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Microanalytical High-Performance Liquid Chromatography Assay for Cefpirome (HR 810) in Serum

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We report a microanalytical high-performance liquid chromatograhy method for quantitation of cefpirome (HR 810) from serum. The drug was extracted from 0.05 ml of serum with 0.2 ml of isopropanol containing β-hydroxypropyltheophylline, the internal standard. Separations were performed on a C_{18} column at ambient temperature with detection at 240 nm. The mobile phase consisted of acetate buffer (0.05 M sodium acetate) containing tetrabutylammonium hydroxide and methanol (pH 5.1; 70:30, vol/vol). The method was linear to 500 μg of cefpirome per ml and had a sensitivity of 0.6 μg/ml. Analytical recovery was >86%, and the between-day coefficient of variation was <4.2%. The stability for 1 week at 4 to 8°C and for 30 days at −20°C was documented. Interference with commonly used antibiotics, analgesics, methylxanthines, and anticonvulsants was not found. The small sample volume and ease of preparation make this method suitable for use in pediatric pharmacokinetic investigations of cefpirome.

Cefpirome (HR 810), a new aminothiazolyl cephalosporin, is reported to have an expanded spectrum of activity against *Pseudomonas* sp., enterococci, and staphylococci, as well as other gram-positive and gram negative bacteria that are resistant to cefotaxime and ceftazidime (1–3, 7). Previous investigations (1–3, 5, 7) have involved various methods, including broth microdilution and agar dilution, to quantitate cefpirome for determination of MICs. Two recent studies (4, 6) report the pharmacokinetics of cefpirome in adults after quantitation of the compound in serum by using a high-performance liquid chromatography (HPLC) assay for which specific details were not provided. Inquiry to the manufacturer of cefpirome (Hoechst-Roussel Pharmaceuticals Inc., Sommerville, N.J.) revealed that an unpublished HPLC method exists that requires a serum sample volume of 1.0 to 2.0 ml, a constraint which limits the utility of this method for pediatric pharmacokinetic investigations.

The expanded activity and safety profile of cefpirome make it a potentially valuable antimicrobial agent for use in infants and children. The pharmacokinetics of this agent in pediatric subpopulations must, however, be characterized before dosing regimens can be adequately designed. To facilitate these endeavors, we have developed a rapid, sensitive microanalytical HPLC method for the quantitation of cefpirome in serum that involves the use of an internal-standard approach.

MATERIALS AND METHODS

Reagents and standards. Acetate buffer (0.05 M) was prepared by dissolving 4.0 g of sodium acetate (Sigma Chemical Co., St. Louis, Mo.) in a total volume of 1 liter with deionized water. Four milliliters of the ion-pairing agent tetrabutylammonium hydroxide (40%, wt/wt; Sigma) was added, and the pH was adjusted to 5.1 with glacial acetic acid. The buffer was then filtered through a GA-8 Metricell membrane filter (pore size, 0.2 μm; Gelman Sciences, Inc., Ann Arbor, Mich.), and the mobile phase, consisting of buffer-methanol (70:30, vol/vol), was prepared. All organic solvents used were of HPLC grade (American Burdick and Jackson, Muskegon, Mich.). The mobile phase was deoxygenated by passing helium through the solution at a rate of 100 ml/min for 15 min. During the analytical run, helium was also passed through the mobile phase at a rate of 5 ml/min.

The internal standard was prepared by dissolving 10 mg of β-hydroxypropyltheophylline (Sigma) in 10 ml of reagent grade water. Two milliliters of this stock solution was diluted to a total volume of 10 ml with isopropanol to yield a working internal-standard concentration of 200 μg/ml. The working internal-standard solution was stable for 2 months at 4°C (retention of >95% of initial concentration).

Analytical-grade cefpirome was graciously donated by the manufacturer (Hoechst-Roussel Pharmaceuticals Inc.). A stock solution of cefpirome was prepared by dissolving 123 mg of cefpirome sulfate in 10 ml of reagent grade water to yield a solution containing 10 mg of free cefpirome per ml. Working standards of 500, 200, 100, 50, 10, 5, 2.5, 1.25, and 0.62 μg/ml were prepared in drug-free human serum.

Apparatus. The HPLC system consisted of a model 600 multisolvent delivery apparatus and model 490 variable-wavelength detector (Waters Associates, Inc., Milford, Mass.). Separations were conducted on a steel μBondapak C_{18} column (30 cm by 3.9 mm; particle size, 10 μm) fitted to a model U6K manual injector (Waters). Assays were performed at ambient temperature at a flow rate of 1.4 ml/min. The effluent was monitored at 240 nm.

Procedure. Serum (0.05 ml) was added to 0.2 ml of working internal standard, vortex mixed for approximately 30 s, and centrifuged at 1,800 × *g* for approximately 1 min. The supernatant was filtered through a 0.2-μm filter (Amicon Corp., Danvers, Mass.) and dried under a stream of nitrogen at ambient temperature. Immediately after drying, the extract was reconstituted in 0.2 ml of the mobile phase and vortex mixed for 15 s, and 0.05 ml was injected onto the HPLC column through the manual injector.

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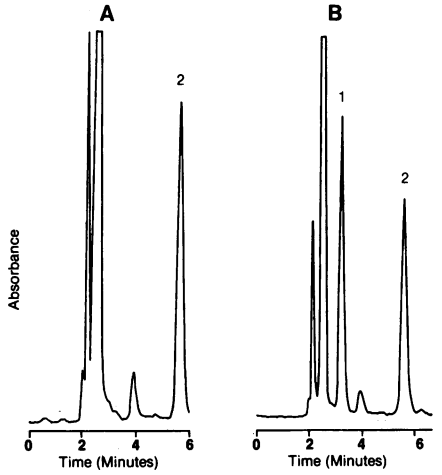

FIG. 1. Chromatograms of serum containing cefpirome (peak 1) and the internal standard, β-hydroxypropyltheophylline (peak 2). (A) Blank serum sample; (B) serum containing 40 μg of cefpirome per ml. The chart recorder attenuation in panel B is increased to keep the cefpirome peak on scale.

RESULTS

A representative chromatogram of serum containing cefpirome and the internal standard is shown in Fig. 1. There was clear resolution of each compound with retention times of 3.2 min (cefpirome) and 5.8 min (β-hydroxypropyltheophylline), respectively. Extraction and chromatographic analysis of five separate blank serum samples confirmed that there were no endogenous peaks that coeluted with cefpirome or the internal standard.

Linearity was established to at least 500 μg of cefpirome per ml, and sensitivity was established to 0.6 μg/ml, thereby demonstrating the broad analytical range of this assay. Analytical recovery was assessed by adding cefpirome at concentrations of 50.0 and 100.0 μg/ml to water and to serum. Recovery from the biological matrix was lower (88.7 and 86.7% for 50.0 and 100.0 μg/ml, respectively) (Table 1). This disparity in recovery suggested that isopropanol protein precipitation does not completely free the drug from protein-binding sites. Between-day precision (Table 2) was assessed at cefpirome concentrations in serum over three orders of magnitude and was consistently <4.2%.

A number of commonly administered antimicrobial agents were examined for possible interference with the HPLC method. None of the parent compounds coeluted with cefpirome or the internal standard (Table 3). Although serum containing 200.0 μg of acetaminophen per ml eluted in close proximity to cefpirome, peak resolution was maintained. Therapeutic acetaminophen concentrations in serum (i.e., 10 to 20 μg/ml) would not be expected to present analytical interference. The methylxanthine metabolites 3-methylxanthine and 3,7-dimethyluric acid had retention times (3.1 and 3.2 min, respectively) similar to cefpirome (retention time, 3.2 min). Extraction of serum samples from two patients with therapeutic theophylline concentrations in serum of 18.2 and 3.3 μg/ml did not reveal the presence of either 3-methylxanthine or 3,7-dimethyluric acid. The storage stability of cefpirome in serum (three samples) at concentrations of 5.0, 50.0, and 500.0 μg/ml was confirmed at 4 to 8°C, with >96% of the original assayed concentrations present at 1 week from preparation. Serum samples stored at −20°C for 1 month (five samples each at cefpirome concentrations of 5.0, 50.0, and 500.0 μg/ml) did not reveal any appreciable degradation of cefpirome, with all samples retaining >94% of their original concentration values.

TABLE 1. Cefpirome analytical recovery

Concn in serum (μg/ml)[a]	Aqueous concn (μg/ml)[b]	Recovery (%)[b]
50.0	56.4 ± 2.1	88.7 ± 2.0
100.0	115.5 ± 4.1	86.7 ± 3.1

[a] Cefpirome at the indicated concentrations in serum was added to water and quantitated as described in the text.
[b] Results are expressed as the mean ± standard deviation of three determinations at each concentration.

TABLE 2. Cefpirome between-day precision[a]

Concn measured (μg/ml)[b]	CV[c] (%)
5.0 ± 0.2	4.0
49.8 ± 2.1	4.2
499.5 ± 20.3	4.1

[a] Cefpirome at concentrations of 5.0, 50, and 500 μg/ml was added to drug-free human serum and was quantitated as described in the text.
[b] Results are expressed as mean ± standard deviation of five determinations on separate days at each concentration. Serum samples were stored at 4 to 8°C and analyzed within 1 week.
[c] CV, Coefficient of variation.

DISCUSSION

HPLC is an analytical approach for quantitating cephalosporin antibiotics in biological fluids that is becoming increasingly popular in clinical laboratories which do not have the capability of microbiological assay (10). A recently published HPLC method for cefotaxime and desacetylcefo-

TABLE 3. Therapeutic agents and metabolites evaluated for chromatographic interference

Agent[a]	Retention time (min)
Antibiotics	
Vancomycin	2.2
Ampicillin	4.5
Tobramycin	6.8
Gentamicin	12.1
Amikacin	ND[b]
Ticarcillin	ND
Nafcillin	ND
Other drugs and metabolites	
Acetaminophen	2.9
Phenobarbital	10.0
Phenytoin	10.0
Carbamazepine	ND
10,11-Carbamazepine epoxide	ND
Theophylline	4.6
Caffeine	6.1
1,7-Dimethylxanthine	4.4
1,3-Dimethyluric acid	3.6
3-Methylxanthine	3.1
3,7-Dimethyluric acid	3.2

[a] Antibiotics, methylxanthines, and 10,11-carbamazepine epoxide were reconstituted in the mobile phase to final concentrations of 1,000.0 μg/ml, and 50 μl was injected. Anticonvulsants and antipyretics were prepared in serum at concentrations of 200.0 μg/ml. Potential interfering substances were extracted with isopropanol not containing the internal standard.
[b] ND, No peaks detected over a 15-min run time.

taxime (8) involved the use of an ion-pairing technique to improve the separation of the cephalosporins. This technique was also found to be useful for cefpirome. The use of β-hydroxypropyltheophylline as an internal standard should improve analytical precision by reducing the sources of error commonly associated with methods involving external standard approaches (9). Although it may be possible to improve analytical sensitivity for cefpirome by increasing the sample volume, the degree of sensitivity accomplished with a 0.05-ml serum sample appears to be more than adequate for use in pharmacokinetic investigations (4, 6). Additionally, this small sample volume requirement will allow multiple blood sampling, which is necessary to characterize cefpirome pharmacokinetics in neonates, infants, and children.

None of the antibiotics likely to be coadministered to patients receiving cefpirome appeared to interfere with this technique. In addition to the antimicrobial agents tested (Table 3), acetaminophen, phenobarbital, phenytoin, carbamazepine, 10,11-carbamazepine epoxide, theophylline, caffeine, 1,7-dimethylxanthine, and 1,3-dimethyluric acid were also found not to coelute with either cefpirome or the internal standard. Consequently, this method should be applicable to investigations of cefpirome disposition in hospitalized pediatric subpopulations.

The extraction procedure was simplified by including the internal standard in the protein-precipitating agent. We chose isopropanol as the precipitating agent after observing cefpirome peak splitting when acetonitrile was used in the extraction. The extraction procedure takes approximately 20 min to perform, half of which is devoted to drying the extract under nitrogen at ambient temperature (a measure instituted to minimize the chance of heat lability which can occur with the cephalosporins [10]). The HPLC run time for this assay is rapid and requires less than 6 min for all peaks to be resolved.

The HPLC method which we have described for cefpirome represents a rapid, sensitive, efficient, and precise analytical approach for quantitating this agent in serum. The characteristics of this method will make it well suited for pharmacokinetic and clinical laboratory investigations of cefpirome in pediatric patients.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Bertran, M. A., D. A. Bruckner, and L. S. Young.** 1984. In vitro activity of HR 810, a new cephalosporin. Antimicrob. Agents Chemother. **26**:277–279.
2. **Jones, R. N., C. Thornsberry, and A. L. Barry.** 1984. In vitro evaluation of HR 810, a new wide-spectrum aminothiazolyl α-methoximino cephalosporin. Antimicrob. Agents Chemother. **25**:710–718.
3. **Jones, R. N., C. Thornsberry, A. L. Barry, L. Ayers, S. Brown, J. Daniel, P. C. Fuchs, T. L. Gavan, E. H. Gerlach, J. M. Matsen, L. B. Reller, and H. M. Sommers.** 1984. Disk diffusion testing, quality control guidelines, and antimicrobial spectrum of HR 810, a fourth-generation cephalosporin, in clinical microbiology laboratories. J. Clin. Microbiol. **20**:409–412.
4. **Maass, L., V. Malerczyk, and M. Verho.** 1987. Pharmacokinetics of cefpirome (HR 810), a new cephalosporin derivative administered intramuscularly and intravenously to healthy volunteers. Infection **15**:207–210.
5. **Maass, L., V. Malerczyk, M. Verho, P. Hajdu, K. Seeger, and N. Klesel.** 1987. Dose linearity testing of intravenous cefpirome (HR 810), a novel cephalosporin derivative. Infection **15**:202–206.
6. **Malerczyk, V., L. Maass, M. Verho, P. Hajdu, N. Klesel, and R. Rangoonwala.** 1987. Single and multiple dose pharmacokinetics of intravenous cefpirome (HR 810), a novel cephalosporin derivative. Infection **15**:211–214.
7. **Seibert, G., M. Limbert, I. Winkler, and T. Dick.** 1983. The antibacterial activity "in-vitro" and β-lactamase stability of the new cephalosporin HR 810 in comparison with five other cephalosporins and two aminoglycosides. Infection **11**:275–279.
8. **Trang, J. M., R. F. Jacobs, G. L. Kearns, A. L. Brown, T. G. Wells, F. L. Underwood, and R. B. Kluza.** 1985. Cefotaxime and desacetylcefotaxime pharmacokinetics in infants and children with meningitis. Antimicrob. Agents Chemother. **28**:791–795.
9. **van der Wal, S., and L. R. Snyder.** 1981. Precision of "high-performance" liquid-chromatographic assays with sample pretreatment. Error analysis for the Technicon "Fast-LC" system. Clin. Chem. **27**:1233–1240.
10. **Ward, J. B., and P. B. Warner.** 1987. Cephalosporin assays. Drugs **34**:247–252.