Journal of Chromatography, 228 (1982) 429—433
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1157

Note

Determination of cefaclor by high-performance liquid chromatography

MILAP C. NAHATA

College of Pharmacy, Ohio State University, 500 West 12th Avenue, Columbus, OH 43210 (U.S.A.)

(Received August 24th, 1981)

Cefaclor, 3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid (Fig. 1A), is a new oral cephalosporin antibiotic. Its advantages over a commonly used oral cephalosporin, cephalexin, include a higher activity in vitro against *Hemophilus influenzae*, Enterobacteriaceae and *Neisseria gonorrhoeae* [1, 2]. Cefaclor has been shown to be effective in the treatment of respiratory and urinary tract infection, and skin infection [3]. In pediatric patients, it is most useful for the therapy of acute otitis media, secondary to nontypable beta-lactamase producing *Hemophilus influenzae* and unresponsive to ampicillin or amoxicillin [4]. It has been suggested that plasma concentration of an oral cephalosporin should be monitored in patients with impaired gastrointestinal absorption, unstable or compromised renal function, and poor compliance [5, 6].

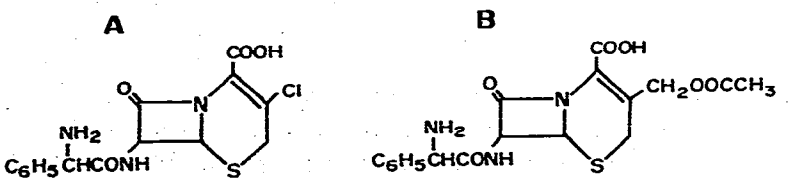

Fig. 1. Chemical structures of cefaclor (A) and cephaloglycin (B).

Most studies have utilized a microbiological assay for the measurement of cefaclor in biologic fluids. A high-performance liquid chromatographic(HPLC) technique for cefaclor is not readily available for wide use because two reports [7, 8] have appeared only in German.

This report describes a rapid, simple, sensitive, specific and reproducible

0378-4347/82/0000—0000/$02.75

HPLC method for cefaclor, using cephaloglycin (Fig. 1B) as an internal standard. To demonstrate its clinical utility, plasma and urinary concentrations of cefaclor in a normal subject are presented.

MATERIALS AND METHODS

Chemicals and reagents

Cefaclor, cephaloglycin and tobramycin were obtained from Eli Lilly (Indianapolis, IN, U.S.A.), carbenicillin from Roerig (New York, NY, U.S.A.), gentamicin from Schering (Kenilworth, NJ, U.S.A.), ampicillin from Wyeth Lab. (Philadelphia, PA, U.S.A.), and chloramphenicol from Parke Davis (Ann Arbor, MI, U.S.A.). Methanol (glass distilled) HPLC grade, was purchased from Mallinckrodt (Paris, KY, U.S.A.).

Chromatographic equipment and conditions

The reversed-phase HPLC system consisted of Consta Metric pump II G (Laboratory Data Control, Riviera Beach, FL, U.S.A.), μBondapak C_{18} column, 30 cm × 3.9 mm, 10 μm (Waters Assoc., Milford, MA, U.S.A.), analytical fixed-wavelength UV detector, Model 153 (Beckman Instruments, Fullerton, CA, U.S.A.) and a recorder, Series 5000 (Fisher Recordall, Houston Instruments, Houston, TX, U.S.A.).

Acetic acid (0.5%) was added to methanol—water (20:80) to prepare the mobile phase, which was pumped at a flow-rate of 2 ml/min. Chart speed of the recorder was set at 0.2 in./min.

Standards

Cefaclor (10 mg) and cephaloglycin (10 mg) were each dissolved in distilled water (10 ml) to yield a concentration of 1 mg/ml. Appropriate amounts of cefaclor solution were added to the plasma to give cefaclor concentrations of 0.5, 1, 2, 3, 5, 10, 15, 20 and 30 μg/ml. Concentrated urine samples were diluted with distilled water to yield the same concentrations of cefaclor. Both plasma and urine samples contained 2.5 μg of cephaloglycin. All samples were stored at −20°C.

Assay procedure

Plasma or urine samples (100 μl) containing known amounts of cefaclor and cephaloglycin were placed in polypropylene microcentrifuge tubes. Methanol, 200 μl was then added to the mixture. The resulting mixture was vortexed for 5 sec and then centrifuged at 9360 *g* for 10 min. The supernatant was transferred to another set of polypropylene test tubes and evaporated at room temperature under a gentle stream of nitrogen. The residue was dissolved in 100 μl of mobile phase, vortex mixed for 30 sec, and 50 μl of this were injected into the HPLC column. The detector was set at 0.005—0.02 a.u.f.s. (wavelength 254 nm).

Calculations

The concentrations of cefaclor in the unknown plasma and urine samples were calculated by comparing the cefaclor:cephaloglycin peak height ratios

with those obtained from cefaclor standard curves for plasma and urine.

Recovery and precision

Cefaclor was added to drug-free plasma and urine and then analyzed by the method described above but without any added internal standard. Fifty microliters of the supernatant were injected and peak heights corresponding to cefaclor measured. Absolute recovery was calculated by comparing these peak heights obtained by direct injection of pure standards.

Precision of the method was evaluated by repeated analysis of plasma and urine standards containing cefaclor and cephaloglycin concentrations of 20, 10 and 1 μg/ml. These samples were analyzed five times.

Drug interference

Commonly used antibiotics such as gentamicin (10 μg/ml), tobramycin (10 μg/ml), carbenicillin (15 μg/ml), ampicillin (20 μg/ml) and chloramphenicol (20 μg/ml) were tested using this method for potential interference with cefaclor and cephaloglycin.

Clinical application

A normal adult volunteer (age 30) received a single dose of cefaclor 1 g, orally (four capsules of Ceclor®, 250 mg, Eli Lilly). Blood samples were obtained at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 h following drug administration. Total urine volumes were collected at 0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 9.0, 12, 14, 17, 20.5, 21 and 24 h after drug administration. The specimens were stored at −20°C and analyzed within three days.

RESULTS AND DISCUSSION

Typical chromatograms of cefaclor and cephaloglycin in plasma and urine are shown in Fig. 2. Detector response (peak height) was linear (correlation coefficients > 0.99) over 0.5—30 μg/ml concentration range for cefaclor, with all curves passing through origin. Peak height ratios of cefaclor:cephaloglycin were also linear over the same concentration range.

The retention times for cefaclor and cephaloglycin were 3.75 and 5.95 min, respectively. Cefaclor was detectable at 0.5 μg/ml in both plasma and urine. Recovery of cefaclor and cephaloglycin ranged from 94—102% and the coefficient of variation for identical samples varied from 2—6%. The assay was specific in that commonly used antibiotics (gentamicin, tobramycin, carbenicillin, ampicillin and chloramphenicol) did not interfere with its measurement. Cefaclor was stable in plasma and urine under the storage conditions.

Fig. 3 shows the plasma concentrations of cefaclor at various times following the drug administration. Cefaclor peak plasma concentration of about 22 μg/ml with a 1-g dose in the present study is proportional to 6 μg/ml achieved with a 250-mg dose [9] and 11—12.5 μg/ml with a 500-mg dose [10—12] but lower than 27.3 μg/ml [13] and 34.6 μg/ml [14] with a 1-g dose. As in other studies [13, 14], the plasma concentration of cefaclor at 6 h following the 1-g dose was undetectable (< 0.5 μg/ml). Elimination half-life of about 43 min is similar to the reported values of 40—60 min in normal subjects [4, 9—15].

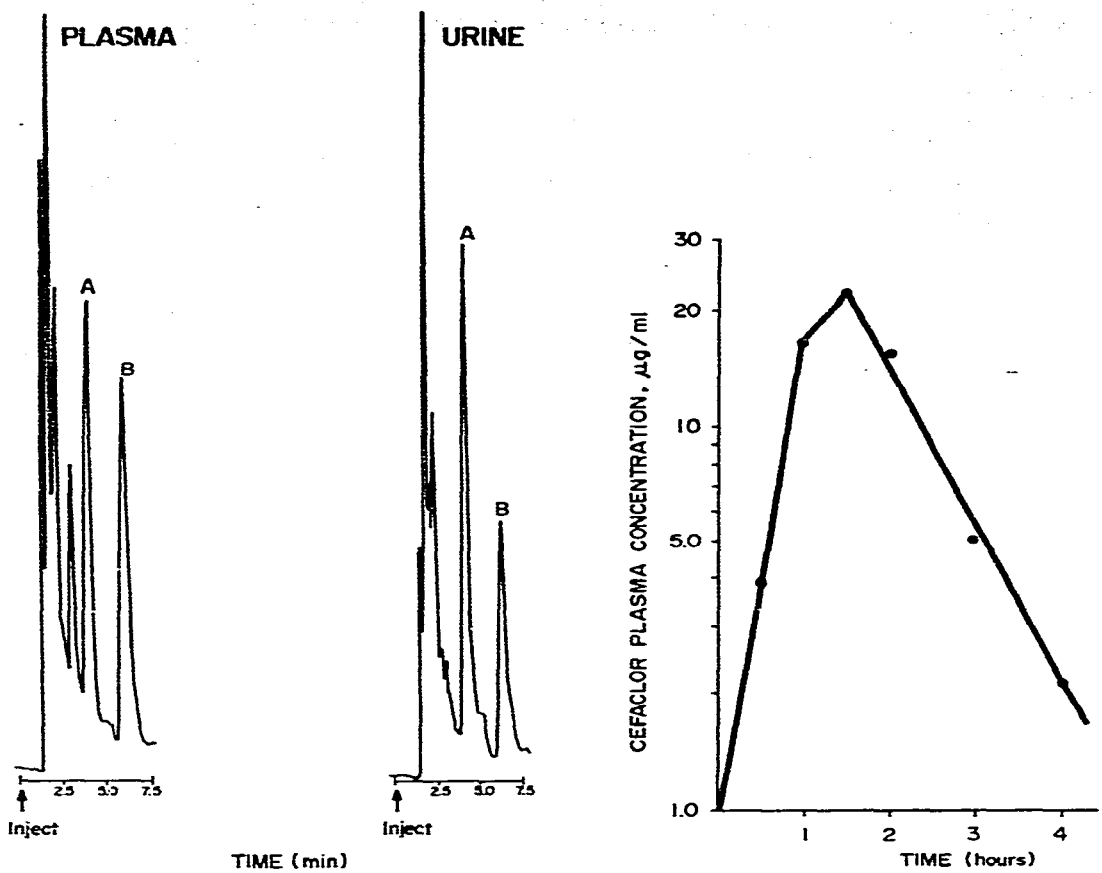

Fig. 2. Chromatograms of cefaclor (A) and cephaloglycin (B) in plasma and urine (A, 20 ml; B, 25 µg/ml). 0.01 a.u.f.s.

Fig. 3. Plasma concentrations of cefaclor following a 1-g oral dose.

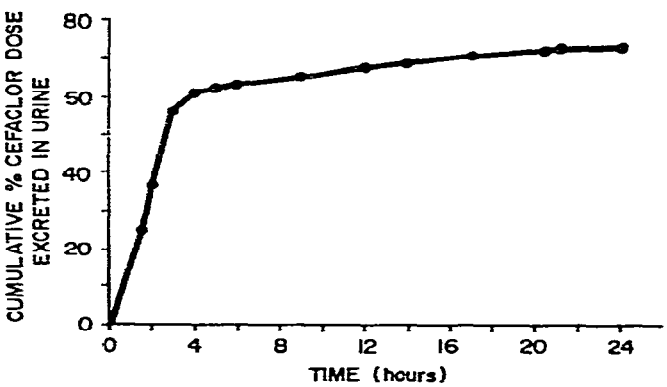

Fig. 4. Cumulative urinary excretion of cefaclor following oral administration of 1 g cefaclor.

Cumulative urinary excretion of cefaclor during a 24-h period following the dose is shown in Fig. 4. About 63% of the dose was excreted within the first 6 h while 77% was recovered within 24 h after cefaclor administration. This finding is consistent with a urinary recovery of about 70% in 6 h [9], 50% in 4 h [12], 65% in 9 h [13], and 50—70% in 8 h [15] following the dose; the percent excreted in the urine was higher than values (45—53% in 24 h) reported in two other studies [11, 14].

The method described has proven simple, rapid, reproducible, sensitive and specific for the determination of cefaclor in plasma and urine. The small sample size required in the procedure makes it suitable for monitoring cefaclor concentration in pediatric patients or for performing pharmacokinetic studies which require multiple samples of biologic fluids.

REFERENCES

1 N.J. Bill and J.A. Washington, Antimicrob. Ag. Chemother., 11 (1977) 470.
2 A.P. Gillett, J.M. Andrews and R. Wise, Postgrad. Med. J. (Suppl.), 55 (1979) 9.
3 R.B. Kammer and L.J. Short, Postgrad. Med. J. (Suppl.), 55 (1979) 93.
4 C.M. Ginsburg and G.H. McCracken, J. Pediatr., 96 (1980) 340.
5 C. Nightingale, M.A. French and R. Quintiliani, in W.E. Evans, J.J. Schentag and W.J. Jusko (Editors), Applied Pharmacokinetics, Applied Therapeutics, San Francisco, CA, 1980, p. 240.
6 J.D. Nelson, J. Pediatr., 92 (1978) 175.
7 U. Ullman and H.W. Diekmann, Infection, 7 (1979) S554.
8 E. Harhausen and C. Simon, Infection, 7 (1979) S603.
9 O.M. Korzeniowski, W.M. Scheld and M.A. Sande, Antimicrob. Ag. Chemother., 12 (1977) 157.
10 D.A. Spyker, B.L. Thomas, M.A. Sande and W.K. Bolton, Antimicrob. Ag. Chemother., 14 (1978) 172.
11 B.R. Meyers, S.Z. Hirschman, G. Wormser, G. Gartenberg and E. Srulevitch, J. Clin. Pharmacol., 18 (1978) 174.
12 J. Santoro, B.N. Agarwal, R. Martinelli, N. Werner and M.A. Levison, Antimicrob. Ag. Chemother., 13 (1978) 951.
13 C. Simon and U. Gatzemeier, Postgrad. Med. J. (Suppl.), 55 (1979) 30.
14 H. Lode, R. Stahlmann and P. Koeppe, Antimicrob. Ag. Chemother., 16 (1979) 1.
15 R. Bloch, J.J. Szwed, R.S. Sloan and F.C. Luft, Antimicrob. Ag. Chemother., 12 (1977) 730.