Simple and Highly Efficient Drugs of Abuse Testing Methods by SPE and LC/MS/MS

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Introduction

New SAMHSA guidelines (October 2010) allow LC/MS/MS methods to be used for confirmation of initial drug tests by government-certified (NLCP) workplace drug testing labs. SAMHSA requires to analyze 6 classes of drugs and metabolites in urine: 1) 6-acetylmorphine; 2) phencyclidine; 3) benzoylecgonine; 4) a group of 5 amphetamines; 5) morphine and codeine, and 6) 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. We present simplified LC/MS/MS confirmation methods, with sample preparation, for all SAMHSA-required categories using the latest instruments and separation tools from Agilent Technologies, including Bond Elut Plexa PCX mixed mode polymeric SPE cartridges and a Poroshell 120 column.

Without elaborate sample pretreatment, solid phase extraction on Plexa PCX provides high extraction efficiency and reduced ion suppression due to a combination of a strong cation-exchange functionality, an amide-free polymer surface and a hydrophilic pore gradient.

The LC method on a superficially porous column provides excellent separation and short retention times; with max pressure < 400 bar, these methods do not require high pressure UHPLC systems.

As required by SAMHSA and NLCP, our MS/MS methods use qualifier ions and are linear across a wide dynamic range.

SAMHSA analytes and cutoffs

Target analyte	Confirmation cutoff, ng/mL	Limit of Detection, ng/mL
Amphetamines		
Amphetamine	250	25
Methamphetamine	250	25
MDA	250	25
MDMA	250	25
MDEA	250	25
Benzoylecgonine (BE)	100	10
11-nor-9-carboxy-Δ^9-THC	15	1.5
Phencyclidine (PCP)	25	2.5
Opiates		
Morphine	2000	200
Codeine	2000	200
6-Acetylmorphine (6-MAM)	10	1

Materials and instrumentation

- Agilent Bond Elut Plexa PCX 30 mg 3 mL cartridges
- Agilent Poroshell 120 EC-C18, 3 x 50 mm, 2.7 µm column
- Agilent 1260 LC
- Agilent 6460 MS with Jet Stream electrospray source

Experimental

	BE	PCP	6-AM	Amphetamines	Opiates	THCA
Sample	1 mL urine spiked with ISTD	1 mL urine spiked with ISTD	1 mL urine spiked with ISTD	0.5 mL urine spiked with ISTD	0.5 mL urine spiked with ISTD	0.5 mL urine spiked with ISTD
Pre-treatment	1 mL 2% formic acid	1 mL 2% formic acid	1 mL 2% formic acid	1 mL 2% formic acid	Add 125 µL conc. HCl Incubate 90 min at 95±5 °C, cool and add 2 mL 0.1 M sodium acetate buffer (pH 4.5). Add 250 µL 7 N KOH Adjust pH <6 Centrifuge	Add 100 µL 7 KOH Incubate 30 min at 60±5 °C, cool and add 125 µL methanol Add 100 µL glacial acetic acid and 1.5 mL 0.2 M sodium acetate buffer (pH 4). Centrifuge
Condition -ing	0.5 mL methanol	0.5 mL methanol	0.5 mL methanol	0.5 mL methanol	0.5 mL methanol	0.5 mL methanol
Wash 1	1 mL 2% formic acid	1 mL 2% formic acid	1 mL 2% formic acid	1 mL 2% formic acid	1 mL 2% formic acid	2 x 2 mL ACN:2% acetic acid (10:90)
Wash 2	1 mL methanol	1 mL methanol	1 mL methanol	1 mL methanol	1 mL methanol	2 mL ACN:2% acetic acid (30:70)
Drying	10 min	10 min	10 min	10 min	10 min	10 min
Rinse						Rinse with 0.2 mL hexane, Pull with low vacuum, dry 3 min
Elution	1 mL MeOH:NH_4OH (100:20)	1 mL Ethyl Acetate:MeOH: NH4OH (80:20:5)	1 mL MeOH:NH4OH (100:10)	1 mL ethyl acetate:MeOH:NH_4OH (50:50:20)	2 mL MeOH:NH_4OH(100:20) Soak with eluent - let eluate drip - apply low vacuum	1.5mL ethyl acetate:IPA (80:20) in 2 steps (0.5+1.0 mL). At each step soak with eluent - let eluate drip - apply low vacuum
Evapora-tion & Reconsti-tution	Evaporate to dryness at <40 °C. Reconstitute in 1 mL initial mobile phase	Evaporate to dryness at <40 °C. Reconstitute in 1mL initial mobile phase	Evaporate to dryness at <40 °C. Reconstitute in 1mL initial mobile phase	Evaporate to 0.2 mL at 37 °C; add 100 µL 0.025 N HCl in MeOH, vortex; evaporate to dryness. Reconstitute in 0.5 mL initial mobile phase	Evaporate to dryness at <40°C. Reconstitute in 0.5 mL initial mobile phase	Evaporate to dryness at <40 °C. Reconstitute in 0.5 mL initial mobile phase

	BE, PCP	6-AM	Amphetamines	Opiates	THCA
Gradient	0 min 10%B 0.5 min 10%B 2.5 min 70%B 2.51 min 90%B	0 min 10%B 1.5 min 25%B 2.0 min 60%B 2.1 min 90%B	0 min 15%B 1.5 min 15%B 3.5 min 30%B 3.6 min 90%B	0-0.5 min 5%B 1.5 min 25%B 2.5 min 55%B 2.6 min 90%B	0 min 30%B 1.0 min 95%B 2.5 min 95%B
Mobile phase	A – 0.1% formic acid in water; B - 0.1% formic acid in methanol				A - 5 mM ammonium formate, B - methanol
Injection volume	2 µL 2 µL	10 µL	2 µL	2 µL	10 µL

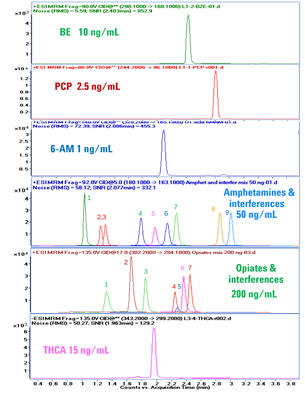
ES Source	BE, PCP	6-AM	Amphetamines	Opiates	THCA
Ionization mode	positive	positive	positive	positive	negative
Capillary voltage	3000 V	2800 V	4000 V	3000 V	3000 V
Drying gas flow	10 L/min	13 L/min	10 L/min	10 L/min	11 L/min
Drying gas temp.	350 °C	350 °C	350 °C	350 °C	350 °C
Nebulizer gas	35 psi	35 psi	35 psi	35 psi	35 psi
Sheath gas flow	12 L/min	12 L/min	12 L/min	12 L/min	12 L/min
Sheath gas temp.	400 °C	400 °C	400 °C	400 °C	320 °C
MS Delta EMV	200 V	200 V	200 V	0	800 V

Experimental

Compound	Parent	Product	Fragmentor	Collision energy
Benzoylecgonine	290.1	168.1	90	15
Benzoylecgonine	290.1	105.1	90	30
Benzoylecgonine-D_3	298.2	171.1	90	15
Benzoylecgonine-D_8	298.2	110.1	90	30
Phencyclidine	244.2	86.1	80	7
Phencyclidine	244.2	159.1	80	7
Phencyclidine-D_5	249.2	86.1	80	7
Phencyclidine-D_5	249.2	164.1	80	7
6-Acetylmorphine	328.2	165.1	140	40
6-Acetylmorphine	328.2	211.1	140	25
6-Acetylmorphine-D_6	334.2	165.1	140	40
6-Acetylmorphine-D_6	334.2	211.1	140	25
Amphetamine	136.1	91.1	64	14
Amphetamine	136.1	119.1	64	4
Amphetamine-D_5	142.1	93.1	66	5
Amphetamine-D_5	142.1	125.1	66	5
MDA	180.1	163.1	92	13
MDA	180.1	105.1	92	17
MDA-D_5	185.1	168.1	68	5
MDA-D_5	185.1	110.1	68	21
MDEA	208.1	163.1	88	8
MDEA	208.1	133.1	88	17
MDEA-D_6	214.2	166.1	90	8
MDEA-D_6	214.2	108.1	90	25
MDMA	194.1	163.1	84	5
MDMA	194.1	135.1	84	17
MDMA-D_5	199.1	165.1	82	4
MDMA-D_5	199.1	107.1	82	25
Methamphetamine	150.1	91.1	80	16
Methamphetamine	150.1	119.1	80	4
Methamphetamine-D_5	159.2	93.1	77	13
Methamphetamine-D_5	159.2	125.1	77	5
Ephedrine-Pseudoephedrine	166.1	133.1	80	21
Phentermine	150.1	133.1	80	6
Phenylpropanolamine	152.1	117.1	80	20
Codeine	300.2	165.1	130	46
Codeine	300.2	215.1	130	23
Codeine-D_6	306.2	165.1	130	44
Codeine-D_6	306.2	218.1	130	23
Morphine	286.1	165.1	130	43
Morphine	286.1	201.1	130	23
Morphine-D_3	292.1	181.1	130	40
Morphine-D_6	292.1	165.1	130	42
Oxycodone	316.2	298.1	130	15
Oxymorphone	302.2	284.1	130	17
Hydrocodone	300.2	199.1	130	30
Norcodeine	286.1	225.1	130	20
Hydromorphone	286.1	185.1	130	28
11-nor-9-carboxy-Δ^9-THC	343.2	299.2	135	18
11-nor-9-carboxy-Δ^9THC	343.2	245.1	135	30
11-nor-9-carboxy-Δ^9THC-D_3	352.2	308.2	145	18
11-nor-9-carboxy-Δ^9THC-D_3	352.2	254.2	145	30

Results and Discussion

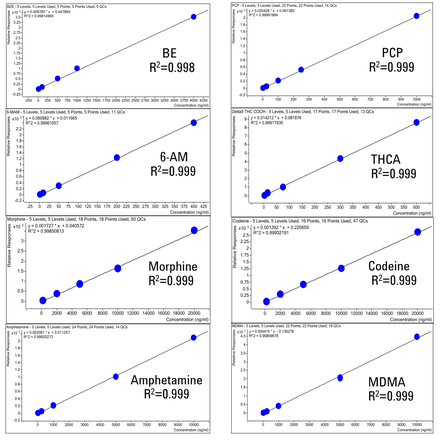
Chromatograms, Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm



Amphetamines and interferences peaks:
1. Phenylpropanolamine 2. Ephedrine 3. Pseudoephedrine
4. Amphetamine 5. Methamphetamine 6. MDA 7. MDMA 8. MDEA
9. Phentermine

Opiates and interferences peaks:
1. Morphine 2. Oxymorphone 3. Hydromorphone 4. Codeine 5. Nor-Codeine (small blue peak) 6. Oxycodone 7. Hydrocodone

Examples of Calibration Curves



Method evaluation, n=5

Compound	Process efficiency * %	Extraction recovery * %	Matrix effect * %	Accuracy* %	Precision* (CV), %
Benzoylecgonine	85	86	99	103**	0.8**
Phencyclidine	83	85	98	93	0.5
6-Acetylmorphine	83	83	100	106**	0.6**
Amphetamine	86	94	91	107**	0.6**
Methamphetamine	93	94	99	105	0.5
MDA	91	95	95	92**	1.1**
MDMA	93	97	96	101**	0.5**
MDEA	95	96	98	106**	0.3**
Morphine	83	85	98	108	0.6
Codeine	85	86	99	108	0.7
11-nor-9-carboxy-Δ^9-THC	73	65	113	98	2.2

Measured according to the method of Matuszewki et al. *Anal. Chem.* **75**: 3019-3030 (2003)

All values except those denoted by * are obtained for the cutoff level

* *Values obtained for 40% cutoff

Conclusions

- Presented methods meet SAMHSA requirements for LOD, linearity, accuracy and precision, and LC separation from possible interferences. Method accuracy is within 10% of target and precision (CV) is within 2.2%.
- High process efficiencies, extraction recoveries and insignificant matrix effects demonstrate excellent quality of Agilent Bond Elut Plexa PCX.
- Extraction methods are simplified to 3 types of pretreatment (simple formic acid pretreatment for four drug classes, acid hydrolysis for opiates and base hydrolysis for THCA) and to combinations of only three organic eluents.
- All LC separations use the same Agilent Poroshell120 EC-C18 column and utilize low backpressure <400 bar, simple mobile phases and no column heating. The longest separation (amphetamines) is completed within 3.2 minutes.
- MS methods use low sample injection volume (2 – 10 µL), no sample pre-concentration, and demonstrate excellent S/N ratio for the lowest SAMHSA-required concentrations due to the enhanced sensitivity of Agilent 6460 QQQ with Jet Stream electrospray source.