Tech Tip: Column Conditioning Ensures Consistent EtG/EtS Results

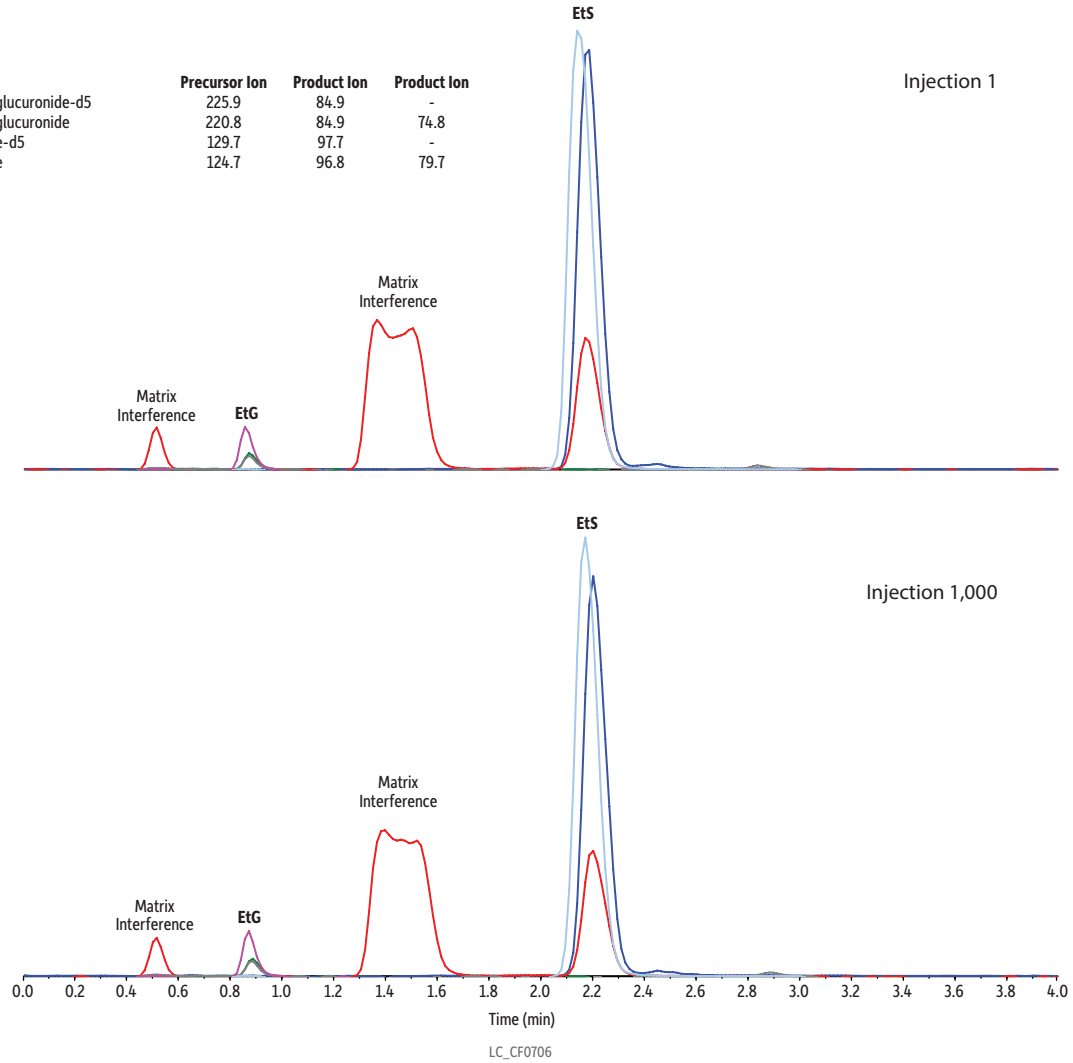
Typical methods for analyzing EtG and EtS in human urine have several limitations: poor retention and resolution of EtG and EtS from matrix components, long run times that limit sample throughput, and short column lifetimes. Restek's chemists have developed a simple, dilute-and-shoot LC-MS/MS method for EtG/EtS analysis using the Raptor EtG/EtS column, which provides consistent separations in a fast, four-minute analysis time.

The Raptor EtG/EtS column features a novel stationary phase developed specifically for this critical application. This method was developed and stringently tested on the Raptor EtG/EtS column because it provides the retention needed to consistently elute the target analytes well away from matrix interferences and is rugged and long-lasting to satisfy the demands of high-throughput labs. As with all new columns, it is good practice to run a number of conditioning or equilibration injections in order to ensure good response, peak shape, and retention time consistency. As part of method development, we determined that running 30 matrix injections through the full gradient program on a new column ensured highly consistent performance during subsequent sample analysis (Figure 1).

By properly conditioning new columns and employing Restek's EtG/EtS method for sample prep and analysis, high-throughput labs testing human urine samples for these alcohol consumption biomarkers can ensure accurate, consistent results with fewer column changes. Full method parameters, validation data, and analyses of patient samples are provided in application note CFAN2736-UNV (to access, visit **www.restek.com** and enter CFAN2736-UNV in the search).

Figure 1: Highly consistent EtG/EtS results are obtained using a preconditioned Raptor EtG/EtS column and the method parameters shown here. Matrix interferences are still well resolved even after 1,000 sample injections.

Peaks	Precursor Ion	Product Ion	Product Ion
1. Ethyl-β-D-glucuronide-d5	225.9	84.9	-
2. Ethyl-β-D-glucuronide	220.8	84.9	74.8
3. Ethyl sulfate-d5	129.7	97.7	-
4. Ethyl sulfate	124.7	96.8	79.7



LC_CF0706

Column
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

Sample
Diluent: 0.1% Formic acid in water
Conc.: A 500 ng/mL QC sample was prepared in urine. 50 µL of the sample was diluted with 950 µL of a working internal standard (25 ng/mL EtS-d5/100 ng/mL EtG-d5 in 0.1% formic acid in water). The sample was vortexed at 3,500 rpm for 10 seconds to mix. The sample was then centrifuged at 3,000 rpm for 5 minutes at 10 °C. The autosampler needle was adjusted to inject from the supernatant.
Inj. Vol.: 10 µL

Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	95	5
2.50	0.5	65	35
2.51	0.5	95	5
4.00	0.5	95	5

Detector MS/MS
Ion Mode: ESI-
Mode: MRM
Instrument HPLC
Notes **Reference Standards**
Ethyl-β-D-glucuronide (cat.# 34101)
Ethyl-β-D-glucuronide-d5 (cat.# 34102)
Ethyl sulfate sodium salt (cat.# 34103)
Ethyl sulfate-d5 sodium salt (cat.# 34104)

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