An Improved Micro-Scale Liquid-Chromatographic Assay for Piperacillin in Plasma and Urine

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An accurate, sensitive, and specific liquid-chromatographic method is described for measuring piperacillin in plasma and urine. Plasma samples deproteinized with two volumes of acetonitrile containing 1.2 mg of the internal standard, p-nitrobenzene sulfonamide, per liter are centrifuged. The clear supernate is evaporated under nitrogen, and the residue is reconstituted in 50 μL of the mobile phase (32/68 by vol acetonitrile/water, adjusted to pH 2.5 with 85% phosphoric acid), of which 10 μL is injected onto a reversed-phase (C-18) column. Urine samples are diluted 10-fold with distilled water, an equal volume of acetonitrile containing 3 mg of the internal standard per liter is added, and 20 μL is chromatographed. Stability studies indicate that storage conditions are critical for both plasma and urine. Piperacillin in plasma is stable at −70°C for at least six weeks, but 100% of it is degraded during the same time at −20°C. Piperacillin in urine is also stable at −70°C for six weeks, but 20% is degraded during six weeks at −20°C.

Additional Keyphrases: neonates · pediatric chemistry · semi-synthetic penicillin · antibiotics

Piperacillin (6-α-(4-ethyl-2,3-dioxo-1-piperazinyl-carbonyl-amino)-α-phenylacetamido penicillinate) is a new semi-synthetic penicillin with greater broad-spectrum in vitro activity than carbenicillin and ticarcillin against Gram-positive and Gram-negative organisms, including Pseudomonas aeruginosa, Proteus, Klebsiella pneumoniae, and Serratia marcescens (1-3). Piperacillin concentrations in plasma should be monitored in cases of meningitis (4), renal failure (5, 6), and chronic renal failure being treated with hemodialysis (6).

Previously, piperacillin has been measured in plasma and urine by nonspecific, time-consuming microbiological methods (7, 8). Such bioassays do not allow rapid determinations. In addition, patients on piperacillin may also be receiving one or more other antibiotics, so the specificity of chromatographic methods may be an advantage.

Some liquid-chromatographic assays for this drug have been reported (4, 9-11). Some (4, 9) require at least 1 mL of plasma, involve an extensive and cumbersome extraction procedure, and in neither is an internal standard used. Another (10) requires a 200-μL sample and a variable-wavelength detector set at 230 nm to achieve the desired sensitivity. Another (11) requires the use of a potassium phosphate buffer at a pH-sensitive region where interfering substances may co-elute with piperacillin.

We describe here a rapid, sensitive, and specific method for the liquid-chromatographic determination of piperacillin in plasma and urine, a procedure that may also be used with neonates and infants.

Materials and Methods

Apparatus

We used a Model 6000A pump and a dual-channel ultraviolet absorption detector, both from Waters Associates, Milford, MA 01757. Samples were injected into a Model 7125 injector valve equipped with a 50-μL sample loop (Rhodyne, Cotati, CA 94928). A 250 × 4.6 mm (i.d.) Partisil ODS-3 (Whatman, Inc., Clifton, NJ 07014) reversed-phase column was fitted to the instrument. Peak recordings were made with a Model 261 strip-chart recorder (Linear Instruments Corp., Irvine, CA 92714). Peak areas and retention times were determined with an HP-3590A computing integrator (Hewlett-Packard Co., Avondale, PA 19311).

Reagents

Chemicals. Sodium piperacillin (lot no. PC-0322-HP103; Lederle Laboratories, Pearl River, NY 10965) and p-nitrobenzene sulfonamide (Aldrich Chemical Co., Milwaukee, WI 53233) were used as received. The chromatographic solvents, acetonitrile ("HPLC" grade; Fisher Scientific Co., Itasca, IL 60143) and glass-distilled water, were filtered through a 0.40-μm filter (nominal pore size) before use.

Mobile phase. The mobile phase, acetonitrile/water (32/68 by vol), was prepared by mixing 680 mL of water with 320 mL of acetonitrile and adjusting the pH of the solution to 2.5 with 85% phosphoric acid. The mobile phase was degassed by sonication under reduced pressure.

Procedures

Plasma. To 100 μL of heparinized human plasma in a 10 × 75 mm test tube, add 200 μL of a 1.2 mg/mL solution of p-nitrobenzene sulfonamide (internal standard) in acetonitrile. Vortex-mix for 10 s and centrifuge (3200 rpm, 12 min). Transfer the resulting supernate to a new test tube and evaporate under a steady stream of nitrogen. Reconstitute the residue in 50 μL of the mobile phase, inject 10 μL of this solution into the chromatograph, and elute with the mobile phase at a flow rate of 2.0 mL/min. Monitor the column effluent at 254 nm with detector sensitivity set between 0.01 and 0.05 A full scale.

Urine. To 100 μL of urine diluted 10-fold with distilled water, add 100 μL of a 3 mg/L solution in acetonitrile of p-nitrobenzene sulfonamide. Vortex-mix for 10 s, centrifuge at 3200 rpm for 12 min, and inject about 20 μL of the clear supernate into the chromatograph. Use the same chromatographic conditions described above for plasma.

Prepare standard curves from data obtained by use of plasma or diluted urine to which known amounts of sodium piperacillin are added to yield final concentrations of 4.7, 9.3, 23.0, 44.9, 85.7, and 188.6 mg of piperacillin (the free acid) per liter and treated as described above. Construct standard curves by plotting the ratios of peak areas for plasma or urine piperacillin to internal standard.

Stability studies. As described above, we prepared 12 solutions each of human plasma and urine containing...
sodium piperacillin to yield piperacillin (free acid) concentrations of 7.8, 72.5, and 134.8 mg/L in plasma and 188.6, 404.1, and 600.1 mg/L in urine. These solutions were stored at –20 and –70 °C, samples being analyzed 0, 1, 2, 5 (or 6), 13, and 43 days later.

Accuracy and precision. Plasma and urine samples containing piperacillin in concentrations of 14.6, 117.9, and 179.2 mg/L and 14.0, 65.8, and 85.7 mg/L, respectively, were prepared in quintuplicate by one person and assayed by another who had no knowledge of the concentrations.

Analytical recovery. Aqueous solutions containing 9.3, 23.0, 85.7, and 188.6 mg/L concentrations of piperacillin were prepared and assayed as described above. Results for these standards were compared with those for standard solutions prepared in plasma and urine to determine analytical recovery.

Data analysis. For all standard curves, the data on peak area ratios vs drug concentration were subjected to linear least squares regression analysis. All results are expressed as mean ± 1 standard deviation (SD).

Results and Discussion

Figure 1 illustrates representative chromatograms of plasma and urine sampled before and after intravenous administration of piperacillin sodium to a pediatric patient. Retention times for piperacillin and the internal standard were 6.0 and 4.2 min, respectively.

The standard curves were consistently linear and highly reproducible. We prepared 19 standard curves for plasma and urine, with piperacillin concentrations of 188.6, 85.7, 44.9, 23.0, 9.3, and 4.7 mg/L, during a period of five months. The slopes of the peak-area ratio of piperacillin to internal standard vs piperacillin free acid concentrations in plasma and urine were 0.0600 ± 0.0029 L/mg and 0.0618 ± 0.0029 L/mg, respectively. The coefficients of variation for the slopes were 4.8% and 5.6% for plasma and urine, respectively. The corresponding intercepts were 0.022 ± 0.033 and 0.053 ± 0.042 mg/L, not significantly different from zero in the case of any curve (p > 0.05).

The precision and accuracy of the method in plasma and urine were determined at three different concentrations, corresponding to the low, medium, and upper range of the piperacillin standard curve. The respective within-run CVs for piperacillin were 4.1% (14.6 ± 0.6 mg/L), 4.0% (114.9 ± 4.6 mg/L), and 2.6% (176.9 ± 4.6 mg/L). For urine, the corresponding CVs were 4.8% (13.9 ± 0.7 mg/L), 1.0% (61.3 ± 0.6 mg/L), and 1.3% (84.6 ± 1.1 mg/L). The run-to-run variation was measured for three plasma and urine controls run on seven different days during a month. The CVs for concentrations of 9.4, 85.7, and 188.6 mg/L of plasma were 7.2%, 3.4%, and 3.0%, respectively. The corresponding CVs for urine concentrations of 23, 85.7, and 188.6 mg/L of urine were 9.0%, 3.6%, and 6.5%. The relative accuracy1 of the method as applied to plasma and urine ranged from 0.2 to 2.6% and 0.7 to 6.8%, respectively.

On comparing drug-supplemented plasma and urine with aqueous standards of piperacillin over a concentration range of 5–200 mg/L, we found an average analytical recovery of 91% for plasma, 104% for urine.

We evaluated interference by 23 other drugs (Table 1). None interfered.

Table 1. Compounds Studied for Chromatographic Interference

| Phenobarbital | Quinidine |
| Primidone | Dipryramide |
| Carbamazepine | Methotrexate |
| Phenytoin | Caffeine |
| Ethosuxinamide | Gentamicin |
| Valproic acid | Tobramycin |
| Lithium | Chloramphenicol |
| Theophylline | Salicylate |
| Digoxin | Acravinophen |
| Lidocaine | Imipramine |
| Procaainamide | Desipramine |
| N-Acetyl-procaainamide |

Piperacillin in plasma samples stored at –20 °C lost about half the drug in 13 days, all of it in 43 days. Piperacillin was stable (less than 10% degradation) in plasma stored at –70 °C for six weeks. In addition, when plasma was allowed to stand at room temperature (25 °C) for 6 h, 20 to 25% of it was lost. Degradation of piperacillin is slower in urine than in plasma: about 20% at –20 °C, less than 5% at –70 °C in 13 days. Evidently, plasma and urine samples should both be processed as promptly as possible and stored at –70 °C.

This method is rapid and has the requisite accuracy, specificity, and sensitivity for routine use in the clinical laboratory. The assay can be done on only 10 μL of plasma, provided the drug concentration exceeds 5 mg/L. This allows the heel-prick method to be used in obtaining plasma samples for piperacillin determination. Thus, this method is suitable for therapeutic monitoring and pharmacokinetic studies of piperacillin in both the adult and pediatric populations. Figure 2 shows a plot of piperacillin plasma concentrations as a function of time after the intravenous administration of a 1.3 g dose of piperacillin sodium to a pediatric patient. The data were best fit by a biexponential equation with a terminal elimination half-life of 30 min.

1 The relative accuracy of a mean is calculated % = 100( X̄ - m)/m, where X̄ is the observed mean of replicate samples and m is the true or correct value for the quantity measured.
Fig. 2. Semilogarithmic plot of piperacillin concentration in plasma as a function of time in a subject who received an intravenous 1.3-g dose of sodium piperacillin

References


