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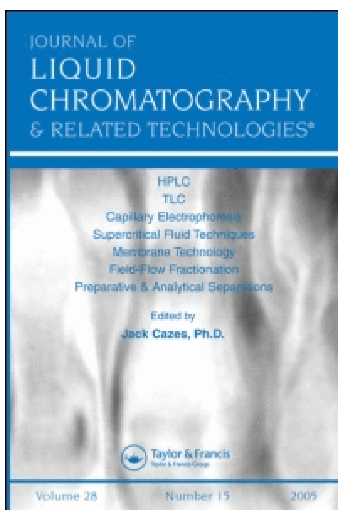

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An Ion-Pairing High-Pressure Liquid Chromatography Assay for the Determination of Cefoperazone in Plasma and Urine

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JOURNAL OF LIQUID CHROMATOGRAPHY, 11(3), 683-700 (1988)

AN ION-PAIRING HIGH-PRESSURE LIQUID CHROMATOGRAPHY ASSAY FOR THE DETERMINATION OF CEFOPERAZONE IN PLASMA AND URINE

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ABSTRACT

A high-performance liquid chromatographic assay for cefoperazone (cfp) in plasma and urine is described. For analysis, the internal standard ticarcillin (ticar) is solvated in acetonitrile, which is then added to plasma or urine. The supernatant is drawn off of the resulting protein precipitate and injected directly onto the reverse-phase C_{18} column, with detection at 254 nm. The mobile phase is composed of phosphate-acetonitrile-tetramethylammonium chloride (TMA). Coefficients of variation for reproducibility were less than 9% for extra-low, low, medium, and high controls. Limits of detection were 0.5 µG/mL for plasma and 1 µG/mL for urine. No interference from other cephalosporins or other antibiotics was found. This high-pressure liquid chromatographic ion-pairing assay is simple, accurate, inexpensive, and requires no extraction.

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INTRODUCTION

Cefoperazone (Fig.1, 7-[D(-)-alpha-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-alpha-(4-hydroxyphenyl) acetamido]-3-[1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid) is a semisynthetic, broad-spectrum, parenteral cephalosporin antibiotic. It is often coadministered with aminoglycosides or imidazoles to treat pseudomonas and mixed (aerobic/anaerobic) infections (1-4).

Cfp has been quantified in biological fluids primarily by microbiological assays (4-6), which suffer from lack of specificity and precision, as well as requiring long periods of time for incubation. Enzymatic degradation has also been noted (6), and an enzymatic assay currently in use (7) experiences competitive inhibition by beta-lactamases. A thioglycolate disk elution technique is reported to have a high false-resistance rate and a poor reproducibility record (8). More recently, rapid and sensitive high-pressure liquid chromatography (HPLC) techniques have been employed for the measurement of cfp in sterile and biological fluids (9-12). All of these assays, however, lack one or more of the following advantages: addition of an internal standard to increase precision and reproducibility; isocratic delivery of mobile phase (vs. gradient) to increase simplicity; no extraction procedure required so as to increase simplicity; short (10 min) run time. To our knowledge, no other reported method to date combines all of the above features.

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EXPERIMENTAL

Apparatus

The HPLC system used consisted of a Waters M-45 pump (Waters Assoc., Milford, MA), WISP 710B or U6K injector (Waters), and a Model 441 fixed-wavelength UV detector (Waters). A Sergovar 120 strip-chart recorder (BBC-Metrawatt/Goerz, Edison, NJ) or HP 3990 integrator (Hewlett Packard, Avondale, PA) were used for quantitation of chromatographic runs. The detector wavelength was 254 nm. The detector signal was evaluated at an absorbance of 0.05 AUFS with a

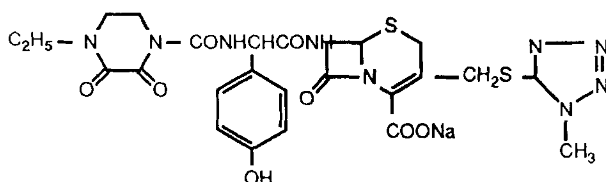

FIGURE 1. Chemical structure of cefoperazone.

filter response time of 0.5 s. Separation was achieved with a C_{18} 5μ, 250 x 4.6 mm reverse-phase column (Alltech Assoc., Deerfield, MI).

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Reagents

Sterile water was deionized and run through a "nanopure" filtration apparatus (Millipore, Milford, MA). Glass-distilled UV grade acetonitrile (CH_3CN) (Burdick & Jackson, product #015, Muskegon, MI), O-phosphoric acid (Nallinckrodt, 85%, lot KTGY, Paris, KY), tetramethylammonium chloride (TMA) (Fluka Chemical Corp., lot 241574 684, Waupaugh, NY), ticarcillin disodium (ticar) (Beecham Laboratories, lot BK3822, Bristol, TN), and cefoperazone dihydrate (cfp) (Pfizer, lot 2J039-35QCS, NY, NY) were used as recieved. Blank plasma was donated by UCSF faculty, staff, and students as needed. Additional plasma was obtained from Irwin Memorial Blood Bank, SF, CA. All plasma not immediately used was stored at -20 degrees C.

Mobile Phase

The mobile phase was composed of 30% CH_3CN, 0.1% o-phosphoric acid, and 0.03% TMA. Mobile phase was prepared in 2 L quantities in the following manner. Six hundred mL of CH_3CN was transferred to a two L graduated cylinder. "Nanopure" water was then poured into the cylinder until the fluid level reached th 1900mL mark. Two mL of o-phosphoric acid were added along with 6 mL of a previously prepared 10% TMA (1 G TMA salt: 9 mL water) solution. Water was then added to the 2 L mark, and the contents filtered under vacuum with a 0.45μ membrane filter. The solution was stirred and pumped through the column at a flow rate of 1.0 mL per minute and at a pressure of 2500 psi.

Sample preparation and analysis

Protein in plasma and urine samples (0.2 mL) was precipitated with 0.5 mL of CH_3CN containing the internal standard ticar (66 µG/mL). After the samples were vortexed for 20 s and centrifuged at 3000xg for 10 min, the resulting supernatant was poured off the protein pellet and evaporated under nitrogen to volume of 0.2 mL. A volume of 8 µL of the concentrated supernatant was then injected onto the column.

RESULTS

Separation

Figures 2 and 3 represent typical chromatograms for human plasma and urine, respectively. The internal standard ticar eluted at 8.8 min, while cfp eluted at 6.8 min.

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TABLE 1
Cefoperazone Standard Curve in Human Plasma
Cfp stock: 2 mG/10 mL MeOH = 0.2 µG/µL x 0.947 (purity factor) = 0.189 µG/µL

µL cfp spiked	conc. cfp (µG/mL)	peak height ratio (cfp/ticar)	calc. conc. (µG/mL)
0.00	0.00	0.00	0.00
0.50	0.47	0.05	0.51
1.0	0.95	0.09	0.98
2.0	1.89	0.16	1.74
5.0	4.74	0.42	4.59
10.0	9.47	0.87	9.50
20.0	18.94	1.72	18.8
30	28.4	2.60	28.4
50	47.4	4.35	47.5
70	66.3	6.13	67.0
100	94.7	8.26	94.2

0 - 100: $r = 1.0000$ $r^2 = 0.9999$ slope = 0.0915 $y_{int} = 0.0004$

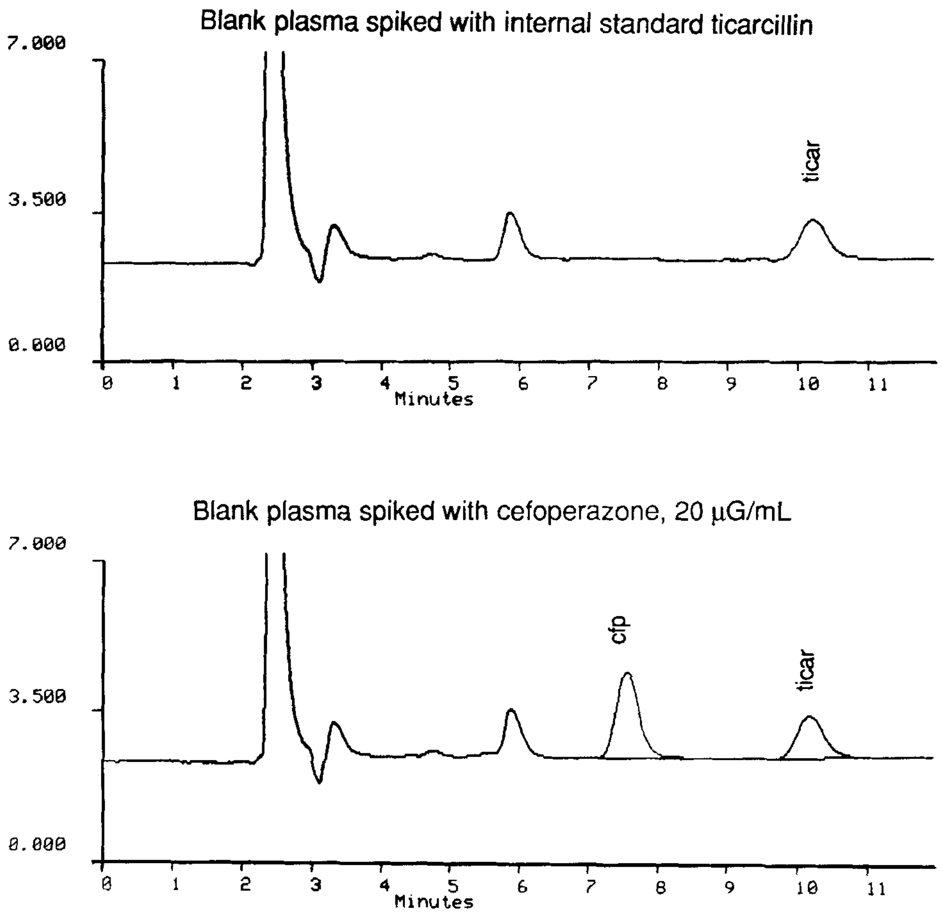

FIGURE 2. Chromatograms of cefoperazone in normal human plasma.

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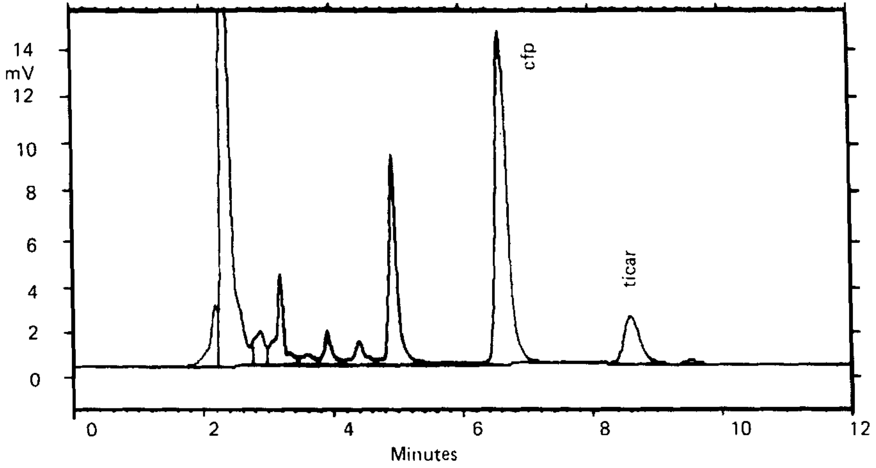
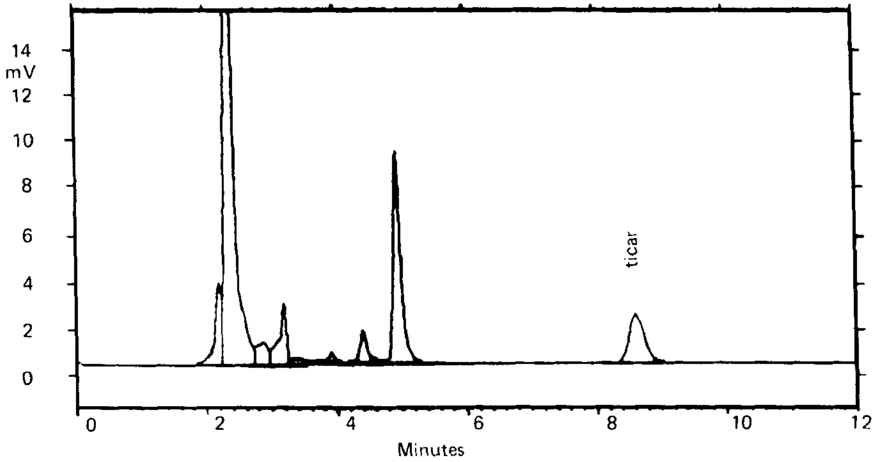


Clinical sample of cefoperazone in urine, concentration 248 μG/ml



Blank urine spiked with internal standard ticarcillin

FIGURE 3. Chromatograpms of cefoperazone in normal human urine.

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Linearity

Table1 and Figure 4 depict the linear relationship between drug plasma concentration and peak height ratios of cfp over ticar. Linear regression of peak height ratio vs concentration gives a typical coefficient of determination (r^2) of 0.999. The standard curve is broken into two linear equations (concentrations of 0-40 µG/mL and 40-200 µG/mL) to allow for better evaluation of lower points. Similar results are shown for urine in Table 2 and Figure 5.

Specificity

Under the above chromatographic conditions, no endogenous peaks interfere with cfp or ticar in plasma or urine. Blank plasma from

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TABLE 2

Cefoperazone Standard Curve in Human Urine
Cfp stock: 4 mG/10 mL MeOH = 0.4 µG/µL x 0.947 (purity factor) = 0.379 µG/µL

µL cfp spiked	conc. cfp (µG/mL)	peak height ratio (cfp/ticar)	calc. conc. (µG/mL)
0.00	0.00	0.00	-1.24
0.50	0.95	0.05	1.19
1.0	1.89	0.07	2.16
2.0	3.79	0.11	4.10
5.0	9.47	0.23	9.93
10.0	18.94	0.42	19.2
20.0	37.9	0.80	37.6

0 - 40: r = 0.9991 r^2 = 0.9982 slope = 0.0206 yint = 0.0254

µL cfp spiked	conc. cfp (µG/mL)	peak height ratio (cfp/ticar)	calc. conc. (µG/mL)
20.0	37.9	0.80	36.0
30	56.8	1.19	57.1
50	94.7	1.91	96.0
70	133	2.63	135
100	189	3.60	187

40 - 200: r = 0.9995 r^2 = 0.9990 slope = 0.0185 yint = 0.1313

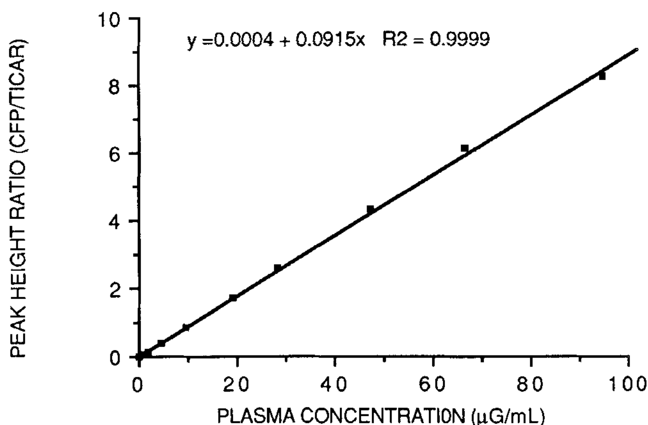

FIGURE 4. Standard curve of cefoperazone in normal human plasma.

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TABLE 3
Cefoperazone in Plasma Intra-day Variation

	CONCENTRATION (μG/mL)			
	HIGH (56.8)	MEDIUM (28.4)	LOW (14.2)	EXTRA-LOW (1.89)
1	60.6	29.1	14.6	1.97
2	60.0	30.4	14.6	1.74
3	60.9	30.2	15.1	2.07
4	61.6	30.5	14.4	2.18
5	64.3	28.4	14.9	2.18
6	60.1	28.3	14.9	2.07
MEAN	61.3	29.5	14.8	2.04
SD	1.60	1.01	0.26	0.16
%CV	2.6	3.4	1.8	8.1

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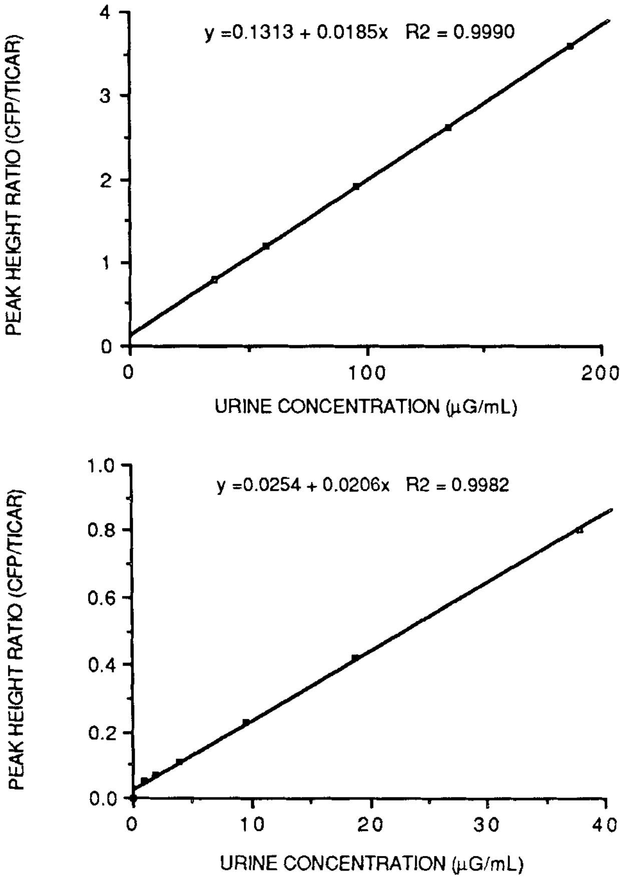

FIGURE 5. Standard curve of cefoperazone in normal human urine. The curve has been split at the 40 µG/mL point so as to improve linearity of lower points.

volunteers also indicated that xanthines, acetylsalicylic acid, and acetaminophen had no influence on cfp quantitation.

Drugs that may be coadministered with cfp were tested for interference. Included were a number of cephalosporins, penicillins, beta-agonists, and benzodiazepines. None coeluted with cfp.

Variation

Intraday and interday variation of the method for extra-low, low, medium, and high plasma and urine concentrations were determined using replicate spiked samples. The ranges for the coefficient of variation (CV) were between 1.3 and 8.1%, as shown in Tables 3-6.

Recovery

Per cent recoveries for cfp in plasma and urine vs. water are shown in Tables 7 and 8. Spiked samples were prepared as described above with the exception that ticar was not added until after centrifugation. The % recovery was determined as follows:

$$\% \text{ Recovery} = \frac{\text{(peak height ratio of cfp/ticar in plasma)}}{\text{(peak height ratio of cfp/ticar in water)}} \times 100$$

Recoveries ranged from 84 to 90% for plasma, and 99 to 105% for urine.

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TABLE 4
Cefoperazone in Plasma Inter-day Variation

CONCENTRATION (µG/mL)

	HIGH (66.3)	MEDIUM (28.4)	LOW (4.74)	EXTRA-LOW (0.94)
1	67.0	29.2	4.79	1.01
2	67.0	28.4	4.59	0.98
3	65.2	27.8	4.85	1.08
4	67.6	28.2	4.60	1.05
5	65.8	28.7	4.69	1.01
6	66.3	28.2	4.60	1.08
MEAN	66.5	28.4	4.69	1.04
SD	0.89	0.48	0.11	0.041
%CV	1.3	1.7	2.4	4.0

TABLE 5
Cefoperazone in Urine Intra-day Variation

	CONCENTRATION (µG/mL)			
	HIGH (113.6)	MEDIUM (56.8)	LOW (28.4)	EXTRA-LOW (3.78)
1	107.3	62.4	26.4	3.46
2	108.9	61.6	25.6	3.25
3	116.2	53.6	26.4	3.25
4	108.6	51.2	30.2	3.66
5	116.3	57.9	29.0	3.21
6	104.5	56.3	29.4	3.83
MEAN	110.3	57.2	27.8	3.44
SD	4.86	4.40	1.92	0.26
%CV	4.4	7.7	6.9	7.4

TABLE 6
Cefoperazone in Urine Inter-day Variation

	CONCENTRATION (µG/mL)			
	HIGH (132.6)	MEDIUM (37.9)	LOW (9.47)	EXTRA-LOW (3.79)
1	134.4	39.9	9.42	3.75
2	134.0	38.7	8.65	3.43
3	134.9	37.6	9.93	4.10
4	129.7	36.8	9.33	3.66
5	133.0	37.9	9.14	3.60
6	133.2	37.2	10.1	3.61
MEAN	133.2	38.0	9.43	3.69
SD	1.86	1.13	0.53	0.23
%CV	1.4	3.0	5.7	6.1

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TABLE 7
Recovery of Cefoperazone From Human Plasma

SAMPLE	PEAK HEIGHT RATIO	
	WATER SAMPLE	PLASMA
LOW CONCENTRATION (2 μG/ml) Recovery (0.204/0.227) = 90%	0.227	0.204
MEDIUM CONCENTRATION (20 μG/mL) Recovery (1.90/2.25) = 84%	2.25	1.90
HIGH CONCENTRATION (70 μG/mL) Recovery (6.93/7.91) = 88%	7.91	6.93

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TABLE 8
Recovery of Cefoperazone From Human Urine

SAMPLE	PEAK HEIGHT RATIO	
	WATER SAMPLE	URINE
LOW CONCENTRATION (4 μG/ml) Recovery (0.189/0.180) = 105%	0.180	0.189
MEDIUM CONCENTRATION (40 μG/mL) Recovery (1.72/1.71) = 101%	1.71	1.72
HIGH CONCENTRATION (140 μG/mL) Recovery (5.75/5.79) = 99%	5.79	5.75

Stability

Tables 9 and 10, and Figures 6 and 7 show stability of cfp when frozen 10 weeks in plasma and 28 weeks in urine. Data was obtained from quality controls prepared in batch lots on one day, and then frozen at -20 degrees C until analysis.

TABLE 9
Cefoperazone in Plasma Stability Study

DAY	CONCENTRATION (μG/mL)			
	HIGH (60.0)	MEDIUM (30.0)	LOW (15.0)	EXTRA-LOW (2.00)
0	70.8	32.7	13.6	2.11
3	69.7	33.1	12.7	2.11
15	61.3	29.9	12.2	2.11
16	60.3	32.0	11.8	2.10
17	58.5	28.0	11.0	2.10
72	58.3	27.2	11.2	2.10

TABLE 10
Cefoperazone in Urine Stability Study

DAY	CONCENTRATION (μG/mL)			
	HIGH (120.0)	MEDIUM (60.0)	LOW (30.0)	EXTRA-LOW (3.5)
0	116.2	57.9	29.4	3.46
1	116.3	57.2	30.2	3.21
18	112.5	57.0	27.6	3.43
46	105.1	49.4	23.5	2.91
92	71.2	41.6	19.3	2.03
181	51.7	21.3	4.4	1.77

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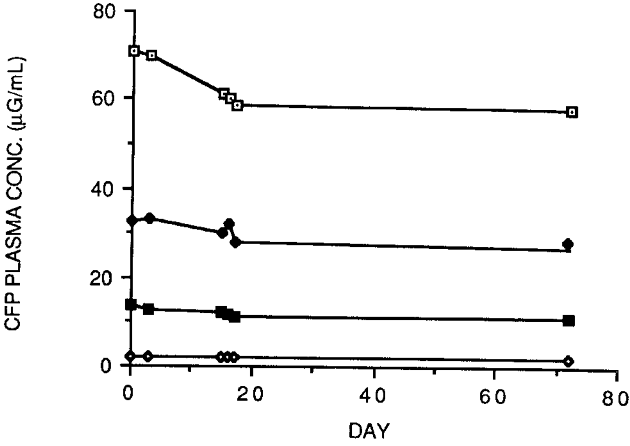

FIGURE 6. Stability of cfp in normal human plasma. The symbols represent the following concentrations: ▣ high, ◆ medium, ■ low, ◇ extra-low (60.0, 30.0, 15.0, and 2.00 µG/mL).

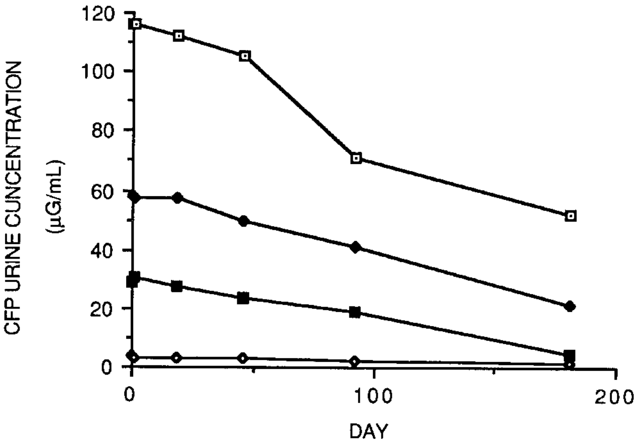

FIGURE 7. Stability of cfp in normal human urine. The symbols represent the following concentrations: ▣ high, ◆ medium, ■ low, ◇ extra-low (120.0, 60.0, 30.0, and 3.5 µG/mL).

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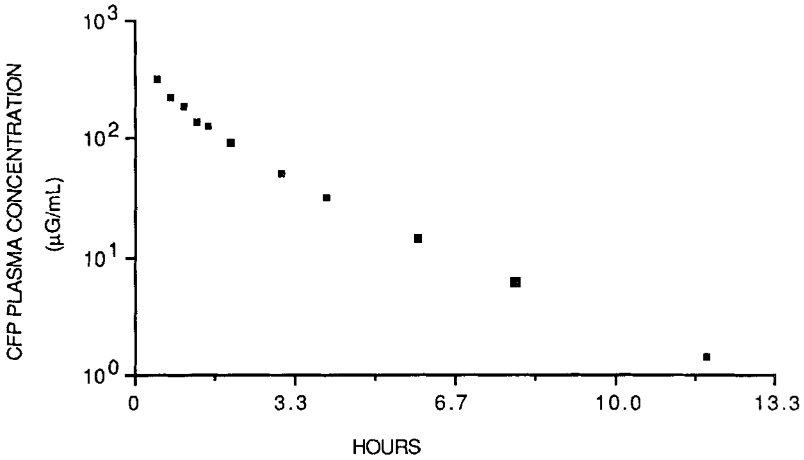

FIGURE 8. Time vs. concentration curve of cfp following a single intravenous dose of 30 mG/kG in a healthy volunteer.

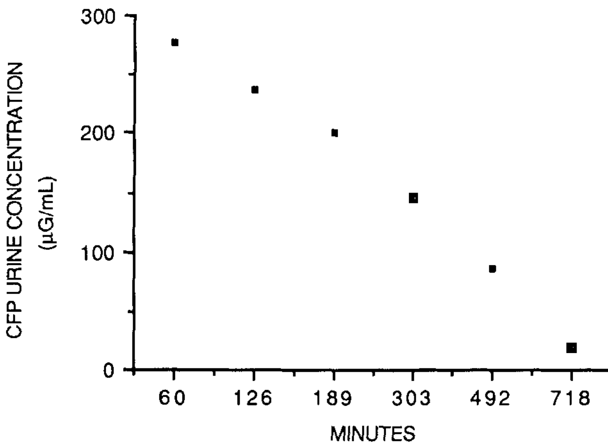

FIGURE 9. Plot of cfp urine excretion rate following a single intravenous dose of 30 mG/kG in a healthy volunteer.

Clinical Studies

This assay has been utilized at the clinical level, including several studies undertaken by our research group (3). Figures 8 and 9 represent time-plots of a healthy volunteer in a pharmacokinetic study who recieved a 30 mG/kG IV bolus dose.

DISCUSSION AND CONCLUSION

When first developing an HPLC assay for cfp in biological fluids, we opted to use the ion-pairing reagent TMA to decrease column affinity of any interferring endogenous peaks. This approach resulted in the elution of all such peaks before 5 min.

Ticar was chosen as the internal standard because of its convenient proximity to cfp. Ticar has a tendency to peak-split, possibly due to the molecule's bifuctionality, when less than 0.01% TMA is used in the moblie phase. The final concentration of 0.03% was arrived at because peak shapes of both cfp and ticar could be maintained at this concentration without changing retention times significantly.

Although we found cfp in urine to be unstable at - 20 degrees C after 6 weeks, Reller et. al. (6) reported that cfp frozen for much longer periods at similar temperatures showed no degradation.

Though not a chemically related species, the use of ticar as an internal standard can be justified because of the method involved. No extraction is required, and precision as well as % recovery are sufficient enough to allow for a high degree of confidence in the use of this assay for clinical samples. The assay is simple, fast, accurate, and inexpensive when compared to other methods of quantitation. We feel this assay offers significant advantage over other methods currently in the literature.

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REFERENCES

1. Barriere, S., and Flaherty, J.F., Third Generation Cephalosporins, Clinical Pharmacy, 3, 351, 1984.

2. Eliopoulos, G.M., and Moellering, R.C., Antibiotic Synergism and Antimicrobial Combinations in Clinical Infections, Review of Infectious Diseases, 4, 282, 1982.

3. Guglielmo, B.J., Flaherty, J.F., Woods, T.M., La Follette, G., and Gambertoglio,J.G., Pharmacokinetics of Cephoperazone and Tobramycin Alone and in Combination, Antimicrobial Agents and Chemotherapy, 31, 264, 1987.

4. Lagast, H., Klastersky, J., Standiford,H., and Viollier, A., ComparativeStudy of the Serum Bactericidal Activity of Cefoperazone Alone and in Combination with Amikacin or Mezlocillin Against Gram-negative Bacilli and Staphylococcus aureus, Infection 12, 3, 190, 1984.

5. Reller, L.B., and Stratton, C.W., Serum Dilution Test for Bactericidal Activity. II. Standardization and Correlation with Antimicrobial Assays and Susceptibility Tests, Journal of Infectious Diseases, 136, 196, 1977.

6. Stankewich, J.P., Stability of T-1551 in Human Serum, Urine and Bile - Final Report (QCSA 60468) for Pfizer, Groton Quality Control, Sept. 10, 1980.

7. Cullman, W., Dick, W., and Edelmann, M., An Enzymatic Assay for Monitoring Serum, Cerebrospinal Fluid, and Urine Concentrations of Beta-lactam Antibiotics, Journal of Clinical Chemistry and Clinical Biochemistry, 23, 151, 1985.

8. Barry, A.L., Packer, R.R., and Jones, R.N., Cefoperazone and Cefoperazone Sublactam Susceptibility Tests with Anaerobic Bacteria by the Thioglycolate Disk Elution Method, Journal of Clinical Microbiology, 22 No. 5, 882, 1985.

9. Determination of Cefoperazone in Human Serum in the Presence of Penicillins by Gradient Elution HPLC, Special Testing and Analytical Developement Laboratories, Pfizer Groton Quality Control, 1982.

10. Cowlishaw, M.G., and Sharman, J.R., Liquid-Chromatographic Assay of Cefoperazone from Plasma and Bile, Technical Brief, Clinical Chemistry, 32 No. 5, 894, 1986.

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11. Dokladalova, J., Quercia, G.T., and Stankewich, J.P., High-Performance Liquid Chromatograhic Determination of Cefoperazone in Human Serum and Urine, Journal of Chromatography, 276, 129, 1983.

12. Schachter, S.H., Spino, M., Tesoro, A., and MacLeod, S.M., High Performance Liquid Chromatographic Analysis of Cefoperazone in Serum and Urine, Journal of Liquid Chromatography, 7, 2421, 1984.

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