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Analysis of ethyl glucuronide and ethyl sulfate in urine with TSQ Quantis MS

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Goal

To develop a robust and sensitive LC-MS/MS workflow for the forensic toxicological analysis of EtG and EtS in urine using liquid chromatographic separation coupled to a triple quadrupole mass spectrometer.

Introduction

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are well-known biomarkers for monitoring ethanol consumption. Although they are minor metabolites of ethanol, their longer half-lives make them useful for detection of past alcohol use in forensic research.

Experimental

Sample preparation

Equal volumes (50 µL) of urine and internal standard (5000 ng/mL of EtG-d5 and 500 ng/mL of EtS-d5) were mixed and then diluted with 900 µL of water for a 20-fold dilution. A 5-µL sample was injected onto the LC-MS/MS system.

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Liquid chromatography

Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Flex HPLC system. The column used was a Phenomenex® Synergi™ Hydro RP column (100 × 3 mm, 2.5 µm particle size). Mobile phases A and B consisted of 0.1% formic acid in Fisher Chemical™ Optima™ grade water and methanol, respectively. The total run time was 6 minutes.

Mass spectrometry

MS analysis was carried out on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer equipped with a heated electrospray ionization probe. Table 1 shows mass spectrometer source parameters.

Table 1. Source parameters for analysis of EtG and EtS on the TSQ Quantis triple quadrupole mass spectrometer.

Ion Source Parameter	Value
Spray Voltage	Static
Negative Ion	2500 V
Sheath Gas	35 Arb
Aux Gas	10 Arb
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	350 °C
Vaporizer Temperature	300 °C

Two selected reaction monitoring (SRM) transitions were monitored for EtG, EtS, and their deuterated internal standards to provide ion ratio confirmations (IRC). The scans were run in timed selected reaction monitoring (t-SRM) mode with a cycle time of 0.4 seconds. Table 2 shows SRM properties used in this analysis.

Data analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software.

Evaluation

Assay precision and accuracy were determined by analyzing a calibration curve in singlicate followed by six replicates of the same samples used as quality controls (QC). Matrix effects were evaluated by observing the internal standard signals in 17 different lots of human urine.

Table 2. SRM transitions for analysis of EtG and EtS.

Compound	Start Time (min)	End Time (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
EtS	0	2	Negative	125.062	79.817	30	75
EtS	0	2	Negative	125.062	96.889	15	75
EtS-d5	0	2	Negative	130.062	79.817	31	81
EtS-d5	0	2	Negative	130.062	98.014	15	81
EtG	2	6	Negative	221.062	74.889	14	92
EtG	2	6	Negative	221.062	84.921	16	92
EtG-d5	2	6	Negative	226.062	74.889	16	94
EtG-d5	2	6	Negative	226.062	84.921	16	94

Results and discussion

Limits of quantitation (LOQ) for EtG and EtS were defined as the lowest concentration at which the back-calculated calibrator concentration on the linear calibration curve was within 20% of theoretical, the ion ratio was within 20% of target, and replicate injections have a %RSD of less than 20%. For EtG, the LOQ was 100 ng/mL in authentic urine; for EtS it was 10 ng/mL. Figure 1 shows representative chromatograms for analytes at their respective LOQs. Figure 2 shows representative calibration curves for both compounds.

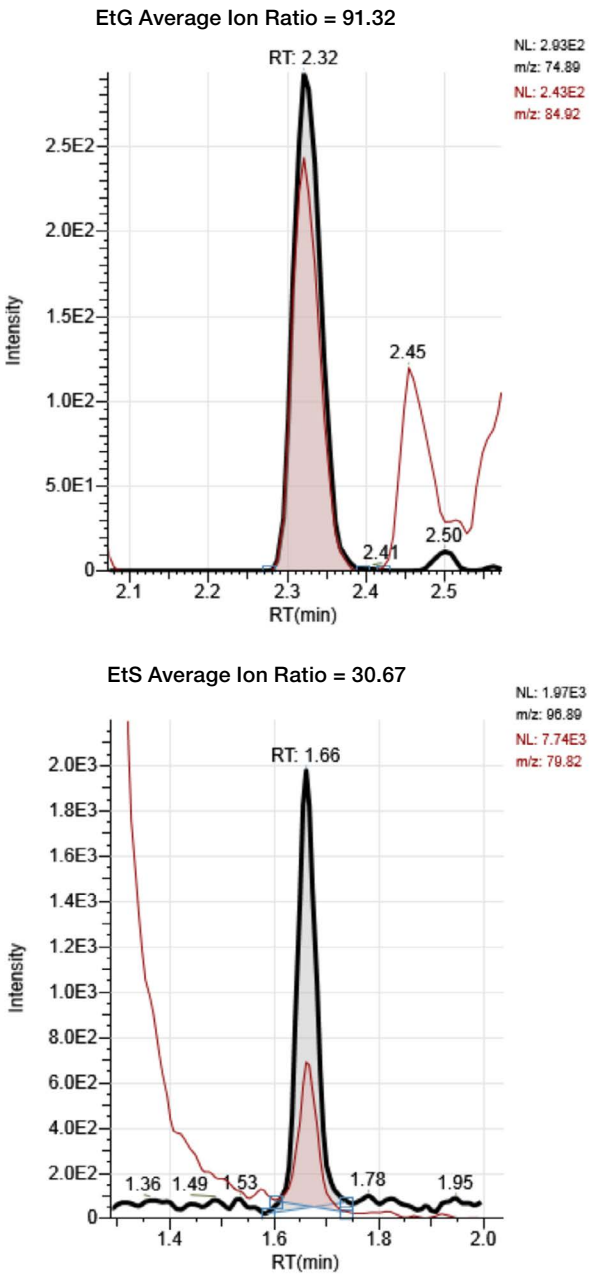

Figure 1. Chromatograms of EtG and EtS at their respective limits of quantitation showing Ion Ratio Confirmation.

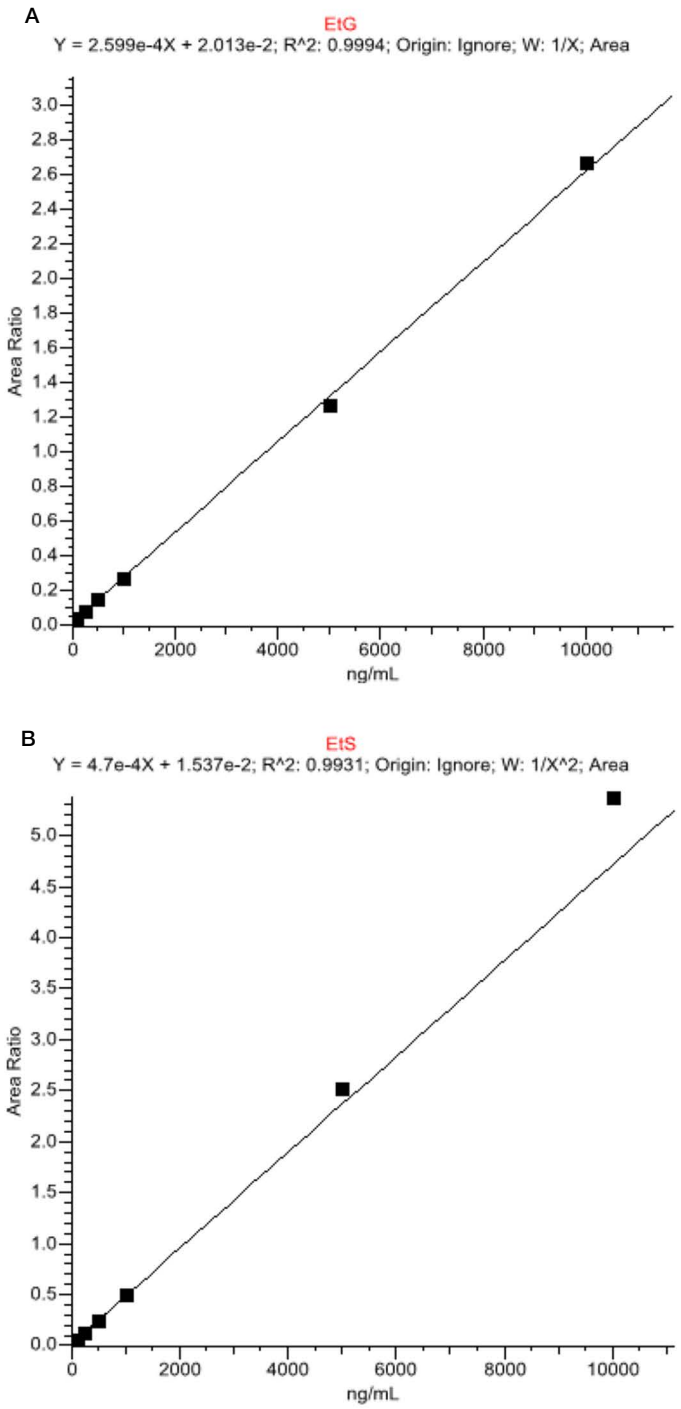

Figure 2. Calibration curves for (A) EtG and (B) EtS in authentic urine.

This method demonstrated excellent precision for replicate injections of the QCs of concentrations spanning the calibration range. Precisions for EtG were less than 9.8% and for EtS were less than 4.0%. Additionally, no significant matrix effects were observed in the 17 different lots of authentic urine tested. Figure 3 shows the recovery of internal standard peak areas of different lots of urine compared to the calibrators.

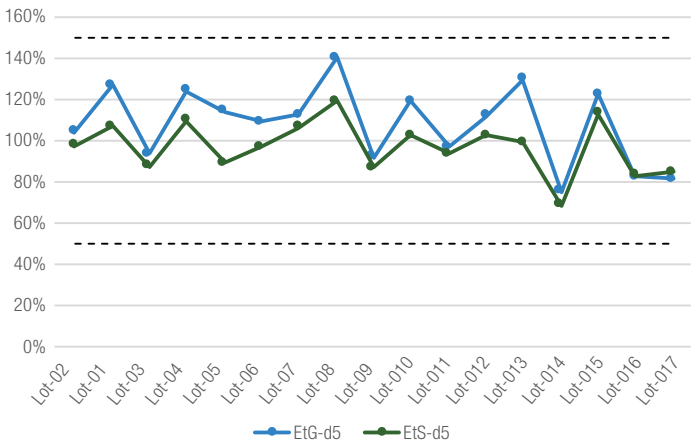

Figure 3. Matrix effects of EtG and EtS internal standards in multiple lots of authentic urine.

Conclusion

- The TSQ Quantis triple quadrupole mass spectrometer provides best-in-class sensitivity and robustness for analysis of EtG and EtS in urine.
- This method gives limits of quantitation in urine of 100 ng/mL for EtG and 10 ng/mL for EtS.
- This method is suitable for use in forensic toxicology.

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