Ethyl Glucuronide and Ethylsulfate

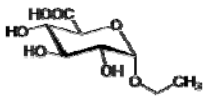
– alcohol abuse biomarkers

Ethyl glucuronide (EtG) is a metabolite of ethyl alcohol which is formed in the body by glucuronidation following exposure to ethanol, such as by drinking alcoholic beverages. The usefulness of ETG as a recent alcohol consumption biomarker has been studied widely.
A disadvantage of the test is that because EtG can be detected in samples at very low levels, it can also be positive after exposure to alcohol from non-beverage sources, or incidental exposure, which can lead to innocent positives. It has been found that EtG can only be formed after alcohol ingestion and has been found not to be formed endogenously. Therefore, the presence of EtG is definitive evidence of anti-mortem alcohol intake. However, negative results of EtG should be interpreted with caution as false negative results may be obtained. There is a time lag between alcohol present in blood and EtG which may lead to false negative results if death happened shortly after alcohol consumption.

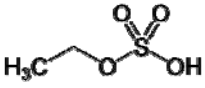
Recently, the stability of EtG has been brought into question which was found to be degraded by bacteria. Therefore, ethylsulfate (ETS) has been introduced as a complementary marker with EtG due to its stable pattern and resistance to bacterial infection; the presence of EtG and ETS provides strong evidence of recent alcohol consumption. An alternative marker for ethanol intake is phosphatidylethanols (PEth), a group of phospholipids formed only in the presence of ethanol via the action of phospholipase D.

EtG and ETS are though promising biomarkers because they are phase two ethanol metabolites and their excretion profiles have been studied and documented. Also, their standards and internal standards are available commercially and can be detected using LC-MS/MS.

The following method was aimed at developing and validating an LC-ESI-ion trap-MS/MS method for identification and quantification of EtG and ETS as ethanol biomarkers from urine samples.*



Ethyl glucuronide (EtG)



Ethyl sulfate (ETS)

* "Direct Determination of Ethyl Glucuronide and Ethyl Sulfate in Postmortem Urine Specimens Using Hydrophilic Interaction Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry "
A.I. Al-Asmari, R.A. Anderson, P. Appelblad
J. Anal. Tox. 34 (2010) 261-272

Ethyl Glucuronide and Ethyl sulfate

SeQuant® ZIC®-HILIC

Recommended column:

SeQuant® ZIC®-HILIC (3µm, 100Å) PEEK 150 × 2.1 mm	(1.50442.0001)
SeQuant® ZIC®-HILIC (guard fitting) PEEK 14 × 1.0 mm	(1.50434.0001)

Recommended solvents and reagents:

Acetonitrile:	hypergrade for LC-MS LiChrosolv®	(1.00029)
Water:	Water for chromatography LiChrosolv®	(1.15333)
	or freshly purified water from Milli-Q® water purification system	

Ammonium acetate (HPLC grade) or in-situ prepared buffer from ammonia and acetic acid

Ammonia solution 28-30% for analysis EMSURE® ACS, Reag. Ph Eur	(1.05423)
Acetic acid 96% for analysis EMSURE®	(1.00062)
Formic acid 98-100% for analysis EMSURE® ACS, Reag. Ph Eur	(1.00264)

Mobile phase:

Time (min)	A (%)	B (%)	Flow Rate (mL/min)
0.00	90	10	0.20
3.00	90	10	0.20
3.01	90	10	0.40
4.00	70	30	0.40
7.00	70	30	0.20
12.00	50	50	0.20
12.01	90	10	0.20
20.00	90	10	0.20

HPLC Sample Preparation

Following addition of pentadeuterated internal standards for ETG and ETS, 200 µl of acetonitrile was added to 0.1 ml of urine and centrifuged at 10000 rpm. The supernatant was then evaporated before reconstituting with 100 µL of initial mobile phase prior to LC-MS/MS analysis.

Ethyl Glucuronide and Ethyl sulfate in Urine

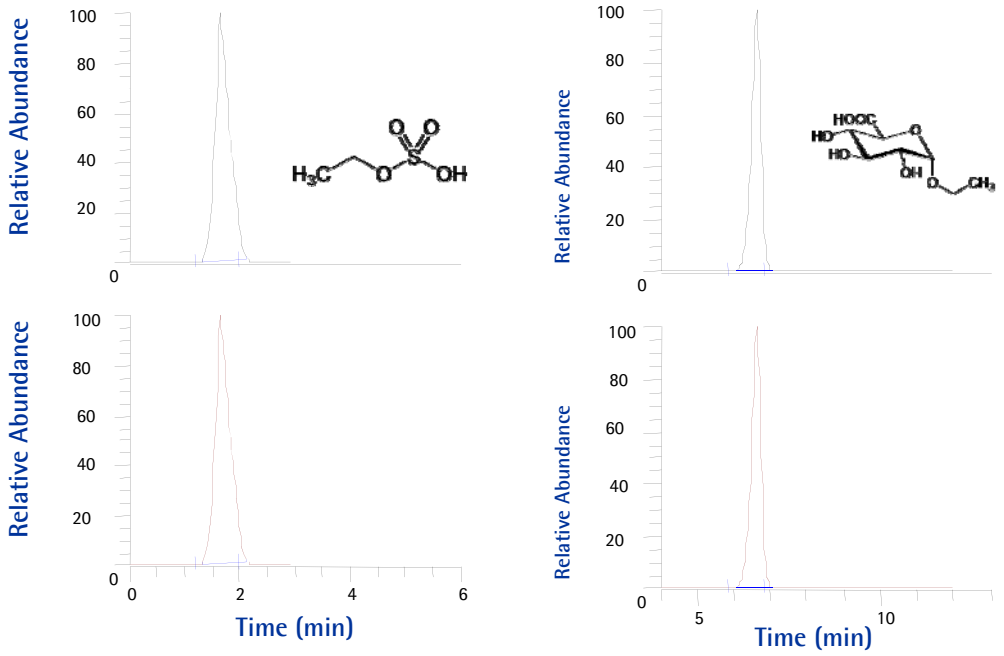
SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (3µm, 100Å) PEEK 150 × 2.1 mm	(1.50442.0001)
Injection:	5 µL in mobile phase	
Detection:	LC-ESI ion trap MS/MS	
Flow Rate:	See table	
Gradient	See table	
Mobile Phase:	A: Acetonitrile B: Ammonium acetate 5mM, pH 6.8	
Temperature:	25 °C (column oven) and 4 °C (autosampler)	
Sample:	Urine samples treated according to sample preparation protocol.	

Quantitation of ES and EtG in Urine

Lower limit of Quantitation = 1 µg/L (1 ppt)



Chromatographic Data

No.	Compound	Retention Time (min)	Precursor ion (m/z)	Product ions (m/z)
1	Void volume	1.3	-	
2	Ethylsulfate	1.7	125.5	97.5
3	Ethyl glucuronide (EtG)	6.7	221.5	103, 113