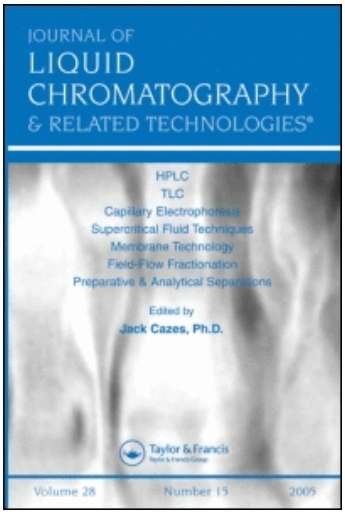

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC MICRO-ASSAY FOR CEFOPERAZONE IN HUMAN PLASMA, URINE AND CSF

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ABSTRACT

A high performance liquid chromatographic method is described for the determination of cefoperazone in plasma, urine and CSF samples. The procedure employs reversed-phase chromatography, using hydrochlorothiazide as an internal standard. The assay only requires 100 µL of sample, with direct injection of diluted urine or CSF, or protein precipitated plasma. Stability studies indicate the need for careful attention to processing and storage conditions of the biologic material. The method is specific for cefoperazone in the presence of kanamycin, tobramycin, gentamicin, ampicillin and penicillin G. The method has been successfully employed in the assay of over 600 samples obtained from human clinical trials.

INTRODUCTION

Cefoperazone is a relatively new, third generation, semi-synthetic cephalosporin antibiotic which is currently undergoing extensive clinical trials in humans. The drug is given primarily

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by intravenous and intramuscular injection, since it is poorly absorbed orally.

Several recent clinical and pharmacokinetic studies of cefoperazone have employed microbiological assays for the drug in biologic fluids (1-6). Because of the required incubation times, such bioassays may be less useful for the rapid determination of drug concentrations in clinical materials. In addition, since studies in patients often involve the concomitant or prior use of other antibiotics, the specificity of chromatographic methods may be advantageous. Three high performance liquid chromatographic methods for serum cefoperazone have recently been developed, using a reversed phase μBondapak C_{18} column. While one method employed a chloroform-1-pentanol extraction (7), the other used a Sep-Pak (Waters Assoc., Milford, MA, USA) sample clean-up step (8). A third procedure separating a number of cefoperazone degradation products involved gradient HPLC (9). None of these methods incorporated an internal standard in the assay procedure and only the latter one was used to analyze low volume (0.1 ml) samples of serum.

The present study concerns the development of a rapid method for the determination of cefoperazone, using hydrochlorothiazide as an internal standard. The method employs the direct injection of diluted urine and CSF, or protein-precipitated plasma into the HPLC. The method has also been applied to human bile.

EXPERIMENTAL

Reagents and Materials

Cefoperazone (T-1551) and T-1551B, a degradation product obtained under acidic conditions (10), as well as kanamycin, tobramycin, gentamicin, ampicillin and penicillin G were supplied by Pfizer, Inc. (New York, NY). Hydrochlorothiazide was obtained from Merck Sharp and Dohme (West Point, PA). Acetonitrile and methanol were HPLC-grade, distilled-in-glass, Burdick and Jackson (Muskegon, MI); glacial acetic acid, ACS grade was from Fisher Scientific (Fairlawn, NJ); and triethylamine was from Matheson Coleman and Bell (Norwood, OH).

Chromatography Equipment and Conditions

The HPLC system (Waters Assoc., Milford, MA) consisted of a model 6000 solvent delivery system, a U6K loop injector, a model 440 UV absorbance detector with a 254 nm filter, a prepacked 30 cm x 3.9 mm i.d. stainless steel column containing 10 μm C_{18} μ-Bondapak, and a guard column with Bondapak phenyl/corasil as packing material. The chromatograms were recorded by a Fisher Recordall Series 500 (Fairlawn, NJ) 10 mv strip chart recorder.

The mobile phase used for the isocratic reversed-phase chromatography consisted of 1.2 ml triethylamine (1 M in acetonitrile), 2.8 ml of 1 M acetic acid and 120 ml acetonitrile in deionized water to a total volume of one liter. The mobile phase was filtered through a Millipore filter, type HA, pore size 0.45 μm (Millipore, Bedford, MA) and deaerated prior to use. The

chromatographic system was operated at ambient temperature, with a flow rate of 2.0 ml/min.

Standard Solutions

Three different concentration ranges of cefoperazone in methanol were prepared for the analysis of plasma, urine and CSF samples. Standard curves for plasma employed methanol solutions containing 25, 50, 100 and 200 µg/ml of cefoperazone. For urine assays 100, 200, 400 and 800 µg/ml methanol standards were prepared, and 1.25, 2.5, 5 and 10 µg/ml methanol standards were used for the CSF assays. These standards were prepared fresh each day. The internal standard, hydrochlorothiazide, was also prepared in methanol at concentrations of 100, 400 and 5 µg/ml for the assay of plasma, urine and CSF samples, respectively. This solution was stable for at least six months, stored at $4^{\circ}C$.

Standard Curves and Patient Samples

A 100 µl aliquot of the biologic sample was transferred to a 15 ml conical centrifuge tube with a micropipet. Similarly, 100 µL of cefoperazone methanol standard and 100 µL of internal standard were added. The mixture was vortexed for 30 sec, allowed to stand for 1 min, and centrifuged at 1400 g and $-5^{\circ}C$ in a refrigerated centrifuge (Beckman, Model J6, Palo Alto, CA) for 15 min. The samples were then stored at $4^{\circ}C$ for no longer than 1 hr prior to analysis. Plasma standard curves employed pooled human plasma. Urine and CSF standard curves were prepared using

deionized water. Blank samples for the standard curves were prepared using 200 µL of methanol instead of the drug and internal standard solutions, and patient samples were assayed by substituting 100 µL of methanol for the drug standard solution. Quantitation of cefoperazone concentrations employed unweighted least-squares regression of plots of peak height ratio (cefoperazone/internal standard) versus cefoperazone concentration.

The detector sensitivity was set at 0.01-0.02, 0.05 and 0.005 a.u.f.s. for the assay of plasma, urine and CSF, respectively. The injection volume was 10-15 µL, 5 µL and 75-100 µL for the plasma, urine and CSF samples, respectively.

Assay Specificity

Pooled human plasma samples were prepared containing 100 µg/ml of kanamycin, tobramycin, gentamicin, ampicillin, penicillin G, and T-1551B, a potential degradation product, in anticipation of the presence of some of these materials in patient samples. These plasma samples were then carried through the assay procedure.

Stability Studies

The effect of storage on the stability of various cefoperazone specimens was also determined. Pooled human plasma was fortified with 50 µg/ml of cefoperazone, and pooled human urine was fortified with 400 µg/ml of cefoperazone. Aliquots of each were then stored at 5°C and -15°C, with assays at 0, 1, 3, 7 and

14 days after preparation. Further, diluted plasma and urine samples, prepared as described earlier up to the point of injection into the HPLC, were stored at 5°C, with assay 0, 24, 48 and 72 hr after preparation. These samples were also stored at room temperature (approximately 25°C) with assay 0, 1, 2, 3, 8, 24 and 48 hr after preparation. In addition, 100 µg/ml methanol solutions of cefoperazone were stored at 5°C, with assay at 0, 1, 3, 7 and 14 days after preparation. These samples were also stored at room temperature and were assayed 0, 1, 2, 3, 8, 24 and 48 hr after preparation.

RESULTS AND DISCUSSION

Figure 1 illustrates typical chromatograms for the assay of patient plasma, urine and CSF samples. The retention times for the internal standard and the cefoperazone were 5-6 and 9-10 min, respectively, depending on the age and condition of the column. Blank samples containing no drug or internal standard were free from any interfering peaks in the vicinity of the drug and internal standard. Peaks observed with plasma samples fortified with 100 µg/ml of other antibiotics were well resolved from both the drug and the internal standard. Ampicillin eluted before the internal standard and penicillin G after the cefoperazone. No peaks were found in samples containing kanamycin, gentamicin or tobramycin and these drugs presumably eluted with the solvent peak or were retained on the column. A large peak was observed

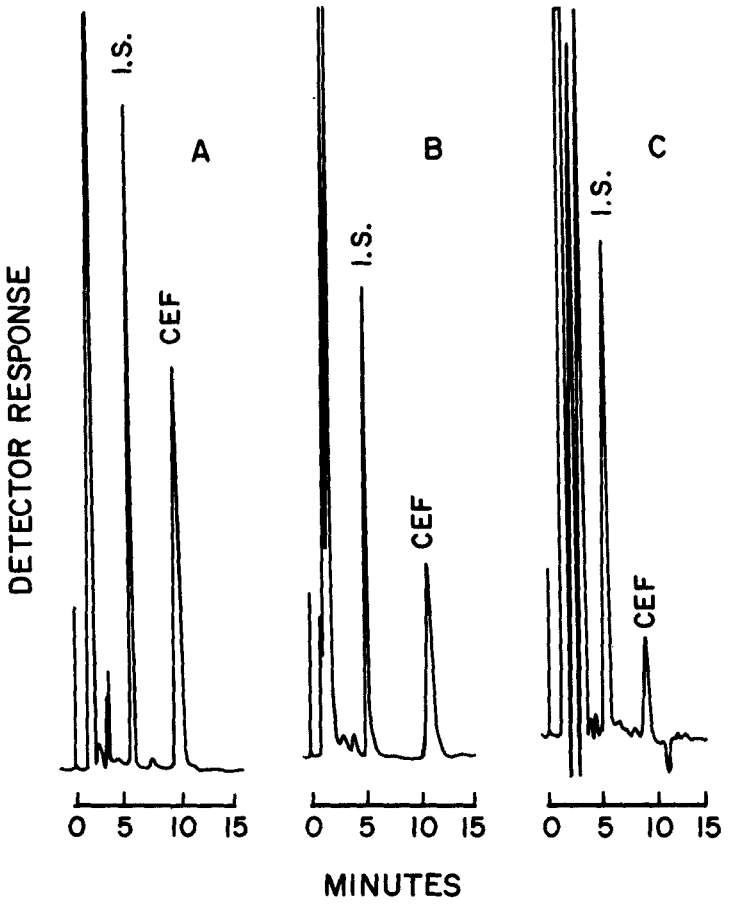

FIGURE 1 - Chromatograms from the assay of plasma, urine and CSF obtained from patients receiving cefoperazone (CEF), with hydrochlorothiazide as the internal standard (IS). (A) Plasma containing 100 µg/ml. (B) Urine containing 269 µg/ml. (C) CSF containing 2 µg/ml.

with T-1551B, a degradation product under acidic conditions, but the retention time for this material was 23 min. Samples containing methicillin exhibited a small peak which overlapped with that of cefoperazone. However, even in serum samples containing 1000 µg/ml of methicillin the peak was less than 10 mm in height.

Plasma, urine and CSF standard curves exhibited excellent linearity, with correlation coefficients greater than 0.99 in each case. Table I summarizes the results of the precision studies for four plasma or urine and three CSF standards assayed on a single day or individually on three to four separate days. In each assay the recoveries were at least 95 percent and the relative standard deviations ranged from 1 to 7 percent.

A series of stability studies were conducted with the plasma, urine and CSF standards. The plasma and urine samples which were stored at -15°C exhibited approximately 2 percent loss after 14 days. In contrast, the extent of degradation for these same samples stored at 5°C ranged from 11 percent after 1 day to 66 percent after 14 days for the plasma, and from 2 percent after 1 day to 16 percent after 14 days for the urine samples. The methanol solutions of cefoperazone showed less than 3 percent degradation for up to 3 hr at room temperature, but a 29 percent loss after 24 hr. When the methanol solutions were stored at 5°C there was less than 8 percent degradation after 24 hr, but 39 percent loss after 14 days. These results clearly indicate the

TABLE I

WITHIN-DAY AND BETWEEN-DAY PRECISION OF CEFOPERAZONE ASSAY*

Within-Day Precision

Serum Standards		Urine Standards		CSF Standards	
Added (μg/ml)	Found ±S.D. (μg/ml)	Added (μg/ml)	Found ±S.D. (μg/ml)	Added (μg/ml)	Found ±S.D. (μg/ml)
23.9	22.7±0.6	95.4	97.6±1.2	1.2	.98±0.02
47.7	44.8±1.1	190.8	192.4±8.2	2.4	2.4±0.1
95.4	91.8±1.0	381.6	385.0±7.5	4.8	4.7±0.3
190.8	189.7±6.0	763.2	767.7±11.4	9.5	9.9±0.1

Between-Day Precision

Serum Standards		Urine Standards		CSF Standards	
Added (μg/ml)	Found ±S.D. (μg/ml)	Added (μg/ml)	Found ±S.D. (μg/ml)	Added (μg/ml)	Found ±S.D. (μg/ml)
23.9	24.3±1.2	95.4	95.2±2.2	1.2	1.0±0.06
47.7	46.7±2.8	190.8	192.4±4.7	2.4	2.4±0.17
95.4	95.2±3.7	381.6	383.8±9.7	4.8	4.7±0.30
190.8	191.8±4.6	763.2	770.4±9.5	9.5	9.6±0.6

* N = 4 replicates for serum and urine, 3 replicates for CSF (±S.D.)

importance of using freshly prepared and properly stored methanol solutions of cefoperazone in the development of standard curves.

Studies were also conducted on the methanol diluted plasma and urine samples stored at room temperature and at 5°C. After 24 hr at 5°C the diluted plasma and urine samples exhibited a 5 and 8 percent loss of cefoperazone, respectively. In contrast a 20 percent degradation was evident in such samples when stored for only 8 hr at room temperature. These results indicate that it is not possible to prepare a large number of plasma or urine samples at one time. Instead it is advisable to prepare only 3-4 methanol diluted samples, and store these samples at 5°C until just before they are to be injected into the HPLC. Kinniburgh et al. (11) have also noted the instability of cefoperazone in methanol and in unbuffered CSF at room temperature. These authors suggest diluting CSF with pH 4.3 acetate buffer to reduce the pH of such samples. However, they did not report the stability of CSF samples which were stored frozen after the addition of the buffer. The lack of a supply of human CSF precluded a stability assessment as part of the present study. However, CSF samples obtained from patients receiving cefoperazone were maintained at -15°C and assayed as soon as possible after collection. Further, only 1-2 samples were carried through the assay at any given time, and these were kept at 5°C for the brief period of time between sample thawing and injection into the HPLC.

Assay of Patient Samples

Thus far the assay method has been employed for the assay of over 500 serum, 60 urine and 60 CSF samples obtained from patients who have received cefoperazone. The majority of these samples exhibited cefoperazone concentrations which fell within the range of concentrations employed for the standard curves. In addition, bile samples obtained from a patient being treated for biliary tract sepsis were assayed using the method and concentrations in excess of 500 μg/ml were observed. The bile was diluted 1:2 with 50 mM, pH 7 phosphate buffer and stored frozen until assayed. Cefoperazone is reported to be extensively excreted in the bile, and less than one percent of the drug has been found as metabolites in the urine of humans (12).

It is concluded the method is rapid, exhibits adequate sensitivity and specificity, and is useful in the analysis of biologic material obtained during the course of treatment of patients with cefoperazone.

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REFERENCES

1. Perfect, J.R. and Durack, D.T., Pharmacokinetics of cefoperazone, moxalactam, cefotaxime, trimethoprim and sulpha-

methoxazole in experimental meningitis, J. Antimicrob. Chemother., 8, 49-58, 1981.

2. Srinivasan, S., Francke, E.L. and Neu, H.C., Comparative pharmacokinetics of cefoperazone and cefamandole, Antimicrob. Agents Chemother., 19, 298-301, 1981.

3. Maksymiuk, A.W., LeBlanc, B.M., Brown, N.S., Ho, D.H. and Bodey, G.P., Pharmacokinetics of cefoperazone in patients with neoplastic disease, Antimicrob. Agents Chemother., 19, 1037-1041, 1981.

4. Hoffler, D., Koeppe, P. and Piper, C., The pharmacokinetics of cefoperazone in normal and impaired renal function, Infection, 9, S24-S29, 1981.

5. Bailey, R.R., Peddie, B. and Blake, E., Serum and urine concentrations of cefoperazone in severe chronic renal failure, Drugs, 22, 46-51, 1981.

6. Marti, M.C., Farquet, C., Fabre, J. and Rudhardt, M., Pharmacokinetics and biliary excretion of cefoperazone in patients with bile duct drainage, Infection, 9, S34-S36, 1981.

7. Brisson, A.M. and Fourtillan, J.B., Determination of cephalosporins in biological material by reversed-phase liquid column chromatography, J. Chromatogr. Biomed. Appl., 223, 393-399, 1981.

8. Dupont, D.G. and De Jager, R.L., High-performance liquid chromatographic determination of cefoperazone in serum, J. Liquid Chromatogr., 4, 123-128, 1981.

9. Dokladalove, J., Quercia, G.T. and Stankewich, J.P., HPLC determination of cefoperazone in human serum and urine, J. Chromatogr., In Press, 1982.

10. Pfizer, Inc., "Cefoperazone Investigator Reference Manual," August 20, 1979, p. 6.

11. Kinniburgh, D.W., Jennison, T.A. and Matsen, J.M., Factors affecting the analysis of cefoperazone in cerebral spinal fluid, J. Analyt. Toxicol., 6, 85-87, 1982.

12. Craig, W.A. and Gerber, A.U., Pharmacokinetics of cefoperazone: A review, Drugs, 22,35-45, 1981.