Quantitation of EtG and EtS in Urine by Ion-Pairing LC-MS/MS

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Key Words
EtG, EtS, ethyl glucuronide, ethyl sulfate, ion pairing, SPE, DHAA, dihexylammonium acetate, TSQ Ultra, HyperSep, Hypercarb, forensic toxicology

Goal
To develop an HPLC-MS/MS method for forensic toxicological analysis of EtG and EtS in urine with limits of quantitation (LOQs) of 100 and 50 ng/mL, respectively.

Introduction
Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are long-term biomarkers for ethanol consumption. Although they are minor metabolites of ethanol, their longer half-lives make them useful for forensic detection of past alcohol use. These compounds are highly polar; they retain poorly on most reversed-phase HPLC columns and elute on or near the chromatographic solvent front. The result is poor peak shape and large matrix effects. This application note demonstrates the use of solid-phase extraction (SPE) sample preconcentration to remove interferences and an ion-pairing reagent to retain these compounds on the HPLC column long enough to move them off the solvent front. This enables better peak shape, less matrix interference, and baseline resolution of both analytes for less risk of ion suppression.

Experimental
Sample Preparation
A 1 mL volume of urine, 25 µL of internal standard solution (50 and 5 ng/mL of EtG–d₅ and EtS–d₅, respectively), and 50 µL of formic acid were mixed. The 200 mg Thermo Scientific™ HyperSep™ Hypercarb™ SPE column (P/N 60106-301) was conditioned with 2 mL of 1% formic acid in water. The sample was loaded at a rate of 1–2 mL/min. Next, the column was washed with 2 mL of water and dried under nitrogen at 10–15 mm Hg for 10 min. The sample was eluted with 2 mL of 1% formic acid in methanol. The eluent was evaporated to dryness under nitrogen at 37 °C and reconstituted in 0.2 mL of water.¹ Finally, 20 µL was injected onto the HPLC-MS/MS.

Liquid Chromatography
Chromatographic separations were performed under gradient conditions using a Thermo Scientific™ Accela™ 1250 pump and Accela Open autosampler. The analytical column was a Thermo Scientific Hypersil GOLD™ column (50 x 3 mm, 5 µm particle size). The column was maintained at room temperature. The injection volume was 20 µL. The flow rate was 1 mL/min, and the total run time was 5 min. Other size columns can be used for this application with the appropriate adjustment in injection volumes and flow rates (as in AN488b²). Mobile phases A and B consisted of 5 mM dihexylammoniumacetate (TCI America) ion pairing reagent in water and acetonitrile (Fisher Chemical), respectively. Mobile phase C was acetonitrile/1-propanol/acetone (45:45:10). Mass Spectrometry
MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra™ triple-stage quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. Two selected-reaction monitoring (SRM) transitions each were monitored for EtG, EtS, and their deuterated internal standards to provide ion ratio confirmations (IRC). Two scan segments, one for EtG and its internal standard and one for EtS and its internal standard, were used to maximize the time the mass spectrometer spent scanning each compound.

Results and Discussion
EtG demonstrated linear response from 100 to 100,000 ng/mL with an LOQ of 100 ng/mL. EtS demonstrated linear response from 25 to 50,000 ng/mL with an LOQ of 25 ng/mL. Figure 1 shows representative calibration curves for both compounds. Figure 2 shows representative chromatograms for EtG and EtS and their respective LOQs.
Conclusion

- This method provides limits of detection in urine of 100 ng/mL for EtG and 25 ng/mL for EtS.
- SPE extraction helps remove interferences in urine.
- Addition of an ion-pairing reagent helps chromatographically separate the compounds from interferences on the solvent front, thereby improving limits of detection.
- This method is suitable for forensic toxicology.

References

1. LC/MS Method For Extracting Ethyl Glucuronides From Urine Using: 200 mg Clean Screen® Extraction Column; Part #: CSETG203; UTC, Inc. Bristol, PA; 2008


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