K2 A Novel Extraction Methodology for the Analysis of Lorazepam and Oxazepam Glucuronide Hydrolysis in Meconium

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The goal of this presentation is to present research studies establishing a novel analytical approach to the analysis of benzodiazepines in meconium using an enzymatic hydrolysis step *in situ*, followed by weak anion exchange clean-up in a disposable pipette, prior to analysis by Liquid Chromatography/Mass Spectrometry (LC/MS). Attendees will learn how the proposed methodology reduces biological matrix effects and minimizes sample preparation time.

This presentation will impact the forensic science community by illustrating how the proposed analytical methodology provides practical advantages over existing methods in terms of rapid sample clean-up and the removal of biological matrix effects that could potentially interfere with the determination of benzodiazepines by LC/MS. This method enables routine rapid monitoring of meconium for cases of suspected drug abuse.

Benzodiazepines are a schedule IV class of psychotropic drugs commonly used for their depressant properties.[1] Monitoring benzodiazepines in meconium is critical both for monitoring abuse by pregnant women and to identify potential health risks for newborns. Benzodiazepines are thought to possibly cause oral clefts.[2] Although analysis of urine can be simple and fast, benzodiazepine metabolites persist in urine only for a few days after use and drug exposure may be underestimated. Meconium is a valuable toxicological matrix because it acts as a repository for xenobiotics including drugs of abuse from the 16th week of gestation until birth; however, the use of meconium as a biological matrix to monitor drug use by liquid chromatography/mass spectrometry is problematic because of the potential for large matrix effects.[1,3] Reducing matrix effects requires extracting target analytes from the endogenous biological interferents, a task that is often time consuming and labor intensive. The objective of the present research is to minimize both meconium matrix effects and sample preparation time by a hydrolysis step *in situ*, followed by weak anion exchange disposable pipette extraction tips for sample clean-up, prior to analysis of benzodiazepines by liquid chromatography/mass spectrometry.

An aliquot of 250µL of water was added to 25mg of blank standards of meconium in an Eppendorf tube. The mixture was vortexed until the meconium became homogenously distributed/dissolved in the water. A solution consisting of 75mL of pH 7.5 potassium phosphate buffer, 50mL of a β-glucuronidase enzyme, and 10mL of 500ppb internal standard in water, was added. This mixture was then incubated for one hour at 55°C. A 600mL aliquot of acetonitrile was added to the mixture to precipitate proteins. This mixture was vortexed and centrifuged. The supernatant was removed and placed into a clean sample vial (~950ul solution). The solution was aspirated into a WAX-S tip (20mg 55-65um resin/40mg salt) twice. This step separates the acetonitrile and water layers and facilitates transfer of the analytes into the cleaner acetonitrile supernatant. The top acetonitrile layer (~500-600ul) was then transferred to a vial suitable for solvent evaporation. This step separates the acetonitrile and water layers and facilitates removal of the analytes in the acetonitrile supernatant. After evaporation in a fresh vial, the residue was reconstituted in 100mL of 10:90 methanol:water.

All analyses were performed using a triple quadrupole system with an Agilent® 1100 HPLC with a C-18 column (3.0 x 50mm, 2.7µm). Sample injections of 20µL were made using an injection valve incorporated on an autosampler. The mobile phase used 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The initial gradient was 70% A for 0.25min, which ramped to 5% A at 2min. The gradient remained at 5% A for 1min, then back to 70% A for a total run time of 6.5min. The eluent was diverted to waste during the intervals of 0-0.5min and 5-6.5min after injection. The column flow rate was 0.4mL/min. The electrospray voltage was 4,000V and the gas pressure was 60psi.

Initial success of hydrolysis was illustrated through analysis of neat meconium samples spiked with lorazepam and oxazepam glucuronides. Post-hydrolysis, a decrease of the glucuronides and concurrent increase in the parent compounds demonstrated that the method was viable. To test the validity of this method further, a blind study was performed with a collaborative laboratory including 35 meconium patient samples tested for ten benzodiazepines and/or metabolites. The blind study resulted in a correlation of approximately 92%. In conclusion, the combination of fast hydrolysis, coupled with a simple clean-up scheme, offers an effective analytical approach for the analysis of benzodiazepines in meconium.

References:

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Meconium, Benzodiazepines, Chromatography