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First- and second-derivative spectrophotometric determination of imipenem and cilastatin in injections

ALBERTO PARRA,*† JAVIER GARCIA-VILLANOVA,‡ VICENTE RÓDENAS‡ and M. DOLORES GÓMEZ§

†*Servicio de Farmacia, Hospital Virgen del Castillo, Avda. de la Feria s/n, 30.510 Yecla (Murcia), Spain*
‡*Servicio de Análisis Clínicos, Hospital Virgen del Castillo, Avda. de la Feria s/n, 30.510 Yecla (Murcia), Spain*
§*Servicio de Microbiología, Hospital Universitario La Fé, Avda. de Campanar 21, 46.009 (Valencia), Spain*

Abstract: First- and second-derivative spectrophotometry has been used for the quantitation of mixtures of imipenem and cilastatin sodium, compounds that have closely overlapping spectral bands. Beer's law was obeyed at concentrations up to 100 μg ml^{-1} of imipenem in both the first- and second-derivative modes and up to 75 μg ml^{-1} of cilastatin in the first-derivative mode. Detection limits at the $P = 0.05$ level of significance were calculated to be 0.40 and 0.52 μg ml^{-1} of imipenem and cilastatin sodium, respectively, in the first-derivative mode, and in a range from 0.45 to 0.68 μg ml^{-1} for imipenem in the second-derivative mode. The method, which is rapid, simple and does not require a separation step, has been successfully applied to the assay of commercial injections.

Keywords: *Imipenem determination; cilastatin determination; derivative spectrophotometry; simultaneous determination; analysis of injections.*

Introduction

Derivative spectrophotometry is a technique first proposed more than 35 years ago [1], that was developed during the 1960s, and improved during the 1970s and 1980s essentially as a result of technological developments [2, 3]. Due to the enhancement of spectral features, it has been employed to determine absorption maxima, to suppress the effect of a broad bandwidth, interfering matrix, and to reduce the interference of overlapping spectral bands [4, 5]. In pharmaceutical analysis, it has proven particularly useful in the assay of single components in the presence of excipients [6–8] or degradation products [13], and in the analysis of two-component mixtures [10–15].

Imipenem/cilastatin sodium is a (1:1, w/w) combination of imipenem (*N*-formimidoyl thienamycin), a crystalline derivative of the novel carbapenem antibiotic thienamycin, and cilastatin, a potent inhibitor of renal dehydropeptidase-I, which prolongs the half-life of imipenem (by preventing its inactivation in the kidney). Its spectrum of action is unusually broad, with activity against the majority of pathogenic bacteria.

The chemical structures of imipenem and cilastatin sodium are shown in Scheme 1. Analytical procedures based on high-performance liquid chromatography (HPLC) have been described for their determination in biological samples for pharmacokinetic studies [16]. However considerable skill is required to carry out this technique successfully. Therefore, in the present study, first- and second-order ultraviolet derivative spectrophotometric methods have been developed, and are proposed for the rapid and reliable quality control assay of commercial injections of imipenem and cilastatin sodium.

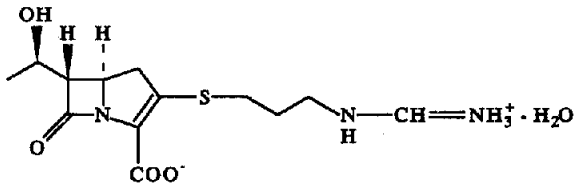
Materials and Methods

Reagents and standard solutions

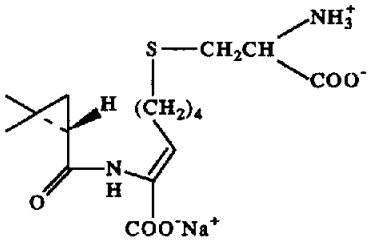
Standardized powders of imipenem (batch C 2250) and cilastatin sodium (batch A 2323) were kindly donated by Merck Sharp and Dohme (Spain).

Stock solutions of imipenem and cilastatin sodium (0.2 mg ml^{-1}) were prepared separately in distilled water. Series of working solutions of imipenem and cilastatin sodium (1–125 μg ml^{-1}, separate and combined) were

* Author to whom correspondence should be addressed.



Imipenem



Cilastatin sodium

Scheme 1
Chemical structures of imipenem and cilastatin sodium.

obtained by dilution and mixing of the stock solutions.

Injectable dosage forms of Tienam (Merck Sharp and Dohme) were used. Aliquots of these solutions, with a nominal concentration of 20 μg ml^{-1} of imipenem and cilastatin sodium, were subjected to the general procedure. The percentage recoveries of the two components were computed from the regression equations.

Apparatus

A Shimadzu UV240 double-beam spectrophotometer with optional program unit model OPI-2 and 1-cm quartz cells was used. Suitable settings were: slit width 2 nm (the response time is automatically adjusted according to the slit width); scan speed, 45 nm min^{-1}; derivative wavelength difference 4 nm. The chart recorder scale expansion was also optimized to facilitate readings on the chart.

Results and Discussion

The simultaneous determination of imipenem and cilastatin using their zero-absorbance spectra cannot easily be carried out due to the large overlap of their spectral bands (Fig. 1). However, first- and second-derivative spectra present spectral features that can be

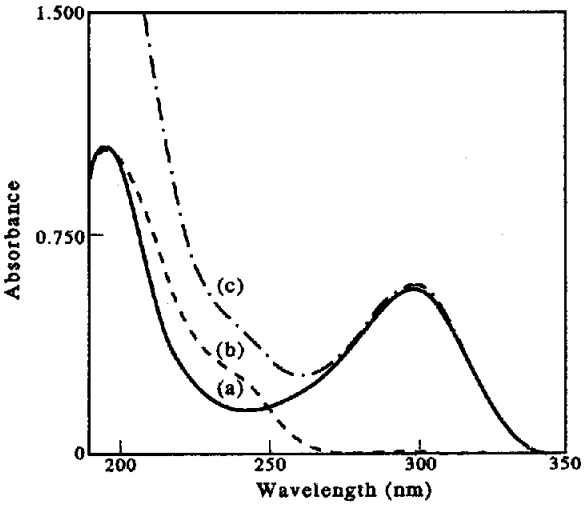


Figure 1
Zero-order spectra of (a) imipenem (20 μg ml^{-1}); (b) cilastatin (20 μg ml^{-1}); and (c) imipenem plus cilastatin (20 μg ml^{-1}, respectively). The reference was water.

used for the simultaneous determination of the two compounds. The suitability of different graphical and zero-crossing measurements (Figs 2, 3) was investigated in the two derivative modes for both compounds. However, as can be seen from Fig. 3, the quantitation of cilastatin was not possible by the second-derivative method.

The most common procedures for the preparation of analytical calibration graphs involve 'peak to peak' and 'base line' measurements (generally called graphical measurements) and 'zero-crossing' measurements made on the chart recording of the spectrum [2].

The heights of each component were not affected by the presence of the other component over the full range of concentrations investigated in the first- (Fig. 4) and the second-derivative modes (Fig. 5).

The spectral measurements at 242 (h_6) and 229 nm (h_7) (i.e. on the slope of derivative spectrum) are reliable [10, 11, 14]. However at 208 nm (h_8) poor results were obtained: the

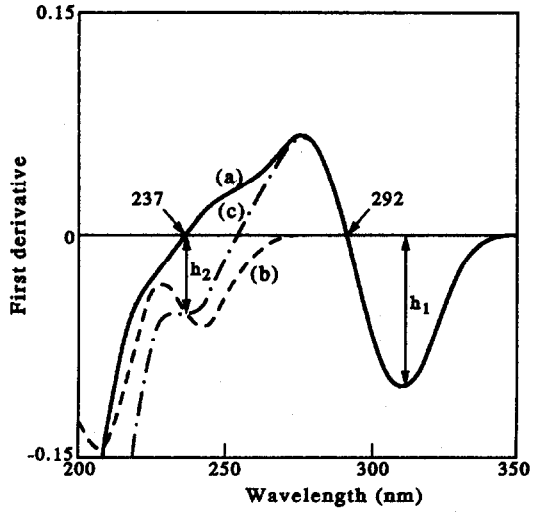


Figure 2
First-derivative spectrum of: (a) imipenem (20 μg ml^{-1}); (b) cilastatin (20 μg ml^{-1}) and (c) a mixture of imipenem (20 μg ml^{-1}) and cilastatin (20 μg ml^{-1}). The arrows indicate the zero-crossing wavelengths of imipenem and cilastatin.

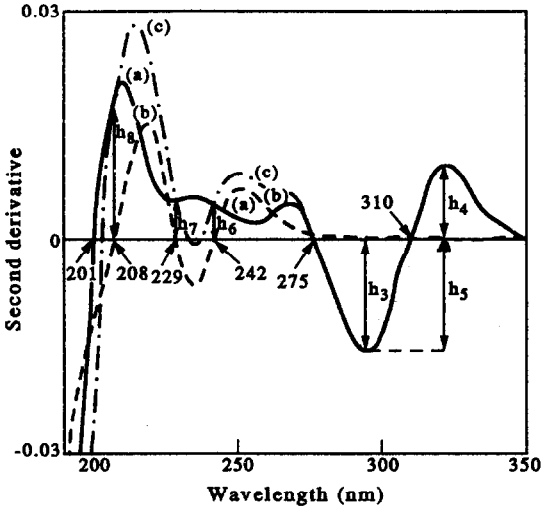


Figure 3
Second-derivative spectrum of: (a) imipenem (20 μg ml^{-1}); (b) cilastatin (20 μg ml^{-1}) and (c) a mixture of imipenem (20 μg ml^{-1}) and cilastatin (20 μg ml^{-1}). The arrows indicate the zero-crossing wavelengths of imipenem and cilastatin.

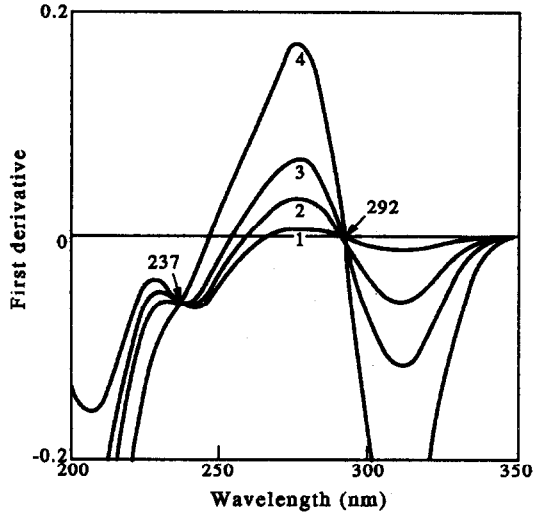


Figure 4
First-derivative spectra of mixtures of cilastatin (20 μg ml^{-1}) and imipenem (2, 10, 20 and 50 μg ml^{-1}: curves 1–4). The reference was water.

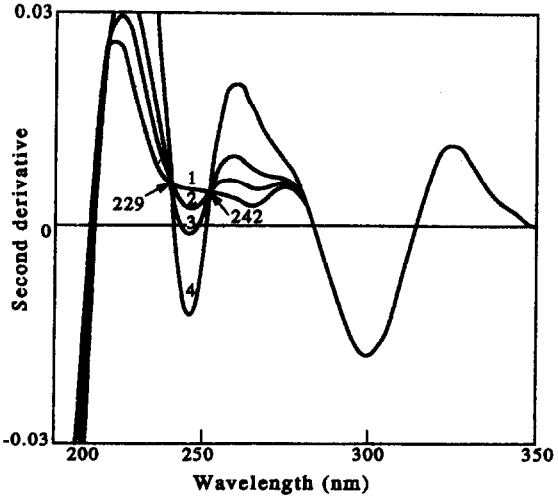


Figure 5
Second-derivative spectra of mixtures of imipenem (20 μg ml^{-1}) and cilastatin (2, 10, 20 and 50 μg ml^{-1}: curves 1–4). The reference was water.

scattering of experimental points was unacceptable and the linearity of the calibration curves was poor.

Statistical analysis of results

Linearity and detection limits. Linear regression equations for mixtures of imipenem and cilastatin are given in Table 1, together with correlation coefficients, variance and detection limits at the $P = 0.05$ level of significance, obtained with 10 measurements. The high values of correlation coefficients indicate the good linearity of all measured values. The small degree of scatter of the experimental data points around the regression line 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. Among the methods utilized for the determination of imipenem, the amplitude giving the largest slope was obtained with the measurement h_1 and so this was preferred. The ordinate values, H, of the equations were calculated from the amplitude measurements (mm) and standardized as follows [17]:

H = recorder divisions (hmm) × scale expansion/100 mm full scale. Beer's law was obeyed by concentrations up to 100 μg ml^{-1} of imipenem in the first- and second-derivative modes and up to 75 μg ml^{-1} of cilastatin in the first-derivative mode.

The detection limit (DL) was calculated by means of the following relationship [18]:

$$\sqrt{\mathrm{DL}} = \sqrt{(s^2 n - 2/n - 1)} \cdot t/b,$$

where: n = number of samples; b = slope of regression line; t = Student's t value at $P = 0.05$ level of significance; s^2 = variance.

Accuracy and precision. To test the accuracy and precision of all the methods proposed, 10 successive determinations of standard mixtures of imipenem and cilastatin were carried out. The 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 imipenem and cilastatin in injections of Tienam 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, which 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) showed a satisfactory recovery and confirmed the accuracy of the method.

In summary, the method has been validated with respect to, and therefore is applicable only to, simple binary mixtures of imipenem and cilastatin sodium, confirming that derivative spectrophotometry offers accuracy and precision with the added advantage of speed, simplicity and low detection limits.

Table 1
Statistical analysis of the determination of imipenem and cilastatin in mixtures by first- and second-derivative spectrophotometry ($n = 10$)

Compound	λ (nm)	Regression equation	r	Variance (s^2)	Detection limit*
Imipenem	311	$h_1 = 2.20 \times 10^{-3} + 4.09 \times 10^{-3} C$	0.9998	5.40×10^{-7}	0.40
Cilastatin	237	$h_2 = 4.15 \times 10^{-3} + 2.13 \times 10^{-3} C$	0.9998	2.60×10^{-7}	0.52
Imipenem	294	$h_3 = 5.30 \times 10^{-4} + 7.23 \times 10^{-4} C$	0.9991	5.12×10^{-8}	0.68
Imipenem	321	$h_4 = 3.18 \times 10^{-4} + 3.93 \times 10^{-4} C$	0.9999	1.00×10^{-8}	0.55
Imipenem	294/321	$h_5 = 5.49 \times 10^{-4} + 1.16 \times 10^{-3} C$	0.9993	4.83×10^{-8}	0.45
Imipenem	242	$h_6 = 3.62 \times 10^{-4} + 1.52 \times 10^{-4} C$	0.9999	1.01×10^{-9}	0.46
Imipenem	229	$h_7 = 5.14 \times 10^{-4} + 2.01 \times 10^{-4} C$	0.9998	2.00×10^{-9}	0.48

r: Correlation coefficient.
C: Concentration of the drug (μg ml^{-1}).
* μg ml^{-1}; $P = 0.05$.

Table 2
Replicate determinations of synthetic mixtures of imipenem and cilastatin

Imipenem/cilastatin	Imipenen*						Cilastatin*
	1st deriv.	2nd deriv.					1st deriv.
I/C†	311 nm	294 nm	321 nm	294/321 nm	242 nm	229 nm	237 nm
20/10	20.09 ± 0.003	19.77 ± 0.015	20.13 ± 0.021	19.81 ± 0.013	19.73 ± 0.009	19.82 ± 0.018	10.12 ± 0.014
10/15	10.07 ± 0.004	9.89 ± 0.010	10.09 ± 0.011	9.93 ± 0.012	9.87 ± 0.007	9.97 ± 0.013	15.08 ± 0.009
15/10	15.08 ± 0.005	14.81 ± 0.013	15.12 ± 0.018	14.90 ± 0.005	14.83 ± 0.011	14.95 ± 0.015	10.03 ± 0.010

*Mean and standard deviation ($\mu g\ ml^{-1}$) for 10 determinations.
†$\mu g\ ml^{-1}$.

Table 3
Determination of imipenem and cilastatin in injections*

	1st deriv.	2nd deriv.				
		Recovery (%)†				
	311 nm	294 nm	321 nm	294/321 nm	242 nm	229 nm
Imipenem	102.7 ± 0.21	103.2 ± 83	104.1 ± 0.45	103.8 ± 0.40	102.8 ± 0.72	103.3 ± 0.55
	237 nm					
Cilastatin	103.4 ± 0.44					

*Tienam injection, 0.5 g imipenem plus 0.5 g cilastatin/vial; supplier Merck Sharp and Dohme.
†Mean and standard deviation for 10 determinations, given as percentage of the declared amount.

Table 4
Recovery of imipenem and cilastatin added to injections

		Imipenem/cilastatin		
Injection	Imipenem/cilastatin content ($\mu g\ ml^{-1}$)*	Added ($\mu g\ ml^{-1}$)	Found† ($\mu g\ ml^{-1}$)	Recovery (%)
Tienam	20.00/20.00	5.00/5.00	25.11/25.13	100.4/100.5
		10.00/10.00	30.18/30.21	100.6/100.7
		15.00/15.00	34.91/35.96	99.7/101.1

*Obtained by dilution of commercial injections.
†Average of three determinations. Imipenem and cilastatin were measured at 311 and 237 nm, respectively.

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