Journal of Chromatography, 182 (1980) 448—453
Biomedical Applications

CHROMBIO. 538

Note

High-performance liquid chromatographic determination of ceftizoxime, a new cephalosporin antibiotic, in rat serum, bile and urine

AKIRA SUZUKI, KOHSEI NODA and HIDEYO NOGUCHI

Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Kashima, Yodogawa-ku, Osaka (Japan)

(Received November 26th, 1979)

Ceftizoxime [(6*R*,7*R*)-7-[(*Z*)-2-(2-imino-4-thiazolin-4-yl)-2-methoxyimino-acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid], a semi-synthetic cephalosporin derivative, is a new broad-spectrum antibiotic developed by Takaya et al. [1]. We required a simple and rapid method of determining ceftizoxime for pharmacokinetic studies in rats and dogs given [^{14}C]ceftizoxime.

Various methods of analysis have been used to determine cephalosporins in body fluids. Concentrations of antibiotic in the serum or other body fluids have traditionally been determined by microbiological assay rather than by chemical methods. However, high-performance liquid chromatography (HPLC) for the quantitative determination of cephalosporin antibiotics in biological fluids has recently been shown to have definite advantages over routine bioassay in rapidity, precision and specificity.

Cooper et al. [2] reported the use of high-speed ion-exchange liquid chromatography for the separation and analysis of cephalothin and deacetylcephalothin in human serum after ion-pair extraction. Rapid analysis of cefazolin in dog and human serum by HPLC has been reported by Wold [3]. In 1978, Nilsson-Ehle and Nilsson-Ehle [4] reported a liquid chromatographic assay of cefuroxime in serum. Direct HPLC determination of cephalexin in human urine has recently been reported by Nakagawa et al. [5].

In the present paper, we describe a method for determining ceftizoxime in rat serum, bile and urine by direct injection of deproteinized biological fluids into a reversed-phase liquid chromatographic column. This technique is more simple and rapid than microbiological assays and is reproducible and sensitive.

EXPERIMENTAL

Reagents and materials

Ceftizoxime (Fig. 1) was prepared by the Fujisawa Pharmaceutical Co. (Osaka, Japan). Acetonitrile of UV grade was used. All the other solvents and reagents were of analytical reagent grade and were used without further purification.

The following aqueous solutions were made in distilled water: 0.2 *M* KH_2PO_4, 0.02 *M* KH_2PO_4, 0.2 *M* H_3PO_4, 0.02 *M* H_3PO_4, 10% acetic acid and 1% acetic acid.

Blank dog serum was obtained from the fresh blood of male beagle dogs. Blank rat serum was obtained from male SD rats fasted overnight. The sera were pooled and stored at −20°C in glass containers until analyzed.

Apparatus

Analyses were made on Waters Assoc. liquid chromatograph equipped with a Model 440 absorbance detector (254 nm or 280 nm fixed wavelength), a Model 6000A pump, a U6K universal injector and a 10-mV recorder.

Chromatographic conditions

A 30 cm × 4 mm I.D. μBondapak Alkyl Phenyl analytical column (Waters Assoc., Milford, MA, U.S.A.; particle size 10 μm) and a 5 cm × 2 mm I.D. precolumn packed with Bondapak Phenyl Corasil (37—50 μm) were used for analysis.

Although a number of mobile phases were tested during the investigation, no single solvent system afforded simultaneous resolution of ceftizoxime and endogenous components of the serum, bile and urine. Therefore, mobile phases of 13% acetonitrile in 0.02 *M* KH_2PO_4—H_3PO_4 buffer (pH 2.6), 11% acetonitrile in 0.02 *M* KH_2PO_4—H_3PO_4 buffer (pH 2.6), and 13% acetonitrile in 1% acetic acid were used for serum, bile and urine samples, respectively. The mobile phase was de-aerated under vacuum for approximately 1 min before use.

Bile and urine samples were detected at 280 nm using sensitivity settings of 0.05—0.2 absorbance unit full scale (a.u.f.s.). Serum samples were detected at 254 nm using sensitivity settings of 0.01 and 0.05 a.u.f.s. The operating temperature was ambient, and the flow-rate was 2.0 ml/min. The chart speed was 0.5 or 1.0 cm/min.

Preparation of standard solutions

All stock solutions of ceftizoxime were prepared by diluting a 50 mg per 100 ml primary standard solution with distilled water to make 0.2, 0.5, 1, 2.5, 5, 10, 25, 50, 100 and 200 μg per 0.1 ml concentrations. The solutions were used

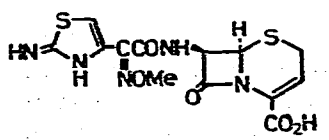

Fig. 1. Chemical structure of ceftizoxime.

for preparing standard curves and in reproducibility studies. The solutions were stored in glass containers at −20°C until analyzed.

Determination of standard curve

Serum. A 0.5-ml sample of serum was placed in a sample tube, and 0.1 ml of acetonitrile, 0.1 ml of 0.2 *M* KH_2PO_4—H_3PO_4 buffer (pH 2.6) and 0.1 ml of ceftizoxime standard solution (0.2—50 μg) were added. After about 10 min, the mixture was filtered through a 0.5-μm membrane filter. Then, 10 μl of the solution were injected into the liquid chromatograph equipped with a UV detector monitoring absorbance at 254 nm at sensitivity settings of 0.01—0.05 a.u.f.s. Standard curves were obtained by plotting peak height against concentration of ceftizoxime.

Bile. A 0.1-ml sample of rat bile was placed in a sample tube, and 0.4 ml of distilled water, 0.1 ml of acetonitrile, 0.1 ml of 0.2 *M* KH_2PO_4—H_3PO_4 buffer (pH 2.6) and ceftizoxime standard solution (1—50 μg) were added. After 10 min, the mixture was filtered through a 0.5-μm membrane filter. Then, 10 μl of the solution were injected into the liquid chromatograph equipped with a UV detector monitoring absorbance at 280 nm at a sensitivity setting of 0.05 a.u.f.s. The peak height was plotted against concentration of ceftizoxime.

Urine. Urine samples of 100 μl volume were diluted ten times with water; a 0.5-ml sample was then placed in a sample tube, and 0.1 ml of acetonitrile, 0.1 ml of 10% acetic acid and 0.1 ml of ceftizoxime standard solution (10—200 μg) were added. After about 10 min, the mixture was filtered through a 0.5-μm membrane filter, and 10 μl of the filtrate were injected into the liquid chromatograph equipped with a UV detector monitoring absorbance at 280 nm at sensitivity settings of 0.05—0.2 a.u.f.s. A standard curve was obtained by adding known amounts of ceftizoxime to the control urine. The peak areas or peak heights of ceftizoxime were plotted against the concentrations of ceftizoxime. Peak areas were obtained from the product of the maximum peak height and width at peak half-height.

RESULTS AND DISCUSSION

Separation

The liquid chromatograms of ceftizoxime from the spiked serum, bile and urine samples are shown in Figs. 2, 3 and 4, respectively. As shown in Fig. 2, the background peaks of the control serum have short retention times and are almost completely separated from those of ceftizoxime. Initially, the bile and urine samples were chromatographed under the same chromatographic conditions as the serum samples. Under those conditions, however, peaks interfering with certizoxime were present on the chromatograms from the control bile and urine. Therefore, a UV detector monitoring absorbance at 280 nm and different mobile phases were used to eliminate the interfering peaks from the chromatogram. The chromatograms of bile and urine samples obtained under these conditions are shown in Figs. 3 and 4. No interfering peaks appeared in any blank bile or urine samples at a retention time similar to that of ceftizoxime. Under these conditions, the retention times for ceftizoxime from serum, bile and urine were 5.0, 6.3 and 6.2 min, respectively.

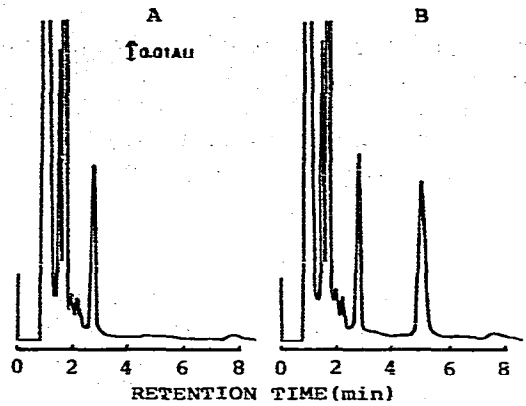

Fig. 2. Chromatograms of blank serum (A) and serum containing 20 μg of ceftizoxime per ml (B). Conditions: column, 30 cm × 4 mm μBondapak Alkyl Phenyl; mobile phase, acetonitrile—0.02 *M* KH_2PO_4—H_3PO_4 (pH 2.6) (13:87, v/v); flow-rate, 2.0 ml/min; detection, UV at 254 nm.

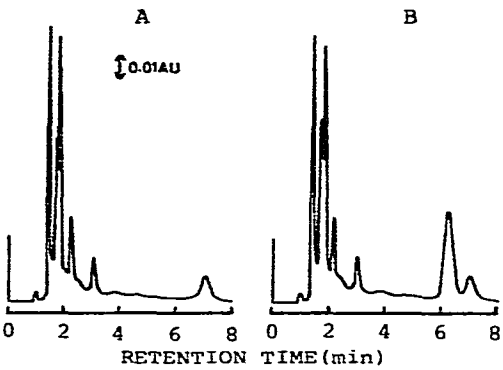

Fig. 3. Chromatograms of blank bile (A) and bile containing 100 μg of ceftizoxime per ml (B). Conditions: column, 30 cm × 4 mm μBondapak Alkyl Phenyl; mobile phase, acetonitrile—0.02 *M* KH_2PO_4—H_3PO_4 (pH 2.6) (11:89, v/v); flow-rate, 2.0 ml/min; detection, UV at 280 nm.

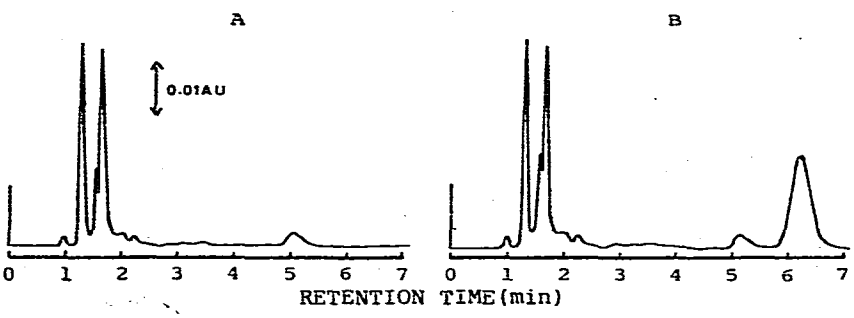

Fig. 4. Chromatograms of blank urine (A) and urine containing 1000 μg of ceftizoxime per ml (B). Conditions: column, 30 cm × 4 mm μBondapak Alkyl Phenyl; mobile phase, acetonitrile—1% acetic acid (13:87, v/v); flow-rate, 2.0 ml/min; detection, UV at 280 nm.

Stability of ceftizoxime

As the assay procedure is extremely simple and does not involve an evaporation step or alkalinity, the degradation of ceftizoxime, which is unstable in alkaline media, is avoided. Ceftizoxime in the serum, bile, urine and mobile phase solutions was stable for more than 6 h at room temperature and for 2 days at 4°C. Ceftizoxime standard solutions were stable for at least one month when the samples were stored at −20°C.

Recovery

Sample recovery of ceftizoxime from spiked solutions prepared with biological fluids was compared with that with water. The results are given in Table I. The peak of ceftizoxime in the spiked urine was low when determined by peak height measurement. It is presumed that this low peak was broadened by urinary components. These results suggest that the peak area measuring method is better than the peak height measuring method for the standard curve for urine.

TABLE I

RECOVERY OF CEFTIZOXIME FROM SPIKED BIOLOGICAL FLUIDS

Recovery = (biological fluid value/water control value) × 100 (n = 5 or 6). Chromatographic conditions are described under Experimental.

Biological fluid	Ceftizoxime range (μg/ml)	Peak measuring method	Recovery (mean ± S.D.)
Serum	0.2—5	Peak height	99.7±2.2
Serum	1—100	Peak height	101.8±5.0
Bile	10—500	Peak height	100.8±3.7
Urine	100—4000	Peak height	87.1±1.9
Urine	100—4000	Peak area	99.3±5.8

Standard curve

Standard curves were obtained by plotting peak height or peak area against concentration of ceftizoxime. A straight line was plotted by least-squares regression analysis, and its slope and intercept at the peak height or peak area was determined (Table II). The correlation coefficients (r) calculated from the regression lines were 0.9998 or better.

TABLE II

LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Conditions for HPLC are given under Experimental.

Biological fluid	Concentration range (μg/ml)	Regression		Correlation coefficient (r)
		Intercept	Slope	
Serum	0.2—5	−0.015	1.004	0.9998
Serum	1—100	+0.047	0.371	0.9999
Bile	10—500	+0.016	0.222	0.9999
Urine	100—4000	+0.018	0.071	0.9998

The lower limit of sensitivity was 10 μg/ml for the bile sample and 100 μg/ml for the urine sample. The determination limit for serum samples can be decreased to 0.2 μg/ml by the use of sensitivity setting of 0.01 a.u.f.s.

Reproducibility

Reproducibility was obtained by adding known amounts of ceftizoxime to the serum and by comparing five samples with a single calibration curve. The results are given in Table III. The data in Table III demonstrate the usefulness of HPLC in the analysis of ceftizoxime in the serum.

The procedure described here was used to obtain an accurate and precise determination of ceftizoxime in dog serum and urine. In this case also was the good accuracy and precision obtained.

TABLE III

REPRODUCIBILITY OF CEFTIZOXIME DETERMINATION IN RAT SERUM

Actual ceftizoxime conc. (μg/ml)	0.60	3.00
Number	5	5
Mean analyzed conc. (μg/ml)	0.57	3.06
Percentage of actual conc.	96.7	102
S.D.	0.02	0.05
Range (μg/ml)	0.55—0.59	3.01—3.10
Coefficient of variation (%)	3.5	1.7

ACKNOWLEDGEMENT

The authors thank Miss Hitomi Ohta for excellent technical assistance.

REFERENCES

1 T. Takaya, H. Takasugi, K. Tsuji and T. Chiba, Pat. Ger. Offen., 2,810,922 (1978); C.A., 90 (1979) 204116k.
2 M.J. Cooper, M.W. Anders and B.L. Mirkin, Drug Metab. Dispos., 1 (1973) 659.
3 J.S. Wold, Antimicrob. Ag. Chemother., 11 (1977) 105.
4 I. Nilsson-Ehle and P. Nilsson-Ehle, Clin. Chem., 24 (1978) 365.
5 T. Nakagawa, J. Haginaka, K. Yamaoka and T. Uno, J. Chromatogr., 147 (1978) 509.