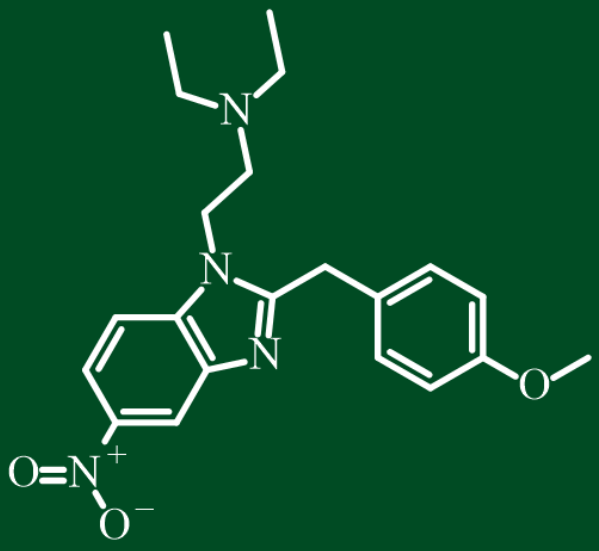
NPS DISCOVERY

NPS Discovery Toolkit

» Metonitazene



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JULY • 2021

Acknowledgements: This report was prepared by Alex J. Krotulski, PhD; Sara E. Walton, BS; 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 (NIJ) of the U.S. Department of Justice (DOJ) (Award Number 2020-DQ-BX-0007). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

Recommended Citation: NPS Discovery (2021), NPS Discovery Toolkit: Metonitazene, Center for Forensic Science Research and Education, United States of America.

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NPS DISCOVERY

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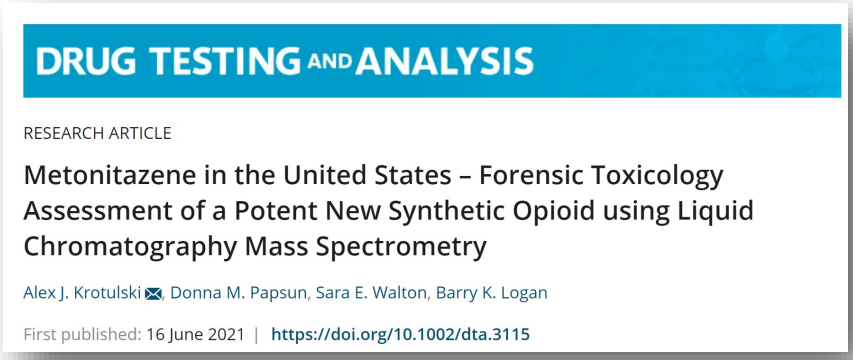

DRUG TESTING AND ANALYSIS

RESEARCH ARTICLE

Metonitazene in the United States – Forensic Toxicology Assessment of a Potent New Synthetic Opioid using Liquid Chromatography Mass Spectrometry

Alex J. Krotulski, Donna M. Papsun, Sara E. Walton, Barry K. Logan

First published: 16 June 2021 | https://doi.org/10.1002/dta.3115

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.

January 2021

Metonitazene Begins Proliferation as Newest Synthetic Opioid Among Latest Cycle of Non-Fentanyl Related Drugs

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 opioid **metonitazene**.

Background: Synthetic opioids are chemically manufactured drugs, often accompanied with unknown potency and adverse effects or health risks. New synthetic opioids may be mixed with more traditional opioids, creating additional risk and danger for recreational drug users. Synthetic opioids may be distributed in powder or tablet form. In the United States (U.S.), an alarming increase in the number of deaths linked to synthetic opioid use has been reported. The primary adverse effect associated with synthetic opioid use is respiratory depression, often leading to death.

Summary: Metonitazene is a potent synthetic opioid bearing structural resemblance to etonitazene, a synthetic opioid that is nationally and internationally controlled. Metonitazene is dissimilar in structure to other synthetic opioids typically encountered in forensic casework (e.g. fentanyl analogues). Metonitazene and similar analogues (e.g. etonitazene, isotonitazene) were first synthesized and reported in the literature in the 1950s. Pharmacological data suggest that this group of synthetic opioids have potency similar to or greater than fentanyl. Metonitazene was first reported by **NPS Discovery** after detection in a seized drug powder in July 2020. To date, metonitazene has been identified in eight blood specimens associated with postmortem death investigations in the U.S. The appearance of metonitazene and its increasing occurrence appears to be linked to recent drug scheduling actions for **isotonitazene** (June 2020) and **brorphine** (December 2020), which are now both past peak positivity based on examination of comprehensive toxicology data. The toxicity of metonitazene has not been extensively studied but recent association with drug user death leads professionals to believe this new synthetic opioid retains the potential to cause widespread harm and is of public health concern. Identifications of metonitazene have also been reported out of Europe.

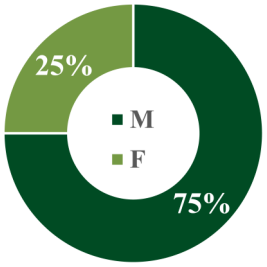
Demographics

Case Type:
- Postmortem (n=8)

Age:
- Range: 30s to 50s

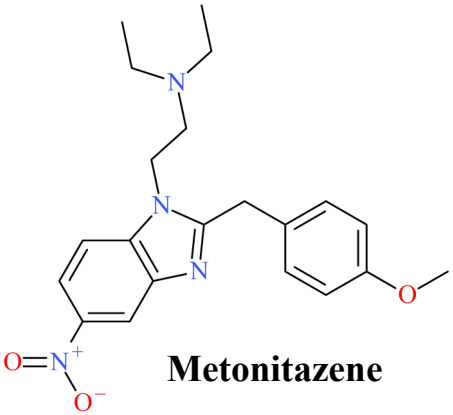
Date of Collection:
- Aug. to Dec. 2020

Other Notable Findings:
- Fentanyl (n=6)
- Cocaine (n=4)
- Methamphetamine (n=4)



Recommendations for Public Health

- Implement surveillance for rapid identification of drug 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 decedents and overdose patients.
- Raise awareness about the risks and dangers associated with opioid use.
- Make naloxone available to recreational drug users.



Metonitazene

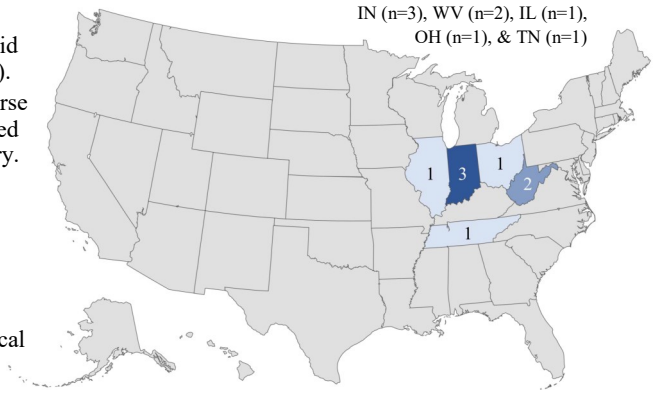
Recommendations for Clinicians

- Become familiar with the signs and symptoms associated with synthetic opioid use (e.g. sedation, respiratory depression).
- Naloxone should be administered to reverse critical respiratory depression and repeated naloxone administration may be necessary. Be aware that clinical conditions may change rapidly and unpredictably after naloxone administration due to precipitation of withdrawal.
- 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 opioid products and other drugs.

Recommendations for MEs & Coroners

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

Geographical Distribution of Metonitazene Positivity



Recommendations for Laboratories

- Utilize analytical data available publicly for the identification of **metonitazene** and synthetic opioids if reference standards are not available.
- Utilize previously developed non-targeted testing protocols or develop sensitive and up-to-date testing procedures for synthetic opioids.
- Prioritize analytical testing of seized drug samples obtained from drug overdose scenes during death investigations.
- Share data on synthetic opioid drug seizures with local health departments, medical examiners and coroners, and related communities.

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References and Related Articles:

• Hunger, A; Kebrle, J; Rossi, A; Hoffmann, K. (1957) Synthesis of analgesically active benzimidazole derivatives with basic substitutions. *Experientia*, **13**, 400-401.

• Hoffmann, K; Hunger, A; Rossi, A. (3 May 1960). Patent US2935514A – Benzimidazoles.

• Vandeputte *et al.* (2020) Synthesis, chemical characterization,

Rapid NPS Testing Now Available:

If your agency suspects synthetic opioid 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.

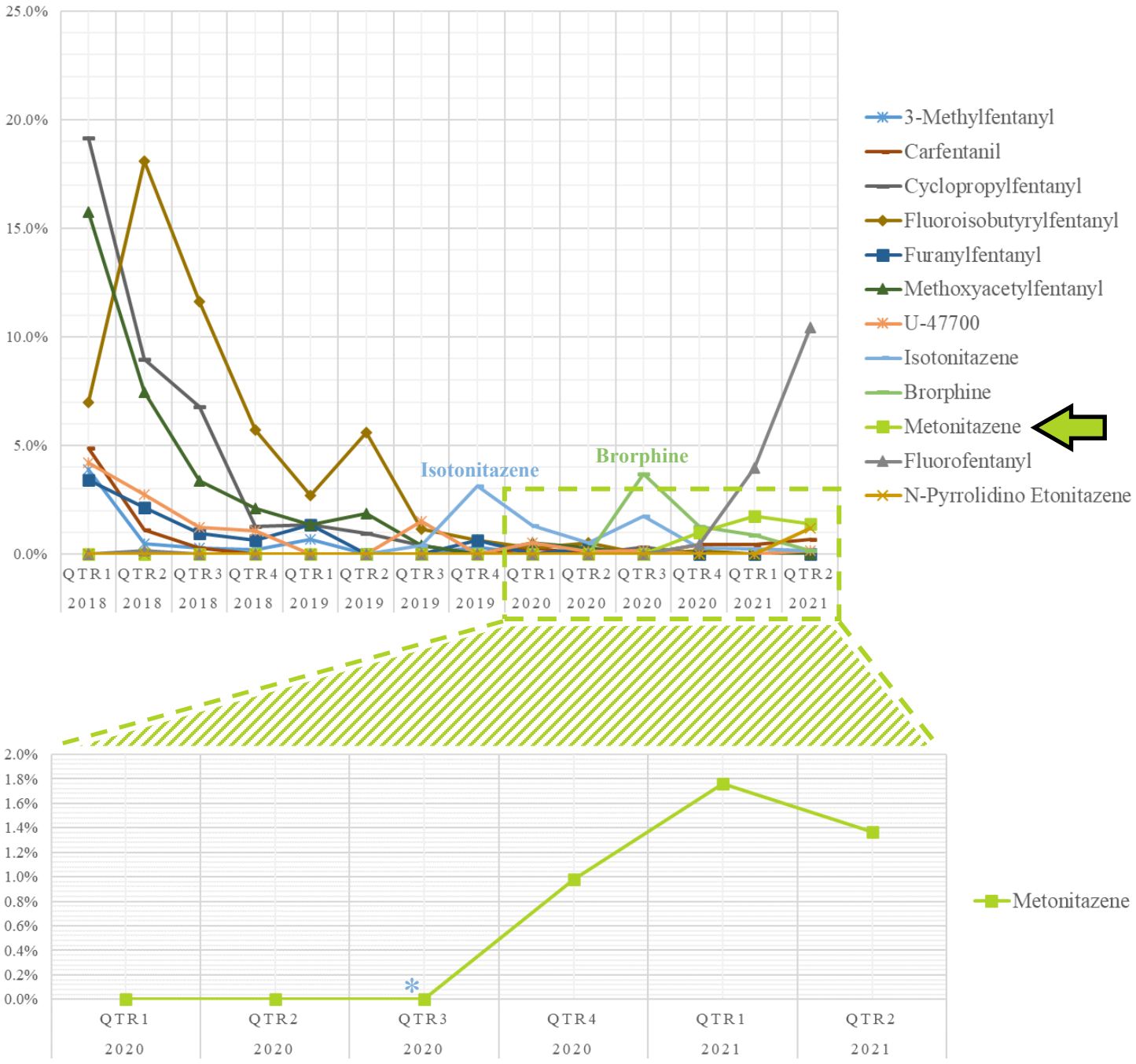
Website: www.npsdiscovery.org **Email:** npsdiscovery@cfsre.org

Trend Plots: NPS Opioids

NEW

METONITAZENE — NPS OPIOID

NPS Opioid Positivity in the United States



* *Metonitazene discovered in the U.S. in July 2020*

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

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NPS DISCOVERY

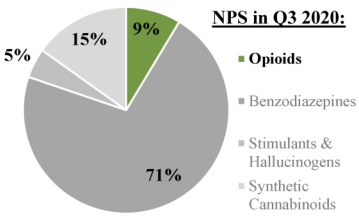
Trend Report: Q3 2020

NPS Opioids in the United States

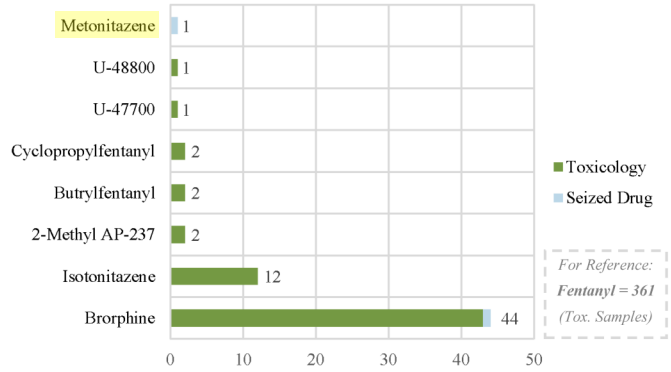
Purpose: This report provides up-to-date information regarding the status of NPS opioid prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including NPS opioids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS opioids have been implicated in an increasing number of emergency room admissions, death investigations, and mass intoxication events, and often appear in combination with other illicit opioids (e.g. fentanyl, heroin). 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 800 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of novel opioids 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 CFSRE during this quarter, including those from sample-mining, data-mining, and/or esoteric testing.

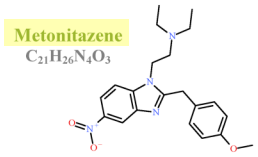


NPS Opioid Positivity



NPS Opioid Combinations

Combination	Frequency
Brorphine + Fentanyl	39
Brorphine + Flualprazolam	39
Brorphine + Stimulant *(Cocaine and/or Methamphetamine)*	18
Brorphine + Tramadol	9
Brorphine + Isotonitazene	6



Analytical Testing and Drug Intelligence Indicate the Appearance of New "Nitazene" Analogues

Acknowledgements: This report was prepared by Alex J. Krotulski, PhD; 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 staff and 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.

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NPS DISCOVERY

Trend Report: Q4 2020

NPS Opioids in the United States

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

Overview: Novel psychoactive substances (NPS), including NPS opioids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS opioids have been implicated in an increasing number of emergency room admissions, death investigations, and mass intoxication events, and often appear in combination with other illicit opioids (e.g. fentanyl, heroin). Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

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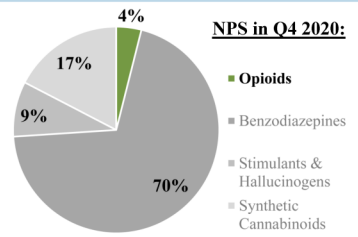
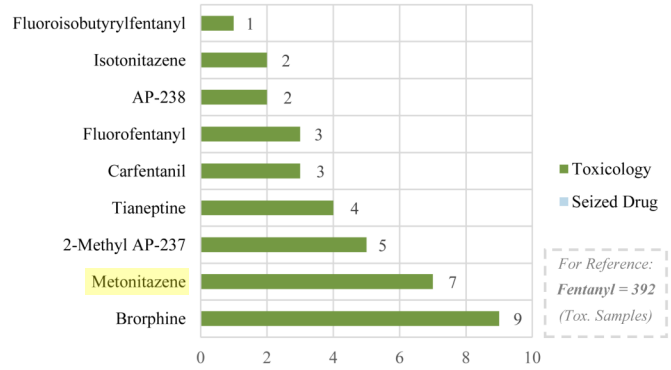


NPS Opioid Positivity

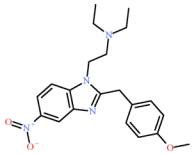


NPS Opioid Combinations

Combination	Frequency
Brorphine + Fentanyl	8
Brorphine + Flualprazolam	8
Brorphine + Stimulant(s) *(Cocaine and/or Methamphetamine)*	7
Metonitazene + Fentanyl	6
2-Methyl AP-237 + Etizolam	4

New Opioid On The Rise:

Metonitazene →



Metonitazene was first identified in July 2020 and increased to seven identifications in Q4 2020.

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.

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NPS DISCOVERY

Trend Report: Q1 2021

NPS Opioids in the United States

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

Overview: Novel psychoactive substances (NPS), including NPS opioids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS opioids have been implicated in an increasing number of emergency room admissions, death investigations, and mass intoxication events, and often appear in combination with other illicit opioids (e.g. fentanyl, heroin). Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

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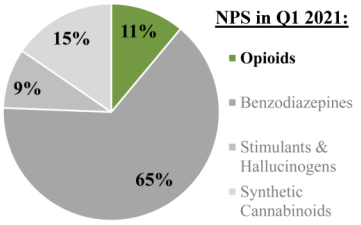
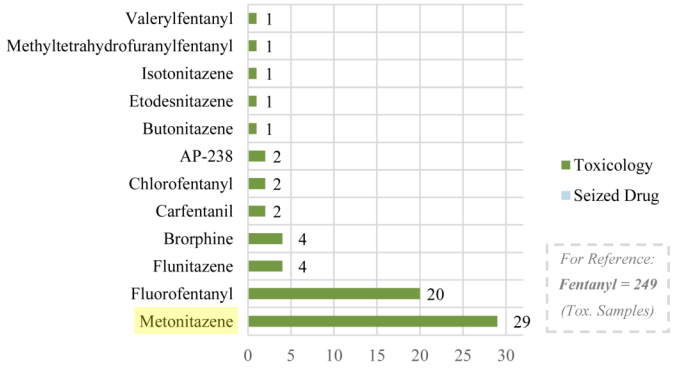


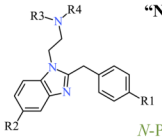
NPS Opioid Positivity



NPS Opioid Combinations

Combination	Frequency
Fluorofentanyl + Fentanyl	18
Fluorofentanyl + NPS Benzodiazepine(s) *(e.g., Etizolam, Flualprazolam)*	16
Metonitazene + NPS Benzodiazepine(s) *(e.g., Clonazolam, Flualprazolam)*	13
Metonitazene + Fentanyl	12
Metonitazene + Flunitazene	4

Continued Emergence of New Opioid "Nitazene" Analogues



"Nitazene" Analogues Reported:
Isotonitazene: 11/19/2019
Metonitazene: 07/30/2020
Butonitazene: 01/15/2021
Etodesnitazene: 02/23/2021
Flunitazene: 03/26/2021
N-Pyrrolidino Etonitazene *(Pending)*

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NPS DISCOVERY

Trend Report: Q2 2021

NPS Opioids in the United States

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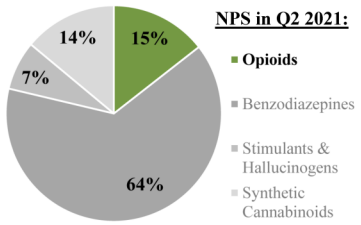
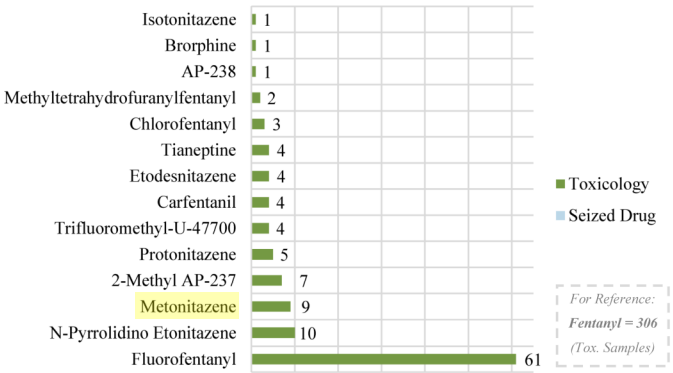


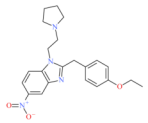
NPS Opioid Positivity



NPS Opioid Combinations

Combination	Frequency
Fluorofentanyl + Fentanyl	59
Fluorofentanyl + NPS Benzodiazepine(s) *(e.g., Etizolam, Flualprazolam)*	46
N-Pyrrolidino Etonitazene + NPS Benzodiazepine(s) *(e.g., Flualprazolam, Etizolam, Clonazolam)*	6
N-Pyrrolidino Etonitazene + Fentanyl	4
2-Methyl AP-237 + Etizolam	4

NPS Discovery Issues a ***Public Alert*** for **N-Pyrrolidino Etonitazene** as Identifications Increase Across U.S. →



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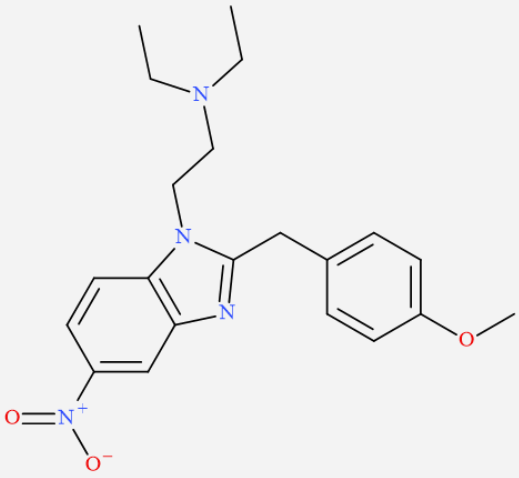
NPS DISCOVERY

New Drug Monograph

METONITAZENE — NPS OPIOID

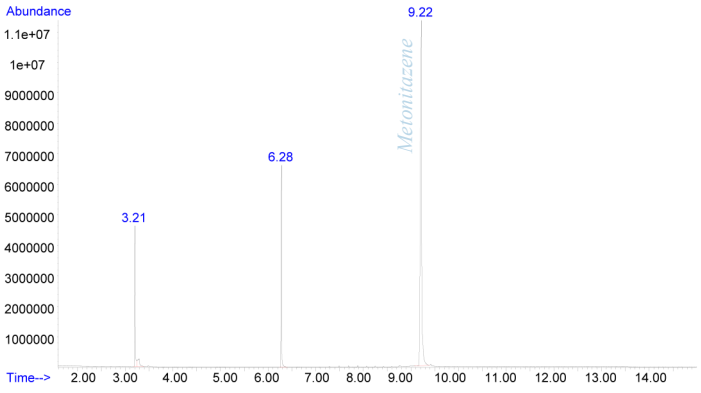
Reference: Information and data figures sourced from **Metonitazene: New Drug Monograph** issued July 30, 2020, by the CFSRE / NMS Labs.

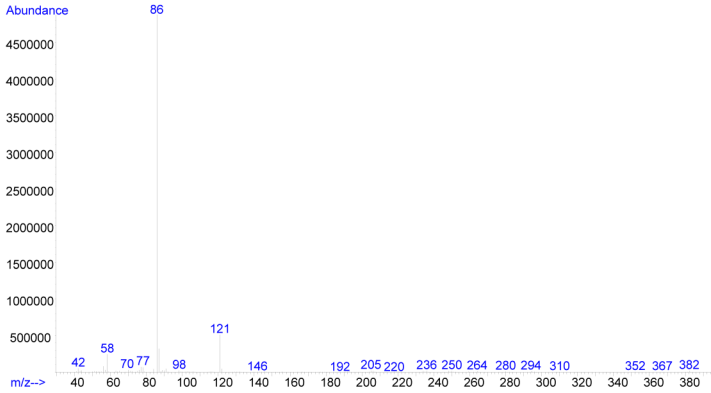
METONITAZENE



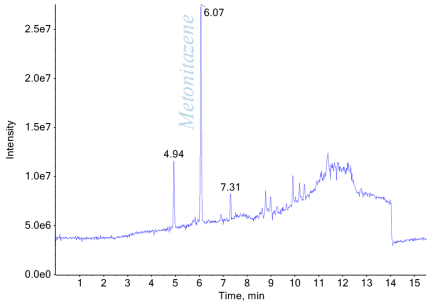
Sample Type	Seized Material
Date Received	July 7, 2020
IUPAC Name	N,N-diethyl-2-[2-[(4-methoxyphenyl)methyl]-5-nitro-benzimidazol-1-yl]ethanamine
CFR	Not Scheduled (07/2021)
CAS#	14680-51-4
Source	NMS Labs – Criminalistic Laboratory
Appearance	White Solid Material
Chemical Formula	$C_{21}H_{26}N_4O_3$
Molecular Weight	382.5
Molecular Ion [M+]	382
Exact Mass [M+H]+	383.2078

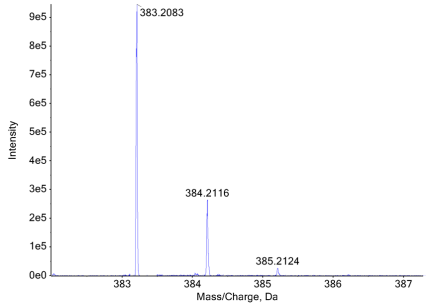
GC-EI-MS DATA

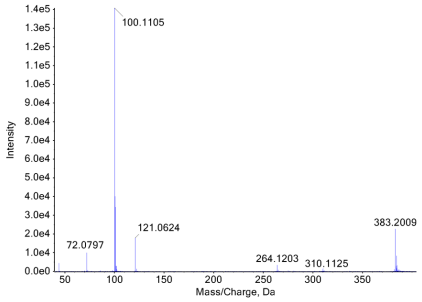




LC-QTOF-MS DATA







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NPS DISCOVERY

Analytical Methods

NEW

METONITAZENE — NPS OPIOID

Purpose: This section provides example analytical methods for the analysis of **metonitazene**. These two instrumental approaches provide 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.

Agilent Technologies (Santa Clara, CA)
Liquid Chromatograph: 1290 UHPLC
Mass Spectrometer: 6495 QQQ-MS

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

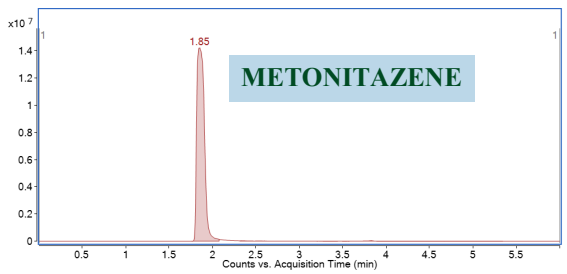
Liquid Chromatograph Parameters	
Column	Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7 µm)
Column Temp.	50 °C
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Flow Rate	0.4 mL/min
Gradient	Initial: 50:50 A:B
	1 min: 50:50 A:B
	4 min: 95:5 A:B
	5 min: 95:5 A:B
	5.1 min: 50:50 A:B
	6 min: 50:50 A:B

Liquid Chromatograph Parameters	
Column	Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 100 mm, 2.7 µm)
Column Temp.	30 °C
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Methanol
Flow Rate	0.4 mL/min
Gradient	Initial: 60:40 A:B
	1 min: 60:40 A:B
	2 min: 70:30 A:B
	5.5 min: 40:60 A:B
	6 min: 60:40 A:B
	7 min: 60:40 A:B

Mass Spectrometer Parameters	
Gas Temp.	250 °C
Gas Flow	16 L/min
Nebulizer	40 psi
Capillary	3,000 V
Nozzle	1,500 V
Sheath Gas Temp.	400 °C
Sheath Gas Flow	12 L/min

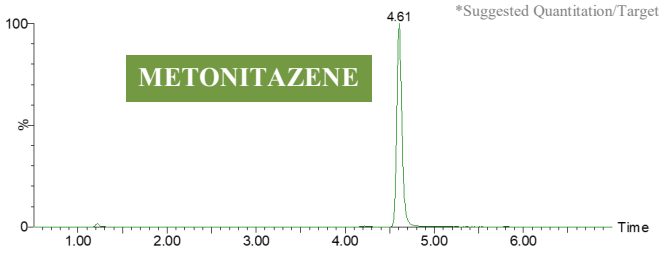
Mass Spectrometer Parameters	
Capillary	2.5 kV
Desolvation Temp.	600 °C
Desolvation Flow	800 L/hr
Cone Flow	60 L/hr
Source Temp.	150 °C

Multiple Reaction Monitoring (MRM) Transitions			
Fragmentor	**MS1**	**MS2**	**Collison**
380	383.2	72.0	20
		100.1*	30
		221.1	30



Multiple Reaction Monitoring (MRM) Transitions			
Cone	**MS1**	**MS2**	**Collison**
48	383.2	72.0	20
		100.0*	22
		221.0	34

*Suggested Quantitation/Target Ion



NEW

Analytical Methods

METONITAZENE — NPS OPIOID

Purpose: Twenty authentic forensic postmortem cases were analysis via LC-QTOF-MS and LC-QQQ-MS to determine quantitative concentrations of metonitazene in biological specimens and poly-drug co-occurrence among these medicolegal death investigations.

Reference: Krotulski AJ, Papsun DM, Walton SE, Logan BK. Metonitazene in the United States – Forensic Toxicology Assessment of a Potent New Synthetic Opioid using Liquid Chromatography Mass Spectrometry. *Drug Test Anal*. 2021. https://doi.org/10.1002/dta.3115

Poly-Drug Co-Occurrence	
Fentanyl	11 (55%)
Quinine	11 (55%)
At Least One Additional NPS	9 (45%)
Only Opioid	6 (30%)
Methamphetamine	5 (25%)
No Additional Drug(s)	3 (15%)

Quantitative Concentration (ng/mL)		
Matrix	Blood (n=18)	Urine (n=14)
Mean	6.3	14
Std. Dev.	7.5	13
Median	3.8	11
Min.	<0.5	0.6
Max.	33	46

Purpose: This section provides two example sample preparation workflows for the extraction of **metonitazene** from biological specimens. These preparation approaches provide a starting point for laboratories looking to assess extraction methods for this new drug, ultimately saving valuable time and resources. These extraction method could serve useful for screening or confirmation, whether quantitate or qualitative.

Liquid-Liquid Extraction (LLE)

1. Aliquot 0.5 mL of sample (e.g., blood, urine)
2. Add internal standard (e.g., isotonitazene-D7)
3. Add 1 mL Borax buffer (pH 10.4), vortex
4. Add 3 mL n-butyl chloride and ethyl acetate (70:30, v:v)
5. Cap and rotate for 10 mins
6. Centrifuge 4600 rpm for 15 mins
7. Transfer supernatant (e.g., freeze pour)
8. Evaporate to dryness at 35 °C (10 psi)
9. Reconstitute for LC-QQQ-MS analysis
10. Transfer to autosampler vials

LLE Assessment (Blood)	
Recovery	97%
Matrix Effects	173%
Process Efficiency	168%

Solid-Phase Extraction (SPE)

1. Aliquot 0.5 mL of sample (e.g., blood, urine)
2. Add internal standard (e.g., isotonitazene-D7)
3. Add 3 mL phosphate buffer (0.1 M, pH 6), vortex, and centrifuge at 3000 rpm for 10 mins
4. SPE with UCT Clean Screen® (130 mg, 3 mL)
5. Condition: 3 mL MeOH, 3 mL H_2O, and 1 mL phosphate buffer (0.1 M, pH 6)
6. Transfer samples to cartridges
7. Wash: 3 mL H_2O, 1 mL acetic acid (0.1 M), and 3 mL MeOH, followed by drying for 5 minutes
8. Elute: Twice with 1 mL ethyl acetate, acetonitrile, and ammonium hydroxide (78:20:2, v:v:v)
9. Evaporate to dryness at 40 °C (10 psi)
10. Reconstitute for LC-QQQ-MS analysis
11. Transfer to autosampler vials

SPE Assessment (Blood)	
Recovery	89%
Matrix Effects	97%
Process Efficiency	87%

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

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NPS DISCOVERY

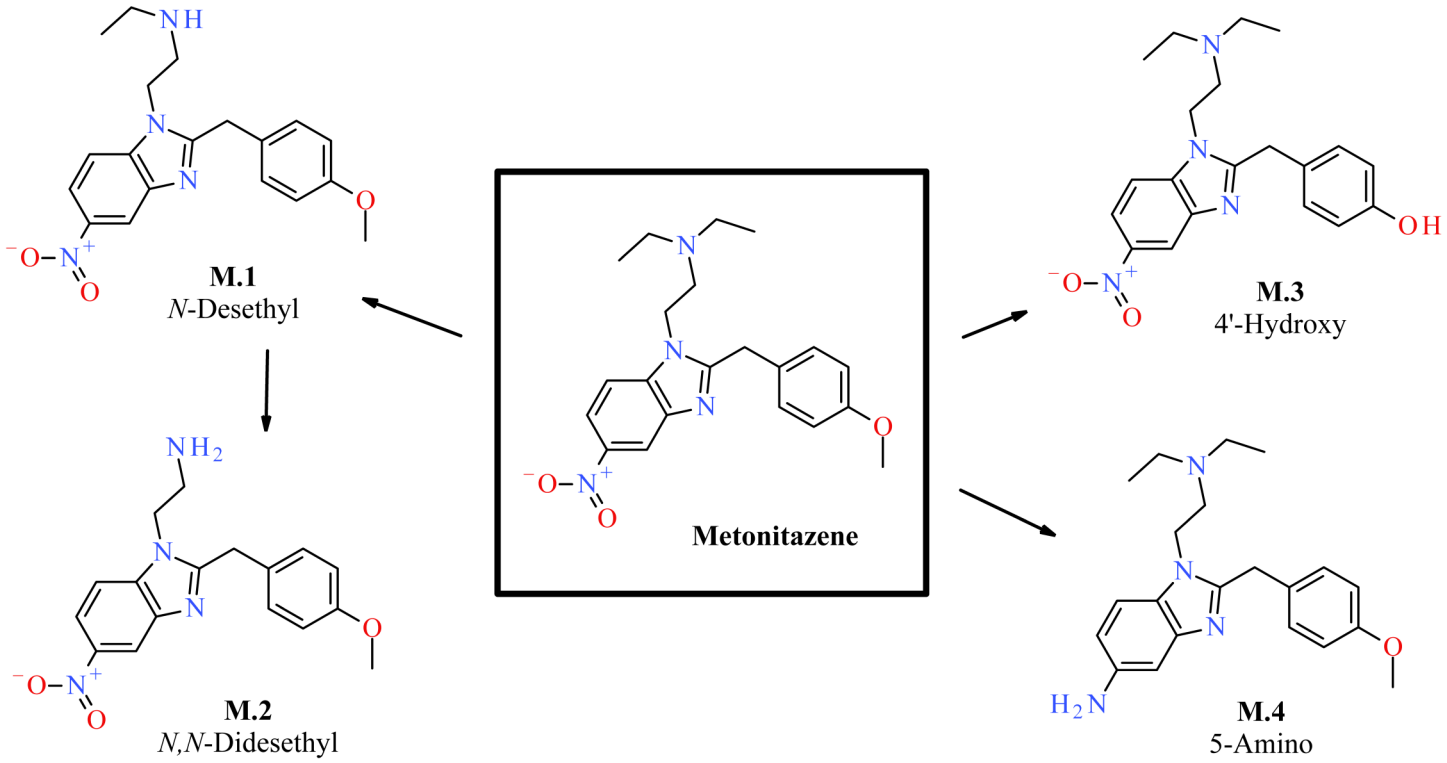
Metabolism

NEW

METONITAZENE — NPS OPIOID

Purpose: The primary metabolites of metonitazene were investigated through *in vivo* experiments. Authentic biological specimens collected after confirmed metonitazene ingestion were examined. Analysis was performed using a SCIEX TripleTOF® 5600+ LC-QTOF-MS (Framingham, MA).

Reference: Krotulski AJ, Papsun DM, Walton SE, Logan BK. Metonitazene in the United States – Forensic Toxicology Assessment of a Potent New Synthetic Opioid using Liquid Chromatography Mass Spectrometry. *Drug Test Anal*. 2021. https://doi.org/10.1002/dta.3115



ID	Biotransformation	Formula	RT (min)	Exact $[M+H]^+$	Measured $[M+H]^+$	Mass Error (ppm)	Diagnostic Product Ions
P.0	Metonitazene	$C_{21}H_{26}N_4O_3$	6.12	383.2078	383.2077	-0.2	100.1121 72.0808
M.1	*N*-Dealkylation	$C_{19}H_{22}N_4O_3$	6.00	355.1765	355.1765	0.2	284.1030 72.0808
M.2	*N,N*-Didealkylation	$C_{17}H_{18}N_4O_3$	5.84	327.1452	327.1447	-1.5	284.1030 44.0500
M.3	*O*-Dealkylation	$C_{20}H_{24}N_4O_3$	5.21	369.1921	369.1925	1.0	100.1121 107.0497
M.4	Nitro Reduction	$C_{21}H_{28}N_4O$	3.53	353.2336	355.2336	-0.7	100.1121 72.0808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