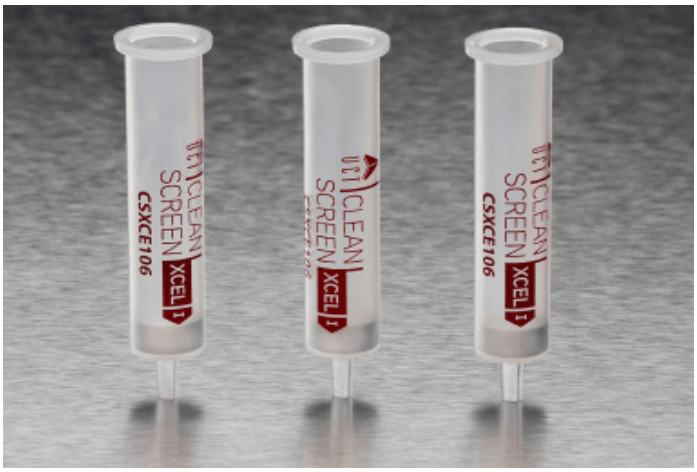
Screening Method for Acidic, Neutral and Basic Drug Analytes in Oral Fluid by LC-MS/MS Using CLEAN SCREEN® XCEL I



UCT Part Numbers

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

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

SPHPHO6001-5
Select pH Buffer Pouches
100 mM Phosphate Buffer pH 6.0

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

SLGRDHLDR-HPOPT
Guard Column Holder

Introduction:

Compared to urine and hair drug tests, oral fluid is a better matrix for detecting recent drug use. It takes time for drugs to pass through the body and present themselves in urine or hair, but oral fluid tests will often detect drugs in the 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.

Drug levels in oral fluid 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 much lower than those for urine and hence more sensitive screening and confirmatory assays are required. A concentration step is often necessary to enhance sensitivity.

The Clean Screen® XCEL I method described in this application note can be utilized to extract a range of different drugs from oral fluid for both screening and confirmatory purposes. By evaporating to completion, final extracts are purified and highly concentrated to achieve required levels of detection/quantitation.

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Sample Pretreatment:

NEAT ORAL FLUID:

- Add 100 - 500 µL of neat oral fluid sample to a clean test 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 1 mL of oral fluid specimen, (diluted in Quantisal™ Buffer), add appropriate internal standards. Mix/vortex for 30 seconds.

SPE Procedure:

1. Load Sample

a) Load at 1 to 2 mL/minute.

2. Wash Column

a) 1 × 3 mL D.I. H_2O
b) 1 × 3 mL 1% HCl solution
Note: A Hexane wash may be added if not looking for parent THC.

3. Dry Column

a) Dry for at least 10 minutes under full pressure or vacuum

4. Elute

a) 1 x 3 mL MeOH:NH4OH (98:2)
Note: Make elution solvent fresh daily.

5. Evaporate

a) Evaporate eluate to dryness
Note: Take care not to overheat or over evaporate. Certain compounds are heat labile, such as amphetamines and phencyclidine. Adding 1 drop of a 1% HCl in MeOH solution before evaporation may be used to prevent volatization.

6. Reconstitute

a) 100 uL of mobile phase or other appropriate solvent or volume

LC-MS/MS Parameters:

LC-MS/MS Parameters	
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**)
Column Temperature	40 °C
Injection Volume	10 µL
Flow Rate	0.3 mL/min

Gradient Program		
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	STOP

Results:

Analyte	Relative Retention Time (min)	Q1	Q3
Ecognine methyl ester	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
Acetaminophen	2	152	110
Gabapentin	2.2	172.1	67.1
MDA	2.5	180.1	105
Atropine	2.5	290.2	124.1
Buspirone	2.5	386.2	122.1
Clonidine	2.5	230	213
Methamphetamine	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-MAM	2.6	328.1	165.1
Naloxone	2.8	328.2	310.2
Methylone	2.8	208	160.1
Phenmetrazine	2.8	178.2	115.1
Phendimetrazine	2.8	192.2	147.1
Caffeine	3	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

Analyte	Relative Retention Time (min)	Q1	Q3
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
Doxepin-D3	5.9	283	107.1
Trazodone	5.9	372.2	176.1
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

Analyte	Relative Retention Time (min)	Q1	Q3
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
Diazepam-D5	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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