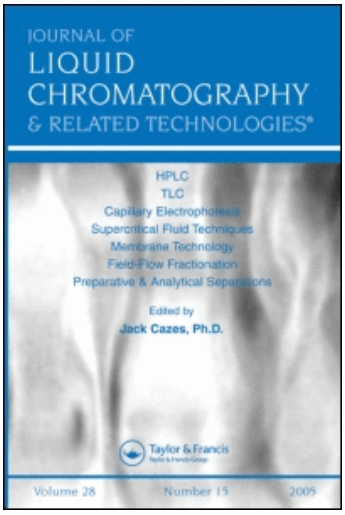

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Measurement of Cefixime in Serum and Cerebrospinal Fluid by High-Performance Liquid Chromatography

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MEASUREMENT OF CEFIXIME IN SERUM AND CEREBROSPINAL FLUID BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Cefixime is a new cephalosporin antibiotic for oral administration. A high-performance liquid chromatographic (HPLC) method was developed to measure cefixime in small volumes of serum and cerebrospinal fluid (CSF) to conduct a pharmacokinetics study in pediatric patients. The assay involved precipitation of serum proteins with 6% trichloroacetic acid, using 7-hydroxycoumarin as an internal standard. Chromatographic separation was accomplished using ultrasphere C8 column and mobile phase containing 15% acetonitrile in a buffer at a detection wave length of 280 nm. The retention time of cefixime and 7-hydroxycoumarin was about 5 and 9 minutes, respectively. The method was suitable for quantitation of cefixime at a concentration ranging from 0.05 to 10 μg/ml. The coefficient of variation was less than 3%. The technique was used successfully to measure cefixime in serum and CSF obtained from an infant receiving cefixime.

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INTRODUCTION

Cefixime is a new semisynthetic, cephalosporin antibiotic for oral administration. It is indicated for the treatment of respiratory infections (otitis media, pharyngitis, tonsillitis, and bronchitis) and uncomplicated urinary tract infections.[1]

A high-performance liquid chromatographic (HPLC) method for the measurement of cefixime has been reported.[2] This method, however, utilized a large volume of serum, which would not be suitable for pediatric application. Furthermore, no method is available to measure cefixime in the cerebrospinal fluid (CSF). We have developed a simple, rapid, accurate, sensitive and reproducible HPLC method for the determination of cefixime in small volumes of serum and CSF. The method was used successfully to measure cefixime in serum and CSF of infants and children.

METHODS

Equipment

High performance liquid chromatography was performed using a Hewlett Packard 1050 series pump, autosampler, and variable wave length detector. A Hewlett Packard 3396 A Integrator was used with Beckman Ultrasphere C8 column (5 micron, 4.6 mm x 25 cm).

Chemicals and Reagents

Cefixime (CL 284,635, PCR-415) was obtained from Lederle Laboratories; and internal standard, 7-hydroxycoumarin (lot no 17F-3428) from Sigma. Potassium phosphate (monobasic; lot no. 7100 KCJV) and phosphoric acid (lot no. 2796 KBRR-5) were purchased from Mallinckrodt. Other reagents included acetonitrile (lot no. AZ 2380; B&J), and trichloroacetic acid (lot no. UN 1839; MCB).

Mobile Phase

The mobile phase consisted of acetonitrile and buffer (15/85: v/v). The buffer was prepared using 0.01 M potassium phosphate acidified with 2.3 mL of phosphoric acid per liter of buffer, and filtered through a 0.45 micron filter.

Standard and Sample Preparation

Cefixime dissolved in methanol was added to the pooled serum samples (100 µl), and mixed with 100 µl (10 µg/ml) of the internal standard, 7-hydroxycoumarin in 6% trichloroacetic acid, to yield concentrations of 0.05, 0.1, 0.5, 1.0, 5.0 and 10 µg/ml.

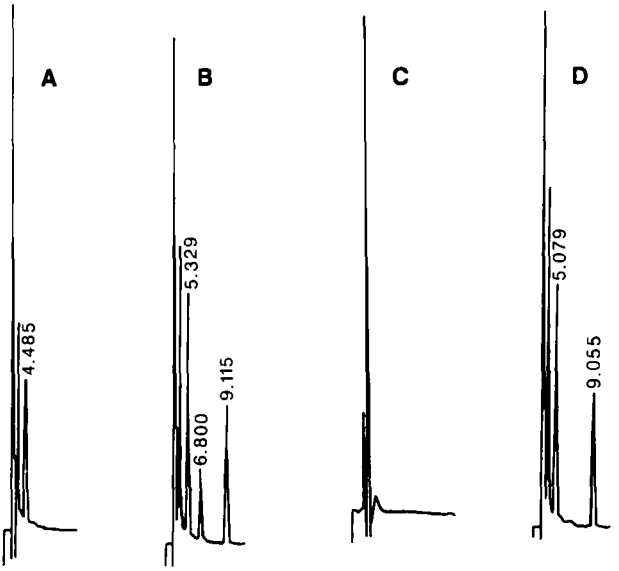

Figure 1. Chromatograms of blank serum (A), spiked serum (B), blank CSF (C) and spiked CSF (D). Cefixime and 7-hydroxycoumarin eluted at about 5 and 9 minutes, respectively.

The samples were vortexed and centrifuged for 5 minutes. The supernatant (100 μl) was injected onto the column. The patient serum and CSF samples were processed similarly except that no cefixime was added.

Chromatographic conditions

The flow rate of mobile phase was maintained at 2 mL/min. The detector was set at 280 nm. All measurements were performed at ambient temperature.

RESULTS AND DISCUSSION

Each chromatographic run required approximately 12 minutes. Cefixime eluted at about 5.33 and 5.08 minutes in serum and CSF, respectively; retention time for the internal standard (7-hydroxycoumarin) was about 9.12 and 9.06 minutes in serum and CSF, respectively. Typical chromatograms of blank serum and CSF,

Cefixime concentration	Ratio in serum	Ratio in CSF
0.05	ND	2.76
0.10	1.07	5.29
0.50	10.45	31.89
1.00	22.63	64.50
5.00	136.46	335.21
10.00	275.92	654.31
Correlation coefficient	0.9996	0.9999

Table 2. Accuracy of cefixime measurement

Known concentration	Percent of known concentration found
0.10	99.3
0.50	104.0
1.00	103.9
5.00	103.0
10.00	99.2

Table 3. Serum concentrations of cefixime in a pediatric patient

Time, hours	Serum concentration, μg/ml
0	0
1.5	1.2
3.0	3.3*
4.5	3.0
6.5	1.8
9.5	0.8

* Concurrent CSF concentration was 0.32 μg/ml

and of serum and CSF containing cefixime and 7-hydroxycoumarin are shown in Figure 1.

Linearity was determined by linear regression analysis of the data (Table 1). The correlation coefficient (r) was greater than 0.999 for both serum and CSF standard curves. It was possible to measure cefixime concentration as low as 0.05 μg/ml. The coefficient of variation was less than 3%. The accuracy of the method ranged from 99.2 to 104% (Table 2). This method was used to determine cefixime concentrations in the serum and CSF of an infant. An infant (age 9 month) undergoing a neurosurgical procedure (CSF shunt placement) received a single oral dose of cefixime, 8 mg/kg. Blood samples (0.5 mL each) were collected at just before (0 hr), and at 1.5, 3.0, 4.5, 6.5 and 9.5 hours after the dose. The peak serum concentration of cefixime was 3.3 μg/ml; the peak occurred at 3 hours after the dose. The CSF concentration was 0.32 μg/ml in a sample collected at 3 hours after the dose.

The HPLC method described here has proved to be simple, accurate, sensitive, and reproducible for the measurement of cefixime in human serum and CSF. Further, it has been used in conducting a pharmacokinetics study in pediatric patients.

ACKNOWLEDGEMENTS

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