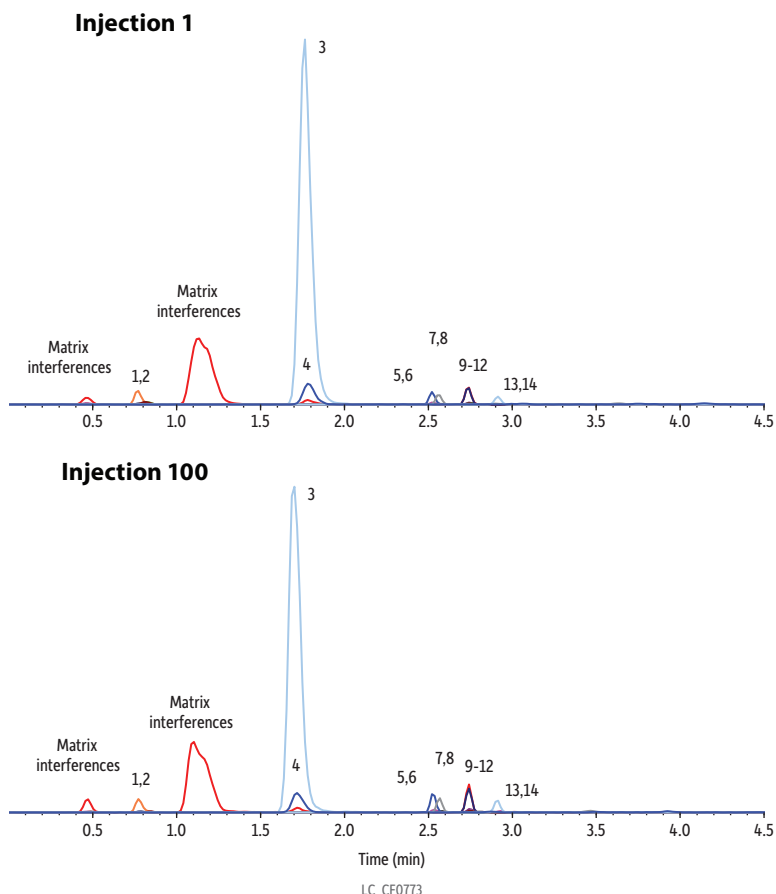


Raptor EtG/EtS Column Robustness (100 Sample Injections)

- Stable peak shape, retention time, and intensity over 100 sample injections.
- Consistent maximum system pressure indicates no column clogging occurred.



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

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

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 at 100 ng/mL to prepare the standard. 50 µL of urine from the 100 ng/mL standard 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.