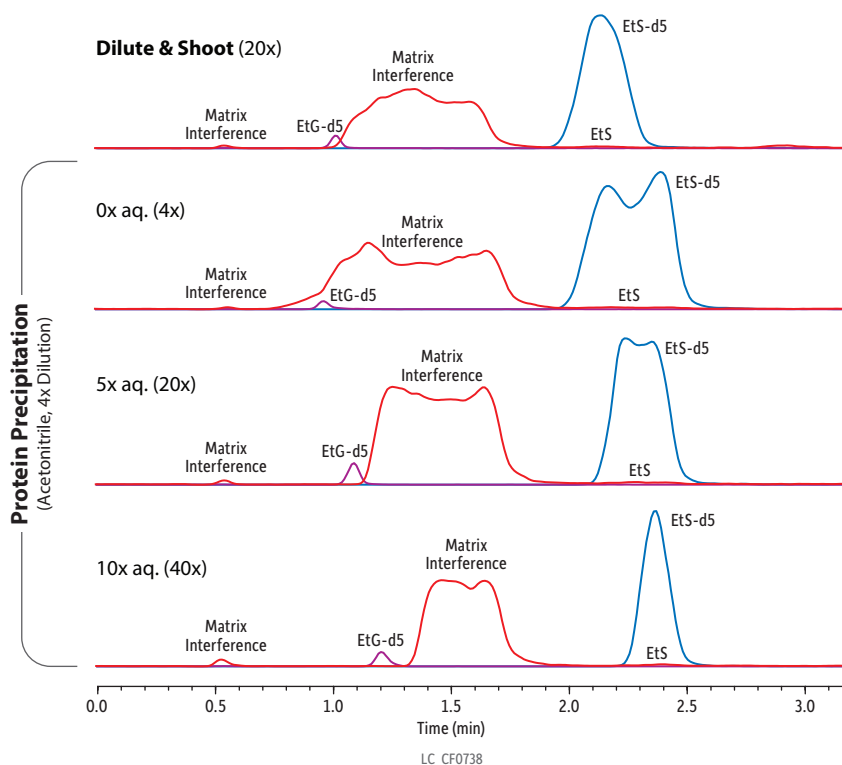


EtG/EtS: Impact of Sample Preparation on Retention and Peak Shape



Peaks	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Ethyl-β-D-glucuronide-d5 (EtG-d5)	200	226.2	85.0	-
2. Ethyl-β-D-glucuronide (EtG)	-	221.2	75.1	85.1
3. Ethyl sulfate-d5 (EtS-d5)	50	130.1	98.0	-
4. Ethyl sulfate (EtS)	-	125.1	97.1	80.0

Differences in sample preparation may cause varying retention times.

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 frit (cat.# 25809)
Temp.:	35 °C
Standard/Sample	Ethyl-β-D-glucuronide-d5 (EtG-d5) (cat.# 34102) Ethyl sulfate-d5 sodium salt (EtS-d5) (cat.# 34104)
Diluent:	0.01% Formic acid in water
Conc.:	Human urine sample containing endogenous EtG and EtS
Inj. Vol.:	10 μL
Mobile Phase	
A:	0.01% Formic acid in water
B:	0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	95	5
3.00	0.5	65	35
3.01	0.5	95	5
4.50	0.5	95	5

Detector	MS/MS
Ion Source:	Electrospray
Ion Mode:	ESI-
Mode:	MRM
Instrument	HPLC

Sample Preparation
Dilute and Shoot: A 50 μL aliquot was taken from a urine sample containing endogenous EtG and EtS and diluted 20 times with 950 μL of a working internal standard (200 ng/mL EtG-d5 and 50 ng/mL EtS-d5 in 0.01% formic acid in water). The sample was vortexed at 3000 rpm for 10 seconds to mix. The sample was then centrifuged at 4300 rpm for 10 minutes at 10 °C and the supernatant was injected.

Acetonitrile Precipitation: A 50 μL aliquot was taken from a urine sample containing endogenous EtG and EtS. 10 μL of internal standard (20 μg EtG-d5 and 5 μg EtS-d5) and 150 μL of acetonitrile were added to the aliquot. The sample was vortexed at 3000 rpm for 10 seconds to mix and centrifuged at 4300 rpm for 10 minutes at 10 °C. After centrifugation, 100 μL of the supernatant was aliquoted and injected for the 4x level (without further dilution) or diluted again with 400 μL (20x) or 900 μL (40x) of 0.01% formic acid in water, respectively. The samples were then vortexed at 3000 rpm for 10 seconds and injected.