

Determination of Gabapentin in Whole Blood Samples Using Solid Phase Extraction and LC-MS/MS



UCT Part Numbers

SLDAGDC21-5UM
SELECTRA® DA (10 mm x 2.1 mm)
Guard Column

SLDA100ID21-5UM
SELECTRA® DA (100 mm x 2.1
mm, 5 µm) LC Column

CSDAU206
CLEAN SCREEN® DAU
200 mg / 6 mL

Procedure:

1. Sample Preparation

- To 0.5-1.0 mL of whole blood add appropriate internal standard* and mix.
- Add 1 mL of acetone dropwise while vortexing.
- Centrifuge at 3000 rpm for 10 minutes.
- Transfer organic phase to a clean glass tube.
- Evaporate to dryness < 40 °C.
- Dissolve residue in 3 mL of 0.1 M HCl.

2. Condition Extraction Column

- Add 1 x 3 mL CH₃OH and draw through the column without allowing the sorbent bed to go dry.
- Add 1 x 3 mL D.I. H₂O and draw through the column without allowing the sorbent bed to go dry.
- Add 1 x 1 mL 0.1M HCl and draw through the column without allowing the sorbent bed to go dry.

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

- Load sample at 1 to 2 mL/minute

4. Wash Column

- Add 1 x 3 mL DI H₂O and draw through the column.
- Add 1 x 3 mL ethyl acetate and draw through the column.
- Add 1 x 3 mL hexane and draw through the column.
- Dry column for 10 minutes at > 10 inches Hg.

5. Elute Gabapentin

- 1 x 3 mL CH₃OH containing 2% NH₄OH.
- Collect eluate at 1 to 2 mL / minute.

6. Dry Eluate

- Evaporate to dryness under nitrogen < 40°C.
- Reconstitute in 100 µL of mobile phase

7. Inject 1-2 µL onto GC-MS



Instrument Conditions

Column	Selectra [®] DA 100 x 2.1 mm; 5 µm
Column Temperature	40°C
Mobile Phase	CH ₃ OH w/ 0.1% Formic acid : DI H ₂ O w/ 0.1% Formic acid (30:70)
Injection Volume	10 µL
Flowrate	0.4 mL / minute
Detector	API 4000 MS/MS

Mass Spec Parameters

Ion Source	ESI
Ion Mode	Positive
Ion Spray Voltage	4500V
Curtain Gas	10
Gas 1	40
Gas 2	40
CAD Gas	Medium
Source Temp	650 °C
Mode	Positive

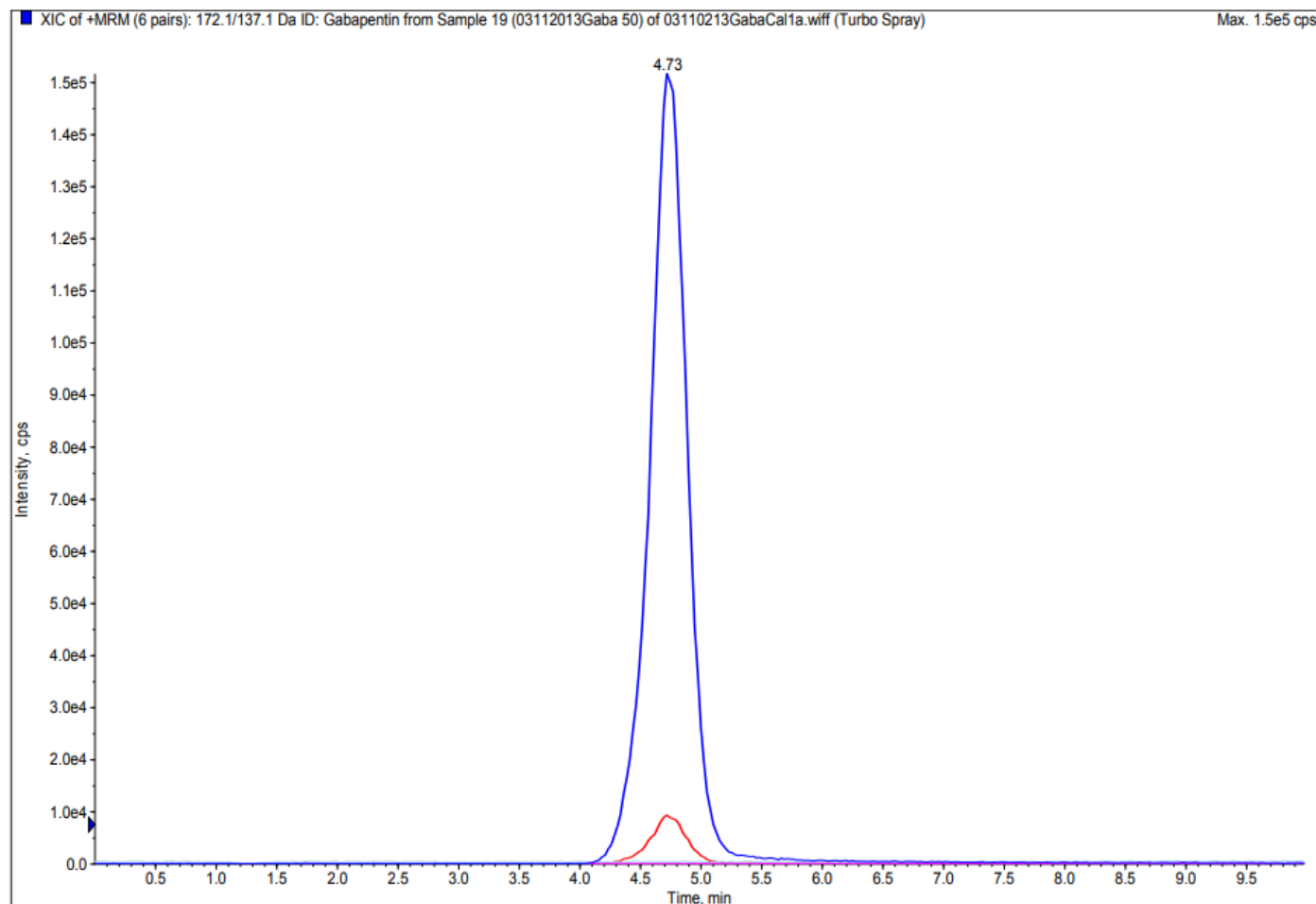
Mass Spec Table

Compound	RT (min)	Q1	Q3	DP	EP	CE	CXP
Gabapentin (1)	4.73	172.1	137.1	50	10	25	8
Gabapentin (2)	4.73	172.1	67.1	50	10	25	8
Gabapentin-D10 (1)	4.68	182.1	147.1	65	12	20	4
Gabapentin-D10 (2)	4.68	182.1	77.1	65	12	20	4

Note: Q1 = Precursor Ion; Q3= Product Ion; DP= Declustering Potential; EP= Entrance Potential; CEP= Collision Entrance Potential; CE= Collision Energy; CXP= Collision Exit Potential.



Chromatogram of Gabapentin

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