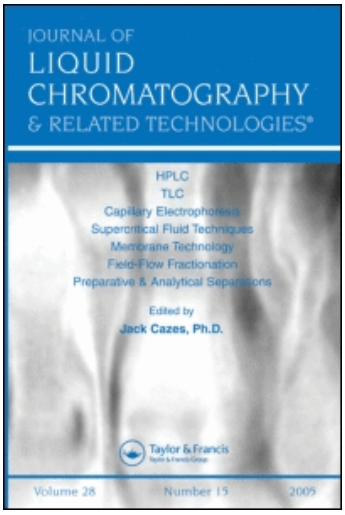

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Human Plasma and Aqueous Humor Determination of Imipenem by Liquid Chromatography with Ultraviolet Detection

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HUMAN PLASMA AND AQUEOUS HUMOR DETERMINATION OF IMIPENEM BY LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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ABSTRACT

A rapid and simple high performance liquid chromatography analytical method is described for the quantitative determination of imipenem in human plasma and aqueous humor. Following methanol extraction, the solution was chromatographed on a reverse-phase 25 cm C_{18} Viosfer (10 μm) column using a mobile phase of 0.1M Borate buffer (pH=7.2)-methanol (90:10, v/v) at a flow rate of 1.5 ml/min. The drug was detected by UV absorption at 313 nm. Total chromatographic analysis time was 15 min. The response was linear from 0.5 to 70 μg/ml for plasma and 0.2 to 5 μg/ml for aqueous humor and the detection limits were 0.4μg/ml for human plasma samples and 0.15 μg/ml for aqueous humor samples, respectively. The method has been applied to the analysis of patients undergoing imipenem therapy and scheduled for cataract surgery.

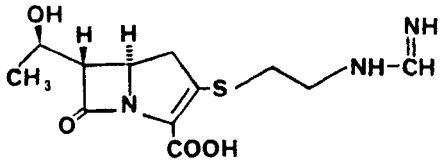

Figure 1. Structure of imipenem.

INTRODUCTION

Imipenem is a beta-lactam antibiotic with a carbapenem nucleus (Figure 1). It has excellent activity against aerobic and anaerobic gram-positive and gram-negative bacteria [1-3]. In vivo the drug is rapidly metabolized by the renal brush border enzyme dehydropeptidase-I (DHP-I). It is therefore co-administered with the DHP-I inhibitor, cilastatin, which results in therapeutic concentrations in the urine [4]. Cilastatin also protects against the potential for renal tubular toxicity observed in experimental animals [5]. Several pharmacokinetic studies of imipenem in normal volunteers [6-8] and in patients [9] have been conducted. Several analytical methods [10-12] have been developed for the quantitative determination of imipenem in human plasma. The reported microbiological assay method [13] seems to be unsuitable for routine analysis because it is time-consuming, and shows a sensitivity which is not sufficient for studies with several biological materials. Therefore, we developed a high performence liquid chromatographic (HPLC) method to determine imipenem in human plasma and aqueous humor.

EXPERIMENTAL

Chemicals

Imipenem or [5R-[5α,6α(R*)]]-6-(1-hydroxyethyl)-3-[[2-[(imino-methyl)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). 4-(2-Hydroxyethyl)piperazine-1-ethanesulphonic acid (HEPES) was purchased from Aldrich (Aldrich Chimica, Milan, Italy). HPLC-grade methanol was purchased from Fluka (Fluka, Buchs, Switzerland). Boric acid, sodium hydroxide and ethylene glycol (all analytical grade reagents) were purchased from Farmitalia Carlo Erba (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).

Standard solutions

Stock solutions (1.0 mg/ml) of imipenem was prepared in a HEPES-ethylene glycol solvent mixture. The solvent was prepared by mixing 5 ml of 0.5M HEPES, pH=6.8, 2.5 ml of ethylene glycol and 2.5ml 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. Working solutions of appropiate concentrations were made by diluting the stock solution of imipenem in the solvent mixture.

Instrumentation and chromatographic conditions

The HPLC instrumentation consisted of a 510 solvent delivery system (Waters Associates, Milford, MA, USA), a UV detector, Lambda Max Model 481 spectrophotometer (Waters) connected to a CC-12 Computing integrator (Perkin-Elmer, Rome, Italy). A Rheodyne Model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 50 μl loop was used. The chromatographic separations were performed at ambient temperature on a Viosfer octadecyl (250 x 4.6 mm i.d.) column (Violet, Rome, Italy) using a mobile phase of 0.1M Borate buffer (pH=7.2)-methanol (90:10, v/v) at a flow-rate of 1.5 ml/min. The HPLC column was preceded by a guard column (Upchurch Scientific) packed with Pellicular ODS, 37-53μm. The UV wavelength was set at 313 nm. All analyses were done at ambient temperature (20°C).

Biological samples

Patients affected by cataract and scheduled for surgery, from whom informed consent had been obtained, were given two 500-mg dose of imipenem at different intervals intramuscularly or intravenously. Plasma was collected at various time intervals afterwards and extracted for HPLC analysis. An aqueous humor sample was also obtained from the surgery department at the same time as one of the plasma samples and was 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 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 4000 **g** for 10 min at 4°C and the supernatant collected. A volume of 50 μl of the supernatant was finally injected onto the HPLC column.

Aqueous humor samples

The extraction procedure was identical to that described above for plasma samples.

Calibration curves

The calibration curve standards were prepared by adding known amounts of imipenem to blank plasma and aqueous humor to provide various concentrations, 0.5-70μg/ml in plasma samples and 0.2 - 5.0 μg/ml in aqueous humor samples. The calibration curves were constructed by plotting the peak-height of imipenem against the concentrations of imipenem. The concentrations of unknown samples were calculated from the calibration curves.

RESULTS AND DISCUSSION

Representative chromatograms obtained from plasma and aqueous humor samples are shown in fig 2 and fig. 3, respectively. The

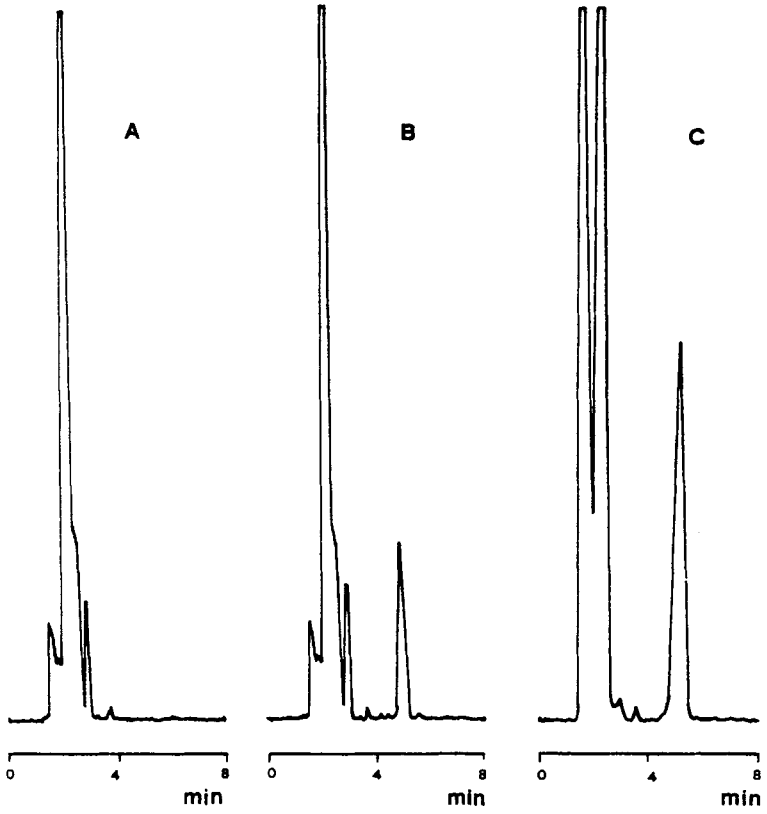

Figure 2. Chromatograms from human plasma extracts: (A) A blank plasma; (B) a blank plasma spiked with 2.0 μg/ml of imipenem; (C) a plasma sample collected 2 h after a 500mg dose of imipenem. All concentrations refer to sample extracts. Horizontal axis, retention time (min).

retention time of imipenem was 5.0 min. No peak derived from endogenous substances in plasma or aqueous humor interfered significantly with the detection of imipenem. The calibration curves for imipenem in human plasma and aqueous humor were linear over

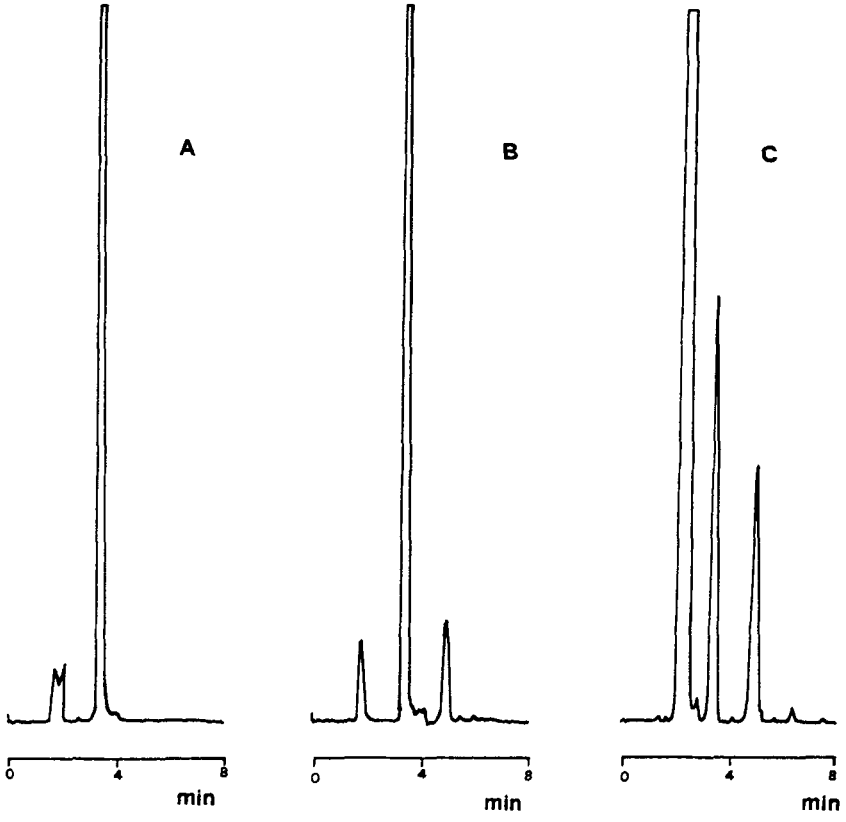

Figure 3. Chromatograms from aqueous humor extracts. (A) A blank aqueous humor; (B) a blank aqueous humor spiked with 1.0 μg/ml of imipenem; (C) a aqueous humor sample collected 1 h after a dose of 500mg of imipenem. All concentrations refer to sample extracts. Horizontal axis, retention time (min).

the range 0.5-70μg/ml and 0.2 - 5.0 μg/ml, respectively. The correlation coefficients of the calibration curves for plasma and aqueous humor were 0.998 and 0.999, respectively. The detection limits (signal to-noise ratio of 3) for imipenem were 0.4μg/ml in

TABLE 1

Within-day precision and accuracy in the calibration standard of imipenem in human plasma.

Theoretical concentration (μg/ml)	Measured concentration* (μg/ml)	CV (%)	Relative error (%)
0.5	0.49±0.02	4.1	2.0
1.0	0.97±0.04	4.1	3.1
5.0	4.95±0.05	1.1	1.0
10	9.78±0.30	3.0	2.2
20	19.50±0.70	3.6	2.5
50	49.15±1.50	3.0	1.7
70	68.84±2.30	3.3	1.7

* Mean of five assays ± S.D.

plasma and 0.15μg/ml in aqueous humor. Reproducibilities for both within-day assay and between-day assay were evaluated to assess the precision and accuracy of this analytical method. The results are shown in Tables 1-4. The coefficients of variation (C.V.) of the five indipendent samples at each concentration in the within-day assay were between 1.1 and 4.1% (Table 1) for plasma samples in the concentration range 0.5-70μg/ml, and between 1.8 and 5.5% (Table 3) for aqueous humor samples in the concentration range 0.2 - 5 μg/ml. The C.V. values in the between-day assay were 1.8 - 4.2% (Table 2) for human plasma samples and 2.1 - 5.2% (Table 4) for aqueous humor samples in the same concentration ranges. The

TABLE 2

Between-day precison in the determination of imipenem in plasma.

Theoretical concentration (μg/ml)	Measured concentration* (μg/ml)	C.V. (%)	Relative Error (%)
0.5	0.47±0.03	6.3	6.3
1.0	0.94±0.04	4.2	6.3
5.0	4.88±0.09	1.8	2.4
10	9.67±0.27	2.8	3.4
20	19.28±0.64	3.3	3.7
50	48.94±1.46	2.9	2.1
70	67.95±2.00	2.9	3.0

*Mean of five assays ± S.D.

Table 3

Within-day precison and accuracy in the calibration standard of imipenem in aqueous humor.

Theoretical concentration (μg/ml)	Measured concentration* (μg/ml)	CV (%)	Relative error (%)
0.2	0.19±0.01	5.5	5.2
0.3	0.29±0.01	3.4	3.4
0.5	0.48±0.02	4.1	4.1
1.0	0.96±0.03	3.1	4.1
2.0	1.90±0.05	2.6	5.2
3.0	2.88±0.06	2.1	4.2
5.0	4.88±0.09	1.8	2.4

*Mean of five assays ± S.D.

TABLE 4

Between-day precision in the calibration standard of imipenem in aqueous humor.

Theoretical concentration (μg/ml)	Measured concentration* (μg/ml)	CV (%)	Relative error (%)
0.2	0.19±0.010	5.2	5.2
0.3	0.29±0.014	4.8	3.4
0.5	0.48±0.019	3.9	4.1
1.0	0.95±0.028	2.9	5.2
2.0	1.94±0.060	3.0	3.0
3.0	2.86±0.080	2.8	4.8
5.0	4.84±0.100	2.1	3.3

*Mean of five assays ± S.D.

accuracy was determined by comparing the nominal concentrations of imipenem with those observed. The relative error in the within-day assay ranged from 4.6 to 2.9% for human plasma samples and 1.9 to 2.2% for aqueous humor samples. In conclusion, the present HPLC method is sufficiently sensitive, rapid and simple. It has proved to be accurate and precise and is thus useful for monitoring imipenem in pharmacokinetic studies and in clinical therapy.

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