Journal of Chromatography, 473 (1989) 215–226
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 452

SCREENING AND CONFIRMATION OF DRUGS IN HORSE URINE BY USING A SIMPLE COLUMN EXTRACTION PROCEDURE

ASHOK K. SINGH*, M. ASHRAF, K. GRANLEY, U. MISHRA and M. MADHUSUDANA RAO
Minnesota Racing Analytical Laboratory, Department of Veterinary Diagnostic Investigation, College of Veterinary Medicine, University of Minnesota, Twin Cities Campus, St. Paul, MN 55108 (U.S.A.)
and
BRAD GORDON
Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Twin Cities Campus, St. Paul, MN 55108 (U.S.A.)
(First received December 29th, 1988; revised manuscript received February 28th, 1989)

SUMMARY

A simple and reproducible column (Clean Screen-DAU, copolymeric bonded-phase silica column) extraction procedure has been described for the screening and confirmation of drugs in horse urine. The recovery of drugs by the column extraction was better than or comparable to the recovery by the liquid–liquid extraction, which is commonly used in the equine analytical laboratories. The column extraction provided broad coverage of drugs, separated extracts into three fractions (acidic/neutral, steroids, basic), produced a cleaner extract, and eliminated the need for special liquid–liquid extraction procedures for different drugs. The column extract was cleaner and did not contain impurities, whereas, the liquid–liquid extract was relatively impure and the extract required further thin-layer chromatographic cleanup. The column extraction procedure was used to confirm the presence of several potent drugs, such as fentanyl, etorphine, and mazindol.

INTRODUCTION

Medication is used in athletes (human and horses) for legitimate treatment as well as for illegal doping, where the intent is to achieve an artificial and unfair increase in performance in a competitive event. In order to discourage the illegal doping in horses, pre- or post-race urine samples from each racing horse is tested for the presence of drugs[1]. The methods which are commonly employed for testing the horse urine involves the extraction of urine by several liquid–liquid extraction procedures, qualitative screening of the extracted urine by thin-layer chromatography (TLC), and confirmation of the drug by gas chromatography–mass spectrometry (GC–MS)[1,2]. Although the liquid–liquid extraction procedure is commonly employed in urine analysis, there is a great deal of concern about the accuracy and reliability of the procedure[3,4]. The extraction procedure is time consuming, expensive, and require

0021-9673/89/$03.50

special procedures for the extraction of several important drugs such as clenbuterol, methylphenidate, opiates and ethacrynic acid[5–8]; and the procedure is not suitable for the confirmation of parent fentanyl, sufentanyl, and mazindol, which are present in urine in trace quantities[9]. Therefore, it is important to develop a sensitive, simple, and universal extraction procedure for the screening and confirmation of drugs in horse urine. Leferink *et al.*[2] have developed a solid-phase extraction procedure which, although provided a clean extract, gave poor recovery for many important drugs. The objectives of this investigation were (i) to develop a simple and reproducible extraction procedure by using a DAU (silica based mixed phase, bonded) chromatography column, and (ii) to compare the performances of the column and liquid–liquid extraction procedures for horse urine.

MATERIALS AND METHODS

The Clean Screen DAU chromatography columns (copolymeric bonded-phase silica columns, CSDAU-505), 2 cm high in a 5-ml disposable syringe, were obtained from Worldwide Monitoring (Horsham, PA, U.S.A.). The drug standards were obtained from Sigma, Aldrich, and the University of Minnesota Veterinary Hospital. Part of the drug-treated horses' urine samples were obtained from the Quality Assurance Program conducted by the Ohio State Racing Laboratories. The radioimmunoassay (RIA) kits for fentanyl and etorphine were obtained from the Diagnostic Products Corporation (DPC) (Los Angeles, CA, U.S.A.). FES spray was prepared by dissolving 500 mg of ferric chloride in 150 ml of ethanol and 40 ml of sulfuric acid (exothermic reaction). N-1-Napthylethylenediamine dihydrochloride (NED) spray was prepared by dissolving 1.0 mg of NED in 10 ml of absolute ethanol. β-D-Glucuronidase (β-D-glucuronide glucuronosohydrolase; E.C. 3.2.1.31) from Limpet (*Patella vulgata*) was obtained from Sigma.

Column extraction procedure (Fig. 1)

For the extraction of basic and glucuronide conjugated drugs, a 5-ml aliquot of urine samples containing drugs were mixed with 2 ml of acetate buffer (pH 5.0, 0.1 *M*) and 1 ml of β-D-glucuronidase (7000 units). The mixture was incubated at 60°C for 2 h. For the extraction of acidic and neutral drugs, a 2–4-ml aliquot of urine was mixed with 1.0 ml of NaOH, (0.1 *M*) and incubated at 25°C for 10 min. After incubations, the pH of the urine samples were adjusted to 6.0 with phosphate buffer (0.1 *M*) (Fig. 1). Both urine aliquots were extracted by using a DAU column as described below.

The DAU column was connected to a vacuum manifold and washed with methanol (5 ml), water (5 ml), and 1.0 *M* acetic acid (5 ml) as shown in Fig. 1. The two urine aliquots were poured into the treated column and the samples were pulled through the column at 2 ml/min. Thereafter, the column was washed with water (5 ml) and acetic acid (5 ml), dried under vacuum, and the drugs were eluted by the procedure described in Fig. 1. The fractions containing the acidic/neutral, steroid, and basic drugs were dried separately at 50°C under nitrogen. The dried residue was redissolved in 50 μl of dichloromethane (DCM) (for acidic drugs) or ethyl acetate (EA) (for basic or steroid drugs).

TLC screening of the column extract. The acidic/neutral extract was spotted on three TLC plates and the plates were developed in solvent 1: chloroform–cyclohex-

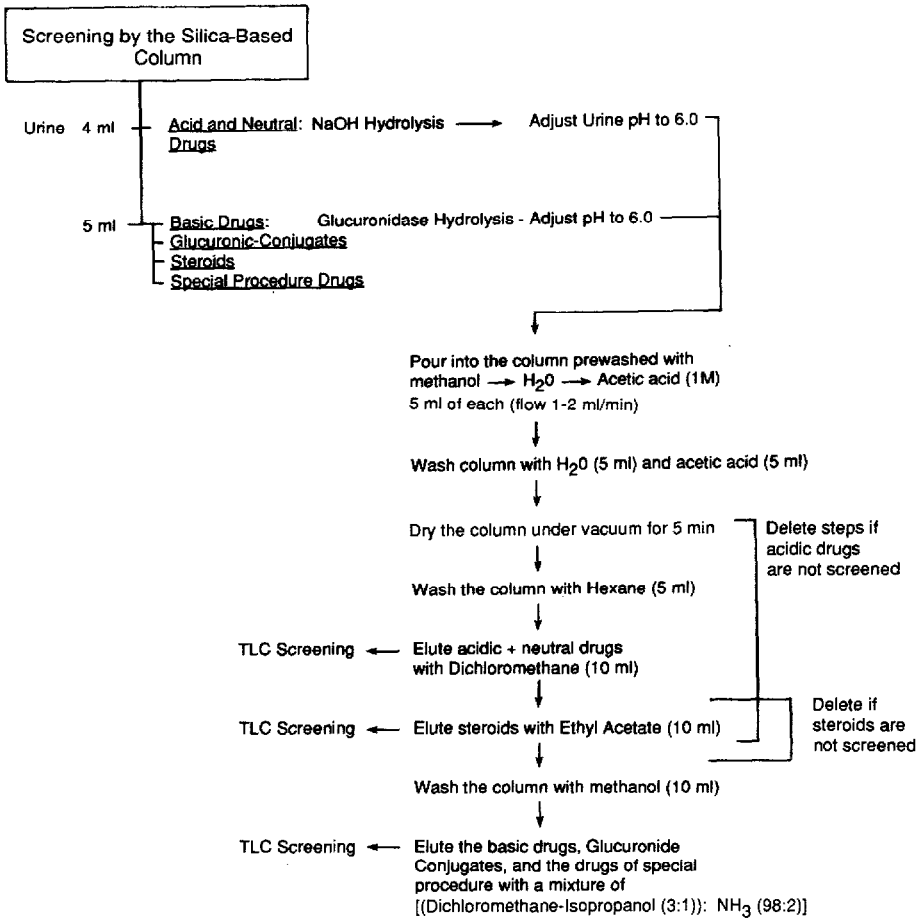

Fig. 1. Procedure for the extraction of drugs from horse urine by using a DAU column.

ane–acetic acid (4:4:2, v/v); solvent 2: chloroform–ethanol (9:1, v/v); and solvent 3: EA–methanol–NH_4OH (85:10:5, v/v/v)[10] respectively. The three plates were sprayed with Mandelin reagent[10] and heated at 60°C for 15 min. The acidic drugs developed a brown color. The plate developed in solvent 3 was further sprayed with the Dragendorff reagent[10] to screen theophylline which appeared as a dark orange spot. The steroid extract was spotted on one TLC plate and the plate was developed in solvent 4 [chloroform–EA–methanol (50:45:5, v/v/v)] followed by solvent 3. Steroids were detected by spraying the plate with H_2SO_4 + ethanol spray and heating it at 60°C until the standards appeared dark brown. The basic extract was spotted on three plates, and one plate was developed in solvent 3 and two plates were developed in solvent 5 [chloroform–methanol–propionic acid (72:18:10, v/v/v)]. The plates developed in solvent 3 were sprayed with Dragendorff reagent and sodium nitrite[10]. The plates developed in solvent 5 were sprayed with Dragendorff[10] or FES reagents. For detecting clenbuterol, plates were exposed to NO_2 gas and then sprayed with NED (Clenbuterol appeared as a pink spot).

Liquid–liquid extraction procedure (Fig. 2)

Acid drugs. A 2-ml aliquot of urine was mixed with 1.0 ml of NaOH (0.1 *M*) and the mixture was incubated at 25°C for 10 min (to hydrolyze the drug–amino acid conjugate). The pH of the mixture was adjusted to 3.0 with saturated phosphate buffer (pH 3.0) and the urine was extracted with 5 ml of DCM. The sample was centrifuged for 5 min at 1500 *g* and the DCM layer was dried at 50°C under nitrogen. For the screening of naproxen, flunixin, and indomethacin, part of the DCM extract was washed with lead acetate (0.1 *M*). We have observed that lead acetate wash also removed furosemide. The dried residue was redissolved in DCM (20 μl) for TLC screening.

Neutral drugs. A 5-ml aliquot of urine was mixed with 3 ml of phosphate buffer (pH 6.0). The mixture was extracted with 5.0 ml of EA. The EA extract was washed with 2.0 ml of $NaHCO_3$ (0.1 *M*) to remove the acidic drugs. The EA extract was dried and the dried residue was redissolved in 30 μl of EA for TLC screening.

Basic drugs. A 9-ml aliquot of urine was mixed with 2 ml of 0.5 *M* sodium carbonate buffer (pH 9.0). The mixture was extracted with DCM–isopropanol (3:1, v/v), and the organic layer was collected after centrifugation at 1500 *g* for 5 min. The organic layer was collected after centrifugation at 1500 *g* for 15 min. The organic layer was dried and the dried residue was redissolved in 30 μl of EA for TLC screening.

Steroids. A 9-ml aliquot of urine was mixed with 2 ml of saturated sodium borate solution and 5 ml of EA. After mixing, the sample was centrifuged for 5 min at

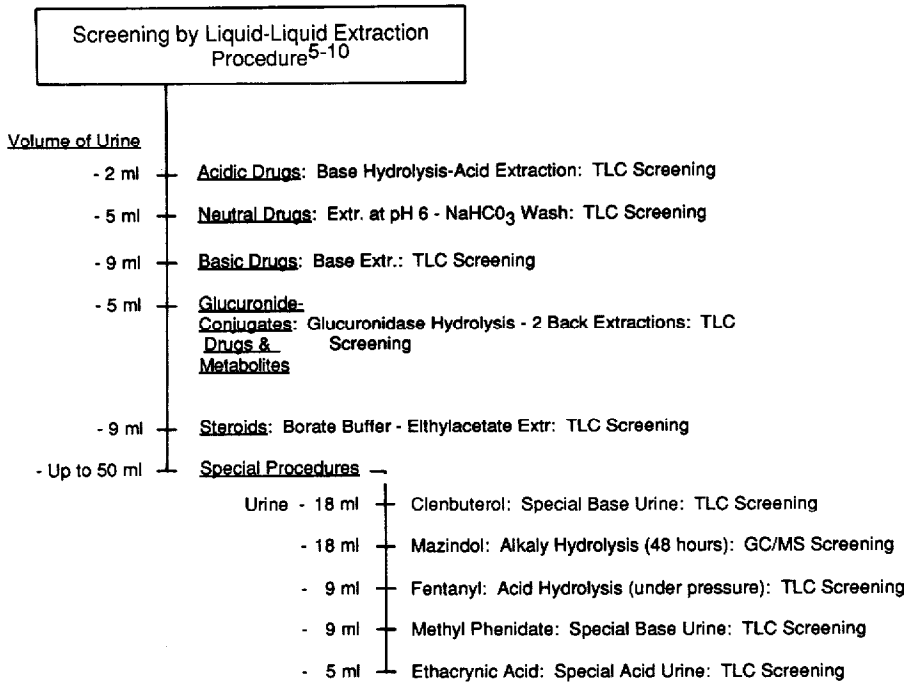

Fig. 2. Summary of the liquid–liquid extraction procedures which are commonly used for the screening and confirmation of drugs in horse urine.

1500 *g*, the organic layer was collected and washed with 1.0 ml of 15% sodium sulfate in 1.0 *M* NaOH. The EA layer was separated and dried with N_2 at 60°C. The dried residue was dissolved in 35 μl of EA.

Glucuronide-bound drugs/metabolites. A 5-ml aliquot of urine was mixed with acetate buffer (pH 5.0) and β-D-glucuronidase (7000 units) and the mixture was incubated at 60°C for 2 h. After incubation, the drugs were extracted from urine by a procedure described previously[11].

Clenbuterol extraction. An 18-ml aliquot of urine was mixed with NaOH (10 *M*) to adjust the pH of the urine to 12.0. The sample was extracted with 10 ml light petroleum (b.p. 34–36°C), the light petroleum layer was collected and dried at 50°C in N_2. The dried residue was redissolved in 20 μl of ethyl acetate for TLC screening.

TLC screening of the liquid–liquid extracts. The TLC screening of the acidic, neutral and steroid drugs were performed as described for the column extraction.

Comparison of the efficiency of column and liquid–liquid extraction procedures

The qualitative extraction efficiency was determined by extracting either the urine samples obtained from the drug treated horse or the urine samples mixed with known amounts of drug as shown in Table I. Each urine sample was extracted by the column and by the liquid–liquid extraction procedures as described earlier. Equivalent amounts of the dried residue were spotted on TLC plates along with the known quantity of each standard. The plates were developed in different solvents and sprayed with different reagents as described previously. The qualitative efficiency was determined by comparing the standard spot with that of the spot obtained from the two extraction procedures. The spots were scored from "++++" to "−": when the spot appearing similar to the standard in intensity it was scored "++++" and the absence of spot was scored "−".

The quantitative recovery of selected drugs was determined by using a GC–MS procedure. The column extracted or liquid–liquid extracted urine samples containing various drugs (Table II) were derivatized by N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and injected into the GC–MS system (HP-5990, DB-5 capillary column) by a procedure described previously[11]. The following GC–MS conditions were used: inlet temperature, 200°C; initial oven temperature 80°C; temperature program 20°C/min to 280°C; run time 35 min. The ion source temperature was 200°C, the electron energy was 70 eV, and the MS source pressure was $2.5 \cdot 10^{-6}$ Torr. The extraction efficiency was determined by comparing the area under the peak for each drug obtained from urine and from known standards.

The quantitative recovery of etorphine and fentanyl was determined by using a RIA kit. Urine samples (5 ml) containing these drugs were extracted by the two extraction procedures. The dried extract was diluted with the RIA buffer (100 μl) supplied by the manufacturer. Volumes of 50 μl of the buffer standards were mixed with [^{3}H]etorphine or [^{3}H]fentanyl and the antibodies. The mixture was incubated at room temperature for 2 h, the free isotope was removed by using dextran coated charcoal and the bound radioactivity was determined by using the scintillation counter. The concentration of the drug was determined by using a built-in RIA program in the counter.

TABLE I

QUALITATIVE TLC SCREENING OF VARIOUS DRUGS BY THE TWO EXTRACTION PROCEDURES

Abbreviations: BU = base urine extraction; AU = acid urine extraction; EH = glucuronide hydrolysis-base urine back extraction; Sp.BU = special base urine extraction; SU = steroid urine extraction; B = base urine column extract, A/N = acid urine column extract; and S = EA column extract. TLC systems; 1 = 4:4:2; 2 = 9:1; 3 = 85:10:5; 4 = 50:45:5; 5 = 72:18:10.

Drug	*Concentration or dose*	*TLC*	*Liquid–liquid extr.*		*Column extr.*	
			Extr.	*Qualitative efficiency*	*Fraction*	*Qualitative efficiency*
Acepromazine	1.0 μg/ml	3	BU	+ +	B	+ + +
Amphetamine	1.0 μg/ml	3	BU	+	B	+ + + +
Apomorphine	1.0 μg/ml	5	EH	+ + + +	B	+ + +
Buprenorphine	0.5 μg/ml	5	EH	+ +	B	+ + + +
Clenbuterol[a]	1.0 mg/horse, 4 h	3	Sp.BU	+ + +	B	+ + + +
Chlorpromazine	1.0 μg/ml	3	EH	+ + +	B	+ + +
Diazepam	1.0 μg/ml	5	EH	+ +	B	+ + + +
Dipyrone	2.0 μg/ml	3	BU	+ +	B	+ + +
Ephedrine	1.0 μg/ml	3	BU	+ +	B	+ + + +
Ethacrynic Acid	2.0 μg/ml	1,2	AU	+ +	A/N	+ +
Etorphine[a]	0.1 mg/horse, *i.m.* 4 h	–	EH	+ +	B	+ + +
Fentanyl[a]	1.0 mg/horse, *i.v.* 0, 2, 4, 6, 8, 24 h	–	EH	–	B	+ + + +
Flunixin	2.0 μg/ml	1,2	AU	+ + +	A/N	+ + +
Guaifenesin	2.0 μg/ml	3	BU	+ + + +	B	–
Hordenine	1.0 μg/ml	5	EH	+ + +	B	+ + + +
Hydrocortisone	2.0 μg/ml	3,4	SU	+ +	S	+ +
Hydromorphone	10 mg/horse, *i.m.* 4 h	5	EH	+ +	B	+ + + +
Hydromorphone	1.0 μg/ml	5	EH	+ +	B	+ + + +
4-Hydroxychlorpromazine	1.0 μg/ml	5	EH	+ +	B	+ + + +
Indomethacin	2.0 μg/ml	1,2	AU	+ + +	A/N	+ + +
Furosemide	2.0 μg/ml	1,2	AU	+ + + +	A/N	+ +
Lidocaine	1.0 μg/ml	5	EH	+ + +	B	+ + + +
Mazindol[b]	20 mg/horse, oral 4 h	–	EH	+ +	B	+ + + +
Meclizine	1.0 μg/ml	5	EH	+ +	B	+ + + +
Mefenamic Acid	2.0 μg/ml	1,2	AU	+ + +	A/N	+ + +
Meprobamate	1.0 μg/ml	3	BU	+ +	B	+ + + +
Methamphetamine	1.0 μg/ml	3	BU	+	B	+ + + +
Methadone	2.0 μg/ml	3	BU	+ +	B	+ +
Methyl phenidate	1.0 μg/ml	3	BU	+ +	B	+ + + +
Metolazone	1.0 μg/ml	3	NU	+ +	A/N	+ +
Nefopam	1.0 μg/ml	3	BU	+ + + +	B	+ + + +
Nalorphine	1.0 μg/ml	5	EH	+ + +	B	+ + +
Naloxane	2.0 μg/ml	3	BU	–	B	+ + + +
Naproxen	2.0 μg/ml	1,2	AU	+ + + +	A/N	+ + +
Nubain	1.0 μg/ml	5	EH	+ + +	B	+ + + +
N-N^1-Diethyltryptamine	1.0 μg/ml	5	BU	+ + + +	B	+ + + +
Oxymorphone[a]	10 mg/horse, *i.m.* 4 h	5	EH	+ +	B	+ +
Pemoline	1.0 μg/ml	3	BU	+ + +	B	+ +
Phenolbutazone	2.0 μg/ml	1,2	AU	+ + +	B	+ + + +
Propionylpromazine[a]	10 mg/horse *i.m.* 4 h	3	BU	+ +	B	+ + + +
Propranolol	2.0 μg/ml	3	EH	+ + +	B	+ + + +
Quinine	1.0 μg/ml	3	BU	+ +	B	+ + + +
Xylazine	1.0 μg/ml	5	EH	+ +	B	+ + + +
Strychnine	0.5 μg/ml	3	BU	+ + +	B	–
Theophylline	1.0 μg/ml	3	NU	+	B	+ + +
Trimethoprime	1.0 μg/ml	3	BU	+ + + +	B	+ + + +
Tetracaine	1.0 μg/ml	5	EH	+ + +	B	+ + + +

[a] Ohio State University Sample. Dose, route and time of urine collection.

[b] Horse injected with mazindol at the University of Minnesota.

TABLE II

PERCENT RECOVERY OF DRUGS EXTRACTED BY USING THE COLUMN AND LIQUID–LIQUID EXTRACTION PROCEDURES

Values are mean ± SD, $n = 4$.

Drug	*Analytical procedures*	*Recovery (%)*	
		Liquid–liquid base extraction	*Column extraction*
Acepromazine	GC–MS	87 ± 10	79 ± 8
Amphetamine	GC–MS	15 ± 3[a]	87 ± 8
Canabinol	GC–MS	15 ± 5[a]	0.3 ± 0.2
Cocaine	GC–MS	8 ± 3[a]	77 ± 10
Diazepam	GC–MS	5 ± 2[a]	51 ± 8
Etorphine	RIA	79 ± 10	83 ± 13
Fentanyl	RIA	0.5 ± 0.2[a]	83 ± 8
Lidocaine	GC–MS	20 ± 6[a]	63 ± 5
Mazindol	GC–MS	50 ± 5	78 ± 5
Methamphetamine	GC–MS	5 ± 3[a]	88 ± 7
Naproxen	GC–MS	60 ± 10	61 ± 9
Phenylbutazone	GC–MS	69 ± 7	75 ± 8
Xylazine	GC–MS	5 ± 3[a]	71 ± 8

[a] $P < 0.05$ when compared with the column-extraction values.

Extraction and confirmation of fentanyl and mazindol in urine samples obtained from the drug-treated horses

For the confirmation of fentanyl, a horse was injected with fentanyl (1.0 mg, *i.v.*) and urine samples were collected at 0 (pre-injection) 2, 4, 6, 8 and 24 h after the injection. The 5-ml urine sample from each time interval was subjected to glucuronidase hydrolysis. At the end of hydrolysis, a 50-μl aliquot was removed from each sample for direct RIA analysis. The remaining samples were extracted by the column and by liquid–liquid extraction procedures. The direct urine, column extracted urine and the liquid–liquid extracted urine were screened for fentanyl by using the RIA kit. The amount of fentanyl present in each sample was calculated from the standard curve. For the GC–MS confirmation of parent fentanyl in horse urine, 10 ml aliquots from 0, 2, 4, and 6 h urine samples and a 30-ml aliquot from the 8-h urine sample were hydrolyzed with glucuronidase and extracted by the column as described previously. The extract was dried and the dried residue was redissolved in 10 μl of EA. Of the EA, 1 μl was injected into the GC–MS system. The oven temperature was programmed as follows: initial temperature, 150°C; final temperature, 280°C (rate, 20°C/min); run time 20 min. The ions monitored for the selected-ion screening were *m/z* 124, 146, 189 and 245. The 30-ml aliquot from the 8-h urine sample was also extracted by the liquid–liquid extraction procedure and analyzed by the GC–MS.

For the confirmation of Mazindol, a Standard Bred horse was fed with 20 mg mazindol and an urine sample was collected at 4 h after the drug feeding (by injecting furosemide, 0.5 mg/kg, *i.m.*). Duplicate 50-ml aliquots of the urine samples were hydrolyzed by glucuronidase. One set of urine sample was extracted by using ten

columns (one for 5 ml of urine). The final extract was pooled and dried. The dried residue was derivatized with BSTFA (10 μl) and 1 μl was injected into the GC–MS system. The GC–MS oven temperature was programmed from 150°C to 280°C with 10°C/min increments. The ions monitored for the quantitation of mazindol were at *m/z* 245, 267, 327, and 356. A 50-ml volume of urine was also extracted by the liquid–liquid extraction procedure and analyzed as described above.

RESULTS AND DISCUSSION

Overall performance of the column and liquid–liquid extraction procedures

This study indicated that the column extraction procedure provided a simple and efficient method for the screening and confirmation of drugs in horse urine. The column extraction separated extracts into three fractions (acidic/neutral, steroids and basics) and produced a clean extract which was suitable for direct GC–MS analysis without further cleanup. The liquid–liquid extraction normally required a TLC cleanup which significantly reduced the recovery of the drugs. For complete screening, the DAU column extraction required 10 ml of urine, whereas, the liquid–liquid extraction required 30–50 ml of urine. By using a vacuum manifold, 30 to 40 samples can be extracted in less than an hour by one technician.

Extraction and recovery of the acidic and neutral drugs

The acidic drugs commonly used in horses are phenylbutazone, fuorsemide, naproxen, flunixin, etc.; and the neutral drugs used in horses are theophylline, theobromine, metolazone, etc.[1,12]. This study indicated that the DAU column provided a relatively clean extraction of the acidic and neutral drugs. The qualitative and quantitative screening has shown that the extraction efficiencies of acidic drugs by the two procedures were similar, except for furosemide which is extracted better by the liquid–liquid extraction procedure (Tables I and II). Unlike the acidic drugs, theophylline exhibited better recovery by the column extraction procedure (Table I). Since the acidic and neutral drugs are used in large quantities, extraction by either procedure may be satisfactory for the screening of these drugs.

Extraction and recovery of the basic drugs and the drugs which required special liquid–liquid extraction procedure

Basic drugs include a broad range of compounds which have different chemical and pharmacological properties. Previous studies have shown that a single liquid–liquid extraction method did not cover all the basic drugs, and that special procedures were necessary to extract certain important drugs such as clenbuterol, methylphenidate and opiates[5–8]. This study indicated that the DAU column extracted a broad range of drugs and the recovery of drugs by the column extraction was better than or similar to the recovery of drugs by the liquid–liquid extraction (Tables I and II). Amphetamine and methamphetamine exhibited $< 25\%$ recovery by the liquid–liquid extraction, whereas, these drugs exhibited $> 85\%$ recovery by the column extraction (Tables I and II). Also the column extraction for these drugs was cleaner and did not contain impurities, whereas the liquid–liquid extraction was relatively impure and the samples required further TLC cleanup (Fig. 3). This study indicated that the column extraction provided an uniform extraction efficiency for a wide range of drugs in-

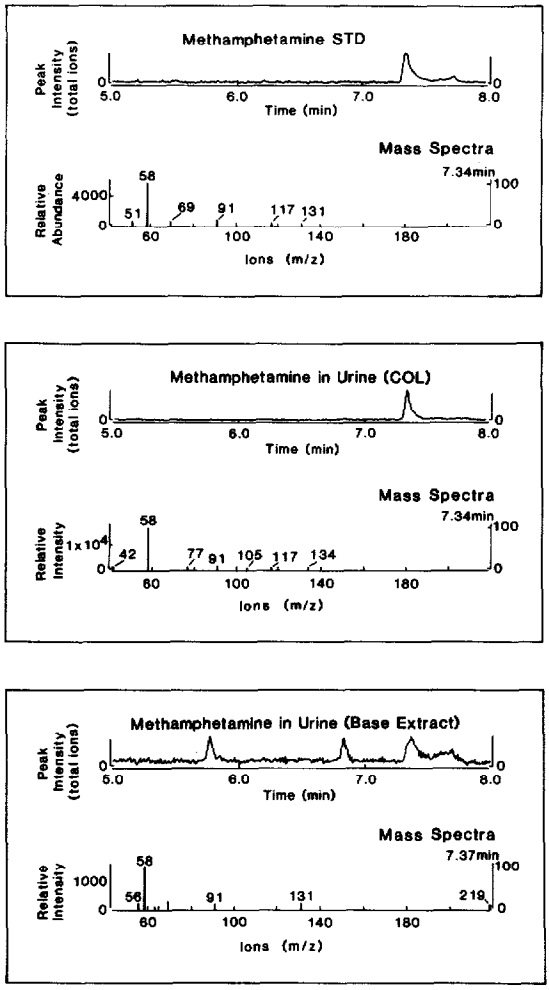

Fig. 3. Confirmation by GC–MS of methamphetamine extracted from horse urine by the column or liquid–liquid extraction procedure. Urine samples of 5 ml (containing 1.0 μg methamphetamine/ml urine) were extracted by the two extraction procedures. The extract was dissolved in 100 μl of ethyl acetate and 1.0 μl of the extract was analyzed by the GC–MS. Standard (STD) samples (containing 10.0 ng of the drug) were also analyzed.

cluding clenbuterol, methylphenidate, diazepam and opiates (Tables I and II). Cone *et al.*[5] have shown that the extraction efficiencies of opiates were significantly different for different solvents used for the liquid–liquid extraction of urine.

Extraction and recovery of steroids

Steroids were eluted from the column by using EA (Fig. 2). Since EA also eluted some non-specific compounds, the steroid extract was relatively dirty and appeared similar to the liquid–liquid extract. The recovery of steroids from the two procedures appeared similar (Table I).

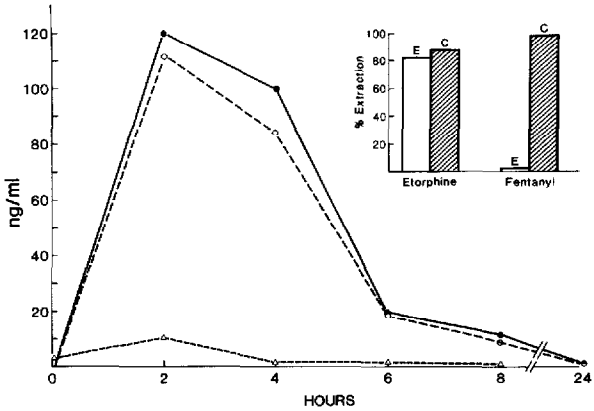

Fig. 4. Comparison of the recoveries of etorphine and fentanyl by the column and liquid–liquid extraction procedures. (●) Analysis of fentanyl by RIA in whole urine; (○) analysis of fentanyl by RIA in column extract; (△) analysis by RIA of liquid–liquid extract. The bar graphs shows the percent recoveries of the two drugs (hatched), column extraction and (open) liquid–liquid extraction.

Extraction, recovery and confirmation of potent drugs such as fentanyl, etorphine and mazindol

The potent drugs included in this study are known to stimulate the central nervous system at lower doses[13–18]. The short duration of action, low urinary concentration, and difficulty in detection have made these drugs attractive doping agents

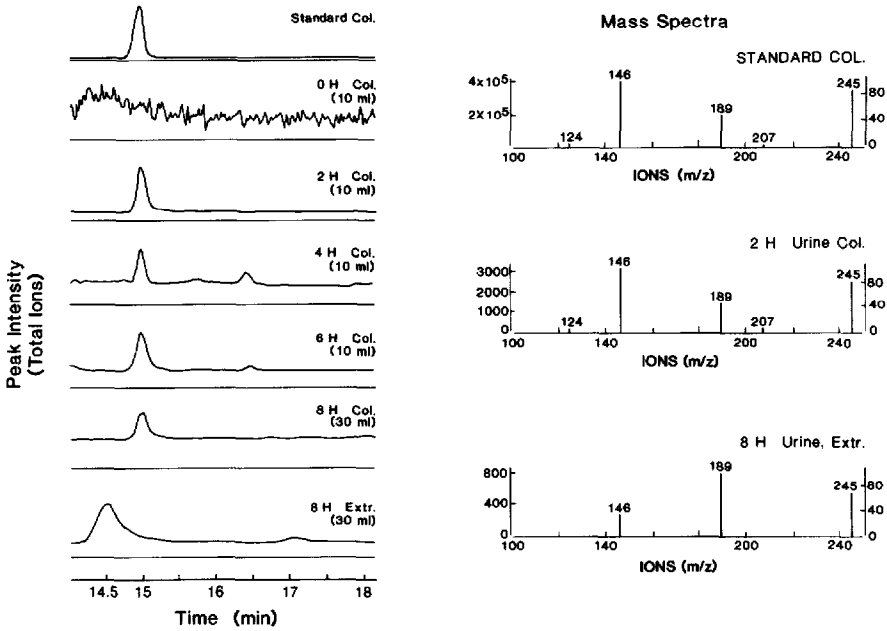

Fig. 5. Screening of fentanyl in urine obtained from fentanyl-injected horse and extracted by using the column (0, 2, 4, 6, 8 h urine) or liquid–liquid extraction (8 h urine) procedures. Parent fentanyl was confirmed by using the selected-ion monitoring GC–MS procedure.

at racetracks[19,20]. The identification of fentanyl and mazindol by the liquid–liquid extraction is particularly difficult because these drugs are first hydrolyzed to despropionyl fentanyl (DPF) and 2-(4-chlorobenzyl)benzoic acid (CBB) respectively[9,21], and then the hydrolyzed product is identified by GC–MS. Since DPF and CBB are not the natural metabolites of fentanyl or mazindol, confirmation of these drugs by confirming the presence of DPF or CBB can be easily challenged in court. This study had indicated that the DAU column selectively extracted etorphine, fentanyl, and mazindol with > 80% recovery (Table II, Fig. 4). The liquid–liquid extraction procedure was not suitable for the extraction of fentanyl and mazindol since the recovery was poor for both drugs (Table II, Fig. 4). Because of the selective and efficient extraction of fentanyl and mazindol by the column, it was possible to identify and confirm the unchanged parent drugs in horse urine. As shown in Figs. 4 and 5, the presence of parent fentanyl was identified by both RIA (Fig. 4) and GC–MS methods (Fig. 5) for up to 8 h after dosing the horse with fentanyl. Presence of parent mazindol was also confirmed by GC–MS of urine samples (Fig. 6) obtained from the mazindol treated horse. Based on these observations it is proposed that (i) the hydrolysis of fentanyl or mazindol was not necessary for the identification of these drugs when the column extraction procedure was used, and (ii) the poor recovery of fentanyl and mazindol by the liquid-liquid extraction procedure may be responsible for the difficul-

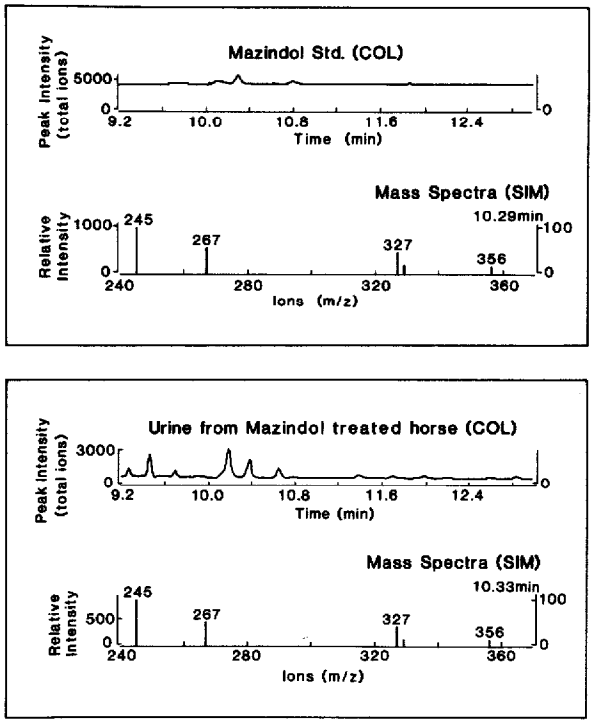

Fig. 6. Screening of mazindol in urine obtained from mazindol-fed horse and extracted by the column extraction procedure. The parent mazindol was confirmed by using a selected-ion monitoring (SIM) GC–MS procedure.

ties previous investigators encountered in the confirmation of parent drugs by GC–MS.

In conclusion, the silica-based DAU column provided a simple and efficient extraction of horse urine for the screening and confirmation of drugs. The recovery of drugs by the column extraction was better than or similar to the recovery by the liquid–liquid extraction procedure. The column extract was clean and could be subjected to direct GC–MS confirmation. The liquid–liquid extract normally required TLC cleanup which reduced the recovery to 25–30%. The column extraction also extracted the potent drugs, such as fentanyl and mazindol which was not possible by the common liquid–liquid extraction; and eliminated the need for special liquid–liquid extraction procedures.

ACKNOWLEDGEMENTS

This project was partially funded by a grant from the Minnesota Racing Commission. Authors are thankful to Mary Thurn for typing the manuscript and to Michelle Mero for preparing the figures.

REFERENCES

1 T. Tobin, *Drugs and the Performance Horse,* Charles C. Thomas Publisher, Springfield, IL, 1981, pp. 5–20.
2 J. G. Leferink, J. Bankers and A. J. H. Schnotman, *Proceedings of the 6th International Conference of Racing Analyst and Veterinarians, Hong Kong, December 1–7, 1985,* Macmillan, New York, 1986, pp. 171–175.
3 H. J. Hansen, S. P. Caudill and J. Boone, *J. Am. Med. Assoc.,* 253 (1985) 2382–2387.
4 D. J. Hanson, *Chem. Eng. News,* 64 (1986) 7–14.
5 E. J. Cone, W. D. Darwin and W. F. Buchwald, *J. Chromatogr.,* 275 (1983) 307–318.
6 E. J. Cone, *J. Chromatogr.,* 129 (1976) 355–360.
7 B. R. Manno, J. E. Manno and C. A. Dempsey, *J. Anal. Toxicol.,* 10 (1986) 116–119.
8 K. S. Patrick and W. H. Elliott, *J. Chromatogr.,* 343 (1985) 329–338.
9 L. M. Young and V. Robillo, *Proceedings of the 6th International Conference of Racing Analyst and Veterinarians, Hong Kong, December 1–7, 1985,* Macmillan, New York, 1986, pp. 257–268.
10 B. Davidow, N. L. Petri and B. Quame, *Am. J. Clin. Pathol.,* 50 (1988) 714–717.
11 A. K. Singh, U. Mishra, M. Ashraf, E. H. Abdennebi, K. Granley, D. Dombrovskis, D. Hewetson and C. M. Stowe, *J. Chromatogr.,* 404 (1987) 223–232.
12 M. Donike, S. Horning, F. Mandel and W. Schanzer, *Proceedings of the 6th International Conference of Racing Analysts and Veterinarians, Hong Kong, December 1–7, 1985,* Macmillan, New York, 1986, pp. 187–193.
13 N. W. Shepard, *The application of Neurolepta-analgesia in anesthesia and other practices,* Pergamon Press, Oxford, 1965, pp. 1–25.
14 J. F. Gardocki and J. A. Yelnosky, *Toxicol. Appl. Pharmacol.,* 6 (1964) 48–53.
15 F. L. Grell, R. A. Koons and J. S. Denson, *Anesth. Analg. (Cleveland),* 49 (1970) 523.
16 J. H. Gagerty and J. H. Trapold, *Triangle Engl. Ed.,* 15 (1976) 25–36.
17 J. H. Gagerty, C. Penberthy, L. C. Iorio and J. H. Trapold, *Arch. Int. Pharmacodyn.,* 214 (1975) 285–307.
18 W. F. M. Van Bever, C. J. E. Niemegeers, K. H. L. Schellekens and P. A. J. Janssen, *Arzeneim.-Forsch.,* 26 (1975) 1548.
19 J. M. Frincke and G. L. Henderson, *Drug Metab. Dispos.,* 8 (1980) 425–427.
20 G. L. Henderson, J. M. Frincke and R. J. Garber, *Proc. West. Pharmacol. Soc.,* 24 (1981) 137–140.
21 J. H. Gagerty and J. H. Trapold, *Triangle Engl. Ed.,* 15 (1976) 25–36.