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Determination of imipenem in plasma by high-performance liquid chromatography for pharmacokinetic studies in patients

L. Garcia-Capdevila, C. López-Calull*, C. Arroyo, M.A. Moral, M.A. Mangues, J. Bonal

Hospital Sta. Creu i St. Pau, Pharmacy Department, Pharmacokinetic Laboratory, Sant Antoni Mª Claret 167, 08025 Barcelona, Spain

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Abstract

A rapid and simple HPLC method is described for the determination of imipenem in human plasma. After blood collection, plasma was separated by centrifugation and immediately stabilized with 3-morpholinopropanesulfonic acid (MOPS) and ethylene glycol solution (1:1). The sample preparation, before injection into HPLC, was ultrafiltration. The mobile phase was boric acid buffer. The imipenem was detected at 300 nm and cilastatin sodium, coadministered, did not interfere. Calibration curves in human plasma were linear from 0.1 to 100 μg/ml. The limit of detection was 0.030 μg/ml. Inter-day precision at 0.1 μg/ml, determined as the coefficient of variation, was 6.26%. Only 250 μl of plasma was required in our assay. Due to the limited stability of imipenem [G.B. Smith et al., J. Pharm. Sci., 79 (1990) 732], stability studies in plasma were done to establish appropriate storage conditions. The assay was applied to pharmacokinetic studies in patients.

Keywords: Imipenem

1. Introduction

Imipenem (*N*-formimidoyl thienamycin) is a ß-lactam antibiotic with a broad spectrum, derived from *Streptomices Cattleya*. Imipenem is metabolized in the kidney by the renal dipeptidase, dehydropeptidase-I. Coadministration of imipenem with the dehydropeptidase inhibitor, cilastatin sodium, increases urinary recovery of the antibiotic. The efficacy of ß-lactamic therapy is related to the length of time that antibiotic levels are above the minimum inhibitory concentration (MIC) [2,3]. Pharmacokinetic studies can help to establish the optimal dosage regimens in specific populations.

Several methods have been described for determination of imipenem in plasma, including HPLC using ultrafiltration [4–7] and microbiological methods [4]. HPLC methods are specific while microbiological assays can not differentiate imipenem from other antibiotics which could be coadministered. The lowest limit of detection reported [4,5] is 0.3 μg/ml, using ultrafiltration and injection volumes of 50 μl or more. The method described in this paper is an HPLC method which also uses ultrafiltration, and gives a low limit of detection with a small injection volume.

2. Experimental

2.1. Reagents and materials

Imipenem standard was provided by Merck, Sharp and Dohme (West Point, PA, USA). 3-Morpholino-

*Corresponding author.

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propanesulfonic acid (MOPS) and ethylene glycol were provided by Fluka (Buchs, Switzerland). Boric acid (GR) and sodium hydroxide (GR) were supplied by Merck (Darmstadt, Germany). HPLC-grade water was provided by Promochem (Wesel, Germany). Ultrafree MC filter units were supplied by Millipore (Bedford, MA, USA). Membrane filters 0.45 μm were provided by Millipore (Cork, Ireland).

2.2. Apparatus

The HPLC system consisted of Waters instruments equipped with a computer system for acquisition and integration of data (Maxima 820 chromatography data station), a 510 pump, a 717 injector autosampler, a 486 UV detector and a Nova Pak C_{18} reversed-phase (4 μm, 150×3.9 mm) column.

2.3. Chromatographic conditions

The mobile phase consisting of 0.2 *M* borate buffer, pH 7.2. It was prepared with 12.4 g boric acid dissolved in 1000 ml of water. The pH was adjusted by adding 1 *M* sodium hydroxide. The solution was filtered and degassed by vacuum filtering through a 0.45-μm membrane filter. Pump flow-rate was 1.0 ml/min. The peak of imipenem was detected by ultraviolet absorbance at 300 nm. The injection volume was 10 μl.

2.4. Drug standards

The stabilizing solution was 0.5 *M* MOPS–water–ethylene glycol (2:1:1). Working stock solutions of imipenem were prepared each day as required in stabilizing solution at a concentration of 1 mg/ml. To test the suitability of the system, a chromatographic control of imipenem was prepared by dilution of the stock solution (1:100) in stabilizing solution. Plasma standards from the stock solutions were prepared using drug-free plasma to obtain final concentrations of 0.1, 0.5, 1.0, 5.0, 10, 50 and 100 μg/ml. An equal volume of stabilizing solution was then added to each standard plasma, mixed and divided into 500-μl aliquots for HPLC assay.

2.5. Plasma samples

Venous blood samples were added to the heparinized Vacutainer tube (Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France). Blood samples were centrifuged at 1000 *g* for 10 min, not later than 15 min after collection. An equal volume of stabilizing solution was added to each plasma sample, vortexed and separated into 500-μl aliquots. All samples were prepared in duplicate and stored at −40°C until analysis.

2.6. Analytical procedure

Aliquots of the stabilized plasma samples and standards were subjected to ultrafiltration, using ultrafree MC units, for 10 min at 6000 *g*. Volumes of 10 μl of filtrates were injected into the HPLC system for analysis. Plasma standard samples (0.1, 0.5, 1.0, 5.0, 10, 50 and 100 μg/ml) were prepared in sextuplicate or triplicate, and were analyzed on 3 separate days during method validation. Revalidation was assessed from the duplicate standard curves made on days when patients' samples were analyzed. Linearity of standard curves, intra- and inter-assay precision and accuracy were determined from these data. The limits of detection (LOD) and quantitation (LOQ) of imipenem were determined from the peak and the standard deviation of the noise level, S_N. The LOD and LOQ were defined as sample concentration of imipenem resulting in peak heights of 3 and 10 times S_N, respectively. The recovery for imipenem by ultrafiltration was determined by comparing the peak heights from processed plasma standard samples to those from imipenem standards in stabilizing solution.

2.7. Stability

The stability of imipenem in plasma was investigated. Plasma samples were stored with stabilizing solution 1:1 at −40°C. A standard of 10 μg/ml was prepared by spiking the appropriate volume of imipenem stock solution in drug-free plasma. An equal volume of stabilizing solution was added, vortexed, separated into 500-μl aliquots and frozen at −40°C. The imipenem concentration was determined in duplicate at 0, 6 and 15 days. The stability

of three plasma samples from a patient at different concentrations (33.3, 19.7, and 9.7 μg/ml) was also analyzed. Aliquots of each sample were analyzed at 0, 3, 10, 15 and 30 days after collection.

2.8. Study protocol

Adult patients with signs of severe infections caused by imipenem-sensitive microorganisms were included in the study. Serial blood samples were obtained during a dose interval for each patient, the first, immediately prior to administration of the dose, the second following the infusion (30 min) and the others at 0.25, 0.75, 1, 1.5, 2.5, 3.5, 4.5 and 5.5 h after infusion was completed.

3. Results

3.1. Chromatography

Typical chromatograms of a blank, spiked plasma and patient sample are shown in Fig. 1. No other interfering peaks were observed. The retention time for imipenem was 4.44 min ±0.53 min ($n=12$).

3.2. Method validation

The linearity between 0.1 and 100 μg/ml was determined by linear regression of log peak-height versus log concentration as the accuracy of the calculated standards is better than direct linear regression, which also showed good linearity ($r=0.9997$). The linear regression, $\ln(\text{conc.})=a+b\ln(\text{peak height})$, was established directly in a Maxima 820 computer system. Results of linearity are shown in Table 1. The precision and accuracy for each standard concentration were calculated inter-day and intra-day. Results of validation and revalidation are shown in Table 2.

3.3. Limits of detection and quantitation

The limits of detection and quantitation were 0.03 and 0.08 μg/ml, respectively, using a 10-μl injection volume.

3.4. Recovery

The losses of imipenem due to adsorption to the ultrafilter processing were determined by comparing peak heights of standards in stabilizer at the same concentration. Triplicate samples were processed for each concentration. Results from this recovery study are given in Table 3. There were no statistical differences between the concentrations studied (0.1, 1.0, 5.0, 50 and 100 μg/ml). Recovery of above 100% can be explained by drug concentration. The final volume sample was approximately 1:10 concentrate.

3.5. Stability

Concentrations of 10 μg/ml standard found at 6 and 15 days were 100.7 and 95.4% of the initial concentration, respectively. No stability differences were observed between concentrations of patient samples assayed. Results of the stability study showed that imipenem losses were <10% for at least 15 days at −40°C. All plasma samples were processed within 10 days after collection.

3.6. Pharmacokinetic results

This analytical method was applied to quantify plasma imipenem concentrations for clinical pharmacokinetic studies. A complete steady-state patient curve after administration of 1.0 g Tienam^{R} i.v. every 6 h is shown in Fig. 2.

4. Discussion

In the method reported here, no internal standard is needed. No advantages were observed with 5-methoxyindole-3-acetic acid (MIAA) [4,6] or other products tested (Resorcin, 3-aminophenol and 4-methylaminophenol) as internal standard. On the contrary, lower recoveries for imipenem were obtained.

The ultrafiltration technique is a direct method of sample preparation which gives good accuracy and excellent precision. In addition, our method achieves a better limit of detection than other methods.

The usual concept of recovery in liquid–liquid

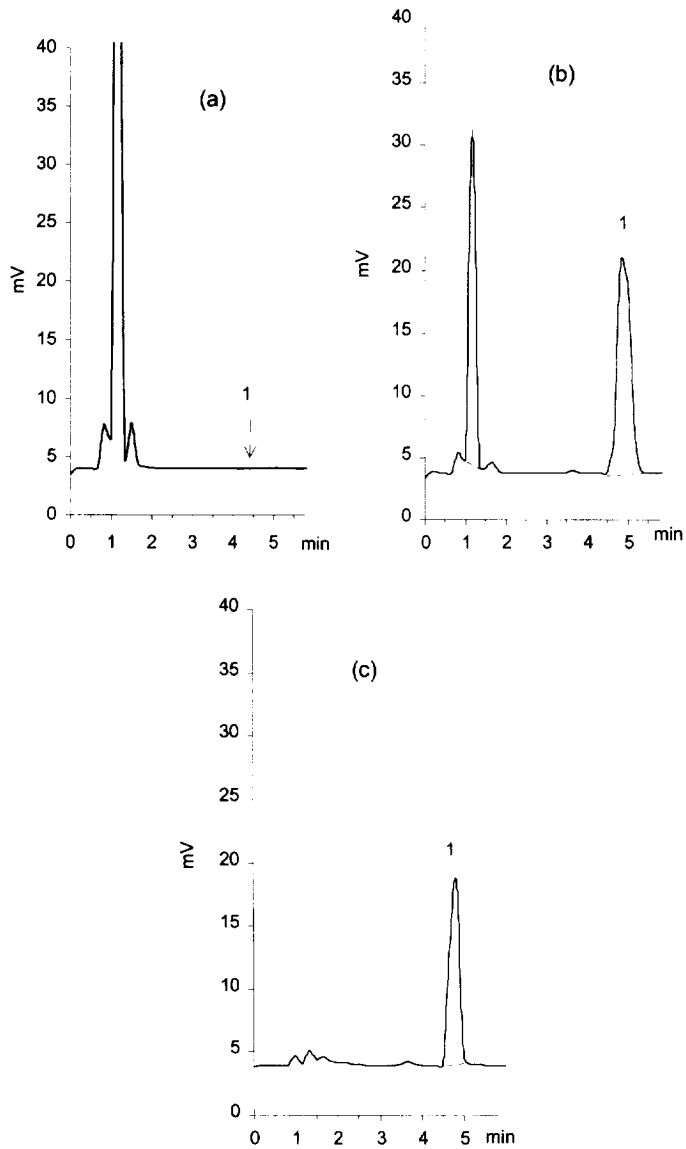

Fig. 1. (a) Chromatogram of blank pooled human plasma. (b) Chromatogram of pooled human plasma spiked with imipenem at a concentration of 50 μg/ml (peak 1=imipenem). (c) Chromatogram of a patient plasma sample at the end of the infusion (peak 1=imipenem).

Table 1
Results of linearity

	Mean	S.D.	C.V. (%)	n
r^2	0.9995	$3.4 \cdot 10^{-4}$	0.03	3
a	3.626	0.055	1.53	3
b	0.9825	$2.55 \cdot 10^{-3}$	0.26	3

S.D.=standard deviation. C.V.%=relative standard deviation. a=y-intercept. b=curve slope.

extraction, can not be applied in the ultrafiltration procedure, as the recovery in this case not only expresses the drug loss, but also the sample concentration. We assume that our method involves sample concentration due to ultrafiltration but the validation method takes this into account.

The protein content of a particular sample has no influence on the final quantitative measurement of

Table 2
Validation of the analytical method

Concentration added (μg/ml)	Concentration found (mean±S.D.) (μg/ml)	C.V. (%)	Accuracy (%)
Intra-assay (n=6)			
0.1	$0.101 \pm 4.0 \cdot 10^{-3}$	3.93	0.75
0.5	0.501±0.016	3.29	0.13
1.0	0.982±0.012	1.18	−1.80
5.0	5.175±0.042	0.81	3.49
10.0	9.559±0.148	1.55	−4.41
50.0	52.69±0.463	0.88	5.37
100.0	96.83±0.734	0.76	−3.17
Inter-assay (n=12)			
0.1	$0.103 \pm 6.4 \cdot 10^{-3}$	6.26	2.86
0.5	0.496±0.015	3.13	−0.43
1.0	0.970±0.033	3.41	−3.04
5.0	5.139±0.061	1.19	2.77
10.0	9.623±0.148	1.54	−3.77
50.0	52.02±0.800	1.54	4.04
100.0	98.58±1.935	1.96	−1.42
Revalidation (n=18)			
0.1	$0.1020 \pm 8.83 \cdot 10^{-3}$	8.66	2.03
0.5	0.4953±0.0175	3.53	−0.93
1.0	0.9780±0.0390	3.99	−2.20
5.0	5.1159±0.0957	1.87	2.32
10.0	9.8007±0.363	3.70	−1.99
50.0	51.3032±2.351	4.58	2.61
100.0	98.656±1.813	1.84	−1.34

S.D.=standard deviation. C.V.%=coefficient of variation.

imipenem, as is evidenced by the results found with standards (50 μg/ml) prepared from plasma of ten different healthy subjects (coefficient of variation of concentration found was 3.7%).

There is a considerable controversy regarding the stability of imipenem (see, e.g., Ref. [1]) in plasma samples. Gravallese et al. [4] reported that imipenem in plasma was stable for more than 42 days, using 4-morpholineethanesulfonic acid (MES) as stabilizer. No significant differences between storage at −20°C or at −70°C were observed. According to Myers et al. [5], imipenem in buffered plasma was completely stable at −70°C over the 90-day study period. However, during the same period at −20°C and 4°C, peak areas decreased more than 90%. Using MES

Table 3
Results of recovery study

Concentration of imipenem (μg/ml)	Recovery (n=3) (%)
0.1	142.9±12.1
1.0	144.3±1.1
5.0	148.1±2.7
50.0	146.5±0.7
100.0	139.6±0.2

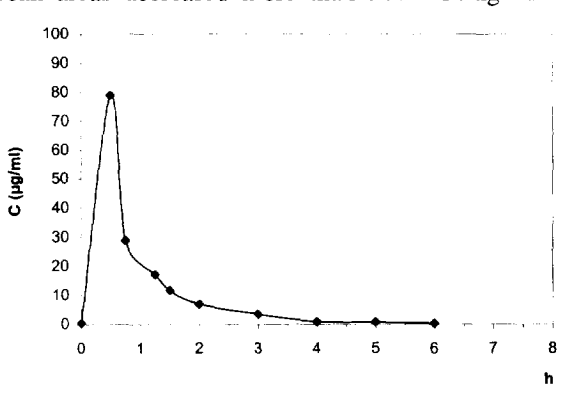

Fig. 2. Plasma imipenem concentrations versus time profile in a patient after administration of a 1.0 g i.v. infusion every 6 h.

and ethylene glycol showed a 15% decrease over 90 days at −20°C. Carlucci et al. [7], who used 4-(2-hydroxyethyl)piperazine-ethanesulfonic acid (HEPES) as stabilizer buffer, reported that imipenem was stable in plasma for at least 2 weeks when stored at −80°C. Results from our stability study show that imipenem in plasma samples, stabilized with MOPS, is stable for more than 15 days at −40°C.

Our method requires only 250 μl of plasma samples, which is a clear advantage for pharmacokinetic studies in critically ill patients and children.

This paper describes a sensitive, specific, rapid and robust reversed-phase HPLC method with ultraviolet detection for determination of imipenem in human plasma. This method has been demonstrated to be suitable for use in pharmacokinetic studies of imipenem.

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