Detection, Quantification, and Pharmacokinetics of Furosemide and Its Effects on Urinary Specific Gravity Following IV Administration to Horses*

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ABSTRACT

Furosemide is a potent loop diuretic used for the prevention of exercise-induced pulmonary hemorrhage in horses. This drug may interfere with the detection of other substances by reducing urinary concentrations, so its use is strictly regulated. The regulation of furosemide in many racing jurisdictions is based on paired limits of urinary SG (<1.010) and serum furosemide concentrations (>100 ng/ml). To validate this regulatory mechanism, a liquid chromatography/mass spectrometry/mass spectrometry method employing a solid-phase extraction procedure and furosemide-d₅ as an internal standard was developed. The method was used to determine the pharmacokinetic pa-

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parameters of furosemide in equine serum samples and its effects on urinary SG after IV administration (250 mg) to 10 horses. Pharmacokinetic analysis showed that serum concentrations of furosemide were well described by a two-compartmental open model. Based on results in this study, it is very unlikely for horses to have serum furosemide concentrations greater than 100 ng/ml or urine SG less than 1.010 at 4 hours after administration (250 mg IV). However, it should be remembered that urine SG is a highly variable measurement in horses, and even without furosemide administration, some horses might naturally have urine SG values less than 1.010.

INTRODUCTION
Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid [Salix, Intervet]) is a potent organic acid loop diuretic used in North America for both control and prevention of exercise-induced pulmonary hemorrhage (EIPH) in horses. Loop diuretics inhibit the active reabsorption of chloride ions in the thick ascending loop of Henle by binding to one of the chloride ion binding sites of the sodium, chloride,
TABLE 1. Multiple Reaction Monitoring Data Acquired by Mass Spectrometry (Negative-Mode Electrospray Ionization) of Furosemide

<table>
<thead>
<tr>
<th>Parent (m/z)</th>
<th>Daughter (m/z)</th>
<th>Dwell (sec)</th>
<th>Cone (V)</th>
<th>Collision Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>329.30</td>
<td>285.00</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>329.30</td>
<td>204.70</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>329.30</td>
<td>77.60</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
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<tr>
<td>330.30</td>
<td>205.80</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>330.30</td>
<td>204.90</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>334.30</td>
<td>290.00</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>334.30</td>
<td>205.80</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>336.30</td>
<td>291.80</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
<tr>
<td>336.30</td>
<td>207.80</td>
<td>0.02</td>
<td>29.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

The regulatory thresholds incorporated into these rules are based on pharmacokinetic studies first reported from this group about 20 years ago and collateral forensic experience. To more accurately define these thresholds, a highly specific and sensitive quantitative analytical method based on liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) for furosemide was developed. A pilot study was performed to redefine, under laboratory conditions, the pharmacokinetics of furosemide in horses and to establish the relationships between serum furosemide concentration and urine SG approximately 4 hours after IV administration of furosemide.

In developing the analytical method, a deuterated analog of furosemide was synthesized to serve as an internal standard, and a solid-phase extraction (SPE) method and a highly sensitive quantitative LC-MS/MS method were developed for furosemide. Following furosemide administration, the pharmacokinetics and the relationship between dose, time after dosing, and serum concentrations of furosemide and urine SG were studied.
MATERIALS AND METHODS

Horses and Sample Collection

Ten mature thoroughbred mares weighing between 542 and 572 kg were used for the study. All horses were acclimated to their stalls 24 hours before initiation of the study. During acclimation and while on study, the animals were given grass hay and water free choice and concentrate feed, which was a 50:50 mixture of oats and an alfalfa-based protein pellet containing 12% protein, was fed twice daily. The animals were vaccinated annually for tetanus and dewormed quarterly with ivermectin. The last treatment with ivermectin was given 2 months prior to this study. A routine clinical examination was performed before each study to assure that these animals were healthy and sound. All animal care was in compliance with the guidelines issued by the Division of Laboratory Animal Resources and approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Horses were provided with approximately 13.2 L of water per day in their stalls during testing. Daily water consumption was not measured during testing, but the buckets were not refilled during the day. Therefore, 13.2 L was the maximum amount of water they could consume daily.

Furosemide was administered as a single IV injection (250 mg) into the right jugular vein. Blood samples were collected from the opposite vein into serum tubes for analyses at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, and 24 hours and were centrifuged at 4°C 800 × g (2000 rpm) for 15 minutes. Separated serum samples were stored at −20°C until assayed. During the first day, complete urine collection was accomplished with a Foley catheter at 0, 1, 2, 3, 4, 5, and 6 hours after drug administration.

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Measured concentration (ng/ml)*</th>
<th>Accuracy</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within-run accuracy and precision</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.31 ± 0.58</td>
<td>93.12 ± 5.81%</td>
<td>15.30%</td>
</tr>
<tr>
<td>100</td>
<td>98.63 ± 1.46</td>
<td>98.63 ± 1.46%</td>
<td>3.63%</td>
</tr>
<tr>
<td>400</td>
<td>397.83 ± 2.69</td>
<td>99.13 ± 0.56%</td>
<td>1.42%</td>
</tr>
<tr>
<td><strong>Between-run accuracy and precision</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.32 ± 0.53</td>
<td>100.32 ± 5.32%</td>
<td>12.86%</td>
</tr>
<tr>
<td>100</td>
<td>103.00 ± 30.00</td>
<td>103.00 ± 3.00%</td>
<td>7.20%</td>
</tr>
<tr>
<td>400</td>
<td>415.00 ± 5.78</td>
<td>104.00 ± 1.48%</td>
<td>3.54%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Extraction efficiency*†</th>
<th>Expanded Uncertainty</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>96 ± 4.1%</td>
<td>25.0%</td>
<td>10.03%</td>
</tr>
<tr>
<td>100</td>
<td>100.8 ± 1.85%</td>
<td>11.4%</td>
<td>4.54%</td>
</tr>
<tr>
<td>250</td>
<td>100.4 ± 1.53%</td>
<td>9.4%</td>
<td>3.75%</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM; n = 6.
†The mean ± the expanded uncertainty equals the 95% confidence range.
hour urine sample was collected with a Harris flush tube (24 Fr × 152.4 cm). Urine was divided into appropriate aliquots (125 ml) and stored at –20˚C until assayed.

SG measurements for all urine samples were determined with a National refractometer (American Optical, Scientific Instrument Division) calibrated to 1.000 with deionized water. The SG of each sample was quantified twice, and between each measurement, the refractometer reading was checked with deionized water to ensure that no change in the control reading had occurred.

Synthesis of Furosemide-d\textsubscript{5} Internal Standard

The synthesis of furosemide-d\textsubscript{5} is based on the method published in the patent literature (Figure 1).\textsuperscript{7} Furosemide was prepared from 4-chloro-2-fluorobenzoic acid by chlorosulfonation and ammonolysis to the corresponding sulfonamide, followed by reaction with furfurylamine.\textsuperscript{8} Later work by Sturm et al\textsuperscript{9} showed a similar, but more practical route using 2,4-dichlorobenzoic acid instead of 4-chloro-2-fluorobenzoic acid.

Sample Preparation

Standard solutions of furosemide (Sigma), found by high-pressure liquid chromatography to have purity greater than 99%, and furosemide-d\textsubscript{5} (internal standard) were prepared in methanol. Extraction standards were prepared by the addition of a known volume of a furosemide solution (0, 5, 25, 50, 100, 250, or 500 ng/ml) to blank serum samples, which were pooled samples collected from untreated horses. A known volume of a furosemide-d\textsubscript{5} standard (10 µl of 10 µg/ml in methanol) was added to each sample, standard, and blank serum sample as an internal standard. The serum standards and blanks (1 ml/sample) were placed in culture tubes. The serum samples were acidified with 1 ml of 3% phosphoric acid in water.

Extraction Procedure

The extraction procedure chosen was based on established SPE methodology. Clean DSC-18 Discovery (Supelco) columns (500 mg bed volume) were conditioned by washing sequentially with 1 ml of methanol, 1 ml of deionized water, and 1 ml of 1.5% (w/v) phosphoric acid. The columns were prevented from drying before applying specimens. Acidified serum samples (1 ml serum and 1 ml 3% phosphoric acid in water) were applied to the preconditioned
columns. The columns were then washed consecutively with 1 ml of acetic acid solution (1 M) and 1 ml deionized water. The columns were eluted with 2 ml of ethylacetate (65%)/dichloromethane (29%)/isopropanol (5%)/hydrochloric acid (1%) (concentrate). The eluent was evaporated to dryness under a stream of nitrogen at room temperature (39°C) and the dried eluents were dissolved in 75 µl of mobile phase consisting of 35% acetonitrile and 65% deionized water containing 1% triethylamine and 5% acetonitrile.

Instrumentation

A Hewlett-Packard Model 1050 LC attached to a Micromass VG Quattro-2 MS/MS in ESI tandem mode was used with a 50 mm × 1.0 mm × 3 µm phenyl-hexyl column (Phenomenex Luna). Each extract sample was transferred to an autosampler vial. A 7-µl
 aliquot was injected into the LC-MS/MS. Detection involved negative-mode electrospray ionization (Table 1).

Fragments arising from \( m/z \) 329.3 and 330.3 are specific to furosemide, whereas those arising from \( m/z \) 334.3 and 336.3 are specific to the internal standard furosemide-\( d_5 \). Quantitation was performed by calculating the ratio of the furosemide \( m/z \) 329.3→204.7 response relative to the internal standard \( m/z \) 334.3→205.8 transition.

**Method Validation**

Linearity of the method was verified from the coefficient of determination \( (r^2) \) of the standard curves from six consecutive runs (MassLynx 3.4 software). Within-run accuracy and precision were determined by analyzing six replicate spiked samples at each of three concentrations (10, 100, and 400 ng/ml; Table 1). The accuracy and precision between runs were determined by analyzing samples at these three concentrations in six consecutive runs (Table 2).

The lower limit of detection (LOD) was calculated from six consecutive runs. The concentration calculated from the mean of the responses at zero concentration (y-intercept) was determined. The LOD was defined as the concentration calculated from the mean response at zero concentration plus two standard deviations (the upper 95% confidence limit for zero).\(^{10}\) In addition to determining the LOD, an alternate calculation was performed utilizing the analyte’s peak height compared with the baseline noise in the \( m/z \) 329.3→204.7 frag-

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**Figure 5.** Typical calibration curve generated by MassLynx 3.4 software, indicating coefficient of determination \( (r^2) \) of 0.999438.
mentation chromatogram. By this method, the LOD was defined as the lowest concentration of analyte producing a peak greater than or equal to three times the baseline noise of the ion chromatogram.

The lower limit of quantitation (LOQ) was defined as the concentration calculated from the mean of the zero responses plus five times the standard deviation. Extraction efficiencies were determined from six runs at three concentrations (25, 100, and 250 ng/ml) (Table 2). Each of the quantitative validation procedures included a concentration of 100 ng/ml, which is a widely used regulatory serum threshold concentration of furosemide.

Pharmacokinetics Analysis
Pharmacokinetic analyses were performed with a nonlinear regression program (Winnonlin, version 3.1, Pharsight Corporation). The goodness of the fit (R²) was evaluated by the Akaike information criterion, residual plots, and visual inspection. The data were weighted as 1/(y_{pred})², where y_{pred} was the model-predicted concentration at the actual time. Area under the curve (AUC) following IV administration was measured by a linear trapezoidal approximation with extrapolation to infinity, and slope of the terminal portion (β) of the log serum drug concentrations versus time curve was determined by the method of least-squares regression.

The compartmental model used is represented by the following equation:

\[ C_p = A e^{-\alpha t} + B e^{-\beta t} \]

where \( C_p \) is plasma concentration of compound at any time (t), A and B are the Y intercepts associated with distribution and elimination phases, respectively, and \( \alpha \) and \( \beta \) represent the rate constant of distribution and terminal elimination phase, respectively. The rate constant of distribution (\( \alpha \)), and distribution half-life (\( t_{1/2,\alpha} \)) were determined using the method of residuals. The terminal half-life (\( t_{1/2,\beta} \)) was calculated as follows:

\[ t_{1/2,\beta} = \ln 2 / \beta \]

Total body clearance (Cl) was calculated as follows:

\[ Cl = IV \text{ dose} / AUC_{0-\infty} \]

The volume of distribution in central compartment (Vd_c) was determined by dose (IV)/A+B; volume of distribution in terminal elimination phase (Vd_β) was calculated as dose (IV)/AUC_{0-\infty}B; and volume of distribution at steady state (Vd_∞) was calculated as dose (IV) × AUMC_{0-\infty}/(AUC_{0-\infty}).
AUMC is area under the first moment curve and is calculated by the trapezoidal method and extrapolated to infinity.\textsuperscript{11}

$K_{10} (\alpha \beta /K_{21})$ is first-order elimination rate constant, which describes elimination of drug from the central compartment. $K_{12} (\alpha + \beta - k_{21} - K_{10})$, and $K_{21} (B\alpha + A\beta / [A+B])$ are distribution rate constants from central to peripheral and from peripheral to central compartments, respectively. The model was selected by examining various parameters such as correlation coefficients ($R^2$), sum of squares, and Schwarz criteria, and, most importantly, by examining the model predicted data versus the observed data graphically to insure that the selected model was realistic.

**Statistical Analysis**

Statistical analyses were performed 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. This allowed for the estimation of the probability for exceeding a threshold concentration of furosemide (100 ng/ml). For determination of the probability of exceeding specified values of serum furosemide concentration and urinary GG, the SAS statistical program (Version 8.1; SAS Institute) was used. The frequency distributions of the serum furosemide concentrations and the urinary SG concentrations at 4 hours were analyzed for normalcy by using the Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling goodness of fit tests incorporated into this program.

**RESULTS**

The total ion chromatographic peaks of furosemide and furosemide-d\textsubscript{5} were superimposable (Figure 2). Ion chromatograms of transitions specific to furosemide and the ion chromatograms of transitions specific to furosemide-d\textsubscript{5}, respectively, are shown in Figures 3 and 4.

The standard curve for the assay was linear from 5 ng/ml to 500 ng/ml, with a mean $r^2$ of $0.9986 \pm 0.00068$ ($n = 6$) (Figure 5). The accuracy within run ranged between 93% and 99% for spiked samples at three concentrations. The precision was determined by the coefficient of variation (CV) for the assay, which ranged from 1.4% to 15.3% (Table 2). The between-run accuracy ranged between 100% and 104%, while the CV ranged from 3.5 to 12.8% (Table 2).

The extraction efficiency, determined in three different concentrations, ranged between 96% and 100% with an expanded uncertainty range of 9.4% to 25% (Table 2). Uncertainty was determined as described by the American Association of Laboratory Accreditation.\textsuperscript{15}

The LOD for furosemide by the LC-MS/MS method, calculated as suggested by Miller
and Miller, was 1.8 ng/ml, and the LOQ was 3.9 ng/ml. Alternately, the LOD calculated from the signal-to-noise ratio of the 329.3→204.7 m/z fragmentation ion chromatogram was 2.2 ng/ml.

Before furosemide administration (0 hour), urine SG ranged from 1.006 to 1.035 g/ml, with a mean of 1.023 g/ml ± 0.0037 (Figure 6). At 1 hour, SG dropped to 1.0093 g/ml ± 0.00073, and steadily increased thereafter through 6 hours. Statistical analysis indicated that horses have less than an 85% chance at 1 hour and less than a 0.05% chance at 2 to 6 hours after treatment to have a urine SG value less than 1.010 g/ml, with t-values increasing with subsequent time points. This suggests that there is less probability for a horse to have a urine SG less than 1.010 at later time points.

Serum furosemide concentrations declined rapidly after administration, presumably associated with the distribution of furosemide from the central compartment (Figure 7); relevant pharmacokinetic parameters are summarized in Table 3. The mean $t_{1/2\alpha}$ was 0.18 ± 0.004 hours, and the mean $t_{1/2\beta}$ was 1.83 ± 0.134 hours. The $t_{1/2\beta}$ was consistent between horses, ranging from 1.24 to 2.75 hours. The $V_{d\alpha}$ and $V_{d\beta}$ were 0.363 L/kg and 1.48 L/kg, respectively, and the mean $V_{dC}$ was 169 ± 12 ml/kg. The systemic clearance was also closely distributed among the 10 horses, ranging from 0.324 L/kg/hr to 0.712 L/kg/hr, with a mean of 0.556 ± 0.039 L/kg/hr.

Because the current regulatory dose of furosemide is 250 mg administered IV 4 hours before post time, and regulation of the use of furosemide in many racing jurisdictions is based on a threshold concentration of 100 ng/ml in serum, the probability of exceeding the serum threshold was estimated from the distribution curve of serum furosemide concentrations 4 hours after administration. This analysis showed that serum furosemide concentrations 4 hours after administration are log normally distributed among the 10 horses studied, as shown in Figure 8 with results summarized in Table 4. From this analysis, it can be estimated that the probability of exceeding a serum furosemide concentration of 31.8 ng/ml at 4 hours after IV administration of 250 mg furosemide is less than 0.001.

Urine SG values were normally distributed at 0, 1, and 4 hours (Figure 9). The probability of exceeding the threshold of 1.010 4 hours after dosing was estimated from that distribution curve. These analyses also show that the probability of a urine SG value of 1.016 at 4 hours after administration is considerably less than 0.001 (Table 4).

**TABLE 3. Pharmacokinetic Parameters for Furosemide in 10 Horses Following a Single IV Dose (250 mg) of Furosemide**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>567.4 ± 6.58</td>
</tr>
<tr>
<td>$K_{10}$ (/hr)</td>
<td>3.31 ± 0.067</td>
</tr>
<tr>
<td>$\alpha$ (/hr)</td>
<td>3.88 ± 0.074</td>
</tr>
<tr>
<td>$\beta$ (/hr)</td>
<td>0.395 ± 0.0267</td>
</tr>
<tr>
<td>$t_{1/2\alpha}$ (hr)</td>
<td>0.179 ± 0.003</td>
</tr>
<tr>
<td>$t_{1/2\beta}$ (hr)</td>
<td>1.83 ± 0.134</td>
</tr>
<tr>
<td>$Cl_s$ (L/kg/hr)</td>
<td>0.556 ± 0.039</td>
</tr>
<tr>
<td>$V_{dC}$ (L/kg)</td>
<td>168.9 ± 12.01</td>
</tr>
<tr>
<td>$V_{d\beta}$ (L/kg)</td>
<td>1.48 ± 0.151</td>
</tr>
<tr>
<td>$V_{dss}$ (L/kg)</td>
<td>0.363 ± 0.032</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.996 ± 0.0008</td>
</tr>
</tbody>
</table>

$\alpha$ = rate constant of distribution; $\beta$ = slope of the terminal portion of the log serum concentration versus time curve; $Cl_s$ = total body clearance; $K_{10}$ = the elimination rate constant; $R^2$ = goodness of fit; $t_{1/2\alpha}$ = distribution half-life; $t_{1/2\beta}$ = terminal half-life; $V_{dC}$ = volume of distribution in the central compartment; $V_{d\beta}$ = volume of distribution in the terminal state; $V_{dss}$ = volume of distribution at steady state.
DISCUSSION

The original research that resulted in the establishment of the existing serum threshold value for furosemide (commonly 100 ng/ml) was done in the authors’ laboratory some 20 years ago.\(^6,16,17\) In those studies, the kinetic parameters of furosemide, which were best described by a three-compartment, open-body model, were established. In addition, the mean plasma concentration of furosemide 4 hours after IV administration (0.5 mg/kg) was determined to be approximately 9.6 ± 3.1 ng/ml. The population distribution of these plasma furosemide values was log normal, and based on this population distribution, the statistical probability of identifying a plasma concentration greater than 24.6 ng/ml 4 hours after dosing was estimated at less than 0.001. This regulatory threshold or limit was then rounded up to 30 ng/ml, and this value was suggested as the regulatory threshold for furosemide.\(^6\)

Practical aspects of equine forensics resulted in the modification of this regulatory threshold in two ways. First, because the principal regulatory concern with furosemide was its ability to dilute equine urine, determination of SG was incorporated into the process. Based on analytical opinion, a regulatory limit of 1.010 was selected as the screening “cut-off” value. Urine SG screening was rapid and inexpensive; therefore, it allowed for highly cost-effective identification of urine samples in which significant dilution of illegal medications may have occurred.

The second modification to the existing protocol was an increase in the regulatory threshold for levels detected in serum. The first state to implement this threshold approach was Oklahoma in the mid 1980s, and the Oklahoma authorities arbitrarily increased the regulatory threshold to 50 ng/ml. Then, as the potential regulatory value of this approach was realized, other states added the SG screening step, and the regulatory limit was further increased, often to 100 ng/ml. Currently, a num-

<table>
<thead>
<tr>
<th>Probability of Exceeding</th>
<th>Serum Furosemide Concentrations (ng/ml)</th>
<th>Urine Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicated Value (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>10.21</td>
<td>1.0093</td>
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<tr>
<td>25</td>
<td>13.08</td>
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<td>1</td>
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<td>1.015</td>
</tr>
<tr>
<td>0.1</td>
<td>31.79</td>
<td>1.016</td>
</tr>
</tbody>
</table>

Figure 8. Frequency distribution of serum furosemide concentrations 4 hours after IV administration of furosemide at 250 mg to 10 horses. Mean value = 10.88 ng/ml.
A number of states, including Kentucky, Ohio, Maryland, and New York, regulate the use of furosemide in this way, using the SG “cut-off” value of 1.010 in combination with a serum threshold of 100 ng/ml for regulatory purposes.

The purpose of this study was to reevaluate these regulatory thresholds using newer and more accurate analytical and statistical techniques. In this regard, the first step was to develop a highly sensitive LC-MS/MS method using SPE to determine the pharmacokinetic parameters and detection times for furosemide in equine serum samples after IV administration (250 mg). Therefore, the method was developed with an LOD of 1.8 ng/ml, and the LOQ was 3.9 ng/ml.

Determination of the pharmacokinetic parameters of furosemide in racing horses provides important information to racing authorities. One objective of this study was to determine the serum furosemide levels that might suggest compliance with a 4-hour furosemide rule. The pharmacokinetic analysis used in the study showed that furosemide is rapidly cleared following IV administration, with a mean t₁/₂ of 1.8 hours. Serum furosemide concentrations 4 hours after administration ranged from 6.6 to 21.5 ng/ml (mean = 10.9 ± 1.4 ng/ml), which was not significantly different from results determined in the previous study conducted 20 years ago.³ Three of the 10 horses had serum furosemide concentrations below the LOQ at 6 hours, and the concentrations of furosemide in the remaining seven horses at 6 hours ranged from 5 to 9.5 ng/ml (mean = 6.8 ng/ml ± 0.66 [SEM]).

By using mean values for serum furosemide concentration and urine SG, horses have criteria (serum furosemide concentration greater than 100 ng/ml and urine SG less than 1.010)
present only at the 1-hour sampling time. Statistical analysis of the data at the 1-hour sampling in this study indicated that there is less than an 85% chance for horses to have a urine SG value less than 1.010 and 75% chance for a serum furosemide concentration to exceed 100 ng/ml. At later times after administration, the chances are less than 0.05% for both urine and serum samples to exceed those thresholds. Based on results in this study, it is very unlikely for horses to have serum furosemide concentrations greater than 100 ng/ml or urine specific gravity less than 1.010 at 4 hours after administration (250 mg IV). On the other hand, it should be remembered that urine SG is a highly variable measurement in horses, and even without furosemide administration, some horses might naturally have urine SG values less than 1.010.

The authors recognize that trainers frequently restrict water from horses treated with furosemide before a race, and water intake was not restricted in this manner in the present study. The reason for this practice of water restriction is that replacement of fluid lost from furosemide diuresis could increase plasma volume (and therefore pulmonary vascular pressures) during periods of intense exercise. However, consumption of 13.2 L or less each day by horses in this study would amount to approximately 2.5% to 3% of their total body water volume and it is not expected that this amount would have a significant effect on serum furosemide concentrations. As for urine SG, the results of the study performed under these conditions would be even more conservative than for a horse that had water withheld. The data developed in this study can be used to develop a range of furosemide serum and urine SG threshold levels. The diuretic activity of furosemide is rapid, occurring within 10 to 15 minutes, and is largely ended within 1 to 3 hours following IV administration.

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