High-Performance Liquid Chromatographic Determination of Piperacillin in Plasma

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Received 3 August 1981/Accepted 21 January 1982

A high-performance liquid chromatographic method has been developed for the quantitative determination of piperacillin in plasma. The compound is first extracted from acid-treated plasma into chloroform: 1-pentanol (3:1) and reextracted into a small volume of an aqueous phase at pH 7, which is injected in the chromatographic column (μ-Bondapack C18; 10 μm). The mobile phase is a mixture of 0.01 M acetate buffer (pH 4.8) and methanol. The method is accurate and reproducible with a sensitivity of about 50 ng of piperacillin per ml of serum.

Piperacillin {sodium 6-[D(-)-α(4-ethyl-2,3-dioxo-1-piperazinyl- carbonyl- amino) -α -phenylacetamido] penicillinate} is a new semisynthetic penicillin with broad spectrum activity against gram-positive and gram-negative organisms (3, 7, 9), including *Pseudomonas aeruginosa*, *Proteus* sp. and *Klebsiella* sp. Pharmacokinetic studies were performed by conventional microbiological assay procedure (2, 4–6, 8). However, a rapid and accurate assay method is essential to such studies.

Recently, we have developed a method for the determination of cephalosporins in biological fluids (1; A. M. Brisson, Ph.D. thesis, University of Poitiers, France, 1980). The present paper describes the application of such a method for the isolation and rapid, specific, quantitative assay of piperacillin by reversed-phase high-performance liquid chromatography.

Pure piperacillin was kindly provided by Lederle Laboratories (Paris, France). Reagent grade acetic acid, sodium acetate, hydrochloric acid, potassium dihydrogen phosphate, disodium hydrogen phosphate, chloroform, 1-pentanol (R. P. Normapur, Prolabo, Paris, France), and methanol (Üvasol grade, E. Merck AG, Darmstadt, Germany) were used without further purification.

A liquid chromatograph (model 1084A Hewlett-Packard, Paris, France) equipped with a 254-nm UV detector was used. The column was a reversed-phase μ-Bondapack C18 (10 μm; 30 cm by 3.9 mm inside diameter; Waters Associates, Paris, France).

A mixture of methanol and 0.01 M (pH 4.8) acetate buffer (40/60, [vol/vol]) was used as the chromatographic eluant at a flow-rate of 1 ml/min (pressure of 175 bars [1.75 × 10^6 dynes/cm²]). In the Hewlett-Packard liquid chromatograph, the solvents were degassed. We filtered (and degassed) the mobile phase with Millipore HA 0.45-μm filters (Millipore Corp. Bedford, Mass.) The mobile phase was maintained at 40°C.

Stock of piperacillin (2 mg/ml) was prepared in double-distilled water by dissolving 22.02 mg/10 ml (potency of the powder, 908 μg/mg). After appropriate dilution in double-distilled water, the piperacillin was added to plasma to prepare standards at levels ranging from 0.25 to 100 μg/ml.

The plasma sample (1 ml) was mixed with 0.50 ml of 0.4 M hydrochloric acid in a 10-ml glassstopped centrifuge tube. Piperacillin was extracted with 7 ml of chloroform–1-pentanol (3:1). After 5 min of extraction, the mixture was centrifuged for 5 min at 1,000 × g, and 5 ml of the organic phase was transferred into another centrifuge tube. Piperacillin was extracted over 5 min in 350 μl of phosphate buffer (pH 7). After centrifugation for 5 min at 1,000 × g, part (20 to 50 μl) of the (upper) aqueous phase was injected into the chromatographic column.

Standards of known piperacillin contents were made up in pooled human serum, and standard curves were prepared by injecting extracts of plasma in the high-pressure liquid chromatographic column and determining peak heights.

The retention time of piperacillin was found to be 5.25 min, and no overlap with peaks of blank plasma was observed. Figure 1 shows three chromatograms obtained with plasma from a healthy adult: blank plasma, plasma containing 50 μg of piperacillin per ml, and plasma drawn 30 min after a 5-min intravenous infusion of 1 g of piperacillin.

The linearity of the method was determined for concentrations ranging from 0.25 to 100 μg/ml. Analysis of plasma samples at concentrations of 2.5 to 20 μg/ml and 0.25 to 2 μg/ml gave linear regression coefficients of 1.00 and 0.9998, respectively, for the lines, with equa-
of us to icrs, prepared with y typtions of y = 6.55x + 1.28 and y = 60x − 1.95, where y is the peak height and x is the piperacillin concentration in micrograms per milliliter of plasma.

The precision of the method was studied by multiple assays performed on plasma samples containing 5 μg of piperacillin per ml. The peak heights for 10 samples ranged from 59.5 to 63 mm (mean ± standard error, 61.5 ± 0.34). The coefficient of variation was 1.76%.

The sample recovery of piperacillin from spiked solutions prepared with plasma was compared with that prepared with water; recovery was about 84%.

Our experience in cephalosporins permitted us to develop the present method for the determination of piperacillin, which is similar in structure to cefoperazone. In the two antibiotics, we found the same substituent: (4-ethyl-2,3-dioxo-1-piperazinylcarbonylamino)-α-phenylacetamido on the cepham nucleus for cefoperazone and on the 6-amino penicillinic acid moiety for piperacillin, except for the hydroxy phenyl.

The other antibiotics, except cephalosporins, are not detected by this chromatographic process. Oral cephalosporins (cefadroxil, cefadroxil, moxalactam, ceforanide, ceftazidime, and cefotiam are not extracted in the same conditions. Interferences with eight cephalosporins, extracted by our two-step technique (cefopeperazone, cefazolin, cephalotin, cefamandole, cefoxitin, cefuroxime, and cefotaxime) were studied; the retention times were lower than those for piperacillin.

In conclusion, the high-pressure liquid chromatographic method described permits the rapid determination of piperacillin in plasma at concentrations as low as 50 ng/ml. The preparation of plasma sample before chromatography is simple, requiring only a two-step extraction. The precision of the method is good, and no interfering peaks are seen with plasma. This method is therefore suitable for the routine determination of piperacillin and in research studies involving pharmacokinetics.

LITERATURE CITED