SCREENING METHOD FOR 121 ACIDIC, NEUTRAL AND BASIC DRUG ANALYTES IN PLASMA, SERUM, URINE, OR TISSUE BY LC-MS/MS

UCT Part Numbers

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

BETA-GLUC-10
SELECTRAZYME®
Beta-glucuronidase

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

SLDA50GDC21-5UM
Selectra® DA Guard Column
10 x 2.0 mm, 5 µm

SLGRDHLDR
Selectra® HPLC Guard Cartridge Holder

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Summary:

Comprehensive screening is often referred to as general unknown or systematic toxicological analysis. With the use of LC/MS/MS the complete detection of both illicit and prescribed drugs can be accomplished easily with a simple solid-phase extraction preparation. This screening tool can give low LOD's as well as specific information as to drugs and metabolites in an unknown sample. The objective of this screening application is to develop a rapid, highly sensitive qualitative method for the simultaneous analysis of acidic, neutral, and basic compounds in biological fluids using Clean Screen XCEL® I solid-phase extraction columns. The sample preparation procedure is minimized while efficiently extracting a large group of representative compounds. Sample analysis was executed using a HPLC column containing a biphenyl stationary phase.

Sample Pretreatment:

To 1 mL of blood, plasma/serum, or 1 g tissue homogenate (1:4) add internal standard(s). Mix/vortex and let stand for 5 minutes. Add 3 mL of 100 mM phosphate buffer (pH 6) and vortex briefly. Centrifuge for 10 minutes at 2000 rpm and discard pellet.

Hydrolysis (for urine samples only):
To 1 mL of urine add 1 mL of acetate buffer (pH 5) containing 5,000 units/mL of Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated enzyme. Vortex briefly and heat for 1-2 hours at 65°C.

SPE Procedure:

1. APPLY SAMPLE DIRECTLY TO SPE Column: Load at 1 to 2 mL/minute

2. WASH:
   a) 1 x 2 mL 0.1M Acetic Acid.
   b) Apply pressure to column for ~1 minute using either full vacuum (≥10mm Hg) or positive pressure (80-100psi).
   c) 1 x 2 mL 2 mL n-Hexane to remove residual aqueous phase [1].
   d) Dry column for 5 minutes at 80-100 psi.

3. ELUTION:
   1 x 2 mL of CH_2Cl_2 / IPA / ammonium hydroxide (78:20:2, v/v).
   *Alternatively, use 1 x 2 mL Methanol + 2% Ammonium Hydroxide.

4. EVAPORATION:
   Evaporate fraction to complete dryness under a gently stream of nitrogen at ~35 °C. Take care not to overheat or over-evaporate the sample as certain compounds are heat labile (e.g amphetamines) [2-3].
   Reconstitute sample in 100 µL of mobile phase.

NOTES:

(1) The hexane wash step can be removed if user is looking to analyze for parent THC.

(2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines. Use 30-50 µL of high purity DMF in the sample before evaporation.

(3) A 1% HCl in CH_3OH solution has been used to prevent volatilization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating then continue to dryness.

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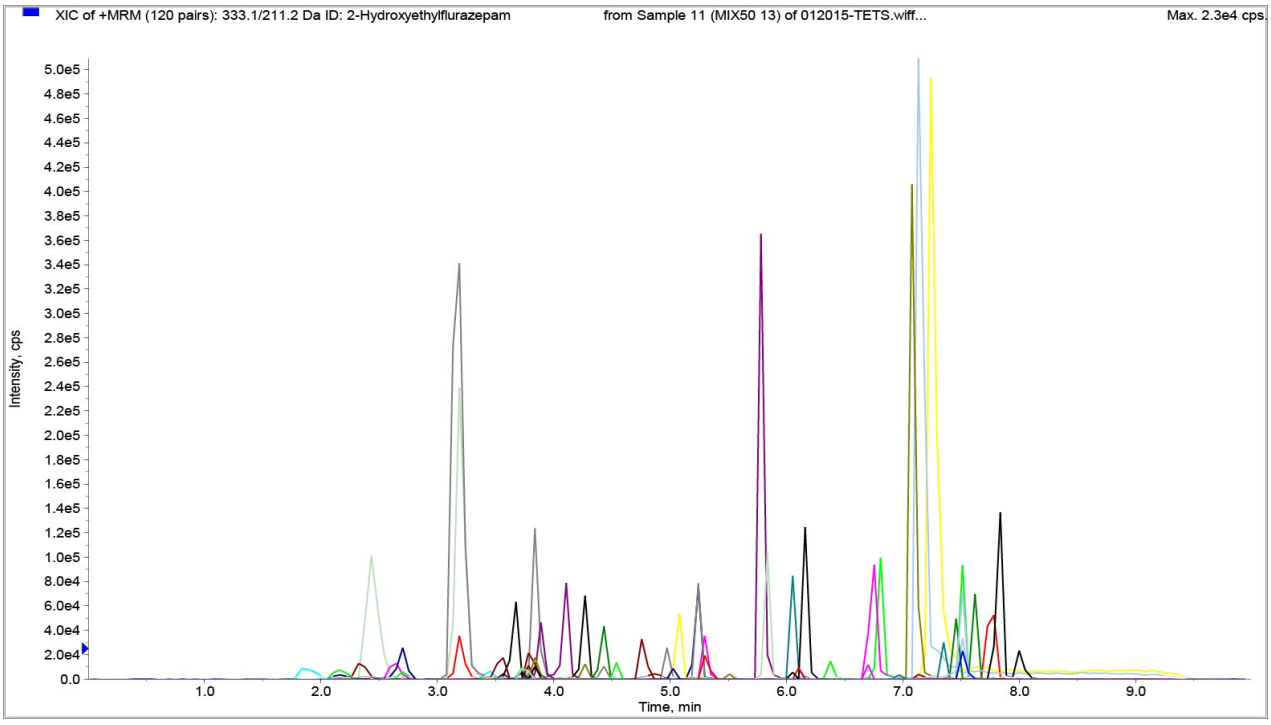
LC-MS/MS Parameters:

Instrumentation	
HPLC system	Shimadzu HPLC 20-AD
MS system	AB Sciex API 3200 Qtrap
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 5 µm (p/n: SLDA50ID21-5UM)
Flow rate	0.6 mL/min
Injection volume	20 µL

Mobile Phase Gradient		
Time (min)	**% Mobile Phase A** (0.1% Formic Acid in Ultrapure 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	



Picture 1: Representative Chromatogram of Standard

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Results:

ANALYTE	Relative Retention Time (min)	Q1	Q3	LOD (ng/mL) (blood samples)
Ecgoninemethylester	0.5	200.1	182.1	5
Phenylpropanolamine	0.9	152.2	134.2	20
Morphine	1.4	286.0	152.0	2
Oxymorphone	1.5	302.0	227.0	2
Pregabalin	1.5	160.2	97.0	50
Pseudoephedrine	1.9	166.1	148.1	20
Hydromorphone	1.9	286.0	185.0	2
Ephedrine	1.9	166.2	148.3	20
Amphetamine	2.0	136.1	91.1	10
Acetaminophen	2.0	152.0	110.0	50
Gabapentin	2.2	172.1	67.1	50
3,4-Methylenedioxyamphetamine	2.5	180.1	105	5
Atropine	2.5	290.2	124.1	50
Buspirone	2.5	386.2	122.1	20
Clonidine	2.5	230.0	213.0	10
Methamphetamine	2.5	150.1	91.1	10
Nicotine	2.5	163.1	132.1	20
Phenylephrine	2.5	168.1	91.1	20
Theobromine	2.5	181.1	138.0	20
Theophylline	2.5	181.1	124.0	20
Mephedrone	2.5	178.2	160.1	10
Phentermine	2.5	150.2	91.2	10
6-O-Monoacetylmorphine	2.6	328.1	165.1	2
Naloxone	2.8	328.2	310.2	2
Methylone	2.8	208.0	160.1	10
Phenmetrazine	2.8	178.2	115.1	20
Phendimetrazine	2.8	192.2	147.1	20
Caffeine	3.0	195.1	122.9	50
Dihydrocodeine	3.0	302.2	199.1	2
Codeine	3.0	300.0	152.0	2
Desmethyltramadol	3.0	250.2	58.2	5
3,4-Methylenedioxymethamphetamine	3.1	194.1	105.1	10
7-Aminonitrazepam	3.1	252.1	121.1	10
Oxycodone-D6 (IS)	3.1	322.3	304.1	-
Oxycodone	3.2	316.1	298.1	2
Hydrocodone	3.4	300.0	199.0	2
Diethylpropion	3.4	206.2	100.2	20
3,4-Methylenedioxyethylamphetamine	3.6	208.1	77.1	10
Naltrexol	3.6	344.3	308.4	5
Pheniramine	3.8	241.2	167.2	10
Olanzapine	4.0	313.1	256.1	20

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ANALYTE	Relative Retention Time (min)	Q1	Q3	LOD (ng/mL) (blood samples)
Norketamine	4.0	224.1	207.1	10
Methylphenidate	4.1	234.1	84.1	20
Norfentanyl	4.1	233.2	84.1	5
Doxylamine	4.1	271.3	167.2	20
Nalbuphine	4.1	358.4	185.2	5
Tramadol	4.3	264.2	58.0	5
Tapentadol	4.3	222.3	107.2	20
Benzoylecgonine	4.4	290.1	168.1	5
7-Aminoclonazepam	4.5	286.1	121.1	5
Ketamine	4.5	238.1	125	10
Meperidine	4.5	248.2	220	20
Meprobamate	4.6	219.1	158.2	25
Normeperidine	4.7	234.1	91.2	20
Cocaine	4.9	304.1	182.1	5
MDPV	5.0	276.2	126.2	10
Midazolam	5.0	326.1	291.3	10
Bupropion	5.0	240.2	184.0	20
alpha-Pyrrolidinopentiophenone	5.0	272.3	110.1	10
5-MeO-DALT	5.0	272.3	110.0	10
7-Aminoflunitrazepam	5.2	284.1	135.1	10
Chlorpheniramine	5.2	275.1	230.1	20
Venlafaxine	5.2	278.2	260.2	25
Mirtazapine	5.3	266.2	195.1	10
Pentazocine	5.3	286.3	175.1	5
Norbuprenorphine	5.4	414.2	187.1	5
Butorphanol	5.4	328.4	131.2	5
Brompheniramine	5.5	319.1	274.1	20
Clozapine	5.5	327.1	270.1	20
Zolpidem	5.6	308.2	235.2	20
Diphenhydramine	5.8	256.2	165.1	10
Buprenorphine	5.8	468.2	396.2	5
Citalopram	5.9	325.2	109.0	10
Doxepin-D3 (IS)	5.9	283.0	107.1	-
Trazodone	5.9	372.2	176.1	5
Doxepin	6.0	280.2	107.1	10
Fentanyl	6.0	337.2	188.2	1
Fluoxetine	6.0	310.1	117.1	20
Haloperidol	6.0	376.1	123.0	10
Clomipramine	6.0	315.2	86.1	10
Phencyclidine-D5 (IS)	6.0	249.2	164.2	-
Dextromethorphan	6.1	272.2	171.2	5
Mianserin	6.1	265.2	208.2	20
Phencyclidine	6.1	244.2	86.1	5
Carisoprodol	6.1	261.2	176.1	100

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ANALYTE	Relative Retention Time (min)	Q1	Q3	LOD (ng/mL) (blood samples)
Quetiapine	6.2	384.2	253.1	20
Zopiclone	6.2	389.1	245.0	25
Dextropropoxyphene	6.3	340.2	266.2	15
Propoxyphene	6.3	340.0	58.0	10
alpha-Hydroxymidazolam	6.3	342.1	168.1	5
Desipramine	6.4	267.2	72.1	10
Imipramine	6.4	281.2	86.1	20
EDDP	6.4	278.2	234.1	20
Cyclobenzaprine	6.4	276.2	215.0	10
Bromazepam	6.5	316.0	182.1	20
Nortriptyline	6.5	264.2	233.1	20
Paroxetine	6.5	330.1	192.1	50
Carbamazepine	6.5	237.1	194.2	50
Amitriptyline	6.6	278.2	233.2	10
Lorazepam	6.8	321.0	229.1	10
Methadone	6.8	310.2	265.2	5
Clonazepam	6.9	316.1	270.1	10
Desalkylflurazepam	6.9	289.0	140.1	10
Oxazepam	6.9	287.1	241.1	5
alpha-Hydroxytriazolam	6.9	359.0	331.1	10
2-Hydroxyethylflurazepam	7.0	333.1	211.2	10
Chlordiazepoxide	7.0	300.1	227.1	10
Triazolam	7.0	343.0	239.0	10
alpha-Hydroxyalprazolam	7.0	325.1	297.2	5
Norfluoxetine	7.0	296.2	134.2	50
Nordiazepam	7.2	271.1	140.1	5
Sertraline	7.2	306.1	159.0	20
Estazolam	7.3	295.1	205.2	5
Flunitrazepam	7.3	314.1	268.1	5
Alprazolam-D5 (IS)	7.3	314.2	286.3	-
Alprazolam	7.4	309.1	281.1	5
Temazepam	7.4	301.1	255.1	10
Diazepam-D5 (IS)	7.5	290.0	198.2	-
Diazepam	7.7	285.1	193.2	5
Methaqualone-D7 (IS)	8.0	259.2	98.2	-
Flurazepam	8.3	388.1	315.1	5

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