



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

Part #

CSDAU – CLEAN SCREEN® DAU

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

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 ANTIDEPRESSANTS:

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

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

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

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

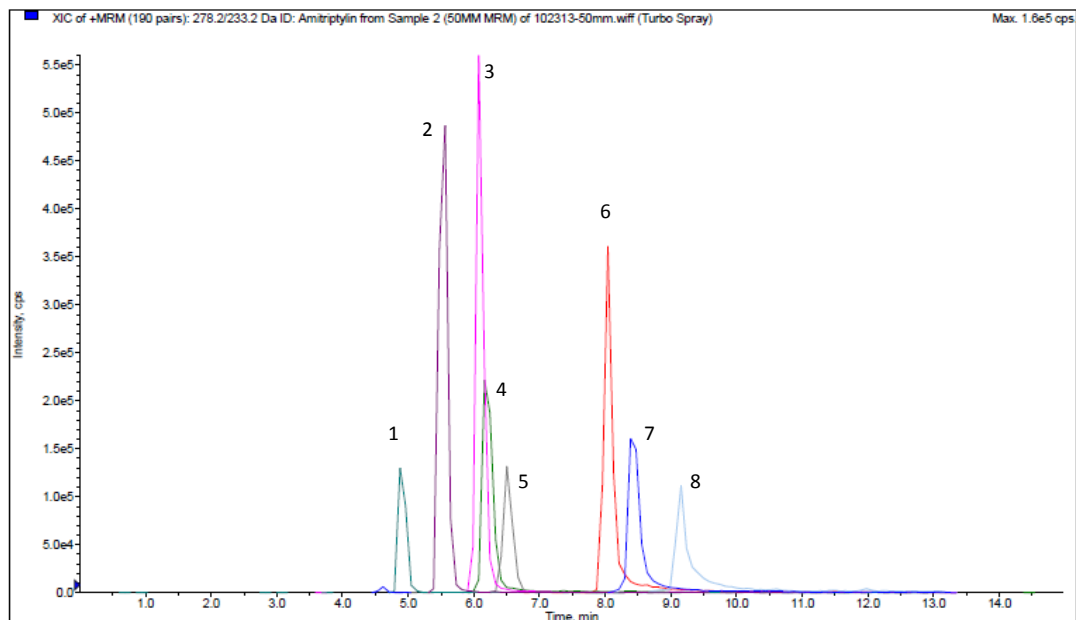
Alternate Derivatization

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

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM

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 |

PARAMETERS

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

Flow Rate: 0.5 mL/minute

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

Mobile Phase B: 0.1% Formic Acid in Methanol

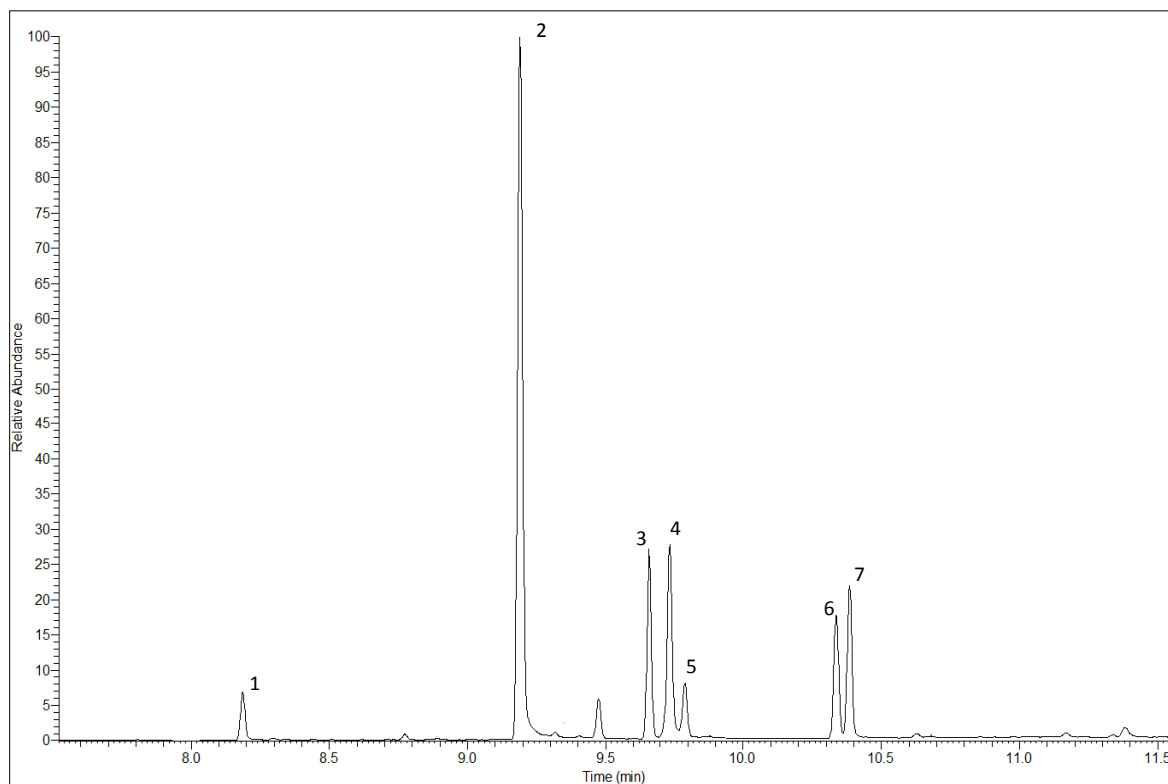
Polarity: Positive

Gradient:

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

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



| Analyte | Quantify Ion | Qualifier Ion 1 | Qualifier Ion 2 | Relative Retention Time minutes |
|------------------|--------------|-----------------|-----------------|---------------------------------|
| 1. Fluoxetine | 309 | 91 | 104 | 8.19 |
| 2. Venlafaxine | 134 | 179 | 202 | 9.19 |
| 3. Amitriptyline | 115 | 203 | 202 | 9.66 |
| 4. Nortriptyline | 189 | 202 | 115 | 9.72 |
| 5. Imipramine | 193 | 280 | 234 | 9.77 |
| 6. Sertraline | 274 | 262 | 159 | 10.34 |
| 7. Citalopram | 324 | 208 | 238 | 10.39 |

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30m x 0.25mm (0.25 μ m) TG-1MS

Injector: 1 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 70 $^{\circ}$ C (0.5) to 320 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (2 minutes)

Carrier gas: Carrier Gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C