K55 Method Development and Validation for the Analysis of Cannabinoids in Meconium Samples

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After attending this presentation, attendees will be able to compare different solid-phase extraction methods for the cleanup of meconium samples prior to Three-Dimensional/Gas Chromatography/Mass Spectrometry (3D/GC/MS) analysis.

This presentation will impact the forensic science community by providing perspectives on how to assess and improve methods for extracting, isolating, and measuring cannabinoids and their metabolites from meconium.

The purpose of this project was to develop an extraction method and instrumental parameters for GC/MS and 3D/GC/MS methods for the analysis of tetrahydrocannabinol (THC), 11-OH-THC, and THC Carboxylic Acid (THCC) that meet laboratory requirements for accuracy and precision and is cost and time efficient. The project resulted in development of an improved screening for the presence of drugs in meconium samples in aid of diagnosing and detecting marijuana use by the mother during gestation.

Collectively, the components of the marijuana plant, *Cannabis sativa*, are known as cannabinoids, and have a variety of pharmacological effects in humans including, but not limited to, analgesia, appetite suppression, hypertension, euphoria, and suppression of nausea. The main active component, THC, is broken down by the liver into a variety of oxidized metabolites. The major route of metabolism is hydrolysis of THC at carbon-11 to form 11-OH-THC which is further oxidized to form 11-Nor-carboxy-THC (THCC). These three components are the major focus of most marijuana testing in human bodily fluids. Prenatal exposure of THC is thought to possibly have detrimental effects, including effects on the systems involved in emotions and maturation. Animal studies have shown that rat pups prenatally exposed to THC could have long-lasting neurological effects. Many drug court and monitoring programs require the mother to abstain from marijuana use while pregnant to avoid exposure of the developing fetus to cannabinoids, and methods are needed to monitor their compliance with those orders.

Comparisons of several variables for the isolation of cannabinoids from meconium were performed, including the comparison of two different brands of THCC-specific Solid Phase Extraction (SPE) columns, Agilent™ and Strata X™, the length of incubation while hydrolyzing the samples, the effect of homogenizing the meconium sample before extraction, the wash solvents used, and the effects of the polarity of the GC/MS columns on which the samples were run.

The significance of these variables was evaluated by performing the same extraction with only the variable in question being altered. The general extraction method includes adding 0.25g of samples to phosphate buffer pH 7.0 and adding 25µL of 12M potassium hydroxide to hydrolyze the samples. After hydrolysis, the samples were neutralized using hydrochloric acid. The neutralized samples were then treated with acetonitrile precipitation and centrifugation before pouring the supernatant onto the SPE columns. The columns were the washed, eluted, and the extract dried down before derivatization with BSTFA.

From the comparison between the two SPE columns, it was determined that the Strata X™ columns had a better recovery of THCC with the extraction method used while the Agilent™ columns had a better 11-OH-THC recovery. The percent recovery of THCC and 11-OH-THC while using the Agilent™ columns was determined to be 91% and 66.5%, respectively. The Strata X™ columns resulted in a 95% and 56% recovery for THCC and 11-OH-THC, respectively. The incubation-time evaluation led to the conclusion that while there was little change in recovery from commercially available control samples (ElSohly Labs), an authentic THC-positive meconium control sample showed significantly increased abundance of free THCC at 30 min incubation at 60°C.

Applying the optimized extraction method using deuterated internal standards for all three analytes generated calibration curves with R^2 values greater than 0.998.

The study concluded that SPE analysis of meconium samples gave cleaner extracts than liquid/liquid extraction, that hydrolysis improved recovery of the drug from the sample, and that combined with GC/MS, SPE produced calibration curves that met laboratory requirements.

Cannabinoids, Meconium, GC/MS

 ** Presenting Author*