



**GABAPENTIN/PREGABALIN/BACLOFEN IN BLOOD,
PLASMA/SERUM BY LC-MS/MS OR GC-MS 200 mg
CLEAN SCREEN® DAU EXTRACTION COLUMN**

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

Or

SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS

SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. PREPARE SAMPLE:

To 0.2-0.5 mL of sample add 1 mL of acetone dropwise while vortexing

Add internal standards

Mix/vortex and let stand for 5 minutes

Transfer organic phase to clean tube

Evaporate to dryness.

Add 3 mL of 100 mM HCl

Vortex mix and centrifuge as appropriate

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

1 x 1 mL 100 mM HCl

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 Ethyl Acetate

1 x 3 mL Hexane

Dry column (10 minutes at full vacuum or pressure)

5. ELUTE GABAPENTIN/PREGABALIN/BACLOFEN:

1 x 3 mL CH₃OH containing 2% NH₄OH

Collect eluate at 1 to 2 mL/minute

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of BSTFA w/1% TMCS;
Cap and heat at 70 °C for 30 minutes;
Remove and allow to cool.
Inject 1-2 µL

Alternate Derivatization

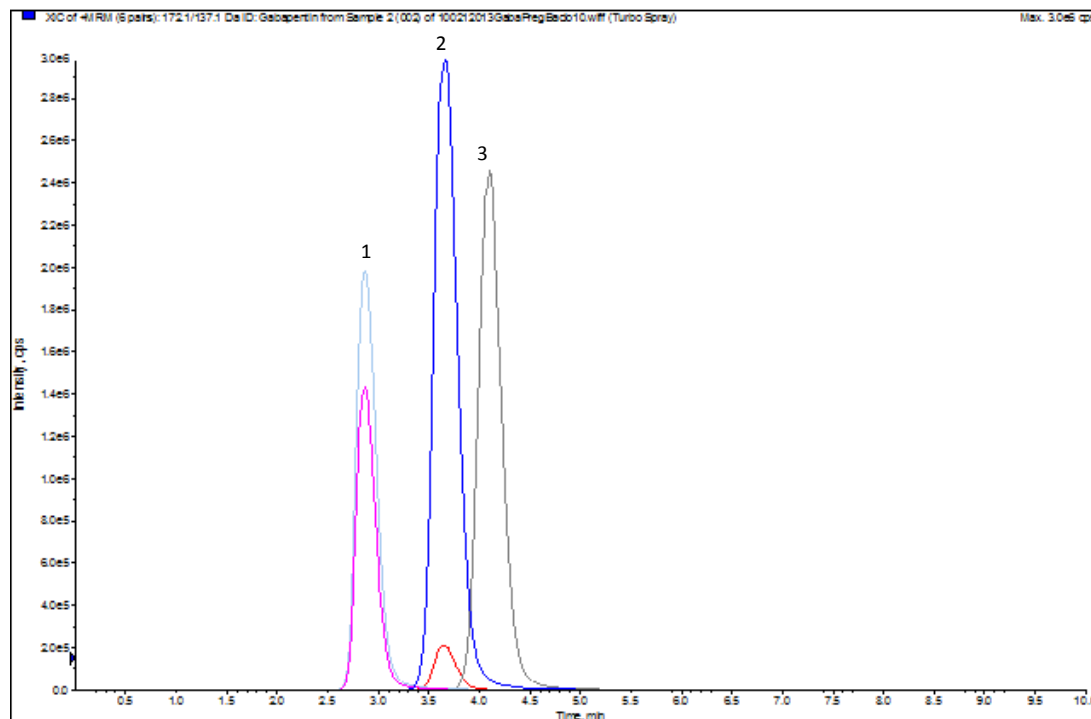
- 50 µL of Ethyl Acetate and 50 µL of MTBSTFA w/1% TBDMCS

GC-MS IONS

Compound	Primary	Secondary	Tertiary
Gabapentin-TMS	210	225	182
Gabapentin D ₁₀ -TMS	220	235	192

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Pregabalin	160.1	97.1	2.96
2. Gabapentin	172.1	67.1	3.66
3. Baclofen	214.0	150.8	4.32

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: 10 µL

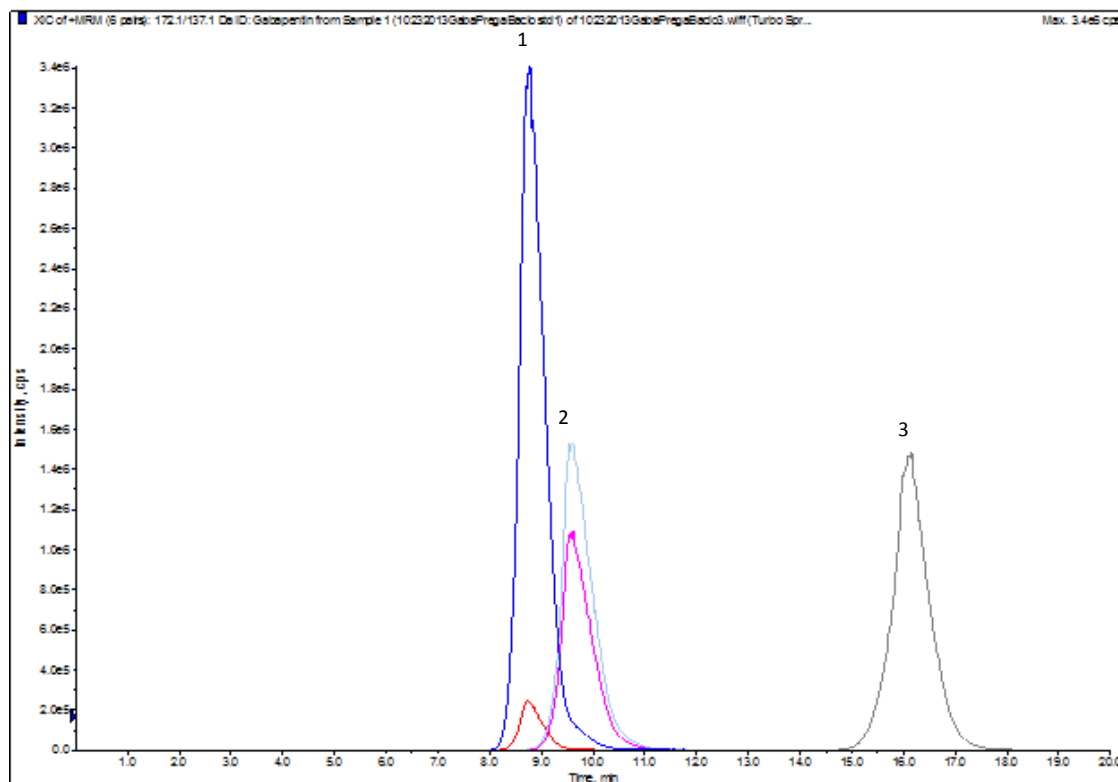
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	85	15
11.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Gabapentin	172.1	67.1	8.78
2. Pregabalin	160.1	97.1	9.56
3. Baclofen	214.0	150.8	16.10

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: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	85	15
20.00	STOP	