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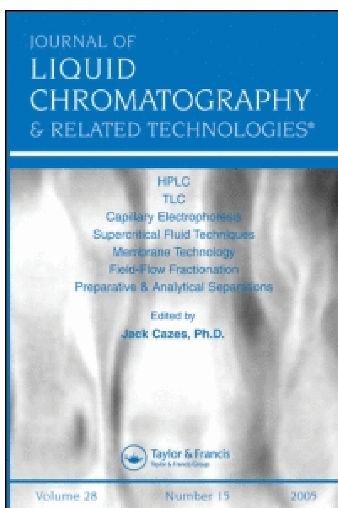

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S. H. Schachter[a]; H. Spino[a]; A. Tesoro[a]; S. M. Macleod[a]
[a] Division of Clinical Pharmacology, Research Institute, The Hospital for Sick Children, and Faculties of Pharmacy and Medicine, University of Toronto, Toronto, Ontario, Canada

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CEFOPERAZONE IN SERUM AND URINE

S.H. Schachter, M. Spino, A. Tesoro, and S.M. MacLeod
Division of Clinical Pharmacology, Research Institute,
The Hospital for Sick Children, and Faculties of Pharmacy
and Medicine, University of Toronto, Toronto, Ontario
Canada M5G 1X8

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ABSTRACT

A high performance liquid chromatographic method was developed for the quantitative analysis of cefoperazone in serum and urine. Standard curves were linear over the range of concentrations 2-30 μg/ml and a good correlation was established between the amount of cefoperazone injected and peak height. The mean percentage analytical recovery of cefoperazone was 96.3% and the mean within day coefficient of variation in serum was 2.8%. Serum and urine components, as well as several beta-lactam antibiotics, did not interfere with the measurement of cefoperazone. This is a rapid, reproducible, and sensitive assay suitable for use in pharmacokinetic studies.

INTRODUCTION

Cefoperazone is a third-generation semisynthetic parenteral cephalosporin (Figure 1) with marked antipseudomonal activity, particularly against Pseudomonas aeruginosa (1). The major route of elimination appears to be biliary secretion with about 15-30% of a dose excreted unchanged in the urine. Less than 1% of an administered dose of the drug is identified as metabolites in the urine (2).

Most published pharmacokinetic data on cefoperazone have been determined using microbiological assay techniques. While bioassays

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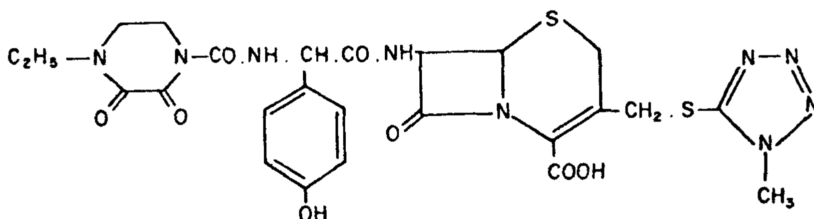

FIGURE 1 Chemical structure of cefoperazone.

offer the advantage of short analysis time and high capacity for analysing a large number of samples, they have the associated disadvantages of lacking specificity and reproducibility. We have developed a rapid, reproducible, and sensitive high performance liquid chromatography (HPLC) assay in order to determine concentrations of cefoperazone in serum and urine. Previously published HPLC assays have cumbersome extraction procedures and/or more complex chromatographic methods of analysis (3,4,5,6). Our assay is simple and its suitability for use in pharmacokinetic analysis has been demonstrated in a study of the disposition of cefoperazone in cystic fibrosis patients and healthy control volunteers (7) (Figure 2).

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MATERIALS AND METHODS

(i) Chemicals:

All reagents and solvents used were HPLC grade. Cefoperazone and Cephalothin were supplied by Pfizer Canada Inc. (Pointe-Claire, Quebec) and Eli Lilly and Co. (Indianapolis, Ind.) respectively. Acetonitrile was obtained from Burdick and Jackson Laboratories, Inc., (Muskegon, Michigan), methanol from Caledon Laboratories, (Georgetown, Ontario), and sodium phosphate monobasic from Fisher Scientific (Fair Lawn, New Jersey).

(ii) Preparation of Buffer and Stock Solutions:

For both the serum and urine assay, 10 mM sodium phosphate monobasic buffer (pH 4.5) was prepared by dissolving 2.7598 g of the buffer in 200 ml of distilled water and bringing the solution to a final vol-

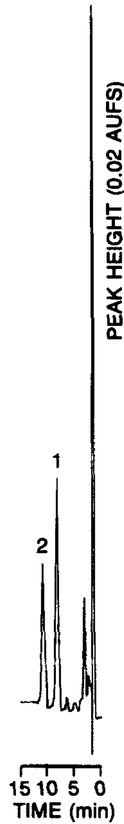

FIGURE 2 Representative chromatogram of cefoperazone (1) and cephalothin (2) in the serum of a cystic fibrosis patient 1 minute following a 1 g/m^2 dose infused over 20 minutes.

ume of 2 L. The pH was then adjusted to 4.5. Stock solutions of cefoperazone and cephalothin were prepared fresh daily. The working cefoperazone standard solution was prepared by dissolving 10 mg of cefoperazone in 10 ml of distilled water and bringing the solution to a final volume of 25 ml. To prepare the working internal standard solution, 10 mg of cephalothin was dissolved in 5 ml of distilled water and an aliquot from this solution was added to a 25 ml volumetric flask containing methanol to reach the desired final concentration of 15 µg/ml.

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(iii) Handling of Samples:

Serum: 200 µl of methanol containing the internal standard cephalothin was added to 100 µl of the serum sample to precipitate any soluble proteins. Each sample was vortexed for 1 min and then centrifuged for 3 min in an Eppendorf 5412 centrifuge. Twenty µl of the resultant supernatant was manually injected directly onto the column.

Urine: No extraction procedure was necessary for the urine and thus 20 µl of the urine sample was injected directly into the HPLC. Appropriate dilutions were prepared to fall within the linear range of the standard curves for both serum and urine using blank serum and a 1:4 ratio of urine to distilled water as diluents.

(iv) Apparatus:

Chromatographic analysis was performed using a Perkin-Elmer series 2 liquid chromatograph, LC-75 variable wavelength detector, and a 023 chart recorder. Separation was achieved using a reverse phase C-18 µ Bondapak column (3.9 mm x 20 cm, 10 µ pore size, Waters Scientific, Milford, MA). The mobile phase consisted of acetonitrile (CH_3CN) and 10 mM sodium phosphate monobasic buffer at pH 4.5 in a ratio of 15%:85% and 12%:88% for the serum and urine respectively. Before use, each mobile phase was degassed using a 0.5 µM Durapore filter system (Millipore Corporation, Bedford, MA).

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(v) Conditions of Analysis:

All serum and urine samples were stored at -20°C prior to analysis. Optimal conditions for the analysis of cefoperazone serum and urine samples included a wavelength of 228 nm based on the absorbance characteristics of the drug. The detector output was attenuated at 0.02 absorbance unit full scale (AUFS) and the mobile phase was delivered at a flow rate of 2.5 ml/min. All analyses were performed at room temperature.

(vi) Calculations:

Serum cefoperazone concentrations were determined using an internal standard technique with cephalothin being the internal standard.

Standard curves were drawn up on each day of analysis based on five standard concentrations ranging from 2-30 µg/ml. A response factor (RF) was then calculated using the following equation:

$$RF = \frac{\text{Peak Height Cephalothin}}{\text{Peak Height Cefoperazone}} \times \frac{\text{Conc. of Cefoperazone}}{\text{Conc. of Cephalothin}}$$

The mean of the response factors of each of the 5 standard concentrations was then used to calculate the serum cefoperazone concentration in the following equation:

$$\text{Conc. of Cefoperazone} = \frac{\text{RF} \times \text{Peak Height Cefoperazone}}{\text{Peak Height Cephalothin}} \times \text{Conc. of Cephalothin}$$

Urine cefoperazone concentrations were determined using an external standard technique. The equation for the line of best fit through 5 urine standard concentrations was calculated by linear least squares regression. The equation was then used to calculate the urine cefoperazone concentrations by substituting in the values for the dependent variable, peak height, to determine the corresponding value for the independent variable, cefoperazone urine concentrations.

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RESULTS AND DISCUSSION

Chromatograms of cefoperazone and cephalothin in serum and cefoperazone in urine are shown in Figures 3 and 4. Endogenous components in the plasma and urine did not interfere with the identification of cefoperazone. Other beta-lactam antibiotics, cefaclor, cephalexin, cefamandole, ticarcillin, ampicillin, and cloxacillin were found to have elution times that differed from those for cefoperazone and cephalothin. In addition, the aminoglycosides, tobramycin and gentamicin did not appear in chromatograms or alter assay results.

Linearity of the standard curve was established by plotting peak height versus cefoperazone concentration at 5 standard concentrations ranging from 2-30 µg/ml ($r^2 = 0.998$, Figure 5). The detection limit for both the serum and urine assay was approximately 2 µg/ml.

The within day precision of the assay was measured by using pooled sera with cefoperazone to produce three different concentrations,

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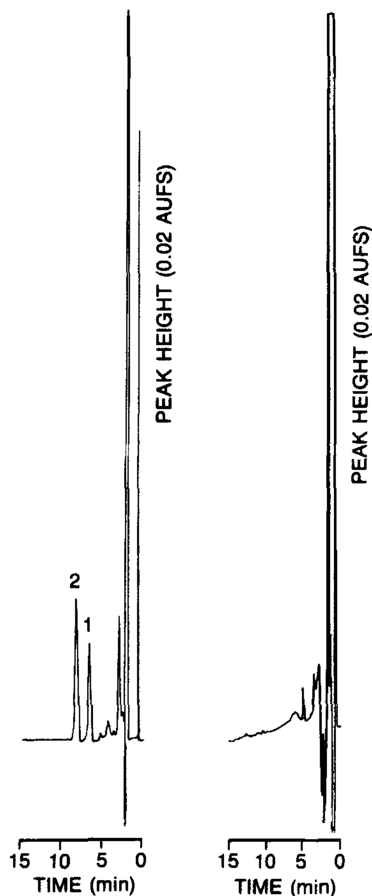

FIGURE 3 (i) Representative chromatogram of cefoperazone (1) and cephalothin (2) in serum. (ii) Representative chromatogram of a blank serum sample.

4.65 μg/ml, 13.95 μg/ml, and 29.45 μg/ml. Six replicates were made for each of the three concentrations involving three sets of six independent extractions. Coefficients of variation were 0.7%, 4.7%, and 3.0% respectively. The percent absolute recovery at low, medium, and high concentrations was 103.2%, 91.8%, and 94.0% with the mean percent recovery being 96.3%.

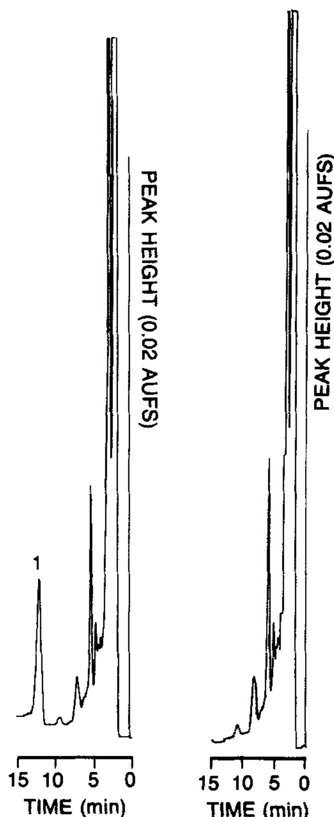

FIGURE 4 (i) Representative chromatogram of cefoperazone (1) in urine. (ii) Representative chromatogram of a blank urine sample.

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Some of the previously described HPLC assays use an external standard technique to quantify serum cefoperazone concentrations (3,5). This method of quantification, although acceptable, is generally not preferred if a suitable internal standard exists. Our current method of quantification, using an internal standard, results in reduced variability since quantitation is independent of the volume injected.

The practical application of certain HPLC assays for cefoperazone to pharmacokinetic analysis may not be suitable since extraction procedures are tedious and time consuming (3,4,5). Furthermore the range of cefoperazone serum concentrations over which the standard

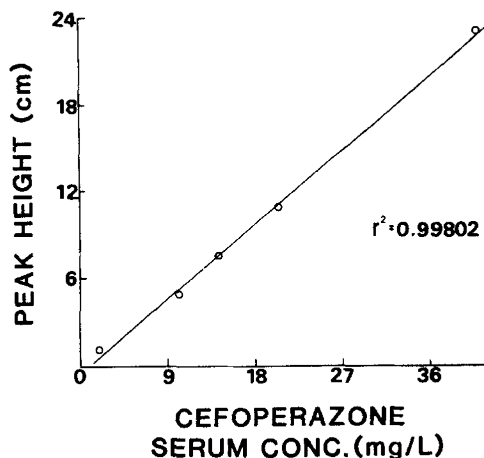

FIGURE 5 Standard curve for cefoperazone over the range of concentrations from 2 - 30 mcg/mL.

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curves are linear is too small to make precise extrapolations for concentrations normally found in the clinical setting. Our assay overcomes these problems and is suitable for use in pharmacokinetic analysis as previously demonstrated (7).

This assay is rapid, reproducible, sensitive, and precise and offers an alternate means of determining cefoperazone concentrations in serum and urine with advantages over traditionally used bioassay techniques.

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