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Determination of imipenem in human plasma, urine and tissue by high-performance liquid chromatography

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Abstract: A simple and reliable HPLC method is described for the new beta-lactam antibiotic imipenem; suitable extraction procedures for the drug in human plasma, urine and prostatic tissue are described. The figures of merit for the assays are reported and examples given of their application.

Keywords: *Imipenem; HPLC; biological fluids; beta-lactam antibiotics; carbapenams.*

Introduction

Imipenem or [5R-[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 is a stabilized amidine derivative or thienamycin, the first member of a new class of beta-lactam antibiotics, the carbapenems, which are characterized by their possession of a desthiocarbapenem nucleus (Fig. 1) [1–3]. Because imipenem is hydrolysed by the kidney enzyme dehydropeptidase-I, it is administered in combination with cilastatin, a specific and highly active dipeptidase inhibitor. This strategy produces improved pharmacokinetics and higher urinary levels of the drug [4, 5].

Some HPLC assays for imipenem have been recently described [6–8]. Published studies have also employed microbiological assay methods for determining the concentration of imipenem in biological fluids [9, 10].

This report describes a new, modified HPLC procedure which compares favourably with previously described methods both as regards simplicity and sensitivity, and as regards the speed of sample measurement. These features allow a rapid, sensitive and specific assay for imipenem levels in biological fluids and tissue samples needed for clinical studies of pharmacokinetics [11].

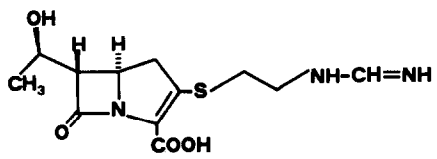

Figure 1
Chemical structure of imipenem.

Experimental

Imipenem was supplied by Merck, Sharp and Dohme Italia (Rome, Italy). HPLC-grade methanol and all other analytical grade reagents (boric acid, sodium hydroxide and ethylene glycol) were obtained from Farmitalia–Carlo Erba (Milan, Italy). 4-(2-Hydroxyethyl)piperazine-1-ethanesulphonic acid (HEPES) was purchased from Fluka (Fluka Chemie, Buchs, Switzerland). 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).

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Stock solutions

The imipenem stock solution contained 1.0 mg ml^{-1} 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. However, stock solutions were stored for not more than 2 weeks at −80°C. 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 0.5–70 μg ml^{-1} when using plasma samples, or 0.5–100 μg ml^{-1} when using urine samples.

Chromatographic system and conditions

The chromatographic apparatus (Waters Assoc. Milford, MA, USA) consisted of a model 6000A solvent delivery system, an U6K injector, fitted with a 100- or 50-μl loop for plasma and urine assays, respectively, and a Lambda Max model 481 LC-spectrophotometer connected to a model 740 Data Module integrator.

The separation was performed on an analytical column (250 × 4.6 mm, i.d.) packed with reversed-phase Ultrasphere Octyl LC-8 material (5 μm particle size; Altex Scientific, CA, USA), connected to a 2-cm Pelliguard (40-μm particle size) precolumn (Supelco Inc., Bellefonte, PA, USA).

The mobile phase consisted of a mixture of sodium borate buffer (pH 7.2)–methanol (90:10, v/v). The mobile phase was prepared daily, sonicated and filtered before use and delivered at a flow-rate of 1.2 ml min^{-1}. The column was maintained at ambient temperature and the compounds thus eluted were recorded by the detector at a constant wavelength of 313 nm. The attenuation was set at 0.01 absorbance units full scale.

Biological samples

Patients affected by benign prostatic hypertrophy and scheduled for surgery, from whom informed consent had been obtained, were given a single 500-mg dose of imipenem either intramuscularly or intravenously. Plasma and urine were collected at various time intervals afterwards and extracted for HPLC analysis. A prostatic tissue sample was also obtained from the surgery department and processed for extraction of imipenem.

Plasma samples

Heparinized blood samples from various patients were centrifuged; 1.0 ml of plasma was collected and placed in a tube containing 1.0 ml of the HEPES buffer (containing ethylene glycol) described above and quickly frozen to −80°C. Samples were thawed just before the extraction procedure and thoroughly agitated.

An aliquot of 0.5 ml of plasma was added to 0.5 ml of methanol and mixed for 15 min with constant shaking. The sample was then centrifuged at 4000 ***g*** for 10 min at 4°C and the supernatant collected.

A volume of 100 μl of the supernatant was finally injected onto the HPLC column.

Urine samples

The extraction procedure was identical to that described above for plasma samples except that the volume of methanol used was 1.0 ml and the HPLC injection volume was 50 μl.

Tissue samples

Tissue samples were placed in tubes previously weighed, containing 10 ml of HEPES-ethylene glycol buffered solvent and frozen at −80°C until extraction commenced.

The weighed samples were homogenized with a Turrax homogenizer (Janke & Kunkel GMBH Co., Staufen, Germany), mixed with an equal volume of HEPES buffer without ethylene glycol, and then centrifuged at 400 ***g*** for 10 min at 4°C. To a sample of 0.5 ml of tissue homogenate was added 0.5 ml of methanol, and this was mixed for 15 min with constant shaking.

The sample was then centrifuged at 400 ***g*** for 10 min at 4°C, and an aliquot of 100 μl of the supernatant was injected onto the HPLC column.

Results and Discussion

Chromatograms of imipenem in human urine, plasma and tissue samples are shown in Figs 2 and 3. They do not contain any interfering peak with a retention time similar to that of imipenem, for which the average retention time was 5.2 min. In these chromatograms a peak attributable to thienamycin is also evident with an average retention time of 3.8 min. Thienamycin is a metabolite of imipenem, possessing a similar biological activity.

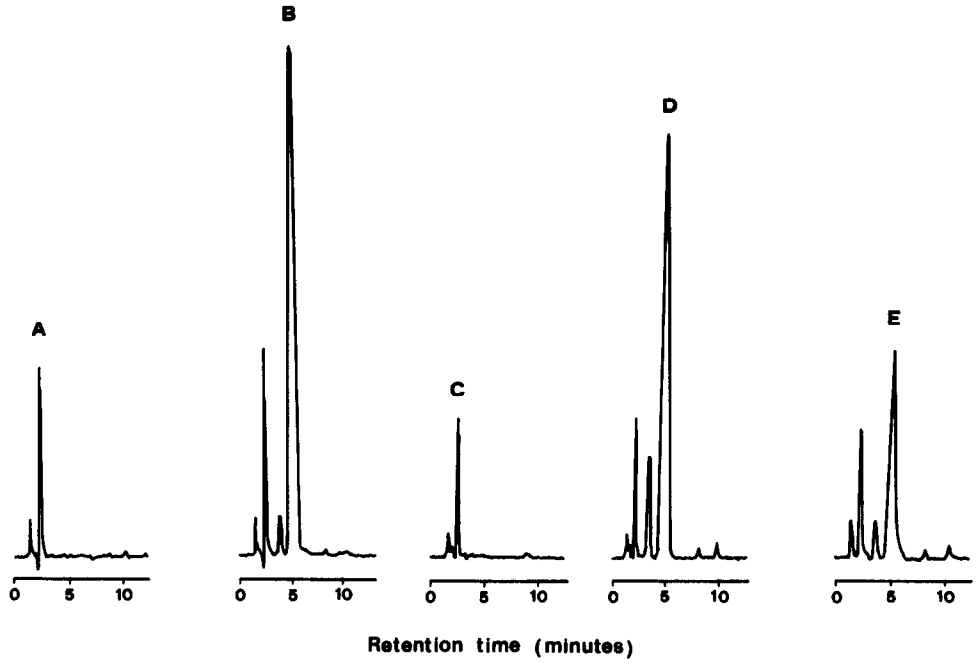

Figure 2
Typical chromatograms obtained for imipenem in urine, plasma and prostatic tissue: (A) drug-free urine extract; (B) spiked urine containing 3.0 μg ml^{-1}; (C) drug-free plasma extract; (D) spiked plasma containing 1.7 μg ml^{-1}; (E) prostatic tissue sample from a patient after a single 500-mg dose (imipenem concentration, 1.2 μg g^{-1}).

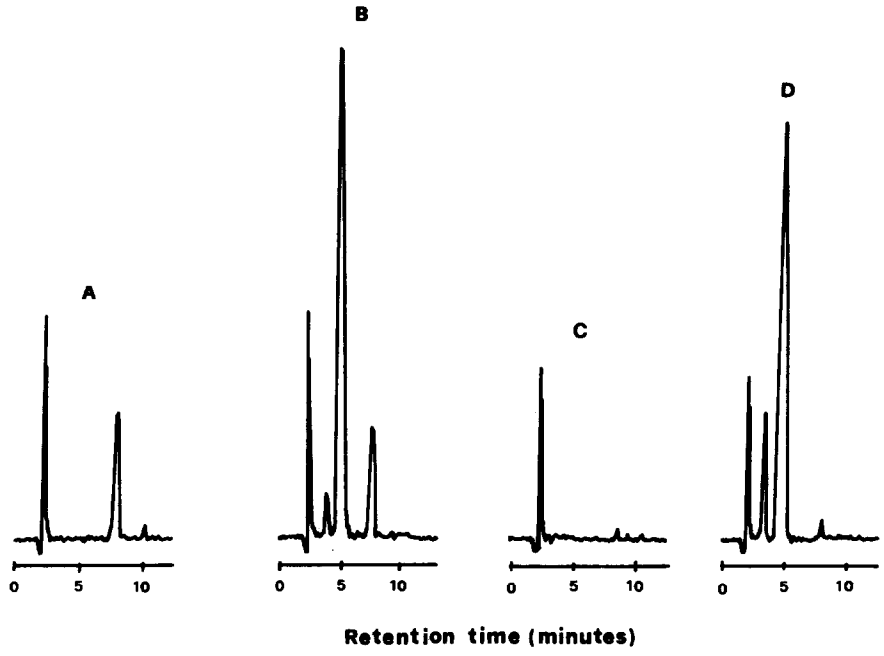

Figure 3
(A) Blank plasma; (B) plasma sample from a patient after the administration of 500 mg dose of drug; (C) blank urine; (D) urine sample from a patient after administration of 500 mg dose of drug.

The standard curve for imipenem in human plasma and urine exhibited good linearity over the ranges 0.5–70 and 0.5–100 μg ml^{-1}, respectively. The slope of the standard curve for plasma by linear regression was 0.960, the *y*-intercept being 0.073, and the correlation coefficient 0.999. The corresponding values for urine were: slope, 0.992; *y*-intercept, −0.062

Table 1
Precision and accuracy of imipenem calibration standard in human plasma and urine

Imipenem in plasma				Imipenem in urine			
Added ($\mu g\ ml^{-1}$)	Found* ($\mu g\ ml^{-1}$)	RSD (%)	Relative error (%)	Added ($\mu g\ ml^{-1}$)	Found* ($\mu g\ ml^{-1}$)	RSD (%)	Relative error (%)
0.5	0.47	3.5	4.8	0.5	0.48	3.8	3.3
1.0	0.95	3.1	3.3	1.0	0.98	2.7	2.8
5.0	4.92	2.7	4.0	5.0	4.95	1.4	2.1
10.0	9.62	2.3	3.8	10.0	9.91	2.9	2.3
20.0	19.93	2.5	3.1	20.0	19.59	2.3	2.4
30.0	28.95	1.9	3.2	30.0	29.73	2.5	3.0
50.0	48.31	1.2	3.7	50.0	49.70	3.3	2.7
70.0	69.71	2.1	3.5	70.0	69.54	3.5	2.3
				100.0	98.91	2.8	3.2

*Mean of five assays.

and correlation coefficient, 0.999. The resulting equation was used to calculate the concentrations of imipenem in the test samples.

The accuracy and precision of the calibration curves were determined from the variation of the standard from the regression line. Precision for the plasma calibration standard ranged from 1.2 to 3.5% (RSD) with relative errors of 1.4 to 3.8% (Table 1). Precision for urine calibration standard ranged from 1.4 to 3.8% (RSD) with relative errors of 2.1 to 3.3% (Table 1). For prostatic tissue the same parameters were found as for plasma.

The detection limits were 0.4 $\mu g\ ml^{-1}$ for plasma (100 μl injected) and 0.3 $\mu g\ ml^{-1}$ (50 μl injected) for urine, at a signal-to-noise ratio of 3:1. This limit is based on the extraction of 0.5 ml for both plasma and urine. Plasma samples were stable for at least 2 weeks when stored at −80°C. An internal standard was not added, due to the simplicity of the sample preparation and to the excellent precision of the data.

This new HPLC method compares favourably with previously published methods for the following reasons. It uses the same chromatographic system for all types of sample examined (plasma, urine and tissue), while other methods use different columns and mobile-phase formulations for urine and plasma [6]. Moreover, the present method shows a sensitivity comparable to that of Gravellese *et al.* [6], or better than that of Wise *et al.* [10]. In addition this is the first method which was shown to be useful for evaluating tissue concentrations of imipenem.

This simple HPLC method should be of value: (a) for monitoring the concentrations of imipenem found 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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