K60 Ethyl Glucuronide, Ethyl Sulfate, and Nicotine and Metabolites Quantified in Human Fetal Liver From Electively Terminated Pregnancies

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After attending this presentation, attendees will be able to describe a simultaneous human fetal liver sample preparation and two Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) quantification methods for hepatic alcohol and tobacco markers. Method validation data and results for eight alcohol and nicotine markers in 118 fetal livers will be presented.

This presentation will impact the forensic science community by providing fetal liver alcohol and, for the first time, tobacco concentrations to document *in utero* exposure and potential related exposure toxicities.

Introduction: Adult health is partly programmed during fetal development. Fetal exposure to tobacco, alcohol, and other drugs alters normal hormone regulation and early development, possibly leading to maladaptations and contributing to adult metabolic syndromes. Mechanisms through which fetal drug exposures result in reduced adult health are poorly understood. The objective was to develop quantitative alcohol and tobacco marker assays for human fetal liver to further laboratory research on fetal endocrine disruption.

Methods: Blank liver (0.25g) was fortified with deuterated internal standards. Samples were bead beater homogenized in methanol 0.01% formic acid and passed through a 10µm reservoir filter. Two filtered liver supernatant portions were aliquoted into separate tubes for supported liquid extraction of nicotine and metabolites and anion-exchange solid phase extraction for Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS). An AB SCIEX™ 5500 Qtrap® mass spectrometer was interfaced with a Shimadzu® UFLCXR system for analysis with gradient chromatographic separation of nicotine, cotinine, 3-*trans*-hydroxycotinine (OHCOT), nicotine-N-glucuronide (NG), cotinine-N-glucuronide (CG), and OHCOT-O-glucuronide (OHCOT-G) on an Agilent® Poroshell 120 EC-C8 column (150 x 2.1mm, 2.7µm). Liver EtG and EtS were separated on a Phenomenex® Kinetex® XB-C18 (100 x 2.1mm, 2.6µm) column. Sensitivity, specificity, linearity, accuracy, imprecision, extraction efficiency, matrix effect, carryover, dilution integrity, and stability were evaluated during method validation. Human fetal liver samples (n=118) from normally progressing, electively terminated pregnancies (11-21 weeks gestation) were analyzed.

Results: Linear ranges were 1-300 (cotinine), 5-1500 (CG, nicotine, EtS), 2.5-750 (NG and OHCOT), 10-2,000 (OHCOT-G), and 20-3,000ng/g (EtG). Calibration employed $1/x^2$ weighting (correlation coefficients ≥0.989). Extraction efficiencies were 72%-87% (EtG and EtS), 80%-94% (nicotine, cotinine, and OHCOT), and 53%-71% (NG, CG, and OHCOT-G). Matrix suppression for all analytes was 17.5%-57.3%, except CG (11%-12.3% enhancement). Overall accuracy was 84.2%-115.4% for all analytes at three Quality Control (QC) concentrations across the linear range; between-run imprecision (%CV) was 3.5%-9.4% (n=20). Five unique negative liver samples had no interfering peaks. None of 90 potential exogenous interferences fortified at 4,000ng/g into low QC samples interfered. No carryover was detected at 1.6 times the upper LOQ. Analytes were stable (≤19% change) after 72h on a 4°C autosampler, for 16h at room temperature, 72 h at 4°C, and three freeze-thaw cycles, and in filtered liver supernatants for five days at 4°C. Of 118 authentic human fetal liver samples, 11% were EtG-positive, 72% EtS-positive, 80% cotinine-positive, 64% OHCOT-positive, 59% nicotine-positive, and 31%-68% positive for nicotine glucuronide metabolites. All EtG-positive samples were also EtS-positive, with EtG concentrations always greater (1.6-4.9 times) than EtS; of 13 samples positive for both, 10 were positive for tobacco markers. In EtS-only positive samples, 78% were positive for tobacco markers. In 33 alcohol-negative samples, 28 were positive for tobacco markers.

Conclusions: The novel bead beater homogenization method with separate sample clean-up and chromatographic methods for acidic EtG and EtS and basic nicotine and metabolites provided accurate quantification in a single 0.25g liver sample. Investigators needing to quantify *in utero* alcohol and tobacco exposure in human fetal liver will find this method simple, effective, and highly reproducible. Fetal liver EtG is most likely of maternal origin, as EtG readily crosses the placenta and fetal glucuronidation capacity is limited. Fetal liver EtS may be from maternal and fetal origin, as this analyte likely crosses the placenta and fetal sulfotransferase activity is variable and significant. These data impact the forensic science community by providing fetal liver alcohol and, for the first time, tobacco concentrations, to document *in utero* exposure, and potential related exposure 1 toxicities.

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Fetal Liver, Ethyl Glucuronide, Nicotine