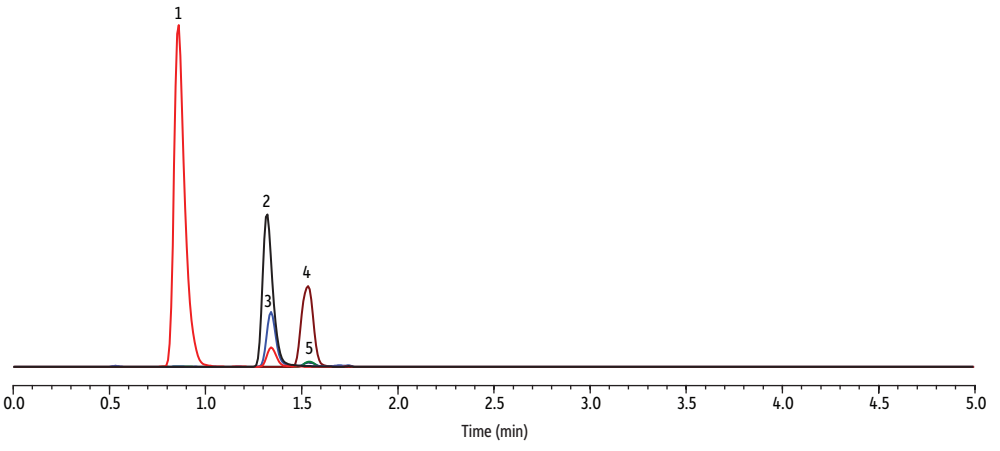
EtG and EtS in Chronic Kidney Disease Urine on Force Biphenyl by LC-MS/MS



Peaks	t_R (min)	Conc. (ng/mL)	Precursor	Product 1	Product 2
1. Urinary interference	0.89	-	125.0	80.1	-
2. EtS-d5	1.34	1000	130.0	97.9	-
3. EtS	1.34	100	125.0	96.8	80.1
4. EtG-d5	1.54	2000	226.0	75.0	-
5. EtG	1.54	100	221.1	75.1	85.2

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)
Diluent: Water
Conc.: 100 ng/mL in urine
Inj. Vol.: 10 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	%A	%B
0.00	100	0
1.74	0	100
3.50	0	100
3.51	100	0
5.00	100	0

Flow: *0.8 mL/min
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 50 ng/mL with EtG and EtS, and mixed with isotopically labeled internal standards (10 µL of 2,500 ppb of EtS-d5 and 5,000 ppb of EtG-d5). 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.
Valve position was directed to waste from 0.00-0.50 minutes and after 1.75 minutes.
*Flow rate was adjusted to 1 mL/min from 1.75 minutes to 3.50 minutes to flush contaminates from the analytical column to reduce matrix effects. Flow rate was switched back to 0.8 mL/min at 3.51 min.