Journal of Chromatography, 339 (1985) 359—365
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2519

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFONICID IN HUMAN PLASMA AND URINE

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(First received August 16th, 1984; revised manuscript received December 18th, 1984)

SUMMARY

A high-performance liquid chromatographic assay for determination of cefonicid concentrations in human plasma and urine samples has been developed using cefazolin as an internal standard. For the analysis of plasma samples two calibration curves were utilized covering the cefonicid concentration ranges of 0.05—1.0 μg/ml and 1.0—50.0 μg/ml, respectively. Coefficients of variation of 7.4% or less were obtained for cefonicid concentrations of 0.05—50.0 μg/ml. Mean bias was +6.0% at 0.05 μg/ml cefonicid and between —2.1% and +1.6% for 1.0—50.0 μg/ml cefonicid. Plasma samples containing 30 ng/ml cefonicid could be well distinguished from blank plasma samples. Urine samples were analysed by using a calibration curve for cefonicid concentrations between 1.0 and 50.0 μg/ml. Coefficients of variation ranged from 8.6% at a cefonicid concentration of 1.0 μg/ml to 0.5% at 50.0 μg/ml with a mean bias between —3.0% and +0.3%.

INTRODUCTION

Cefonicid is a new parenterally active cephalosporin which has a prolonged plasma elimination half-life ($t_{1/2}$) of 3.5 h and of 4.8 h after intravenous and intramuscular administration [1], respectively, when compared to cephalothin, cefazolin, cefamandole or cefoxitin with a respective $t_{1/2}$ of 0.5 h, 1.8 h, 50 min and 40 min to 1.0 h [2—4].

The pharmacokinetics of cefonicid was determined by microbiological assay [1, 5—8] for analysis of plasma, urine and tissue samples. However, the microbiological assay has two disadvantages. Firstly, the assay is time consuming as an incubation time of 18 h is required. Secondly, the assay has in principle no inherent potential for detection and quantitation of metabolic derivatives of the parent compound. For further intensive pharmacokinetic investigations of

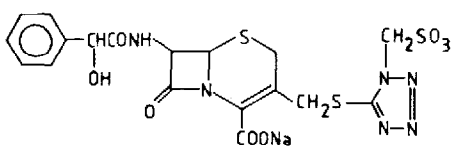
0378-4347/85/$03.30

cefonicid a rapid, sensitive and specific assay was needed. Although several high-performance liquid chromatographic (HPLC) assays for various cephalosporins have been developed (ref. 9 and references cited therein), an assay for cefonicid using an HPLC technique has not been published yet. We report here an HPLC assay for plasma and urine samples sensitive enough to detect concentrations of 30 ng/ml.

EXPERIMENTAL

Materials

Cefonicid (I) was synthesized by Smith Kline & French Laboratories (Lot X-1-2X80). Cefazolin (II) was received from Fujisawa Pharmaceutical Co. (Lot 100110G). Methanol HPLC grade was delivered by J.T. Baker (Deventer, The Netherlands), acetonitrile (Chromasolv^{TM}) by Riedel-de-Haen (Seelze, F.R.G.) and triethylamine by Ega Chemie (Steinheim, F.R.G.). Human serum (Human-serum Biotest^{TM}) for preparation of standards was purchased from Biotest Pharma (Dreieich, F.R.G.). For the extraction of cefonicid from plasma or urine 1-ml Bond Elut^{TM} extraction columns (C_{18} bonded phase, part no. 607101) from Analytichem International (Harbor City, U.S.A.) were used.



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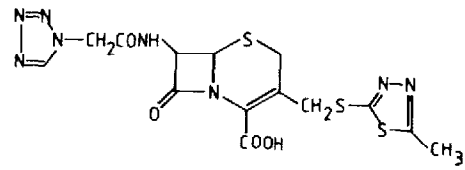

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Apparatus and chromatographic conditions

The chromatographic system consisted of a M 6000 A pump from Waters Assoc. (Eschborn, F.R.G.), an Altex Ultrasphere ODS column (5 μm particle size, 250 × 4.6 mm I.D.) from Beckman Instruments (Munich, F.R.G.), a Spectroflow 773 variable-wavelength UV detector from Kratos (Karlsruhe, F.R.G.), an automatic sample injection system WISP 710 B (Waters Assoc.) and a Data Module integrator (Waters Assoc.). The wavelength of the UV detector was set at 265 nm. Two different mobile phases were used: phase A for the concentration range of 50.0—1.0 μg/ml cefonicid, and phase B for the concentration range 1.0—0.05 μg/ml cefonicid. Mobile phase A was composed of 85% 0.05 *M* phosphate buffer containing 0.01 *M* triethylamine (the pH of the mixture was adjusted to 7.2), 9% acetonitrile and 6% methanol. The range of the UV detector was set at 0.01 a.u.f.s. Mobile phase B consisted of 87% 0.05 *M* phosphate buffer containing 0.01 *M* triethylamine (pH of the mixture was adjusted to 5.0), 11% acetonitrile and 2% methanol. The range of the UV detector was set at 0.002 a.u.f.s.

Preparation of samples

Plasma samples. For conditioning the Bond Elut^{TM} extraction columns, 5 ml

of methanol and then 5 ml of water were passed through within 6 min. A plasma sample (500 µl) containing 50—1.0 µg/ml cefonicid was mixed with 50 µl of a solution of 55 µg/ml cefazolin in 0.05 *M* phosphate buffer pH 6.5 (this solution is stable in the refrigerator for two days). A 500-µl volume of this mixture was pipetted onto the conditioned extraction column and passed through within 1 min under suction. After washing the column with 500 µl of 0.05 *M* phosphate buffer pH 6.5, cefonicid and cefazolin were eluted with 300 µl of methanol of which 15 µl were injected into the HPLC system.

For assay of plasma samples containing 1.0—0.03 µg/ml cefonicid, 500 µl of the sample were mixed with 50 µl of a solution of 5.5 µg/ml cefazolin in 0.05 *M* phosphate buffer pH 6.5; 500 µl of this mixture were worked up as described above. The methanol eluate was then evaporated to dryness under nitrogen at ambient temperature. The residue was dissolved in 100 µl of 0.05 *M* phosphate buffer pH 6.5 and 25 µl of this solution were injected into the HPLC system.

Urine samples. Urine samples were diluted either 1:20 or 1:100 with 1.8 *M* aqueous urea solution containing 0.1 mol/l sodium chloride to result in cefonicid concentrations within the calibration range. A 500-µl volume of the diluted sample was mixed with 50 µl of a solution of 55 µg/ml cefazolin in 0.05 *M* phosphate buffer pH 6.5. Thereafter 500 µl of this mixture were worked up identically to plasma samples containing 50—1.0 µg/ml cefonicid (as described above).

Calibration curves for plasma samples

Standard solutions of 1 mg/ml, 250, 10, 5, and 1 µg/ml cefonicid in 0.05 *M* phosphate buffer pH 6.5 were prepared and stored in the refrigerator (the solutions are stable for two days). Appropriate volumes of these standard solutions were adjusted to 500 µl with human serum (human serum Biotest™ was used instead of pooled plasma as it is homogeneous and of a standardized quality) to result in cefonicid concentrations of 50.0—1.0 µg/ml and 1.0—0.05 µg/ml, respectively. The standards thus obtained were then worked up according to the procedure described for plasma samples and chromatographed. For each concentration range a linear regression line was calculated as a function of the peak area ratios of cefonicid to internal standard versus concentration units of cefonicid. These calibration curves were prepared daily.

Calibration curve for urine samples

Standard solutions of 1 mg/ml, 250 and 10 µg/ml in 0.05 *M* phosphate buffer pH 6.5 were prepared and stored in the referigerator. Appropriate volumes of these standard solutions were adjusted to 500 µl with a 1.8 *M* aqueous urea solution containing 0.1 mol/l sodium chloride, resulting in cefonicid concentrations of 50—1 µg/ml (the aqueous urea solution was taken for preparation of standards instead of water in order to use a matrix similar to urine). These standards were worked up according to the procedure described above and then chromatographed. A regression line was calculated as a function of the peak area ratios of cefonicid to internal standard versus concentration units of cefonicid. This calibration curve was prepared daily.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of a blank plasma standard and of a 1.0 μg/ml cefonicid standard. These chromatograms demonstrate that the cefonicid peak is not completely separated to baseline from the preceding broad peak at 4.50 min. However, within the concentration range of 1.0—50.0 μg/ml cefonicid the influence of this interference peak is negligible. The calibration curve of peak area ratio was linear with a correlation coefficient of $r = 0.999$. Mean recoveries from spiked samples were 86% ($n = 6$, S.D. = ± 7%) at a cefonicid concentration of 1.0 μg/ml and 77% ($n = 3$, S.D. = ± 4%) at a concentration of 50.0 μg/ml by comparison with chromatograms of cefonicid standard solutions of equivalent concentrations.

The accuracy and precision of the HPLC assay for cefonicid in plasma was determined by adding known concentrations of 0.05, 1.0, 7.5, and 50.0 μg/ml cefonicid to blank human serum. The coefficients of variation ranged from 5.0 to 7.4%. Mean bias ranged from +6.0% at 0.05 μg/ml cefonicid to —2.1% at 7.5 μg/ml cefonicid (Table I).

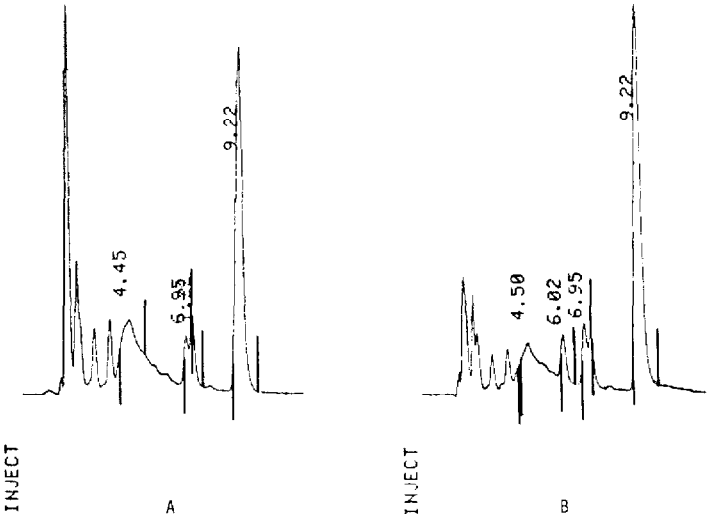

Fig. 1. Chromatograms of a blank plasma standard (A) and of a plasma standard containing 1.0 μg/ml cefonicid (B) with mobile phase A at 0.01 a.u.f.s. Peak at 6.02 min is cefonicid, peak at 9.22 min is internal standard.

TABLE I

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR CEFONICID IN PLASMA

Plasma concentration of cefonicid (μg/ml)	n	Mean concentration found (μg/ml)	Coefficient of variation (%)	Bias (%)
0.05	4	0.053	7.4	+6.0
1.0	5	0.99	6.4	—1.0
7.5	5	7.34	5.0	—2.1
50.0	5	50.81	6.6	+1.6

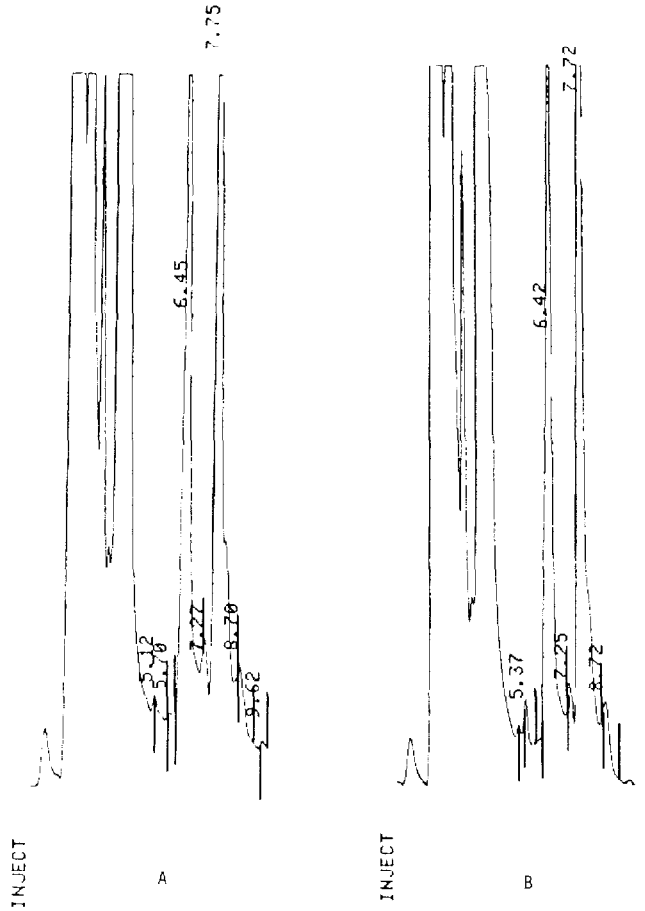

Fig. 2. Chromatograms of a blank plasma standard (A) and of a plasma standard containing 0.03 μg/ml cefonicid (B) with mobile phase B at 0.002 a.u.f.s. Peak at 5.37 min is cefonicid, peaks at 7.75 min and 7.72 min are internal standard.

Fig. 1 indicates that in chromatograms of plasma samples containing less than 1.0 μg/ml cefonicid the interference between the cefonicid peak and the preceding peak can not be neglected. For such cases, the mobile phase was slightly altered. Fig. 2 demonstrates that a concentration of 0.03 μg/ml can be well distinguished from a blank sample. The calibration curve of peak area ratio in the concentration range of 0.05—1.0 μg/ml was linear with a correlation coefficient of $r = 0.999$. However, at plasma concentrations above 1.0 μg/ml cefonicid, interference between the cefonicid peak and the following peak at 6.42 min could not be avoided. Therefore, mobile phase B can only be used for low cefonicid concentrations.

For analysis of cefonicid concentrations in urine a linear calibration curve was obtained within the concentration range of 1.0—50.0 μg/ml with a correlation coefficient of $r = 0.999$. Accuracy and precision data for cefonicid in urine are summarized in Table II. The coefficient of variation ranged from 0.5 to 8.6%. Mean bias varied from +0.3 to −3.0%.

This assay was used for the analysis of plasma and urine samples from a

TABLE II

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR CEFONICID IN URINE

Urinary concentration of cefonicid (μg/ml)	n	Mean concentration found (μg/ml)	Coefficient of variation (%)	Bias (%)
1.0	5	0.97	8.6	−3.0
7.5	5	7.39	3.3	−1.6
50.0	4	50.17	0.5	+0.3

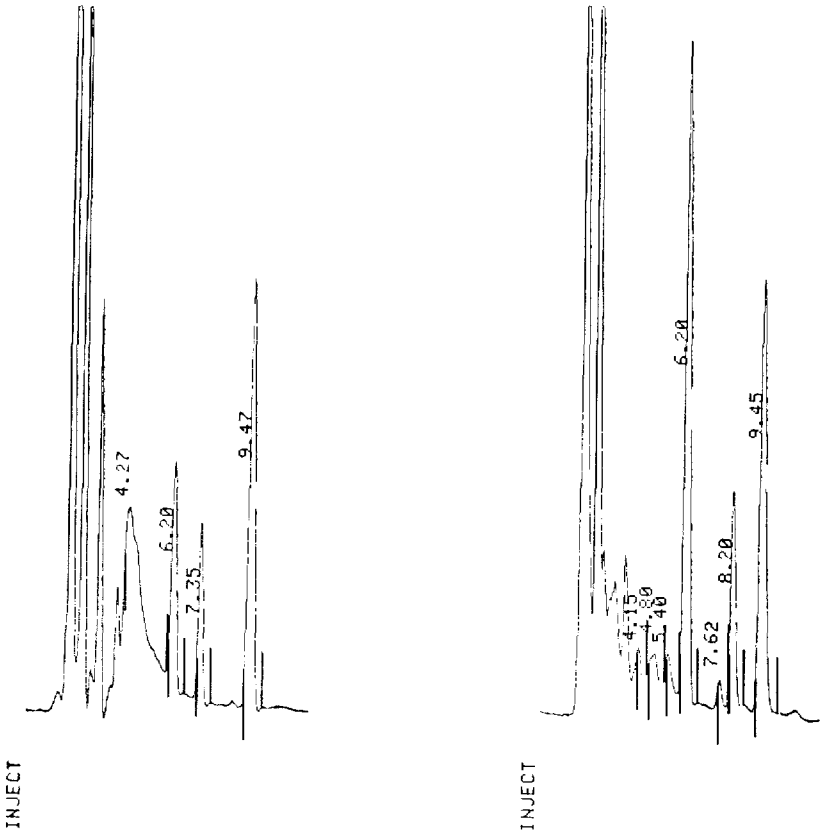

Fig. 3. Typical chromatogram of a plasma sample taken 12 h after intramuscular administration of 20 mg/kg body weight cefonicid to a healthy subject. Mobile phase A at 0.01 a.u.f.s. Peak at 6.20 min is cefonicid, peak at 9.47 min is internal standard. Cefonicid concentration = 4.6 μg/ml.

Fig. 4. Typical chromatogram of a urine sample (diluted 1:20 with 1.8 *M* aqueous urea solution) from the 12—24 h urine fraction after intravenous administration of 20 mg/kg body weight cefonicid. Mobile phase A at 0.01 a.u.f.s. Peak at 6.20 min is cefonicid, peak at 9.45 min is internal standard. Cefonicid concentration = 10.8 μg/ml.

24 h pharmacokinetic study in which either an intravenous or an intramuscular dose of 20 mg/kg body weight cefonicid was administered to healthy subjects. The results of this study are still to be published. Plasma concentrations of cefonicid ranged from about 140 μg/ml to about 1 μg/ml, indicating that in

general the calibration curve from 1.0 to 50.0 μg/ml can be used for most of the samples. Plasma samples containing concentrations of cefonicid higher than 50.0 μg/ml had to be diluted because otherwise the Bond Elut™ columns did not extract cefonicid completely from the sample. Figs. 3 and 4 demonstrate typical chromatograms of a plasma sample and a urine sample, respectively.

The results indicate that the HPLC assay for cefonicid described in this paper is more sensitive than the microbiological assay for which a limit of determination of 0.4 μg/ml is described [7]. This HPLC assay is rapid and provides the possibility of analysing at least 40 samples plus standards during a normal working day. It may therefore be particularly useful for extensive pharmacokinetic studies.

REFERENCES

1 D.H. Pitkin, J. Dubb, P. Actor, F. Alexander, S. Ehrlich, R. Familiar and R. Stote, Clin. Pharmacol. Ther., 30 (1981) 587.
2 I.W. Fong, E.D. Ralph, E.R. Engelking and W.M.M. Kirby, Antimicrob. Agents Chemother., 9 (1976) 65.
3 W.M.M. Kirby and C. Regamey, J. Infect. Dis., 128 (Suppl.) (1973) 341.
4 J.J. Schrogie, R.O. Davies, K.C. Yeh, D. Rogers, G.I. Holmes, H. Skeggs and C.H. Martin, J. Antimicrob. Chemother., 4 (1978) 69.
5 D.H. Pitkin, P. Actor, F. Alexander, J. Dubb, R. Stote and J.A. Weisbach, in J.D. Nelson and C. Grassi (Editors), Current Chemotherapy and Infectious Diseases, Vol. I, American Society of Microbiology, Washington, DC, 1980, p. 252.
6 D.H. Pitkin, P. Actor and J.A. Weisbach, J. Pharm. Sci., 69 (1980) 354.
7 S.L. Barriere, G.J. Hatheway, J.G. Gambertoglio, E.T. Lin and J.E. Coute, Jr., Antimicrob. Agents Chemother., 21 (1982) 935.
8 R.P. Sterlin, D.J. Connor, J.C. Norman and D.A. Cooley, Antimicrob. Agents Chemother., 23 (1983) 790.
9 I. Nilsson-Ehle, J. Liquid Chromatogr., 6 (1983) 251.