DETECTION, QUANTIFICATION AND PHARMACOKINETICS OF FUROSEMIDE (SALIX®) FOLLOWING INTRAVENOUS ADMINISTRATIONS IN HORSES[§]

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Furosemide (Salix®) is a potent loop diuretic commonly used in North America for prevention of Exercise-Induced Pulmonary Hemorrhage (EIPH) in horses. Since furosemide is a diuretic, it may interfere with detection of other drugs or drug metabolites by urinary dilution, thereby reducing their urinary concentrations. The regulation of furosemide in many racing jurisdictions is based on a threshold concentration of 100 ng/ml in blood samples obtained between 4 and 5.5 hours following intravenous administration of the drug. In this study, we developed a sensitive LC-MS/MS method with solid phase extraction (SPE) to determine the pharmacokinetic parameters and "detection times" for furosemide in equine serum samples after 250 mg intravenous administration to help regulatory agencies control this agent in racing horses.

Modification of existing SPE methodologies was carried out. Briefly, this consisted of conditioning Supelco DSC-18 Discovery columns (500 mg bed volume) with methanol, water, and 1.5% (v/v) phosphoric acid in water, application of phosphoric acid-acidified serum samples, cleaning the columns with 0.5 M citric acid and water and elution with ethyl acetate:dichloromethane:HCl (conc.), 70:29:1. Dried eluents were resuspended in a mobile phase combination consisting of 35%A:65%B, where A was acetonitrile, B was deionized water containing 1% triethylamine and 5% acetonitrile. Chromatography involved 0.15 mL/min isocratic elution with the 35%A:65%B mobile phase using a 50 x 1.0 mm x 3 micron phenyl-hexyl column (Phenomenex Luna). Detection involved negative mode electrospray with measurement of negative ions as follows: those fragments arising from m/z 329.3 $(M-H)^-$ and m/z 330.3 were specific to furosemide, whereas those arising from 334.3 $(M-H)^-$ and m/z 336.3 were specific to the furosemide-d5 (internal standard), synthesized in house. The standard curve was linear from 1 to 50 ng/ml, with an *r*-value of 0.996.

Furosemide was administered intravenously at 250 mg to 10 thoroughbred mares and post-administration serum and urine samples were collected at various time points. An appropriate model was selected to determine pharmacokinetic parameters of furosemide. Statistical analyses were done by applying the central limit theorem of statistics to a sampling distribution of the sample mean in a *t*-test distribution with the single assumption that the low sample number is representative of the population in a binomial distribution to estimate the probability for exceeding a threshold concentration of furosemide (100 ng/ml) used for the regulation of this compound in horses. We also established the distribution of specific gravities of the urine samples at various post-administration times.

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