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Note

Column liquid chromatographic determination of cefpiramide in human serum and urine

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Cefpiramide (Fig. 1) sodium is a water-soluble semisynthetic third-generation cephalosporin antibiotic which is effective against the majority of gram-positive organisms and has moderate activity against gram-negative organisms including most strains of *Pseudomonas aeruginosa*. In over 1300 patients with a variety of infections, the overall clinical efficacy is 72% [1]. Cefpiramide is highly and reversibly protein-bound, with a serum half-life of 4–5 h and non-renal clearance of 75% of a given dose [2].

This paper describes a reversed-phase high-performance liquid chromatographic (HPLC) method for the rapid, sensitive and accurate determination of cefpiramide in human plasma, serum and urine. The technique is based upon methodology which has been useful for the determination of other cephalosporins [3]. A method for cefpiramide HPLC assay has not been published.

EXPERIMENTAL

HPLC method

Acetonitrile, methanol and sodium acetate were HPLC-grade chemicals from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Cefamandole (Mandol®, Eli Lilly, Indianapolis, IN, U.S.A.) was obtained commercially and reagent-grade cefpiramide (Wyeth Labs., Philadelphia, PA, U.S.A.) was supplied by the company.

Chromatography was performed at room temperature since cephalosporins are heat-labile. A Waters Model M-45 solvent delivery system was connected to a Model 710B WISP sample injector (Waters Assoc., Milford, MA, U.S.A.). A Waters Model 441 fixed-wavelength UV detector was used at 254 nm with a sen-

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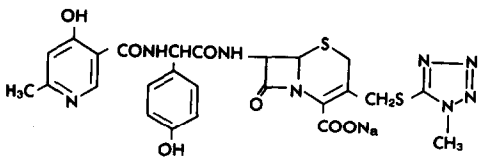

Fig. 1. Chemical structure of cefpiramide.

sitivity of 0.02 absorbance units full scale (a.u.f.s.). The mobile phase was acetonitrile–methanol–0.1 *M* sodium acetate–water (pH 5.2) (14.4:0.6:10:75, v/v), which was degassed and filtered through a Millipore filter (0.22 μm). The mobile phase was passed through a Waters C_{18} 10-μm particle reversed-phase column (30 cm × 4 mm I.D.) at a flow-rate of 2 ml/min. Chromatograms were integrated on a Shimadzu GR3A Chromatopac integrator. The retention times for cefpiramide and internal standard were 6.5 and 9.5 min, respectively. The following drugs, which are likely to be used clinically in conjunction with cefpiramide, did not interfere with the assay: metronidazole, vancomycin, nafcillin, ticarcillin, clindamycin and gentamicin.

Preparation of serum and plasma standards

Cefpiramide stock solution was prepared by accurately weighing 50 mg reagent-grade cefpiramide in 50 ml phosphate-buffered saline (pH 7.4). Aliquots of 1.0 ml were stored at −70°C, used once and discarded. This solution was added in varying amounts to 0.3 ml serum to yield concentrations of 0.92–153 μg/ml. Disposable borosilicate culture tubes were used.

The cefamandole internal standard stock solution was prepared by reconstituting a 2-g vial of Mandol with 20 ml of water. This solution (100 mg/ml) was stored at −70°C in 1-ml aliquots, used once and discarded. The stock solution was diluted in pure methanol to yield a concentration of 38.5 μg/ml. An internal standard solution was prepared consisting of cefamandole diluted stock solution (38.5 μg/ml)–0.1 *M* sodium acetate pH 5.2 (80:20).

Spiked serum samples (0.3 ml) were deproteinated by adding 0.3 ml of cold (iced) internal standard solution, then vortexed for 30 s and incubated at −20°C for 10 min. The mixture was centrifuged at 1500 *g* for 10 min and 10 μl of supernatant were injected into the column.

The standard curve was constructed by plotting cefpiramide/cefamandole peak-height ratios against the spiked concentration of cefpiramide in the serum. Eight or nine concentrations were used to construct the standard curve.

Preparation of serum or plasma samples

Serum or plasma samples from subjects receiving cefpiramide were prepared and analyzed in the same way as the spiked specimens used to construct the standard curve. Samples containing drug concentrations higher than those used in the standard curve were diluted with internal standard solution.

Preparation of urine standard

Cefpiramide stock solution (1 mg/ml) was prepared as described above. This solution was added to 0.1-ml blank human urine samples to yield cefpiramide

concentrations of 12.9–367 μg/ml. The cefamandole stock solution (100 mg/ml) prepared as described above was diluted in methanol to yield a working stock solution of 76.9 μg/ml. The internal standard solution was prepared as described for serum.

Spiked urine samples were deproteinated by adding 1.0 ml of internal standard solution to 0.1 ml urine, vortexed for 30 s and 5 μl of the mixture were injected into the column.

Preparation of samples for quality control

All assays were run with a standard curve.

Samples of serum and urine were spiked at various concentrations, aliquoted and frozen at −70°C and then used for the stability study. Aliquots were analyzed in duplicate at seven to twelve days intervals over a six-week period. At three concentrations, six separately spiked samples of serum and urine were prepared simultaneously and analyzed immediately to determine reproducibility of results within one day.

To assess inter-day reproducibility, eight serum standard curves were compared over a period of five weeks and seven urine standard curves were compared over a period of four weeks. Serum and urine were also spiked at three concentrations for five and six different days, respectively (throughout a period of one month and two months, respectively), and immediately analyzed in duplicate.

Statistical analysis and curve fitting were performed with the assistance of Prophet Computer Resource at the University of California, San Francisco [4, 5]. Linearity (r^2), precision (coefficient of variation), recovery [3] (relation of test result to the true concentration) and percentage accuracy [6] were analyzed.

Detection limit was defined as the smallest peak height that was three times the baseline noise level.

RESULTS

Sample chromatograms of serum and urine containing cefpiramide are shown in Fig. 2. The detection limits for cefpiramide were 0.92 μg/ml for serum and 12.85 μg/ml for urine. The statistical analysis of serum and urine standard curves is summarized in Table I.

Results for assay precision, recovery and accuracy assessments in serum and urine are summarized in Tables II and III. The mean coefficients of variation for all serum and urine assays were 3.2% (range 0.8–9.4%) and 7.0% (range 4.04–10.6%), respectively.

For both serum and urine, there are no statistically significant differences among the means (for each concentration), when comparing intra- and inter-day precision assessment ($P > 0.05$; Student's t-test).

The mean recoveries of the assay for all determinations in serum and urine were 98.3% (range 95.4–100.3%) and 101.2% (range 97.5–104.0%), respectively. The accuracy ranges for all determinations in serum and urine were −4.6 to 0.29% and −2.4 to 3.9%, respectively.

The results of six weekly determinations of cefpiramide in spiked serum and

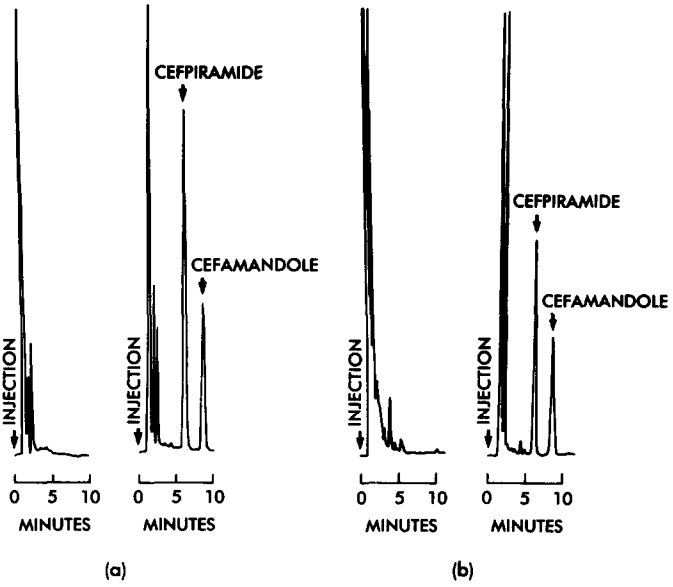

Fig. 2. (a) Chromatograms of patient's serum before and after receiving 2.0 g cefpiramide intravenously. (b) Chromatograms of patient's urine before and after receiving 2.0 g cefpiramide intravenously.

urine stored at −70°C revealed no significant degradation of the drug.

Fig. 3 illustrates a time–concentration curve following a 2-g intravenous infusion of cefpiramide in a patient with normal renal function. A computer-generated fitted curve was obtained using an open, two-compartment pharmacokinetic model [5]. Elimination half-life ($t_{1/2}$) was 4.48 h.

DISCUSSION

This reversed-phase HPLC assay for cefpiramide provides selective, rapid and reliable determinations in serum and urine. Preparation of samples requires a simple deproteinization step. Use of a methanol–aqueous extraction medium at pH 5.2 not only precipitates protein but also releases drug from protein, thereby maximizing drug recovery [3]. In addition to prolonging the life of the column, deproteinization also assures chromatographic resolution. Sharp peaks representing the internal standard and cefpiramide are easily discernible.

TABLE I

STANDARD CURVE STATISTICAL SUMMARY

Sample	n	Correlation coefficient (r^2) (mean ± S.D.)	Standard deviation of regression (mean ± S.D.)	F value★ (mean ± S.D.)
Serum	8	0.999 ± 0.000	0.07 ± 0.02	24062 ± 16691
Urine	7	0.999 ± 0.001	0.02 ± 0.01	7828 ± 4789

★F value = ratio of the regression mean square to the residual mean square.

TABLE II

ASSAY PRECISION, RECOVERY AND ACCURACY FOR CEFPIRAMIDE DETERMINATION IN SERUM

Spiked concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Coefficient of variation (%)	Recovery★ (%)	Accuracy★★ (%)
Intra-day★★★ (n=6)				
9.18	9.07 ± 0.28	3.09	98.8	−1.2
45.9	43.80 ± 1.31	2.99	95.4	−4.6
137.7	138.10 ± 1.25	0.90	100.3	0.29
Inter-day§ (n=5)				
9.18	9.07 ± 0.85	9.4	98.8	−1.2
45.9	44.70 ± 0.95	2.1	97.4	−2.6
137.7	136.68 ± 1.13	0.82	99.3	0.7

★Measured/spiked × 100% [3].

★★Measured − spiked/spiked × 100% [6].

★★★Six separately spiked samples at each of three concentrations.

§On five different days, plasma spiked at three concentrations and immediately analyzed in duplicate.

The stability study indicates that no significant drug degradation occurs in plasma or urine stored at −70°C for six weeks.

The linearity of the standard curve, in the range described, is excellent. Assay precision is high for serum and urine. Recovery and percentage accuracy are within previously published acceptable limits [3, 6], therefore making the method suitable for clinical and pharmacological studies.

TABLE III

ASSAY PRECISION, RECOVERY AND ACCURACY FOR CEFPIRAMIDE DETERMINATION IN URINE

Spiked concentration (μg/ml)	Measured concentration (mean ± S.D.) (μg/ml)	Coefficient of variation (%)	Recovery (%)	Accuracy (%)
Intra-day★ (n=6)				
36.72	37.85 ± 2.12	5.60	103	3.08
183.6	186.55 ± 7.54	4.04	102	1.61
550.8	569.93 ± 25.66	4.50	103	3.47
Inter-day★★ (n=6)				
36.72	38.18 ± 4.04	10.59	104	3.9
183.6	180.63 ± 15.8	8.74	98.4	−1.6
550.8	536.57 ± 45.8	8.54	97.5	−2.4

★Six separately spiked samples at each of three concentrations.

★★On six different days, urine spiked at three concentrations and immediately analyzed in duplicate.

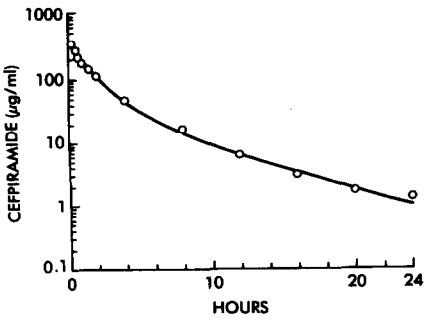

Fig. 3. Serum concentration-time curve following intravenous injection of cefpiramide (2 g) in a patient with normal renal function. Elimination half-life, 4.5 h.

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