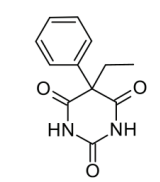
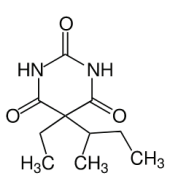
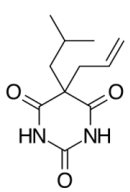
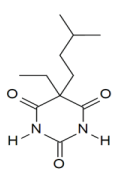
Barbiturate and THCA in Urine

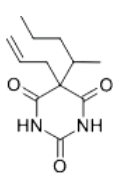
Compounds :

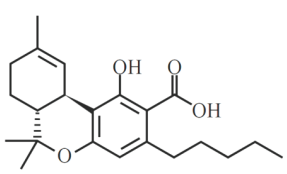

Phenobarbital


Butabarbital


Butalbital


Amobarbital


Secobarbital


THCA

OROCHEM
Molecular Purification by Design

For Free Consultation,
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Molecular Purification by Design

SPE EXTRACTION METHOD:

Panthera Deluxe 30 mg/cc SPE cartridge
(catalog # SYPA6030-01)

HPLC METHOD:

Column:	Orochem Gazelle C18 UHPLC column 2.1 x 50 mm, 1.5 µm
Catalog Number:	G17BQ-931
Mobile Phase:	A: 0.1 % formic acid B: 0.1 % formic acid in methanol
Gradient:	Initial 30% B then gradient to 40% B at 0.5 min, to 60% B at 2.5 min, to 85% B at 4.0 min, hold until 4.7 min, then switch to 95% B at 4.71 min, hold until 5.6 min, then switch to 30% B at 5.61 min and hold until 7.0 min
Flow rate:	0.3 mL/min
Injection volume:	20 uL
SMRM Detection:	API 4500

Enzyme Hydrolysis:
Pipette 100 µL urine into each collection plate well. Add 50 µL of Internal Standard spiking solution, 20 µL of glucoronidase solution and 50 µL of 200 mM pH 6.8 ammonium acetate buffer. Mix. Incubate at 55 ^{O}C for 30 minutes.

▼

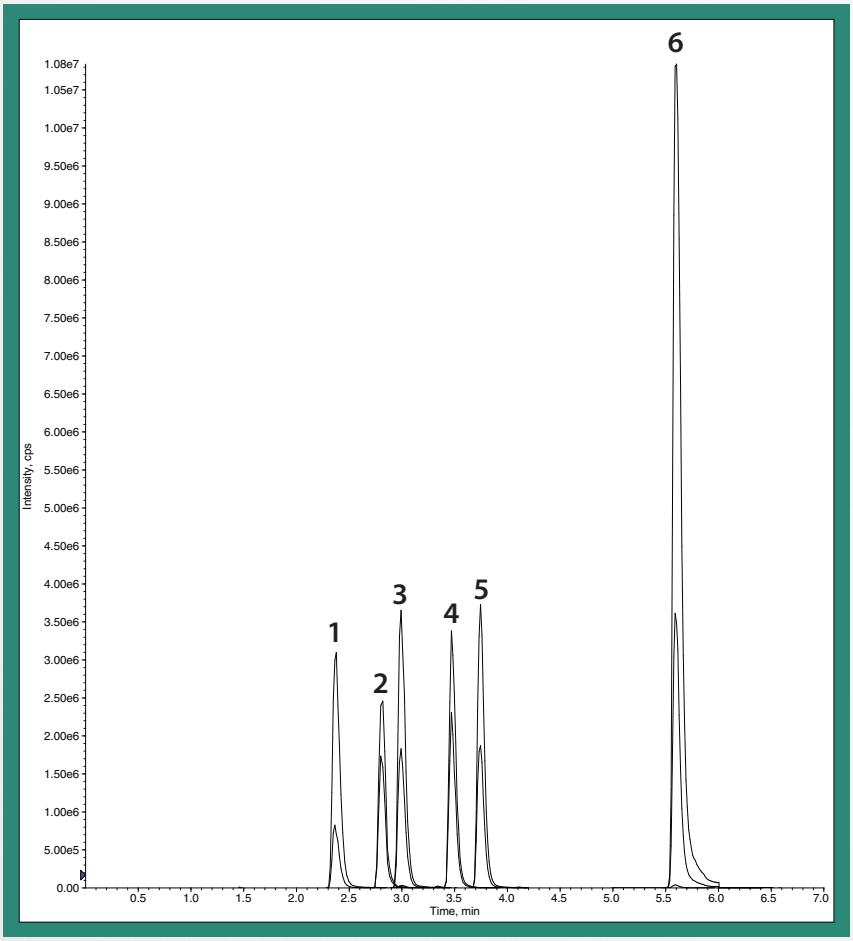
SPE Condition:
1 ml of methanol, followed by 1 ml of water

▼

Load:
Load hydrolyzed sample solution on SPE cartridge

▼

Wash, Elute, Evaporate and Reconstitute:
Wash SPE with 1 mL of water followed by 1 mL of 20% methanol and thoroughly flush with air. Elute with two batches of 0.75 mL of methanol. Evaporate to dryness and reconstitute in 0.37 mL of 30% methanol.



1	Phenobarbital
2	Butabarbital
3	Butalbital
4	Amobarbital
5	Secobarbital
6	THCA

Results:

	Recovery%	CV%
Phenobarbital	101.3	5.2
Butabarbital	95.9	6.4
Butalbital	97.9	6.2
Amobarbital	104.1	5.6
Secobarbital	100.5	6.9
THCA	88.5	7.5

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