



## BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL<sup>®</sup> I EXTRACTION COLUMN

Part #

CSXCE111 – CLEAN SCREEN XCEL<sup>®</sup> I 130 mg, 1 mL Tube

BETA-GLUC-10 – Selectrazyme<sup>®</sup> Beta-glucuronidase

SLDA50ID21-5UM – Selectra<sup>®</sup> DA HPLC Column, 50 x 2.1 mm, 5  $\mu$ m

### 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  $6.0 \pm 0.5$ .  
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 1-2mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme<sup>®</sup>  $\beta$ -glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50  $\mu$ L of concentrated  $\beta$ -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. 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 x 3 mL 98% Methanol: 2% Acetic Acid  
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

### 4. ELUTION

1 x 3 mL CH<sub>2</sub>Cl<sub>2</sub>/ IPA/ NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2 mL/minute.

**NOTE:** Prepare elution solvent daily.  
Add IPA/ NH<sub>4</sub>OH, mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12).

### 5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

### 6. RECONSTITUTE / DERIVATIZE

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

#### Alternate Derivatization

Dissolve residue in 50  $\mu$ L of Ethyl Acetate and 50  $\mu$ L of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2  $\mu$ L

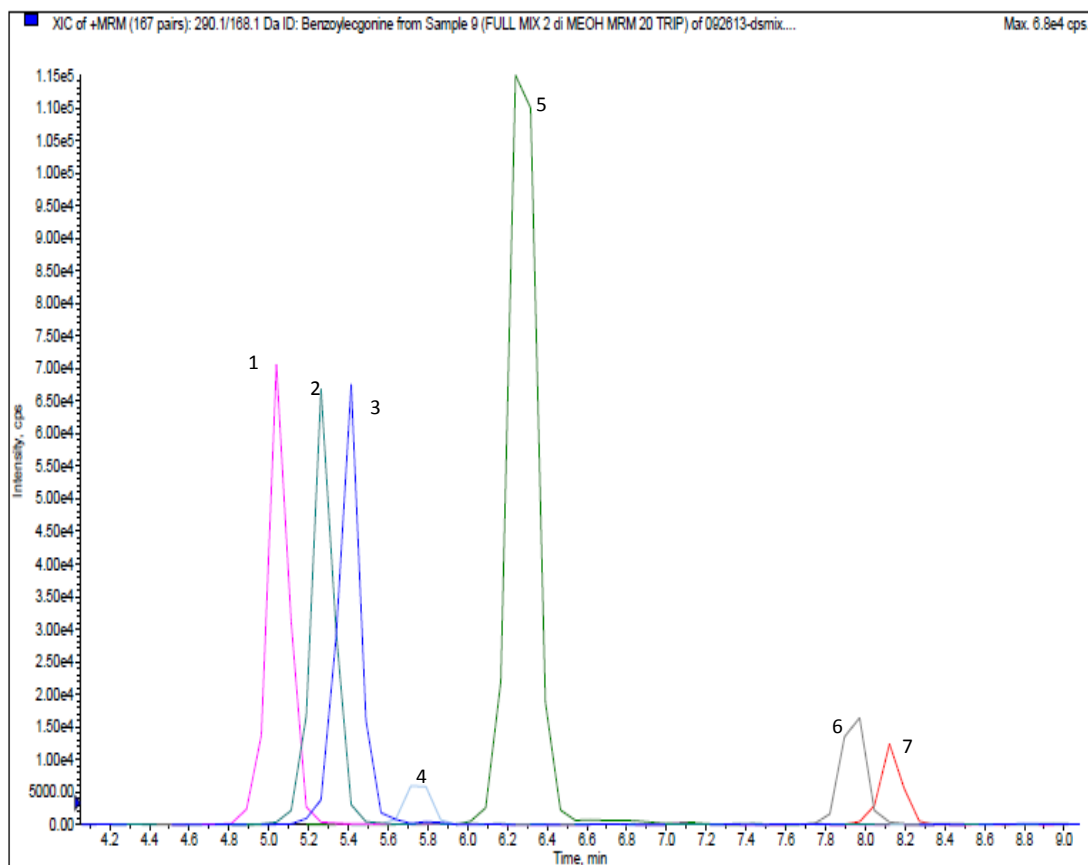
## NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

## INSTRUMENT CONDITIONS (LC-MS/MS):

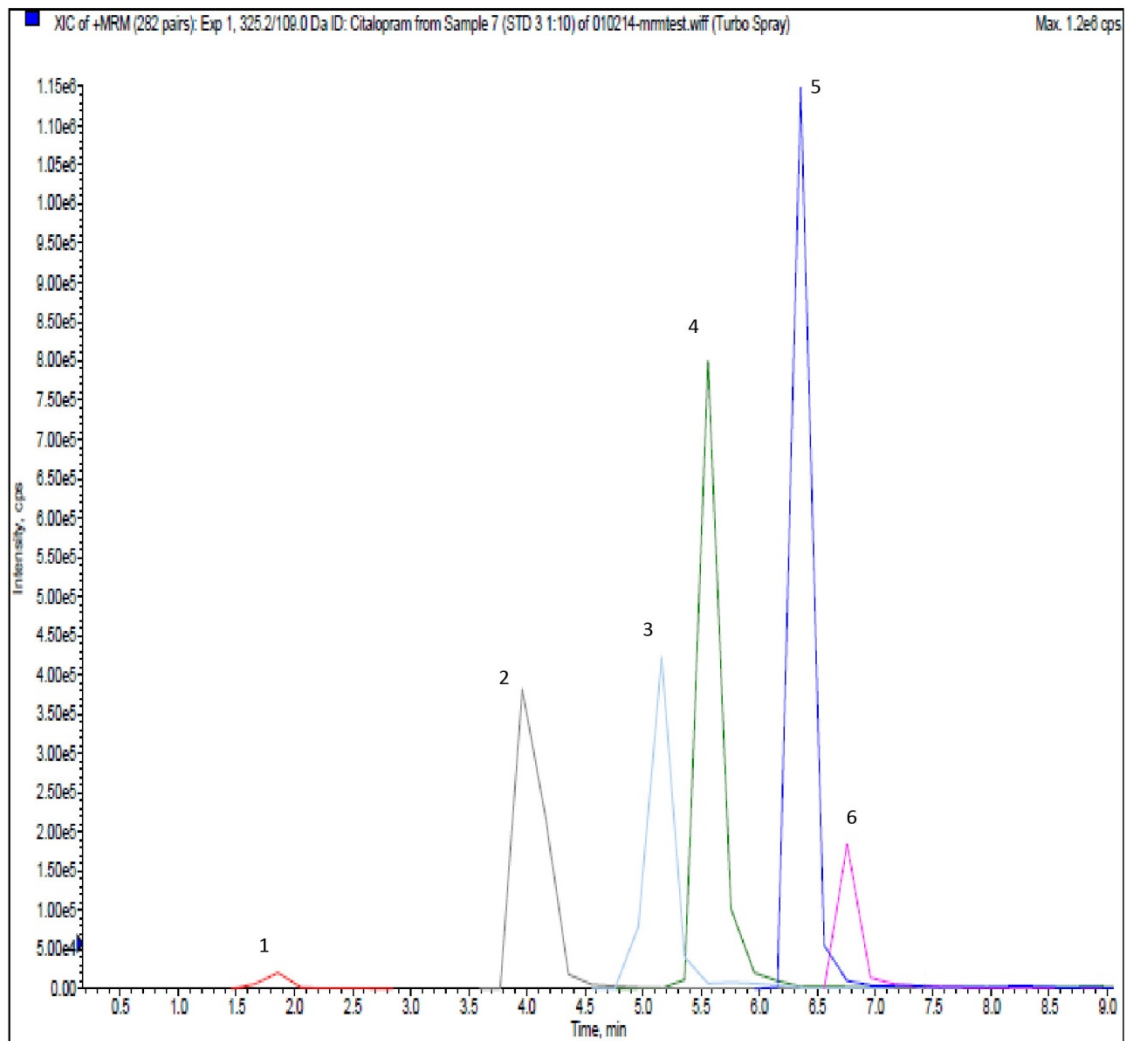
### CHROMATOGRAMS

#### Basic Panel 1



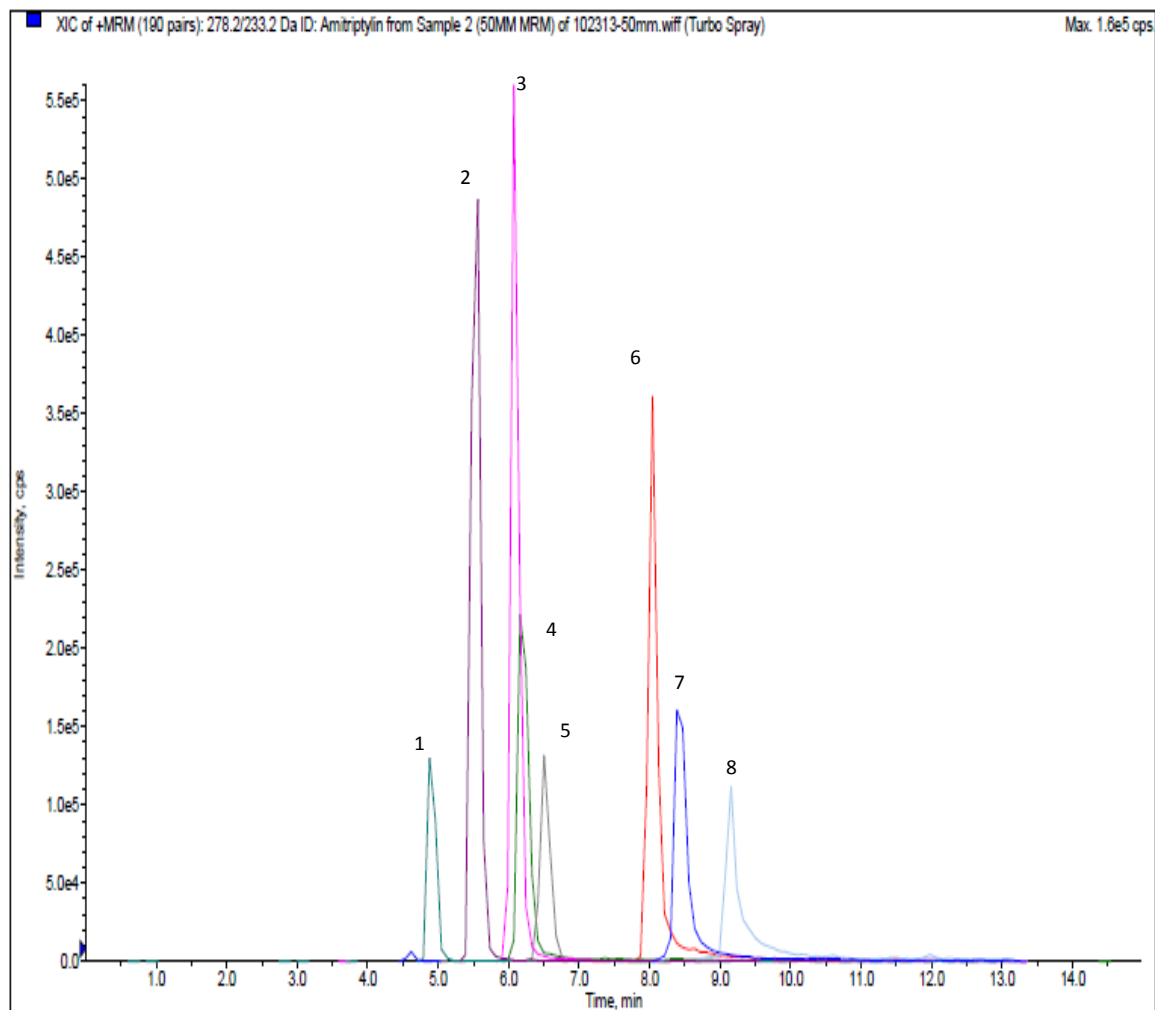
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylcegonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

## Basic Panel 2



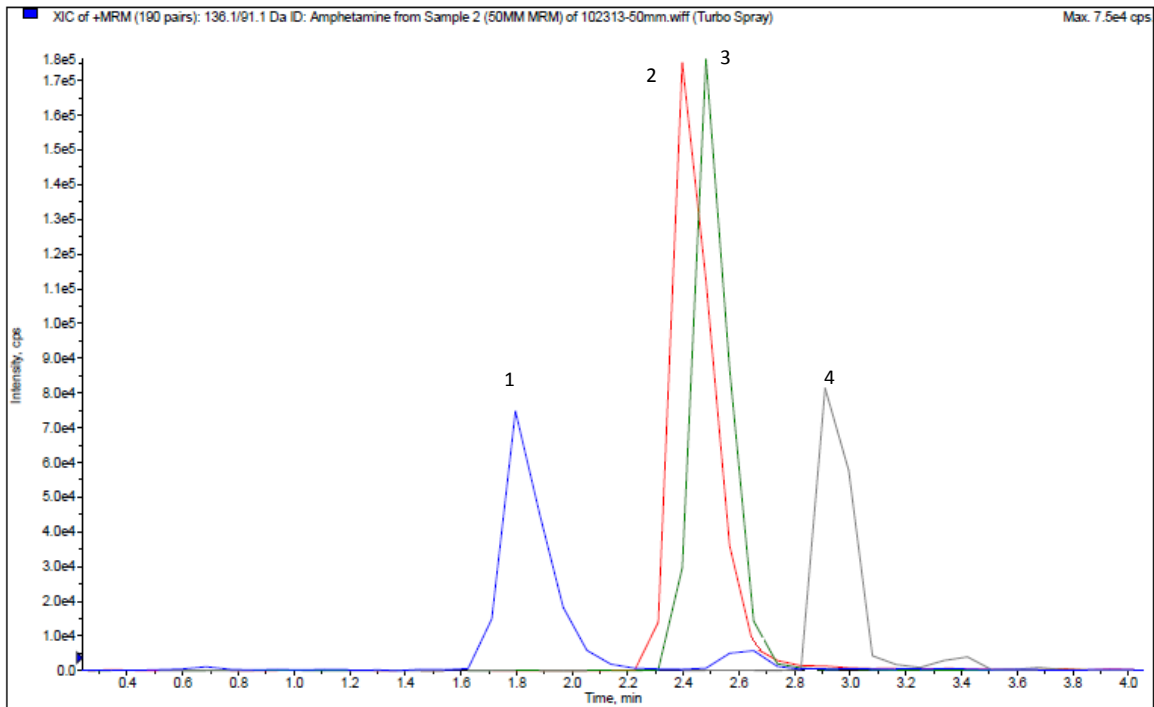
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

## 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. Quetiapine	384.2	253.1	6.50
6. Imipramine	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. H<sub>2</sub>O

**Flow Rate:** 0.5 mL/minute

**Injection Volume:** 20 µL

**LC Column:** Selectra<sup>®</sup> DA HPLC Column 50 x 2.1 mm 5 µm

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

**Mobile Phase B:** 0.1% Formic Acid in Methanol

**Polarity:** Positive

### 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/NORMEPIRIDINE  
CYCLOBENZAPRINE  
DIPHENHYDRAMINE

OPIATES(7)  
PCP  
FENTANYL/NORFENTANYL  
CITALOPRAM

METHADONE/EDDP  
COCAINE/BZE  
SERTRALINE TRAMADOL/NORTRAM  
CLONIDINE