Poster Reprint

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Quantitative LC/MS/MS Analysis of Ethyl Glucuronide and Ethyl Sulfate using Charged Surface-C18 Column

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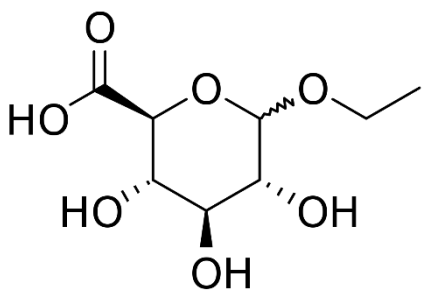
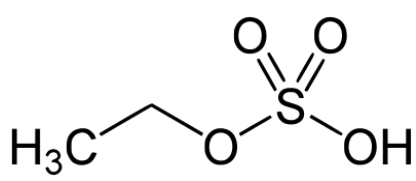
Introduction

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) biomarkers are formed by the liver after ethanol ingestion. Eliminated by the kidneys, they can be detected in urine for approximately 2-80 hours post-imbibing. Due to the polar nature of these metabolites, they are difficult to retain on a reversed-phase column. This study tested if a hybrid end-capped charged surface C18 column would retain them well enough for detection and quantification by LC/MS/MS.

Experimental



Ethyl Glucuronide
Log P = - 1.4
pKa = 3.4



Ethyl Sulfate
Log P = -0.5
pKa = 2.1

A simple “dilute and shoot” sample prep was used

- Urine was spiked with standards made from a working stock solution.
- Calibrators, controls, and samples were spiked with internal standard, diluted 1:50 in mobile phase A
- Samples were injected onto an Agilent InfinityLab Poroshell 120 CS-C18 column

Experimental

LC Configuration				
1290 Infinity II Binary Pump (G7120A)				
1290 Infinity II Multisampler (G7167B)				
1290 Infinity II Multicolumn Thermostat Column Compartment (G7116B)				
Needle wash	IPA:MeOH:ACN:Acetone (50:20:20:10)			
Autosampler temperature	4°C			
Injection volume	5 µL			
Analytical column	Agilent Poroshell CS-C18 (2.1x50 or 2.1x100 2.7µm) (p/n 679775-942 or 675775-942)			
Column temperature	40 °C			
Mobile phase A	0.01% Formic Acid in Water			
Mobile phase B	Methanol			
Flow rate	0.350 mL/minute			
Gradient	2.1x50mm column		2.1x100mm column	
	Time	%B	Time	%B
	0.00	2	0.00	2
	3	2	1.20	2
	3.01	98	3.0	50
	3.99	98	3.01	98
	4.0	2	3.99	98
			4.00	2
Stop Time	4.0 minutes		4.0 minutes	
Post Time	1.0 minutes		1.4 minutes	

Triple Quadrupole Mass Spectrometer Configuration		
	6470 LC/TQ (G6470B)	Ultivo LC/TQ (G6465B)
Ionization mode	Negative	Negative
Drying gas temperature	200 °C	200 °C
Drying gas flow	12 L/min	12 L/min
Nebulizer pressure	50 psi	50 psi
Sheath gas temperature	350 °C	350 °C
Sheath gas flow	12 L/min	12 L/min
Nozzle voltage	2000 V	2000 V
Capillary voltage, negative	5000 V	5000 V
Delta EMV, negative	300 V	0 V
CAV:	3 V	3 V

Compound	Precursor	Product		Dwell (msec)	Frag (V)	CE (V)
EtG	221.1	75		50	100	16
EtG	221.1	85		50	100	20
EtG-d5	226.1	75		50	100	16
EtS	125.1	97		50	80	20
EtS	125.1	80		50	80	40
EtS-d5	130.1	98		50	80	20

Segment #	Time (2.1x50)	Time (2.1x100)	Scan Type	Diverter Valve	Data Store
1	0.0 min	0.0 min	MRM	Waste	No
2	0.8 min	1.0 min	MRM	MS	Yes
3	3.2 min	3.4 min	MRM	Waste	No

Figure 1. Mass spectrometer analytical method conditions.

Results and Discussion

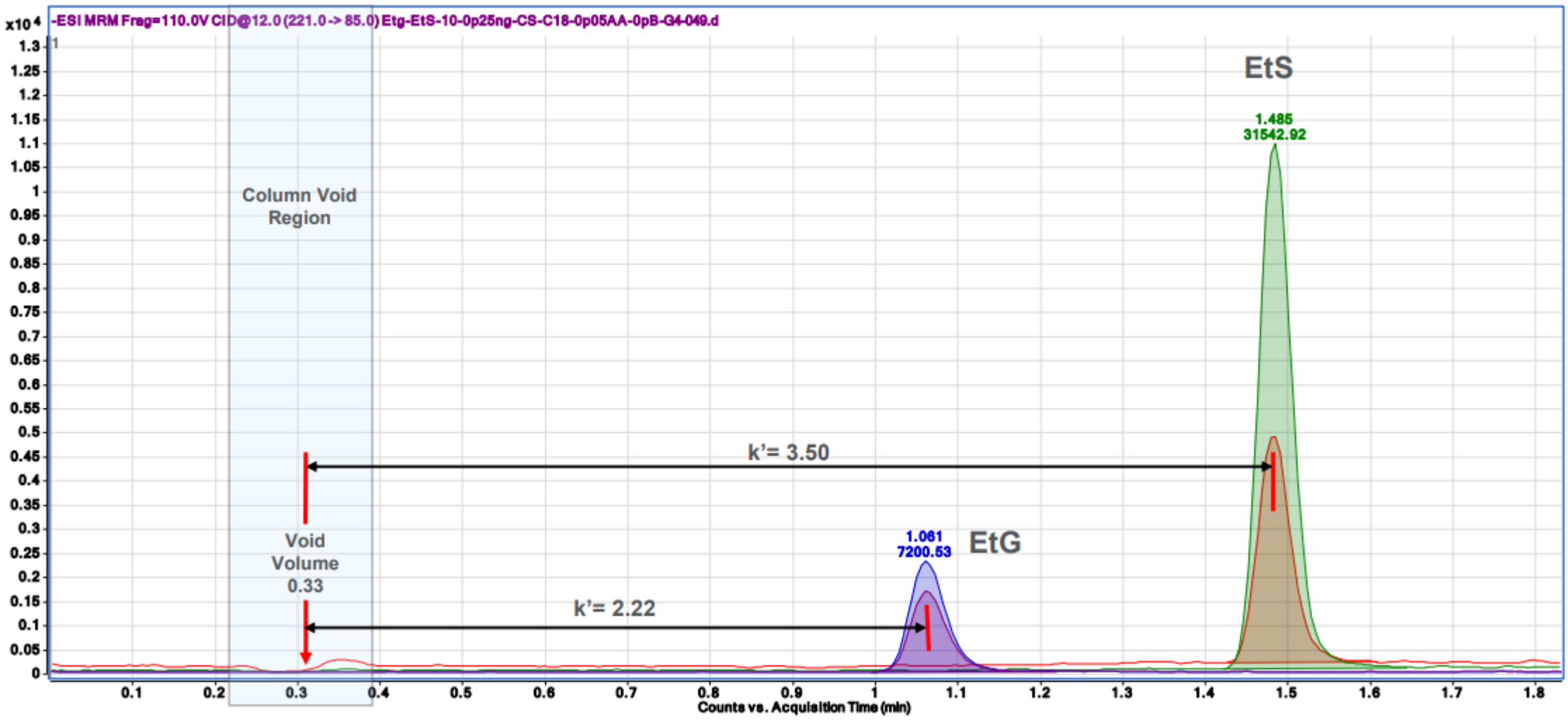
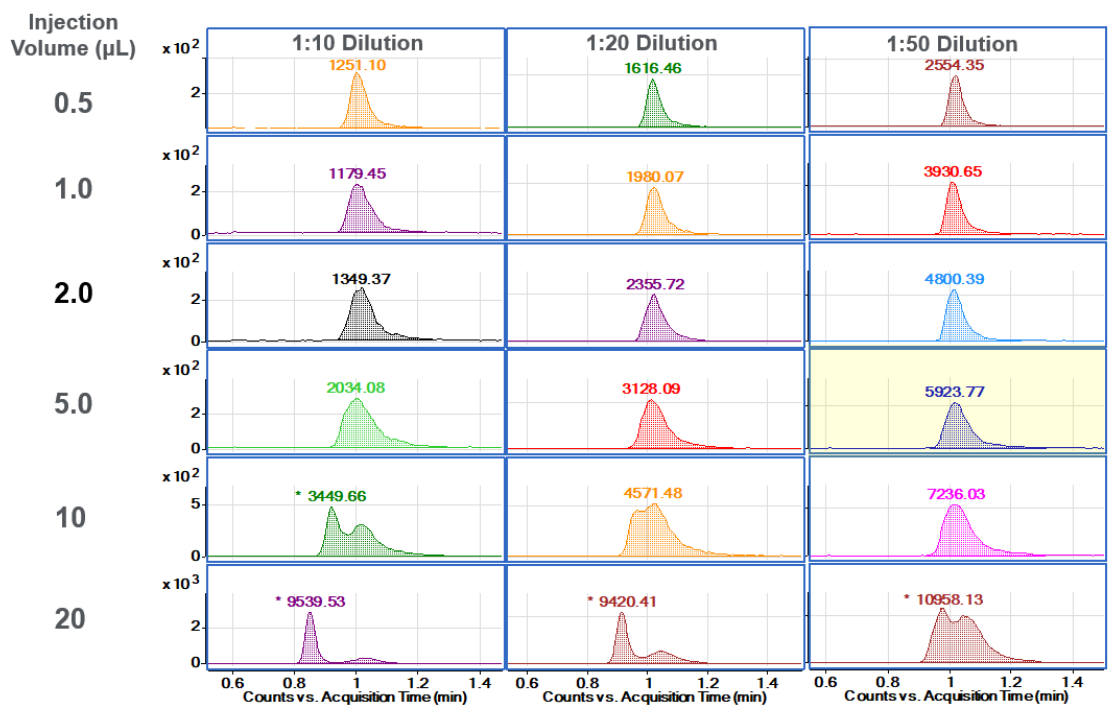


Figure 2. Ethyl Glucuronide and Ethyl Sulfate are adequately retained and separated.

A dilution study was performed testing dilutions of 1:10, 1:20 and 1:50. In addition to assessing the dilutions, the injection volume was also tested at 0.5, 1, 2, 5, 10, and 20 µL.

For this study, a urine sample was spiked at 2000 ng/mL EtG and 200 ng/mL EtS to ensure adequate response. By comparing area count and peak shape, it was determined that a 1:50 dilution with a 2µL injection volume yielded the best results for both ethyl glucuronide and ethyl sulfate.



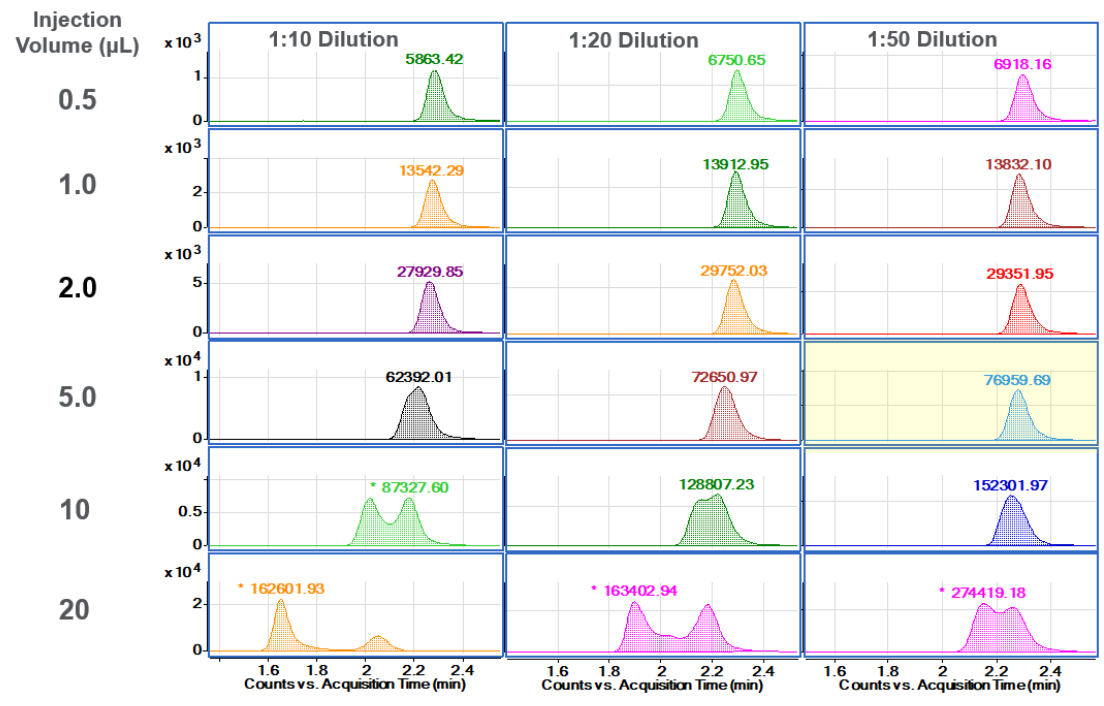


Figure 3. Dilution is the solution to pollution, in this case. A high dilution and low injection volume yielded the best peak shape and response. Left – EtG at 2000 ng/mL. Right – EtS at 200 ng/mL.

Results and Discussion

Quantification was based on a 7-point calibration curve ranging from 100 to 10,000 ng/mL for EtG and 10 to 1,000 ng/mL EtS. Both compounds were linear within their analysis range with 1/x weighting. R^2 were > 0.998.

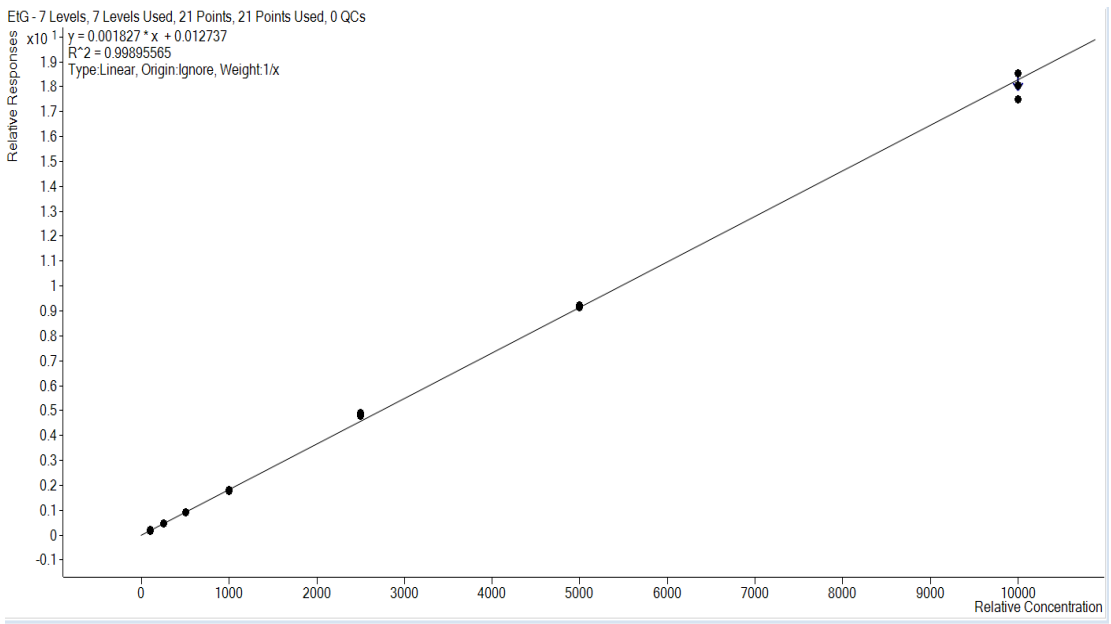
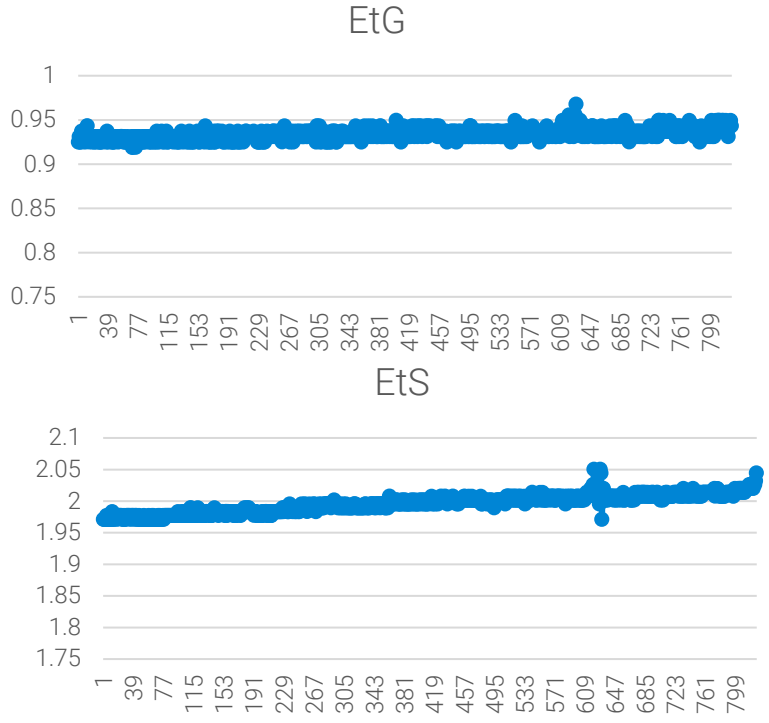


Figure 4. 7-point calibration curve injected in triplicate.

Over 800 injections, retention time and peak area were very stable.



Excellent reproducibility was observed for both analytes over 1000 injections as seen in Figure 4. Five samples with different EtG and EtS concentrations were injected 200 times each. Samples 1-4 had reportable EtG and EtS, while sample 5 was a negative with no alcohol consumption within 72 hours.

Figure 5. 1000 injections, 200 per sample showed reproducible response across the run.

6470 LC/TQ (Left) and Ultivo LC/TQ (Right)

Conclusions

The data indicate the CS-C18 column adequately retains ethyl glucuronide and ethyl sulfate. A high dilution and low injection volume produce the best peak shape. Retention times and peak areas were stable across hundreds of injections. While further experiments are needed to determine column lifetime and method robustness, preliminary data indicate this method would work well in a high-throughput environment due to its short run time and simple sample preparation.

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