Pyrilamine and *O*-Desmethylpyrilamine Detection in Equine Serum and Urine

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Abstract

Pyrilamine (mepyramine) is an H_1-receptor antagonist used in human and veterinary medicine. It has the potential to produce central nervous system effects in horses and therefore may have some impact on an outcome of a horse race. A single oral dose of pyrilamine (300 mg/horse) was given to three animals. Serum samples were collected before drug administration and at 0.25, 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, and 144 h, and 7, 8, 9, 10, 11, 12, and 13 days post-administration. Urine samples were collected at 0–1, 1–2, 2–4, 4–6, 24, 48, 72, 96, 120, and 144 h, and 7, 8, 9, 10, 11, 12, 13 days post-administration. Urine and serum samples were initially screened by the pyrilamine enzyme-linked immunosorbent assay (ELISA) kit with subsequent confirmation and quantitation utilizing a newly developed and validated gas chromatography–mass spectrometry (GC–MS) method for pyrilamine and its major metabolite *O*-desmethylpyrilamine with chlorpromazine as an internal standard. Prior to the basic extraction, urine specimens were hydrolyzed using β-glucuronidase. The urine extracts as well as the serum samples were then subjected to solid-phase extraction on Bond Elut LRC-PRS columns. Pyrilamine was not found in any of the urine samples but it was present in serum in low concentrations (4–123 ng/mL) up to 6 h after drug administration. The limit of detection and limit of quantitation for the GC–MS method for pyrilamine in serum were 1.5 and 3.1 ng/mL, respectively, and for *O*-desmethylpyrilamine in urine were 5 and 6.2 ng/mL, respectively. Pyrilamine concentration in serum peaked at 15 min, 30 min, and 1 h in horse #1, #2, and #3, respectively. Urine specimens were screened positive for pyrilamine and its metabolites using ELISA for extended periods of time (4 days in one horse and 9 days in two other animals). Using GC–MS, *O*-desmethylpyrilamine was detected in urine for 11 days in horse #1, 4 days in horse #2, and 9 days in horse #3. While pyrilamine was eliminated from the bloodstream rather quickly, the metabolite level remained in the urine for days after administration. When evaluating laboratory results, regulators must take into account that a urine sample positive for *O*-desmethylpyrilamine does not necessarily indicate that the drug remains active in the horse's system, possibly affecting the outcome from the race.

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Introduction

Pyrilamine (mepyramine) {*N*-[(4-methoxyphenyl)methyl]-*N*',*N*'-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine} is an ethylenediamine first generation antihistamine used to provide symptomatic relief of allergic reactions by acting as an H_1-receptor antagonist with some local anesthetic action (1). Conjugated metabolites identified in human urine include pyrilamine, *O*-desmethylpyrilamine, and some hydroxylated derivatives of pyrilamine, as well as a very small quantity of free *O*-desmethylpyrilamine (2). In horses, urinary metabolites include pyrilamine, *O*-desmethylpyrilamine-glucuronide (major urinary metabolite), 5'-hydroxypyrilamine, *N*,*O*-desmethylpyrilamine, pyrilamine *N*-oxide, and possibly other metabolites (3,4). Though the effects of pyrilamine on horse behavior have not been studied, antihistamines (e.g., H_1 antagonists) have the potential to affect the central nervous system in horses, causing, like in humans, sedation or central nervous system stimulation, depending on the dose and route of administration (1). For that reason, the Association of Racing Commissioners International (ARCI) has classified pyrilamine as a Class 3 agent (5). The ARCI document states that, "Drugs placed in this class may or may not have an accepted therapeutic use in the horse. They all have a potential of affecting the performance of a racing horse". Examples include bronchodilators, local anesthetics, antihistamines, and potent diuretics (5). This makes detection of pyrilamine and its metabolites necessary for proper regulation of the horse-racing industry.

The ARCI guidelines were developed to aid interpretation of racehorse drug screening results and include suggested penalties for each class violation (5). These penalty guidelines allow for mitigating circumstances to be taken into account when the racing authorities issue a ruling. The penalty may include everything from a small fine to the purse redistribution. Therefore, before imposing a penalty, the regulators must examine all information to determine the significance of laboratory findings in each case, including whether the drug has a legitimate therapeutic purpose, the time the drug was last admin-

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istered before a race, the route of administration, and the likelihood that the drug was affecting the horse at the time of the race.

A search of the scientific literature revealed a paucity of information concerning the pharmacokinetics of pyrilamine in the horse, such as elimination half-life, serum clearance, period of detection in serum and urine of either the parent drug or its primary metabolite, an analytical methodology for the confirmation and quantification of the parent drug and metabolite amenable to a production-type laboratory environment, etc. The purpose of this study, therefore, was to monitor the concentration of pyrilamine and its major metabolite *O*-desmethylpyrilamine in equine serum and urine over an extended period of time after the oral administration of a single dose of the drug. In the equine testing laboratories, the immunoassay screening is typically applied to all samples in order to quickly eliminate all negative samples from any batch of specimens and to select presumptive positive specimens for further confirmation. An important objective of the study was to evaluate the detection times of pyrilamine and its metabolites in urine and serum samples of horses when using commercially available enzyme-linked immunosorbent assay (ELISA) kits. In addition, the authors developed a simple and sensitive gas chromatography–mass spectrometry (GC–MS) method for the confirmation and quantitation of pyrilamine and its major metabolite in biological fluids such as those collected in the study. The parent drug and metabolite detection times determined in this study will provide both horse owners and veterinarians with valuable information concerning when the administration of pyrilamine should be discontinued before an event in order to prevent any question of doping the animal in an effort to affect race time. This information can also aid in the evaluation of mitigating circumstances in the event of a positive result in a post-race blood or urine sample.

Materials and Methods

Animals and drug administration

Three healthy, non-competing mares were used in this study. A 15-year-old thoroughbred, a 9-year-old thoroughbred, and an 11-year-old quarter horse were each dosed orally with 0.5 oz of Tri-Hist® Granules (SmartPak Equine LLC; each ounce of the product contained 600 mg of pyrilamine and 600 mg of pseudoephedrine). The administered dose equaled 300 mg pyrilamine and 300 mg pseudoephedrine. There are no commercially available oral formulations for horses containing pyrilamine alone, and there is no data in the literature on potential interaction between pyrilamine and pseudoephedrine in horses. Blood samples for each time point were collected from the jugular vein of the horse's neck into two 12.5-mL serum separator tubes. After 25–30 min at room temperature, the blood samples were centrifuged and serum was transferred to one 12.5-mL red-top container (unpreserved) and kept frozen prior to analysis. Samples were taken before drug administration and at 0.25, 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, 144 h, and 7, 8, 9, 10, 11, 12, 13 days post administration. For the urine collection, the Foley catheter was introduced into the horse's urinary bladder and the sterile urine collection bag was attached to the end of the catheter. One urine sample was collected prior to the drug administration by emptying the bag and transferring all its content to the individually labeled urine collection cup. Urine 0–1, 1–2, 2–4, and 4–6 h pools were collected by taking all urine from the bag at approximately 1, 2, 4, and 6 h and transferring it to the individually labeled urine collection containers. After 6 h, the catheter was removed. At 24 h, the catheter with the urine collection bag attached was introduced again and approximately 1 h later, all urine from the bag was transferred to the urine collection cup and the catheter was removed. The same procedure was repeated at 48, 72, 96, 120, and 144 h, and 7, 8, 9, 10, 11, 12, and 13 days post-administration. Immediately after collection, all urine samples were frozen unpreserved until analyzed.

Reagents

Pyrilamine maleate and chlorpromazine hydrochloride were obtained from Fisher Scientific (Hanover Park, IL) and prepared as standard stock solutions at the concentration of 1 mg/mL in methanol. All stock solutions were stored in tightly sealed glass vials and refrigerated at 4°C (± 2°C). *O*-Desmethylpyrilamine was obtained from Neogen (Lexington, KY) as a 200 µg/mL solution in methanol. Type H-2, β-glucuronidase from *Helix pomatia* with 98,800 units/mL glucuronidase activity and 702 units/mL sulfatase activity was purchased from Sigma-Aldrich (St. Louis, MO). Sodium phosphate buffer (100 mM, pH 5) was prepared by mixing 470 mL of a 27.6 mg/mL monobasic sodium phosphate solution with 30 mL of a 28.4 mg/mL dibasic sodium phosphate solution and diluting to 1 L (each solution made with deionized water). All reagents used, including glacial acetic acid, ascorbic acid, ammonium hydroxide (NH_4OH), dichloromethane (DCM), isopropyl alcohol (IPA), sulfuric acid (H_2SO_4), methanol (MeOH), ethyl acetate (EtAc), *N*,*O*-bis (trimethylsilyl) trifluoroacetamide 1% trichloromethylsilane (BSTFA 1% TCMS), and acetonitrile (ACN) were HPLC-grade or better, and they were purchased from Fisher Scientific.

Preliminary screening

All urine pools and serum samples were analyzed by pyrilamine ELISA (Neogen). Each urine or serum sample was buffered at a dilution of one part urine or plasma in nine parts phosphate buffered saline. The ELISA test was developed according to the manufacturer's instructions and the absorbance of each sample was measured at 650 nm using an ASYS Hitech Expert 96 microplate reader (Phenix Research Products, Hayward, CA).

Instrumentation

A Hewlett-Packard model 6890 GC equipped with a model 5973 mass selective detector operated with Enhanced Chemstation Ver. D.02.00.275 was used for all GC–MS analyses (Hewlett-Packard, Wilmington, DE). A J&W DB-5MS (15.0 m × 250.00 µm × 0.25 µm) capillary column was used (Agilent Technologies, Wilmington, DE). Helium (ultra-high purity; Progressive Industries, Chicago, IL) was used as the carrier gas,

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at a flow rate of 1.5 mL/min. Two microliters of each sample were injected on column, in splitless mode, with an inlet temperature of 270°C. The initial oven temperature was 70°C, held for 1 min, followed by a ramp of 15°C per min to a final temperature of 280°C, held for 3 min for a total run time of 18 min. Data was collected using selected ion monitoring mode on the MS. Pyrilamine was detected using ions 58, 121, and 215 (*m/z*). The derivatization product of *O*-desmethylpyrilamine (*O*-desmethylpyrilamine-TMS) was detected using ions 58, 179, and 272 (*m/z*), and for chlorpromazine 58, 86, and 318 (*m/z*) (underlined ions were used for quantitation).

Urine sample preparation, enzyme hydrolysis, and base extraction

Five milliliters of equine urine were transferred to a 20-cm × 125-mm test tube and spiked with 25 µL of 100 µg/mL of chlorpromazine (internal standard) working stock solution to give a final concentration of 500 ng/mL. One milliliter of sodium phosphate buffer (pH 5) was added, and if necessary, the sample was adjusted to pH 5 (± 0.5) with glacial acetic acid using a pH meter. One-hundred microliters of β-glucuronidase were added (9880 units of activity), and the sample incubated at 37°C for 24 h (experimentally determined and optimized). After incubation, 5 drops of 10% ascorbic acid were added, and then the pH of each sample was adjusted to 10 (± 0.5) with NH_4OH using a pH meter. Eight milliliters of 9:1 DCM/IPA was then added, and the sample was mixed by a rotorack (Glas-Col®) for 15 min, followed by the centrifugation for 15 min to achieve phase separation. The top aqueous layer was aspirated to waste, and the solvent layer transferred to a clean glass tube. Three milliliters of 0.5 N H_2SO_4 was added to the organic layer, and the tube was mixed by a rotorack for 15 min and centrifuged for 5 min to achieve phase separation. The top layer (aqueous) was transferred to a clean glass tube, and three drops of 10% ascorbic acid were added. The pH of each sample was adjusted to 10 (± 0.5) with NH_4OH, and 5 mL of 9:1 DCM/IPA was added. The sample was mixed by a rotorack for 15 min and centrifuged for 5 min. The top layer (aqueous) was aspirated to waste. The solvent layer was transferred to a drying tube and evaporated to dryness under a nitrogen stream in a 40°C water bath. The residue was dissolved in 5 drops of methanol and mixed with 5 mL of sodium phosphate buffer (pH 5) for solid-phase extraction.

Serum sample preparation

One microliter of equine serum was spiked with 5 µL of 100 µg/mL of chlorpromazine working standard solution to give a final concentration of the internal standard 500 ng/mL. The sample was vortex mixed and buffered with 4 mL of sodium phosphate buffer (pH 5) for solid-phase extraction.

Solid-phase extraction and derivatization

A Bond Elut LRC-PRS, 500-mg bonded solid-phase extraction cartridge (Varian, Harbor City, CA) was conditioned with 4 mL of methanol, followed by 2 mL of deionized water, and then 2 mL of sodium phosphate buffer (pH 5) passed through under vacuum. The urine extract or serum sample (see previous) was then added, followed by 2 mL of sodium phosphate buffer (pH 5). The cartridge was then allowed to dry under vacuum for 5 min. The cartridge was next washed with 2 mL of methanol and allowed to dry for 5 min. The sample was eluted with 2 mL of 85:10:15 EtAc/MeOH/NH_4OH into a clean glass tube. This step was repeated, and the eluents combined in the same tube. The eluent was evaporated to dryness under a nitrogen stream in a 40°C water bath. One milliliter of 1:1 DCM/MeOH was added to the residue and vortex mixed. It was then transferred to an autosampler vial and evaporated to dryness. Twenty microliters of BSTFA containing 1% TCMS and 20 µL of ACN were added to the residue, and the vials were sealed. The samples were incubated at 65°C for 30 min and then injected onto the GC–MS.

Method validation

Quantitation of pyrilamine in serum and its major metabolite *O*-desmethylpyrilamine in urine was performed using an internal standard method. A six-point standard curve for pyrilamine in blank serum and a seven-point standard curve for *O*-desmethylpyrilamine in blank horse urine were prepared by linear least-squares regression analysis of the ratio of the peak area of analytes to the peak area of the internal standard (chlorpromazine) as a function of concentration. Urine standards were prepared at concentrations of 6.2, 12.5, 25, 50, 100, and 200 ng/mL of *O*-desmethylpyrilamine, and serum standards were prepared at concentrations of 3.1, 6.2, 12.5, 25, 50, 100, and 200 ng/mL of pyrilamine. All urine or serum samples with concentrations of analytes greater than the highest calibrator were reanalyzed. The aliquots were taken from the original specimens, diluted, extracted, and requantitated. In addition, two levels of controls of pyrilamine in negative serum and *O*-desmethylpyrilamine in negative urine were prepared using control stock solution of pyrilamine (1 mg/mL) and a solution of *O*-desmethylpyrilamine (200 µg/mL). The concentrations of pyrilamine controls in serum were 15 and 70 ng/mL, and *O*-desmethylpyrilamine in urine 35 and 70 ng/mL. Serum and urine controls were analyzed on three different days to determine inter- and intraday variability. The respective standard curves were run on each day of analysis of controls. The percent coefficient of variation was determined by dividing the standard deviation by the mean measured concentration and multiplying by 100%. The percent relative accuracy was determined using the following equation:

[(Mean Measured Concentration – Theoretical Concentration)/ Theoretical Concentration] × 100%.

Results and Discussion

The ELISA screening results of urine samples collected from three horses are presented in Table I. The sensitivity of the standard Neogen ELISA pyrilamine kit with respect to detection times can be evaluated, according to the manufacturer, by identifying the urine samples that have 50% (I-50) less absorbance (measured at 650 nm) than the negative control. In this study, the ELISA kit was able to detect pyrilamine equiv-

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alents for approximately 9 days in horse #1 and #3, and up to 4 days in horse #2. All serum samples screened by ELISA were positive for pyrilamine for only up to 6 h after the oral administration of the 300 mg pyrilamine/300 mg pseudoephedrine combination product. Another controlled administration study (4) provided the supporting information to our findings. In that study, pyrilamine and its metabolites were detected for up to 11 h in serum and 69 h in urine following the IV administration of 100 mg pyrilamine using a similar ELISA kit. The same report showed that pyrilamine and *O*-desmethylpyrilamine were detectable in post-administration samples for up to one week after a single oral (300 mg/horse) or IV dose.

In order to quantify pyrilamine and its major metabolite in serum and urine, a sensitive GC–MS method was developed and validated. Method validation data are presented in Tables II and III. The limits of detection (LODs) and limits of quantitation (LOQs) were calculated in the following manner. The mean noise was measured using 20 points before the calibration curve peaks and 20 points after, as well as blanks. For *O*-desmethylpyrilamine in urine, the lowest calibration curve point (6.2 ng/mL) had a signal-to-noise ratio of approximately 11:1. The lowest calibration curve point for pyrilamine in serum (3.1 ng/mL) had a signal-to-noise ratio of approximately 30:1. These values were, therefore, used as the LOQ for each matrix. Approximate LODs were calculated by extrapolating calibration curve data to determine what concentrations might yield a 3:1 signal-to-noise ratio. These calculations indicate an LOD of approximately 1.5 ng/mL of pyrilamine in serum and approximately 5.0 ng/mL of *O*-desmethylpyrilamine in urine. In the study by Stevenson et al. (3), the LOD for pyrilamine in plasma and urine using GC–MS was 5.0 and 10.0 ng/mL, respectively. In this study, the standard curves for pyrilamine in serum and for *O*-desmethylpyrilamine in urine were linear over the concentration range and had correlation coefficients of 0.9992 and 0.9995, respectively. The full scan mass spectra of pyrilamine, *O*-desmethylpyrilamine-TMS, and chlorpromazine are presented in Figure 1. The representative chromatograms for *O*-desmethylpyrilamine derivative in urine and pyrilamine in serum are presented in Figures 2 through 4. Sufficient resolution was achieved between pyrilamine and *O*-desmethylpyrilamine derivative, as shown in Figure 2. Pyrilamine was not detected in any of the urine samples; only the metabolite, *O*-desmethylpyrilamine, was identified (Figure 3). *O*-Desmethylpyrilamine was not identified in any of the serum samples analyzed. The metabolite concentrations in pooled urine samples were the highest between one and four hours post drug administration. Horse #2 had the highest *O*-desmethylpyrilamine concentration, greater than 140,000 ng/mL, vastly higher than the peak level of the other two horses (Table IV). However, *O*-desmethylpyrilamine levels for this horse decreased rapidly and reached the quantitation limit sooner (96 h) than in the other horses. In horse #1, *O*-desmethylpyrilamine was detected up to 11 days after the administration of a single dose of pyrilamine/pseudoephedrine with the maximum concentration greater than 9000 ng/mL. In

Table I. ELISA Absorbance at 650 nm in Urine Samples Collected from Three Horses After Pyrilamine Administration*

Urine Collection Pool	Horse 1	Horse 2	Horse 3
0–1 h	0.072	0.052	0.064
1–2 h	0.055	0.077	0.059
2–4 h	0.061	0.051	0.051
4–6 h	0.051	0.051	0.060
24 h	0.055	0.064	0.052
48 h	0.057	0.063	0.063
72 h	0.064	0.093	0.118
96 h	0.096	0.402†	0.133
120 h	0.181	0.859	0.409
144 h	0.232	1.207	0.300
7 days	0.207	1.024	0.148
8 days	0.305	1.133	0.478
9 days	0.260†	1.052	0.524†
10 days	0.651	1.064	0.715
11 days	0.597	1.042	0.677
12 days	0.623	1.095	0.725
13 days	0.571	1.155	0.975

* Positive control (pyrilamine concentration 20 ng/mL): 0.154 (absorbance at 650 nm); negative control: 1.101 (absorbance at 650 nm), I-50 = 0.55).
† The last sample positive for pyrilamine equivalents.

Table II. Accuracy and Precision of Pyrilamine Control Serum Preparations (ng/mL)

Parameter	Low Control	High Control
Theoretical concentration	15	70
Intraday variability		
Mean measured concentration (± SD)	16.7 (± 0.6)	76.4 (± 5.2)
% Coefficient of variation	3.6	6.8
% Relative accuracy	11.3	9.1
Interday variability		
Mean measured concentration (± SD)	15.7 (± 1.6)	79.7 (± 5.7)
% Coefficient of variation	10.2	7.1
% Relative accuracy	4.7	13.8

Table III. Accuracy and Precision of *O*-Desmethylpyrilamine Control Urine Preparations (ng/mL)

Parameter	Low Control	High Control
Theoretical concentration	35	70
Intraday variability		
Mean measured concentration (± SD)	37.1 (± 0.3)	74.2 (± 5.1)
% Coefficient of variation	0.8	6.9
% Relative accuracy	6.0	6.0
Interday variability		
Mean measured concentration (± SD)	37.9 (± 4.7)	73.2 (± 6.1)
% Coefficient of variation	12.4	8.3
% Relative accuracy	8.3	4.6

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horse #3, the metabolite was detected up to 9 days with the maximum concentration greater than 22,000 ng/mL. As demonstrated in Table V, pyrilamine serum levels peaked at 1 h following drug administration in horse #3, 30 min in horse #2, and 15 min in horse #1. The maximum concentrations ranged from 38 to 123 ng/mL. For all three horses, serum levels dropped below the LOQ by 6 h. These relatively low serum concentrations can be explained by the extensive first-pass metabolism of pyrilamine after oral administration, significantly impacting the bioavailability (only 20%) of the drug (4). Although pyrilamine leaves the bloodstream between 4 and 6 h post-drug administration, the metabolite presence can be detected in the urine even up to 9 days post-administration.

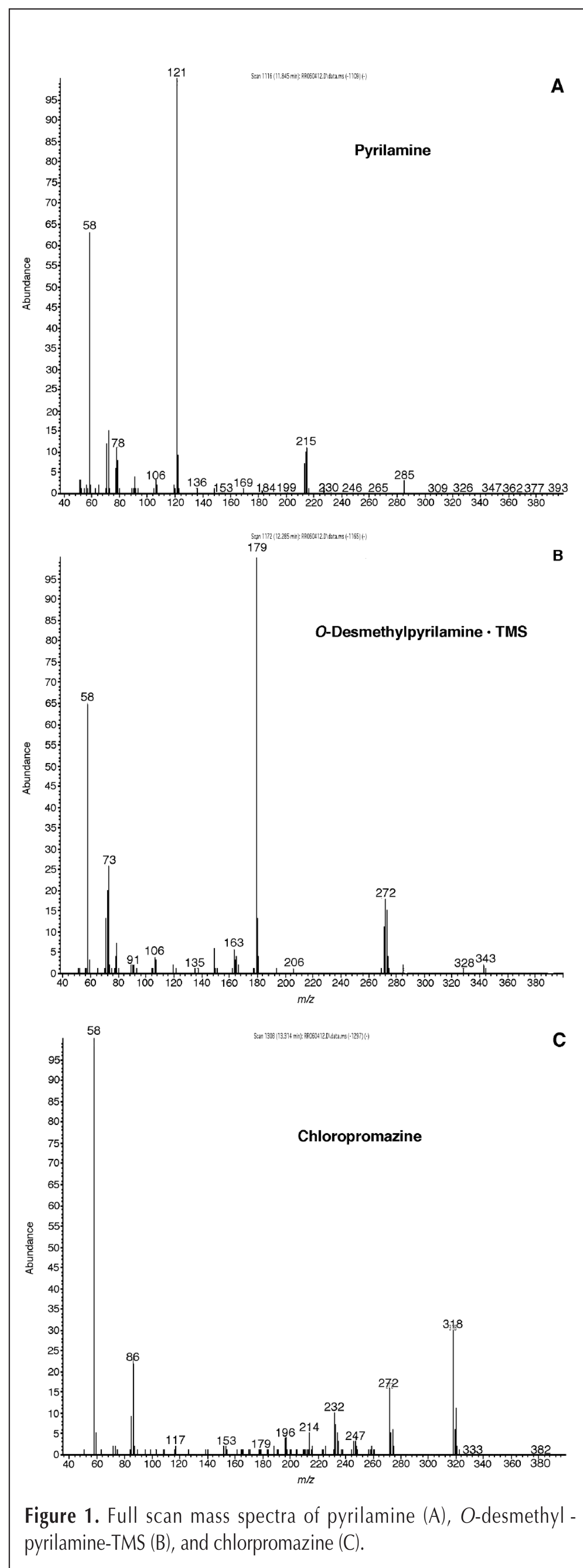

Figure 1. Full scan mass spectra of pyrilamine (A), *O*-desmethyl - pyrilamine-TMS (B), and chlorpromazine (C).

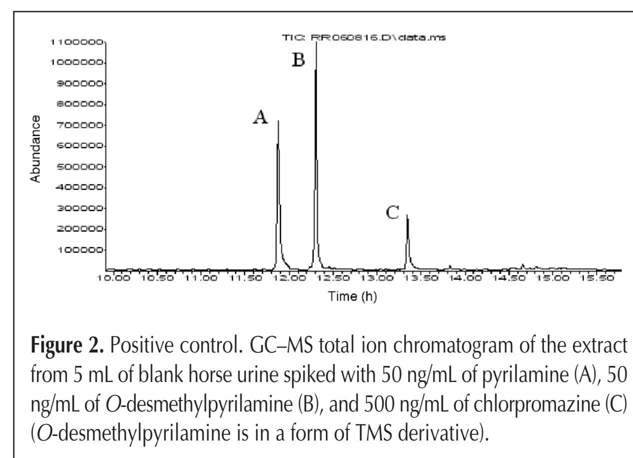

Figure 2. Positive control. GC–MS total ion chromatogram of the extract from 5 mL of blank horse urine spiked with 50 ng/mL of pyrilamine (A), 50 ng/mL of *O*-desmethylpyrilamine (B), and 500 ng/mL of chlorpromazine (C) (*O*-desmethylpyrilamine is in a form of TMS derivative).

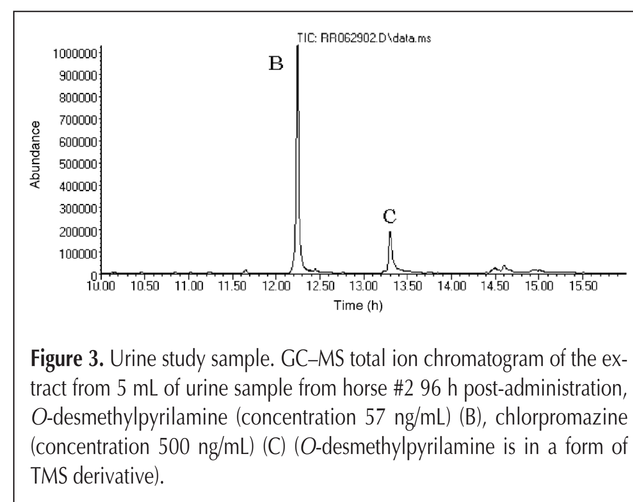

Figure 3. Urine study sample. GC–MS total ion chromatogram of the extract from 5 mL of urine sample from horse #2 96 h post-administration, *O*-desmethylpyrilamine (concentration 57 ng/mL) (B), chlorpromazine (concentration 500 ng/mL) (C) (*O*-desmethylpyrilamine is in a form of TMS derivative).

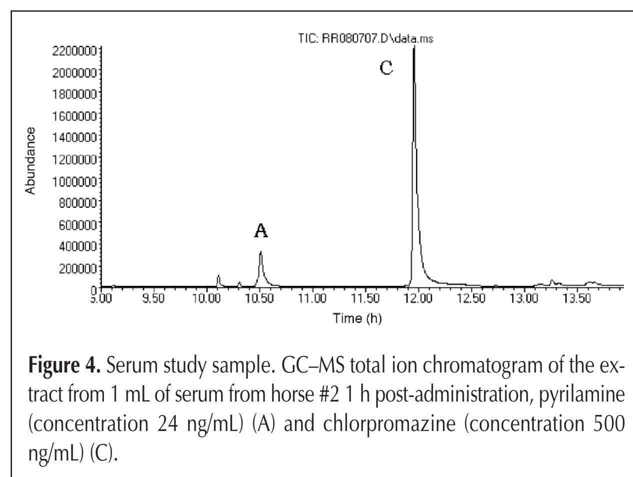

Figure 4. Serum study sample. GC–MS total ion chromatogram of the extract from 1 mL of serum from horse #2 1 h post-administration, pyrilamine (concentration 24 ng/mL) (A) and chlorpromazine (concentration 500 ng/mL) (C).

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Table IV. Urine *O*-Desmethylpyrilamine Concentrations in Study Horses (ng/mL)

Time	Horse 1	Horse 2	Horse 3
0–1 h	150	986	ND*
1–2 h	9203	140,837	10,736
2–4 h	2235	81,342	22,674
4–6 h	2645	28,825	18,316
24 h	1844	7112	6951
48 h	332	981	643
72 h	169	334	160
96 h	57	18	61
120 h	26	BQL†	8
144 h	18	BQL	8
7 days	17	BQL	12
8 days	9	ND	BQL
9 days	12	ND	8
10 days	7	BQL	ND
11 days	7	ND	BQL
12 days	BQL	ND	BQL
13 days	BQL	ND	BQL

* Not detected.
† Below quantitation limit (< LOQ).

For instance, at 24 h, when pyrilamine is not detected in serum any more, the metabolite is still present at approximately 2–7 µg/mL in the urine.

These data indicated that the owners should discontinue orally administered pyrilamine/pseudoephedrine at least 24 h before a race to ensure that the drug has been eliminated from the bloodstream. To ensure that a horse has clean urine, pyrilamine use must be discontinued several days before a race. However, when evaluating laboratory results, regulators must take into account that a urine sample containing minute quantities of *O*-desmethylpyrilamine does not necessarily indicate that the drug remains active in the horse's system.

Conclusions

Commercially available ELISA may be employed for the detection of pyrilamine and its metabolites for extended periods of time in horse urine and only for a few hours in serum after administration of a single oral dose of the drug. The concentrations of pyrilamine in serum were much lower than the concentrations of its metabolite in urine. In the case of the therapeutic use of pyrilamine in horses, because the metabolite can be detected for several days post-drug administration, it is rather unrealistic to require that the major metabolite be absent or undetectable in the urine to adhere to the so-called "zero tolerance" policy. The state regulators need to evaluate positive results issued by the equine testing laboratories with caution before regulatory penalties are imposed.

Table V. Serum Pyrilamine Concentrations in Study Horses (ng/mL)

Time	Horse 1	Horse 2	Horse 3
0	ND*	ND	ND
15 min	96	15	78
30 min	80	38	95
1 h	63	24	123
2 h	22	4	37
4 h	6	ND	11
6 h	BQL†	ND	5

* Not detected.
† Below quantitation limit (< LOQ).

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References

1. T.J. Haley. Physical and biological properties of pyrilamine [literature survey]. *J. Pharm. Sci.* **72:** 3–11 (1983).
2. B.C. Chung, D.H. Kim, B.H. Jung, K. Eom, W. Slikker Jr., and J. Park. Identification of urinary metabolites of pyrilamine after oral administration to man. *Xenobiotica* **24(5):** 451–459 (1994).
3. Pyrilamine. In *Analytical Methodology for Detection and Confirmation of Drugs in Equine Body Fluids, Vol. II: Respiratory Drugs*, A.J. Stevenson, M.P. Weber, and S. Kacew, Eds. Canadian Equine Drug Evaluation Program, Canadian Pari-Mutuel Agency, Agriculture and Agri-Food Canada, 1996, pp 171–179.
4. W.E. Woods, J.D. Harkins, A.F. Lehner, L. Dirikolu, W. Karpiesiuk, J. Boyles, W.G. Carter, M. Fisher, T. Haller, and T. Tobin. Detection and disposition of pyrilamine and its metabolite O-desmethylpyrilamine in equine blood and urine: a preliminary report. Proceedings of the 13th International Conference of Racing Analysts and Veterinarians, 2000, pp 472–477.
5. Association of Racing Commissioners International, Inc. Uniform Classification Guidelines for Foreign Substances and Recommended Penalties and Model Rule. http://www.arci.com/druglisting.pdf, July 2007.

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