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## Note

# High-performance liquid chromatographic determination of cefmenoxime (AB-50912) in human plasma and urine

IRIS A. NOONAN, JOHN G. GAMBERTOGLIO, STEVEN L. BARRIERE, JOHN E. CONTE, Jr. and EMIL T. LIN*

*Infectious Disease Research Unit: Division of Clinical Pharmacy, Department of Pharmacy and the Clinical Infectious Diseases Unit, Department of Medicine, University of California, San Francisco, CA 94143 (U.S.A.)*

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Cefmenoxime (Fig. 1) is a new semisynthetic third generation cephalosporin developed by Takeda Chemical Industries. Currently, it is under clinical evaluation by Abbott Laboratories. Cefmenoxime has a broad antibacterial spectrum against many gram-positive and gram-negative bacteria including *Haemophilus influenzae*, indole-positive *Proteus*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter cloacae*, and many strains of *Pseudomonas aeruginosa*. Recently, a high-performance liquid chromatographic (HPLC) procedure for the determination of cefmenoxime in plasma has been developed [1]. This procedure involves special treatment of samples prior to analysis followed by ultrafiltration.

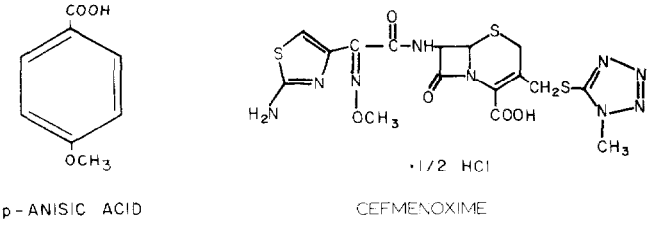


Fig. 1. Chemical structure of cefmenoxime and internal standard *p*-anisic acid.

We report the development of a simple, rapid HPLC assay requiring only deproteination of plasma with addition of acetonitrile containing internal standard, *p*-anisic acid (Fig. 1) prior to injection. In addition, this assay is applicable for analysis of cefmenoxime in urine. This assay has been used to

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analyze plasma and urine samples obtained following pharmacokinetic studies in healthy volunteers and renal failure patients. The stability of cefmenoxime in frozen plasma and urine has also been determined.

## MATERIALS AND METHODS

### *Reagents*

Cefmenoxime (Lot No. 17-961-AR) and *p*-anisic acid (Aldrich, Lot No. 092581) were both supplied by Abbott Laboratories (North Chicago, IL, U.S.A.). All organic solvents used were HPLC grade (Alltech, Arlington Heights, IL, U.S.A.). Reagents used were analytical grade. Water was doubly distilled and purified.

### *Chromatographic system*

The chromatographic equipment consisted of an M-45 solvent delivery system connected to a Model 710A WISP sample injector (Waters Assoc., Milford, MA, U.S.A.). A Model 441 fixed-wavelength UV detector, 254 nm (Waters Assoc.) was used at a sensitivity of 0.005 absorbance units full scale (a.u.f.s.) for plasma samples and 0.05 a.u.f.s. for urine samples. The mobile phase consisted of 14% acetonitrile and 0.2% phosphoric acid, which was filtered through a Millipore filter (0.45 $\mu$m) and degassed prior to use. Minor changes in the acetonitrile content or the pH of the mobile phase may be necessary due to column efficiency loss or interference from plasma samples. This eluent was pumped through a $\mu$Bondapak Phenyl column (30 cm × 3.9 mm, particle size 10 $\mu$m, Waters Assoc.) at 2 ml/min. Chromatograms were recorded on a Model

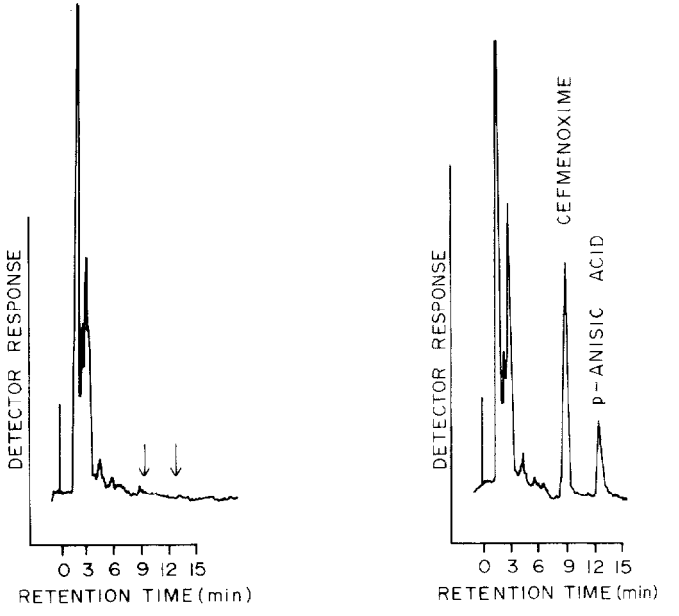



Fig. 2. Typical chromatograms of cefmenoxime and *p*-anisic acid in blank (left) and spiked plasma (right).

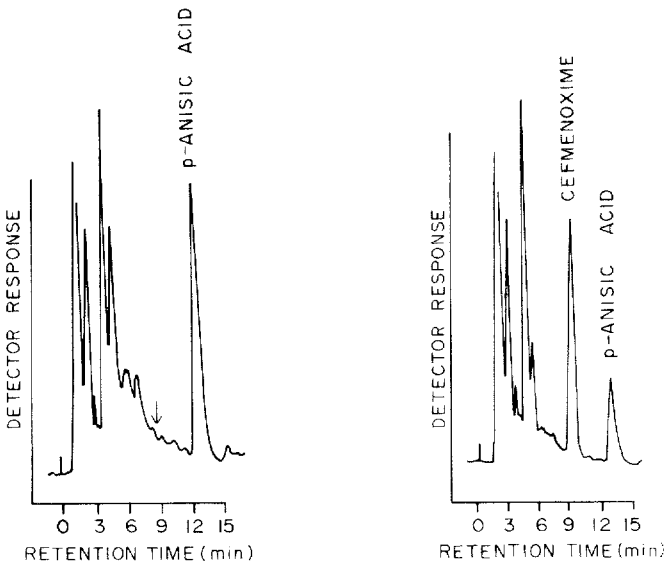



Fig. 3. Typical chromatograms of cefmenoxime and *p*-anisic acid in blank urine with internal standard (left) and spiked urine (right).

37 MR dual-pen strip-chart recorder, 10 mV (Pedersen Instruments, Lafayette, CA, U.S.A.) at a chart speed of 10 in./h. The retention time was 10 min for cefmenoxime and 14 min for the internal standard (Figs. 2 and 3).

*Preparation of plasma samples and standards*

*Plasma samples.* A plasma sample, 0.2 ml, was transferred into a disposable culture tube (12 × 75 mm) and deproteinated by adding 0.2 ml of acetonitrile containing the internal standard (0.8 μg/ml, *p*-anisic acid). The mixture was vortexed for 10 sec and centrifuged (700 *g*) for 10 min. The supernatant was decanted into a clean culture tube, evaporated under nitrogen to 0.1 ml, vortexed briefly, and approximately 25—50 μl were injected onto the column. Plasma samples at drug concentrations higher than those used in preparing the standard curve were diluted and evaporated as necessary.

*Plasma standards.* Cefmenoxime stock solution was prepared by accurately weighing 2—3 mg of cefmenoxime into a 100-ml volumetric flask and dissolving in 0.07 *M* phosphate buffer (pH 7.0). Blank human plasma was spiked with cefmenoxime stock solution to yield concentrations ranging from 0.2—6 μg/ml and prepared and treated identically to that described above. A standard curve was constructed by plotting the peak height ratios of cefmenoxime to *p*-anisic acid against the concentrations of cefmenoxime in plasma.

*Preparation of urine samples and standards*

*Urine samples.* Urine samples were diluted to the desired concentration and injected directly onto the column after addition of internal standard (0.2 mg/ml).

*Urine standards.* Cefmenoxime stock solution was prepared by accurately weighing 8—9 mg of cefmenoxime into a scintillation vial and dissolving with

5 ml of 0.07 *M* phosphate buffer (pH 7.0). Blank human urine was spiked with cefmenoxime stock solution to yield concentrations ranging from 16—210 μg/ml. Urine samples were prepared as described above. A standard curve was constructed by plotting the peak height ratios of cefmenoxime to *p*-anisic acid against the concentrations of cefmenoxime in plasma. Correlation coefficients for 10 plasma standard curves averaged 0.999±0.0004 (±S.D.) and for ten urine standard curves, 0.999±0.001.

## RESULTS

### *Assay precision*

Six plasma and six urine samples were prepared, analyzed and compared to the control (spiked) plasma and urine samples. The data are listed in Table I. The assay variability was less than 5% for both concentrations in plasma and urine.

### *Stability*

The stability of cefmenoxime in plasma frozen at —20°C was studied at different concentrations. Duplicate plasma samples containing either 0.7 or 3 μg/ml of cefmenoxime were prepared and assayed. Results are shown in Table II. Duplicate urine samples containing either 44 or 222 μg/ml of cefmenoxime were prepared and assayed. Results are shown in Table III.

TABLE I

ASSAY PRECISION FOR CEFMENOXIME IN PLASMA AND URINE

| | Spiked concentration (μg/ml) | Measured concentration ($n$ = 6) | |
|---|---|---|---|
| | | Mean ± S.D. (μg/ml) | C.V. (%) |
| Plasma | 0.755 | 0.804 ± 0.038 | 4.73 |
| | 3.02 | 3.14 ± 0.054 | 1.72 |
| Urine | 44.45 | 43.8 ± 1.41 | 3.22 |
| | 222.25 | 224.72 ± 6.67 | 2.97 |

TABLE II

STABILITY OF CEFMENOXIME IN PLASMA FROZEN AT —20°C AT DIFFERENT CONCENTRATIONS

Values are the average of two measurements.

| Day | Cefmenoxime concentration (μg/ml) | |
|---|---|---|
| | Low (0.7 μg/ml) | High (3.0 μg/ml) |
| 0 | 0.72 | 3.07 |
| 1 | 0.76 | 3.01 |
| 2 | 0.74 | 2.86 |
| 3 | 0.72 | 2.83 |
| 4 | 0.79 | 2.98 |
| 7 | 0.74 | 2.78 |
| 14 | 0.70 | 2.69 |
| 21 | 0.73 | 2.67 |

TABLE III

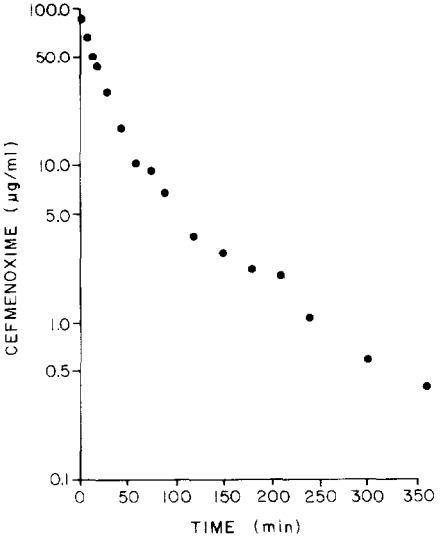
STABILITY OF CEFMENOXIME IN URINE FROZEN AT −20°C AT DIFFERENT CONCENTRATIONS

Values are the average of two measurements.

| Day | Cefmenoxime concentration (μg/ml) | |
|---|---|---|
| | Low (44.0 μg/ml) | High (222.0 μg/ml) |
| 0 | 41.5 | 198.93 |
| 1 | 40.7 | 186.05 |
| 3 | 42.3 | 196.86 |
| 7 | 42.1 | 192.01 |
| 14 | 41.6 | 187.93 |

*Pharmacokinetic study*

Fig. 4 shows a plasma concentration versus time plot following cefmenoxime intravenous infusion (10 mg/kg) to a healthy volunteer. A urine excretion rate curve following a cefmenoxime intravenous infusion (10 mg/kg) to a healthy volunteer is shown in Fig. 5.



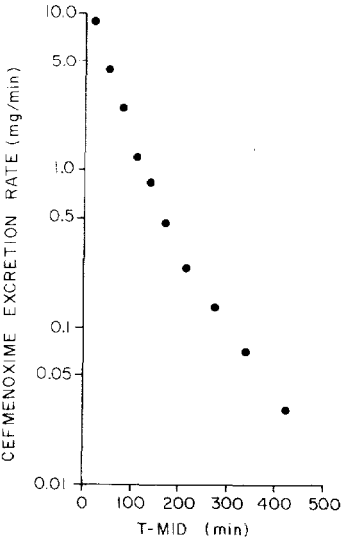




Fig. 4. Plasma concentration versus time plot following the administration of cefmenoxime by intravenous infusion (10 mg/kg) to a healthy volunteer.

Fig. 5. Urine excretion rate curve following the administration of cefmenoxime (10 mg/kg) by intravenous infusion to a healthy volunteer.

## DISCUSSION

An HPLC assay has been developed for the analysis of cefmenoxime in human plasma and urine samples. This method which does not require extraction is simple, selective and sensitive and requires only 0.2 ml of biological fluid. Both cefmenoxime and the internal standard, *p*-anisic acid, are resolved

from endogenous plasma and urine components. A previously described HPLC technique for analysis of the drug in plasma requires ultrafiltration of the samples using the Amicon Centriflow system [1]. Our procedure requires only the deproteination of plasma samples by simple addition of acetonitrile prior to HPLC injection. No previous HPLC urine assay has been described. Our procedure utilizes direct injection of urine samples onto the column after addition of internal standard. The assay proved to be quite sensitive with the lower detection limits of 0.2 $\mu$g/ml for plasma and 5 $\mu$g/ml for urine samples. Assay precision was evaluated and variability was less than 5%. Stability was also determined for cefmenoxime in plasma and urine frozen at $-20^{\circ}$C. The results showed less than 10% degradation for plasma samples at one week and for urine samples, two weeks. The HPLC assay described here has shown to be rapid and reproducible and due to its specificity allows cefmenoxime to be analyzed in the presence of other antibiotics. This assay is currently being utilized in pharmacokinetic studies of healthy subjects and renal failure patients.

ACKNOWLEDGEMENTS

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REFERENCE

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