IMMUNOASSAY DETECTION OF DRUGS IN RACING HORSES
VII.   DETECTION OF ACEPROMAZINE IN EQUINE URINE AND BLOOD
BY ELISA AND PCFIA

S. Kwiatkowski*, L. Sturma, M.R. Dai, H.-H. Tai, D.S. Watt,
C.L. Tai, W.E. Woods, T.J. Weckman, J.-M. Yang, T. Wood, 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
Departments of Veterinary Science and Chemistry
and the School of Pharmacy
University of Kentucky
Lexington, Kentucky 40546-0099
and
C.A. Prange, C. Brockus, D. Stobert, S. Wie and R.A. Chung
International Diagnostics Systems Corporation
2614 Niles Avenue
P.O. Box 799
St. Joseph, Michigan 49085
and
J. McDonald, V.D. Bass, S. Merchant, M. Artemenko,
and B. DeLeon
Illinois Racing Board Laboratory
750 South State Street
EMCH Mendel Building
Elgin, Illinois 60120

SUMMARY

We have developed and evaluated a one step enzyme-linked immunosorbent assay (ELISA) test and a particle concentration fluorescence immunoassay (PCFIA) test for acepromazine as part of a panel of pre- and post-race tests for illegal medications in racing horses. These tests are rapid, sensitive and economical and development of the tests occurred in less than seven months. The ELISA test detects acepromazine with an I-50 of about 150 pg/ml. In vivo, it readily detects the presence of acepromazine or its metabolites in equine blood and urine from 8 to 72 hours or longer, respectively, after administration of sub-therapeutic doses. In vitro, the ELISA test cross-reacts with analogs of acepromazine, suggesting that it will also detect the use of other phenothiazine tranquilizers. The PCFIA test detects acepromazine with an I-50 of about 10 ng/ml. When applied to pre-race screening of serum samples as part of the pre-race testing program at a midwestern racetrack, the PCFIA test detected a number of cases of acepromazine abuse. Screening of stored post-race urine samples from associated horses by the ELISA test 'flagged' numerous samples for acepromazine, suggesting a pattern of acepromazine abuse. To date about twenty of these acepromazine flagged samples have been confirmed positive on mass spectrometry. As such the ELISA and PCFIA tests described in this communication are capable of substantially improving the quality of pre- and post-race testing programs for phenothiazine tranquilizers in racing horses.

INTRODUCTION

Acepromazine (acetylpromazine or 1-[10-[3-(dimethylamino)propyl]-10H-phenothiazin-2-yl]ethanone) is an acetylated derivative of the phenothiazine tranquilizer promazine. Acepromazine is commonly used for sedation in equine medicine with a recommended dose of between 0.5 mg/kg and 0.1 mg/kg (Tobin and Ballard, 1979). The pharmacological properties of acepromazine

include sedation, reduction of respiratory rate, penile protrusion, reduction of hematocrit, and hypothermia (Ballard et al., 1982).

Acepromazine and related phenothiazine tranquilizers have been used to illegally affect the performance of both racing and show horses. A very small dose of acepromazine can help smooth a nervous jumper's approach to and performance over the obstacles in jumping events. In other show horse events a tranquilizer can assist a horse by rendering him less sensitive to disturbing influences. The ability to withstand such incidents in the "standing" portions of the event may add substantially to the scores assigned by show judges.

On the other hand, some racing horses tend to become overly excited in the paddock prior to a race, and therefore do not put in a good racing performance. These horses are said to run their race in the paddock rather than on the track, and as a group they are referred to as "washy horses." One approach to this problem is to treat these horses with a small amount of tranquilizer prior to a race. If the dose is chosen correctly it is believed that the tranquilizer will "take the edge off" a horse and allow him to settle sufficiently for a concentrated race effort. The phenothiazine derivatives, particularly acepromazine and propiopromazine, have been commonly used in this regard. They are readily available in veterinary circles and as extremely potent drugs are difficult to detect with currently available analytical methodology (Tobin et al., 1979; Tobin, 1981).

Regulation of the use of the phenothiazine tranquilizers is also complicated by the fact that these drugs have legitimate veterinary uses (Davies et al., 1983; Nolan et al., 1984; Taylor, 1985). They may be used in "breaking" young horses, to calm horses in loading and transporting them and also in association with minor surgical and other interferences with the

horse. For this reason traces of these tranquilizers may be found in horse urine after a routine medical administration of these agents, posing a problem for horsemen and regulators.

One approach to the problem of the illegal use of these drugs is to develop a more sensitive test for these agents. To this end we have raised a series of antibodies to acepromazine and incorporated these antibodies into a one step enzyme-linked immunosorbent assay (ELISA) and particle concentration fluorescence immunoassay (PCFIA) test for acepromazine. In this report we detail the development and evaluation of these tests for acepromazine.

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 hours prior to dosing for acclimatization.

Dosing and Sampling

Acepromazine maleate was obtained as the authentic drug standard and as an injectable preparation PromAce^R from Aveco Co., Inc. (Fort Dodge, IA). The drug (either 5, 1, 0.1 or 0.05 mg/horse) was administered by rapid IV injection into the jugular vein.

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 into serum separation vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ), and centrifuged. All samples were stored frozen until assayed.

One Step ELISA

Acepromazine was derivatized, linked to BSA and antibodies to this drug raised as described by Kwiatkowski et al. (in preparation) and Tai et al. (1978).

The one step ELISA tests were performed as previously described (Yang et al., 1987; McDonald et al., 1988; Tobin et al., 1988). Briefly, anti-acepromazine antibody was linked to flat bottom Immulon Removawells[R] (Dynatech, Chantilly, VA) (Voller et al., 1976). The acepromazine derivative was linked to horse radish peroxidase (HRP) (Wie et al., 1982), to give rise to a covalently bound acepromazine-HRP complex.

The assay was started by adding 20 ul of the standard, test, or control samples to each well, along with 100 ul of the acepromazine-HRP solution. Acepromazine 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 acepromazine-HRP conjugate. The degree of the antibody-acepromazine-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 least 15 min after addition of substrate.

Urine samples were analyzed directly. Blood samples were diluted 1:3 with PBS buffer before assay.

Particle Concentration Fluorescence Immunoassay (PCFIA)

Pre-race testing of track serum samples was performed by PCFIA as previously described (Jolley et al., 1984; McDonald et al., 1987; Yang et al., 1987) on a Pandex Fluorescence Concentration Analyzer 15-010-1 (Pandex,

Mundelein, IL). Anti-morphine antibody and reagents were supplied by International Diagnostics Systems.

<u>Mass Spectral Confirmation of Acepromazine</u>

Gas chromatography/mass-spectroscopy (GC-MS) was performed on a Hewlett-Packard GC model 5890 equipped with a Hewlett-Packard MS-5970 detector and a 15 meter SPB-1 (dimethylpolysiloxane) capillary column (Supelco, Bellefonte, PA). The samples were injected directly into the detector in the non-split mode. GC oven temperature was programmed from $100\,^{\circ}C$ to $280\,^{\circ}C$ at $30\,^{\circ}C/min$. Urine samples were subjected to enzymatic hydrolysis as previously described (Combie <u>et</u> <u>al</u>., 1982). Urine samples (25 ml) were incubated ($65\,^{\circ}C$, 3 hr) at pH 5 with β-glucuronidase from <u>Patella</u> <u>vulgata</u> (Sigma Chemicals, St. Louis, MO) (5,000 units/5ml urine). The samples were made basic (pH 9.0) with ammonium hydroxide and extracted into dichloromethane (DCM)/isopropanol (9:1) (12 ml solvent/25 ml urine). The samples were then back extracted into 7 ml 0.5N sulfuric acid, made basic (pH 9.0) with 0.6N ammonium hydroxide, and re-extracted into DCM/isopropanol (10 ml solvent/25 ml urine). The samples were evaporated to dryness, redissolved in methyl acetate, and purified by preparative thin layer chromatography (E Merck F-254 silica gel-60 plates, Alltech-Applied Science, Deerfield, IL). The solvent system was ethyl acetate: methanol: conc. ammonium hydroxide (85:10:5)/cyclohexane (3/1). The plates were scraped and the samples were eluted into 1 ml isopropanol and extracted with 2 ml 0.2N sulfuric acid. The samples were made basic with 2 ml 0.6N ammonium hydroxide (pH 9.0) and extracted into 6 ml DCM. The samples were evaporated to dryness and redissolved in ethyl acetate. Samples were injected on the GC/MS with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierre Chemicals, Rockford, IL).

RESULTS

The time course and sensitivity to added acepromazine of our one step ELISA test are shown in Figure 1. The standards were added in PBS buffer. In the absence of added acepromazine the reaction reached an apparent optical density value of about 0.75 after 60 minutes of reaction time. The addition of increasing concentrations of acepromazine acted to inhibit the reaction, with virtually complete inhibition of the reaction occurring after addition of 20 ul of 100 ng/ml acepromazine standard.

The data of Figure 2 show standard curves of the acepromazine ELISA test run in PBS buffer with increasing reaction reading times. After 15 minutes there appeared to be no significant improvement of apparent sensitivity of the assay. Therefore ELISA tests in buffer or urine were read after at least 15 minutes of reaction time. The apparent I-50 for acepromazine in this assay was about 150 pg/ml.
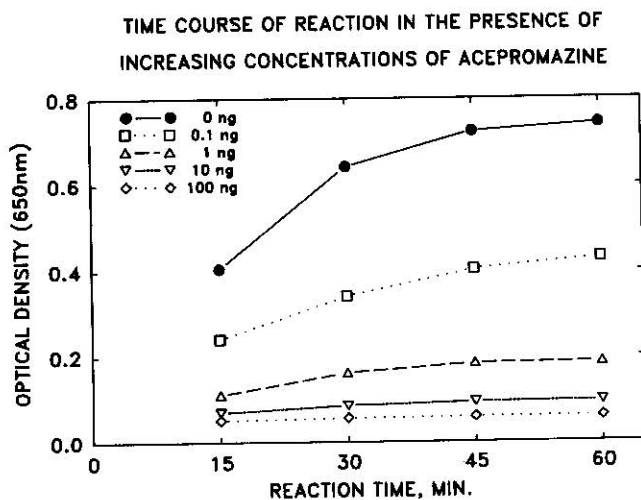
TIME COURSE OF REACTION IN THE PRESENCE OF
INCREASING CONCENTRATIONS OF ACEPROMAZINE

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

397

SENSITIVITY OF ACEPROMAZINE ELISA
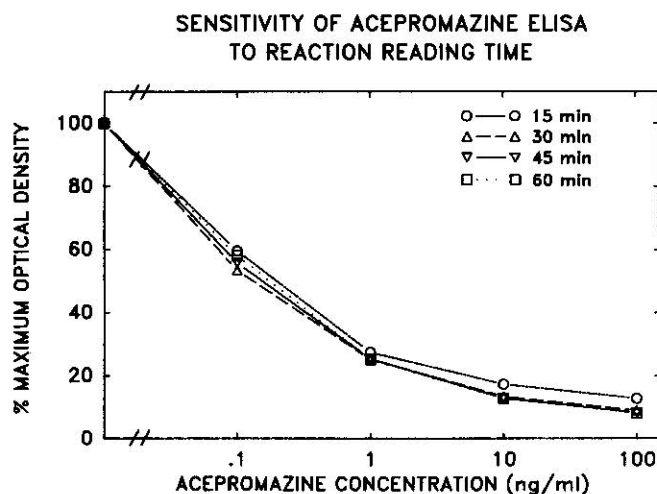TO REACTION READING TIME

Figure 2. The symbols show the family of acepromazine inhibition curves throughout the first 60 minutes of the assay. Maximal inhibition, with an I-50 of about 150 pg/ml, was found after 15 minutes incubation. Maximum optical density was the reading for PBS buffer with no drug added.

The ability of the anti-acepromazine antibody to detect several phenothiazine congeners was evaluated in vitro. Tests showed strong cross-reactivity of the antibody to the promazine family of phenothiazines. In fact, the antibody was even more sensitive to the tested promazine analogs than to acepromazine (Figure 3). The antibody was less reactive with some other phenothiazine congeners (Figure 4). These results suggest that an ELISA assay based on an anti-acepromazine antibody should be able to detect phenothiazine congeners in horses dosed with these drugs.

The ability of the ELISA test to detect acepromazine and its metabolites in the blood and urine of horses dosed intravenously with the drug was investigated. Acepromazine was administered at dose levels ranging from 5 to 0.05 mg/horse to either of two horses.

CROSS–REACTIVITY OF ANTI–ACEPROMAZINE
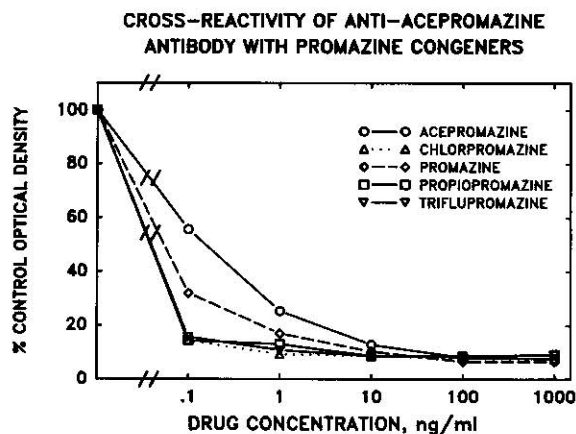ANTIBODY WITH PROMAZINE CONGENERS

Figure 3. The symbols show the ability of the indicated promazine congeners to inhibit ELISA reactions based on anti-acepromazine antibody. Maximum optical density was the reading for PBS buffer with no drug added.

CROSS–REACTIVITY OF ANTI–ACEPROMAZINE
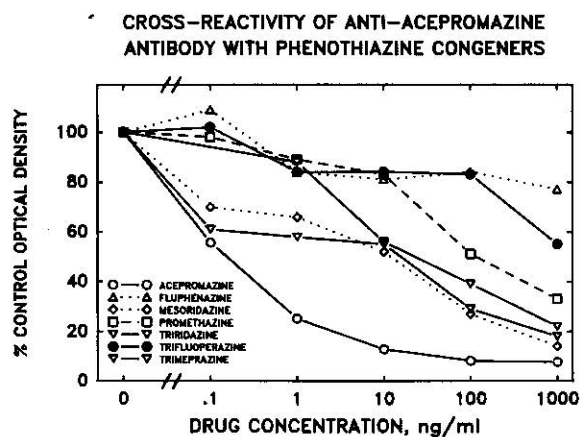ANTIBODY WITH PHENOTHIAZINE CONGENERS

Figure 4. The symbols show the ability of the indicated phenothiazine congeners to inhibit ELISA reactions based on anti-acepromazine antibody. Maximum optical density was the reading for PBS buffer with no drug added.

Acepromazine equivalents were readily detectable in urine at these dose levels. Inhibition of the ELISA reaction was essentially complete from 30 minutes to 8 hours at dose levels from 5 mg/horse to 0.1 mg/horse. Inhibition was still evident at 24 hours at all dose levels, and at 72 hours for 5 mg/horse and 1 mg/horse doses (Figure 5).

In blood, acepromazine was assayed at the 5 mg/horse and 1 mg/horse dose levels. The ELISA reaction readily detected the drug at both dose levels in serum for up to 4 hours post-dose and at the 5 mg/horse level up to 8 hours (Figure 6). In plasma the ELISA reaction detected acepromazine in samples for up to 8 hours at the 5 mg/horse dose level (Figure 7).

DETECTION OF ACEPROMAZINE IN EQUINE
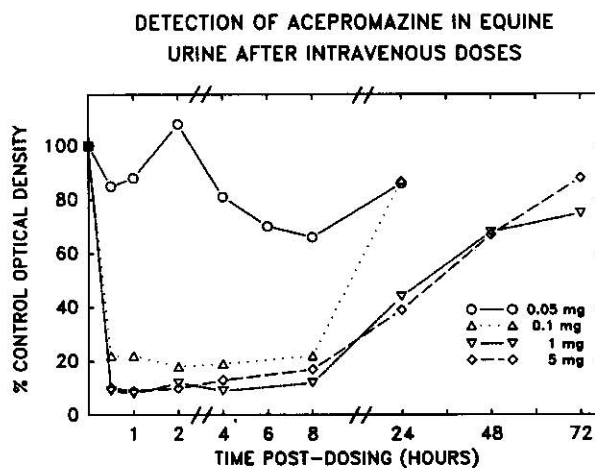URINE AFTER INTRAVENOUS DOSES

Figure 5. In urine samples, inhibition of the ELISA reaction was evident for up to 24 hours at all dose levels, and at 72 hours for 5 mg/horse and 1 mg/horse dose levels. Control optical density was the reading for pre-dose urine.

DETECTION OF ACEPROMAZINE IN EQUINE
SERUM AFTER INTRAVENOUS DOSES
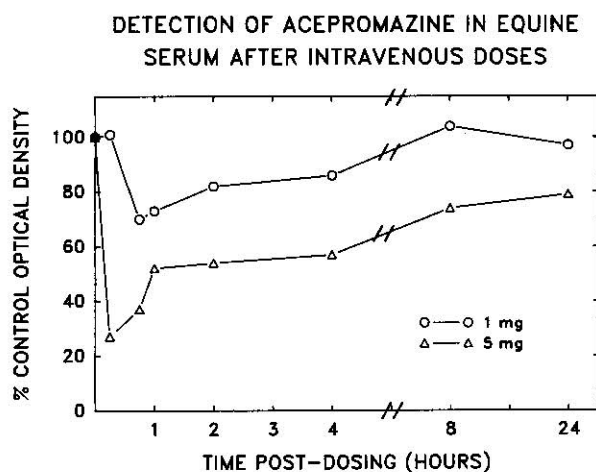
Figure 6. In serum samples, inhibition of the ELISA reaction was evident for up to 4 hours at 5 mg/horse and 1 mg/horse dose levels and up to 8 hours at the 5 mg/horse level. Control optical density was the reading for pre-dose serum.

DETECTION OF ACEPROMAZINE IN EQUINE
PLASMA AFTER INTRAVENOUS DOSES
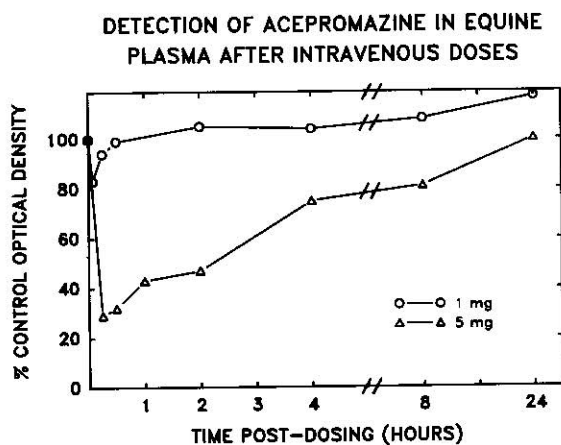

Figure 7. In plasma samples, inhibition of the ELISA reaction was evident for up to 8 hours at the 5 mg/horse dose level. Control optical density was the reading for pre-dose plasma.

The PCFIA format of this test is shown in Figure 8. In this format acepromazine displaced the acepromazine BPE conjugate with a half maximal inhibition of binding occurring at about 10 ng/ml. This assay was then made part of a pre-race panel of drug tests that have been developed by International Diagnostic Systems Corp. and the Illinois Racing Board laboratory as part of a new pre-race testing initiative.
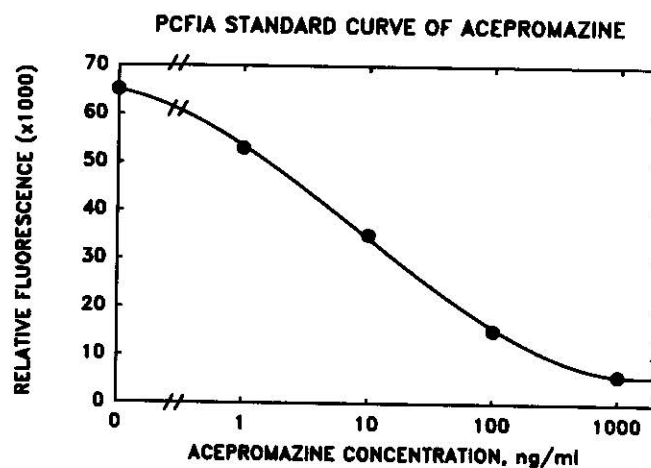
PCFIA STANDARD CURVE OF ACEPROMAZINE

Figure 8. The symbols show the inhibition of PCFIA fluorescence in PBS buffer observed in the presence of the indicated concentrations of acepromazine. I-50 for acepromazine in this PCFIA system is approximately 10 ng/ml. Control fluorescence value is for buffer with no acepromazine added.

Shortly after introduction of this test into pre-race testing it began to indicate the use of promazine or a structurally similar agent in certain horses. Table I shows the PCFIA readouts from such a pre-race test. A panel of pre-race tests is involved, and the two vertical left hand columns (#1 and #2) represents the tests for acepromazine. The first figure (A) represents a known negative serum sample, the second (B) a known positive sample and the subsequent (C, D, etc.) figures represent live pre-race samples. Inspection of left hand column shows that the fifth pre-race (G) sample (*) clearly resembles the positive control rather than the negative control, and is thereby flagged for acepromazine.

Table I.   PCFIA readouts for a panel of pre-race drug tests.

Channel Report: 545/575 Gain Setting: 25 Read Time: 100 ms
Plate ID# 00001423

	1	2	3	4	5	6	7	8	9	10	11	12
A	40758	38376	0	23884	22812	0	17032	9878	0	16754	15732	0
B	14944	36316	0	10538	21146	0	5560	7326	0	4380	13132	0
C	41990	33200	0	23484	20220	0	14110	8604	0	16572	13656	0
D	40234	40784	0	23708	22044	0	11520	11916	0	15882	14666	0
E	40162	0	8	21012	0	0	9048	0	0	12358	0	0
F	38228	0	6	22884	0	0	10674	0	0	12378	0	0
G	*19112	0	0	23210	0	0	10396	0	0	13820	0	0
H	39342	18	0	23114	0	0	8582	0	0	13784	0	0

The table represents PCFIA readouts (in relative fluorescence units) for a panel of pre-race drug tests on serum samples. The two left hand columns show data for acepromazine. The first figure is for a known negative control sample, the second for a known positive control. Subsequent figures are for pre-race serum samples. The sample for the fifth horse (*) in this race is "flagged" for acepromazine. Data in other columns are for three other drugs which were simultanously screened.

The initial action taken on this presumptive positive was to flag the urine samples from this and associated horses for more detailed examination. Evaluation of these urines soon showed evidence for the presence of acepromazine metabolites in these samples, supporting the evidence developed by the pre-race test. Based on this evidence retained samples were selected for more detailed examination.

The technical approach to this examination was as follows. Since these samples had already passed the standard NASRC Quality Assurance TLC examination, more sensitive detection methods were required. All of these retained samples were first screened by the acepromazine ELISA test, which in its current format is substantially more sensitive than the PCFIA test. All "hits" on these ELISA screens were then taken for special extraction, and confirmation by GC/MS (Figure 9). As shown in Table II, of the 19 samples selected by the ELISA test and subjected to GC/MS analysis, all confirmed positive for the presence of acepromazine or a metabolite of acepromazine.

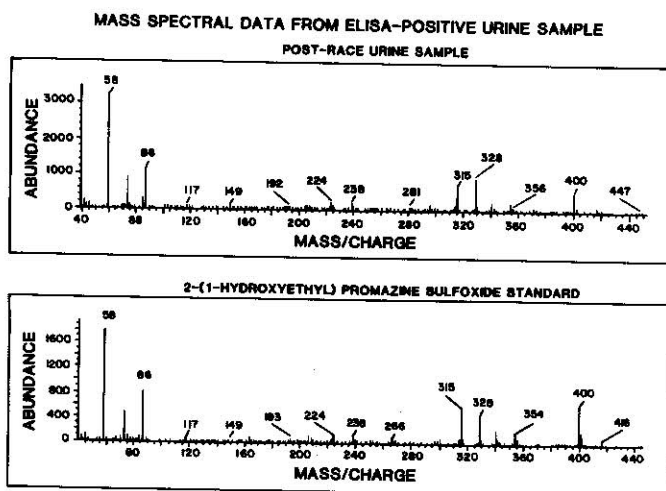MASS SPECTRAL DATA FROM ELISA-POSITIVE URINE SAMPLE

Figure 9. The upper panel shows the mass spectrum of a material recovered from a track urine sample flagged positive for acepromazine by ELISA. The lower panel shows the mass spectrum of the BSTFA derivative of the reduced sulfoxide of acepromazine as a standard.

Table II. Acespromazine ELISA results of urine samples from a racetrack screening assay.

Sample #	Race Date	ELISA Optical Density	Confirmation GC/MS (Pos./Neg.)
1	3/17/88	0.168	Pos.
2	3/12/88	0.224	Pos.
3	3/18/88	0.193	Pos.
4	3/26/88	0.132	Pos.
5	3/30/88	0.158	Pos.
6	3/17/88	0.115	Pos.
7	3/2/88	0.267	Pos.
8	3/16/88	0.163	Pos.
9	3/26/88	0.164	Pos.
10	2/26/88	0.303	Pos.
11	3/22/88	0.210	Pos.
12	2/25/88	0.224	Pos.
13	3/7/88	0.177	Pos.
14	3/7/88	0.177	Pos.
15	12/13/87	0.121	Pos.
16	12/11/87	0.291	Pos.
17	3/24/88	0.274	Pos.
18	11/14/87	0.379	Pos.
19	4/1/88	0.135	Pos.

Control dosed 3 mg IV acespromazine (2-4 hr. sample)

($\bar{x} \pm$ SD)   0.120 $\pm$ 0.020   n = 10

Aceromazine ELISA optical densities are shown for 19 post-race urine samples obtained from two racetracks in Illinois. These 19 samples were considered to be suspect with a relative optical density reading approximating that of a positive control. The positive control as an equine sample taken from 2-4 hrs after IV administration of 3 mg aceromazine. The $\bar{x} \pm$ SD represent 10 different ELISA assays. All 19 ELISA positive samples were confirmed by GC/MS to contain either 2-(1-hydroxyethyl) promazine or 2-(1-hydroxyethyl) promazine sulfoxide.

DISCUSSION

The one step ELISA test for acepromazine described in this report is the most sensitive test for acepromazine developed to date. In vitro it can detect levels of acepromazine of less than 0.1 ng/ml, and the antibody is not yet mature. It appears likely that as the antibody response in our rabbits develops that the assay will become even more sensitive. As such this ELISA will easily detect clinically effective doses of acepromazine in horses, and will also likely detect sub-therapeutic doses of acepromazine of the kind used in performance horses. These tests can also detect phenothiazine tranquilizers in the blood stream of horses after both clinical and subclinical doses, which to date has not been possible with any testing technology.

In its current configuration the ELISA test is able to detect administration of less than 100 ug of acepromazine/horse using "raw" or unconcentrated urine. This is a very small dose of acepromazine indeed, and raises the question of whether or not such a sensitive test is necessary for a drug such as acepromazine, particularly in view of the legitimate uses of this drug in veterinary medicine. The answer to this question depends at least in part on the minimum dose of acepromazine that will produce a pharmacological effect, and on how this dose relates to any other testing methods that may be available for acepromazine.

The currently used testing methodology for acepromazine is based on thin layer chromatography. Based on the samples used in interlaboratory trials, the minimum dose of acepromazine that standard TLC methodology will detect is about 2.5 mg/horse. Since our ELISA test is able to detect doses of 100 ug/horse and possibly less, our ELISA test is at least twenty-five times more sensitive than the currently used thin layer chromatographic technology

and approximately equivalent to the limit of HPTLC technology (100 ug/horse) under optimal conditions (Blake, 1988 unpublished data).

There is good evidence that acepromazine can produce subtle pharmacological effects in horses at doses of less than 2.5 mg/horse. Studying the effects of acepromazine on penile protrusion we found that this relatively crude indicator of a tranquilizing effect of acepromazine was half maximally affected after a dose of about 5 mg/horse, and that the threshold for this effect was about 2.5 mg/horse (Ballard et al., 1982). However, when we examined the effects of acepromazine on hematocrit, a much more sensitive indicator of an adrenergic blocking effect, we found that doses of 1 mg/horse produced good pharmacological effects. Since at that time (1982) we had no method of detecting such low doses of acepromazine, we did not pursue this effect to determine its threshold. However, it appears clear to us that the threshold dose for a pharmacological effect of acepromazine in the horse is less than 500 ug/horse, and likely close to the 0.1 mg/horse sensitivity of this test. There appears, therefore, to be a need for a test with the sensitivity of our ELISA test in the regulation of the use of acepromazine in performance horses.

It should also be borne in mind that the sensitivity of this test can be substantially increased if it is required. In the same way that drug extracts can be concentrated up to 100 fold for thin layer chromatography, samples from blood and urine can also be extracted and concentrated prior to immunoassay if increased sensitivity is required. In this way the sensitivity of this ELISA test can be increased by 10 or 100 fold, virtually ensuring that any pharmacologically effective dose of acepromazine in the horse can be detected.

The abuse of acepromazine in racing horses has a long history and has been only partially controlled by thin layer chromatography. As well as

being used for "washy" horses as outlined in the introduction, acepromazine is used in very small doses to aid in the control of excitable horses during a race. In this pattern of abuse the horse is given a small but pharmacologically effective dose of acepromazine shortly prior to the race. Such a horse is more easily controlled by the jockey during the race, and can more easily be held to the optimal pace for the conditions of any particular race. This can be a considerable advantage if the horse is naturally nervous and tends to fight the jockey and is difficult to control during a long race.* In this regard the sensitivity of this ELISA test is of critical importance since the pharmacological effects of acepromazine are found at doses that cannot be detected by the currently available thin layer chromatographic techniques.

An ELISA test for acepromazine has been described in the veterinary literature by an Australian group (Smith and Chapman, 1987). Since this test will not detect less than 240 ng/ml of acepromazine or its metabolites, it is, in the form that has been reported, much less sensitive than our test, and would not seem to offer any advantages above either of the technologies discussed in this report.

The analysis presented here assumes the patterns of phenothiazine tranquilizer use is limited to the administration of one phenothiazine at a time only. It would be relatively simple matter, however, for an individual who wished to circumvent the current TLC technology to combine one or more phenothiazine tranquilizers. Since TLC detection technology depends on separating these drugs prior to their identification, administration of three different phenothiazine tranquilizers would give rise to a three fold greater pharmacological effect without increasing the risk of detection.

*Personal communication to John McDonald from D. E. Dooley, D.V.M., California Horse Racing Board, Sacramento, California.

With the immunoassay technology described in this report, however, there is a good probability that the different drugs will each cross-react with our anti-phenothiazine antibody, thereby increasing the probability of detection of the medication offense.

One final point about the development of this test is the time schedule over which it was developed. Acepromazine was identified as a problem drug that we committed to develop a test for in September of 1987. Within about one month we had derivatives of acepromazine formed and were ready to inject our first rabbits. Just prior to Christmas 1987 we bled our first rabbits and found good indications of antibody formation. In the early part of 1988 the antibody matured and development of the ELISA and PCFIA tests commenced. In late March these tests were introduced into pre-race testing in Illinois and within days the first pre-race "hits" for acepromazine had been "flagged". Throughout April and May both pre- and post-race samples were screened for acepromazine using the PCFIA and ELISA tests and mass spectral confirmation of the immunoassay "hits" developed. As this paper goes to press about 20 acepromazine positives have been called and a substantially larger number of suspects await confirmation. This sequence of events shows that an immunoassay test can be developed for a drug, the test put into place, and abuse of the drug controlled in a period of somewhat over six months between identification of the drug as a problem and the deployment of the test and development of the first positives for the drug.

In summary, therefore we have developed very sensitive ELISA and PCFIA tests for acepromazine. The tests are sufficiently sensitive to detect acepromazine or its metabolites in the blood and urine of horses dosed with very small amounts of acepromazine. The test cross-reacts well with other promazine analogs that are likely to be used in horses. The tests are much more sensitive than the currently used thin layer chromatographic tests on

which most pre- and post-race drug testing is currently based. When the tests were introduced into pre- and post-race testing in Illinois they rapidly identified a pattern of abuse of acepromazine, initially in pre-race testing and then in retained post-race urine samples. Using this technology large numbers of pre- and post-race samples were rapidly, accurately, and inexpensively screened for acepromazine and its metabolites, suspects flagged, and about twenty positives confirmed. In this way a pattern of drug abuse was controlled that was impossible to counteract with the previously available technology. Additionally, this acepromazine test was developed and brought on line within about a seven month period from selection of the drug as a test candidate to the calling of the first positive. Beyond this these are the first positives for a high potency drug ever called in pre-race testing, and also the first positives called on the new immunoassay based technology.

REFERENCES

Ballard, S., Shultz, T., Kowanacki, A., Blake, J.W., Tobin, T. (1982) The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse. J. Vet. Pharmacol. Therap., 5, 21-31.

Combie, J., Blake, J.W., Nugent, T.E., Tobin, T. (1982) Morphine glucuronidase hydrolysis: superiority of glucuronidase from Patella vulgata. Clin. Chem., 28, 83-86.

Davies, J.V. and Gerring, E.L. (1983) Effect of spasmolytic analgesic drugs on the motility patterns of the equine small intestine. Res. Vet. Sci., 34(3), 334-339.

Jolley, M.E., Wang, C.-H., Ekenberg, S.J., Zuelke, M.S., Kelso, D.M. (1984) Particle concentration fluorescence immunoassay (PCFIA): a new, rapid immunoassay technique with high sensitivity. J. Immunol. Meth. 67, 21-35.

McDonald, J., Gall, R., Wiedenbach, P., Bass, V.D., DeLeon, B., Brockus, C., Stobert, D., Wie, S., Prange, C.A., Yang, J-M., Tai, C.L., Weckman, T.J., Woods, W.E., Tai, H.-H., Blake, J.W., Tobin, T. (1987) Immunoassay detection of drugs in horses. I. Particle concentraiton fluoroimmunoassay detection of fentanyl and its congeners. Res. Comm. Chem. Pathol. Pharmacol., 57, 389-407.

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(2), 259-278.

Nolan, A.M., Hall, L.W. (1984) Combined use of sedatives and opiates in horses. Vet. Record, 114(3), 63-67.

Smith, M.L., Chapman, C.B. (1987) Development of an enzyme-linked immunosorbent assay for the detection of phenothiazine tranquilizers in horses. Res. Vet. Sci., 42, 415-417.

Tai, H.-H. and Yuan, B. (1978) Development of radioimmunoassay for thromboxane B_2. Anal. Biochem., 87, 343-349.

Taylor, D.M. (1985) Chemical restraint of the standing horse. Eq. Vet. J., 17(4), 269-273.

Tobin, T. and Ballard, S. (1979) Pharmacology review: The phenothiazine tranquilizers. J. Eq. Med. Surg., 3, 460-466.

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. (1981) Drugs and the Performance Horse, Charles C. Thomas, Publisher, Springfield, IL, pp. 228-244.

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.