectively). The reference was water.

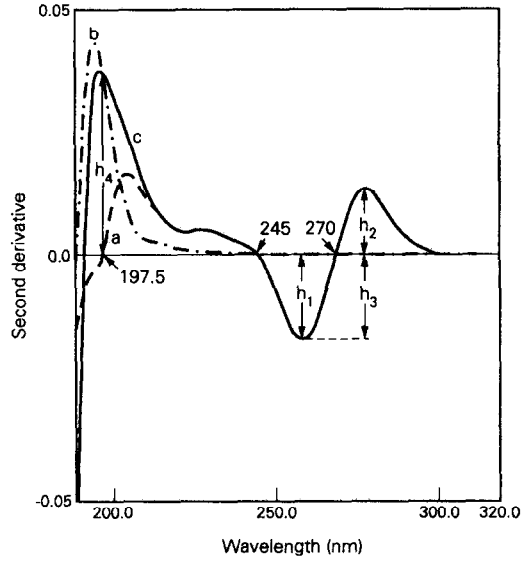

Figure 2
Second derivative spectrum of (a) cephradine (20 μg ml^{-1}); (b) L-arginine (10 μg ml^{-1}) and (c) a mixture of cephradine (20 μg ml^{-1}) and L-arginine (10 μg ml^{-1}). The arrows indicate the zero-order crossing wavelengths of cephradine.

arginine (10 μg ml^{-1}) and (c) a mixture of cephradine and L-arginine (20 and 10 μg ml^{-1}, respectively). The large overlap of the spectral bands of the two components at 190–300 nm obscures any spectral feature utilizable for the determination of cephradine. The profiles of the first derivative spectra are not adequate for the application of this method. However, the second derivative spectra allowed the simultaneous determination of both components in a mixture.

The commonest procedure for the preparation of analytical calibration graph are 'peak-to-peak' and 'baseline' measurements (generally called graphical measurements) and 'zero-crossing' measurements. They are obtained by means of graphical construction on the chart recording of the spectrum [1].

Figure 2 shows the second derivative spectra of cephradine and L-arginine, the zero-crossings of cephradine occur at 197.5, 245 and 270 nm. It is important to note that the value of the second derivative of L-arginine is near to zero from 350 to 225 nm (whereas the absorbance of cephradine in the same region is significantly different from zero). Hence, in this range, is possible to take derivative measurements of the mixture proportional to the cephradine concentration only.

Figure 3 shows a typical set of second derivative spectra of mixtures of 4 μg ml^{-1} of L-arginine plus increasing amounts of cephradine (from 20 to 50 μg ml^{-1}). Analagously the measure of cephradine is independent of L-

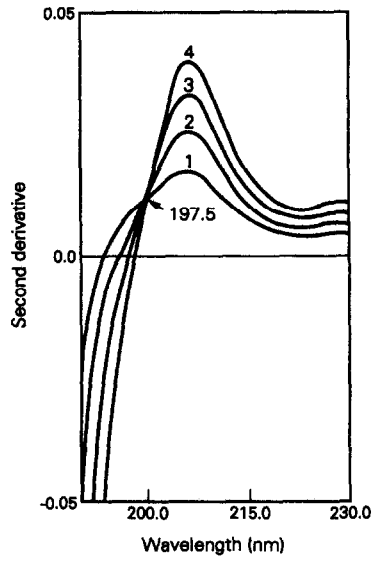

Figure 3
Second derivative spectra of mixtures of L-arginine, 4 μg ml^{-1} and cephradine, 20, 30, 40 and 50 μg ml^{-1} (curves 1–4). The reference was water.

arginine concentration (from 5 to 240 μg ml^{-1}). The heights at 258.5 and 278.5 were denoted h_1 and h_2 (Fig. 2). The vertical distance between the adjacent maximum and minimum at 258.5 and 278.5 (i.e. peak-to-baseline and peak-to-peak measurements, respectively [1]) was denoted h_3. The variation

of h_1, h_2 and h_3 was not affected by the presence of arginine over the full range of concentrations investigated. The height at 197.5 nm was denoted h_4, this height is independent of the derivative amplitude of cephradine and was proportional to the L-arginine concentration. The second derivative of L-arginine is zero or near to zero at the other zero-crossing wavelengths of cephradine as previously seen, which prevents suitable use of these wavelengths for determining L-arginine. The spectral measurements at 197.5 nm (i.e. on the slope of derivative spectrum) is reliable [14, 15, 18, 22] as shown by accuracy and repeatability in the experimental results.

Statistical analysis of results

Linearity. By using the derivative spectra, linear regression equations for mixtures of cephradine and L-arginine were established [23]. These are given in Table 1, together with correlation coefficients, and variances at $P = 0.05$ level of significance and for $n = 10$ samples. The high values of correlation coefficients indicate the good linearity of all calibrations. Because the intercepts on the y axis are close to zero, a single-point calibration was justified and for cephradine the largest slope amplitude was obtained for measurement h_3 and so this was preferred. The ordinate values, H, of the equations were calculated from the amplitude measurements (mm) and standardized as follows [24]: H = recorder divisions (hmm) × scale expansion/100 mm full scale. Beer's law was followed for concentrations up to 100 μg ml^{-1} of cephradine and 20 μg ml^{-1} of L-arginine in the presence of each other.

Accuracy and precision. To test accuracy and precision of all methods proposed, 10 successive determinations of mixtures of cephradine and L-arginine were carried out. The results reported in Table 2 show that the accuracy and precision were satisfactory.

Determination of cephradine and L-arginine in injections

The method was applied to the recovery of cephradine and L-arginine in injections of Velocef and Septacef which comprise only this simple binary mixture (and no other added excipients, e.g. buffering 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. 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) were satisfactory 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 cephradine and L-arginine, confirming that second derivative spectrophotometry is a simple, rapid and selective technique.

Table 1
Linearity of the determination of cephradine and L-arginine in mixtures by second derivative spectrometry

Compound	λ (nm)	Regression equation	r	Variance
Cephradine	258.5	$h_1 = 2.32 \times 10^{-4} + 6.42 \times 10^{-4}$ C	0.9997	2.5×10^{-6}
Cephradine	278.5	$h_2 = 2.35 \times 10^{-4} + 4.87 \times 10^{-4}$ C	0.9999	5.0×10^{-7}
Cephradine	258.5/278.5	$h_3 = 4.73 \times 10^{-4} + 1.13 \times 10^{-3}$ C	0.9998	4.9×10^{-6}
L-Arginine	197.5	$h_4 = 1.67 \times 10^{-3} + 2.67 \times 10^{-3}$ C	0.9973	7.8×10^{-6}

Number of samples, $n = 10$; level of significance, $P = 0.05$; r = correlation coefficient.

Table 2
Replicate determinations of mixtures of cephradine and L-arginine. All values are in μg ml^{-1}

Actual content		Found			
		Cephradine			L-Arginine
Cephradine	L-Arginine	258.5 nm*	278.5 nm*	258.5/278.5 nm*	197.5 nm*
20.0	10.0	20.13 ± 0.043	20.15 ± 0.045	20.18 ± 0.045	10.10 ± 0.049
10.0	15.0	10.05 ± 0.035	10.08 ± 0.043	10.03 ± 0.030	15.16 ± 0.008
30.0	6.0	30.22 ± 0.036	30.35 ± 0.041	30.21 ± 0.035	6.01 ± 0.034

*Mean and standard deviation (μg ml^{-1}) for 10 determinations.

Table 3
Recovery* of cephradine and L-arginine from injections

Injection	Composition (mg)	Cephradine 258.5 nm	Cephradine 278.5 nm	Cephradine 258.5/278.5 nm	L-Arginine 197.5 nm
Velocef	Cephradine (1000) L-Arginine (500)	102.3 ± 0.87	101.5 ± 0.72	102.4 ± 0.65	102.9 ± 0.85
Velocef	Cephradine (500) L-Arginine (250)	102.1 ± 0.45	101.8 ± 0.83	102.2 ± 0.40	102.7 ± 0.71
Septacef	Cephradine (500) L-Arginine (250)	98.5 ± 0.50	98.6 ± 0.86	98.0 ± 0.83	105.0 ± 0.93

*Mean and standard deviation for 10 determinations, given as a percentage of the declared content.

Table 4
Recovery of cephradine and L-arginine added to injections

Injection	Cephradine/L-arginine ($\mu g\ ml^{-1}$)*	Cephradine/L-arginine Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)†	Recovery (%)
Velocef	20.00/10.00	4.00/2.00	23.90/11.95	97.5/97.5
		8.00/4.00	28.10/14.05	101.3/101.3
		16.00/8.00	36.15/18.00	100.9/100.0
Septacef	20.00/10.00	4.00/2.00	23.95/12.00	98.8/100.0
		8.00/4.00	28.04/13.95	100.5/98.8
		16.00/8.00	36.10/18.10	100.6/101.3

*Obtained by dilution of commercial injections.
†Average of three determinations. Cephradine and L-arginine were measured at 258.5/278.5 and 197.5 nm, respectively.

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