Determination of EtG and EtS in human serum and urine by LC-MS/MS

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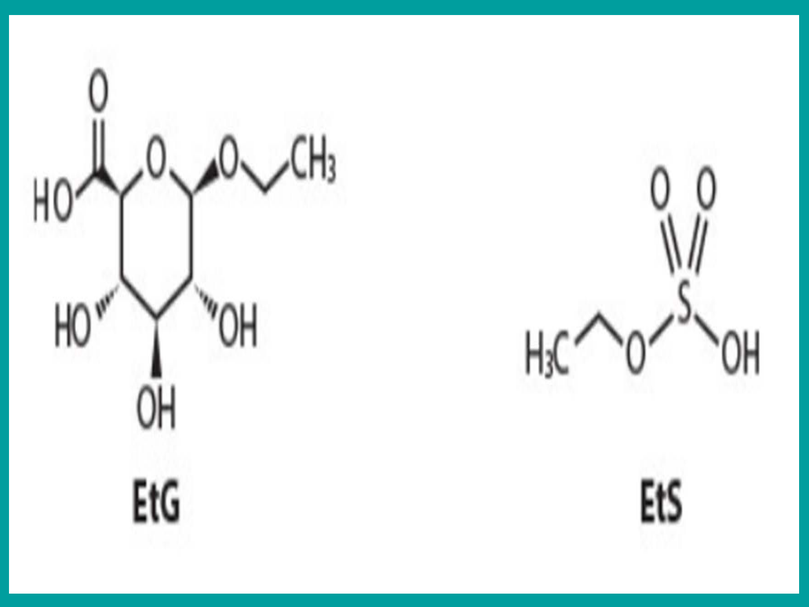
SUMMARY

Alcohol is the most commonly abused substance worldwide. Alcohol abuse claims thousands of lives every year. Drinking alcohol during pregnancy can cause fetal alcohol spectrum disorder. More than 95% of ethanol ingested is rapidly metabolized in the liver. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are direct ethanol metabolites widely used as biomarkers for alcohol intake and abuse because of their longer half-lives than the parent compound. In this study, we developed a dilute-and-shoot method for analysis of EtG and EtS in urine and serum by LC-MS/MS.

DRUG STRUCTURES AND PROPERTIES

Figure 1: Structures of Analytes



METHOD DETAILS

Sample Preparation

Urine samples: 50 µL of internal standard in DI was added into 50 µL of urine, then further diluted with 2.3 mL of 0.1% acetic acid in water, and filtered using 0.2 µm PTFE.

Serum samples: 100 µL of internal standard in DI was added into 100 µL of serum followed by protein precipitation using 1.8 mL acetonitrile. The supernatant was evaporated and reconstituted using 1 mL of 0.1% acetic acid containing 5% methanol.

10 µL of the final solutions were analyzed.

Table 1: HPLC Gradient for serum

Total Time(min)	B (%)	Flow Rate (µL/min)
0	1	600
3.5	5	600
4.5	95	1000
4.6	100	1200
9	100	1200
9.01	1	1200
13	1	1200
13.01	1	600
14.5	1	600

Mobile Phase: A: 0.1% Formic acid B: ACN
Column Temperature: 40° C
Injection Volume: 10 mL

Table 2: HPLC Gradient for urine

Total Time(min)	B (%)	Flow Rate (µL/min)
0	1	600
3.5	5	600
4.5	95	1000
4.6	100	1200
6.6	100	1200
6.61	1	1200
8.6	1	1200
8.61	1	600
10	1	600

METHOD PERFORMANCE

Table 3: Analyte MS Parameters and RT

Compounds	Retention time (min)	DP*	Quant Ion			Qual Ion		
			MRM 1	CE**	CXP***	MRM 2	CE	CXP
EtS	2.22	-35	125/97	-25	-8.0	125/79.9	-40	-9.0
EtS-d5	2.20	-40	130/97.9	-27	-6.0	130/79.9	-42	-9.0
EtG	2.95	-35	221/74.9	-20	-10.0	221/85	-22	-9.0
EtG-d5	2.93	-40	226/75	-21	-11.0	226/85	-23	-12.0

DP*: Declustering Potential CE**: Collision Energies CXP***: Collision Cell Exit Potential

Table 4: Method performance

Parameters	Unit	EtG		EtS	
		Urine	Serum	Urine	Serum
Linear range	ng/mL	10 - 10,000	10 - 1000	1 - 1000	1 - 100
LoD & LoQ	ng/mL	10		1	
Intra-precision	%	1.2 - 4	2 - 5.4	2.7 - 5.8	1.7 - 4.4
Inter-precision	%	2.5 - 6.4	2.4 - 6.4	3.5 - 5.7	3.1 - 4.3
Accuracy	%	86.5 - 112.8	92.7 - 117.8	92.3 - 117.1	92.3 - 119.3
Interference	%	94.4 - 106.7	89.3 - 102.4	100.9 - 109.6	90.4 - 99.5
Matrix effect	%	-16.5 ~ 4.4	9.1 ~ 15.6	-13.9 ~ 6.2	-12.9 ~ -1.5
Recovery	%	94.7 - 109.4	89.2 -105.5	89.4 - 113.6	92.7 - 107.4
Extract Stability	%	4 days	7 days	4 days	7 days
No carryover observed after		10000	5000	500	1000

Table 5: Urine sample proficiency test Results

Urine sample proficiency test results on Mar 2020					
ID	Report results (ng/mL)	Mean (ng/mL)	Accuracy (%)	Acceptable reange Lower	Acceptable reange Upper
EtG_1	759.2	704.41	**107.8**	453.6	955.2
EtG_2	2592.6	2318.81	**111.8**	1670.3	2967.3
EtG_3	5084.8	4798.75	**106.0**	3685.8	5911.7
EtS_1	na	na	**na**	na	na
EtS_2	152.9	134.91	**113.3**	0	279.5
EtS_3	930.1	899.47	**103.4**	602.6	1196.4

Figure 2: Chromatogram of EtG and EtS

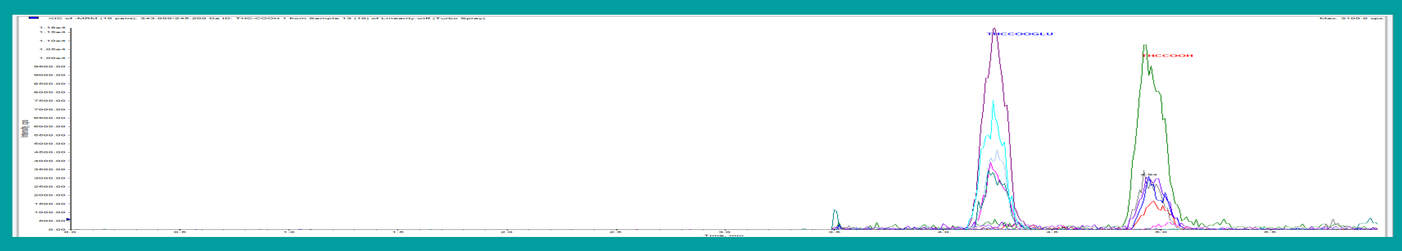
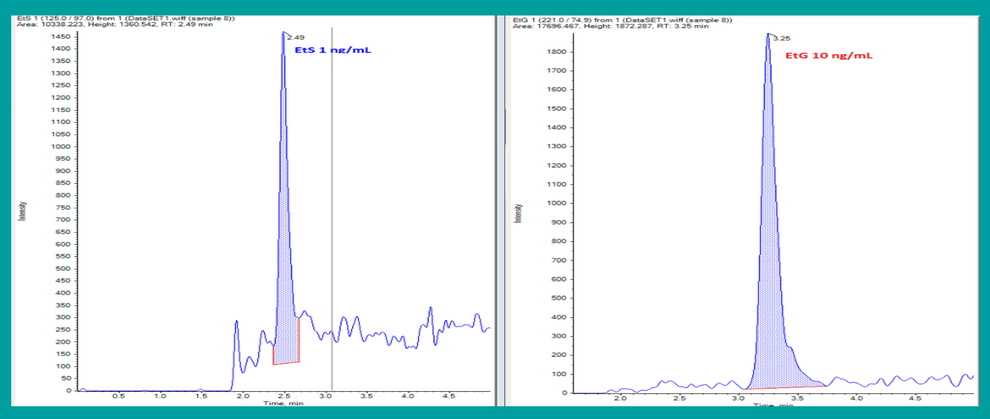


Figure 3: Extracted ion chromatography at LoQ (EtS 1 ng/mL and EtG 10) ng/mL)



CONCLUSIONS

A fast, simple, sensitive, robust, and high-throughput LC-MS/MS method for EtG and EtS determination in urine and serum was developed and validated. The method has very low limit of quantification (LoQ) compared to published methods. It can be used for the identification of ethanol abstinence and meets the high sensitivity requirement for biomonitoring applications

ACKNOWLEDGEMENT

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