



cfsre

The Center for Forensic
Science Research & Education



NPS
DISCOVERY

The Use of Combined Seized Drug and Toxicology Workflows for Rapid Identification of Emerging Substances in Response to Public Health Threats

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Workshop I — Identification, Characterization, and Analysis of NPS (Friday November 19, 2021)

The VIII International Conference on Novel Psychoactive Substances (NPS) – Virtual

Introduction

- **Center for Forensic Science Research & Education**

- Associate Director
 - Toxicology & Chemistry
- Program Manager
 - NPS Discovery

- **Thomas Jefferson University**

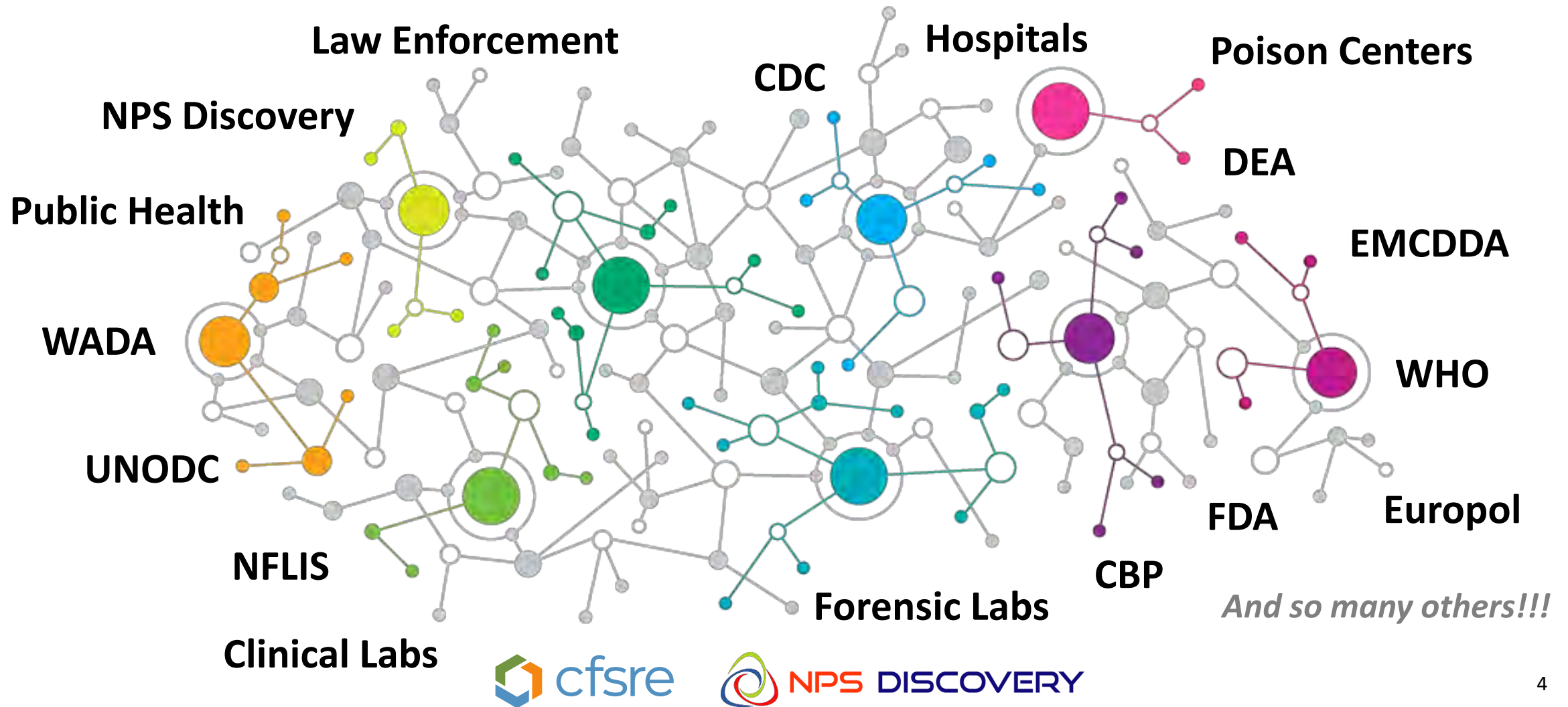
- Assistant Program Director
 - MS in Forensic Toxicology
- Faculty / Lecturer



Disclosure and Introduction

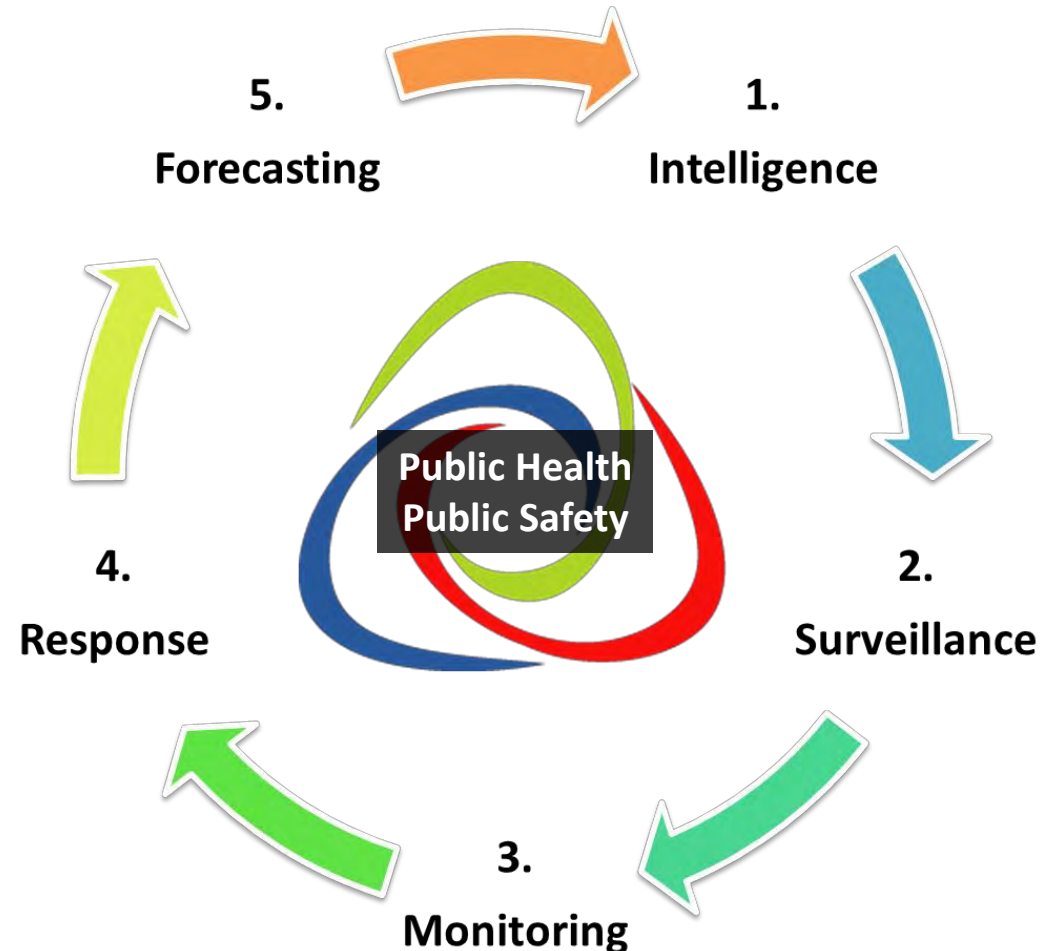
- I have no conflicts of interest to disclose.
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
- I have worked in collaboration with:
 - NMS Labs – private toxicology laboratory
 - U.S. Customs and Border Protection
 - U.S. Department of Justice
 - Emergency Departments / Hospitals / Poison Control Centers
 - Medical Examiner and Coroner Offices / Forensic Laboratories

Drug Testing Network



NPS Discovery

- Open-access national drug early warning system in United States
- Launched in 2018
- Multidisciplinary program
- Focus on dissemination and outreach
- Intersection between:
 - Forensic Toxicology
 - Drug Chemistry
 - Clinical Intoxications



Presentation Overview

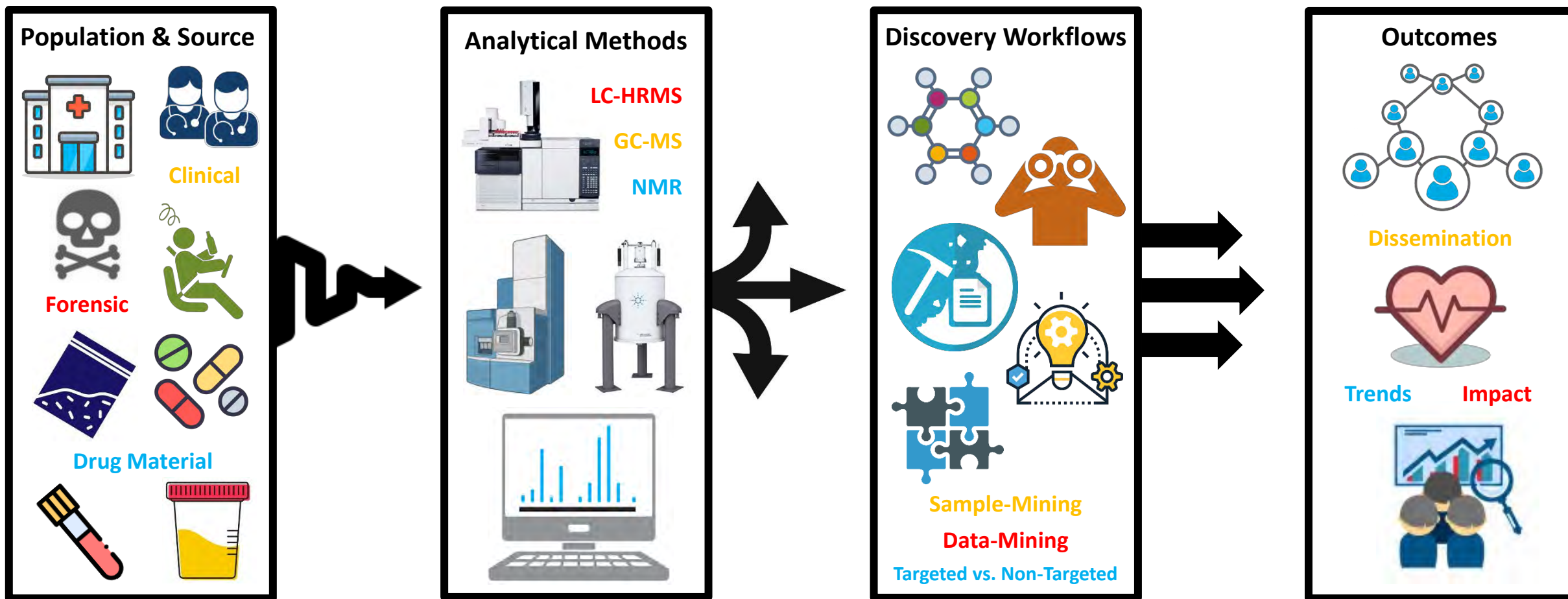
**Sample
Source**

**Analytical
Methods**

**Discovery
Workflows**

**Results &
Outcomes**

Our Process Overview



SAMPLE SOURCE

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes





Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

- 
BLOOD
 WITHIN 24 HOURS FROM THE LAST USE
- 
URINE
 2 TO 5 DAYS FROM THE LAST USE
- 
HAIR
 THREE MONTHS (90 DAYS) FROM THE LAST USE
- 
SALIVA
 UP TO 1 DAY TO 5DAYS
- 
SYSTEM
 UP TO 3 DAYS

ANALYICAL METHODS

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes



Non-Exclusive List of Instruments

- Immunoassays (EMIT, ELISA, etc.)
- **Mass spectrometry***
 - Gas chromatography mass spectrometry (GC-MS)
 - Liquid chromatography tandem mass spectrometry (LC-MS/MS)
 - High resolution mass spectrometry (HRMS)
 - Example: LC-TOF-MS, LC-QTOF-MS, LC-Orbitrap-MS
- Nuclear magnetic resonance (NMR) spectroscopy



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Sensitivity vs. Specificity

- **Sensitivity:**

- Limits of detection
- NPS concentration <1 ng/mL

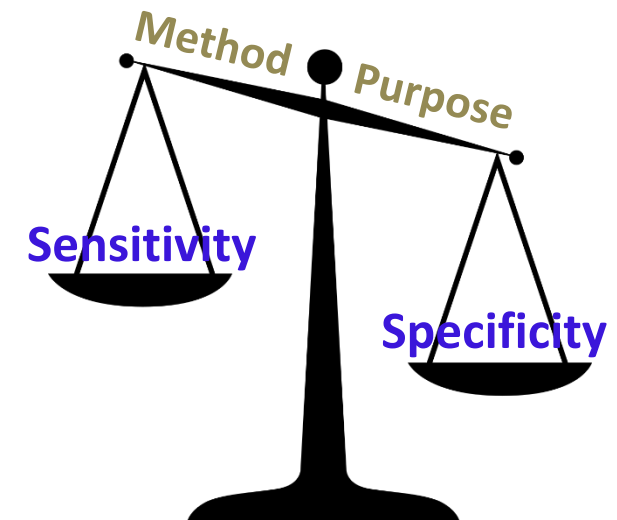
- **Both dictate instrument to use and interpretation of results**

- Run time / acquisition time
- Sample throughput

- **Specificity:**

- Differentiation power
- NPS isomers
- Retention time
- Fragmentation pattern

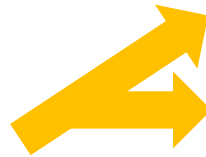
Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes



“Targeted” vs. “Non-Targeted”

- **Instrumental Analysis**

- Data (or information) dependent acquisition (DDA or IDA)
 - Targeted analysis
 - Sample dictates outcome of results
 - “If x → then y” (mass)
- Data independent acquisition (DIA)
 - Non-targeted analysis
 - Outcome (acquisition) regardless



- **Data Processing**

- Targeted
 - Defined scope of testing
 - Library/database (mass list, XIC list)
 - Processing criteria
- Non-targeted
 - “Suspect screening”...*
 - Unknown searching
 - Difficult and time consuming

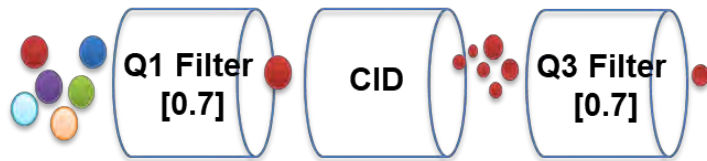
- **... Interpretation / Discovery**

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

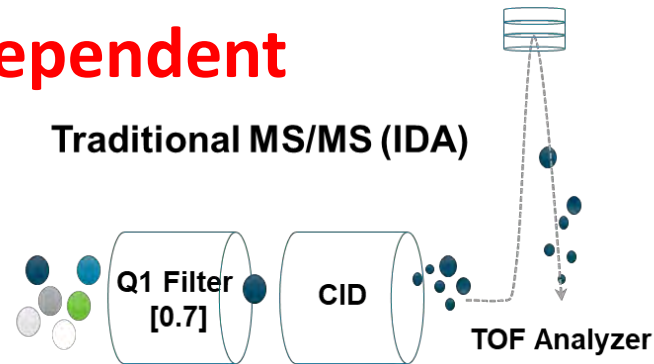
“Targeted” vs. “Non-Targeted”

Targeted / Data Dependent

Traditional “Triple Quad”



Traditional MS/MS (IDA)

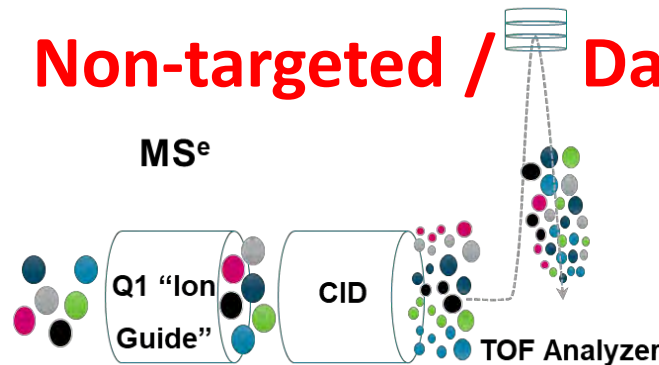


Non-targeted / Data Independent

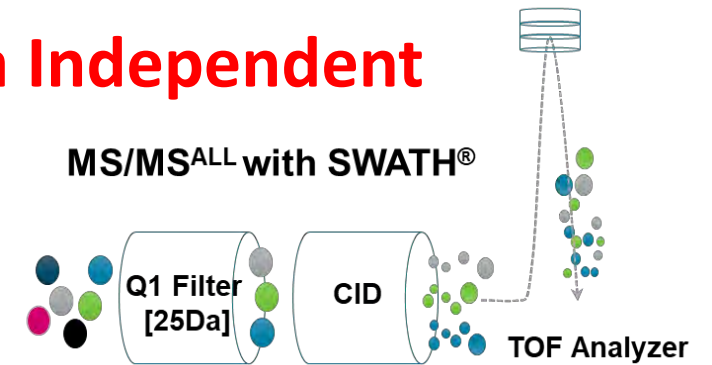
“Single Quad” / Scan



MS^e



MS/MS^{ALL} with SWATH®



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Instruments and Utility

- **Screening**

- Low specificity
- Low accuracy

- GC-MS*
- LC-TOF-MS

- Drug discovery
- “Non-targeted”

- **Identification**

- Moderate specificity
- High accuracy

- GC-MS*
- LC-QTOF-MS

- Drug discovery
- Drug characterization
- “Targeted/Non-targeted”

- **Confirmation**

- High specificity
- High accuracy

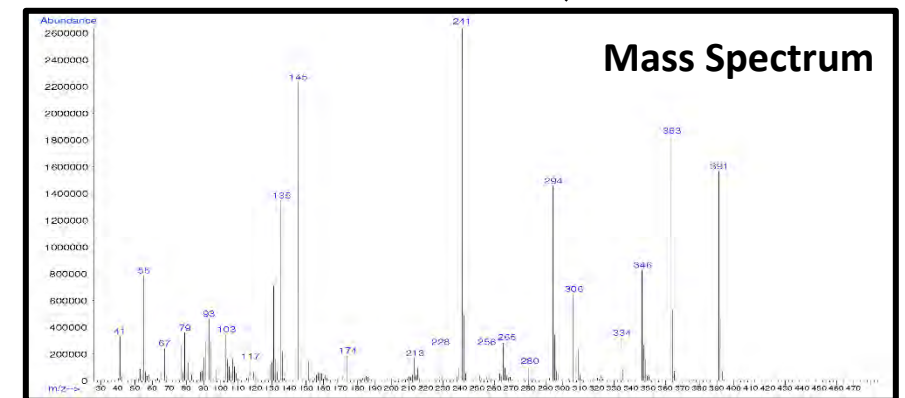
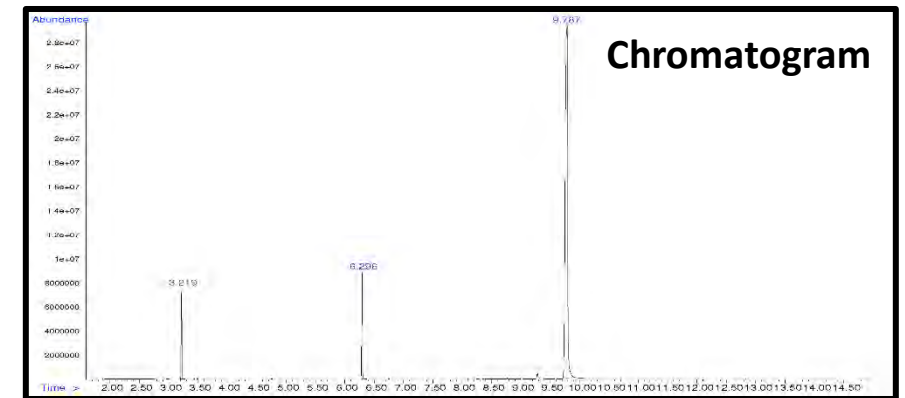
- LC-MS/MS
- NMR

- Drug characterization
- Absolute identify
- Quantitation
- “Targeted”

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

GC-MS Method

- **Sample Preparation:**
 - Drug samples – acid/base extraction
- **Instrument:**
 - Agilent 5975 Series GC/MSD →
- **Column & Carrier Gas:**
 - Zebron™ Inferno™ ZB-35HT
 - Helium (Flow: 1 mL/min)
 - Temperature program
- **MS Parameters: 40-550 m/z**

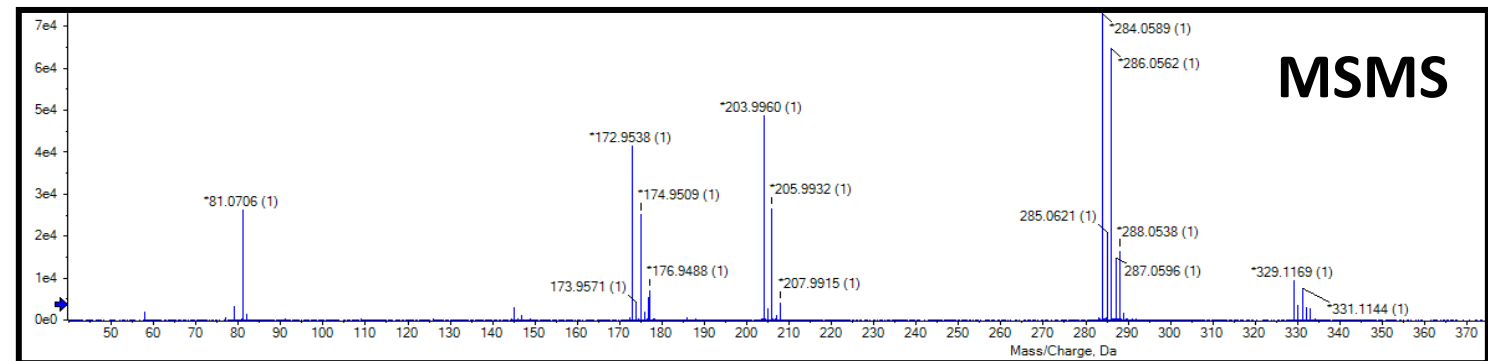
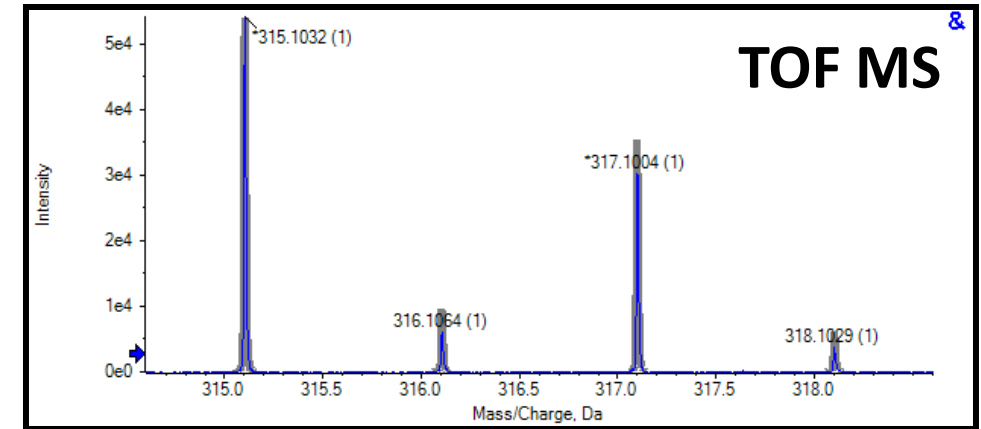


Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

General LC-QTOF-MS Parameters

- **Sample Preparation:**
 - 1:100 dilution of GC-MS extract
 - Extraction of biological samples
- **Instrument:**
 - Sciex TripleTOF® 5600+ →
- **Column and Mobile Phase:**
 - Phenomenex® Kinetex C18
 - A: Ammonium formate (10 mM, pH 3.0)
 - B: Methanol/acetonitrile (50:50)

• **MS Parameters: 100-510 Da**

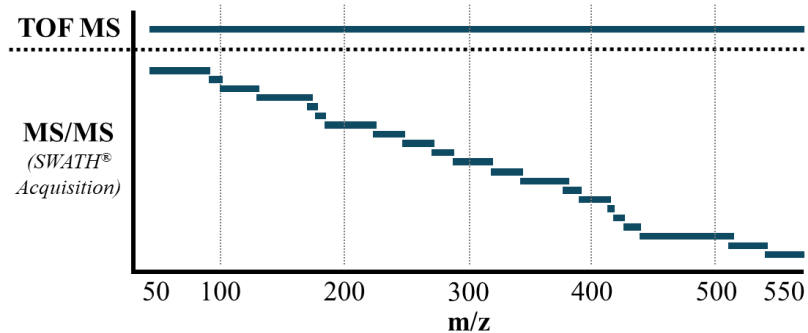


Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Two LC-QTOF-MS Methods

- **Basic Drug Method**

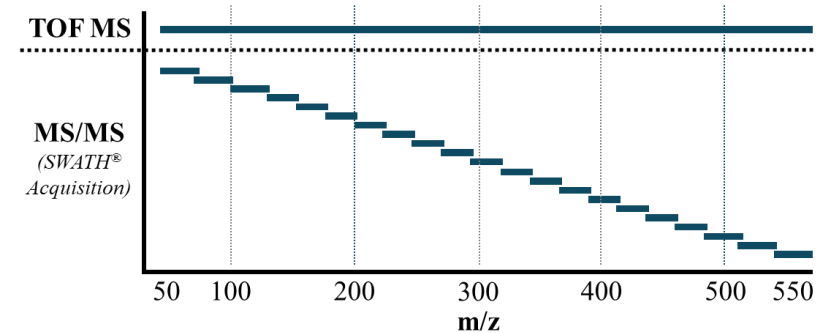
- Generic LC gradient
- SWATH® Acquisition



- 15.5-minute method
- **800+ drugs of abuse, NPS, metabolites, etc.**

- **Synthetic Cannabinoid Method**

- Generic LC gradient
- SWATH® Acquisition

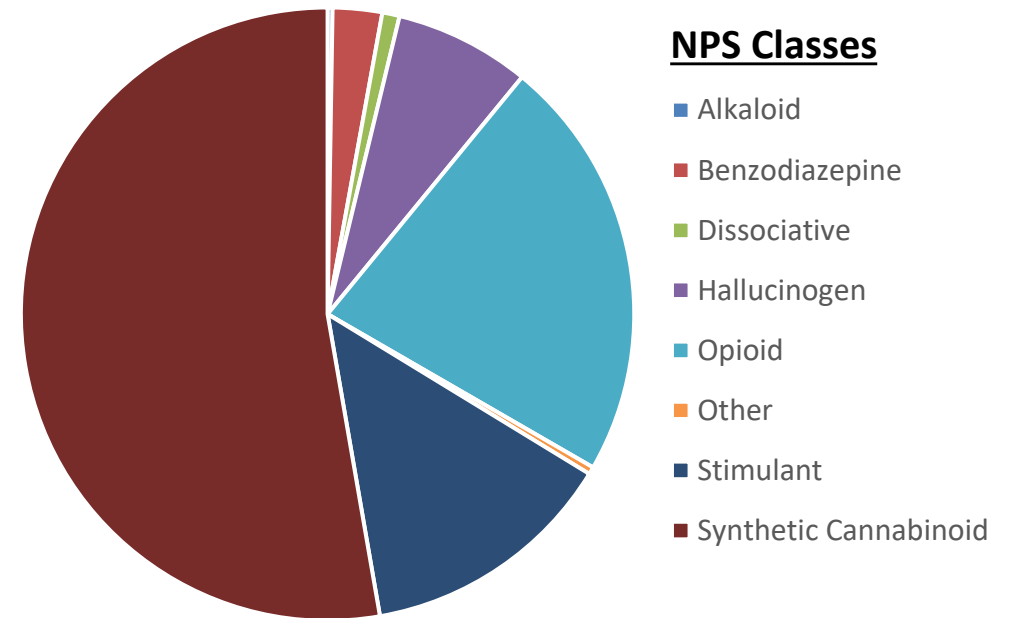
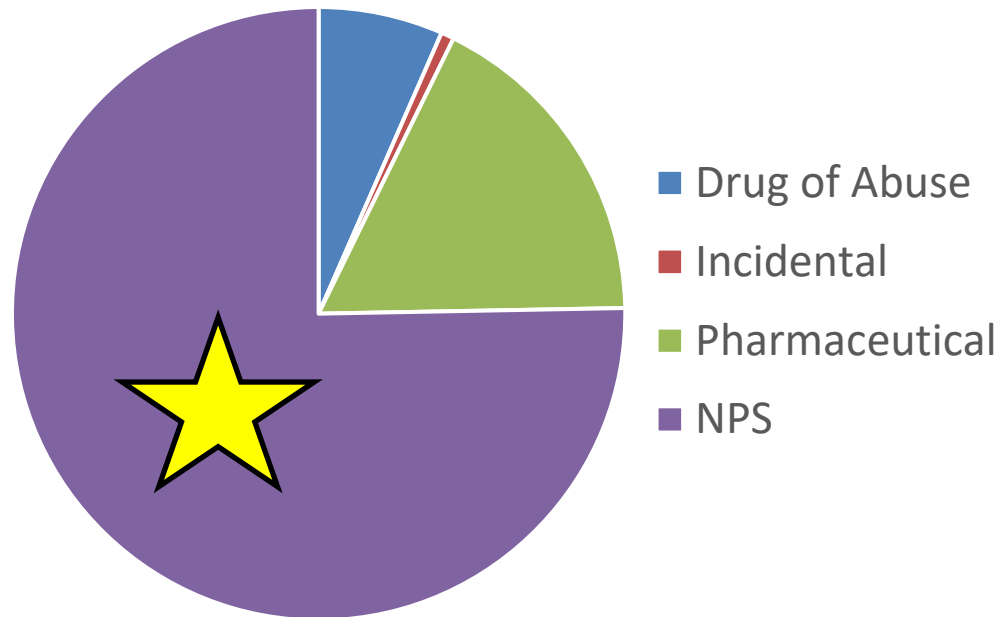


- 7-minute method
- **250+ synthetic cannabinoids (parent and metabolites)**

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Standards / Library Database

- >950 standards in library database



HRMS Identification Criteria

- Criteria should be consistent across industries
- Setpoints can be related to certainty of the method (screen vs. identification)
- Evaluated based on experimental data

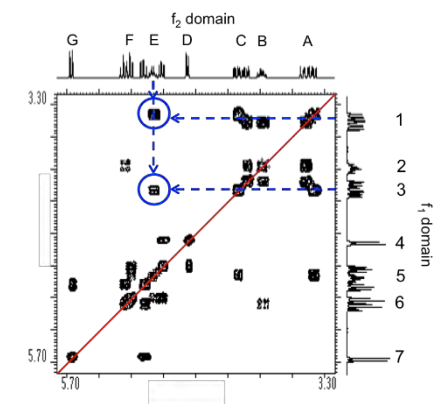
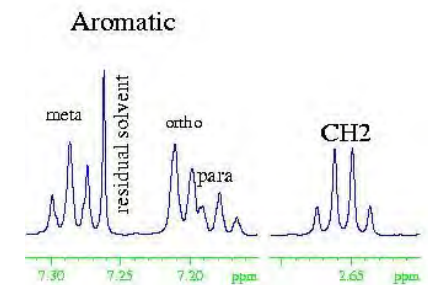
- Strict vs. lenient
- Manual vs. automated

	Mass Error Mass Error (ppm)	Retention Time Delta (min)	Isotope Isotope Ratio % Difference	Library Hit Library Score
✓	< 5.0	< 0.25	< 30.0	> 90.0
▲	< 10.0	< 0.35	< 50.0	> 70.0
●	>= 10.0	>= 0.35	>= 50.0	<= 70.0

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

NMR Method

- **Sample Preparation:**
 - Dilute powder in CDCl₃
- **Instrument:**
 - 300 MHz INOVA VARIAN Spectrometer →
- **Pulse Sequence:** Proton
- **Spectral Width:** 4798.5 Hz for 1D (-2 – 14 ppm) and 3773.6 for 2D
- **Delay between pulses:** 1st delay, d1 = 1.000



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Example LC-MS/MS Method

- **Sample Preparation:**

- Extraction of biological samples

- **Instrument:**

- Waters Xevo TQ-S micro →



- **Column and Mobile Phase:**

- Agilent InfinityLab Poroshell 120 EC-C18
- (3.0 x 100 mm, 2.7 μm)
- A: 0.1% formic acid in water
- B: 0.1% formic acid in methanol

Table I. LC Gradient Conditions

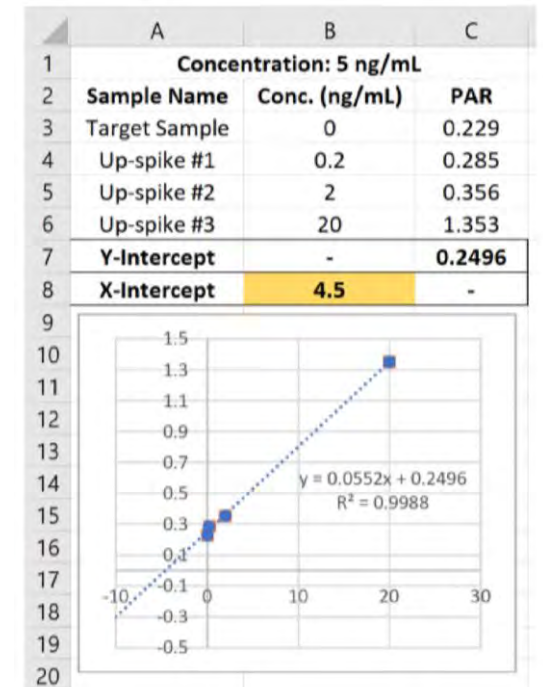
Time (min)	%A	%B	Flow (mL/min)
Initial	50	50	0.4
1.0	50	50	0.4
4.0	5	95	0.4
5.0	5	95	0.4
5.1	50	50	0.4
6.0	50	50	0.4

Table II. MRM Parameters

Analyte	Cone (V)	Precursor (m/z)	Collision (V)	Product (m/z)	Dwell (s)
Isotonitazene	50	411.2	46	106.9	0.053
			22	100.0	0.053
			44	72.0	0.053
Fentanyl-d ₅	56	342.2	24	188.0	0.053
			40	105.0	0.053

- **MS Parameters: MRM**

- **Quantitation: Standard Addition**



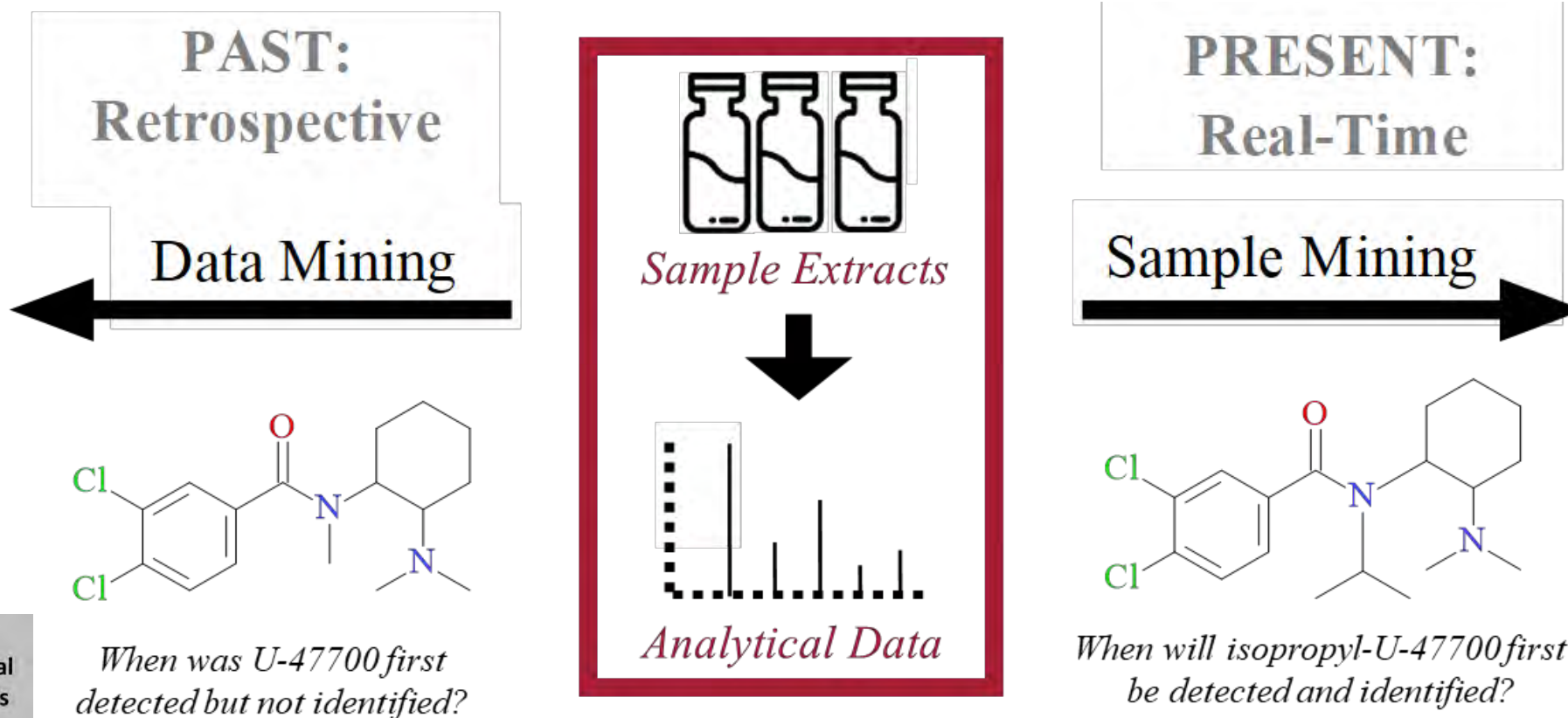
Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

DISCOVERY WORKFLOWS

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes



Sample-Mining vs. Data-Mining



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

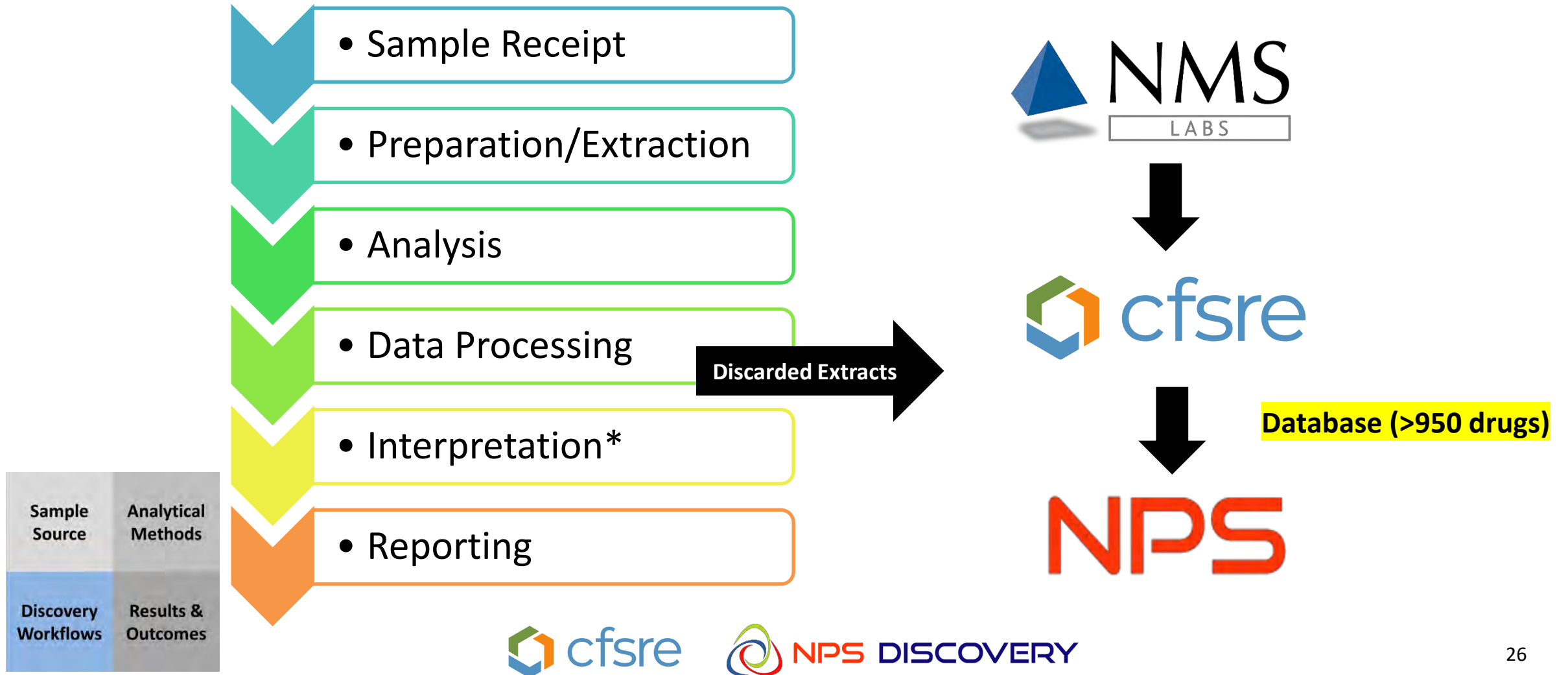
Sample-Mining vs. Data-Mining

- **Considerations for successful SM and DM:**
 - Scope of testing → Differs from standard laboratory scope of testing
 - Comprehensive in nature – not limited by drug class (but, limited by sample preparation)
 - Consistency in methods and sample acquisitions, especially for data-mining
 - Need software or application to assist with data processing and storage

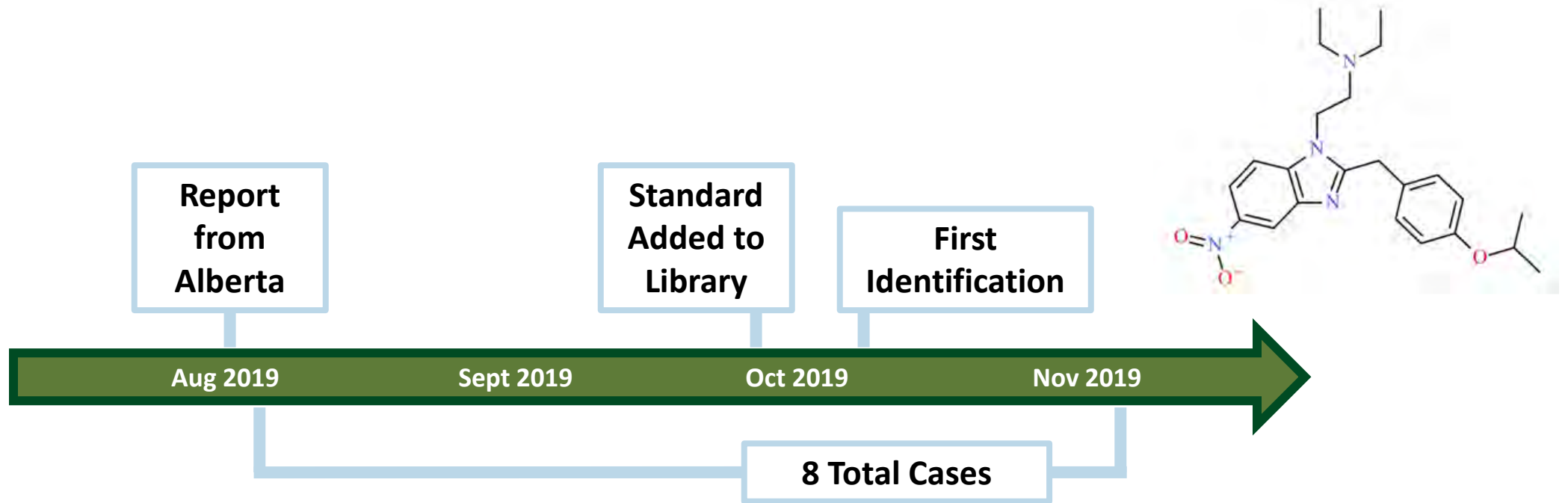
Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes



Sample-Mining



Data-Mining Example: Isotonitazene



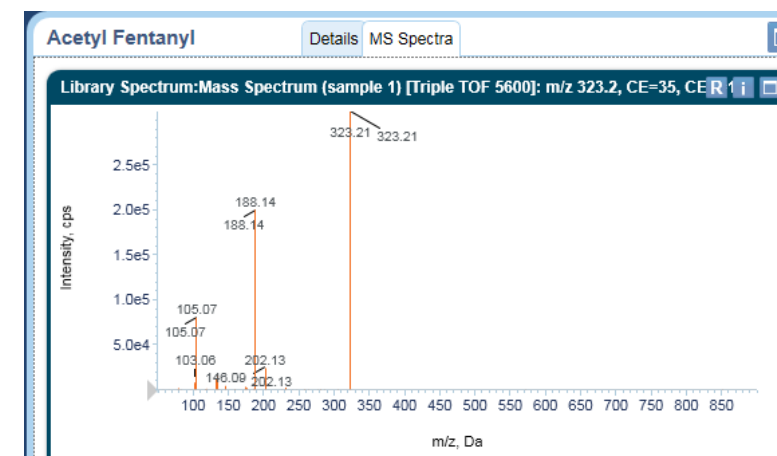
Toxicologically relevant identifications that were “missed” during initial data processing

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Library Databases

- Three main types:
 - In-house
 - Commercial
 - Publicly available
- Used for:
 - Targeted data processing
 - “Suspect screening”
- Information included:
 - Analyte name
 - Formula → exact mass
 - Retention time
 - Fragmentation pattern
- ***Results are only as good as their identifications...***

Name	Extraction Mass (Da)	Expected RT (min)	Fragment Mass (Da)
Alpha-PVP	232.16959	5.1	
Alpha-PVP	232.16959	5.1	232.1703
Alpha-PVP	232.16959	5.1	91.0556
Alpha-PVP	232.16959	5.1	126.1281
Alpha-PVP	232.16959	5.1	105.0344
Alpha-PVP	232.16959	5.1	161.0958



Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

Additional Lab Discovery Workflows

- **In-Scope Findings vs. Out-of-Scope Findings (OSF) at NMS Labs**

- In-scope findings correlate to the laboratories standard scope of testing
- Out-of-scope findings are additional drugs above and beyond the standard scope
 - Tier 1: Confirmation methods available
 - Tier 2: Intelligence gathering purposes

- **Added MRM Transitions to LC-MS/MS Methods (OCME's)**

- Laboratory may have several different confirmations panels
- Based on intelligence, the lab might add suspected or confirmed precursor-product ion transitions to methods
- “Background drug monitoring” helps drive future testing development
 - Reportable results???

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

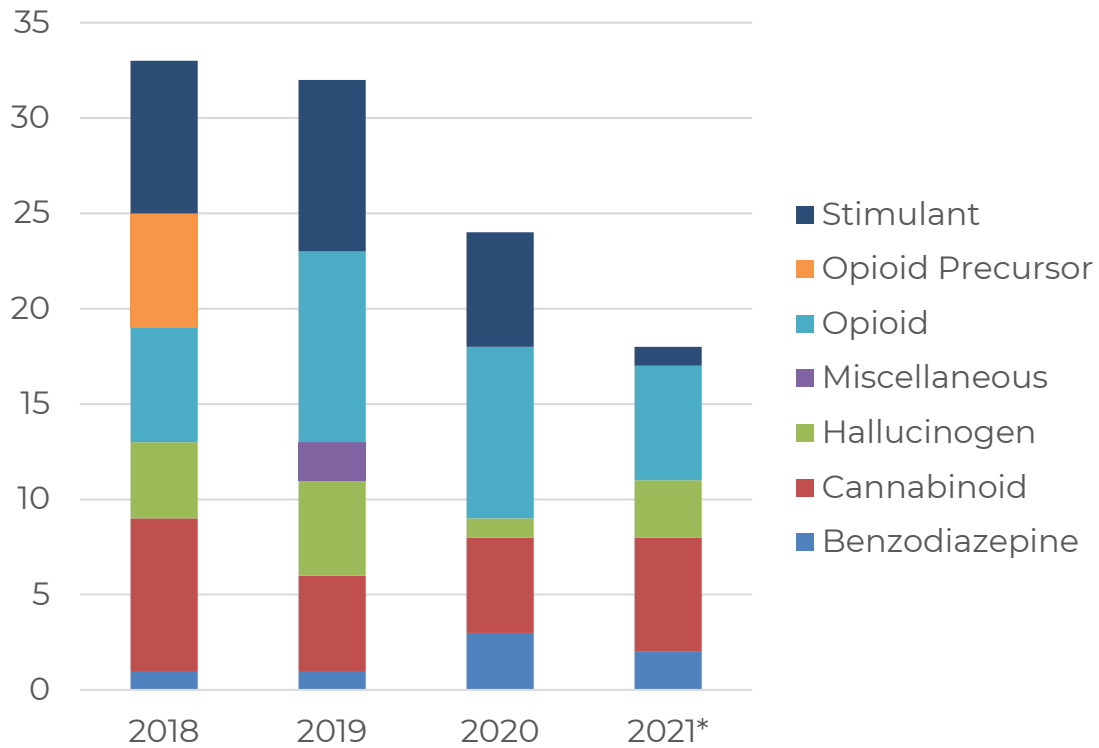
OUTCOMES & SUCCESS STORIES

Sample Source	Analytical Methods
Discovery Workflows	Results & Outcomes

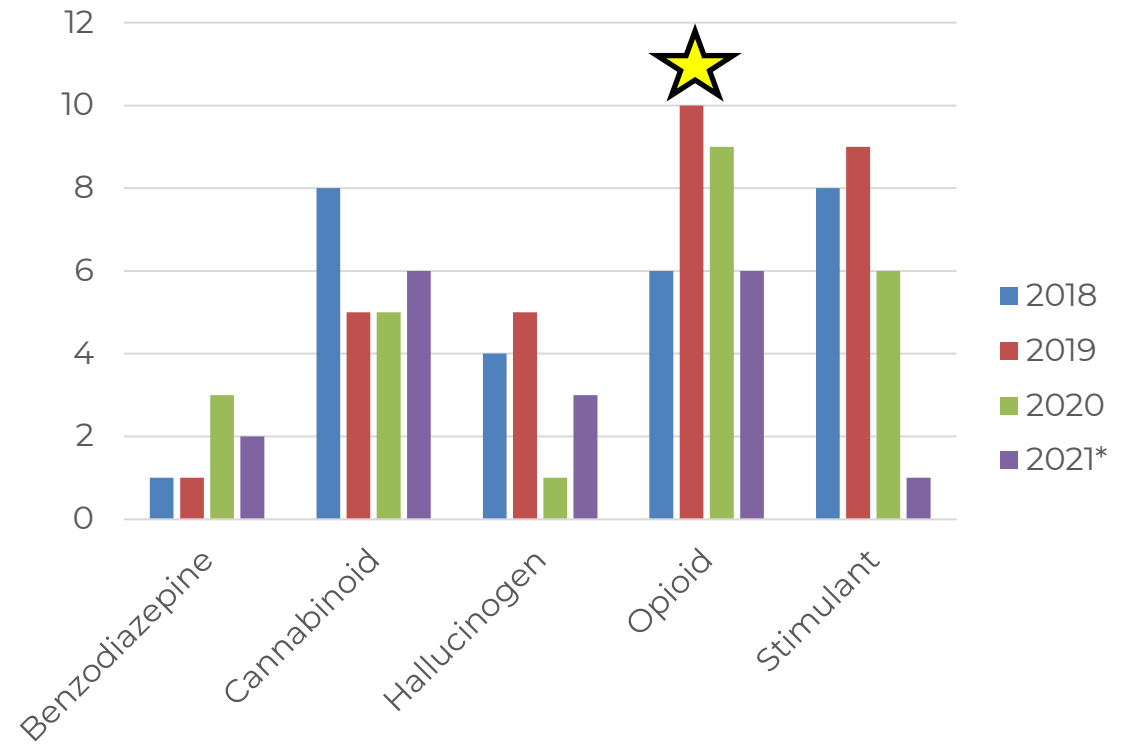


Discovery of New NPS

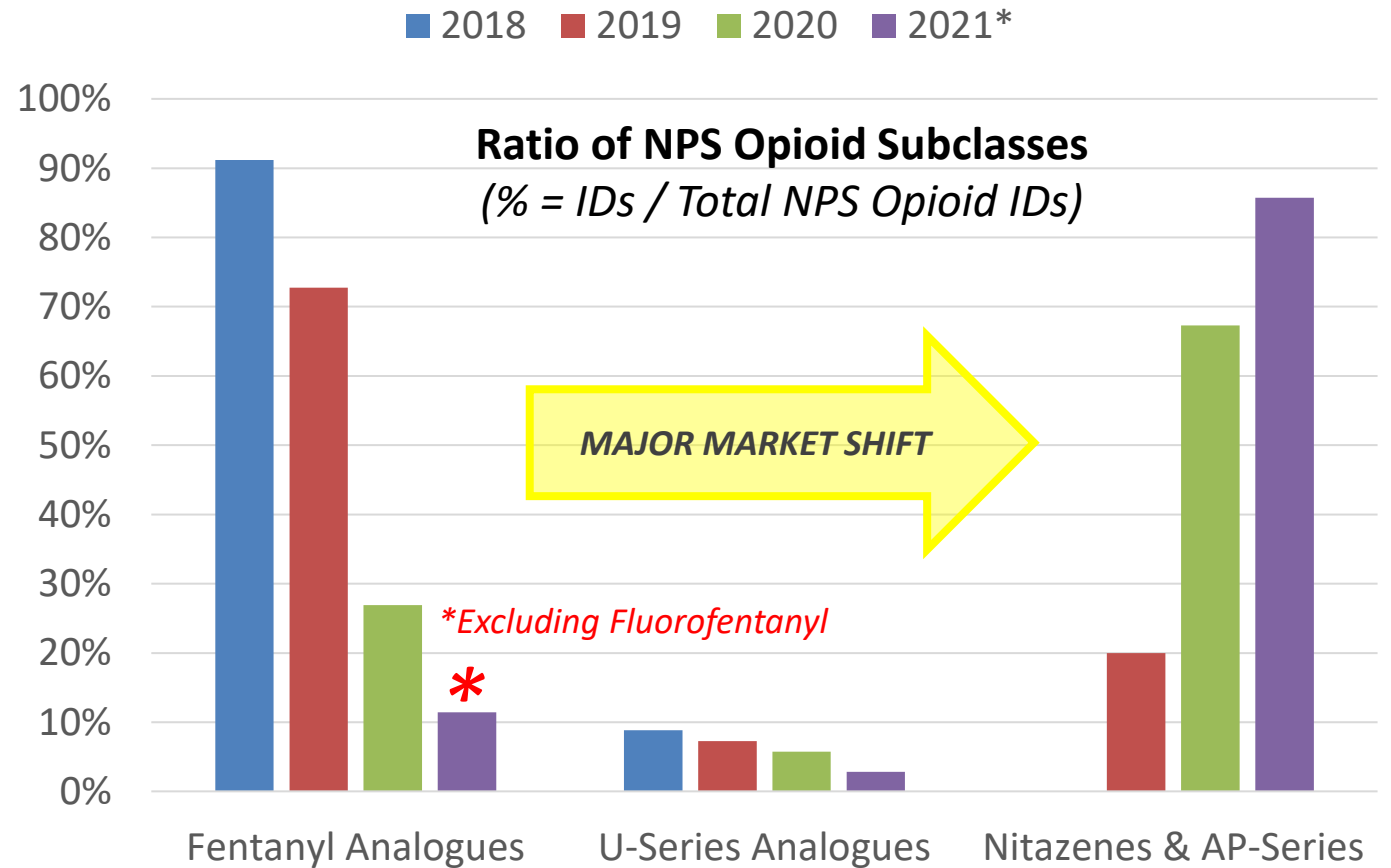
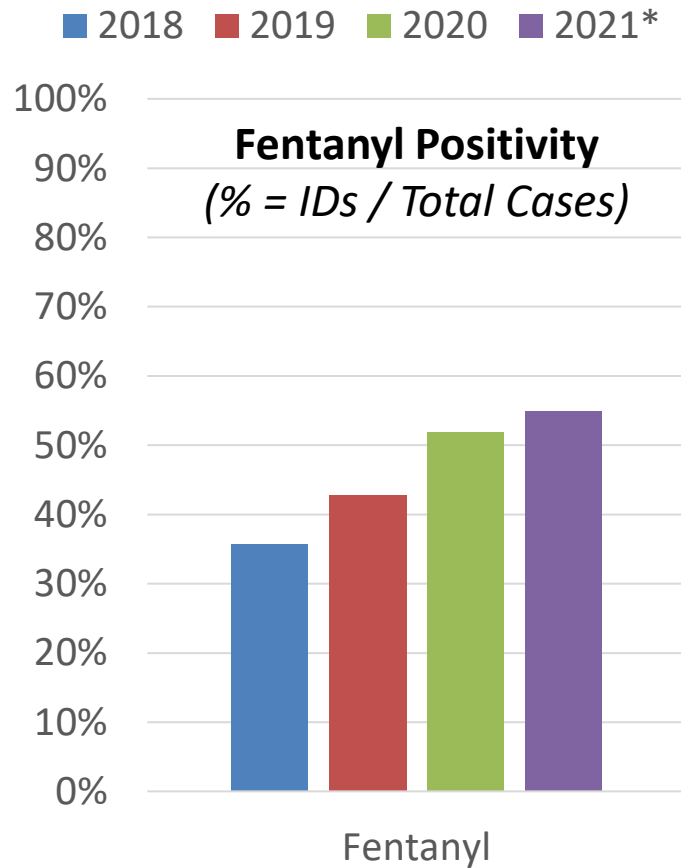
NPS Reported Per Year By Class



Class of NPS Reported Per Year



Positivity of NPS Opioids



“Santa Muerte” Outbreak in Philadelphia, PA

- Circumstances:
 - Late July weekend in Philadelphia (2018)
 - >160 overdoses and >10 deaths
 - “Santa Muerte” stamped heroin bags →
 - Believe to be the last of the “pure” heroin
 - EMS responded and several local hospitals were admitting patients
 - Patients were administered naloxone, but became agitated and confused
 - Scopolamine/anticholinergic toxidrome?
- CFSRE received seized “heroin” powder and biological samples
 - Testing by GC-MS and/or LC-QTOF-MS

Philly overdose drug may have contained toxic designer drug

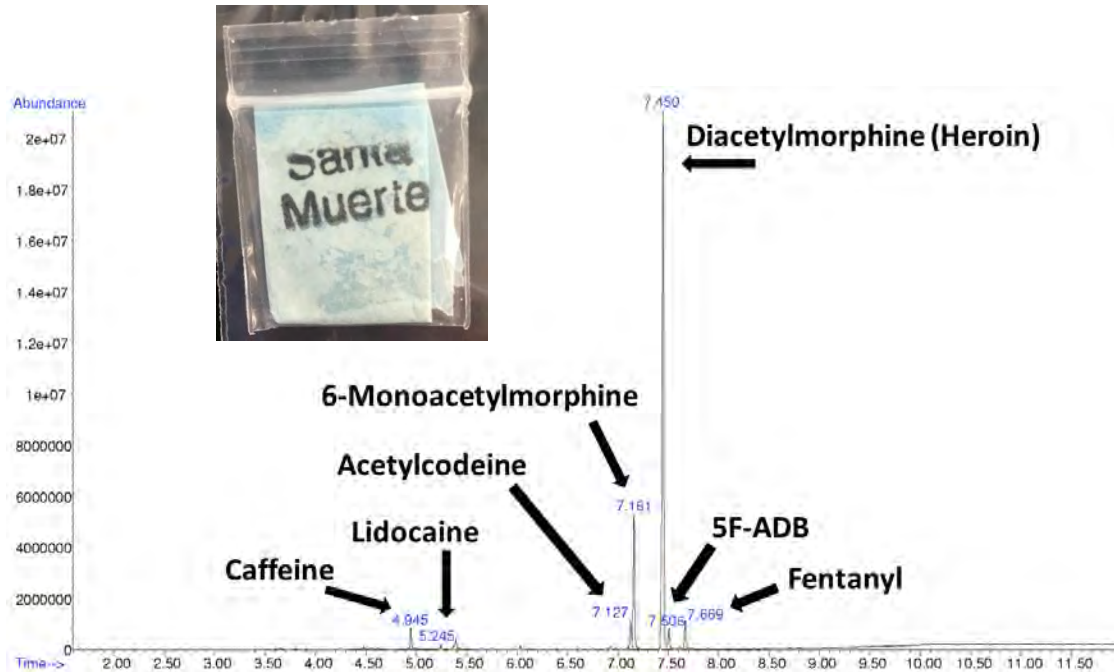
by [Aubrey Whelan](#) and [Mari A. Schaefer](#), Updated: July 26, 2018



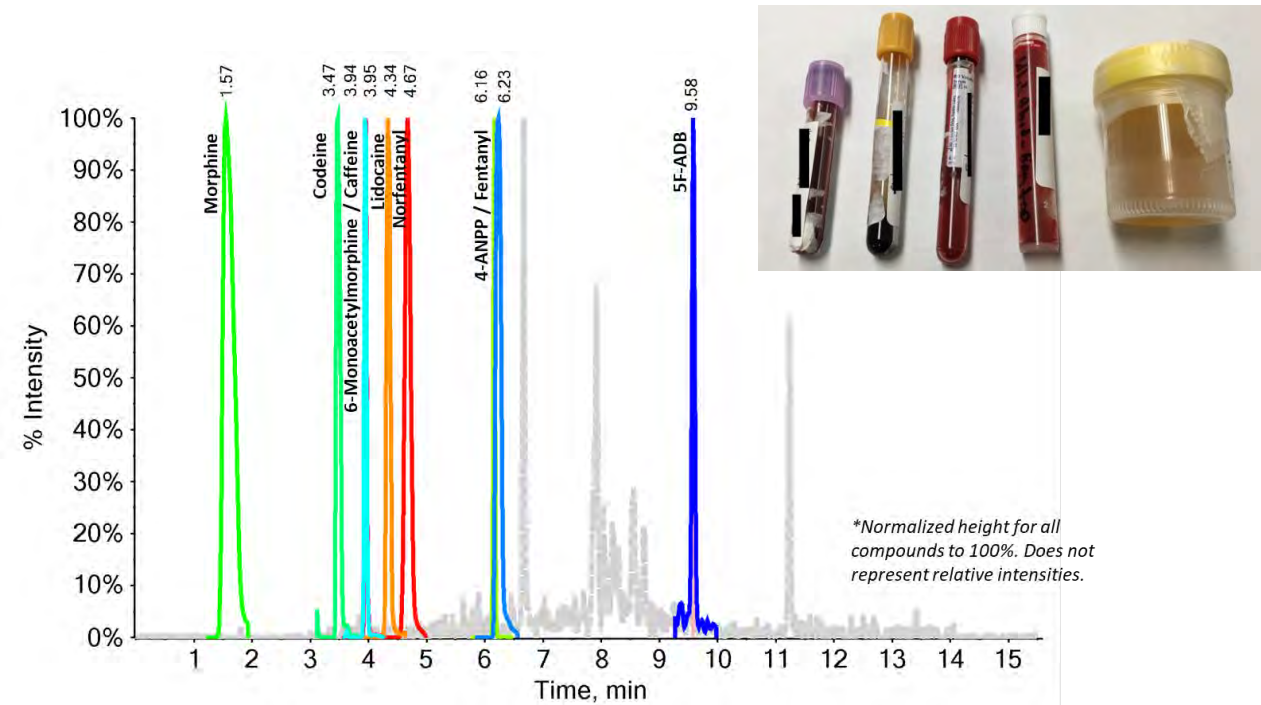
DAVID MAIALETTI

“Santa Muerte” Outbreak in Philadelphia, PA

- Seized drug sample



- Biological samples



***First time finding synthetic cannabinoids with heroin/fentanyl. Changes to future treatment/administration of naloxone?**

Partnerships with Clinicians & Hospitals

- Patients present to ED after overdose
- Clinician suspect synthetic drug use
 - Outside hospital scope of testing
- Waste/residual biological samples
 - Sent to CFSRE
- Analyzed via LC-QTOF-MS
- Results reported back within ~2 weeks; Clinicians can adjust future treatment, etc.



Partnerships with Clinicians & Hospitals

- **Case 1:**

- 63 y/o male behaving erratically in public
- Tachycardic, hypertensive, and hyperthermic
- Required multiple doses of antipsychotics and benzodiazepines to sedate
- Acute kidney injury and rhabdomyolysis
- Admitted to taking “everything under the sun” on a multi-day binge
- Urine drug screen was positive for amphetamines and opiates
- Toxidrome was most consistent with sympathomimetics or synthetic cannabinoids
- **Result: 4F-MDMB-BICA (cannabinoid)**

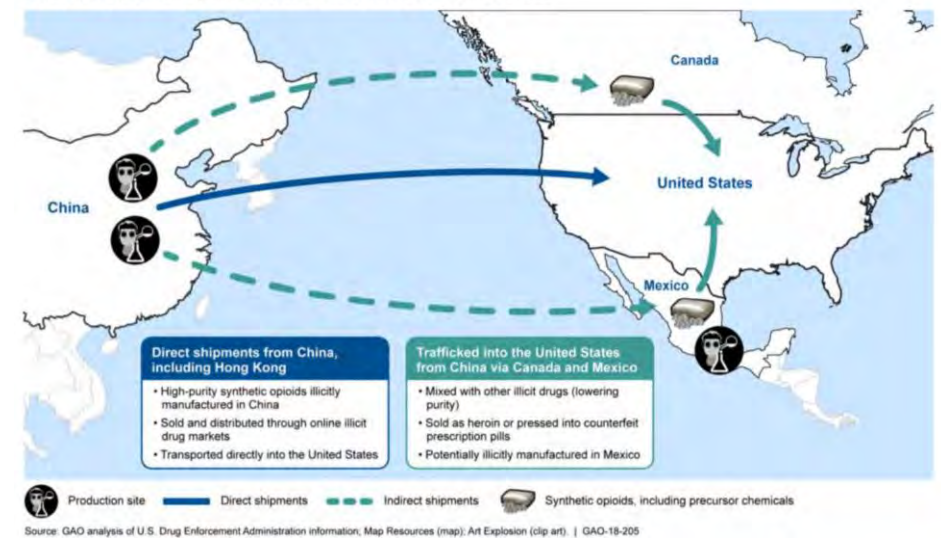
- **Case 2:**

- 31 y/o found unresponsive at home
- Respiratory depression
- EMS administered naloxone
- Transported to hospital
- Vitals: HR 65, BP 122/71, RR 18, 97.9°F
- 24 later, opioid toxidrome resolved
- Hospital drug screens negative for opioids
- Patient admitted to taking “2-methyl AP-237” which he purchases and mixed with water
- **Results: 2-Methyl AP-237 (opioid) and concomitant use of NPS benzodiazepines**

New Drugs Entering the United States

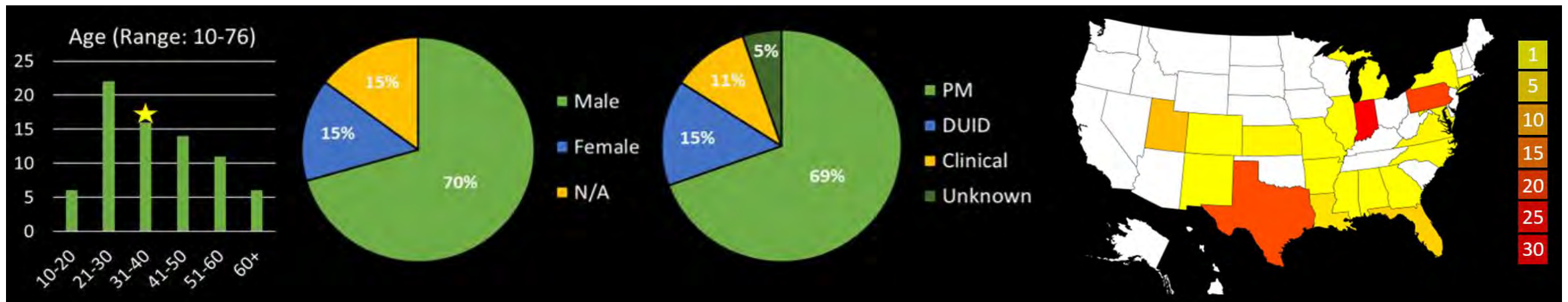
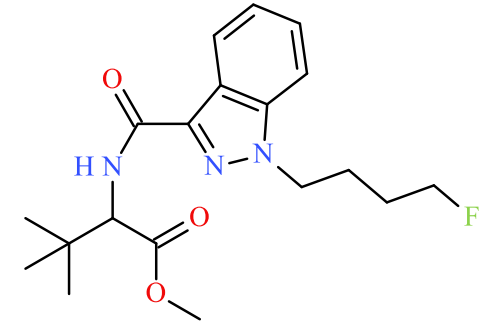
- Unidentified powders
- Agents suspect synthetic drugs present
- Powders sent to CFSRE
- Analyzed via GC-MS, LC-QTOF-MS, & NMR
- Correlation to street drugs supply and toxicology samples

Figure 4: Flow of Illicit Synthetic Opioids from China to the United States



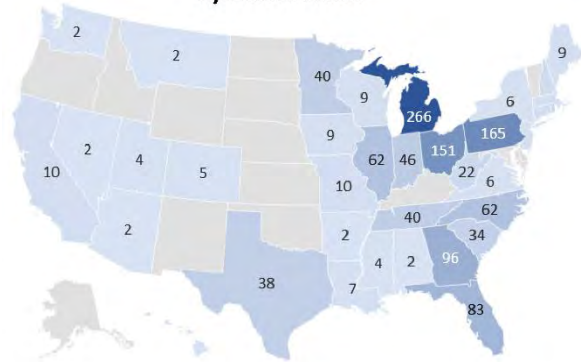
New Drugs Entering the United States

- Example 1: 2-Methyl AP-237 (opioid)
- Example 2: 4F-MDMB-BINACA (cannabinoid)

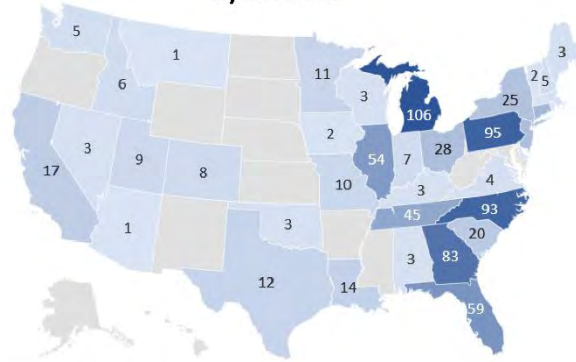


Geographical Differences Observed

a) Carfentanil



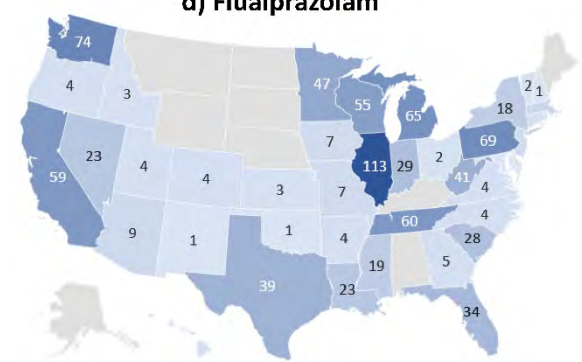
b) U-47700



c) Eutylone



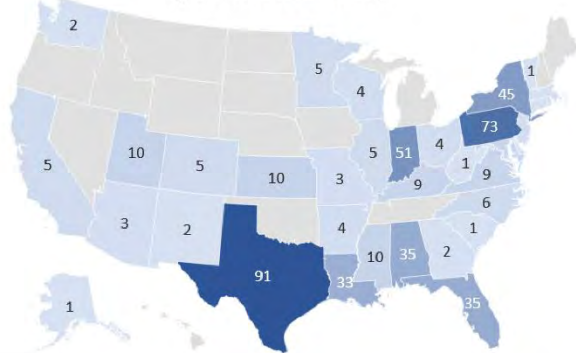
d) Flualprazolam



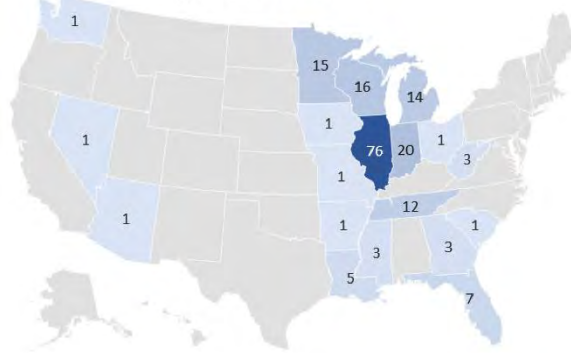
e) *N*-Ethylpentylone



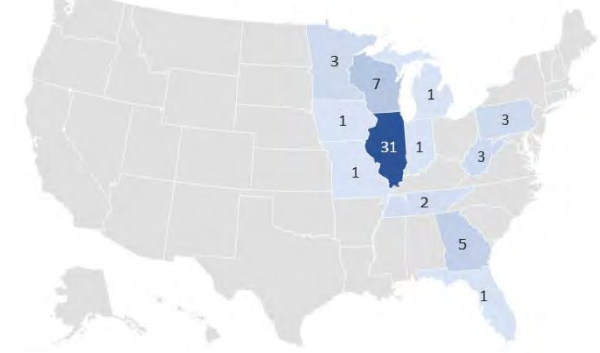
f) 5F-MDMB-PICA



g) Isotonitazene



h) Brorphine



DEA Scheduling of New Synthetic Drugs

- **8-Factor Analysis:**

1. Its actual or relative potential for abuse.
2. Scientific evidence of its pharmacological effect, if known.
3. The state of current scientific knowledge regarding the drug or other substance.
4. **Its history and current pattern of abuse.**
5. **The scope, duration, and significance of abuse.**
6. **What, if any, risk there is to the public health.**
7. Its psychic or physiological dependence liability.
8. Whether the substance is an immediate precursor of a substance already controlled under this subchapter.



Federal Register / Vol. 85, No. 118 / Thursday, June 18, 2020 / Proposed Rules

36819

DEPARTMENT OF JUSTICE
Drug Enforcement Administration
21 CFR Part 1308

[Docket No. DEA-631]

**Schedules of Controlled Substances:
Temporary Placement of Isotonitazene
in Schedule I**

AGENCY: Drug Enforcement Administration, Department of Justice.

ACTION: Proposed amendment; notice of intent.

SUMMARY: The Acting Administrator of the Drug Enforcement Administration is issuing this notice of intent to publish a temporary order to schedule *N,N*-diethyl-2-(2-(4 isopropoxybenzyl)-5-nitro-1*H*-benzimidazol-1-yl)ethan-1-amine (commonly known as isotonitazene), including its isomers, esters, ethers, salts, and salts of isomers, esters, and ethers whenever the existence of such isomers, esters, ethers, and salts is possible, in schedule I of the Controlled Substances Act. When it is issued, the temporary scheduling order will impose the regulatory controls and administrative, civil, and criminal sanctions applicable to schedule I controlled substances on persons who handle (manufacture, distribute, reverse distribute, import, export, engage in research, conduct instructional activities or chemical analysis, or

Factor 5. Scope, Duration, and Significance of Abuse

Isotonitazene, similar to etonitazene (schedule I), has been described as a potent synthetic opioid and evidence suggests it is being abused for its opioidergic effects (see Factor 6). The abuse of isotonitazene, similar to other synthetic opioids, has resulted in adverse health effects. Isotonitazene has been positively identified in 18 death investigation cases spanning between August 2019 and January 2020. These reports were from four states—Illinois (9), Indiana (7), Minnesota (1), and Wisconsin (1). Most (n = 12) of the decedents were male. The ages ranged from 24 to 66 years old with an average age of 41. Other substances identified in postmortem blood specimens obtained from these decedents include etizolam (6); flualprazolam, a nonscheduled benzodiazepine (7); fentanyl (6); heroin (3); tramadol, a schedule IV substance

* Krotulski AJ, Papsun DM, Kacinko SL, and Logan BK (2020). Isotonitazene Quantitation and Metabolite Discovery in Authentic Forensic Casework. *Journal of Analytical Toxicology*. [Epub ahead of print].

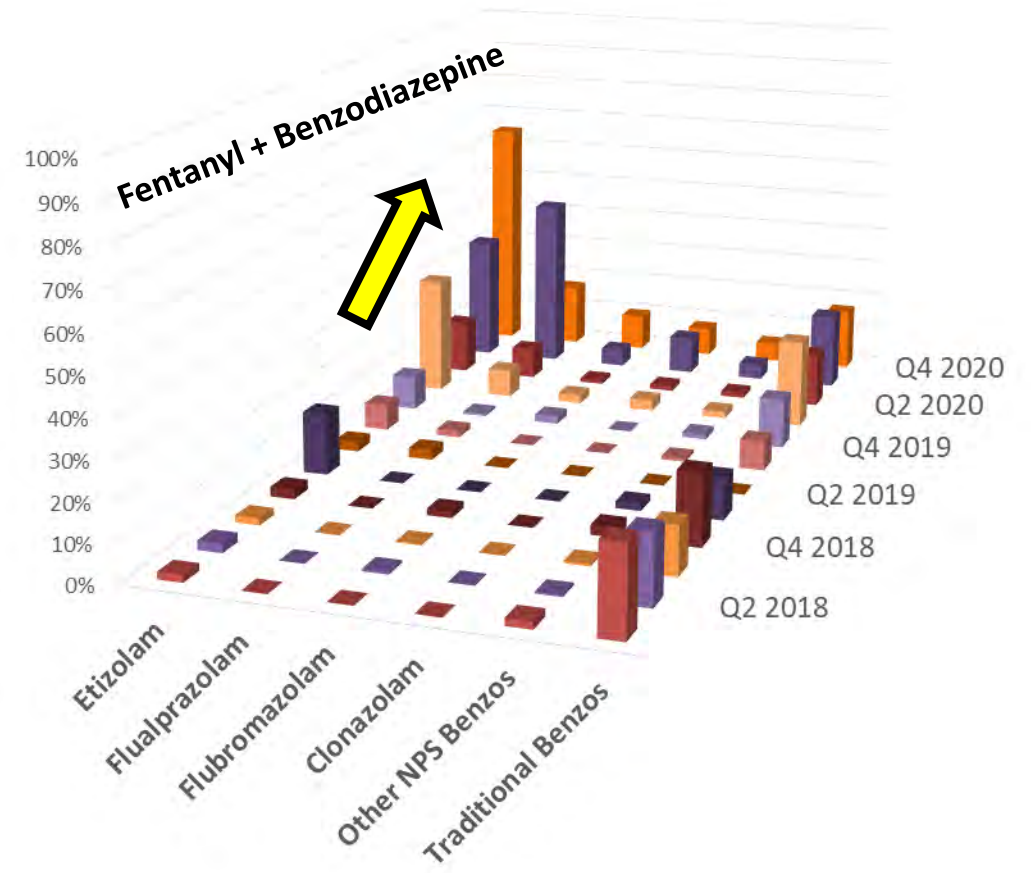
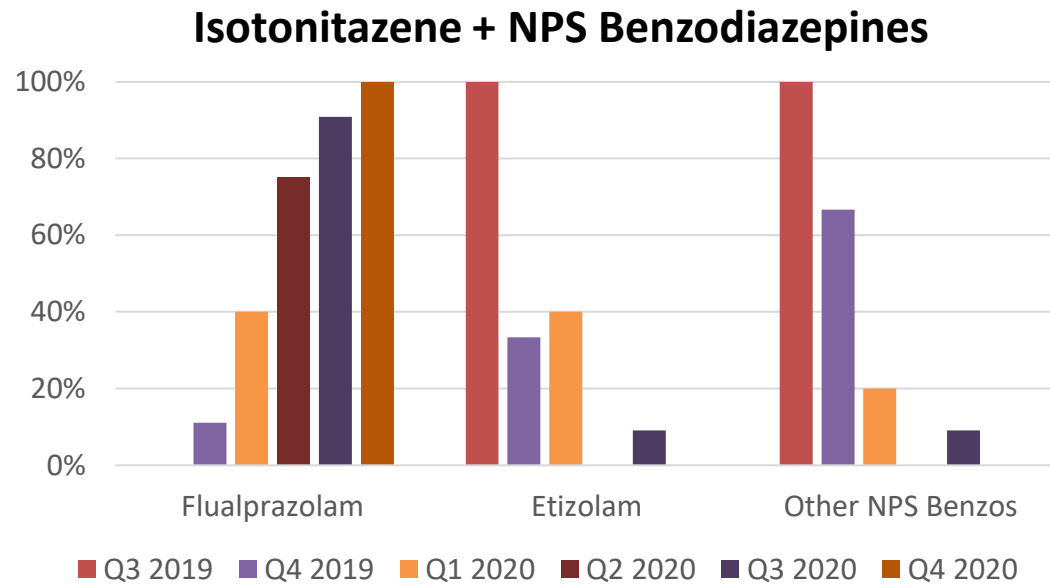
Poly-Drug Use

- Poly-drug use continues to increase, especially with opioids and benzodiazepines
 - Toxicology testing vs. drug material testing
- It is rare to find single-drug toxicology cases involving NPS
 - Drug products are more frequently mixed, cut, diluted, adulterated, etc.
 - Toxicology testing has become more comprehensive
 - Complicates interpretation
- Common combinations:
 - Benzodiazepines and fentanyl
 - Fentanyl and stimulants (coc. and/or meth.)
 - NPS opioids and NPS benzodiazepines



Concurrent Use: Benzodiazepines and Opioids

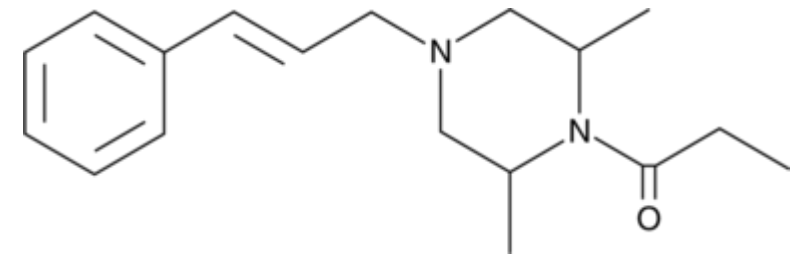
- NPS benzodiazepines are increasingly being found in combination with opioids (mostly fentanyl)



Crude Pharmacological Assessments?

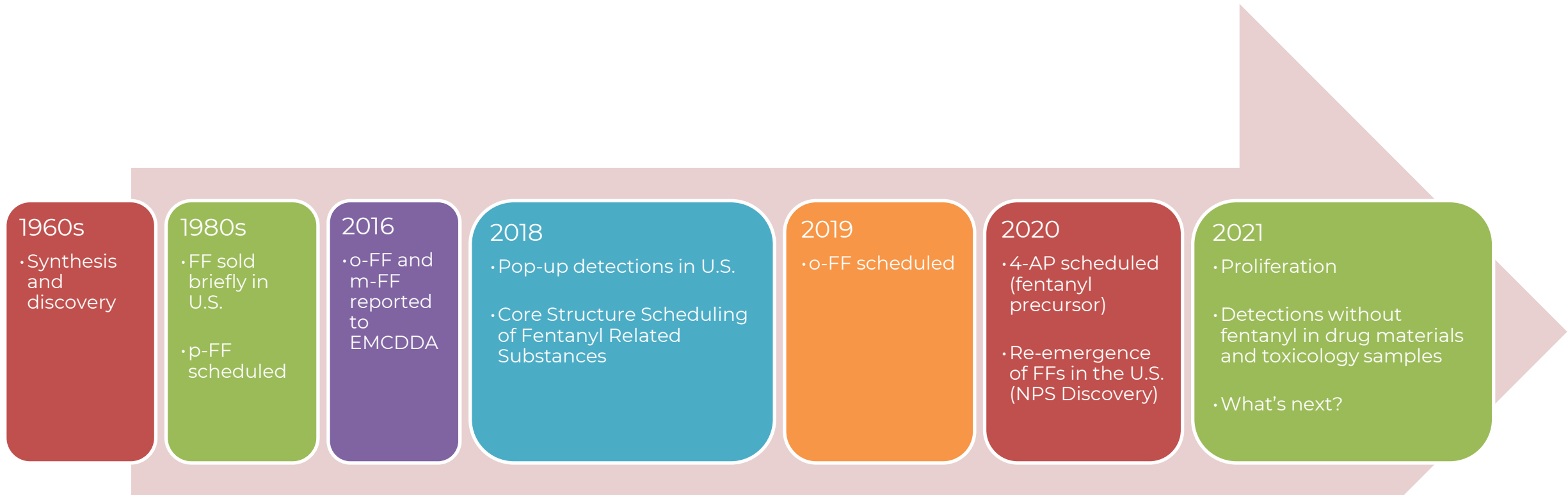
- **Real Case Example:**

1. Medical examiner reaches out about a death
2. Routine toxicology testing is negative (no cause/manner of death)
3. Autopsy shows signs of suspected drug death (e.g., pulmonary edema)
4. Toxicology samples sent for expanded testing
5. New opioids “AP-238” discovered
6. Laboratory quantifies the drug in blood sample

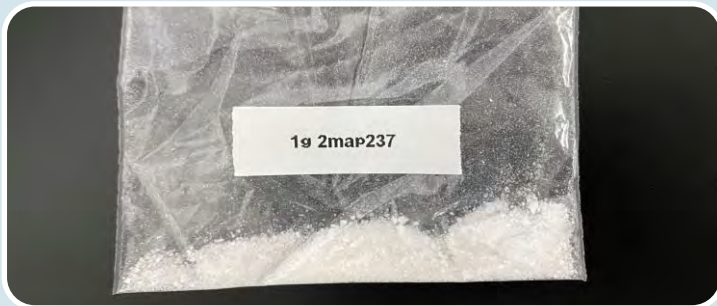


- **Interpretation:** Active drug, Opioid-like, Less potent than fentanyl*

Timeline of Fluorofentanyl



But ... Not All NPS Opioids Are Created Equal



Gray Market Vendors & Research Chemicals

I.e., buying drugs online

E.g., 2-Methyl AP-237



Sold Recreational as "Heroin/Fentanyl"

I.e., buying drugs on street

E.g., Metonitazene (Midwest)



Combined With The Larger Drug Supply*

I.e., buying drugs on street

E.g., *para*-Fluorofentanyl with Fentanyl (Widespread)

**Excluding cases where drug found alone*

New Drug Monographs

Public Alerts

Scientific Publications

Trend Reports

And So Much More !!!

Sample Source Analytical Methods

Discovery Workflows Results & Outcomes

www.npsdiscovery.org



NMS LABS
 2300 Stratford Ave
 Willow Grove, PA 19090

4F-MDMB-BINACA

Sample Type: Seized Material

Latest Revision: January 11, 2019
 Date Received: December 21, 2018
 Date of Report: January 11, 2019

CC(C)(C)C(NC(=O)c1cnc(CCC)cn1)C(=O)OC

January 2019 New Synthetic Cannabinoid: 4F-MDMB-BINACA

Purpose: The objective of this public announcement is to notify public health and public safety, law enforcement, clinicians, medical examiners and coroner, laboratory personnel, and all other related communities about new information surrounding the emergent synthetic cannabinoid 4F-MDMB-BINACA.

Summary: 4F-MDMB-BINACA, first identified in seized drug casework in the United States in December of 2018, has been identified in eight blood specimens associated with post-mortem investigations and driving under the influence of drugs (DUID) investigations. 4F-MDMB-BINACA is very similar in structure to the popular synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA), differing by the removal of one carbon (-CH₂-) linkage from the molecule. 5F-ADB has been associated with a large number of adverse events, injury and toxicity of 4F-MDMB-BINACA have not been explicitly studied, but its association with drug user deaths lead professionals to believe this new synthetic novel psychoactive substance (NPS) and retain the potential to cause adverse events.

Demographics

- Age: Adolescent to Adult
- Sex: Male (n=5), Female (n=1)
- Case Type: Death (n=5), DUID (n=3)
- Specimen Type: Blood (n=8)
- Date of Collection: Dec. 2018, Jan. 2019
- Other Notable Findings: 5F-MDMB-PINACA (n=4), 5F-ADB (n=2), No Other Findings (n=3)

Trend Report: Q2 2020 NPS Stimulants & Hallucinogens in the United States

Purpose: This report provides up-to-date information regarding NPS stimulant & NPS hallucinogen prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including NPS stimulants and NPS hallucinogens, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. Both NPS stimulants and NPS hallucinogens have been implicated in emergency room admissions, death investigations, and/or situations events associated with night clubs and music festivals. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification.

Objective: Our laboratory employs 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 800 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of emerging stimulants and hallucinogens, and further data analysis of important trends. This project was conducted in collaboration with the toxicology and criminology laboratories of NMS Labs. Forensic case types linked to these results include illicit drug investigations, methodical 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 CFSRE during this quarter, including those from sample-testing, data-mining, and/or casework testing.

NPS in Q2 2020:

- 9% Stimulants & Hallucinogens
- 59% Synthetic Cannabinoids
- 25% Benzodiazepines
- 7% Opioids

NPS Stimulant & Hallucinogen Positivity

Substance	Count
N-Ethyl Propylone	1
N-Ethyl Heptedrone	1
HO-PCP	1
Ethylphenidate	1
Dimethylone	1
Dibutylone	1
Cathinone	1
Alpha-PVP	1
5-MeO-DMT	1
4-HO-DPT	1
4-HO-DET	1
Cacore 907	2
4F-Methylphenidate	2
Alpha-PIP/Alpha-PIHP	3
2F-Deschloroketamine	4
Eutylone	5

NPS Stimulant Combinations

Combination	Frequency
Eutylone + Etizolam + Fentanyl + Cocaine	1
Eutylone + Etizolam + Cocaine	1
Eutylone + Fentanyl + Cocaine	1
Eutylone + Fentanyl	1

New Discoveries in Q2 2020

α-PCVP, Hexabrom, 4F-APH

Recommendations for Clinicians

- Become familiar with the signs and symptoms associated with synthetic cannabinoid use; can range from profound agitated delirium to sedation, difficulty in arousal, and bradycardia. Symptoms can alternate and overlap.
- Be aware that clinical conditions may change rapidly and unpredictably.
- Be mindful that illicit drugs have limited quality control, containing undeclared substances that impact the expected clinical effects or findings.
- Counsel about the dangers of synthetic cannabinoid products and other drugs.

Recommendations for ME's & Coroners

- Test for new synthetic cannabinoids and their biomarkers in suspected synthetic cannabinoid overdose cases.
- Consider testing for synthetic cannabinoids if circumstances result in an unspecified drug fatality.
- Be aware that ELISA screening for synthetic cannabinoids may not be specific or specialized for the newest generation of compounds; consider mass spectrometry-based screening.
- Be aware that concentrations of synthetic cannabinoids in biological specimens can be very small in comparison to other drugs or NPS; GC-MS sensitivity may not be adequate.

Recommendations for Laboratories

- Utilize analytical data available publicly for the identification of 4F-MDMB-BINACA and other synthetic cannabinoids if reference standards are not available to your laboratory.
- Develop sensitive and up-to-date testing procedures for synthetic cannabinoids.
- Prioritize analytical testing of seized drug samples taken from drug overdose scenes during death investigations.
- Share data on synthetic cannabinoid drug seizures with local health departments, medical examiners, and coroners.

Map of the United States

Legend:

- Green: Seized
- Red: Death
- Blue: DUID

Sample Source Analytical Methods

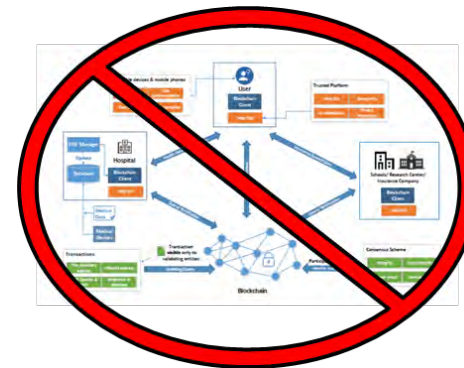
Discovery Workflows Results & Outcomes

TAKE AWAYS



Persistent Challenges

- Synthetic drugs present in forensically relevant samples often remain **unidentified** due to scope of analysis and analyst experience/expertise
- First identification are often **lagging or delayed**
 - Analytical capabilities, testing performed
 - Standard reference material
 - Relation to previously identified NPS
- There is **no centralized reporting system** within the United States



Considerations for Identifying NPS

- **Sample Source:**
 - Understand population, effects, etc.
 - Are you testing the correct matrix?
- **Analytical Methods:**
 - Mass spectrometry
 - Sensitive and specific instruments
 - Non-targeted data acquisition
- **Discovery Workflows:**
 - Does your laboratory have a strategy?
 - NPS do not just *appear*
 - Non-targeted data processing
 - Appropriate acceptance criteria
- **Results & Outcomes:**
 - Public and private partnerships foster generation of important data
 - Dissemination is key!
 - Forecasting future trends?

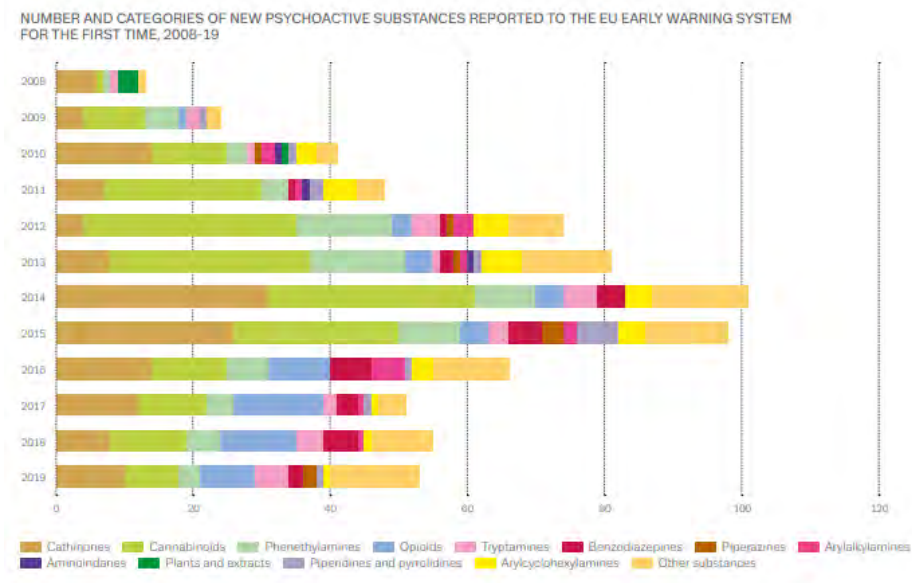


Recommendations

- Implement data independent acquisition (“non-targeted”) strategies
- Use high resolution mass spectrometry for drug screening
- Develop appropriate data processing workflows
- Maintain updated and accurate scopes of testing
- Partner with laboratories outside your field for testing
- Collaborate with other scientists to develop meaningful results
- Dissemination and information sharing!
- Seek opportunities for funding and support

Conclusions

- Drug testing for new synthetic substances is not easy... and it takes an army
- Constantly evolving NPS trends lead to constant need for adjusting/adapting
- Expertise and advanced skill sets are needed
- State-of-the-art instrumentation is preferred
- Resources are available
 - Method development, test design, etc.



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 - Carolina Noble
- **Forensic and Clinical Partners**
- **Medical Examiner and Coroner Partners**
- **Federal, State, and Local Partners**



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Click Presentation Title

The screenshot shows a web browser at the URL <https://www.npsdiscovery.org/resources/presentations/>. The page features the CFSRE logo (Redefining Excellence in Forensic Science) and a navigation menu with 'Resources' expanded to show 'Presentations'. A list of presentations is displayed, with the first one titled 'Synthetic Cannabinoids – Novel Psychoactive Substances (NPS): Analytical Methods and En...' by Alex J. Krotulski, dated 04/28/2021. A second presentation is listed below it, dated 02/18/2021, titled 'Quantitative Forensic Toxicology by Standard Addition: Consideration, Experimentation, and Implementation' by Alex J. Krotulski, Sherri Kacinko, Joseph Homan, and Barry K Logan. Red circles with numbers 1, 2, and 3 are overlaid on the browser address bar, the 'Presentations' menu item, and the first presentation title, respectively.





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