



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

Part #

CSDAU - CLEAN SCREEN® DAU SPE cartridge

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50x2.1 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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE BASIC ANALYTES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L.
- **GC-MS:** Dissolve residue in 100 μ L of Ethyl Acetate

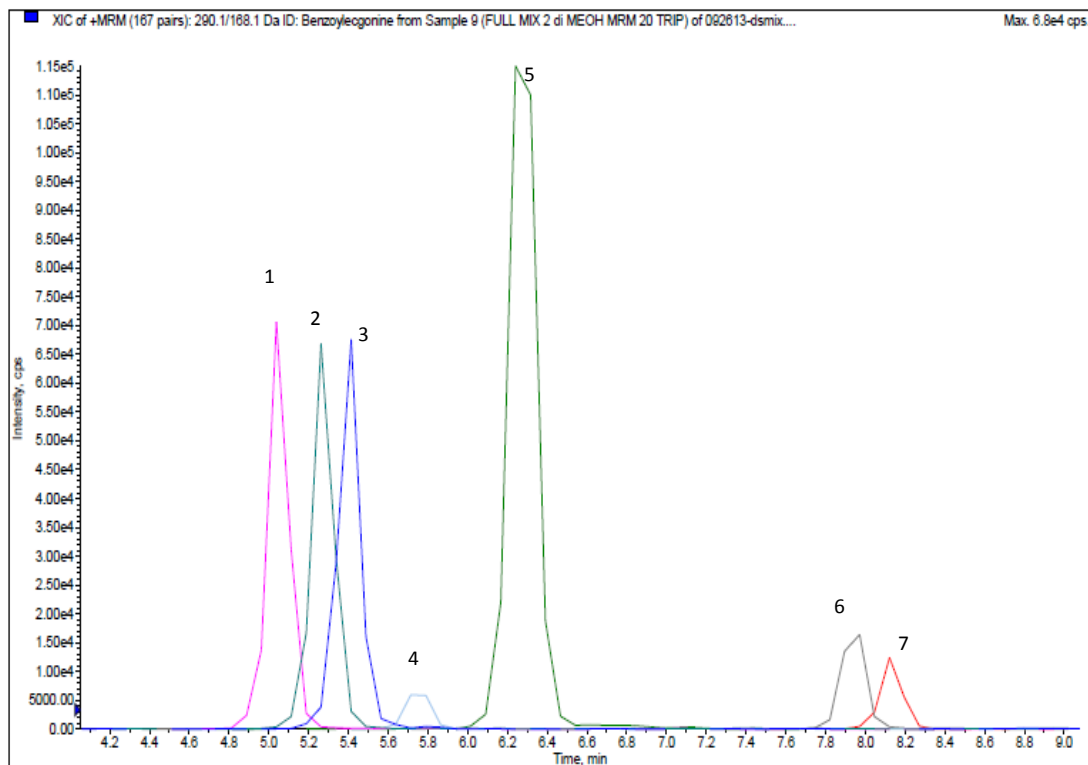
Alternate Derivatization

Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L of derivatizing reagent and react at 70 $^{\circ}$ C for 30 minutes; Cool and inject 1-2 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

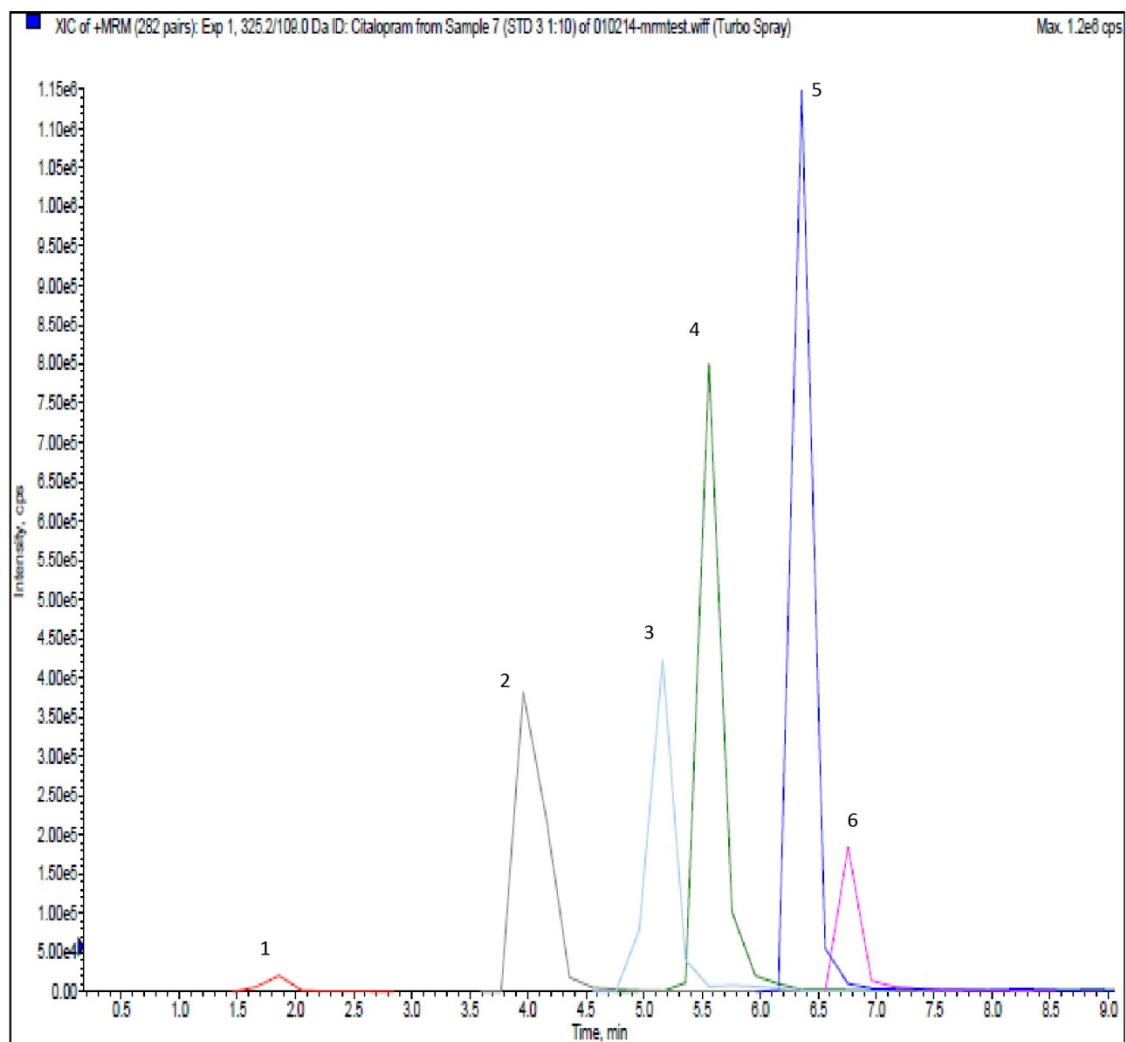
CHROMATOGRAMS

Basic Panel 1



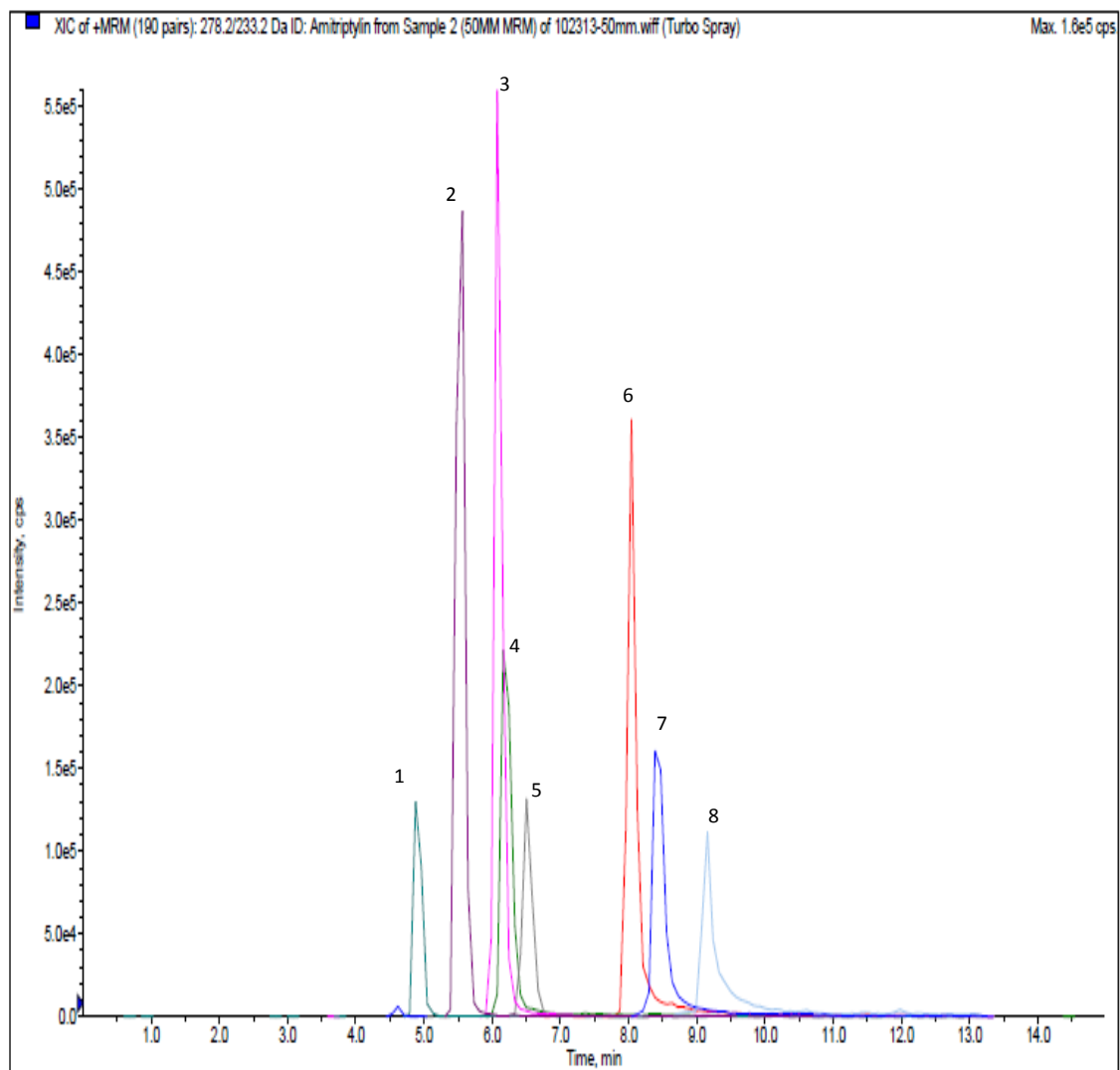
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Tapentadol	222.2	107.2	5.10
2.Tramadol	264.2	58.0	5.25
3.Benzoylcegonine	290.1	168.1	5.40
4.Meperidine	248.2	220.0	5.75
5.Cocaine	304.1	182.1	6.30
6.Fentanyl	337.2	188.2	7.90
7.Buprenorphine	468.3	396.3	8.15

Basic Panel 2



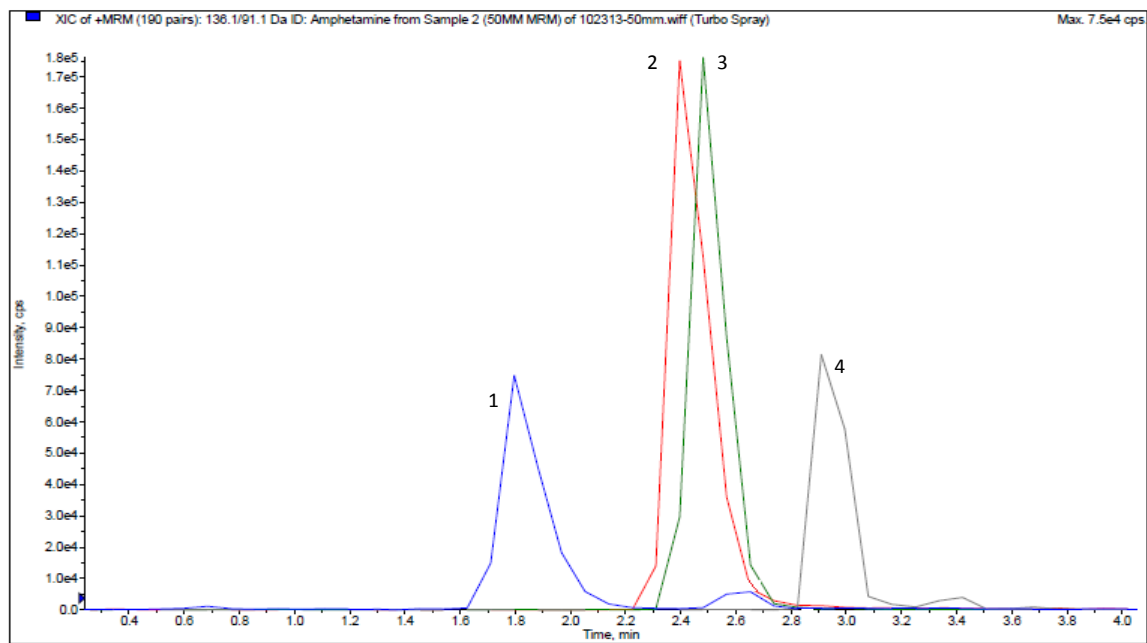
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 µL

L 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	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)

MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE

OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM

METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE