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

UCT Featured Products

ZSDAU020
CLEAN SCREEN® DAU
200 mg, 10 mL Tube

STMPAH-0-1
SELECTRA-SIL®
TMPAH

SLDA50ID21-5UM
Selectra® DA HPLC Column,
50 x 2.1 mm, 5 µm

Procedure:

1) Prepare Sample

a) To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
b) Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate.
c) Mix/vortex and let stand for 5 minutes.
d) Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
e) Sample pH should be 6.0 ± 0.5.
f) Sample pH should be 6.0 ± 0.5. Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
g) Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2) Condition Clean Screen® Extraction Column

a) 1 x 3 mL CH_3OH.
b) 1 x 3 mL D.I. H_2O.
c) 1 x 3 mL 100 mM phosphate buffer (pH 6.0).
NOTE: Aspirate at full vacuum or pressure.

3) Apply Sample

a) Load at 1 to 2 mL/minute.

4) Wash Column

a) 1 x 3 mL D.I. H_2O.
b) 1 x 1 mL 100 mM Acetic Acid.
c) Dry column (5 minutes at full vacuum or pressure).
d) 1 x 2 mL hexane.

5) Elute Barbiturates

a) 1 x 3 mL Ethyl Acetate: Hexane (50:50).
b) Collect eluate at 1 to 2 mL/minute.

6) Dry Eluate

a) 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 100 µL of Ethyl Acetate.

Alternate Derivatization
Add 25 µL of 0.2 M TMPAH.
Reaction occurs in injection port.

FORENSICS

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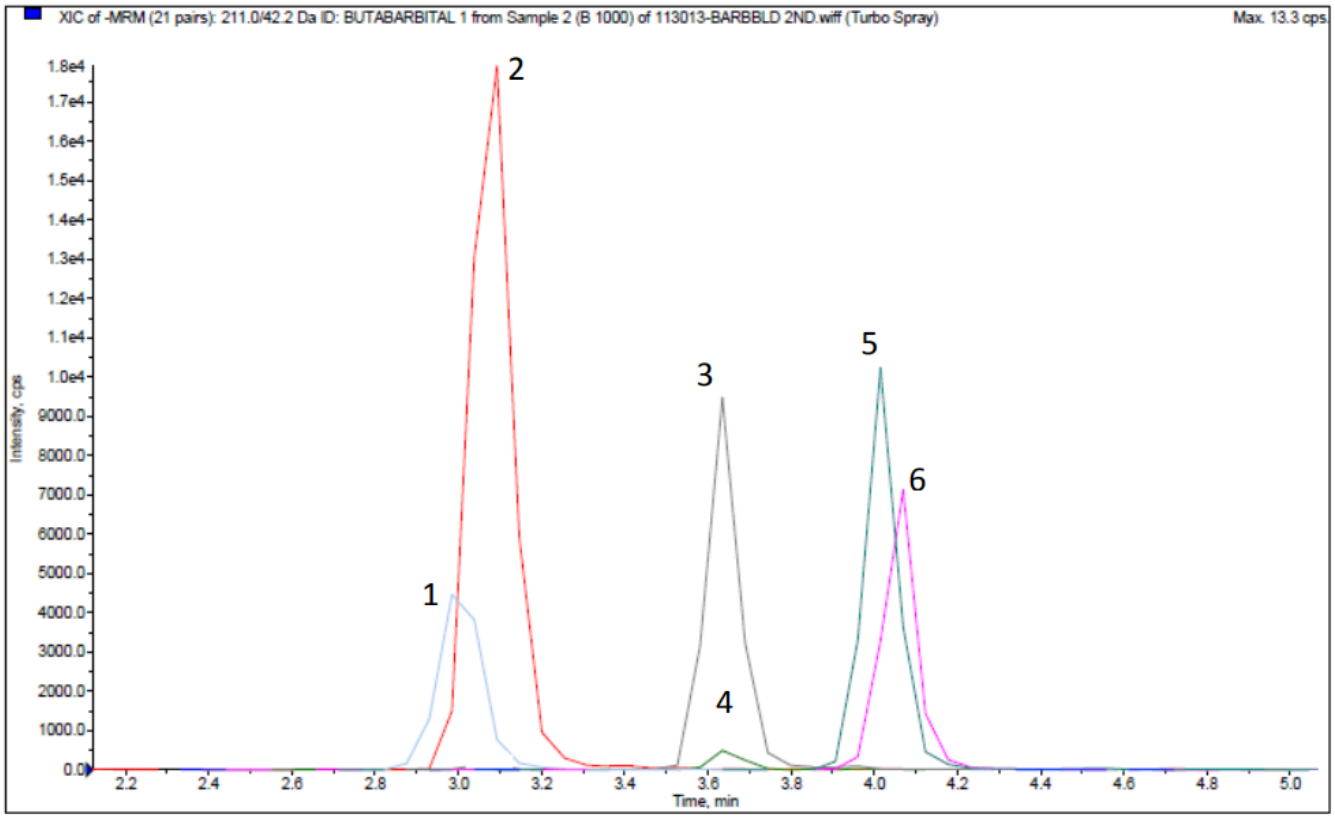
INSTRUMENT CONDITIONS (LC-MS/MS):



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

LC-MS/MS Parameters	
Mobile Phase A	0.1% Formic Acid in D.I. H_2O
Mobile Phase B	0.1% Formic Acid in Methanol
Flow Rate	0.6 mL/minute
Flow Rate	100 µL
Injection Volume	10 µL
Instrument	API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC
LC Column	Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Polarity	Positive

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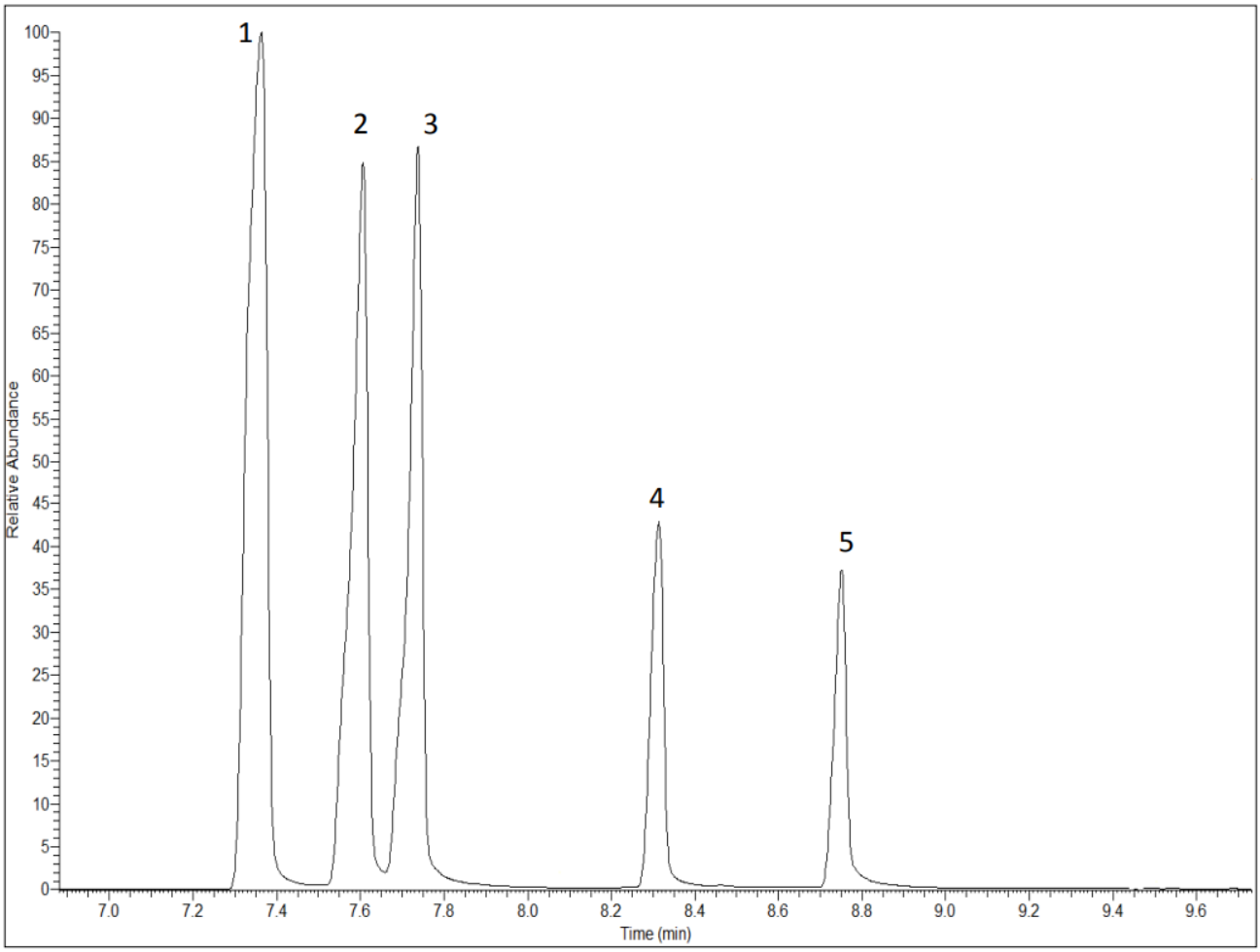
GRADIENT:

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

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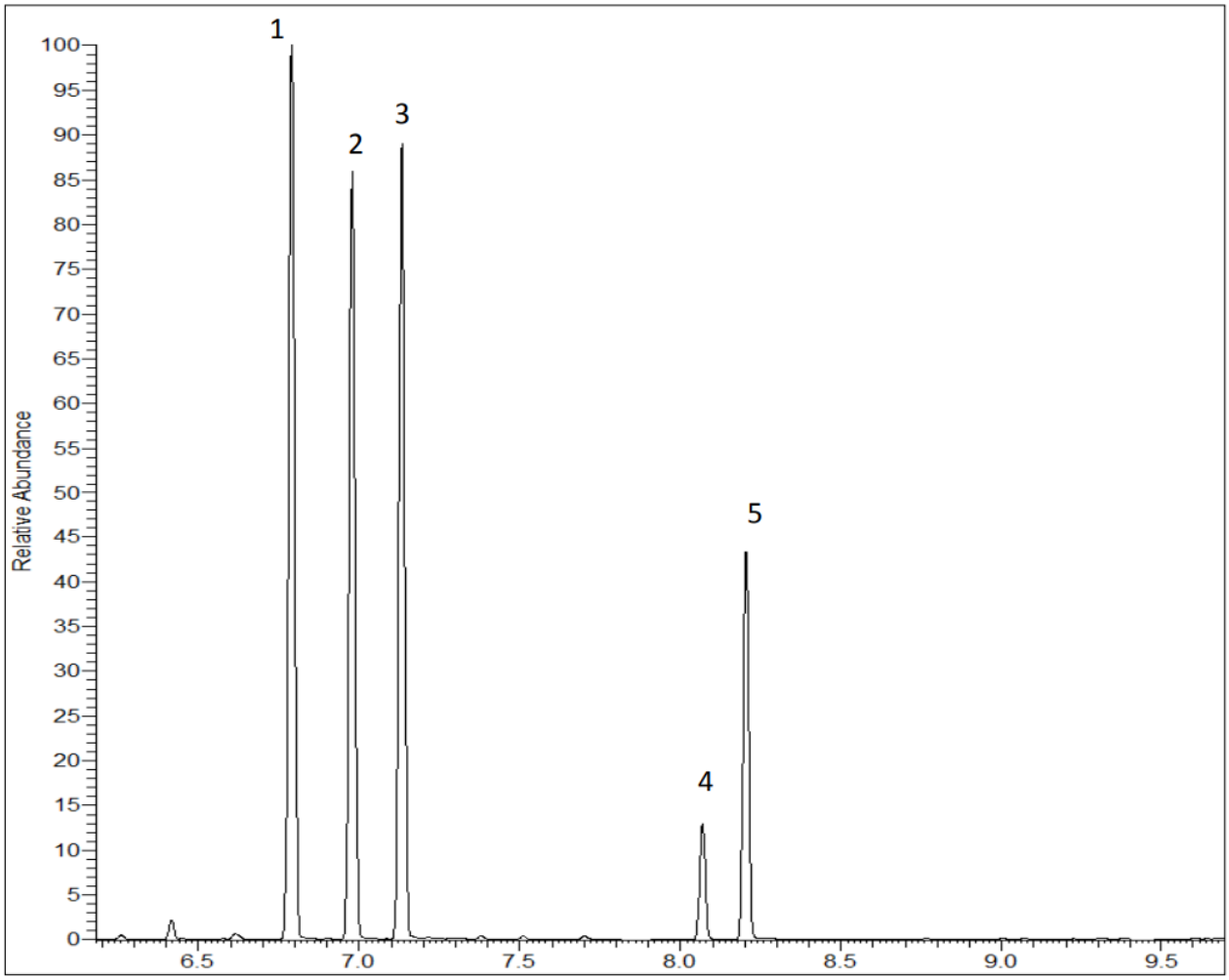
CHROMATOGRAM 2 (TMPAH)



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	-	-

GC-MS Parameters	
GC/MS	0.1% Formic Acid in D.I. H_2O
GC capillary column	0.1% Formic Acid in Methanol
Injector	0.6 mL/minute
Oven temperature program	100 µL
Carrier gas	10 µL
MSD condition	API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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