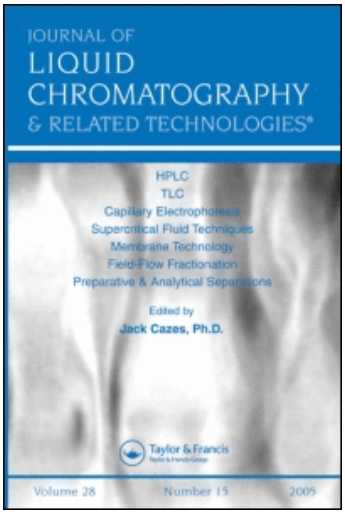

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LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF CEFADROXIL IN ITS SUSPENSION AND IN SERUM

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Abstract

We have described a simple, accurate, sensitive and reproducible reverse phase HPLC method for the measurement of cefadroxil in the commercially available suspension and in serum. The mobile phase consisted of sodium phosphate buffer and methanol and the internal standard was cefaclor. The samples were injected onto an Ultrasphere C_8 column and the detector was set at 230 nm. The standard curves were linear and the detection limit was 0.5 mcg/ml. The interday and intraday coefficient of variation was <4.7%. The method was successfully used to: 1) determine the stability of cefadroxil in the commercially available suspension; and, 2) measure serum cefadroxil concentration in a pediatric patient with osteomyelitis.

Cefadroxil is a first-generation oral cephalosporin, which is indicated for the treatment of pharyngitis and tonsillitis, skin and skin structure infections, and urinary tract infections caused by susceptible microorganisms.[1] It appears to possess an antistaphylococcal activity

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similar to semisynthetic oral penicillins e.g. dicloxacillin and cloxacillin, and other first generation cephalosporins e.g. cephalexin and cephradine.

The attractive features of cefadroxil include: 1) it can be given less frequently than the penicillins and cephalexin, which may improve patient compliance; 2) its absorption is affected much less by food than is the case with penicillins and other cephalosporins; and, 3) it has a lower incidence of adverse effects than semisynthetic oral penicillins. These potential benefits have led to an increased interest in exploring its pharmacokinetics in humans so that optimal dosage guidelines can be developed for patients.

Limited data are available about the pharmacokinetics of cefadroxil in infants and children.[2] However, the investigators have utilized a microbiological assay, which would be less accurate and specific than a high-performance liquid chromatographic (HPLC) method. There were two HPLC methods available but these could not be reproduced in our laboratory.[3,4] Both had unknown peaks interfering with cefadroxil in the serum obtained from pediatric patients. Further, these analytical methods were not developed using small volumes for application to pediatric patients.

With the recent efforts to contain health care costs, an increasing number of patients are being sent home with a supply of cefadroxil suspension. Thus, it would be of interest to evaluate its chemical stability over an extended period. However, no HPLC method has been described to measure cefadroxil in a suspension formulation.

This article describes a simple, accurate, sensitive and reproducible reverse-phase HPLC method for the determination of cefadroxil in the commercially available suspension and in serum of a pediatric patient with osteomyelitis.

Materials and Methods

Equipment:

The chromatographic system (Waters Associates, Milford, MA) consisted of an automated sample injector (WISP 712, Waters Associates), a model 110A Beckman pump, Ultrasphere C8 column (5mcm, 4.6mm x 250mm, Beckman Instruments, Inc., San Ramon, CA 94583) at room temperature (22^0C), a detector (V^4 Variable Wavelength Absorbance Detector, ISCO Inc., Lincoln, Nebraska 68505), and an integrator (Hitachi Model D-2000 Chromato-Integrator, Hitachi, Ltd., Tokyo, Japan).

Chemicals and Reagents:

These included, sodium phosphate monobasic granular (Mallinckrodt Inc., Paris, Kentucky 40361), methanol (JT Baker Inc., Phillipsburg, New Jersey 08865), phosphoric acid (Mallinckrodt Inc., Paris, Kentucky 43061), cefadroxil standard powder (Bristol-Myers, Evansville, Indiana 47721), and cefaclor standard powder (Eli Lilly & Co., Indianapolis, Indiana) as an internal standard.

Mobile Phase:

The mobile phase consisted of a mixture of sodium phosphate monobasic granular in deionized water (12.5 mmol/L) and methanol (85:15 v/v). It was adjusted to a pH of 2.6 with concentrated phosphoric acid. The mobile phase was then filtered through a 0.2 um membrane and degassed prior to use.

Standards:

A stock standard solution of cefadroxil (1 mg/ml) was prepared from cefadroxil standard powder dissolved in deionized water. Standard solutions of 1, 2.5, 5, 10, 15, 25 ug/ml were made by pipetting 10, 25, 50, 100, 150, 250, 350 and 500 ul into 10 ml volumetric flasks and diluting with serum. These solutions were stored at -74^{0}C for future use.

Standard solutions of cefadroxil were also prepared in serum. Concentrations of 1, 2.5, 5, 10, 15, 25, 35 and 50 ug/ml were made by pipetting 10, 25, 50, 100, 150, 250, 350 and 500 ul into 10 ml volumetric flasks and diluting with serum. These solutions were stored at -74^{0}C for future use.

A stock standard solution of cefaclor (1 mg/ml) was made from cefaclor powder. From the stock solution, a working solution of 25 ug/ml was prepared by pipetting 625 ul of stock solution into a 25 ml volumetric flask and diluting with deionized water. Cefaclor was utilized as the internal standard for the chromatographic procedure. This solution was stored at 4^{0}C for future use.

Sample Preparation:

1. Standard Curve (Aqueous): 100 ul of each standard solution of cefadroxil was mixed with 100 ul of cefaclor solution. The mixture was vortexed for ten seconds.

2. Standard Curve (Serum): 100 ul of each standard serum solution was mixed with 100 ul of 6% perchloric acid and 100 ul of cefaclor solution. The mixture was vortexed for 20 seconds and centrifuged for 10 minutes. The supernatant was used for analysis.

3. Suspension samples: 100 ul of clear, diluted commerically available (10 ug/ml) suspension was mixed with 100 ul of cefaclor solution. The mixture was vortexed for 10 seconds.

4. Patient serum samples: 100 ul of serum was mixed with 100 ul of 6% perchloric acid and 100 ul cefaclor solution. The mixture was vortexed for 20 seconds and centrifuged for 10 minutes. The supernatant was used for analysis.

Chromatographic Procedures:

The mobile phase flow rate was maintained at 2 ml/min. The column was equilibrated with mobile phase for 30 minutes before each run. The detector was set at 230 nm. The chart speed was 2.5 mm/min. Samples were injected onto the column by means of the automated sampler which withdrew 50 ul for analysis from each vial.

Results and Discussion

Each chromatographic run required approximately 12 minutes. Cefadroxil eluted at about 4 minutes and the internal standard at 10 minutes (Figure 1.I). A typical chromatogram of cefadroxil and the internal standard in serum is shown in Figure 1.II.

Linearity was determined by linear regression analysis of the data. The r values were 0.996 and 0.999 for standard curves of aqueous and serum specimens, respectively. The limit of detection was 0.5 mcg/ml. The interday and intraday coefficient of variation was <4.7%.

The analytical method was found to be simple, accurate, sensitive and precise for the measurement of cefadroxil. The method can be utilized for cefadroxil measurement in either aqueous solutions or serum.

Applications

The chemical stability of cefadroxil was studied in six commercially available bottles after reconstituting the drug, as recommended by the manufacturer. The potency of >95% of the original concentration was retained for six weeks at 4^0C. This is an important finding for patients, including those with osteomyelitis, who normally require 4 to 6 weeks

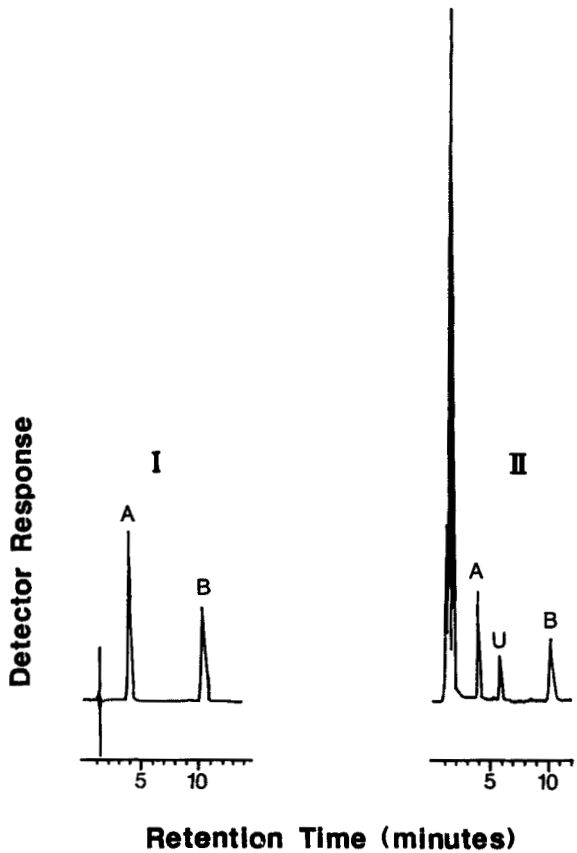

Figure 1.I. Chromatograms of cefadroxil (A) and internal standard cefaclor (B) in a suspension of cefadroxil;

II. Chromatograms of cefadroxil (A) and cefaclor (B) in a serum specimen. An unknown peak (U) was present in blank and patient's serum.

of oral antibiotics. It is possible that cefadroxil could be prepared for the complete course of therapy for a patient and thus, avoid the need for return visits to the pharmacy. It may also be possible to reconstitute and store cefadroxil in the department of pharmacy without loss of potency. This may reduce pharmacy time spent in reconstitution for each individual cefadroxil prescription.

One patient (age 22 months, weight 10 kg) with osteomyelitis was studied after cefadroxil oral dose of 60 mg/kg every 12 hours. Blood samples (0.50 ml) were collected, just prior to the dose (0 hr), and at 1, 2, 6, and 12 hours after the dose. The peak serum concentration (Cmax) of cefadroxil in this patient was 35.4 mcg/ml at a 60 mg/kg dose, compared with a previously reported peak concentration of 11.0 ug/ml at the dose of 15 mg/kg and 7.4 mcg/ml at 10 mg/kg dose.[2] The peak in our patient occurred at 1 hour after the dose, as was previously reported.[2] The lowest serum concentration (Cmin) in our patient was 0.5 mcg/ml, and it occurred at the end of 12-hour dosage interval. Thus, our HPLC method proved simple, sensitive, accurate and reproducible for the measurement of cefadroxil in the commercially available suspension formulation, as well as in the serum of a pediatric patient. This could be used to conduct the pharmacokinetics and pharmacodynamics studies for the development of optional dosage guidelines of cefadroxil in pediatric patients.

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