Screening Method for Acidic, Neutral and Basic Drug Analytes in Oral Fluid by LC-MS/MS Using CLEAN SCREEN® XCEL I

UCT Part Numbers

CSXCE106
Clean Screen® XCEL I
130mg / 6mL SPE Cartridge

SPHPHO6001-5
Select pH Buffer
100 mM Phosphate pH 6.0

SLDA50ID21-5UM
Selectra® DA LC column
50 x 2.1 mm, 5 µm

SLDAGDC21-5UM
Selectra® DA guard column
10 x 2.1 mm, 5µm

SLGRDHLDR
Guard Column Holder

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Summary:

When compared to urine and hair drug tests, oral fluid is best at detecting recent drug use. Drugs take time to metabolize and pass through the system in a urine test, and the same drugs are incorporated as hair grows and it takes time for the drug to be present in the hair above the scalp. But an oral fluid test will often detect drugs in a donor's system immediately after use. This makes oral fluid testing ideal for a broad range of situations ranging from pre-employment, to reasonable suspicion, to post-accident testing where the employer is interested in assessing what's in the donor's system at the time of the drug test collection.

Oral fluid levels largely correlate with the amount of drug in the blood (dependent on the saliva/plasma ratio for each drug). Higher drug and drug metabolite levels are found in urine because they are concentrated by the kidneys during the excretion process. The SAMHSA cut-off levels for oral fluid are very much lower than those for urine and hence more sensitive screening and confirmatory assays are required for oral fluid analysis often times with the incorporation of a concentration step to enhance sensitivity.

The below Clean Screen® XCEL I method can be utilized for both universal screening and confirmatory purposes when extracting various drugs from oral fluid prior to analysis by LC-MS/MS. By evaporating to completion, final extracts are purified and highly concentrated to achieve required levels of detection/quantitation.

Sample Pretreatment:

NEAT ORAL FLUID:

Add 100 - 500 µL of neat oral fluid sample to a clean tube.
Add internal standard(s) and let sit for 10 minutes at room temperature.
Add 800 µL of 100 mM phosphate buffer (pH= 6.0).
Mix/vortex for 10 seconds. Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

ORAL FLUID COLLECTION DEVICE:

To 1mL of oral fluid specimen, (diluted in Quantisal™ Buffer), add appropriate internal standards. Mix/vortex for 30 seconds.

SPE Procedure:

1. **Sample Extraction**
   a) Apply the sample directly to the SPE cartridge (if required, use a low vacuum to draw the sample through at ≤3 mL/min).

2. **Wash cartridge**
   a) 1 × 3 mL D.I. H_2O.
   b) 1 × 3 mL 1% HCl Solution.
   c) Dry cartridges for ~10 minutes under a high vacuum.

   Note: A Hexane wash may be added if not looking for parent THC.

3. **Elution**
   a) Elute with 1 × 3 mL MEOH containing 2% Ammonium Hydroxide (MEOH: NH_4OH, 98:2 v/v).
   b) Evaporate the sample to dryness under a gentle stream of nitrogen. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine. A 1% HCl in MEOH solution may be used to prevent volatization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating than continue to dryness.
   c) Reconstitute in 100 µL of Mobile Phase and vortex for 1 minute.
   d) Transfer sample to an autosampler vial containing a low volume insert.

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LC-MS/MS Parameters:

Instrumentation	
HPLC system	Agilent 1200 Binary Pump SL
MS system	API 4000 QTRAP (MS/MS)
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 5 µm (p/n: SLDA50ID21-5UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 5 µm (p/n: SLDAGDC21-5UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	40°C
Flow rate	300 µL/min
Injection volume	10 µL

Mobile Phase Gradient		
Time (min)	**% Mobile Phase A** (0.1% Formic Acid in Water)	**% Mobile Phase B** (0.1% Formic Acid in MEOH)
0.0	90	10
0.5	90	10
4.0	60	40
7.5	15	85
8.5	10	90
8.51	90	10
10.0	STOP	

Results:

ANALYTE	Relative Retention Time (min)	Q1	Q3
Ecgoninemethylester	0.5	200.1	182.1
Phenylpropanolamine	0.9	152.2	134.2
Morphine	1.4	286	152
Oxymorphone	1.5	302	227
Pregabalin	1.5	160.2	97
Pseudoephedrine	1.9	166.1	148.1
Hydromorphone	1.9	286	185
Ephedrine	1.9	166.2	148.3
Amphetamine	2	136.1	91.1
Paracetamol	2	152	110
Gabapentin	2.2	172.1	67.1
3,4-Methylenedioxyamphetamine	2.5	180.1	105
Atropine	2.5	290.2	124.1
Buspirone	2.5	386.2	122.1
Clonidine	2.5	230	213
Metamphetamine	2.5	150.1	91.1
Nicotine	2.5	163.1	132.1
Phenylephrine	2.5	168.1	91.1
Theobromine	2.5	181.1	138
Theophylline	2.5	181.1	124
Mephedrone	2.5	178.2	160.1
Phentermine	2.5	150.2	91.2
6-O-Monoacetylmorphine	2.6	328.1	165.1
Naloxone	2.8	328.2	310.2
Methylone	2.8	208	160.1

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ANALYTE	Relative Retention Time (min)	Q1	Q3
Phenmetrazine	2.8	178.2	115.1
Phendimetrazine	2.8	192.2	147.1
Caffeine	3.0	195.1	122.9
Dihydrocodeine	3	302.2	199.1
Codeine	3	300	152
Desmethyltramadol	3	250.2	58.2
MDMA	3.1	194.1	105.1
7-Aminonitrazepam	3.1	252.1	121.1
Oxycodone D6	3.1	322.3	304.1
Oxycodone	3.2	316.1	298.1
Hydrocodone	3.4	300	199
Diethylpropion	3.4	206.2	100.2
MDEA	3.6	208.1	77.1
Naltrexol	3.6	344.3	308.4
Pheniramine	3.8	241.2	167.2
Olanzapine	4	313.1	256.1
Norketamine	4	224.1	207.1
Methylphenidate	4.1	234.1	84.1
Norfentanyl	4.1	233.2	84.1
Doxylamine	4.1	271.3	167.2
Nalbuphine	4.1	358.4	185.2
Tramadol	4.3	264.2	58
Tapentadol	4.3	222.3	107.2
Benzoylecgonine	4.4	290.1	168.1
7-Aminoclonazepam	4.5	286.1	121.1
Ketamine	4.5	238.1	125
Meperidine	4.5	248.2	220
Meprobamate	4.6	219.1	158.2
Normeperidine	4.7	234.1	91.2
Cocaine	4.9	304.1	182.1
MDPV	5	276.2	126.2
Midazolam	5	326.1	291.3
Bupropion	5	240.2	184
alpha-pyrrolidinopentophenone	5	272.3	110.1
5-methoxy DALT	5	272.3	110
7-Aminoflunitrazepam	5.2	284.1	135.1
Chlorpheniramine	5.2	275.1	230.1
Venlafaxine	5.2	278.2	260.2
Mirtazapine	5.3	266.2	195.1
Pentazocine	5.3	286.3	175.1
Norbuprenorphine	5.4	414.2	187.1
Butorphanol	5.4	328.4	131.2
Brompheniramine	5.5	319.1	274.1
Clozapine	5.5	327.1	270.1
Zolpidem	5.6	308.2	235.2
Diphenhydramine	5.8	256.2	165.1
Buprenorphine	5.8	468.2	396.2
Citalopram	5.9	325.2	109
D3-Doxepin	5.9	283	107.1
Trazodone	5.9	372.2	176.1

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ANALYTE	Relative Retention Time (min)	Q1	Q3
Doxepin	6	280.2	107.1
Fentanyl	6	337.2	188.2
Fluoxetine	6	310.1	117.1
Haloperidol	6	376.1	123
Clomipramine	6	315.2	86.1
Phencyclidine-D5	6	249.2	164.2
Dextromethorphan	6.1	272.2	171.2
Mianserin	6.1	265.2	208.2
Phencyclidine	6.1	244.2	86.1
Carisoprodol	6.1	261.2	176.1
Quetiapine	6.2	384.2	253.1
Zopiclone	6.2	389.1	245
Dextropropoxyphene	6.3	340.2	266.2
Propoxyphene	6.3	340	58
alpha-hydroxymidazolam	6.3	342.1	168.1
Desipramine	6.4	267.2	72.1
Imipramine	6.4	281.2	86.1
EDDP	6.4	278.2	234.1
Cyclobenzaprine	6.4	276.2	215
Bromazepam	6.5	316	182.1
Nortriptyline	6.5	264.2	233.1
Paroxetine	6.5	330.1	192.1
Carbamazepine	6.5	237.1	194.2
Amitriptyline	6.6	278.2	233.2
Lorazepam	6.8	321	229.1
Methadone	6.8	310.2	265.2
Clonazepam	6.9	316.1	270.1
Oxazepam	6.9	287.1	241.1
alpha-Hydroxytriazolam	6.9	359	331.1
2-Hydroxyethylflurazepam	7	333.1	211.2
Triazolam	7	343	239
alpha-Hydroxyalprazolam	7	325.1	297.2
Norfluoxetine	7	296.2	134.2
Nordiazepam	7.2	271.1	140.1
Sertraline	7.2	306.1	159
Estazolam	7.3	295.1	205.2
Flunitrazepam	7.3	314.1	268.1
Alprazolam-D5	7.3	314.2	286.3
Alprazolam	7.4	309.1	281.1
Temazepam	7.4	301.1	255.1
D5-Diazepam	7.5	290	198.2
Diazepam	7.7	285.1	193.2
Methaqualone-d7	8	259.2	98.2
Flurazepam	8.3	388.1	315.1
THC-COOH	8.4	345.1	299.1
THC	8.5	315.2	193.2

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