ACIDIC/NEUTRAL/BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:

CSDAU – CLEAN SCREEN® DAU

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

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

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: 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.
(Hydroxylamine can be added to sample here if oxime derivative is preferred.)
Allow sample to cool

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH_3OH
1 x 3 mL D.I. H_2O
1 x 3 mL 100 mM phosphate buffer (pH 6.0)
NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute

4. WASH COLUMN:

1 x 3 mL D.I. H_2O
1 x 1 mL 100 mM acetic acid
Dry column (10 minutes at full vacuum or pressure)
1 x 2 mL hexane to remove residual aqueous phase

5. ELUTE ACIDIC AND NEUTRAL DRUGS (FRACTION 1):

1 x 3 mL Hexane: Ethyl Acetate (50:50)
Collect eluate at 1 to 2 mL/minute

6. DRY ELUATE:

Evaporate to dryness at < 40°C
Reconstitute with 100 µL of Ethyl Acetate or Mobile Phase

7. WASH COLUMN:

1 x 3 mL CH_3OH
Dry column (5 minutes at full vacuum or pressure)

8. ELUTE BASIC ANALYTES:

1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute
NOTE: Prepare elution solvent daily
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

9. DRY ELUATE:

Evaporate to dryness at < 40°C. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine. Reconstitute with 100 µL Ethyl Acetate or Mobile Phase

Notes:

(1) Fraction 1 (Acid Neutrals) and Fraction 2 (Bases) can be combined together if need be. The Acid/ Neutral fraction tends to be dirtier than the Basic one, so for more effective results, keep fractions separate.

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

(3) A 1% HCl in CH_3OH solution has been 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.

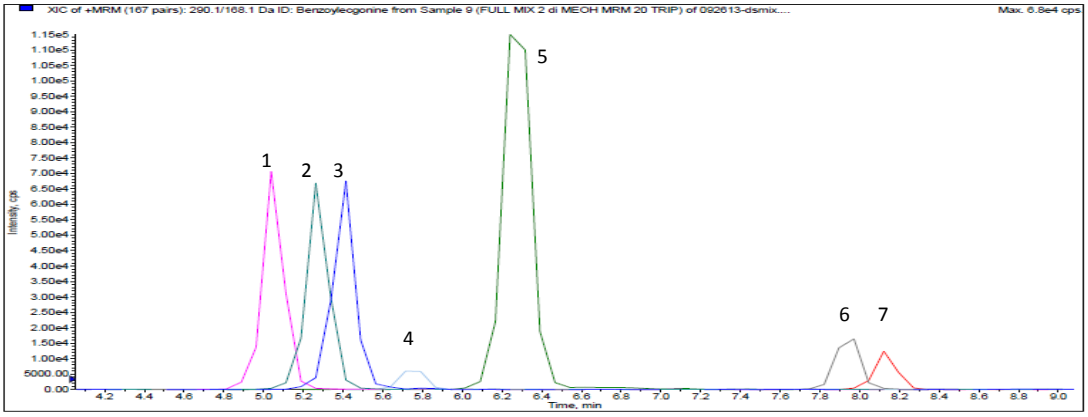
(4) The hexane wash step can be removed if user is looking to analyze for Parent THC

(5) To extract the benzodiazepine group at higher recovery, following the elution of the acidic/neutral drugs, a second elution can be done prior to moving on to the second wash phase. The second elution solvent would consist of 98% Ethyl Acetate/ 2% Ammonium Hydroxide.

INSTRUMENT CONDITIONS (LC-MS/MS):

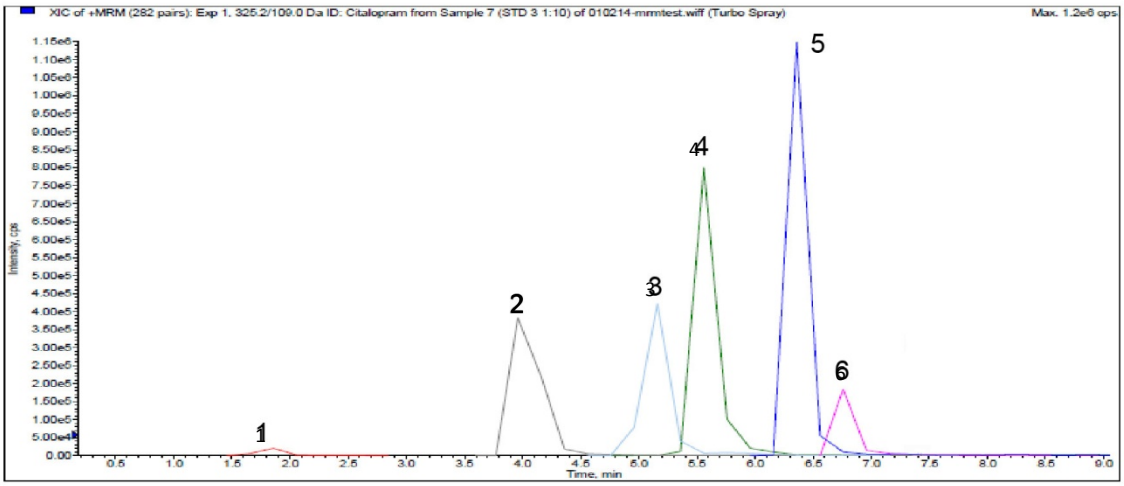
CHROMATOGRAMS

Basic Panel 1



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Clonidine	**230.0**	**213.0**	**1.80**
2. Ketamine	**238.1**	**125.0**	**4.00**
3. Mirtazepine	**266.2**	**195.1**	**5.10**
4. Clozapine	**327.1**	**270.1**	**5.60**
5. Citalopram	**325.2**	**109.0**	**6.40**
6. Norfluoxetine	**296.2**	**134.2**	**6.80**

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O **Mobile Phase B:** 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute **Polarity**: Positive

Injection Volume: 20 µL

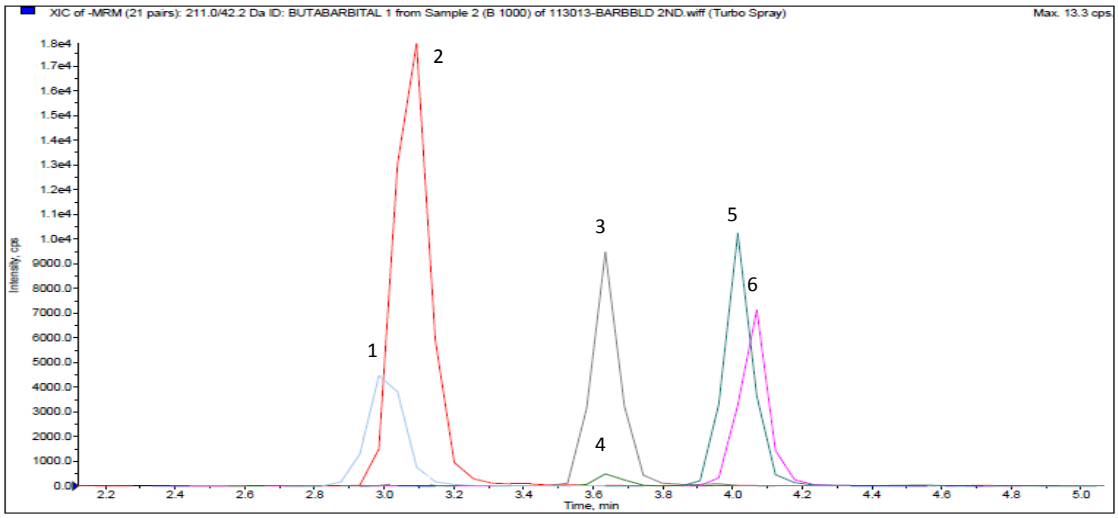
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

Barbiturates



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Phenobarbital	**230.8**	**42.0**	**3.0**
2. Butalbital	**223.0**	**42.1**	**3.1**
3. Amobarbital	**225.0**	**42.0**	**3.6**
4. Pentobarbital	**225.0**	**42.1**	**3.6**
5. Secobarbital D5	**242.1**	**42.0**	**4.0**
6. Secobarbital	**237.0**	**42.0**	**4.1**

PARAMETERS

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 **Polarity**: Positive

Reconstitute: 100 µL **Injection Volume**: 10 µL

LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	90	10
6.00	50	50
6.01	10	90
7.00	90	10
7.50	STOP	

CLEAN SCREEN® DAU
Forensic Applications

Data Provided By:
City of Philadelphia,
Department of Public Health Office of the Medical Examiner
321 University Avenue Philadelphia, Pennsylvania 19104

The following are some of the many compounds that have been extracted from forensic samples with the CLEAN SCREEN® DAU bonded silica extraction cartridge (Part #: CSDAU303):

I. ACIDIC / NEUTRAL DRUG FRACTION (A)

Acetaminophen
Barbiturates
Benzoic acid
Caffeine
Carbamazepine
Carisoprodol
Chlorpropamide
Clonazepam
Cotinine
Diazepam
Glutethimide and metabolite
Ibuprofen
Meprobamate
Methyl salicylate
Nordiazepam
Phenytoin
Primidone
Salicylic acid
Theophylline
Thiopental

II. BASIC DRUG FRACTION (B)

Amantadine
Amitriptyline and metabolite
Amphetamine
Benzocaine
Benzoylecgonine
Benztropine
Bromodiphenhydramine
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine and metabolite
Codeine
Cresol
Dextromethorphan
Dextrorphan
Thioridazine
Dihydrocodeine
Dihehydramine
Doxepin and metabolite
Ephedrine
Fluoxetine
Imipramine and metabolite
Ketamine
Lidapine
Loxapine
Meperidine
Methadone and metabolite
Methamphetamine
Methyl ρ-aminobenzoate
Methyl benzoate
Methyl ecgonine
Methylparaben
Trazodone
Methylphenidate
Methyprylon and metabolites
Morphine
Nicotine
Oxycodone
Pentazocine
Phencyclidine
Phenethylamine
Phentermine
Phenylpropanolamine
Procaine
Propoxyphene and metabolite
Propylparaben
Tranylcypromine
Trifluoperazine
Trimipramine