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DETERMINATION OF IMIPENEM (N-FORMIMIDOYL THIENAMYCIN) IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, COMPARISON WITH MICROBIOLOGICAL METHODOLOGY AND STABILITY

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SUMMARY

High-performance liquid chromatographic (HPLC) methods using ultraviolet (UV) detection have been developed for the assay of the antibiotic imipenem (N-formimidoyl thienamycin) in human plasma and urine. A reversed-phase analytical column is employed in the plasma assay method and a cation-exchange column is used in the urine assay method. Both methods use borate buffer in the mobile phase. The method of preparation of human fluid samples for HPLC injection has been optimized with respect to the stability of imipenem in aqueous buffers, in morpholine buffer—ethylene glycol stabilizer, and in urine and plasma. Preparation of the samples before injection into the HPLC systems involves deproteination/filtration of the plasma/urine samples. The open lactam metabolite and the coadministered dehydropeptidase inhibitor, cilastatin sodium, do not interfere with the 313-nm detection of imipenem in either the plasma or the urine assay. Thienamycin, the precursor of imipenem and an impurity in imipenem formulations, is separated from the drug using both of these methods. Concentrations generated from the HPLC analysis of plasma and urine samples from two healthy volunteers compare favorably with results using a microbiological assay method. Correlation of the two methods gives $r \geq 0.990$ for both fluids.

INTRODUCTION

Imipenem* (N-formimidoyl thienamycin) (I) is a stable derivative of thienamycin (II), a naturally produced carbapenem antibiotic [1]. Chemical structures are shown in Fig. 1. It has an unusually high degree of activity against a broad spectrum of bacteria [2, 3].

*The Merck Sharp & Dohme Research Laboratories corporate identification code for imipenem is MK0787.

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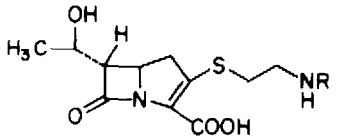
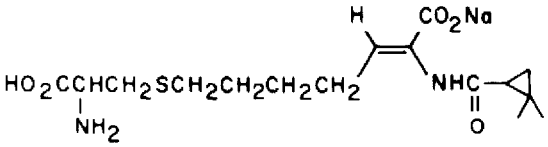


I. Imipenem R = CH=NH

II. Thienamycin R = H



III Cilastatin Sodium

Fig. 1. Chemical structures of imipenem, thienamycin and cilastatin sodium.

The urinary recovery of imipenem following intravenous administration in humans is low, typically 6—38% of the dose [4]. This is because imipenem is highly metabolized in the kidney by the renal dipeptidase, dehydropeptidase-I (DHP-I) [4, 5]. The renal metabolism occurs by cleavage of the beta-lactam. Coadministration of imipenem with the dehydropeptidase inhibitor, cilastatin sodium [(*Z*)-S-(6-carboxy-6-[([2,2-dimethyl-(*S*)-cyclopropyl]carbonyl)amino]-5-hexenyl)-L-cysteine monosodium salt*, (III)], increases the urinary recovery of the antibiotic and makes inter-subject urinary excretion more uniform at about 72% [6].

The determination of imipenem in human fluids in the presence and absence of cilastatin sodium has been achieved by a large plate, disk-diffusion, microbiological method [4, 6]. Although sensitive, the microbiological assay is not specific and does not differentiate imipenem from other antibiotics which could be coadministered. High-performance liquid chromatographic (HPLC) assay provides an alternative method for the determination of imipenem in urine and plasma. HPLC analysis is sensitive, rapid and specific, permitting the separation of imipenem from thienamycin, cilastatin sodium and the open lactam form of the drug. The HPLC methods used involve reversed-phase chromatography for plasma and cation-exchange chromatography for urine, both using UV detection at 313 nm. The UV $(H_2O)\lambda_{max}$ of imipenem is 298 nm [1]. Good agreement between the microbiological and the HPLC assays would indicate the absence of significant quantities of active metabolites or precursor.

*The Merck Sharp & Dohme Research Laboratories corporate identification code for cilastatin sodium MK0791.

EXPERIMENTAL

Materials and reagents

HPLC-grade methanol used in the mobile phase was purchased from Bodman Chemical (Burdick & Jackson Labs.). Boric acid, ethylene glycol, citric acid, sodium phosphate dibasic, sodium phosphate monobasic and anhydrous sodium carbonate were obtained from Fisher Scientific. Sodium bicarbonate was obtained from J.T. Baker. 4-Morpholinepropanesulfonic acid (MOPS), 4-morpholineethanesulfonic acid (MES), serotonin creatinine sulfate monohydrate and 5-methoxyindole-3-acetic acid (MIAA) were procured from Aldrich. Sodium hydroxide (pellets) was purchased from Mallinkrodt, and triethylamine from Pierce. Amicon's Centriflo ultrafiltration membrane cones (CF50A) were used for deproteination of urine and plasma samples, and Milli-Q water was supplied from the Millipore Reagent Water System.

Imipenem, thienamycin and cilastatin sodium were supplied by Merck Sharp and Dohme Research Labs. (Rahway, NJ, U.S.A.).

Apparatus

The HPLC system consisted of a fixed-wavelength absorbance detector (Waters Assoc., Model 440) using a 313-nm filter for the detection of imipenem. A variable-wavelength detector (Schoeffel Instruments Spectroflow monitor SF770 and monochromator GM770) was used to determine the UV absorption of the open ring product of imipenem at 214 nm. The solvent delivery system used was a double-reciprocating pump (Waters Model 6000A). Injections were made with an automated injector (Waters Intelligent Sample Processor, WISP Model 710A). A microprocessor (Waters System Controller 720) provided automated control of the pump and the injector. Integration of peak area or height detected at 313 nm was performed by a computing integrator (Spectra Physics Model SP4100). A chart recorder (Houston Instruments Omniscribe) was used to record the chromatographic profile detected at 214 nm.

Centrifugation was carried out in a refrigerated table top centrifuge (International Equipment Company Centra 7R).

Chromatographic conditions

Plasma assay was performed on a reversed-phase, 5-μm ODS Hypersil, 100 mm × 5 mm I.D., analytical column (Shandon Southern Instruments). A 30 mm × 4.6 mm I.D. Brownlee 10-μm LiChrosorb C_{18} guard column cartridge (Rainin Instrument Co.) fitted into an MPLC guard holder (Rainin) was placed in-line before the analytical column. Pump flow-rate was set at 4 ml/min, injection volume was 10 μl, and run time was 6.0 min. Integration parameters were set at 0.5 cm/min for chart speed and 8 mV full scale for attenuation.

Chromatographic conditions for the urine assay consisted of a 250 mm × 4.6 mm I.D. analytical column packed with Altex 10-μm Ultrasil CX cation-exchange packing (Rainin). A 30 mm × 4.6 mm I.D. Brownlee 10-μm Partisil SCX guard column cartridge (Rainin) was fitted into an MPLC guard holder and placed in-line prior to the analytical column. A 250 mm × 4.6 mm I.D.

stainless-steel preconditioning column dry packed with 37—53 μm silica particles (Whatman Chemical Separation) was used to saturate the mobile phase with silica and was fitted between the pump and the injector. (The pH of the buffer used in the urine assay (pH 7.5) borders on the maximum suggested pH for buffers used with silica-based column packings. A silica preconditioning column is used to saturate the mobile phase and prevent breakdown of the silica backbone of the cation-exchange packing in the analytical column.) To protect the automated injector from particulates from the preconditoning silica, a 2-μm Hastelloy C filter (Scientific Systems) was inserted between the silica column and the injector. Mobile phase flow-rate was 2 ml/min, injection volume was 30 μl and run time was 10 min. Chart speed of the integrator was 0.5 cm/min and attenuation was 16 mV full scale. The recorder chart speed was set at 0.5 cm/min.

Prepared solutions

Stabilizing solutions. (Imipenem is most stable at neutral pH. MES (pK_a = 6.15) and MOPS (pK_a = 7.2) buffers are used to bring the pH of plasma and urine, respectively, to approximately 6.8. Ethylene glycol is essential to the stability of imipenem for long-term storage because it disrupts the organized water structure that leads to breakdown of beta-lactams in the frozen state.) Plasma stabilizer was prepared by dissolving 194.2 g of MES in 700 ml Milli-Q water, adjusting the pH to 6.0 with 1.0 *M* sodium hydroxide and diluting to 1 l with Milli-Q water. The MES buffer was then diluted 1:1 with ethylene glycol. Similarly, 209.3 g of MOPS were dissolved in 700 ml Milli-Q water, adjusted to pH 6.8, diluted to 1 l, and diluted again 1:1 in ethylene glycol. This solution was used as the urine stabilizer.

Mobile phase. Mobile phase for the plasma assay was 0.2 *M* boric acid, adjusted to pH 7.2 with 1.0 *M* sodium hydroxide. The solution was degassed by vacuum filtering through a 0.45-μm membrane filter (Waters Assoc.).

The urine assay mobile phase was prepared by mixing 52 ml methanol with 2 l of 0.5 *M* boric acid containing 130 μl of triethylamine and adjusting to pH 7.5 with 1.0 *M* sodium hydroxide. Degassing was carried out as described above for the plasma assay mobile phase.

Standard solutions

Urine and plasma imipenem calibration standards in the range of 0.30—72 μg/ml for plasma and 1—100 μg/ml for urine were prepared daily from stock solutions of the antibiotic in stabilizer. Standards were prepared by spiking the appropriate volume of stock solution into 500 μl of biological fluid mixed with 500 μl of appropriate stabilizer.

Analytical procedure

Samples were thawed and prepared for HPLC analysis as follows. To 1.0 ml of stabilized plasma sample (500 μl plasma in 500 μl stabilizer) was added the internal standard MIAA to a final concentration of 168 μg/ml. Serotonin (136 μg/ml final concentration) was used as the internal standard in 1 ml stabilized urine. The same concentrations of each internal standard were also added to urine and plasma standards. Samples and standards were mixed on a

Vortex mixer and deproteinated through Amicon CF50A ultrafilters by centrifugation at 1500 *g* (2700 rpm) for 10 min at 5°C. The filtrates were analyzed by direct injection into the HPLC system. Drug concentrations were calculated from the linear regression of the standards using peak height response ratio (imipenem to internal standards) vs. concentration of imipenem. Urine samples with imipenem concentrations of more than 100 μg/ml were diluted appropriately with a 1:1 mixture of urine and its stabilizer. All plasma sample concentrations fell below the 75 μg/ml upper limit of the standard curve.

Study protocol

Urine and plasma samples from an imipenem clinical study which were analyzed by a microbiological assay [4] were re-analyzed using the HPLC method. All samples were assayed by the microbiological method in February and March 1981. Plasma samples were analyzed by HPLC in April 1981. The HPLC analysis of urine samples was completed in May 1983. All samples had been stored at −70°C to −80°C in the interim period.

The samples were from an open study conducted to determine the tolerance, safety and pharmacokinetic properties of single doses of imipenem per se and imipenem coadministered with a dehydropeptidase inhibitor (cilastatin sodium). Subjects were given an intravenous infusion of 500 mg of imipenem in treatment 1, and 500 mg of imipenem plus 250 mg of cilastatin sodium were given in the same manner in treatment 2. There was seven-day washout period between the two treatments. Blood samples were collected at 0 (preinfusion), 20 and 40 min, and 1, 2, 4, 6 and 8 h after infusion. The blood samples were chilled immediately and centrifuged at 2–5°C; the plasma was collected. Urine collections were made at the following time intervals: 0–1, 1—2, 2—3, 3—4, 4—5, 5—6, 6—8 and 8—10 h after infusion. The volumes of the urine samples were recorded. A 2-ml aliquot of both urine and plasma from each collection period was stabilized by a 1:1 dilution with the appropriate stabilizer to decrease the possibility of degradation. All samples were stored at −70° to −80°C until just prior to analysis.

Stability

The short-term stability of imipenem in plasma and urine at 24°C was investigated using the following four preparation methods:

(a) Unstabilized, unfiltered sample. A stock solution of 2 mg/ml imipenem was spiked into a blank pool of plasma to a concentration of 100 μg/ml. A 500-μl aliquot of plasma was removed from the pool, and internal standard and stabilizer were added to each sample. The sample was mixed on a Vortex mixer, filtered (ultrafiltration), and peak height ratio was determined by HPLC analysis. This procedure was repeated every hour up to 5 h.

(b) Stabilized, unfiltered sample. A second pool was prepared by adding stabilizer (1:1) to a portion of original pool. The final concentration was 50 μg/ml. Every hour for 5 h 1 ml of stabilized plasma was removed from the pool and prepared and analyzed as indicated in *a*.

(c) Unstabilized, filtered (deproteinated) sample. Imipenem was spiked into 1 ml of plasma to a concentration of 50 μg/ml. After the addition of internal

standard, the sample was filtered at zero hour of the study and analyzed. The same sample was then analyzed hourly for 5 h.

(d) Stabilized, filtered (deproteinated) sample. Imipenem was spiked into 1 ml of stabilized plasma to a concentration of 50 μg/ml. The sample was then prepared and analyzed as indicated in *c*.

All samples in this study were prepared in duplicate. The stability of imipenem in urine was determined similarly to that described for plasma substituting the urine stabilizer for the plasma stabilizer where necessary.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms of blank and spiked plasma and urine matrices are shown in Figs. 2 and 3, respectively. The detection of imipenem in urine and plasma subject samples has been determined to be specific. No background peaks which would interfere with imipenem or internal standards are present. The stabilizers also do not interfere. The renal dehydropeptidase inhibitor, cilastatin sodium, does not absorb at 313 nm and does not appear in the chromatograms under the assay conditions used for plasma and urine.

An investigation to identify the metabolites and degradation products of imipenem is currently being conducted. The major metabolite, formed by hydrolysis or enzymatic reaction is known to be the open lactam [5]. It has been established that the hydrolysis product can be formed in dilute acid [5].

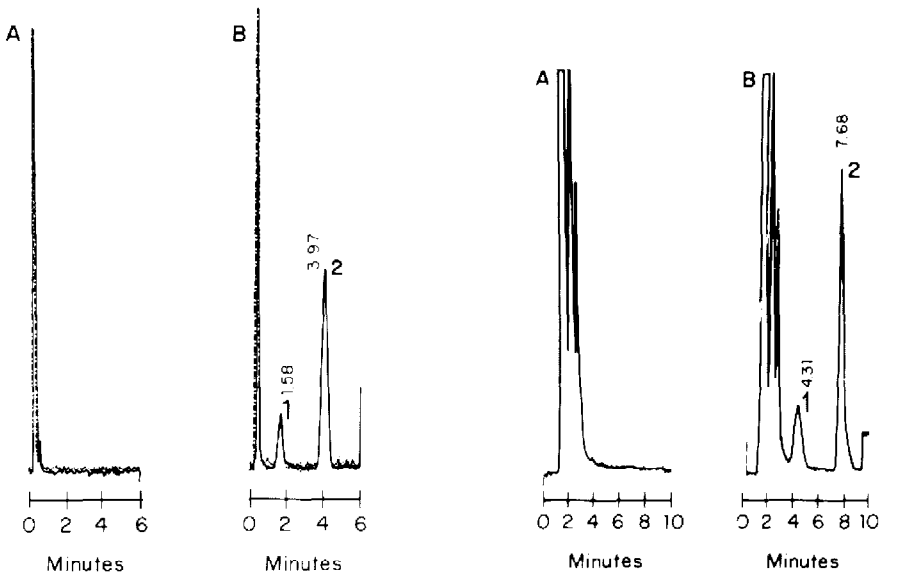


Fig. 2. Chromatograms of (A) a blank plasma—stabilizer matrix and (B) of imipenem, 7.2 μg/ml (1) and internal standard, 168 μg/ml (2) in stabilized plasma. The injection volume was 10 μl. Chromatographic conditions are given in the text.

Fig. 3. Chromatograms of (A) a blank urine—stabilizer matrix and (B) of imipenem, 10 μg/ml (1) and internal standard, 136 μg/ml (2) in stabilized urine. The injection volume was 30 μl. Chromatographic conditions are given in the text.

Therefore, to determine specificity, imipenem (1.5 m*M*) was reacted in citrate—phosphate buffer at pH 3.1. The same concentration was also reacted in 0.05 *M* carbonate buffer, pH 10.8 and 0.1 *M* phosphate buffer, pH 7.25. The degradation of imipenem and the formation of the reaction products were followed simultaneously by HPLC analysis with detection at 313 nm and 214 nm, respectively, as described for urine analysis using a flow-rate of 2.5 ml/min (Fig. 4). A schematic diagram showing the possible reaction sequence is given in Fig. 5. None of the reaction products, A—E, interfere with the detection of imipenem. Product II, which is detected at both 313 nm and 214 nm (as is imipenem) and is present at time zero of the study, has been identified as thienamycin by HPLC analysis of pure thienamycin. Thienamycin is an impurity found in

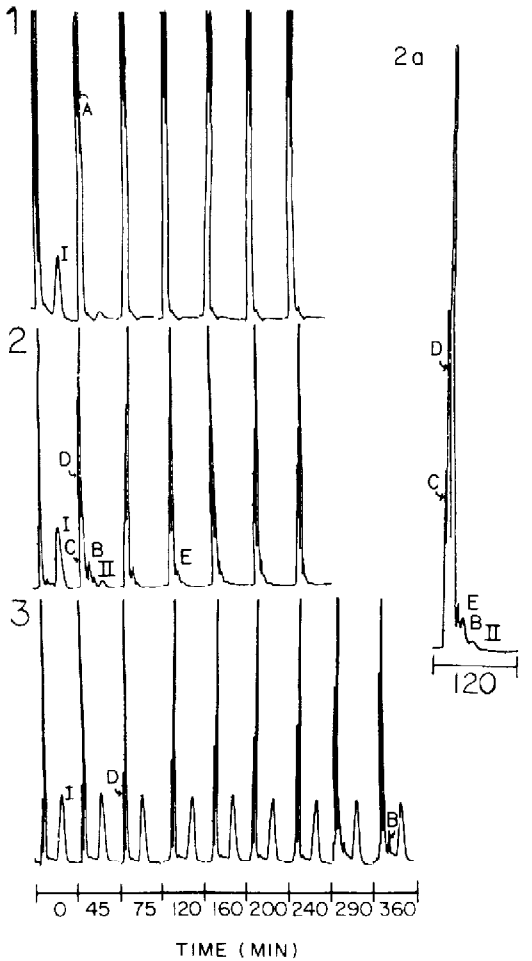
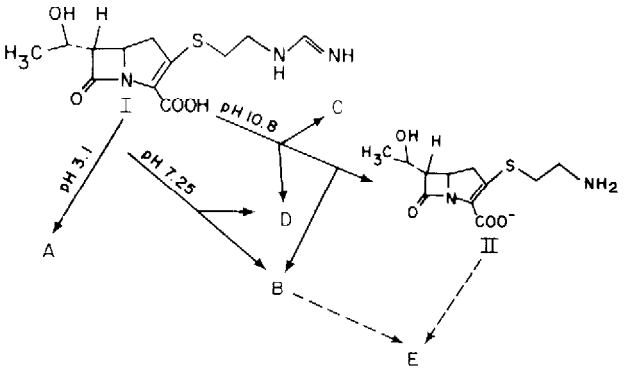


Fig. 4. Reaction of imipenem in (1) citrate—phosphate buffer, pH 3.1, (2) carbonate—bicarbonate buffer, pH 10.8 over a 4-h period, and (3) phosphate buffer, pH 7.25 over 6 h. Inset (2a) is an enlargement of the chromatogram of the reaction products of imipenem in carbonate—bicarbonate after 2 h of reaction time. Imipenem and reaction products are detected at 214 nm after separation on an Altex Ultrasil CX column using the chromatographic conditions described in the text. I = imipenem; II = thienamycin and all other products; A—E = unknowns.

imipenem formulations and this may account for its presence in all solutions at zero hour (Fig. 6). The concentration of thienamycin increases in basic buffer after 45 min of reaction time then slowly degrades.

As indicated in Fig. 5, products A–D and II appear to be directly related to the degradation of imipenem. Product E does not appear in the chromatograms of Fig. 4 until imipenem is no longer detected and as products II and B begin to decrease. It may be possible that product E is a degradation product of II or B or both.



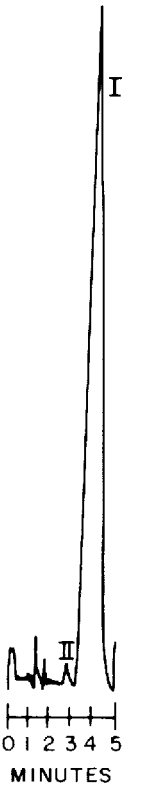


Fig. 5. Possible reaction sequence of imipenem under acidic, neutral and basic conditions. Refer to Fig. 4 for chromatographic peaks of products A—E.

Fig. 6. Chromatogram of imipenem (I) in phosphate buffer detected at 313 nm at zero hour of the reaction study. Thienamycin (II) is also detected.

There appears to be no common products in the acid- and base-catalyzed reaction mixtures. The base-catalyzed reaction of imipenem yields multiple peaks (products B—D and II) and the acid treatment seems to yield only one product (A). The neutral reaction mixture yields two by-products (products D and B) with retention times concurrent with two of the base catalyzed products.

Linearity and reproducibility

The linearity and reproducibility of peak height ratio response with respect to concentration of imipenem was determined by multiple replicate analysis at

each standard concentration. The results were analyzed by linear regression. Both assays were found to be linear, with correlation coefficients (r) of greater than 0.999. Over the linear concentration range, the average coefficient of variation (C.V.) was 4.98 ± 2.3% (n = 3 or 4) for the plasma assay and 6.57 ± 3.0% (n = 5 or 6) for the urine assay.

Sensitivity

The limit of detection of the assay was determined as the lowest detectable concentration above background with a variance of less than 10%. The sensitivity limit of the plasma assay was 0.3 μg/ml (C.V. = 6%) using a 75-μl injection volume, while that of the urine assay was 1.0 μg/ml (C.V. = 9.8%) using a 75-μl injection volume, while that of the urine assay was 1.0 μg/ml (C.V. = 9.8%) using a 30-μl injection volume.

Recovery

The losses of imipenem and internal standard due to adsorption to the ultrafilter or degradation during sample processing were determined by comparing peak heights of authentic standards in stabilizer per se vs. stabilized plasma and stabilized urine standards processed as described. Results from this recovery study are given in Table I.

TABLE I

RECOVERY OF IMIPENEM AND INTERNAL STANDARD FROM PLASMA AND URINE

Compound	Concentration (μg/ml)	Recovery (%)	Standard deviation (%)	n
Plasma				
Imipenem	20	48.7	3.29	5
	80	53.1	4.43	5
Internal standard	136	45.9	6.16	10
Urine				
Imipenem	20	83.8	3.66	5
	80	87.4	4.16	5
Internal standard	136	70.3	2.90	10

Stability

The storage of plasma and urine samples containing imipenem presents stability problems arising from the accelerated inactivation of the antibiotic in the frozen state at conventional temperatures, e.g. −10°C to −20°C, and from its sensitivity to pH extremes both above and below neutrality [7]. Accelerated inactivation in the frozen state has also been reported for penicillins and could be minimized by inclusion of high concentrations of agents disrupting organized water structure (e.g. glycerol or ethylene glycol) [8]. The stability of imipenem has been characterized in a variety of buffers [9, 10]. Stability of imipenem in pH 5—8, non-nucleophilic, inert buffers depends upon the concentration of the drug and upon the concentration of the buffer. Increasing the concentration of a phosphate buffer, pH 7.2 from 0.5 to 1.0 M decreases the half-life of imipenem by a factor of two [9]. The most

effective stabilizing buffers for storage of thienamycin are the zwitterionic substituted morpholines such as 2-(N-morpholino)-ethanesulfonate (MES) and 3-(N-morpholino)propanesulfonate (MOPS), probably because they do not promote cleavage of the beta-lactam [10]. These buffers together with 25% ethylene glycol were used to investigate the stability of imipenem in urine and plasma and to determine the best method for handling and storing the drug.

Aqueous buffer

The N-formimidoyl derivative of thienamycin was developed to circumvent the bimolecular reaction which causes the instability of thienamycin in aqueous solutions. Aminolysis occurs when one molecule of thienamycin undergoes beta-lactam cleavage by the primary amine of another [10]. Beta-lactam cleavage in both thienamycin and imipenem is also promoted by certain aqueous buffers like borate and concentrated (1 *M*) phosphate buffers [9, 10]. Compared to other beta-lactam antibiotics, imipenem has been found to be more susceptible to breakdown in aqueous buffers [9]. Imipenem is less stable than the penicillins and the cephalosporins (which are stable) in phosphate buffer. In dilute phosphate buffer the stability of imipenem is comparable to that found in morpholine buffer—ethylene glycol stabilizer when monitored over a 5-h period at room temperature (Fig. 7). Imipenem reacts more extensively in borate buffer. The degradation of imipenem in 0.2 *M* borate buffer, pH 7.0 is first order with a half-life of 1.82 h.

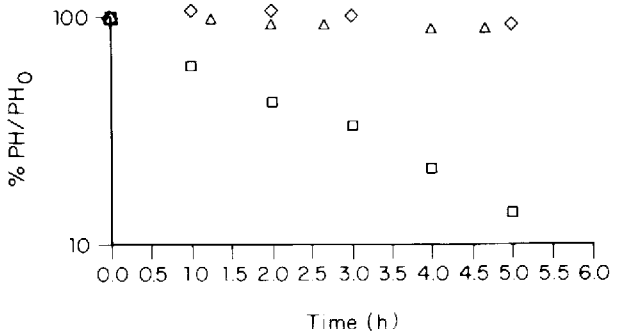


Fig. 7. Stability of 50 μg/ml imipenem in 0.2 *M* borate buffer, pH 7.0 (□), 0.1 *M* phosphate buffer, pH 7.25 (△), and 1.0 *M* MES—ethylene glycol, pH 6.0 (◇) at 24°C.

Short-term plasma and urine stability

The semilogarithmic plot of time vs. percentage of unreacted imipenem (Fig. 8) for deproteinated unstabilized imipenem plasma standards shows first-order breakdown of the drug at 24°C with a half-life of 1.65 h and an 84.1% loss over 5 h. Stabilization of deproteinated plasma standards more than doubles the stability of the drug with only 38.3% lost in 5 h. The results indicate that in handling imipenem plasma samples the greatest degree of stability is achieved by stabilizing the samples upon collection and only deproteinating immediately prior to analysis. Only 5.9% of the drug is lost over 5 h under these optimum conditions, almost fifteen times less than in the case of deproteinated, unstabilized samples.

As in the case of plasma, stabilizing the urine samples and filtering immediate-

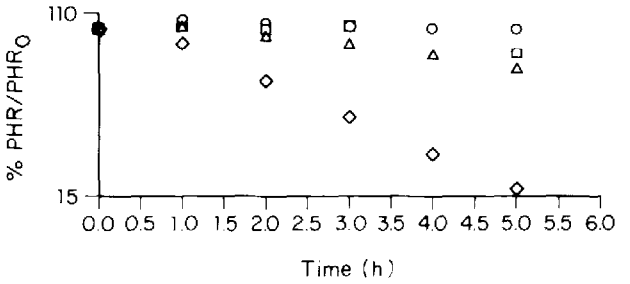


Fig. 8. Stability of imipenem in plasma (□), stabilized plasma (○), deproteinated plasma (◇), and stabilized, deproteinated plasma (△) at 24° C.

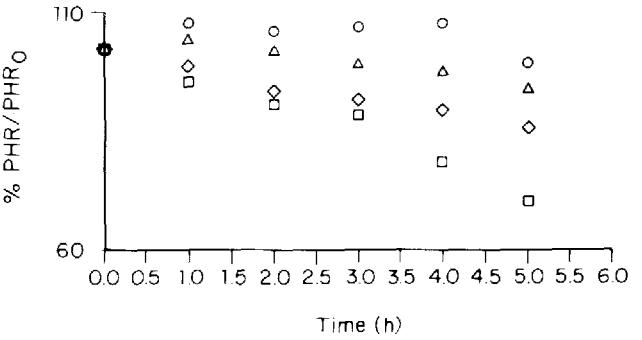


Fig. 9. Stability of imipenem in urine (□), stabilized urine (○), deproteinated urine (◇), and stabilized, deproteinated urine (△), at 24° C.

ly prior to analysis is the most reliable method of handling imipenem samples since only 3.7% of the drug was lost over a 5-h period (Fig. 9). This compared to 32.0% lost from unstabilized urine after 5 h at 24° C. Though there is loss of imipenem from the unstabilized, deproteinated standards in urine, the removal of high-molecular-weight molecules from urine affects imipenem stability in unstabilized urine less adversely than in unstabilized plasma. Only 18.2% of imipenem is lost in urine in this case as compared to 84.1% in plasma. Urine pH was slightly acidic (pH 5.3). It has been found that imipenem breakdown increases as urine pH decreases [4].

Long-term stability

A long-term stability study of imipenem in urine was conducted by storing spiked stabilized urine quality control standards at −70° C and −20° C. The low concentration standard was spiked to contain 19.8 μg/ml while the high concentration standard was spiked to contain 76.9 μg/ml. Results of the analysis of these standards over a 37-day interval are given in Table II. The interassay C.V. for the low concentration standard was 2.88% at −70° C and 7.18% at −20° C. For the high concentration standard the difference in the C.V. value was not as great, i.e. 6.22% at −70° C and 9.62% at −20° C.

The stability of imipenem in stabilized plasma over a 42-day period showed no significant difference between storage at −20° C and storage at −70° C. The mean concentration of a 10 μg/ml imipenem control standard was 9.85 μg/ml (C.V. = 2.59%) at −70° C and 9.82 μg/ml (C.V. = 3.38%) at −20° C. The 40

TABLE II

COMPARISON OF IMIPENEM STABILITY IN STABILIZED URINE AFTER STORAGE AT −70° C AND −20° C

Day	−70° C		−20° C	
	19.8 μg/ml	76.9 μg/ml	19.8 μg/ml	76.9 μg/ml
0	18.2	75.0	18.2	75.0
2	18.5	71.6	17.5	67.9
4	19.6	81.8	17.9	72.9
22	19.1	71.3	15.2	58.0
37	18.8	70.8	18.1	67.7
Mean	18.8	74.1	17.4	68.3

μg/ml control standard stored at −70° C had a mean concentration of 40.0 μg/ml (C.V. = 6.38%). When stored at −20° C, the mean concentration was 38.3 μg/ml (C.V. = 3.00%).

It is recommended, however, that urine and plasma imipenem samples be stabilized and stored at −70° C to −80° C over the long term.

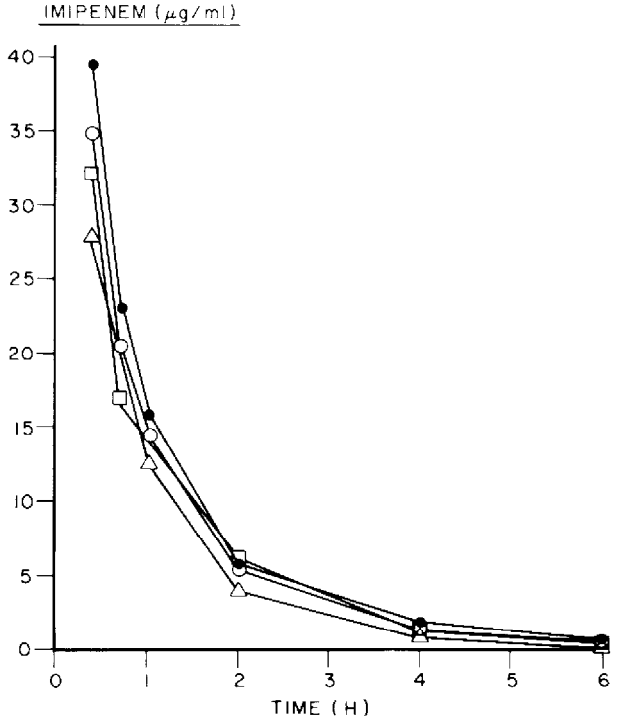


Fig. 10. Plasma concentration of imipenem after intravenous administration of 500 mg of imipenem to subject 1B (△) and subject 5B (○) or of 500 mg of imipenem combined with 250 mg of cilastatin sodium administered to subject 1B (□) and subject 5B (●).

Clinical study assay results

Plasma concentrations of imipenem. The plasma concentration—time curve in Fig. 10 shows the elimination of imipenem from the plasma of two subjects after intravenous infusion of imipenem and imipenem coadministered with cilastatin sodium. Plasma concentrations of the drug after infusion of the imipenem—cilastatin sodium regimen were slightly higher than those following imipenem alone.

Renal excretion of imipenem. The recovery of imipenem from subject urine after administration of imipenem alone is low (Table III). Only 6.45% of the dose is recovered from subject 1B and 20.3% from subject 5B. A dramatic increase in urinary recovery results from the coadministration of cilastatin sodium. The dehydropeptidase inhibitor increased the urinary recovery in both subjects to about 70%.

TABLE III

URINARY EXCRETION OF IMIPENEM ADMINISTERED ALONE (TREATMENT 1) AND WITH CILASTATIN SODIUM (TREATMENT 2)

Collection period (h)	Imipenem (mg)			
	Treatment 1		Treatment 2	
	Subject 1B*	Subject 5B**	Subject 1B***	Subject 5B§
0—1	19.5	54.8	182	168
1—2	5.43	22.5	67.9	59.5
2—3	2.23	9.48	31.7	45.2
3—4	1.35	4.48	11.3	15.3
4—5	0.362	1.83	4.68	6.24
5—6	0.277	0.987	1.92	3.85
6—8	0.355	0.954	1.00	1.39
8—10	0	0.180	0.033	0.492
Total	29.5	95.2	300	300

*Imipenem dose = 457.2 mg.
**Imipenem dose = 469.0 mg.
***Imipenem dose = 422.3 mg (+250 mg cilastatin sodium).
§Imipenem dose = 463.3 mg (+250 mg cilastatin sodium).

Comparison of microbiological and HPLC methods

Good correlation was obtained in comparing the HPLC method to the microbiological method for the analysis of imipenem in urine and plasma samples (Figs. 11 and 12). The linear regression of HPLC results vs. microbiological results gave a correlation coefficient (r) of 0.990 (slope = 1.05) for the plasma assay. The r value for the urine assay was 0.997 (slope = 0.995) over the whole range of values and 0.995 (slope = 1.15) for the lower concentrations (0—70 μg/ml). The close correlation of the two assays indicates that the microbiological assay is specific for imipenem with respect to the HPLC conditions discussed in the text. This suggests that interference due to thienamycin in subject samples is insignificant.

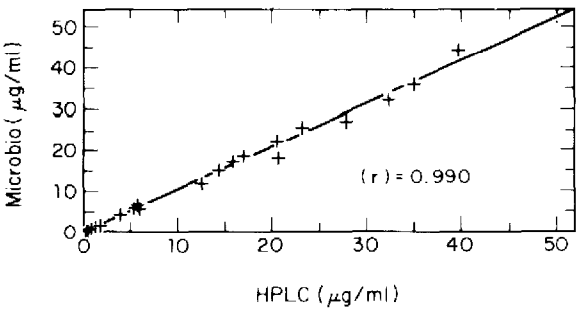


Fig. 11. Comparison of HPLC and microbiological methods for the determination of imipenem in plasma. Individual measurements were plotted for 22 samples.

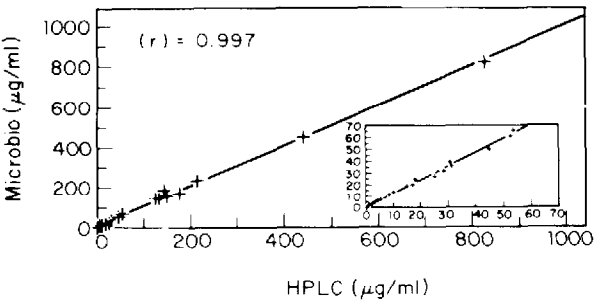


Fig. 12. Comparison of HPLC and microbiological methods for the determination of imipenem in urine. Individual measurements were plotted for 32 samples. The insert is an enlargement of the 0—70 μg/ml area of the correlation plot.

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