Determination of Cefoperazone Concentration in Serum and Muscle Tissue with a Versatile High-Pressure Liquid Chromatographic Method

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A rapid, specific, and reproducible high-pressure liquid chromatographic method was developed for the determination of cefoperazone concentration in serum and tissue. The assay uses a simple methanol extraction, with cefoxitin as the internal standard. The limits of detection are 1 to 150 μg/ml; the maximum coefficient of variation is 7.4%. Using the same chromatography column, μBondapak phenyl, and mobile-phase 0.005 M tetrabutylammonium buffer-acetonitrile (80:20), the method can be easily adapted for the analysis of cefoxitin and moxalactam.

Cefoperazone is a third-generation cephalosporin with antimicrobial activity distinguished from currently available cephalosporins by its increased activity against Pseudomonas aeruginosa (2, 3, 5). Pharmacokinetic data obtained by bioassay show that after the infusion of a 2-g intravenous dose, mean serum levels of 108 to 134 and 11 to 18.5 μg/ml are found at 1 and 6 h, respectively (4, 6). Bioassay procedures, in general, lack specificity and are cumbersome and time consuming when applied to a large number of samples. Rapid, accurate methods for the analysis of serum cefoperazone levels in this range would be of clear value. Thus, we sought to develop a rapid, simple, and sensitive assay for cefoperazone in serum and to test the applicability of this method for determining the concentration of the antibiotic in tissues. In this report, we present a reverse-phase high-pressure liquid chromatographic (HPLC) method for the determination of cefoperazone concentration in serum and tissue.

Cefoperazone was supplied by Pfizer Inc. (New York, N.Y.); cefoxitin was supplied by Merck Sharp & Dohme (West Point, Pa.). Organic solvents were of HPLC grade (Fisher Scientific Co., Pittsburgh, Pa.). Water was glass distilled and filtered through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). Chromatography was performed on a Waters model ALC-GPC 204 liquid chromatograph equipped with a U6K injector, a 440-fixed-wavelength (254 nm) absorbance detector, and an Ominiscribe recorder (Houston Instruments, Houston, Tex.). A μBondapak phenyl column (3.9 mm by 30 cm; Waters Associates, Milford, Mass.) was used with a guard column packed with Bondapak C-18 (Waters Associates). The mobile phase consisted of water-acetonitrile (80:20) to which tetrabutylammonium phosphate buffer (Pic A; Waters Associates) was added to achieve a final concentration of 0.005 M. Chromatography was carried out at room temperature. At a flow rate of 2.7 ml/min, column pressure was approximately 2,500 lb/in².

Cefoperazone was weighed on an analytical balance and dissolved in filtered, distilled water to achieve desired final concentrations. A 0.1-ml sample of each standard solution was placed in a test tube, and a 0.1-ml sample of methanol containing the internal standard (cefoxitin, 75 μg/ml) was added. The tubes were then blended in a Vortex mixer for 20 s, and 20 μl of solution was introduced into the injector. A standard curve was constructed, using the ratio of cefoperazone to cefoxitin peak heights plotted against cefoperazone concentration.

Patients undergoing neck dissections received cefoperazone as part of a randomized controlled trial. After giving written, informed consent, patients received 2 g of cefoperazone intravenously before entering the operating room. Samples of muscle tissue were obtained upon operation, and a simultaneous serum sample was drawn. Samples were stored at −70°C until assayed. Samples of muscle tissue were rinsed in sterile saline and blotted dry to remove blood; minced portions weighing 0.1 to 0.2 g were placed in glass tubes, and distilled water equal to approximately twice the weight of the sample was added. The tissue was homogenized in a Potter-Elvehiem homogenizer at 1,200 rpm, and
the whole homogenate was extracted. A 100-μl portion of serum or tissue homogenate was placed in a centrifuge tube, and 100 μl of methanol containing the internal standard (cefoxitin, 75 μg/ml) was added. The samples were blended in a Vortex mixer for 30 s, then allowed to incubate at room temperature for 10 min. The tubes were centrifuged at 2,000 rpm in a Sorvall GSA centrifuge for 10 min, whereupon 20 μl of supernatant was injected onto the column.

Control samples were prepared by adding cefoperazone to outdated human plasma to yield final concentrations of 25, 50, and 100 μg/ml. These samples were prepared for analysis in a manner identical to patient samples and were used to determine assay reproducibility.

Figure 1 shows a typical standard curve for cefoperazone with concentrations ranging from 5 to 150 μg/ml. The lower limit of detection is approximately 1 μg/ml. At concentrations below 5 μg/ml, the adjustment of recorder sensitivity (from 0.05 to 0.01 absorbance units, full scale) or the injection of larger volumes was required. The typical retention times for cefoperazone and cefoxitin are approximately 10.5 and 5.8 min, respectively. Plasma components did not interfere with the detection of cefoperazone or cefoxitin. Figure 2A shows a chromatogram of blank plasma treated with methanol as described above, but containing no internal standard. Figure 2B shows a chromatogram of a control plasma sample. Table 1 demonstrates the reproducibility of the method, using 12 control plasmas assayed on 6 different days. The addition of the internal standard significantly improved accuracy; the analysis of loaded plasma without the addition of cefoxitin yielded an average recovery of cefoperazone 11 to 16% less than that obtained using the internal standard (P ≤ 0.001 for all concentrations tested). Plasma samples were stable after storage for 4 weeks at −70°C. Gentamicin at a level of 12 μg/ml did not interfere with the determination of either cefoperazone or cefoxitin (data not shown).

The results of the analysis of intraoperative serum samples plus simultaneously collected muscle samples are shown in Table 2. Experi-

![FIG. 1. Typical standard curve, showing the ratio of cefoperazone peak height to cefoxitin peak height plotted against cefoperazone concentration.](image)

![FIG. 2. (A) Chromatogram of blank plasma carried through methanol deproteinization. (B) Chromatogram of plasma containing cefoperazone (100 μg/ml) and cefoxitin (the internal standard; 75 μg/ml). (a) Cefoxitin peak; (b) cefoperazone peak.](image)

### Table 1. Reproducibility of reverse-phase HPLC for measuring cefoperazone concentrations in control sera

<table>
<thead>
<tr>
<th>Cefoperazone concn (μg/ml) added to sample</th>
<th>No. of samples</th>
<th>Concn (μg/ml)</th>
<th>Coefficient of variation (%)</th>
<th>Mean analytical recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>12</td>
<td>25.2 ± 1.4</td>
<td>5.4</td>
<td>100.8</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>48.9 ± 3.6</td>
<td>7.4</td>
<td>97.8</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>96.9 ± 7.2</td>
<td>7.4</td>
<td>96.9</td>
</tr>
</tbody>
</table>

* A total of 12 samples at each concentration were analyzed on 6 separate days.
* a Mean ± 1 standard deviation.
* c (Mean/loaded value) × 100.

### Table 2. Concentration of cefoperazone in human serum and muscle tissue obtained at operation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time after cefoperazone injection</th>
<th>Serum concn (μg/ml)</th>
<th>Tissue concn (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 h, 25 min</td>
<td>120</td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td>2 h, 55 min</td>
<td>71.5</td>
<td>9.3</td>
</tr>
<tr>
<td>3</td>
<td>2 h, 10 min</td>
<td>48.0</td>
<td>20.7</td>
</tr>
</tbody>
</table>
ments using muscle tissue homogenates to which cefoperazone had been added indicated that recovery was 87 to 103%. We have successfully applied this method to the determination of antibiotic concentrations in tissue and wound drainage (R. Muder, unpublished data).

The development of numerous third-generation cephalosporins with overlapping, broad antimicrobial spectra has complicated the criteria used for deciding which cephalosporin to employ in various clinical situations. Since differences among many of these cephalosporins are minor, the consideration of pharmacokinetics and the concentration achieved at the site of infection may provide a rational basis for drug selection.

We present a rapid, reverse-phase HPLC method for the detection of cefoperazone in serum and tissue. This method was shown to have good accuracy and reproducibility. It has a technical advantage in that the same instrumentation and similar chromatographic conditions are used as in a moxalactam analysis previously reported from this laboratory (1), a considerable convenience for investigators studying both drugs. Furthermore, the method can also be extended to the analysis of cefoxitin, using cefoperazone as an internal standard.

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LITERATURE CITED