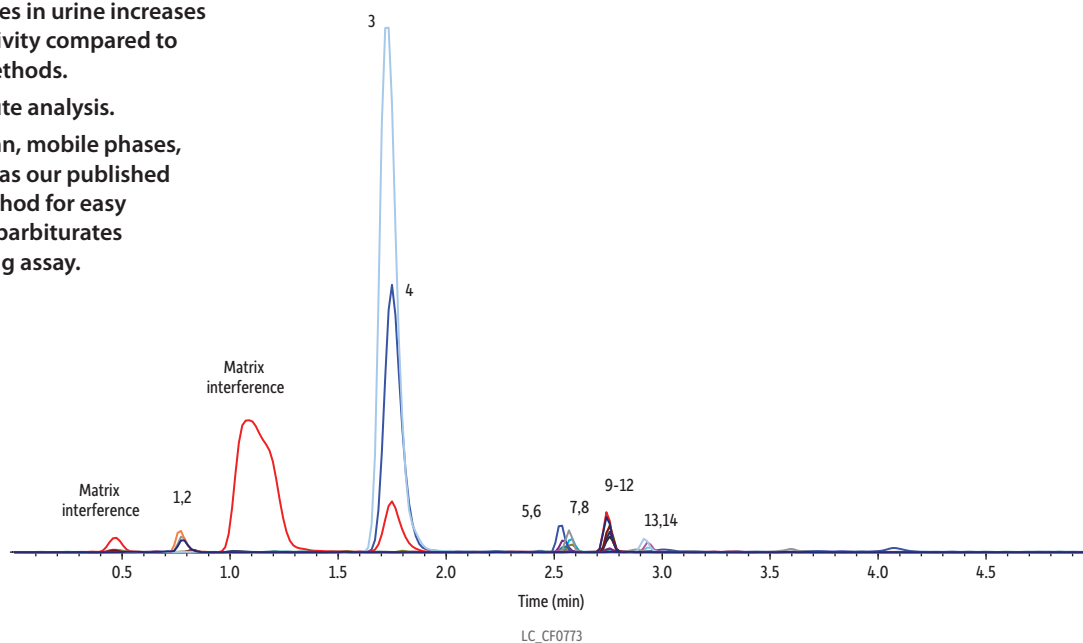


Simultaneous Analysis of Alcohol Metabolites and Barbiturates in Urine by LC-MS/MS

- Single method for EtG/EtS and 5 barbiturates in urine increases lab productivity compared to separate methods.
- Fast, 5-minute analysis.
- Same column, mobile phases, and buffers as our published EtG/EtS method for easy addition of barbiturates to an existing assay.



Peaks	tr (min)	Conc. (ng/mL)	Precursor Ion	Product Ion 1	Product Ion 2
1. EtG-d5	0.76	100	225.9	84.7	-
2. EtG	0.80	500	220.9	74.9	85
3. EtS-d5	1.70	100	129.7	97.7	-
4. EtS	1.78	500	124.8	96.8	79.7
5. Phenobarbital-d5	2.54	100	236.0	42.0	-
6. Phenobarbital	2.55	500	231.2	188.0	42.0
7. Butalbital-d5	2.57	100	228	42.0	-
8. Butalbital	2.58	500	223	180.0	42.0
9. Amobarbital-d5	2.74	100	230	42.0	-
10. Pentobarbital-d5	2.74	100	230	42.0	-
11. Amobarbital	2.75	500	225	182.0	42.0
12. Pentobarbital	2.76	500	225	182.0	42.0
13. Secobarbital-d5	2.93	100	242	42.0	-
14. Secobarbital	2.93	500	237	194.0	42.0

Column Raptor EtG/EtS (cat.# 9325A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: UltraShield UHPLC PreColumn filter 0.2 µm (cat.# 25809)
Temp.: 30 °C

Standard/Sample Ethyl-β-D-glucuronide (cat.# 34101)
 Ethyl-β-D-glucuronide-d5 (cat.# 34102)
 Ethyl sulfate sodium salt (cat.# 34103)
 Ethyl sulfate-d5 sodium salt (cat.# 34104)
 Phenobarbital (cat.# 34037)
 Pentobarbital (cat.# 34036)
 Butalbital (cat.# 34032)
 Secobarbital (cat.# 34038)

Diluent: Water, 0.1% formic acid
Inj. Vol.: 10 µL

Mobile Phase
A: Water, 0.1% formic acid
B: Acetonitrile, 0.1% formic acid

Detector MS/MS
Ion Source: Electrospray
Ion Mode: ESI-

Sample Preparation Human urine (alcohol free) was fortified with EtG, EtS, phenobarbital, butalbital, amobarbital, pentobarbital, and secobarbital in order to prepare the calibration standards and QC samples. 50 µL of urine was diluted with 950 µL of the working internal standard (100 ng/mL EtG-d5, EtS-d5, and barbiturates-d5 in 0.1% formic acid in water). Samples were vortexed at 3500 rpm for ten seconds to mix, centrifuged at 3000 rpm for five minutes at 10 °C, and injected for LC-MS/MS analysis.

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	95	5
1.20	0.5	80	20
1.21	0.5	65	35
3.00	0.5	55	45
4.00	0.5	95	5
5.00	0.5	95	stop