



Determination of 78 Banned or Controlled Racing Industry Drugs in Horse Urine Using SPE and LC-MS/MS

UCT Part Numbers

XRDAH203

Gravity Flow XtrackT[®] DAU
200 mg, 3 mL column

SPPHO6001-5

Select pH Buffer Pouches
100 mM Phosphate pH 6.0

ASFBETA-GLUC-25

Abalonase[™] +
Purified Beta-glucuronidase
Enhanced sulfatase activity

SLDA100ID21-3UM

Selectra[®] DA HPLC
100 X 2.1 mm, 3 μm

SLDAGDC21-3UM

Selectra[®] DA Guard Column
10 X 2.1 mm, 3 μm

SLGRDHDR

Guard Column Holder



Summary:

The world of horse race drug testing is constantly evolving. New regulations regarding banned and controlled substances can be found throughout the United States, Europe, Asia and Australia [1,2]. The need for a reliable and quick SPE method for the determination and separation of a wide variety of drug classes is critical to laboratories certified in this realm of veterinary medicine. In addition to traditional drugs of abuse, horse race drug testing also encompasses drug classes such as bronchodilators, diuretics, antihistamines and non-steroidal anti-inflammatory medications.

The use of Gravity Flow XtrackT[®] DAU Sorbent reduces the challenges traditionally associated with extracting horse urine. It allows for one elution step that results in a clean extract and contains acidic, neutral and basic drugs. Abalonase[™] + Purified Beta-glucuronidase with enhanced sulfatase activity is utilized rather than traditional abalone derived enzyme due to the high abundance of sulfate-bound conjugates in horse urine. This method can easily be adapted to encompass other animal racing industries as well.



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Sample Pretreatment:

To 250 μ L horse urine add 250 μ L of Abalonase™ + working solution (see Abalonase™ + package insert for working solution preparation instructions) and appropriate amount of internal standard. Vortex for 30 seconds.

SPE Procedure:

1. SPE Conditioning

- 1 x 3 mL MeOH.
- 1 x 3 mL D.I. H₂O.
- 1 x 3 mL 100 mM Phosphate Buffer (pH 6.0).

2. Sample Extraction

- Load sample onto pre-conditioned SPE cartridge.

3. Wash Cartridge

- 1 x 3 mL D.I. H₂O.
- 1 x 3 mL 100mM Acetic Acid.
- 1 x 3 mL Hexane.
- Dry column under high vacuum for 1 minute to remove residual Hexane.

4. Elution

- 1 x 3 mL MeOH/NH₄OH (98:2).

5. Concentration

- Evaporate the sample to dryness at 35-40°C under a gentle stream of nitrogen.
- Reconstitute in 100 μ L of mobile phase starting gradient.

LC-MS/MS Parameters:

HPLC Parameters		
HPLC: Agilent 1200 Series		
Column: UCT, Selectra®, DA, 100 x 2.1 mm, 3 μ m		
Guard column: UCT, Selectra®, DA, 10 x 2.1 mm, 3 μ m		
Column temperature: 40 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 10 μ L		
Gradient program:		
Time (min)	A% (0.1% formic acid in H ₂ O)	B% (0.1% formic acid in MeOH)
0	95	5
5	40	60
10	40	60
10.1	0	100
16.5	0	100
17	95	5
22	95	5

MS Parameters	
Instrumentation	AB Sciex 4000 Q Trap
Polarity	ESI +
Spray voltage	5000 V
Vaporizer temperature	650 °C
Collision gas	Medium
Cycle time	6.2 sec
Acquisition method	Scheduled MRM

SRM Transitions							
Analyte	t _R (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	DP(V)
Acetaminophen	6.4	153.0	116.0	29	115.1	43	96
Albuterol	5.3	241.0	149.1	25	223.1	15	41
Alprazolam	13.0	308.9	205.3	17	281.2	17	31
Amitriptyline	11.9	278.2	91.1	17	105.1	17	46
Amphetamine	6.0	136.1	91.2	23	119.1	13	51
Atenolol	6.3	267.1	145.0	35	190.0	25	61
Baclofen	6.4	214.1	150.9	23	196.9	17	36
Benzoyllecognine	9.2	209.1	168.0	25	105.1	39	76
Benzocaine	9.6	166.2	138.0	15	120.0	23	31
Bupivacaine	9.1	289.0	140.3	35	84.1	59	51
Buprenorphine	10.6	468.4	55.1	87	83.2	87	81
Butorphanol	9.7	329.2	311.2	33	158.2	61	71
Caffeine	10.0	195.0	138.0	27	110.0	33	36
Carprofen	13.8	274.2	228.0	15	193.2	43	36
Chlordiazepoxide	10.2	300.0	227.1	21	283.0	35	56
Chlorothiazide	14.2	295.9	215.2	29	250.0	19	31
Citalopram	10.0	325.0	109.1	39	262.0	27	46
Clenbuterol	8.3	277.1	202.9	25	259.0	15	66
Clonazepam	13.4	316.1	270.2	39	241.2	37	56
Clonzapine	9.5	327.0	270.0	35	192.0	63	56
Cocaine	9.2	304.1	182.0	30	95.1	73	46
Cyclothiazide	10.7	390.1	317.2	33	100.0	49	41
Dextrophan	8.6	258.1	157.1	53	199.1	37	91
Diazepam	14.5	285.1	193.2	43	154.1	37	56
Dothiepin	11.1	296.1	251.1	25	225.1	25	61
Ephedrine	5.5	166.2	148.0	11	117.0	25	36
Estazolam	12.5	295.3	100.2	31	208.1	31	61
Fentanyl	10.5	338.3	189.1	29	105.0	57	71
Flunitrazepam	14.1	313.9	268.2	33	239.2	45	36
Flurazepam	10.9	388.1	315.1	37	134.1	67	46
Furosemide	14.2	332.1	91.1	57	119.0	33	66
Gabapentin	6.2	172.0	154.0	15	137.0	25	61
Hexobarbital	10.7	273.0	157.0	17	81.0	29	41
Hydrochlorothiazide	14.3	297.9	252.2	19	217.1	29	36
Hydrocodone	8.0	300.0	199.0	39	128.0	50	46
Hydromorphone	6.7	286.0	185.0	41	157.0	50	46
Imipramine	11.7	280.9	85.9	25	58.1	63	61
Ketamine	8.8	238.1	125.0	30	220.0	21	36
Levorphanol	8.7	258.1	157.2	59	199.1	35	61



SRM Transitions							
Analyte	t _R (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	DP (V)
Lidocaine	7.4	235.1	86.0	27	58.1	55	36
Loratadine	14.9	383.1	337.2	31	267.2	45	76
Lorazepam	13.1	321.0	303.5	21	275.0	29	46
Lormetazepam	14.0	334.9	289.1	31	317.2	17	76
Maprotiline	11.8	278.1	250.1	27	117.0	33	41
MDA	7.1	180.2	163.1	15	105.0	31	46
MDEA	8.3	208.1	163.0	19	105.1	37	41
MDMA	7.7	194.2	163.1	19	105.1	33	76
Meprobamate	11.3	219.0	191.0	31	178.0	31	126
Methadone	13.2	310.1	265.0	19	105.0	35	56
Methamphetamine	7.0	150.0	91.1	27	119.1	15	46
Mianserin	10.3	265.0	208.0	31	91.0	61	66
Midazolam	10.8	326.0	291.0	50	222.0	50	60
Mirtazepine	9.4	266.1	72.0	27	195.1	61	61
Morphine	6.2	286.3	152.0	79	165.0	50	46
Nalbuphine	8.4	359.2	341.1	31	255.0	43	71
Nalorphine	13.0	311.3	266.1	25	105.1	41	71
Naloxone	7.5	328.0	310.0	27	211.9	55	46
Naltrexone	14.3	343.0	308.0	35	315.3	35	46
Naproxen	13.7	231.1	185.0	21	170.2	37	36
Nitrazepam	11.7	281.1	86.0	31	208.1	31	36
Nortriptyline	11.7	264.1	190.9	33	105.1	33	51
Olonzapine	7.1	313.0	256.0	33	84.0	35	66
Orphenadrine	10.5	270.1	166.0	37	165.1	63	26
Oxazepam	13.2	287.0	241.3	31	104.2	21	46
Oxycodone	7.9	316.0	140.0	39	256.0	50	31
Oxymorphone	6.3	302.0	227.0	37	198.0	50	36
Paroxetine	12.4	330.0	192.0	27	123.0	37	61
Prazepam	15.1	325.1	271.0	29	140.0	57	56
Promethazine	11.2	285.1	86.0	29	71.1	61	41
Propranolol	9.6	261.1	116.1	27	184.1	25	66
Protriptyline	11.5	264.2	191.1	29	91.2	29	51
Pseudoephedrine	6.0	166.2	148.0	11	117.0	25	36
Risperidone	10.6	411.1	191.1	39	69.0	81	61
Sertraline	12.9	305.9	274.9	17	159.1	33	31
Theophylline	8.2	180.9	123.9	27	95.9	33	31
Trazodone	10.3	372.1	148.1	47	95.9	85	56
Triazolam	14.3	343.0	308.1	39	315.0	37	81
Trimipramine	12.4	295.1	100.2	51	58.1	51	51
Venlafaxine	11.9	278.2	233.1	25	105.2	29	31



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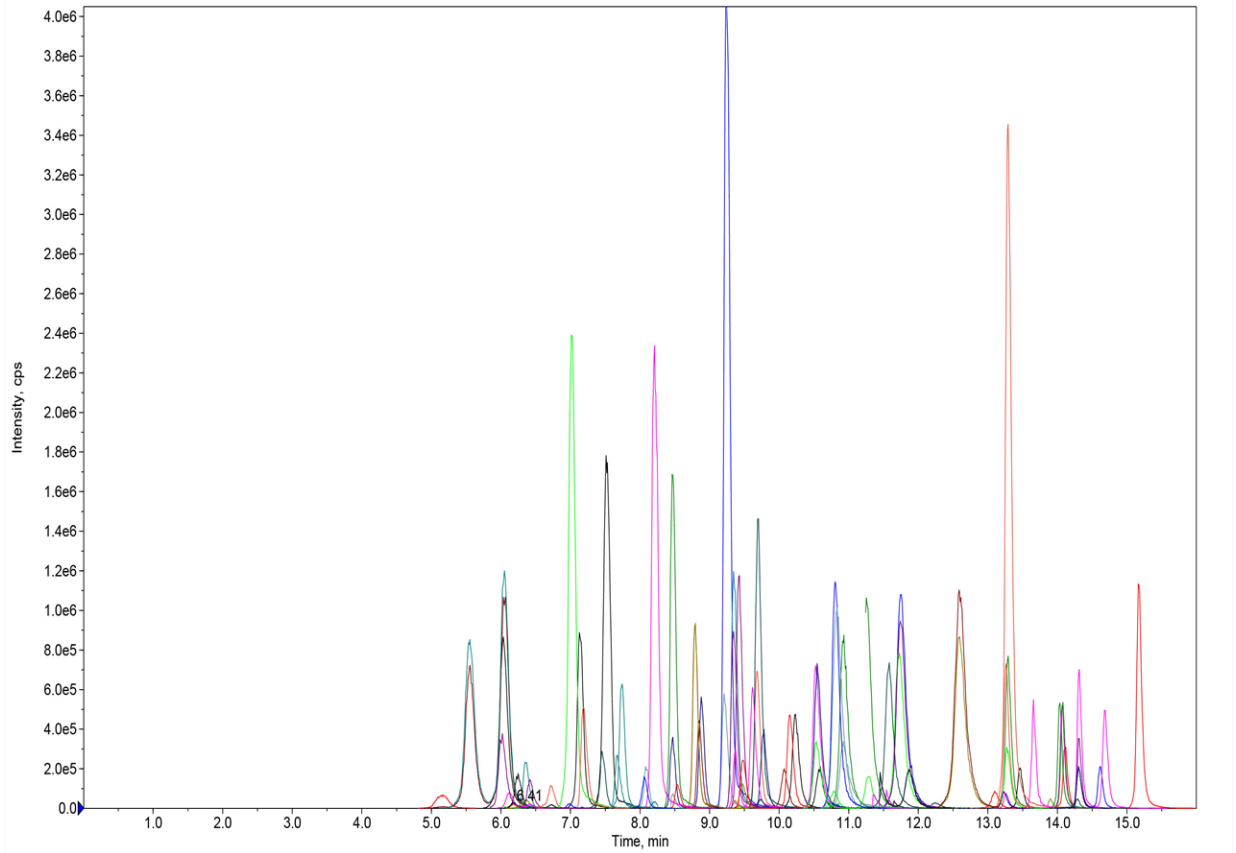


Figure 1. Chromatogram of an extracted sample fortified at 50 ng/mL.

Results:

FEI Controlled Substances		
Compound	Relative Recovery (%) 250 ng/mL (n=4)	ARCI Classification
Albuterol	70	3
Benzocaine	82	4
Bupivacaine	114	2
Buprenorphine	97	2
Butorphanol	84	3
Caffeine	72	2
Carprofen	79	4
Chlorothiazide	70	4
Clenbuterol	79	3
Diazepam	81	2
Fentanyl	111	1
Furosemide	120	N/A
Hydrochlorothiazide	70	4
Ketamine	81	2
Lidocaine	87	2
Loratadine	105	4
Methadone	102	1
Morphine	116	1
Oxazepam	72	2
Promethazine	98	3



FEI Banned Substances					
Drug	Rel. Recovery (%)	ARCI Classification	Drug	Rel. Recovery (%)	ARCI Classification
Acetaminophen	53	4	MDMA	77	1
Alprazolam	89	2	Meprobamate	84	2
Amitriptyline	98	2	Methamphetamine	76	1
Amphetamine	89	1	Mianserin	97	N/A
Atenolol	77	3	Midazolam	112	2
Baclofen	57	4	Mirtazepine	127	2
Benzoyllecgonine	75	1	Nalbuphine	70	2
Chlordiazepoxide	85	2	Nalorphine	114	2
Citalopram	97	2	Naloxone	87	3
Clonazepam	79	2	Naltrexone	86	3
Clozapine	85	2	Nitrazepam	99	2
Cocaine	90	1	Nortriptyline	86	2
Cyclothiazide	108	4	Olonzapine	89	2
Dextrorphan	71	4	Oxycodone	80	1
Dothiepin	94	N/A	Oxymorphone	71	1
Ephedrine	120	2	Paroxetine	86	2
Estazolam	109	2	Prazepam	93	2
Flunitrazepam	82	2	Propranolol	87	3
Flurazepam	113	2	Protriptyline	95	2
Gabapentin	61	4	Pseudoephedrine	119	3
Hydrocodone	76	1	Risperidone	77	2
Hydromorphone	75	1	Sertraline	84	2
Imipramine	91	2	Trazodone	107	2
Levorphanol	128	1	Triazolam	86	2
Lorazepam	91	2	Trimipramine	90	2
Lormetazepam	87	2	Venlafaxine	104	2
Maprotiline	82	2	Warfarin	75	5
MDA	72	1	Zolpiclone	88	2
MDEA	84	1	Zolpidem	130	2

REFERENCES:

1. http://www.fei.org/sites/default/files/FEI_Prohibited_Substances_List_and_DB.pdf
2. <http://arcicom.businesscatalyst.com/model-rules---standards.html>



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