K8 Development of a Quantitation Method for Synthetic Cannabinoid Metabolites in Urine Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will be able to perform a Liquid-Liquid Extraction (LLE) method followed by an LC/MS/MS method to extract, detect, and quantify synthetic cannabinoid metabolites of JWH-018, UR-144, AKB-48, AB-PINACA, ADB-PINACA, ADBICA, PB-22, 5F-PB-22, and BB-22 from urine.

This presentation will impact the forensic science community by demonstrating a validated method for the extraction and quantitation of metabolites of nine synthetic cannabinoids from urine samples. The parent drugs are currently scheduled by the Drug Enforcement Administration (DEA) as Schedule I compounds or have been recently identified as emerging cannabinoid agents being sold in synthetic cannabis blends. Development of an updated assay to detect and quantify the metabolites is important for forensic and toxicology laboratories for the assessment of prior consumption of the latest generation of synthetic cannabinoids.

Over the past few years, synthetic cannabinoids have become increasingly popular and prevalent in an effort by drug users to bypass current legislation and achieve a "legal" high. At the federal level in the United States, most are classified Schedule I substances by the DEA as they have a high potential for abuse and no medical purpose, so in an attempt to escape legal consequences, "manufacturers" produce compounds that are structurally different from currently scheduled drugs, but still give similar effects to achieve that high. JWH-018, UR-144, ADB-PINACA, AKB-48, PB-22, and 5F-PB-22 have been scheduled by the DEA while AB-PINACA, ADBICA, and BB-22 are currently not scheduled but have recently been identified as being components in synthetic cannabis blends in Japan and the United States. The structures of emerging synthetic cannabinoid compounds are believed to have similar properties to previously recognized compounds. As the number of compounds continues to increase, it is essential to develop a method that is versatile and can keep up with the rapidly emerging compounds as these newly emerged compounds may not register a positive result in common drug screening procedures.

An updated method was developed and validated to extract, identify, and quantify the N-pentanoic acid metabolites of JWH-018, UR-144, AKB-48, AB-PINACA, ADB-PINACA, and ADBICA; and the 3-carboxyindole metabolites of PB-22, 5F-PB-22, and BB-22 from urine, using LLE followed by LC/MS/MS. The metabolites were chosen based on the possibility of being active metabolites based on recent publications and also since there would be no detection issues as there could be with the various hydroxylated metabolites.

The liquid chromatograph and mass spectrometer conditions were optimized for the nine synthetic cannabinoid metabolites, including the mobile phase gradient and Multiple Reaction Monitoring (MRM) transitions. The LC conditions included a ten-minute run with initial conditions of 70:30 ratio of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). Over eight minutes, the ratio switched to 10:90 of A:B and was then held for one minute, before re-equilibrating to the starting conditions at 9.1 minutes. The method achieved baseline separation and identification was based on their MRM transitions. Calibration models were produced in the range of 1ng/mL-100ng/mL for JWH-018, UR-144, AKB-48, PB-22, and 5F-PB-22 metabolites; 1ng/mL-50ng/mL for ADBICA, AB-PINACA, and ADB-PINACA metabolites; and 5ng/mL-100ng/mL for BB-22 metabolite (R^2>0.98). The limits of detection were at or less than 2ng/mL and limits of quantitation were at or less than 5ng/mL. The method was validated using Scientific Working Group for Toxicology (SWGTOX) guidelines for quantitative methods and once the method was validated, it was applied successfully to authentic urine samples. The range for authentic samples was 2ng/mL-250ng/mL. Specificity was determined by testing possible compounds that could produce a possible interference and then comparing the true negatives to the sum of samples that were true negatives and false positives. This method will be of use to the field of forensic toxicology as it incorporates a method to test for the consumption of currently scheduled compounds and recently emerging synthetic cannabinoids.

Synthetic Cannabinoids, Urine, LC/MS/MS

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