Pergamon

0731-7085(94)00067-0

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 10, pp. 1243–1248, 1994

0731-7085/94 $7.00 + 0.00

Determination of imipenem and cilastatin sodium in Primaxin® by first order derivative ultraviolet spectrophotometry

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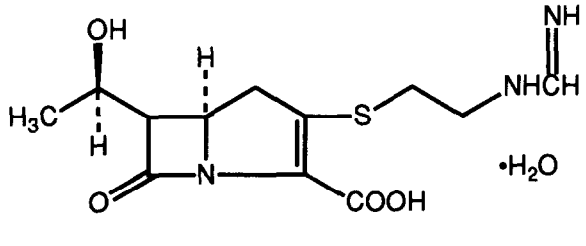
Abstract: A UV-spectrophotometric assay to measure the concentrations of the active drug components (imipenem and cilastatin) or Primaxin® for routine release testing is described. The assay is based on the use of first order derivative spectrophotometry. The trough amplitudes in the first derivative spectrophotometric spectra at 243 and 318 nm were selected to determine cilastatin and imipenem, respectively. A linear relationship ($R > 0.99$) between the trough amplitudes and concentrations was demonstrated over the range 14–42 μg ml^{-1} for both drug components. Commercial IV formulations and laboratory prepared mixtures containing both drugs in different proportions were assayed using the developed method with good recoveries (ave. 100.6%). The method is rapid, precise, accurate and was shown to be equivalent to the more time consuming LC method; which is currently used for routine release testing. The specificity and stability indicating properties of the method will also be addressed.

Keywords: *Primaxin®; imipenem; cilastatin sodium; derivative UV spectrophotometry.*

Introduction

Imipenem (I) is a broad spectrum injectable antibiotic which has been shown to provide effective treatment of infections caused by Gram-negative and Gram-positive bacteria [1–3]. Since imipenem is known to be metabolized in the kidney [4], it has been administered in conjunction with the renal dehydropeptidase inhibitor cilastatin sodium (II) to retard its inactivation [5–7]. Primaxin® is a powder combination product of Merck & Co., Inc. consisting of 500 mg of each component in a single vial with sodium bicarbonate 2.0% present (on a weight basis) as a buffer. The product is reconstituted with 100 ml of normal saline or other appropriate infusion solution to yield a preparation which is 5 mg ml^{-1} of each component.

The primary means of analysis of Primaxin® has been by liquid chromatography (LC) using an ion pair reagent [8]. Due to the limited solution stability of imipenem which has been reported for intravenous [8] and aqueous [9, 10] solutions, LC analysis cannot be conducted over extended intervals as is typically performed for release testing of large numbers of samples. The primary means of degradation at pH 7 is hydrolysis of the β-lactam group. Although solutions of imipenem are most



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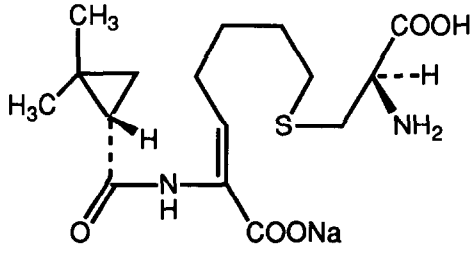

II

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stable at pH 7, the rate constant measured at pH 7 is 0.00403 h^{-1} [9]. Consequently LC analysis sets which do not exceed 8 h are typically recommended. With the use of a spectrophotometric assay, large numbers of samples can be assayed during a single experimental run with adequate solution stability.

Derivative UV spectrophotometry has been employed to analyse imipenem in biological fluids [11]. The method uses third derivative UV at 306 and 312 nm as a means to suppress broad background matrix absorption. Cilastatin is not assayed by this method.

This study reports a direct UV assay for release testing of Primaxin® (imipenem and cilastatin). The specificity and stability indicating properties of the method were also investigated.

The UV spectra of imipenem and cilastatin in MOPS [3-(N-Morpholino)-poropanesulphonic acid] buffer overlap between 200 and 270 nm (Fig. 1). The absorbance of imipenem exhibits a minimum at 243 nm. Therefore, the first derivative of imipenem absorbance at the trough amplitude of 243 nm is zero (Fig. 2) and any absorbance in the derivative spectrum at 243 nm is due to the absorbance of cilastatin.

The absorbance of imipenem at 300 nm can be used for quantitation since there is no interference from cilastatin. There is also an inflection point in the absorption spectrum of imipenem at 318 nm. Therefore, imipenem exhibits a minimum at 318 nm in the first derivative spectrum. The amplitudes of the first derivative spectrum of Primaxin® at 243 and 318 nm can conveniently be used to simultaneously determine the concentrations of cilastatin and imipenem, respectively (Figs 3, 4).

Experimental

Materials

Primaxin®, a combination of imipenem (N-formimidoylthienamycin monohydrate), cilastatin sodium (the sodium salt of a derivatized heptanoic acid) and sodium bicarbonate is a marketed product of Merck & Co., Inc. Normal sodium chloride solution (0.9%) was obtained from Abbott Laboratories, 3-(N-Morpholino)-propanesulphonic acid (MOPS) was obtained from The Sigma Chemical Co., 1-hexanesulphonic acid, sodium salt was obtained from Eastman Kodak and sodium hydroxide was obtained from Fisher Scientific.

The MOPS buffer solution was prepared by adding sufficient sodium hydroxide to 0.1 M MOPS to adjust the pH to 7.0. Mobile phase for the LC analysis was prepared by mixing 800 ml of water, 40 ml of the (pH 7.0, 0.1 M) MOPS buffer, and 2.0 g of 1-hexanesulphonic acid. The pH was adjusted to 7.0 with sodium

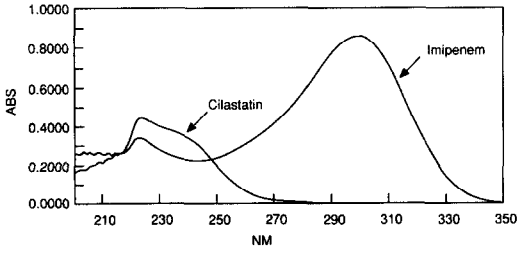

Figure 1
Ultraviolet spectra of imipenem and cilastatin from 200 to 350 nm.

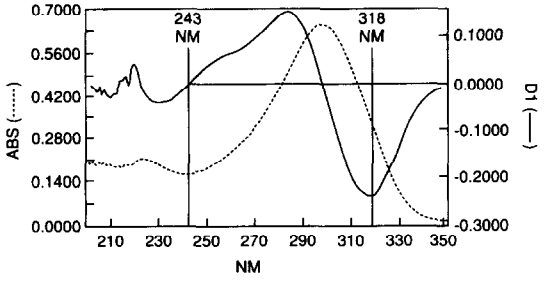

Figure 2
Imipenem UV absorbance and first derivative.

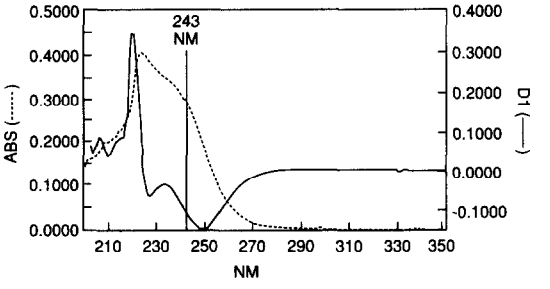

Figure 3
Cilastatin UV absorbance and first derivative.

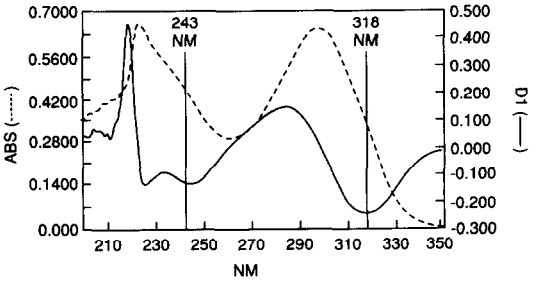

Figure 4
Imipenem/cilastatin UV absorbance and first derivative.

hydroxide and was brought to 1000 ml with water.

Stock solutions of imipenem and cilastatin sodium were prepared in MOPS buffer (25 and 30 μg ml^{-1}, respectively) for both the UV and the LC analyses.

Apparatus

Spectrophotometry

Spectrophotometric analyses were performed with a microcomputer-based Perkin–Elmer Lambda-6 UV-Visible double beam spectrophotometer equipped with an Epson printer. It was interfaced with an Epson Equity III+ Data Station (Q201A) via a standard RS232C interface for storage of spectra. The Epson printer (Model EX-800) was linked to the data station. Suitable settings are: slit width 2 nm, response time 5 s, scan speed 60 nm min^{-1} and first derivative mode.

A pair of matched 10-mm silica cuvettes suitable for the far-UV region was used in this study. A Sartorius Model R160P electronic, analytical balance was used for weighing samples.

Liquid chromatography

The LC separations were performed on a Hewlett–Packard 1090 liquid chromatograph with a LUSI controller and equipped with a Hewlett–Packard autosampler and a thermostated oven compartment. Measurements were made with a 10 μl injection volume at 50°C using a variable wavelength detector (Kratos Model 773) which was equipped with an 8-μl flow cell, an absorbance scale ranging from 0.005 to 1.25 a.u.f.s. and connected to a Hewlett–Packard Model 3390A integrator. The detector wavelength was set at 250 nm (Attenuation 32). Routine analyses were carried out isocratically on a Hewlett–Packard RP-8 (C-8) column (200 × 3.9 mm i.d.) at a flow rate of 4.0 m, min^{-1}.

Calibration

Spectrophotometric method. Imipenem and cilastatin sodium samples, weighed on the Sartorius R160P electroanalytical balance were dissolved in (pH 7.0, 0.1 M) 3-(N-Morpholino)-propanesulphonic acid (MOPS) buffer.

Linearity of cilastatin was established by preparing one series of five solutions containing a fixed concentration of imipenem (25 μg ml^{-1}) with concentrations of cilastatin ranging from 15 to 45 μg ml^{-1}. After verifying that there was no UV interference from sodium bicarbonate, the appropriate amount was also added to mimic the commercial formulation (20 mg sodium bicarbonate per 500 mg imipenem/500 mg cilastatin sodium). The first derivative spectra were recorded using the MOPS buffer solution as a blank. All solutions were measured from 200 to 350 nm. Using regression analysis the following equation was obtained for cilastatin: $y = 5.66x + 8.7 \times 10^{-4}$ ($r = 0.9998$), where y = the absolute value of the first derivative of cilastatin absorbance at 243 nm and x = the concentration of cilastatin (μg ml^{-1}). Recoveries and lack of interference (bias) from imipenem were confirmed with solutions of cilastatin alone over the same concentration range. All linearity and recovery solutions were concurrently assayed using the LC method.

Linearity of the imipenem was established by preparing another series of five solutions containing a fixed concentration of cilastatin (30 μg ml^{-1}) and sodium bicarbonate with concentrations of imipenem ranging from 14 to 42 μg ml^{-1}. The first derivative spectra were obtained between 200 and 350 nm. Using regression analysis the following equation was obtained for imipenem: $y = 11.27x + 6.2 \times 10^{-3}$ ($r = 0.9998$), where y = the absolute value of the first derivative of imipenem absorbance at 318 nm and x = the concentration of imipenem (μg ml^{-1}). Recoveries and lack of interference (bias) from cilastatin were confirmed using solutions of imipenem alone over the same concentration range. Again, all recovery and linearity solutions were concurrently assayed using the LC method.

Chromatographic method. The same solutions that were prepared for the spectrophotometric method were concurrently assayed using the LC assay. A 10-μl aliquot of each solution was injected onto the chromatographic column in duplicate. The area counts of the analytes (imipenem and cilastatin) were calculated against the corresponding concentrations to provide a comparison to the spectrophotometric method.

Determination of imipenem and cilastatin in pharmaceutical formulations

A series of nine commercial samples of Primaxin® IV formulation containing 500 mg each of dry powder imipenem and cilastatin

and 20 mg of sodium bicarbonate were dissolved in 100 ml of 0.9% sodium chloride solution. A 1.0-ml aliquot of each sample solution was transferred into a 200 ml volumetric flask and diluted to volume with 0.1 M MOPS buffer. The solutions were used to obtain both the first derivative UV spectra and the LC chromatograms concurrently with the appropriate standard solution (25 $\mu g\ ml^{-1}$ of each active). Results are shown in Table 1.

Results and Discussion

Derivative UV spectroscopy was used to develop a method for the routine release testing of Primaxin®. The UV spectrum of imipenem shows an absorbance between 200 and 350 nm. Cilastatin exhibits a UV absorbance between 200 and 270 nm (Fig. 1). For a UV analysis, imipenem can be assayed directly at its maximum at 300 nm, but in combination there is no wavelength where only cilastatin absorbs.

The UV spectrum of imipenem exhibits another maximum at 223 nm and a minimum at 243 nm (Fig. 2). At these two points the first derivative of the UV absorbance of imipenem is zero. Consequently in the combination product, any contribution to the first derivative at these wavelengths is only from cilastatin (Figs 3 and 4). Of the two wavelengths, it was decided to work at 243 nm. The trough amplitude around this minimum absorbance is shallower than is the maximum at 223 nm and any error due to instrument drift or solvent effects would be lessened. The first derivative can also be used to quantitate imipenem which exhibits a minimum at 318 nm where there is no interference from cilastatin.

The analysis for replicates in Table 1 compares assay results by derivative UV and LC for Primaxin® IV (Lot 9049R). There is excellent agreement between the two methods for both drug components of Primaxin. The LC generated data averaged 101.0% of the UV testing for imipenem and averaged 100.2% of the UV testing for cilastatin. The average of nine analyses are within 1.0% for both components. Comparable average RSD values (1–2%) for the UV and LC assays were obtained for both components. The deviations are a combination of the analytical deviation and the weight deviation of the dry powder fill in the vials. It is thus concluded that the derivative UV method is equivalent to the more time consuming LC method.

Specificity

Both imipenem and cilastatin degrade in intravenous [8] and aqueous [9, 10] solution with imipenem degrading at a much more rapid rate than cilastatin. Degradation products do not interfere with the analysis of samples in the LC method because they are separated on the LC column using the described assay conditions [9]. The stability indicating properties of the derivative UV assay were investigated by the analysis of stressed product and reconstituted product.

Samples of Primaxin® powder were stressed at 80°C for 2 weeks. Assay of these samples using both the UV and LC methods showed no

Table 1
Assay precision and method comparison

	*Imipenem (mg/vial) (First derivative at 318 nm)			*Cilastatin (mg/vial) (First derivative at 243 nm)		
	UV	LC	LC/UV (%)	UV	LC	LC/UV (%)
	529	542	102.5	529	534	100.9
	520	524	100.8	520	503	96.7
	525	540	102.9	533	528	99.1
	532	538	101.1	537	534	99.4
	511	518	101.4	533	534	100.2
	522	518	99.2	513	523	101.9
	548	543	99.1	544	544	100.0
	524	541	103.2	533	549	103.0
	521	513	98.5	533	534	100.2
Ave.	525.8	530.8	101.0	530.6	531.4	100.2
RSD	1.9%	2.3%	1.7	1.7%	2.5%	1.8

*5% overage for container withdrawal.

Table 2
Stability and method specificity

Time (h)	Imipenem mg/vial (% initial) (First derivative at 318 nm)		Cilastatin mg/vial (% initial) (First derivative at 243 nm)	
	UV	LC	UV	LC
0	526	514	533	531
2	524 (99.6)	528 (102.7)	525 (98.5)	531 (100.0)
4	511 (97.1)	506 (98.4)	528 (99.4)	534 (100.6)
6	510 (97.0)	469 (91.2)	533 (100.0)	518 (97.6)
8	501 (95.2)	486 (94.2)	533 (100.0)	507 (95.5)
24	460 (87.5)	391 (76.1)	532 (99.8)	458 (86.3)

significant degradation (less than 2% by LC). Therefore the approach of stressing Primaxin® powder to study the stability indicating properties of the UV method was abandoned.

The stability of reconstituted Primaxin® solutions was next evaluated. Samples were tested using both the UV and LC methods at regular time intervals after reconstitution. The stability samples were taken from the initial dilution of the Primaxin® vial to 100 ml resulting in a 5 mg ml^{-1} solution. The rapid degradation over several hours of the imipenem is verified by the LC assay but the apparent drop in potency is much slower for the derivative UV assay (Table 2). The results of the derivative UV assay indicate that cilastatin is stable. However, the LC method shows that cilastatin also degrades in solution although at a slower rate than imipenem.

Degradation has been shown to be concentration dependent [9]. At higher concentrations (3–30 mg ml^{-1}) decomposition was progressively more repid as a second reaction dominates. Degradation is pseudo-first-order for solutions of lower concentration (1–2 mg ml^{-1} and lower) and analytical solutions in MOPS buffer at 0.5 mg ml^{-1} (thc normal LC method concentration) have typically been tested for up to 8 h with satisfactory results using the LC method. At the assay concentration (25 μg ml^{-1} of each active) of the derivative UV assay, samples are stable and can be assayed for up to 8 h.

This data confirms that in aqueous solution Primaxin® forms UV absorbing degradates that interfere at the wavelength used for the UV assay of imipenem and cilastatin. Other studies [9, 10] carried out at pH 4, pH 9 and at pH 7 have established the identity of these UV absorbing degradates.

It can be concluded that the derivative UV assay is not appropriate for stability testing of Primaxin®. However, due to the ease and speed of the UV assay, its demonstrated equivalence to the LC assay and the demonstrated instability of Primaxin®, the UV assay would be appropriate for the routine release testing of Primaxin®.

Conclusions

A rapid, precise and accurate UV assay has been developed for Primaxin® which employs the first derivative amplitudes at a UV minimum to eliminate interference in a multicomponent drug. This method has been shown to be suitable for release testing for concentration and gives comparable results to the currently used LC assay. The method offers a considerable time saving over the LC method. This allows assay results to be calculated and verified before the assay solutions degrade, thus avoiding costly and time consuming retesting.

Acknowledgements — We would like to thank D. Haynes for performing solution assays, and Dr M. Brooks for his suggestions and guidance.

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[Received for review 21 March 1994; revised manuscript received 16 May 1994]