First-order derivative ultraviolet spectrophotometry of imipenem–cilastatin formulations

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Abstract

Objective: Imipenem–cilastatin is a well-established broad-spectrum intravenous antibiotic used for the treatment of life-threatening infections worldwide. The preparation has been studied by various methods but not by ultraviolet (UV) spectroscopy, although this technique remains one of the simplest, most accurate and precise validated quality control laboratory methods. The aim of this study was to validate a method for separating the intercalating peaks of the UV spectra of imipenem and cilastatin and to compare the purity and quantities of these two active ingredients in different marketed brands. No previous studies have been conducted to compare the generic product (Cilanem®) with the original (Tienam®).

Methods: First-order derivative UV spectrophotometry was used to separate the intercalating peaks of imipenem and cilastatin by measuring absorbance at 243 nm and 300 nm, respectively.

Results: Cilanem® had better physical characteristics and a higher cilastatin concentration, while the brand product Tienam® contained a higher concentration of imipenem.

Conclusion: The generic product Cilanem® is at least non-inferior to the brand product Tienam®.

Keywords: Cilanem; Imipenem–cilastatin; Tienam®; UV assay

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Introduction

Imipenem (N-formimidoylthienamycin monohydrate) is an off-white, non-hygroscopic crystalline derivative of thienamycin with the empirical formula $C_9H_{12}N_3O_4S\cdot H_2O$ and a relative molecular mass of 317.37 g. Its structural formula is shown in Figure 1.\textsuperscript{1,2} Cilastatin sodium is an off-white to yellowish-white, hygroscopic, amorphous compound, which is the sodium salt of a derivatized heptenoic acid. It has the empirical formula $C_{16}H_{25}N_2O_5SNa$ and a relative molecular mass of 380.43 g. Its structural formula is shown in Figure 2.\textsuperscript{1,2}

Imipenem was the first carbapenem to be developed, with a broad spectrum of activity against resistant Gram-positive, Gram-negative and anaerobic bacteria. Since it was marketed in the 1980s, it has played a central role in the treatment of severe community- and hospital-acquired infections caused by multi-drug-resistant microorganisms including *Pseudomonas aeruginosa* and β-lactamase-producing bacteria, such as penicillins and cephalosporins.\textsuperscript{5} As the mechanism of action of imipenem is similar to that of penicillins and cephalosporins, it is a potent inhibitor of bacterial cell wall synthesis and exerts a bactericidal effect against all sensitive microorganisms. Imipenem is rapidly degraded by the renal enzyme dehydropeptidase I; therefore, an inhibitor of this enzyme, cilastatin, was added to the formulation to prevent renal degradation of imipenem and prolong its action.\textsuperscript{6,7}

As there is no Government quality control laboratory in Lebanon, many drugs obtain official approval for introduction onto the Lebanese market without being subjected to robust pharmaceutical quality control testing. Much greater effort is required to ensure the quality, effectiveness and safety of most of the drugs currently available in Lebanon.

The clinical efficacy and the susceptibility and resistance of bacteria to the imipenem–cilastatin formulation have been widely studied; however, ultraviolet (UV) spectroscopy, one of the simplest, most accurate and precise validated quality control laboratory methods, has not been used for pharmaceutical quality control for a long time. The main objective of our study was to compare the quality of the two brands of imipenem–cilastatin present on the Lebanese market.

To avoid interference between the peaks of imipenem and cilastatin, which intercalate at 250–350 nm, we combined zero-order with first-order derivative UV spectroscopy.\textsuperscript{8} Published studies and international pharmacopoeia have validated use of the maximum absorbance wavelengths 243 nm and 300 nm for the assay of cilastatin and imipenem, respectively.\textsuperscript{1–4} Imipenem alone shows peak absorbance at 300 nm, while cilastatin alone does not absorb UV light above 270 nm. At a wavelength of 243 nm, imipenem shows minimum UV absorbance in the zero-order derivative and a zero trough amplitude in the first-order derivative.

Materials and Methods

Experimental

All experiments were conducted on a Genesys 10S UV–Vis spectrophotometer (Thermo Scientific\textsuperscript{TM}) with 1-cm quartz cuvettes (Hellma\textsuperscript{TM} Analytics, Germany).

The samples were donated by MSD Inc. and Ranbaxy Scientific in Lebanon. The two products were Tienam\textsuperscript{®} (MSD, Netherlands, batch no. 2048000) and Cilanem\textsuperscript{®} (Ranbaxy, India, batch no. 2233459).

The formulations, containing a powder equivalent to 500 mg imipenem monohydrate and 500 mg cilastatin sodium, were diluted in 100 ml of distilled water to obtain stock solutions of 5 mg/ml for each compound. Seven dilutions were prepared in distilled water: 5, 10, 15, 20, 25, 30 and 40 $\mu$g/ml.

UV spectrophotometry

Imipenem was assayed at 300 nm and cilastatin at 243 nm. Standard slopes (Figures 3 and 4) were constructed with the seven concentrations of each product. Each sample was tested in triplicate throughout the study.

The concentrations of active ingredients were calculated from the equation for the standard slope, $y = f(x)$, and the percentages were calculated from $\% = 100 \times (\text{calculated concentration})/(\text{theoretical concentration})$ with Tienam\textsuperscript{®} as the reference product (100%).

![Figure 1: Structural formula of imipenem.](image1)

![Figure 2: Structural formula of cilastatin.](image2)

![Figure 3: Standard slope for imipenem at 300 nm for both products.](image3)
Results

Appearance: All the vials of Tienam\textsuperscript{®} tested had powder caked onto the walls, with considerable amounts of powder stuck to the edges, a problem that was not encountered with Cilanem\textsuperscript{®}. The caking may be due to the manufacturing process, from excess humidity inside the vial or the quality of the glass or seal.

Purity: The two products showed similar purity, with peak UV absorbance at the same wavelength.

Solubility: Both products were highly soluble in water. The reconstitution time was 65 s for Tienam\textsuperscript{®} and 87 s for Cilanem\textsuperscript{®}.

The quantities of imipenem and cilastatin in each product were determined at 243 nm and 300 nm, respectively. The average absorbance values are shown in Tables 1 and 2, with calculated concentrations. The average percentages of the two active ingredients were 98.8% imipenem and 109.0% cilastatin in Cilanem\textsuperscript{®}. The quantitative assays showed that Tienam\textsuperscript{®} contains slightly more imipenem, while Cilanem\textsuperscript{®} contains slightly more cilastatin. The two products showed similar linearity at different concentrations, which was within the range 5–40 μg/ml.

Absorbance scanning at wavelengths of 200–350 nm is shown in Figure 5. First-order derivatives were determined at the same wavelengths to remove interference between the two active ingredients (Figure 6). The UV spectrum shows absorbance values over wavelengths 1 nm apart, ranging from 200 nm to 350 nm. Zero- and first-order scanning curves showed that the two products have similar spectral pathways at all wavelengths. In the zero-order derivative curve, both products showed one peak at 300 nm, which reflects the purity of imipenem. The difference in absorbance values was not significant. The highest peak was observed with Tienam\textsuperscript{®}. The first-order derivative scanning curve showed zero trough amplitude at 300 nm for both products, which corresponds to the peak on the zero-order scanning curve, and two peaks at \( \lambda_{\text{max}} \), which correspond to the inflections of the zero-order scanning curve.

Discussion

As our present study shows, first order derivative spectroscopy is of importance in the assay of Imipenem/
Cilastatin formulations to separate the intercalating UV spectrograms of the two present APIs.

The two products had comparable quality profiles, each having slight advantages in certain criteria over the other. The larger amount of cilastatin in Cilanem® may prolong the duration of action of the imipenem in the formulation.

**Conclusion**

We conclude that the generic formulation Cilanem® has an acceptable, competitive quality profile in comparison with that of the original product Tienam®.

**Conflict of interest**

The authors have no conflict of interest to declare.

**References**