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Sherry Cox  
*University of Tennessee, Knoxville*

Molly White  
*University of Tennessee, Knoxville*

Kristen Gordon  
*University of Tennessee, Knoxville*

Joan Bailey  
*University of Tennessee, Knoxville*

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Determination of Ceftiofur and its Metabolites in Plasma Using Reverse Phase Liquid Chromatography

Sherry Cox, Molly White, Kristen Gordon, Joan Bailey, University of Tennessee College of Veterinary Medicine, Department of Biomedical & Diagnostic Sciences, Knoxville, TN 37996

ABSTRACT
A high performance liquid chromatography procedure for the determination of ceftiofur and all its desfuroylceftiofur metabolites in plasma has been developed and validated using reverse phase liquid chromatography. Following a derivatization method that converts ceftiofur and all desfuroylceftiofur metabolites to desfuroylceftiofur acetamide, separation was attained on a Symmetry C18 column and quantification occurred using UV detection at 265 nm. The mobile phase was a mixture of (A) 0.1% trifluoracetic acid (TFA) in water and (B) 0.1% TFA in acetonitrile. The mixture was pumped at a starting gradient of 90% A and 10% B and was adjusted to 75% A and 25% B over 25 min, and back to initial conditions over 3 min. With a flow-rate of 1.0 ml/min. The procedure produced a linear curve over the concentration range of 0.1 – 100 µg/mL with a lower limit of quantification of 0.1 µg/mL. Intra-assay variability ranged from 0.7 to 8.8% for desfuroylceftiofur acetamide, respectively, and the average recovery was 99%. This method could be useful to those investigators dealing with small sample volumes, particularly when conducting pharmacokinetics studies which require multiple sampling from the same animal.

INTRODUCTION
Ceftiofur is a broad spectrum, third-generation cephalosporin antibiotic used in a variety of gram-positive, gram-negative, and anaerobic infections in domestic animal species including many species of Pasterurella spp., Streptococcus spp., Staphylococcus spp., Salmonella spp., and Escherichia coli. Ceftiofur is cleaved into furonic acid and desfuroylceftiofur, an active metabolite. Like most cephalosporins, its is bactericidal and acts by inhibiting cell wall synthesis. The sodium salt of ceftiofur is approved for use in the US for a variety of domestic species including cattle, swine, sheep, goats, horses, dogs, and poultry. The hydrochloride salt of ceftiofur is approved for use in cattle and swine. Ceftiofur crystalline-free acid (CCFA, Excede) is a long-acting preparation of ceftiofur labeled for use as a single dose in cattle and swine for treatment of certain bacterial pathogens. CCFA is also labeled for treatment of lower respiratory infections in horses and is used off label in many non-domestic species, including birds kept as companion animals or in zoological collections.

MATERIALS AND METHODS
Reagents and Standards
Ceftiofur and cefotaxime were purchased from U.S. Pharmacopeia. All other reagent grade chemicals and solvents were purchased from Fisher Scientific. Stock solutions of ceftiofur and cefotaxime (100 µg/ml) were prepared in methanol. Dilutions in methanol were prepared to produce 1 and 10 µg/ml working stocks. The standards were stored at 4°C and were stable for a minimum of 6 months. For preparation of calibration standards and quality control samples, appropriate aliquots of the working stock solutions were added to untreated plasma. The final concentrations were 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 µg/ml for calibration standards and 0.35, 3.5, 35 and 75 µg/ml quality control samples. Calibration standards and control samples were treated the same as test samples. Linearity was assessed by linear regression analysis. The calibration curve had to have a correlation coefficient of 0.98 or higher. The acceptance criterion for each back calculated standard concentration was 15% deviation from the nominal value except lower limit of quantification (LOQ), which was set at 20µg/mL.

RESULTS AND DISCUSSION
Blank plasma samples were prepared in the same manner as study samples and no endogenous plasma components interfered with the elution of the peaks of interest. The plasma peak ratio (area of Ceftiofur divided by the IS area) versus the concentration was plotted and produced a linear curve, over the concentration range of 0.1 – 100 µg/ml, with resulting correlation coefficients of >0.999. A typical equation for the calibration curve was y = 0.039x + 0.660, where y represents the peak area ratios of ceftiofur to internal standard and x represents the plasma concentration of ceftiofur in µg/ml. Intra and inter-assay RSD for plasma spiked with specific concentrations of ceftiofur ranges from 0.7 to 7.7% (Tables 1 and 2). The precision was below the set ± 15% for all quality control samples. The recovery (Table 2) for ceftiofur from spiked plasma was determined by comparing extracted areas with directly injected analyte areas and ranged from 95% to 104%. The relative standard deviation was less than 10% for all values. The recovery of cefotaxime was 98% and its relative standard deviation was 3.3%. The addition of cefotaxime as an internal standard corrects for intra- and inter-day assay variability in the assay. The lower limit of quantification (LOQ) was 0.1 µg/mL and represents a peak approximately five times baseline noise.

CONCLUSIONS
In conclusion, this analytical procedure was validated in terms of recovery, linearity, LOD, precision, and accuracy. The limit of quantification and recovery are more than adequate for use in pharmacokinetic studies. Our results indicate that this HPLC procedure is a reproducible method that provides consistent quantification of ceftiofur and all its desfuroylceftiofur metabolites in plasma. The addition of cefotaxime as an internal standard also allows for the correction of intra- and inter-assay variability. This method has been used successfully to determine ceftiofur concentrations in plasma samples at this institution.