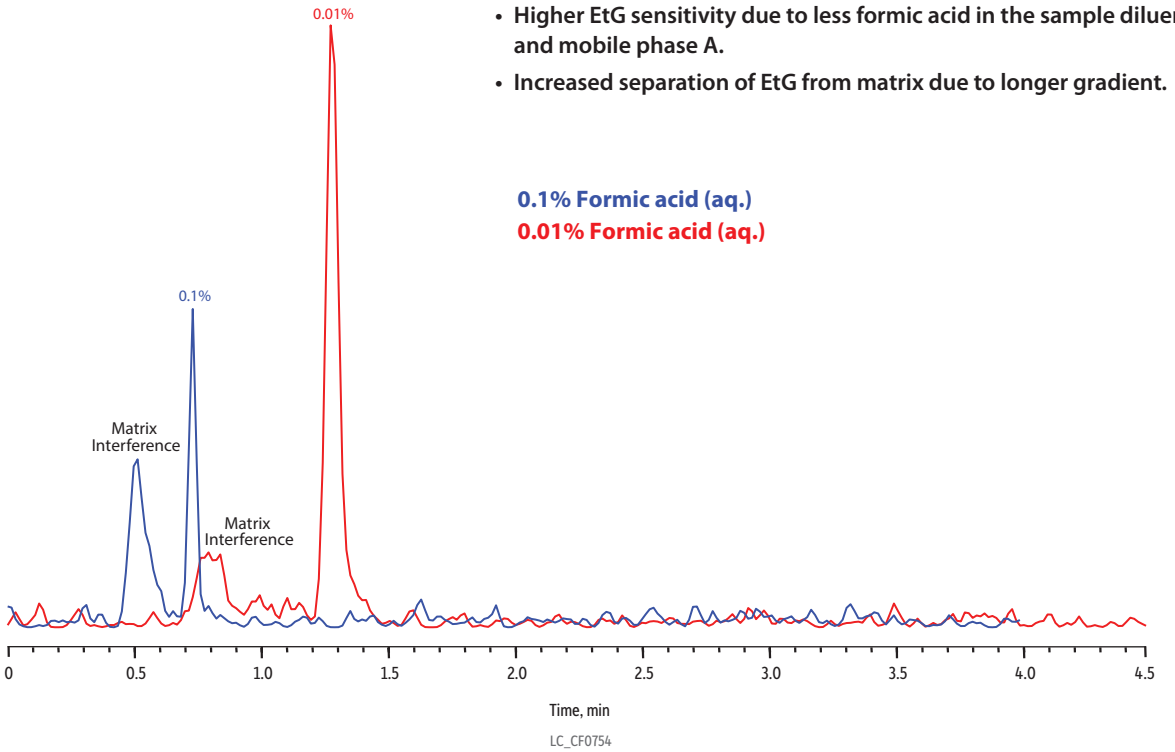
EtG Signal Sensitivity Comparison at 25 ng/mL in MS Gold Urine



- Higher EtG sensitivity due to less formic acid in the sample diluent and mobile phase A.
- Increased separation of EtG from matrix due to longer gradient.

0.1% Formic acid (aq.)
0.01% Formic acid (aq.)

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 (EtG) (cat.# 34101)
Conc.:	25 ng/mL
Inj. Vol.:	10 µL
Mobile Phase	
Flow:	0.5 mL/min
Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	HPLC
Sample Preparation	A 25 ng/mL standard was prepared in MS Gold urine. 50 µL aliquots of the standard were diluted with 950 µL of working internal standard (50 ng/mL EtS-d5/200 ng/mL EtG-d5 in either 0.1% formic acid or 0.01% formic acid in water to match the respective aqueous mobile phases). The samples were vortexed at 3500 rpm for 10 seconds to mix. The samples were then centrifuged at 3000 rpm for 5 minutes at 10 °C and analyzed by LC-MS/MS using their respective mobile phase conditions to compare EtG signal sensitivity between the methods.
Notes	**Method Comparison Details**

0.1% Aqueous Formic Acid Method (Blue)
- Sample diluent: 0.1% formic acid in water
- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in acetonitrile
- Gradient (%B): 0.00 min (5%), 2.50 min (35%), 2.51 min (5%), 4.00 (5%)

0.01% Aqueous Formic Acid Method (Red)
- Sample diluent: 0.01% formic acid in water
- Mobile phase A: 0.01% formic acid in water
- Mobile phase B: 0.1% formic acid in acetonitrile
- Gradient (%B): 0.00 min (5%), 3.00 min (35%), 3.01 min (5%), 4.50 (5%)