Journal of Chromatography, 147 (1978) 509–512

CHROM. 10,441

Note

Direct high-speed liquid chromatographic determination of cephalexin in urine

TERUMICHI NAKAGAWA, JUN HAGINAKA, KIYOSHI YAMAOKA and TOYOZO UNO
Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606 (Japan)
(Received June 13th, 1977)

It has been reported that cephalexin, a semisynthetic derivative of cephalosporin C, is rapidly absorbed following oral administration to give a high serum level and urine concentration[1]. Several analytical methods have been used for the quantitation of this drug in aqueous solution and biological fluids. Each method has its characteristic advantages and disadvantages; microbioassay[1–3] is highly sensitive but specificity, and fluorimetry[4,5] is more sensitive than polarography[6], or ultraviolet (UV)[7] or infrared[8] spectroscopy, but requires tedious procedures including solvent extraction and chemical pretreatment. High-speed liquid chromatography (HSLC), which combines good reproducibility and specificity with simple procedure, has been used for the determination of cephalexin in aqueous solution[9,10]. Prompted by a literature survey, we have developed this method to incorporate direct injection of the urine specimen. This paper describes the method, which should markedly facilitate analyses of the excretion rate and pharmacokinetic profile of cephalexin, and also be applicable to analogous compounds.

EXPERIMENTAL

Chromatography

A high-speed liquid chromatograph ALG/GPC 204 (Waters Assoc., Milford, Mass., U.S.A.) equipped with UV detector (254 nm, Waters Assoc. 440) was used in a reversed phase with a μBondapak C_{18} column (30.5 cm × 4.0 mm I.D., Waters Assoc.) and a mobile phase of methanol–water (1:5, v/v) containing 0.5% acetic acid, the flow-rate of which was maintained at 2.0 ml/min (1800 p.s.i.). Samples of 5 μl were used in the injector (Waters Assoc. U6K).

Reagents and materials

Distilled water and analytical grade methanol were used after micropore filtration and degassing. Reagent grade acetic acid was used as supplied. The monohydrate form of cephalexin (928 μg/mg potency), which was used as standard, and cephalexin capsules (Keflex®, 250 mg as potency) were gifts from Shionogi Seiyaku, Osaka, Japan. The potency of the standard cephalexin was regarded as the chemical purity, because no impurity peaks were found on the chromatogram. Hence, the results given below (Table I, Figs. 2 and 3) are corrected to this purity (92.8%).

Standard solution and calibration graph

Standard solutions were prepared by dissolving known amounts of standard cephalexin in human urine to make 1, 5, 10, 50, 100, 500 and 1000 μg/ml concentrations. The peak height was plotted against the concentration. The calibration graph thus obtained showed good linearity through the origin (correlation coefficient 0.999, $n = 3$ for each plot), and the average standard deviation of each plot was ±0.374.

Urinary excretion

The cephalexin capsules were orally administered to five healthy male volunteers after the control urine was taken. Urine samples were collected according to planned schedule (see Table I) and their volumes were measured. A 1–2-ml aliquot of the urine was filtered through 0.45-μm pore size triacetylcellulose membrane (Fuji Photo Film, Tokyo, Japan) and 5.0 μl of the filtrate was injected into the liquid chromatograph using a high-precision microsyringe (Precision Sampling, Baton Rouge, La., U.S.A.). The urine concentration was calculated from the peak height using the calibration graph.

RESULTS AND DISCUSSION

Prior to the urinary excretion experiments, the stability of cephalexin in water, human urine and the mobile phase was examined. It is known that cephalexin is stable in acidic media and its acidic degradation is pH independent, even in strong acid solution, whereas it suffers significant degradation in alkaline solution[9]. The pH values of the media used in this study were 3.1 for the mobile phase, 5.5 for distilled water and 5.5–6.5 for urines. No peaks due to degradation products of cephalexin were observed on the chromatograms obtained from standard urine solution and water solution. The UV response was reproducible except in case of fluctuation of the mobile-phase flow-rate. As shown in Fig. 1, the background peaks due to control urine have short retention times and are almost completely separated from that of cephalexin under the conditions used. In the very low concentration region of *ca.* 1 μg/ml, however, a minor background peak overlapped visibly with that of cephalexin, which increased the apparent peak height and caused error. This, however, was not a problem because such a low concentration is almost below the urinary excretion level of cephalexin administered to humans in therapeutic doses. It was found that an increase in the water content in the mobile phase produced a greater increase in the retention time of cephalexin than in those of background peaks. Thus, the ratio of water to methanol in the mobile phase was chosen to give satisfactory separation in as short a time as possible. The background peaks differed only in their intensities, which did not affect the separation. It is recommended, however, that the column be cleaned with a large volume of water and methanol to avoid depression of column efficiency.

Table I lists the results for the urinary excretion of caphalexin in each of the five subjects, and the average values. Figs. 2 and 3 illustrate the time courses of average excretion rate and of average cumulative recovery of cephalexin excreted in urine, respectively. These data indicate that the urinary excretion of cephalexin reaches a maximum rate between 1.5 and 2.5 h after a single oral administration of

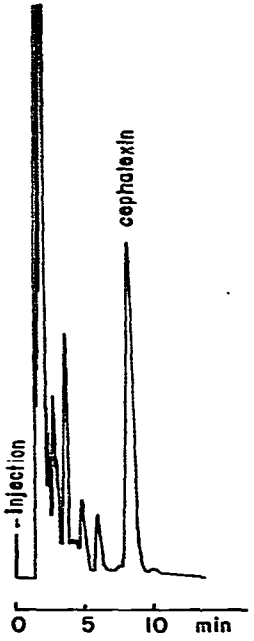

Fig. 1. HSLC separation of cephalexin from ordinary excretions in human urine. Concentration of cephalexin, 100 μg/ml; injected volume, 5 μl; a.u.f.s., 0.1. The background peaks are due to ordinary urine. Liquid chromatographic conditions are described in the Experimental section.

TABLE I

URINARY EXCRETION (mg) OF CEPHALEXIN FOLLOWING A SINGLE ORAL ADMINISTRATION OF ONE CAPSULE (250 mg) TO HEALTHY VOLUNTEERS

Subject	T.N.	J.H.	M.M.	N.H.	K.Y.		
Age (year)	37	23	28	27	28		
Body weight (kg)	79	62	68	57	68		
Time (h)						*Average*	*S.E.*
0 –0.5	0.0	0.69	0.80	0.0	0.0	0.30	0.19
0.5–1.0	35.37	12.88	68.55	70.12	14.57	40.30	12.50
1.0–1.5	55.32	20.48	70.98	74.36	34.83	51.19	10.39
1.5–2.0	36.70	47.22	29.44	38.44	47.83	39.93	3.45
2.0–2.5	21.31	35.09	16.42	19.81	57.17	29.96	7.51
2.5–3.0	14.85	30.08	9.46	9.03	28.62	18.41	4.59
3.0–3.5	7.62	15.89	5.72	5.19	13.19	9.52	2.13
3.5–4.0	8.35	10.47	4.14	2.64	8.21	6.76	1.46
4.0–5.0	7.42	6.66	6.09	1.76	6.05	5.60	0.99
5.0–6.0	2.00	2.70	0.78	0.0	5.03	2.10	0.87
Total	188.94	182.16	212.38	221.35	215.50	204.07	

the capsule, and almost half of dosed amount is excreted within 2 h and 81.6% in 6 h, where the ceiling effect is observed. Earlier reports[1,2,4] stated that an average of more than 90% of the dose was excreted in urine in 6 h. However, investigation of the data[1,2] revealed that the total urinary recovery of cephalexin obtained by micro-

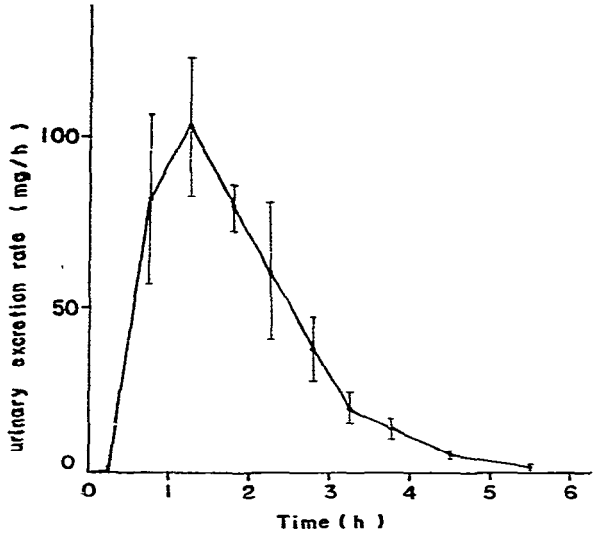

Fig. 2. Urinary excretion rate of cephalexin following oral administration of one 250-mg capsule.

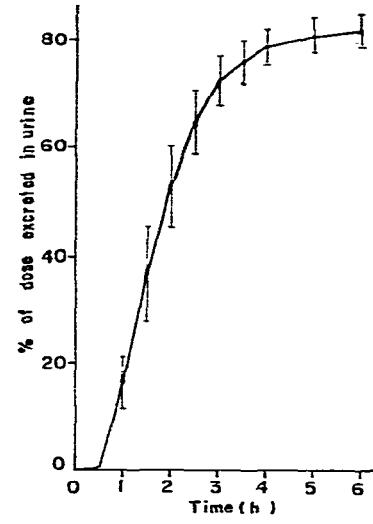

Fig. 3. Cumulative urinary excretion of cephalexin following oral administration of one 250-mg capsule.

bioassay sometimes considerably exceeded 100%, though the average percentage values did not, probably owing to errors in the analytical method. The present method is more specific and accurate than micribioassay and simpler than fluorimetry.

Some kinetic parameters were calculated from the average values in Table I, using a one-compartment open model and the least-squares method. The values for the apparent first-order rate constants of absorption and of urinary excretion, and the absorbed fraction of the dose are 3.46 h^{-1}, 0.553 h^{-1} and 0.852, respectively. A comparative discussion on the pharmacokinetic profiles of cephalexin with those described in a review article[11] will be given in a future paper.

REFERENCES

1 P. E. Gower and C. H. Dash, *Brit. J. Pharmacol.*, 37 (1969) 738.
2 R. L. Perkins, H. N. Carlisle and S. Saslaw, *Amer. J. Med. Sci.*, 256 (1968) 122.
3 C. H. O'Callaghan, S. M. Kirby and D. R. Wishart, *Antimicrob. Agents Chemother.*, (1968) 716.
4 R. Aikawa, M. Nakano and T. Arita, *Chem. Pharm. Bull.*, 24 (1976) 2350.
5 A. B. C. Yu, C. H. Nightingale and D. R. Flanagan, *J. Pharm. Sci.*, 66 (1977) 213.
6 E. J. Benner, *Antimicrob. Agents Chemother.*, (1971) 201.
7 L. P. Marrelli, in E. H. Flynn (Editor), *Cephalosporins and Penicillins*, Academic Press, New York, 1972, p. 630.
8 B. Casu and P. Ventura, *J. Pharm. Sci.*, 65 (1976) 211.
9 T. Yamana and A. Tsuji, *J. Pharm. Sci.*, 65 (1976) 1563.
10 E. R. White, M. A. Carroll, J. E. Zarembo and A. D. Bender, *J. Antibiot.*, 28 (1975) 205.
11 C. H. Nightingale, D. S. Greene and R. Quintiliani, *J. Pharm. Sci.*, 64 (1975) 1899.