Multidrug Analytical Method using Polarity Switching and Scheduled MRM on the Sciex Triple Quad 7500 for Equine Doping Control

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Overview

Purpose: To develop a screening method to simultaneously screen for a variety of compounds at various concentrations in a short amount of time.

Method: A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the simultaneous detection of more than 100 compounds in equine plasma was developed. The samples were prepared by liquid-liquid extraction using methyl tert-butyl ether (MTBE). An Ace 5 C18 column (75 x 2.1mm) was used for the analysis. 5mM ammonium formate and acetonitrile were used for the mobile phases. Analytes were monitored using scheduled multiple-reaction monitoring (MRM) on a Sciex Triple Quad™ 7500 mass spectrometer using both negative and positive ionization. Total analysis time was 7.5 minutes per sample.

Results: Liquid-liquid extraction using MTBE provided efficient extraction recoveries for all compounds in this study, ensuring compliance with the required sensitivities for equine doping control regulatory agencies. Polarity switching increased compound coverage in both positive and negative modes, improving screening selectivity and sensitivity. The method demonstrated excellent sensitivity for all compounds studied. Among the compounds in this study, 43% had a limit of detection (LOD) less than 10 pg/mL, 22% had a LOD between 10-50 pg/mL, and 32% had a LOD between 100-500 pg/mL. Although there are over three hundred MRM transitions built into this method, the scheduled multiple-reaction monitoring (MRM) allowed for the acquisition of sufficient points across the peaks for peak integration. On average, there were approximately 6 data points across a peak. For the majority of the compounds in this method, three transitions were included in the mass table to allow reliable screening results, significantly reducing the false positive screening rate. Compounds eluted throughout the LC gradient, with retention times ranging from 1.00 min at the earliest to 6.30 min at the latest. Stable retention times were observed from run to run, demonstrating the robustness of the LC method. The developed method has successfully screened hundreds of samples and has proven to be a sensitive, reliable, and robust screening method.

Method

Sample Preparation: 1 mL of plasma or serum is extracted using 5 mL of methyl tert-butyl ether.

Instrumentation:

Sciex Triple Quad™ 7500 triple quadrupole mass spectrometer with an ExionLC™ liquid chromatographic system

LC Column: Ace 5 C18 column (75 x 2.1mm).

Mobile Phase: 5 mM NH_4FA (A) and Acetonitrile (B).

The gradient is shown in table 1 and total analysis time is 7.5 minutes.

Injection Volume: 20 µL.

Scan Type: Scheduled Multiple reaction monitoring (sMRM) with polarity switching.

Table 1. LC Gradient Program for Analysis

Time (min)	A(%)	(B%)
0	95	5
5	10	90
6	10	90
6.5	95	5
7.5	95	5

Table 2. sMRM Parameters of Analytes and LOD in Negative Mode

Analytes	Retention Time (min)	Retention Time Tolerance (±s)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (CE, V)	Collision Cell Exit Potential (CXP, V)	Entrance Potential (EP,V)	Limit of Detection (LOD, ng/mL)
Amobarbital	3.98	30	225.0	42.0	-36	-15	-10	0.1
Andarine	4.37	30	440.2	150.0	-30	-15	-10	
	4.37	30	440.2	261.1	-20	-15	-10	
	4.37	30	440.2	205.0	-35	-15	-10	0.05
Beclomethasone	3.98	30	453.1	377.2	-19	-9	-10	
	3.98	30	453.1	341.2	-27	-8	-10	
	3.98	30	453.1	297.2	-35	-21	-10	0.01
Butalbital	3.57	30	223.0	42.0	-45	-15	-10	
	3.57	30	223.0	180.1	-17	-13	-10	
	3.57	30	223.0	85.0	-16	-4	-10	0.1
Chlorthalidone	3.25	30	337.0	146.0	-35	-21	-10	
	3.25	30	337.0	80.0	-35	-21	-10	
	3.25	30	337.0	283.0	-35	-21	-10	0.1
Ostarine	4.70	30	388.0	269.1	-25	-18	-10	
	4.70	30	388.0	185.1	-48	-22	-10	
	4.70	30	388.0	90.0	-99	-10	-10	0.01
Pentobarbital	3.99	30	225.0	182.0	-18	-15	-10	
	3.99	30	225.0	138.0	-18	-15	-10	
	3.99	30	225.0	42.0	-18	-15	-10	0.1
Phenobarbital	3.52	30	231.0	42.0	-33	-15	-10	0.1
Phenytoin	3.91	30	251.0	208.0	-13	-17	-10	
	3.91	30	251.0	102.0	-21	-17	-10	
	3.91	30	251.0	180.0	-20	-17	-10	0.1
Prednisolone	3.58	30	405.0	172.0	-55	-13	-3	
	3.58	30	405.0	171.0	-69	-13	-3	0.05
Secobarbital	4.13	30	237.0	42.0	-35	-15	-10	0.1
Zeranol	4.28	30	321.0	277.1	-31	-17	-10	
	4.28	30	321.0	303.1	-30	-19	-10	
	4.28	30	321.0	259.1	-34	-17	-10	0.1

Table 3. sMRM Parameters of Analytes in Positive Mode

Analytes	Retention Time (min)	Retention Time Tolerance (±s)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (CE, V)	Collision Cell Exit Potential (CXP, V)	Entrance Potential (EP,V)	Limit of Detection (LOD, ng/mL)
16b-OH-Stanazolol	4.03	30	345.4	95.0	10	50	5	
	4.03	30	345.4	175.1	10	49	5	
	4.03	30	345.4	345.4	10	49	5	0.01
3-OH Lidocaine	2.37	30	251.1	86.1	10	22	15	
	2.37	30	251.1	58.1	10	55	15	0.01
3-OH Mepivacaine	2.43	30	263.2	70.2	10	62	15	
	2.43	30	263.2	98.2	10	26	15	
	2.43	30	263.2	68.2	10	84	15	0.01
4-Aminoantipyrine	2.89	30	204.1	56.0	6	40	10	
	2.89	30	204.1	159.0	6	40	10	
	2.89	30	204.1	94.0	6	40	10	0.01
4-Methylaminoantipyrine	2.65	30	218.0	56.0	10	30	15	
	2.65	30	218.0	97.0	10	30	15	
	2.65	30	218.0	159.0	10	30	15	0.01
7-Carboxy-CBD/11-Nor-THC	5.20	45	345.2	299.2	10	29	16	
	5.20	45	345.2	193.0	10	38	9	
	5.20	45	345.2	257.1	10	29	14	0.25
Acetazolamide	2.36	30	223.0	181.1	10	21	15	
	2.36	30	223.0	163.9	10	28	15	0.5
ADB-Fubinaca	4.70	30	383.2	253.0	10	34	18	
	4.70	30	383.2	338.0	10	21	23	
	4.70	30	383.2	109.0	10	55	9	0.002
Albuterol	2.19	30	240.0	148.0	10	25	15	
	2.19	30	240.0	222.0	10	14	15	
	2.19	30	240.0	166.0	10	18	15	0.01
Altrenogest	4.80	30	311.0	227.0	10	25	15	
	4.80	30	311.0	269.0	10	25	15	
	4.80	30	311.0	251.0	10	25	15	0.01
Ambroxol	3.24	30	376.9	262.0	10	20	42	
	3.24	30	376.9	116.0	10	20	42	
	3.24	30	376.9	183.0	10	50	42	0.01
Amiloride	2.25	30	230.0	171.0	10	30	11	
	2.25	30	230.0	60.0	10	40	11	0.1
Androsterone	5.07	30	291.0	255.0	10	21	15	
	5.07	30	291.0	147.0	10	21	15	
	5.07	30	291.0	199.0	10	21	15	5
Anileridine	3.66	30	353.2	120.0	10	15	15	
	3.66	30	353.2	103.0	10	65	15	0.01
Benzoylecgonine	2.84	30	290.0	168.0	10	26	15	
	2.84	30	290.0	105.0	10	35	15	
	2.84	30	290.0	82.0	10	34	15	0.2
Benzylpiperazine	2.57	30	177.0	91.0	10	32	15	
	2.57	30	177.0	85.0	10	32	15	
	2.57	30	177.0	56.0	10	20	15	0.01
Boldenone	4.15	30	287.0	173.0	10	23	42	
	4.15	30	287.0	135.0	10	21	42	
	4.15	30	287.0	121.0	10	32	42	0.025
Boldione	4.34	30	285.2	121.0	10	20	15	
	4.34	30	285.2	147.0	10	20	15	
	4.34	30	285.2	151.0	10	20	15	0.01
Bromhexine	4.49	30	377.0	114.0	10	28	15	
	4.49	30	377.0	264.0	10	28	15	
	4.49	30	377.0	83.0	10	28	15	0.01
Buprenorphine	3.83	30	468.0	84.0	10	60	15	
	3.83	30	468.0	101.0	10	47	15	
	3.83	30	468.0	414.0	10	47	15	0.01
Butorphanol	3.34	30	328.0	185.0	10	50	7	
	3.34	30	328.0	157.0	10	61	7	
	3.34	30	328.0	160.0	10	48	7	0.01
Cathinone	2.43	30	150.0	132.0	10	17	15	
	2.43	30	150.0	117.0	10	32	15	
	2.43	30	150.0	105.0	10	25	15	0.01
Clenbuterol	3.11	30	277.0	203.0	10	22	12	
	3.11	30	277.0	168.0	10	41	12	
	3.11	30	277.0	132.0	10	38	13	0.0005
Clenpenterol	3.31	30	291.1	203.0	10	15	15	
	3.31	30	291.1	132.0	10	30	15	0.05
Clostebol	4.87	30	323.0	143.0	10	36	15	
	4.87	30	323.0	131.0	10	36	15	
	4.87	30	323.0	269.0	10	36	15	0.01
Codeine	2.54	30	300.1	215.1	10	50	15	
	2.54	30	300.1	183.1	10	50	15	
	2.54	30	300.1	58.1	10	50	15	0.01
Cyproheptadine	3.93	30	288.1	191.1	10	30	15	
	3.93	30	288.1	96.0	10	45	15	0.05
Des-Ciclesonide	5.10	30	471.6	323.2	10	17	48	
	5.10	30	471.6	151.9	10	159	18	0.01
Detomidine	3.23	30	187.0	81.0	10	25	15	
	3.23	30	187.0	119.0	10	28	15	
	3.23	30	187.0	54.0	10	53	15	0.01
Dexamethasone/ Betamethasone	3.89	30	393.0	373.2	10	10	22	
	3.89	30	393.0	355.2	10	15	23	
	3.89	30	393.0	337.1	10	17	22	0.002
Diazepam	4.69	30	285.1	193.0	10	41	42	
	4.69	30	285.1	154.0	10	36	42	
	4.69	30	285.1	222.2	10	37	42	0.01
Doxapram	3.37	30	379.0	292.0	10	31	15	
	3.37	30	379.0	167.0	10	31	15	
	3.37	30	379.0	97.0	10	31	15	0.01
Ergonovine	2.63	30	326.0	180.0	10	50	15	
	2.63	30	326.0	208.0	10	40	15	
	2.63	30	326.0	223.0	10	35	15	0.05
Ethylphenidate	3.37	30	248.1	84.0	10	15	15	
	3.37	30	248.0	56.0	10	50	15	0.01
Fenspiride	2.69	30	261.0	79.0	10	50	15	
	2.69	30	261.0	169.0	10	40	15	
	2.69	30	261.0	105.0	10	37	15	0.01
Firocoxib	4.43	30	337.2	282.9	10	15	12	
	4.43	30	337.2	265.0	10	26	12	
	4.43	30	337.2	209.0	10	28	12	0.25
Fludrocortisone	3.63	30	381.1	239.0	6	32	15	
	3.63	30	381.1	181.0	6	40	20	
	3.63	30	381.1	91.0	6	88	10	0.1
Flumethasone	3.93	30	411.0	371.0	10	15	42	
	3.93	30	411.0	391.0	10	11	42	
	3.93	30	411.0	253.0	10	20	42	0.05
Guaifenesin	3.08	30	199.0	125.0	10	11	7	
	3.08	30	199.0	75.0	10	12	7	
	3.08	30	199.0	151.0	10	13	7	0.02
GW 501516	5.37	30	454.0	257.0	10	43	16	
	5.37	30	454.0	188.0	10	59	18	
	5.37	30	454.0	256.0	10	67	17	0.05
Hydroxyamphetamine	1.74	30	152.0	135.0	10	15	15	
	1.74	30	152.0	107.0	10	15	15	0.5

Table 3(cont'd). sMRM Parameters of Analytes in Positive Mode

Analytes	Retention Time (min)	Retention Time Tolerance (±s)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (CE, V)	Collision Cell Exit Potential (CXP, V)	Entrance Potential (EP,V)	Limit of Detection (LOD, ng/mL)
Hydroxyzine	4.00	30	375.0	201.0	10	28	15	
	4.00	30	375.0	173.0	10	25	15	
	4.00	30	375.0	166.0	10	40	15	0.01
Imipramine	4.00	30	281.0	58.0	10	35	15	
	4.00	30	281.0	208.0	10	35	15	
	4.00	30	281.0	86.0	10	25	15	0.1
Ligandrol (LGD-4033)	5.01	30	339.0	240.0	10	35	15	
	5.01	30	339.0	220.0	10	35	15	
	5.01	30	339.0	199.0	10	35	15	0.05
Lormetazepam	4.41	30	335.0	177.0	10	60	15	
	4.41	30	335.0	289.0	10	30	15	
	4.41	30	335.0	253.0	10	50	15	0.01
Mazindol	3.37	30	285.1	256.1	10	50	15	
	3.37	30	285.1	242.0	10	50	15	
	3.37	30	285.1	130.0	10	50	15	0.5
Medroxyprogesterone Acetate	5.24	30	387.5	327.0	10	18	15	
	5.24	30	387.5	285.0	10	24	15	
	5.24	30	387.5	123.0	10	33	15	0.05
Mepivacaine	2.92	30	247.2	68.0	10	86	15	
	2.92	30	247.2	70.2	10	64	15	
	2.92	30	247.2	98.2	10	24	15	0.01
Methandrostenolone	4.36	30	301.0	173.0	10	23	42	
	4.36	30	301.0	149.0	10	21	42	
	4.36	30	301.0	121.0	10	32	42	0.05
Methaqualone	4.27	30	251.0	132.0	10	39	15	
	4.27	30	251.0	91.0	10	39	15	
	4.27	30	251.0	117.0	10	39	15	0.01
Methylenedioxymethamphetamine (MDMA)	2.76	30	194.0	133.0	10	30	15	
	2.76	30	194.0	105.0	10	50	15	
	2.76	30	194.0	163.0	10	17	15	0.01
Methylhexanmine	2.88	30	116.0	57.0	10	17	10	
	2.88	30	116.0	41.0	10	32	19	
	2.88	30	116.0	99.0	10	11	13	0.5
Methyltestosterone	4.65	30	303.0	109.0	10	32	15	
	4.65	30	303.0	97.0	10	30	15	
	4.65	30	303.0	79.0	10	69	15	0.01
Minoxidil	2.94	30	210.0	193.0	10	22	15	
	2.94	30	210.0	164.0	10	22	15	
	2.94	30	210.0	110.0	10	22	15	0.01
Nandrolone	4.26	30	275.4	109.0	10	34	6	
	4.26	30	275.4	145.1	10	32	8	
	4.26	30	275.4	135.0	10	29	9	0.03
Pentoxifylline	3.10	30	279.1	138.0	10	32	15	
	3.10	30	279.1	181.1	10	20	15	
	3.10	30	279.1	99.1	10	25	15	0.05
Phenazocine	3.63	30	322.3	175.1	10	39	15	
	3.63	30	322.3	105.0	10	47	14	
	3.63	30	322.3	115.0	10	98	14	0.05
Phencyclidine	3.60	30	244.0	86.0	10	19	15	
	3.60	30	244.0	159.0	10	19	15	
	3.60	30	244.0	91.0	10	19	15	0.01
Phenylpropanolamine	2.38	30	152.0	115.0	10	32	15	
	2.38	30	152.0	91.0	10	36	15	
	2.38	30	152.0	117.0	10	23	15	0.1
Propantheline	4.03	30	369.0	327.1	10	27	22	
	4.03	30	369.0	100.1	10	28	13	
	4.03	30	369.0	152.0	10	104	8	5
Remifentanil	3.27	30	377.2	317.2	10	20	15	
	3.27	30	377.2	228.1	10	25	15	0.05
Reserpine	4.10	30	609.3	397.2	10	20	15	
	4.10	30	609.3	448.2	10	20	15	
	4.10	30	609.3	195.1	10	20	15	0.05
Selegiline	3.10	30	188.0	91.0	10	30	15	
	3.10	30	188.0	65.0	10	70	15	
	3.10	30	188.0	119.0	10	30	15	0.2
Sotalol	2.27	30	273.1	255.1	10	20	15	
	2.27	30	273.1	213.0	10	20	15	
	2.27	30	273.1	133.0	10	30	15	0.05
Stanozolol	4.71	30	329.0	121.0	10	48	13	
	4.71	30	329.0	109.0	10	48	13	
	4.71	30	329.2	329.2	10	50	13	0
Tamoxifen	4.96	30	372.2	70.0	10	60	15	
	4.96	30	372.2	72.0	10	15	15	
	4.96	30	372.2	129.1	10	25	15	0.2
Terbutaline	2.15	30	226.0	152.0	10	23	15	
	2.15	30	226.0	125.0	10	23	15	
	2.15	30	226.0	135.0	10	23	15	0.01
Testosterone	4.42	30	289.0	97.0	10	27	13	
	4.42	30	289.0	109.0	10	26	13	
	4.42	30	289.0	79.0	10	68	13	0.01
THC\CBD	6.15	45	315.0	193.1	10	30	11	
	6.15	45	315.0	259.1	10	26	18	
	6.15	45	315.0	123.0	10	47	12	0.5
Thebaine	3.12	30	312.1	249.0	10	15	15	
	3.12	30	312.1	58.0	10	20	15	0.05
THG	4.97	30	313.0	266.0	10	31	13	
	4.97	30	313.0	241.0	10	31	13	
	4.97	30	313.0	239.0	10	27	13	0.01
Tolfenamic Acid	5.31	30	262.1	229.0	10	25	15	
	5.31	30	262.1	209.1	10	25	15	
	5.31	30	262.1	244.1	10	30	15	0.5
Trenbolone	4.09	30	271.0	115.0	10	92	10	
	4.09	30	271.0	199.1	10	33	15	
	4.09	30	271.0	178.1	10	73	10	0.05
Trendione	4.41	30	269.2	192.0	10	39	10	
	4.41	30	269.2	207.1	10	31	13	
	4.41	30	269.2	210.1	10	36	11	0.01
Triamcinolone acetonide	4.00	30	435.0	415.0	10	15	15	
	4.00	30	435.0	397.0	10	15	15	
	4.00	30	435.0	339.0	10	15	15	0.002
Triflupromazine	4.31	30	353.1	86.0	10	25	15	
	4.31	30	353.1	58.0	10	25	15	0.05
Verapamil	3.95	30	455.3	303.2	10	30	15	
	3.95	30	455.3	165.1	10	30	15	
	3.95	30	455.3	260.2	10	30	15	0.01
Zilpaterol	2.18	30	262.0	185.0	10	32	11	
	2.18	30	262.0	244.1	10	18	16	
	2.18	30	262.0	202.1	10	27	13	0.1
Zomepirac	4.44	30	292.0	246.0	10	10	15	
	4.44	30	292.0	139.0	10	20	15	
	4.44	30	292.0	108.1	10	20	15	0.5
Zuclopenthixol	4.25	30	401.5	221.0	10	71	14	
	4.25	30	401.5	202.1	10	100	12	
	4.25	30	401.5	231.0	10	41	13	0.5

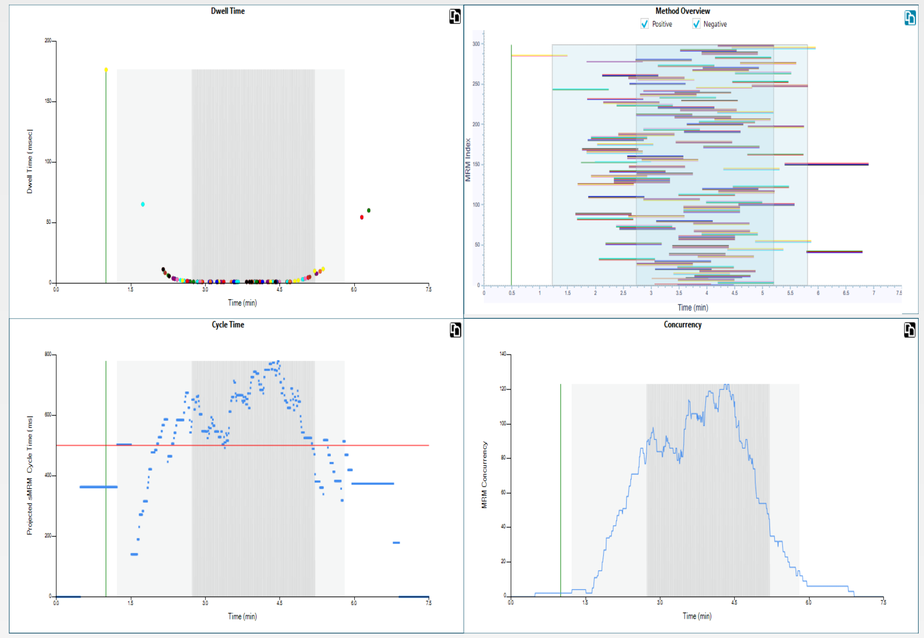

Figure 1. sMRM Method Overview, Dwell Time, Cycle Time and Concurrency

Results

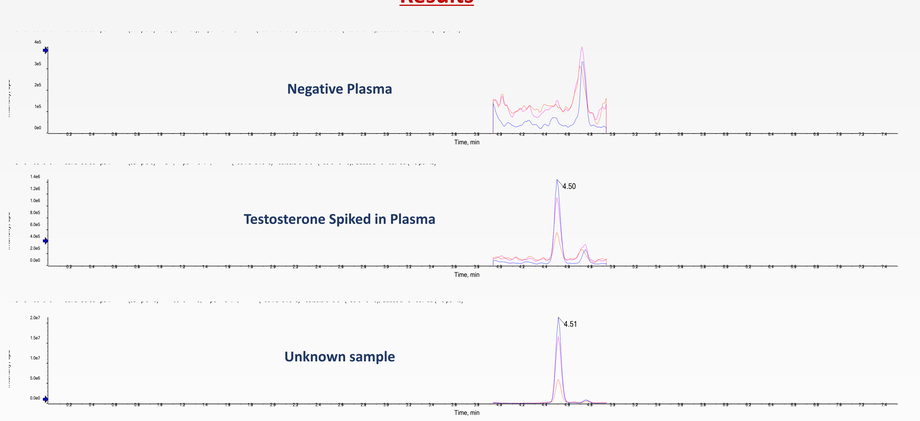

Figure 2. Chromatogram comparison of a Sample Suspect for Testosterone

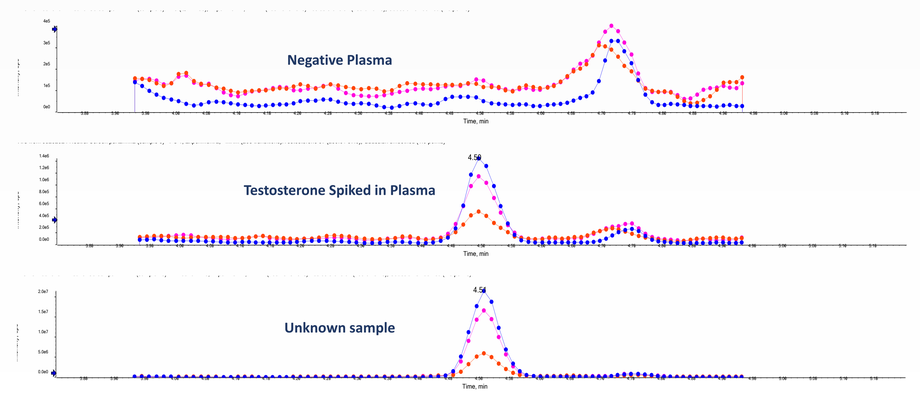

Figure 3. Chromatogram comparison of a Sample Suspect for Testosterone showing the number of points across the peaks

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