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

Ye J., Zhang X., Huang D. Y., Kinniburgh D. W.

Alberta Centre for Toxicology, Physiology & Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

Background: 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.

Methods: Urine samples were prepared by a 50-fold dilution using 0.1% acetic acid in water and filtered by 0.2 µm PTFE. Serum samples were prepared by protein precipitation using acetonitrile. The supernatant was evaporated and reconstituted at a 10-fold dilution using 0.1% acetic acid containing 5% methanol. 10 µL of the final solutions were analyzed. The LC-MS/MS analysis was carried out on a Sciex Qtrap 5500 in negative MRM mode coupled with an Agilent 1260 Infinity HPLC. The product ions traces m/z 221.0→85.0, m/z 221.1→74.9, m/z 125.0→97.0, m/z 125.0→79.9, 226.1→75.1, m/z 226.0→75.0, m/z 226.0→85.0, m/z 130.0→79.9, m/z 130.0→97.9, were used as identifiers for EtG, EtS, EtG-d_5 and EtS-d_5. Separation was performed using an Eclipse Plus Phenyl-Hexyl column (4.6mm×100 mm, 5µm). Gradient elution was done in 0.1% acetic acid in water and methanol at an initial flow-rate of 600 µl/min. Retention time was 2.4 and 3.2 mins for EtS and EtG with a total analysis time of 10 mins.

Results: Calibration curves were established for EtG (10–10000 ng/mL in urine and 10–1000 ng/mL in serum) and EtS (1–1000 ng/mL in urine and 1–100 ng/mL in serum) with $r^2 > 0.999$. Intra and inter-day precision CV% was less than 7% for both compounds. The accuracy was between 86.5 to 118.3%. The extraction yield was satisfactory (86.3–102.8%, IS corrected). Serum matrix effect was -1.5 to 15.2%, while urine matrix effect was -16.5~4.4%. No carryover was observed even after the highest calibrator. No endogenous or exogenous interference was observed. LoD and LoQ were 1 ng/mL for EtS and 10 ng/mL for EtG.

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 compared to published methods. It can be used for the identification of ethanol abstinence and meets the high sensitivity requirement for biomonitoring applications

Key words: EtG; EtS; Dilute-shoot; LC-MS/MS