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Short Communication

Imipenem in biological fluids analysed by derivative UV-spectrophotometry*

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Introduction

Imipenem (Fig. 1) is the *N*-formimidoyl derivative of thienamycin, the first member of a new class of β-lactam antibiotics, the carbapenems, having a broad-spectrum activity against aerobic and anaerobic bacteria. Because imipenem is hydrolysed by the renal brush border enzyme dehydropeptidase-I (DHP-I) [1], it is co-administered in clinical usage with cilastatin, a specific and highly active dipeptidase inhibitor, which improves the plasma concentration and markedly increases the urinary excretion of the unmetabolized drug [2]. Published studies have also employed microbiological [3, 4] and HPLC [5–8] assay methods for determining the concentration of imipenem in biological fluids. In this work a simple derivative UV-spectrophotometric method for the quantitation of imipenem in human plasma, urine and tissue is described. Derivative spectrophotometry is a well-established technique for resolution enhancement; it allows selective discrimination of sharp bands over broad in UV spectra offering an effective approach to the suppression of broad background matrix absorption [9–11].

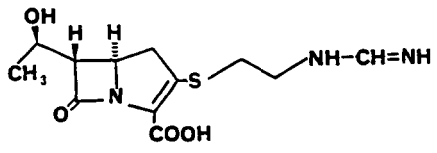

Figure 1
Chemical structure of imipenem.

Experimental

Reagents and chemicals

Imipenem or [5*R*-[5α,6α(*R**)]]-6-(1-hydroxyethyl)-3-[[2-[(iminomethyl) amino]-ethyl]thio]-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid monohydrate was supplied by Merck Sharp & Dohme Italia (Rome, Italy). Methanol (spectroscopic reagent grade) and ethylene glycol (analytical reagent grade) were purchased from Fluka Chemie (Buchs, Switzerland). 4-(2-Hydroxyethyl)piperazine-1-ethanesulphonic acid (HEPES) was purchased from Aldrich (Milan, Italy). Water was purified and deionized using a Milli-Q ion-exchange filtration system (Millipore, Bedford, MA, USA). Water was filtered through HA 0.45 μm filters, while methanol was filtered through FA 0.5 μm filters (Millipore).

Apparatus and conditions

A Perkin–Elmer UV–vis spectrophotometer Model Lambda 5 was used. Zero-order spectra: scan speed 60 nm min^{-1}; spectral slit width 2 nm. Derivative conditions: scan speed 60 nm min^{-1}; spectral slit width 2 nm; Δλ 6.

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Stock solutions

The stock solution contained 1.0 mg ml^{-1} imipenem in a HEPES–ethylene glycol solvent mixture. The solvent was prepared by mixing 5 ml of 0.5 M HEPES, pH 6.8, 2.5 ml of ethylene glycol and 2.5 ml of HPLC grade water; this buffered solution was shown to preserve imipenem unaltered for several days. A working standard was prepared daily by diluting an aliquot of the stock solution with an appropriate volume of the solvent mixture to a concentration range of 2.0–50 μg ml^{-1} when using plasma samples or 2.0–70 μg ml^{-1} when using urine samples.

The calibration curve was derived from human plasma by adding appropriate amounts of imipenem. The calibration curve for urine was prepared similarly.

Patients

A single 500 mg dose of imipenem was given, either intramuscularly or intravenously, to patients affected by benign prostatic hypertrophy and scheduled for surgery, from whom informed consent had been obtained. Plasma and urine were collected at various times afterwards and extracted for UV analysis.

Plasma samples

Heparinized blood samples from various patients were centrifuged. A 1.0 ml volume of plasma was collected and placed in a tube containing 1.0 ml of the solvent mixture described above and quickly frozen to −80°C. Samples were thawed just before the extraction procedure. An aliquot of 0.5 ml of plasma was added to 0.5 ml of methanol and mixed for 15 min. The sample was then centrifuged at 3000**g** for 10 min at 4°C and the supernatant collected. The supernatant was transferred to a semimicro cell (internal width = 4 mm) and analysed using the peak–trough amplitude between 306 and 312 nm in the third-derivative UV spectrum.

Urine samples

Urine samples were stored frozen (−80°C) until required for the assay. Samples were thawed just before the extraction procedure, which was identical to that described for plasma samples.

Tissue samples

Tissue samples were placed in tared tubes, containing 2.5 ml of HEPES–ethylene glycol buffered solvent and frozen at −80°C until required for the extraction. The weighed samples were homogenized, mixed with an equal volume of HEPES buffer without ethylene glycol and then centrifuged at 3000**g** for 5 min at 4°C. A 1.5 ml volume of methanol was added to a sample of 1.5 ml of tissue homogenate and this was mixed for 10 min. The sample was then centrifuged at 3000**g** for 5 min at 4°C and the supernatant was transferred to a semimicro cell and analysed.

Results and Discussion

Typical derivative UV spectra of the blank plasma, plasma sample, blank urine, urine sample and tissue sample are shown in Figs 2 and 3. The determination of imipenem could be carried out, in the concentration range

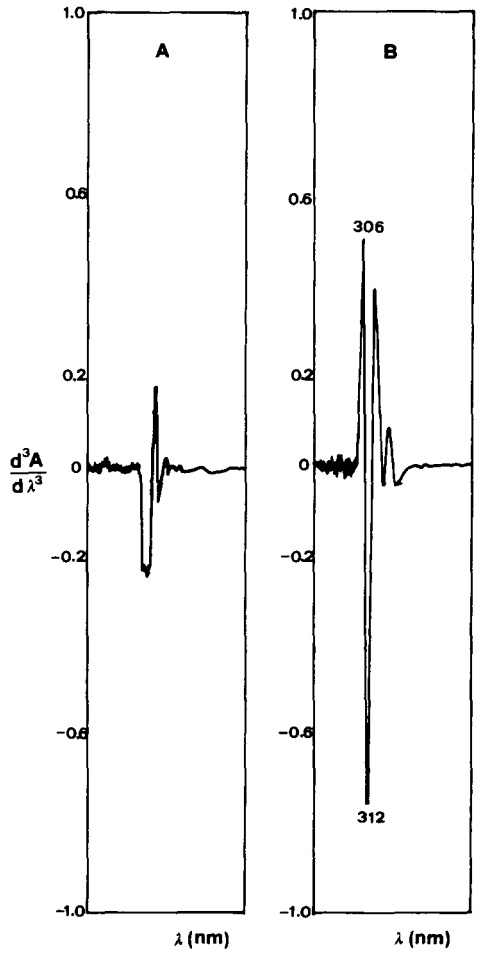

Figure 2
Third-derivative UV spectra of extracted samples of (A) blank plasma; (B) plasma of treated patient containing 4.7 μg ml^{-1} of imipenem.

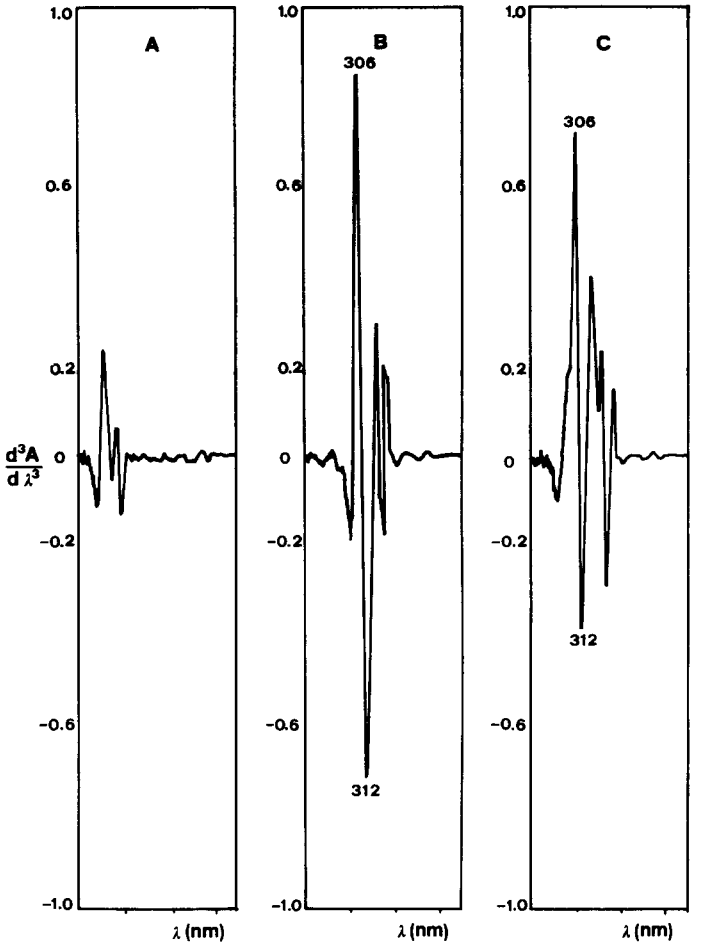

Figure 3
Third-derivative UV spectra of extracted samples of (A) blank urine; (B) urine and (C) tissue of treated patient containing 5.4 and 2.3 μg ml^{-1} of imipenem.

examined, by utilizing the peak–trough amplitude between 306 and 312 nm in the third-derivative spectrum and by using the following equations, obtained through regressional analysis of data for the standard solutions previously reported: $y = 2.7x + 4.2 \times 10^{-3}$ ($r = 0.9999$) for plasma extracted; while for urine, $y = 0.26x + 3.5 \times 10^{-3}$ ($r = 0.9998$), where y = peak–trough amplitude between 306 and 312 nm in the third-derivative spectrum, measured on the scale ±1.0 and x = concentration of imipenem (μg ml^{-1}). The data employed for the calibration curves were the average of a minimum number of five determinations for each sample. The minimum concentration of imipenem detectable by the described procedure was 3 μg ml^{-1} for plasma and 2 μg ml^{-1} for urine, respectively. The RSD of the results was approximately 3% in the concentration range examined for plasma and urine. Recovery of imipenem, calculated by comparison of peak–trough amplitude before and after the extraction, was 70% for plasma and 76% for urine, respectively. Derivative UV spectrophotometry was a suitable technique for the reliable analysis of biological fluids containing imipenem. Using the derivative spectrophotometric procedure, the residual background absorption could be suppressed and selective drug identification and estimation accomplished. This spectrophotometric method should be of value: (a) for monitoring the concentrations of imipenem in biological fluids *in vivo*; (b) for assessing the tissue concentrations of the drug and comparing them with the minimum inhibitory concentrations of relevant pathogens of each anatomical area; (c) for assessing patient compliance during the prescribed imipenem regimes; and (d) for examining the relationship between imipenem concentration in plasma and its antimicrobial effect in clinical trials.

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References

[1] H. Kropp, J.G. Sundelof, J.S. Kahan, F.M. Kahan and J. Birnbaum, *Antimicrob. Agents Chemother.* **17**, 993–1000 (1980).

[2] S.R. Norrby, K. Alestig, B. Björnegard, L.A. Burman, F. Ferber, J.L. Huber, K.H. Jones, F.M. Kahan, J.S. Kahan, H. Kropp, M.A.P. Meisinger and J.G. Sundelof, *Antimicrob. Agents Chemother.* **23**, 300–307 (1983).

[3] S.R. Norrby, K. Alestig, F. Ferber, J.L. Huber, K.H. Jones, F.M. Kahan, M.A.P. Meisinger and J.D. Rogers, *Antimicrob. Agents Chemother.* **23**, 293–299 (1983).

[4] R. Wise, I.A. Donovan, M.R. Lockley, J. Drumm and J.M. Andrews, *J. Antimicrob. Chemother.* **18**, Suppl, E93–101 (1986).

[5] D.A. Gravallese, D.G. Musson, L.T. Pauliukonis and W.F. Bayne, *J. Chromatogr.* **310**, 71–84 (1984).

[6] C.M. Myers and J.L. Blumer, *Antimicrob. Agents Chemother.* **26**, 78–81 (1984).

[7] G. Carlucci, L. Biordi, C. Vicentini and M. Bologna, *J. Pharm. Biomed. Anal.* **8**, 283–286 (1990).

[8] W.J. Ebey, B.A. Boucher and J.A. Piper, *J. Liq. Chromatogr.* **11**, 3471–3481 (1988).

[9] G. Talsky, L. Maysing and H. Kreuzer, *Angew. Chem. Int. Ed. Engl.* **17**, 785–799 (1978).

[10] A.F. Fell, *Proc. Anal. Div. Chem. Soc.* **115**, 260–267 (1978).

[11] P. Levillain and D. Fompeydie, *Analusis* **14**, 1–20 (1986).

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