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Liquid Chromatographic Assay of Ceftizoxime in Sera of Normal and Uremic Patients

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The application of high-pressure liquid chromatography assays for cephalosporin serum concentrations is difficult in uremic patients because of interference from nondialyzable substances. We developed a high-pressure liquid chromatography method for determining the serum concentration of ceftizoxime in normal and uremic patients. The method involves protein precipitation with acetonitrile, followed by removal of the acetonitrile with dichloromethane. Separation was accomplished with a reverse-phase (C-18) column and a mobile phase of 13% acetonitrile and 2.8% acetic acid. UV detection at 310 nm was used to monitor the peaks. This assay produced a linear relationship between peak height ratio and ceftizoxime concentration from 1.5 to 100 μg/ml. Samples from 30 patients were assayed by this method and by a bioassay, with a good correlation of results ($r = 0.9832$). The method was applicable equally to normal and uremic serum samples.

Ceftizoxime (FK-749) is a new broad-spectrum semisynthetic cephalosporin antibiotic with beta-lactamase stability. Ceftizoxime is not metabolized and is cleared from the body primarily by the kidneys by both filtration and secretion. For this reason, measurement of serum levels may be desirable in the management of patients with decreased renal function.

Various biological assays have been reported for the quantitation of ceftizoxime in serum; however, this type of assay is generally time-consuming and subject to interference from other antibiotics. High-pressure liquid chromatography (HPLC) assays for the measurement of antibiotics have definite advantages over bioassays in precision, specificity, and rapidity.

Numerous HPLC assays (8) have been developed for the currently available cephalosporins; most of these use UV detection and either reverse-phase or ion-pair chromatography. The major differences are in sample preparation procedures. Protein precipitation with acids or organic solvents (7), anion-exchange columns (3), and extraction with various solvents (2) have all been used in sample clean-up. In addition to well-described difficulties with incomplete recovery and sample dilution, others have shown that serum samples from patients with end-stage renal disease (ESRD) have significant interferences when UV detection methods (5) are used.

The purpose of the present report is to describe an HPLC method for the quantitation of ceftizoxime which is simple, rapid, and, due to the wavelength used for detection, free from interference in samples from ESRD patients.

MATERIALS AND METHODS

Reagents. Acetonitrile, dichloromethane (both HPLC grade), and glacial acetic acid were obtained from Fisher Scientific Co., Pittsburgh, Pa. Ceftizoxime (free acid reference powder) was provided by Smith Kline & French Laboratories, Philadelphia, Pa. The internal standard, cefotaxime, was obtained from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Stock solutions (1,000 μg/ml) of both drugs were prepared in 0.1 M phosphate buffer, pH 6.0, and stored in aliquots at −70°C.

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Standards were prepared in normal human serum (Flow Laboratories, McLean, Va.) over a range from 1.5 to 100 μg/ml. A working internal standard solution containing 200 μg of cefotaxime per ml was prepared fresh daily by diluting the stock solution with distilled water.

Controls for assessing precision were prepared in pooled human sera at concentrations of 5.0 and 85.0 μg/ml and frozen at −70°C in aliquots.

Chromatography. Assays were performed on a Waters HPLC system. Components included an M-45 pump, a model 480 variable wavelength UV absorbance detector, and a C-18 μBondapak 30-by-3.9-cm analytical column (Waters Associates, Milford, Mass.). Injection was accomplished with a 20-μl loop injector (model no. 7125; Rheodyne Inc., Cotati, Calif.) and a 50-μl adjustable-volume, blunt-needle glass syringe (Hamilton Co., Reno, Nev.). Peaks were recorded on an Omniscribe strip chart recorder (Houston Instruments, Austin, Tex.). A 5 cm guard column packed with CO:PELL ODS (Whatman, Clifton, N.J.) was placed between the injector and the analytical column.

The mobile phase consisted of 130 ml of acetonitrile and 28 ml of glacial acetic acid diluted to a final volume of 1,000 ml with distilled water. This was prepared fresh daily and degassed under vacuum just before use. The flow rate was 1.5 ml/min, and the eluant was monitored at 310 nm with a sensitivity setting of 0.05 absorbance units, full scale. Operations were carried out at room temperature.

Procedure. A 200-μl volume of serum, 100 μl of working internal standard, and 1.0 ml of acetonitrile were placed in a disposable glass tube (13 by 100 mm). The mixture was vortexed for 5 s and centrifuged for 5 min at 30 × *g*. The protein-free supernatant was decanted into another tube (13 by 100 mm), 1.5 ml of dichloromethane was added, and the mixture was vortexed for 5 s and centrifuged for 5 min. A sample of 10 to 20 μl of the upper aqueous layer was injected.

Peak heights were measured manually, and linear regression analysis of the peak height ratio of ceftizoxime to internal standard was used to form a standard curve from which an unknown sample concentration could be determined.

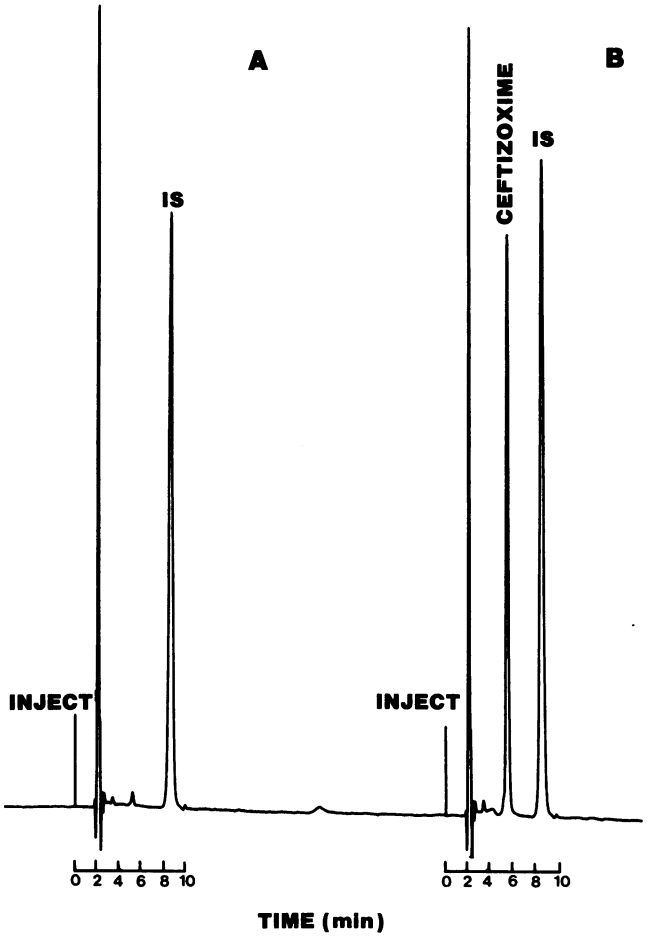

FIG. 1. HPLC analysis of ceftizoxime in sera of normal volunteers. (A) Predose sample with internal standard (IS); (B) postdose sample containing ceftizoxime at a concentration of 73 μg/ml.

RESULTS

Chromatography. Figure 1 shows typical chromatograms of predose and postdose sera of patients with normal kidney function. The retention times were 6.0 min for the ceftizoxime and 9 min for the internal standard. Fifteen sera from patients not receiving ceftizoxime were analyzed for possible interferences, and none was found. Figure 2 shows typical chromatograms of patients with ESRD. Fifteen sera from ESRD patients were tested, and all had interferences at 254 nm which could be eliminated by changing the wavelength to 310 nm.

Linearity. There was a linear relationship between peak height ratios and ceftizoxime concentration from 1.5 to 100 μg/ml. Samples with higher amounts of ceftizoxime could be diluted before assay. Linear regression analysis of a typical standard curve resulted in a line with the equation $y = 0.018x + 0.0162$; $r = 0.993$.

Precision. The precision of the method was determined by assays of pooled human sera spiked with ceftizoxime on the same day and over a period of several weeks (Table 1).

Specificity. Table 2 lists several cephalosporins that could also be detected by this assay. Other compounds which could not be detected at a concentration of 100 μg/ml were aminoglycosides (gentamicin, tobramycin, and amikacin), clindamycin, erythromycin, vancomycin, penicillin, cefoperazone, piperacillin, ticarcillin, carbenicillin, and apalcillin.

Method comparison. Results obtained by the HPLC method were compared with those previously obtained by bioassay for 30 serum samples (4) which had been stored at −70°C for up to 2 years. No change in bioactivity was detected. Bivariate regression analysis of the paired results revealed good correlation between the two methods ($r = 0.9832$) and insignificant bias (0.46 μg/ml).

Recovery studies. Relative and analytical recovery rates of ceftizoxime were evaluated by adding 50 μg of ceftizoxime per ml to distilled water and to serum and assaying each six times. The spiked water was assayed before and after extraction. Analytical recovery, as calculated by peak height ratio in a serum versus water matrix, was 98 ± 2%. The relative recovery of ceftizoxime was 106 ± 3% and the relative recovery of the internal standard was 107 ± 3.5%.

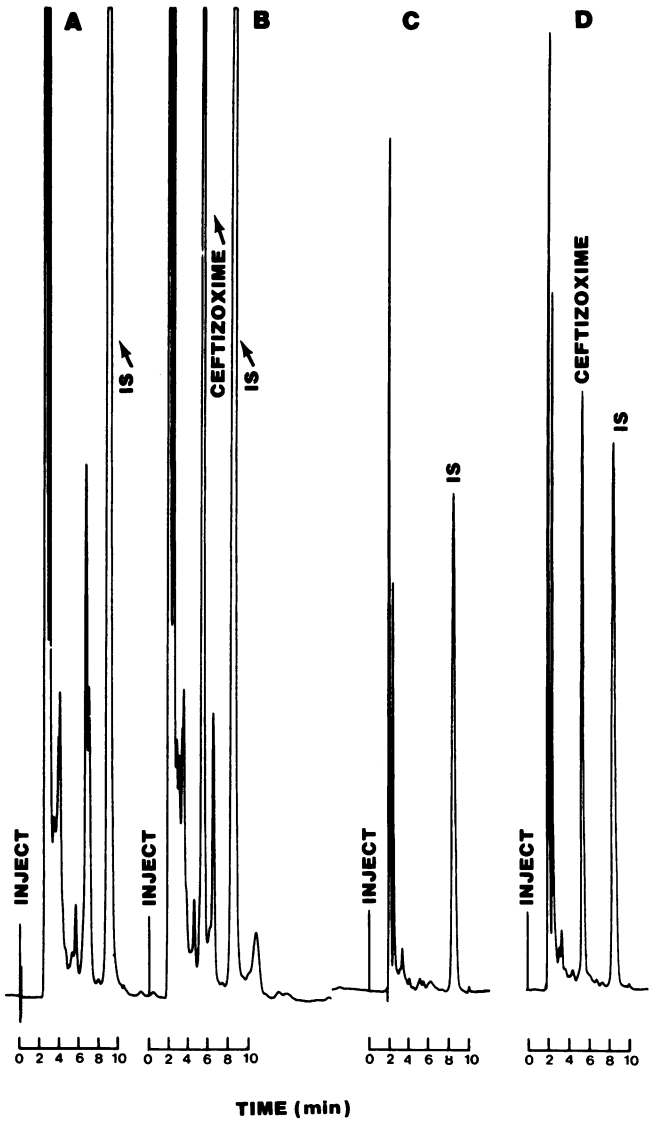

FIG. 2. HPLC analysis of ceftizoxime in sera of ESRD patients. Peaks are of (A) predose samples, and (B) postdose samples (λ = 254 nm), and (C and D) same samples analyzed at 310 nm, showing the elimination of interferences. Concentration of ceftizoxime is 87 μg/ml.

TABLE 1. Precision of ceftizoxime determination

Run	Ceftizoxime added (μg/ml)	Ceftizoxime detected (μg/ml)			
		Mean	SD	Coefficient of variation (%)	n
Within day	5.0	5.2	0.17	3.2	15
	85.0	84.0	2.3	2.7	15
Day to day	5.0	5.3	0.23	4.3	11
	85.0	84.0	2.8	3.3	12

TABLE 2. Retention times of ceftizoxime and other antibiotics

Antibiotic	Retention time (min)
Ceftriaxone	4.4
Cephalexin	5.2
Cephapirin	5.5
Cefazolin	6.0
Ceftizoxime	6.0
Moxalactam	7.5
Cephaloridine	8.0
Cefotaxime	9.0
Cefoxitin	18
Cefamandole	32

DISCUSSION

We found that the development of an HPLC assay for ceftizoxime in the sera of ESRD patients was complicated by interfering peaks. Two previous reports of HPLC assays for ceftizoxime (3, 6) do not discuss any interference found in sera of ESRD patients; however, neither stated whether any such specimens were assayed. A similar problem was observed in measuring moxalactam in the sera of ESRD patients by HPLC (5). The interfering peaks were observed in the sera of all ESRD patients and did not seem to be related to medications, but rather to the inefficiency of dialysis. In our assay, optimization of mobile-phase pH and detector wavelength was used to eliminate these interferences. Decreasing the pH of the mobile phase to 2.8 (below the pK_a [1] of ceftizoxime) resulted in an increase in the retention time of the ceftizoxime without affecting the interfering peaks, and changing the wavelength from 254 to 310 nm eliminated any interferences from endogenous compounds (1). Although the absorbance of ceftizoxime at 310 nm is only about one-third of that at 254 nm, the loss in sensitivity was easily compensated for by the increased peak efficiency obtained with the low pH mobile phase. Use of a mobile phase with a higher pH resulted in considerable peak tailing. The low pH had not affected column efficiency after 400 samples were analyzed.

Deproteinization of serum with acetonitrile has been used by other workers to avoid any pH extremes of acid precipitation which might effect the stability of ceftizoxime. The protein-free extract containing acetonitrile must be modified to reduce the percentage of acetonitrile. We chose to remove the acetonitrile with dichloromethane because dichloromethane extraction did not dilute the sample. The resulting aqueous layer contained ceftizoxime in approximately the same concentration as that of the original serum specimen, and therefore allowed a more sensitive determination of ceftizoxime.

Analytical evaluation of the assay demonstrates the precision, linearity, accuracy, and recovery of the method. The only antibiotics tested that interfered with the assay were cefazolin and cephapirin, which would not normally be administered concomitantly with ceftizoxime. The proposed method is applicable to therapeutic monitoring of ceftizoxime as well as to pharmacokinetic studies.

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