Liquid Chromatographic Assay of Cefuroxime in Serum

Ingrid Nilsson-Ehle and Peter Nilsson-Ehle

We describe a procedure for determining cefuroxime concentrations in serum by using high-performance liquid chromatography. The drug is extracted from serum with dimethylformamide and separated from other substances in the extract by reversed-phase chromatography. The ultraviolet (280-nm) absorption of the effluent was monitored and quantitated based on the height of the cefuroxime peak. Intra- and inter-assay imprecision (CV) was less than 3.1 and 4.3%, respectively. Serum concentrations as low as 1.0 mg/liter could be accurately measured. No interference from various other drugs and antibiotics was found. A biological half-life of 52 min in serum was observed after intravenous injection of the drug into a human volunteer.

High-performance liquid chromatography (HPLC), used to determine various antibiotics in biological fluids (1–7), has shown definite advantages over routine microbiological assays with regard to rapidity, precision, specificity, and sufficient sensitivity for clinical use. This report describes such a procedure for determining cefuroxime (a new cephalosporin derivative with high stability to β-lactamases) in serum. Briefly, the assay involves extraction of drug from samples with dimethylformamide, reversed-phase partition HPLC, and detection by on-line monitoring of the effluent absorption at 280 nm.

Materials and Methods

Materials

Water used throughout the procedure was freshly distilled and de-ionized. Solvents and chemicals were analytical grade.

Apparatus. An ALC/GPC 204 liquid chromatograph (Waters Associates AB, Gothenburg, Sweden) was used. It was equipped with a Model 440 uv absorbance detector, operated at 280 nm at an attenuation of 0.01 absorbance unit full scale. A recorder (Vitatron Scientific Instruments, Maidenhead, England) was used to register ultraviolet absorption.

In vitro serum samples. Approximately 1 g of cefuroxime sodium (Glaxo Laboratories, Greenford, England) was accurately weighed and dissolved in distilled water to give concentrations ranging from 10 to 200 mg/liter. Volumes of 0.1 ml of these aqueous solutions were added to 0.9-ml samples of pooled human serum and incubated at ambient temperature for at least 20 min. We prepared control samples identically, substituting water for serum.

In vivo serum samples. A healthy human volunteer (body weight, 55 kg) was given 1 g of cefuroxime (Glaxo Laboratories) intravenously. Blood was drawn at various times up to 6 h and sera obtained after centrifugation were assayed for cefuroxime.

Sera were collected from 10 patients who were undergoing treatment with various commonly used drugs and antibiotics but not receiving cefuroxime therapy, to assess the specificity of the developed assay.

Assay Procedure

Extraction: One milliliter of dimethylformamide was added to 1 ml of serum sample, stirred rapidly on a vortex-type mixer, and the mixture was heated for 10 min in a water bath set at 60 °C. After centrifugation at 2000 × g for 10 min, 0.8 ml of the viscous supernatant fluid was diluted with an equal volume of distilled water to facilitate the subsequent passage through 0.6-μm filters (Solvinit; Millipore Corp., Bedford, Mass. 01730).

Separation and quantitation: A 50-μl aliquot of the extraction filtrate was injected into the liquid chromatograph and separated on a μBondapak C18 column, 30 × 4 mm i.d. (Waters Associates AB). The mobile phase consisted of acetic acid/water/methanol (179/20 by vol), which was filtered through 0.6-μm filters (Solvinit), and de-aerated under reduced pressure. The flow rate was 2.0 ml/min.

Control samples with known concentrations of cefuroxime were taken through the assay procedure and a standard curve was constructed expressing peak heights for cefuroxime as a function of concentration of drug (Figure 1). Cefuroxime concentrations in serum samples were determined by comparing peak heights obtained when analyzing these samples with the standard curve.

Results

In the chromatographic system we used, cefuroxime had a retention time of 8 min 45 s (Figure 2a). It was well separated from other substances in the dimethylformamide extract, and no interfering absorption was found in extracts of sera containing no drug (Figure 2b).

The modification of the extraction procedure originally described by Hoehn et al. (8) for cephalothin was introduced to more completely precipitate serum proteins, thus facilitating HPLC separation. For cefuroxime, this extraction was found to give quantitative recovery as determined by assaying samples of known concentrations (Table 1).

Imprecision was calculated for two concentrations. Two serum pools, containing 4.0 and 18.0 mg of cefuroxime per liter, were divided into 12 portions each. Six of these were assayed separately on the same day, the other six were stored at −20 °C and quantitated separately during one month. Intra-assay imprecision (CV) for concentrations of 4.0 and 18.0 mg/liter was 3.1% (mean 3.89 mg/liter, range 3.73–4.10 mg/liter) and 1.7% (mean 18.17 mg/liter, range 17.75–18.50 mg/liter), respectively. Inter-assay imprecision for the same two
Antibiotics administration

Fig. 1. Standard curve for cefuroxime

![Graph](image1.png)

Fig. 2. Chromatograms of extracts of serum
(a) Serum to which cefuroxime was added (20 mg/liter). (b) Serum without cefuroxime. Chromatographic conditions: column: μBondapak C18, 30 cm X 4 mm i.d.; mobile phase: acetic acid/water/methanol, 1/79/20, by vol; flow rate: 2.0 ml/min; detection: spectrophotometry at 280 nm; recorder chart speed: 5 mm/min; sample volume: 50 μl

![Graph](image2.png)

Fig. 3. Serum concentrations of cefuroxime after intravenous administration of 1 g of the drug to a healthy human volunteer
Insert shows semilogarithmic representation of the same data. t1/2, biological half-life of cefuroxime in serum

Table 1. Analytical Recovery of Cefuroxime from Serum

<table>
<thead>
<tr>
<th>Conc added to serum (mg/liter)</th>
<th>Conc measured (mg/liter)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>100.0</td>
</tr>
<tr>
<td>5.0</td>
<td>4.75</td>
<td>95.0</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td>15.0</td>
<td>14.51</td>
<td>96.7</td>
</tr>
<tr>
<td>20.0</td>
<td>19.75</td>
<td>98.8</td>
</tr>
</tbody>
</table>

* Each value represents the mean of duplicate analyses.

Table 2. Drugs Not Interfering with Determination of Cefuroxime in Serum

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Other drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl penicillin</td>
<td>Bromhexine</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>Clemastin</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Diazepam</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>Digoxin</td>
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<tr>
<td>Gentamicin</td>
<td>Dimeticon</td>
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<tr>
<td>Lincomycin</td>
<td>Dizyrazin</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Emepron bromide</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>Furosemide</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Levothyroxin</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Nitrazipam</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Oxazepam</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Potassium citrate</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Terbutalin</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Thiodizine</td>
</tr>
</tbody>
</table>

concentrations was 4.3% (mean 3.95 mg/liter, range 3.70–4.20 mg/liter) and 2.1% (mean 18.18 mg/liter, range 17.70–18.80 mg/liter).

Specificity of the method was studied by similarly assaying serum samples from 10 patients receiving various commonly used drugs and antibiotics (Table 2) but no cefuroxime. No peaks were detected at the retention time of cefuroxime.

Intravenous administration of 1 g of cefuroxime to the human volunteer yielded a concentration of 433 mg/liter 15 min after the injection. One hour after injection there was a linear relationship between time and log concentration (Figure 3). The biological half life calculated from this part of the curve was found to be 52 min.

Discussion

Cefuroxime, a new cephalosporin antibiotic with high stability against β-lactamases, offers a definite advantage over the currently used β-lactamase-susceptible cephalosporins. Reports on its metabolism, pharmacokinetic behavior, and toxicity are as yet scarce. No renal toxicity has hitherto been reported; but in some cases increased liver aminotransferase activity has been observed. Small children and patients with hepatic or renal insufficiency should be treated with caution and the concentrations of the drug in serum should be monitored.

We found no evidence of metabolites in the serum from the healthy volunteer. However, the situation might be different in cases with renal or hepatic dysfunction, and the pharmacokinetics of cefuroxime in such patients need to be studied further.
Our assay allows accurate determinations of serum concentrations down to 1.0 mg/liter (peak height in chromatograms 4 mm; maximal baseline fluctuation 1.5 mm). It is thus sensitive enough for clinical use, and appears suitable for pharmacokinetic studies.

We thank Waters Associates AB, Gothenburg, Sweden, for putting the chromatograph at our disposal.

References


Spectrophotometry of Theophylline-7-acetic Acid and Theophylline

James A. Owen and Kanji Nakatsu

A spectrophotometric assay procedure is described for individually measuring theophylline-7-acetic acid and theophylline in 2.0 ml of serum. Absorbance is a linear function of concentration over a range of 0.5–50.0 mg/liter for theophylline-7-acetic acid and 0.5–40 mg/liter for theophylline. No endogenous or exogenous compounds were found that interfere with theophylline-7-acetic acid determinations. As with other spectrophotometric theophylline assays, theobromine and, to a slight extent, phenobarbital interfere with theophylline determinations.

Additional Keyphrase: drug assay

In practice, theophylline is not a particularly easy drug to use because of its physical and pharmacological properties. It is poorly soluble in water; furthermore, it is frequently associated with gastric upset and palpitations, and sometimes causes convulsions. In an attempt to obviate these problems, many theophylline derivatives have been tested. One of these analogs, theophylline-7-acetic acid, was synthesized by Baise in 1949 (1) and is currently marketed as an alternative to theophylline. This acidic analog is often combined with piperazine to form a neutral preparation for both oral and parenteral administration. This preparation produces little or no gastric irritation when given orally, or pain on injection (2). On the other hand, there is little information on its therapeutic effectiveness, the concentration required in plasma, and its kinetics (2, 3). Before an attempt can be made to gather kinetic data it was necessary to develop an assay for theophylline-7-acetic acid because this drug cannot be assayed by the spectrophotometric theophylline assay procedure of Schack and Waxler (4). We describe here a specific spectrophotometric assay in which the serum concentrations of both theophylline-7-acetic acid and theophylline are individually determined. The method requires 2.0 ml of serum and is reliable at concentrations as low as 1 mg/liter.

Materials and Methods

Equipment. Absorbance data were obtained with a Coleman Model 124UV-Visible spectrophotometer (Perkin-Elmer Corp., Maywood, Ill. 60153) scanning from 310 to 250 nm, connected to a Recordall Series 5000 chart recorder (Fisher Scientific, Whity, Ontario). For dispensing volumes of 1.0 ml or less, "pipetman" (Mandel Scientific Co., Ville St. Pierre, Quebec H9R 1A3) adjustable pipets were used. "Repipet" dispensers (Labindustries, Berkeley, Calif. 94710) were used for volumes greater than 1.0 ml.

Chemicals and Reagents. All reagents, reagent-grade unless otherwise noted, were used without pretreatment or special precautions, and can be stored at room temperature, except for citrate buffer, which is stored at 4 °C.

The following chemicals were used in preparation of standards and controls; theophylline (as aminophylline), caffeine, theobromine (Sigma Chemical Co., St. Louis, Mo. 63178), theophylline-7-acetic acid (Adams Chemical Co., Round Lake, Ill. 60073), phenobarbital (Allen and Hanburys, Toronto), and 3-methylxanthine (Aldrich Chemical Co., Milwaukee, Wis. 53233).

Extraction and analytical procedure. The extraction is done as follows: Pipet a 2.0-ml aliquot of sample or standard

CLINICAL CHEMISTRY, Vol. 24, No. 2, 1978 367