K12 The Detection and Quantitation of Ten Synthetic Cannabinoid Metabolites in Human Urine Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)

Cassandra A. Swart, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Daniel Lee, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Mikayla Caldwell, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Katherine N. Moore, MS, Research Triangle Park, NC 27709; Nichole D. Bynum, MS, RTI International, Johnson Building, Research Triangle Park, NC 27709; Sabra R. Botch-Jones, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118*

Learning Overview: After attending this presentation, attendees will be able to use the method described, or develop their own singular method, for analysis of urine for selected synthetic cannabinoid metabolites.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the analysis of selected synthetic cannabinoid metabolites.

Background/Introduction: Synthetic cannabinoids remain in the top 25 drug-encountered analytes in seized drug evidence based on the United States Drug Enforcement Administration's National Forensic Laboratory Information System 2017 mid-year report. Despite efforts to control synthetic cannabinoids, illicit manufacturers continue to modify their structures to avoid legal regulations, creating an ever-changing analytical target for forensic laboratories. In addition, due to structural modifications of these synthetic cannabinoids, many of these compounds can bind to endogenous CB1 and CB2 receptors with greater affinity, causing severe adverse and life-threatening effects. Because of their structural dissimilarity to Δ^{9}-Tetrahydrocannabinol (THC), combating the rapid growth and emergence of synthetic cannabinoids with conventional THC-based methods is often not effective.

Objective: With a focus on synthetic cannabinoids of different core structures such as naphthoylindole, admantoylindole, quinolinyl, and carboxamide, the purpose of this research was to develop and validate a robust and reliable method to accurately identify and quantify ten synthetic cannabinoid metabolites in human urine.

Method: Using HPLC with a 4000 QTRAP® Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS) in positive ionization mode, samples were extracted using supported liquid extraction using ISOLUTE® cartridges. The method was validated in accordance to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for quantitative analysis using the following analytes: UR-144 degradant N-pentanoic acid, UR-144 N-(5-hydroxypentyl), PB-22 N-(5-hydroxypentyl), MDMB-FUBICA metabolite 3, JWH 250 N-pentanoic acid, ADB-PINACA pentanoic acid, ADB-PINACA N-(4-hydroxypentyl), AB-FUBINACA metabolite 3, 5-fluoro PB-22 3-carboxyindole, and 5-fluoro MDMB-PICA metabolite 7.

Results: With this developed method, total analysis time was eight minutes with samples eluting within 3.26 to 4.47 minutes. Calibration curves for each analyte had accepted R^2 values > 0.99. The calibration model was established to be linear using a weighting factor of 1/x. A linear dynamic range of 5ng/mL –40ng/mL was used for all analytes. Extraction of analytes using Supported Liquid Extraction (SLE) cartridge improved sample-prep time by more than half, compared to traditional solid phase extraction methods. Percent recovery of analytes using the SLE was determined to be from 68.4% to 90.7%. Bias and precision was assessed at 5ng/mL, 25ng/mL, and 30ng/mL for all analytes. Samples had acceptable calculated percent bias and percent coefficient of variation within ±20%. No carryover was observed. No interference was observed for other commonly encountered drugs clonazepam, diazepam, (+) methadone, morphine, fentanyl, cocaine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 25I-NBOMe, and Phencyclidine (PCP) at 2,000ng/mL.

Conclusion/Discussion: The overall development and validation of this method demonstrates a sensitive and reliable way to positively identify ten different synthetic cannabinoid metabolites in human urine in rapid time.

Synthetic Cannabinoids, Supported Liquid Extraction, Metabolites