

Liquid-Chromatographic Determination of Five Orally Active Cephalosporins—Cefixime, Cefaclor, Cefadroxil, Cephalixin, and Cephadrine—in Human Serum

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We report an isocratic "high-performance" liquid-chromatographic (HPLC) procedure for measurement of five orally administered cephalosporins (cefixime, cefaclor, cefadroxil, cephalixin, and cephradine) in 0.1 mL of human serum. Serum protein is precipitated with acetonitrile, the sample is centrifuged, and the supernate is evaporated under nitrogen. The residue is reconstituted in 0.1 mL of mobile phase, and 50 to 80 μ L of this is injected onto a reversed-phase Altex Ultrasphere™ Octyl (C₈) column. The five cephalosporins are resolved by elution with a pH 2.6 mobile phase of methanol/monobasic phosphate buffer (20/80) by vol, flow rate 2 mL/min. The column effluent is monitored at 240 nm. Cefixime serves as the internal standard for the analysis of the four other compounds, cephalixin as the internal standard for cefixime. We used two standard curves for all compounds: a low-range curve for concentrations commonly observed clinically and a higher-range curve for higher concentrations. The former were linear from 1.0 to 10 mg/L for cefaclor, cefadroxil, cephalixin, and cephradine and from 0.1 to 1 mg/L for cefixime. The high-concentration curves were linear from 1 to 10 mg/L for cefixime and from 10 to 100 mg/L for the other compounds. The detection limits were 0.1 mg/L for cefixime, 1 mg/L for the other cephalosporins. Mean within-run and day-to-day CVs were always <15% for all compounds studied.

Additional Keyphrases: chromatography, reversed-phase · antibiotics

Several cephalosporin antibiotics (cefaclor, cefadroxil, cephalixin, and cephradine) have been introduced in this country for oral treatment of infectious diseases. Cefixime is a new, orally active, broad-spectrum cephalosporin intended for the treatment of otitis media and of urinary and respiratory-tract infections. Several "high-performance" liquid-chromatographic (HPLC) methods have been described for the assay of the above-mentioned cephalosporins in biological fluids (1-9). Although many HPLC assays have been developed, some are limited to the detection of only one or two drugs, or do not measure drug concentrations that are clinically significant (3-5, 8).

Here we describe a rapid, sensitive, and precise reversed-phase HPLC assay that allows for the quantification of all five cephalosporins in human serum. This assay may be preferable to other methods because the same column, buffer system, and flow rate are used for all five analytes. Therefore, all five cephalosporin antibiotics can be routinely measured with one HPLC system, which should result in a shorter turnaround time and greater efficiency of analysis.

Materials and Methods

Apparatus: We used a Waters HPLC system (Millipore, Waters Division, Milford, MA) consisting of a Model 590 pump, a Model 481 variable-wavelength detector set at 240 nm, and a WISP™ Model 710B autosampler. The column system comprised a 4.6 mm \times 15 cm Altex Ultrasphere™-Octyl (C₈) column (5- μ m particles; Beckman Instruments Inc., Berkeley, CA), with a Waters RCSS Silica Guard Pak™ precolumn (Millipore, Waters Division).

Reagents: Methanol and acetonitrile were "HPLC" grade; monobasic sodium phosphate (monohydrate) and phosphoric acid were reagent grade (all from J. T. Baker Chemical Co., Phillipsburg, NJ). De-ionized water was further purified in a Milli-Q™ Water Purification System (Millipore Corp., Bedford, MA).

Drug standards: Cefixime, cephradine, and cefaclor reference standards were obtained from American Cyanamid Co., Pearl River, NY. Cephalixin and cefadroxil were purchased from Sigma Chemical Co., St. Louis, MO.

The internal standard solutions were prepared as follows: dissolve 5 mg of cefixime in 10 mL of methanol to yield a 500 mg/L stock solution. After serial 10-fold dilutions of the 500 mg/L stock solution, add a known aliquot of the resulting 5 mg/L working standard to all samples. Prepare a stock solution of cephalixin by dissolving 5 mg in 10 mL of methanol. Serial 10-fold dilutions of this working stock yield a 50 mg/L stock solution, used for assays in the higher concentration range (1.0 to 10 mg/L), and a 5 mg/L working standard, used for the low-concentration-range assays (0.1 to 1.0 mg/L).

Prepare stock solutions of cefadroxil, cefaclor, and cephradine by dissolving 5 mg of each compound in 10 mL of methanol to give 500 mg/L stock solutions. For each compound, make serial 10-fold dilutions of the stock solution to yield a 50 mg/L and a 5 mg/L working standard.

Mobile phase: The mobile phase consisted of methanol/monobasic sodium phosphate buffer, 12.5 mmol/L (20/80) by vol). The pH was adjusted to 2.6 with concentrated phosphoric acid. The mobile phase was filtered through a 0.5- μ m pore size filter (Type FH; Millipore Corp.) and degassed before use. The mobile phase was delivered at ambient temperature at a flow rate of 2 mL/min.

Blood samples: Whole blood was obtained from healthy drug-free volunteers from the medical department of American Cyanamid Co. and allowed to clot. Serum was harvested and used in the preparation of all standards.

Assay procedure: To 1.9-mL polyethylene microcentrifuge tubes add aliquots of the working standard solutions to give the desired drug concentrations, then evaporate the aliquots under a gentle stream of nitrogen. Reconstitute the residue with 0.1 mL of human serum, a known volume of internal standard, and 0.1 mL of acetonitrile, to precipitate serum proteins. Vigorously vortex-mix for approximately 15 s, then centrifuge at 14 000 \times g (Eppendorf centrifuge Model 5414; Brinkmann Instruments, Westbury, NY) for 2 min.

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Transfer the clear supernate to another microcentrifuge tube and remove the solvent by evaporation under a gentle stream of nitrogen. Reconstitute the residue with 0.1 mL of mobile phase and inject 50 to 80 μ L into the chromatograph. Measure the absorbance of the effluent at 240 nm.

Results and Discussion

Figure 1 shows a typical chromatographic separation of the five cephalosporins (at their clinically significant concentrations), obtained by subjecting 0.1 mL of the human serum samples to the analytical procedure.

In this assay, both cefixime and cephalixin served as internal standards. Cefixime was used as the internal standard for the determination of cefaclor, cefadroxil, cephalixin, and cephradine, while cephalixin was used as the internal standard for the determination of cefixime.

For the linearity studies we assayed seven different concentrations (1, 2, 5, 10, 20, 50, and 100 mg/L) of cefaclor, cefadroxil, cephalixin, and cephradine, and of cefixime (0.1, 0.2, 0.5, 1, 2, 5, and 10 mg/L). The detector response (peak height ratio) was linear over the concentration ranges studied. Standard curves were obtained by plotting the added drug concentration (mg/L) vs the peak-height ratio (drug:internal standard). For linear least-squares regression analysis of the data we used a laboratory automation system (Model 3357; Hewlett-Packard, Paramus, NJ) and SAS[®] (SAS Institute Inc., Cary, NC); the results are presented in Table 1.

Precision and accuracy. Analytical recovery experiments were performed by adding the individual cephalosporins to serum or methanol samples. Serum samples were extracted according to the procedure previously described. The recovery of each cephalosporin from the drug-supplemented human serum samples, calculated with respect to methanol samples similarly prepared, was 81.4% over the concentration ranges studied for all compounds except cefaclor (Table 2). The lower mean net recovery for cefaclor may result from its limited solubility in mobile phase. This could present a problem at concentrations >50 mg/L, but such concentrations of cefaclor are not normally observed clinically (10).

Within-day CV, over the concentration range studied, was

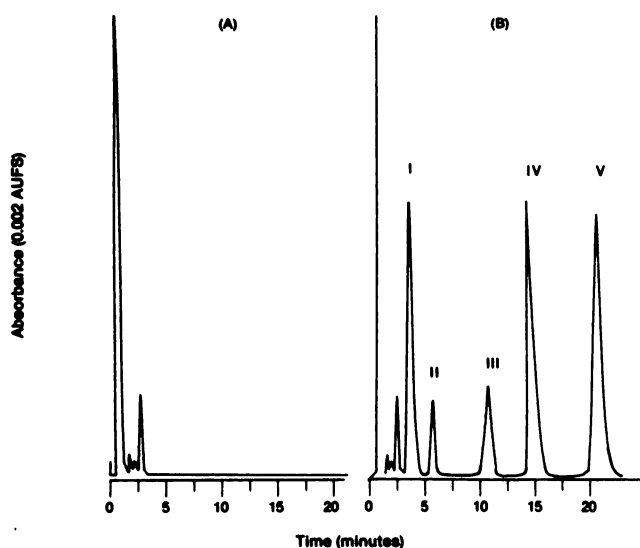


Fig. 1. Typical chromatographic separation of the five cephalosporins: (left) serum blank; (right) serum supplemented with cefadroxil, 1.0 mg/L (I); cefaclor, 1.0 mg/L (II); cefixime, 1.0 mg/L (III); cephalixin, 5.0 mg/L (IV); and cephradine, 5.0 mg/L (V)

Table 1. Linear Regression (Least-Squares Fit) Data for Calibration Curves

| Concn range, mg/L | Slope | Intercept ^a | r | S _{yx} ^b |
|-------------------|-------|------------------------|-------|------------------------------|
| <i>Cefixime</i> | | | | |
| 0.01–1.0 | 0.717 | –0.003 | 0.999 | 0.190 |
| 1–10 | 0.063 | –0.024 | 0.998 | 0.160 |
| <i>Cefaclor</i> | | | | |
| 1–20 | 0.145 | 0.025 | 0.998 | 0.464 |
| 10–100 | 0.021 | 0.187 | 0.988 | 0.400 |
| <i>Cefadroxil</i> | | | | |
| 1–20 | 0.754 | –0.076 | 0.999 | 0.576 |
| 10–100 | 0.283 | 3.99 | 0.964 | 1.77 |
| <i>Cephalixin</i> | | | | |
| 1–20 | 0.121 | –0.042 | 0.998 | 0.297 |
| 10–100 | 0.013 | –0.002 | 0.998 | 0.166 |
| <i>Cephradine</i> | | | | |
| 1–20 | 0.088 | –0.019 | 0.998 | 0.191 |
| 10–100 | 0.012 | 0.001 | 0.996 | 0.213 |

^aNone of the intercept values was significantly different from zero ($P > 0.05$). ^bStandard deviation of the residuals from the linear least-squares regression. Linear regression data were calculated from plot of peak height ratio vs concentration. Therefore values for intercept and S_{yx} are unitless.

<15% for all compounds, based on at least three measurements each. The between-day CV, determined from measurements made over three to five days, was also <15% for all compounds (Table 2).

Interference. Because the ultimate goal of many HPLC assays is clinical monitoring of drug therapy, we tested for potential interferences by assaying some commonly administered drugs at their maximum therapeutic concentrations (10): acetaminophen, 30 mg/L; phenytoin, 20 mg/L; cimetidine, 1 mg/L; diazepam, 1000 μ g/L; ibuprofen, 500 μ g/L; propranolol, 200 μ g/L; warfarin, 2 mg/L; digoxin, 4 μ g/L; salicylic acid, 300 mg/L. Salicylic acid, which would be present after doses of aspirin, was the only compound that absorbed at 240 nm and was measurable with this assay. However, it eluted at 22.1 min, which was not in the same time frame as any of the five cephalosporins studied (see Figure 1).

This procedure has several distinct advantages over previous methods. The same HPLC column, buffer system, and flow rate are used for all five cephalosporins, making it possible to quantify all five cephalosporins in human serum. Any delay due to column re-equilibration resulting from the use of different mobile phases and (or) switching of columns is eliminated, which allows for a shorter turnaround time and more efficient analyses. This method should provide for rapid monitoring of the concentrations of these compounds in clinics and hospitals.

Table 2. Performance Data for the Antibiotics Studied

| Drug | Concn range, mg/L | Mean CV, % | | Mean net recovery, % |
|------------|-------------------|------------|-------------|----------------------|
| | | Within-day | Between-day | |
| Cefixime | 0.1–1 | 5.8 | 8.3 | 81.4 |
| | 1–10 | 4.7 | 4.2 | |
| Cefaclor | 1–20 | 5.7 | 6.0 | 72.6 |
| | 10–100 | 5.2 | 9.8 | |
| Cefadroxil | 1–20 | 8.8 | 7.1 | 90.1 |
| | 10–100 | 11.4 | 14.2 | |
| Cephalixin | 1–20 | 7.6 | 7.2 | 82.3 |
| | 10–100 | 4.0 | 5.6 | |
| Cephradine | 1–20 | 6.9 | 7.7 | 84.2 |
| | 10–100 | 7.3 | 8.6 | |

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