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Note

Analysis of piperacillin using high-performance liquid chromatography

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Piperacillin sodium, a new semisynthetic penicillin derivative, has been shown to be effective in the treatment of many serious infections associated with gram-positive and gram-negative organisms including anerobes [1,2]. The ability to study the disposition and elimination of this antibiotic is dependent on accurate, sensitive, and specific methods to measure it in biological fluids. It is also desirable to monitor levels of piperacillin during therapy of certain serious infections caused by organisms in which the inhibitory concentration is relatively high. In some instances piperacillin concentration must be determined in the presence of other antimicrobial agents. Published studies [3—5] have employed microbiological assays to measure piperacillin in biological fluids. However, these assays are relatively cumbersome and potentially subject to interference from concurrently administered antibiotics.

The purpose of this paper is to describe a rapid high-performance liquid chromatographic (HPLC) assay which requires 200 μl of sample and is not subject to interference from other commonly used antibiotics such as penicillin-G, ampicillin, chloramphenicol, gentamicin, and kanamycin.

MATERIALS AND METHODS

Chromatography

Assays were performed using a Perkin-Elmer Series 2 liquid chromatograph equipped with an LC75 variable-wavelength detector and interfaced to a Sigma-10B data system (Perkin-Elmer Corporation, Norwalk, CT, U.S.A.). All analyses were performed on a 10-μm Waters μBondapak C_{18} 30 × 3.9 cm column (Waters Assoc., Milford, MA, U.S.A.) maintained at 50°C. A guard column packed with 10 μm size C-18 pellicular material (Supelco, Bellefonte, PA,

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U.S.A.) was installed between the injector port and the analytical column. The flow-rate was 2.0 ml/min and the effluent was monitored at 230 nm.

Reagents

Piperacillin sodium salt was supplied by Lederle Labs. (Pearl River, New York, NY, U.S.A.). 5-Ethyl-5-p-tolylbarbituric acid (ETBA) was purchased from Applied Science Labs. (State College, PA, U.S.A.). Methanol and acetonitrile were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). All other chemicals were reagent grade.

The mobile phase was 22% acetonitrile in 0.1 *M* sodium acetate buffer, adjusted to a pH of 4.6 with a few drops of glacial acetic acid. This solution was freshly prepared and degassed under vacuum just prior to use.

A stock solution of 1.0 g/l piperacillin was prepared by dissolving an appropriate amount of piperacillin salt in methanol. The stock standard was then diluted with drug-free serum, urine, or cerebrospinal fluid (CSF) to provide assay standards over a concentration range of 1.0—200 mg/l. The internal standard (20 mg/l ETBA in methanol) was also used as the protein-precipitating agent. This solution was stored at −10°C and kept ice cold during sample processing.

Procedure

Two hundred microliters of sample (serum, CSF, or diluted urine) were placed in a 10 × 75 mm disposable glass tube. Depending on the time of urine collection in relation to the time of the dose, urine dilution ranged from 1:100 at peak time (0.1 h post dose) to 1:10 (3—4 h post dose). Two hundred microliters of ice-cold methanol containing the internal standard were added to the tube, which was then vortexed for 30 sec. The samples were placed on ice for 5 min, then centrifuged for 15 min at 1600 *g* in a refrigerated centrifuge. The clear supernatant was transferred to a clean 10 × 75 mm test tube and kept on ice until analyzed. Thirty microliters of supernatant were injected onto the column.

RESULTS

Fig. 1 shows typical chromatograms of (A) drug-free serum containing the internal standard; (B) drug-free serum reconstituted with 20 mg/l piperacillin and the internal standard; and (C) a patient sample in which the determined concentration of piperacillin was 43.8 mg/l. The retention times for piperacillin and internal standard were 3.7 and 6.5 min, respectively. The concentration of piperacillin was calculated from the integrated area under the peaks and was linearly related to the internal standard area over the concentration range 1.0—200 mg/l. Standard curves were identical with serum, urine, or CSF. The mean recovery of piperacillin from serum samples was 85%.

Within-run precision was evaluated by processing aliquots of a prepared piperacillin serum pool and day-to-day precision was evaluated by assaying samples on consecutive days (Table I). Stability studies were also conducted using drug-free sera reconstituted with the drug. Aliquots of these samples were frozen at −70°C and analyzed over a 3.5-month period. As shown in Table II,

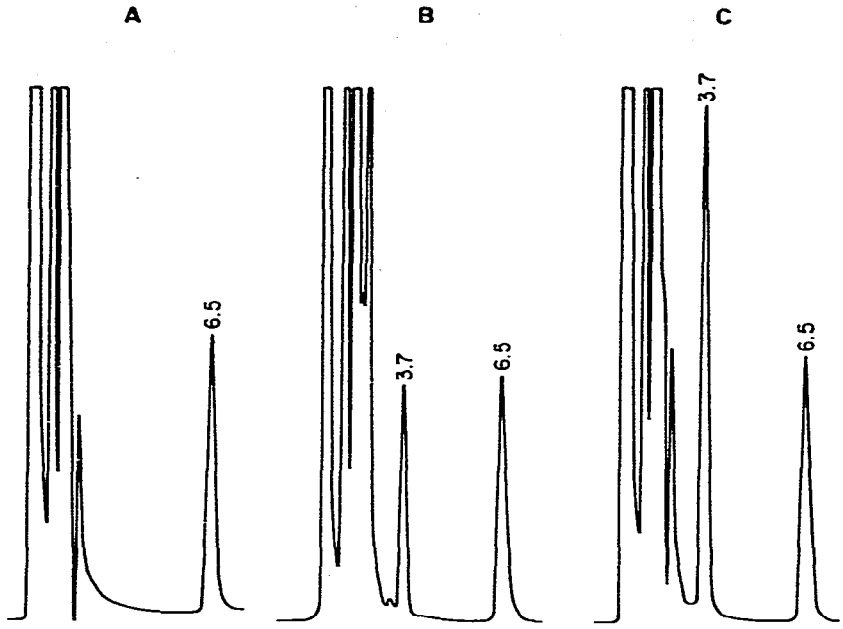

Fig. 1. Chromatograms obtained from (A) drug-free serum containing the internal standard, (B) drug-free serum reconstituted with 20 mg/l piperacillin, and (C) patient's serum in which the determined concentration of piperacillin was 43.8 mg/l. The retention times for piperacillin and the internal standard were 3.7 and 6.5 min, respectively. The early eluting peaks are unidentified extractants which do not interfere with the assay.

TABLE I

PRECISION OF SERUM PIPERACILLIN ANALYSIS

	Within-run	Day-to-day
Amount added (mg/l)	20.0	50.0
Amount found		
Mean (mg/l)	20.7	50.0
S.D. (mg/l)	1.3	3.7
C.V. (%)	6.1	7.4
n	14	32

TABLE II

STABILITY OF PIPERACILLIN

The samples were stored frozen at −70°C.

Day	Value (mg/l)	Day		Value (mg/l)
1	20.0	48		20.7
8	22.3	50		24.3
9	19.1	73		25.7
10	18.6	106		25.0
31	20.8		Mean:	21.8
			S.D.:	2.6

there was no appreciable change in drug concentration. The accuracy of the method was further validated by blindly assaying five serum samples containing 0.0—133.0 mg/l piperacillin which were supplied by Lederle Labs. The results of these analyses are shown in Table III.

TABLE III

ANALYSIS OF PIPERACILLIN BLIND-CHECK SAMPLES

$r = 0.99$.

	Assay concentration (mg/l)	Actual concentration (mg/l)
Sample 1	142.2	133.0
Sample 2	13.2	13.0
Sample 3	86.8	83.0
Sample 4	60.0	60.0
Sample 5	N.D.	N.D.

DISCUSSION

Measurement of antibiotic concentrations has traditionally depended on bioassays using a sensitive strain of bacteria. However, bioassays are increasingly being replaced by non-biological assays which offer distinct advantages. HPLC has proved to be one of the more practical and popular assay tools for this purpose. Antibacterial bioassays generally require a period of incubation by which the assay time can range from 4 to 12 h or more. Sample preparation and chromatography, however, typically require no more than 10 to 20 min per sample. Bioassays tend to be relatively nonspecific and subject to random variation inherent in biological systems. In contrast, HPLC conditions can be standardized to provide a high degree of reproducibility and specificity. This is particularly advantageous when the compound in question must be measured in the presence of concurrently administered antibiotics.

The method described here offers several advantages over traditional bioassays. The required sample size is small, a particularly important consideration when working with infants and children. The specificity, sensitivity, and accuracy of the method make it useful for pharmacokinetic studies. In addition, short assay time and simplicity make the method attractive for the routine monitoring of piperacillin levels during therapy. This method may be readily instituted in any laboratory with HPLC capability.

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