



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

Part #

ZSDAU020 CLEAN SCREEN[®] DAU 200 mg, 10 mL Tube

STMPAH-0-1 – SELECTRA-SIL[®] TMPAH

SLDA501D21-5UM – Selectra[®] DA HPLC Column, 50 x 2.1 mm, 5 μ 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

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 1 mL 100 mM Acetic Acid
Dry column (5 minutes at full vacuum or pressure)
1 x 2 mL hexane

5. ELUTE BARBITURATES:

1 x 3 mL Ethyl Acetate: Hexane (50:50)
collect eluate at 1 to 2 mL/minute

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

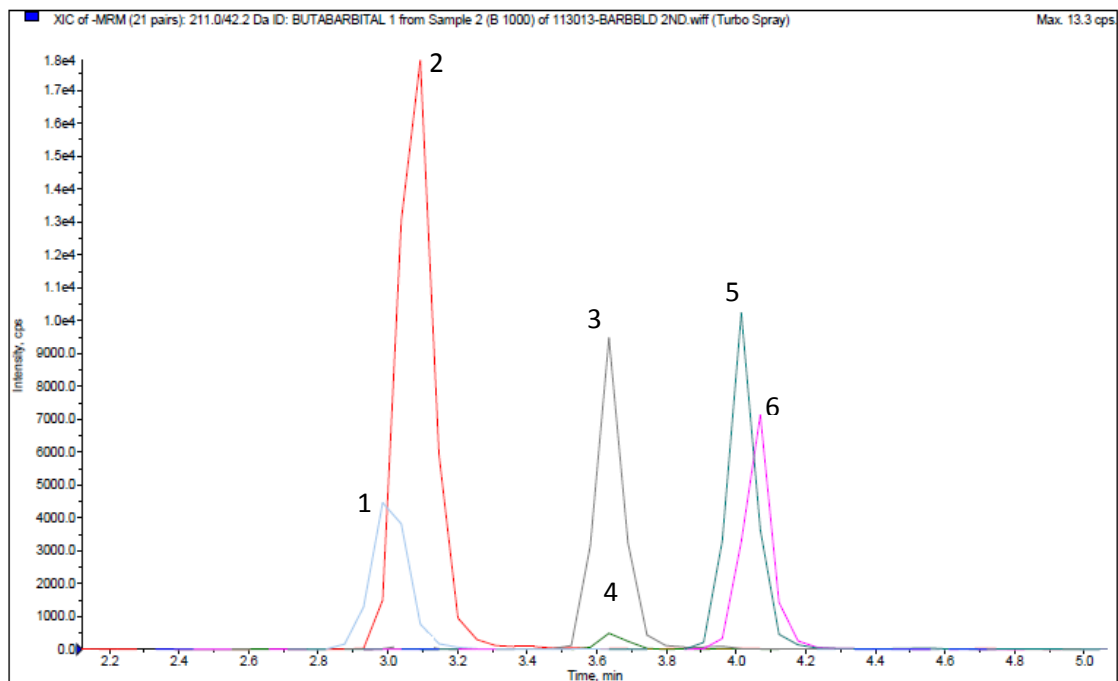
- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 100 μ L of Ethyl Acetate

Alternate Derivatization

Add 25 μ L of 0.2 M TMPAH
Reaction occurs in injection port

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Phenobarbital	230.8	42.0	3.0
2. Butalbital	223.0	42.1	3.1
3. Amobarbital	225.0	42.0	3.6
4. Pentobarbital	225.0	42.1	3.6
5. Secobarbital D5	242.1	42.0	4.0
6. Secobarbital	237.0	42.0	4.1

PARAMETERS

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

Flow Rate: 0.6 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

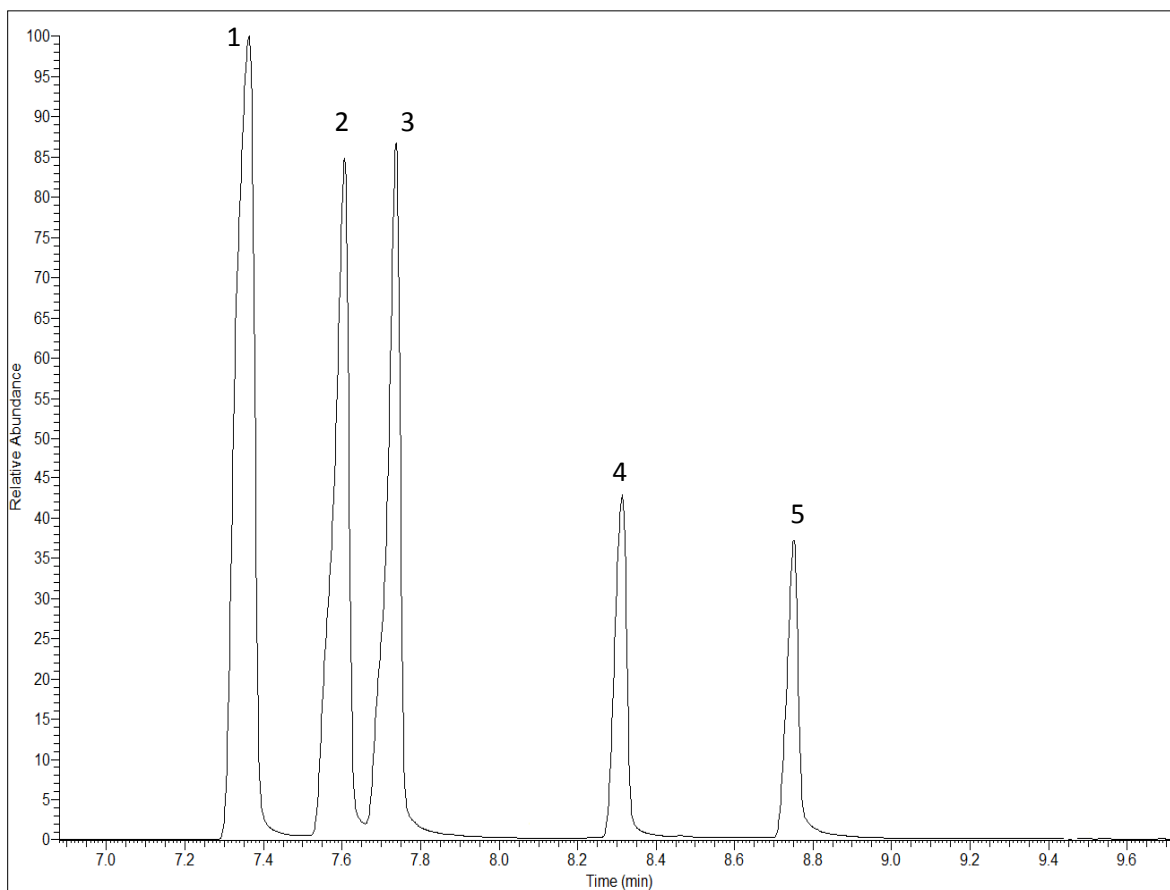
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	90	10
6.00	50	50
6.01	10	90
7.00	90	10
7.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

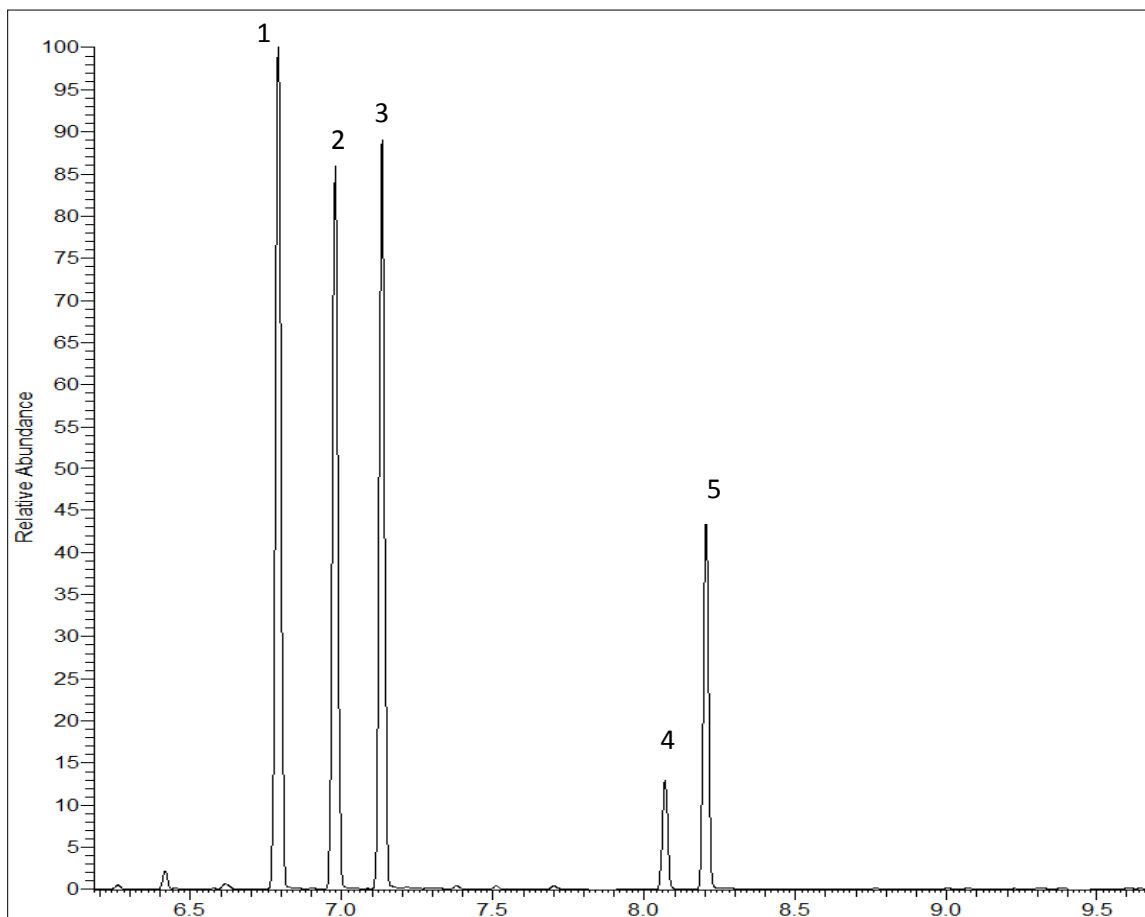
CHROMATOGRAM 1 (UNDERIVATIZED)



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Butabarbital	156	141	157	7.36
2. Amobarbital	156	141	157	7.61
3. Pentobarbital	156	141	197	7.74
4. Hexobarbital*	221	157	236	8.31
5. Phenobarbital	204	232	117	8.75

*Suggested internal standard for GC/MS

CHROMATOGRAM 2 (TMPAH)



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Butabarbital	169	184	211	6.79
2. Amobarbital	169	184	185	6.98
3. Pentobarbital	169	184	112	7.13
4. Hexobarbital	235	251	171	8.07
5. Phenobarbital	232	146	175	8.21
Phenobarbital D ₅	237	151	-	

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 °C

Oven temperature program: 70 °C (0.5) to 320 °C (25 °C/minute): hold (2 minutes)

Carrier gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 °C, MS Source: 350 °C, MS Quad: 150 °C