macmod

SMARTER CHROMATOGRAPHY

FORENSIC TOXICOLOGY APPLICATIONS GUIDE

INFORMATION & RESEARCH COLLECTED IN COLLABORATION WITH:

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LC-MS SEPARATION OF PAIN MANAGEMENT OPIATES

The 2 µm HALO Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between codeine and hydrocodone, (peaks 1 and 3, respectively) and morphine and hydromorphone (peaks 5 and 8, respectively).

TEST CONDITIONS

Columns:	HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm
Part Number:	91812-611
Mobile Phase	A: water/0.1% formic acid B: acetonitrile/0.1% formic acid

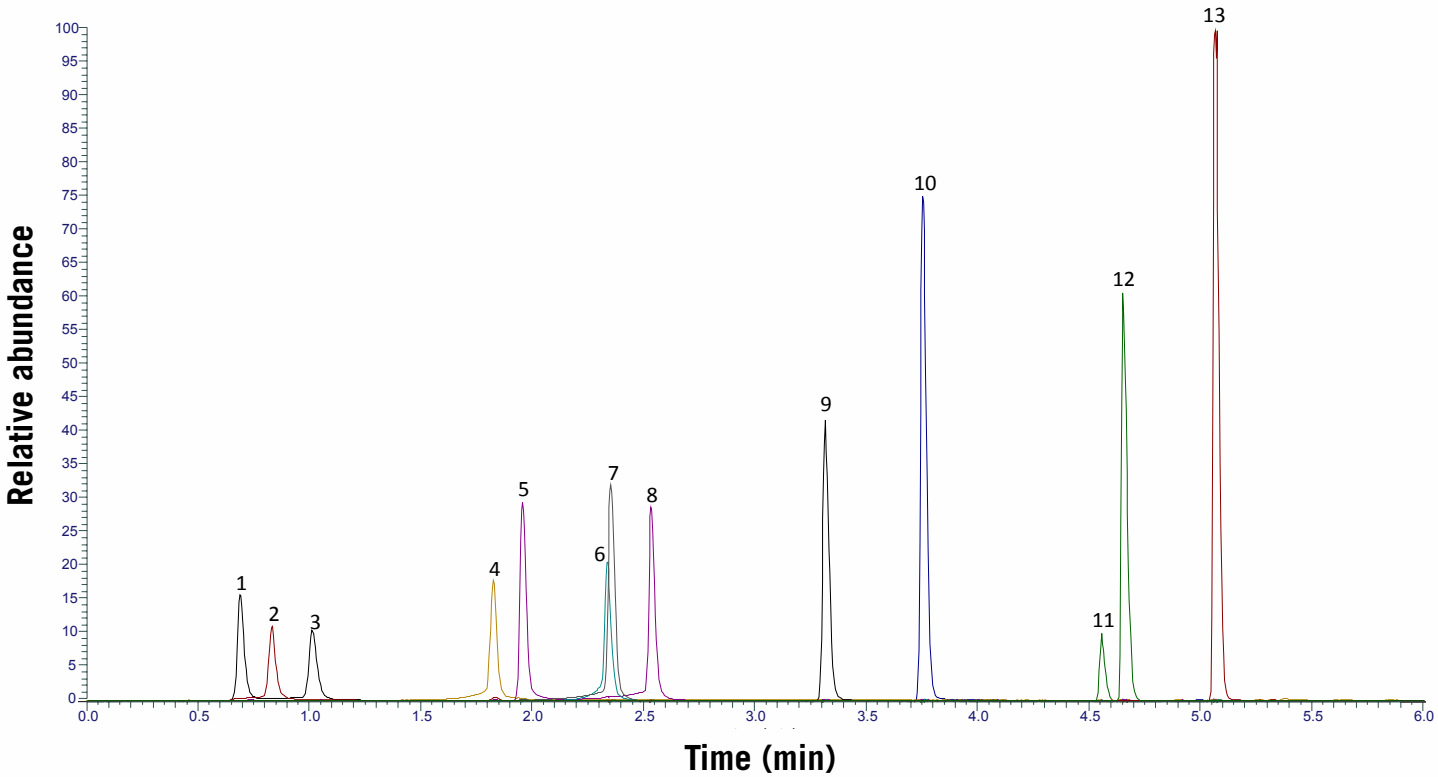
Flow Rate:	0.4 mL/min
Initial Pressure:	325 bar
Temperature:	40 °C
Injection Volume:	1 µL
Sample Solvent:	95/5 water/acetonitrile
LC System:	Shimadzu Nexera X2
Detection:	+ESI MS

Gradient:

Time	%B
0.0	10
2.22	20
5.00	60
5.50	60
5.51	10
6.50	END

PEAK IDENTITIES

1. Morphine (m/z 286)
2. Oxymorphone (m/z 302)
3. Hydromorphone (m/z 286)
4. Naloxone (m/z 328)
5. Codeine (m/z 300)
6. Naltrexone (m/z 342)
7. Oxycodone (m/z 316)
8. Hydrocodone (m/z 300)
9. cis-Tramadol (m/z 264)
10. Meperidine (m/z 248)
11. Fentanyl (m/z 337)
12. Buprenorphine (m/z 468)
13. (+/-) - Methadone (m/z 310)



LC-MS SEPARATION OF SUBSTANCE ABUSE & MENTAL HEALTH - 5 DRUG PANEL

The resolution between methamphetamine and phentermine, (peaks 3 and 5, respectively). The SAMHSA 5 panel consists of amphetamines, cocaine, marijuana, opiates, and phencyclidine (PCP).

TEST CONDITIONS

Column:	HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm
Part Number:	91812-611
Mobile Phase	A: water/0.1% formic acid B: methanol/0.1% formic acid

Gradient:

Time	%B
0.0	5
4.00	98
5.00	98
5.01	5
7.00	END

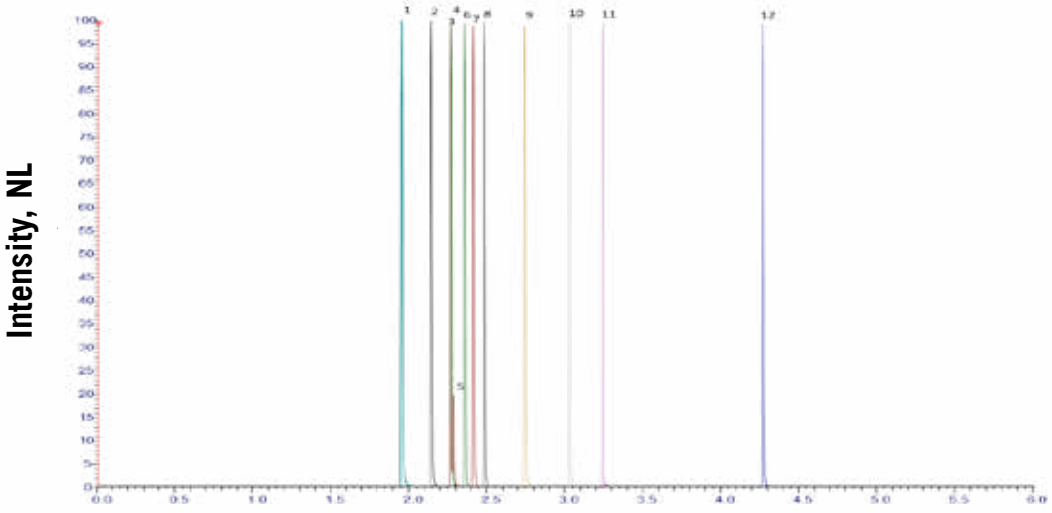
Flow Rate:	0.4 mL/min
Initial Pressure:	325 bar
Temperature:	40 °C
Injection Volume:	2 µL
Sample Solvent:	95/5 MeOH/Water
LC System:	Shimadzu Nexera X2

MS CONDITIONS

Detection:	+ESI MS
Mass Spectrometer:	Thermo Exactive HF
Sheath Gas Flow Rate:	50 (arbitrary units)
Aux Gas Flow Rate:	13 (arbitrary units)
Sweep Gas Flow Rate:	0 (arbitrary units)
Spray Voltage:	3.50 k V
Cap Temp:	263 °C
S-Lens RF Level:	70 V
Aux Gas Heater Temperature:	425 °C

PEAK IDENTITIES

1. Morphine (MH^+= 286.341 g/mol)
2. Amphetamine (MH^+= 136.206 g/mol)
3. Methamphetamine (MH^+= 150.237 g/mol)
4. MDA (MH^+= 180.221 g/mol)
5. Phentermine (MH^+= 150.233 g/mol)
6. Codeine (MH^+= 300.364 g/mol)
7. 6-MAM (MH^+= 328.380 g/mol)
8. MDMA (MH^+= 194.246 g/mol)
9. MDEA (MH^+= 208.271 g/mol)
10. Benzoylecgonine (MH^+= 290.331 g/mol)
11. PCP (MH^+= 244.387 g/mol)
12. THC-COOH (MH^+= 345.415 g/mol)



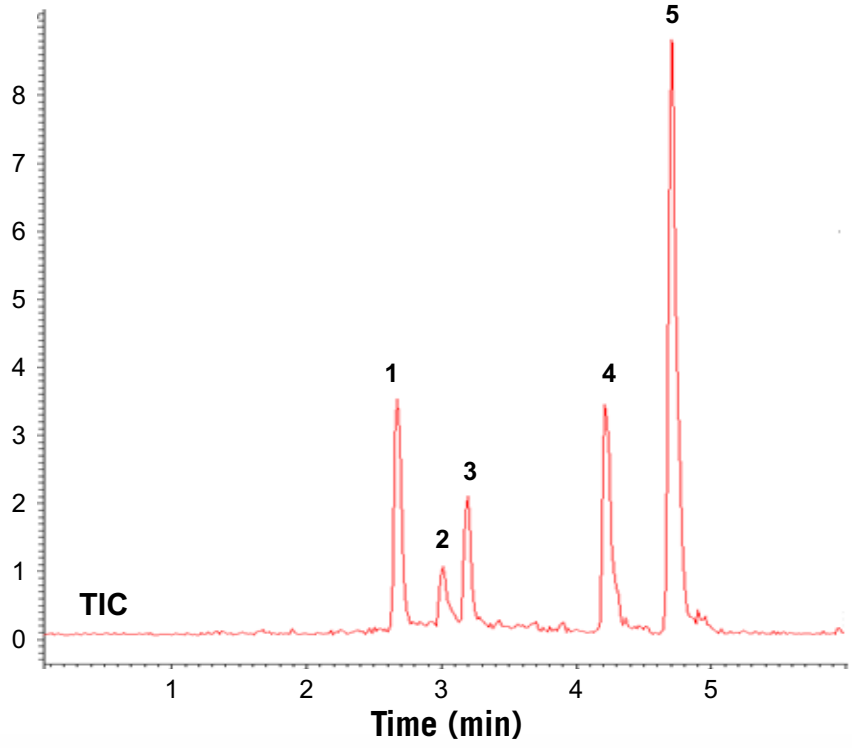
OPIATES IN URINE BY LC-MS/MS

TEST CONDITIONS

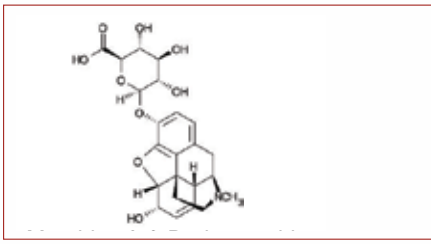
Column:	ACE Excel 3 µm SuperC18
Dimensions:	75 x 2.1 mm
Part Number:	EXL-1111-7502U
Mobile Phase:	A: 5 mM ammonium hydroxide pH 10.8 in H_2O
	B: 5 mM ammonium hydroxide pH 10.8 in MeOH/H_2O (90:10 v/v)

Time (mins)	%B
0	5
5	95

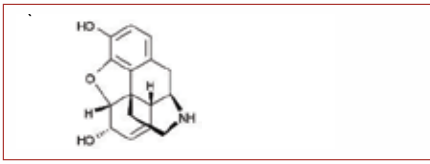
Flow Rate:	0.6 mL/min
Injection:	2 µL
Temperature:	60 °C
Detection:	Varian 320 Triple Quadrupole MS
	Electrospray Voltage: +5 kV
	Inlet Capillary Voltage: 30 V
	CID with argon at 1.5 mTorr
	Collision cell potential ranges from 5 to 17 V
	Drying Gas (Nitrogen) Temperature: 325 °C
	Nebulizing Gas (Nitrogen) Pressure: 35 psi
	Extended Dynamic Range



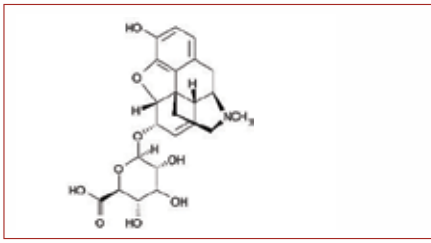
1. Morphine 3-ß-D-glucuronide
LOD (est) 100 ppb
(m/z 462.0 → 285.9)



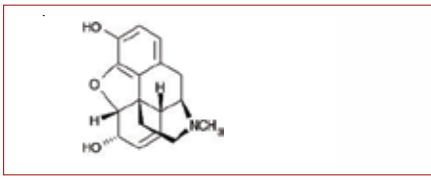
2. Normorphine
LOD (est) 100 ppb
(m/z 272.0 → 165.0)



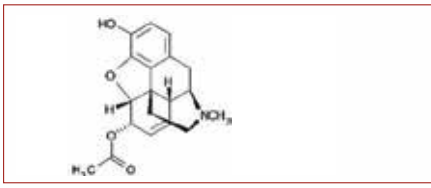
3. Morphine 6-ß-D-glucuronide
LOD (est) 100 ppb
(m/z 462.0 → 285.9)



4. Morphine
LOD (est) 20 ppb
(m/z 286.0 → 200.9)



5. 6-Acetylmorphine
LOD (est) 10 ppb
(m/z 328.0 → 164.9)



50-COMPOUND DRUGS OF ABUSE SCREEN BY UHPLC-MS/MS

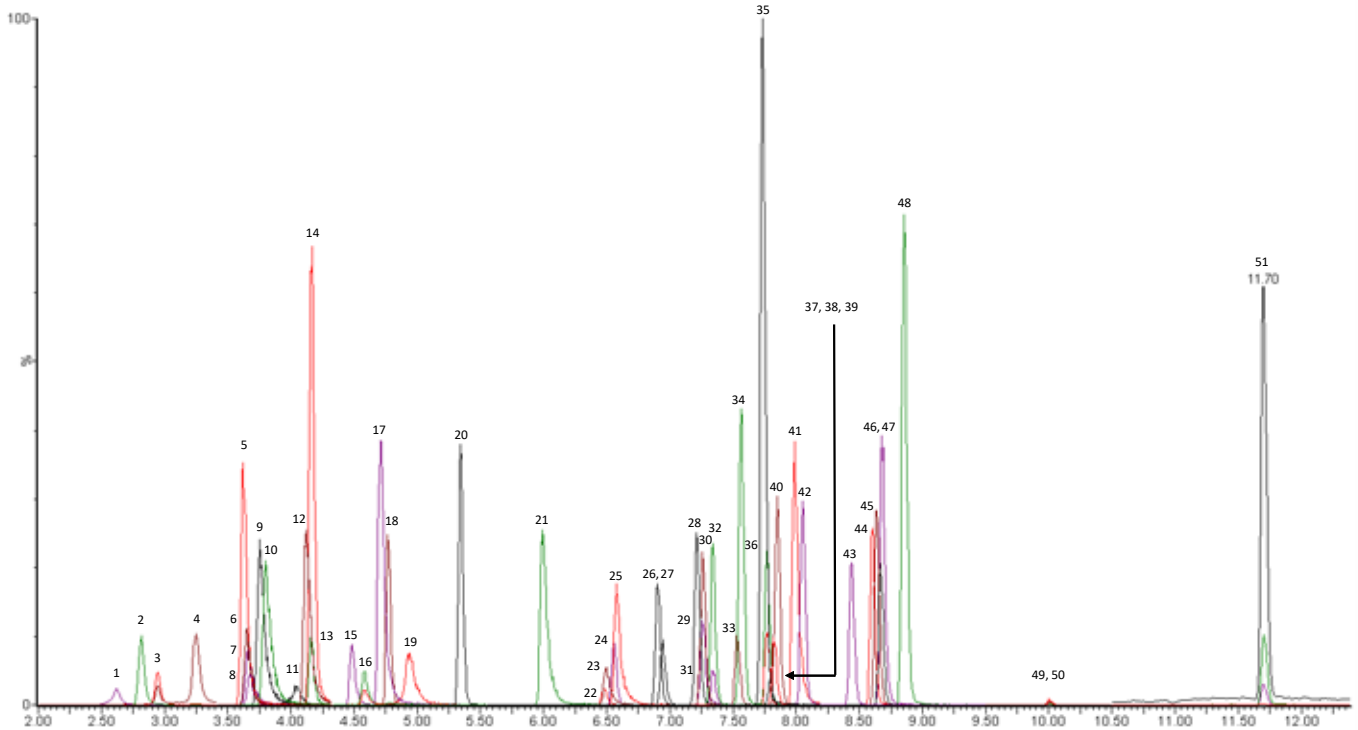
TEST CONDITIONS

Column: ACE Excel 1.7 µm C18
Dimensions: 100 x 2.1 mm
Part Number: EXL-171-1002U
Mobile Phase: A: 5 mM ammonium acetate in H_2O
B: 5 mM ammonium acetate in MeOH

Time (mins)	%B
0.0	10
10.0	90
11.9	90
13.4	10
15.5	10

Flow Rate: 0.3 mL/min
Injection: 10 µL
Temperature: 40 °C

Detection: MS Quattro Premier XE triple quad
MRM, positive, and negative ESI mode
Desolvation Temperature: 450 °C
Ion Source Temperature: 150 °C
Collision Gas Pressure: 3.5 x 100^{-4} mbar



Time (min)

50-COMPOUND DRUGS OF ABUSE SCREEN BY UHPLC-MS/MS

Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)	Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)
1	Oxymorphone	2.62	302.2	198.1	23	PCP	6.49	244.2	159.9
2	Morphine-D3	2.82	289.2	201.0	24	Zaleplon	6.55	306.2	264.2
3	Morphine	2.95	286.2	201.0	25	EDDP	6.58	278.2	234.2
4	Hydromorphone	3.25	286.2	185.1	26	Norketamine	6.90	224.1	124.9
5	Amphetamine-D5	3.62	141.0	123.9	27	Bromazepam	6.95	316.1	182.1
6	Amphetamine	3.65	136.0	118.9	28	Ketamine	7.21	238.1	124.9
7	Dihydrocodeine	3.66	302.2	199.1	29	Clonazepam	7.26	316.1	270.1
8	MDA	3.67	180.1	105.0	30	Nitrazepam	7.26	282.2	236.1
9	MDMA	3.75	194.1	163.0	31	α-hydroxytriazolam	7.34	359.1	331.1
10	Methamphetamine	3.80	150.0	90.9	32	Flunitrazepam	7.34	314.2	268.2
11	Oxycodone	4.03	316.2	241.2	33	α-hydroxyalprazolam	7.54	325.2	297.1
12	MDEA	4.12	208.2	163.0	34	Estazolam	7.56	295.2	267.2
13	BZE-D3	4.15	293.1	171.0	35	Zolpidem	7.73	308.2	235.1
14	BZE	4.17	290.1	168.0	36	Triazolam	7.77	343.0	308.1
15	6-MAM	4.48	328.2	165.1	37	2-Hydroxyethylflurazepam	7.77	333.2	109.0
16	Codeine	4.59	300.3	215.1	38	Lorazepam	7.80	321.1	275.1
17	Norfentanyl	4.71	233.1	84.0	39	Oxazepam	7.82	287.2	241.0
18	7-Amino-clonazepam	4.77	286.2	121.0	40	Alprazolam	7.85	309.2	281.2
19	Hydrocodone	4.94	300.2	199.1	41	Methadone	7.99	310.2	265.2
20	7-Amino-flunitrazepam	5.34	284.4	135.0	42	Temazepam	8.05	301.1	255.1
21	Cocaine	5.99	304.2	182.0	43	Nordiazepam	8.44	271.1	139.9
22	Norbuprenorphine	6.47	414.3	101.0	44	Midazolam	8.61	326.2	291.2

50-COMPOUND DRUGS OF ABUSE SCREEN BY UHPLC-MS/MS

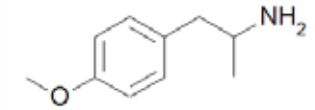
Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)	Peak	Analyte	Rt	Q1 (Da)	Q3 (Da)
45	Diazepam-D5	8.63	290.2	154.0	49	THC-COOH-D3	9.98	348.2	302.2
46	Diazepam	8.67	285.2	154.0	50	THC-COOH	10.01	345.2	299.2
47	Flurazepam	8.68	388.2	315.1	51	Buprenorphine	11.70	468.3	101.0
48	Fentanyl	8.85	337.3	105.0					

MDMA (ECSTASY) & PMA (DR. DEATH) SEPARATION

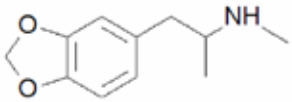
TEST CONDITIONS

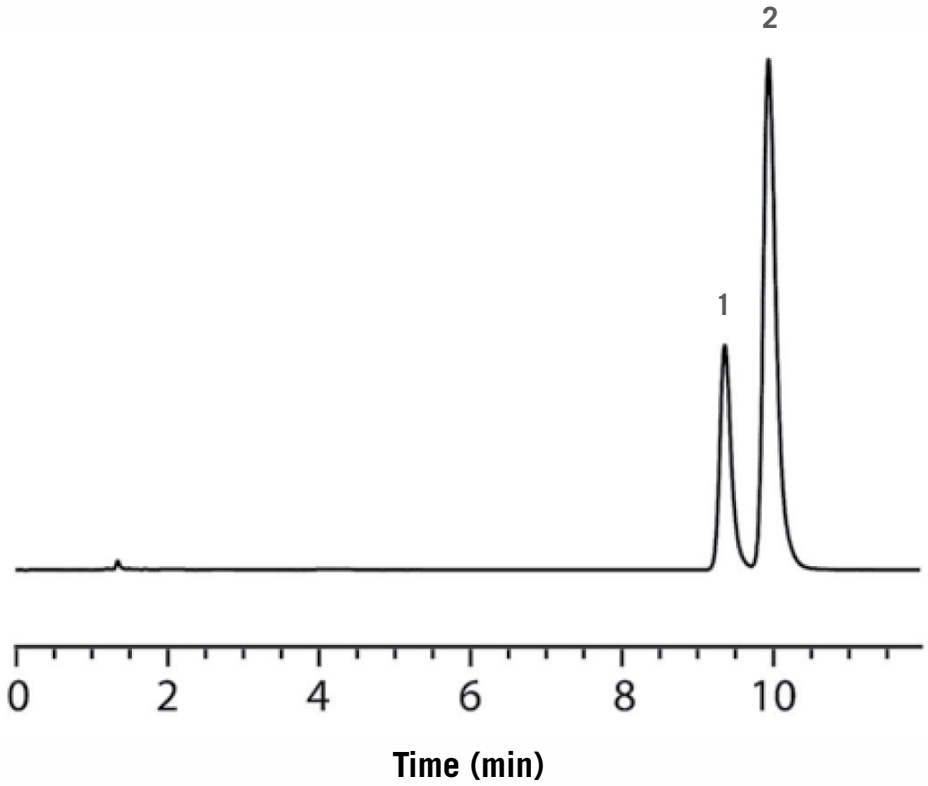
Column:	ACE 3 µm C18
Dimensions:	150 x 4.6 mm
Part Number:	ACE-111-1546
Mobile Phase:	0.05 mM KH_2PO_4 pH 3.2 in H_2O/MeCN (90:10 v/v)
Flow Rate:	1.2 mL/min
Injection:	10 µL
Temperature:	22 °C
Detection:	UV, 210 nm

1. PMA (4-Methoxyamphetamine)
LOD 0.08 µg/mL
LOQ 0.26 µg/mL



2. MDMA (3,4-Methylenedioxy methamphetamine)
LOD 0.04 µg/mL
LOQ 0.12 µg/mL





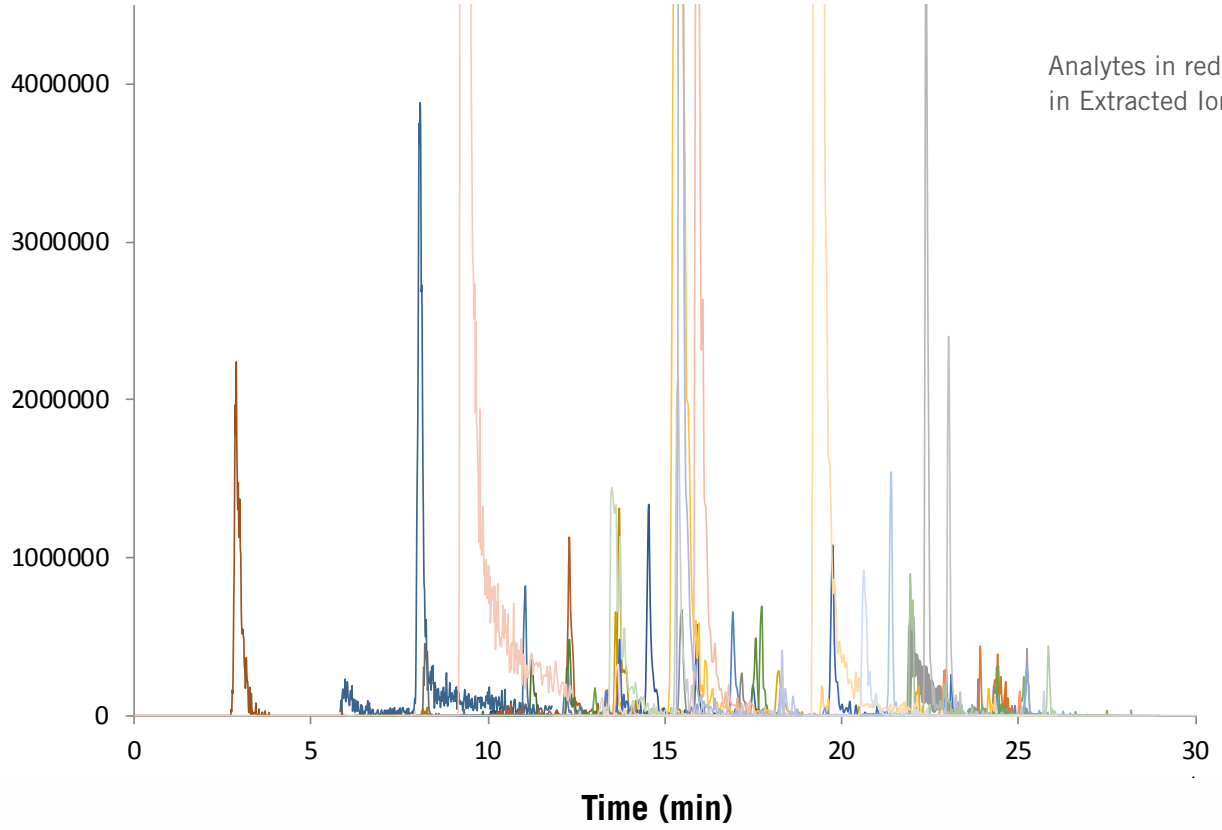
250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

TEST CONDITIONS

Column: ACE Excel 2 µm C18-PFP
Dimensions: 100 x 2.1 mm
Part Number: EXL-1010-1002U
Mobile Phase: A: 2 mM ammonium acetate + 0.1% formic acid in H_2O
B: 2 mM ammonium acetate + 0.1% formic acid in MeOH

Time (mins)	%B
0	2
4	2
34	100
38	100
40	2

Flow Rate: 0.3 mL/min
Injection: 10 µL
Temperature: 37 °C
Detection: Thermo Quantum Ultra MS
ESI in positive ion mode



Analytes in red are included in Extracted Ion Chromatography.

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

Analyte	R_t	MRM Transitions	Analyte	R_t	MRM Transitions
6-Acetylcodeine	16.9	342.2 > 165.1	Benzylpiperazine	5.1	177.2 > 65.1
Alfentanil	21.1	417.4 > 197.2	Bisoprolol	19.9	326.3 > 116.1
Alprazolam	23.3	309.11 > 205.1	Bromazepam	20.4	316 > 182.1
Aminoclonazepam	13.9	286.2 > 222.3	Buphedrone	14.2	178.2 > 160.1
Aminodesmethylflunitrazepam	20.0	270.1 > 121.1	Buphedrone ephedrine	13.5	180.4 > 162.2
Aminoflunitrazepam	16.2	284.1 > 135.1	Bupivacaine	19.5	289.2 > 84.2
Aminonitrazepam	10.9	252.1 > 94.1	Buprenorphine glucuronide	18.1	644.4 > 468.4
Aminorex	12.2	163 > 120.1	Bupropion	19.5	240.1 > 131.1
Amiodarone	31.3	646.2 > 100	Butylone	14.7	222.1 > 131.1
Amisulpride	16.2	370.1 > 242	Caffeine	13.1	195.1 > 110.1
Amitriptyline	24.4	278.2 > 91.1	Camfetamine	18.7	202.2 > 67.1
Amlodipine	25.3	409.3 > 238.2	Carbamazepine	21.5	237.1 > 192.2
Amlodipine met	22.9	407 > 318	Carbamazepine 10,11-Epoxide	18.6	253.2 > 180.1
Amphetamine	11.6	136.1 > 65.2	Cathine	20.0	134.2 > 91.1
Amphetamine-d6	11.6	142.2 > 67.2	Cathinone	9.5	150.2 > 117.1
Anhydroecgonine methyl ester	9.8	182.2 > 91.1	2C-B-FLY	20.1	286 > 269.1
Aripiprazole	25.5	448.1 > 285.1	Chlordiazepoxide	19.5	300.1 > 227.1
Atenolol	10.7	267.1 >145.1	Chloroquine	18.5	320.1 > 142.1
Atomoxetine	23.1	256.2 > 44.1	Chlorpheniramine	20.4	275.1 > 167.2
Atomoxetine metabolite	22.5	242.2 > 44.1	Chlorpromazine	25.9	319.2 > 58.1
Benzedrone	21.7	254 > 65.1	Citalopram	22.2	325.1 > 246.1
Benzoylecgonine	15.6	290.1 > 77.2	Clobazam	22.8	301.1 > 259.2
Benzoylecgonine-d3	15.6	293.2 > 77.2	Clomethiazole	17.3	161.9 > 113

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

Analyte	R_t	MRM Transitions	Analyte	R_t	MRM Transitions
Clomipramine	27.0	315.1 > 86.1	Desomorphine	13.1	272.1 > 152.1
Clonazepam	23.1	316.1 > 214.2	Desoxypipradrol	20.5	252.1 > 91.1
Clonidine	13.2	230 > 44.2	Dextromethorphan	20.0	272.2 > 171.1
Clozapine	21.7	327.1 > 192.1	Diamorphine	16.9	370.1 > 165.1
Cocaethylene	19.5	318.2 > 82.2	Diazepam	25.5	285.1 > 154.1
Cocaine	18.0	304.2 > 82.2	Didesmethylcitalopram	22.2	297 > 262.1
Codeine	11.9	300.2 > 153.2	Digoxin	24.2	781.2 > 97
Cotinine	2.5	177.1 > 80.1	Dihydrocodeine	11.7	302.2 > 128.1
Creatinine	1.3	114 > 44	Diltiazem	22.7	415.1 > 178.1
Cyclizine	22.5	267.1 > 167.1	Dimethocaine	16.3	279.3 > 92.1
D2PM (Diphenylprolinol)	7.3	254.1 > 130.1	Dinitrophenol	18.4	183 > 109
Dehydroaripiprazole	25.3	446.1 > 285.1	Diphenhydramine	21.2	256.1 > 167.1
Desimipramine	24.3	267.1 > 72.2	Dipipanone	25.0	350.2 > 265.2
N-Desmethylclozapine	21.0	313 > 192.1	Donepezil	22.2	380.1 > 91
Desmethylcitalopram	22.4	311.1 > 109.1	Dothiepin	23.7	296.2 > 202.2
Desmethylflunitrazepam	22.5	300.1 > 254.2	Ecgonine ethyl ester	2.5	214.1 > 196.1
Desmethylfluoxetine	25.4	296.2 > 134.1	Ecgonine methyl ester	1.0	200.1 > 182.1
N-Desmethylmirtazapine	16.1	252.1 > 195.1	EDDP	21.4	278.2 > 219.2
Desmethylolanzapine	12.6	299.1 > 198.1	Estazolam	22.5	295.1 > 267.1
N-Desmethyltramadol	16.7	250.1 > 44.2	Ethylamphetamine	14.5	164.1 > 91.2
O-Desmethyltramadol	12.8	250.1 > 58.2	Ethylmethcathinone	15.8	192.2 > 131.2
Desmethylvenlafaxine	15.5	264.3 > 58.1	Ethylphenidate	19.2	248.1 > 56.2
N-Desmethylzopiclone	20.0	375.1 > 245.1	Etizolam	23.5	343.1 > 314.2

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

Analyte	R_t	MRM Transitions	Analyte	R_t	MRM Transitions
Fenfluramine	20.3	232.1 > 159.1	Hydroxybupropion	18.5	253.1 > 130.1
Fentanyl	21.4	337.2 > 105.1	4-Hydroxymethamphetamine	20.0	166.1 > 107.1
Flubromazolam	23.2	371.1 > 223.1	8-Hydroxymirtazapine	15.6	282.1 > 211
Flunitrazepam	23.5	314.1 > 269.3	7-Hydroxymitragynine	18.2	415.3 > 175.1
2-Fluoroamphetamine	13.2	154.1 > 83.1	3-Hydroxyphenazepam	23.3	366.9 > 320.8
Fluoroamphetamine interferent	10.6	154 > 67.1	7-Hydroxyquetiapine	15.3	400.3 > 208.1
3-Fluoromethcathinone	20.0	182.1 > 149.1	9-Hydroxyrisperidone	19.5	427.2 > 69.1
4-Fluoromethcathinone	20.0	182 > 148.1	Imipramine	24.2	281.1 > 86.2
Fluoxetine	25.3	310.1 > 44.2	5-Iodo-2-aminoindane	18.4	260.1 > 115.1
Fluphenazine	28.1	438.3 > 143.1	Ketamine	15.4	238.1 > 125.1
Flurazepam	21.6	388 > 315	Lamotrigine	16.8	256.1 > 211.1
Fluvoxamine	26.0	319.1 > 71	Levamisole	13.3	205.1 > 91.1
Gabapentin	10.5	172.1 > 67.2	Levetiracetam	8.9	171.2 > 126.1
Glibenclamide	28.2	494.1 > 168.9	Lidocaine	14.8	235.1 > 86.2
Gliclazide	24.6	324.1 > 110	Lorazepam	22.8	321 > 229.1
Glimepiride	28.4	491.1 > 126	Lormetazepam	23.8	335 > 289.1
Glipizide	24.5	446.1 > 286	LSD	20.1	324.3 > 223.1
Haloperidol	23.1	376.1 > 95.1	MCAT	5.4	164.2 > 130.1
Hippuric acid	10.9	180 > 77	mCPP	17.0	197.1 > 118.1
Hydrocodone	13.1	300.1 > 199.1	MDA	13.4	180.1 > 133.1
Hydromorphone	10.4	286.2 > 185.1	MDAI	12.3	178.19 > 161.1
Hydroxyalprazolam	22.4	325.1 > 216.1	MDEA	15.2	208.1 > 135.1
4-Hydroxyamphetamine	5.4	152.1 > 107.1	MDMA	14.1	194.1 > 135.2

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

Analyte	R_t	MRM Transitions	Analyte	R_t	MRM Transitions
MDPV	18.1	276.1 > 135.1	Mitragynine	22.6	399.3 > 174.1
MEGX	13.4	207.1 > 58.1	Modafinil	21.1	296.1 > 129
MeOPP	13.3	193.2 > 133.1	6-Monoacetylmorphine	12.9	328.1 > 165.1
Mephedrone	14.6	178.1 > 144.2	Mono-N-desethylamiodarone	30.8	618.2 > 547.2
Mescaline	12.7	212.1 > 165.1	Morphine	7.7	286.1 > 152.2
Metformin	2.5	130 > 60.1	Morphine glucuronide	2.7	462.2 > 201.1
Methadone	24.0	310.2 > 105.1	Morphine-d3	7.7	289.2 > 152.2
Methadone-d3	24.0	313.2 > 268.2	Naloxone	20.0	328.3 > 212.1
Methamphetamine	12.8	150.1 > 91.2	Naphyrone	23.2	282.2 > 141.1
Methaqualone	22.8	251.2 > 91.1	Nefopam	19.5	254.9 > 166.1
Methedrone	13.9	194.1 > 146.1	Nifoxipam	20.9	316.05 > 298.1
Methiopropamine	10.6	156.1 > 97	Nimetazepam	23.6	296.1 > 250.2
Methocarbamol	16.8	242.1 > 118.1	Nitrazepam	22.8	282.1 > 236.1
Methoxetamine	17.2	248.2 > 121.1	Noralfentanil/sufentanil	19.4	277.1 > 245.1
3-Methoxytyramine	6.6	168 > 91	Norbuprenorphine glucuronide	15.5	590.3 > 414.3
Methylethcathinone	20.0	192.1 > 144.2	Norcyclizine	21.9	253.2 > 167.1
Methylhexanamine	13.4	116.1 > 57.3	Nordiazepam	24.4	271.1 > 140.1
Methylone	12.7	208.1 > 132.1	Nordothiepin	24.4	282.1 > 202.1
Methylphenidate	16.5	234.1 > 56.2	Norfentanyl	15.6	233.2 > 56.2
5-Methyltryptamine	16.9	175.1 > 143	Norketamine	14.8	224.1 > 125.1
Metoclopramide	17.2	300.1 > 227.1	Normorphine	3.8	272.1 > 165.2
Midazolam	20.9	326.1 > 249.1	Nornefopam	19.7	240.9 > 166.1
Mirtazapine	16.6	266.1 > 72.2	Noroxycodone	13.0	302.1 > 284.1

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

Analyte	R_t	MRM Transitions	Analyte	R_t	MRM Transitions
Norpropoxyphene	23.0	308 > 44.2	Promethazine	20.0	285.1 > 86.2
Norsertraline	20.0	275.3 > 159	Propofol	23.9	179 > 137
Nortriptyline	24.6	264.1 > 91.1	Propofol glucuronide	20.1	372.2 > 148.1
Olanzapine	13.4	313.1 > 84.1	Propoxyphene	23.6	340.2 > 58.2
Orphenadrine	23.4	270.1 > 181.1	Propranolol	23.0	260.1 > 157.1
Oxazepam	23.5	287.1 > 104.1	Quetiapine	21.7	384.1 > 221.1
Oxybutynin	24.6	358.1 > 141.9	Remifentanil	18.4	377.3 > 113.1
Oxycodone	12.9	316.1 > 241.1	Risperidone	20.4	411.1 > 190.8
Oxymorphone	20.0	302 > 227	Ritalinic acid	14.4	220.2 > 56.1
Paracetamol	7.8	152.1 > 65.1	Sertraline	25.6	306.1 > 159
PCP	20.5	244.3 > 86.2	Sildenafil	23.6	475.4 > 58.1
Pentazocine	19.5	286.3 > 175.2	Sildenafil N-oxide	23.9	491.4 > 312.3
Pentedrone	16.0	192.2 > 131.1	Sufentanil	22.9	387.3 > 140.2
Phenazepam	24.5	350.9 > 206	Temazepam	24.2	301.1 > 177.1
Pheniramine	15.1	241.2 > 196.2	Temazepam-d5	24.1	306.1 > 260.2
Phenytoin	20.0	253.1 > 77	Tetrazepam	24.5	289.2 > 225.2
Pholcodine	9.2	399.2 > 114.1	TFMPP	7.9	231.1 > 118.1
PMA	13.8	149.2 > 91.1	Theophylline	11.2	181.1 > 124.1
PMMA	14.6	180.2 > 121.1	Tramadol	16.9	264.2 > 58.2
Powder 20140730	18.7	248.3 > 84.2	Trazodone	23.9	372.2 > 179.2
Prazepam	27.0	325 > 140	Trifluoperazine	28.8	408.2 > 113.2
Pregabalin	10.4	160.1 > 97.2	Trihexyphenidyl	23.7	302.1 > 70.1
Procyclidine	22.4	288.3 > 42	Varenicline	12.3	212.2 > 168.1

250-COMPOUND DRUGS OF ABUSE SCREEN IN URINE BY LC-MS/MS

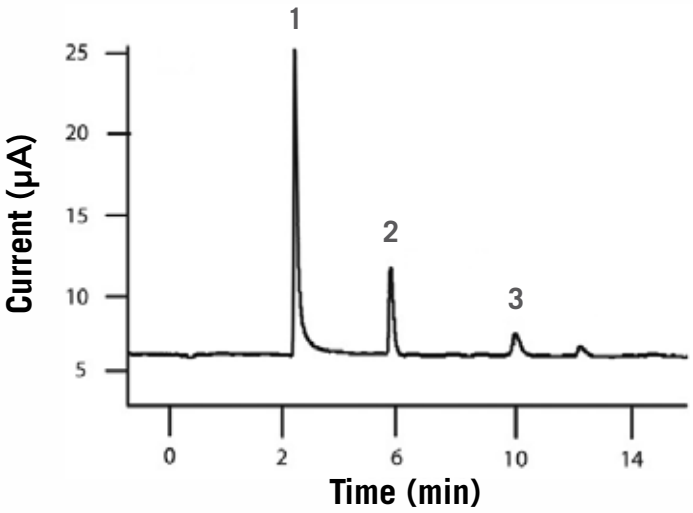
Analyte	R_t	MRM Transitions
Venlafaxine	19.1	308 > 44.2
Verapamil	23.9	275.3 > 159
Vigabatrin	2.5	264.1 > 91.1
Warfarin	25.7	313.1 > 84.1
Zaleplon	21.5	270.1 > 181.1
Zolpidem	18.8	287.1 > 104.1
Zolpidem phenyl COOH	15.3	358.1 > 141.9
Zopiclone	16.5	316.1 > 241.1
Zopiclone N-oxide	17.6	302 > 227

CATHINONE PSYCHOACTIVE SUBSTANCES BY LC-UV & LC-UV & LC-AMPEROMETRY

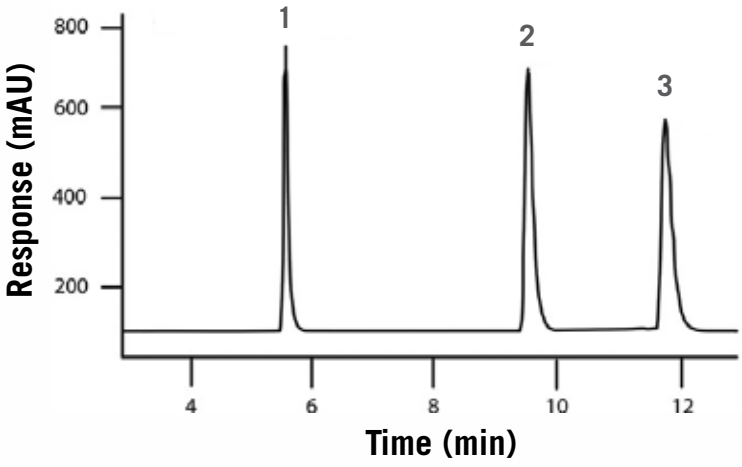
TEST CONDITIONS

Column:	ACE 3 µm C18
Dimensions:	150 x 4.6 mm
Part Number:	ACE-111-1546
Mobile Phase:	10 mM ammonium acetate-100 mM KCl pH 4.3/MeOH (70:30 v/v)
Flow Rate:	0.8 mL/min
Injection:	10 µL
Temperature:	22 °C
Detection:	UV, 264 nm Amperometric Potential +1.4 V

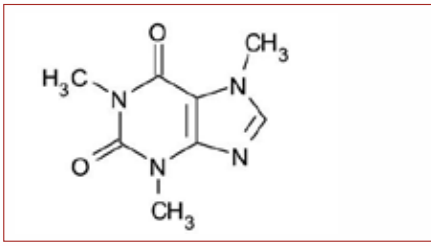
Amperometric Detection



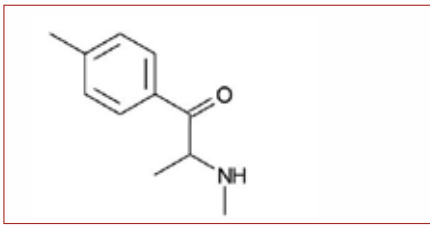
UV Detection



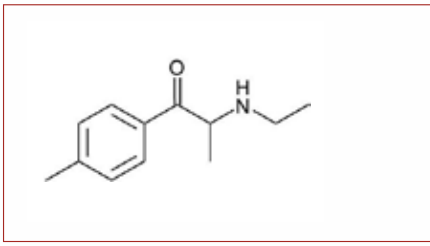
1. Caffeine



2. 4-Methylmethcathinone
(4-MMC, mephedrone)



3. 4-Methylethcathinone
(4-MEC)

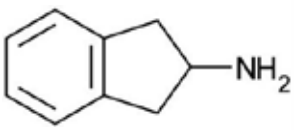


PSYCHOACTIVE SUBSTANCES IN 'SYNTHACAINE' BY LC-UV

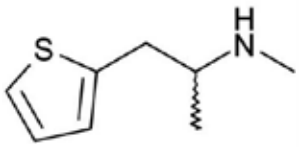
TEST CONDITIONS

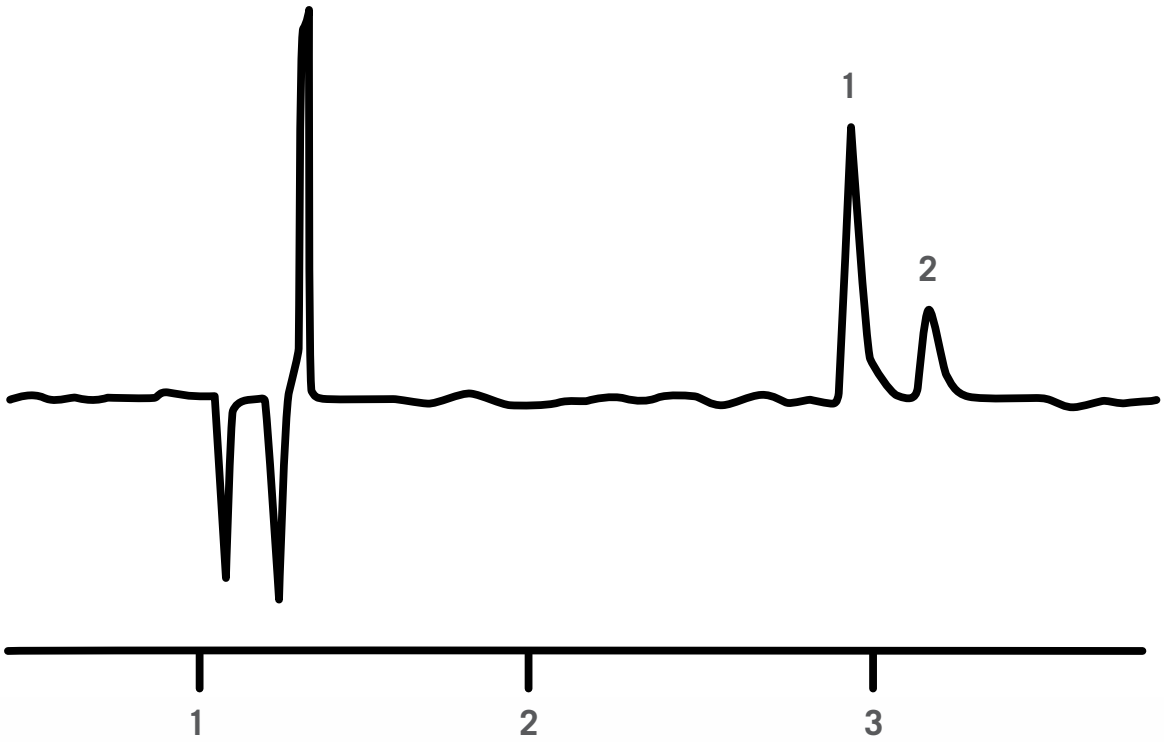
Column:	ACE 3 µm C18
Dimensions:	150 x 4.6 mm
Part Number:	ACE-111-1546
Mobile Phase:	10 mM ammonium formate pH 3.5/MeCN (90:10 v/v)
Flow Rate:	1.2 mL/min
Temperature:	22 °C
Detection:	UV, 207 nm (2-Aminoindane) and 233 nm (Methiopropamine)
Sample:	Synthacaine 40 µg/mL

1. 2-Aminoindane
LOD 0.83 µg/mL



2. Methiopropamine
LOD 0.31 µg/mL





AMATOXINS FROM AMANITA PHALLOIDES MUSHROOMS BY LC-HRMS

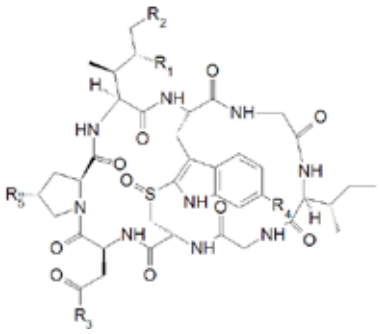
TEST CONDITIONS

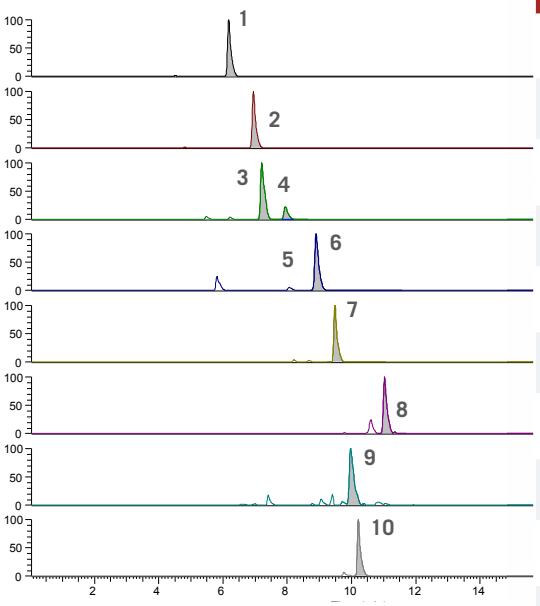
Column: ACE 3 µm AQ
Dimensions: 150 x 3.0 mm
Part Number: ACE-116-1503
Mobile Phase: A: 0.1% formic acid in H_2O
B: 0.1% formic acid in MeOH

Time (mins)	%B
0	15
17	100
22	100
22	15
30	15

Flow Rate: 0.4 mL/min
Injection: 10 µL
Temperature: 50 °C
Detection: Thermo Exactive MS
ESI in positive ion mode

Amatoxins





Peak	Analyte	R1	R2	R3	R4	R5	Exact Mass
1	α-Amanitin	OH	OH	NH2	OH	OH	918.35417
2	ß-Amanitin	OH	OH	OH	OH	OH	919.338182
3	γ-Amanitin	H	OH	NH2	OH	OH	902.359252
4	Amaninamide	OH	OH	NH2	H	OH	902.359252
5	Amanin	OH	OH	OH	H	OH	903.343267
6	ε-Amanitin	H	OH	OH	OH	OH	903.343267
7	Amanullin	H	H	NH2	OH	OH	886.364337
8	Proamanullin	H	H	NH2	OH	H	870.369423
9	Amanullinic acid	H	H	OH	OH	OH	887.348353
10	Bacitracin (IS)	-	-	-	-	-	1421.7489

LC-MS SEPARATION OF FENTANYL & ANALOGUES IN SYNTHETIC URINE

A mixture of fentanyl and some of its analogues spiked into synthetic urine are separated on a HALO Biphenyl column using LC-MS detection. These opioids are known to be much more potent than heroin and have become a significant contributor toward the opiate crisis in America.

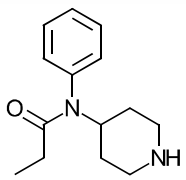
TEST CONDITIONS

Column:	HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 50 mm
Part Number:	92812-411
Mobile Phase	A: water/0.1% formic acid/10mM ammonium formate B: methanol/0.1% formic acid/10mM ammonium formate
Gradient:	40-90% B in 3 minutes
Flow Rate:	0.8 mL/min
Initial Pressure:	380 bar
Temperature:	30 °C
Injection Volume:	0.5 µL
Sample Solvent:	Surine Negative Urine
LC System:	Shimadzu Nexera
MS:	Shimadzu LCMS 2020 (single quadrupole)
ESI:	4.5 kV
Heat Block:	300 °C
Nebulizing Gas Flow:	1.3 L/min

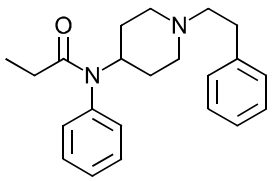
PEAK IDENTITIES

1.	Norfentanyl	TIC/233
2.	Acetyl Fentanyl	TIC/323
3.	Fentanyl	TIC/337
4.	Sufentanil	TIC/387

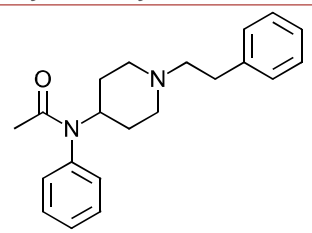
Norfentanyl



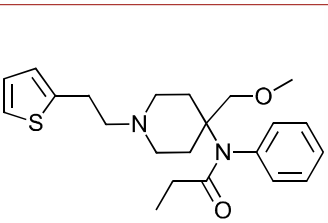
Fentanyl

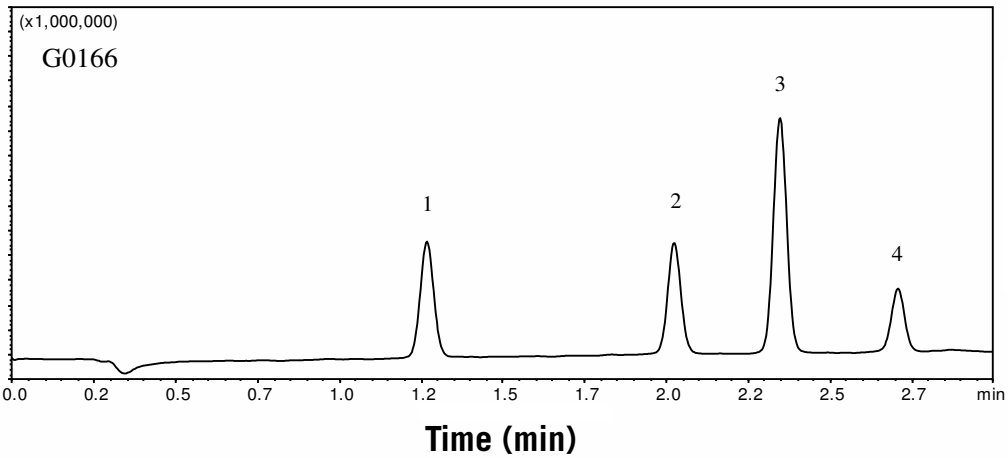


Acetyl Fentanyl



Sufentanil



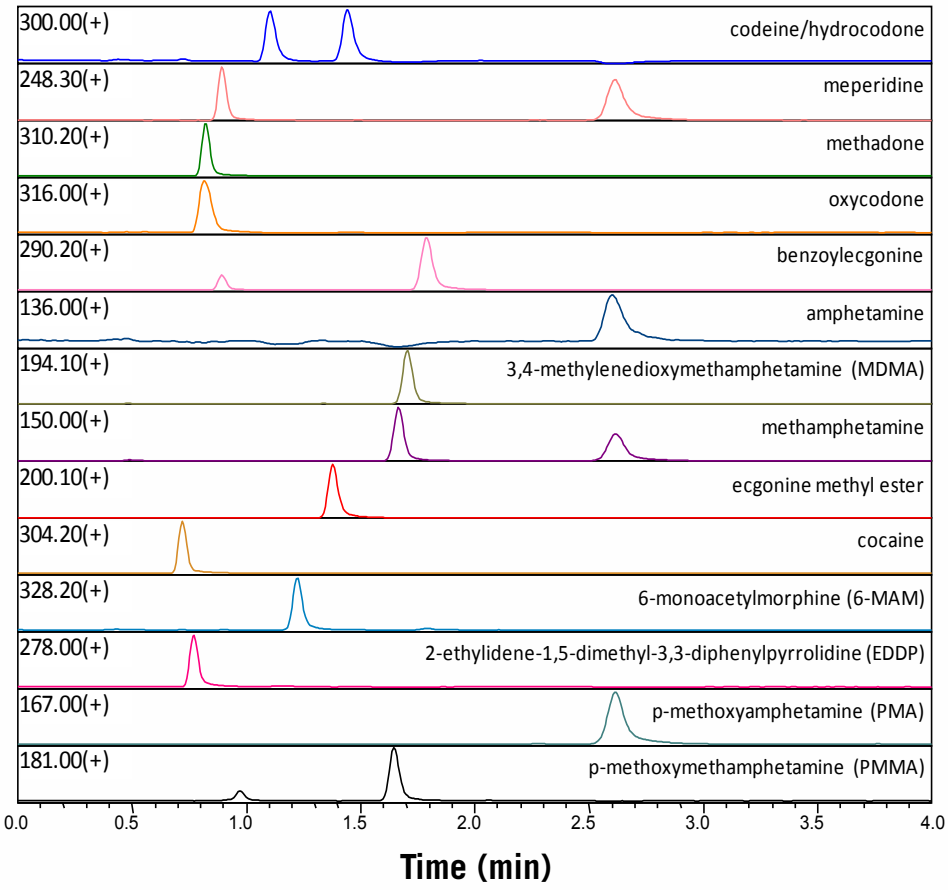


LC-MS SEPARATION OF DRUGS OF ABUSE & METABOLITES

This mixture of drugs of abuse and metabolites is quickly identified using a HALO Penta-HILIC column and selected ion monitoring (SIM) for improved sensitivity. Adapted from J. Pharm. Anal. 2013; 3 (5): 303-311.

TEST CONDITIONS

Column:	HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 100 mm
Part Number:	92812-605
Mobile Phase	A: 5 mM ammonium formate, pH 3
	B: Acetonitrile
Isocratic:	Premixed 5/95 A/B
Flow Rate:	0.5 mL/min.
Pressure:	149 bar
Temperature:	60 °C
Detection:	Selected Ion Monitoring as indicated
Injection Volume:	1 µL
Sample Solvent:	90/10 ACN/water
MS Parameters:	Positive ion mode, 2 kV, 400 °C heat block 225 °C capillary
LC-MS System:	Shimadzu Nexera and LCMS-2020 (singlequadrupole MS)



LC-MS ANALYSIS OF KRATOM ALKALOIDS

The 2 µm HALO C18 is an ideal choice for analysis of kratom and its metabolite. Kratom is an herbal extract that comes from the leaves of an evergreen tree (Mitragyna speciosa) grown in Southeast Asia. Believed to act on opioid receptors, kratom has been used by people to mitigate the symptoms of opioid withdrawal. However, studies on the effects of kratom have identified many safety concerns and no clear benefits, and kratom is not currently regulated by the United States.

TEST CONDITIONS

Column:	HALO 90 Å C18, 2 µm, 2.1 x 50 mm
Part Number:	91812-402
Mobile Phase	A: water/0.1% formic acid
	B: ACN/0.1% formic acid

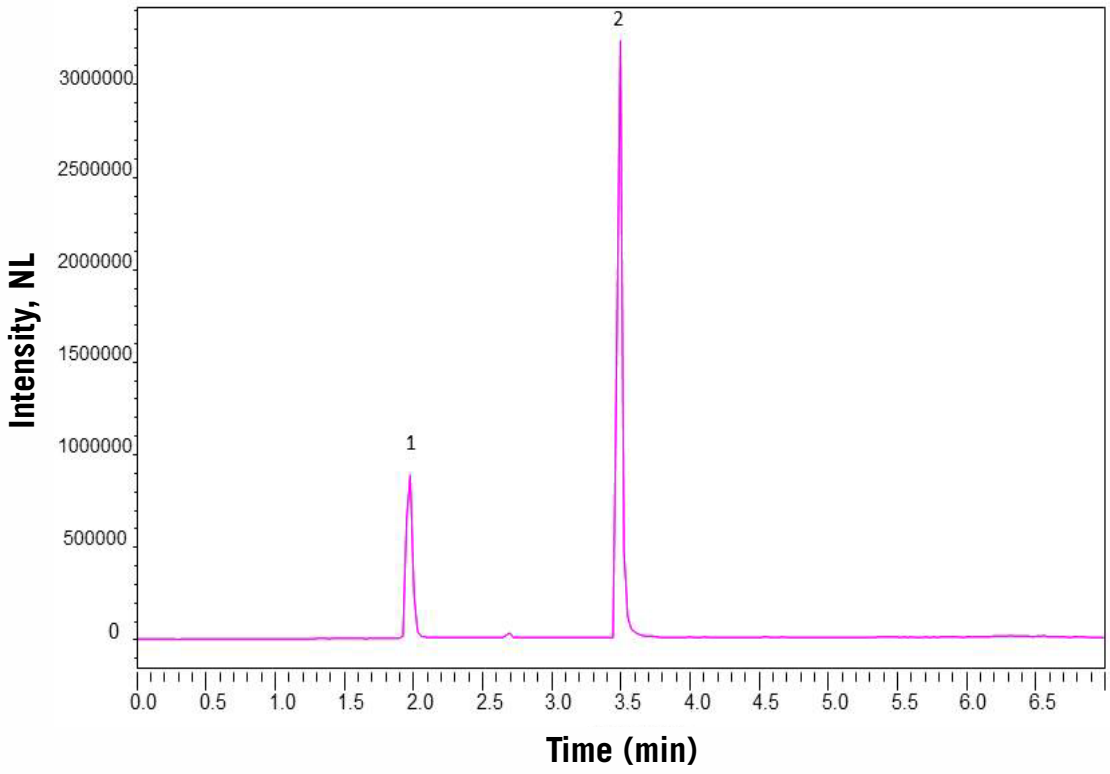
Gradient:

Time	%B
0.0	10
4.00	95
5.00	95
5.01	10
7.00	END

Flow Rate:	0.4 mL/min
Initial Pressure:	315 bar

PEAK IDENTITIES

1. 7-OH Mitragynine (MH^+=415.502 g/mol)
2. Mitragynine (MH^+=399.453 g/mol)



LIDOCAINE IN SALIVA BY LC-MS/MS

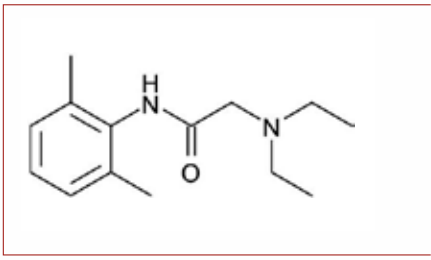
TEST CONDITIONS

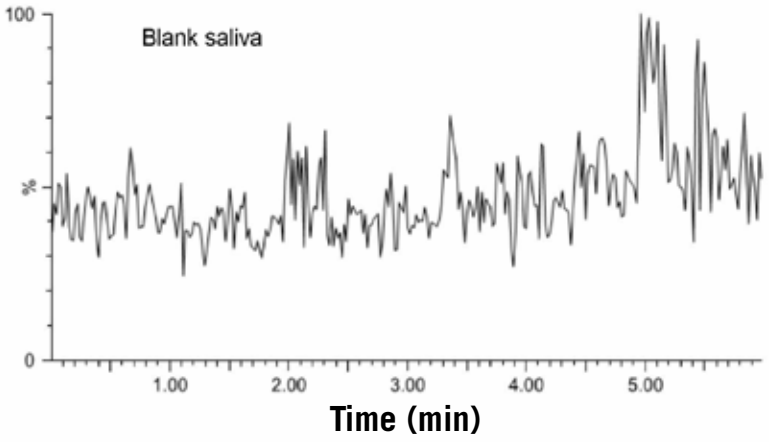
Column:	ACE 3 µm C18
Dimensions:	100 x 3.0 mm
Part Number:	ACE-111-1003
Mobile Phase:	A: 0.1% formic acid in MeCN/H_2O (20:80 v/v)
	B: 0.1% formic acid in MeCN/H_2O (80:20 v/v)

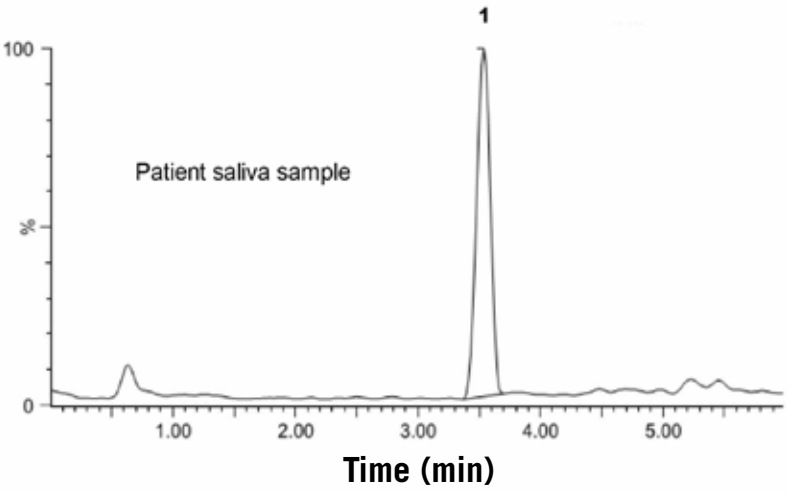
Time (mins)	%B
0.0	20
1.0	20
3.0	80
4.5	80

Flow Rate:	0.3 mL/min
Injection:	10 µL
Detection:	Quattro-Micro triple quad MS
	Positive ion mode ESI

1. Lidocaine
(m/z 235 → 86)







Saliva samples taken after
"Emla 5%" application to skin

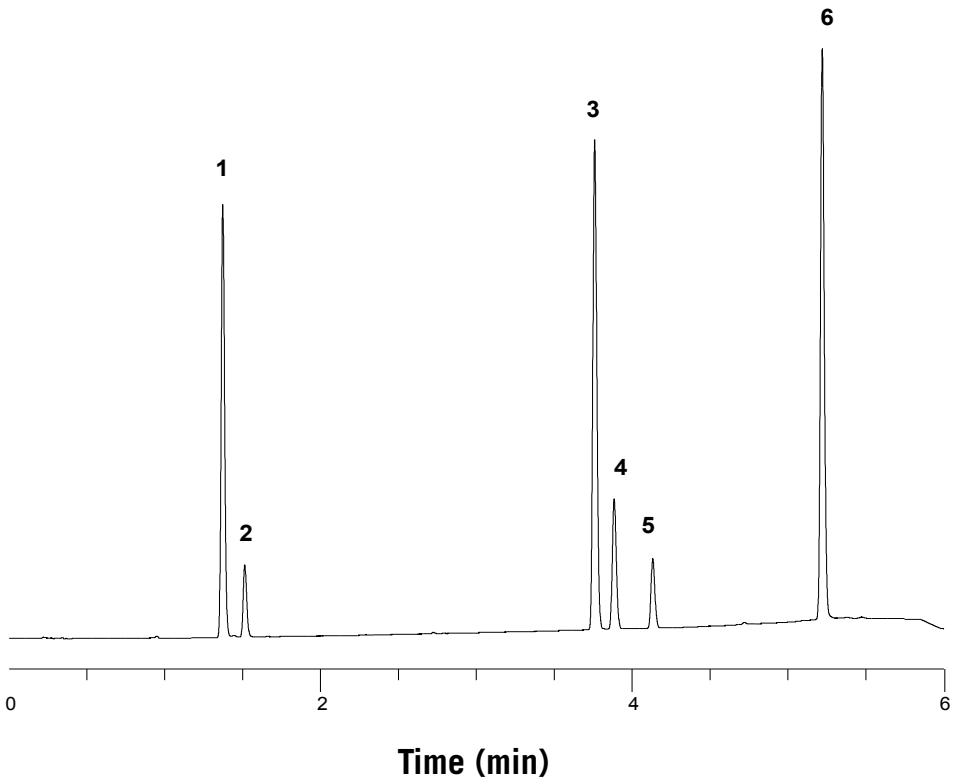
DIURETICS

TEST CONDITIONS

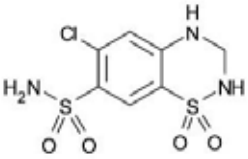
Column:	ACE Excel 2 µm C18-PFP
Dimensions:	50 x 3.0 mm
Part Number:	EXL-1010-0503U
Mobile Phase:	A: 10 mM ammonium formate pH 3.0 in H_2O
	B: 10 mM ammonium formate pH 3.0 in MeOH/H_2O (9:1 v/v)

Time (mins)	%B
0.0	5
0.5	5
5.0	70
5.5	70
6.0	5

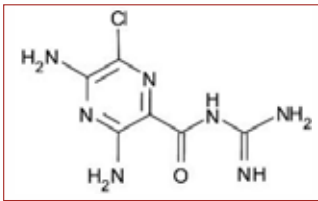
Flow Rate:	1 mL/min
Injection:	2 µL
Temperature:	60 °C
Detection:	UV, 254 nm



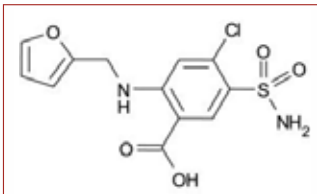
1. Hydrochlorothiazide



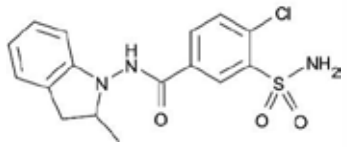
2. Amiloride



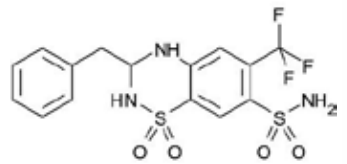
3. Furosemide



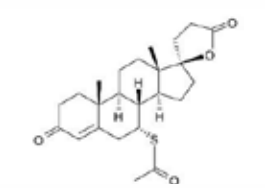
4. Indapamide



5. Bendroflumethiazide



6. Spironolactone



DERMORPHIN OPIOID PEPTIDE IN EQUINE URINE BY LC-MS/MS

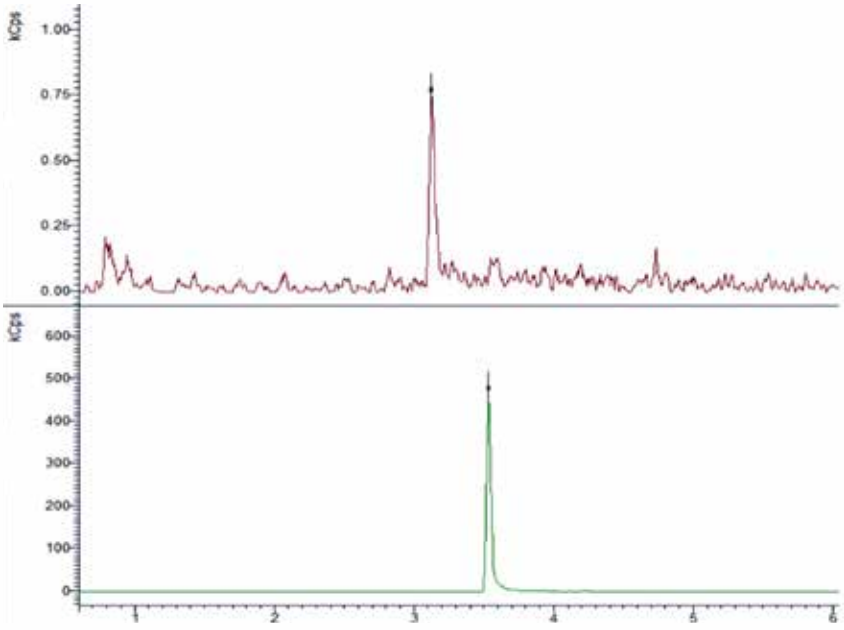
TEST CONDITIONS

Column:	ACE 3 µm C18
Dimensions:	100 x 2.1 mm
Part Number:	ACE-111-1002
Mobile Phase:	A: 0.2% formic acid in H_2O
	B: 0.2% formic acid in MeCN

Time (mins)	%B
0.00	5
0.20	5
8.00	95
8.50	95
8.51	5
12.50	5

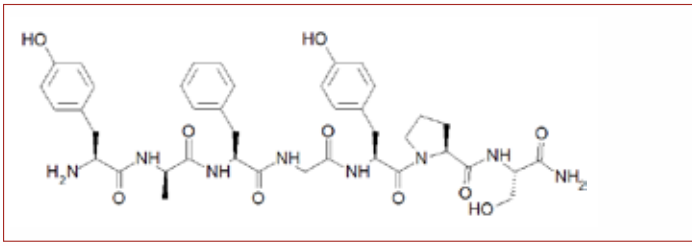
Flow Rate:	0.4 mL/min
Injection:	40 µL
Detection:	Bruker EVOQ Elite triple quad MS
	VIP Heated-ESI Temperature: 350 °C
	Cone Gas Temperature: 250 °C
	Spray Voltage: +4000 V

Accurate quantification of dermorphin in equine urine in range 0.05 – 100 ng/mL
LLOQ = 0.05 ng/mL

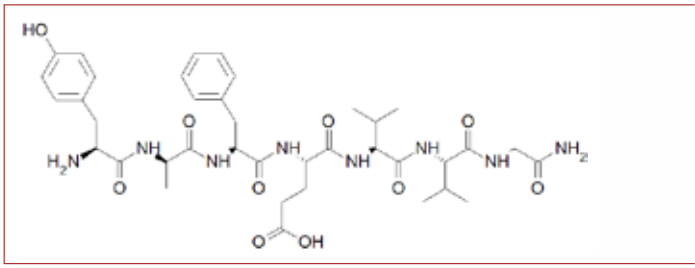


Time (min)

1. Dermorphin
(m/z 803.4 → 602 (Quantifier ion))
(m/z 803.4 → 202 (Qualifier ion))



2. Deltorphin-II (IS)
(m/z 783 → 277)

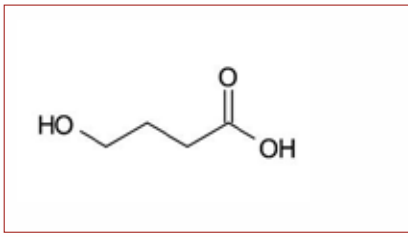


GAMMA-HYDROXYBUTYRIC ACID (GHB) & GAMMA-BUTYROLACTONE (GBL) SEPARATION

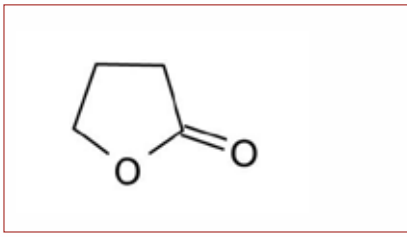
TEST CONDITIONS

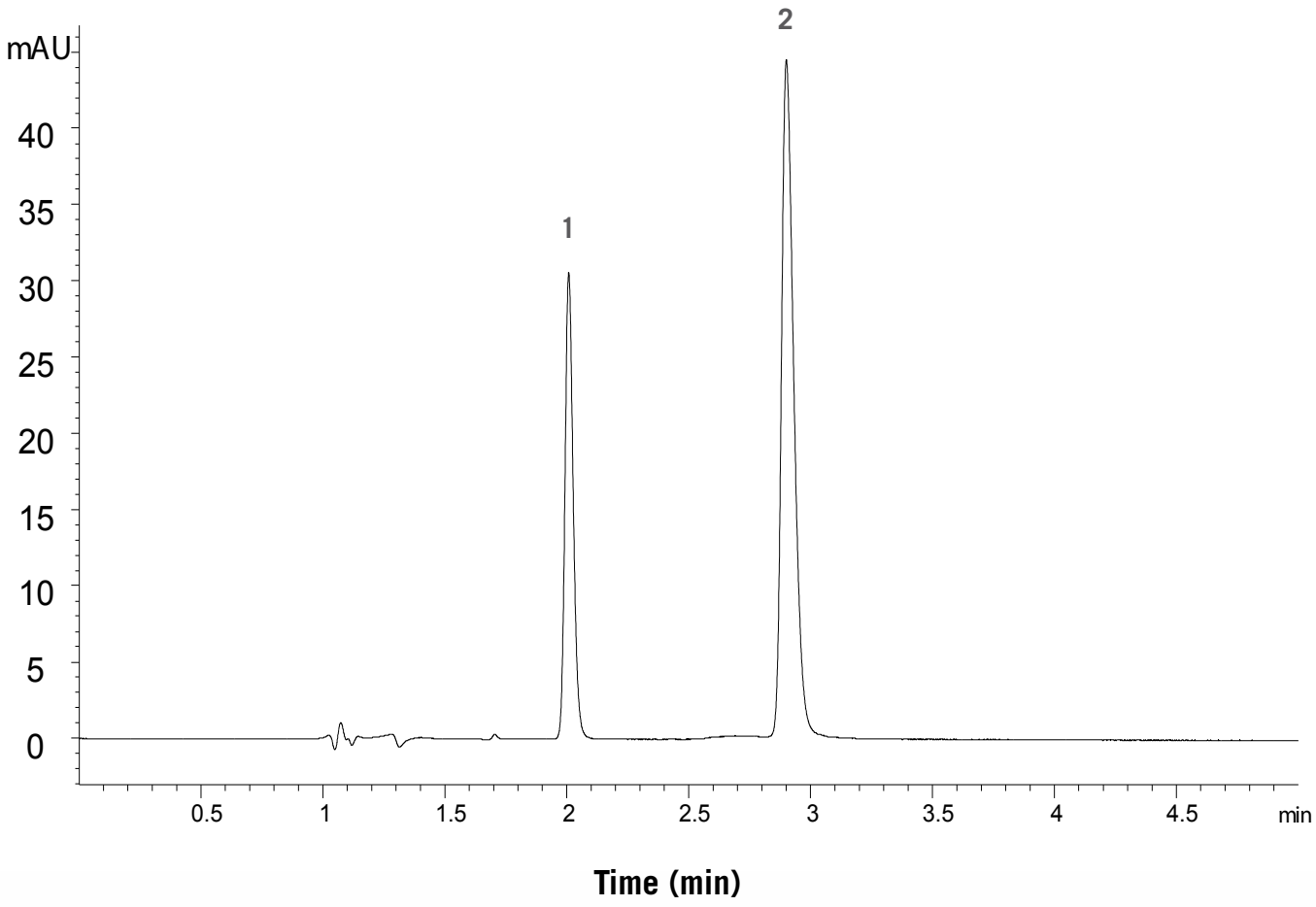
Column:	ACE Excel 2 µm C18-AR
Dimensions:	100 x 3.0 mm
Part Number:	EXL-109-1003U
Mobile Phase:	20 mM KH_2PO_4 pH 2.5 H_2O/MeCN (98:2 v/v)
Flow Rate:	0.43 mL/min
Injection:	2 µL
Temperature:	30 °C
Detection:	UV, 215 nm

1. GHB



2. GBL





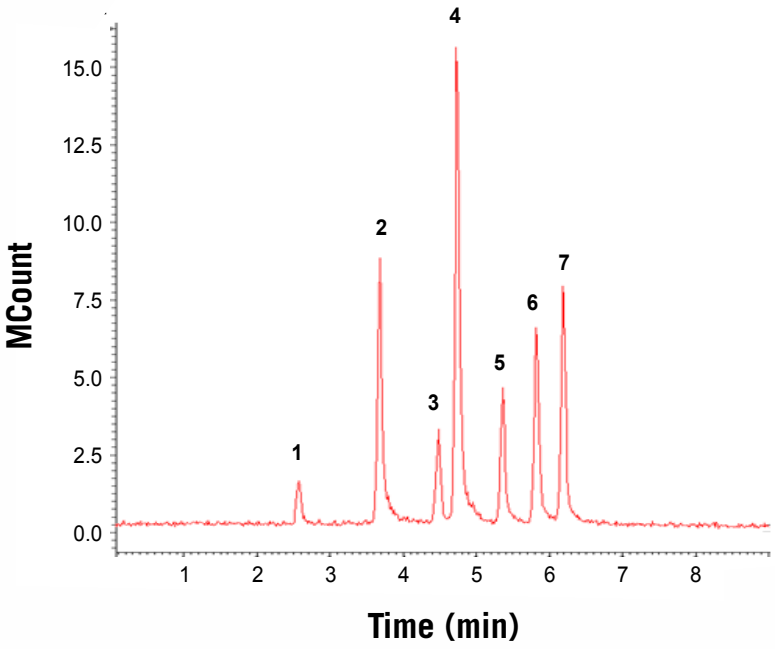
AMPHETAMINES IN URINE BY LC-MS/MS

TEST CONDITIONS

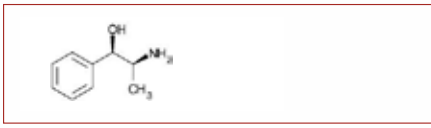
Column:	ACE Excel 3 µm SuperC18
Dimensions:	75 x 2.1 mm
Part Number:	EXL-1111-7502U
Mobile Phase:	A: 5 mM ammonium hydroxide pH 10.8 in H_2O
	B: 5 mM ammonium hydroxide pH 10.8 in MeOH/H_2O (90:10 v/v)

Time (mins)	%B
0	30
8	95

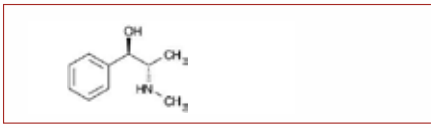
Flow Rate:	0.6 mL/min
Injection:	2 µL
Temperature:	60 °C
Detection:	Varian 320 Triple Quadrupole MS
	Electrospray Voltage: +5 kV
	Inlet Capillary Voltage: 30 V
	CID with Argon at 1.5 mTorr
	Collision cell potential ranges from 5 to 17 V
	Drying gas (nitrogen) Temperature: 325 °C
	Nebulizing Gas (Nitrogen) Pressure: 35 psi
	Extended Dynamic Range



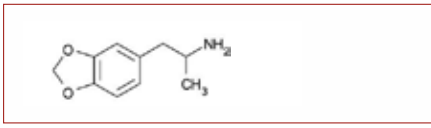
1. Phenylpropanolamine
LOD (est) 4 ppb
(m/z 151.6 → 134.0)



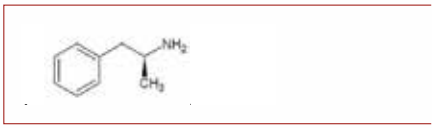
2. (l)-Ephedrine
LOD (est) 2 ppb
(m/z 166.2 → 148.0)



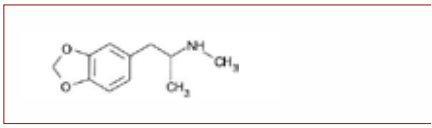
3. (dl)-3,4-MDA
LOD (est) 30 ppb
(m/z 179.7 → 163.0)



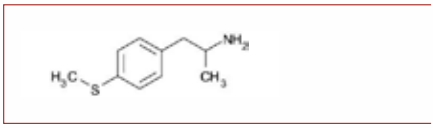
4. (d)-Amphetamine
LOD (est) 4 ppb
(m/z 135.8 → 90.9)



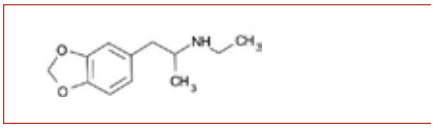
5. (dl)-3,4-MDMA
LOD (est) 2 ppb
(m/z 193.7 → 163.0)



6. 4-Methylthioamphetamine
LOD (est) 10 ppb
(m/z 182.2 → 165.0)



7. (±)-MDEA
LOD (est) 1 ppb
(m/z 207.7 → 165.0)



ISOCRATIC SEPARATION OF 14 CANNABINOIDS

A HALO C18 column is used to separate a mixture of 14 cannabinoids, showing fast results and high resolution critical pairs. Cannabinoids are a class of chemical compounds primarily found in the marijuana plant. Many of these compounds have been found to provide medicinal benefits such as reduction in pain and inflammation.

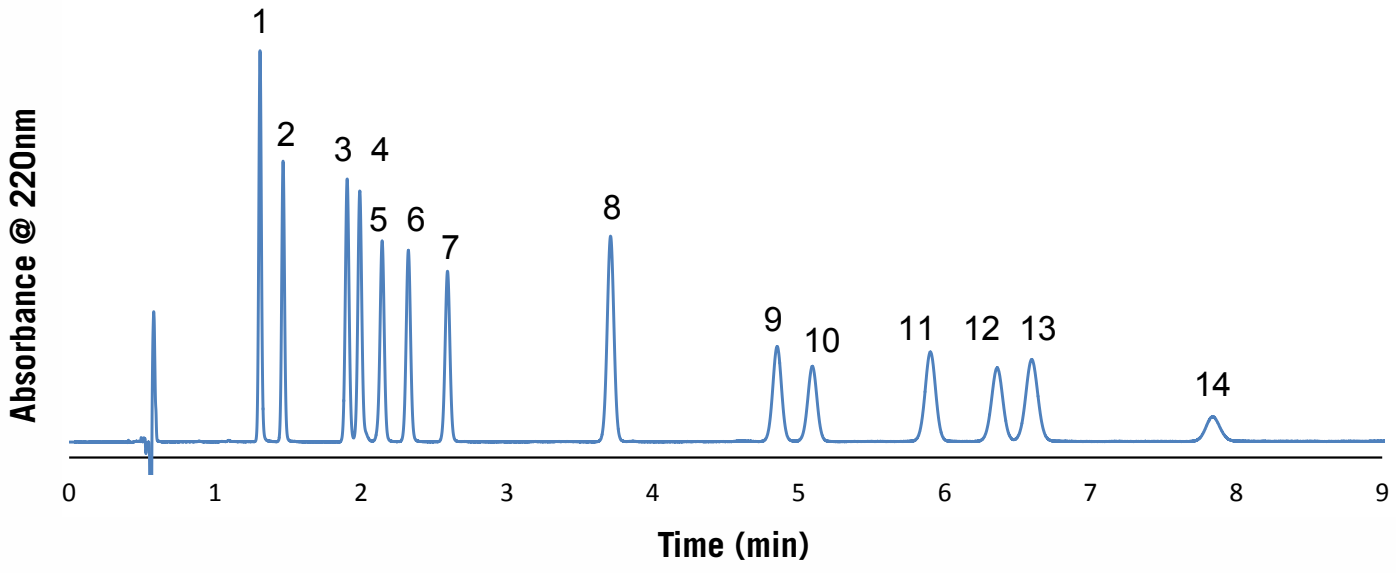
TEST CONDITIONS

Column:	HALO 90 Å C18, 2.7 µm, 3.0 x 150 mm
Part Number:	92813-702
Mobile Phase:	A: water/0.1% formic acid B: acetonitrile/0.085% formic acid
Isocratic:	75%B
Flow Rate:	1.0 mL/min
Initial Pressure:	350 bar
Temperature:	30 °C
Detection:	UV 220 nm, PDA
Injection Volume:	0.6 µL
Dwell Volume:	0.471 mL
Sample Solvent:	75/25 methanol/water
Response Time:	0.025 sec
Data Rate:	100 Hz
LC System:	Shimadzu Nexera X2
Flow Cell:	1 µL

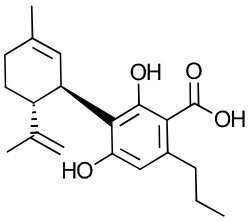
PEAK IDENTITIES

1. Cannabidivarinic acid (CBDVA)
2. Cannabidvarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Cannabinol (CBN)
9. delta-9- Tetrahydrocannabinol (Δ9-THC)
10. delta-8-Tetrahydrocannabinol (Δ8-THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

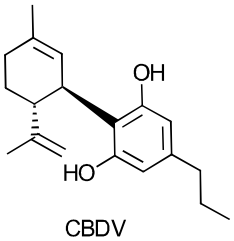
STRUCTURES ON PAGE 27



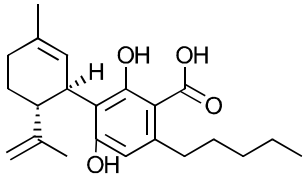
ISOCRATIC SEPARATION OF 14 CANNABINOIDS



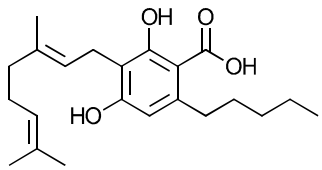
CBDVA



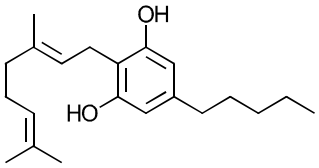
CBDV



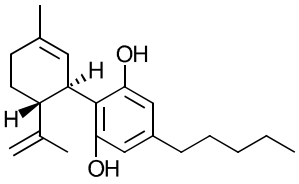
CBDA



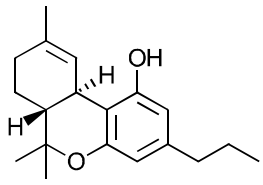
CBGA



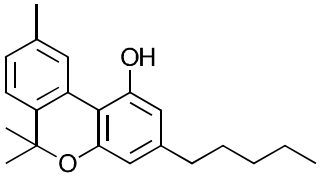
CBG



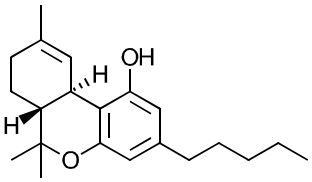
CBD



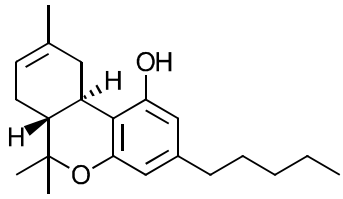
THCV



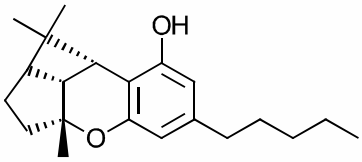
CBN



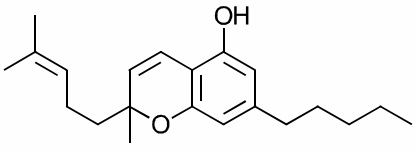
Δ 9-THC



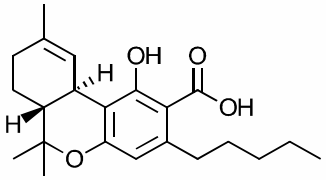
Δ 8-THC



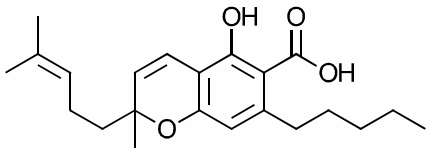
CBL



CBC



THCA



CBCA

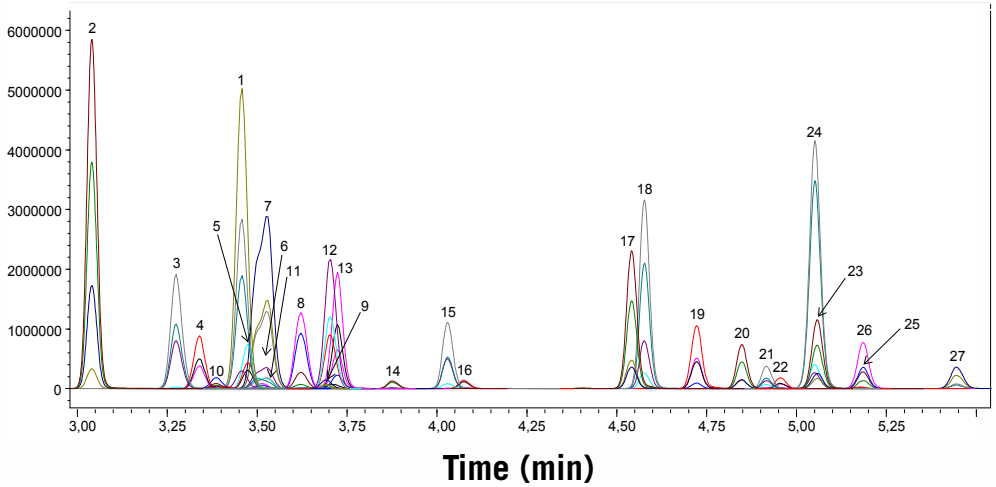
CANNABINOIDS (SYNTHETIC) BY LC-MS/MS

TEST CONDITIONS

Column: ACE Excel 3 µm C18-AR
Dimensions: 100 x 3.0 mm
Part Number: EXL-119-1003U
Mobile Phase: A: 15 mM ammonium formate pH 4.0 in H_2O
B: 0.1% formic acid in MeCN

Time (mins)	%B
0.00	40
3.74	90
8.00	90
8.50	40

Flow Rate: 0.5 mL/min
Injection: 10 µL
Temperature: 40 °C
Detection: Shimadzu LCMS 8040 MS
Positive ion ESI



PEAK IDENTITIES

1. JWH-018 N-5-OH-pentyl-d5
(m/z 362.90 → 155.05; 127.00; 128.05)
2. JWH-250 N-5-OH-pentyl
(m/z 352.20 → 121.15; 91.10; 186.05)
3. JWH-073 N-4-OH-butyl
(m/z 344.20 → 155.00; 127.10; 54.95)
4. JWH-018 N-pentanoic
(m/z 372.20 → 155.05; 127.10)
5. JWH-018 N-5-OH-pentyl
(m/z 357.80 → 155.05; 127.05)
6. AM2201 N-4-OH-pentyl
(m/z 376.40 → 155.00; 127.00; 144.00)
7. AM2201 5/6-OH-indole
(m/z 375.90 → 155.05; 127.05; 248.10)
8. JWH-081 N-5-OH-pentyl
(m/z 388.20 → 185.05; 157.05; 114.15)
9. MAM2201 N-4-OH-pentyl
(m/z 389.60 → 169.00; 141.05; 115.15)
10. AB-CHMINACA
(m/z 356.70 → 241.05; 312.20; 340.15)
11. UR-144 N-pentanoic
(m/z 341.60 → 125.10; 55.05; 57.10)
12. JWH-019 N-6-OH-hexyl
(m/z 371.80 → 155.05; 127.00; 144.00)
13. JWH-122 N-5-OH-pentyl
(m/z 372.20 → 169.05; 141.05; 115.15)
14. AKB48 N-pentanoic
(m/z 395.60 → 135.00; 93.10; 79.05)
15. JWH-018 5-OH-indole
(m/z 358.20 → 155.00; 127.05; 230.05)
16. AKB48 N-5-OH-pentyl
(m/z 381.60 → 135.10; 93.10; 79.05)
17. JWH-210 5-OH-indole
(m/z 386.10 → 183.05; 153.10; 155.05)
18. PB-22
(m/z 358.80 → 214.05; 144.05; 116.00)
19. JWH-073
(m/z 328.20 → 127.10; 155.05; 200.10)
20. EAM2201
(m/z 387.70 → 183.10; 232.10; 155.10)
21. JWH-122 N-4-pentenyl
(m/z 353.70 → 169.05; 141.10; 115.10)
22. JWH-018
(m/z 341.70 → 155.00; 127.05; 214.10)
23. JWH-081
(m/z 372.10 → 185.05; 157.15; 127.10)
24. AKB48F
(m/z 384.30 → 135.15; 107.10; 93.10)
25. THJ-018
(m/z 342.60 → 215.10; 145.05; 90.00)
26. JWH-122
(m/z 356.30 → 169.05; 141.10; 115.15)
27. JWH-210
(m/z 370.10 → 183.10; 155.10; 153.10)

ISOCRATIC SEPARATION OF SYNTHETIC CANNABINOIDS USING MS CONFIRMATION

Synthetic cannabinoids are man-made compounds that act like the chemicals found in the marijuana plant. The four compounds in this mixture represent only a small number of the variations that exist. Just as one compound is made illegal, another variation will be synthesized to take its place. This represents a growing challenge for law enforcement agencies. Using a HALO C18 column gives a fast, efficient separation of these cannabinoids with ample resolution for the next generation of illegal species.

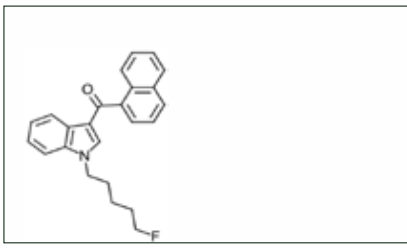
TEST CONDITIONS

Column:	HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number:	92812-602
Mobile Phase:	Isocratic: 25/75 A/B A: 5 mM ammonium formate B: 95/5 acetonitrile/water with 5 mM ammonium formate
Flow Rate:	0.6 mL/min
Pressure:	279 bar
Temperature:	30 °C
Injection Volume:	0.5 µL
Sample Solvent:	50/50 water/acetonitrile
Detection:	UV 200 nm, VWD
Data Rate:	100 Hz
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

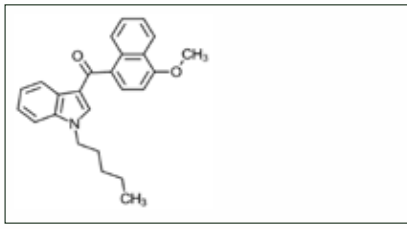
PEAK IDENTITIES

1. AM2201 (359.44 g/mol)
2. JWH-081 (371.47 g/mol)
3. JWH-122 (355.47 g/mol)
4. JWH-019 (355.47 g/mol)

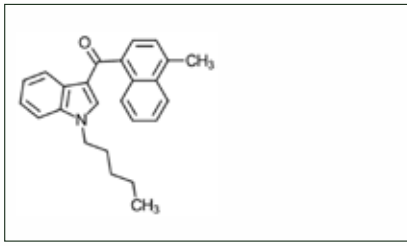
AM2201



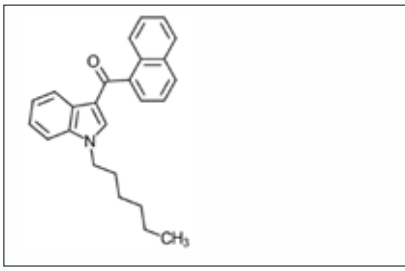
JWH-081

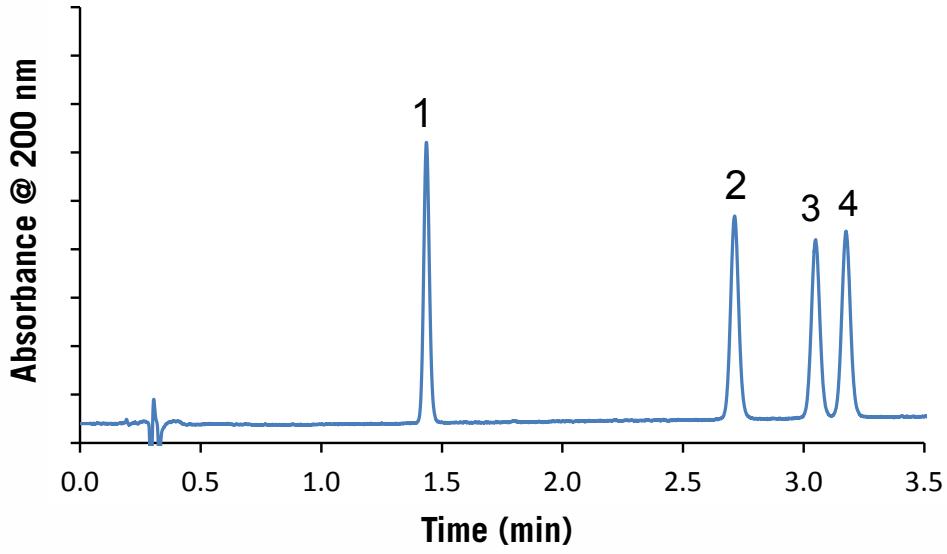


JWH-122



JWH-019





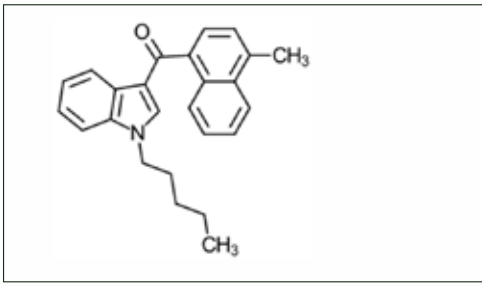
ISOCRATIC SEPARATION OF SYNTHETIC CANNABINOIDS USING MS CONFIRMATION

Synthetic cannabinoids can be very similar in their chemical structure. In fact, many of these cannabinoids are analogs or isomers of each other and can be difficult to distinguish. Two homologues in this particular sample were fraction collected and then identified using an orbital ion trap MS system. The Orbitrap allows us to see signature fragmentations of a particular compound, allowing positive identification of each homologue.

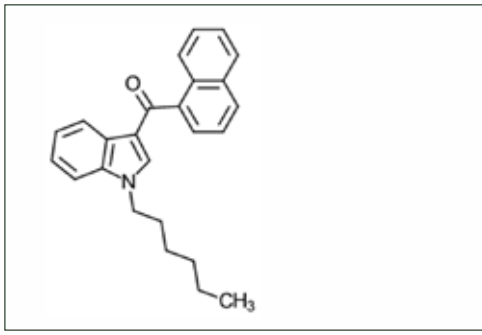
MS TEST CONDITIONS

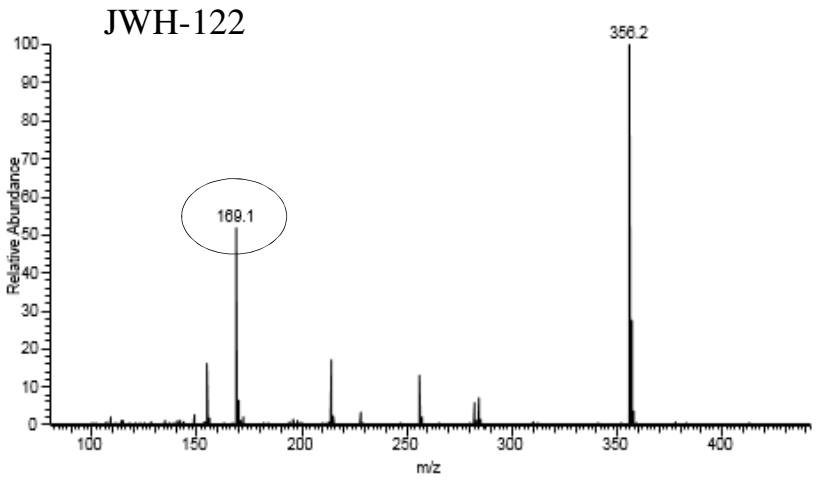
MS System:	Thermo Fisher Orbitrap VelosPro ETD
Scan Time:	6 µscans/250 ms max inject time
Scan Range:	50-2000 m/z
MS Parameters:	Positive ion mode, ESI at +4.0 kV, 225 °C capillary

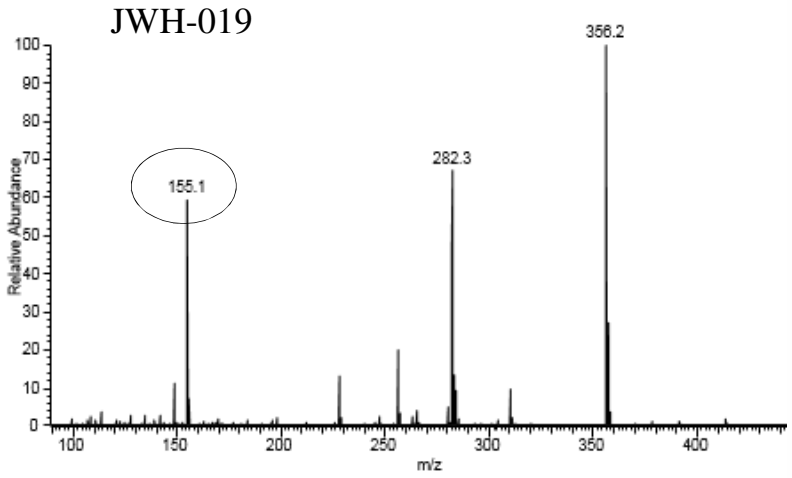
JWH-122 (m/z= 356.47)



JWH-019 (m/z= 356.47)







ISOCRATIC SEPARATION OF SYNTHETIC CANNABINOIDS

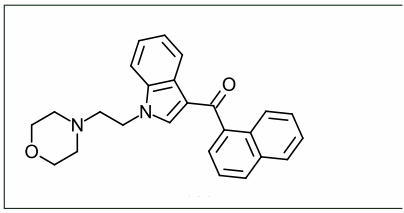
TEST CONDITIONS

Column:	HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number:	92812-602
Mobile Phase:	Isocratic: 25/75 A/B A: 5 mM ammonium formate, pH unadjusted B: 95/5 acetonitrile/water with 5 mM ammonium formate
Flow Rate:	0.6 mL/min
Pressure:	247 bar
Temperature:	30 °C
Injection Volume:	0.5 µL
Sample Solvent:	50/50 water/acetonitrile
Detection:	UV 200 nm, VWD
Data Rate:	50 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR

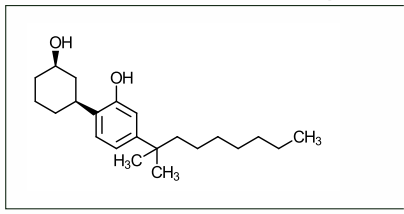
PEAK IDENTITIES

1. JWH-200
2. (±)-CP 47, 497
3. (±)-CP 47, 497 C8 Homologue
4. JWH-250
5. HU-211

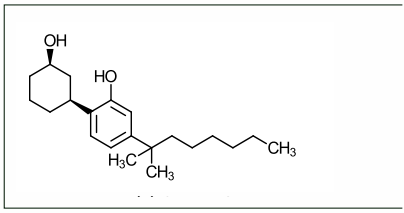
JWH-200



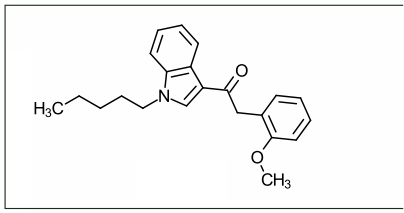
(±)-CP 47, 497 C8 Homologue



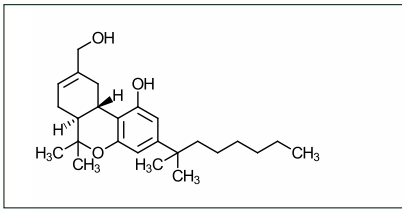
(±)-CP 47, 497

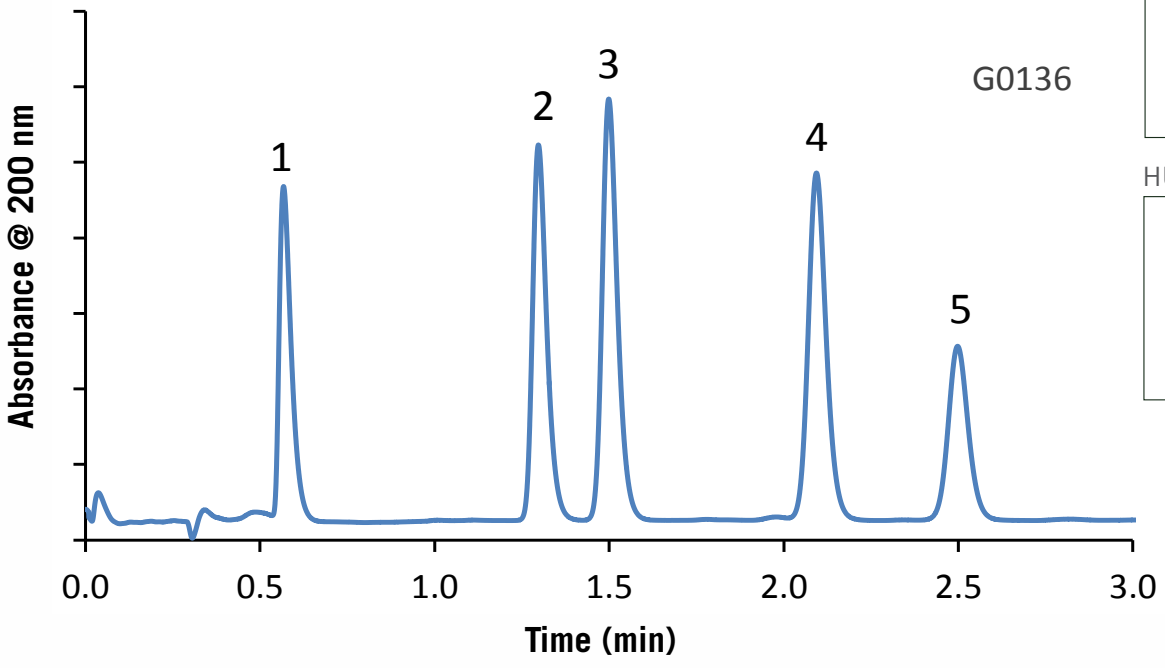


JWH-250



HU-211



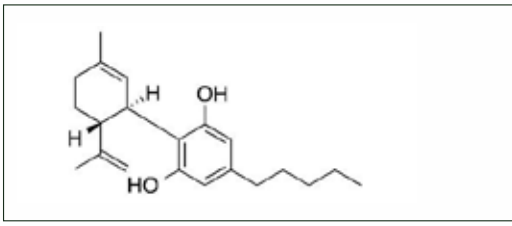


CANNABINOIDS IN RAT PLASMA

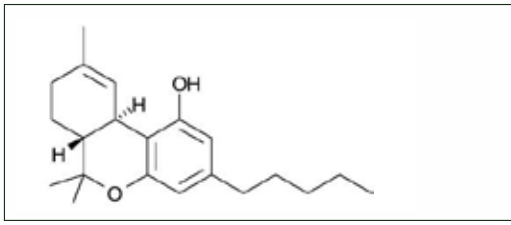
TEST CONDITIONS

Column:	ACE 3 µm C18-PFP
Dimensions:	150 x 4.6 mm
Part Number:	ACE-1110-1546
Mobile Phase:	H_2O/MeCN (38:62 v/v)
Flow Rate:	1 mL/min
Injection:	30 µL
Temperature:	55 °C
Detection:	UV, 220 nm

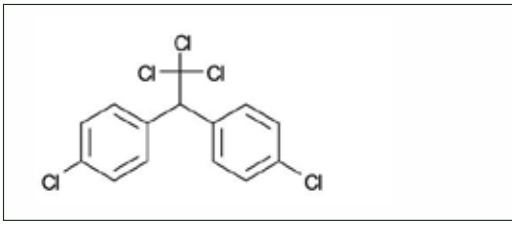
1. Cannabidiol (CBD)

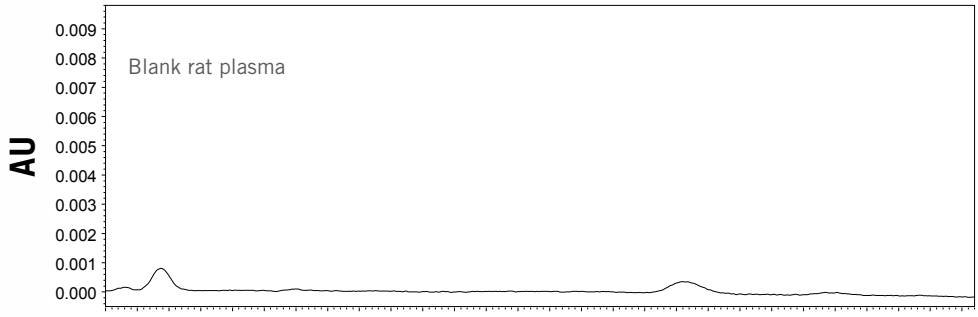


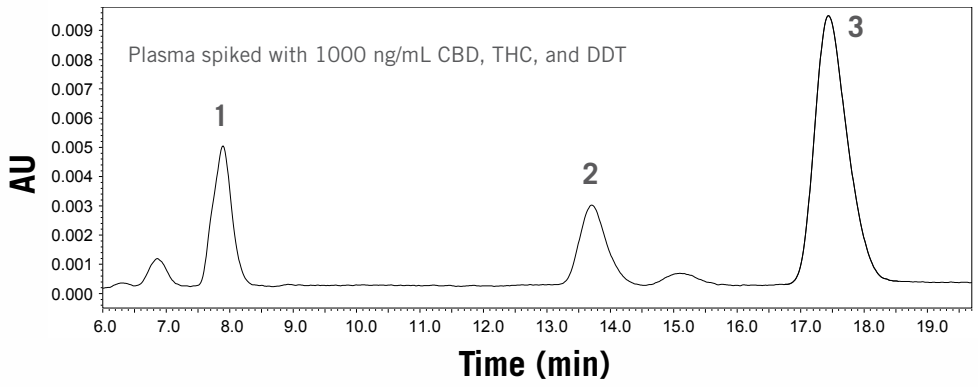
2. Δ^9-Tetrahydrocannabinol (THC)



3. 4,4-Dichlorodiphenyltrichloroethane (DDT) (IS)







LLOQ 10 ng/mL for both cannabinoids
Method linearity 10 – 10,000 ng/mL

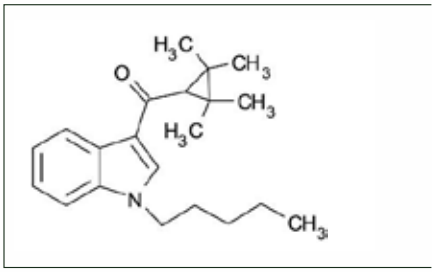
SYNTHETIC CANNABINOIDS (SPICE) FROM ORAL FLUIDS

TEST CONDITIONS

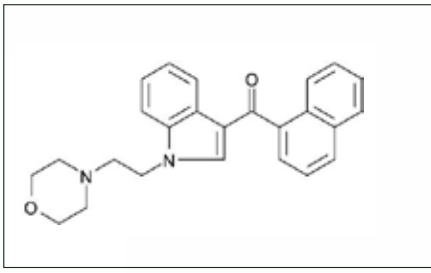
Column:	ACE Excel 2 µm C18-AR
Dimensions:	100 x 2.1 mm
Part Number:	EXL-109-1002U
Mobile Phase:	0.1% formic acid in MeOH/H_2O (85:15 v/v)
Flow Rate:	0.3 mL/min
Temperature:	Ambient
Detection:	Applied Biosystems/MDS Sciex 4000 Q-Trap Positive mode Turbo Ionspray

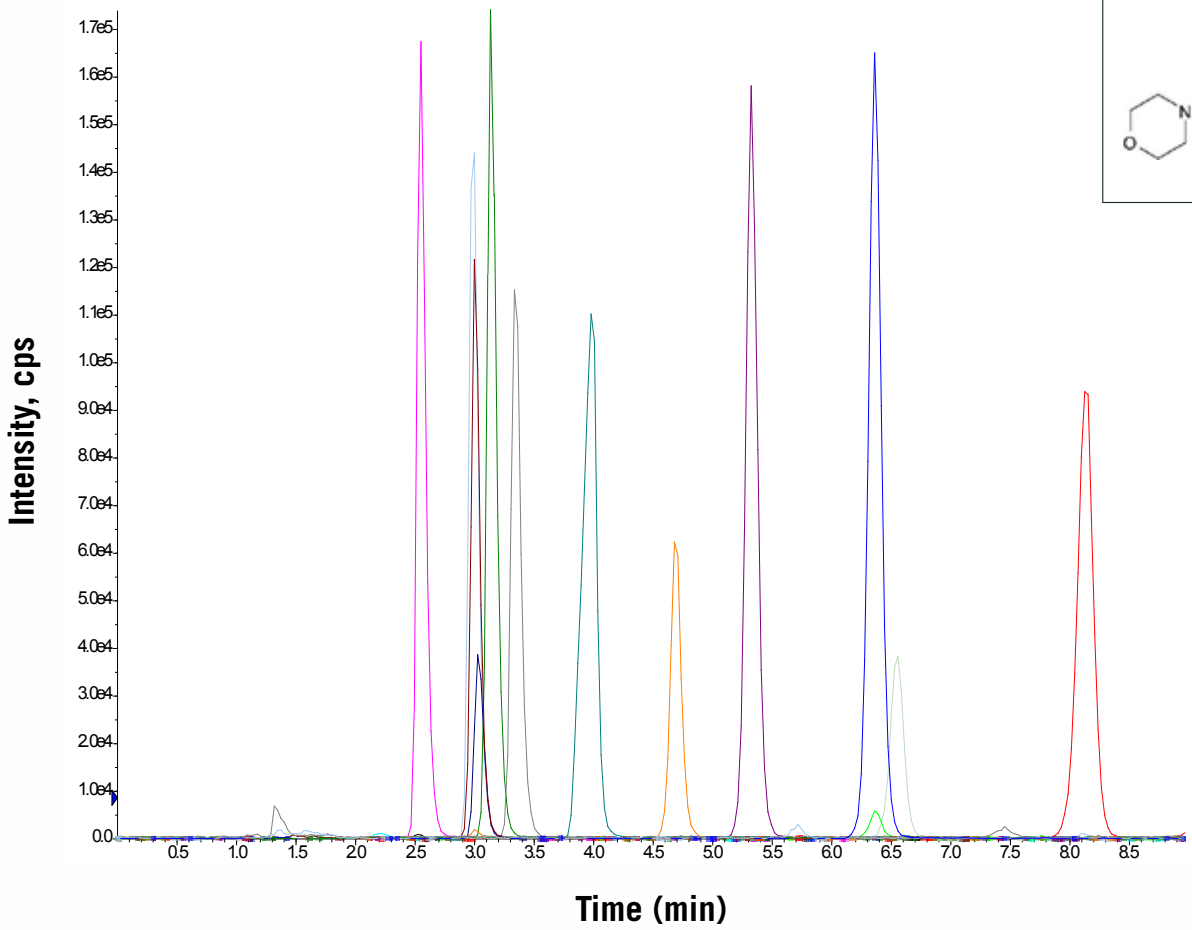
Extracted ion chromatogram for SPICE analytes fortified in neat oral fluid at 20 ng/mL

UR-144



JWH-200





Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	30	35	16
3.14	d5-JWH-018 N- (4-hydroxypentyl)	363.5>155	40	35	16
3.14	JWH-018 N- (4-hydroxypentyl)	358>155	40	30	16
3.34	JWH-018 5-pentanoic acid	372>155	40	30	16
3.98	JWH-200	385>155	40	30	16
4.69	XLR-11	330>125	30	35	16
5.32	JWH-250	336>121	40	30	16
6.36	JWH-073	328>155	40	30	16
6.37	UR-144 5-Chloro-pentyl	346.9>125	30	35	16
6.55	UR-144	312.5>125	30	35	16
8.14	JWH-018	342>155	40	30	16

RAPID ANALYSIS OF CANNABINOIDS

TEST CONDITIONS

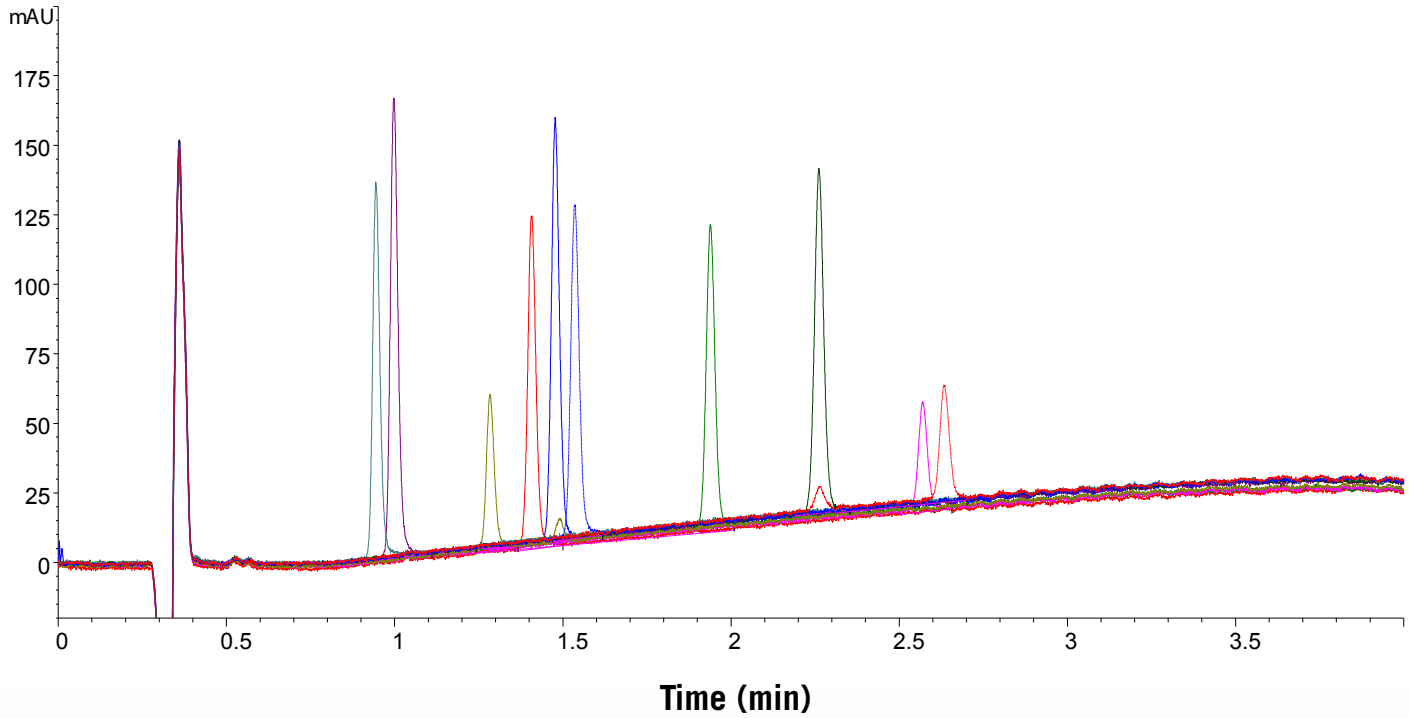
Column:	ACE UltraCore 2.5 µm SuperC18
Dimensions:	50 x 3.0 mm
Part Number:	CORE 25A 0503U
Mobile Phase:	A: 0.075% formic acid in H_2O
	B: 0.1% formic acid in MeCN

Time	%B
0.00	70
0.25	70
4.18	100
5.05	100

Flow Rate:	0.6 mL/min
Injection:	2 µL
Temperature:	40 °C
Detection:	UV, 210 nm
Mass On Column:	20 ng per analyte

PEAK IDENTITIES

1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC COOH)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerol (CBG)
5. Cannabidiol (CBD)
6. Tetrahydrocannabivarin (THCV)
7. Cannabinol (CBN)
8. (-)-trans-Δ^9-Tetrahydrocannabinol (THC)
9. Cannabichromene (CBC)
10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A)



CANNABINOIDS

TEST CONDITIONS

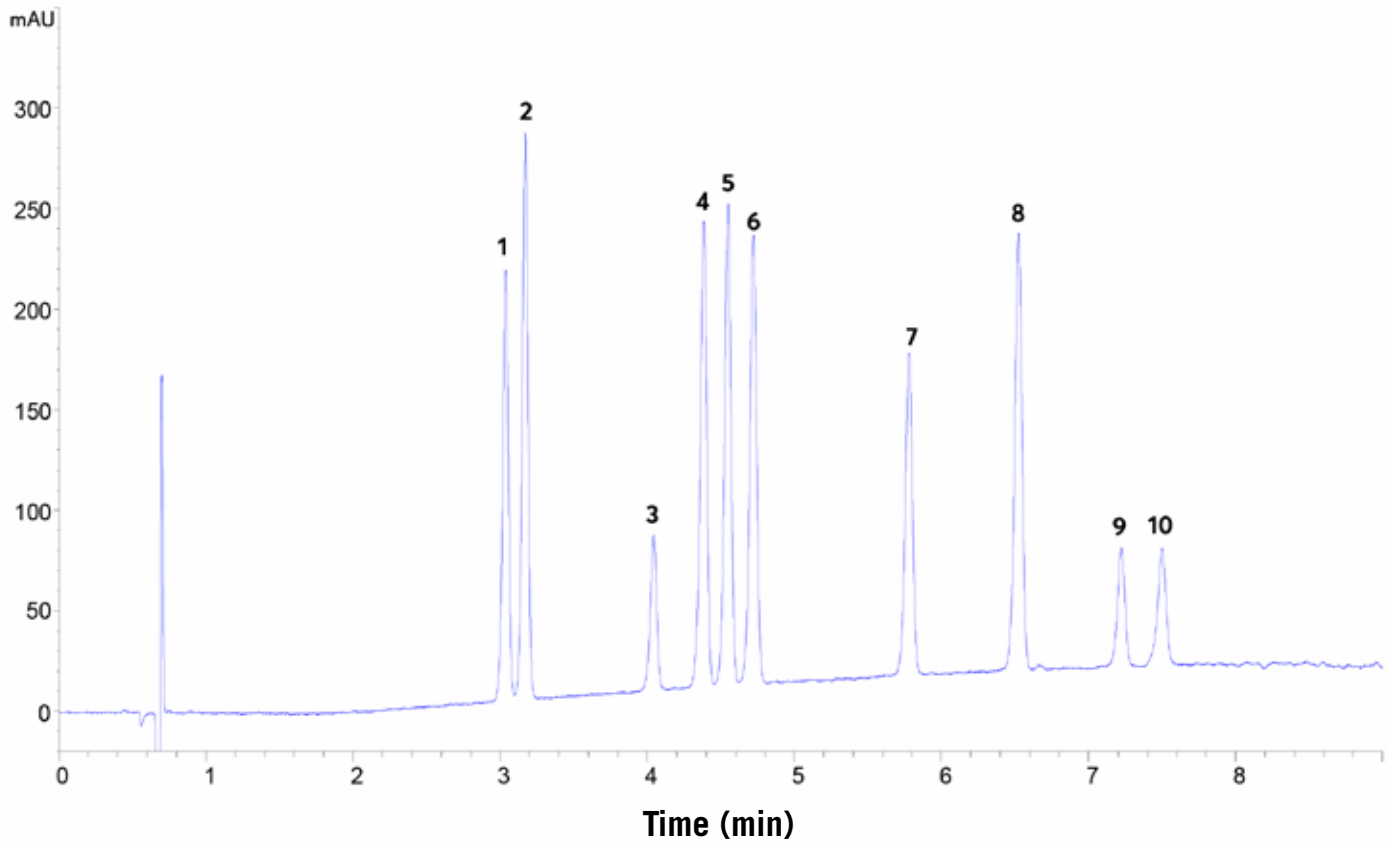
Column:	ACE Excel 2 µm SuperC18
Dimensions:	100 x 3.0 mm
Part Number:	EXL-1011-1003U
Mobile Phase:	A: 0.075% formic acid in H_2O
	B: 0.1% formic acid in MeCN

Time (mins)	%B
0.0	70
1.0	70
10.0	100
12.0	100

Flow Rate:	0.6 mL/min
Injection:	5 µL
Temperature:	40 °C
Detection:	UV, 210 nm

PEAK IDENTITIES

1. (-)-1-Nor-9-carboxy-Δ^9 tetrahydrocannabinol (THC-COOH)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerol (CBG)
5. Cannabidiol (CBD)
6. Tetrahydrocannabivarin (THCV)
7. Cannabinol (CBN)
8. (-)-trans-Δ^9-Tetrahydrocannabinol (THC)
9. Cannabichromene (CBC)
10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A)



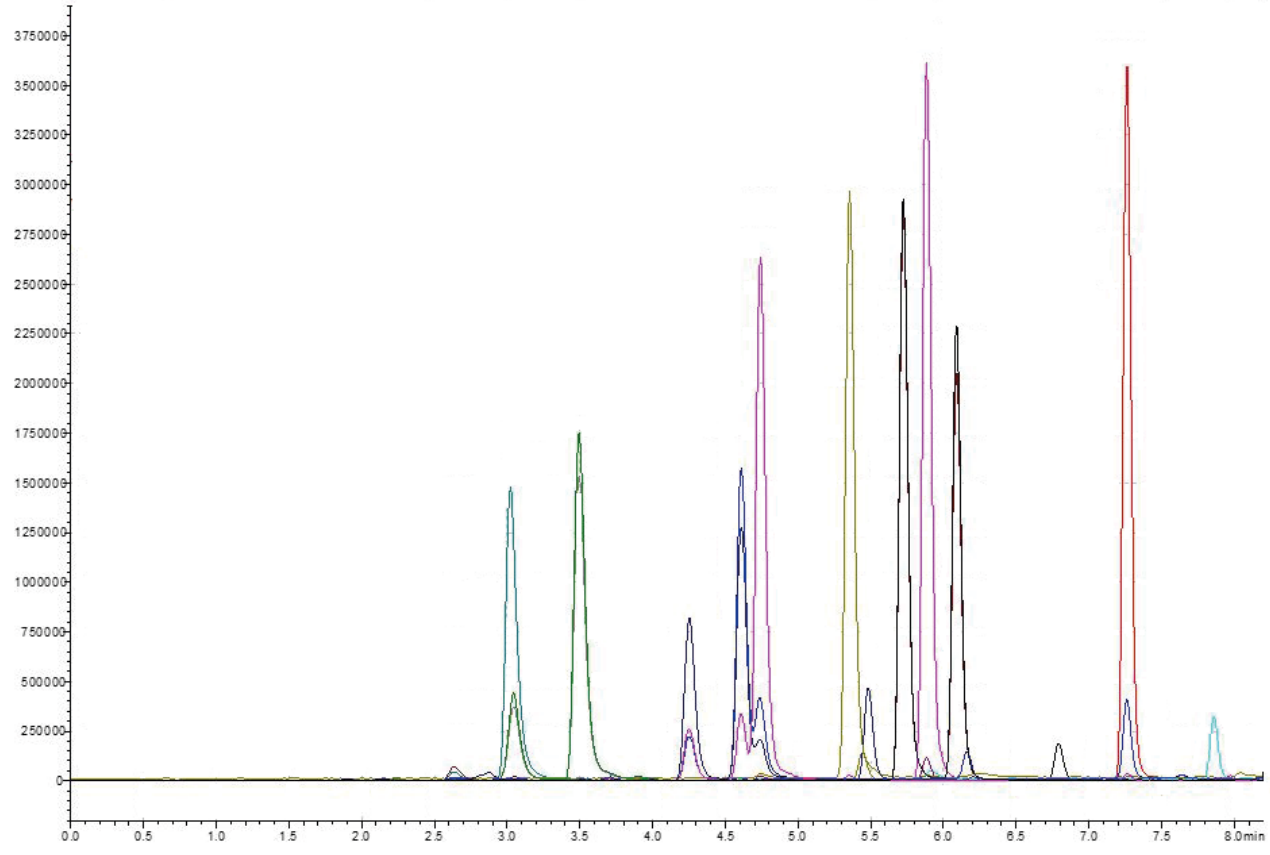
DETECTION OF 21 ENDOGENOUS STEROIDS IN SERUM BY UHPLC-MS/MS

TEST CONDITIONS

Column:	ACE Excel 1.7 µm C18
Dimensions:	100 x 2.1 mm
Part Number:	EXL-171-1002U
Mobile Phase:	A: 0.2 mM ammonium fluoride in water B: Methanol

Time (mins)	%B
0	50
3	60
8	90
9	95
9.1	95
9.5	50

Flow Rate:	0.4 mL/min
Injection Volume:	10 µL
Temperature:	40 °C
Detection:	Shimadzu 8060 Triple Quad MS MRM, positive, and negative ESI mode
Sample:	Endogenous Steroids extracted from human serum using supported liquid extraction or polymer based SPE prior to LC/MS MS



Time (min)

Analyte	Rt (Mins)	MRM Transitions	Ion Mode
Aldosterone-d4	2.62	363.1 → 190.3	-
Aldosterone	2.65	359.1 → 189.3	-
Cortisone	3.03	361.3 → 163.2	+
18-OH-Corticosterone	3.06	363.3 → 269.2	+
Cortisol	3.51	363.4 → 121.3	+
DHEAS	3.76	367.1 → 97.1	-
21-Deoxycortisol	4.27	347.1 → 311.2	+
Corticosterone	4.63	347.3 → 329.3	+
11-Deoxycortisol	4.76	347.3 → 109.3	+
Androstenedione	5.38	287.3 → 97.2	+
Estradiol	5.47	271.1 → 145.2	-
Esterone	5.51	269.2 → 145.2	-
11-Deoxycorticosterone	5.75	331.3 → 109.1	+
Testosterone	5.91	289.3 → 97.1	+
17-OH-Progesterone	6.11	331.3 → 97.1	+
17-OH-Pregnenolone	6.18	315.3 → 297.2	+
DHEA	6.23	289.3 → 253.2	+
DHT-d3	6.80	294.4 → 258.3	+
DHT	6.82	291.3 → 255.3	+
Progesterone	7.29	315.2 → 97.2	+
Pregnenolone	7.89	299.3 → 159.3	+

CORTICOSTEROIDS BY LC-MS/MS

TEST CONDITIONS

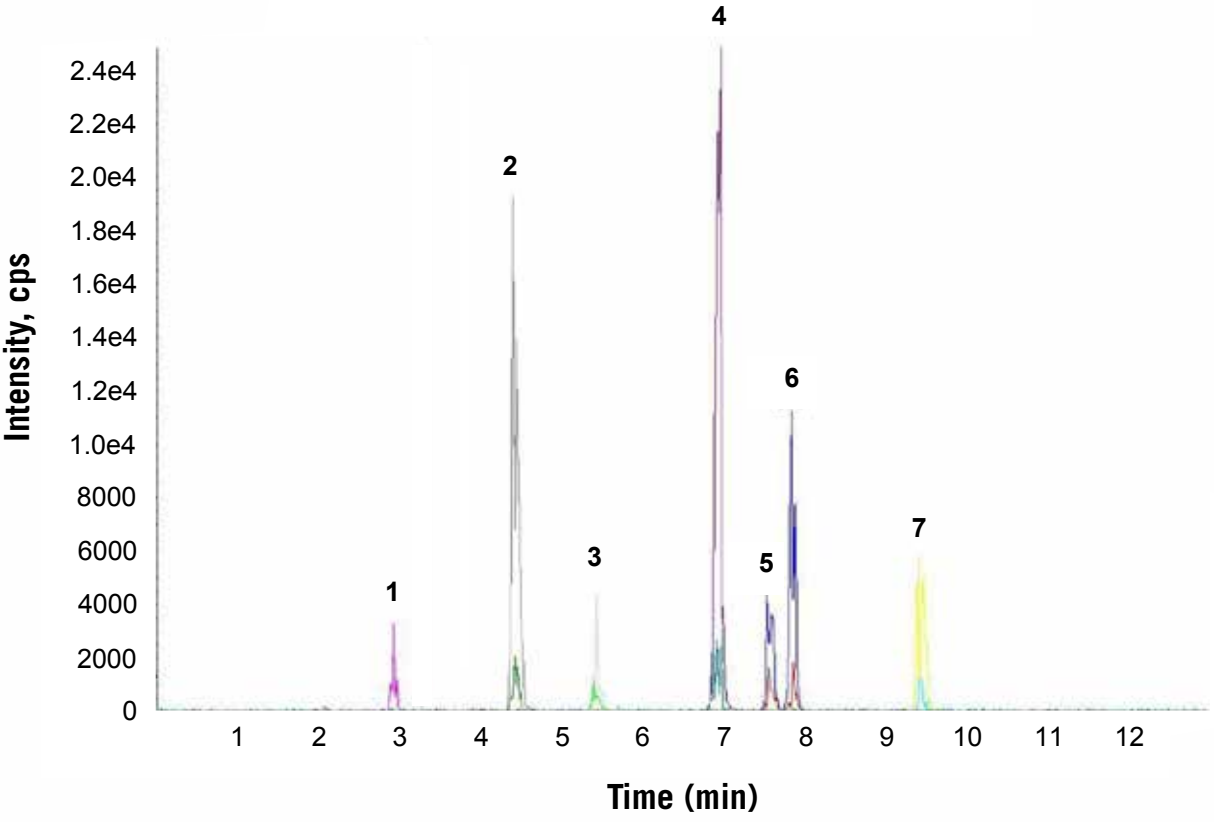
Column:	ACE 3 µm C18-PFP
Dimensions:	150 x 2.1 mm
Part Number:	ACE-1110-1502
Mobile Phase:	A: 0.1% formic acid in H_2O B: MeCN/0.1% formic acid in H_2O (90:10 v/v)

Time (mins)	%B
0.0	30
14.0	50
17.0	95
20.0	30

Flow Rate:	0.3 mL/min
Injection:	25 µL
Temperature:	15 °C
Detection:	Turbospray, MRM

PEAK IDENTITIES

1. Triamcinolone
2. Prednisolone
3. Fluoroprednisolone
4. Methylprednisolone
5. Betamethasone
6. Dexamethasone
7. Flumethasone



SEPARATION OF STEROIDS

A mixture of 11 steroids is separated using a 6-minute gradient on a HALO 90 Å Biphenyl column. The chromatogram shows very good resolution between all peak pairs with excellent peak shape and high efficiency.

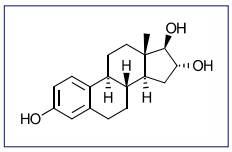
TEST CONDITIONS

Column:	HALO 90 Å Biphenyl, 2.7 µm, 4.6 x 50 mm
Part Number:	92814-411
Mobile Phase	A: Water B: Acetonitrile
Gradient:	20-60% B in 6 minutes
Flow Rate:	1.85 mL/min
Initial Pressure:	344 bar
Temperature:	30 °C
Detection:	UV 215 nm, PDA
Injection Volume:	4 µL
Sample Solvent:	Acetonitrile:water, 37.5:62.5
Data Rate:	100 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

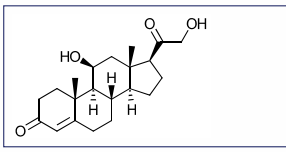
PEAK IDENTITIES

1. Estriol
2. Hydrocortisone
3. Prednisone
4. Cortisone
5. Corticosterone
6. ß-Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17-α-Hydroxyprogesterone
10. 11-Deoxycorticosterone
11. Progesterone

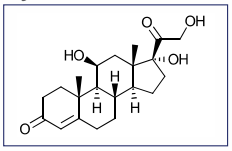
Estriol



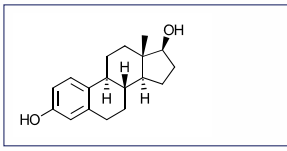
Corticosterone



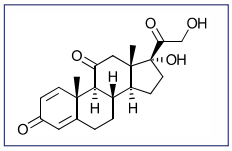
Hydrocortisone



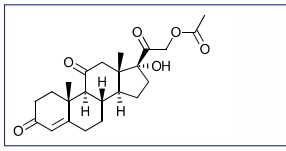
ß-Estradiol



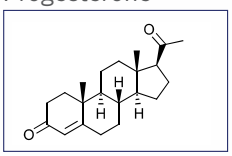
Prednisone



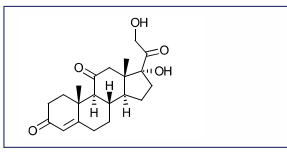
Cortisone Acetate



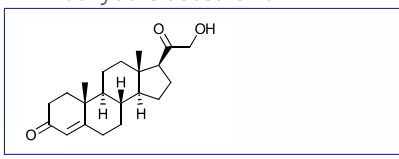
Progesterone



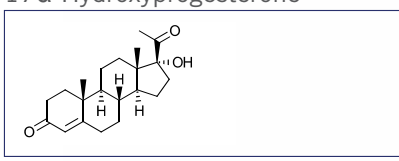
Cortisone



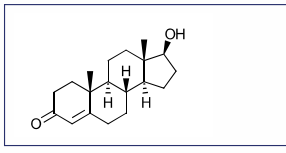
11-Deoxycorticosterone

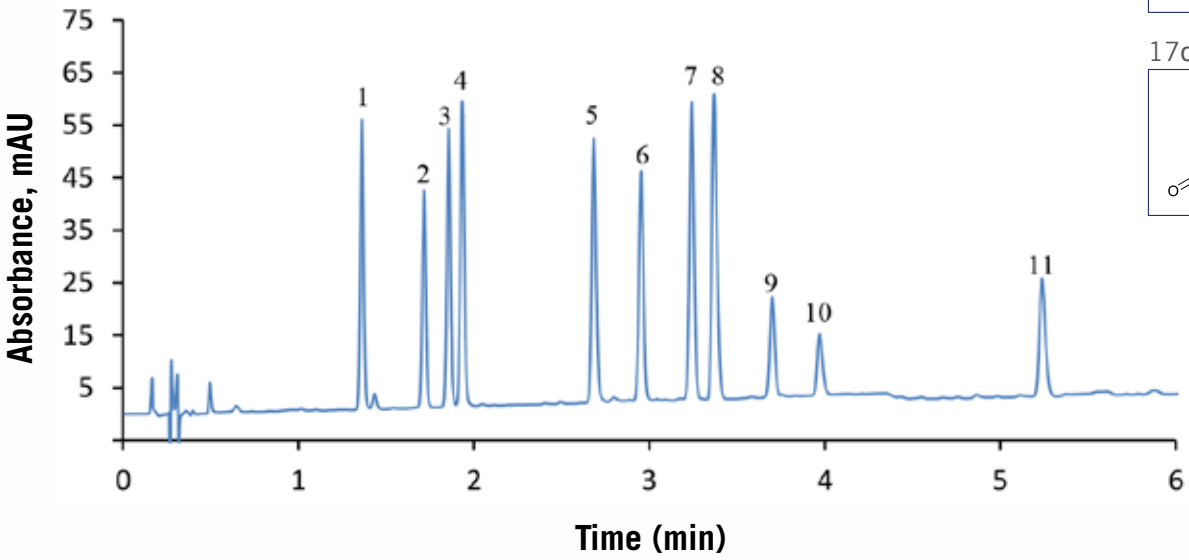


17α-Hydroxyprogesterone



Testosterone





ANABOLIC STEROIDS FROM HORSE URINE BY LC-MS/MS

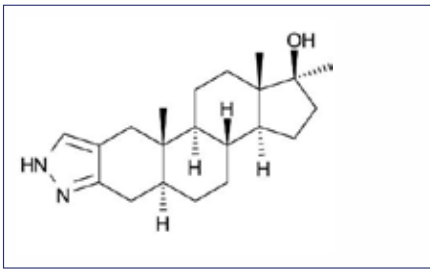
TEST CONDITIONS

Column: ACE Excel 2 µm C18
Dimensions: 50 x 2.1 mm
Part Number: EXL-101-0502U
Mobile Phase: A: 2 mM ammonium acetate, 0.1% formic acid in H_2O
B: 2 mM ammonium acetate, 0.1% formic acid in MeOH

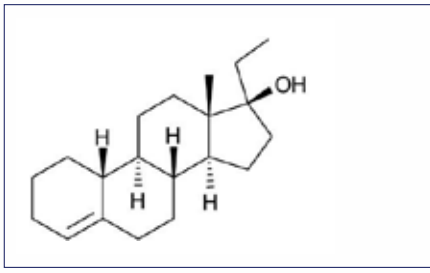
Time (mins)	%B
0.00	75
0.25	75
1.50	90
1.51	100
3.50	100
3.51	75
4.00	75

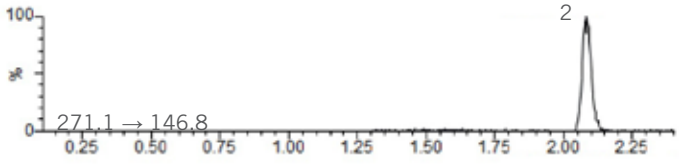
Flow Rate: 0.4 mL/min
Injection: 10 µL
Temperature: 40 °C
Detection: Premier XE triple quad MS
MRM positive ion mode
Desolvation Temperature: 450 °C
Ion Source Temperature: 120 °C

1. Stanozolol
(m/z 329.0 → 94.8)
(m/z 329.0 → 106.8)

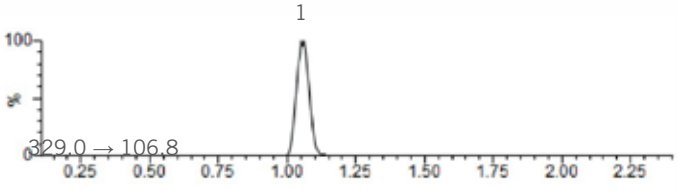


2. Ethylestrenol
(m/z 271.1 → 120.8)
(m/z 271.1 → 146.8)

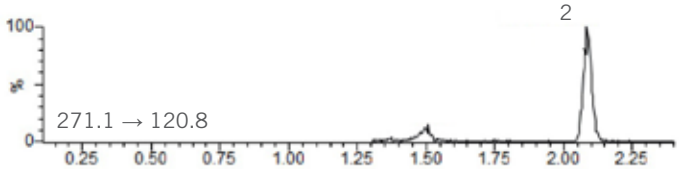




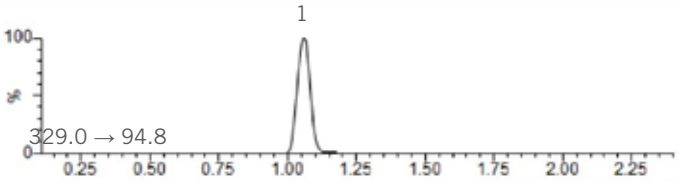
Time (min)



Time (min)



Time (min)



Time (min)

CREATINE & METABOLITE CREATININE USING HILIC MODE

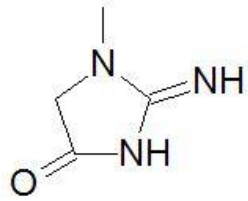
TEST CONDITIONS

Column: ACE 5 µm HILIC-A
Dimensions: 150 x 4.6 mm
Part Number: HILA-5-1546U
Mobile Phase: A: 2 mM ammonium formate pH 3.0 in MeCN/H_2O (95:5 v/v)
B: 2 mM ammonium formate pH 3.0 in MeCN/H_2O (50:50 v/v)

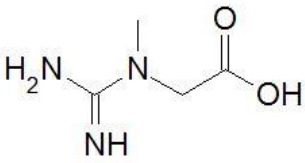
Time (mins)	%B
0	5
10	5
11	55
12	55
31	5

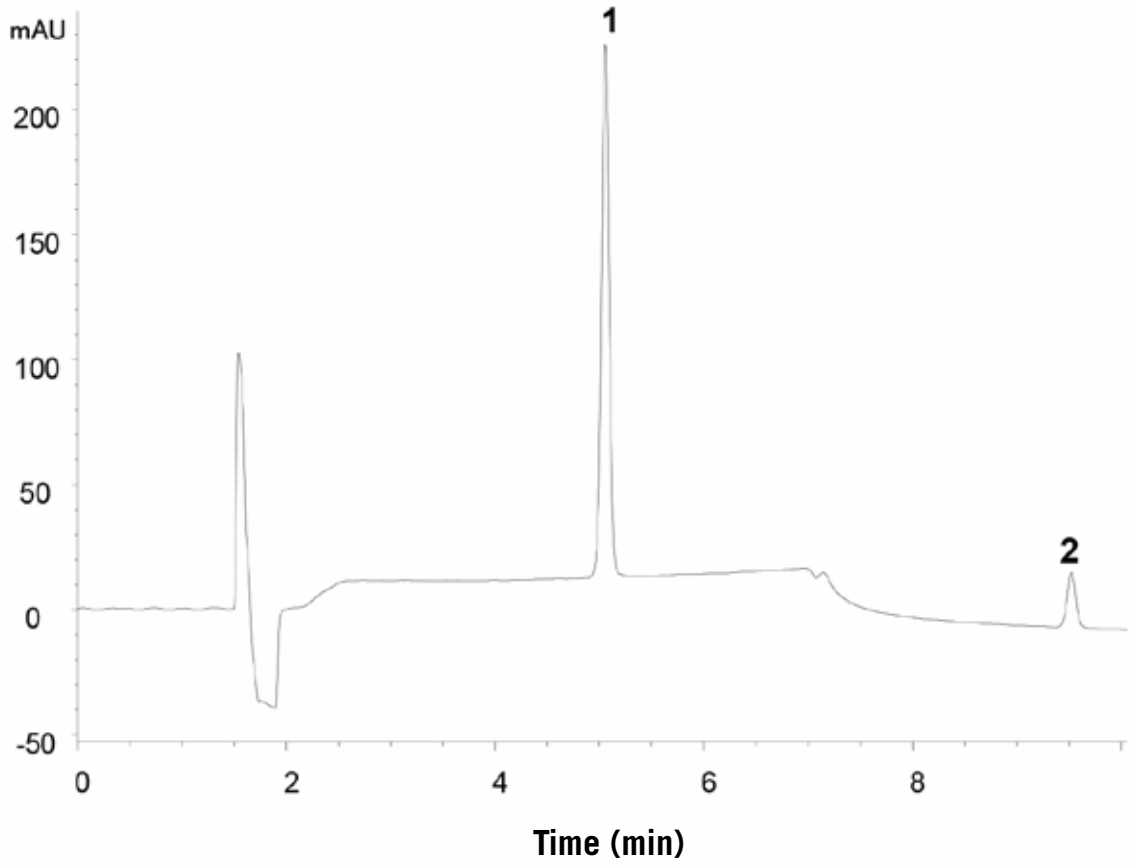
Flow Rate: 1.5 mL/min
Injection: 5 µL
Temperature: 25 °C
Detection: UV, 230 nm

1. Creatinine



2. Creatine





LC-MS ANALYSIS OF ETHYL GLUCURONIDE (ETG) & ETHYL SULFATE (ETS)

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are metabolites of ethanol that are found in urine. The presence of these can be used to determine if an alcoholic beverage was ingested. Zero tolerance programs often use this test.

TEST CONDITIONS

Column: HALO 90 Å Penta-HILIC, 2 µm, 2.1 x 100 mm
Part Number: 91812-605
Mobile Phase: A: 5 mM ammonium formate/0.1% formic acid in 95:5 ACN/water
B: 5 mM ammonium formate/0.1% formic acid in 80:20 ACN/water

Gradient:

Time	%B
0.00	0
1.00	100
5.00	100
5.01	0
7.00	END

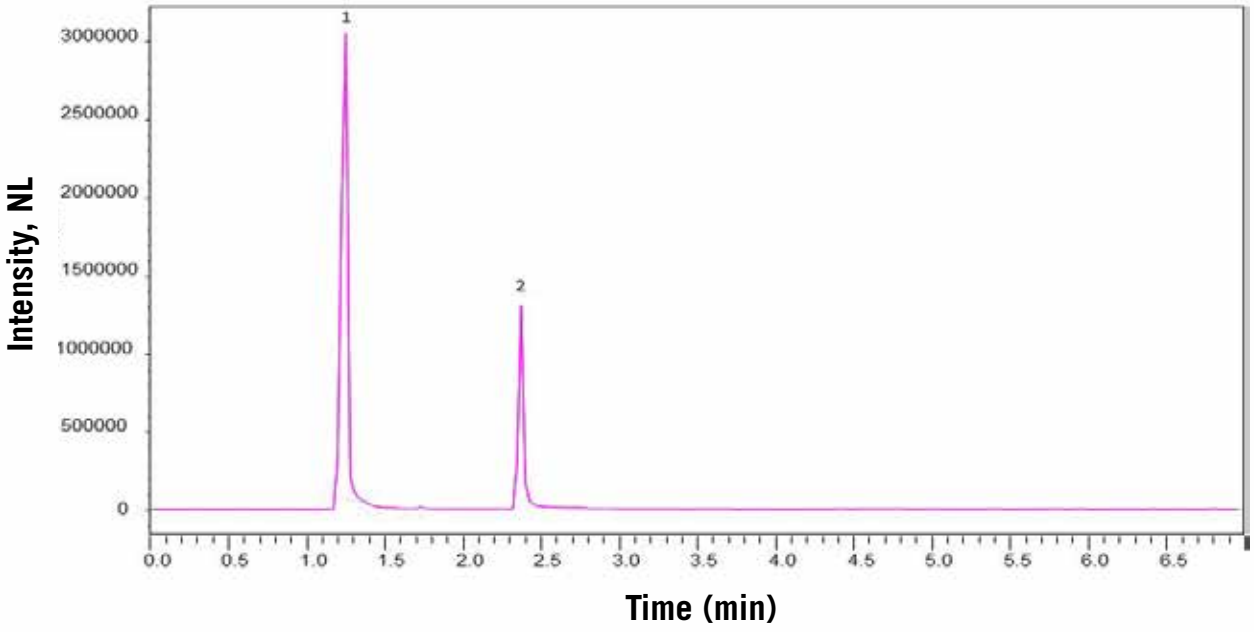
Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Injection Volume: 2 µL
Sample prep: 5ng/mL EtG/EtS in 20 uL of synthetic urine
10 fold dilution with mobile phase A

PEAK IDENTITIES

1. EtS (MH^-=125.120 g/mol)
2. EtG (MH^-=221.193 g/mol)

MS CONDITIONS

LCMS system: Shimadzu LCMS-2020
Detection: -ESI MS
Spray Voltage: 4.50 kV
Drying Line Temp: 300 °C
Heat Block: 450 °C



PHOSPHATIDYLETHANOL BIOMARKER ANALYSIS

Phosphatidylethanol (PEth) measurement in blood is used as a biomarker of chronic alcohol use/abuse.

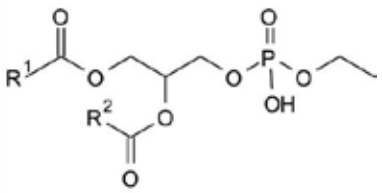
TEST CONDITIONS

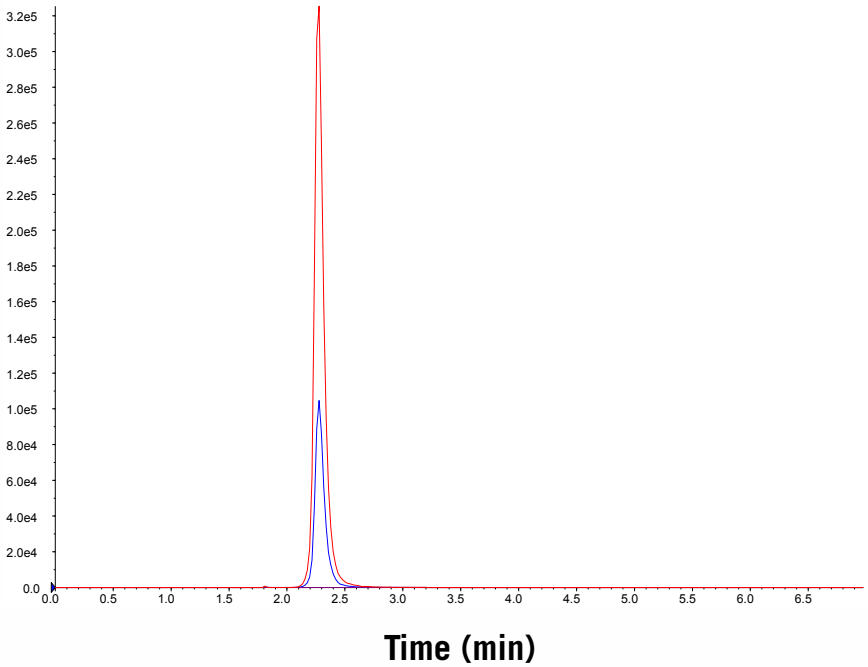
Column: ACE Excel 2 µm C4
Dimensions: 100 x 2.1 mm
Part Number: EXL-103-1002U
Mobile Phase: A: 2 mM ammonium acetate/MeCN (20:80 v/v)
B: IPA

Time (mins)	%B
0.00	10
1.00	10
3.00	60
3.01	100
5.00	100
5.10	10

Flow Rate: 0.4 mL/min
Injection: 5 µL
Temperature: 40 °C
Detection: AB SCIEX triple quad 5500
Turbo IonSpray negative mode ESI
IonSpray Voltage: -4500 V
Temperature: 650 °C

Phosphatidylethanol





1. R1/R2 = 18:1/18:1
(m/z 701.4 → 281.2)

2. R1/R2 = 16:1/16:1
(m/z 701.4 → 255.1)

ALCOHOL BIOMARKERS BY UHPLC-MS/MS

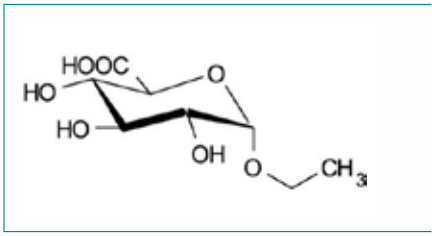
TEST CONDITIONS

Column:	ACE Excel 1.7 µm C18
Dimensions:	100 x 2.1 mm
Part Number:	EXL-171-1002U
Mobile Phase:	A: 1 mM ammonium fluoride
	B: MeCN

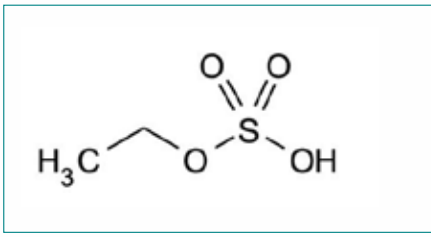
Time (mins)	%B
0.0	0
0.5	20
1.5	20
2.0	100
4.0	100
4.5	0

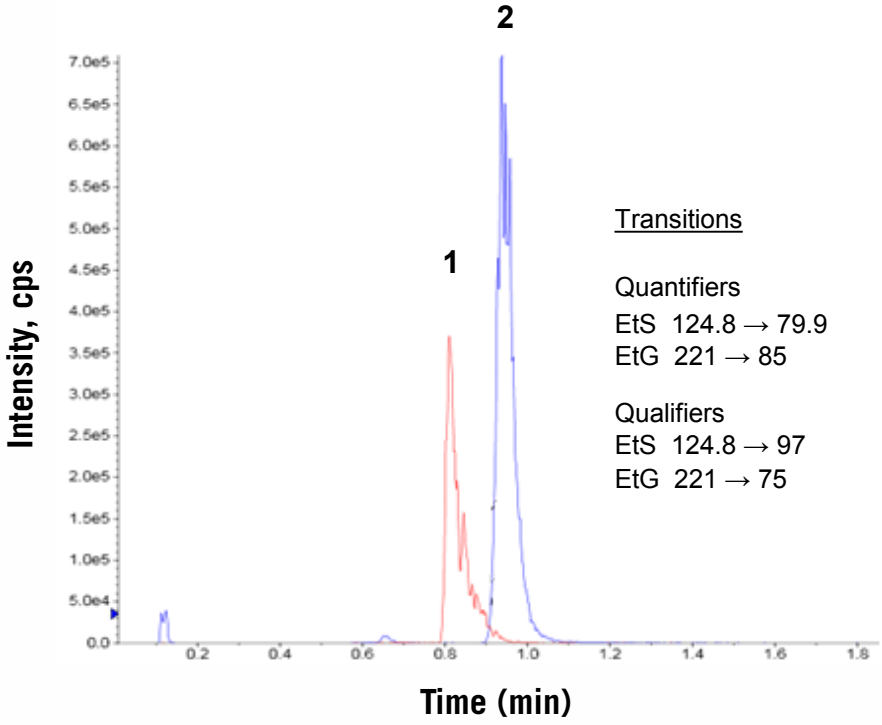
Flow Rate:	0.4 mL/min
Injection:	1 µL
Temperature:	40 °C
Detection:	AB SCIEX triple quad 5500
	Negative ESI MRM
	Source Temperature: 750 °C
	IonSpray Voltage: -4500 V

1. Ethyl glucuronide (EtG)



2. Ethyl sulfate (EtS)





Transitions

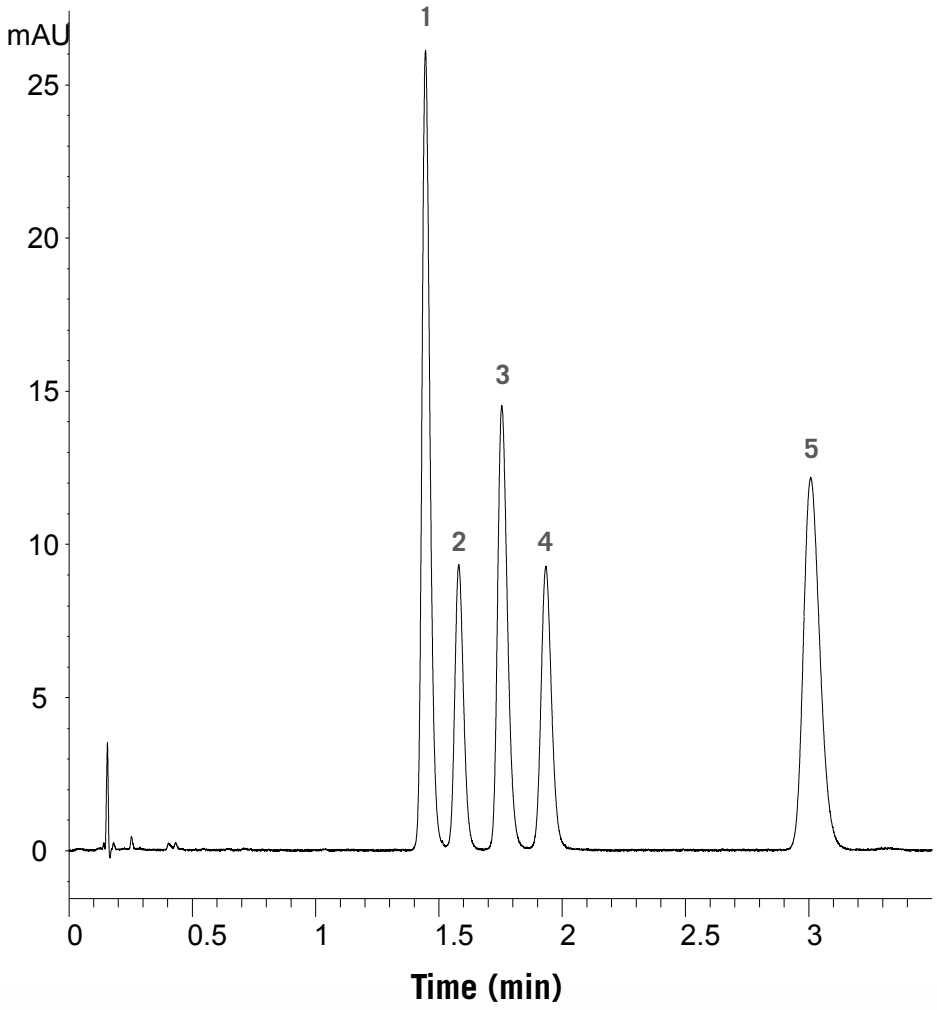
Quantifiers
EtS 124.8 → 79.9
EtG 221 → 85

Qualifiers
EtS 124.8 → 97
EtG 221 → 75

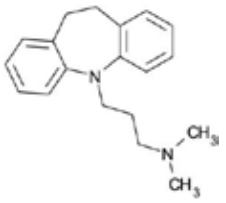
UHPLC ANALYSIS OF TRICYCLIC ANTIPRESSANTS

TEST CONDITIONS

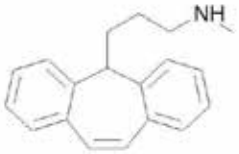
Column:	ACE Excel 1.7 µm SuperC18
Dimensions:	50 x 3.0 mm
Part Number:	EXL-1711-0503U
Mobile Phase:	20 mM ammonium formate pH 3.0 in MeOH:H_2O 45:55 v/v
Flow Rate:	1.25 mL/min
Injection:	0.7 µL
Temperature:	60 °C
Detection:	UV, 260 nm
Instrument:	Chromaster UltraRs



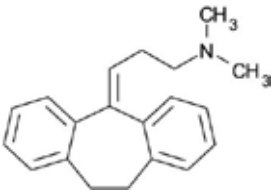
1. Imipramine



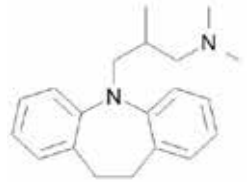
2. Protriptyline



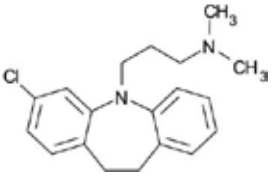
3. Amitriptyline



4. Trimipramine



5. Clomipramine



ANTIDEPRESSANTS

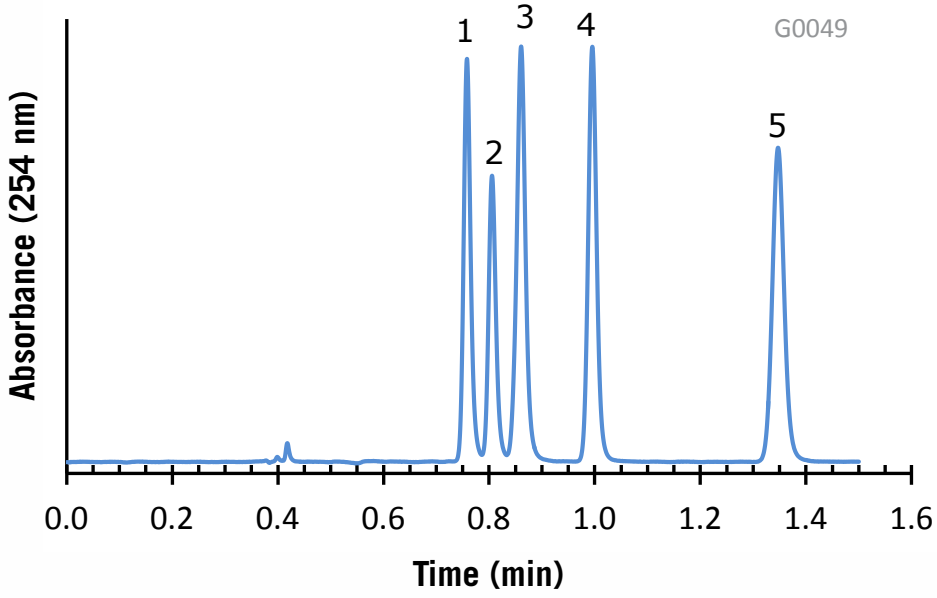
Basic drugs such as antidepressants can be separated rapidly under HILIC conditions with good peak shape using HALO Penta-HILIC stationary phase.

TEST CONDITIONS

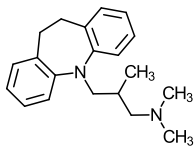
Column:	HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number:	92814-605
Mobile Phase:	7/93: A/B
	A: 0.1 mM ammonium formate, pH=3.5 (adj.)
	B: Acetonitrile
Flow Rate:	2.5 mL/min
Pressure:	165 bar
Temperature:	30 °C
Detection:	UV 254 nm, VWD
Injection Volume:	0.5 µL
Sample Solvent:	10/90: Water/Acetonitrile
Response Time:	0.02 sec
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
ECV:	~14 µL

PEAK IDENTITIES

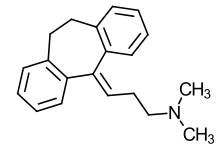
1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine



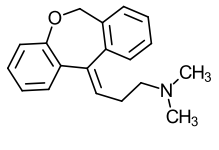
Trimipramine



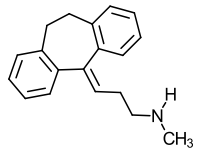
Amitriptyline



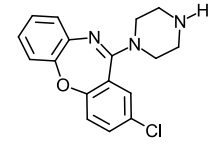
Doxepin



Nortriptyline



Amoxapine



AMINE MEDICATIONS

A mixture of amines including antihistamines, decongestants, and other medications is separated on a HALO 5 µm C18 column. The column shows excellent peak shapes for basic compounds using an ammonium formate buffer at low pH.

TEST CONDITIONS

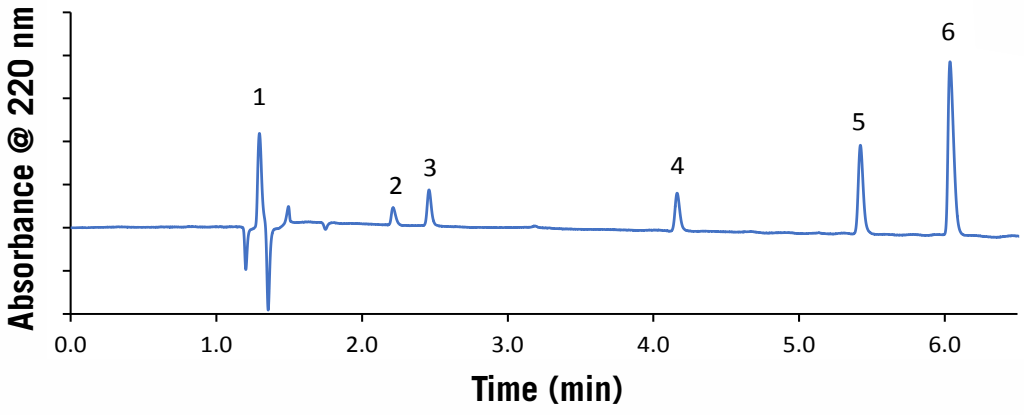
Column:	HALO 90 Å C18, 5 µm, 4.6 x 150 mm
Part Number:	95814-702
Mobile Phase:	A: 50 mM ammonium formate/0.1% formic acid
	B: 50/50 MeOH:Acetonitrile/0.1% formic acid

Gradient:	Time (min.)	%B
	0.0	20
	6.5	60

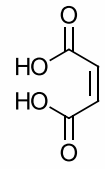
Flow Rate:	1.0 mL/min
Initial Back Pressure:	190 bar
Temperature:	30 °C
Detection:	220 nm, PDA
Injection Volume:	3 µL
Sample Solvent:	80/20 Mobile Phase A/B
Data Rate:	40 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

PEAK IDENTITIES

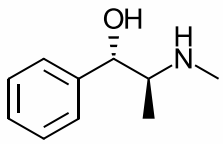
1. Maleic Acid
2. Pseudoephedrine
3. Scopolamine
4. Doxylamine
5. Chlorpheniramine
6. Diphenhydramine



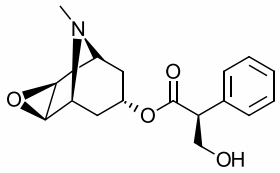
Maleic Acid



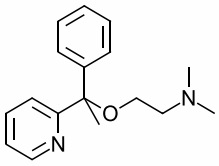
Pseudoephedrine



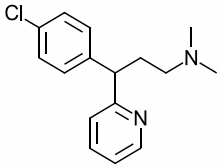
Scopolamine



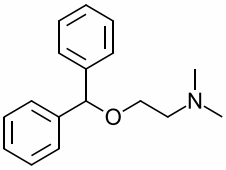
Doxylamine



Chlorpheniramine



Diphenhydramine



NICOTINE & METABOLITES

TEST CONDITIONS

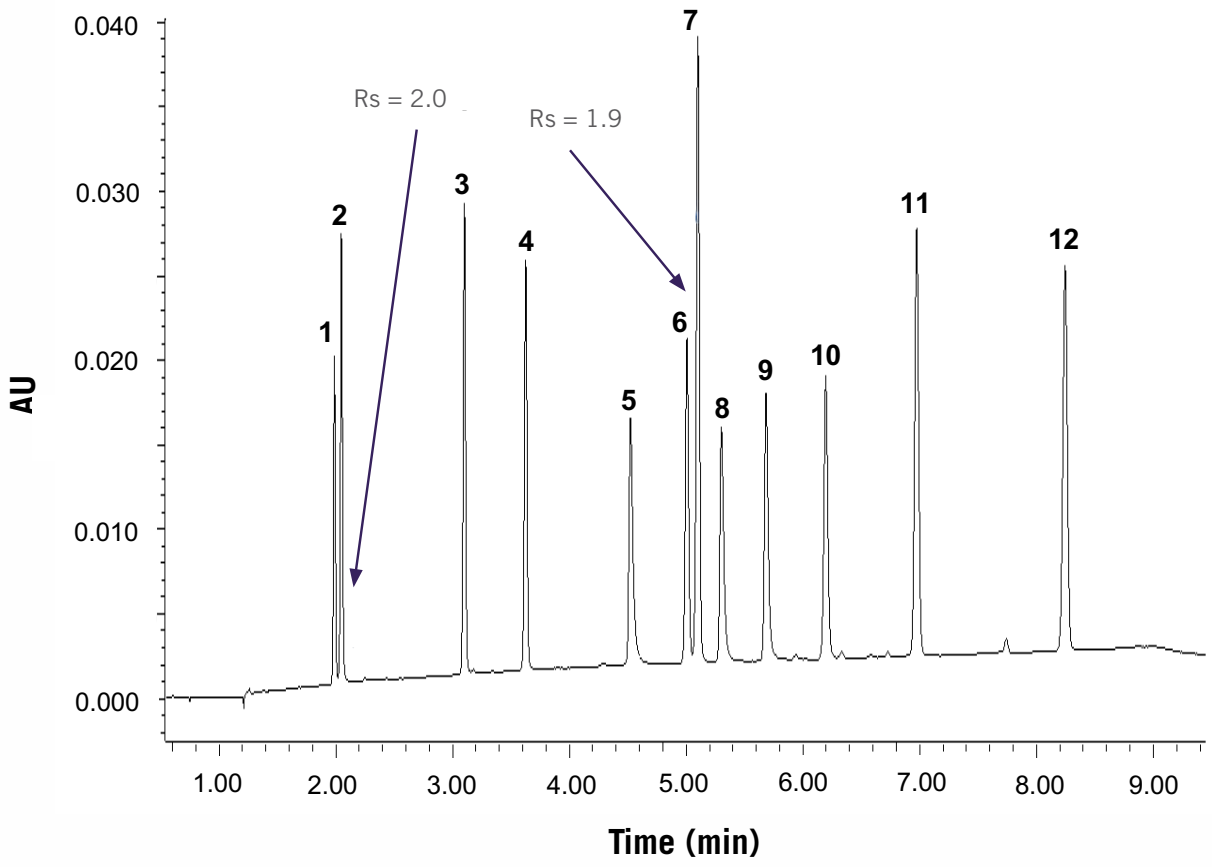
Column:	ACE Excel 2 µm SuperC18
Dimensions:	100 x 2.1 mm
Part Number:	EXL-1011-1002U
Mobile Phase:	A: 10 mM ammonium acetate, pH 10
	B: MeCN

Time (mins)	%B
0.0	0
8.5	34
10.0	0
11.5	0

Flow Rate:	0.6 mL/min
Injection:	5 µL
Temperature:	30 °C
Detection:	UV, 260 nm
Sample:	Nicotine and related substances

PEAK IDENTITIES

1. Nicotine-cis-N-oxide
2. Nicotine-trans-N-oxide
3. Norcotinine
4. Cotinine
5. Nornicotine
6. Anatabine
7. Myosmine
8. MNP
9. Anabasine
10. Nicotine
11. ß nornicotyrine
12. ß nicotyrine



SNAKE VENOM FROM CROTALUS DURISSUS TERRIFICUS

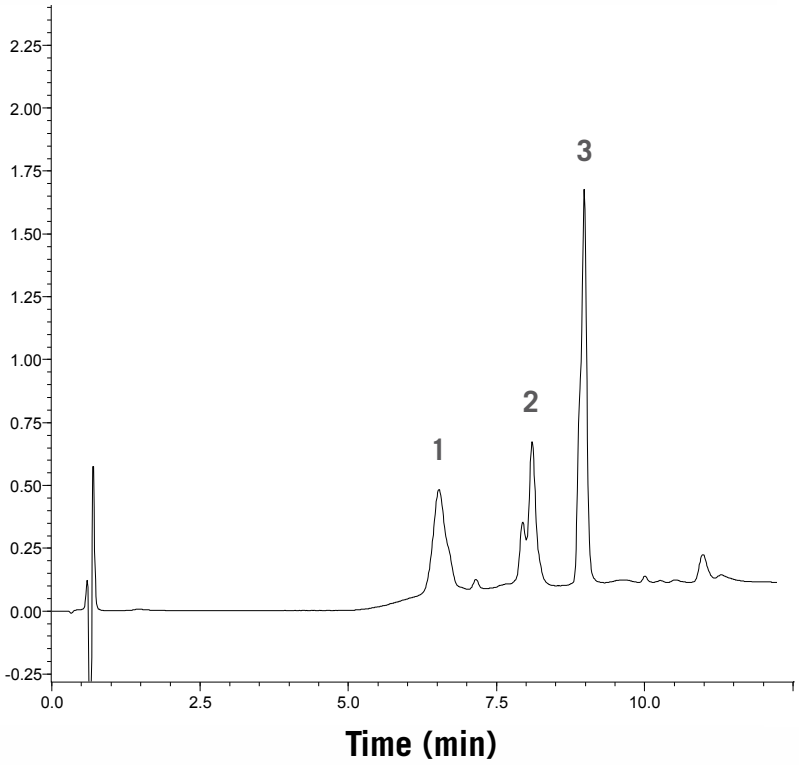
TEST CONDITIONS

Column:	ACE 3 µm C8-300
Dimensions:	50 x 4.6 mm
Part Number:	ACE-212-0546
Mobile Phase:	A: 0.1% TFA in H_2O
	B: 0.1% TFA in MeCN/H_2O (90:10 v/v)

Time (mins)	%B
0.0	20
2.0	20
27.0	70

Flow Rate:	1.2 mL/min
Injection:	20 µL
Detection:	UV, 214 nm
Sample:	Lyophilised crude venom powder was solubilised (1 mg/mL) in 0.1% TFA. Resulting solutions were centrifuged and the supernatant used for analyses.

Crotamine is a 42 residue long cationic polypeptide containing 11 basic residues (9 lysines, 2 arginines) and 6 cysteines



PEAK IDENTITIES

1. Crotamine
2. Crotapotin
3. Phospholipase A2 (PLA_2)

SEPARATION OF 17 EXPLOSIVES

The determination of explosives in the environment is outlined in EPA method 8330B and, under the conditions recommended, requires two column phases to determine 17 compounds. However, all 17 explosive compounds can be separated on a HALO C18, 2.7 µm column in less than 20 minutes using a water/methanol gradient.

TEST CONDITIONS

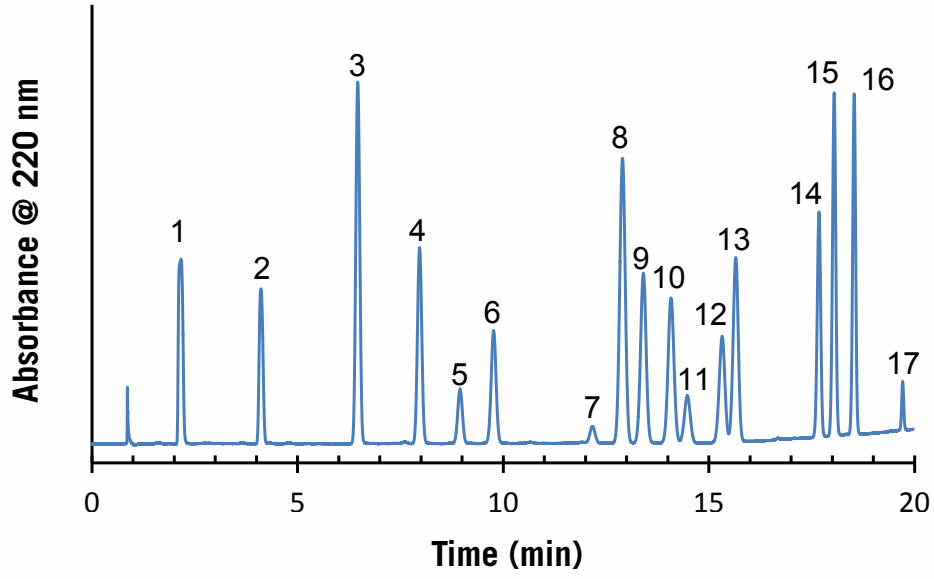
Column:	HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number:	92814-702
Mobile Phase:	A: Water
	B: Methanol

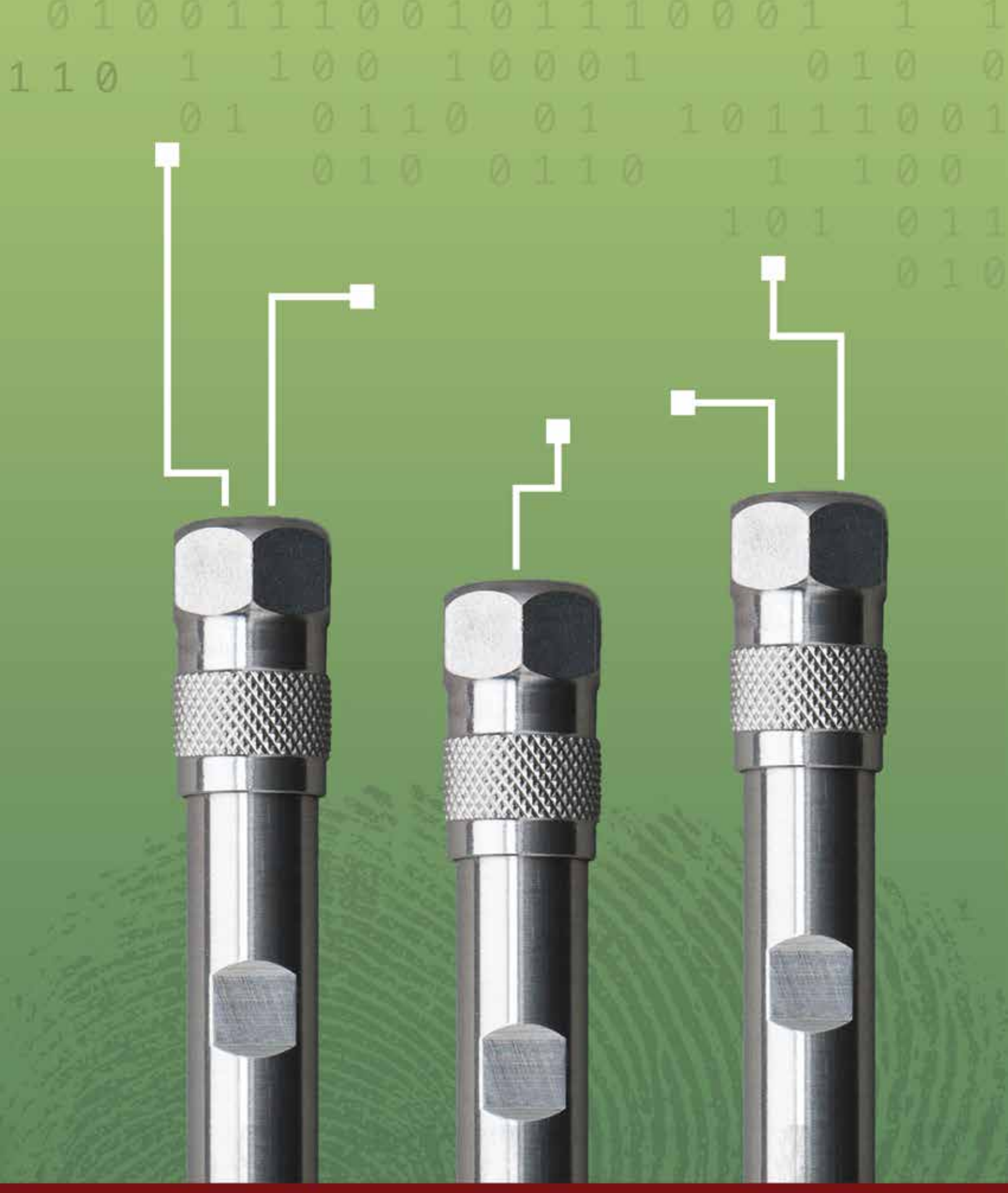
Time (mins)	%B
0.0	25
14.0	35
20.0	62

Flow Rate:	1.5 mL/min
Pressure:	366 bar to start, maximum: 405 bar
Temperature:	43 °C
Detection:	UV 220 nm, VWD
Injection Volume:	40 µL
Sample Solvent:	50/50: water/methanol
Response Time:	0.02 sec
Data rate:	25 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
ECV:	~14 µL

PEAK IDENTITIES

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. 3,5-Dinitroaniline
6. Nitrobenzene
7. Nitroglycerin
8. Tetryl
9. 2,4,6-Trinitrotoluene
10. 2-Amino-4,6-Dinitrotoluene
11. 4-Amino-2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2,6-Dinitrotoluene
14. 2-Nitrotoluene
15. 4-Nitrotoluene
16. 3-Nitrotoluene
17. PETN (pentaerythritol tetranitrate)





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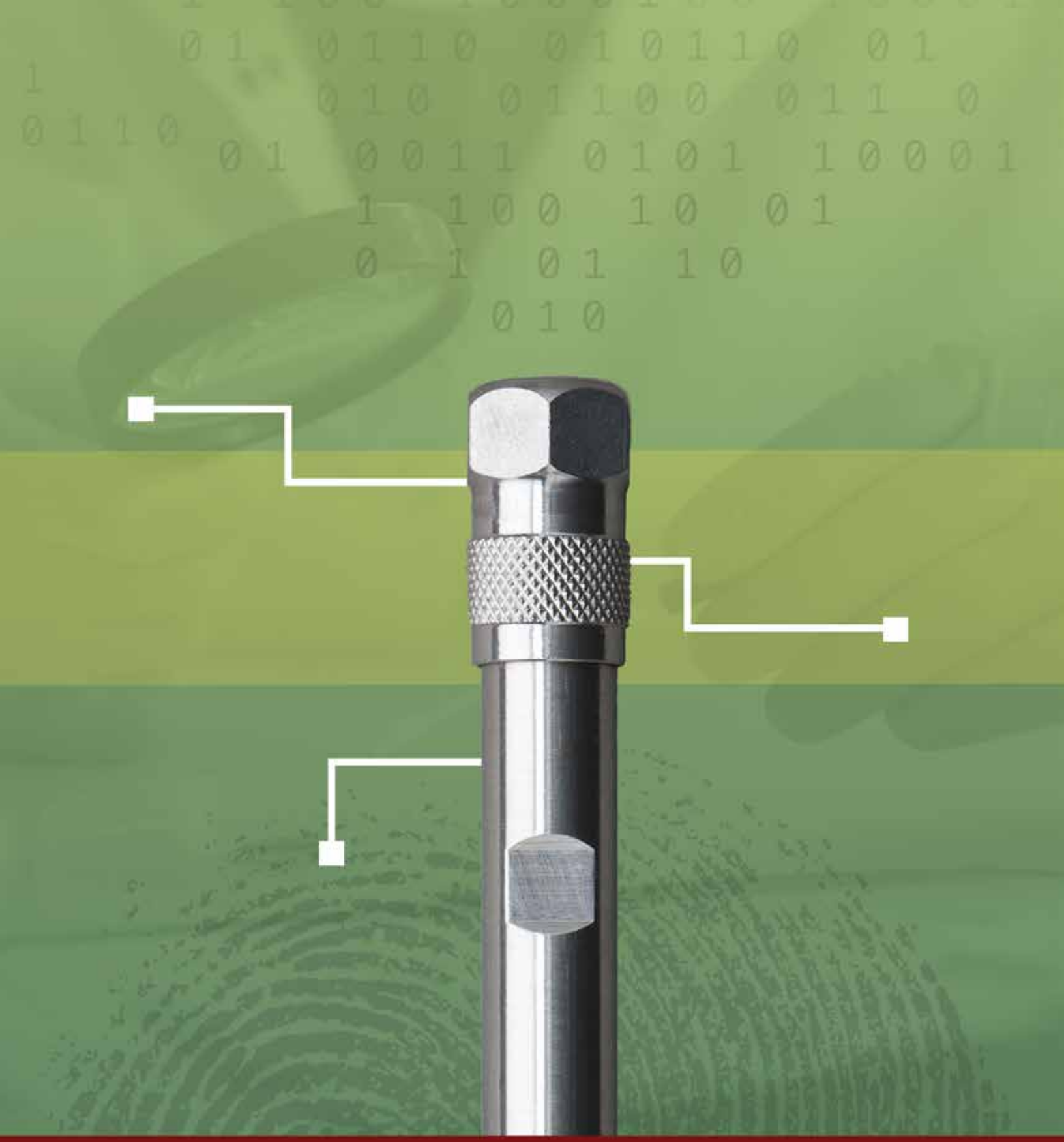

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