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Contents

The Detection and Biotransformation of Guanabenz in Horses: A Preliminary Report*

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ABSTRACT

Guanabenz (2,6-dichlorobenzylidene-aminoguanidine) is a centrally acting antihypertensive drug whose mechanism of action is via α_2 adrenoceptors or, more likely, imidazoline receptors. Guanabenz is marketed as an antihypertensive agent in human medicine (Wytensin tablets, Wyeth Pharmaceuticals). Guanabenz has reportedly been administered to racing horses and is classified by the Association of Racing Commissioners International as a class 3 foreign substance. As such, its identification in a post-race sample may result in significant sanctions against the trainer of the horse. The present study examined liquid chromatographic/tandem quadrupole mass spectrometric (LC-MS/MS) detection of guanabenz in serum samples from horses treated with guanabenz by rapid IV injection at 0.04 and 0.2 mg/kg. Using a method adapted from previous work with clenbuterol, the parent compound was detected in serum with an apparent limit of detection of approximately 0.03 ng/ml and the limit of quantitation was 0.2 ng/ml. Serum concentrations of guanabenz peaked at approximately 100 ng/ml after the 0.2 mg/kg dose, and the parent compound

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was detected for up to 8 hours after the 0.04 mg/kg dose. Urine samples tested after administration of guanabenz at these dosages yielded evidence of at least one glucuronide metabolite, with the glucuronide ring apparently linked to a ring hydroxyl group or a guanidinium hydroxylamine. The LC-MS/MS results presented here form the basis of a confirmatory test for guanabenz in racing horses.

■ INTRODUCTION

Guanabenz (2,6-dichlorobenzylidene-aminoguanidine) is a centrally acting antihypertensive drug whose mechanism of action is through either the activation of α_2 adrenoceptors or, as more recent research suggests, imidazoline receptors.[1] Guanabenz has been marketed in human medicine as an antihypertensive agent for many years (Wytensin, Wyeth Pharmaceuticals).

Anecdotal reports suggest that racetrack veterinarians have been dissolving 8 mg tablets of guanabenz in a vitamin B_{12}/alcohol solution and injecting this mixture IV into horses before a race.[2] The rationale for this administration is to reduce pulmonary blood pressure, thereby reducing the incidence and severity of exercise-induced pulmonary hemorrhage (EIPH). Conversely, as an α_2 agonist agent, guanabenz may have the ability to tranquilize or sedate a horse and may also have some bronchodilator activity.

Medications capable of improving the success of racehorses are classified by the Association of Racing Commissioners International (ARCI) based on their performance-enhancing potential. Guanabenz is currently classified as an ARCI class 3 agent[3] and, as such, its identification in postrace samples can result in significant penalties. More recently, attempts have been made to reclassify guanabenz as an approved therapeutic medication. However, such applications have not been pursued, and guanabenz is currently a medication whose administration to a horse shortly before racing is considered inappropriate.

No useful postrace confirmation method for guanabenz has been previously reported in the literature, apparently because the effective dose of guanabenz is relatively small (8 to 24 mg/horse) and also because guanabenz is extensively metabolized. Therefore, the objective of the present study was to develop an LC-MS/MS–based multiple-reaction monitoring (MRM) serum method, chromatographically similar to a highly sensitive LC-MS/MS–based serum method for clenbuterol recently reported from this laboratory.[4]

■ MATERIALS AND METHODS

Horses

Two mature Thoroughbred mares weighing 468 and 499 kg were used for this study. The animals were fed grass hay and a concentrate feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet, twice daily. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin (200 µg/kg). A routine clinical examination was performed to assure that the animals were healthy and sound. Animals used in this study were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee, which also approved the experimental protocol. Horses received one dose of guanabenz (0.04 or 0.2 mg/kg), and the observed and predicted serum values were determined.

Sample Collection

Whole-blood samples (10 ml) were collected from the jugular vein into serum collection tubes at 0, 5, 10, 15, 20, and 30 minutes and 1, 2, 4, 8, and 24 hours after administration. Serum was separated from each sample and divided into five aliquots, which were frozen in

at –20°C until assayed.

Analytical Detection of Guanabenz

Guanabenz acetate was obtained from RBI Pharmaceuticals and clenbuterol hydrochloride from Sigma Chemical Company. Guanabenz (1 mg/ml) and clenbuterol (10 µg/ml) were dissolved in methanol and stored in a freezer. These standards were allowed to come to room temperature (23°C) before use. With each analytical run, serial dilutions were made from the stock guanabenz standard and added to blank serum to prepare a standard curve in the range of 0.5 to 100 ng/ml. Prior to extraction, 10 µl of clenbuterol (10 µg/ml) was added to each sample as an internal standard.

Extraction of Guanabenz

For guanabenz recovery, serum samples (2 ml) were transferred by pipette into 15-ml screw-cap tubes. Clenbuterol internal standard, 200 µl of ammonium hydroxide (concentrate), and 5 ml of dichloromethane were added to each tube. Tubes were shaken on a reciprocating shaker for 30 minutes and then centrifuged at 730 ×*g* for 20 minutes. If excessive emulsion was present following centrifugation, the tubes were mixed gently by inversion then recentrifuged to better separate the layers. The aqueous layer was then aspirated to waste. The dichloromethane extracts were decanted into silanized test tubes and evaporated to dryness under a stream of gaseous nitrogen below 40°C in a water bath. A quantity (80 µl) of the initial mobile phase was added to each tube, which was then agitated on a vortex mixer to redissolve the dried residue. This solution was transferred to a microinsert in an automatic liquid sampler vial from which 25 µl was injected for chromatography.

Liquid Chromatography

High-pressure liquid chromatography (HPLC) was accomplished on a Model 1050 HPLC (Agilent) with a Luna (Phenomenex) (5 µ × 250 mm × 2 mm) phenyl/hexyl column. The column was maintained at 30°C. A water–formic acid–acetonitrile gradient was applied at a flow rate of 0.45 ml/minute, producing a pressure of approximately 200 bar. The mobile phase is described in Table 1. Both guanabenz and clenbuterol were eluted as discrete symmetric peaks between 6 and 7 minutes.

Mass Spectrometry/Mass Spectrometry

Full-scan electrospray ionization (ESI) mass spectra were obtained on analytical standards at

TABLE 1. Description of Mobile Phase for Liquid Chromatographic Evaluation of Guanabenz

...utes	*Action*	*Mix*	*Mobile Phase*
		100%	Water, 0.05% formic acid, 5% acetonitrile
	linear gradient to:	10.5%	Water, 0.05% formic acid, 5% acetonitrile
		89.5%	Acetonitrile, 0.05% formic acid
0	maintain:	10.5%	Water, 0.05% formic acid, 5% acetonitrile
		89.5%	Acetonitrile, 0.05% formic acid
0.5	linear gradient to:	100%	Water, 0.05% formic acid, 5% acetonitrile
-15	maintain:	100%	Water, 0.05% formic acid, 5% acetonitrile

10 µg/ml in acetonitrile:0.05% formic acid (aqueous), 50:50 by infusion at 1.2 ml/hour via a Harvard syringe pump into the electrospray probe of a Quattro II (Micromass) MS/MS set in positive ion mode. All spectra were optimized by combination of 1 to 2 minutes of uniformly acquired data, background subtraction, and peak smoothing. Both daughter and parent ion analyses were also performed.

Mass Spectrometer Tuning

The mass spectrometer was tuned by direct injection of 10 ng/µl guanabenz in acetonitrile:0.05% formic acid (aqueous), 50:50. The peak shape and intensity of the monoprotonated guanabenz *m/z* 231 ion were optimized by adjustment of capillary, HV lens, cone voltage, skimmer lens, and RF lens settings. Skimmer lens offset was left at 5 V. Collision gas (argon) and collision energy were adjusted for collisionally induced dissociation in the central hexapole by optimizing settings for the *m/z* 172 daughter ion from 231 in MS2. Generally, the collision gas was set to 3 × 10^{-3} mbar. Increasing the photomultiplier setting 100 to 150 V above the usual setting (650 V) was found to sufficiently increase the sensitivity. In general, the source cone voltage was set at +26 V; the collision energy was set between −22 and −24 V; the capillary of the ESI probe was set at +3.1 KV; and the HV lens was set at +0.52 KV. Source temperature was set at 120°C.

Mass Spectrometry of Guanabenz

Mass spectral data were acquired on a Quattro-II MS/MS operated in ESI positive mode. (ESI[+]) MRM data were acquired as in Table 2, with an interchannel delay of 0.01 seconds.

Quantitation was based on the guanabenz *m/z* 231→172 transition and its ratio to the clenbuterol *m/z* 277→203 transition. Additional guanabenz confirmatory ion transitions monitored included *m/z* 231→214, 233→216, 233→174, and 235→176. Calculations were performed using the Quattro MassLynx software (version 3.1), fitting the unknowns to a linear regression standard curve ($r > .98$) with 1/x weighting (giving more significance to lower values).

Guanabenz Glucuronide

For glucuronide analyses, urine was collected from horses treated 2 hours previously with guanabenz (0.04 mg/kg) by IV injection. The urine was passed through a filter with a cutoff of 3,000 molecular weight to remove high molecular weight materials, and diluted 1:10 with

TABLE 2. Multiple Reaction Monitoring Data Acquired by Mass Spectrometry of Guanabenz, With an Interchannel Delay of 0.01 Seconds

Parent (m/z)	*Daughter (m/z)*	*Dwell (sec)*	*Purpose*
231.0	172.0	0.10	Guanabenz quantitative transition
231.0	214.0	0.05	Guanabenz qualifier
233.0	216.0	0.05	Guanabenz qualifier
233.0	174.0	0.05	Guanabenz qualifier
235.0	176.0	0.05	Guanabenz qualifier
277.0	203.0	0.10	Clenbuterol (internal standard) quantitative transition
279.0	205.0	0.05	Clenbuterol qualifier

acetonitrile:0.05% formic acid (aqueous), 50:50. The mixture was infused at 1.0 ml/hr via a Harvard syringe pump into the electrospray probe of the instrument set in positive mode.

Pharmacokinetics of Guanabenz

Pharmacokinetic analyses were performed using a nonlinear regression program (Winnonlin version 3.1; Pharsight Corp). Area under the curve (AUC) was measured with a linear trapezoidal approximation with extrapolation to infinity, and slope of the terminal portion (β) of the log serum concentration versus time curve was determined by the method of least-squares regression.

The compartmental model used is represented by the following general equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is plasma concentration of compound at any time (t), and A and B are the Y-intercepts associated with distribution and elimination phases, respectively. The rate constant of distribution (α) 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 $t_{1/2}\ \beta = \ln 2/\beta$ and total body clearance (Cl_s) was calculated as $Cl_s = \text{dose}/AUC_{0-\infty}$.

The volume of distribution in the central compartment (Vd_c) was calculated using the formula $Vd_c = \text{dose}/A + B$. Volume of distribution at steady state (Vd_{ss}) was calculated according to the formula $Vd_{ss} = Vd_c[K_{12} + K_{21}/K_{21}]$, where K_{12} and K_{21} are distribution rate constants from central to peripheral and from peripheral to central compartments, respectively. K_{10} is the elimination rate constant and is calculated as $K_{10} = \alpha\beta/K_{21}$.

TABLE 3. Pharmacokinetic Parameters of Guanabenz in Two Horses Following Single Administration (0.04 and 0.2 mg/kg) by IV Injection

Parameter	*0.04 mg/kg*	*0.2 mg/kg*
K_{10} (/min)	0.0453	0.056
α (/hr)	0.2518	0.2512
β (/hr)	0.0059	0.012
$t_{1/2}\ K_{10}$ (min)	15.28	12.43
$t_{1/2}\ \alpha$ (min)	2.75	2.76
$t_{1/2}\ \beta$ (min)	118	56
$AUC_{0-\infty}$ (ng/ml/min)	998.54	3542.3
Cl_s (ml/h)	334	471
Vd_c (L/kg)	0.88	1.013
Vd_{ss} (L/kg)	5.72	3.76
R^2	0.97	0.99

α = rate constant of distribution; $AUC_{0-\infty}$ = area under the serum concentration versus time curve; β = 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; $t_{1/2}$ = distribution half-life; $t_{1/2}\ \beta$ = terminal half-life; Vd_c = volume of distribution in the central compartment; Vd_{ss} = volume of distribution at steady state.

■ RESULTS

HPLC chromatograms of the guanabenz standard and 8-hour serum sample after guanabenz dosing (0.04 mg/kg IV) are shown in Figures 1A and 1B. The upper panels in each chromatogram trace the *m/z* 277→203 fragmentation for the clenbuterol internal standard, and the lower panels follow the *m/z* 231→172 fragmentation for guanabenz. Chromatographic simultaneity of product ion transitions acquired for guanabenz, including the *m/z* 231→172 transition for guanabenz quantitation and four qualifier ion transitions observed as nested sets during gradient HPLC, is also shown in Figure 1C. It is recommended

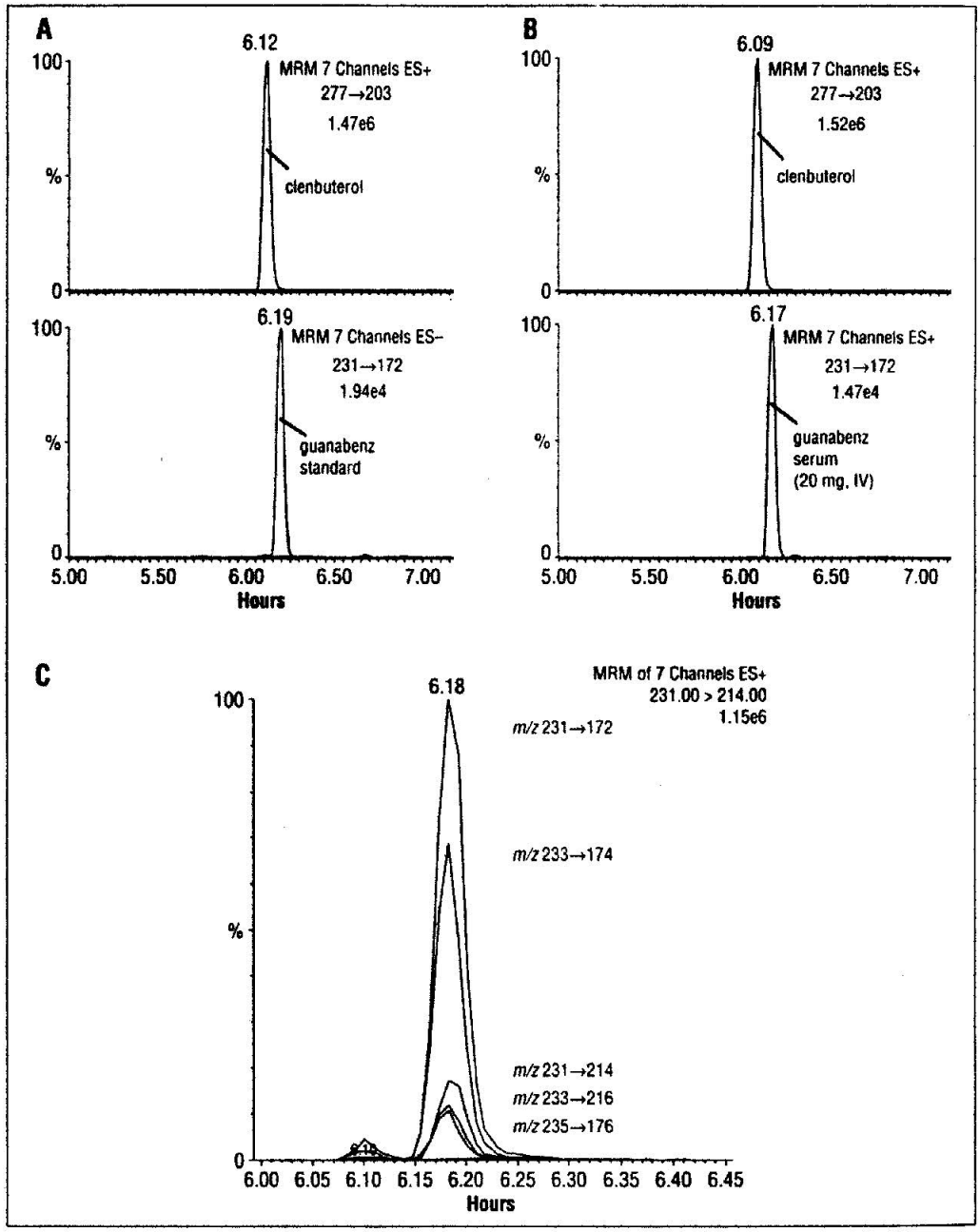

Figure 1. *HPLC chromatograms of guanabenz standard* **(A)** *and 8-hour serum sample* **(B)** *from one horse after dosing (0.04 mg/kg). Upper panels show the 277→203 transitions for the clenbuterol internal standard, and lower panels show the 231→172 transitions for guanabenz standard and 8-hour serum of a treated horse, respectively. HPLC chromatogram of guanabenz standard* **(C)** *showing* m/z *231→172 transition for quantitation and four qualifier ion transitions specific to guanabenz.*

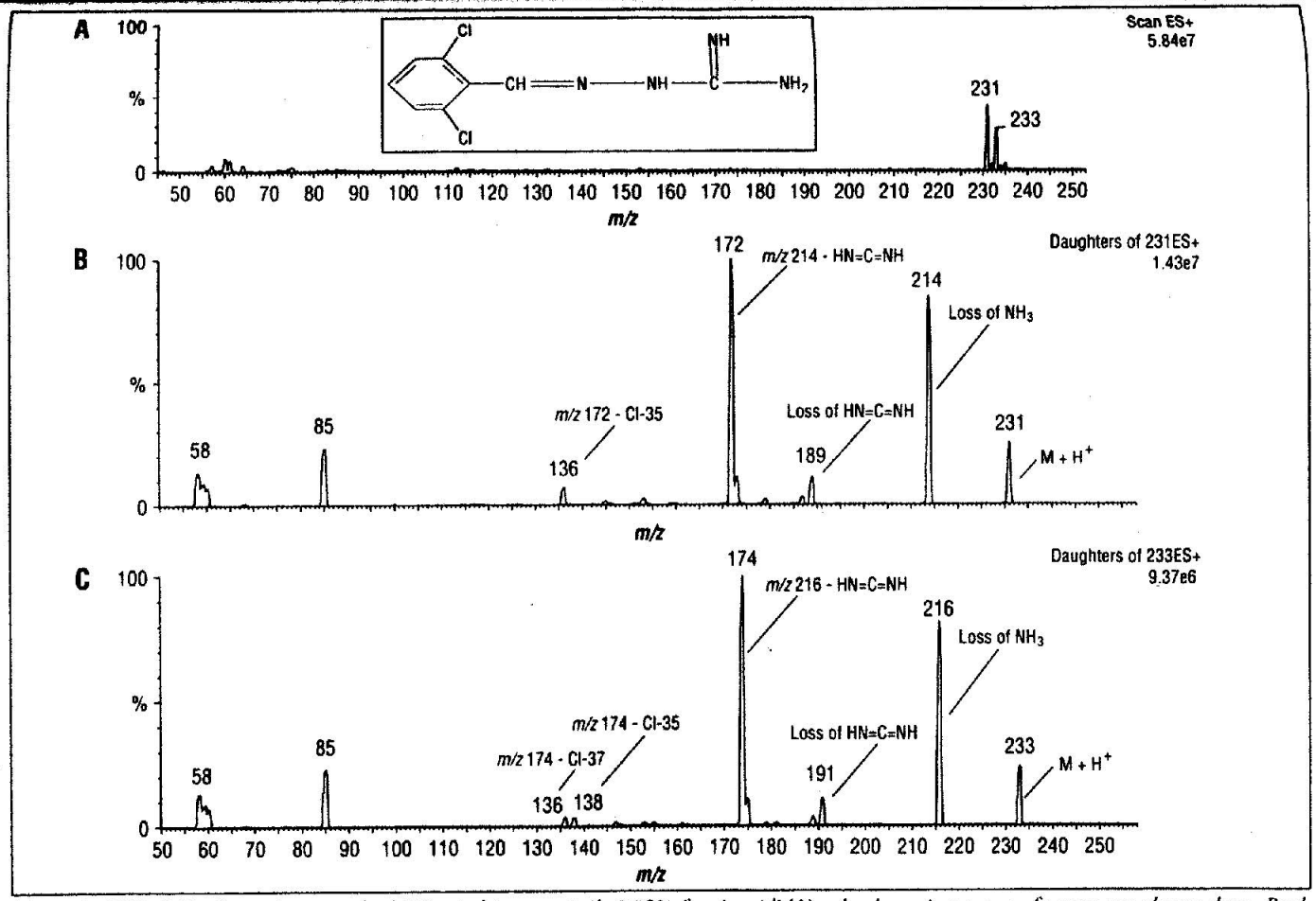

Figure 2. *ESI[+]MS of guanabenz standard (10 μg/ml in acetonitrile:0.05% formic acid)* **(A)**; *also shown is structure of unprotonated guanabenz. Product ion spectra for the principal ion* m/z *231* **(B)** *and the principal ion* m/z *233* **(C)**. *Product ions differing due to chlorine atoms are labeled with interpretations. The common m/z 58 and 85 ions represent* $HN=C(NH)NH_2$ *and loss of* $C_6H_4Cl_2$, *respectively.*

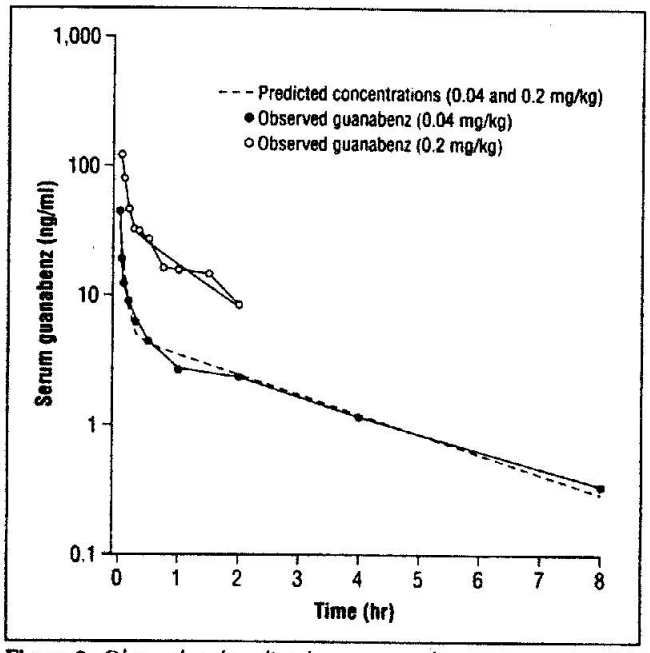

Figure 3. *Observed and predicted serum guanabenz concentrations following IV injection of guanabenz (0.04 and 0.2 mg/kg) in horses.*

that guanabenz confirmation include several such qualifier transitions to serve as diagnostics for accuracy in assignment of the presence of guanabenz, as is typically done according to laboratory standard operating procedures during selected ion monitoring mass spectrometry. The areas for the specific product ions measured relative to the largest transition were as follows (measurements summarized from 19 standards and unknowns): *m/z* 231→172, 100%; *m/z* 233→174, 61.2% ± 8.4%; *m/z* 231→214, 17.8% ± 1.7%; *m/z* 233→216, 12.1% ± 3.2%; *m/z* 235→176, 10.1% ± 1.8% (Figure 1C).

The mass spectrum of the guanabenz standard (Figure 2A) shows peaks at *m/z* 231, 233, and 235 in the isotopic ratio pattern characteristic of dichloronated organic compounds (unprotonated structure of guanabenz overlayed in Figure 2A). Product ion spectra for the principle ions (*m/z* 231 and 233, respectively) seen in the mass spectrum are shown in Figures 2B and 2C. Interpretation of the product ion fragments is noted.

Predicted logarithmic serum guanabenz concentrations and the actual rapid decline in serum guanabenz concentration after IV administration at two dosages (0.04 and 0.2 mg/kg) are presented in Figure 3. Initial concentrations of parent compound were approximately 120 ng/ml for the 0.2 mg/kg dosage and 50 ng/ml for 0.04 mg/kg. The predicted line was generated by assumption of a two-compartment model and application of the Winnolin pharmacokinetics program. Pharmacokinetic parameters of guanabenz are shown in Table 3.

Mass spectra for the 0 hour (control) and the 2-hour urine samples after guanabenz dosing (20 mg) are presented in Figures 4A and 4B. There is an increased abundance of the *m/z* 423, 424, 425, 426, and 427 ions relative to control; the 423, 425, and 427 ions suggest the isotopic pattern of a dichlorinated organic compound, implying the presence of a guanabenz metabolite. One reasonable, predicted structure of a high-molecular weight metabolite would be a glucuronide of a hydroxylated guanabenz species. Mass Spec Calculator Pro software predicts the isotopic pattern for such a compound in Figure 4C. The chart insert in Figure 4C compares the calculated ion pattern with the observed pattern. The relative congruence of the two clusters suggested the need to examine the product ions of the major fragments.

Product ion spectra for the *m/z* 423, 425, and 427 ions shown in Figure 4B are present-

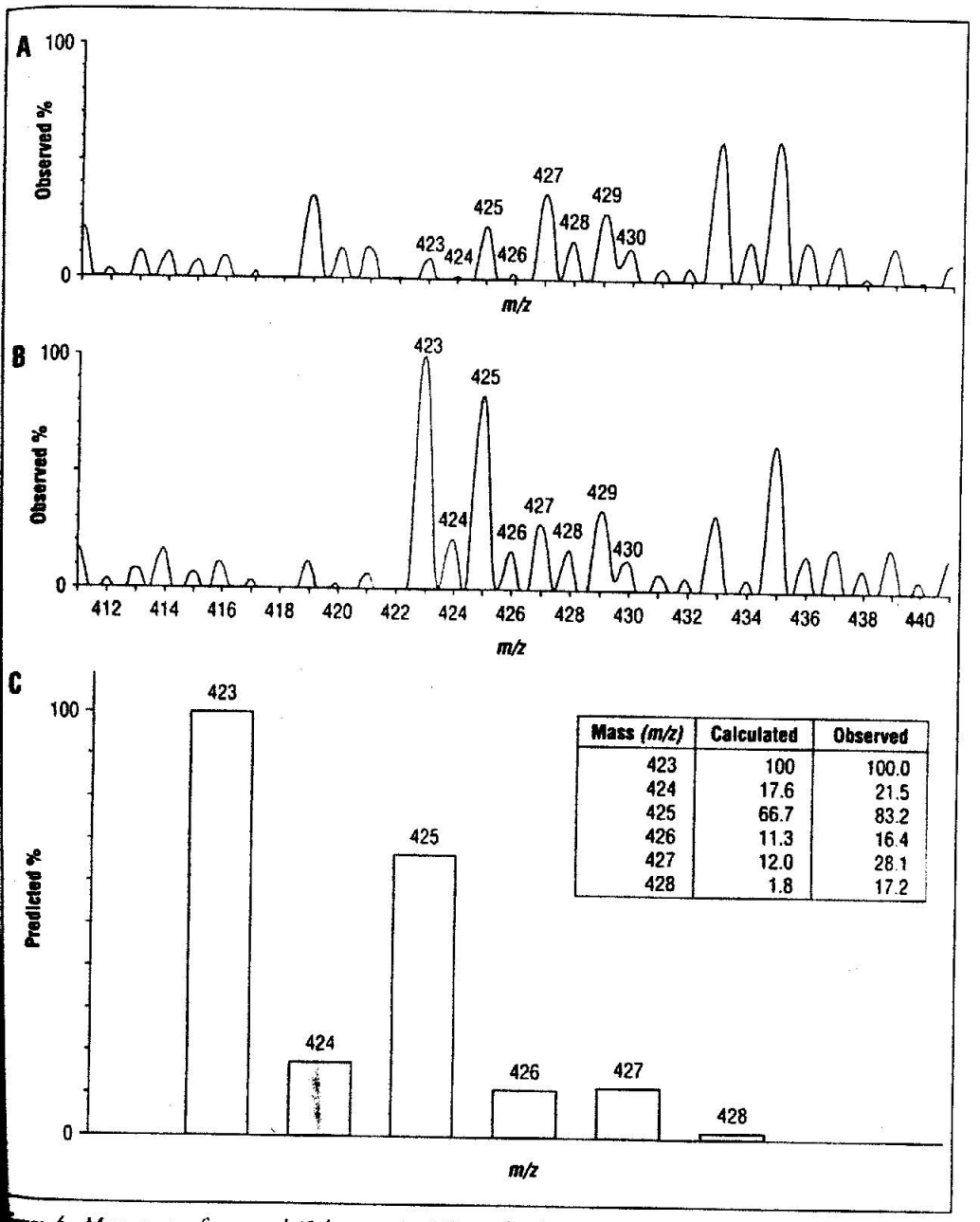

Figure 4. *Mass spectra for control (0 hour) urine* **(A)**, *and 2-hour samples* **(B)** *after IV dosing of guanabenz (2.04 mg/kg). Predicted molecular ion isotope for the glucuronic acid metabolite of 3-hydroxyguanabenz, 4-hydroxyguanabenz, or guanoxabenz, with identical molecular formulas* **(C)** *(see Figure 6).* **Inset chart** *shows the calculated abundance (in Figure 4C) and the observed abundance (in Figure 4B) of the various [M+H] isotopes.*

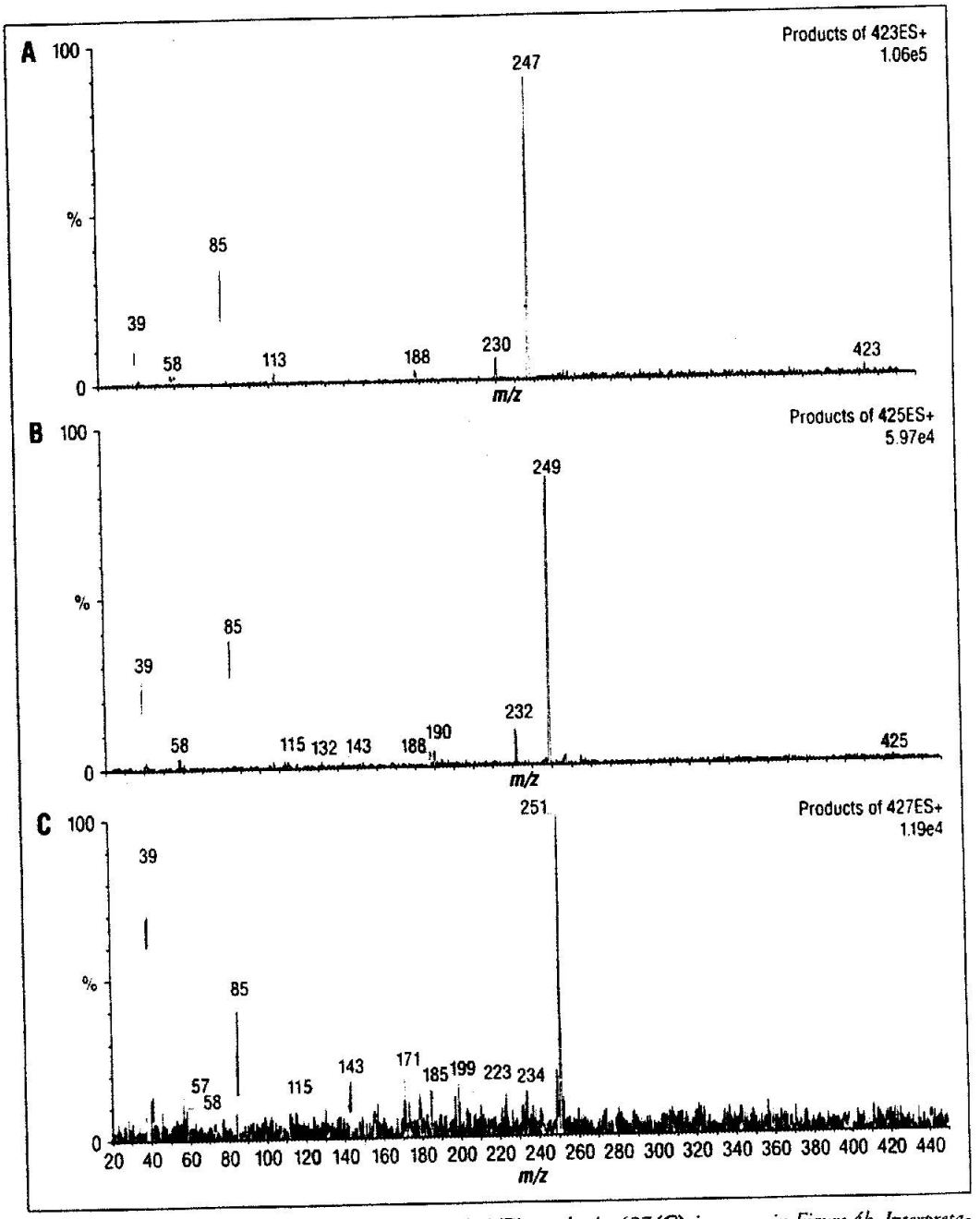

Figure 5. *Product ion spectra for* m/z *423* **(A)** *m/z 425* **(B)**, *and* m/z *427* **(C)** *ions seen in Figure 4b. Interpretation of the ESI[+]MS product ion fragments consistent with putative hydroxyguanabenz glucuronide structures evident in Figure 6 are shown in Table 4.*

ed in Figure 5. The principal M+H pseudomolecular precursor ion at *m/z* 423 has major product ions at *m/z* 247, 230, 188, 113, 85, 58, and 39 (Figure 5A). The other two major M+H isotopic peaks at *m/z* 425 and 427 have many of the same ions with +2 or +4 increases in mass/charge ratios, respectively. This is true for *m/z* 425, 249, and 232, in Figure 5B, and 427, 251, and 234 in Figure 5C. Other ions (*m/z* 85, 58, and 39) are also in common. These relationships suggest that *m/z* 423, 425, and 427 are isotopic variants of the same structure. This self-consistent data set is summarized in Table 4, including corresponding peak and fragment structure assignments, consistent with an assigned glucuronide structure.

Three alternative structures for guanabenz-O-glucuronide deduced from the evidence in Figures 4 and 5 are shown in Figure 6.

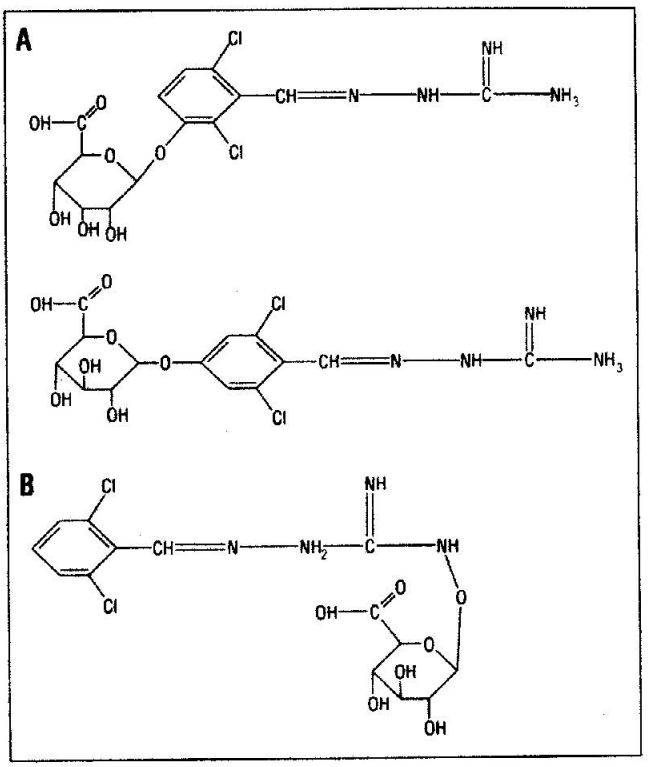

Figure 6. *Alternative structures for the guanabenz-O-glucuronide from evidence provided in Figures 4 and 5. Possible structures include 423 MW ($^{35}Cl_2$) as drawn ($C_{14}H_{17}N_5O_7Cl_2$) and are monoprotonated [M+H]$^+$ ions: 3- and 4-hydroxyguanabenz-O-glucuronide* **(A)**, *and guanabenz hydroxylamine O-glucuronide* **(B)**, *which is supported by the literature for human metabolism of guanabenz and by the assignments in Figure 5.*

Assay Validation

The level of detection (LOD) for guanabenz in serum was 0.3 ng/ml. The LOD is defined as the concentration below which the ion transition ratios for the analyte fail to meet the criteria proposed by the Association of Racing Chemists. Ion transitions used for determining the LOD were 231→172, 231→214, 233→174, 233→216, and 235→176.

The limit of quantitation (LOQ) for guanabenz in serum was 3 ng/ml. The LOQ is defined as the concentration below which the coefficient of variation (CV) of the assay exceeds 20%. This value was determined by analysis of 10 serum samples of different concentrations on three different days. A plot of CV versus concentration showed increasing CV with decreasing concentration, with the CV exceeding 20% at a concentration of 3 ng/ml.

Precision and accuracy of the assay were determined from analysis of spiked samples on five different days. Analysis at a concentration of 50 ng/ml yielded a value of 49.8 ± 3.98, and at 5 ng/ml the value was 4.8 ± 6.44 for between-run CVs of 8.0% at 50 ng/ml and 13.5% at 5 ng/ml.

TABLE 4. Interpretation of the Electrospray Ionization (ESI[+]MS) Product Ion Fragments of the Putative Hydroxyguanabenz Glucuronide Structures*

Fragment	$^{35}Cl_2$	$^{35}Cl\,^{37}Cl$	$^{37}Cl_2$
$[M+H]^+$ precursor	423	425	(427)
Loss of glucuronic $C_6H_8O_6$	247	249	251
Loss of glucuronic $C_6H_9O_7$	230	232	234
CH_2=C(OH)-C(Cl)=C-CH=N-NH-C(NH_2)=NH	188	188/190	(190)
Ring scission to CH=CHC(Cl)=CCH=NH	113	113/115	115
C/N-NH-C(NH_2)$_2$	85	85	85
Guanidinium N/C(NH_2)NH_3	58	58	58
Ring scission to CH=CH–CH	39	39	39

*Refer to Figures 4 and 5.

■ DISCUSSION

This study was conducted to develop a highly sensitive LC-MS/MS method for detection of guanabenz in serum following administration of a clinical dose of this agent to mature horses. Preliminary work suggested that guanabenz was not readily recovered by solid-phase extraction, and a simple trichloroacetic acid protein precipitation method was not particularly productive in the authors' experience. Therefore, a dichloromethane-based liquid/liquid extraction method was used, with clenbuterol as the internal standard. Based on previous work with clenbuterol,[4] an LC-MS/MS method was developed for guanabenz. The method allowed detection of guanabenz in equine serum with a limit of detection of 0.03 ng/ml. Detection at this level of sensitivity should be sufficient for regulatory control of this agent.

To test this hypothesis, guanabenz was administered at 0.2 and 0.04 mg/kg by rapid IV injection to two horses. Initial concentrations of parent guanabenz were approximately 120 ng/ml for the high dose and 50 ng/ml for the low dose. Serum concentrations of guanabenz dropped rapidly, with an extremely rapid distribution half-life (approximately 2.5 minutes), followed by a much slower elimination half-life. These pharmacokinetic characteristics suggest that guanabenz is a highly lipid-soluble agent, rapidly distributing throughout the body, followed by a much slower elimination phase. Consistent with these interpretations, the apparent Vd_{ss} was 5.72 L/kg after administration at 0.04 mg/kg and 3.76 L/kg after 0.2 mg/kg. The relatively low serum concentrations of guanabenz found 1 hour after administration are representative of the difficulties racing chemists have in detecting guanabenz in postrace blood or serum samples after its administration at clinically effective doses.

Conversely, the sensitivity of this method allowed for detection and confirmation of guanabenz in equine serum for up to 8 hours after administration at doses of approximately 20 mg per horse (0.04 mg/kg). Given the fact that the duration of pharmacologic effects of these low doses are relatively subtle when guanabenz was administered at 0.04 mg/kg, lower doses were not administered. It is likely that doses at the low end of those being used at racetracks would also be detectable because the LOD is 0.03 ng/ml. As observed in this study, there was a fivefold decrease in serum concentrations between the 0.2-

and 0.04-mg/kg dosages. Serum concentrations following dose reduction of another 2.5-fold should be well above the LOD for several hours.

The study also evaluated the forms in which guanabenz appears in urine samples following IV administration. Analysis of urine samples yielded evidence of at least one glucuronide metabolite, with the glucuronide ring apparently linked to a ring hydroxyl group or a guanidinium hydroxylamine, as suggested by the literature for human metabolism of guanabenz[5] and by the data generated in the analyses in this study.

Although the use of blood or serum testing is nontraditional in racing chemistry, it has many advantages over urine testing for regulatory control of an agent. First, suitable standards of parent drug are usually readily available, and the stability of the parent drug is usually well characterized. Secondly, the recovery and quantification of parent drug from plasma or serum is generally more straightforward than the recovery of metabolites from equine urine, which usually involves a hydrolysis step. Beyond this, even if the urinary metabolites are confidently identified, their synthesis and characterization must be accomplished before their quantification in urine can be achieved. As well, suitable analytical standards of these metabolites must be made available to racing chemists, which presents additional difficulties. Finally, the relationship between serum or plasma concentrations of drug and its pharmacological effects can be much more accurately and confidently characterized than can the relationship between urine concentrations of drug metabolites and pharmacological effects. All of these considerations suggest that, wherever possible, racing chemists should focus on the detection and quantification of drugs and medications in plasma or serum, rather than in equine urine as has been done previously.

Further work is in progress using a highly sensitive ELISA test for detecting guanabenz and its metabolites in equine urine samples. Preliminary results indicate this ELISA test allows for rapid identification of urine samples that have a high probability of containing guanabenz and its metabolites, thereby directing attention to the corresponding blood sample. Findings in that study will be reported at the completion of testing.

■ CONCLUSION

Application of the highly sensitive LC-MS/MS method for guanabenz in serum, as described here, allows rapid detection, unequivocal identification, and confident quantification of guanabenz in serum. Together with the ELISA test for detecting the presence of guanabenz and its metabolites equine urine samples, these technologies have the potential to provide racing chemists with the requisite methodologies for effective control of guanabenz in competition horses.

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