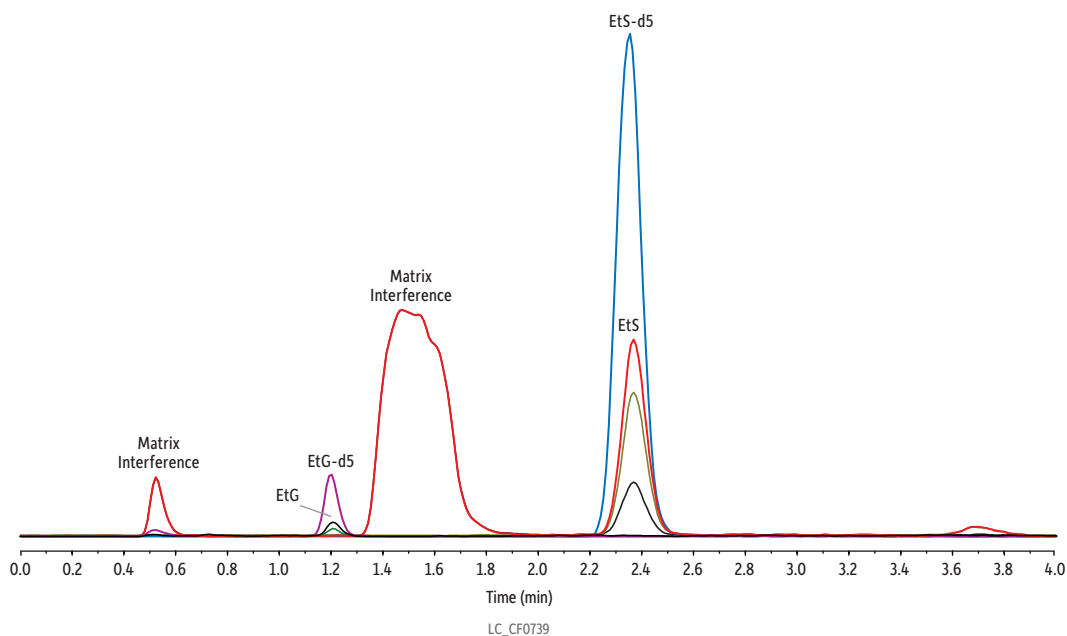


# EtG/EtS on Raptor EtG/EtS: Updated Method Conditions (Mobile Phase and Gradient)



Peaks	tr (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Ethyl-β-D-glucuronide-d5 (EtG-d5)	1.21	200	226.2	85.0	-
2. Ethyl-β-D-glucuronide (EtG)	1.23	500	221.2	75.1	85.1
3. Ethyl sulfate-d5 (EtS-d5)	2.36	50	130.1	98.0	-
4. Ethyl sulfate (EtS)	2.38	500	125.1	97.1	80.0

**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-β-D-glucuronide (EtG) (cat.# 34101)  
 Ethyl sulfate-d5 sodium salt (EtS-d5) (cat.# 34104)  
 Ethyl sulfate sodium salt (EtS) (cat.# 34103)

**Diluent:** 0.01% Formic acid in water  
**Conc.:** 500 ng/mL (40x acetonitrile precipitation sample prep)  
**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** A 500 ng/mL standard was prepared in urine. A 50 μL aliquot was mixed with 10 μL of internal standard (20 μg/mL EtG-d5 and 5 μg/mL EtS-d5 in water) and 150 μL of acetonitrile by vortexing at 3000 rpm for 10 seconds and centrifuged 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 for LC-MS/MS analysis.