Synthetic Cannabinoids

Alex J. Krotulski, PhD, Program Manager – NPS Discovery, Associate Director – CFSRE

Novel Psychoactive Substances (NPS): Analytical Methods and Encountered Challenges (Part 1)

SOFT NPS Webinar – Wednesday April 23, 2021

Disclosure

- I have no conflicts of interest to disclose.

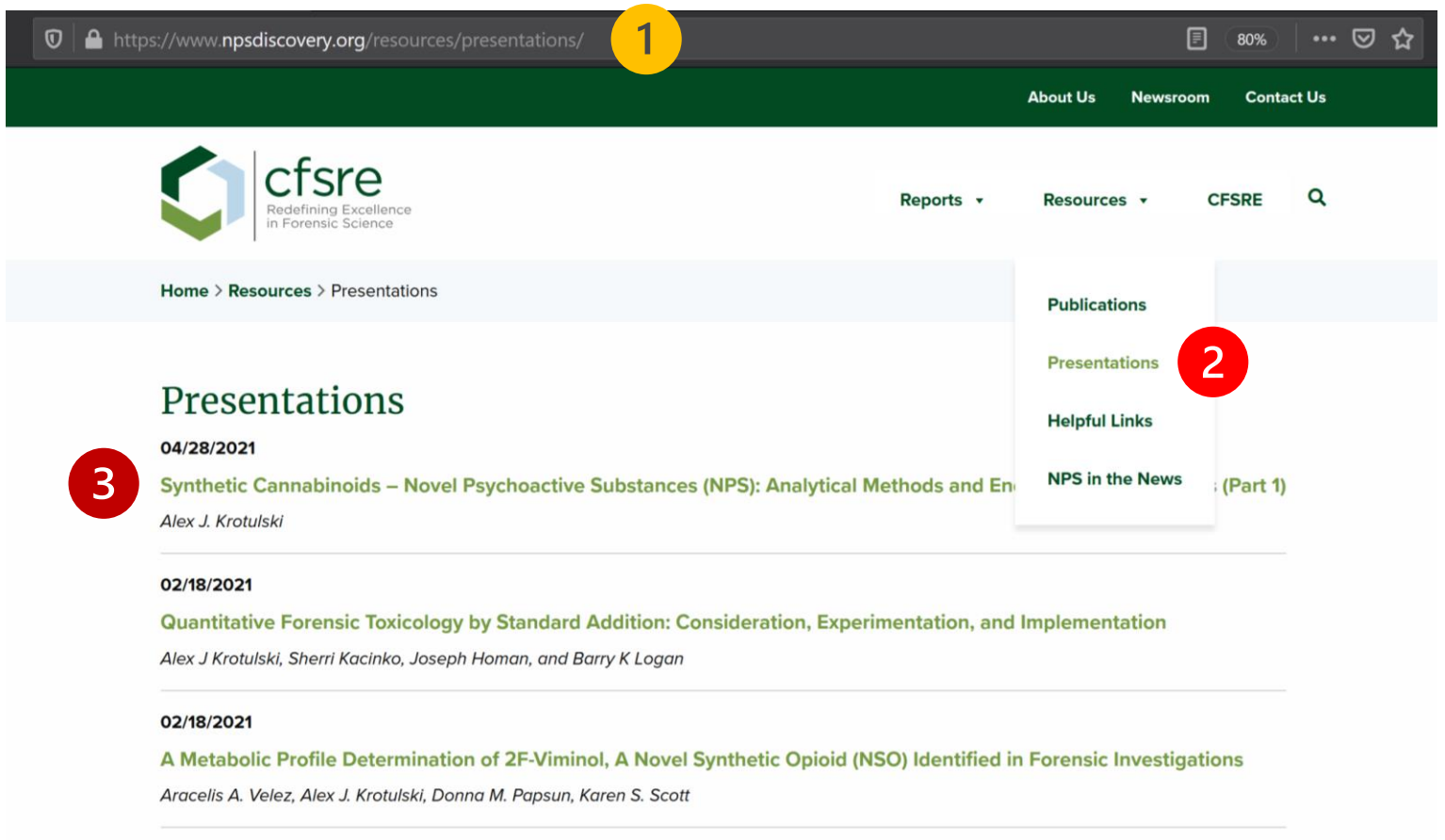
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.

Download A Copy of This Presentation!

1 **www.npsdiscovery.org**

2 **Resources → Presentations**

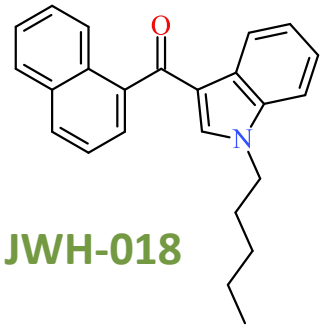
3 **Click Presentation Title**

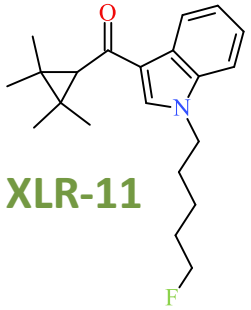


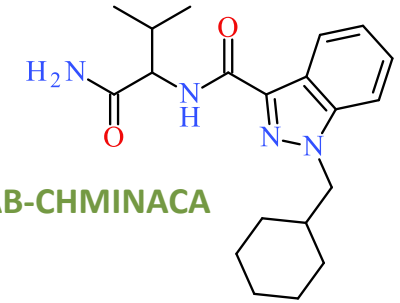
BACKGROUND

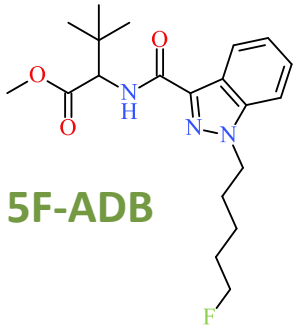
Synthetic Cannabinoids, Diversity, & Naming

- Synthetic drugs that mimic effects of THC / target endogenous CB receptor system

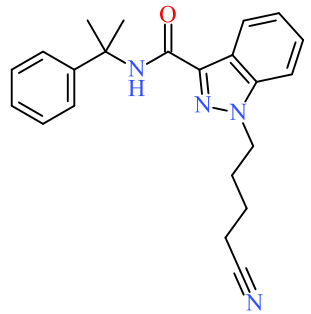

JWH-018

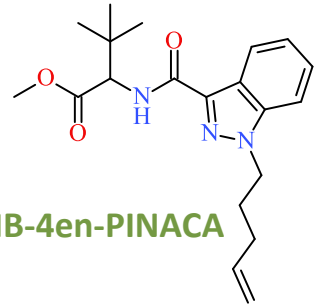

XLR-11

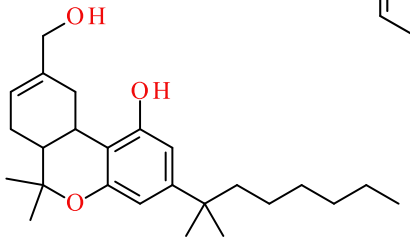

AB-CHMINACA

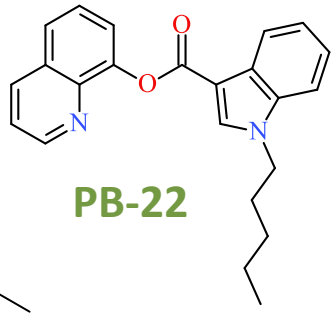

5F-ADB

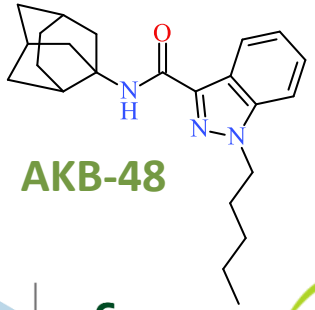
4CN-CUMYL-BINACA

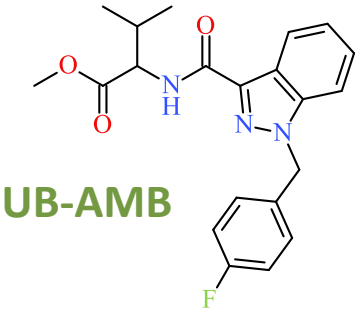



MDMB-4en-PINACA

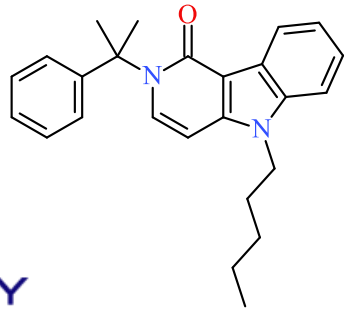

HU-210

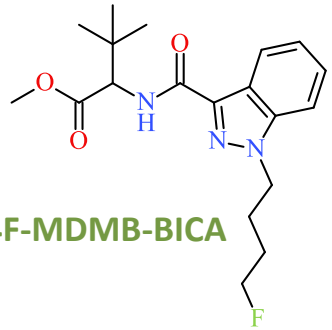

PB-22


AKB-48


FUB-AMB

CUMYL-PeGaCLONE



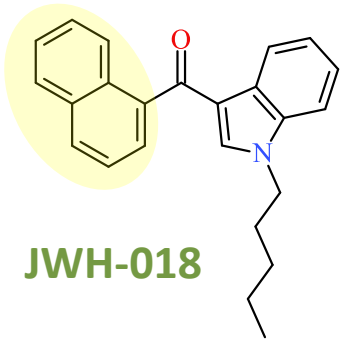

4F-MDMB-BICA

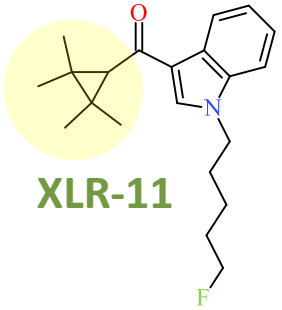
cfsre

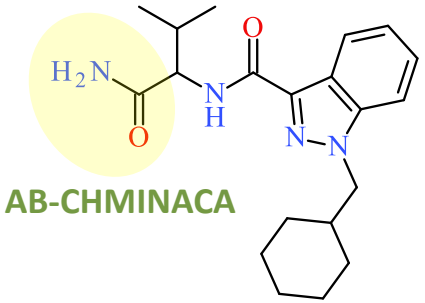
NPS DISCOVERY

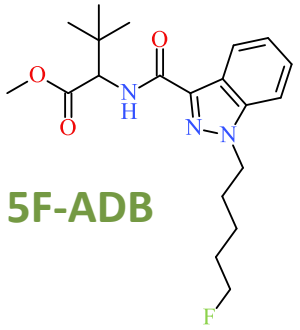
Synthetic Cannabinoids, Diversity, & Naming

- Synthetic drugs that mimic effects of THC / target endogenous CB receptor system

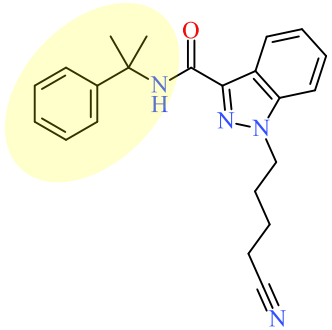

JWH-018

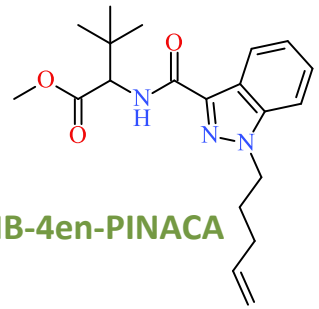

XLR-11

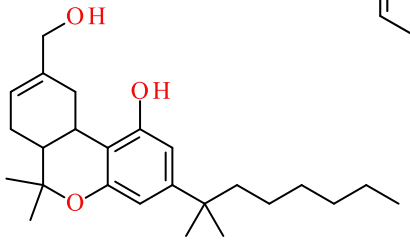

AB-CHMINACA

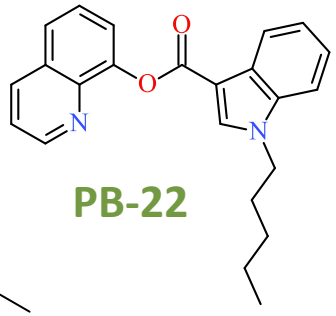

5F-ADB

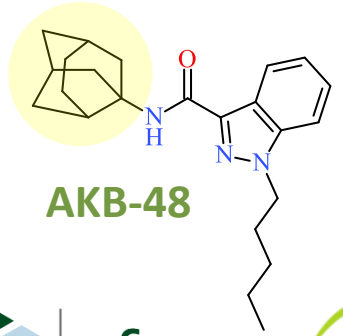
4CN-CUMYL-BINACA

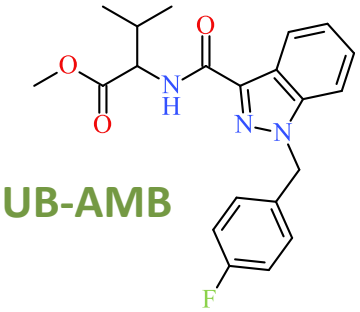



MDMB-4en-PINACA

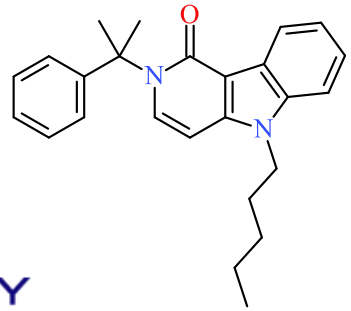

HU-210

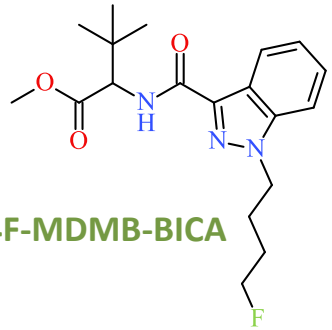

PB-22


AKB-48


FUB-AMB

CUMYL-PeGaCLONE



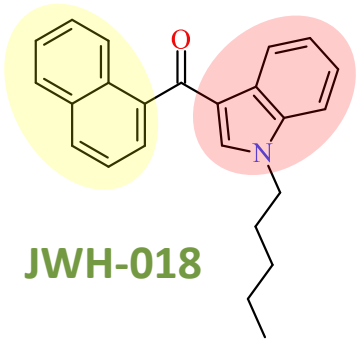

4F-MDMB-BICA

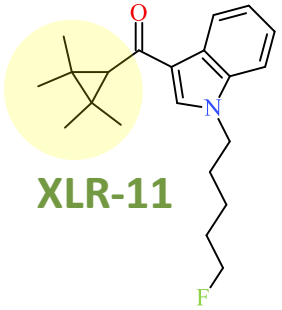
cfsre

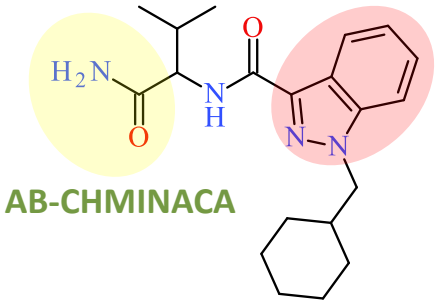
NPS DISCOVERY

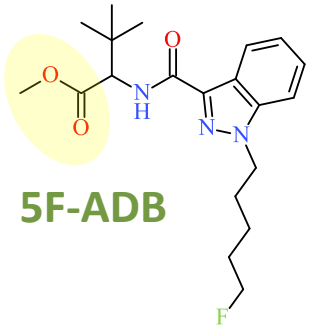
Synthetic Cannabinoids, Diversity, & Naming

- Synthetic drugs that mimic effects of THC / target endogenous CB receptor system

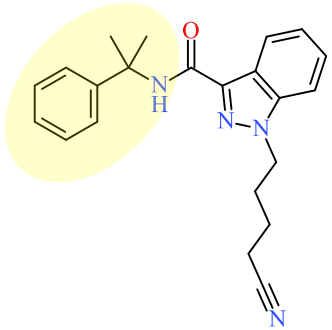

JWH-018

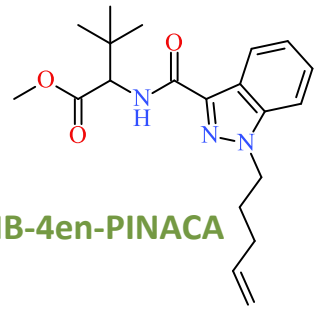

XLR-11

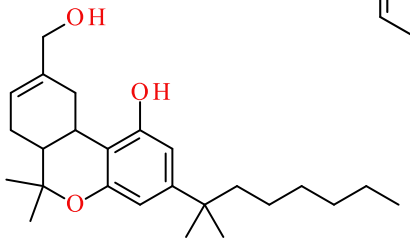

AB-CHMINACA

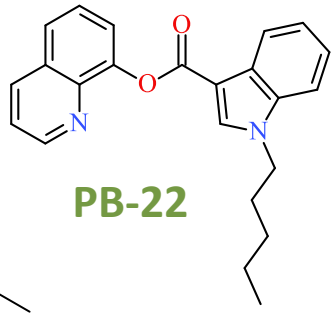

5F-ADB

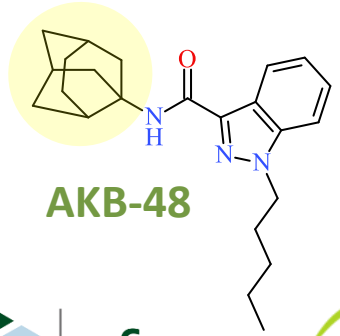
4CN-CUMYL-BINACA

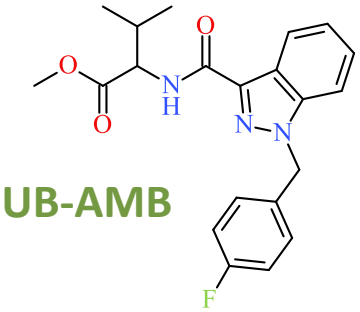



MDMB-4en-PINACA

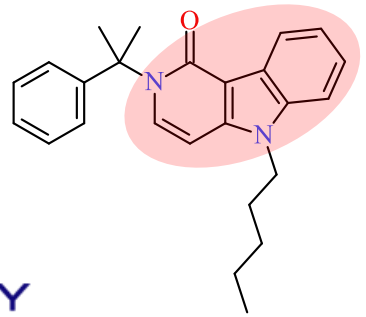

HU-210

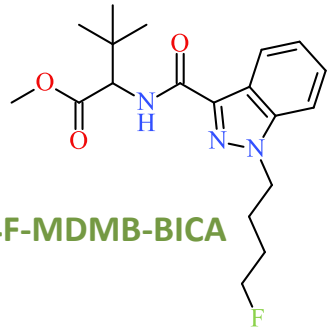

PB-22


AKB-48


FUB-AMB

CUMYL-PeGaCLONE



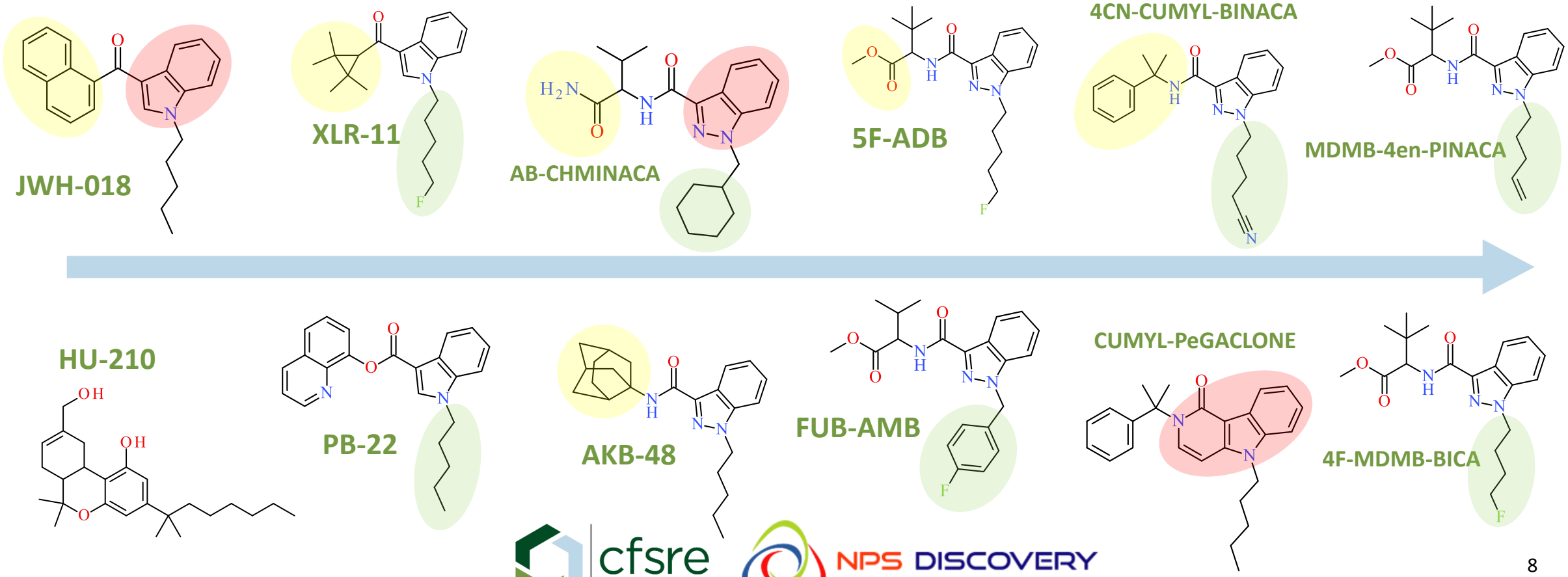

4F-MDMB-BICA

cfsre

NPS DISCOVERY

Synthetic Cannabinoids, Diversity, & Naming

- Synthetic drugs that mimic effects of THC / target endogenous CB receptor system



cfsre

NPS DISCOVERY

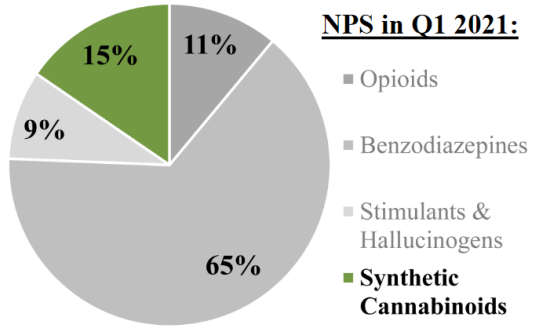
Trend Report: Q1 2021

Synthetic Cannabinoids in the United States

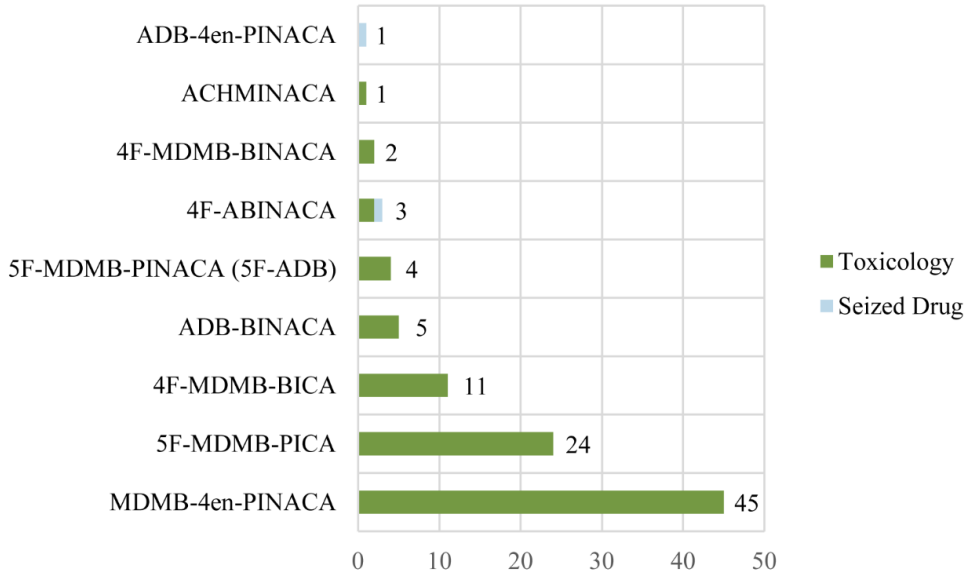
Purpose: This report provides up-to-date information regarding the status of synthetic cannabinoid prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including synthetic cannabinoids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. Synthetic cannabinoids have been implicated in an increasing number of emergency room admissions, death investigations, and intoxication events in corrections populations. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

Objective: Our laboratory utilizes novel approaches for the analysis of drugs in biological samples and seized materials using comprehensive non-targeted data acquisition by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The scope of analysis contains more than 900 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of novel synthetic cannabinoids and further data analysis of important trends. This project was conducted in collaboration with the toxicology and criminalistics laboratories of NMS Labs. Forensic case types linked to these results include illicit drug investigations, medicolegal death investigations, and/or driving under the influence of drugs (DUID) investigations. The results in this report represent the total number of NPS identifications at the CFSRE during this quarter, including those from sample-mining, data-mining, and/or esoteric testing.

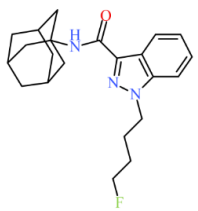


Synthetic Cannabinoid Positivity



Synthetic Cannabinoid Combinations

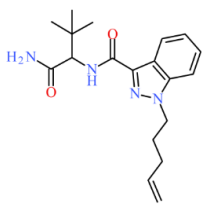
Combination	Frequency
MDMB-4en-PINACA + 5F-MDMB-PICA	11
MDMB-4en-PINACA + 4F-MDMB-BICA	8
5F-MDMB-PICA + 4F-MDMB-BICA	3
MDMB-4en-PINACA + 4F-MDMB-BINACA	2
4F-MDMB-BICA + 4F-MDMB-BINACA	2



New Identifications:

ADB-4en-PINACA →

← 4F-ABINACA



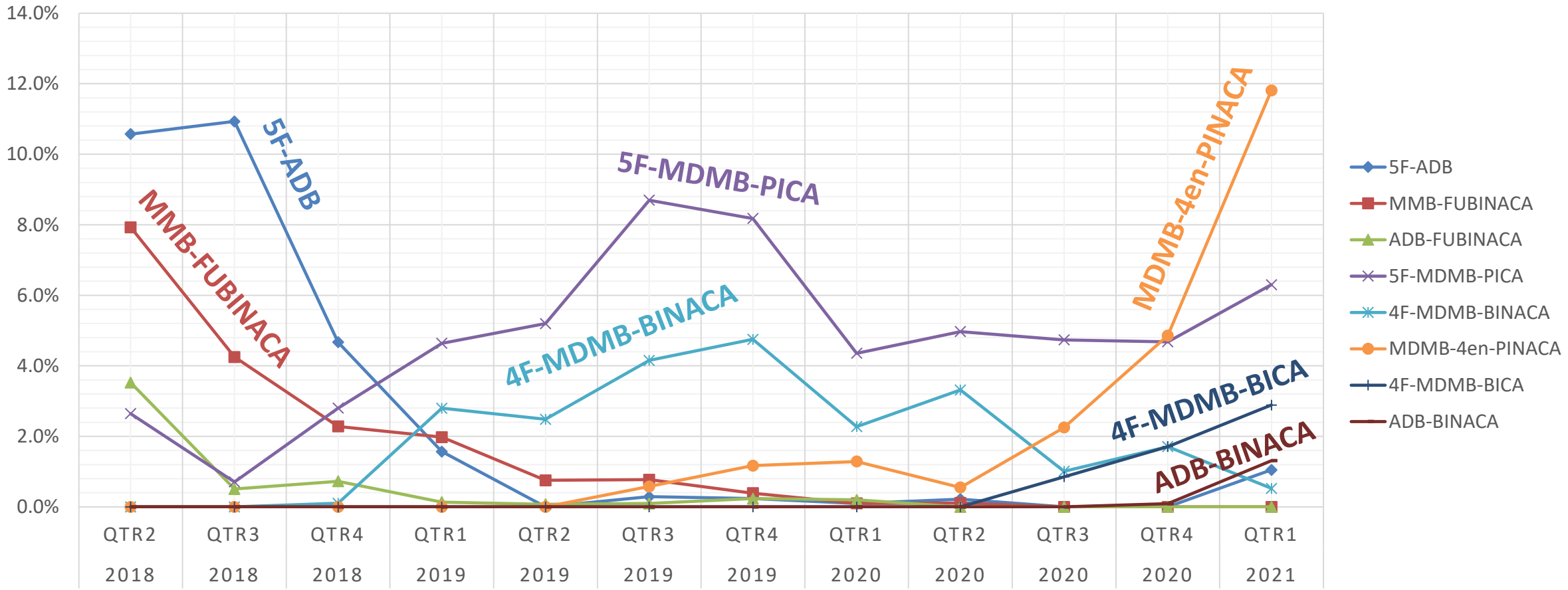
Acknowledgements: This report was prepared by Alex J. Krotulski, PhD; Sara E. Walton, BS; Amanda L.A. Mohr, MSFS, D-ABFT-FT; and Barry K. Logan, PhD, F-ABFT at the Center for Forensic Science Research and Education (CFSRE) at the Fredric Rieders Family Foundation. NPS Discovery would like to acknowledge scientists at CFSRE and NMS Labs for their involvements and contributions. For more information about our programs and reports, please contact NPS Discovery at npsdiscovery@cfsre.org or visit our website at www.npsdiscovery.org.

Funding: NPS Discovery at the CFSRE is supported in part by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (Award Number 2020-DQ-BX-0007, "Real-Time Sample-Mining and Data-Mining Approaches for the Discovery of Novel Psychoactive Substances (NPS)"). The opinions, findings, conclusions and/or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

cfsre Redefining Excellence in Forensic Science

NPS DISCOVERY

Synthetic Cannabinoid Trends



cfsre NPS DISCOVERY

METHODS

Methods: Extraction

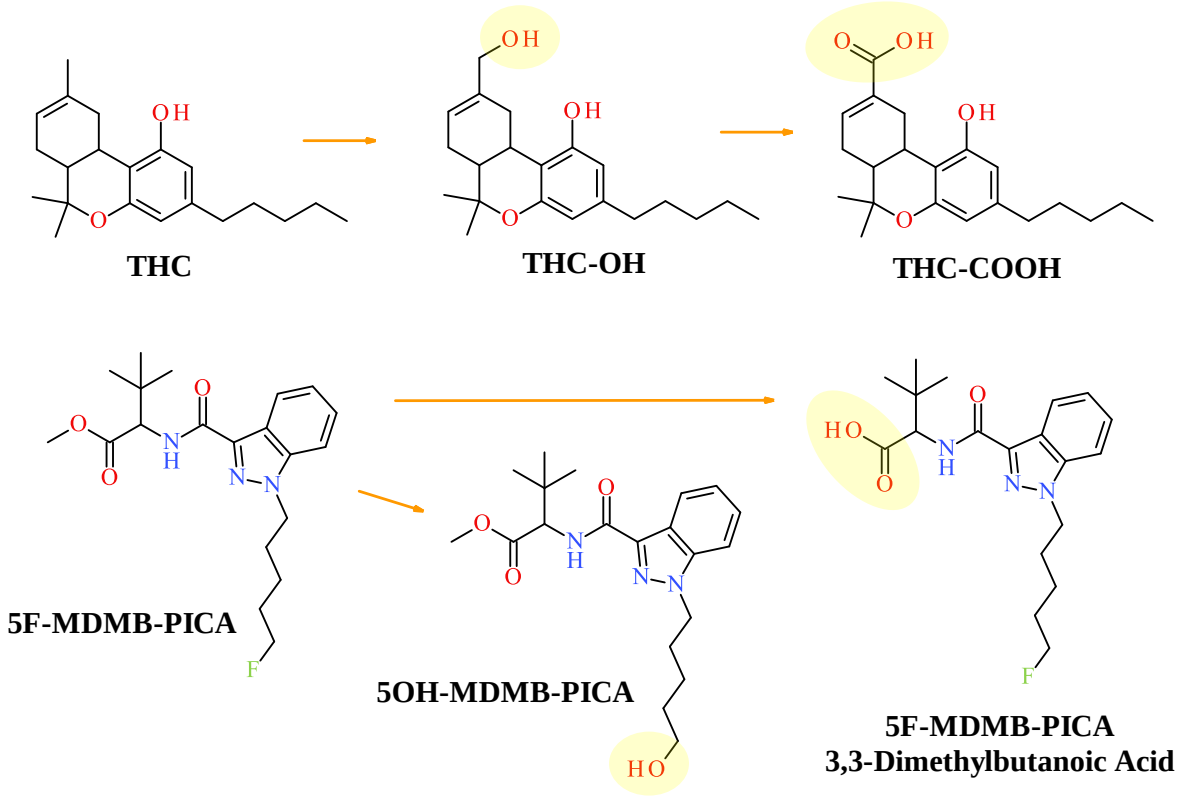
- **Blood – Liquid-Liquid Extraction (LLE)**
  - "Targeted" for parent drugs
  - 0.5 mL of blood sample
  - 0.5 mL of Tris HCl Buffer (pH 10.2)
  - 3 mL of methyl tert-butyl ether (MTBE)
  - Cap, rotate, and centrifuge
  - Remove organic layer and dry down
  - Reconstitution for LC-MS analysis

- **Urine – Solid Phase Extraction (SPE)**
  - "Targeted" for acidic metabolites
  - 1 mL of urine sample
  - Hydrolysis*
  - Ammonium Carbonate Buffer (pH = 9.3)
  - Agilent Plexa PAX (60mg/3mL)
    - Condition, wash, elute
  - Dry down and reconstitution for LC-MS

cfsre NPS DISCOVERY

Methods: Extraction

- **What if you want to detect both parent drugs and metabolites?**
  - THC extraction method
- **Any Matrix – Liquid-Liquid Extraction**
  - "Non-targeted"
  - 0.5 mL of blood sample
  - 0.5 mL of 5% phosphoric acid in H20
  - 3 mL of hexane / MTBE / EtOAc (80:10:10)
  - Cap, rotate, centrifuge, remove, dry down



cfsre NPS DISCOVERY

Methods: Instrumentation

CONFIRMATION – MORE COMMON

- **LC-MS/MS (Triple Quad / Ion Trap)**
  1. Infusion of drug into MS
  2. Setup MS parameters
     - MRM method
  3. Assessment of chromatography
     - Analytical column
     - Mobile phase
     - Separation of isobars
  4. Develop process method
  5. Verification or validation
  6. Authentic sample analysis

SCREENING – INCREASINFLY USED

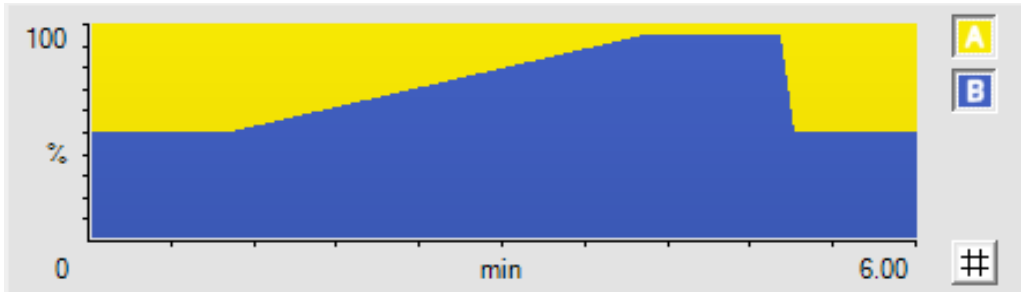
- **LC-HRMS (TOF / TOF / Orbitrap)**
  1. Infusion of drug into MS
  2. Assess MS setpoints
     - Targeted acquisition method
     - *Add fragment spectrum to library
  3. Assessment of chromatography
     - Retention time
  4. Develop processing method
     - Name, formula, exact mass, and RT
  5. Verification or validation
  6. Authentic sample analysis

***BOTH CAN BE QUAL OR QUANT**

cfsre NPS DISCOVERY

Methods: Instrumentation

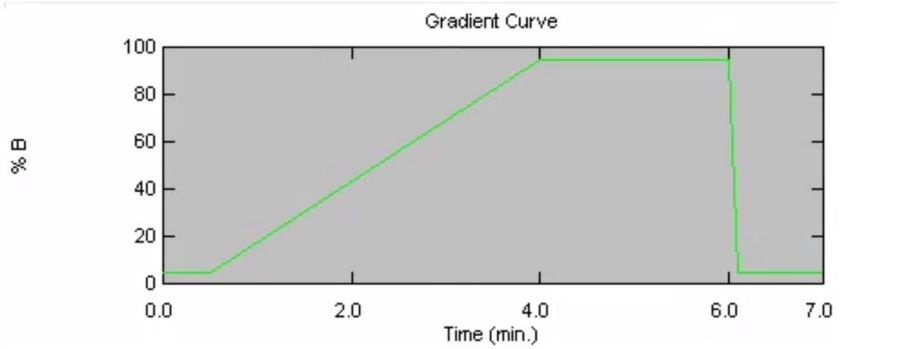
- **LC-MS/MS (Triple Quad / Ion Trap)**
  - 5F-MDMB-PICA and Metabolites

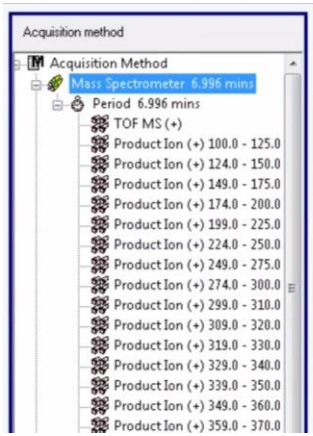


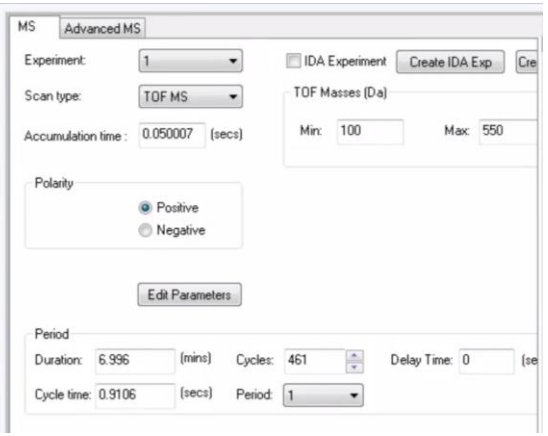
Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Auto Dwell	Dwell (s)	Cone (V)	Collision (V)	PIC
1	2COOH-MDMB-PICA	361.2000	144.0000	☑	0.018	34	42	☐
2	2COOH-MDMB-PICA	361.2000	216.1000	☑	0.018	34	16	☐
3	5F-MDMB-PICA Butanoic Acid	363.2000	144.0000	☑	0.018	34	42	☐
4	5F-MDMB-PICA Butanoic Acid	363.2000	232.1000	☑	0.018	34	18	☐
5	5F-MDMB-PICA Butanoic Acid-D5	368.2000	149.0000	☑	0.018	34	42	☐
6	5F-MDMB-PICA Butanoic Acid-D5	368.2000	237.1000	☑	0.018	34	18	☐
7	5OH-MDMB-PICA	375.2000	144.0000	☑	0.018	34	36	☐
8	5OH-MDMB-PICA	375.2000	230.1000	☑	0.018	34	16	☐
9	5F-MDMB-PICA	377.2000	144.0000	☑	0.018	32	42	☐
10	5F-MDMB-PICA	377.2000	232.1000	☑	0.018	32	16	☐
11	4OH-5F-MDMB-PICA Butanoic Acid	379.2000	144.0000	☑	0.018	32	34	☐
12	4OH-5F-MDMB-PICA Butanoic Acid	379.2000	248.1000	☑	0.018	32	14	☐
13	5F-MDMB-PICA-D5	382.2000	149.0000	☑	0.018	32	42	☐
14	5F-MDMB-PICA-D5	382.2000	237.1000	☑	0.018	32	16	☐
15	6'OH-5F-MDMB-PICA	393.2000	160.0000	☑	0.018	32	38	☐
16	6'OH-5F-MDMB-PICA	393.2000	248.1000	☑	0.018	32	14	☐

- **LC-HRMS (TOF / TOF / Orbitrap)**
  - Synthetic Cannabinoid Screen







cfsre

NPS DISCOVERY

CHALLENGES

Challenges: Sensitivity and Quantitation

- Generally lacking information about synthetic cannabinoid concentrations
  - Few labs test for / quantitate these NPS
- Synthetic cannabinoids are **increasing in potency** over time leading to **decreases in concentration** of drug in biological specimens
- Low to sub-ng/mL concentrations are not uncommon
  - Need for **LC-MS**-based methods

- **Case Study:** 5F-MDMB-PICA

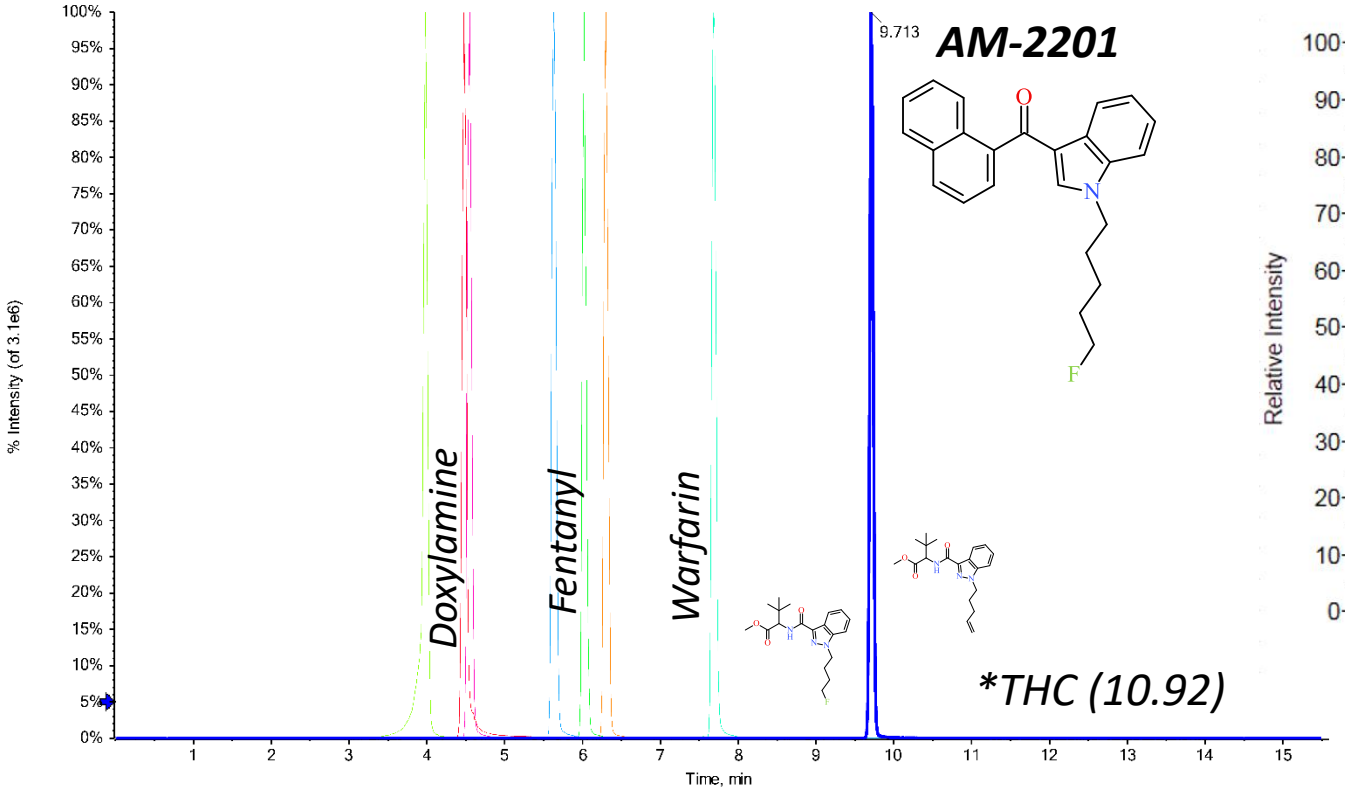
Quantitation via LC-MS/MS	5F-MDMB-PICA
Quantifiable Samples (N=44)	24
Mean (±SD)	**2.2 ± 1.8**
Median	1.4
Range	0.5 – 6.2
Positive Cases (<LOQ)	16

(Unpublished Data / Krotulski et al. 2021)

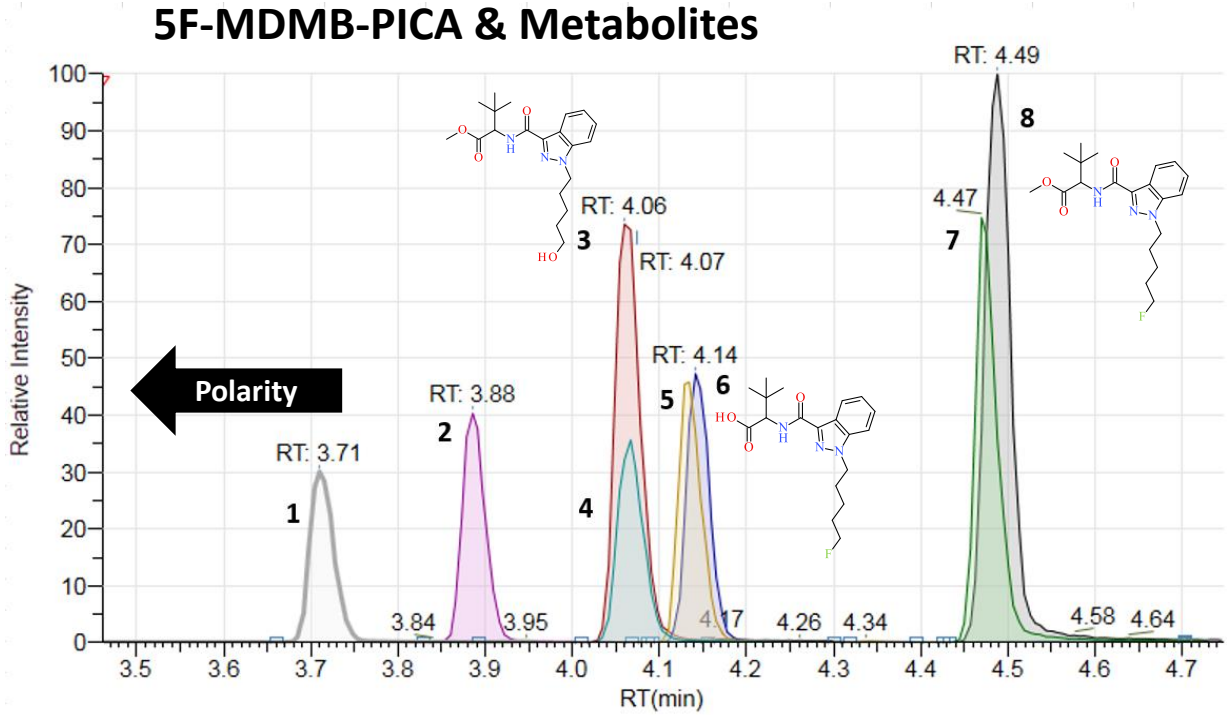
cfsre NPS DISCOVERY

Challenges: Chemical Behavior

General Screening Method



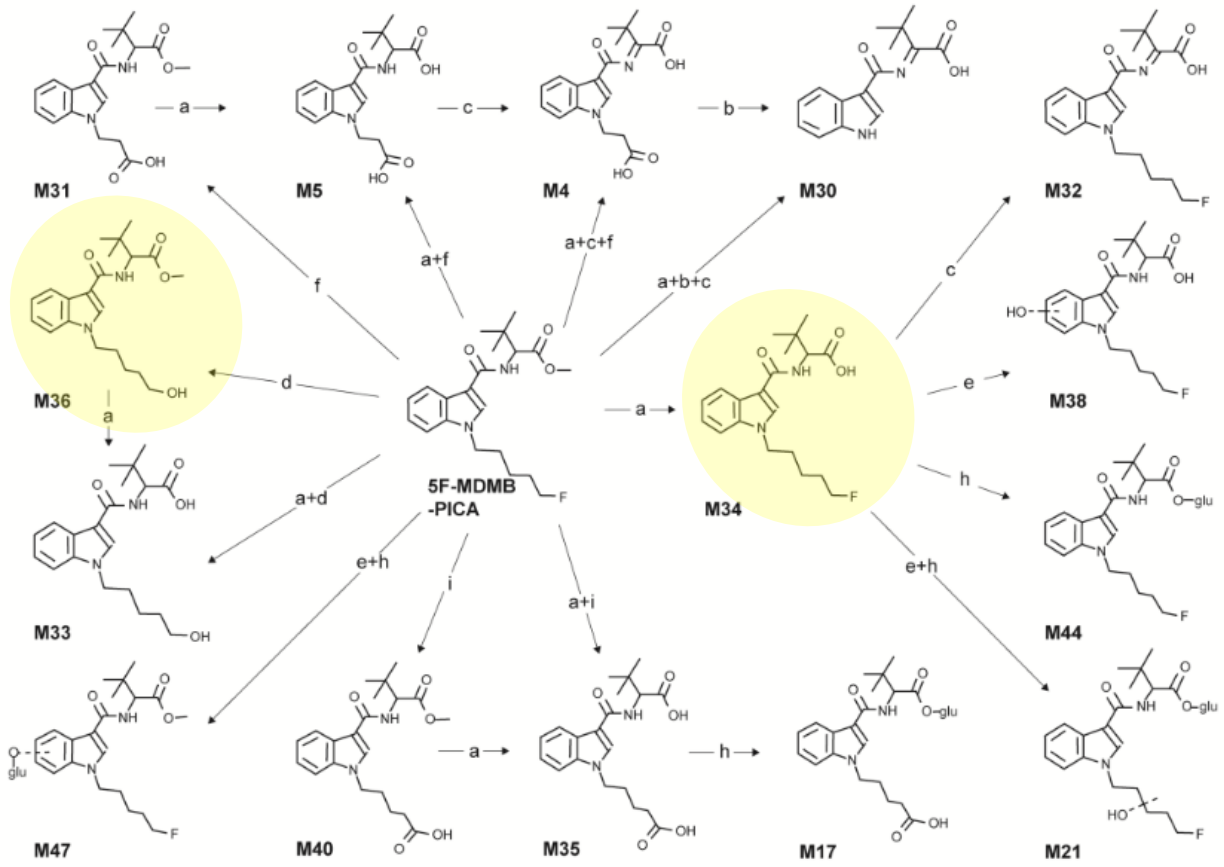
5F-MDMB-PICA & Metabolites



cfsre NPS DISCOVERY

Challenges: Metabolism

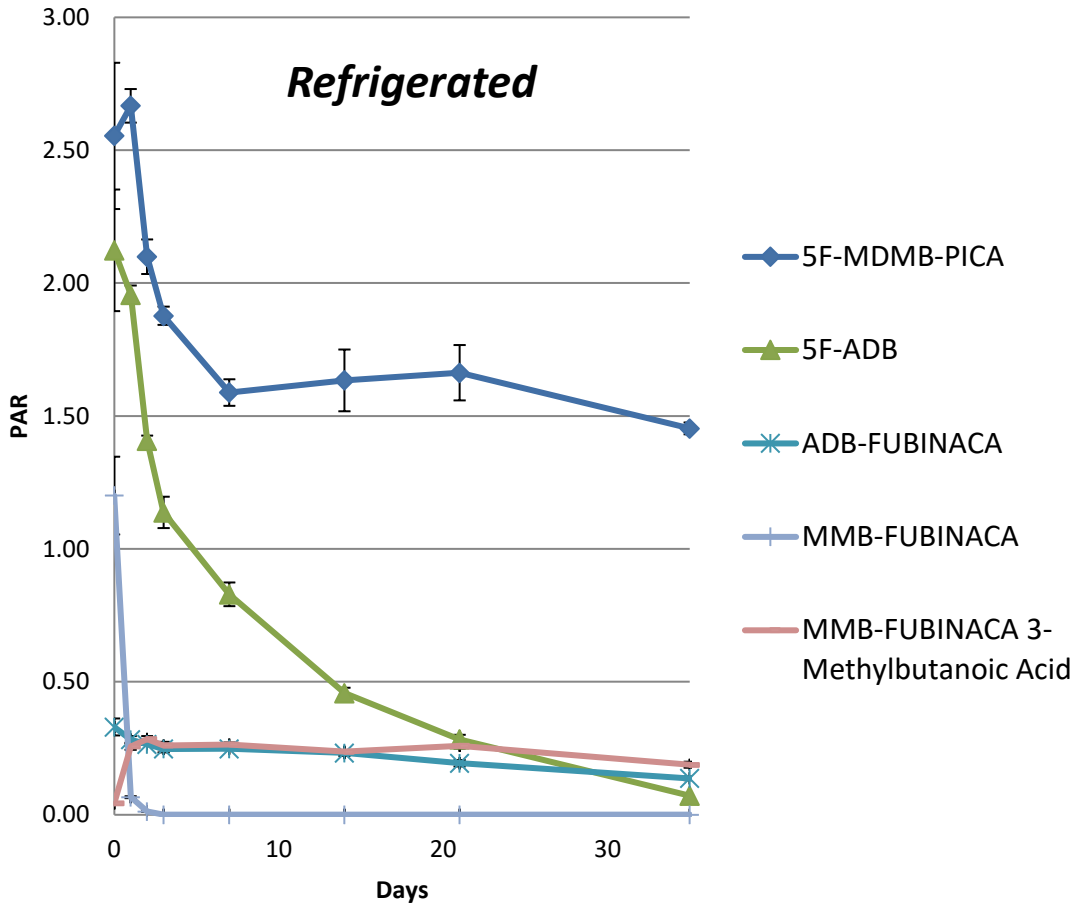
- **Synthetic cannabinoid** are unique from other **NPS** classes specifically relating to metabolism:
  - **No parent** drug found in urine
  - More complex / more metabolites →
  - **Common metabolites**
  - **Active metabolites**
  - Toxic metabolites / byproducts
  - **We need data to decipher!**



https://doi.org/10.1093/jat/bkab034

Challenges: Instability

- Synthetic cannabinoids have been shown to be unstable in biological samples
- *tert*-Leucine derived parent drugs
  - Also known as: Methyl / alkyl esters
  - 5F-MDMB-PINACA (5F-ADB), 5F-MDMB-PICA, MMB-FUBINACA (FUB-AMB), 4F-MDMB-BINACA, 4F-MDMB-BICA, 5F-EDMB-PICA
  - **MDMB-4en-PINACA**
- Breakdown via same pathway as metabolism (ester hydrolysis / butanoic acid "metabolites")
- No parent compound? Recent use?
- **Need methods for parent & metabolites?**



https://doi.org/10.1093/jat/bkaa054

cfsre NPS DISCOVERY

CONCLUSIONS

Conclusion – Where to from here?

- **Synthetic cannabinoids are different**
  - They don't behave like other NPS
  - Need separate workflows and methods
- **Synthetic cannabinoids are challenging**
  - Low concentrations
  - Parent drug vs. metabolites
  - Instability
- **Synthetic cannabinoids continue to appear in forensic toxicology casework**
- **What challenges does your lab face?**

cfsre NPS DISCOVERY

Acknowledgements

- **Center for Forensic Science Research & Education (CFSRE)**
  - Barry Logan
  - Mandi Mohr
  - Melissa Fogarty
  - Judith Rodriguez-Salas
  - Sara Walton
- **NMS Labs**
  - Donna Papsun
  - Sherri Kacinko

Thank You!

Contact Information

Alex J Krotulski, PhD

alex.krotulski@cfsre.org

Website/Social Media

www.npsdiscovery.org

Twitter: @NPSDiscovery