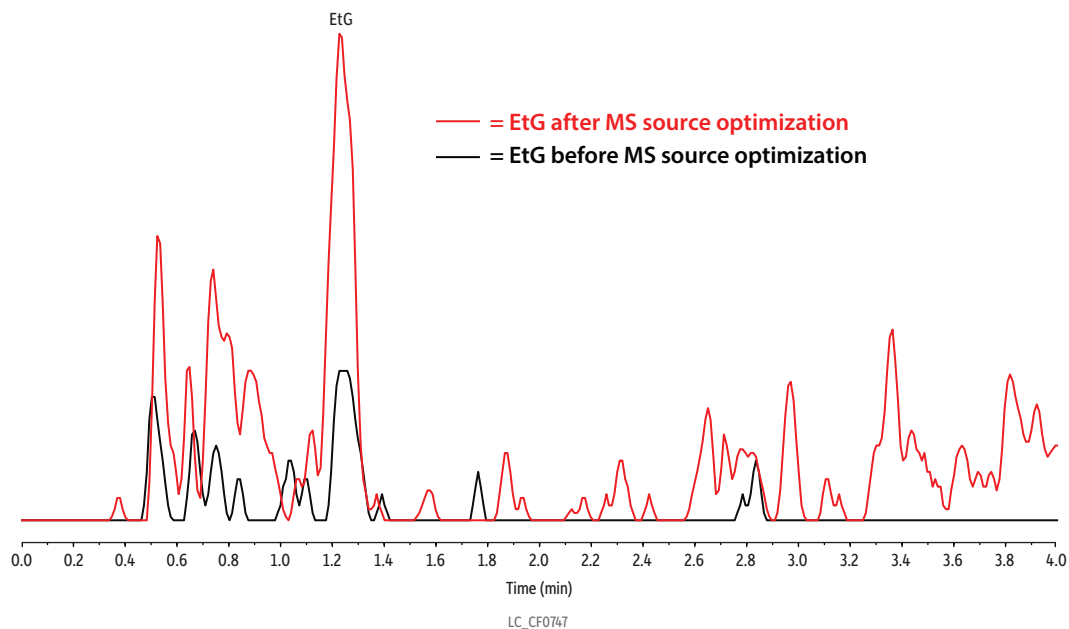


EtG Sensitivity Before and After Source Optimization



Peaks	Retention Time (min)	Concentration (ng/mL)	Precursor Ion	Product Ion
1. Ethyl-β-D-glucuronide (EtG)	1.23	100	221.2	85.1

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)
 Diluent: 0.01% Formic acid in water
 Conc.: 100 ng/mL (40x acetonitrile precipitation sample cleanup)
 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
3.5	0.5	65	35
3.51	0.5	95	5
4.5	0.5	95	5

Detector MS/MS
 Ion Source: Electrospray
 Ion Mode: ESI-

Instrument HPLC
Sample Preparation Acetonitrile Precipitation: A 100 ng/mL standard was prepared in urine. A 50 μL aliquot of the standard solution was mixed with 10 μL of internal standard (20 μg/mL EtG-d5 and 5 μg/mL ETS-d5) and 150 μL of acetonitrile by vortexing at 3000 rpm for 10 seconds, followed by centrifugation at 4300 rpm for 10 minutes at 10 °C. After centrifugation, 100 μL of the supernatant was diluted with 900 μL (40x dilution) of 0.01% formic acid in water. The sample was then vortexed at 3000 rpm for 10 seconds and injected.