cfsre The Center for Forensic Science Research & Education

NPS DISCOVERY

NPS Discovery Toolkit

» *N,N*-Dimethylpentylone

DEC • 2022

Acknowledgements: This report was prepared by Sara E. Walton, MS; Alex J. Krotulski, PhD; Melissa F. Fogarty, MSFS, D-ABFT-FT; Donna M. Papsun, MS, D-ABFT; and Barry K. Logan, PhD, F-ABFT. Funding was received from 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 authors and do not necessarily represent the official position or policies of the U.S. Department of Justice.

Recommended Citation: NPS Discovery (2022), NPS Discovery Toolkit: *N,N*-Dimethylpentylone, Center for Forensic Science Research and Education, United States of America.

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NPS DISCOVERY

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Publication Pending: Journal of Analytical Toxicology

Fogarty MF, Krotulski AJ, Papsun DM, Walton SE, Lamb M, Truver M, Chronister CW, Goldberger BA, Logan BK. New Synthetic Cathinone *N,N*-Dimethylpentylone (Dipentylone) Implicated in Forensic Postmortem Case Series.

Purpose: The NPS Discovery Toolkit is a consolidation of our program outcomes into a comprehensive document detailing relevant information about the characterization of a specified novel psychoactive substance (NPS). This *toolkit* includes basic drug information, date of first appearance, prevalence, temporal trends, geographical trends, demographics, poly-drug combinations (including with other NPS), metabolism, methods for identification and confirmation, reference concentration ranges, and much more. This toolkit is designed to serve as a one-stop resource for scientists and interested individuals looking for all-inclusive information about a new drug.

About Us: The Center for Forensic Science Research and Education (CFSRE, Willow Grove, PA) is a non-profit organization that operates a state-of-the-art laboratory with a mission to advance forensic science testing and knowledge. In 2018, the CFSRE launched "NPS Discovery" as a response to increased emergence and proliferation of new synthetic drugs, including those associated with increasing harms and adverse effects. NPS Discovery has grown to become a premier open access drug early warning system for timely information sharing among public health and public safety stakeholders.

▸ NPS Discovery welcomes collaborative partnerships with engaged agencies and communities impacted by the use of NPS. Individuals can contact our program to learn more about our advanced testing capabilities, to request information regarding sample submissions, and/or to join our growing dissemination networks.

April 2022

Synthetic Stimulant Market Rapidly Changing as *N,N*-Dimethylpentylone Replaces Eutylone in Drug Supply Typically Sold as "Ecstasy" or "Molly"

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NPS DISCOVERY

Purpose: The objective of this announcement is to notify public health and safety, law enforcement, first responders, clinicians, medical examiners and coroners, forensic and clinical laboratory personnel, and all other related communities about new information surrounding the emergent synthetic stimulant ***N,N*-dimethylpentylone**.

Background: Synthetic stimulants are chemically manufactured drugs with sub-classifications based on their structural relation to amphetamine or cathinone. Synthetic stimulants, including substituted cathinone analogues (e.g., eutylone), can retain both stimulant and hallucinogenic properties, and can cause associated health risks. Synthetic stimulants are often prepared and distributed in powder, capsule, or tablet form, and may be sold as "Ecstasy", "Molly", or "MDMA" (3,4-methylenedioxymethamphetamine) on recreational drug markets. In the United States (U.S.), synthetic stimulants have been associated with adverse effects and linked to cardiac effects resulting in death. Adverse effects can include hyperthermia, dehydration, arrhythmias, hallucinations, and serotonin syndrome.

Summary: In 2020 and 2021, the substituted cathinone **eutylone** was the most commonly encountered synthetic stimulant to appear in forensic casework, despite the drug being considered federally scheduled as an isomer of pentylone since March 2017 according to the U.S. Drug Enforcement Administration (DEA). In September 2021, eutylone was recommended for international control. It is this notice that likely created a shift in the NPS drug market, which would later be noted by declining eutylone positivity and increasing *N,N*-dimethylpentylone positivity. *N,N*-Dimethylpentylone was first identified in toxicology samples in the U.S. in Q3 2021, marking the initial insurgence of this drug into the supply and the beginning of its proliferation. To date, *N,N*-dimethylpentylone has been identified in 32 toxicology cases, including antemortem and postmortem investigations, in addition to drug material cases. *N,N*-Dimethylpentylone is not explicitly scheduled in the U.S.; however, it could be considered an isomer of ***N*-ethyl pentylone** (Schedule I). Of note, pentylone is a metabolite of *N,N*-dimethylpentylone.

Case Breakdown

Case Type:

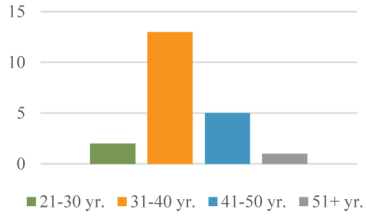
- Postmortem (n=26)
- DUID (n=1)
- Unknown (n=5)

Date of Collection:

- August 2021 to March 2022

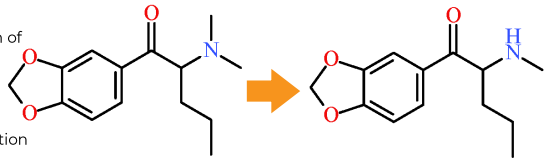
Other Notable Findings:

- Pentylone (n=23)
- Eutylone (n=5)
- Methamphetamine (n=11)
- Fentanyl / Opioids (n=13)
- No Other Drugs (n=8)



Recommendations for Public Health

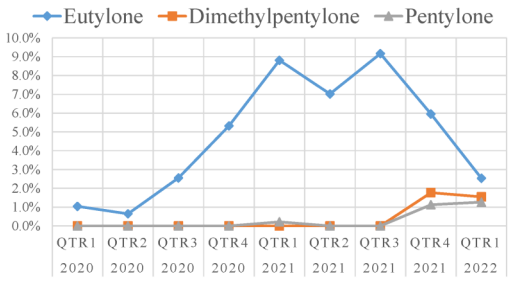
- Implement surveillance for rapid identification of drug use and overdose outbreaks.
- Engage local poison centers and clinicians to assist with treatment of affected patients.
- Track and monitor geographical drug distribution and trends.
- Track demographics and known risk factors for people who use stimulant/hallucinogen drugs.
- Raise awareness about the risks and dangers associated with synthetic stimulant use.



***N,N*-Dimethylpentylone → Pentylone**

Recommendations for Laboratories

- Utilize analytical data available publicly for the identification of ***N,N*-dimethylpentylone**.
- Utilize non-targeted testing protocols or develop sensitive and up-to-date testing procedures.
- Prioritize testing of drug material samples.
- Share data on synthetic stimulant identifications with local health departments, forensic scientists, and related communities.

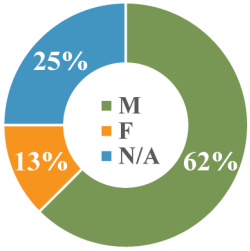


Recommendations for Clinicians

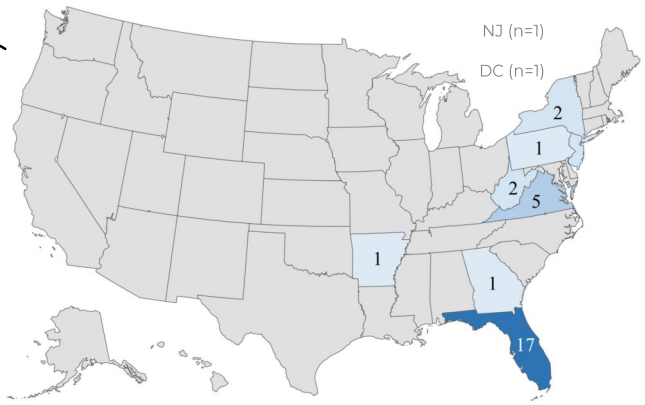
- Become familiar with the signs and symptoms of synthetic stimulant use (e.g., agitation, hallucinations, excitement, elevated pulse, arrhythmias, serotonin syndrome).
- Be mindful that recreational drugs have limited quality control, containing undeclared substances that impact expected clinical effects or findings.
- Counsel about the potential harms of "Ecstasy", "Molly", and "MDMA" products and use.

Recommendations for MEs & Coroners

- Test for new synthetic stimulants and their biomarkers in suspected stimulant-related cases.
- Be aware that ELISA screening for synthetic stimulants may not be specific or specialized for the newest generation of drugs; consider mass spectrometry-based screening.
- Be aware that concentrations of synthetic stimulants in biological specimens can vary; however, GC-MS sensitivity may be adequate.



Geographical Distribution of N,N-Dimethylpentylone in the U.S.



Conc. in Postmortem Blood [ng/mL] (n=5)			
***N,N*-Dimethylpentylone**		**Pentylone**	
Mean (±S.D.)	270 ± 400	**Mean (±S.D.)**	120 ± 170
Median	87	**Median**	37
Range	33 - 970	**Range**	10 - 420

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References and Related Articles:

- Koppe, H.; Ludwig, G.; and Zeile, K. (1964) Aryl-alpha-Aminoketone Derivatives CH Boehringer Sohn AG and Co KG, Boehringer Ingelheim GmbH, Assignee. Patent GB1085135A.
- World Health Organization. (2021) Critical Review Report: Eutylone Expert Committee on Drug Dependence 44th Meeting.
- Krotulski *et al.* (2021) Eutylone Intoxications—An Emerging Synthetic Stimulant in Forensic Investigations *Journal of Analytical Toxicology*, **45 (1),** 8-20.

Rapid NPS Testing Now Available:

If your agency suspects synthetic stimulant toxicity with no identifiable cause of death or your jurisdiction is noticing an increase in overdose patients requiring analytical testing, contact NPS Discovery at the Center for Forensic Science Research and Education (CFSRE); a non-profit organization in collaboration with local and federal agencies which can provide rapid testing after novel drug outbreaks in the United States.

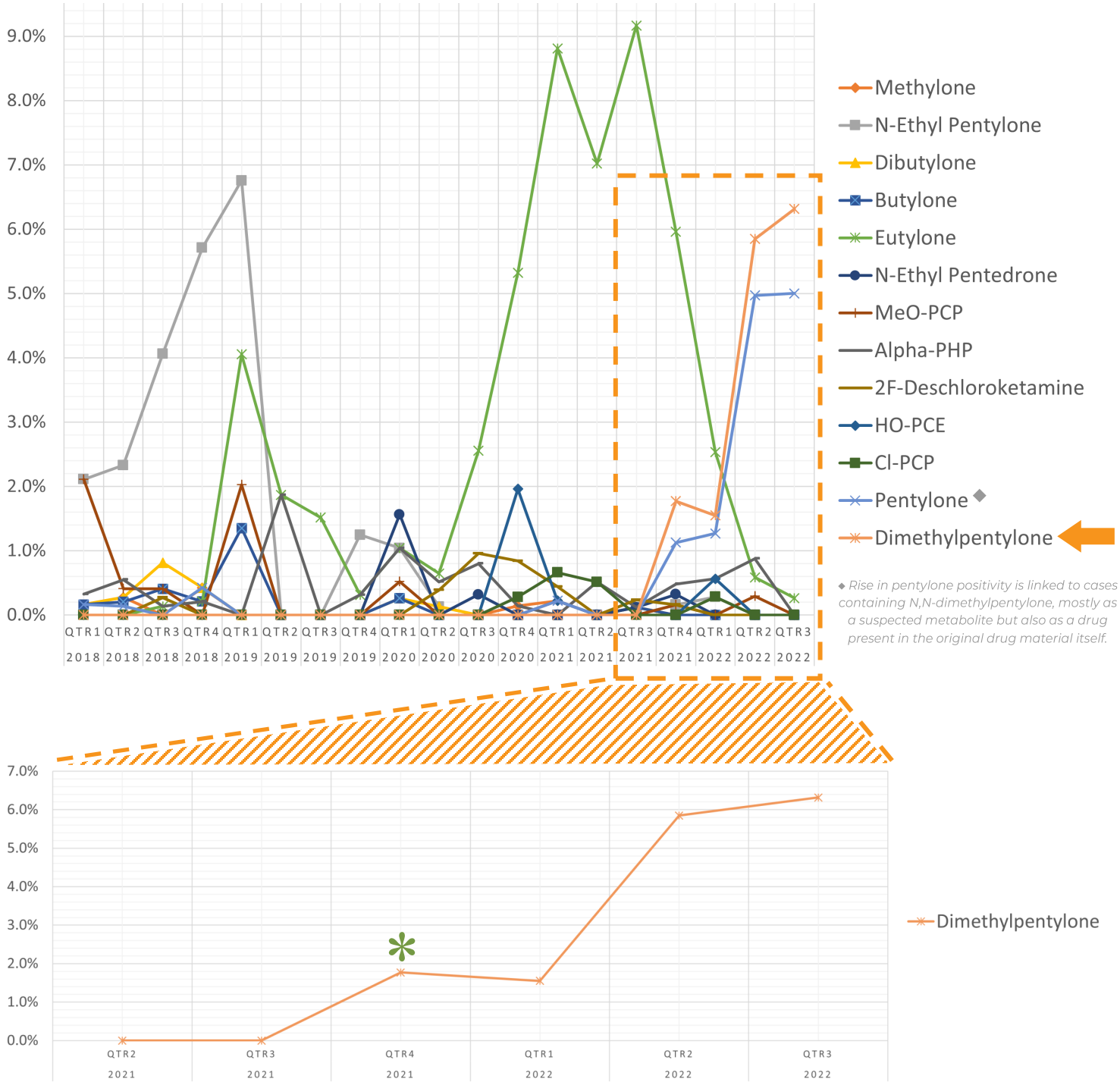
Website: www.npsdiscovery.org **Email:** npsdiscovery@cfsre.org

Trend Plots: NPS Stimulants

NEW

N,N-DIMETHYLPENTYLONE — NPS STIMULANT

NPS Stimulant Positivity in the United States



* *N,N-Dimethylpentylone discovered in the U.S. in November 2021*

Note: *Data generated by NPS Discovery at the CFSRE. Percent positivity (%) calculated by samples analyzed per quarter.*

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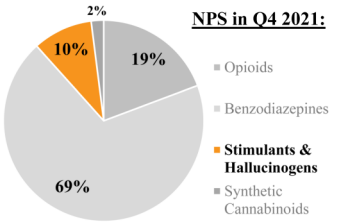
Trend Report: Q4 2021

NPS Stimulants & Hallucinogens in the United States

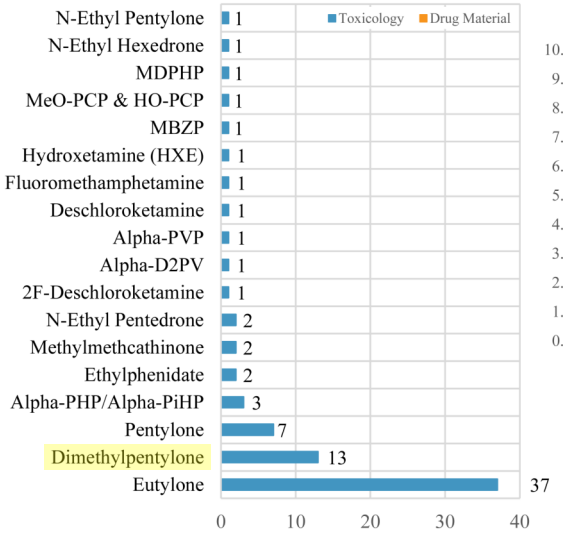
Purpose: This report provides up-to-date information regarding NPS stimulant & NPS hallucinogen prevalence and positivity within the United States.

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 intoxication 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(s).

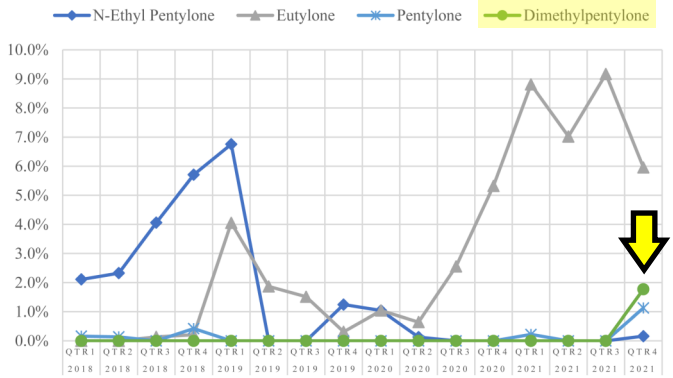
Objective: Our laboratory utilizes novel approaches for the analysis of drugs in biological samples and seized materials using comprehensive non-targeted data acquisition by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). 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 emerging stimulants and hallucinogens, 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.



NPS STIMULANTS & HALLUCINOGENS IDENTIFIED



SELECT POSITIVITY: Q1 2018 to Q4 2021



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Trend Report: Q1 2022

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 intoxication 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(s).

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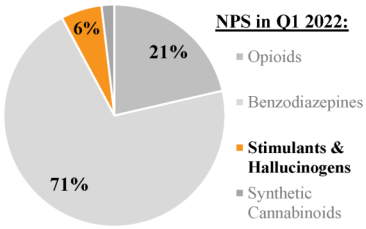
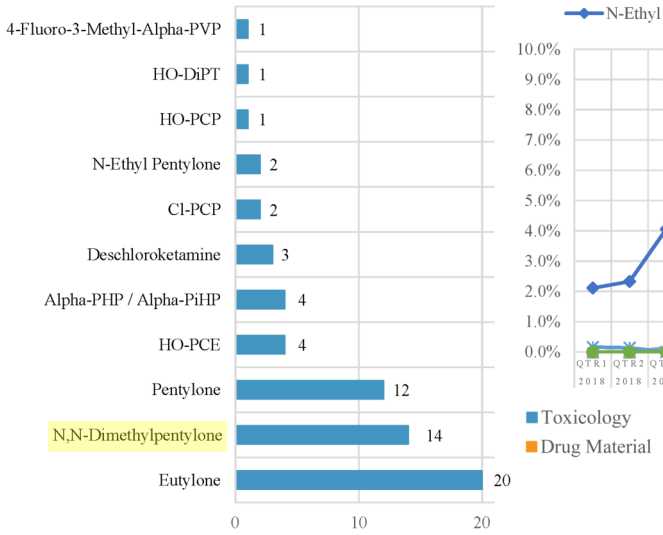


NPS STIMULANTS & HALLUCINOGENS IDENTIFIED



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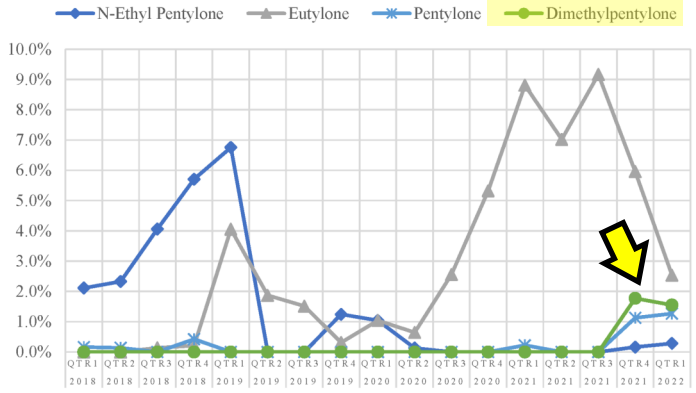

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Trend Report: Q2 2022

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 intoxication 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(s).

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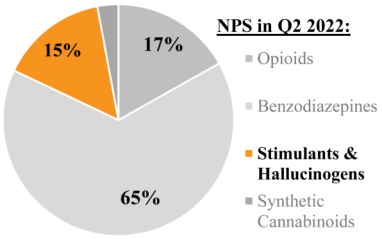
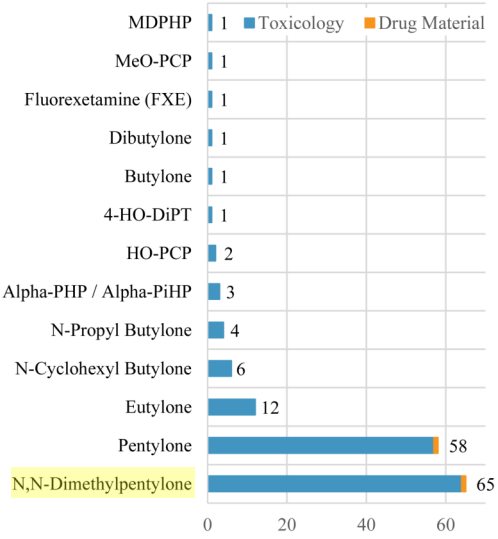
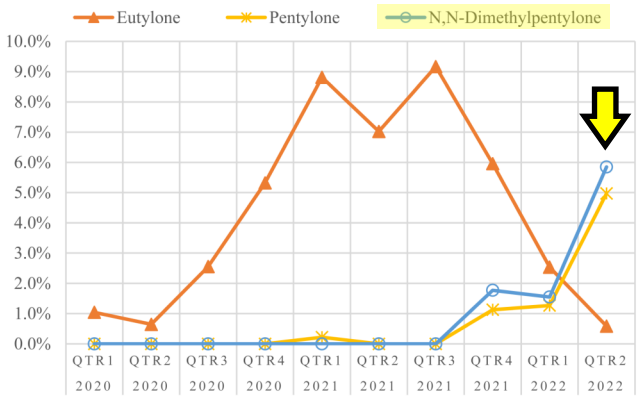


NPS STIMULANTS & HALLUCINOGENS IDENTIFIED



SELECT POSITIVITY: Q1 2018 to Q2 2022



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NPS Stimulant & Hallucinogens in the United States

TREND REPORT | Q3 2022

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 intoxication 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(s).

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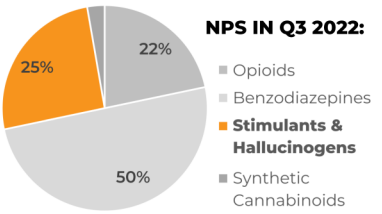
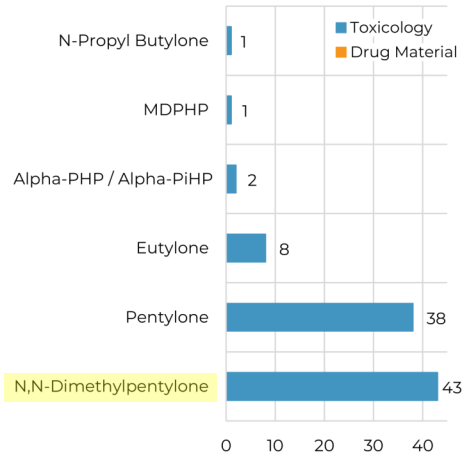
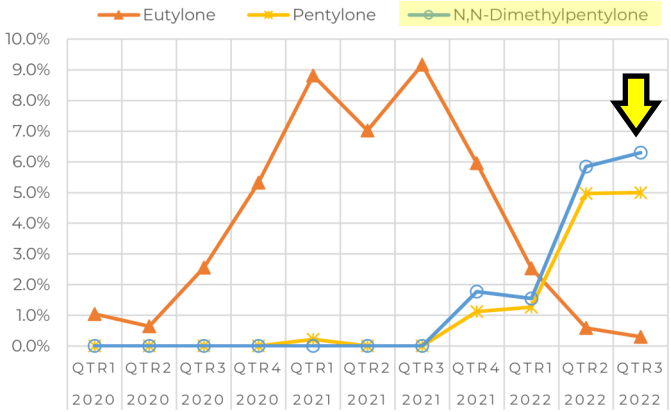


NPS STIMULANTS & HALLUCINOGENS IDENTIFIED



SELECT POSITIVITY: Q1 2020 to Q3 2022



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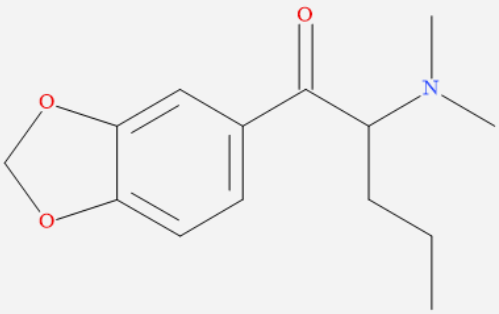
New Drug Monograph

N,N-DIMETHYLPENTYLONE — NPS STIMULANT

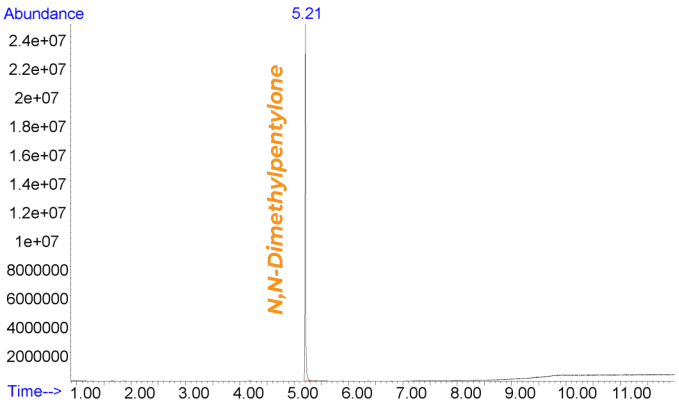
Reference: Information & data figures sourced from ***N,N*-Dimethylpentylone: New Drug Monograph** issued Dec. 17, 2021, by the CFSRE.

N,N-Dimethylpentylone

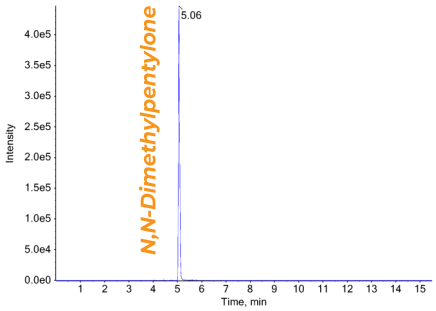


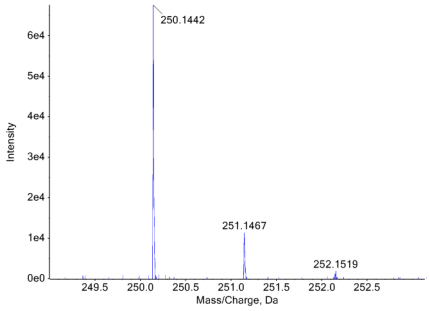
Sample Type	Toxicology Sample
Date Received	November 1, 2021
IUPAC Name	1-(1,3-benzodioxol-5-yl)-2-(dimethylamino) pentan-1-one
CFR	Not Scheduled* (12/2021)
CAS#	17763-13-2
Source	NMS Labs – Toxicology Department
Chemical Formula	$C_{14}H_{19}NO_3$
Molecular Weight	249.3
Molecular Ion [M+]	249
Exact Mass [M+H]+	250.1438

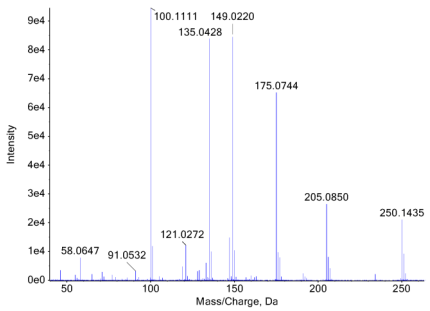
GC-EI-MS DATA



LC-QTOF-MS DATA







cfsre NPS DISCOVERY

Analytical Methods

NEW

N,N-DIMETHYLPENTYLONE — NPS STIMULANT

Purpose: This section provides an analytical method for the analysis of *N,N*-dimethylpentylone. This instrumental approach provides a starting point for laboratories looking to development methods for this new drug, ultimately saving valuable time and resources. In addition, mass spectrometer setpoints could be used to initiate ion monitoring or novel surveillance prior to availability of reference material in the laboratory.

Waters™ Corporation (Milford, MA)
Liquid Chromatograph: ACQUITY UPLC I-Class
Mass Spectrometer: Xevo TQ-S micro QQQ-MS

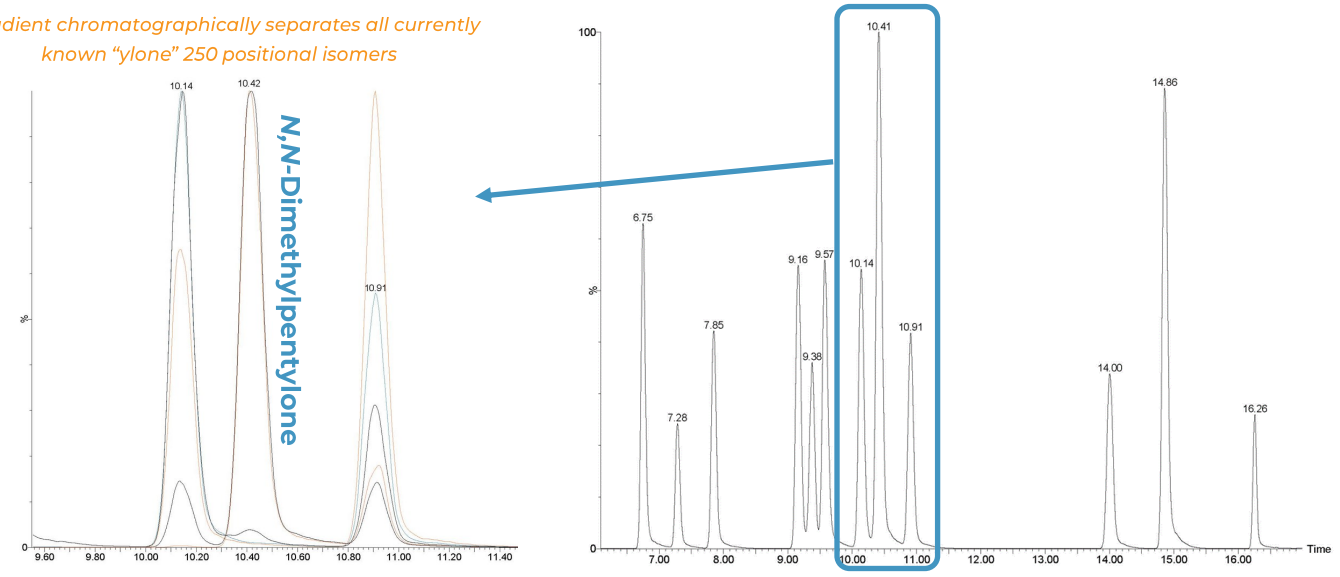
Liquid Chromatograph Parameters	
Column	Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 150 mm, 2.7 µm)
Column Temp.	60 °C
Mobile Phase A	5 mM Ammonium Formate in Water, pH 3
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Flow Rate	0.4 mL/min
Gradient	Initial: 90:10 A:B
	0.5 min: 90:10 A:B
	12 min: 80:20 A:B
	15 min: 70:30 A:B
	15.1 min: 5:95 A:B
	15.75 min: 5:95 A:B
	16 min: 90:10 A:B
	17 min: 90:10 A:B

Mass Spectrometer Parameters	
Capillary	2.5 kV
Desolvation Temp.	600 °C
Desolvation Flow	800 L/hr
Cone Flow	60 L/hr
Source Temp.	150 °C

Multiple Reaction Monitoring (MRM) Transitions			
Cone	**MS1**	**MS2**	**Collison**
24	250.1	175.1*	16
		100.1	22
		205.1	14

*Suggested Quantitation/Target Ion

**Gradient chromatographically separates all currently known "ylone" 250 positional isomers*



NEW

Analytical Methods

N,N-DIMETHYLPENTYLONE — NPS STIMULANT

Purpose: Ninety-one authentic forensic postmortem cases were analyzed via LC-QTOF-MS and LC-QQQ-MS to determine quantitative concentrations of ***N,N*-dimethylpentylone** in biological specimens and poly-drug co-occurrence among these medicolegal death investigations.

Poly-Drug Co-Occurrence	
Pentylone	80 (87%)
Additional "ylones" (e.g., Butylone, Eutylone, N-Propyl Butylone)	68 (74%)
Fentanyl	54 (59%)
Traditional Stimulants (*e.g., Methamphetamine, Cocaine*)	53 (58%)
Other Opioids (*e.g., Morphine, Methadone, Tramadol*)	44 (48%)
Only Stimulant	22 (24%)
Benzodiazepines (*e.g., Diazepam, Alprazolam, Flualprazolam*)	20 (21%)

Quantitative Concentrations* (ng/mL)		
Matrix ▸	Blood (n=91)	Urine (n=5)
Mean	275	1,970
Std. Dev.	335	1,809
Median	160	1,515
Min.	3.3	250
Max.	2,200	4,600

**Excluding outliers in blood (4,600 ng/mL) and urine (16,200 ng/mL)*

Purpose: This section provides an example sample preparation workflow for the preparation and extraction of ***N,N*-dimethylpentylone** from biological specimens. This approach provides a starting point for laboratories looking to assess extraction methods for this new drug, ultimately saving valuable time and resources. The extraction method could serve useful for screening or confirmation, whether quantitate or qualitative.

LLE Assessment (Blood)	
Recovery	97%
Matrix Effects	96%
Process Efficiency	94%

Calculations (Using Peak Area Ratio)

Recovery: (Pre-spike / Post-Spike) x 100
Matrix Effects: (Post-spike / Unextracted) x 100
Process Efficiency: (Pre-spike / Unextracted) x 100

Liquid-Liquid Extraction (LLE)

1. Aliquot 0.5 mL of sample (e.g., blood, urine)
2. Add internal standard (e.g., *N*-Ethyl Pentylone-D5)
3. Add 1 mL Borax buffer (pH 10.4), vortex
4. Add 3 mL *N*-butyl chloride & ethyl acetate (70:30, *v:v*)
5. Cap and rotate for 15 mins
6. Centrifuge 4600 rpm for 10 mins
7. Transfer supernatant (e.g., freeze-pour)
8. Add 100 µL of 10% HCl in MeOH, vortex
9. Evaporate to dryness at 35 °C (10 psi)
10. Reconstitute for LC-QQQ-MS analysis
11. Transfer to autosampler vials

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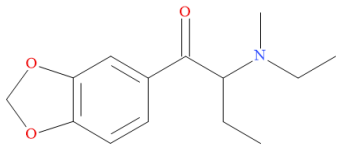
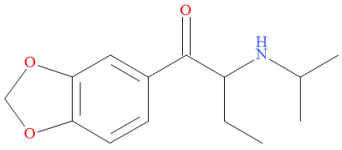
Isomeric Issues

NEW

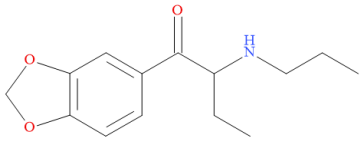
N,N-DIMETHYLPENTYLONE — NPS STIMULANT



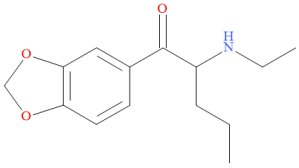
N-Ethyl *N*-Methyl Butylone



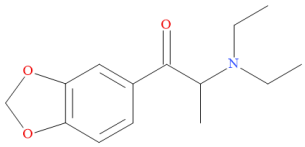
N-Isopropyl Butylone



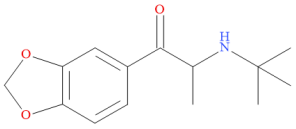
N-Propyl Butylone



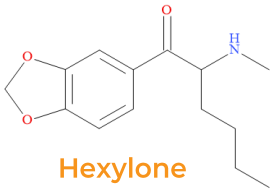
N-Ethyl Pentylone



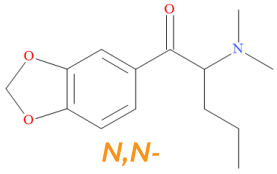
Diethylone



Tertylone



Hexylone



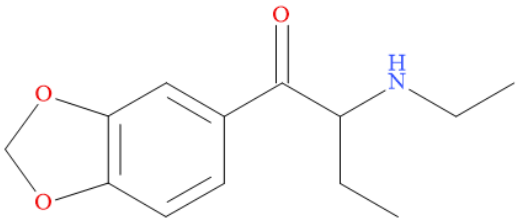
N,N-Dimethylpentylone

250 *m/z*

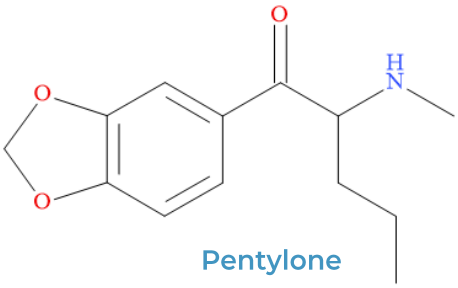
236 *m/z*

276 *m/z*

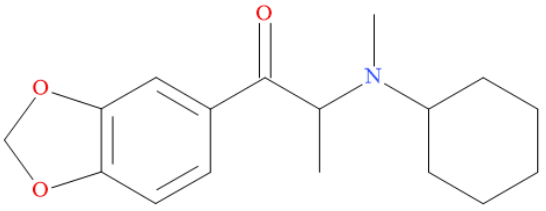
290 *m/z*



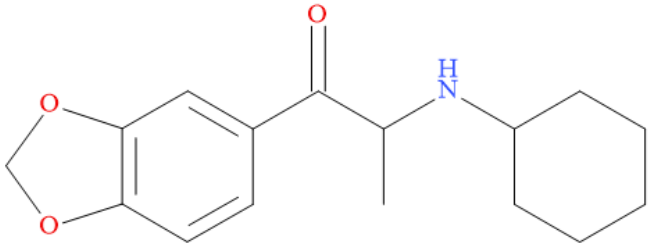
Eutylone



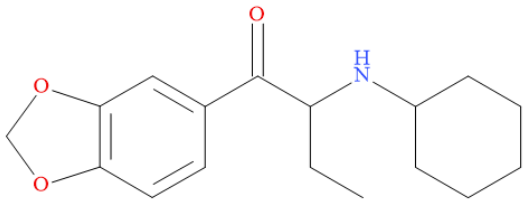
Pentylone



N-Methyl *N*-Cyclohexyl Methylone



N-Cyclohexyl Methylone



N-Cyclohexyl Butylone

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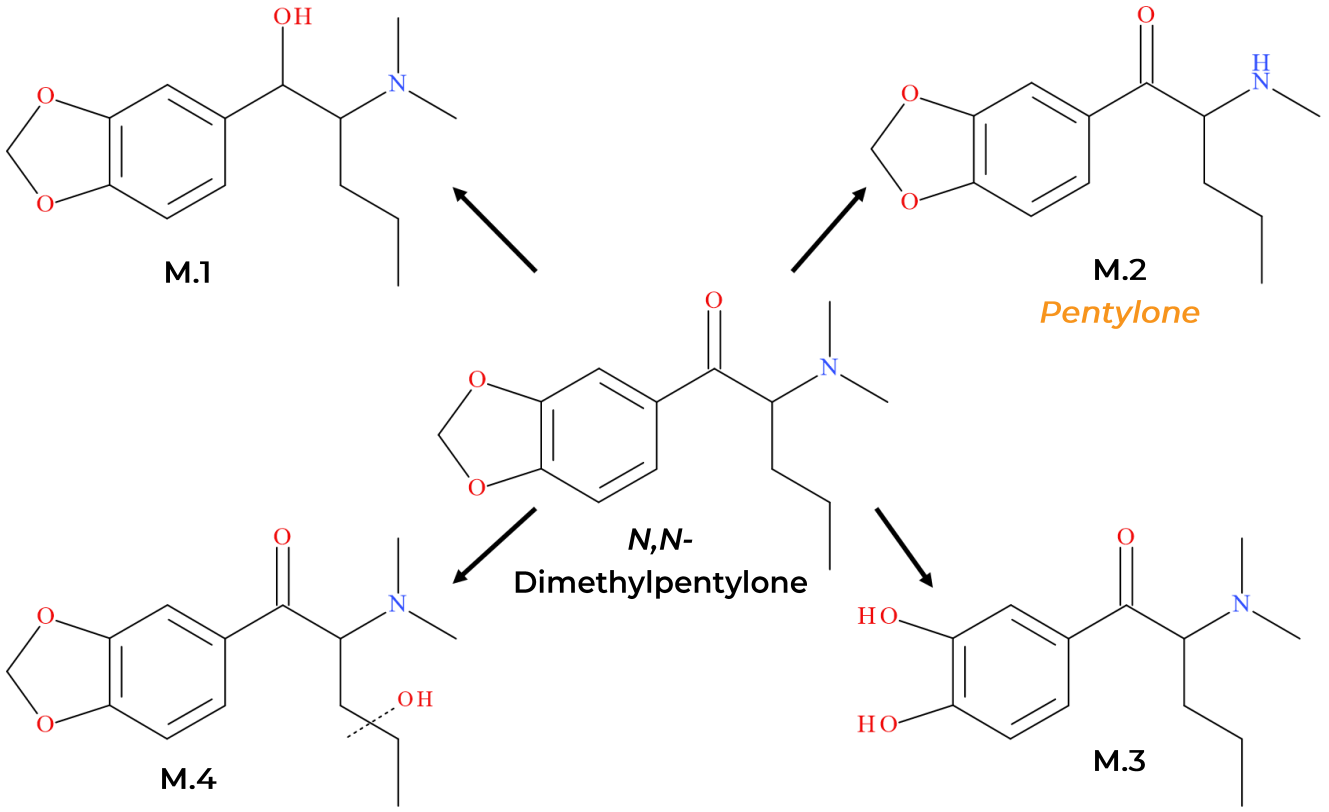
Suspected Metabolism

NEW

N,N-DIMETHYLPENTYLONE — NPS STIMULANT

Purpose: The primary metabolites of *N,N*-dimethylpentylone were evaluated through *in vivo* experiments using human liver microsomes. Analysis was performed using a SCIEX TripleTOF® 5600+ LC-QTOF-MS (Framingham, MA) and data was processed using SCIEX MetabolitePilot™ (Version 2.0).



ID	Biotransformation	Formula	Exact [M+H]⁺	Diagnostic Product Ions
P.0	*N,N*-Dimethylpentylone	$C_{14}H_{19}NO_3$	250.1438	149.0239 135.0446
M.1	Hydrogenation	$C_{14}H_{21}NO_3$	252.1594	149.0239 135.0446
M.2	*N*-Deethylation (**Pentylone**)	$C_{13}H_{17}NO_3$	236.1281	188.1073 175.0647
M.3	Demethylenation	$C_{13}H_{19}NO_3$	238.1438	137.0239 121.0290
M.4	Hydroxylation	$C_{14}H_{20}NO_4$	267.1465	149.0239 135.0446

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NPS DISCOVERY