High-Performance Liquid Chromatographic Method for Determination of Cefmetazole in Human Serum

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A fast, specific, sensitive high-performance liquid chromatographic method for the determination of cefmetazole in human serum was developed. The serum samples were deproteinized by adding 5% trichloroacetic acid in methanol containing barbital as an internal standard and were injected onto a reverse-phase column (μ-Bondapak C₁₈) with a mobile phase of 10 to 15% acetonitrile in 0.005 M citrate buffer (pH 5.4). Eluted components were detected by UV absorption at 254 nm. Cefmetazole and the internal standard were separated from interfering serum components by this method. The peak height ratio of cefmetazole to the internal standard was proportional to the cefmetazole concentration in the range from 0.4 to 100 μg/ml. Serum samples obtained from three patients after a single intravenous injection of cefmetazole were assayed by this method and by a microbiological method. There was a good correlation between the two assay methods (correlation coefficient, 0.98). The stability of cefmetazole in human serum was also determined by this method. Cefmetazole was stable in human serum for 2 weeks at 4°C or for at least 8 weeks if it was kept frozen. As the high-performance liquid chromatography method is simple, specific, accurate, and reproducible, it appears to be more suitable for routine assay of cephalosporins than other assay methods.

Cefmetazole sodium (7-β-cyanomethylthioacetamide-7-α-methoxy-3-[(1-methyl-1H-tetrazol-5yl)-thiomethyl]-3-cephem-4-carboxylate), a new semisynthetic derivative of cephamycin developed by Sankyo Co., Ltd., Tokyo, Japan, has broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria, in addition to high resistance to inactivation by several β-lactamases. It also has high antibacterial activity against indole-positive Proteus and Serratia strains, against which commercial cephalosporin and penicillin antibiotics are ineffective. Cefmetazole has been used widely since it was introduced in 1980 and has proved to be an excellent antibiotic (3–5, 7).

Although body fluid levels of cefmetazole have been determined by a microbiological assay (7), rapid monitoring of this drug in body fluids is often necessary in clinical situations. Therefore, we used high-performance liquid chromatography (HPLC) to determine cefmetazole levels in human serum. This technique requires extraction of the bound drug from human serum proteins, which was readily achieved with 5% trichloroacetic acid in methanol.

HPLC assay procedure. (i) Instruments and operations. The HPLC instrument used in this study was a model ALC/GPC 2000 liquid chromatograph (Waters Associates, Milford, Mass.). It was equipped with a model 6000A solvent delivery system, a model U6K universal injector, and a model 440 absorbance detector. The eluant was monitored at 254 nm. Separation was accomplished on a μ-Bondapak C₁₈ column (Waters Associates), and samples were injected with a 25-μl syringe (Precision Sampling Co., Baton Rouge, La.). The mobile phase consisted of 10 to 15% acetonitrile in 0.005 M citrate buffer (pH 5.4). The concentration of acetonitrile in the mobile phase was adjusted daily for optimum separation and peak shape. The flow rate was maintained at 1.0 ml/min at pressures of 70 to 150 kg/cm². Operations were carried out at room temperature. Calibration of cefmetazole levels was carried out based on the peak height ratio of cefmetazole to the internal standard.

(ii) Preparation of standard solutions. Cefmetazole was dissolved in distilled water to a concentration of 1,000 μg/ml. This solution was diluted successively with distilled water to concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 μg/ml. Then 50 μl of each diluted solution was mixed with an equal volume of human serum. The standard solution was freshly prepared each day.

(iii) Preparation of sample solutions. A 50-μl portion of each serum sample was mixed with an equal volume...
of internal standard solution. This mixture was kept in an ice bath for 10 min and centrifuged at 3,000 rpm for 5 min. The resulting supernatant was removed from the test tube to another test tube in an ice bath with a Pasteur pipette and without delay was injected onto a reverse-phase liquid chromatography column. The injection volume was 10 to 20 μl.

**Microbiological assay.** The microbiological assay for determining the cefmetazole concentrations in sera was performed by the thin-layer disk plate method, using *Micrococcus luteus* ATCC 9341 as the test organism. The standard solutions were prepared by using Monitrol IX (DADE, Div. American Hospital Supply Corp., Miami, Fla.).

**Test for stability of cefmetazole.** Sodium cefmetazole (10 and 100 μg/ml) was dissolved in sera from healthy volunteers who had not received any drug. These serum samples were divided into test tubes and stored at 4 and −20°C. The amount of residual cefmetazole was determined after 1, 2, 3, 4, and 8 weeks by the HPLC method.

**Determination of serum levels.** Serum samples were obtained from three patients who received 2 g of sodium cefmetazole intravenously, and these samples were stored at −20°C until they were assayed.

## RESULTS

**Chromatograms of cefmetazole.** Figure 1 shows typical chromatograms of patient serum samples and serum blanks with and without antibiotic supplementation. The retention times of cefmetazole and the internal standard were 7.2 and 12.0 min, respectively. Figure 1 shows that the cefmetazole and internal standard peaks were not disturbed by other serum components.

**Standard curve.** Figure 2 shows a standard curve obtained by HPLC assay of human sera containing various amounts of cefmetazole. There was a good linear relationship between the peak height ratio and the cefmetazole concentration from 0.4 to 100 μg/ml. When a high serum concentration was expected, standard curves were prepared with larger amounts of internal standard added to serum samples and standard solutions.

**Precision.** The precision of the HPLC method was determined by assaying five replicate serum samples containing 25 or 50 μg of cefmetazole per ml (25 to 50 μg/ml is the therapeutic concentration range of this antibiotic) (Table 1). The low variation coefficients (1.72 and 2.40%) indicate that the HPLC method was suitable for assay of serum levels of cefmetazole.

### TABLE 1. Precision of cefmetazole determinationa

<table>
<thead>
<tr>
<th>Cefmetazole concn added to serum (μg/ml)</th>
<th>Mean concn (μg/ml)</th>
<th>Variation coefficient (%)</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>25.00 ± 0.43b</td>
<td>1.72</td>
</tr>
<tr>
<td>50</td>
<td>49.99 ± 1.20</td>
<td>2.40</td>
</tr>
</tbody>
</table>

a Values represent five determinations at each concentration.

b Mean ± standard deviation.
Specificity. Commonly used antibiotics, such as gentamicin (5 μg/ml), netilmicin (5 μg/ml), and amikacin (5 μg/ml), were tested with the HPLC method for interference with cefmetazole and barbital. However, this method was specific for cefmetazole in that aminoglycosides did not interfere with the measurement of this drug.

Comparison of the HPLC assay with the microbiological assay. Serum samples from three patients given 2 g of cefmetazole intravenously were assayed by both the HPLC method and the microbiological method. Curves of serum level versus time obtained by the two methods are shown in Fig. 3, and the correlation between the two methods is shown in Fig. 4. Although in general the microbiological assay showed a slightly lower level, there was a good correlation between the assay values of the two methods. The linear regression equation was as follows: \( y = 1.06x - 4.91 \) (correlation coefficient, 0.98).

Stability of cefmetazole. Figure 5 shows the effect of temperature on the stability of cefmetazole in human serum. More than 98% of the initial cefmetazole in a preparation remained unchanged when it was stored for 2 weeks at 5°C or for 8 weeks at -20°C. We observed no difference in stability between 100- and 10-μg/ml cefmetazole preparations.

**DISCUSSION**

Cephalosporins are the most commonly used antibiotics in the treatment of infectious diseases. Large amounts of cephalosporins are often administered to save heavily infected patients. However, the risk of side effects and the potential toxicity due to overdose, especially in patients with hepatic dysfunction or renal dysfunction or both (2, 6, 8, 9), could be prevented by rapid monitoring of serum drug levels.

Several analytical methods for the determination of cephalosporins in body fluids have been described previously. However, each method has its advantages and disadvantages. Microbiological assays (7) are time consuming, but they determine the antimicrobial serum activity. Fluorimetric assays (1, 10, 11) require cumbersome pretreatment of serum samples, although they are very sensitive methods. Therefore, we developed an HPLC method for determining cefmetazole in body fluids. Compared with the
other methods available, the HPLC method is convenient for determining cephalosporins in body fluids for the following reasons: (i) it is rapid; (ii) it is a simple and reliable procedure; (iii) it has good reproducibility and sensitivity; and (iv) it is highly specific.

Under the experimental conditions which we used, the cefmetazole peak was well separated from the peaks of the other serum components (Fig. 1). Moreover, high reproducibility was demonstrated by the variation coefficients (1.72 and 2.40%), and the assay had a sensitivity of 0.4 \( \mu \text{g/ml} \). Therefore, determination of cefmetazole by this method was satisfactory within the therapeutic concentration range. Cefmetazole concentrations determined by the HPLC method were in good agreement with microbiological assay values.

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LITERATURE CITED