



## BASIC ANALYTES IN BLOOD/URINE/SERUM BY LC-MS/MS OR GC-MS CLEAN SCREEN<sup>®</sup> XCEL I 96 WELLPLATE

Part #

WSH96EXE11 – CLEAN SCREEN XCEL<sup>®</sup> I 130 mg, 96 well plate

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 1-2 mL whole blood, plasma/ serum or urine add 500  $\mu$ L 100mM phosphate buffer (pH 6.0)

Add appropriate volume and concentration of internal standard.

**Note:** See Hydrolysis step if required

**Hydrolysis:** To 1-2 mL of urine sample, add 500  $\mu$ L of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme<sup>®</sup>  $\beta$ -glucuronidase. Optionally, add 500  $\mu$ L of acetate buffer and 25  $\mu$ 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  
Do not adjust pH- sample is ready to be added to the extraction plate.

### 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 1 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 1 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

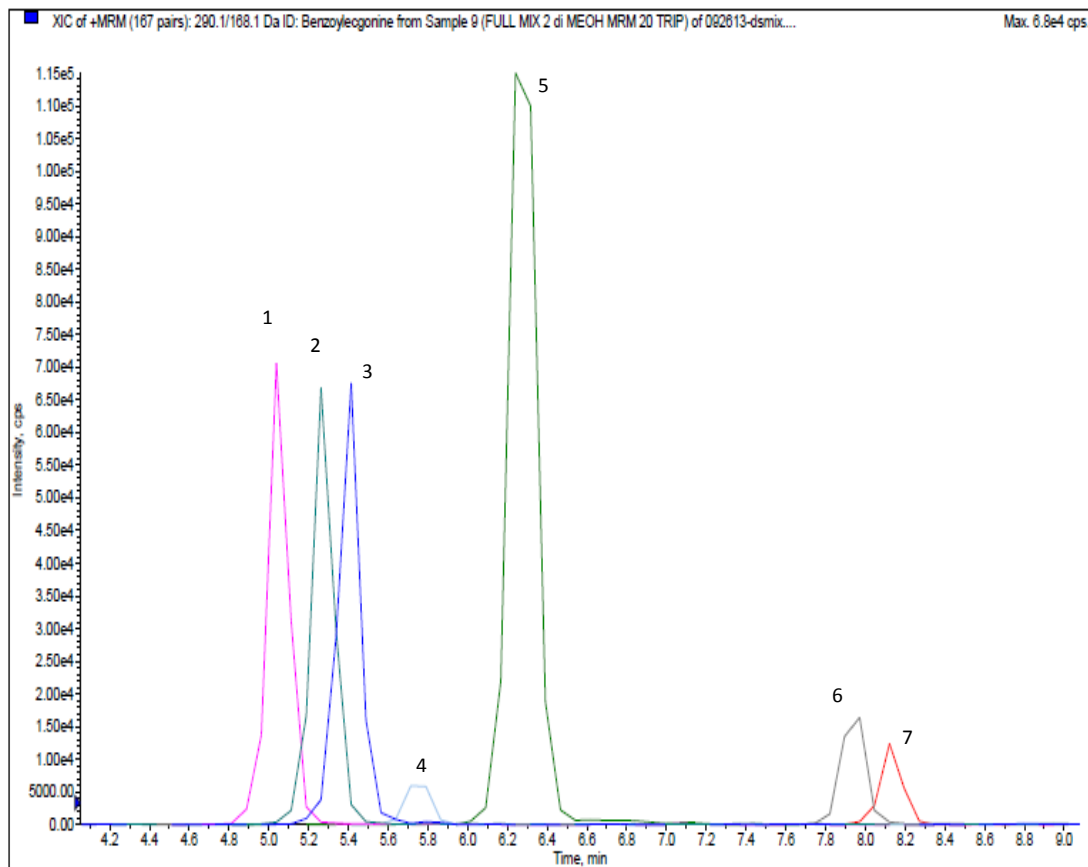
## NOTES

*(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):

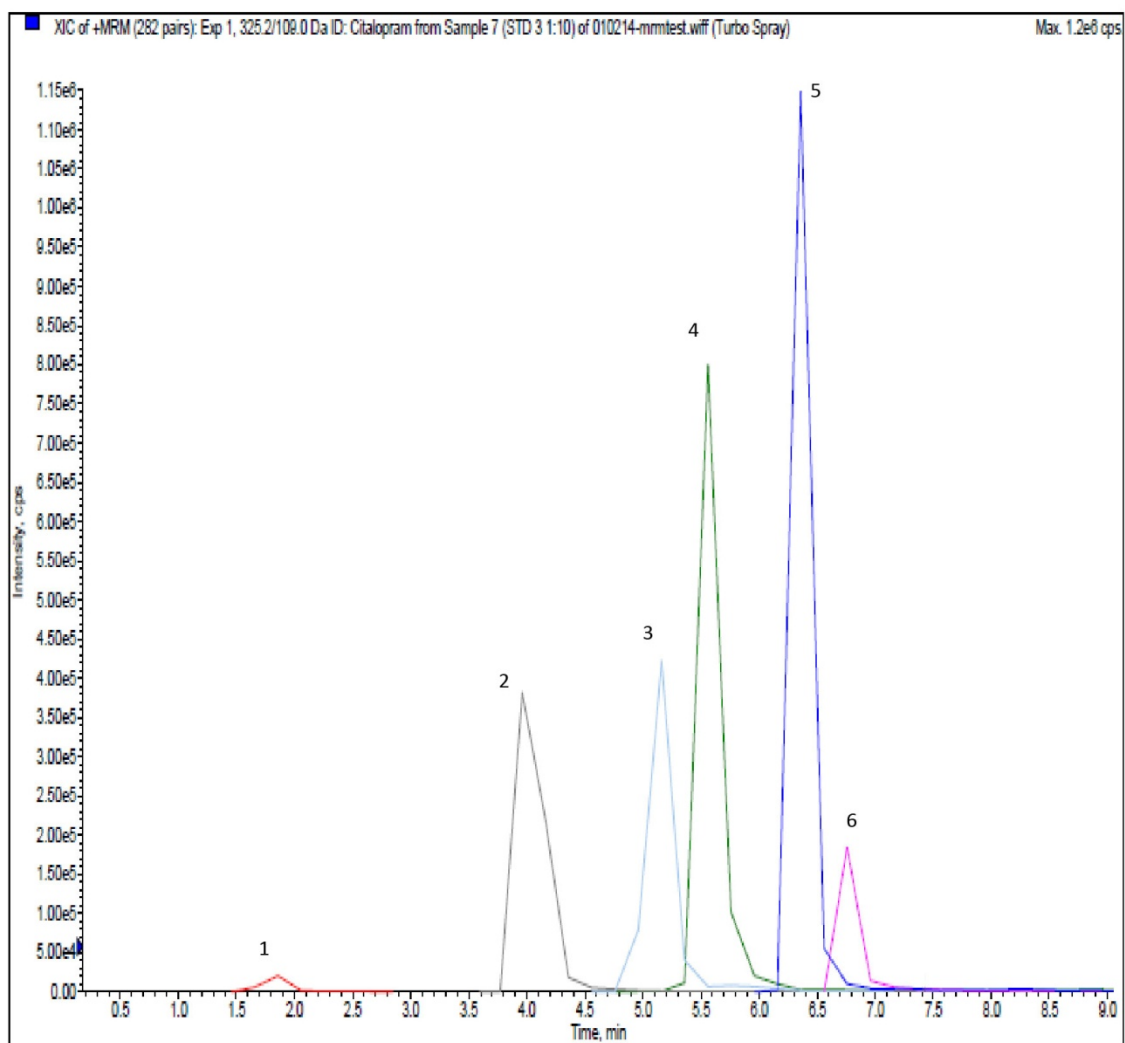
### CHROMATOGRAMS

#### Basic Panel 1



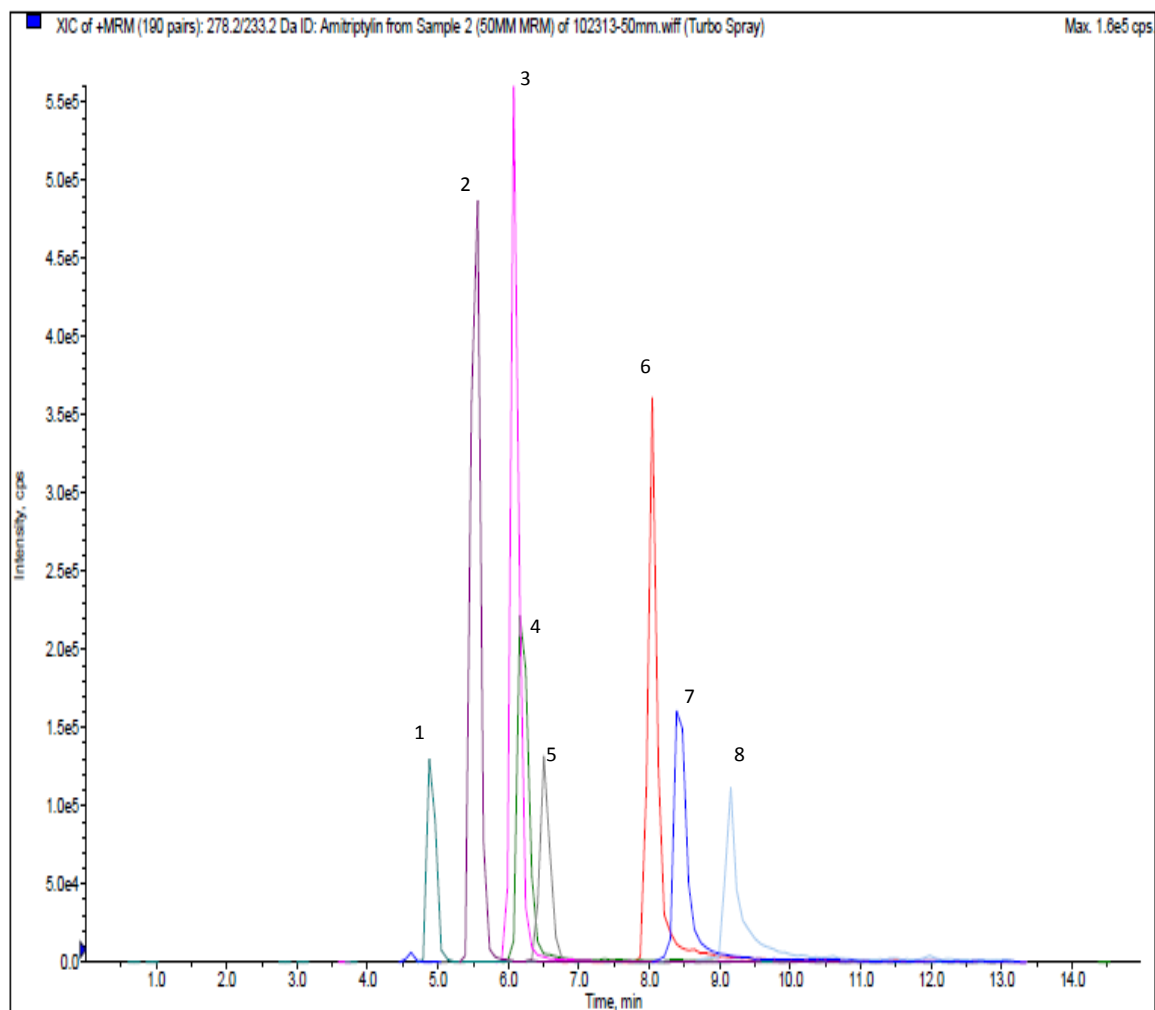
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylcegonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

## Basic Panel 2



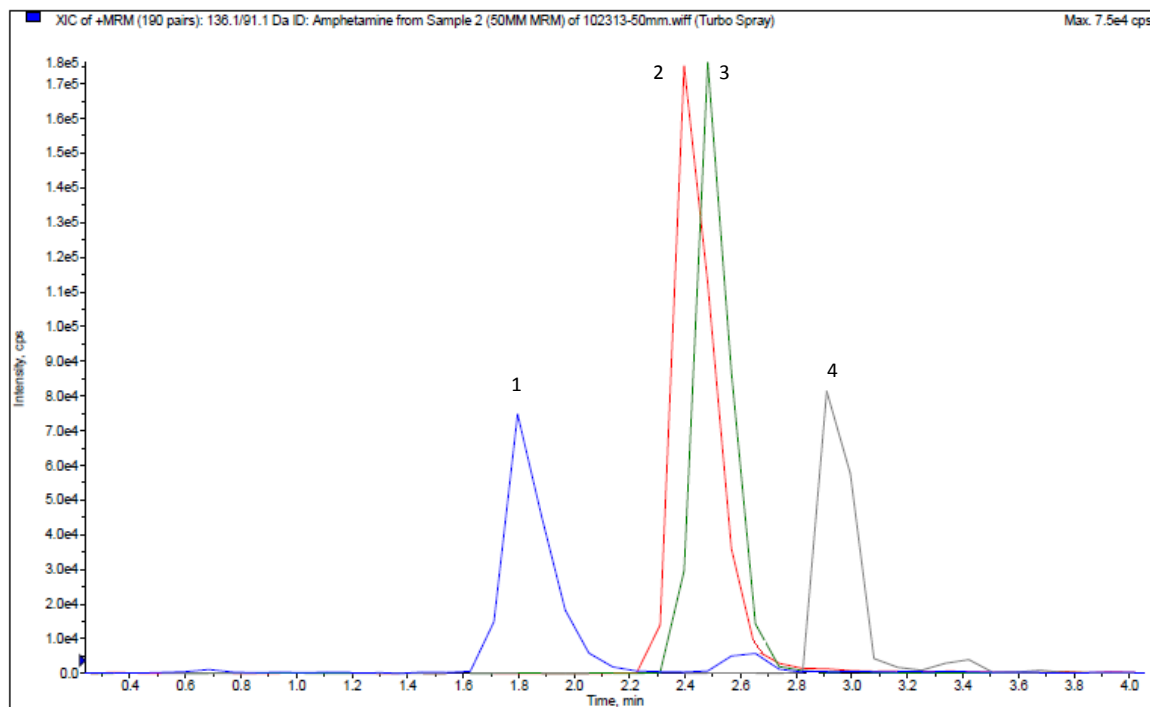
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

## Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxine	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

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

**Flow Rate:** 0.5 mL/minute

**Polarity:** Positive

**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

### 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

MDMA/MDA/MDEA

OPIATES(7)

METHADONE/EDDP

SYMPATHOMIMETICS

MEPERIDINE/NORMEPERIDINE

PCP

COCAINE/BZE

TCA'S(7)

CYCLOBENZAPRINE

FENTANYL/NORFENTANYL

SERTRALINE TRAMADOL/NORTRAM

DIPHENHYDRAMINE

CITALOPRAM

CLONIDINE