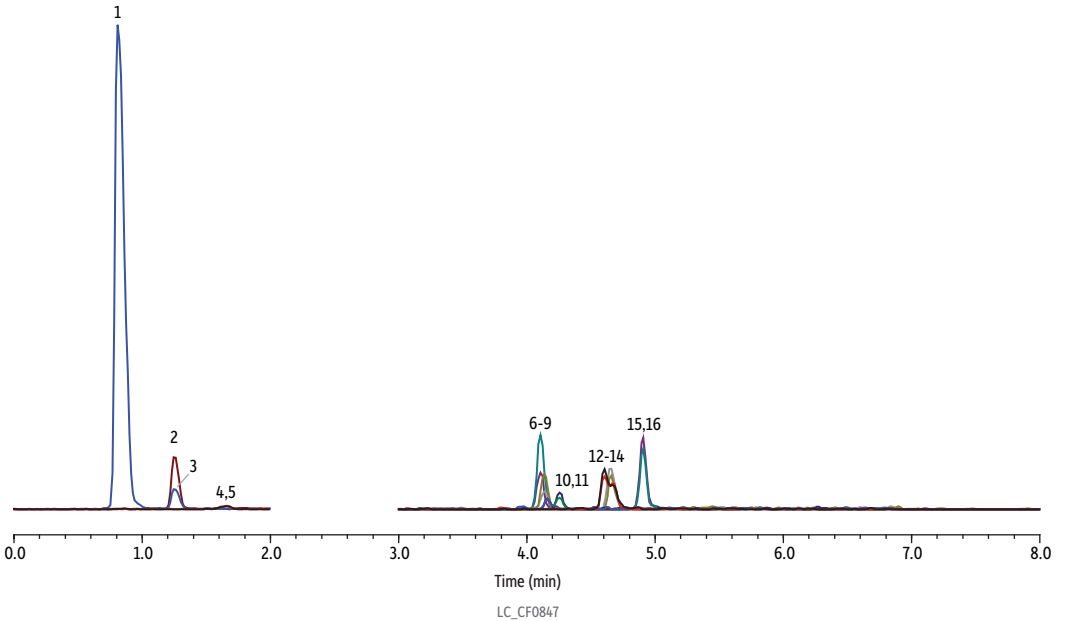
EtG, EtS, and Barbiturates in Chronic Kidney Disease Urine on Force Biphenyl by LC-MS/MS



Peaks	t_R (min)	Conc. (ng/mL)	Precursor	Product 1	Product 2
1. Urinary interference	0.89	-	125.0	80.1	-
2. EtS	1.22	100	125.0	96.8	80.1
3. EtS-d5	1.21	1000	130.0	97.9	-
4. EtG	1.65	100	221.1	75.1	85.2
5. EtG-d5	1.64	2000	226.0	75.0	-
6. Phenobarbital-d5	4.15	4000	236.0	42.0	-
7. Phenobarbital	4.18	200	231.2	42.1	188.1
8. Butabarbital-d5	4.18	4000	216	41.9	-
9. Butabarbital	4.19	200	211.0	168.3	42.0
10. Butalbital-d5	4.23	4000	228.0	185.3	-
11. Butalbital	4.27	200	223.0	42.1	180.3
12. Amobarbital	4.63	200	225.0	182.3	42.1
13. Amobarbital-d5	4.69	4000	230.0	42.0	-
14. Pentobarbital	4.71	200	225.0	182.3	42.1
15. Secobarbital-d5	4.92	4000	242.0	199.3	-
16. Secobarbital	4.94	200	237.0	42.1	194.2

Column Force Biphenyl (cat.# 962931E)
Dimensions: 100 mm x 3 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Guard Column: Force Biphenyl EXP guard cartridge 5 mm, 3 mm ID, 3 µm (cat.# 962950253)
Temp.: 30 °C
Standard/Sample
Ethyl sulfate sodium salt (EtS) standard (cat.# 34103)
Ethyl sulfate-d5 sodium salt (EtS-d5) standard, 1000 µg/mL, methanol, 1 mL/ampul (cat.# 34104)
Ethyl-beta-D-glucuronide (EtG) standard, 1000 µg/mL, methanol, 1 mL/ampul (cat.# 34101)
Ethyl-beta-D-glucuronide-d5 (EtG-d5) standard, 1000 µg/mL, methanol, 1 mL/ampul (cat.# 34102)
Butalbital standard, 1000 µg/mL, P&T methanol, 1 mL/ampul (cat.# 34032)
Phenobarbital standard, 1000 µg/mL, P&T methanol, 1 mL/ampul (cat.# 34037)
Pentobarbital standard, 1000 µg/mL, P&T methanol, 1 mL/ampul (cat.# 34036)
Secobarbital standard, 1000 µg/mL, P&T methanol, 1 mL/ampul (cat.# 34038)
Diluent: Water
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.8	100	0
1.75	0.8	50	50
5.50	0.8	15	85
6.00	0.8	0	100
6.01	0.8	100	0
8.00	0.8	100	0

Max Pressure: 427 bar
Detector Shimadzu 8060 MS/MS
Ion Source: Electrospray
Ion Mode: ESI-
Mode: MRM
Instrument Shimadzu Nexera X2
Sample Preparation Urine (50 µL) was aliquoted to a 2 mL centrifuge tube, fortified at either 100 ng/mL for EtG and EtS or 200 ng/mL for barbiturates, and was mixed with isotopically labeled internal standards (10 µL). Cold acetonitrile (150 µL) was added to the tube, vortexed for ~30 seconds, and centrifuged for 10 minutes at 4200 rpm. The supernatant (100 µL) was aliquoted to an autosampler vial (cat.#21143) and water (900 µL) was added before the sample was capped (cat.#24498), vortexed for ~30 seconds, and 10 µL injected onto the LC-MS/MS for analysis.
Notes An Ultra Shield UHPLC PreColumn Filter 0.2 µm frit (cat.#25811) was installed before the guard cartridge.