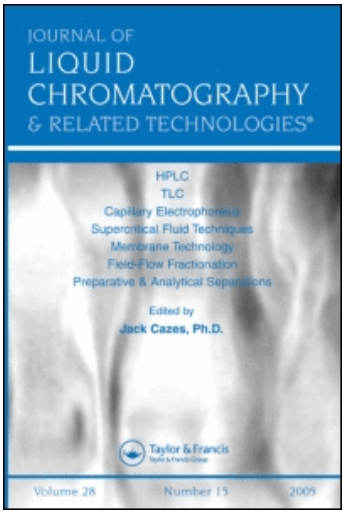

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ION-PAIRED LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFAZOLIN IN CANINE SERUM AND TISSUES

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ABSTRACT

Cefazolin is commonly used as a prophylactic antibiotic in dogs undergoing total hip arthroplasty.

A sensitive high-performance liquid chromatographic method was developed for the determination of cefazolin in canine serum and tissues. The tissues were those in contact with the hip prothesis; namely, the coxofemoral joint capsule, cancellous bone from the acetabulum and bone marrow from the femoral canal.

The method involved filtration of diluted serum or tissue extracted with ethanol:acetonitrile:water (40:10:50) through a 30,000 molecular weight cut-off filter. Separation of cefazolin from other components was by ion-paired (dodecanosulfonate) high performance liquid chromatography using a reversed-phase column eluted with acetonitrile/water solution. The ultraviolet absorbance of the column effluent was monitored at 230 nm. Recovery of cefazolin spiked at 10 μg/ml from serum was 89.8% with a coefficient of variation (CV) of 5.3% (n=10). Recovery of cefazolin spiked at 5 μg/ml from tissue extracts of joint capsule, cancellous bone and bone marrow was: 93.9%, 98.2%, and 104.2% respectively, with a CV of 6.7%, 10.2% and 10.6% respectively (n=10). A correlation coefficient of 0.9999 occurred with cefazolin in aqueous solution (n=5). The limit of detection for cefazolin was approximately 10 ng/ml of serum or tissue extract.

INTRODUCTION

Cefazolin is one of the cephalosporin β-lactam antibiotics commonly used for the treatment of certain infections caused by gram-positive and -negative bacteria (1).

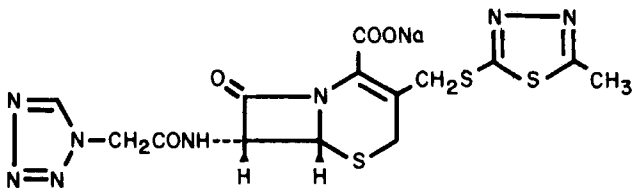
As are all the cepholosporins, cefazolin is produced semisynthetically, with its primary structure (Fig. 1) based on 7-aminocephalosporanic acid. Its use as an intraoperative antibiotic in orthopedic procedures involving prosthetic joint implants is due to its excellent antistaphylococcal activity. The dosing schedule needed to maintain therapeutic concentrations at the site of the implant is not known. Therefore, a method was devised to measure cefazolin in canine patients undergoing total hip arthroplasty.

In recent years, a considerable number of HPLC procedures have been reported for the quantitative determination of cephalosporins in body fluids. (2, review). Among them are a few reports concerning LC determination of cefazolin in serum and plasma (3-5). Analysis of this antibiotic in tissues has not been reported. This paper describes an HPLC method for the determination of cefazolin in serum and from the tissues exposed during a total hip arthoplasty in the canine.

MATERIALS AND METHODS

Apparatus

a) Liquid chromatograph - Model 590 pump, Model 710B Wisp automatic sample injector, Model 730 Data Module recorder/ integrator, Model 721 System Controller, Model 481 variable



CEFAZOLIN SODIUM

FIGURE 1.

wavelength UV detector, column temperature control accessory (Waters Chromatography Division, Milford, MA).

b) LC column - Brownlee Phenyl Spheri-5 MPLC analytical cartridge, 4.6 mm id X 22 cm long, in 22 cm MPLC holder (Sci-Con, Winter Park, FL).

c) Microseparation system - Centricon-30, molecular weight cut-off filter at 30,000 Daltons (Amicon Corp, Danvers, MA).

d) Centrifuge - IEC Centra-7R equipped with an 831 fixed-angle rotor (International Equipment Corp., Nesseham Heights, MA).

e) Ultrasonic Bath, Model 450 (E/MC Corp., Division of RAI Research).

Reagents

a) Solvents - LC grade acetonitrile, methanol, 85% phosphoric acid and trielthylamine (Fisher Scientific Co., Raleigh, NC).

b) Ion Pair Reagent - Dodecanesulfonate, 0.5 M solution. (Bodman Chemicals, Media, PA).

c) LC grade water - Obtained from Model 1000 Hydro Ultrapure Water System (Hydro Services and Supplies, Research Triangle Park, NC).

d) Cefazolin Standard - The sodium salt of cefazolin was used as the standard. (Sigma Chemical Co., St. Louis, MO). Stock solutions (1 mg/ml) were made in 40% ethanol and stored for no longer than 2-3 days at - 20°C. Working standards were prepared daily.

e) Mobile phase - In a 1 L graduated cylinder: 150 ml acetonitrile, 20 ml methanol, 10 ml dodecanasulfonate (0.5 M), 5 ml triethylamine, 5 ml 85% phosphoric acid and LC grade water added to a total volume of 1 L. The mobile phase was deareated under vacuum before use.

Sample Preparation

A. Serum

Serum samples (0.5 ml) were diluted with 0.5 ml of a mixture of ethanol, acetonitrile and water (40:10:50) in the micro-separation system and Vortexed for 15 seconds. Samples were centrifuged for 30 min. at 3500 rpm in a fixed-angle rotor. The colorless filtrate was transfered to LC vials.

B. Tissues

Tissue samples were minced using a # 10 Bard-Parker scalple blade and carefully weighed. A mixture of ethanol, acetontrile and water (40:10:50) was added to 0.1-03. g of minced tissue to a total volume of 1 ml and ultrasonicated for 1 hour. The extract was then transfered to a microseparation system and centrifuged for 30 min at 3500 rpm in fixed-angle rotor. The colorless filtrate was transfered to LC vials.

LIQUID CHROMATOGRAPHIC DETERMINATION

The mobile phase flow rate was 0.8 ml/min. The UV detector was set at 0.01 absorbance units full scale (AUFS) for tissues and 0.05 for serum samples. Absorption wavelength was set for 230 nm, with a time constant at 1.0 and the column temperature maintained at 50°C. Integration parameters for the Waters Data Module were peak width = 30 and noise rejection = 10. A 10 µl injection volume of the standard solution at the desired concentration was used to calibrate using the single-point calibration system. Standard curves were based on peak area.

10 µl of tissue extract or 5 µl of serum filtrate were injected and quantitated using the calibration system.

RESULTS AND DISCUSSION

Previous works from this laboratory (6-8) have shown the usefulness of the microseparation system in removing macromolecules and eliminating the need for time-consuming solvent extraction procedures for quantitative analysis of penicillins and tetracyclines by HPLC. This method of purification has also been shown to be applicable for the analysis of several cephalosporins, including cefazolin, in serum and tissues.

Based on previous studies with other β - lactam antibiotics, the Spheri-5 Phenyl column was determined to be optimum for separation and was therefore chosen for use in this system.

Several elution systems consisting of two ion-pair reagents were investigated: octanesulfonate and dodecanesulfonate and different amounts of acetonitrile, methanol and phosphoric acid.

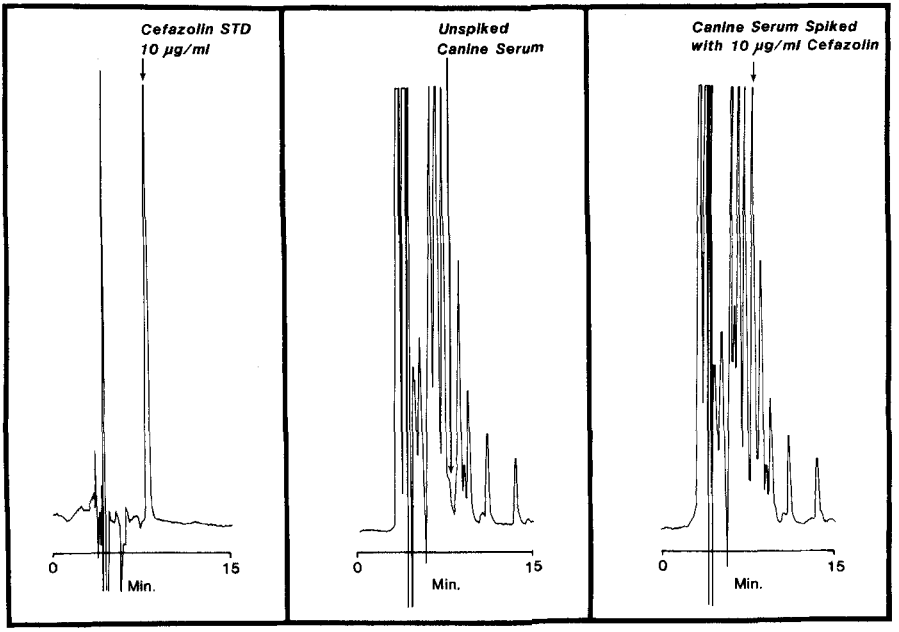


FIGURE 2. Reversed-Phase Chromatograms Obtained from 10 µg/ml Cefazolin Standard Solution, Unspiked and Spiked Canine Serum. Injection Volumes were 10 µl.

An optimal separation of cefazolin from endogenous constituents present in serum and tissue extracts was obtained with a reversed mobile phase consisting of acetonitrile-methanol-phosphoric acid-water (15:2:0.5:82.5 v/v) in 0.005 M dodecanosulfonate solution with pH adjusted to 2.8 with triethylamine.

Cefazolin, like many β-lactam antibiotics, exhibits significant binding to serum proteins (84%) (9). Several solutions were tested for their ability to free cefazolin from serum protein binding. For our purpose, the ethanol, acetonitrile and water

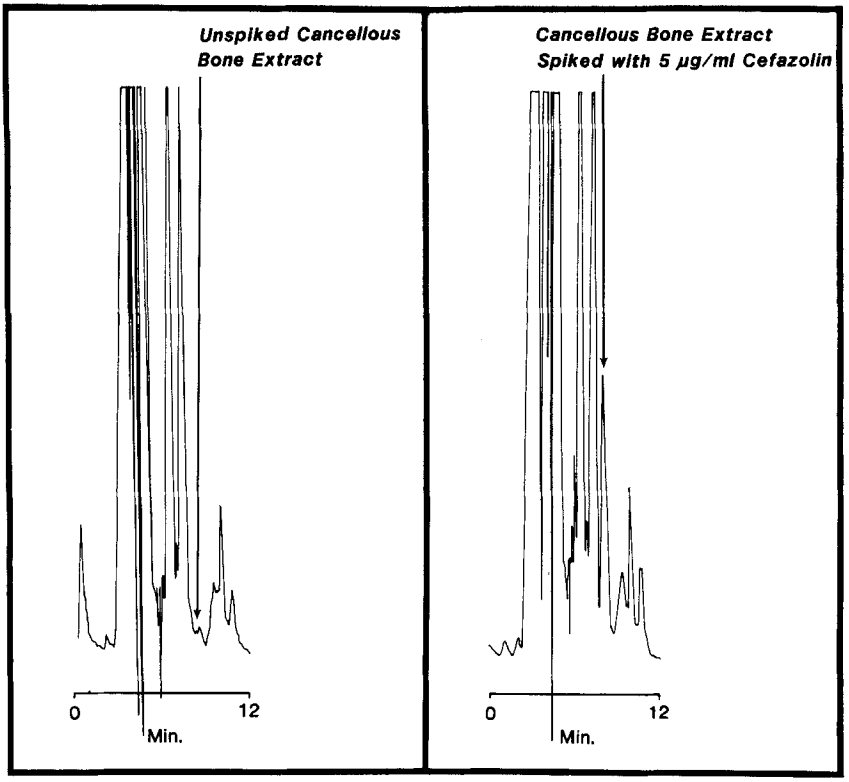


FIGURE 3. Reversed-Phase Chromatograms Obtained from Unspiked and Spiked Cancellous Bone Extracts.

(40:10:50) solution was chosen. We assumed that the same solution would be applicable as an extractor of cefazolin from tissues as well.

Cefazolin has 2 absorption maximas in the working mobile phase, 230 nm and 272 nm. The 230 nm wavelength was selected due to the greater sensitivity (2-3 fold) seen at this wavelength compared to the other.

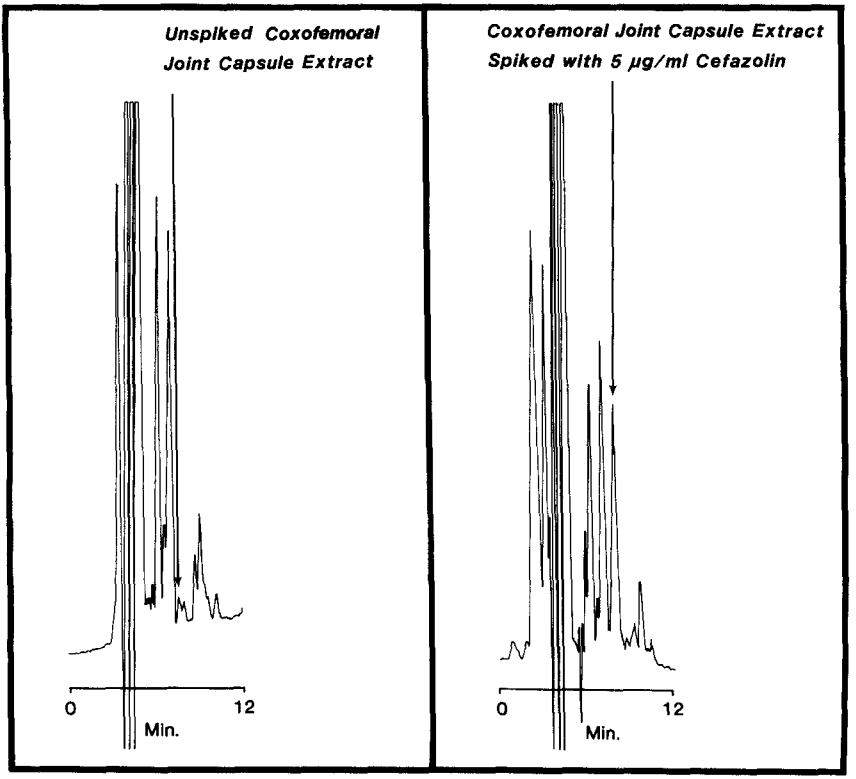


FIGURE 4. Reversed-Phase Chromatograms Obtained from Unspiked and Spiked Coxofemoral Joint Capsule Extracts.

Cefazolin has a retention time just under 8 minutes for the system described. The response for 5 cefazolin standard solutions was plotted vs concentration with a resulting correlation coefficient of 0.9999. The range of detection was linear to 300 µg/ml for tissue extracts and 1000 µg/ml for serum samples. Typical chromatograms obtained from 10 µg/ml cefazolin standard solution and canine serum are shown in Figure 2. The comparison of a serum

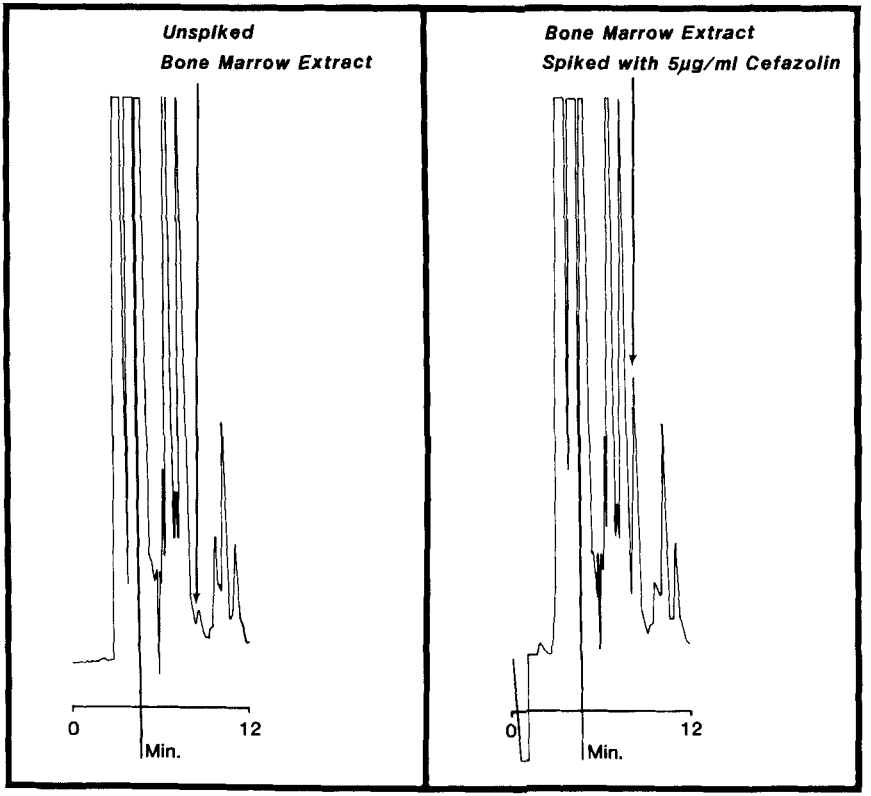


FIGURE 5. Reversed-Phase Chromatograms Obtained from Unspiked and Spiked Bone Marrow Extracts.

spiked with 10 µg/ml cefazolin to that of a serum blank indicates that the analyte was almost completely separated from endogenous substances, with a coefficient of variation of 5.3% and a recovery of 89.8% (n=10), (Table 1). Figure 3 shows chromatograms of blank and spiked (5 µg/ml) extracts of cancellous bone from a canine acetabulum. The chromatogram of the blank extract shows the analytical window for the cefazolin peak, which is baseline-

TABLE 1.

Cefazolin in Canine Serum and Tissues. Statistics.

	Serum	Coxofemoral Joint Capsule	Cancellous Bone	Bone Marrow
Amount Spiked (μg/ml)*	10	5	5	5
Number of Reps (n)	10	10	10	10
Range	8.00-9.43	4.25-5.17	4.41-6.07	4.41-5.52
Mean ($\bar{x}$)	8.98	4.69	4.91	5.21
Standard Deviation (SD)	0.4762	0.3137	0.5036	0.5535
Coefficient of Variation (CV)	5.3	6.7	10.2	10.6
Recovery (%)	89.8	98.8	98.2	104.2

* For tissues: 1 ml extract contained 0.1 to 0.3 g tissue.

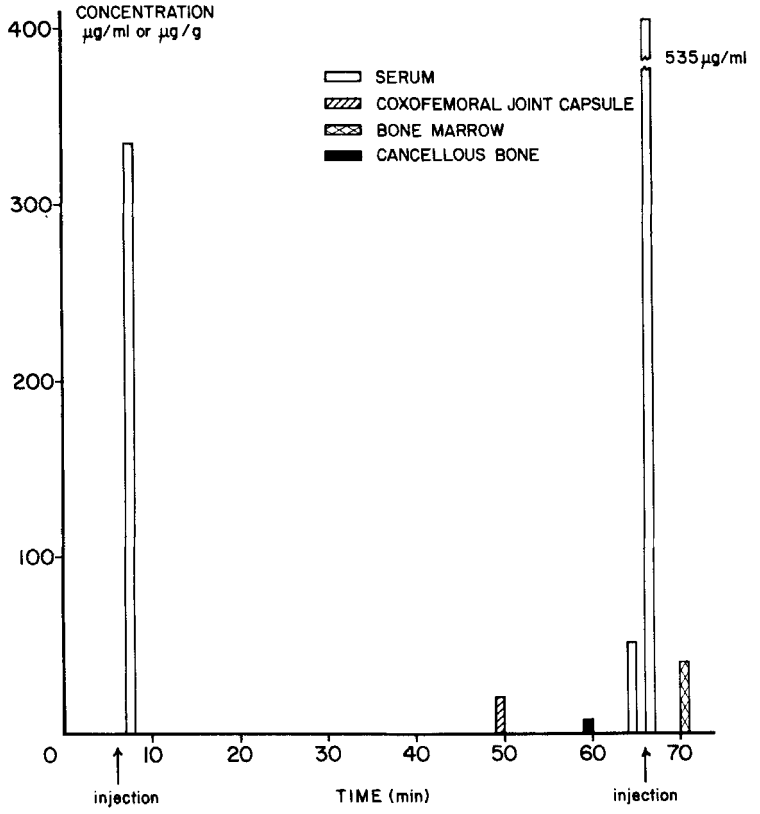


FIGURE 6. Cefazolin Concentration in Canine Tissues During Total Hip Surgery Arthoplasty.

resolved, on the chromatogram of a spiked extract. Figures 4 and 5 show chromatograms of blank and spiked (5 µg/ml) cefazolin extracts of bone marrow from the femoral canal and coxofemoral joint capsule. The blank extract gave a baseline that was virtually free of interfering peaks for both tissue extracts.

Recovery of cefazolin spiked at 5 μg/ml from tissue extracts of joint capsule, cancellous bone and bone marrow was: 93.9%, 98.2% and 104.2% respectively with CV of 6.7%, 10.2% and 10.6% respectively (Table 1). The limit of detection, defined as a peak at least 3 times the height of baseline noise, was 10 ng/ml serum or tissue extract. Figure 6 shows the cefazolin concentration overtime in canine tissues during a total hip arthoplasty. The concentrations assayed in tissues are above those considered of therapeutic importance (>0.1 μg/ml).

In conclusion, the described method for determination of cefazolin in serum and tissues was simple, sensitive, precise and reliable.

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