A GC-MS Method for the Determination of Isoxsuprine in Biological Fluids of the Horse Utilizing Electron Impact Ionization

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Abstract

Isoxsuprine is used to treat navicular disease and other lower-limb problems in the horse. Isoxsuprine is regulated as a class 4 compound by the Association of Racing Commissioners, International (ARCI) and, thus, requires regulatory monitoring. A gas chromatography–mass spectrometry method utilizing electron impact ionization was developed and validated for the quantitation of isoxsuprine in equine plasma or equine urine. The method utilized robotic solid-phase extraction and tri-methyl silyl ether products of derivatization. Products were bis-trimethylsilyl (TMS) isoxsuprine and tris-TMS ritodrine, which released intense quantifier ions m/z 178 for isoxsuprine and m/z 236 for ritodrine that were products of C–C cleavage. To our knowledge, this procedure is faster and more sensitive than other methods in the literature. Concentrations in urine and plasma of isoxsuprine were determined from a calibrator curve that was generated along with unknowns. Ritodrine was used as an internal standard and was, therefore, present in all samples, standards, and blanks. Validation data was also collected. The limit of detection of isoxsuprine in plasma was determined to be 2 ng/mL, the limit of quantitation of isoxsuprine in plasma was determined to be < 5 ng/mL. The mean coefficient of determination for the calibrator curves for plasma was 0.9925 ± 0.0052 and for calibrator curves for urine 0.9904 ± 0.0075. The recovery efficiencies at concentrations of 50, 200, and 300 ng/mL were 76%, 73%, and 76%, respectively, in plasma and 92%, 89%, and 91% in urine.

Introduction

Isoxsuprine continues to be recommended and used by equine veterinary practitioners in the treatment of navicular disease and other lower-limb problems (1). Isoxsuprine is accepted to have α1 adrenergic sympatholytic activity (2). Post-performance drug screening of urine samples readily discloses the presence of isoxsuprine (3,4) for as long as six weeks after administration of the drug (5).

Doses of isoxsuprine are large relative to doses of other drugs and can be as large as 4 mg/kg/day orally. Long regimens of treatment from months to years are not uncommon. In addition, tritiated-isoxsuprine has demonstrated binding to melanin and keratin (6). These factors lead to the forensic detection of the drug in body fluids for an extended period of time after administration.

Methodology for isoxsuprine detection in equine urine includes hydrolysis of samples and then recovery of the parent compound (7). The major material present in urine is the O-glucuronide metabolite of isoxsuprine (8). The likelihood of a sulfate metabolite is small, as hydrolysis with a partially purified sulfatase from *Aerobacter aerogenes* yielded no free isoxsuprine (9). In addition, earlier studies using electrospray ionization–mass spectrometry (MS) yielded no products that could be identified as a sulfate metabolite before hydrolysis with a β-glucuronidase (*H. pomatia*) preparation that contains sulfatase activity (Lehner & Bosken, unpublished).

Several methods currently exist for the determination of isoxsuprine in biological fluids. These methods include high-performance liquid chromatography (HPLC)–UV detection and HPLC–thermospray MS (9), HPLC utilizing electrochemical detection (3), gas chromatography (GC)–MS (10), and atmospheric pressure chemical ionization mass spectrometric determination utilizing single reaction monitoring mode (5). The procedure reported here is performed via GC–MS analysis utilizing electron impact (EI) ionization.

Materials and Methods

Horses, drug administration, and sample collection

Animals used in these experiments were managed according to the rules and regulations of the Institutional Animal Care Use Committee at the University of Kentucky, Lexington, KY, which also approved the experimental protocol. Ten mature Thoroughbred mares weighing 490–604 kg were dosed with 2 mg/kg isoxsuprine, either orally or intravenously. Intravenous doses were administered as a single bolus. Horses were provided hay and water ad libitum during experimentation. Twice-a-day feedings, including hay and a 50:50 mixture of oats and alfalfa-based protein pellet (12% protein), were performed. The animals were dewormed quarterly with ivermectin (MSD Agvet, Rahway, NJ) and vaccinated annually for tetanus. Each mare served as its own control.

Isoxsuprine-HCl for oral dosing was obtained from Integrity Pharmaceutical Corporation (Indianapolis, IN). Isoxsuprine-HCl for intravenous dosing was from Sigma (St. Louis, MO). Collection of urine samples took place immediately before and at time points after administration using a Harris flush tube (24 Fr × 60 in., Seamless, Ocala, FL). Urine samples were aliquoted and stored at –20°C until assayed. Blood samples were taken immediately before and after administration into heparinized tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The samples were inverted and then stored refrigerated before centrifugation to remove the plasma.
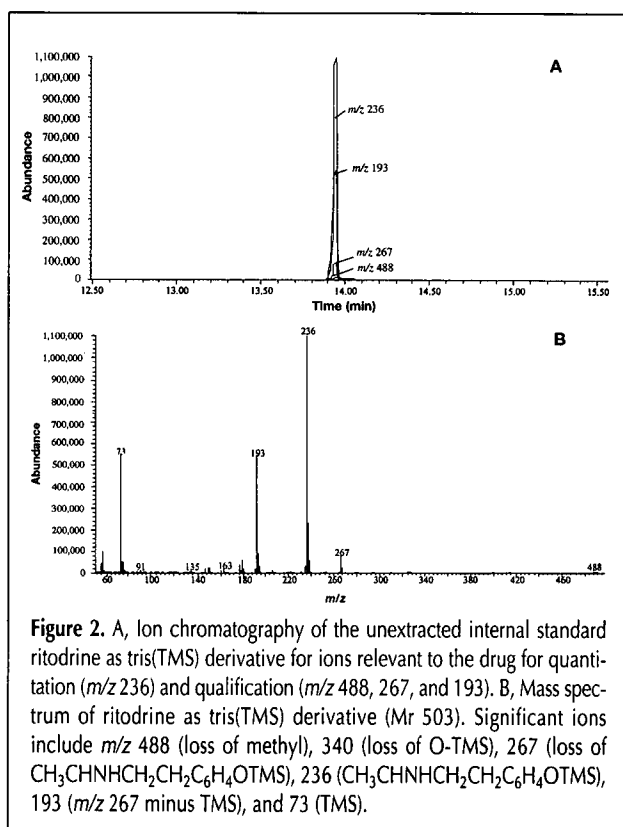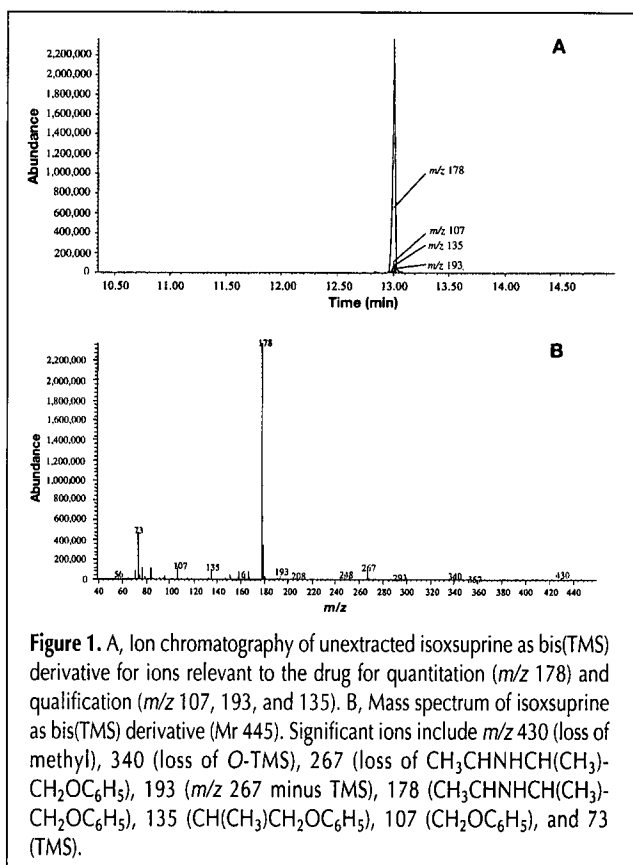
Sample preparation

Isoxsuprine for calibration curves and ritodrine for the in-ternal standard were both purchased from Sigma (isoxsuprine-HCl, I-0880 and ritodrine-HCl, R-0758). Chromatograph responses from Sigma isoxsuprine were compared with those from a certified standard (USP #1354003, the United States Pharmacopeial Convention, Inc. Rockville, MD) to verify that it was appropriate for quantitative studies. The certified isoxsuprine standard (peak apex spectrum) provided an 86% match to the AORC_R3.L library entry for isoxsuprine-TMS2 (445 M_r). The spectrum was entered into an in-house library where it provided a 94% match to itself. The Sigma isoxsuprine (peak apex spectrum) gave a 91% match to the in-house library entry. Spectra from the side of the peak, as well as the average spectrum, ranged a 90–91% match. Calibrator solutions of isoxsuprine and ritodrine were prepared in methanol at 1 mg/mL free drug. Extraction calibrators were prepared for matrix (plasma or urine) in concentrations including 10, 40, 50, 100, 200, 300, and 400 ng/mL. A known amount of a ritodrine calibrator (200 ng) was added to each sample, standard, and blank as an internal standard.

Sample hydrolysis

The urine samples, standards (unsupplemented), and blanks (2 mL/sample) were placed in culture tubes. To each sample, 0.8 mL of 1M sodium acetate buffer (pH 5.0) and 0.4 mL β-glucuronidase solution (Sigma Type H-5, 5000 units/mL) were added to be hydrolyzed. The samples were mixed briefly by vortex and incubated (water bath) at 37°C overnight.

For plasma samples, 0.7 mL of 1M sodium acetate buffer (pH 5.0) and 0.3 mL of β-glucuronidase solution (Sigma Type H-5, 5000 units/mL) were added to the 2-mL sample volume for hydrolysis. After vortex mixing, hydrolysis was performed under

Figure 1. A, Ion chromatography of unextracted isoxsuprine as bis(TMS) derivative for ions relevant to the drug for quantitation (m/z 178) and qualification (m/z 107, 193, and 135). B, Mass spectrum of isoxsuprine as bis(TMS) derivative (Mr 445). Significant ions include m/z 430 (loss of methyl), 340 (loss of O-TMS), 267 (loss of CH₃CHNHCH(CH₃)-CH₂OC₆H₅), 193 (m/z 267 minus TMS), 178 (CH₃CHNHCH(CH₃)-CH₂OC₆H₅), 135 (CH(CH₃)CH₂OC₆H₅), 107 (CH₂OC₆H₅), and 73 (TMS).

Figure 2. A, Ion chromatography of the unextracted internal standard ritodrine as tris(TMS) derivative for ions relevant to the drug for quantitation (m/z 236) and qualification (m/z 488, 267, and 193). B, Mass spectrum of ritodrine as tris(TMS) derivative (Mr 503). Significant ions include m/z 488 (loss of methyl), 340 (loss of O-TMS), 267 (loss of CH₃CHNHCH₂CH₂C₆H₄OTMS), 236 (CH₃CHNHCH₂CH₂C₆H₄OTMS), 193 (m/z 267 minus TMS), and 73 (TMS).

the same conditions as for urine for the measurement of total drug. Plasma samples were also processed without hydrolysis to measure free drug. Total drug was defined as free drug plus conjugated drug. Each plasma or serum sample had 1 mL of 0.1M sodium phosphate buffer added to it, and the sample pH was adjusted to 6.0 ± 0.5 with 1M sodium hydroxide or 1M hydrochloric acid.

After cooling the urine hydrolysates, the isoxsuprine and ritodrine supplements were added as required. The samples were next sonicated for 90 s, followed by centrifugation. The samples were placed in a 4°C Beckman Coulter Allegra 6R (Palo Alto, CA) centrifuge equipped with a GH-3.8 rotor then centrifuged for 15 s at 1430 × g. After the addition of 2 mL of 0.1M sodium phosphate buffer (pH 6), the sample pH was adjusted to 6.0 ± 0.5 with 1M sodium hydroxide or 1M hydrochloric acid, as measured using pH indicator strips (pH 5–10, Merck, Darmstadt, Germany).

Solid-phase extraction (SPE) and derivatization

SPE was accomplished on the Rapid Trace automatic workstation (Zymark Corp., Hopkinton, MA). Clean Screen SPE columns (CSDAU203, 3 mL, 200 mg bed, United Chemical Technologies, Bristol, PA) were conditioned by sequential additions of 2 mL methanol, 2 mL water, and 2 mL 0.1M sodium phosphate buffer (pH 6.0). Next, the samples were loaded onto the columns at a flow rate of 1.0 mL/min. The columns were then washed sequentially with 2 mL water, 2 mL 1M acetic acid, and 2 mL methanol. The columns were dried for 1 min with high-purity nitrogen gas. The analyte was eluted with 3 mL of dichloromethane/isopropanol/ammonium hydroxide mixture (78:20:2, v/v) at a flow rate of 1.0 mL/min. The effluent was suspended in a water bath (< 40°C) and evaporated to dryness under a stream of nitrogen gas. For derivatization, each sample was dissolved in 50 µL N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA/1% TMCS, Pierce, Rockford, IL), vortex mixed 15 s, and incubated at 75°C for 45 min.

GC–MS analysis

Each derivatized sample was transferred to an autosampler vial equipped with a spring-loaded 300-µL insert (type 7673,
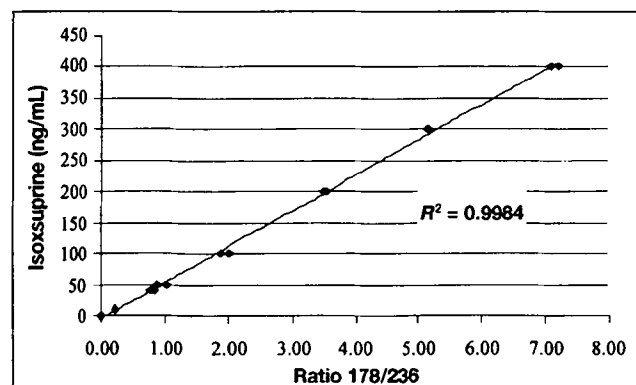

Figure 3. Standard curve of isoxsuprine in plasma based on quantitation of ion m/z 178 set relative to that of the internal standard ritodrine and its quantitative ion m/z 236. The r^2 for this standard curve was 0.9984, which was typical for the assay.

National Scientific Co., Lawrenceville, GA). The instrument employed was a Hewlett-Packard model 6890 GC (Agilent Technologies, Palo Alto, CA) equipped with a model 5972A mass selective detector (MSD). The column was an HP-5MS (30-m × 250-µm i.d., 0.25-µm film thickness). The carrier gas was helium with a flow of 1 mL/min maintained by electronic flow control.

The volume injected was 1 µL and was run in splitless mode at an injector temperature of 250°C. The initial oven temperature was 180°C (held 2 min), increasing at 20°C/min to 280°C (held 12 min). The interface temperature was 280°C. The solvent delay was 6.0 min. The MS was run in selected ion monitoring (SIM) mode. Integrated areas of ion m/z 178 [isoxsuprine-bis(TMS), see ion chromatogram, Figure 1A] and ion m/z 236 [ritodrine-tris(TMS), Figure 2A] were used to generate a standard curve (Figure 3) and quantitate isoxsuprine concentration by interpolation on a plot of (area m/z 178)/(area m/z 236) as a function of known concentration. Qualifier ions for isoxsuprine included m/z 107, 135, and 179 (see EI mass spectrum, Figure 1B). Qualifier ions for ritodrine included m/z 193, 267, and 488 (Figure 2B). The dwell time for both quantitative ions was 50 ms and 10 ms for the qualifier ions.

Validation of method

The limit of detection (LOD) of isoxsuprine was determined by spiking blank matrix samples with decreasing concentrations of isoxsuprine until the point was reached at which the specific ion chromatograms for diagnostic ions had a signal-to-noise ratio (S/N) of ≥ 3. The LOD of the analyte in matrix was the point at which the relative ion ratios for diagnostic ions exceeded the Association of Official Racing Chemists (AORC) criteria specified in Appendix A, while maintaining an S/N ratio of ≥ 3.
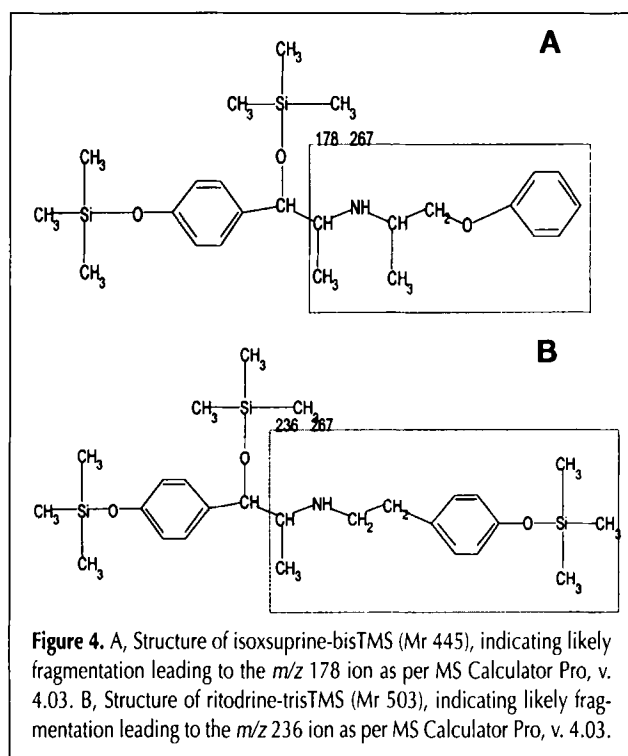

Figure 4. A, Structure of isoxsuprine-bisTMS (Mr 445), indicating likely fragmentation leading to the m/z 178 ion as per MS Calculator Pro, v. 4.03. B, Structure of ritodrine-trisTMS (Mr 503), indicating likely fragmentation leading to the m/z 236 ion as per MS Calculator Pro, v. 4.03.

The limit of quantitation (LOQ) was determined for samples in matrix. All samples were processed by the method previously described. Five spiked replicates at each of four concentrations were analyzed on each of three days. A separate calibrator curve was prepared each day. After the third day, the data was analyzed to determine the coefficients of variation (CV) values for each concentration. As the concentration of analyte decreased, the CV increased. The LOQ was the concentration at which the CV for the assay exceeded 20%.

The recovery of an analyte was the detector response obtained from an amount of the analyte added to and extracted from the biological matrix compared to the detector response obtained for the true concentration of the pure authentic standard (11). Three sets of blank matrix samples in triplicate were spiked
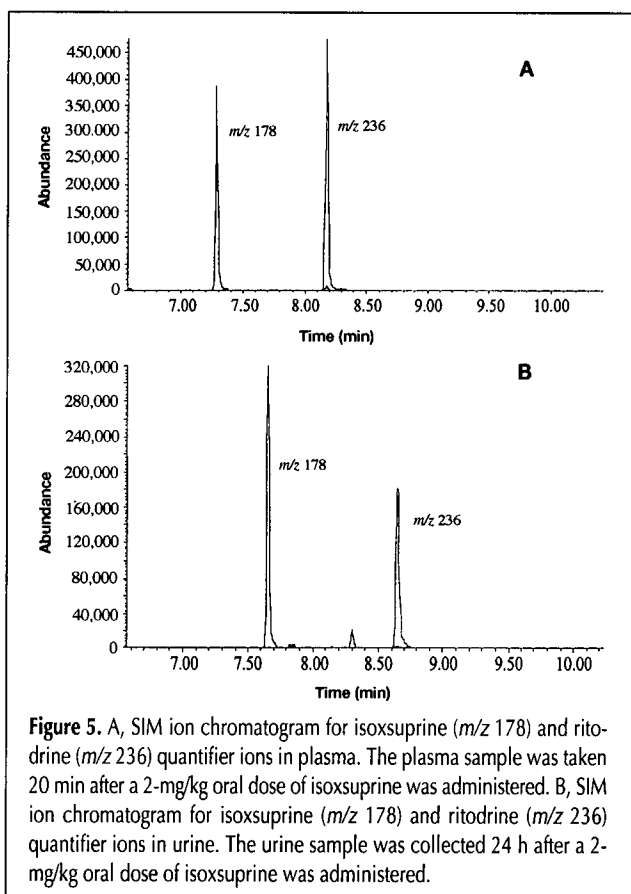

Figure 5. A, SIM ion chromatogram for isoxsuprine (m/z 178) and ritodrine (m/z 236) quantifier ions in plasma. The plasma sample was taken 20 min after a 2-mg/kg oral dose of isoxsuprine was administered. B, SIM ion chromatogram for isoxsuprine (m/z 178) and ritodrine (m/z 236) quantifier ions in urine. The urine sample was collected 24 h after a 2-mg/kg oral dose of isoxsuprine was administered.

with analyte and internal standard, yielding concentrations at low, medium, and high values within the range of the calibrator curve (50, 200, and 300 ng/mL). A duplicate set of blank matrix samples was generated without the addition of either analyte or internal standard. All samples were extracted. The unsupplemented samples were then supplemented with equivalent amounts of analyte and internal standard. A calibrator curve was run concurrently to provide for quantitative results. SIM peak area ratios for extracted analyte/internal standard were divided by the area ratios for the nonextracted samples.

Within-run and between-run accuracy was determined by analyzing five spiked samples of three different concentrations on each of three days. Each day, an independent calibrator curve was constructed. The accuracy was calculated by dividing the calculated value by the nominal value and multiplying this by 100 to report the result in percent. Precision was determined using the analysis of variance (single factor) procedure from Microsoft Excel (Microsoft Corp., Redmond, WA). Acceptable precision values were CV values less than 20%. The within-run and between-run CV values, respectively, were calculated using the following equations:

$$CV = \frac{\sqrt{MS_{wg}}}{GM} \times 100 \qquad \text{Eq. 1}$$

$$CV = \frac{\sqrt{\dfrac{MS_{bg} - MS_{wg}}{n}}}{GM} \times 100 \qquad \text{Eq. 2}$$

where MS_{wg} is the means squared within group, MS_{bg} is the means squared between groups, and GM is the grand mean.

Results

Development of the isoxsuprine method

Reaction of isoxsuprine with BSTFA + 1% (TMCS) was found to yield a single peak on GC–MS (Figure 1A), the mass spectrum of which was found to be consistent with a bis-TMS derivative. The mass spectrum (Figure 1B) had its highest visible fragment at m/z 430, consistent with loss of a methyl group from calculated M⁺ molecular ion of m/z 445, as well as an intense base peak at m/z 178, which MS Calc Pro 4.03 software (Quadtech Associates, Inc., Fairfield, CA) indicated as likely to have arisen from the $CH_3CHNHCH(CH_3)CH_2OC_6H_5$ fragment (Figure 4A). This was supported by the corresponding presence of the remainder of the molecule at m/z 267, with both fragments arising from cleavage of a C–C bond. The base peak was chosen to be utilized for quantitative studies, because of its intensity, and m/z 107, 135, and 179 were chosen as qualifier ions, as they were the next largest peaks in the spectrum.

The TMS derivatives gave excellent chromatography with maximized responses and

Table I. Compiled Results of Accuracy Trials in Plasma and Urine Collected from Three Consecutive Days of Analysis

			Accuracy (%)						
	Plasma					Urine			
Conc. ng/mL	Between Run	Within Run			Conc. ng/mL	Between Run	Within Run		
		Day	Day	Day			Day	Day	Day
		1	2	3			1	2	3
15	109	94	121	109	15	113	111	118	114
150	105	100	105	110	150	112	107	111	113
350	109	99	111	116	350	106	99	108	110

minimal tailing of the base peak or qualifier ions (shown nested within the base peak ion chromatograms in Figure 1). Figure 5 shows ion chromatography for matrix extracts with GC conditions starting at elevated initial temperatures, which provided rapid peak elution and relatively close retention times for isoxsuprine and its internal standard ritodrine.

The internal standard was chosen based on its structural similarity to isoxsuprine, its ability to yield a single chromatographic peak in relatively close proximity to isoxsuprine (within 1 min retention time) (Figure 2A), its ability to undergo reaction with the derivatizing agent, and its intense base peak at m/z 236 (mass spectrum in Figure 2B). This base peak, found suitable for quantitative work, arose from the equivalent C–C bond cleavage, as that in isoxsuprine-bisTMS, reassuring our earlier assignment as to its origin (Figure 4B). The largest visible peak in the ritodrine mass spectrum, m/z 488, again arose from loss of methyl from a calculated M+ molecular ion of m/z 503.

Extraction of series of standards from matrix with quantitation based on the ratio of the isoxsuprine m/z 178 peak area to the ritodrine m/z 236 peak area gave excellent linearity, with an example of such a standard curve shown for plasma in Figure 3.

Hydrolysis recovery

The stability of the isoxsuprine molecule to the conditions of enzyme hydrolysis employed was determined. A known quantity (160 ng/mL) of isoxsuprine was added to blank horse urine. Aliquots of the supplemented urine were incubated for 18 h at 37°C. The GC–MS-determined concentrations of isoxsuprine in the incubated samples were compared to the concentration determined for the corresponding standard (160 ng/mL) of the standard curve, which was supplemented immediately prior to extraction. The recovered isoxsuprine concentration after incubation at 37°C was 94.3% of the control value.

Validation of GC–MS-SIM method

The instrumental LOD in plasma was determined to be 2 ng/mL. The lower LOQ in plasma was determined to be < 5 ng/mL. The instrument LOD in urine was determined to be 4 ng/mL, and the lower LOQ was determined to be 7 ng/mL.

The recovery efficiencies for plasma in the 50-, 200-, and 300-ng/mL samples were 76%, 73%, and 76%, respectively. The recovery efficiencies for urine in the 50-, 200-, and 300-ng/mL samples were 92%, 89%, and 91%, respectively. The mean coefficient of determination (r^2) for the standard curves for plasma ($n = 19$) was 0.9925 ± 0.0052 and 0.9904 ± 0.0075 for urine ($n = 22$).

Accuracy and precision values for both plasma and urine are presented in Tables I and II. The between-run accuracy values for the 15-, 150-, and 350-ng/mL plasma samples were 109%, 105%, and 109%, repectively.

The within-run precision value (CV) for the 15-ng/mL plasma sample was 16.4%. The between-run precision value for the 15-ng/mL plasma sample was 9.2%. The within-run and between-run precision values for the 150-ng/mL plasma samples were 10.5% and 1.2%, respectively. The precision values for

Table II. Compiled Results of Precision Trials in Plasma and Urine Collected from Three Consecutive Days of Analysis

	Precision, CV (%)				
	Plasma			Urine	
Conc. (ng/mL)	Between Run	Within Run	Conc. (ng/mL)	Between Run	Within Run
1			1		
15	9.2	16.4	15	6.1	12.4
150	1.2	10.5	150	10.4	11.0
350	6.6	10.7	350	5.0	9.3

Figure 7. Time vs. concentration plot of total isoxsuprine in equine urine after a 2-mg/kg intravenous dose. Urine concentrations of total isoxsuprine remained above the LOQ in the urine through at least 72 h.

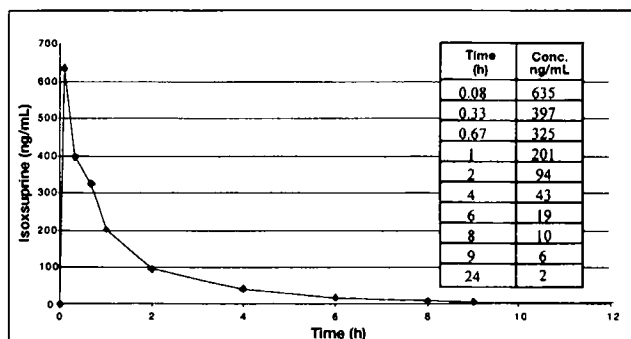| Time (h)	Conc. ng/mL
0.67	699,705
2	385,902
4	325,969
6	137,789
8	65,591
9	43,604
24	801

Figure 6. Time vs. concentration plot of free isoxsuprine in equine plasma after a 2-mg/kg intravenous dose. Samples were collected for 72 h, but values went below the LOQ after 24 h.
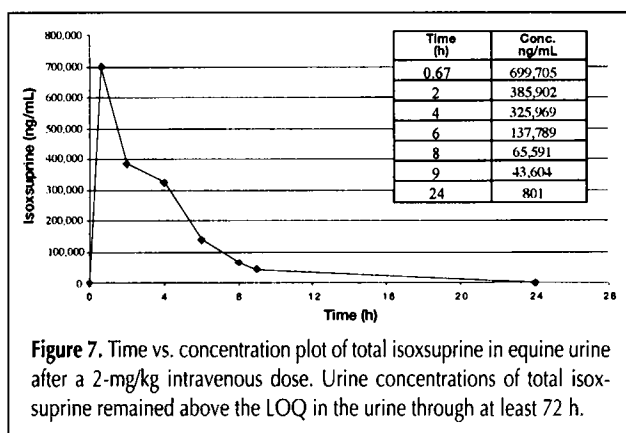
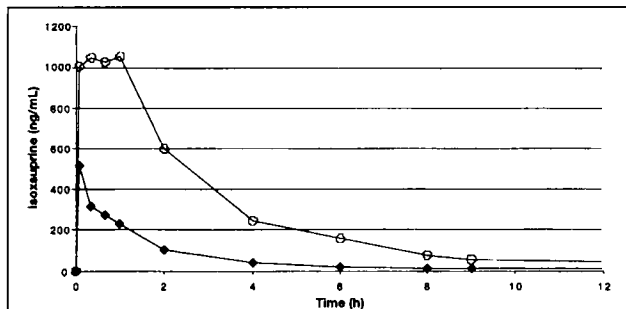| Time (h)	Conc. ng/mL
0.08	635
0.33	397
0.67	325
1	201
2	94
4	43
6	19
8	10
9	6
24	2

Figure 8. Time vs. concentration plot of both free and total plasma isoxsuprine in a mare dosed with 2 mg/kg isoxsuprine, intravenously. Total isoxsuprine concentration values are denoted by open circles, and free drug concentratons are denoted by solid squares.

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the 350-ng/mL plasma sample were 10.7% within run and 9.2% between run.

Analytical results

Typical time versus concentration plots of isoxsuprine in the plasma and urine in intravenously dosed mares are presented in Figures 6 and 7. In Figure 6, the values of free isoxsuprine in plasma from a mare dosed with 2 mg/kg of isoxsuprine are presented. The free drug plasma concentration appears to diminish rapidly. Figure 7 is the time versus concentration plot for isoxsuprine in the urine of a mare dosed with 2 mg/kg of isoxsuprine. This graph illustrates the extremely large concentration of isoxsuprine that can be excreted in the urine after dosing.

Figure 8 is a time versus concentration plot of both free and total isoxsuprine in the plasma of a mare intravenously dosed with 2 mg/kg isoxsuprine. It appears that the majority of isoxsuprine present in the bloodstream is in the conjugated form. The x-axis (time) is truncated to better illustrate the initial

Figure 9. Overview of reactions relevant to the processing of isoxsuprine from samples through an analytical GC–MS procedure as narrated in the Materials and Methods section. Note: Isoxsuprine-glucuronide metabolite is theoretically possible at either the phenolic or benzylic hydroxyl groups.

profile of the excretion of the drug from the blood. The 24-h plasma isoxsuprine sample concentrations, both free and total, were below the LOQ.

Discussion

The reported GC–MS method for the quantitation of isoxsuprine in equine plasma and urine appeared to be efficient in use of laboratory resources and repeatable during its application. This reported assay did not appear to be labor intensive in our hands. The use BSTFA/1% TMCS alleviated concerns with volatility, polarity, and repeatability of the injected samples. The chemistry involved in this method is summarized in Figure 9, showing the metabolic fate of the racemic dosage form as a glucuronide, its analytical hydrolysis, extraction and derivatization in the lab, and its cleavage in the mass spectrometer to yield the m/z 178 qualifier ion.

One aspect of the assay found to be of particular importance was the pH of the samples that were extracted. Much care was taken to verify that the pH of each sample was, indeed, in the specified range, owing to variation in the urine sample pH values.

The LODs (≤ 2 ng/mL) and LOQs (≤ 5 ng/mL) in plasma reflected the sensitivity of the assay. The LOD for this procedure was much less than the thresholds adopted by several racing jurisdictions (12). Administration of isoxsuprine yielded urinary concentrations greater than the LOD in horses dosed with varying amounts of the drug, and the drug was present in detectable levels in the urine for as long as 72 h.

This retention of isoxsuprine in the body may be caused by several factors. Isoxsuprine will bind to melanin and, despite its polarity, may be stored in body fat. In addition, glucuronide conjugates of several drugs are known to bind to tissue proteins (13). Isoxsuprine has been found in the urine of horses that have not been exposed to the drug for months. This lends credence to the theories of body storage (6). Also, entero-hepatic circulation is likely involved in the continued detection of the metabolite in urine for extended periods of time. Another scenario leading to the detection of isoxsuprine in horse urine could be from environmental contamination (14). These factors may also be the cause of post-race samples that test positive for isoxsuprine.

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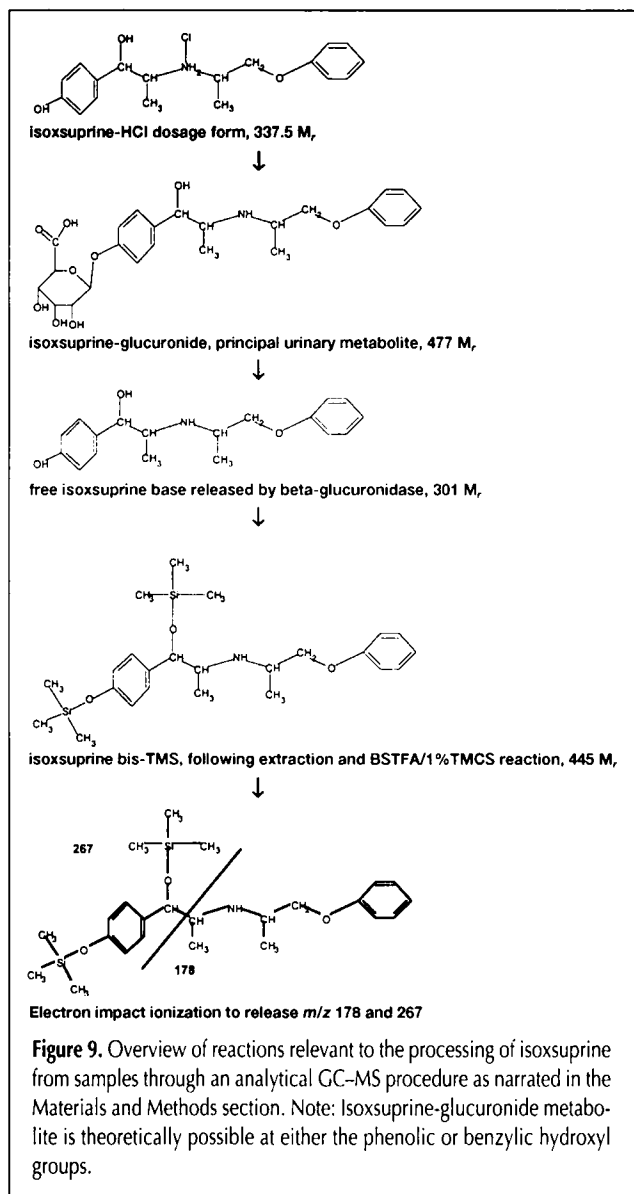
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Appendix A

Summary of AORC-proposed criteria for identification by chromatographic methods coupled with Mass Spectrometry (MS) (AORC, 2001)

Chromatography. Unknown and standard retention times must be in agreement.

MS. A minimum number of three specified diagnostic ions should be obtained for any technique. A diagnostic ion is defined as a molecular or fragment ion for which the presence and abundance are characteristic of the analyte.

The molecular ion should be included among the diagnostic ions if it is present at a relative abundance greater than 5% of the base peak.

The signal-to-noise ratio of the diagnostic ions must be equal to or greater than 3:1, measured by integrated single mass traces (single ion chromatograms).

Measured diagnostic ions with a relative intensity > 10% of base peak in the reference spectrum must be present in the test spectrum.

Maximum permitted difference (tolerance) for matching diagnostic ions.

The relative abundance (intensity) is the abundance of a particular ion relative to the most abundant diagnostic ion monitored expressed as a percentage.

Relative intensities are calculated by integrating the signals of single ion chromatograms.

The maximum permitted differences in relative abundance should be as follows:

Low resolution MS or selected ion monitoring, the greater of 5% absolute or 30% relative.

MS–MS and related techniques, the greater of 15% absolute or 35% relative.

The maximum permitted difference (tolerance) in relative abundance may be absolute or relative.

An absolute tolerance range of 5% is defined as the standard ion's relative abundance ± 5. For example, the acceptable absolute range for a relative abundance of 56% would be 56 ± 5, or a range of 51–61%.

A relative tolerance of a range of 30% is defined as the standard ion's relative abundance ± 30% of that abundance. For example, the acceptable relative tolerance range for a relative abundance of 60% would be 60 ± (30% of 60), or a range of 42–78%.

Extraneous ions in the test spectrum should not exceed 15% relative abundance.

More rigorous criteria may be appropriate for SIM data; if possible, a minimum of four diagnostic ions or stricter limits on relative intensities.