K47 The Identification of Synthetic Cannabinoids in Forensic Toxicology Casework Using an Archived High Resolution Mass Spectrometry Data System

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Learning Overview: After attending this presentation, attendees will be able to describe a rapid Liquid Chromatography (LC) high resolution Time-Of-Flight Mass Spectrometry (TOF/MS) method for the analysis of synthetic cannabinoids and workflow for the retrospective datamining of previously acquired sample extracts and TOF data.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a novel approach for creating a historical data archive for biological specimens that will be continually re-interrogated as new synthetic cannabinoids and their metabolites are discovered to determine retrospectively the date of first appearance and spread of new compounds as they appear on the drug market.

Since 2008, synthetic cannabinoids have continued to infiltrate the drug market and have been implicated in an increasing number of emergency room admissions, death investigations, and high-profile intoxication events in corrections populations. Understanding the scale and scope of these events requires the availability of comprehensive analytical testing, which is currently lacking due to the speed with which new compounds appear. From a laboratory standpoint, the challenges of remaining current with synthetic cannabinoids stem from the diversity of compounds in the class, the large number of analogs and configurations, and delays in availability of analytical standards.

For the extraction of synthetic cannabinoids and their metabolites, blood samples (0.5mL) were prepared using liquid-liquid extraction with Tris hydrochloride buffer (pH 10.2) and MTBE. Urine samples (1mL) were prepared using Solid Phase Extraction (SPE) with ammonium carbonate buffer (pH=9.3) and elution with formic acid in methanol. All sample extracts were evaporated to dryness and reconstituted in 200µL of mobile phase.

Samples were acquired using a SCIEX® TripleTOF® 5600+ quadrupole TOF/MS coupled to a Shimadzu® Nexera® ultra high-performance liquid chromatograph. A reverse phase gradient using ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) was employed for chromatographic separation on a Phenomenex® Kinetex® C18 analytical column (50mm x 3.0mm, 2.6µm) at a flow rate of 0.5mL/min. The total run time for analysis was seven minutes. Precursor ions were acquired by TOF/MS scan (100m/z–550m/z) via positive electrospray ionization. Precursor isolation was performed using SWATH® acquisition with overlapping windows from 10-25 Da in width, and fragmentation was achieved using a rolling collision energy of 35eV±15eV.

Data processing was performed using PeakView software with an Extracted Ion Chromatogram (XIC) list containing 262 synthetic cannabinoid parent compounds, metabolites, and internal standards. New standards are continually being added to the library as they become available. All previously analyzed casework data files are reprocessed using PeakView to investigate if other synthetic cannabinoids are present that were not known about at the time of initial testing.

This synthetic cannabinoid screening method was qualitatively validated for 19 parent compounds in blood and 19 metabolites in urine. These compounds were determined to be representative of the overall library database (*n*=262), spanning a range of generations and diverse chemistries based on standard availability, and reflected the most currently prevalent drugs and their metabolites. Following three days of qualitative validation, all analytes met method validation performance criteria for precision/accuracy, limits of detection, interferences, processed sample stability, and carryover.

To date, 227 extracts from NMS Labs have been analyzed for synthetic cannabinoids. In total, 44 extracts were positive for at least one synthetic cannabinoid (parent or metabolite) for a positive rate of 19.4%. Table 1 shows all positive parent compounds identified and their rates of occurrence. This initial analysis has resulted in the identification of newly emergent synthetic cannabinoids, including 4-cyano-CUMYL-BUTINACA, 5F-EDMB-PINACA, and 5F-MDMB-PICA, which were not previously included in the scope at the time of testing.

Table 1: Synthetic Cannabinoid Parent Positivity (June and July 2018)

Parent Compound	Occurrence
5F-ADB (5F-MDMB-PINACA)	20
MMB-FUBINACA (FUB-AMB)	9
ADB-FUBINACA	8
5F-MDMB-PICA	6
AB-PINACA	2
4-cyano-CUMYL-BUTINACA	2
5F-EDMB-PINACA	2
5F-PB-22	1
AB-CHMINACA	1
5F-AMB	1

A rapid method for analysis of synthetic cannabinoids has been developed, validated, and successfully applied to forensic sample extracts. Additional populations, such as corrections and emergency room patients, are also being tested. As new synthetic cannabinoids appear on the market, they will be added to the library database and all previously acquired data will be re-interrogated for the new analyte. Data will be continually tabulated to create timelines and trend reports within these populations.

Synthetic Cannabinoids, LC/qTOF, 5F-ADB