Development of a quantitation method for synthetic cannabinoid metabolites in urine using liquid chromatography tandem mass spectrometry (LC-MS/MS)

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Over the past few years, synthetic cannabinoids have become increasingly popular and prevalent in an effort from drug users to bypass current legislation and achieve a "legal" high. At the federal level in the United States, most are Schedule I substances by the Drug Enforcement Administration (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, 5F-PB-22, and AB-PINACA have been scheduled by the DEA; while 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 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 liquid-liquid extraction followed by liquid chromatography-mass spectrometry/mass spectrometry.

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. Three different sources of β-glucuronidases, which included E. coli, Helix pomatia, and red abalone were also compared to optimize the hydrolysis step of the method and since the three sources were not significantly different in their effectiveness, red abalone was selected based on cost and availability. The liquid chromatograph 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). The method achieved baseline separation and identification was based on the MRM transitions of each analyte. Calibration curves for each analyte showed acceptable correlation over the range of 1-100 ng/mL ($R2 > 0.99$) and the limits of detection and quantitation were equal to or less than 1 ng/mL. Validation of the method was started using SWGTOX guidelines and the method was applied successfully to blind samples that were independently prepared.