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

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SUMMARY

A rapid high-performance liquid chromatographic technique is described for the determination of cefonicid, a recently licensed cephalosporin antibiotic Quality-control assessment indicates a high level of accuracy and precision, stability data demonstrate no significant degradation of cefonicid in plasma and urine when stored at −70°C for six weeks

INTRODUCTION

Cefonicid (Fig. 1) is a recently licensed cephalosporin with antibacterial activity similar to that of cefamandole [1] It has been used successfully in the therapy of patients with a variety of infections, including urinary-tract infections [2, 3], community-acquired pneumonia [4], gonorrhea [5], osteomyelitis [6] and soft-tissue infections [7] Favorable results have been obtained with use of the drug for prophylaxis of infections associated with surgical procedures [8—10]. Cefonicid is highly protein bound (98%) and has a prolonged serum half-life (4 5 h) in patients with normal renal function [11, 12] In patients with impaired renal function, total clearance and renal

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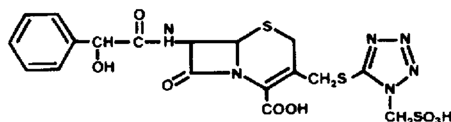
CEFONICID



CEPHALOTHIN

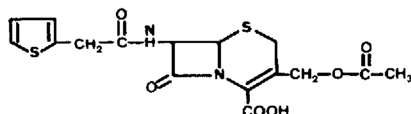


Fig 1 Chemical structures of cefonicid and the internal standard, cephalothin

clearance of the drug decrease linearly with a decline in creatinine clearance [13].

In two published papers [11, 13] cefonicid concentrations were determined using a reversed-phase, ion-pair, high-performance liquid chromatographic (HPLC) technique developed by one of the authors (E L.) at the University of California, San Francisco. This paper describes the details and quality-control assessment of the assay and compares plasma with serum determinations It is (with minor modifications) the same technique previously presented as an abstract [14].

EXPERIMENTAL

Reagents

Reagent-grade cefonicid sodium (supplied as 94.2% pure, Lot No X1-2X80, IPO No. 82053) was obtained from Smith Kline and French Labs (Phildadelphia, PA, U.S A.). Cephalothin sodium used for the internal standard was the commercially available preparation for injection (Keflin, Eli Lilly, Indianapolis, IN, U.S A). Acetonitrile and phosphoric acid were HPLC grade (Fisher Scientific, Fair Lawn, NJ, U S A). The ion-pair reagent, tetrabutylammonium hydrogen sulfate, was analytical grade (Aldrich, Milwaukee, WI, U S A.). Water was doubly distilled Human plasma used for standard curves was obtained from Irwin Memorial Blood Bank (San Francisco, CA, U.S A) Serum and urine used for standard curves were obtained from healthy volunteers. Plasma and serum specimens were obtained from volunteers participating in a cefonicid pharmacokinetic study [15].

HPLC system

The chromatographic equipment consisted of a Model M-45 solvent delivery system connected to a Model 710B WISP sample injector (Waters Assoc , Milford, MA, U.S.A.) A Waters Model 441 fixed-wavelength UV detector was used at 254 nm with a sensitivity of 0 005 absorbance units full scale (a.u.f.s.). The mobile phase consisted of 25% (v/v) acetonitrile, 0 1% (v/v) phosphoric acid and 0.3% (w/v) tetrabutylammonium hydrogen sulfate, which was filtered

and degassed through a Millipore filter (0.22 μm) prior to use. This eluent was pumped through a Waters μBondapak phenyl column (30 cm $\times$ 3.9 mm, particle size 10 μm) at a flow-rate of 2 ml/min. Chromatograms were recorded on a Model 37 MR dual-pen strip-chart recorder, 10 mV (Pedersen Instruments, Lafayette, CA, U.S.A.) at a chart speed of 20 cm/h. All steps of the chromatography were performed at room temperature. The retention time was 11.5 min for the internal standard and 13.5 min for cefonicid

Preparation of plasma and serum standards

Cefonicid stock solution was prepared by accurately weighing 400 mg of cefonicid and dissolving it in 40 ml water. Aliquots of 0.5 ml were stored at −70°C, used once and discarded. A working stock solution was prepared by diluting the above solution with mobile phase to yield a final cefonicid concentration of 100 μg/ml.

This solution was added in varying amounts to 0.2-ml samples of human plasma (or serum) to yield concentrations of 4.71—117.7 μg/ml. Disposable centrifuge tubes (Corning, 15 ml) were used.

A cephalothin stock solution was prepared by reconstituting a 1-g vial of cephalothin sodium with 50 ml water to yield a final concentration of 20 mg/ml. Aliquots of 0.5 ml were stored at −70°C, used once and discarded. This cephalothin stock solution was diluted with water to yield a 12.5 μg/ml working stock solution. The working stock solution was diluted 1:5 with acetonitrile to prepare the final internal standard solution.

Spiked plasma (or serum) samples were deproteinated by adding 0.4 ml of internal standard solution. The mixture was vortexed 10 s and centrifuged (1500 *g*) for 10 min. The supernatant was decanted into a glass tube (12 $\times$ 75 mm), evaporated under nitrogen to 0.2 ml, vortexed briefly and injected onto the column. Injection volumes were 5—20 μl.

The standard curve was constructed by plotting cefonicid/cephalothin peak-height ratios against the spiked concentration of cefonicid in the plasma (or serum). Five or six concentrations were used to construct the standard curves.

Preparation of plasma and serum samples

Plasma and serum samples from subjects receiving cefonicid were prepared and analyzed in the same way as the spiked specimens which were used to construct the standard curve. Samples containing drug concentrations higher than those used in preparing the standard curve were diluted with internal standard and not evaporated.

Preparation of urine standard

The cefonicid working stock solution (100 μg/ml) was prepared as described above. This solution (100 μg/ml) was added to 0.2-ml blank human urine samples to yield cefonicid concentrations of 4.71—141.2 μg/ml.

The cephalothin stock solution (20 mg/ml), prepared as described above, was diluted with water to yield a 50 μg/ml working stock solution. The working stock solution was diluted 1:5 with acetonitrile to prepare the final internal standard solution.

Spiked urine samples were deproteinated by adding 0 4 ml internal standard. The mixture was treated as described for plasma but not evaporated. Injection volumes were between 7 and 15 μl Standard curves were constructed as described for plasma and serum.

Preparation of samples for quality control

Samples for quality control were prepared using low (9.42 μg/ml), medium (47 1 μg/ml) and high (94.2 μg/ml) concentrations of cefonicid. Aliquots (0 5 ml) of these solutions were frozen and stored at —70°C. For the intra-day comparison one aliquot of each concentration was removed at hourly intervals, each was prepared and analyzed separately.

For the inter-day comparison, one aliquot for each concentration was thawed and analyzed on six separate days within a ten-day period. For the inter-week comparison, two aliquots for each concentration were thawed and analyzed at seven- to eight-day intervals over a six-week period. Plasma, serum and urine standard curves were prepared usually within one to two days, but not more than nine days, of sample analysis.

Statistical analysis and curve fitting were performed with the assistance of the Prophet Computer Resource at the University of California, San Francisco [16, 17] Linearity (r and r^2 from regression analysis of standard curve data), precision (coefficient of variation), recovery [18] (relation of test result to the true concentration) and percentage accuracy [19] were analyzed.

Detection limit was defined as the smallest peak height which was three times the baseline noise level. A standard curve was also constructed at very low concentrations of cefonicid (four points from 0.5 to 4.0 μg/ml, each point done in quadruplicate), and linearity of these data (r and r^2) was determined

RESULTS

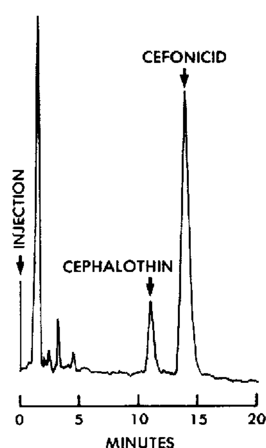
Figs 2 and 3 are sample chromatograms of plasma and urine, respectively. Small variation in the retention time in plasma and urine are the result of column variation

Using our defined detection limit, the limit of determination in plasma is 0 5 μg/ml. The r and r^2 of the very low concentration standard curve are 0 997 and 0 994, respectively

The statistical analyses for the mean r and r^2 for the standard curves are summarized in Table I

The results of the precision, recovery and accuracy assessments in plasma and urine are summarized in Tables II and III The mean coefficients of variation for plasma and urine are 3 83% (range 1 11—7 38%) and 4 91% (range 1 47—14 5%), respectively For plasma, there are no statistically significant differences among the means (at each concentration) for the intra- versus inter-day, intra- versus inter-week, or inter-day versus inter-week comparisons In each case, $p > 0.05$ by Newman—Keuls multiple-range test

For urine, there is no significant difference among the means (at low concentrations) for the intra- versus inter-day, intra-day versus inter-week, or inter-day versus inter-week comparisons In each case, $p > 0\ 05$ by Newman—Keuls multiple-range test For medium and high concentrations,



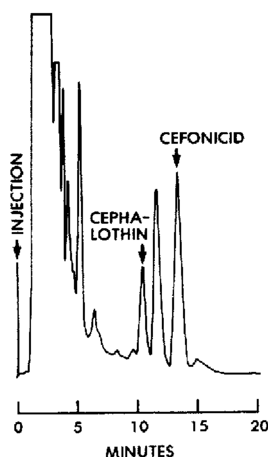


Fig 2 Chromatogram of plasma from patient receiving cefonicid intravenously (cefonicid concentration 45 5 μg/ml)

Fig 3 Chromatogram of spiked urine (cefonicid concentration 123 8 μg/ml)

TABLE I

SUMMARY OF STANDARD CURVE DATA

	Plasma		Serum		Urine	
	r	r^2	r	r^2	r	r^2
n	17	17	7	7	8	8
Mean	0 999	0 997	0 999	0 998	0 999	0 997
S D	0 001	0 003	0 001	0 002	0 001	0 002
Coefficient of variation (%)	0 135	0 272	0 076	0 152	0 094	0 188
99% Confidence interval	0 001	0 002	0 001	0 002	0 001	0 002
Linear equation	$y = -0\ 077+0\ 09(x)$		$y = -0\ 038+0\ 083(x)$		$y = -0\ 194+0\ 108(x)$	

there is no difference between the intra-day and inter-week results. In each case, $p > 0\ 05$ by Newman—Keuls multiple-range test. However, the intra- versus inter-day and inter-day versus inter-week comparisons revealed statistically significant ($p < 0\ 05$ by Newman—Keuls multiple-range test) but not clinically or kinetically significant differences (range of differences 4 49—10 2%, mean difference 6 73%) Difference here defined as (difference between mean results) divided by (lower of two mean results) × 100%

The mean recoveries of the assay for all determinations in plasma and urine are 106% (range 100—111%) and 111% (range 97—124%), respectively Mean accuracies for all determinations in plasma and urine are 6% (range 0—11%) and 11% (—3—24%), respectively

Table IV shows the results of six weekly determinations of cefonicid concentrations (at three concentrations) in plasma and urine Within the limits of the coefficients of variation of the test, there is no degradation of the drug over six weeks.

Simultaneous plasma and serum specimens were obtained at various times from patients receiving the drug intravenously In patients on chronic hemo-

dialysis, for sixteen pairs of values (range 30 3—194.9 μg/ml) the mean plasma and serum concentrations are 85.7 and 90 3 μg/ml, respectively The difference (5 3%) is not statistically different ($p > 0$ 05 by t-test for paired data) In non-dialysis patients, for 29 paired values (range 2 8—279 5 μg/ml) the mean plasma

TABLE II

ASSAY PRECISION, RECOVERY AND ACCURACY OF THE DETERMINATION OF CEFONICID IN PLASMA (n = 6)

Spiked concentration (μg/ml)	Measured concentration (mean ± S D) (μg/ml)	Coefficient of variation (%)	Recovery* (%)	Accuracy** (%)
Intra-day				
9 42	10 3 ± 0 19	1 84	109	9
47 1	48 6 ± 0 54	1 11 (n = 5)	103	3
94 1	96 5 ± 2 60	2 69	102	2
Inter-day				
9 42	10 5 ± 0 73	6 99	111	11
47 1	50 2 ± 1 10	2 18	107	7
94 2	99 5 ± 4 26	4 28	106	6
*Inter-week****				
9 42	10 2 ± 0 76	7 38	108	8
47 1	48 9 ± 1 65	3 37	104	4
94 2	94 4 ± 4 38	4 64	100	0

*Measured/spiked × 100% [18]
**(Measured — spiked)/spiked × 100% [19]
***Results are the means of duplicate preparations and analyses

TABLE III

ASSAY PRECISION, RECOVERY AND ACCURACY OF THE DETERMINATION OF CEFONICID IN URINE

All results are the means of duplicate preparations and analyses

Spiked concentration (μg/ml)	Measured concentration (mean ± S D) (μg/ml)	Coefficient of variation (%)	Recovery (%)	Accuracy (%)
Intra-day				
9 42	11 7 ± 0 30	2 60	124	24
47 1	48 9 ± 0 72	1 47	103	3
94 1	105 5 ± 2 54	2 41	112	12
Inter-day				
9 42	11 5 ± 0 80	7 00	122	22
47 1	45 8 ± 4 52	4 52	97	—3
94 2	95 8 ± 5 98	6 24	102	2
Inter-week				
9 42	11 7 ± 1 70	14 5	124	24
47 1	47 9 ± 1 25	2 62	102	2
94 2	101 1 ± 2 84	2 81	107	7

TABLE IV

WEEKLY DETERMINATIONS OF CEFONICID CONCENTRATIONS IN SPIKED SAMPLES OF PLASMA AND URINE

All results are the means of duplicate preparations and analyses

Week	Measured concentration (μg/ml)		
	9 42 μg/ml Spiked	47 1 μg/ml Spiked	94 2 μg/ml Spiked
Plasma			
1	10 7	48 7	93 9
2	9 6	47 4	88 2
3	9 2	48 6	94 5
4	11 3	51 7	100 2
5	10 4	47 3	98 2
6	10 2	50 0	91 2
Urine			
1	11 4	47 6	101 7
2	12 4	48 0	97 2
3	11 3	45 6	98 4
4	14 2	49 3	104 5
5	9 0	48 1	103 6
6	11 8	48 6	101 1

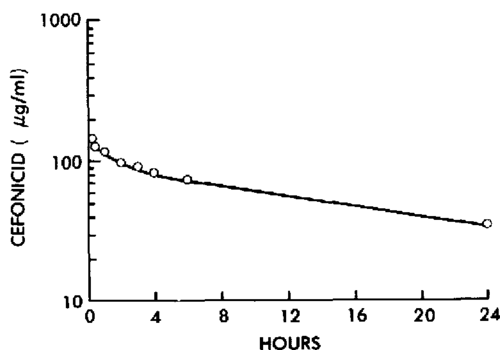


Fig 4 Plasma concentration– time curve following intravenous injection of cefonicid (15 mg/kg) in a patient with impaired renal function (corrected creatinine clearance, 62 2 ml/min) Elimination half-life, 16 6 h

and serum concentrations are 121 4 and 132.9 μg/ml, respectively This difference (9.4%) is statistically ($p < 0$ 01 by both t-test and Wilcoxon's sign-rank test for paired data) but not clinically significant

Fig. 4 depicts the plasma concentration– time curve following an intravenous injection of cefonicid (15 mg/kg) in a patient with impaired renal function (corrected creatinine clearance 62 2 ml/min)

DISCUSSION

An HPLC assay has been developed that allows fast and reliable determination of cefonicid concentrations in plasma, serum and urine This method requires less than 1 ml of biological fluid and is simple to perform No prepara-

tion of the specimen is necessary other than deproteination with acetonitrile. Sharp peaks representing the internal standard and cefonicid are easily discernible

Both the linearity of the standard curve data and the precision of all plasma and urine determinations are excellent and compare favorably with those reported by Signs et al. [18]. Recovery and percentage accuracy for the assay are within previously published acceptable limits [18, 19], therefore making it suitable for clinical and pharmacological studies.

Study of the stability of cefonicid in plasma and urine frozen at − 70°C indicates that, within the limits of the coefficients of variation of the test, there is no clinically significant decrease in concentration over six weeks

Serum concentrations are significantly higher than simultaneous plasma concentrations in non-dialysis patients Serum concentrations on the average are also higher than simultaneous plasma concentrations in dialysis patients, but the difference is not statistically significant. The explanation for these observations is unclear but is possibly related to the high protein binding of cefonicid.

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