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Determination of Ceftazidime in Biological Fluids by Using High-Pressure Liquid Chromatography

CAROLYN M. MYERS AND JEFFREY L. BLUMER*

Department of Pediatrics, Division of Pediatric Pharmacology and Critical Care, Rainbow Babies and Childrens Hospital, and Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

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Ceftazidime is a new β-lactamase-stable third-generation cephalosporin with a broad spectrum of antimicrobial activity. To evaluate the biodisposition of the drug in infants and children, a rapid and simple high-pressure liquid chromatographic technique was developed. The method is useful for both serum and urine and involves methanol precipitation followed by reverse-phase chromatography on MicroPak MCH 10. The mobile phase, consisting of 20% methanol and an 80% aqueous solution of 50 mM ammonium dihydrogen phosphate and 117 μM perchloric acid, is pumped at 1 ml/min through the column which is maintained at 50°C. The drug was detected at 257 nm with a variable-wavelength UV detector. A good linear correlation was observed between the peak area and the ceftazidime concentration at 0.3 to 500 μg/ml ($r = 0.999$). Since an equal volume of cold methanol is used to precipitate proteins from serum samples and only 20 μl of the resultant supernatant is injected into the column, samples as small as 50 μl may be routinely analyzed. This method has been used to study ceftazidime pharmacokinetics in more than 30 patients and has proven to be rapid and reproducible.

Ceftazidime (Fig. 1) is a third-generation parenteral cephalosporin which is highly stable to bacterial β-lactamases. It was discovered as part of a research program designed to find an antibiotic for the treatment of serious infections due to a wide range of bacteria, including indole-positive proteus species and *Pseudomonas aeruginosa* (2). The favorable properties of ceftazidime include efficient penetration of the bacterial cell wall, resistance to bacterial enzyme degradation, a high intrinsic activity against the bacterial cell targets (3–5), a broad spectrum of activity, very low toxicity, extensive tissue penetration, metabolic stability, and a low degree of serum protein binding (2).

For proper use of the drug in treating serious bacterial infections in pediatric patients, a thorough understanding of the biodisposition and pharmacokinetics of ceftazidime is mandatory. The purpose of the present study is to describe a new micromethod for the determination of ceftazidime concentrations in biological fluids. The high-pressure liquid chromatography (HPLC) method reported herein has proven to be rapid mination of ceftazidime concentrations in the blood and urine of more than 30 patients.

MATERIALS AND METHODS

Reagents. Ceftazidime (GR 20263) was supplied by Glaxo Group Research Ltd., Greenford, Middlesex, England. Ammonium dihydrogen phosphate and perchloric acid were reagent grade and from Fisher Scientific Co., Pittsburgh, Pa. Glass-distilled methanol (HPLC grade) was purchased from Burdick and Jackson Laboratories, Muskegon, Mich.

Apparatus. Analyses were performed on a Varian model 5040 ternary liquid chromatograph controlled by a Vista model 401 data system which includes a printer-plotter (Varian, Palo Alto, Calif.). Samples were injected into a Rheodyne model 7030 column-switching valve fitted with a 20-μl loop and pumped at 1 ml/min through a MicroPak MCH 10 reverse-phase column (4 mm by 30 cm). The column was temperature controlled at 50°C with a column heater and was preceded by a guard column (4 mm by 4 cm) filled with Vydac 40-μm pellicular reverse-phase packing. Peaks were detected at 257 nm (8-nm slit width) with a Vari-Chrom variable-wavelength UV detector. A water thermostat (Radiometer type VTS13, The London Co., Cleveland, Ohio) circulated water throughout the jacketed flow cell to increase base-line stability. Chromatograms were printed at an attentuation of 32 and a chart speed of 0.2 cm/min.

Sample preparation. All specimens were stored frozen at −70°C and kept on ice during other manipulations. Serum was extracted by adding 0.15 ml of cold 100% methanol to 0.15 ml of serum in a 1.5-ml capped microtube. After the samples were vortexed for 10 s, they were allowed to sit for at least 5 min on ice. The protein precipitate was centrifuged at 12,800 × *g* in a microfuge equilibrated at 4°C. These low-temperature precautions maximized the protein precipitation and minimized column clogging. Capped tubes were used

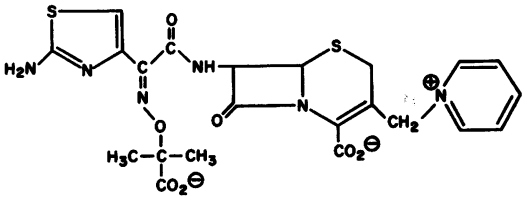

FIG. 1. Chemical structure of ceftazidime (GR 20263), (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop - 2 - oxyimino)acetamido] - 3 - (pyridinium-1-ylmethyl)ceph-3-em-4-carboxylate.

to minimize methanol evaporation. The supernatant solution was immediately transferred to another 1.5-ml capped microtube and stored on ice. Before injection, samples were warmed to room temperature and vortexed.

Urine was prepared for injection by sedimenting particulate matter with centrifugation and then making dilutions of the supernatant solution in 0.9% NaCl ranging from 1:6 to 1:144.

HPLC. Two reservoirs were employed for the mobile phase (method A). Methanol at 20% was proportioned from one reservoir. An 80% aqueous solution composed of 50 mM ammonium dihydrogen phosphate and 117 μM perchloric acid was proportioned from a second reservoir.

An alternate method (method B) for ceftazidime determination in serum was devised if somewhat sharper peaks were desired. See Fig. 2 for a comparison of the two methods. For this method a flow gradient capacity is necessary, and the detector flow cell should have water circulating through the tubing jacket to minimize any resulting base-line alteration. In this case the mobile phase was composed of 9% acetonitrile and 91% of the phosphate-perchloric acid solution described above. At time zero a flow of 0.5 ml/min was selected, and a linear gradient was set to reach a flow of 1.5 ml/min at 4 min. Both chromatographic methods were designed so that a serum peak with an area equivalent to 3.0 μg of ceftazidime per ml would elute before the ceftazidime peak.

Standard stock solutions were prepared in 2.7 mM sodium carbonate. The standards for all studies were prepared in pooled human serum or urine which accounted for 95% of the final total volume. Between-day standards were measured into ready-to-assay amounts and stored at −70°C in the 1.5-ml capped microtubes.

RESULTS AND DISCUSSION

The data presented below were obtained with method A. However, there is no demonstrable difference in range, linearity, or precision between the two methods.

The analysis was linear to concentrations of 500 μg of ceftazidime per ml. Duplicate serum samples at 0, 5, 20, 50, 100, 300, and 500 μg/ml yielded a coefficient of linearity between the peak area and the serum concentration of 0.999. The mean absolute variation from a straight line was equivalent to 1.4 μg/ml. The limit of detection for this method was 0.3 μg/ml. In several patients a peak with the same retention volume as ceftazidime was detected in the zero time sample; its equivalent ceftazidime area was 0 to 2 μg/ml and was most frequently at the 0.3-μg/ml limit of detection. A somewhat larger peak oc-

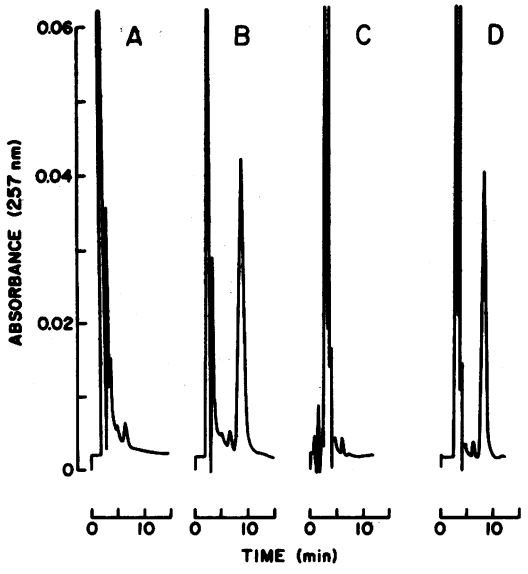

FIG. 2. HPLC of ceftazidime in serum. (A and B) Method A—0 and 100 μg/ml, respectively; (C and D) method B—0 and 100 μg/ml, respectively.

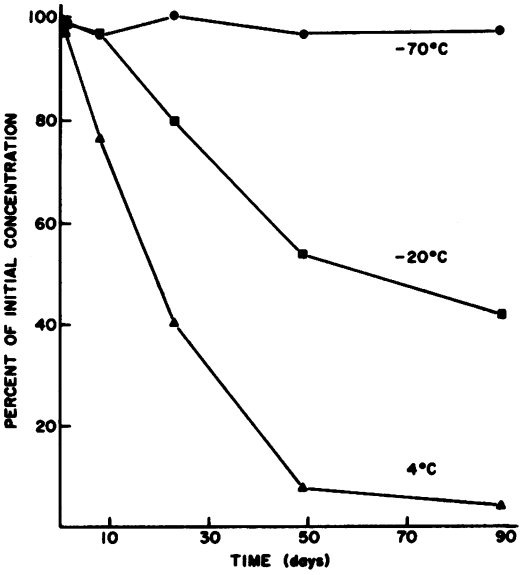

FIG. 3. Stability of ceftazidime in serum at different temperatures.

TABLE 1. Capacity factors of drugs tested for interference in high-pressure liquid chromatographic assays for ceftazidime

Drug	k'[a]		
	Method A		Method B (9% acetonitrile)
	20% Methanol	45% Methanol[b]	
Morphine	1.4, 4.4	0.4, 1.1	1.0, 3.7
Acetaminophen	1.5	ND[c]	1.2
Moxalactam	2.2	ND	1.7
Ceftazidime	2.3	0.3	1.9
Cimetidine	2.3	ND	1.8
Salicylic acid	2.5	ND	2.1
Theobromine	2.8	ND	1.5
Cephazolin	3.6	ND	3.6
Ticarcillin	4.0	ND	2.9
Diazepam	4.3, 6.2	0.9, 1.3	3.0, 4.3
Theophylline	4.3	ND	2.4
Carbenicillin	4.9	ND	3.0
Sulfamethoxazole	5.6	ND	7.3
Ceftriaxone	6.1	1.0	4.9
Cephamandole	8.0, 21.5	ND	9.5
Trimethoprim	8.3	ND	8.0
Chloramphenicol	8.8	ND	10.8
Chloramphenicol succinate	11.4, 13.5	ND	17.6, 21.6
Caffeine	12.1 8	ND	6.7
Phenobarbital	12.5	ND	11.5
Piperacillin	ND[d]	1.2	ND
Oxacillin	ND	2.3	ND
Prednisone	ND	3.4	ND
Nafcillin	ND	5.0	ND
Methyl prednisolone	ND	7.4	ND

[a] $k' = (t_D - t_M)/t_M$, where t_D is the elution time of the drug and t_M is the elution time of the mobile phase.
[b] Mobile phase was 45% methanol and 55% aqueous solution of 50 mM ammonium hydrogen phosphate and 117 μM perchloric acid.
[c] ND, Not determined.
[d] Peak did not elute within 1.5 h.

curred in urine; the range was 0 to 0.5 mg/ml, and the mean was 0.01 mg/ml. Therefore, care should be taken to obtain predose serum and urine to use as a blank and to serve as a preliminary screen of late peaks which might interfere with subsequent chromatograms.

The within-day coefficients of variation for ceftazidime serum standards ($n = 15$) at 100 and 5 μg/ml were 1.4 and 5.1, respectively. The between-day coefficients of variation at 100 ($n = 23$) and 5 ($n = 10$) μg/ml were 2.3 and 12.6, respectively. The recovery of ceftazidime from serum was determined by adding methanol to saline standards in the same manner as it was added to serum standards (see above). From 16 paired serum and saline samples and recovery was 95.8%, with a coefficient of variation equal to 1.5.

The stability of ceftazidime solutions (100 μg/ml) stored for 3 months at various low temperatures is shown in Fig. 3. In serum at 4°C, ceftazidime was unstable, and during the first 3 weeks the peak lost almost 3% of its area daily. At −20°C the drug was relatively stable for 1 week, but over the subsequent 6 weeks 1% of the peak area was lost daily. At −70°C, serum, saline, and urine solutions were stable throughout the study, losing overall only 3, 4, and 9% of their peak areas, respectively. It was consequently clear that specimens must be stored at −70°C unless they are to be immediately analyzed.

Extracts of ceftazidime from serum which are stored for 6 h on ice retain 98% of their peak area, suggesting that serum extracts can be prepared in advance.

The elution times for various substances in both chromatographic systems are presented in Table 1. There are no compounds which present a serious interference. Although moxalactam

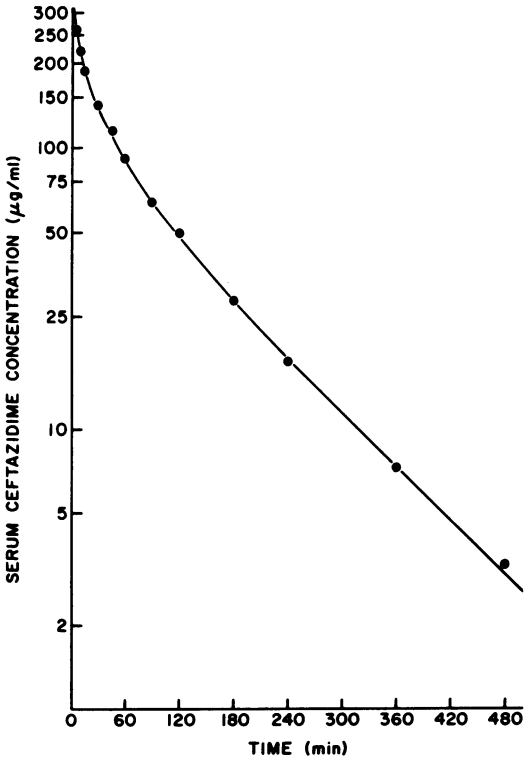

FIG. 4. Curve of overall ceftazidime concentration in serum versus time. Ceftazidime concentrations in serum were determined using the HPLC assay described in the text. Serum samples were obtained at the times indicated, and the data were analyzed using a two-compartment pharmacokinetic model. Each point represents the mean of determinations on nine patients. The coefficient of variation at each point never exceeded 18%.

elutes at the same time as ceftazidime, the two drugs are not given together. Cimetidine, due to its low therapeutic concentration in serum and low extinction, will not be detected by either method. Salicylic acid, the most prevalent serum component resulting from aspirin ingestion, may appear as a small postceftazidime shoulder: at 50 μg/ml, salicylic acid would give an equivalent ceftazidime area of 4 μg/ml. Finally, theobromine may appear as a small peak after ceftazidime in method A and before ceftazidime in method B; at 10 μg/ml, theobromine would be given an equivalent ceftazidime area of 6 μg/ml.

To ascertain the usefulness of this assay in a clinical pharmacokinetic study, serum and urine were obtained from nine patients, 6 to 15 years of age, after a single 50-mg/kg intravenous dose of ceftazidime (M. D. Reed, C. M. Kercsmar, C. M. Myers, R. C. Stern, D. Murdell, and J. L. Blumer, Abstr. Annu. Meet. Am. Soc. Clin. Pharmacol. Ther. 33:246, 1983). A summary of the serum elimination data is depicted in Fig. 4. Two-compartment pharmacokinetic analysis of these data revealed an average serum half-life of 1.59 h, with a clearance of 137 ml/min per 1.73 m^2. Urinary excretion data revealed an average recovery of 87% during the first 8 h after the dose. Of this, almost 65% was eliminated during the first two h.

The assay described above provides a rapid and reproducible means for the detection of ceftazidime concentrations in biological fluids. One method published previously (1) has not been reproducible by us because the perchloric acid (0.8 M) used for protein precipitation caused variable chromatographic elution times. In addition, a serum peak consistently interfered with the ceftazidime peak. Our micromethod appears to be well suited for the determination of ceftazidime pharmacokinetics in infants and children.

ACKNOWLEDGMENTS

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