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Short communication

# Determination of cefoxitin in serum and tissue

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**Abstract**

A simple, rapid and sensitive method for the clean-up and analysis of cefoxitin in serum and tissue is described. Serum (0.5 ml) and tissue (100 mg) samples after homogenization underwent high speed centrifugation. Chromatography was performed on a μBondapak $C_{18}$ cartridge using a mobile phase of 0.005 *M* potassium dihydrogen phosphate–acetonitrile–glacial acetic acid (77.5:22:0.5, v/v/v) with a flow-rate of 2.0 ml/min. Ultraviolet detection occurred at 235 nm. The procedure produced a linear curve for the concentration range 100–5000 ng/ml. The assay produced accurate, repeatable and rapid results for both tissue and serum samples without the need for chemcial extraction. © 1998 Elsevier Science B.V.

*Keywords:* Cefoxitin

## 1. Introduction

Cefoxitin (Fig. 1) is classified as a semisynthetic second generation cephalosporin [1]. It is derived by a chemical modification of the naturally-occurring cephamycin, which gives cefoxitin its high degree of resistance to inactivation by bacterial cephalosporinases. Cefoxitin has been proven to be effective against such bacteria as: *Serratis marcescens*, *Bacteroides fragilis* and *Proteus morganii* [2,3].

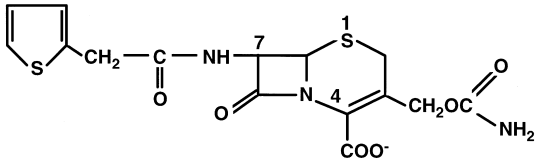


Fig. 1. Chemical structure of cefoxitin.

Because of its effectiveness against many aerobic and anaerobic bacterial infections, cefoxitin is widely used in human and veterinary surgery to aid in the prevention of post-operative infections [4]. It remains a mainstay of prophylactic therapy in surgeries dealing with infections of mild to moderate severity [5] and is part of the treatment regimen recommended by the Centers for Disease Control and Prevention (CDC) for the treatment of women with pelvic inflammatory disease (PID) [6]. Techniques for measurement of cefoxitin in blood and tissues are necessary for determination of antibiotic dosages and dosing rates that maintain effective minimum inhibitory concentrations (MICs).

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In the past decade, many high-performance liquid chromatography (HPLC) methods have been reported for the determination of cefoxitin in plasma, serum and tissue. The procedures used to extract cefoxitin from serum/plasma and tissue use chemicals such as trichloroacetic acid [3,7,8], chloroform–pentanol [9], acetonitrile [10] and methanol [1]. The present article describes a rapid and efficient HPLC procedure for analysis of cefoxitin in plasma, serum, or tissue samples using high speed centrifugation.

## 2. Experimental

### 2.1. Reagents and standards

Methanol and acetonitrile were ''HPLC'' grade (Burdick and Jackson Laboratories, Muskegon, MI, USA), potassium dihydrogen phosphate (Sigma Chemical St. Louis, MO, USA) and glacial acetic acid (Mallinckrodt, Paris, KY, USA) were reagent grade.

Cefoxitin was a gift from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ, USA) and cephalothin was purchased from the United States Pharmacopeia Convention, (Rockville, MD, USA).

Stock standard solutions of cefoxitin (25 and 1 μg/ml) in water were prepared and stored at 4°C. The solutions were confirmed to be stable for 6 months. Working standards were prepared fresh daily by dilution of stock standards. A stock standard solution of cephalothin (25 μg/ml, internal standard) in water was prepared and stored at 4°C. This solution was also confirmed to be stable for six months.

### 2.2. Apparatus

The analytical system consisted of a model 600 solvent delivery system, a model 712 WISP autosampler, a 100×8 mm μBondapak $C_{18}$ cartridge (10 μm particle size) and a μBondapak $C_{18}$ Guard-Pak precolumn insert, a model 481 absorbence detector and a computer equipped with 820 Maxima software (Waters, Milford, MA, USA).

### 2.3. Chromatography

The mobile phase was an isocratic mixture of 0.005 *M* potassium dihydrogen phosphate–acetonitrile–glacial acetic acid (77.5:22:0.5, v/v/v). It was prepared using double-distilled, deionized water, filtered (0.22 μm) and degassed before use. The flow-rate was 2.0 ml/min, column temperature was ambient and UV absorbence was measured at 235 nm. AUFS was set at 0.005 and response time was 1.

### 2.4. Serum extraction procedure

Previously frozen serum samples were thawed and vortexed before use. The sample (0.5 ml) was placed in a test tube (100×13 mm) and 25 μl of cephalothin (25 μg/ml), the internal standard, was added. The sample was vortex-mixed and placed in an ultrafree MC filter unit with a 10 000 MW cutoff (Millipore, Bedford, MA, USA). The sample was centrifuged for 30 min at 16 000 *g* and a 180 μl aliquot of the filtrate was injected onto the liquid chromatograph.

### 2.5. Tissue extraction procedure

The tissue (100 mg) was placed in a 30 ml corex test tube and homogenized in 1 ml of water using a polytron homogenizer (Brinkman Instruments, Westbury, NY, USA). The internal standard (25 μl of a 25 μg/ml solution) was added to the homogenate and vortexed. Samples were placed in the centrifuge for 15 min at 1000 *g*. The supernatant was then filtered through a Acrodisc CR PTFE 0.2 μm filter (Gelman Sciences, Ann Arbor, MI USA), prewet with water and methanol. A volume of 180 μl of the filtrate was then injected onto the liquid chromatograph.

### 2.6. Determination of recovery and precision

Analytical recovery experiments were performed by comparing serum or tissue samples spiked with cefoxitin to water standards similarly prepared. Precision was determined by performing replicate analyses of either four tissue or serum samples spiked with a known concentration of the compound. Standard curves were obtained by plotting the added drug concentration (ng/ml or ng/mg) vs. the peak area ratio (drug/internal standard).

## 3. Results

Representative chromatograms of feline serum and tissue (colon) samples following cefoxitin administration (22 mg/kg, intravenously) are shown in Fig. 2b (serum) and Fig. 3b (tissue). The retention times were 8.75 min for cefoxitin and 13.55 min for cephalothin in serum and 8.55 and 13.95 min in tissue, respectively. Endogenous components from blank serum or tissue did not interfere with the elution of cefoxitin or the internal standard (Fig. 2a serum and Fig. 3a tissue).

The method produced a linear curve over the concentration range used in this study with correlation coefficients ranging from 0.994 to 0.998 for tissue and from 0.993 to 0.999 for serum. The mean percentage recoveries ranged between 84 and 96% for tissue and between 79 and 100% for serum. The intra- and inter-assay coefficients of variation in tissue were 8.5 and 8.1%, respectively, at a mean concentration of 350 ng/mg ($n$=4). Corresponding coefficients of variation for serum at a mean concentration of 350 ng/ml were 6.7 and 6.2%, respectively. The limit of cefoxitin detection in tissue was 50 ng/mg, which represents a peak area approximately three times baseline noise, compared to 10 ng/ml for serum, which also represents a peak area approximately three times baseline noise.

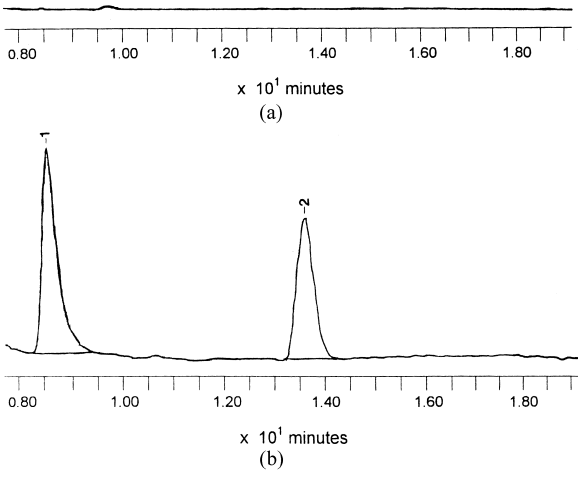


Fig. 2. (a) Chromatogram of blank feline serum – no peaks. (b) Chromatogram of a feline serum sample collected 2 min after administration of 22 mg/kg intravenously. Peak: 1=cefoxitin; 2=cephalothin.

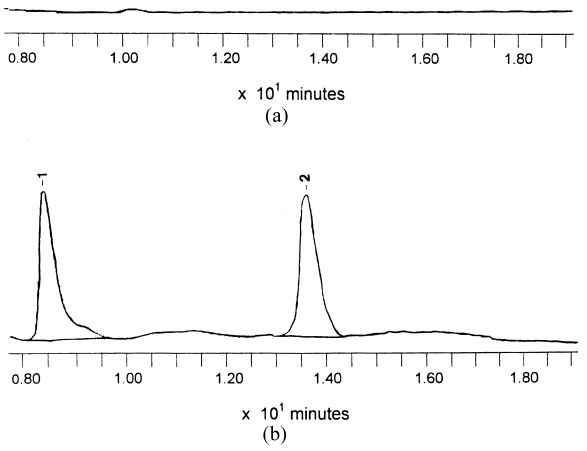


Fig. 3. (a) Chromatogram of blank feline colon – no peaks. (b) Chromatogram of a feline colon tissue sample collected after administration of 22 mg/kg intravenously. Peak: 1=cefoxitin; 2=cephalothin.

## 4. Discussion

The risk of wound infection after abdominal surgery in both humans and animals remains high. Pre-operative antibiotic prophylaxis, especially with cephalosporin derivatives, has been shown to be efficacious in humans [8]. The procedure described in this paper is a rapid, sensitive and reliable method that could be used in determining cefoxitin concentrations in surgery patients.

The detection limits and recoveries for both serum and tissue samples are of equal sensitivity to or better than existing methods for extraction and analysis of cefoxitin. This method should be appropriate for patients and experimental animals administered therapeutic dosages of cefoxitin, since the recommended dose for humans is much higher than the dose for felines. The use of cephalothin as an internal standard corrects for intra- and inter-assay variability in the extraction and chromatography steps.

Peak absorption of cefoxitin occurs at 235 nm. Because regions of strong absorption by proteins lie between 180–220 and 270–290 nm [11], the cefoxitin spectrum overlaps with the sides of these strong absorption bands. Therefore, proteins must be removed from serum samples prior to quantitation of cefoxitin. Previously described procedures used sol-

vents that denatured and precipitated proteins. Wheeler et al. [3], Robbs et al. [8] and Charles and Ravenscroft [7] used trichloroacetic acid, while Brisson and Fourtillan [9] used chloroform–pentanol, Regazzi et al. [1] used methanol and Dresse et al. [10] used acetonitrile. In the present study, filtration was used to remove proteins from serum samples. Cefoxitin was filtered freely through the ultrafree MC filter unit which Hunt et al. [12] and Peelen et al. [13] also used to demonstrate that proteins can be removed from samples using this filter unit prior to instrumental analysis.

The method described here requires that the serum sample undergo high speed centrifugation followed by injection onto the HPLC, while the tissue is homogenized in water, centrifuged, filtered and then injected onto the HPLC. This eliminates the need for organic or aqueous solvents for extraction, thus making the procedure easier and safer to use in a clinical setting. The procedure described here used 0.5 ml of serum and 100 mg of tissue; however, the analysis should be appropriate for reduced volumes. This procedure may also be useful for detection of low protein binding drugs with similar structures such as cefotaxime, ceftizoxime, cephalothin and cefuroxime.

In conclusion, a rapid, sensitive and clinically useful HPLC procedure has been developed for analysis of cefoxitin in plasma or serum and small tissue samples.

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