Development of Analytical Methods for the Detection of Metaraminol in the Horse*

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Abstract

Aramine® (metaraminol bitartrate) has been found in the possession of horse trainers and veterinarians who have been investigated for possible inappropriate drug administration to racing horses. Metaraminol (3-hydroxyphenylisopropanolamine) is a sympathomimetic amine that directly and indirectly affects adrenergic receptors, with alpha effects being predominant. Because it has the potential to affect the performance of a racing horse, its use is prohibited. In the present study, methods for the detection of metaraminol were developed. Metaraminol was found to be extracted with poor recovery (< 50%) from aqueous solutions by routine basic extraction or cation exchange/reversed-phase solid-phase extraction techniques. However, an extractive acetylation method gave good (> 90%) recovery of metaraminol from aqueous samples. Sequential urine samples collected from horses administered metaraminol intramuscularly at 0.02, 0.10, and 0.23 mg/kg were extracted by the developed extractive acetylation procedure and analyzed by gas chromatography-mass spectrometry (GC-MS) in full-scan and selected ion monitoring modes. Norphenylephrine was used as an internal standard for quantitative analysis. The maximum concentration of metaraminol occurred between 1 and 2 h postadministration. Metaraminol was detected in the 0.23 mg/kg administration urine for 24 h postadministration. Metaraminol was detected for the 0.10 and 0.02 mg/kg doses for approximately 8 h postadministration. No apparent biotransformation products were observed in a reaction mixture of metaraminol and horse liver microsomal reaction mixture. Comparison of gas chromatograms of the extracts of the postadministration urine samples with those of the preadministration samples failed to reveal any exogenous compound other than metaraminol.

Introduction

Metaraminol (3-hydroxyphenylisopropanolamine) is a sympathomimetic amine that has a pharmacological action that is similar to, but less potent and more prolonged than that of norepinephrine. In humans, it increases cardiac output, peripheral resistance, and blood pressure. Metaraminol is devoid of central nervous system stimulatory effects (1,2).

Metaraminol would be expected to cross-react with the sympathomimetic amine immunoassays currently in use in horse drug testing laboratories. Among the immunoassays used, the ELISA tests for sympathomimetic amines (amphetamine, methamphetamine, phentermine, mephentermine) have a high rate of false-positive reactions. Extensive analytical investigation of these samples have failed to detect any sympathomimetic amines or related metabolites. The horseracing industry has felt that metaraminol might be the cause of some of these false-positive ELISA results.

The detection of metaraminol and/or its metabolite in the urine of horses administered this drug has been unsuccessful. To regulate the use of this stimulant, analytical methods need to be developed to identify metaraminol and/or its metabolites in horse urine.

The purposes of this study were to 1. determine the metabolic fate of metaraminol in the horse, 2. determine the temporal urinary excretion profile of metaraminol and its major metabolites, and 3. develop analytical methods for the confirmation of the presence of metaraminol and/or its metabolites in horse urine.

Materials and Methods

Materials

Metaraminol tartrate (M-4773), β-glucuronidase (G-8132) from Putella vulgata, and trifluoroacetic acid (TFA, F1647) were obtained from Sigma Chemical Co. (St. Louis, MO). Norphenylephrine HCl (11,372-7) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Triethylamine (TEA, reagent grade, 04885-4), acetonitrile (OPTIMA®, A996-4), ethyl acetate (OPTIMA, E196-4), chloroform (OPTIMA, C297-4), isopropanol (OPTIMA, A464-4), acetic anhydride (reagent grade, A-10), sodium bicarbonate (certified grade, S233-500), potassium carbonate (certified grade, P179), and sodium chloride (certified grade,
S271-1) were obtained from Fisher Scientific Co. (Pittsburgh, PA). Hydrochloric acid (reagent grade, 95%) and ammonium hydroxide (reagent grade, 97-01-33) were obtained from J.T. Baker (Phillipsburg, NJ). Zorbas® Stable Bond C18 (2.1 mm x 150 mm, 3.5-µm particle size) high-performance liquid chromatography (HPLC) columns were obtained from Mac-Mod Analytical (Chadds Ford, PA). HP5 (30 m x 0.25 mm, 0.25-µm film thickness) GC columns were obtained from Hewlett-Packard (Wilmington, DE). Reversed-phase/cation-exchange (RP/CE, Xtract® R, XRDAP206, 200 mg/6 mL) extraction columns were obtained from United Chemical Technologies, Inc. (Brstol, PA). Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA, 3-3148) and trifluoroacetic anhydride (TFAA, 3-3165) were obtained from Supelco (Bellefonte, PA). Octyldecyl reversed-phase (Sep-Pak®, 36905) extraction columns were obtained from Waters Associates (Milford, MA).

### Preparation of metaraminol and norphenylephrine standard solutions

Stock aqueous and methanol solutions of metaraminol and norphenylephrine were prepared at 500 µg/mL in the appropriate solvent containing 10 µg/mL phenylpropanolamine. Working metaraminol analytical standards were prepared from the stock solution by appropriate dilutions with the solvent containing 10 µg/mL phenylpropanolamine. The working internal standard solution of norphenylephrine was prepared at 1.0 µg/mL by dilution of the stock aqueous solution with 10 µg/mL phenylpropanolamine/H₂O. The phenylpropanolamine helped prevent loss of standard by adsorption to the glassware.

### Gas chromatographic–mass spectrometric (GC–MS) system

For GC–MS analysis, a Hewlett-Packard HP5890 series II GC interfaced to a Hewlett-Packard HP5970 mass selective detector (MSD) was used. The injector temperature was maintained at 275°C, the interface temperature at 300°C, and the source temperature at 200°C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The MS was operated in the electron ionization (EI) mode. Full-scan spectra were collected from 43 to 543 da at 1 scan/s. Selected ion monitoring (SIM) experiments were performed by monitoring specific ions at a dwell time of 100 ms. One-microliter aliquots of samples were injected in the splitless mode.

### HPLC–MS system

HPLC analyses were performed on an HP1090 HPLC system interfaced to an atmospheric pressure chemical ionization (APCI) source on a Quatro MS. Solvent A consisted of 0.05% (v/v) TFA in reagent-grade water and solvent B consisted of 0.05% (v/v) TFA in acetonitrile. The mobile phase was delivered to the Zorbas® Stable Bond C18 (2.1 mm x 150 mm, 3.5-µm particle size) column at a flow rate of 0.417 mL/min. For extraction recovery experiments, metaraminol was eluted in an isocratic mobile phase consisting of 5% B/A. For the analysis of acetylated metabolites extracted from biological samples, the composition of the mobile phase was changed from 100% A to 100% B in 15 min with a 5.0-min isocratic hold at 100% B. Metaraminol, acetylated metaraminol, and acetylated norphenylephrine were detected in the MS in the APCI positive ionization mode (APCI+). The source temperature was 150°C, and the probe temperature was 450°C. Selected fragmentation ions (m/z 150 and m/z 168) were monitored by single ion reaction (SIR) at a cone potential of 20 V for the detection of metaraminol. Selected product ions (Ar, 30 eV) of the protonated molecular ions [M+H]⁺ were generated in the multiple reaction monitoring mode (MRM) at a cone potential of 5 V for the detection of acetylated metaraminol and acetylated norphenylephrine. Ion ratio spectra were constructed by plotting the ion chromatogram peak-area ratios to the base ion chromatogram peak area against the ion's mass-to-charge ratio.

### Metaraminol administration

Metaraminol administration

Aramine (metaraminol tartrate) was administered by intramuscular injection to three Standardbred horses; one at 0.23, one at 0.10, and one at 0.02 mg metaraminol/kg body weight. Urine samples were collected continuously via urethra catheter pre-administration and at timed segments postadministration. The urine samples were stored frozen until use.

### Evaluation of common drug-screening methods to detect metaraminol in horse urine

The 8 h post 0.23 mg/kg administration horse urine sample was processed by common drug-screening procedures used in racing chemistry laboratories. These included thin-layer chromatography (TLC) analysis of a paired ion and a base extraction, sympathomimetic amine enzyme linked immunosorbant assay (ELISA) and a GC–MS analysis of the TFAA derivatized base fraction from an RP/CE solid-phase extraction. Fluorescamine, Dragendorff, copper chloride, sodium nitrite, and ferric chloride reagents were used for visualization in the TLC analysis.

### Investigation of in vitro biotransformation of metaraminol

Horse liver was obtained from Michael Court at Tufts University (Boston, MA). Microsomes were isolated from 205 g of liver (3). The isolated microsomes were assayed for total protein (4) and cytochrome P450 (5) concentrations. Thirty micro liters of 1.25-mg/mL methanolic metaraminol or testosterone were added to each of two culture tubes. Testosterone was used as a positive standard to verify microsomal activity. The methanol was evaporated to dryness at 65°C under a stream of nitrogen. Co-factor solution (100 µL), 0.05M phosphate buffer (50 µL), and microsome solution (100 µL) were added to each culture tube. A matrix control sample was prepared by mixing the cofactor solution, the phosphate buffer, and the microsomes in a tube without substrate. A negative reaction mixture was prepared by adding the cofactor solution, the phosphate buffer and heat-denatured microsomes to tubes containing either testosterone or metaraminol. All microsomal mixtures were incubated at 37°C for 15 min. The enzyme reactions were terminated by heat denaturation at 100°C. Denatured protein was removed from the solution by centrifugation at approximately 1000 x g for 10 min. Ten microliters of the supernatant phase from the metaraminol incubation solution was analyzed on the HPLC–MS system in the full-scan mode using an isocratic mobile phase of 3% B/A. Ten microliters of the supernatant phase from the testosterone incubation solution was analyzed on the HPLC–MS system in the full-scan mode using
the solvent gradient mobile phase. The [M+H]+ ion peak area of the substrate in the negative reaction mixture and in the 15-min reaction mixture was used to calculate the percent change in substrate concentration.

**Determination of analytical detection characteristics**

Trimethylsilyl (TMS) derivatives of the phenolic and aliphatic alcohol functional groups on metaraminol were prepared by reacting 100 μL of BSTFA in 100 μL of ethyl acetate with metaraminol at 85°C for 1 h. The reagents were evaporated to dryness at 65°C under a stream of nitrogen. The acetyl derivative of the amine function was then prepared by reacting the residue with 100 μL of trifluoroacetic anhydride in 100 μL of ethyl acetate at 75°C for 30 min. The solvent was evaporated to dryness and the residue dissolved in 50 μL of BSTFA and 50 μL of ethyl acetate. One microliter of the TFA/TMS derivative was analyzed on the GC–MS system in the full-scan mode.

The acetyl derivatives of metaraminol and norphenylephrine were prepared by reacting the parent compound with 100 μL acetic anhydride and 10 μL TEA in 100 μL of ethyl acetate at 75°C for 30 min. The solvent was evaporated and the residue dissolved in 100 μL of ethyl acetate for GC–MS analysis or 100 μL of methanol for HPLC–MS analysis. One microliter of each derivative was analyzed on the GC–MS system in the full-scan mode. Five microliters of the derivative solutions were analyzed on the HPLC–MS system in the [M+H]+ product ion mode.

The up-front collision-induced (UPCID) spectrum of metaraminol was obtained by analyzing 5 μL of a metaraminol standard on the HPLC–MS system using an isocratic 5% B mobile phase and a cone potential of 15 and 50 V, respectively.

**Investigation of extraction efficiencies**

Several methodologies were evaluated for their ability to extract metaraminol from an aqueous solution. A 0.64-μg/mL concentration of metaraminol in reagent-grade water was used as the test solution. The following extraction procedures were performed in triplicate on this solution, and 5 μL of the recovered metaraminol (dissolved in 100 μL reagent-grade water) was analyzed on the HPLC–MS system using a mobile phase of 5% B. The peak area response of the SIR m/z 150 ion chromatogram was used to calculate metaraminol recovery.

**Weak base extraction, pH ~ 10.** Five milliliters of 0.64-μg/mL metaraminol was mixed with 0.5 mL of concentrated ammonium hydroxide. The solution was extracted twice with 5 mL of chloroform/isopropanol (3:1). The combined extract was evaporated to dryness under a stream of nitrogen at 65°C.

**Strong base extraction, pH > 12.** Five milliliters of 0.64-μg/mL metaraminol was mixed with 0.1N NaOH. The solution was extracted twice with 5 mL of chloroform/isopropanol (3:1). The combined extract was evaporated to dryness under a stream of nitrogen at 65°C.

**Diocetyl sulfoxoccinate ion paired extraction.** Five milliliters of 0.64-μg/mL metaraminol solution was mixed with 1 mL of 0.6-phosphoric acid diocetyl sulfoxoccinate in 0.5M pH 7.0 phosphate buffer (6). The solution was extracted twice with chloroform/isopropanol (3:1). The combined extract was evaporated to dryness under a stream of nitrogen at 65°C.

**Potassium carbonate salting-out extraction.** Potassium carbonate (2.5 g) was dissolved in 5.0 mL of the 0.64-μg/mL metaraminol solution. The solution was extracted three times with ethyl acetate. The combined extract was evaporated to dryness under a stream of nitrogen at 65°C.

**Reversed-phase/cation exchange solid-phase extraction.** World Wide Monitoring XtrackT solid-phase extraction columns were conditioned by passing 6 mL of methanol and 6 mL of 0.1M phosphate buffer (pH 6) through the column. Five milliliters of the 0.64-μg/mL metaraminol solution was mixed with 1.0 mL of the 0.1M phosphate buffer (pH 6). The total solution was transferred to the solid-phase extraction column and allowed to pass through the column under vacuum. The column bed was vacuum-dried for 5 min. Six milliliters of methanol was passed over the column under gravity feed and then pulled dry under vacuum. The column was then eluted with methylene chloride/isopropanol/ammonium hydroxide (78:20:2.0) under gravity feed. The collected eluent was evaporated to dryness under a stream of nitrogen at 65°C.

**Extractive acetylation.** Sodium bicarbonate (1 g) was dissolved in 5.0 mL of the 0.64-μg/mL metaraminol solution in a culture tube. Acetic anhydride (0.4 mL) was added slowly down the side of the tube so that it formed a layer on top of the aqueous solution. The solution was allowed to react at room temperature for 20 min. Another 0.5 g of sodium bicarbonate was added to the solution. The tube was capped, and the contents were mixed on a vortex-type mixer. (Note: this mixture has a tendency to effervesce and bubble over if the cap is not removed slowly.) The cap was carefully removed from the tube, and 3 mL of reagent-grade water and 3 g of sodium chloride were added. The solution was then extracted twice with 5.0 mL of ethyl acetate. The extracts were combined and evaporated to dryness under a stream of nitrogen at 65°C. The dried extract residue and the residue of known amounts of metaraminol were dissolved in 100 μL of acetic anhydride, 100 μL of ethyl acetate, and 10 μL of triethylamine. The mixture was heated at 75°C for 30 min and then evaporated to dryness under a stream of nitrogen at 65°C. The directly acetylated metaraminol standard residues were used to construct the standard curve and calculate the recovery of metaraminol from the aqueous solution.

**Determination of metaraminol excretion form(s).** Five milliliters of the pre-administration urine and 5 mL of the 8 h post 0.23 mg/kg metaraminol administration urine samples were processed by the extractive acetylation procedure. The pre-administration urine sample and the postadministration urine sample were also treated with either 0.38 mL of concentrated HCl or with 1.0 mL of 10,000 units/mL of β-glucuronidase prior to extractive acetylation. The extracts were dissolved in 100 μL of ethyl acetate and analyzed by GC–MS. The total and selected ion chromatograms of the 8-h urine extracts were compared to the respectively treated pre-administration urine chromatogram for the presence of peaks unique to the postadministration urine samples.

**Detection and quantitation of metaraminol in horse urine**

Each of the pre-administration and postadministration urine samples from the 0.23, 0.10, and 0.02 mg/kg-dosed horses were processed by an extractive acetylation procedure. The procedure was similar to that already described, except that 1.0 mL of
urine was used instead of 5.0 mL, 0.2 mg of sodium bicarbonate was used instead of 1 g, and 1.0 mL of 1.0-μg/mL norphenylephrine was added as an internal standard. Aqueous standards were co-processed with the samples, and the relative peak-area response of metaraminol in these known solutions was used to calculate the concentration of metaraminol in the urine samples. The residues from the sample and standard extracts were dissolved in 200 μL of ethyl acetate and analyzed by the GC–MS procedure, either in the full-scan mode or the SIM mode depending on the concentration range. Between each urine extract, 1 μL of 50% BSTFA in ethylacetate was injected to reduce the active sites in the injector and column.

Results and Discussion

The failure of horseracing laboratories to detect metaraminol in horse urine could have been due to its not being used in racing horses or that the methods commonly used to screen horse urine for drugs were not capable of detecting the presence of this drug or its metabolite(s). Analysis of the 8 h postadministration urine sample from a horse administered 0.23 mg/kg metaraminol by procedures commonly used in horse racing laboratories (TLC analysis of basic and ion-paired extracts, sympathomimetic amine ELISA analysis, and automated GC–MS analysis of the TFA/TMS derivative of RP/CE extracts) failed to detect metaraminol. Metaraminol was found to be present in this sample at 3.5 μg/mL by the developed extractive acylation/GC–MS procedure. These data suggested that methods currently used in racing chemistry laboratories would not detect the presence of metaraminol.

Another reason that metaraminol administration might not have been detected was that it was metabolized to another compound that the screening procedure did not detect. These rationale led to our investigation of the metabolism of metaraminol. When metaraminol was incubated with horse hepatic microsomes, no change in metaraminol concentration was observed after 15 min. The incubation of testosterone in a similar microsomal reaction medium resulted in a 48% reduction in the testosterone concentration and the detection of 17-ketotestosterone at a concentration similar to that of the remaining testosterone. Metaraminol did not appear to be metabolized by in vitro horse hepatic microsomal enzymes. The only species in which an oxidative metabolite of metaraminol has been reported was the guinea pig (7,8). In this species hepatic microsomal enzymes catalyzed the methylation of the meta phenolic functional group.

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Figure 1 shows the structures of metaraminol and norphenylephrine, which was used as an internal standard for quantitative analysis. The presence of primary amine, aliphatic hydroxyl, and phenolic functional groups suggested that these molecules were very polar, which might pose problems in their extraction from aqueous solutions. Additionally, it was apparent that efficient analysis of these compounds by gas chromatography would require the derivatization of these functional groups. Two different derivatives of metaraminol were evaluated for the quality of their EI mass spectral profiles. Figure 2 shows the EI spectrum of the metaraminol trifluoroacetyl/trimethylsilyl (TFA/TMS) derivative. Silylation was performed first, so the
aliphatic alcohol and phenolic functional groups were silylated. Treatment of the TMS derivative with TFAA should have resulted in the acetylation of the primary amine functional group. It is possible that the resultant amide function was also silylated; however, this is not usually a stable bond. Figure 3 shows the suggested structure of this derivative and a possible fragmentation pathway to the m/z 267 ion. The m/z 337 ion probably resulted from the loss of CF3 plus a hydrogen ion. These two ions, at reasonably high mass values, appear to be composed of significant portions of the original metaraminol structure and have relatively good (> 10%) intensity. This mass spectrum should be relatively diagnostic for the metaraminol structure.

Figures 4A and 4B show the EI mass spectra of the acetyl derivatives of metaraminol and norphenylephrine, respectively. Low collision energy APCl+ of these derivatives gave mass spectra with base ions at m/z 294 and m/z 280, respectively. This is consistent with the molecular weight of compounds resulting from the acetylation of the primary amine, aliphatic alcohol, and phenolic functional groups of these compounds. The ions at m/z 44 and m/z 86 in the acetylmethamphetamine spectrum appear to be indicative of the presence of a methyl group on the carbon atom bonded to the primary amine. In the case of norphenylephrine, where no additional alkyl group is bound to this carbon atom, the correlative ions were m/z 43 and m/z 73, respectively. Unfortunately, the higher mass ions, which would incorporate a greater amount of the compound's structure, were of relatively low intensity and might be difficult to detect or obtain reproducible intensities at low concentrations of metaraminol.

Analysis of metaraminol by HPLC did not require derivatization to obtain good chromatographic characteristics and APCl+ mass spectrometry could be used as a detector. Additionally, the collision energy in APCl+ MS could be adjusted so that the molecular ion and major diagnostic fragment ions could be generated. Figure 5 shows the APCl+ mass spectrum of metaraminol at two different collision energies.

The mass spectral profiles of compounds eluting from a chromatographic column contain the ions of all the compounds that elute into the source at any given time. If the chromatographic resolution is such that only one compound enters the source at a time, then the mass spectral profile should be that compound alone. If more than one compound elutes from the column at a given time, the mass spectral profile will be a mixture of the spectra of all the compounds. If, however, low collision energy is used in the APCl+ source so that the pseudomolecular ion is generated in high yield, it can be uniquely fragmented in a reaction chamber and its mass spectral profile obtained. Figure 6A and 6B show the [M+H]+ product ion spectra of acetylmethamphetamine and acetylnorphenylephrine, respectively. Each spectrum contains the respective [M+H]+ ion as well as several relatively intense fragment ions.

The ability to extract metaraminol from aqueous solutions was investigated using HPLC separation of the underivatized compound and detection by APCl+ MS. Table I shows the recovery of metaraminol by various extraction procedures. The greatest recovery of underivatized metaraminol was obtained by the RP/CE solid-phase extraction procedure. This method averaged just over 50% recovery. Brooks and Smith (9) reported a method of reducing the polarity of amine and alcoholic functional groups by acetylation prior to extraction. Under basic conditions, amine and alcoholic functional groups become acetylated. The less polar derivative is then extracted into an organic solvent. GC–MS analysis of the reaction products of this procedure revealed the presence of two peaks. If the residue from the extraction was subjected to further acetylation in acetic anhydride and triethylamine, one peak was observed. Experience in our laboratory has shown that primary amines and phenolic

![Figure 5. APCl+ mass spectrum of metaraminol at a cone potential of 15 V (A) and at a cone potential of 50 V (B).](https://academic.oup.com/jat/article-abstract/24/4/281/885320)

![Figure 6. Product ions of the m/z 280 [M+H]+ ion of the triacetyl metaraminol derivative (A) and product ions of the m/z 280 [M+H]+ ion of the triacetyl norphenylephrine derivative (B). The [M+H]+ ion was generated in the APCl+ mode using a cone potential of 5V. The daughter ions were generated by collision induced dissociation using argon gas and collision energy of 30 eV.](https://academic.oup.com/jat/article-abstract/24/4/281/885320)

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<th>Table I. Recovery of Metaraminol from Aqueous Solutions by Various Extraction Methods.</th>
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<td><strong>Extraction method</strong></td>
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<tr>
<td>Basic (pH ~ 10) into CIP* (3:1)</td>
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<tr>
<td>Basic (pH &gt; 12) into CIP (3:1)</td>
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<tr>
<td>K₂CO₃, salting out into EtOAc*</td>
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<tr>
<td>DSS–IP* into CIP (3:1)</td>
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<tr>
<td>RP/CE into MCIPAH*</td>
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<td>Extractive acetylation into EtOAc</td>
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* CIP = chloroform/isopropanol (3:1).
* DSS–IP = ion-pair extraction with dioctylsulfoxonate as counter-action.
* EIOAc = ethyl acetate.
* MCIPAH = methylene chloride/isopropanol/ammonium hydroxide (7:8:2:0.1:0.2).
functional groups tend to acetylate readily by extractive acetylation, whereas aliphatic alcohols and secondary amines tend to be only partially derivatized. Figure 7 shows a schematic of the probable reaction products of the procedure used for complete derivatization. Using this procedure metaraminol recovery was 104 ± 8% from an aqueous solution (Table I).

Based on the extraction recovery experiments and the GC–MS analysis, a procedure for the qualitative and quantitative analysis of metaraminol in horse urine using GC–MS was developed. Norphenylephrine was used as the internal standard. Metaraminol and the internal standard were extractively acetylated and concentrated prior to analysis. The compounds were detected by full-scan or selected ion monitoring of diagnostic ions for acetylmetharaminol and acetylnorphenylephrine.

The GC–MS and HPLC–MS procedures were applied to the pre-administration urine and the 8 h 0.23 mg/kg postadministration urine samples. Metaraminol was the only compound detected in the postadministration sample that was not detected in pre-administration samples. The concentration of metaraminol in this postadministration urine sample was similar whether or not the sample was pretreated with $\beta$-glu-

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**Figure 7.** Proposed products in the extractive acetylation/acetylation reaction with metaraminol.

![Diagram](https://example.com/diagram7.png)

**Figure 8.** GC–EI–SIM m/z 234 ion chromatogram of the extractive acetylated residues of 8 h postadministration (0.02-mg/kg metaraminol i.m.) horse urine (A), 0.50-μg/mL metaraminol aqueous standard (B), pre-administration horse urine (C), and 0.50-μg/mL metaraminol spiked horse urine (D).

![Graphs](https://example.com/graph8.png)

**Figure 9.** EI–SIM ion ratio of compound at Rt-13.76 min in 8 h postadministration (0.02-mg/kg metaraminol i.m.) horse urine GC chromatogram (A) and triacetyl derivative of metaraminol (B).

![Graphs](https://example.com/graph9.png)

**Figure 10.** HPLC–APCI–MRM m/z 294 $\rightarrow$ m/z 192 ion chromatograms of acetylated extract of 8 h postadministration (0.02-mg/kg metaraminol i.m.) horse urine (A), 0.50-μg/mL metaraminol aqueous standard (B), and 0.50-μg/mL spiked horse urine (C).
curonidase or subjected to acid hydrolysis. This suggested that in vitro metabolites (including glucuronide and sulfate conjugates) were not a major excretion form of metaraminol.

Figure 8B shows a m/z 236 reconstructed ion chromatogram of an extractive acetylated metaraminol standard. A peak at the same retention time (13.6 min) was detected in the extract of a horse urine sample collected 8 h following a 0.02-mg/kg metaraminol administration (Figure 8A). However, a peak with the same retention time also occurred in the extract of the pre-administration horse urine sample (Figure 8C). In the extract of the pre-administration horse urine sample spiked at 0.5 μg/mL metaraminol, the retention time of the apparent peak for acetylated metaraminol was shifted to 13.77 min, which was consistent with a peak in the 8 h postadministration urine sample (Figure 8D). The ion ratios of the compound at Rt 13.76 min in the 8 h postadministration urine extract (Figure 9A) were consistent with the ion ratios of the acetylated metaraminol standard at Rt 13.63 min (Figure 9B). It appeared that the GC retention time of acetylated metaraminol was influenced by the presence of compounds co-extracted from the urine samples.

The extractive acetylated metaraminol residues were dissolved in methanol and analyzed by HPLC–MS. Metaraminol was detected by monitoring diagnostic product ions of the m/z 294 [M+H]+ ion. Figure 10 shows m/z 194 reconstructed ion chromatograms of the 0.02 mg/kg 8 h postadministration urine extract, an acetylated metaraminol standard and a pre-administration urine spiked at 0.5 μg/mL metaraminol. The retention times in the sample and the spiked sample were consistent with that of the acetylmetharaminol standard. The m/z 294 product ion ratios from the compound at Rt 7.76 min in the 8 h postadministration sample extract were consistent with those of the acetylmetharaminol at Rt 7.81 min in the standard extract (Figure 11).

The probability of correct identification of a drug increases with the amount of structural information obtained. The use of GC–MS and LC–MS for the analysis of metaraminol results in greater confidence in its identification. These techniques are complimentary to each other. The resolution of GC is greater than that of the HPLC so there is less chance of co-eluting interferences. The tandem MS system has the advantage of verifying the molecular weight of metaraminol, and its collision-induced fragment ions are more intense than those in the electron ionization spectrum.

For quantitative analysis, aqueous standard curves were analyzed from 0.10 μg/mL to 10.0 μg/mL in the full-scan mode and from 0.025 μg/mL to 0.50 μg/mL in the SIM mode. The m/z 236 peak-area ratio of metaraminol to that of the m/z 234 peak area of the internal standard was used as the quantitative response. The slope of the standard curve (Figure 12 and Figure 13) of the concentration versus the relative peak area was used to calculate the concentration of metaraminol in the pre- and

![Figure 11. MRM m/z 294 product ion ratios for 8 h postadministration (0.02 mg/kg metaraminol) acetylated extract HPLC 7.76 min peak (A) and 0.5 μg/mL metaraminol aqueous standard acetylated extract HPLC 7.81 min peak (B). Ion at m/z 294 generated in APCI+ mode at a cone potential of 5 V. Daughter ion generated by collision-induced dissociation in argon at a collision potential of 30 eV).](https://academic.oup.com/jat/article-abstract/24/4/281/885320)

![Figure 12. Standard curve of metaraminol concentration versus peak area at m/z 234 of acetyl derivative relative to internal standard peak area at m/z 236 (RPA). Compounds were detected in the full-scan mode.](https://academic.oup.com/jat/article-abstract/24/4/281/885320)

![Figure 13. Standard curve of metaraminol concentration versus peak area at m/z 234 of acetyl derivative relative to internal standard peak area at m/z 236 (RPA). Compounds were detected in the SIM mode.](https://academic.oup.com/jat/article-abstract/24/4/281/885320)

![Figure 14. Urinary excretion profile of metaraminol in horse urine at various intramuscular administration doses.](https://academic.oup.com/jat/article-abstract/24/4/281/885320)
postadministration urine samples and in the control urine samples. The accuracy of the assay was approximately 95% at 0.5 
µg/mL and 1.0 µg/mL and approximately 84% at 0.1 µg/mL. A 
signal-to-noise ratio was used for the detection limit for the 
GC–MS analysis. By this criteria the detection limit was 0.03 
µg/mL in the SIM mode and 0.5 µg/mL in the full scan mode.

Figure 14 shows the temporal urinary excretion profile for 
metaraminol administered by intramuscular injection at 0.23, 
0.10, and 0.02 mg/kg i.m. The maximum concentration of 
metaraminol occurs between 1 and 2 h postadministration. 
Metaraminol was detected in the 0.23 mg/kg administration 
urine for 24 h postadministration. Metaraminol was detected in 
the 0.10- and 0.02-mg/kg dose for approximately 8 h postad-
ministration.

Conclusions

The data collected in this study suggest that metaraminol is 
not metabolized to oxidative, reductive, or conjugated products 
but is primarily excreted as the parent drug in horses. Proce-
dures commonly used in drug-testing laboratories for the general 
detection of drugs failed to detect the presence of metaraminol in horse urine. Using extractive acetylation and 
GC–MS or HPLC–MS analysis, metaraminol was detected up to 
24 h in the urine of a horse given a high i.m. dose of metaraminol. 
At lower doses, metaraminol was detected in the urine for approxi-
mately 8 h.

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