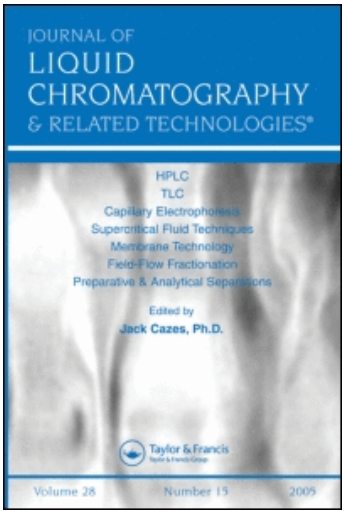

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MEASUREMENT OF CEFAZOLIN IN PLASMA AND PERITONEAL DIALYSIS FLUID BY HPLC

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ABSTRACT

Cefazolin is a commonly used antibiotic in infants and children. We have described a reverse phase HPLC method for the measurement of cefazolin in plasma and peritoneal dialysis solutions. The mobile phase consisted of sodium phosphate buffer and acetonitrile, and the internal standard was 7-hydroxycoumarin. The standard curves were linear over 0.25-200 mcg/ml, and the detection limit was 0.25 mcg/ml. The coefficient of variation was <6%. The method was successfully used to: 1) measure the plasma concentrations of cefazolin in small volumes of blood collected from an infant; and, 2) determine the stability of cefazolin in the peritoneal dialysis solutions over a 14-day period.

INTRODUCTION

Cefazolin is one of the most commonly used antibiotics for the treatment of infections caused by a variety of gram-positive and gram-negative microorganisms.[1]

This is perhaps the most widely used prophylactic antibiotic to prevent infection in patients undergoing surgery.[2]

We routinely use cefazolin as a prophylactic antimicrobial in infants and children undergoing gastrointestinal operations. However, no specific dosage guidelines are available, probably due to limited pharmacokinetics data in the pediatric population.[1] Thus, it would be desirable to have an HPLC method, which could be used to determine cefazolin in small volumes of plasma to characterize the pharmacokinetics in infants and children.

Continuous ambulatory peritoneal dialysis (CAPD) is being used with increasing frequency in pediatric and adult patients with end-stage renal disease.[3] Infection is one of the major complications in these patients; 60% of patients undergoing CAPD may develop peritonitis during the first year of dialysis.[3] Cefazolin is one of the commonly used antibiotics in CAPD patients.[4,5] The drug is added to the dialysis solutions and the dialysis may be carried out with a dwell time of 6 hours per exchange with several exchanges daily. Since many of these patients are being treated at home, it is important to assure the stability of cefazolin in peritoneal dialysis solutions during the storage period in the refrigerator and room temperature. It would be best to use a specific HPLC method to determine the stability of cefazolin in the dialysis solutions.

The purpose of this article it to describe a simple, rapid, accurate, sensitive, and reproducible HPLC method to measure cefazolin in plasma and peritoneal dialysis fluids.

MATERIALS AND METHODS

Equipment

The HPLC analysis was performed using Hewlett Packard 1050 series chromatographic system including a pump, autosampler, detector and 3369A integrator (Hewlett Packard Co., Westerville, OH). An ultrasphere ODS C_{18} column was used for plasma samples and C_8 column for peritoneal dialysis fluid samples (Beckman Instruments, San Ramon, CA). The pH was measured by a digital pH meter (Model 701A, Orion Research, Cambridge, MA).

Chemicals and Reagents

These included cefazolin sodium (Lot No. 86PAD70, Eli Lilly & Co., Indianapolis, IN), 7-hydroxycoumarin as an internal standard (Cat.No. 4-7626, Sigma Chemicals, St. Louis, MO), reagent grade sodium phosphate dibasic and monobasic, and phosphoric acid 85% (Fisher Chemicals, Fair Lawn, NJ), and HPLC grade acetonitrile (J.T. Baker, Phillipsburg, NJ).

Dialysis solutions

Two concentrations of dextrose containing dialysate were studied, Dianeal PD1-1.5% dextrose 500-ml bags (Travenol Labs Inc., Deerfield, IL 60015, lot CO84111) and Dianeal PD-1 4.25% dextrose 500-ml bags (Travenol Labs Inc,. Deerfield, IL 60015, lot CO92221). Each ml of dialysate contained the following: sodium chloride 567 mg, sodium lactate 392 mg, calcium chloride 25.7 mg, and magnesium chloride 15.2 mg.

Chromatographic conditions

The mobile phase consisted of 0.01 M sodium phosphate buffer, pH 5.3 and acetonitrile (v:v 85:15 for plasma samples and 82:18 for peritoneal dialysis fluid samples). The pH was adjusted with phosphoric acid and measured with a calibrated digital pH meter. The prepared mobile phase was filtered and degassed by vacuum through a 0.22 mcm filter. The flow rate was 2 ml/min and the chart speed was 2.5 mm/min. The detector was set at 254 nm for plasma samples and 230 nm for peritoneal dialysis fluid samples.

Sample preparation and analysis

Standards in plasma and peritoneal dialysis solutions were prepared to yield cefazolin concentrations of 0.25 to 200 mcg/ml. One hundred microliters of plasma or peritoneal dialysis solutions was used. One hundred microliters of 6% perchloric acid in methanol containing 10 mcg/ml of internal standard, 7-hyroxycoumarin was added. The samples were vortexed for 10 seconds, chilled in ice for 5-10 minutes, and then centrifuged at 11,000 g for 5 minutes. A 25 mcl aliquot of the resulting supernatant was injected onto the column.

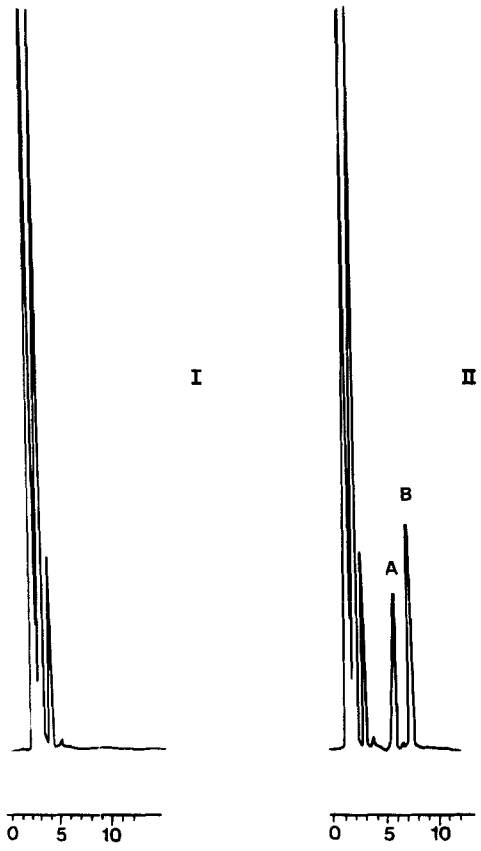

Figure 1. I. Chromatogram of plasma containing no drug (blank)

II. Chromatograms of cefazolin (A) and internal standard, 7-hydroxycoumarin (B) in plasma

RESULTS

Each chromatographic run required about 8 minutes. The retention time of cefazolin and the internal standard was 5.42 and 6.65 minutes in plasma (Figure 1), and 5.61 and 6.77 minutes in the peritoneal dialysis samples (Figure 2). The standard curves were linear; the r values ranged from 0.995 to 0.999. The limit of detection was 0.25 mcg/ml, and the interday and intraday coefficient of variation was <6%. The accuracy ranged from 95-99%.

Figure 2 I. Chromatograms of cefazolin (A), and 7-hydroxycoumarin (B) in peritoneal dialysis solution.

II. Chromatograms of cefazolin (A), 7-hydroxycoumarin (B), and degradation products at pH 12.

APPLICATIONS

A 10-month old infant undergoing a gastrointestinal operation received cefazolin for surgical prophylaxis. A single dose of cefazolin, 20 mg/kg was given intravenously over 2 minutes at the time of induction of anesthesia. Blood samples (0.5 ml each) were obtained just prior to the dose (0 hr), and at 0, 0.25, 0.5, 1, 2, 4 and 6 hours after the dose. The plasma was separated and stored at -70^{0}C until analyzed. The peak

plasma concentration was 190 mcg/ml, and the elimination half-life was 2.1 hours. The half-life was slightly longer in this infant than 1.7-1.8 hours reported in a study of older children, which utilized a microbiological assay.[6]

Cefazolin stability was determined in the two commercially available peritoneal dialysis solutions. Cefazolin was measured on days 0, 3, 7, 10, and 14 at 4^0C and on day 0, 1, 2, 3, 8, and 11 at 24^0C. The cefazolin concentration remained above 90% of the initial concentration throughout the study except on day 11 at 24^0C. Thus, the peritoneal dialysis solutions containing cefazolin can be stored for 14 days in the refrigerator and 8 days at room temperature without significant drop in its concentration.

This method was proved stability-indicating. The peritoneal dialysis fluid sample was intentionally degraded by raising pH. The cefazolin peak decreased and the peaks of degradation products did not interfere with the quantitation of cefazolin (Figure 2).

The HPLC method described here was found to be simple, rapid, sensitive, accurate and reproducible for the determination of cefazolin in small volumes of plasma and peritoneal dialysis fluids. It can be used to conduct pediatric clinical pharmacology and stability studies of cefazolin.

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