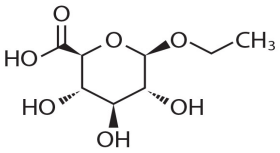
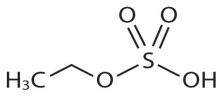
Quantitative Analysis of EtG and EtS in Urine Using Clean Screen® ETG and LC-MS/MS



EtG



EtS

UCT Part Numbers

CSETG203
Clean Screen ® ETG 200 mg in a 3 mL SPE cartridge

SLGRDHLDR-HPOPT
Guard Column Holder

SLETG100ID21-3UM
Selectra® ETG HPLC column 100 x 2.1 mm, 3 µm

SLETGGDC20-3UM
Selectra® ETG guard column 10 x 2.0 mm, 3 µm

Procedure:

1. Prepare Sample

a) To 200 µL of urine sample with 5% formic acid add appropriate deuterated analogues of EtG/EtS.
b) Vortex for 30 seconds.

2. Condition Clean Screen® ETG Extraction Column

a) 1 x 2 mL MEOH containing 1% formic acid.
b) 1 x 2 mL D.I. H_2O containing 1% formic acid.

Note: Aspirate at < 3 inches Hg to prevent sorbent from drying out.

3. Apply Sample

a) Load sample at 1-2 mL / minute.

4. Dry Column

a) 10 minutes at full vacuum or pressure.

5. Elute EtG/EtS

a) 1 x 2 mL MEOH containing 1% formic acid.
b) Collect eluate at 1-2 mL /minute.

6. Evaporate / Reconstitute

a) Evaporate eluate under a gentle stream of nitrogen < 40°C.
b) Dissolve the residue in 200 µL of D.I. H_2O.

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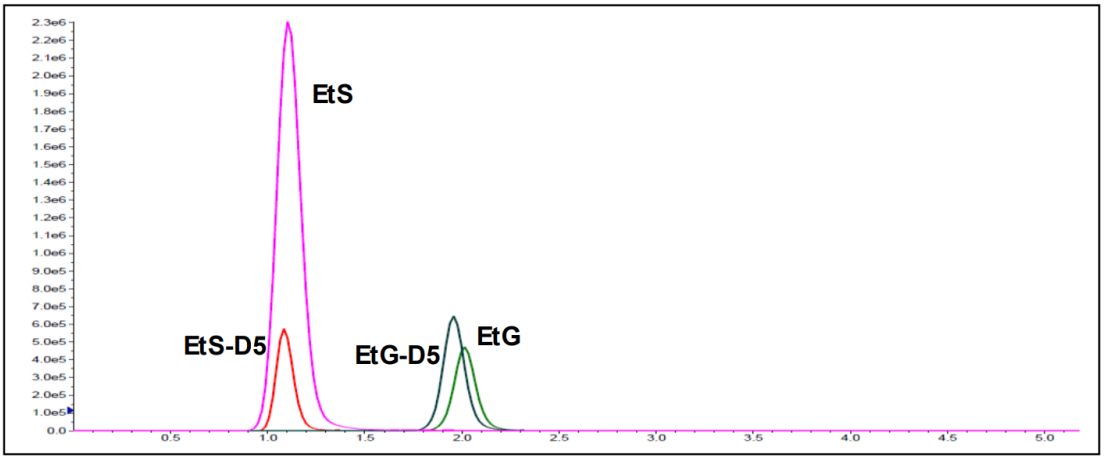
LC-MS/MS Method:

Instrumentation	
Instrument	Agilent 1200 Binary Pump SL
Detector	AB Sciex API 4000 Q Trap MS/MS
Column	UCT Selectra® ETG HPLC column, 100 x 2.1 mm, 3 µm
Guard Column	UCT Selectra® ETG, 10 x 2.0 mm, 3 µm
Column Temperature	30°C
Column Flow Rate	0.3 mL/min
Injection Volume	10 µL

Gradient Program		
Time (min)	**% Mobile Phase A** (0.1% Formic Acid in water)	**% Mobile Phase B** (0.1% Formic Acid in ACN)
0	100	0
1.5	100	0
1.7	0	100
2.7	0	100
3.0	100	0
6.0	100	0



MRM transitions (ESI⁻, 50 ms dwell time)				
Compound	**Rt (min)**	**Q1 ion**	**Q3 ion 1**	**Q3 ion 2**
EtS-D5	1.28	130.1	97.8	79.7
EtS	1.31	125.1	95.8	96.9
EtG -D5	1.66	226.1	85.1	74.9
EtG	1.69	220.9	85.1	75.1

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Results:

Excellent recoveries were achieved with EtG at 96% and EtS at 98.3%.The extraction efficiency was evaluated by fortifying samples at two concentrations (250 ng/mL and 2500 ng/mL). RSD values were less than 5.3% (n= 4 at each concentration).

Recovery and RSD% from Urine Spiked at 2 Levels				
	Spiked at 250 ng/mL		Spiked at 2500 ng/mL	
Compound	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
EtG	96.0	4.8	102.9	4.4
EtS	98.3	6.5	109.6	3.9
Overall mean	97.15	5.65	106.25	4.15

Discussion:

Upon re-evaluation of UCT's original EtG extraction method utilizing Clean Screen® ETG columns, it was noted that the previously employed aqueous wash step resulted in significant loss of both EtG and EtS. Also, it was discovered that there was significant sample breakthrough on the carbon-based extraction column using 0.5 mL of sample or higher due to a lack of sufficient capacity. As a result, the method was modified using decreased sample volume as to not overload the column and w ithout the use of the aqueous wash step. Surprisingly, the cleanliness of the extract was not compromised and excellent recoveries were achieved.

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