Determination of Ticarcillin Levels in Serum by High-Pressure Liquid Chromatography

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A high-pressure liquid chromatographic method for determining the concentrations of ticarcillin in serum was developed and compared, with 93 patient sera, to a standard agar well diffusion bioassay. For analysis, serum plus temocillin, the internal standard, were extracted with chloroform-n-amyl alcohol and back extracted into phosphate buffer. A reverse-phase C18 column and an ammonium acetate-methanol mobile phase were used with detection at 242 nm. Reproducibility studies yielded coefficients of variation ranging from 2.4 to 4.7% for low, mid, and high controls. Although cefoxitin, cephalothin, and cefuroxime exhibited retention similar to that of ticarcillin, a wide range of commonly administered antibiotics and other drugs did not interfere. The high-pressure liquid chromatographic assay is an accurate, reproducible method for determining the concentration of ticarcillin in serum during multiple antibiotic therapy or when rapid results are required.

Ticarcillin (α-carboxy-3-thienylmethylpenicillin) is a semisynthetic penicillin exhibiting a high degree of activity against Pseudomonas aeruginosa and other gram-negative organisms (5, 9, 10, 12, 15, 18). In addition to being used to treat proven Pseudomonas infections, ticarcillin is also used empirically in the immunocompromised host. Often, in both cases, ticarcillin is concurrently administered with aminoglycosides or cephalosporins or both. Toxicity associated with the use of penicillins is usually minimal; however, central nervous system side effects can be seen with high levels in serum (14). Although not required routinely, ticarcillin levels in serum should be monitored in patients with renal insufficiency, particularly when other beta-lactam antibiotics are being coadministered, and during the treatment of infections caused by organisms with high MICs to the antibiotic.

Traditionally, ticarcillin levels in serum have been determined by microbiological assays, primarily the agar well diffusion (AWD) bioassay (1, 5, 6, 8, 11, 13, 15–18). Although cost-effective, these assays often lack the specificity and precision associated with biochemical assays or immunoassays for determining levels of antibiotics and require a minimum of 8 h of incubation, eliminating the possibility of dosage adjustment within the dosage interval. High-pressure liquid chromatographic (HPLC) procedures for determining ticarcillin concentrations have been reported for the quantitation of ticarcillin and other selected penicillins and cephalosporins in pharmaceutical preparations (4) and for the measurement of ticarcillin in serum and urine (7). While both assays are useful for the quantitation of ticarcillin, the former does not employ an internal standard to reference for quantitation, and the latter assay is cumbersome for application in a clinical laboratory. We therefore developed an HPLC method with a reverse-phase C18 µBondapack column with internal standard quantitation which is applicable for routine use in the clinical laboratory.

MATERIALS AND METHODS

Reagents. Ticarcillin and temocillin were provided by Beecham Laboratories, Bristol, Tenn. Methanol and chloroform were obtained from Mallinkrodt, Inc., St. Louis, Mo. Glacial acetic acid, hydrochloric acid, n-amyl alcohol, ammonium acetate, and sodium dihydrogen phosphate were purchased from Fisher Scientific Co., Fair Lawn, N.J. Dibasic sodium phosphate was obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. The mobile phase for HPLC analysis was an 85:15 volume mixture of ammonium acetate-methanol. The ammonium acetate was prepared at a concentration of 0.1 M and adjusted to pH 4.0 with glacial acetic acid. Reagent solutions for sample extraction were prepared in bulk and stored at room temperature. These included the following: 0.4 N hydrochloric acid, chloroform-n-amyl alcohol (3:1), and 0.1 M phosphate buffer (pH 7.0). The phosphate buffer was diluted 1:10 with water before use. All deionized water used in reagent preparation was further purified by passage through a Nanopure water purification system (Sybron/Barnstead, Boston, Mass.).

Standards and controls. Ticarcillin disodium (potency, 787 µg/ml) was reconstituted in sterile deionized water and diluted in heat-inactivated pooled normal human serum (Flow Laboratories, McLean, Va.) to prepare standards with the following concentrations: 50, 100, 200, and 400 µg/ml. A control serum, containing 250 µg of ticarcillin per ml, was prepared in the same manner. Standards and control sera were dispensed into 0.5-ml aliquots and stored at −70°C. Temocillin (potency, 730 µg/ml) was reconstituted and diluted in sterile deionized water to a final concentration of 150 µg/ml. This was divided into aliquots that were stored at −70°C. Standards, control serum, and internal standard solution of temocillin were thawed on the day of use and discarded at the end of the day.

Sample preparation. A modification of the procedure of Brisson and Fourtillan (2, 3) for the extraction from serum of piperacillin and several cephalosporins was used. Serum samples, standards, and control serum were prepared for analysis by combining 0.35 ml of sample, 0.15 ml of internal standard solution, 0.25 ml of 0.4 N HCl, and 3.5 ml of chloroform-n-amyl alcohol in a screw-cap test tube (16 by 100 mm), which was covered with a Teflon-lined screw cap. Samples were mixed for 5 min and then centrifuged for 5 min. The upper aqueous layer was discarded, and the organic layer was transferred to a screw-cap test tube (113 by
100 mm). Phosphate buffer (0.35 ml) was added to the organic layer, and the samples were capped with Teflon-lined caps. Samples were again mixed for 5 min and centrifuged for 5 min. The upper aqueous layer was removed to a 500-μl polypropylene tube and stored at 4°C until analysis.

**HPLC instrumentation and parameters.** A Waters solvent delivery system (model 6000A) and a Waters variable wavelength detector (model 450) were used with a reverse-phase Waters µBondapak C18 column (10 μm; inner diameter, 30 cm by 3.9 mm) (Waters Associates, Inc., Milford, Mass.). Samples were injected with a Rheodyne syringe loading sample injector (model 7125) fitted with a 20-μl loop (Alltech Associates, Inc., Applied Science Div., State College, Pa.). Flow rate was set at 1.8 ml/min, and A242 was monitored. Detector sensitivity was set at 0.04 absorbance units full scale. A linear recorder (Alltech Associates) set at a chart speed of 30 cm/h was used. A 20-μl sample was injected for analysis.

**Quantitation.** A standard curve was generated by plotting the ratio of the height of the ticarcillin peak to that of the internal standard peak against the established concentrations of the standards. Least squares linear regression analysis was used to generate the curve and calculate concentrations in the control and patient samples.

**Extraction efficiency.** Absolute recovery of both ticarcillin and the internal standard was evaluated by comparing peak heights from extracted sera with peak heights of aqueous preparations of ticarcillin and the internal standard at the same concentrations. Percent recovery was calculated by dividing the height of the serum sample peak height by that of the aqueous sample peak. Relative recovery was evaluated by assaying sera spiked with known ticarcillin concentrations. Percent recovery was calculated by dividing the assayed concentrations by the target concentrations in the samples.

**Specificity.** Ninety-three compounds, consisting of antibiotics and other drugs, were evaluated for possible interference. When the aqueous or methanolic preparation of a compound exhibited possible interference, then serum, spiked with the compound, was prepared for analysis and examined for the presence of interfering peaks. Also, sera from patients not receiving ticarcillin but receiving a variety of other drugs were extracted and examined for the presence of interfering peaks.

**Precision.** Intraday reproducibility was evaluated by assaying at least 10 times each low (75 μg/ml), mid (150 μg/ml), and high (300 μg/ml) concentration of ticarcillin in pooled normal human sera. Interday reproducibility was evaluated by assaying 10 times these same concentrations over a 3- to 4-week period.

**Microbiological assay.** Ticarcillin was assayed by an agar well diffusion assay, with a clinical isolate of *Providencia stuartii* as the test organism. The assay medium was antibiotic medium 1 (Difco Laboratories, Detroit, Mich.) to which p-aminobenzoic acid (Sigma Chemical Co., St. Louis, Mo.) and thymidine (Sigma) were added. Standards and control serum were prepared as described above for the HPLC assay.

**Specimens.** Patient sera utilized in this study were those on which ticarcillin levels had been ordered as part of the clinical management of the patients. Sera were analyzed by both methods on the same day. Sera from patients not receiving ticarcillin were obtained from specimens sent to the laboratory for other drug level determinations.

**Data analysis.** Least squares linear regression analysis was used to determine the correlation between the microbiological assay results and the HPLC assay results.

**RESULTS**

Typical chromatograms of the internal standard and ticarcillin extracted from serum are shown in Fig. 1. The retention times for the internal standard and ticarcillin were 5.4 and 6.8 min, respectively. Of the 93 compounds tested for interference, three cephalosporins were found to elute with ticarcillin or close to it. Cefuroxime exhibited a retention time identical to that of ticarcillin, while cefoxitin eluted with a retention time of 7.4 min. Complete base-line resolution between the ticarcillin peak and the cefoxitin peak did not occur, but ticarcillin levels could be determined in the presence of cefoxitin. The first of two peaks associated with cephalothin exhibited a retention time similar to that of ticarcillin. Some of the more commonly administered drugs were tested without indication of interference. Included were amikacin, gentamicin, tobramycin, chloramphenicol, vancomycin, rifampin, clindamycin, trimethoprim, nafcillin, ampicillin, penicillin, methicillin, moxalactam, cefotaxime, cefamandole, cefusoladin, cefazidime, cefotizoxide, cefoperazone, 5-fluorocytosine, metronidazole, sulfamethoxazole,
piperacillin, mezlocillin, dicloxacillin, thienamycin, allopurinol, acetazolamide (Diamox), acetaminophen, caffeine, flurazepam, chlorpromazine, procainamide, theophylline, and phenobarbital. No endogenous interferences were found in sera from patients not receiving ticarcillin.

Absolute recovery of ticarcillin from serum over the concentration range of 29 to 385 μg/ml averaged 71%, while the recovery of the internal standard averaged 67%. Relative recovery of ticarcillin from serum over the concentration range of 75 to 300 μg/ml was found to average 97%. Reproducibility studies yielded coefficients of variation of less than 4% for low, mid, and high concentrations of ticarcillin in serum (Table 1). Figure 2 shows a typical standard curve generated by the HPLC assay. During the 3-month period of the correlation study, the correlation coefficients for the HPLC standard curves ranged from 0.997 to 1.000, whereas the AWD standard curve correlation coefficients ranged from 0.990 to 1.000. Coefficients of variation for the control serum containing 250 μg/ml of ticarcillin, ranged from 3.37 to 5.70% for the HPLC assay and 6.29 to 6.76% for the AWD assay.

Figure 3 shows the correlation between the HPLC assay and the AWD assay. Linear regression analysis of the data yielded a correlation coefficient of 0.9885 for the 93 sera assayed by both methods. The slope of the regression line was 0.85, and the intercept was 13.51.

### TABLE 1. Ticarcillin HPLC assay reproducibility studies

<table>
<thead>
<tr>
<th>Ticarcillin target value (μg/ml)</th>
<th>Intraday reproducibility</th>
<th>Interday reproducibility</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean (μg/ml)</td>
</tr>
<tr>
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<td>11</td>
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<tr>
<td>150</td>
<td>10</td>
<td>140.9</td>
</tr>
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<td>300</td>
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*CV, Coefficient of variation.

### DISCUSSION

An accurate, reproducible HPLC method for determining levels of ticarcillin in serum has been developed. Although the AWD assay is adequate for the majority of routine ticarcillin level determinations in our laboratory, the HPLC method has been particularly useful when patients are receiving multiple antibiotic therapy or when rapid result turnaround is necessary.

Prior to developing this assay, several HPLC procedures for ticarcillin quantitation were evaluated. In addition to being accurate, precise, and specific, the assay needed to be easily incorporated into the routine work flow of the laboratory and readily performed by several technologists. The HPLC assay of Kwan et al. (7) offered accuracy and precision, but we felt that there were certain disadvantages in the methods. The relatively low and very precise pH (2.05) of the mobile phase necessary to separate ticarcillin and the internal standard was one such disadvantage as was the extraction procedure which involved multiple steps, including evaporation of the extract and shaking in a water bath. This procedure also resulted in ticarcillin appearing as only one peak on the chromatogram, whereas with the mobile phase described by Das Gupta and Stewart (4), ticarcillin presented as two peaks, possibly representing positional isomers of the drug. Several of the mobile phases tried in the developmental stages of our work resulted in double peaks...
for ticarcillin. Lowering of the pH to 4.0 resulted in the fusion of the two peaks, which facilitated chromatogram interpretation.

The use of the internal standard method for quantitation was necessary to overcome sample-to-sample extraction variations. Temocillin was first evaluated as an internal standard because of its structural similarity to ticarcillin. The chromatographic characteristics and recovery in the extraction procedure were also found to be comparable to ticarcillin, thus making it an excellent choice for the internal standard. Although the extraction procedure presented here recovers only approximately 70% of ticarcillin and temocillin, this method was chosen because of its applicability for the extraction of other penicillins and cephalosporins as described by Brisson and Fourtillan (2, 3). This permits multipurpose reagents and supplies and reduces teaching time needed for training staff members in the HPLC procedures.

The procedure allows for the quantitative analysis of ticarcillin in serum in patients receiving multiple antibiotics with analysis times which permit dose adjustments within the dosing interval. The assay permits the monitoring of ticarcillin for both efficacy in maintaining serum concentrations above the MICs for infecting organisms and for toxicity in patients receiving multiple beta-lactam antibiotics during renal insufficiency.

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LITERATURE CITED