ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1983, p. 720–724
0066-4804/83/110720-05$02.00/0

Vol. 24, No. 5

High-Pressure Liquid Chromatographic Analysis of Ceftazidime in Serum and Urine

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Received 28 June 1983/Accepted 1 September 1983

A rapid and sensitive high-pressure liquid chromatographic procedure was developed for quantitative analysis of a new semisynthetic cephalosporin, ceftazidime, in serum and urine. A good linear relationship was established between peak height and the amount of ceftazidime injected over a concentration range of 1.9 to 30 μg/ml. Recovery was approximately 90% at concentrations of 3, 15, and 30 μg/ml. The method is specific for ceftazidime, with no interference noted from 11 other β-lactam antibiotics tested. The assay is accurate, reproducible, and useful for pharmacokinetic studies in humans as demonstrated in two subjects.

Ceftazidime [(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] is a recently developed semisynthetic cephalosporin antibiotic with enhanced in vitro activity against a wide variety of gram-negative organisms, particularly *Pseudomonas aeruginosa* and *Pseudomonas cepacia* (2–7).

Traditionally, microbiological assays have been used to determine antibiotic concentrations in biological fluids. Even though this method is sensitive, has a high capacity for processing samples, and measures only the biologically active component in the test system, lack of specificity, limited reproducibility, and an inability to detect biologically inactive metabolites are major disadvantages. An additional disadvantage is the length of time required for sample analysis. Microbiological assays for ceftazidime utilizing *Bacillus subtilis* 1904E spore suspension and *Proteus morganii* NCTC 235 as test organisms both require incubation periods of 18 h (Laboratory Manual-Ceftazidime, Glaxo Canada Ltd.). High-pressure liquid chromatography (HPLC) provides a sensitive and specific alternative with the additional advantages of high precision and rapid turn-around time. An HPLC assay for ceftazidime has been previously published (1) but is difficult to reproduce since analysis is performed on a packed column not commercially available. The purpose of this report is to describe an HPLC assay for the measurement of ceftazidime in serum and urine developed on a commercially available, prepacked HPLC column. The suitability of this method for use in pharmacokinetic studies is confirmed by analysis of serial serum samples obtained after administration of ceftazidime (50 mg/kg) infused intravenously over 20 min in two healthy volunteers.

MATERIALS AND METHODS

Reagents. Ceftazidime pentahydrate (lot number GCR 2191/D/1) was obtained from Glaxo Group Research Ltd., Ware, Hertfordshire, United Kingdom. The internal standard, 8-chlorotheophylline, was purchased from Sigma Chemical Co., St. Louis, Mo. Potassium phosphate monobasic (KH_2PO_4) was acquired from Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, N.J., and HPLC grade methyl alcohol (UV cutoff, 203 nm) was obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. The mobile phase for analysis of serum samples was an 82:18 volume mixture of 150 mM KH_2PO_4 buffer, pH 6.5, and methanol, which was filtered and degassed by passing through a 0.5-μm Durapore filter in an all-glass filter apparatus (Millipore Corp., Bedford, Mass.). The solvent mixture for urine analysis (88:12 volume mixture of 50 mM KH_2PO_4, pH 6.5, and methanol) was filtered and degassed in a similar manner. Stock solutions of drug and internal standard were prepared daily by dissolving 10 mg of ceftazidime acid and 5 mg of 8-chlorotheophylline in 10 and 50 ml of distilled water, respectively. Dissolution of the 8-chlorotheophylline was aided by sonication for 2 to 3 min.

Sample preparation. To a 100-μl volume of serum in a 0.5-ml propylene microcentrifuge tube was added an equal volume of internal standard solution (equivalent to 3 μg of 8-chlorotheophylline) prepared from the stock solution. The proteins were precipitated by the addition of 100 μl of methanol.

The mixture was then blended with a Vortex mixer for 15 sec and centrifuged at 12,800 × *g* for 3 min in an Eppendorf model 5412 centrifuge. A 20-μl volume of the supernatant was then injected manually with a 25-μl syringe through a Rheodyne model 7105 valve injector with a 175-μl capacity loop.

A 100-μl portion of urine containing ceftazidime (diluted with distilled water to within the concentration range of the standards) was mixed with 200 μl of internal standard solution and injected directly into the system.

Apparatus and chromatographic conditions. The HPLC system consisted of a Perkin Elmer series 2/2 pump (Perkin Elmer Corp., Norwalk, Conn.) and a Perkin Elmer model LC-75 variable wavelength detector set at 255 nm based on the ceftazidime spectrum (Fig. 1) obtained with a Beckman DU-8 UV-visible computing spectrophotometer (Beckman Instruments, Inc., Scientific Instruments Division, Irvine, Calif.). A 3.9-mm by 30-cm μBondapak C_{18} column (Waters Associates, Inc., Milford, Mass.) preceded by a guard column filled with μBondapak C_{18}/Corasil 37- to 50-μm particles (Waters Associates, Inc.) was used to achieve separation. The absorbance detector output was monitored with a Perkin Elmer model 023 linear recorder. The attenuation was set at 0.02 absorbance units full scale for serum analysis and 0.08 absorbance units full scale for urine analysis. The mobile phase was delivered at a flow rate of 1.2 ml/min (1,200 lb/in^2) for the serum assay and 2.0 ml/min (2,000 lb/in^2) for the urine analysis. All analyses were carried out at ambient temperature.

Method of calculation. Chromatogram peaks for ceftazidime and 8-chlorotheophylline were identified by their retention times. Ceftazidime concentrations in serum and urine were calculated on the basis of the ratio of ceftazidime and 8-chlorotheophylline peak heights. On each day of analysis, standards of three different ceftazidime concentrations were prepared in blank human serum and extracted as previously described. For each standard concentration, a response factor (RF) was calculated in the following manner:

$$RF = \frac{\text{Peak height internal standard}}{\text{Peak height ceftazidime}} \times \frac{[\text{ceftazidime}]}{[\text{internal standard}]}$$

The average of the three response factor determinations was used to calculate samples of unknown ceftazidime concentration according to the following equation:

$$[\text{Ceftazidime}] = \frac{\text{Peak height ceftazidime in unknown} \times RF}{\text{Peak height internal standard in unknown}} \times [\text{internal standard}]$$

Ceftazidime concentrations (in micrograms per milliliter) were expressed as the acid equivalent, with a factor of 0.859 used to convert to ceftazidime acid the amount of ceftazidime pentahydrate used in the preparation of the stock solutions previously described.

Recovery study. Ceftazidime serum samples were spiked at concentrations of 3, 15, and 30 μg/ml. The recovery of ceftazidime from the serum was determined by comparing the peak heights from the extracted serum samples with those obtained from unextracted aqueous standards of the same concentrations. The percent recovery was determined as the mean of six replications at each concentration.

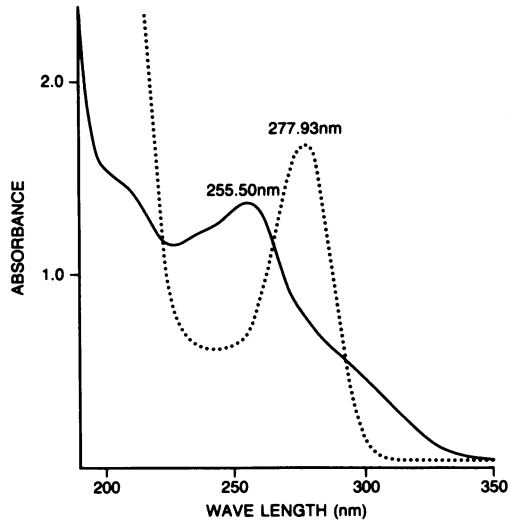

FIG. 1. Absorbance scan for 36.6 mg of ceftazidime acid (—) and 26.1 mg of 8-chlorotheophylline (· · ·) dissolved in 1 liter of distilled water.

RESULTS AND DISCUSSION

Serum assay. Figure 2 shows the typical chromatogram of ceftazidime and the internal standard in human serum compared with a blank serum sample. An unknown endogenous peak separates the peaks produced by ceftazidime and 8-chlorotheophylline. The elution times for ceftazidime and 8-chlorotheophylline were 5.0 and 10.7 min, respectively. The retention times for a number of β-lactam antibiotics and other commonly administered drugs were determined under these assay conditions. None of these were found to interfere with the measurement of ceftazidime or internal standard (Table 1).

Linearity of the detector response was established by preparing a standard curve from an aqueous ceftazidime solution (Fig. 3). This was obtained by plotting the peak height of ceftazidime (ordinate axis) against the amount of the drug injected (abscissa). The plot was linear over the interval 12.5 to 200.0 ng per sample (r^2 = 0.9999). Assuming a constant injection volume of 20 μl, this corresponds to a concentration range of 1.9 to 30.0 μg/ml. The sensitivity of the assay (using an injection volume of 20 μl) is estimated to be 1.0 μg/ml. Serum samples containing less than this concentration can be quantified by increasing the injection volume or the attenuation or both.

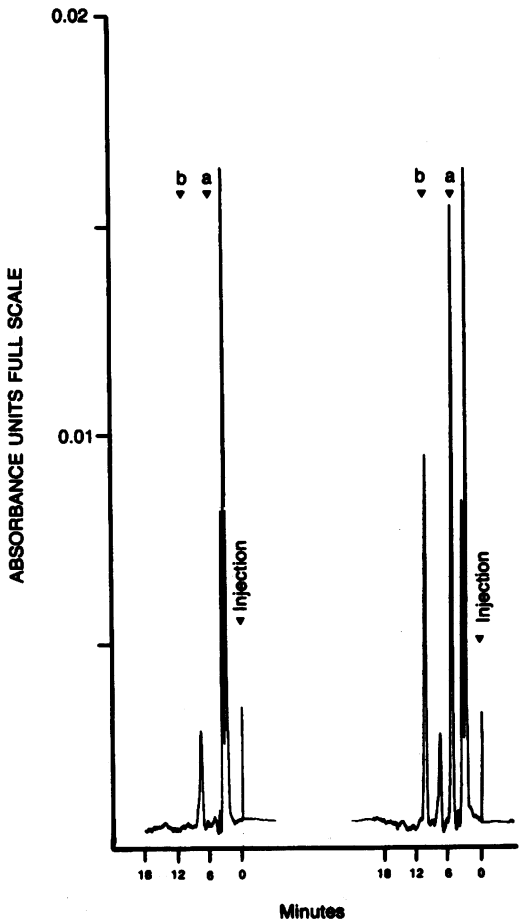

FIG. 2. Typical HPLC chromatogram of control human serum and serum containing 30 μg of ceftazidime (peak a) per ml and 30 μg of 8-chlorotheophylline (peak b) per ml.

TABLE 1. Retention times of ceftazidime, some β-lactam antibiotics, and other commonly administered drugs

Compound	Retention time (min)
Ceftazidime pentahydrate	5.0
8-Chlorotheophylline (internal standard)	10.7
Cefaclor	11.7
Cefamandole nafate	>15[a]
Cefazolin sodium	>15
Cefoperazone sodium	>15
Cefotaxime sodium	13.7
Cephalexin	12.3
Cephalothin sodium	>15
Amoxicillin trihydrate	4.4
Ampicillin sodium	14.0
Cloxacillin sodium	>15
Ticarcillin monosodium	>15
Acetaminophen	6.0
Amikacin	>15
Caffeine	>15
Clindamycin	>15
Gentamicin	>15
Tobramycin	>15

[a] No peak apparent in chromatogram at 15 min; retention time greater than 15 min or minimal absorptivity at 255 nm or both.

The recovery (mean ± standard deviation) of ceftazidime from the serum was found to be 89.6 ± 4.4, 91.3 ± 3.5, and 89.2 ± 2.1% at 3, 15, and 30 μg/ml, respectively.

The reproducibility of the assay method within the same day was determined at three different concentrations of ceftazidime. Six serum samples at each concentration (2.92, 15.29, and 30.35 μg/ml) were extracted and injected on the

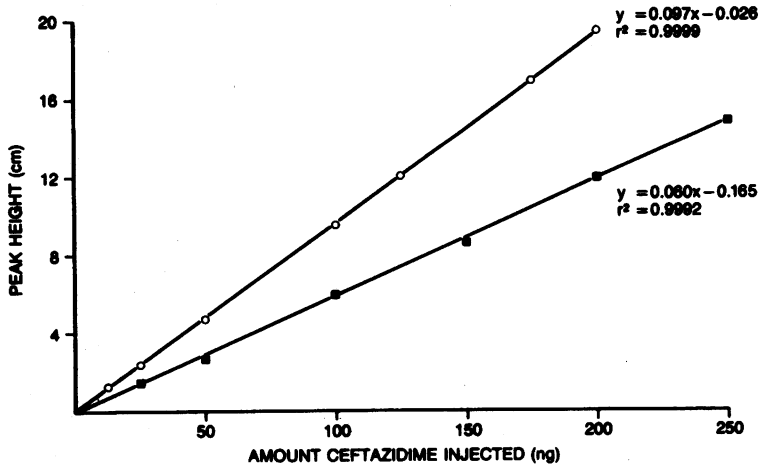

FIG. 3. Linear relationship between amount of ceftazidime injected (in nanograms) and the resultant peak height (in centimeters) under serum assay (○) and urine assay (■) conditions.

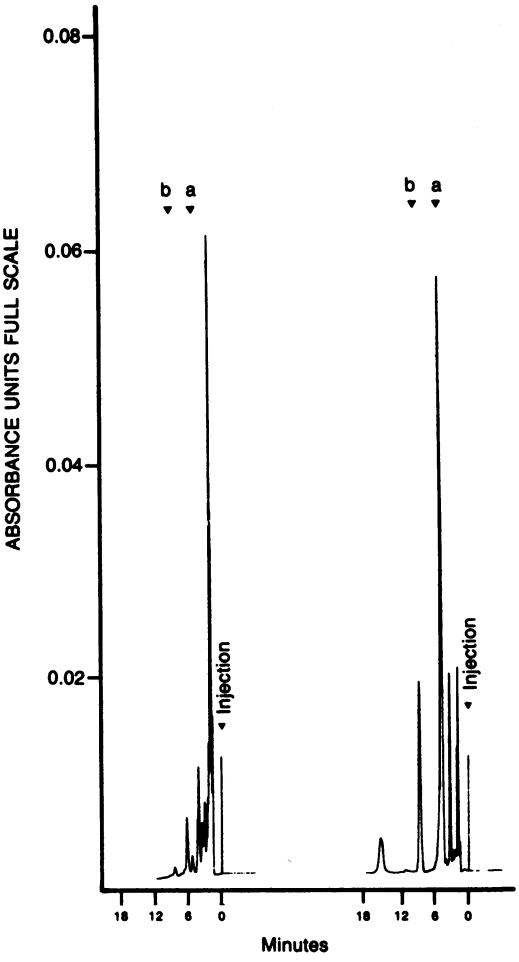

FIG. 4. Typical HPLC chromatogram of blank urine and a subject urine sample containing 240 μg of ceftazidime (peak a) per ml and 135 μg of 8-chlorotheophylline (peak b) per ml at 0.08 absorbance units full scale.

same day. This resulted in mean (± standard deviation) concentrations of 2.90 ± 0.15, 15.45 ± 0.82, and 30.34 ± 0.86 μg/ml. The coefficients of variation were 5.2, 5.3, and 2.8% respectively, demonstrating that the method is accurate and reproducible.

Urine assay. The presence of excessive endogenous interfering substances when analyzing urine samples under serum assay conditions made it necessary to alter the composition of the mobile phase (88:12 volume mixture of 50 mM KH_2PO_4 and methanol). The typical chromatograms of blank urine diluted 1:10 and diluted urine containing ceftazidime and 8-chlorotheophylline are shown in Fig. 4. The urinary ceftazidime concentrations observed in samples obtained from patients required a 20- to 200-fold dilution to be quantified. Retention times were 5.0 and 9.2 min for ceftazidime and the internal standard, respectively.

Linearity of the detector response was established for ceftazidime under the urine assay conditions in a manner analogous to that previously described (Fig. 3). The resultant plot was linear from 25.0 to 250.0 ng per sample (r^2 = 0.9992). As a result of the decreased methanol content and faster flow rate used for urine sample analysis, the peak heights for a given amount of ceftazidime injected were lower than under the comparable serum assay conditions.

Pharmacokinetic study. The feasibility of using this HPLC method for analysis of human serum samples was determined after a 20-min intravenous infusion of ceftazidime (50 mg/kg) in two volunteers. Serial serum samples obtained postinfusion were analyzed for ceftazidime content by the method described. The data were fitted to a two-compartment open model by using a computerized nonlinear least-squares regression analysis technique. Coefficients of determination for the fitted curves were 0.9986 and 0.9988 for subjects A and B, respectively. Relevant pharmacokinetic parameters are presented in Table 2, whereas the serum concentration-time profiles are depicted in Fig. 5. Urine samples collected up to 6 h postinfusion ranged in concentration from 2,370 to 11,340 μg/ml with 72.5 and 74.9% of the ceftazidime being recovered unchanged in the urine of subjects A and B, respectively, at the end of the collection period.

The HPLC assay presented is a rapid, sensitive, accurate, and reproducible method of determining the ceftazidime concentration in both serum and urine. It appears to be specific for ceftazidime since all the β-lactam antibiotics and

TABLE 2. Pharmacokinetic parameters obtained from nonlinear least-squares regression analysis of postinfusion data[a]

Subject	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	Vc (liter/kg)	Vss (liter/kg)	AUC_∞ (mg · min/liter)	TBC (ml/min)	RCl (ml/min)
A	6.7	88.2	0.133	0.218	31,230	91.8	72.4
B	14.3	108.7	0.167	0.254	35,794	121.2	99.8

[a] Abbreviations: $t_{1/2\alpha}$, distribution phase half-life; $t_{1/2\beta}$, terminal phase half-life; V_c, volume of distribution of the central compartment; V_{ss}, volume of distribution at steady state; AUC_∞, area under the curve; TBC, total body clearance; RCl, renal clearance.

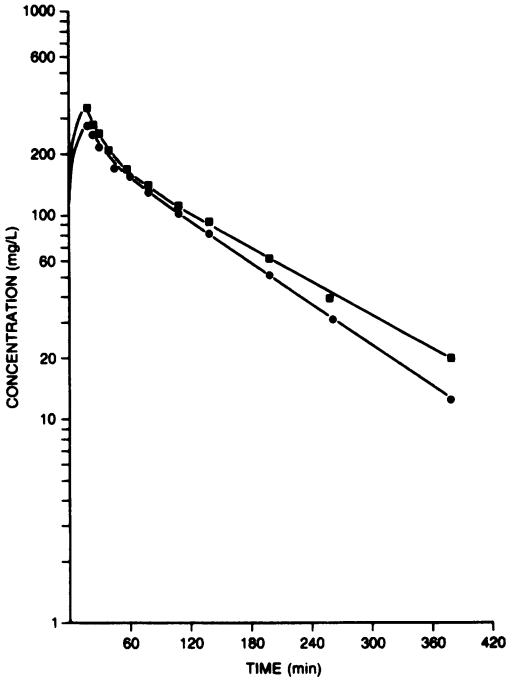

FIG. 5. Serum concentration-time curves for subject A (●) and subject B (■) after a 20-min intravenous infusion of ceftazidime.

other common drugs tested do not interfere with it. Coadministration of dimenhydrinate (Gravol, Dramamine), the 8-chlorotheophylline salt of diphenhydramine, could potentially interfere with this procedure. Should this problem present itself, cefaclor would be an appropriate substitute for the present internal standard (unpublished data). This method is also suitable for pharmacokinetic studies in humans and is likely to prove useful in further clinical investigation of ceftazidime.

ACKNOWLEDGMENTS

J.S.L. is a Fellow of the Ontario Ministry of Health, Health Research Personnel Development Program.

This work was supported in part by grant MA6831 from the Medical Research Council of Canada.

LITERATURE CITED

1. **Ayerton, J.** 1981. Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. J. Antimicrob. Chemother. 8(Suppl. B):227–231.
2. **Fleming, P. C., and B. Knie.** 1981. Activity of ceftazidime against *Pseudomonas aeruginosa* from bacteraemic and fibrocystic patients. J. Antimicrob. Chemother. 8(Suppl. B):169–173.
3. **Greenwood, D., and A. Eley.** 1982. Comparative antipseudomonal activity of some newer β-lactam agents. Antimicrob. Agents Chemother. 21:204–209.
4. **Neu, H. C., and P. Labthavikul.** 1982. Antibacterial activity and β-lactamase stability of ceftazidime, an aminothiazolyl cephalosporin potentially active against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 21:11–18.
5. **Reeves, D. S., H. A. Holt, and M. J. Bywater.** 1983. Comparative activity *in vitro* of ceftazidime and nine other antibacterial agents. Infection 11(Suppl. 1):S3–S11.
6. **Verbist, L.** 1981. Comparison of in vitro activities of eight β-lactamase-stable cephalosporins against β-lactamase-producing gram-negative bacilli. Antimicrob. Agents Chemother. 19:407–413.
7. **Verbist, L., and J. Verhaegen.** 1980. GR-20263: a new aminothiazolyl cephalosporin with high activity against *Pseudomonas* and *Enterobacteriaceae*. Antimicrob. Agents Chemother. 17:807–812.