Novel Utility of High Resolution Mass Spectrometry (HRMS) for the Discovery of Emerging Synthetic Cannabinoids and their Metabolites in Forensic Casework

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Overview

- Synthetic cannabinoids continue to cause adverse events and deaths among recreational drug users, as emergent synthetic cannabinoids often go undetected or unidentified
- Our laboratory developed a UHPLC-QTOF-MS method with SWATH® Acquisition to conduct sample mining and data mining to characterize emergent synthetic cannabinoids
- The UHPLC-QTOF-MS method was qualitatively validated for blood and urine samples
- Authentic biological samples from forensically relevant casework were analyzed
- Sample mining: several new synthetic cannabinoids were detected, including 5F-MDMB-PICA, 4F-MDMB-BINACA, APP-BINACA, and 5F-EDMB-PINACA
- Data mining: several metabolites for 4F-MDMB-BINACA were identified

Introduction

Synthetic cannabinoids have infiltrated a variety of illicit drug products in recent years. Proliferation of synthetic cannabinoid use has been made apparent by the increasing number of emergency department admissions, death investigations, and high-profile intoxication events, specifically those in correctional facilities.

Synthetic cannabinoids provide great analytical challenges to laboratory personnel and forensic scientists across the county. The rate at which new synthetic cannabinoids are discovered leads to the need for updated methods of testing on a monthly or weekly basis. Traditional targeted analyte detection does not allow for the constant evolution necessary to keep up with expanding synthetic cannabinoid drug markets. Therefore, newer technologies utilizing non-target acquisition approaches are vital to remaining at the forefront of forensic drug testing.

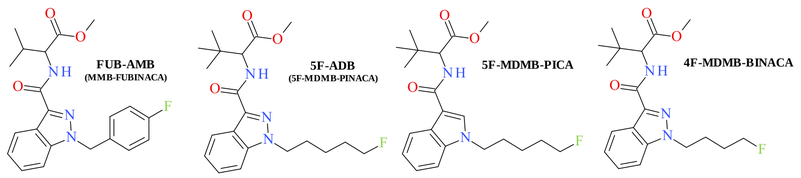

Figure 1: Popular Synthetic Cannabinoids in Current Recreational Drug Supplies

Objective

This study sought to evaluate innovative methodologies (e.g. UHPLC-QTOF-MS with SWATH® Acquisition) for the timely identification of emerging synthetic cannabinoids in relation to first incident and/or first detection. To accomplish this objective, a drug-rich dataset (e.g. sample extracts) was used for comprehensive sample mining and the resulting datafiles were used for comprehensive data mining, specifically to study metabolic profiles.

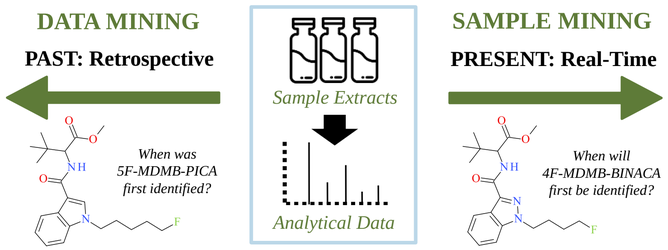

Figure 2: Sample Mining vs. Data Mining

Methods

Sample Preparation and Acquisition

1. Blood samples were prepared by liquid-liquid extraction (LLE)
2. Urine samples were prepared by solid phase extraction (SPE)
3. Discarded de-identified sample extracts were obtained from NMS Labs (Horsham, PA) correlating to authentic biological samples from forensic investigations

Method Validation

1. Qualitative validation included 19 synthetic cannabinoid parent compounds in blood and 19 synthetic cannabinoid metabolites in urine (prepared in triplicate over five days)
2. Evaluated validation performance characteristics including precision/accuracy, limits of detection (LOD), interferences, processed sample stability, and carryover

Analysis by UHPLC-QTOF-MS

LC: Shimadzu Nexera XR Ultra High Performance Liquid Chromatograph (UHPLC)

MS: Sciex TripleTOF® 5600+ Quadrupole Time-of-Flight Mass Spectrometer (QTOF-MS)

- Column: Phenomenex® Kinetex C18 (2.6µm, 3.0 x 50mm)
- Mobile Phase A (MPA): 10mM Ammonium Formate in Water, pH 3 with Formic Acid
- Mobile Phase B (MPB): 0.1% Formic Acid in Acetonitrile and Methanol (50:50)
- Injection Volume: 20 µL, Flow Rate: 0.5 mL/min, Total Run Time: 7 minutes
- Positive Electrospray Ionization, SWATH® Acquisition

Table 1: LC Gradient

Time (min)	% MPA	% MPB
0	95	5
0.5	95	5
4	5	95
6	5	95
6.1	95	5
7	95	5

Table 2: MS Acquisition Parameters

Parameter	Set Point
TOF MS Scan Range	100-550 Da
Precursor Ion Isolation	SWATH®
Q1 Isolation Windows	Variable: 10-25 Da
Collision Energy	35±15 eV
Fragment Scan Range	40-550 Da
Cycle Time	0.91 seconds

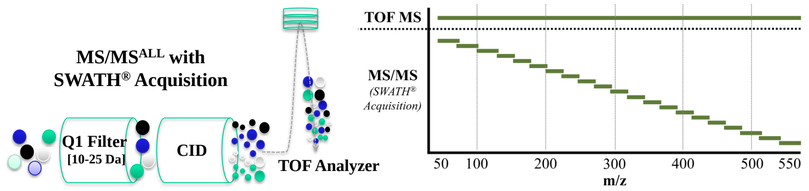

Figure 3: Illustrations of SWATH® Acquisition

Data Processing

Software: PeakView® (Sciex, Version 2.2) and MasterView™ (Sciex, Version 1.1)

Table 3: Processing Criteria

Criteria	Pass	Review*	Fail
Mass Error (ppm)	<5	<10	>10
Retention Time Error (min)	<0.25	<0.35	>0.35
Isotope Ratio (% Difference)	<30	<100	>100
Library Score	>90	>50	<50
Signal-to-Noise Ratio	>10	-	<10
Peak Intensity (counts)	>800	-	<800

Table 4: Library Database

Category	# Analytes
Parent Compounds	>240
Metabolites	23
Internal Standards	16
Total	***>275***

*Additional criteria for analyst review included acceptable chromatography, acceptable chromatographic and mass spectral peak shape, acceptable library spectra, and control comparison

Results

Table 5: Synthetic Cannabinoid Positivity (Parent Compounds)

Synthetic Cannabinoid (Parent)	Positive Samples (n=454)	% Positivity (n=4,743)
5F-ADB	149	3.1
5F-MDMB-PICA	**124**	**2.6**
4F-MDMB-BINACA	**58**	**1.2**
FUB-AMB	48	1.0
ADB-FUBINACA	22	0.5
APP-BINACA	**10**	**0.2**
5F-EDMB-PINACA	**6**	**0.1**
4-CN-CUMYL-BINACA	**5**	**0.1**
FUB-AKB-48	5	0.1
AB-FUBINACA	5	0.1
AB-CHMINACA	3	0.1

• *2 Identifications*: **5Cl-AKB-48**, AB-PINACA, 5F-ADBICA, 5F-AMB, 5F-AB-PINACA MAB-CHMINACA
• *1 Identification*: **MFUBINAC**, **5Cl-AB-PINACA**, HU-331, 5F-PB-22, 5F-ADB-PINACA, MMB-CHMICA, MDMB-FUBINACA

Table 6: Synthetic Cannabinoid Positivity (Metabolites)

Synthetic Cannabinoid (Metabolites)	Positive Samples (n=282)	% Positivity (n=4,743)
5F-ADB Metabolite	114	2.4
FUB-AMB Metabolite	107	2.3
5F-MDMB-PICA Metabolite	**40**	**0.8**
4F-MDMB-BINACA Metabolite	**15**	**0.3**
4-CN-CUMYL-BINACA Metabolite	**2**	**0.04**

• *1 Identification*: ADB-PINACA Metabolite, 5F-AMB Metabolite, 5F-NPB-22 Metabolite, MDMB-FUBICA Metabolite

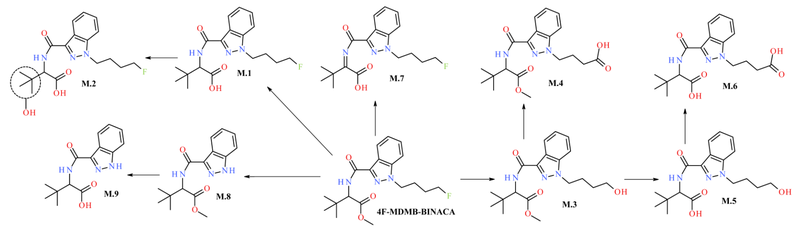

Figure 4: In Vivo Metabolites of 4F-MDMB-BINACA

Conclusions

- UHPLC-QTOF-MS with SWATH® Acquisition is a valuable tool for the characterization of emergent synthetic cannabinoids (and other drug species) in forensic samples
- Sample mining and data mining provide useful insight into drug use patterns and biological breakdown (e.g. metabolism) for new substances previously unidentified

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