K3 Simultaneous Quantification of Twenty Common Drugs of Abuse and Metabolites in Human Meconium by LCMSMS

Teresa R. Gray, MS, National Institute on Drug Abuse, Chemistry and Drug Metabolism, IRP, NIH, 251 Bayview Boulevard, Suite 05A406, Baltimore, MD 21224; Diaa M. Shakleya, PhD, National Institute on Drug Abuse, 251 Bayview Boulevard, Suite 05A729.02, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, National Institute on Drug Abuse, Chemistry & Drug Metabolism, Intramural Research, National Institute on Drug Abuse, NIH, 251 Bayview Boulevard, Suite 05A721A, Baltimore, MD 21224*

After attending this presentation attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

This presentation will impact the forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings.

Drug abuse during pregnancy is associated with adverse obstetrical and neonatal outcomes. Detection of *in utero* drug exposure is often accomplished by meconium analysis due to ease and non-invasiveness of specimen collection and a long window of drug detection. However, the amount of meconium is often limited, prohibiting multiple assays for different drugs of abuse. Attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

An LCMSMS method for the simultaneous quantification of amphetamine (AMP), methamphetamine (MAMP), *p*-hydroxymethamphetamine (pOHMAMP), cocaine (COC), benzoylecgonine (BE), cocaethylene (CE), *m*-hydroxybenzoylecgonine (mOHBE), nicotine (NIC), cotinine (COT), 3'-*trans*-hydroxycotinine (OHCOT), morphine (MOR), 6-acetylmorphine (6AM), codeine (COD), hydromorphone (HYM), hydrocodone (HYC), oxycodone (OXY), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine (BUP), and norbuprenorphine (NBUP) in meconium in only 0.25 g of meconium was developed and validated.

Meconium specimens (0.25 g) fortified with deuterated internal standards were homogenized in acidic methanol. After centrifugation and supernatant evaporation, analytes were isolated using mixed mode solid phase extraction and analyzed by LCMSMS operating in positive multiple reaction monitoring (MRM) mode. Two analytical runs utilizing the same extract were required: a 5-µL injection, 18 minute run with gradient elution that quantified all analytes except BUP and NBUP. These two analytes were measured in a second 5 min isocratic run with a 10-µL injection volume to enhance sensitivity. The analytical method was validated over four days for limits of quantification, recovery, imprecision, extraction efficiency, matrix effects, carryover, and endogenous and exogenous interference.

Limits of quantification were 1 ng/g for COT, CE, BE, and COC, 2.5 ng/g for MAMP, EDDP, MTD, and pOHMAMP, 5 ng/g for AMP, mOHBE, NIC, OHCOT, MOR, 6-AM, HYM, HYC, OXY, and 25 ng/g for BUP and NBUP. The upper limit of quantification for all analytes was 500 ng/g, except for pOHMAMP at 250 ng/g. Correlation coefficients for each calibration curve were >0.996 with all calibrators quantifying within ±20% of target when calculated against the calibration curve. Validation parameters were tested at three concentrations spanning the linear dynamic range. Intra- and inter-day recovery ranged from 83.3 – 126.6% and 80.1 – 129.0%, respectively. Inaccuracies of up to 30% were considered acceptable due to meconium's complexity. Intra- and inter-day imprecision ranged from 0.9 – 16.9% relative standard deviation (RSD) and 3.1 – 9.8% RSD, respectively. Extraction efficiencies ranged from 46.7 – 96.0%. Matrix effects ranged from -305.7 – 40.7%, depending on the analyte, with negative values indicating ion enhancement. Matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds, highlighting the importance of employing matched deuterated internal standards in LCMSMS quantification procedures, especially with complex matrices. Similar results were observed for matrix effects determination in seven different blank meconium sources fortified with low quality control concentrations; while matrix effects varied between meconium specimens, matrix enhancement or suppression of related native, and deuterated compounds were similar and quantification was within acceptable limits. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 48 h on the 15°C autosampler. Losses of less than 34.0% were observed for each condition, except for 6AM and MOR. After room temperature, 4°C, and three freeze-thaw cycles, up to 85.8% of 6AM was lost; however, MOR concentrations under these conditions increased by up to 31.2%. In cases of suspected heroin exposure, meconium should be immediately frozen and repeated freeze thaw cycling should be avoided. No analyte carryover was observed at two times the upper limit of quantification. No interference by 57 illicit and therapeutic drugs or endogenous meconium compounds was observed. Method applicability for all analytes except 6AM, BUP, and NBUP was demonstrated by analysis of meconium from drug-exposed neonates.

 ** Presenting Author*

The most comprehensive chromatographic method for the identification and quantification of drugs and metabolites in meconium is described. This LCMSMS method will impact the clinical and forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings. This method will be employed in prenatal drug exposure studies to correlate meconium concentrations to neonatal outcome measures.

LCMSMS, Drugs of Abuse, Meconium

 ** Presenting Author*