

TEMPLE  
UNIVERSITY®

# **Sample Mining** – The Identification of Emerging Novel Psychoactive Substances (NPS) through Re-Analysis of Biological Extracts from Forensic Toxicology Casework

**Alex J. Krotulski, Dr. Susan Jansen Varnum, and Dr. Barry K. Logan**

*AAFS 2019 – Thursday February 21<sup>st</sup>, 2019*

# Disclosure

- This project was supported by Award Number 2017-R2-CX-0002, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication, program, or exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.



# Conflicts of Interest

---

- Nothing to report.



# BACKGROUND

---

# Background

- Novel psychoactive substances (NPS) continue to pose health and safety threats within the United States

Morning Mix

**‘It is taking people out’: More than 70 people overdose on K2 in a single day in New Haven**



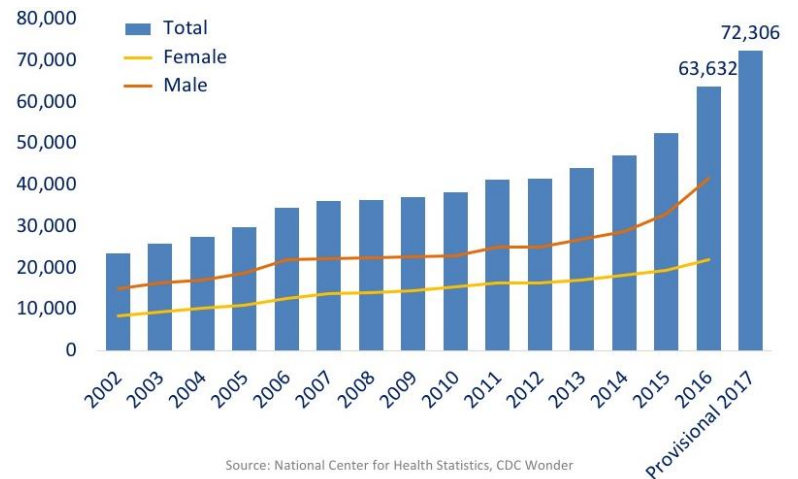
**Over 200 Philly Heroin Users Poisoned Over the Weekend, Potentially Due to “K2”**



music → music festivals

**Seven dead: Mass deaths at music festivals from suspected drug overdoses**

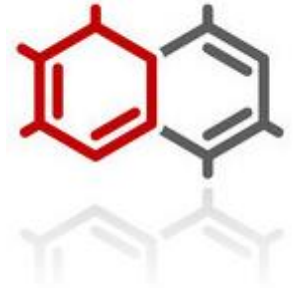
SEVEN young people have died from suspected drug overdoses in what could be one of the largest mass deaths at a music festival anywhere in the world.



Source: National Center for Health Statistics, CDC Wonder

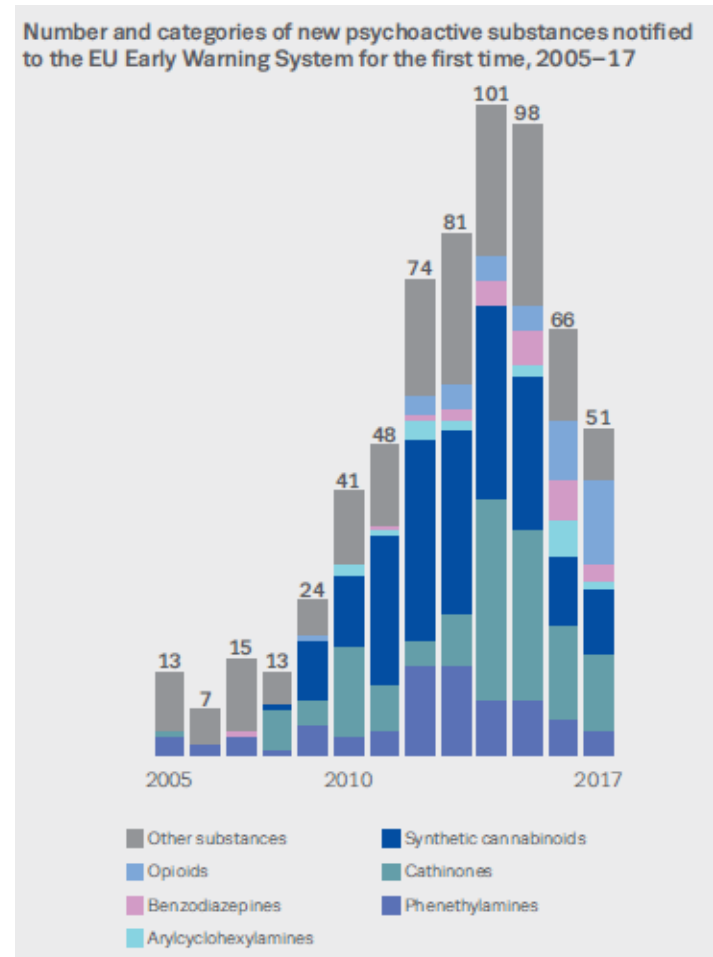
# Background

- Effecting numerous communities
  - Public health and public safety
  - Clinicians and medical toxicologist
  - Law enforcement
  - Forensic toxicologist and drug chemists
  - Medical examiners and coroners
  - Lawyers



# Background

- New NPS identified each year
  - European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) →
  - National Forensic Laboratory Information System (NFLIS)
  - DEA Emerging Treat Reports
  - NPS Discovery
- Mostly reporting systems for seized drug identifications



# Background

---

- New NPS are present in forensically relevant samples and they often go unidentified
  - Scope of analysis/approach of analysis
  - Analyst experience/expertise
  - Lack of standard reference materials
- First identification are often lagging or delayed
- There is no centralized reporting system within the United States



# Purpose

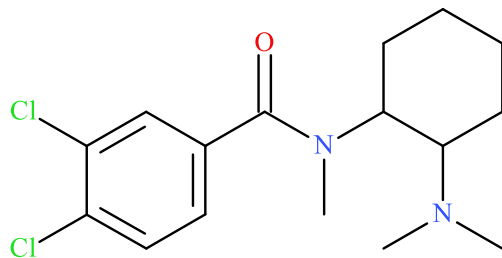
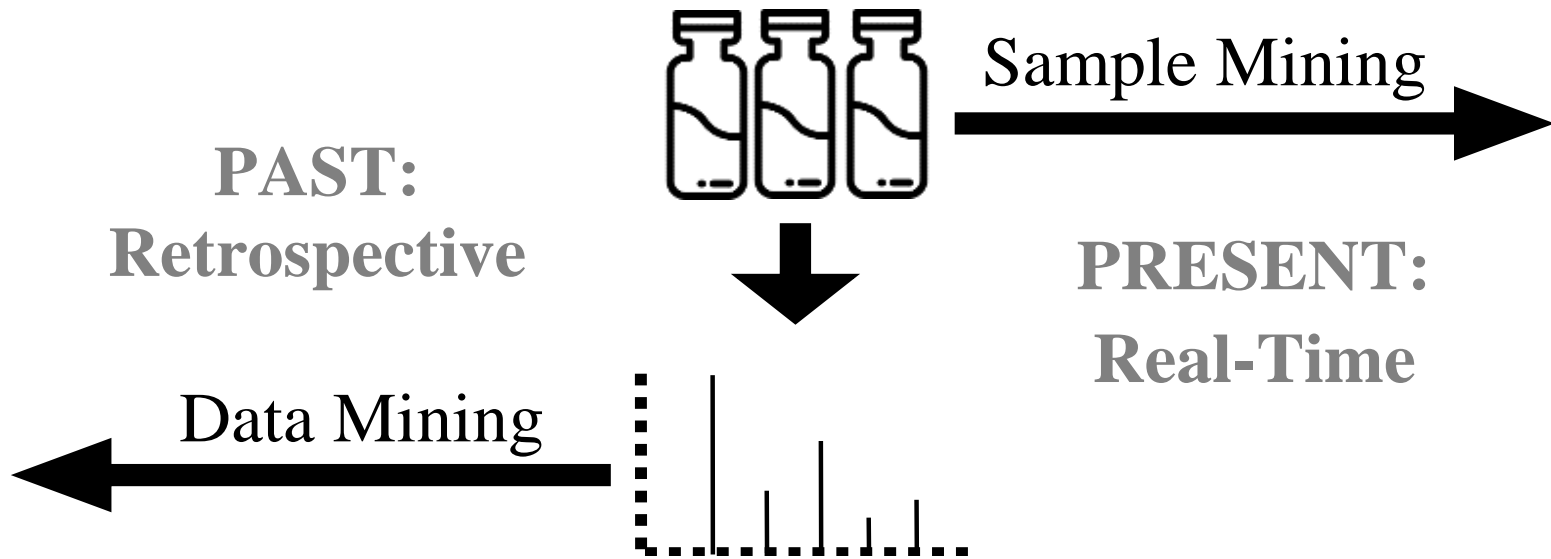
- Develop an approach for timely identification of NPS and subsequent dissemination
  - *“Sample mining”*
- Instrumentation that provided necessary analytical data for positive identification
  - LC-MS/MS vs. LC-TOF vs. LC-QTOF



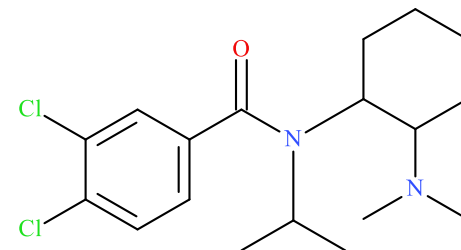
# Sample Mining

- Definition: process of identifying NPS through real-time analysis/re-analysis of samples
  - Sample extracts, biological specimens, datafiles, etc.
- Differs from traditional drug testing
  - Non-targeted acquisition for the discovery of NPS
  - Focuses on drugs not within the scope of testing and/or the predictable drug using population.
- Mining → analytical expertise and data processing required to discover new substances within data-rich sample set

# Sample Mining



When was the first identification?



When will the first identification be?

# METHODS

---

# Sample Extracts

- De-identified, discarded sample vial extracts from a large forensic toxicology laboratory
  - NMS Labs (Willow Grove, PA)
- Prepared using standard extraction protocols
- Target procedures for the analysis of NPS
  - Data-rich sample set
  - Novel Opioids
  - Designer Benzodiazepines
  - Novel Stimulants



# Analysis

- Liquid chromatography quadrupole time-of-flight (LC-QTOF) mass spectrometry
  - Sciex TripleTOF<sup>®</sup> 5600+
  - Shimadzu Nexera XR UHPLC
- 15.5 minute method
- Simple reverse phase gradient
  - Ammonium formate (10mM, pH 3); MeOH/ACN (50:50)
  - Phenomenex<sup>®</sup> Kinetex C18 (50mm x 3.0mm, 2.6 $\mu$ m).
- Non-targeted mass acquisition (SWATH<sup>®</sup>)



Ionization	Precursor Ion Range (Da)	Collision Energy (eV)	Product Ion Range (Da)
ESI+	100-510	35 $\pm$ 15	50-510

# Data Processing

- Targeted data processing
  - MasterView and PeakView
  - >800 compounds in database

Category	Analytes
NPS	>600
Abused Drugs	66
Other	175

	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	> 50.0
●	>= 10.0	>= 0.35	>= 50.0	<= 50.0

- Non-targeted data processing\*
  - Case-by-case basis

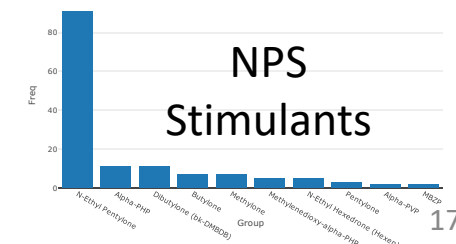
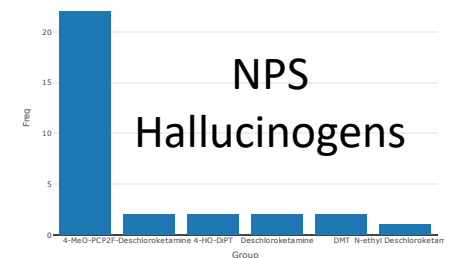
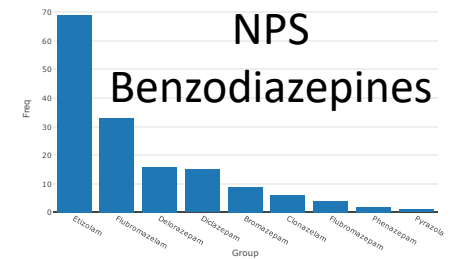
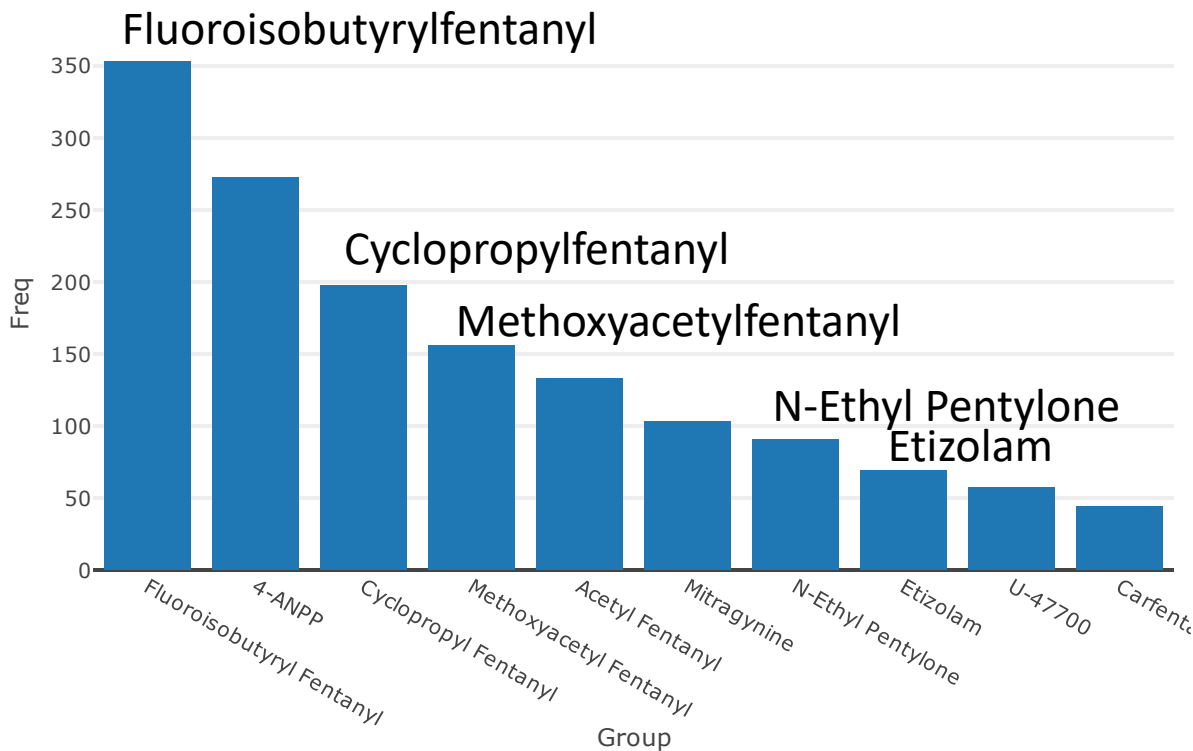
# RESULTS

---



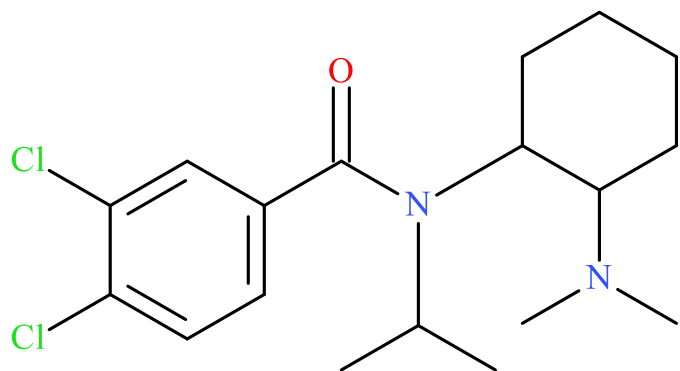
# Results

- To date, more than 3,300 extracts have been re-analyzed using LC-QTOF

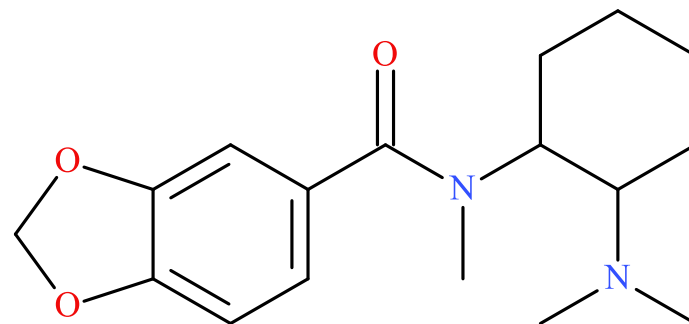


# Novel Opioids: U-Series Analogues

- Isopropyl-U-47700 and 3,4-MD-U-47700
  - First identifications in toxicology casework
    - May 2018
  - In combination with other novel opioids
  - More recently identified in seized drug material
  - Suspected opioid activity



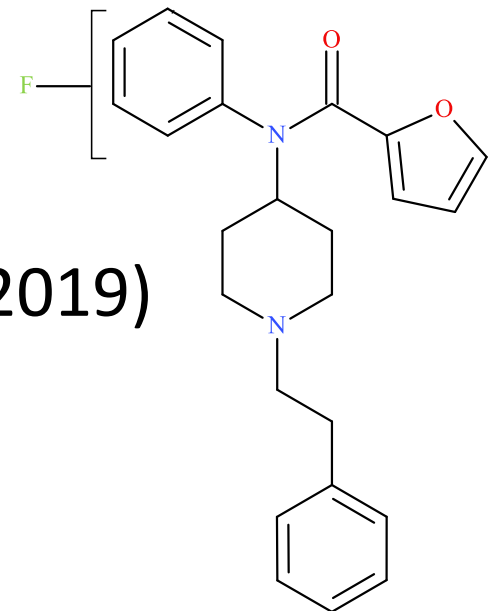
*Isopropyl-U-47700*



*3,4-MD-U-47700*

# Novel Opioids: Fentanyl Analogues

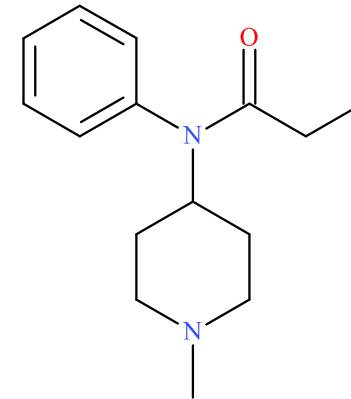
- Phenylfentanyl
  - Identified in August 2019
- Fluorofuranylfentanyl
  - Identified in blood sample (January 2019)
    - Additional identifications in FL and OH
  - Found with Fluoro-4-ANPP
    - Metabolite and/or precursor



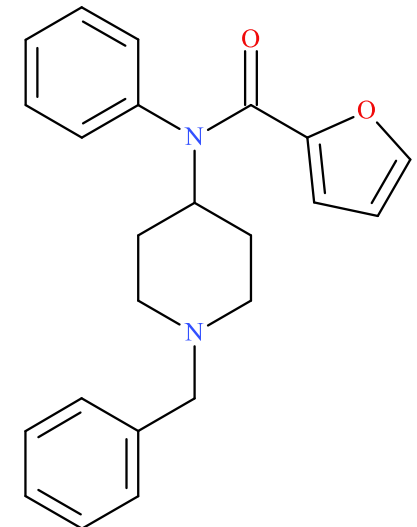
*Fluorofuranylfentanyl*

# Novel Opioids: Fentanyl Precursors

- N-methyl Norfentanyl
  - Inactive; found with fentanyl
- Benzylfuranylfentanyl
  - Hypothesized to be inactive
  - Not found with furanyl fentanyl
- Despropionyl-ortho-Methylfentanyl
- Despropionyl-3-Methylfentanyl



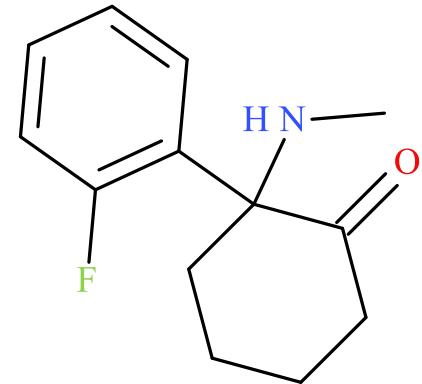
*N-methyl Norfentanyl*



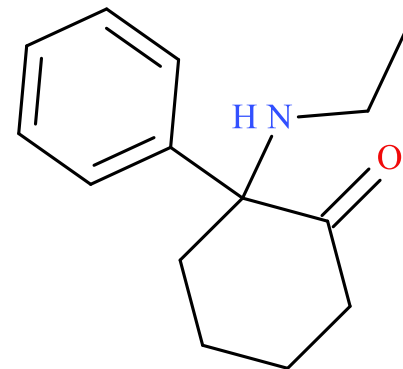
*Benzylfuranylfentanyl*

# Novel Hallucinogens

- 2F-Deschloroketamine
  - Identified in August 2018
    - First seized drug identification in December 2016
  - Deschloro-norketamine
    - Non-targeted data processing
- *N*-Ethyl Deschloroketamine
  - Identified in December 2018



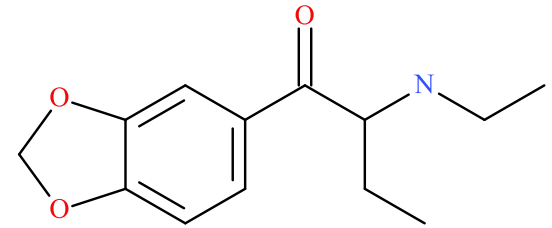
*2F-Deschloroketamine*



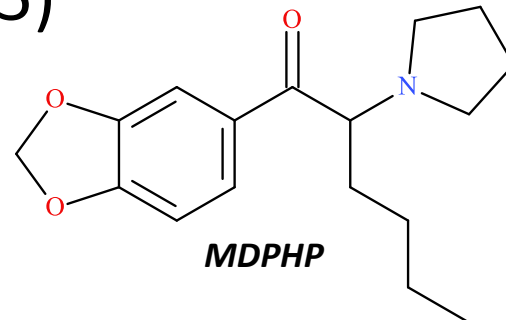
*N-Ethyl Deschloroketamine*

# Novel Stimulants

- Eutylone (bk-EBDB)
  - First identification in August 2018 (5 total cases)
  - In combination with Fluoroisobutyrylfentanyl (FIBF)
- 2018 ... the year of the “hex’s”
  - $\alpha$ -PHP (n=11)
  - 3,4-MD- $\alpha$ -PHP (MDPHP; n=5)
  - N-Ethyl Hexylone (n=1)
  - N-Ethyl Hexedrone (n=5)



*Eutylone*



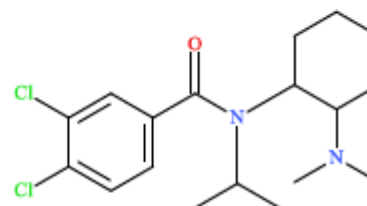
*MDPHP*

# Dissemination

- Monograph
  - Chemical data
  - Analytical data
  - Case information
  - Resources
- Listserv and Website
  - [www.npsdiscovery.org](http://www.npsdiscovery.org)
  - SWGDRUG Website
  - UNODC Portal



## Isopropyl-U-47700



Sample Type: Biological Fluid

Latest Revision: May 18<sup>th</sup>, 2018

Date of Report: May 18<sup>th</sup>, 2018

### 1. GENERAL INFORMATION

IUPAC Name: 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-isopropylbenzamide

InChI String: InChI=1S/C18H26Cl2N2O/c(1-12)(2)22(17-8-6-5-7-16(17)21(3)4)18(23)13-9-10-14(19)15(20)11-13/h9-12,16-17H,5-8H2,1-4H3

CFR: Not Scheduled (05/2018)

CAS# Not Available

Synonyms: Isopropyl-U47, IP-U47

Source: NMS Labs

### 2. CHEMICAL AND PHYSICAL DATA

#### 2.1 CHEMICAL DATA

Analyte	Chemical Formula	Molecular Weight	Exact Mass [M+H] <sup>+</sup>
Isopropyl-U-47700	C <sub>18</sub> H <sub>26</sub> Cl <sub>2</sub> N <sub>2</sub> O	357.3	357.1495

# CONCLUSIONS

---



# Conclusions

---

- Non-targeted data acquisition by SWATH<sup>®</sup> has been highly successful for identification of emerging NPS in toxicology samples
- Extracts provide a data-rich sample set for discovery of emerging NPS
- Timely dissemination of data regarding emerging NPS greatly benefits laboratory personnel, medical provider, and public health and safety agencies

# Future Work

---

- Novel data processing strategies
- Trends and demographics
- Drug combinations
- Metabolite identifications
- Quantitation by SWATH<sup>®</sup> acquisition

# Acknowledgements

- NMS Labs
  - Donna Papsun
  - Meredith Atkins, Linay Williams, and Kristina Long
  - Department Staff



- Sciex
  - Dr. Adrian Taylor
  - Dr. Oscar Cabrices



- CFSRE
  - Meaghan Drumm





## Questions?

Alex J. Krotulski, MSFS

Graduate Student – Department of Chemistry

[alex.krotulski@frfoundation.org](mailto:alex.krotulski@frfoundation.org)