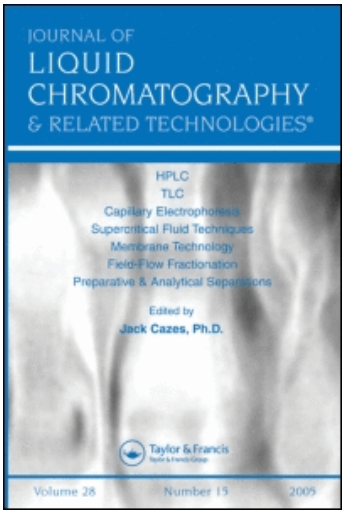

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Determination of Cefpirome in Human Plasma by High-Performance Liquid Chromatography

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DETERMINATION OF CEFPIROME IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Cefpirome is an investigational third-generation cephalosporin, which appears promising for the treatment of various pediatric infections. A high performance liquid chromatographic method was developed to measure cefpirome in small volumes of plasma for conducting pharmacokinetics studies in infants and children. The assay involved precipitation of plasma proteins with acetonitrile, using cefaclor as an internal standard. Chromatographic separation was accomplished using a reverse-phase

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C18 micro Bondapak column, and mobile phase consisting of 18% methanol in 0.05 M acetate buffer at a detection wave length of 240 nm. The retention time of cefpirome and cefaclor was 8.2 and 9.7 minutes, respectively. The method was suitable for quantitation of cefpirome at concentrations ranging from 0.5 to 150 mcg/ml. The interday and intraday coefficient of variation was <6%. Several commonly used drugs did not interfere with the measurement of cefpirome. The method is being used to measure cefpirome in 50 microliters of plasma samples obtained from infants and children.

INTRODUCTION

Cefpirome is an investigational third-generation cephalosporin. Based on the antimicrobial spectrum, pharmacological properties, and clinical experience in adult patients,[1,2] cefpirome appears to be a very promising antibiotic for the treatment of various systemic infections in infants and children. However, the pharmacokinetics of cefpirome must be evaluated in the neonatal and pediatric patients to develop dosage guidelines before its clinical efficacy can be investigated in these populations.

Two high-performance liquid chromatographic (HPLC) methods have been described.[3,4] One method utilized a large volume of serum, which would not be suitable for pediatric application;[3] cefpirome could not be separated from the plasma/solvent peaks using the other method.[4] We have developed a simple, rapid, accurate, sensitive, reproducible and specific HPLC method for the determination of cefpirome in small volumes of plasma, which could be utilized for conducting pharmacokinetics and pharmacodynamics studies in infants and children.

Materials and Methods

Equipment

High performance liquid chromatography was performed using a Hewlett Packard 1050 Series Quaternary Pump, autosampler and variable wave length Detector. A Hewlett Packard 3396A Intergrator and 9114B disc drive was used with C18 micro Bondapak column (Waters, Model #27324 Millipore, Milford, MA) and a New Guard cartridge (7 mcm) and holder (Brownlee Labs, Santa Clara, CA). The buffer portion of the mobile phase was filtered and degassed by vacuum through a 0.22 mcm filer.

Chemicals and Reagents

Cefpirome was obtained from Hoechst Roussel Pharmaceuticals Inc. (Sommerville, NJ). Cefaclor was received from Eli Lilly and Company (Indianapolis, IN). Reagent grade sodium acetate (Mallinckrodt Inc., Paris, KY), tetrabutyl ammonium hydroxide, (Aldrich Chemical Company, Inc., Milwaukee, WI), glacial acetic acid (Mallinckrodt Inc., Paris, KY), methanol, acetonitrile (JT Baker Inc., Phillipsburg, NJ), and deionized water were used without further purification.

Mobile Phase

An ion pair mobile phase was used containing 0.05 M acetate buffer with the ion-pairing agent tetrabutyl ammonium hydroxide (40%, wt/wt) and methanol (82% acetate buffer, 18% methanol). The buffer was prepared by adding 4 g sodium acetate to 1L of deionized water and adding 4 mL tetrabutyl ammonium hydroxide. The pH was adjusted to 5.1 with glacial acetic acid using a calibrated pH meter (Orion Research model 701A, Cambridge, MA).

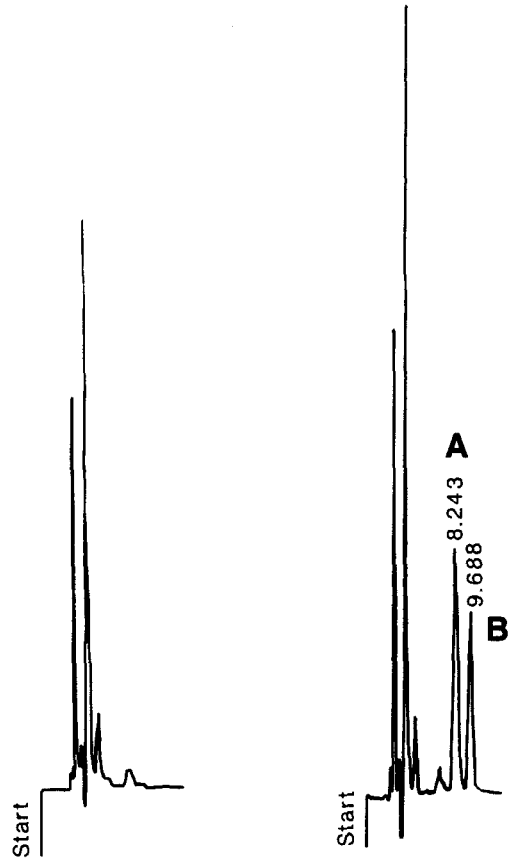

Figure 1. Chromatograms of plasma without any drug (left) and with cefpirome (A) and cefaclor (B).

Sample Preparation

Pooled plasma was spiked with cefpirome in concentrations ranging from 1 to 150 mcg/ml and stored at -70°C until analyzed. A 50 mcl plasma sample was pipetted into a 1.5 mL conical, polypropylene, Eppendorf microtainer. A 300 mcl of acetonitrile, containing 20 mcg/mL of cefaclor as an internal standard was added

Table 1. Ratios of peak height for cefpirome/cefaclor at various cefpirome concentrations.

Concentrations mcg/ml	Ratio
1	0.066
5	0.129
10	0.531
25	1.339
50	2.776
75	4.754
100	6.287
150	9.687

to each sample and vortexed for 30 seconds. All samples were then centrifuged (Fisher Scientific, micro-centrifuge, model 59A) for 1 minute. The supernatant was then transferred to clean microtainers and evaporated to dryness (N-EVAP analytical evaporator, Organomation Associates Inc., South Berlin, MA). Each sample was reconstituted with 125 mcl of mobile phase and vortexed for 15 seconds. Resultant supernatant was injected directly onto the column.

Chromatographic procedure

The mobile phase flow rate was maintained at 1.3 mL/min. The detector was set at 240 nm. The chart speed was 2.5 mm/min. Samples (25 mcl each) were injected onto the column for analysis.

Table 2. Accuracy of cefpirome assay

Known concentration mcg/ml	Determined concentration mcg/ml	Percent found
5	5.05	101
100	97.9	98

Table 3. Retention times of various drugs

Drug	Retention time (min.)
Cefotaxime	9.9
Cefazolin	9.5
Cephalexin	7.3
Ibuprofen	15.6
Theophylline	7.5
Gentamicin	No response*
Phenobarbital	No response
Ampicillin	7.3
Vancomycin	6.2
Acetaminophen	6.0

* No peaks observed during a 21 min. time

Results and Discussion

Each chromatographic run required approximately 12 minutes. Cefpirome eluted at 8.24 minutes and the internal standard (Cefaclor) at 9.69 minutes (Figure 1). Typical chromatograms of blank plasma, and the plasma containing cefpirome and cefaclor are shown in Figure 1.

Linearity was determined by linear regression analysis of the data (Table 1). The r value was 0.999 for the standard curve. It was possible to measure cefpirome concentration as low as 0.5 mcg/mL. The interday and intraday coefficient of variation was <5%.

The accuracy of the method ranged from 98% to 101% (Table 2). The method was considered specific based on the fact that the presence of several commonly used drugs (antibiotics, methylxanthine, anticonvulsants, analgesics and antipyretics) at clinically relevant concentrations in plasma did not interfere with the quantitation of cefpirome and cefaclor (Table 3).

The analytical method has been found to be simple, accurate, sensitive, reproducible, and specific for the measurement of cefpirome in small volumes of plasma. Thus, it can be used to conduct the pharmacokinetics and pharmacodynamics studies of cefpirome in infants and children for the development of optimal dosage guidelines.

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