K59 Development and Validation of Two Methods for the Analysis of Synthetic Cannabinoids in Whole Blood

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After attending this presentation, attendees will better understand the Scientific Working Group for Forensic Toxicology (SWGTOX) -compliant approach to method validation for the analysis of two classes of synthetic cannabinoid compounds in forensic whole blood samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology.

This presentation will impact the forensic science community by providing information about two complementary analytical methods used to analyze 34 synthetic cannabinoid compounds of the Indazole Carboxamide (NACA) and Indole classes, many of which have only recently appeared on the market. This presentation will also inform attendees about recent trends in positivity rates for these compounds in forensic samples.

Since the mid to late 2000s, an increasing number of New Psychoactive Substances (NPS), including synthetic cannabinoids, have been appearing on the illicit drug market in the United States. In 2011, one such compound, AM-2201 was ranked at number 21 in the National Forensic Laboratory Information System (NFLIS) ranking of most frequently encountered drugs in the nation's crime laboratories. In the same year, looking in the hallucinogen category, seven unique synthetic cannabinoids appeared in the top 16 rankings. By 2013, there were 17,242 synthetic cannabinoids cases in the United States. In 2014, XLR-11 was joined by a new category of NACA compounds including AB-FUBINACA,AB-PINACA, ADBICA, 5F-ADBICA, ADB-PINACA, ADB-FUBINACA, 5F-ADB-PINACA, 5F-APICA, PB-22, 5F-PB-22, BB-22, FUBIMINA, THJ-018, 5F-AB-001, AB-PINACA AB-CHMINACA, and ADB-CHMINACA, in addition to the more traditional indole class members including the JWH, and AM series, and XLR-11.

This presentation describes two complementary methods for the analysis of 34 currently popular synthetic cannabinoids compounds in whole blood using Liquid-Liquid Extraction (LLE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The compounds were segregated into two groups depending on their chemistry (NACA and Indole classes) for analyses, which were individually optimized for extraction conditions and performance. The cutoff concentration for the NACA compounds in the NACA group were 1ng/mL for AB-FUBINACA, ADBICA, 5F-ADBICA, ADB-FUBINACA, 5F-ADB-PINACA, and AB-CHMINACA and 0.2ng/mL for ADB-PINACA, AB-PINACA, and ADB-CHMINACA. The cutoff concentration for the Indole compounds were 0.1ng/mL for JWH-018, AM-2201, JWH-122, JWH-081, MN-18, 5F-MN-18, MN-25, FUB-PB-22, MDMB-CHMINACA, and AB-001 and 0.2 ng/mL for JWH-210, UR-144, XLR-11, FUB-AKB48, and APICA and 1ng/mL for 5F-APICA. Since this is a qualitative test, any samples with analyte about the cutoff concentration are considered positive. The methods were subject to a SWGTOX-compliant validation procedure that evaluated precision around the decision concentration (cut-off) , stability in matrix and on-instrument, sensitivity and specificity, robustness, an evaluation of interfering compounds, matrix effect, and extraction efficiency. After the validation was complete, authentic patient samples were analyzed using the method and positivity trends were evaluated.

Sample preparation consisted of single-step LLEs using 3mL Methyl Tertiary Butyl Ether (MTBE) for the NACA group (*n*=9) and 3mL 99% Hexane/1% Ethyl Acetate for the Indole group (*n*=25). The analytical method consisted of separation using an ACQUITY® UPLC BEH C18 (100mm x 2.1mm, 1.7-micron) column coupled with a VanGuard™ BEH C18 1.7-micron guard column and a gradient elution. The NACA class started with an initial mixture of 55% mobile phase A (0.1% formic acid in water) and 45% mobile phase B (80% acetonitrile/20% methanol), while the Indole class started with an initial mixture of 50% mobile phase A and 50% mobile phase B, both transitioned to a final mixture of 5% mobile phase A and 95% mobile phase B. Both NACA and Indole classes had a total runtime of eight minutes. Both methods were run on a Waters® Xevo-TQS®.

This method produced data that met the acceptance criteria for precision around the cutoff concentration and was shown to be 100% sensitive and specific in blinded spiked controls in diverse whole blood samples. The method was also shown to meet validation criteria for precision around the decision concentration, stability in matrix and on instrument, robustness, interference, matrix effect, and extraction efficiency.

Subsequently, the method has been in production for four months, during which time a total of 661 samples have been tested. Switching from the previous scope to the scope described in this presentation, the positivity rate for the test increased from approximately 7% on the prior method to 35% on the method described herein. The most prevalent compounds in casework samples during the period March-June 2015 were AB-CHIMINACA, XLR-11, and ADB-CHMINACA.

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Over the four months the method has been in place, the number of positives has demonstrated a decline, suggesting further evolution of the synthetic cannabinoid illicit market. The laboratory is currently validating a revised scope developed using various drug intelligence sources and has committed to a nine-month update on the scope of testing to keep pace with the changing market.

Synthetic, Forensic Toxicology, Cannabinoids

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