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Note

Reversed-phase high-performance liquid chromatographic method to determine ceftriaxone in biological fluids

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Ceftriaxone is a member of the "third generation" of cephalosporin antibiotics characterized by a relatively broad antibacterial spectrum, including moderate activity against many strains of *Pseudomonas aeruginosa*. In addition to its broad antibacterial spectrum, ceftriaxone exhibits a uniquely long elimination half-life, is resistant to β-lactamase producing organisms, and diffuses well into extravascular spaces, including the cerebrospinal fluid (CSF). These characteristics offer potential clinical advantages which may lead to increasing use of this drug to treat bacterial meningitis and a variety of other serious infections.

Current published methods for determining ceftriaxone in biological fluids include bioassay of total antibacterial activity [1] and high-performance liquid chromatography (HPLC) [2—4]. The bioassays tend to be cumbersome, require a relatively long turnaround time, are susceptible to interference from concurrently administered antibiotics, and exhibit considerable random variation inherent in the assay system. Two of the previously described HPLC methods [2, 3] require a different mobile phase for determining the ceftriaxone concentration in each of these biological specimens: bile, serum, and urine. Another recent method uses normal-phase column chromatography [4].

We describe here a reversed-phase HPLC method using ion-pair chromatography to quantitate ceftriaxone in body fluids. This method utilizes the same chromatographic conditions and column for serum and urine. Additionally, by use of this same method, ceftriaxone can be measured in CSF as well. The

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method requires a small sample volume, provides sensitivity and precision, simplifies sample preparation and analysis, and allows rapid turnaround time.

EXPERIMENTAL

Materials and reagents

Ceftriaxone analytical standard was supplied by Hoffmann-LaRoche (Nutley, NJ, U.S.A.). Monobasic and dibasic potassium phosphate (Baker Analyzed) were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Fisher Scientific (Fairlawn, NJ, U.S.A.) was the source of HPLC-grade acetonitrile, methylene chloride, and the paired-ion reagent, hexadecyltrimethylammonium bromide (HDTMAB).

A stock standard solution of 1.0 mg/ml ceftriaxone in deionized water was prepared monthly and stored at 5°C. The mobile phase was prepared in the following sequence to prevent the buffer salts from crystallizing out of solution. Acetonitrile (400 ml) was added to 2.73 g of HDTMAB followed by the addition of 200 ml of deionized water. HDTMAB was completely dissolved prior to the addition of 7.5 ml of a 1 *M* monobasic—dibasic potassium phosphate buffer (pH 7.0). The volume was made up to 1 l with deionized water and degassed under vacuum prior to use.

Chromatography

Chromatography was performed utilizing a Waters Assoc. (Milford, MA, U.S.A.) M-45 solvent delivery system in conjunction with a Perkin-Elmer (Norwalk, CT, U.S.A.) oven and LC-55 UV/VIS variable-wavelength spectrophotometer. The detector was interfaced with a Perkin-Elmer Sigma 10 data system which provided the retention times and the integrated area under the peaks. A 5-μm 25 × 0.45 cm LiChrosorb RP-8, reversed-phase column (Supelco, Bellefonte, PA, U.S.A.) was used for analysis. The effluent was monitored at 280 nm at a flow-rate of 1.6 ml/min, while the oven temperature was maintained at 40°C.

Procedure

Standard curves were prepared by adding ceftriaxone stock solutions (1 mg/ml) to drug-free serum or urine to yield final concentrations of 5, 20, 50, 100, 200, and 400 μg/ml. A 200-μl aliquot of sample or standard (serum or urine diluted 1:10 with deionized water) was added to 200 μl of acetonitrile in a 1.5-ml Eppendorf tube. The tubes were vortexed and centrifuged for 5 min at 21,000 *g* in an Eppendorf 3200 centrifuge. The supernatant was transferred into a clean tube and extracted with 500 μl of methylene chloride. Each tube was vortexed for 20 sec and centrifuged for 5 min. A 10-μl volume of the upper aqueous phase was injected onto the column.

The concentration of ceftriaxone in CSF was determined by calibrating with aqueous standards in the range 0.5—50 μg/ml. CSF specimens were only centrifuged and 10 μl was injected onto the column without prior sample preparation.

RESULTS

Typical chromatograms obtained from serum are shown in Fig. 1. The retention time of ceftriaxone was 7.0 min. Similar chromatograms were obtained from CSF and urine specimens. Concentrations and peak areas were linearly related over the respective ranges in serum, urine, and CSF. Least-squares regression of the ceftriaxone standard curve generated the line $Y = 0.98X - 0.15$ with a correlation coefficient of 0.99.

Day-to-day variation was established by analyzing freshly prepared serum ceftriaxone concentrations of 5, 10, 20, 50, 100, 200 and 400 μg/ml. The average coefficient of variation (C.V.) was 5.5% with a range of 2.5—11.3% (Table I).

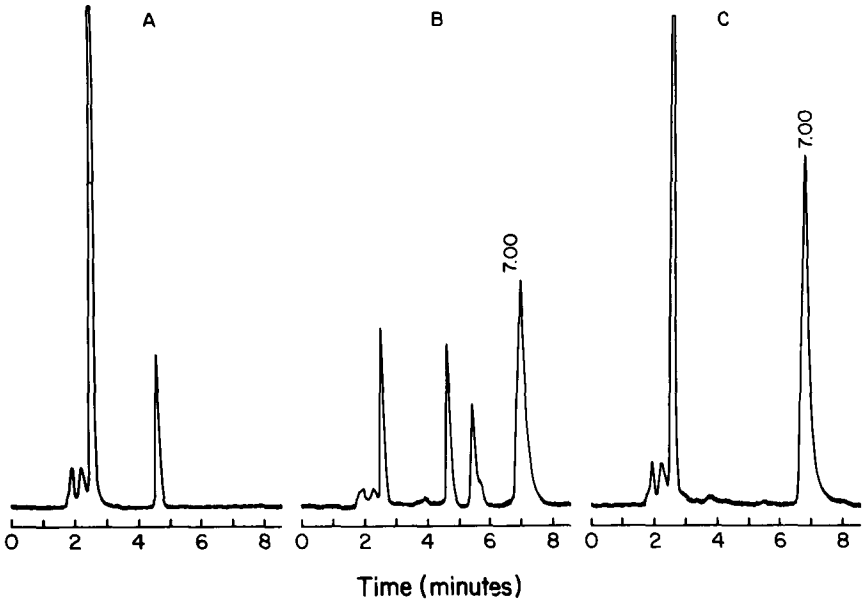

Fig. 1. Chromatograms of (A) drug-free serum; (B) a patient's serum sample in which the determined concentration of ceftriaxone was 29.4 μg/ml; (C) a control serum containing 50 μg/ml ceftriaxone. The retention time of ceftriaxone was 7.00 min.

TABLE I

DAY-TO-DAY VARIATION OF FRESHLY PREPARED SERUM CEFTRIAXONE STANDARDS

In all cases $n = 10$.

Ceftriaxone added (μg/ml)	Mean ± S.D. (μg/ml)	C.V. (%)
5	4.9 ± 0.6	11.3
20	19.5 ± 1.5	7.7
50	49.5 ± 1.5	3.0
100	100.5 ± 2.7	2.7
200	196.8 ± 11.4	5.8
400	395.0 ± 9.8	2.5

TABLE II

STABILITY OF CEFTRIAXONE IN SERUM AT −20° C OVER A FIVE-WEEK PERIOD

Week	Determined concentration of ceftriaxone (μg/ml)
1	52.1
2	59.8
3	57.6
4	54.8
5	53.3
Mean	55.5
± S.D.	3.2
C.V. (%)	5.7

The stability of ceftriaxone in serum was determined by freezing aliquots of a pooled serum containing ceftriaxone which were assayed over a five-week period. There was no significant loss of ceftriaxone during storage at −20° C (Table II). Analytical recovery of ceftriaxone from serum samples was determined to be 96% and 95% at concentrations of 50 and 400 μg/ml, respectively, when compared with aqueous standards.

Commonly co-administered antibiotics, such as methicillin, penicillin, ampicillin, sulfamethoxazole, gentamicin, tobramycin, and chloramphenicol, did not produce interfering peaks.

DISCUSSION

Ceftriaxone promises to be a very useful antibiotic to treat serious bacterial infections because of its broad spectrum of activity, prolonged half-life, and diffusion characteristics. The clinical efficacy of the drug can be further enhanced by the ability to accurately and rapidly determine concentrations in biological fluids thereby ensuring that bactericidal levels are achieved.

In the method presented here, we have taken advantage of using an RP-8 column, an ion-paired mobile phase, and a single, unique sample preparation step to obtain chromatograms free of peaks that interfere with the analysis of ceftriaxone. This clean chromatogram is obtained from serum, CSF, and, most importantly, urine. The method of Trautmann and Haefelfinger [2] used a separate, different mobile phase for serum and urine, while our method uses the same chromatographic conditions for serum, urine, and CSF. Using our method, specimens from CSF were injected directly onto the column, without prior sample processing. The use of one set of analytical conditions decreases the time and cost required for analysis and eliminates the change-over time from one set of chromatographic conditions to another. This method has a sample volume requirement of 200 μl which makes it suitable for use in pediatric patients as well as adults.

The range of pediatric ceftriaxone concentrations in our experience during 6 h after a dose was from 31 to 227 μg/ml in serum and from 0.7 to 20.6 μg/ml in CSF. If these values can be considered as an indication of the therapeutic range for these two fluids, then the method described here has the required

sensitivity and linearity (5.0—400 μg/ml in serum; 0.5—50 μg/ml in CSF) to be used in the routine therapeutic monitoring of ceftriaxone.

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