Liquid-Chromatographic Assay of Cefamandole in Serum, Urine, and Dialysis Fluid

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We describe a "high-performance" liquid-chromatographic assay for quantifying cefamandole in biological fluids from patients with renal impairment. Serum samples are deproteinized with acetonitrile, then extracted with dichloromethane; dialysis-fluid samples are injected directly; urine samples are diluted appropriately before injection onto the reversed-phase column. The mobile phase is a methanol/aqueous solution (31/69 by vol) containing 500 μL of phosphoric acid, 20 mmol of sodium sulfate, and 200 μL of triethylamine per liter, the mixture being adjusted to pH 6.0 with NaOH. Retention time for cefamandole is 12 min. Its peak is well resolved in highly contaminated samples from renally impaired subjects. The assay's selectivity, reproducibility (within-day and between-day CVs <8% in all three sample fluids), and sensitivity—0.5 mg/L in serum, 1.0 mg/L in dialysis fluid, and 5.0 mg/L in urine—make it applicable to pharmacokinetic studies.

Additional Keyphrases: chromatography, reversed-phase, cephalosporin antibiotics, pharmacokinetics, kidney disease

When the second-generation cephalosporin antibiotic cefamandole was introduced, it was quantified in biological samples by microbiological techniques (1–3). Subsequently, "high-performance" liquid-chromatographic procedures for this assay have been developed (4–11). These procedures are useful in measuring cefamandole concentrations in subjects with normal renal function, or in the one case reported (9), to measure relatively high concentrations of cefamandole in dialysis fluid from renally impaired subjects. Using these existing procedures, we were unable to quantify this drug in biological fluids from subjects who were undergoing continuous ambulatory peritoneal dialysis when cefamandole was administered intravenously, because of the high concentrations of interfering components relative to the concentrations of cefamandole. Assay was especially difficult in dialysis fluid, where peak concentrations of cefamandole are usually <15 mg/L after a 1.0-g intravenous infusion. The concentrations of interfering compounds in dialysis fluid are similar to those found in urine samples; the high cefamandole concentration in the latter, however, allows dilution, helping to minimize background.

The technique described here is highly reproducible and specific for the drug. For urine and dialysis fluid there is no extraction procedure. For serum, extraction with dichloromethane after a protein-precipitation step obviates sample dilution, thus increasing sensitivity. No internal standard is required, and the same mobile phase can be used to measure cefamandole in serum, urine, and dialysis fluid. We also present data to show the utility of this assay for pharmacokinetic studies in renally impaired patients.

Materials and Methods

Reagents. Cefamandole lithium and cefamandole nafate were obtained from Eli Lilly & Co., Indianapolis, IN 46205; sodium sulfate and triethylamine from Sigma Chemical Co., St. Louis, MO 63178; phosphoric acid from J.T. Baker Chemical Co., Phillipsburg, NJ 08865; and methanol from Burdick & Jackson Labs., Muskegon, MI 49442.

Apparatus. We used a Model 100A pump, a Model 504 autosampler equipped with a 20-μL loop, and a Model 160 fixed-wavelength (254 nm) ultraviolet detector (all from...
Beckman Instrument Co., Fullerton, CA 92634. A Model 3390-A integrator (Hewlett-Packard, Phoenix, AZ 85304) was used to quantify peak heights or areas. The analytical column was a 25 cm × 4.6 mm reversed-phase column packed with 5-μm "Ultrasphere ODS" (Beckman) and combined with a Co:Well 10-μm ODS guard column (Whatman Chemical Separation Division, Clifton, NJ 07014).

Standards and samples. Aqueous 10 g/L standards of cefamandole lithium were prepared and portions were frozen at −4 °C. They were thawed on the day they were used. Working standards with concentrations ranging from 25 to 500 mg/L were prepared by diluting with doubly distilled water; we added 20 μL of each to 480-μL samples of drug-free serum, "used" dialysis fluid, or 10-fold diluted urine to prepare standard curves ranging from 1.0 to 20.0 mg/L. "Used" dialysis fluid was drained from the peritoneal cavity of a renally impaired subject not receiving cefamandole.

Procedure. Proteins were removed from serum samples by the method of Bawdon et al. (10); i.e., add 2 mL of acetonitrile to 500 μL of serum, vortex-mix, then centrifuge (1500 × g, 5 min). Decant the acetonitrile/aqueous mixture into a clean tube and add 4 mL of dichloromethane. Again vortex-mix and centrifuge as before. Transfer 250 μL of the aqueous supernate to an autosampler vial and inject 20 μL of it onto the analytical column.

We injected dialysis fluid and diluted urine samples without further preparation.

The mobile phase, which was filtered through a 0.45-μm (pore-size) nylon 66 membrane (Rainin Instrument Co., Woburn, MA 01801) before use, consisted of methanol and water (31:69 by vol) to which 500 μL of phosphoric acid, 2.84 g of sodium sulfate, and 500 μL of triethylamine per liter of mobile phase was added. The pH of the mobile phase was adjusted to 6.0 with 2.0 mol/L NaOH. At a flow rate of 1.1 mL/min, corresponding to a pressure of about 24 000 kPa (3500 psi), the retention time for cefamandole was about 12.0 min.

Analytical performance. To a 10-mL sample of each fluid we added aqueous cefamandole lithium to obtain cefamandole concentrations of 0.5, 5.0, and 15.0 mg/L in serum; 1.0, 5.0, and 15.0 mg/L in used dialysis fluid; and 5.0, 15.0, and 30.0 mg/L in diluted urine. We used these samples—15 at each concentration for each fluid—to assess intra- and inter-assay reproducibility, and also storage stability. We assayed cefamandole in aliquots stored frozen for 60 days to determine sample storage stability. Standard curves prepared in serum, used dialysis fluid, diluted urine, or water were compared to gauge the analytical recovery of cefamandole from serum and possible interference from constituents in any of the three fluids. Additionally, we obtained serum samples from 20 hemodialysis or peritoneal dialysis patients. We supplemented these samples to give a known concentration of cefamandole and analyzed by the procedure for serum standards to assess any possible differences in recovery between various samples from patients with renal impairment.

Clinical studies. In another study we obtained patients' samples containing cefamandole. We administered a 1.0-g intravenous infusion of cefamandole nafate over 30 min to six volunteers who were undergoing continuous ambulatory peritoneal dialysis and who did not, at the time of the study, have peritonitis. Blood was sampled at regular intervals during the next 72 h, and the serum was frozen shortly after collection. The volume of dialysis fluid collected at each exchange (average interval 4.5 h) was measured and three portions were frozen. Urine samples were collected and pooled to include 3- to 12-h collection intervals; the volume was measured and three portions were stored frozen. The total collection time for urine and dialysis fluid was 72 h.

We collected samples from each of the six subjects before the administration of cefamandole, to assess possible interference, especially from co-administered medications.

Results and Discussion

Our goal was to develop a liquid-chromatographic assay for cefamandole in samples from renally impaired subjects that would have the necessary sensitivity, selectivity, and reproducibility for pharmacokinetic studies. Figures 1–3

![Fig. 1. Typical chromatograms of serum samples obtained from a subject before (A) and after (B) cefamandole dose](image)

Arrow: the cefamandole peak, corresponding to a concentration in serum of 2.6 mg/L

![Fig. 2. Typical chromatograms of fivefold-diluted urine sample obtained from a subject before (A) and after (B) cefamandole dose](image)

Arrow: the cefamandole peak, corresponding to an original (predilution) concentration in urine of 6.4 mg/L
show typical chromatograms of serum, dialysis fluid, and urine samples obtained from a patient who was undergoing continuous ambulatory peritoneal dialysis, assayed before and after the administration of cefamandole. The baseline resolution for cefamandole was complete, the retention time was approximately 12 min, primarily a function of the methanol concentration and ionic strength of the mobile phase; increasing the proportion of methanol and decreasing the ionic strength shorten this time.

Analysis of samples obtained from six subjects before they received cefamandole demonstrated no interference from co-administered medications that included allopurinol, cimetidine, clonidine, digoxin, furosemide, hydralazine, ketoconazole, L-thyroxin, metoclopramide, nifedipine, propranolol, and quinine.

Our comparisons of curves prepared with aqueous standards or standards in used dialysis fluid, diluted urine, or serum showed that, in practice, curves prepared with standards in the same matrix as the samples being analyzed should be used. For example, standards prepared in serum and pretreated for analysis according to Bawdon et al. (10) averaged 37.9% (SD 5.67%) greater apparent cefamandole concentration than standards prepared in water and injected directly onto the analytical column. This discrepancy is not ascribable to matrix interference, but instead to either a concentrating effect by the dichloromethane extraction, or a volume contraction owing to the loss of protein, or both. This effect allowed a more sensitive analysis of serum samples than for dialysis fluid or urine, but does not alter the validity of this method's lack of an internal standard because serum samples are always compared with standards made in serum. Results of a study comparing the analytical recovery of cefamandole from 20 separate serum samples from subjects undergoing either hemodialysis or peritoneal dialysis indicate that potential between-subject differences in matrix, due to renal impairment, do not affect the recovery of cefamandole. The average peak height, in arbitrary (integrator) units, was 83 986 (SD 3088; CV 4.83%). Typical slopes and intercepts of curves for standards in water, serum, used dialysis fluid, and urine are: 0.999 ×
Thus we recommend that standard curves be prepared in the same matrix as the sample being analyzed, and if multiple assays are to be performed on the same sample, multiple aliquots should be frozen to obviate freeze/thaw instability. We also recommend that samples obtained from patients receiving a drug not listed in this text should be analyzed before cefamandole is administered, to rule out any potential interference.

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References