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High-Pressure Liquid Chromatographic Assay of Ceftizoxime with an Anion-Exchange Extraction Technique

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An anion-exchange extraction method was used in conjunction with high-pressure liquid chromatography for assay of ceftizoxime in 181 serum samples. Comparison of this method with bioassay gave a linear regression line described by $Y = 1.11 + 0.98\,X$, with a correlation coefficient of 0.984. The anion-exchange extraction method is a fast, reliable method of preparing serum samples containing ceftizoxime for assay by liquid chromatography.

Ceftizoxime (FK 749) is a new, extended-spectrum cephalosporin which is prepared as the desacetoxymethyl derivative of cefotaxime (2). We have previously developed a technique of anion-exchange extraction for high-pressure liquid chromatography (LC) of cephapirin, cefotaxime, and cefoxitin in serum (1). The purpose of this report is (i) to apply this LC method to ceftizoxime and (ii) to compare quantitative results of the LC and microbiological assays.

Anion-exchange columns were prepared by using 6.0-ml syringe barrels (Monoject, St. Louis, Mo.) with a 12.7-mm filter paper disk (Schleicher & Schuell Co., Keene, N.H.) placed to retain Sephadex. DEAE–Sephadex A-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) in pH 7.2 phosphate-buffered saline (PBS) (8.0 g of NaCl, 1.15 g of Na_2HPO_4, 0.2 g of KCl, and 0.2 g of KH_2PO_4 dissolved in 1 liter of distilled water) was used to fill the syringe barrels, which were packed to a bed volume of 3.0 ml and washed with PBS. Another 12.7-mm filter paper disk was added to the top of the column to prevent the bed from being disturbed when sample or reagents were added. After column preparation, 0.5 ml of a serum sample was placed on the column, followed by 0.5 ml of PBS. Protein and other serum interferences were removed by the addition of 4.0 ml of PBS (pH 7.2). The ceftizoxime retained on the Sephadex column was eluted by using 5.0 ml of 1.0 M sodium chloride. A 100-μl volume of this final eluate was injected directly into the chromatographic system via a completely filled fixed-loop sampling valve (1). The result for each sample was the average value of two injections. Ceftizoxime (20 μg/ml) in pooled human serum, prepared by passing through anion-exchange columns in the same manner as the unknown samples, was used as the external standard. The detector response was linear to a concentration of at least 100 μg/ml. Recovery of ceftizoxime from the DEAE–Sephadex A-25 columns was >95%, which is similar to that for cephapirin, cefotaxime, and cefoxitin (1). A Varian LC 5020 (Varian Associates, Inc., Walnut Creek, Calif.) liquid chromatograph with a Varichrom variable-wavelength detector and a CDS 111L peak integrator with a strip chart recorder was used in this investigation. The analytical column was a μ-Bondapak-C18 (Waters Associates, Inc., Milford, Mass.) with a 30-cm length, 3.9-mm inside diameter, and 10-μm particle size. The mobile phase had a flow rate of 2.0 ml/min at isocratic conditions of 13% acetonitrile (high-pressure liquid chromatography grade) and 87% dilute acetic acid (pH 2.8). The acetic acid component was prepared by adding enough acetic acid to deionized water to reach pH 2.8 (approximately 1.5 ml/liter) and then filtered, using a 0.45-μm MF-Millipore membrane (Millipore Corp., Bedford, Mass.). The detector monitored column eluate at 270 nm, using 0.05 absorbance units as the full-scale sensitivity setting. With these conditions, ceftizoxime gave a retention time of 4.2 min. The microbiological assay for ceftizoxime was performed in triplicate by using an agar-well technique (3) with a 24-h incubation at 37°C. The indicator organism was *Escherichia coli* ATCC 10536 (American Type Culture Collection, Rockville, Md.) seeded as a 0.25% suspension into antibiotic medium no. 1 at pH 6.6 (Difco Laboratories, Detroit, Mich.). Ceftizoxime standards were prepared in pooled human serum.

A total of 181 samples were analyzed from 13 subjects who received ceftizoxime intravenously. Each serum sample collected was divided in two and frozen. These samples were stored at −80°C until the assays were performed. Samples were diluted before assay when they were esti-

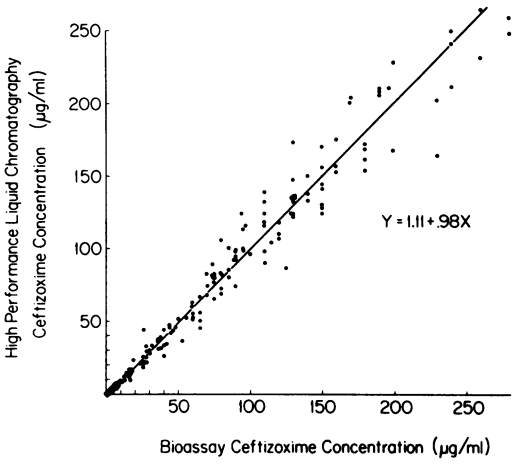

FIG. 1. Scatter plot comparison of assay methods and analysis, using least squares regression line.

mated to contain an antibiotic concentration greater than 100 μg/ml for the LC assay or 40 μg/ml for the bioassay. Linear regression analysis is shown in Fig. 1. The correlation coefficient was 0.984, and the paired t test gave $t = 0.6382$ with 180 df. This is equivalent to no significant difference between the methods ($P = 0.05$).

The results of the linear regression analysis demonstrate the LC assay has a statistically equivalent direct relationship with the microbiological assay. The correlation coefficient reflects the close agreement of sample results obtained by the two methods. Our previous investigation of the anion-exchange extraction technique indicated that this method reliably removed serum interferences and that 22 commonly used drugs did not give any interferences during the LC separation (1). These findings indicate that the LC assay method, using the same anion-exchange column extraction described for cephapirin, cefotaxime, and cefoxitin, can be successfully applied to the assay of ceftizoxime in human serum.

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