VOL. 9, NO. 1
1988

Research Communications in
Substances of Abuse

IMMUNOASSAY DETECTION OF DRUGS IN RACING HORSES
V. DETECTION OF MAZINDOL IN EQUINE BLOOD AND URINE BY A ONE STEP ELISA ASSAY

Charles A. Prange, Catherine Brockus, Diana Stobert and Siong Wie
International Diagnostics Systems Corporation
2614 Niles Avenue
P.O. Box 799
St. Joseph, Michigan 49085
and
John McDonald, Roy Gall, Paul Wiedenbach, Vivian D. Bass,
and B. DeLeon
Illinois Racing Board Laboratory
750 South State Street
EMCH Mendel Building
Elgin, Illinois 60120
and
F.J. Ozog and M.T. Green
Industrial Laboratories Co.
1450 East 62nd Avenue
Denver, Colorado 80216
and
W.E. Woods, C.L. Tai, M.R. Dai, T.J. Weckman, H.-H. Tai, J.-M. Yang,
S.-L. Chang, J.W. Blake, and T. Tobin
The Maxwell H. Gluck Equine Research Center and the
Kentucky Equine Drug Research and Testing Programs
Department of Veterinary Science and School of Pharmacy
University of Kentucky
Lexington, Kentucky 40546-0099

Published as Kentucky Agricultural Experiment Station Article #88-4-42 with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #141 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky.

Supported by a grant entitled "Immunoassay Tests for High Potency Narcotic Analgesics in Racing Horses" from the Kentucky Equine Drug Research Council and the Kentucky State Racing and Harness Racing Commissions.

Correspondence should be addressed to Dr. Tobin

SUMMARY

We have developed and evaluated a one step enzyme-linked immunosorbent assay (ELISA) test for mazindol as part of a panel of pre- and post-race tests for illegal medications in racing horses. This ELISA test detects mazindol with an I-50 of about 10 ng/ml. The test is economical in that it can be read with an inexpensive spectrophotometer, or even by eye. The test is rapid, and 10 samples, a normal pre-race complement, can be analyzed in about 20 minutes. The test readily detects the presence of mazindol or its metabolites in equine blood and urine for 6 to 72 hours, respectively after administration of sub-therapeutic doses. No information is currently available on the cross-reactivity of this test with analogs of mazindol. When introduced into routine screening, this test yielded 10 confirmed positives in preliminary trials. As such this test is capable of both improving the quality and reducing the cost of pre-race and post-race testing for mazindol in racing and show horses. The series of ELISA tests reported in this series of papers has therefore to date resulted in approximately 100 confirmed positives for members of four different families of drugs used illegally in racing horses.

INTRODUCTION

Mazindol, an imidazoline derivative [5-(4-chlorophenyl)-2,5-dihydro-3H-imidazol(2,1a) isoindol-5-OL], is a central nervous system stimulant and anorexigenic agent used in humans for the control of narcolepsy and obesity (Iijima et al., 1986; Gogerty and Trapold, 1976). Although structurally dissimilar to amphetamine, mazindol exhibits several pharmacological properties common to the phenethylamine drugs. These properties include the ability to stimulate dose-related locomotor and general psychomotor activity in the rat (Gogerty et al., 1975; Angel et al., 1987). Mazindol and

amphetamine also are throught to have similar hypothalamic binding sites (Angel et al., 1986). Oral doses of mazindol have been observed to cause a marked "uneasiness" in the horse (Mendonca and Todi, 1984). In humans, excessive use of mazindol may lead to tolerance and physical dependence.

The metabolism and pharmacokinetics of mazindol in the horse have been studied (Timmings et al., 1985). After an oral dose of 50 mg of mazindol per horse, the parent compound was detected by gas chromatography (GC) in the urine for up to 12 hrs post-dose and in plasma up to 6 hrs after administration. The peak urine concentration was about 100 ng/ml at about 6 hrs post-dose. The peak plasma level was about 35 ng/ml between 1 and 2 hrs post-dose. Comparison of this data to a tritiated mazindol administration study (Mendonca and Toci, 1984) indicates that most of the drug is excreted in the horse as unidentified metabolites. As many as nine metabolites of mazindol have been reported in the rat (Dugger et al., 1976).

Due to the central nervous stimulant properties of mazindol, it has a high potential for abuse in racing horses. Detection of mazindol in post-race urine samples in the Western United States has been recently reported (Sams, 1987). These "positives" were detected by thin layer chromatography (TLC) screening followed by gas chromatography/mass spectroscopy (GC/MS) confirmation. Illegal race-track dosages are thought to be about 2-4 mg by intravenous (IV) injection (Timmings et al., 1985).

Recently, interest in pre-race drug testing in blood has increased. TLC screening methods do not have the sensitivity to detect basic illegal drugs such as mazindol in blood. Furthermore, these methods tend to be slow, labor intensive and expensive (Tobin et al., 1979; Tobin, 1981). We are currently developing simple, rapid, inexpensive and sensitive enzyme immunoassay detection methods for drugs of abuse in racing horses (Yang

et al., 1987; McDonald et al., 1988, Tobin et al., 1988). In this report we outline the development of an enzyme-linked immunosorbent assay (ELISA) test for mazindol and we evaluate its application to both urine and blood testing for mazindol in horses.

MATERIALS AND METHODS

Horses

Mature Thoroughbred, half Thoroughbred and Standardbred mares (400-600 kg) were used throughout. The animals were kept at pasture and allowed free access to food and water. The horses were placed in standard box stalls (17 m^2) approximately 12 hrs prior to dosing for acclimatization.

Dosing and Sampling

Mazindol pure drug standard was obtained from Sandoz Research Institute (East Hanover, NJ). Due to the general insolubility of mazindol, the pure drug standard was dissolved in 0.1 M hydrochloric acid (HCl) at a concentration of 4 mg/1.5 ml. PBS buffer (phosphate buffered saline, 0.10 M sodium phosphate, pH 7.4, Sigma Diagnostics, St. Louis, MO) (2 ml) was added to adjust the pH of the solution to 5.0 for IV injections. Mazindol (4 mg/horse) was administered by rapid IV injection into the jugular vein. For the oral dosing (50 mg/horse), crushed mazindol tablets (Sanorex®, Sandoz Pharmaceuticals, East Hanover, NJ) were administered by stomach tube.

All urine samples were collected by bladder catherization. Blood samples were collected by venipuncture into vacuum blood tubes containing potassium oxalate and sodium fluoride (for plasma) or serum separation vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ). The blood tubes were centrifuged to collect the plasma and the serum. All samples were stored frozen until assayed.

One Step ELISA

The one step ELISA tests were performed as previously described (Yang et al., 1987; McDonald et al., 1988; Tobin et al., 1988). Briefly, anti-mazindol antibody was linked to flat bottom Immulon Removawells® (Dynatech, Chantilly, VA) (Voller et al., 1976). Mazindol was linked to horse radish peroxidase (HRP) (Wie and Hammock, 1982), to give rise to a covalently bound mazindol-HRP complex. All reactions were run at room temperature.

The assay was started by adding 20 ul of the standard, test, or control samples to each well, along with 100 ul of the mazindol-HRP solution. Mazindol standards were prepared in PBS buffer. During this step, the presence of free drug or cross-reacting metabolites competitively prevented the antibody from binding to the mazindol-HRP conjugate. The degree of the antibody-mazindol-HRP binding was therefore inversely proportional to the amount of drug in the sample. After 25 min of incubation the fluid was removed from the microtiter wells and the wells washed three times with buffer. TMB Microwell Peroxidase substrate solutions (Kirkegaard and Perry, Gaithersburg, MD) were then added to all wells and their optical density read at 650 nm in an Ultrascan microwell reader (International Diagnostic Systems Corp., St. Joseph, MI) at 15 to 60 min after addition of substrate. Tests run with standards in PBS buffer or plasma were read at 15 to 20 min; tests run with urine samples usually required longer reaction times for maximum sensitivity (Tobin et al., 1988).

Kits for this mazindol test and for other tests reported in this series of papers are commercially available from International Diagnostic Systems Corporation, St. Joseph, Michigan.

Mass Spectral Confirmation of Mazindol

The urine sample (50 ml) was adjusted to pH 9 with sodium carbonate/sodium bicarbonate buffer. The sample was extracted three times with 15 ml each of dichloromethane (DCM): isopropanol (IPA) (10:1). The DCM fractions were combined and washed with 5 ml water with one drop of 50% sodium hydroxide (NaOH). The organic layer was removed and dried with a small amount of sodium sulfate. The sample was then extracted with 5 ml of 1% sulfuric acid.

The aqueous phase was washed with 10 ml of DCM and then made basic with 50% NaOH and extracted with 20 ml DCM. The sample was dried in a conical tube at 60°C. The residue was dissolved in DCM and spotted on a preparative silica gel TLC plate. The plate was developed in chloroform:methanol: propionic acid (72:18:10). A mazindol standard was also run on the plate to locate the mazindol in the sample. The spots were scraped and the silica gel pulverized with a spatula.

To this powder, 1 ml of 0.1M HCl was added and the sample was warmed 10 min in a water bath. After cooling, the pH of the sample was adjusted to 9 with 1M NaOH and extracted with DCM:IPA (10:1) on a rotating mixer for 20 min. The organic phase was separated and evaporated in a 60°C water bath under a stream of nitrogen. The sample was dissolved in ethyl acetate and injected into the GC/MS with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce Chemicals, Rockford, IL).

The GC/MS system employed consisted of a Hewlett-Packard Model #5890 capillary gas chromatography equipped with a Hewlett-Packard Model #5970 mass spectroscopy detector and a Hewlett-Packard data station (Hewlett-Packard Instruments, Palo Alto, CA). GC/MS conditions were similar to those previously described (McDonald et al., 1988).

RESULTS

Data of Fig. 1 show the time course and sensitivity to added mazindol of our one step ELISA test. The standards were added in PBS buffer. In the absence of added mazindol the reaction reached an apparent optical density value of about 1.7 after 25 min of reaction time. The addition of increasing concentrations of mazindol acted to inhibit the reaction, with virtually complete inhibition of the reaction occurring after the addition of 20 µl of 100 ng/ml mazindol standard.

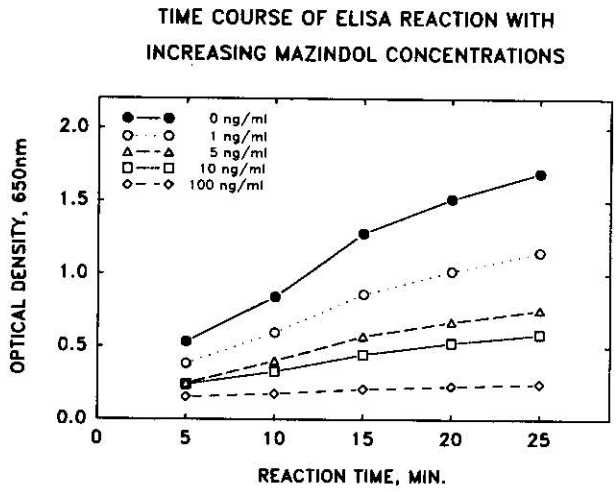

Figure 1. The symbols show the time course of the inhibition of the ELISA in the presence of increasing concentrations of added mazindol.

Standard curves of the mazindol ELISA test run in PBS buffer with increasing reaction reading times are shown in Fig. 2. After 15 min there appeared to be no significant improvement of apparent sensitivity of the assay. Therefore ELISA tests in buffer or plasma were read after 15 or 20

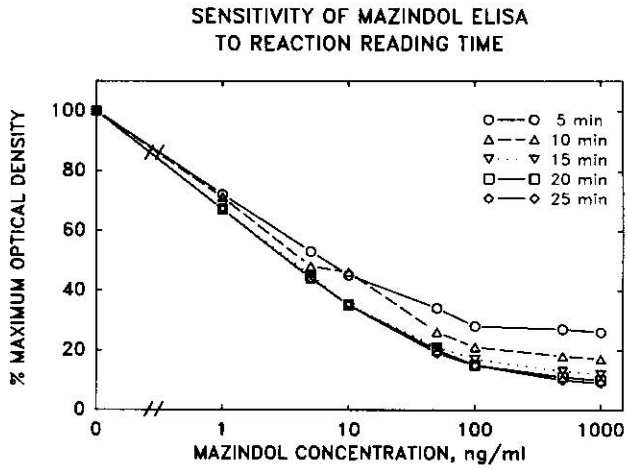

Figure 2. The symbols show the family of mazindol ELISA standard curves throughout the first 25 minutes of the assay. Maximal inhibition, with an I-50 of about 3.5 ng/ml, was found after 15 minutes incubation. Maximum optical density was the reading for PBS buffer with no drug added.

min reaction time. The apparent I-50 for mazindol in this assay was about 3.5 ng/ml.

The ability of the ELISA test to detect mazindol in the blood and urine of horses dosed orally with the drug was examined. Mazindol or its metabolites were detected in the plasma of two horses dosed with 50 mg mazindol/horse (Fig. 3). Inhibition of the reaction was noted 30 min post-dose, peaked at about 2 hrs, and was still evident after 6 hrs. Mazindol equivalents were quite readily detectable in the urine from these two horse (Fig. 4). The reaction was essentially totally inhibited in samples taken up to 48 hrs in one horse and 72 hrs in the other, at which time the sampling had been discontinued.

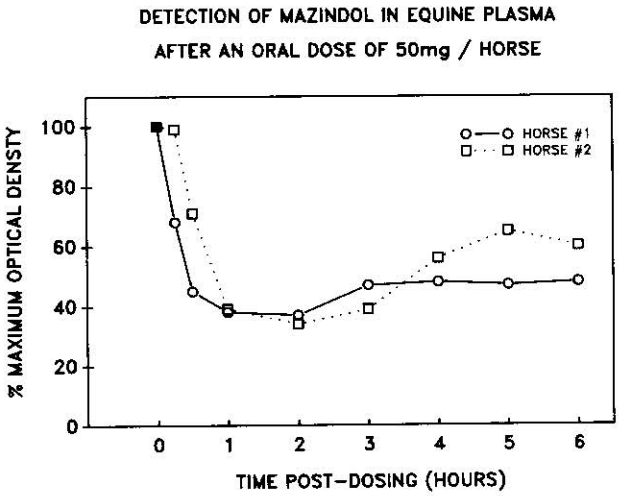

Figure 3. The figure shows ELISA activity in plasma samples from two horses prior to (solid symbol) and after dosing (open symbols) with 50 mg mazindol/horse orally. Reaction reading time = 15 min. Maximum optical density was the reading for the pre-dose control sample for each horse.

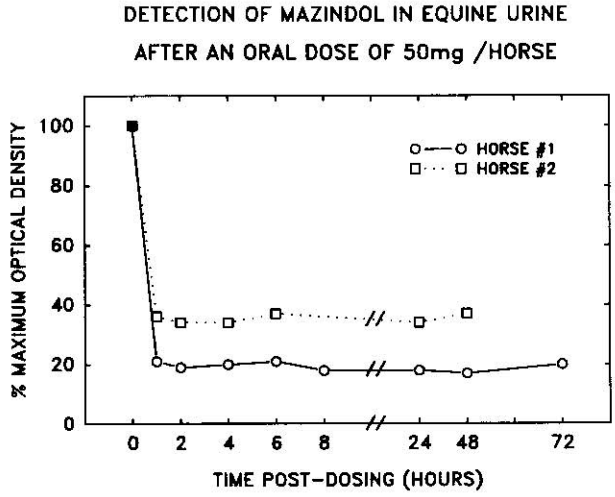

Figure 4. The figure shows ELISA activity in urine samples from two horses prior to (solid symbols) and after dosing (open symbols) with 50 mg mazindol/horse orally. Reaction reading time = 20 min. Maximum optical density was the reading for the pre-dose sample for each horse.

Mazindol or its metabolites were also detected in the blood and urine of three horses dosed by IV injection (4 mg/horse). Mazindol equivalents were readily detected in the plasma (Fig. 5) and serum (Fig. 6) for at least 2 hrs in these horses. Some inhibition of the reaction was noted in blood samples taken at 4 hrs and 8 hrs post-dose, although the amount of this inhibition varied between horses.

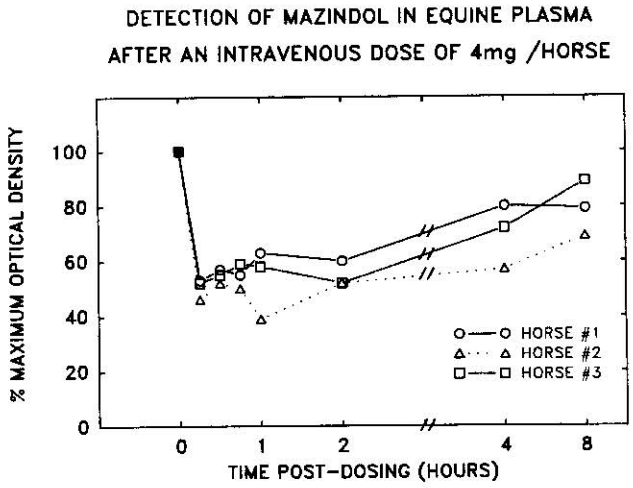

Figure 5. The figure shows ELISA activity in plasma samples from three horses prior to (solid symbols) and after dosing (open symbols) with 4 mg mazindol/horse IV. Reaction reading time = 20 min. Maximum optical density was the reading for the pre-dose sample for each horse.

The ELISA test was able to detect mazindol or its metabolites in the urine from the horses dosed orally (Fig. 7). Significant inhibition of the reaction was noted in samples taken 15 min post-dose. The inhibition peaked at 2 to 4 hrs, and was still evident at 24 hrs after administration. In the two horses sampled at 48 hrs, the reaction was back to control (pre-dose) levels.

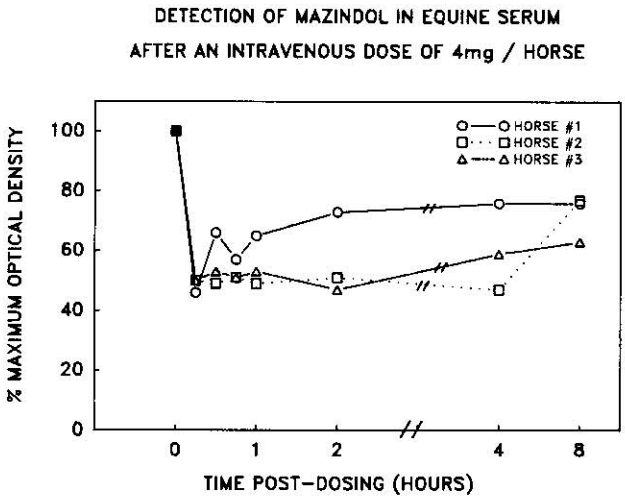

Figure 6. The figure shows ELISA activity in serum samples from three horses prior to (solid symbol) and after dosing (open symbols) with 4 mg mazindol/horse IV. Reaction reading time = 20 min. Maximum optical density was the reading for the pre-dose sample for each horse.

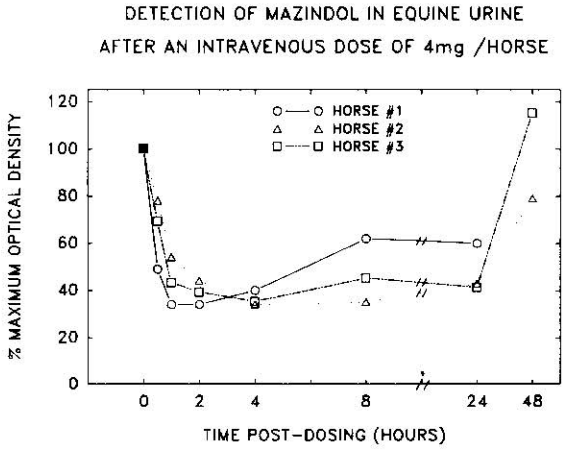

Figure 7. The figure shows ELISA activity in urine samples from three horses prior to (solid symbol) and after dosing (open symbols) with 4 mg mazindol/horse. Reaction reading time = 60 min. Maximum optical density was the reading for the pre-dose sample for each horse.

Equine urine samples provided by the Quality Assurance Program of the National Association of State Racing Commissioners (NASRC) were assayed for mazindol equivalents by the mazindol ELISA test. These samples were collected from horses dosed orally with 400 mg mazindol/horse. Mazindol or its metabolites were easily detected by the ELISA. The inhibition of the reaction was nearly total in the 2 hr sample with no significant recovery of activity by the time of the 24 hr sampling. No Quality Assurance samples beyond 24 hours were apparently collected, even though mazindol may likely be detected in horses treated with this dose of mazindol for one week.

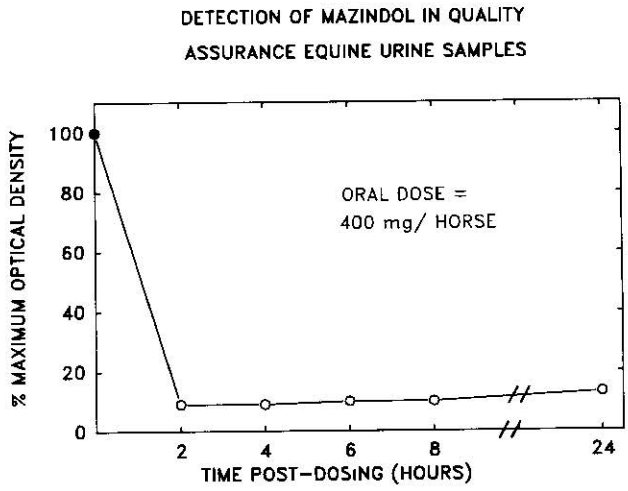

Figure 8. The figure shows ELISA activity in NASRC Quality Assurance Program urine samples from a horse dosed with 400 mg mazindol. The solid circle (●) shows the ELISA response in the pre-dose control sample and the open circles (○) show the responses post-dose. Maximum optical density was the reading for the pre-dose sample.

This ELISA test for mazindol has been used recently to screen for mazindol abuse in racing horses in the Western United States. Table 1 shows

the ELISA results from the samples collected on one racing day at a Western track. Results are also shown for a GC/MS confirmed mazindol urine standard. Four urine samples were flagged for mazindol by the ELISA, all of which were confirmed by GC/MS as illustrated in Fig. 9.

Table 1. Mazindol ELISA results of urine samples from a race track screening assay.

Sample #	Race Date	ELISA Optical Density	Results (Pos./Neg.)	Comments
		0.183		Mazindol Standard
1	1/10/88	0.371	Neg.	---
2	1/10/88	0.401	Neg.	---
3	1/10/88	0.202	Pos.	Confirmed by GC/MS
4	1/10/88	0.219	Pos.	Confirmed by GC/MS
5	1/10/88	0.648	Neg.	---
6	1/10/88	0.562	Neg.	---
7	1/10/88	0.640	Neg.	---
8	1/10/88	0.559	Neg.	---
9	1/10/88	0.522	Neg.	---
10	1/10/88	0.553	Neg.	---
11	1/10/88	0.381	Neg.	---
12	1/10/88	0.195	Pos.	Confirmed by GC/MS
13	1/10/88	0.364	Neg.	---
14	1/10/88	0.399	Neg.	---
15	1/10/88	0.452	Neg.	---
16	1/10/88	0.363	Neg.	---
17	1/10/88	0.550	Neg.	---
18	1/10/88	0.206	Pos.	Confirmed by GC/MS

Mazindol ELISA optical densities are shown for 18 horse urine samples taken post-race one day on one race track in the Western United States. The results were flagged "negative" or "positive", and the positive samples were subjected to GC/MS analysis. Samples flagged "positive" were those with optical density readings near that of the mazindol GC/MS confirmed standard.

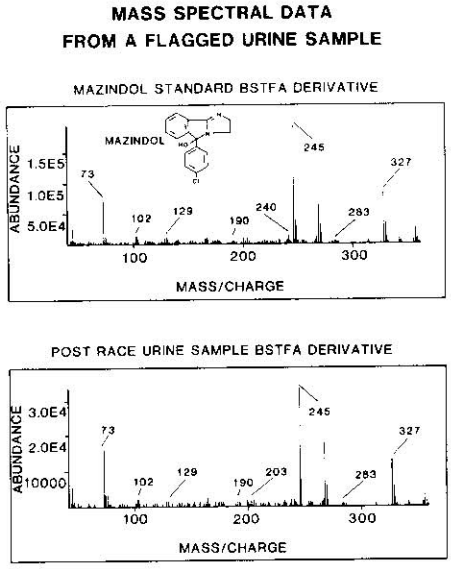

Figure 9. The upper panel shows the mass spectrum of the BSTFA derivative of a mazindol standard, while the lower panel shows the mass spectrum of a material recovered from a track urine sample flagged positive for mazindol by the ELISA.

Few behavioral effects were noted in the horses after administration of mazindol. The mares dosed with 50 mg mazindol orally showed transient excitability 30 min after administration which lasted for 1.5 hrs. No effects were observed in the horses dosed IV.

DISCUSSION

As a potent central stimulant drug mazindol has the potential to be abused in racing and show horses and there is good evidence that this abuse occurs. For example, research projects on the detection of mazindol in

horses have been financed by the American Horse Shows Association, and also by the Quality Assurance Program of the NASRC. To date however, these approaches have not produced a testing methodology sufficiently sensitive to detect the amounts of mazindol being abused in racing and show horses.

This situation is highlighted by the doses of mazindol used in the Quality Assurance Program tests. In these experiments horses were dosed orally with 400 mg of mazindol, a relatively large dose of this drug. Since the dose of mazindol for a human is about 2 mg, the dose for an adult horse may be estimated at about five times this dose or about 10 mg/horse. This value is in good agreement with the doses reportedly administered to racing horses and to show horses, which are in the range of about 10 mg/horse or less. To effectively control abuse of this drug in racing horses, therefore, a method which will detect doses of 10 mg or less of mazindol/horse is required.

The ELISA test reported here is readily able to detect such doses of mazindol when they are administered to horses. As shown in Figs. 1 and 2, this test is able to detect levels of parent mazindol of about 1 ng/ml relatively easily. This level of sensitivity is sufficient to detect mazindol in the blood and urine of horses after doses of mazindol of as little as 4 mg/horse. As shown in Figs. 5 and 6 plasma and serum from horses dosed IV with these levels of mazindol inhibited this ELISA test about 50%, more than sufficient to allow flagging of forensic samples for confirmatory analysis for mazindol.

Mazindol was similarly detected in equine urine after this dose of mazindol. As shown in Fig. 7, urine from horses dosed with this dose of mazindol inhibited the ELISA reaction by up to 60% at one hour after IV administration of the drug. These samples remained inhibited at

approximately this level for 24 hours, but had returned to control values by 48 hours after dosing. The data show that doses of mazindol of as little as 4 mg are readily detectable in urine. Beyond this, the ease with which the 4 mg dose was detected in urine and the fact that it was also detected in blood at this dose suggests that much lower doses of mazindol are likely to be detectable by this test.

Not unexpectedly higher doses of mazindol were also very easily detected. We started our mazindol experiments with a dose of 50 mg orally, because this was the dose that was used in the previously most sensitive analytical report on mazindol (Timmings et al., 1985). In this work, Beaumier and co-workers showed that mazindol at a dose of 50 mg/horse was detectable in urine for about 13 hours. In contrast, the data reported here, which shows that mazindol is likely detectable in urine for at least 72 hours after a 50 mg dose further highlights the sensitivity of this ELISA test for mazindol and its metabolites.

In the Quality Assurance Program an oral dose of 400 mg/horse was administered and shipped to participating laboratories for analysis. These unrealistically high levels of mazindol were readily detected in urine samples from the Quality Assurance Program (Fig. 8). No plasma samples were provided for analysis for mazindol by the Quality Assurance Program. Further, samples were not provided beyond 24 hours, while it appears possible that mazindol remains in the urine of a horse for possibly 2 days after this dose.

When this test was introduced into racing, it readily detected mazindol in racing samples. As shown in Table 1, mazindol containing samples were readily flagged for further examination based on this ELISA test. Based on this test about ten mazindol positives have been detected at this time,

including five from the same trainer. The data suggest that this test is able to detect levels of mazindol abuse currently occurring and that are not being detected by the current Quality Assurance methods. In this regard, this ELISA for mazindol is rather like our ELISA for morphine, which has detected major patterns of oxymorphone abuse, including the winner of a million dollar futurity. Despite the fact that a Quality Assurance test for oxymorphone also existed, it was not sufficiently sensitive to detect the levels of drug being abused on the race track. However, these ELISA tests that we are developing have to date detected well over 100 confirmed positives and have stopped patterns of abuse for buprenorphine, sufentanil, oxymorphone, and, most recently, mazindol.

REFERENCES

Angel, I., Kiss, A., Stivers, J., Skirboll, L., Crawley, J.N., Paul, S.M. (1986) Regulation of [^{3}H]mazindol binding to subhypothalamic area: involvement in glucoprivic feeding. Brain Res. Bull. 17, 873-877.

Angel, I., Luu, M.-D., Paul, S.M. (1987) Characterization of [^{3}H]mazindol binding in the rat brain: sodium-sensitive correlates with the anorectic potencies of phenylethylamines. J. Neurochem. 48, 491-497.

Dugger, H.A., Coombs, R.A., Schwarz, H.J., Migdalof, B.H., Orwing, B.A. (1976) Biotransformation of mazindol. I. Isolation and identification of some metabolites from rat urine. Drug Metab. Dispos. 4, 262-268.

Gogerty, J.H., Penberthy, C., Iorio, L.C., Trapold, J.H. (1975) Pharmacological analysis of a new anorexic substance: 5-hydroxy-5-(4-chlorophenyl)-2,3-dihydro-5H-imidazo-(2,1-a)isoindole (mazindol). Arch. Int. Pharmacodyn. 214, 287-307.

Gogerty, J.H., Trapold, J.H. (1976) Chemistry and pharmacology of mazindol. Triangle 15, 25-36.

Iijima, S., Sugita, Y., Hishikawa, Y. (1986) Therapeutic effects of mazindol on narcolepsy. Sleep 9, 265-268.

McDonald, J., Gall, R., Wiedenbach, P., Bass, V.D., DeLeon, B., Brockus, C., Stobert, D., Wie, S., Prange, C.A., Ozog, F.J., Green, M.T., Woods, W.E., Tai, C.L., Weckman, T.J., Tai, H-H., Yang, J-M., Chang, S.-L., Blake, J.W., Tobin, T. (1988) Immunoassay detection of drugs in horses. III. Detection of morphine in equine blood and urine by a one step ELISA assay. Res. Comm. Chem. Pathol. Pharmacol, 59, 259-278.

Mendonca, M. and Todi, F. (1984) Excretion studies with H^3-mazindol. In Johnston, G. (ed.) Proceedings of the 38th Meeting, Association Official Racing Chemists, Denver, CO. pp. 246-252.

Sams, R.A. (1987) Medication case reports. Association Official Racing Chemists, Bulletin No. 431, p. 27.

Timmings, S., Beaumier, P., Sutherland, D., Nikolajev, J. (1985) Metabolism and elimination of mazindol by the horse after oral administration. In Crone, D.L. (ed.) Proceedings 6th Int. Conf. Racing Anal. Vet., Hong-Kong, pp. 247-255.

Tobin, T. (1981) Drugs and the Performance Horse, Charles C. Thomas, Publisher, Springfield, IL. pp 363-365.

Tobin, T., Maylin, G.A., Henion, J., Woodward, C., Ray, R., Johnston, J., Blake, J.W. (1979) An evaluation of pre- and post-race testing and blood and urine testing in horses. J. Equine Med. Surg., 3, 85-90.

Tobin, T., Tai, H.-H., Tai, C.L., Houtz, P.K., Dai, M.R., Woods, W.E., Yang, J.-M., Weckman, T.J., Chang, S.-L., Blake, J.W., McDonald, J., Gall, R., Wiedenbach, P., Bass, V.D., Ozog, F.J., Green, M., Brockus, C., Stobert, D., Wie, S., Prange, C.A. (1988) Immunoassay detection of drugs in racing horses. IV. Detection of fentanyl and its congeners in equine blood and urine by a one step ELISA assay. Res. Comm. Chem. Pathol. Pharmacol., 60, 97 - 116.

Voller, A., Bidwell, D.W., Bartlett, A. (1976) The enzyme linked immunosorbent assay (ELISA). Bull. Wld. Hlth. Org., 53, 55-56.

Wie, S.I., Hammock, B.D. (1982) The use of enzyme-linked immunosorbent assay (ELISA) for the determination of Triton X nonionic detergents. Anal. Biochem., 125, 168-176.

Yang, J-M., Tai, C.L., Weckman, T.J., Woods, W.E., Tai, H-H., Blake, J. W., Tobin, T., McDonald, J., Gall, R., Wiedenbach, P., Bass, V.D., DeLeon, B., Brockus, C., Stobert, D., Wie, S., Prange, C.A., (1987) Immunoassay detection of drugs in horses. II. Detection of carfentanil in equine urine by RIA, PCFIA and ELISA. Res. Comm. Subst. Abuse 8, 59-75.