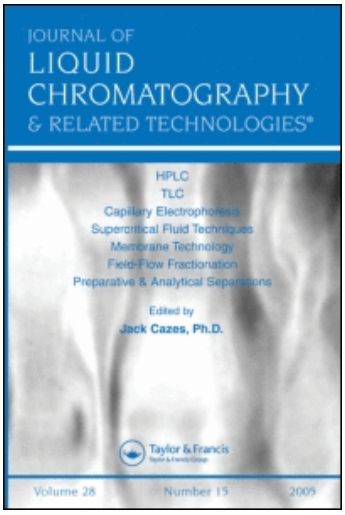

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ebey, Walter J. , Boucher, Bradley A. and Pieper, John A.(1988) 'A Rapid HPLC Method for Determination of Imipenem in Plasma', Journal of Liquid Chromatography & Related Technologies, 11: 16, 3471 — 3481
To link to this Article: DOI: 10.1080/01483918808082269
URL: http://dx.doi.org/10.1080/01483918808082269

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(16), 3471-3481 (1988)

A RAPID HPLC METHOD FOR DETERMINATION OF IMIPENEM IN PLASMA

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ABSTRACT

A high performance liquid chromatographic method was developed for the determination of imipenem concentrations in plasma. This method involves stabilization of plasma with a mixture of 2-(N-morpholine)-ethanesulfonic acid and ethylene glycol (1:1). Samples are ultrafiltered using a membrane separation system and the ultrafiltrate injected directly onto a octyldecyl column. Chromatography is performed in the reverse-phase mode with a mobile phase of acetonitrile-phosphate buffer-triethylamine (pH 7.0). The lower limit of sensitivity was 1mcg/ml using UV detection at 300nm. Recovery and reproducibility results, and interferences from other therapeutic agents are presented and discussed. The assay procedure is applied to estimate pharmacokinetic parameters of imipenem in a thermally injured patient.

INTRODUCTION

Imipenem is the N-formimidoyl derivative of thienamycin (see figure 1). It is the first carbapenem antibiotic approved for use in the United States. Imipenem is a potent antimicrobial with a very broad spectrum of activity including gram negative and gram positive aerobic bacteria, and anaerobic bacteria (1). It is used for the treatment of a variety of serious infections.

Several pharmacokinetic studies of imipenem in normal volunteers (2-4) and in patients (5) have been conducted. Imipenem is primarily renally excreted and when administered alone undergoes extensive metabolism by the renal enzyme dehydropeptidase-I (6). In order to provide increased urinary concentrations of imipenem, it is formulated as a fixed combination with cilastatin, a renal dipeptidase inhibitor (Primaxin^{R}). The elimination half-life of imipenem is approximately 55 to 60 minutes in volunteers with normal renal function (2,3). It distributes readily into a variety of body fluids and tissues with a mean steady-state volume of distribution of 0.23l/kg in normal volunteers (3). Mean peak plasma concentration of imipenem following a single intravenous 500mg dose range from 30 to 35mcg/ml (1).

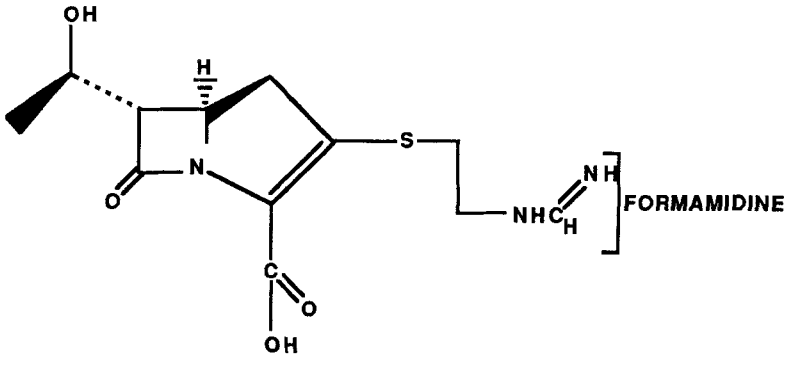

FIGURE 1. Structure of imipenem (N-formimidoylthienamycin).

Various disease states may significantly alter the disposition of drugs and thereby affect their efficacy and toxicity. Pharmacokinetic studies in patients with thermal injury, for example, have demonstrated changes in the disposition of aminoglycosides (7), vancomycin (8), diazepam (9), and cimetidine (10). The pharmacokinetic profile of imipenem has not been examined in this patient population. This information is essential for designing optimal drug regimens of this antibiotic in these patients. In order to conduct research of this nature, it is necessary to have a specific, reliable method of quantitation of imipenem plasma concentrations.

A simple high performance liquid chromatographic technique is described for the quantitation of imipenem in plasma. This technique is a modification of previously described assays for the antibiotic (11-13). These modifications were incorporated to optimize the analyical procedure rendering it more efficient and reproducible in our hands. The primary alterations from earlier assays are changes in the composition of the mobile phase and elimination of an internal standard because of the direct method of sample preparation as suggested by Myers and Blumer (12). The assay was used to describe the concentration-time profile of imipenem in a patient with a burn wound infection. The wide variety and number of concurrent medications used in this patient and other critically ill patients presents a challenge for any analytical method. This assay could readily be used for parallel pharmacokinetic studies in other patient populations. Extension of this technique to clinical monitoring of imipenem concentrations is also feasible.

MATERIALS

Instrumentation

The HPLC system consisted of a constaMetric III metering pump (LDC/Milton Roy, Riviera Beach, FL) fitted with a six-port

rotary injection valve (Model 7125, Rheodyne, Cotati, CA) and a 50µl capillary loop; a spectroMonitor II variable wavelength UV detector (LDC/Milton Roy) operated at 300nm; a CI-10B integrator (LDC/Milton Roy); and an octyldecyl 10µm silica column, 3.9x300mm (Waters µBondapack, Milford MA. All separations were performed isocratically at a flow rate of 1.0ml/min (1000psi) with the column maintained at ambient temperature.

Chemicals and Reagents

Potassium phosphate monobasic, phosphoric acid (Fisher Scientific, Fairlawn, NJ), 2-(N-morpholine)-ethanesulfonic acid (MES), ethylene glycol (Sigma Chemical Co., St. Louis, MO) and triethylamine (Aldrich Chemical Co., Milwaukee, WI) were reagent grade. Acetonitrile was HPLC grade (Burdick and Jackson, Muskegon, WI). Analytical grade imipenem (N-formimidoyl thienamycin monohydrate) was obtained from Merck, Sharp and Dohme Research Lab (Rahway, NJ). All water used in the HPLC system and in the preparation of solutions was deionized and passed through a 0.22µm filter (Milli-Q System, Millipore Corp., Bedford MA).

Mobile Phase

Potassium phosphate monobasic and triethylamine were added to water to produce a 0.01M and 0.25% solution, respectively. The pH was adjusted to 7.00 with 2.0M phosphoric acid, filtered and degassed. The mobile phase consisted of 2% acetonitrile and 98% phosphate buffer/triethylamine solution.

Stabilizing and Stock Solutions

A stabilizing solution of MES buffer and ethylene glycol (1:1) was used in preparation of all standards, unknowns, and patient samples as recommended by Gravallese et. al. (11). This solution provides stability for imipenem at a near neutral pH for at least 37 days at -70°C (11). The MES buffer was prepared by

adding 48.55g MES to 175ml of water. The pH was adjusted to 6.0 with 10M NaOH and the solution diluted to 250ml with water. A stock solution was prepared by dissolving 10mg of imipenem in 100ml of the MES/ethylene glycol stabilizing solution.

METHODS

Validation Procedure

The performance of the assay was assessed on ten different days to validate between-day reproducibility. This consisted of analyzing: 1)a daily standard curve in plasma (blank, 1, 2, 3, 10, 50, 100mcg/ml), and 2) ten replicate validation samples at 1, 10, 100mcg/ml. Within-day reproducibility was assessed by analyzing a standard curve in plasma and seven replicate validation samples at each concentration. Precision of the method was measured by determining the standard deviation and coefficient of variation on validation samples between-day and within-day. Accuracy was evaluated by comparing measured values to the known values of 1, 10 and 100mcg/ml.

Preparation of Standards and Unknown Samples

Aliquots of the imipenem stock solution and the MES/ethylene glycol stabilizing solution (500µl) total volume were added to 500µl of blank plasma to prepare a standard curve (blank, 1, 2, 3, 10, 50, 100mcg/ml) and validation samples (1, 10, 100mcg/ml). Each mixture was vortexed for 1 minute and 900µl transferred to a Centrifree micropartition system reservoir fitted with a MPS-1 14mm YMT membrane (Amicon Corp., Danvers, MA). The ultrafiltration system was then centrifuged at 1500g for thirty minutes in a centrifuge equipped with a 35° fixed angle rotor. The ultrafiltrate was injected directly onto the chromatographic column. No internal standard was used because of the direct method of sample preparation (12).

Patient blood samples were collected into heparinized glass tubes over five hours following a one hour intravenous infusion of imipenem/cilastatin (Primaxin^{R}). Samples were immediately centrifuged for ten minutes. The plasma was collected and 2.0ml added to 2.0ml to the MES/ethylene glycol stabilizing solution. The samples were vortexed for 15 seconds and separated into 1ml aliquots. The stabilized samples were stored at -70°C until analysis within seven weeks. On the day on analysis, samples were thawed to room temperature and vortexed for one minute. Samples were thereafter handled as outlined above for the imipenem standards.

Standard Curve and Concentration Calculations

The peak height of imipenem was determined by the integrator using the perpendicular peak height to baseline for each standard curve concentration and a linear regression analysis performed. The concentration of each validation sample and each patient sample was calculated from the peak height and the linear regression function generated from the respective standard curve.

Recovery

Percent recovery of imipenem following ultrafiltration was assessed by comparing the peak heights of chromatograms obtained from the stock solution ultrafiltrates (1 to 100mcg/ml) to those of the unfiltered 100mcg/ml standard stock solution in the MES/ethylene glycol stabilizing solution on three separate occasions.

Application of Methods

The concentration-time profile of imipenem was determined for a 32 year old male admitted to the Burn ICU of the Regional Medical Center at Memphis. This patient received imipenem 500mg

as imipenem/cilastatin (Primaxin^{R}) intravenously every six hours infused over one hour for the treatment of a burn wound infection. Blood samples were obtained over five hours after the first and ninth dose. Concurrent medications administered during the study period included: acetaminophen, amikacin, amphotericin B, butorphanol, cimetidine, diphenhydramine, midazolam, ticarcillin, tobramycin, and vancomycin.

RESULTS AND DISCUSSION

Imipenem was well resolved from endogenous and exogenous substances under the chromatographic conditions. Chromatograms of blank plasma, an imipenem standard, and a patient sample are shown in figure 2. Mean retention time for imipenem was 6.7 minutes. No interference was apparent from any of the concurrent medications received during the study.

Mean recovery of imipenem was near 100% which is consistent with the value reported by Myers and Blumer (12). This is in contrast to recovery of approximately 50% (11) and 45% (13) by two other investigators. The lower limit of detection was 0.2mcg/ml for our assay defined as a signal to noise ratio of 4 to 1. The chromatographic conditions did not appear to limit the lifetime or performance of the octyldecyl stationary phase. No decrease in resolution or sensitivity was observed during the validation procedure.

The standard curve was linear from 1 to 100mcg/ml for imipenem during the entire validation procedure. The lower limit of sensitivity of 1mcg/ml was selected since the variance of concentrations 0.2 to 1.0mcg/ml exceeded 10%. The mean(sd) correlation coefficient and Y-intercept value over the 10 day validation period were 0.9999(0.0005) and -0.16(0.49)mcg/ml, respectively.

The results of the validation procedure are presented as within-day and between-day comparisons (Table 1). The within-day

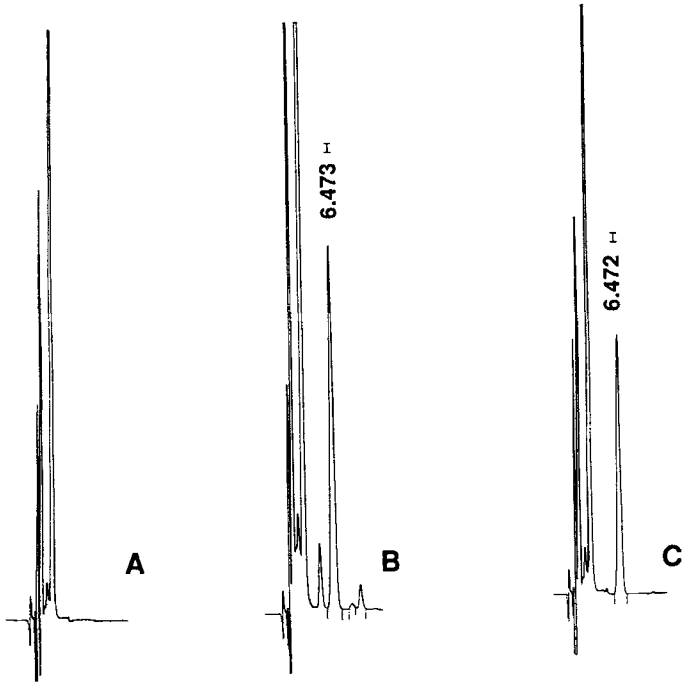

FIGURE 2. Chromatograms of stabilizing solution and: A) blank plasma, B) plasma spiked with imipenem 100mcg/ml, C) burn patient plasma after administration of Primaxin^{R} (imipenem/cilastatin). Peak I=imipenem.

TABLE 1

Imipenem Precision and Accuracy (mean±sd)

Concentration(C) (mcg/ml)	Within-day C (mcg/ml)	%CV	Between-day C (mcg/ml)	%CV
1	1.06±0.057	5.40	0.98±0.092	9.38
10	9.56±0.339	3.54	10.13±0.26	2.56
100	100.17±2.99	2.98	98.61±2.77	2.81

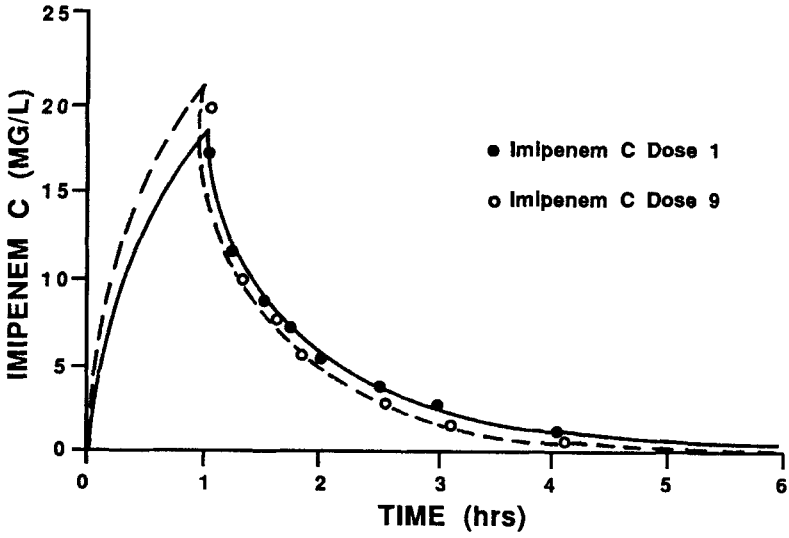

FIGURE 3. Plasma concentration-time profile following the first and ninth dose of imipenem/cilastatin 500mg (Primaxin^{R}) administered intravenously over one hour in a burn patient with 60% total body surface area burns.

coefficient of variation for imipenem ranged from 3.0% to 5.4%. The between-day coefficient of variation ranged from 2.6% to 9.4% over the same concentration range.

A representative plasma concentration-time plot following the first and ninth 500mg dose of imipenem in a patient with 60% total body surface area burns is shown in figure 3. Estimated terminal half-life of imipenem in this patient was 0.89 and 0.68 hours for the two respective doses which is more rapid relative to mean data from normal volunteer studies (2,3). The volume of distribution estimates are also altered relative to mean data from normal volunteers (3).

CONCLUSIONS

The described HPLC method for the determination of imipenem offers excellent separation and resolution. It is linear and reproducible over a range of concentrations expected after 500mg to 1000mg doses of the antibiotic. The selectivity and sensitivity of the assay are suitable for pharmacokinetic and possible clinical applications. This may be particularly appropriate for patient subsets such as the burn patient described.

ACKNOWLEDGMENTS

Supported by a grant from Merck, Sharp and Dohme, A Division of Merck and Co., Inc. The supply of analytical grade imipenem by Merck, Sharp and Dohme Research Lab is gratefully acknowledged.

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