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

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INTRODUCTION

Comprehensive screening is often referred to as general unknown or systematic toxicological analysis. With the use of LC-MS/MS and full screen (QTrap methodology) 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 analysis of acidic, neutral, and basic compounds in biological fluids using Clean Screen XCEL® I solid phase extraction columns. The sample preparation is minimized while efficiently extracting a large group of representative compounds. Sample analysis was executed using the Selectra® DA a unique polyaromatic phase HPLC column.

APPLICATION

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
   Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate. Centrifuge for 10 minutes at 2000 rpm and discard pellet
   NOTE: See Hydrolysis step if required

   Hydrolysis (for urine samples only): To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65 °C.

2. **APPLY SAMPLE:**
   Load sample directly to column without any preconditioning
   Pull sample through at a rate of 1-2 mL/ minute
   Dry column thoroughly under full vacuum or positive pressure for 1 minute

3. **WASH 1 – ACIDIC & NEUTRAL COMPOUNDS (FRACTION 1):**
   Add 1 x 1 mL of DI H_2O
   Apply pressure to column for ~1 minute (either vacuum (10mm Hg) or positive pressure(~80-100 psi).
   This ensures that the entire sample and any residual is pulled through to waste

   Add 1 x 1 mL of 0.1M Acetic Acid
   Apply pressure to column for ~1 minute (either vacuum (10mm Hg) or positive pressure(~80-100 psi).
   Add 1 x 2 mL Hexane to remove residual aqueous phase
   Dry column (5 minutes at full vacuum or pressure)

4. **ELUTION 1 – ACIDIC & NEUTRAL COMPOUNDS (FRACTION 1):**
   Add 1 x 1 mL Ethyl Acetate: Hexane (50:50) Collect eluate at 1 to 2 mL/minute

5. **DRY ELUTE:**
   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~35 °C Reconstitute with 100 µL of Mobile Phase

6. **WASH 2 - BASIC COMPOUNDS (FRACTION 2):**
   Add 1 x 1 mL of 2% Acetic Acid/98% Methanol
   Dry column 5 minutes at full vacuum (10mm Hg) or positive pressure (~80-100 psi)

7. **ELUTION 2 - BASIC COMPOUNDS (FRACTION 2):**
   1 x 1 mL of CH_2Cl_2/ IPA/ Ammonium Hydroxide (78/20/2).

8. **DRY ELUTE:**
   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines. Reconstitute with 100 µL Mobile Phase.

INSTRUMENT CONDITIONS (LC-MS/MS)

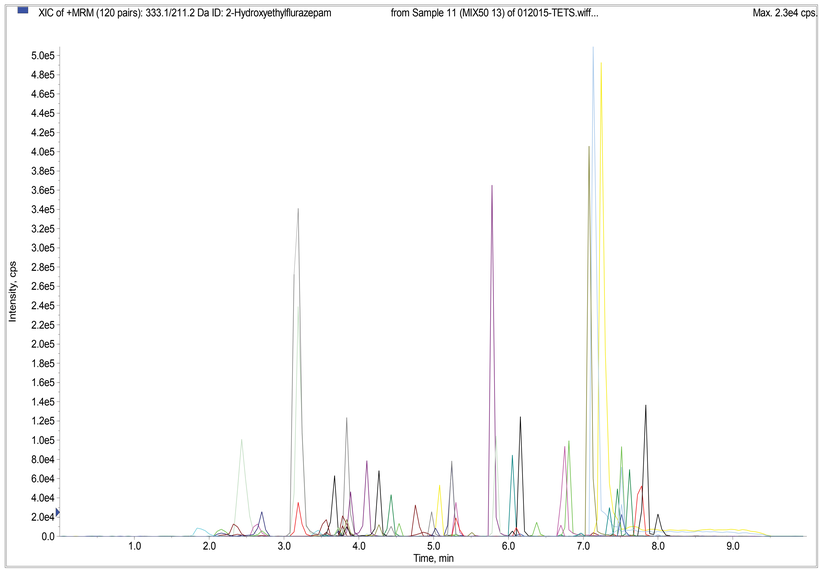
PARAMETERS

Instrument: Shimadzu HPLC 20-AD
Detector: AB Sciex API 3200 Qtrap MS/MS
LC Column: UCT Selectra® DA HPLC Column 50 x 2.1mm, 5 µm
Polarity: ESI +
Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.6 mL/minute
Injection Volume: 20 µL

Gradient

Time	%A	%B
0.00	90	10
0.50	90	100
4.00	60	40
7.50	15	85
8.50	10	90
8.51	90	10
10.00	STOP	

CHROMATOGRAM



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RESULTS

ANALYTE TABLE

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	152	2
Oxymorphone	1.5	302	227	2
Pregabalin	1.5	160.2	97	50
Pseudoephedrine	1.9	166.1	148.1	20
Hydromorphone	1.9	286	185	2
Ephedrine	1.9	166.2	148.3	20
Amphetamine	2	136.1	91.1	10
Acetaminophen	2	152	110	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	213	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	20
Theophylline	2.5	181.1	124	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	160.1	10
Phenmetrazine	2.8	178.2	115.1	20
Phendimetrazine	2.8	192.2	147.1	20
Caffeine	3	195.1	122.9	50
Dihydrocodeine	3	302.2	199.1	2
Codeine	3	300	152	2
Desmethyltramadol	3	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	3.1	322.3	304.1	NA
Oxycodone	3.2	316.1	298.1	2
Hydrocodone	3.4	300	199	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
Phenixamine	3.8	241.2	167.2	10
Olanzapine	4	313.1	256.1	20
Norketamine	4	224.1	207.1	10
Methylphenidate	4.1	234.1	84.1	20
Norfentanyl	4.1	231.2	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	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	276.2	126.2	10
Midazolam	5	326.1	291.3	10
Bupropion	5	240.2	184	20
alpha-pyrrolidinopentophenone	5	272.3	110.1	10
5-methoxy DALT	5	272.3	110	10
7-Aminoflunitrazepam	5.2	284.1	135.1	10

ANALYTE	Relative Retention Time (min)	Q1	Q3	LOD (ng/mL) (blood samples)
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	10
D3-Doxepin	5.9	283	107.1	NA
Trazodone	5.9	372.2	176.1	5
Doxepin	6	280.2	107.1	10
Fentanyl	6	337.2	188.2	1
Fluoxetine	6	310.1	117.1	20
Haloperidol	6	376.1	123	10
Clomipramine	6	315.2	86.1	10
Phencyclidine-D5	6	249.2	164.2	NA
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
Quetiapine	6.2	384.2	253.1	20
Zopiclone	6.2	389.1	245	25
Dextropropoxyphene	6.3	340.2	266.2	15
Propoxyphene	6.3	340	58	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	10
Bromazepam	6.5	316	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	229.1	10
Methadone	6.8	310.2	265.2	5
Clonazepam	6.9	316.1	270.1	10
Desalkylflurazepam	6.9	289	140.1	10
Oxazepam	6.9	287.1	241.1	5
alpha-Hydroxytriazolam	6.9	359	331.1	10
2-Hydroxyethylflurazepam	7	333.1	211.2	10
Chlordiazepoxide	7	300.1	227.1	10
Triazolam	7	343	239	10
alpha-Hydroxyalprazolam	7	325.1	297.2	5
Norfluoxetine	7	296.2	134.2	50
Nordiazepam	7.2	271.1	140.1	5
Sertraline	7.2	306.1	159	20
Estazolam	7.3	295.1	205.2	5
Flunitrazepam	7.3	314.1	268.1	5
Alprazolam-D5	7.3	314.2	286.3	NA
Alprazolam	7.4	309.1	281.1	5
Temazepam	7.4	301.1	255.1	10
D5-Diazepam	7.5	290	198.2	NA
Diazepam	7.7	285.1	193.2	5
Methaqualone-d7	8	259.2	98.2	NA
Flurazepam	8.3	388.1	315.1	5

CONCLUSIONS

The use of the XCEL® I solid phase extraction cartridges in conjunction with the Selectra® DA polyaromatic HPLC column has permitted for the expedient analysis of 121 acidic, neutral, and basic compounds from blood samples. Acceptable limits of detection have been established allowing for the implementation of this rapid procedure into production use as a general unknown comprehensive screen.

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