K47 LC/MS/MS Quantification of Ethyl Glucuronide and Ethyl Sulfate in Meconium

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After attending this presentation, attendees will be able to describe sample preparation, Limits Of Quantification (LOQ) and Detection (LOD), extraction efficiency, matrix effect, accuracy, and imprecision of this Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) method.

This presentation will impact the forensic science community by describing an analytical method that offers new approaches to sample clean-up and chromatographic separation of EtG and EtS in meconium. Investigators need alternatives to Fatty Acid Ethyl Esters (FAEE) to identify and quantify *in utero* alcohol exposure; this method for quantifying EtG and EtS in meconium offers a less problematic approach to identify *in utero* alcohol exposure.

Introduction: Maternal alcohol consumption during pregnancy is associated with fetal alcohol spectrum disorder that encompasses growth retardation, craniofacial dysmorphology, cognitive disorders, and social impairments. According to the 2011National Surveys of Drug Use and Health report, 9.4% of pregnant respondents 15-44 years old admitted to current alcohol use, with 2.6% reporting binge drinking. A small percentage of ingested ethanol undergoes non-oxidative metabolism yielding FAEEs, EtG, and EtS. These chemical ethanol derivatives are detectable longer than ethanol in several matrices and are, therefore, clinically useful in identifying recent alcohol exposure. Alcohol marker testing in meconium, the first neonatal feces, can identify *in utero* alcohol exposure during the 3rd and perhaps 2nd trimesters. EtG and EtS are thought to prevent false positive reporting of maternal alcohol consumption during pregnancy, whereas FAEE may be present in meconium due to olive oil intake during pregnancy, and are highly unstable in meconium unless specimens are frozen from collection to analysis.[1] Several methods describe meconium FAEE quantification, although currently only one published method reports quantification of EtG and EtS in meconium. This study proposed to develop and validate a selective and sensitive LC/MS/MS assay for EtG and EtS in meconium.

Methods: Blank meconium (0.1 ± 0.003g) was fortified with d5-EtG and d5-EtS internal standards. Specimens were homogenized, vortexed, centrifuged, and supernatant transferred to clean tubes. Meconium supernatant was loaded onto a pre-conditioned Biotage anion-exchange column (100mg/3mL). Columns were washed with acetonitrile and methanol, and analytes eluted with 1% hydrochloric acid in acetonitrile. Extracts were dried under nitrogen at 40°C and reconstituted in water with 0.1% formic acid. An ABSciex 5500 Qtrap® mass spectrometer was interfaced with a Shimadzu UFLCXR system for analysis with gradient chromatographic separation of EtG and EtS on a Phenomenex Kinetex® XB-C18 column (2.1x 100mm, 2.6µm). Mobile phase was water and methanol, modified with 0.1% formic acid. Sensitivity, specificity, linearity, accuracy, imprecision, extraction efficiency, matrix effect, carryover, and dilution integrity were included in the method validation. Meconium samples from infants whose mothers self-reported alcohol consumption during pregnancy were analyzed, demonstrating method applicability.

Results: Linear ranges for EtS and EtG were 2.5-500 and 5-1000ng/g, respectively. Calibration curves employed $1/x^2$ weighting (correlation coefficients ≥0.990). Extraction efficiencies across the linear range were 56.5-84.6% for EtS and 60.6-72.1% for EtG. Matrix effects were -18.6% to 28.9% for EtG and EtS. Analytical accuracy was 101.9-112.6% for both analytes at three quality control (QC) concentrations across the linear range; inter-day imprecision (%CV) was 2.2-11.0% for both analytes (N=18). Meconium from six different negative pools contained no interfering peaks. None of 92 potential exogenous interferences fortified at 10,000ng/g into low Quality Control (QC) samples caused quantification criteria or transition ratios to fail for either analyte. No carryover was detected after a specimen containing two times the upper LOQ. Analytes were stable (≤15% concentration change) on the autosampler at 4°C after 72h.

Conclusions: This analytical method offers new approaches to sample clean-up and chromatographic separation of EtG and EtS in meconium. Investigators need alternatives to FAEE to identify and quantify *in utero* alcohol exposure; this method for quantifying EtG and EtS in meconium offers a less problematic approach to identify *in utero* alcohol exposure.

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Reference:

1. Chan, D, et al. (2003). "Population baseline of meconium fatty acid ethyl esters among infants of nondrinking women in Jerusalem and Toronto." Ther Drug Monit 25(3): 271-278.

Meconium, Ethyl Glucuronide, FAEE

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