K34 An Analysis of Alcohol Metabolites Ethyl Glucuronide and Ethyl Sulfate in Human Umbilical Cord Samples

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Learning Overview: After attending this presentation, attendees will be able to discuss a method for detecting ethyl glucuronide in human umbilical cord samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the capability of an analytical method that can be used to analyze ethyl glucuronide in umbilical cord samples as a biomarker of alcohol use during pregnancy.

Alcohol is the most popular legal psychoactive drug used in the United States. Due to its use and abuse across a variety of ages, races, and genders, the concern for prenatal exposure to ethanol is one to be taken seriously. While approximately 90% of pregnant women between the ages of 15 and 44 years old report they are abstaining from alcohol, maternal self-report is not always reliable. Many women do not want to self-incriminate or face the social stigma of reporting alcohol use; also many women reporting data may inadvertently have recall bias skewing the overall percentages. A more reliable and objective method to identify prenatal ethanol exposure would be beneficial to allow more timely intervention if needed.

The two alcohol metabolites focused on in this project are ethyl glucuronide and ethyl sulfate. These metabolites are formed by ethanol undergoing conjugation with either a glucuronide or a sulfate group, respectively. The detection of ethyl glucuronide has been reported in multiple newborn specimen types; however, there is very little data on ethyl sulfate.

This presentation describes a screening and confirmation method for the analysis of ethyl glucuronide and ethyl sulfate using a homogenizing-crash extraction with Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

The method was validated according to a Scientific Working Group for Forensic Toxicology (SWGTOX) -compliant procedure for a qualitative assay including experiments that evaluated precision around the decision concentration (cut-off), sensitivity and specificity, robustness, evaluation of interfering compounds, matrix effect, and extraction efficiency and stability of the analyte in extract and pot-extraction stability.

Sample preparation for both the screening and confirmation methods consisted of an external cleaning process to remove contamination from the samples, followed by homogenizing the sample coupled with a protein precipitation. The extract was then diluted prior to instrumental analysis. The analytical method was performed on an AB SCIEX™ 6500+ triple quadrupole mass spectrometer equipped with an Electrospray Ionization (ESI) source for both methods. Separation was achieved using a Waters® CSH C18 (3mm x 100mm, 3.5micron) column coupled with 2 Phenomenex Security Guard Cartridges Polar-RP for the screening assay and a Waters® HSS T3 (3mm x 100mm, 3.5micron) column coupled with 2 Phenomenex Security Guard Cartridges Polar-RP for the confirmation assay.

These methods produced data that met the acceptance criteria established for the validation. Both methods produced 94% sensitivity and 100% specificity during the validation. It was also determined that there was no significant carry over after a sample of high concentration, and that there were no interferences when looking at 39 related compounds and common drugs of abuse. The analyte was stable in the extract for seven days, and stable in the auto-sampler vial for three days post-initial analysis. During development and validation, 138 patient cases were analyzed for ethyl glucuronide, and 124 cases for ethyl sulfate, and it was determined the estimated positivity rate for ethyl glucuronide was 4.35% and 100% for ethyl sulfate. Due to the 100% positivity rate for ethyl sulfate, it was removed from the scope while more information can be obtained.

Ethyl Glucuronide, Umbilical Cord, Forensic Toxicology