The Newest Wave of Nitazene Analogues

What's Trending: NPS Discovery Webinar Series – Friday July 7, 2023

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Center for Forensic Science Research and Education

Disclosures

- 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.
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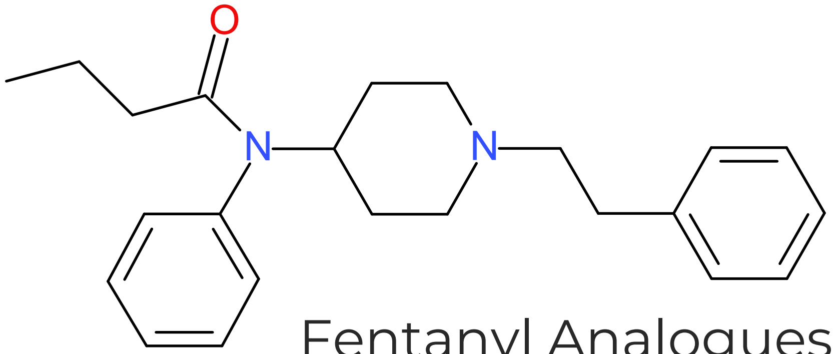
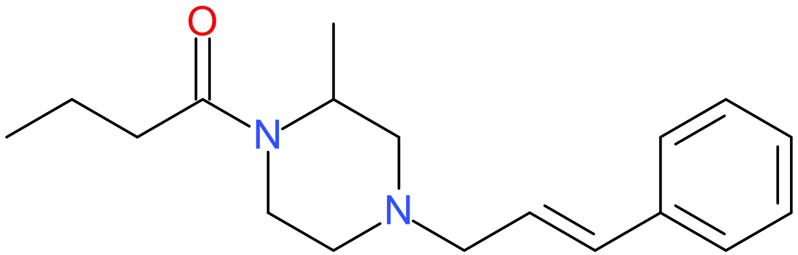
History

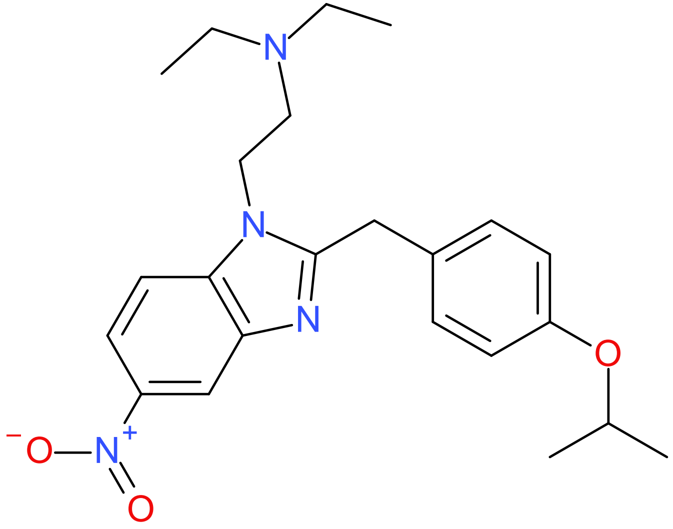
Types of Novel Synthetic Opioids



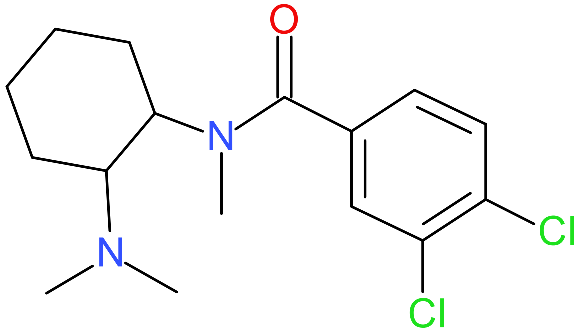
Fentanyl Analogues



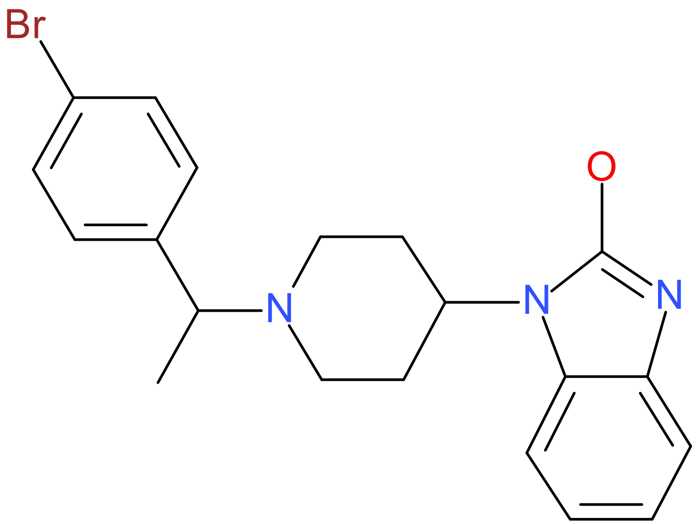
Cyclohexylbenzamides
(AP-Series)



2-Benzylbenzimidazoles
(Nitazene Analogues)



Cinnamylpiperazines
(U-Series)



Benzimidazolones
(Brorphine)

2-Benzylbenzimidazoles

- Synthesized in 1957
  - Hunger, Rossi, and Hoffman (Switzerland)
  - Intended for opioid analgesic use
- Increasing polydrug use
  - Benzo-dope: causes increased effects on breathing and sedation
- Produce analgesia, euphoria, and sedation by activating the μ-opioid receptor (MOR)
  - Easily pass through the blood-brain barrier
- Adverse effects:
  - Respiratory depression*
  - GI effects (nausea, vomiting), reduced blood pressure and heart rate
  - Dependence/tolerance from repeated use

Kurze Mitteilungen | Published: 01 October 1957

Synthese basisch substituierter, analgetisch wirksamer Benzimidazol-Derivate

A. Hunger, J. Kebrle, A. Rossi & K. Hoffmann

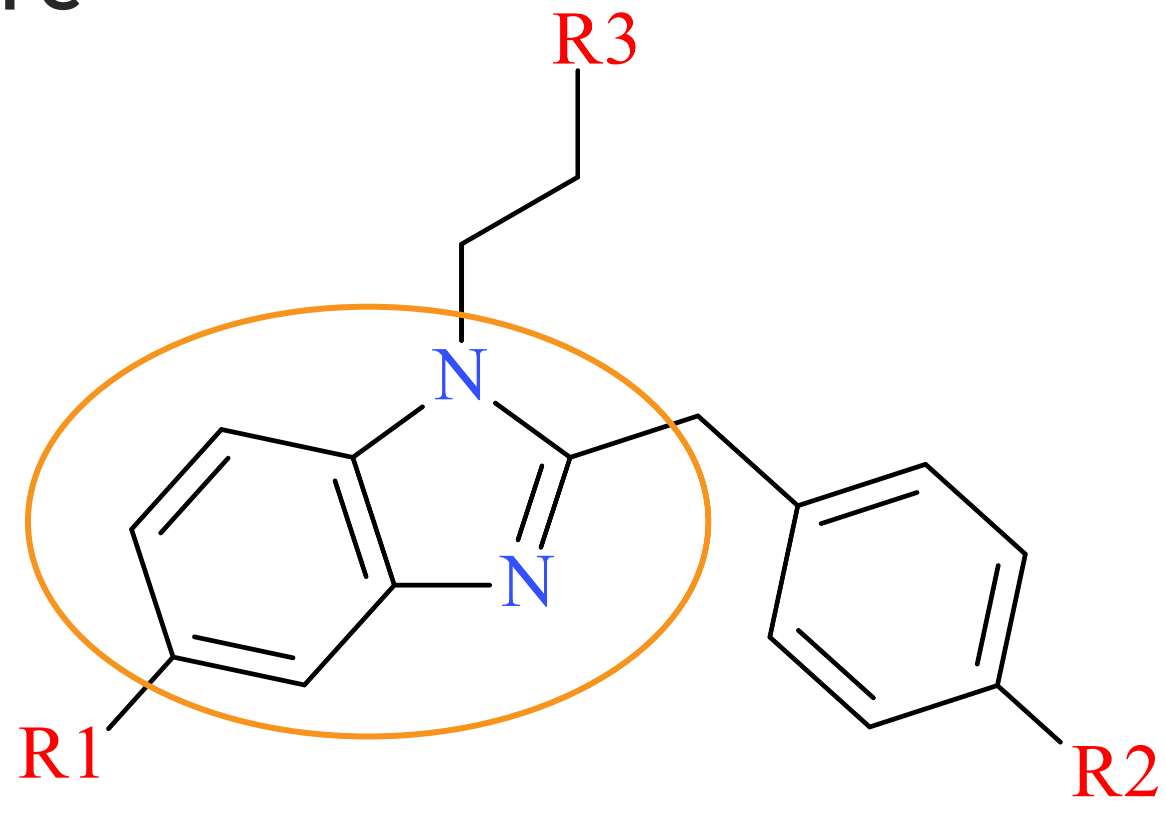
Experientia 13, 400–401 (1957) | Cite this article

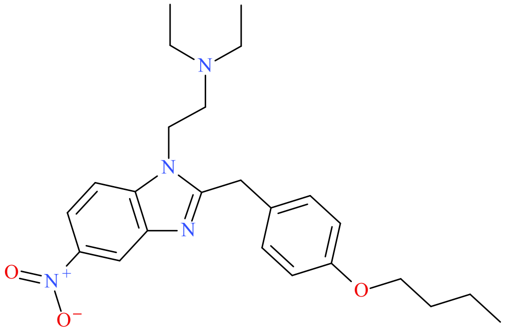
Summary

The syntheses of 1-(β-dialkylamino-ethyl)-2-benzyl-5-nitro-benzimidazoles, a new series of powerful analgesics, are described.

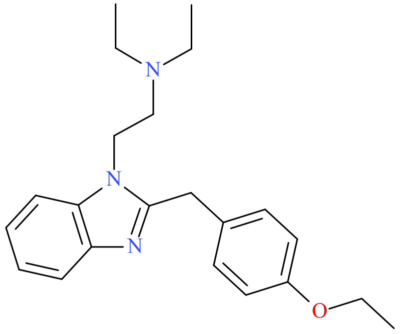
“Nitazene” Core Structure

- Benzimidazole core
- R1:
  – Nitro group
  – No modification (desnitazenes)
- R2:
  – Benzyl side chain
- R3:
  – *N*,*N*-Diethylamine
  – *N*-Pyrrolidino ring
  – *N*-Piperidinyl ring

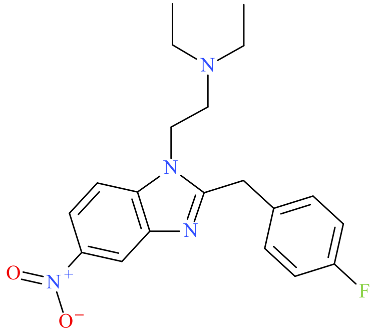




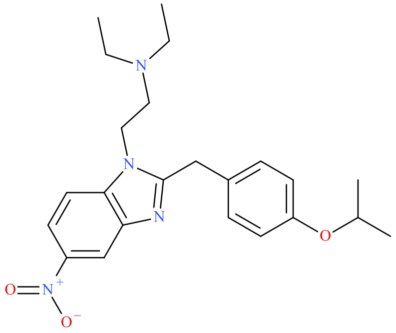
Butonitazene



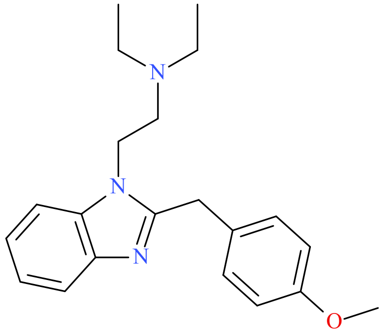
Etodesnitazene



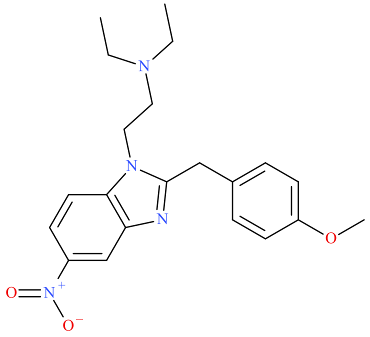
Flunitazene



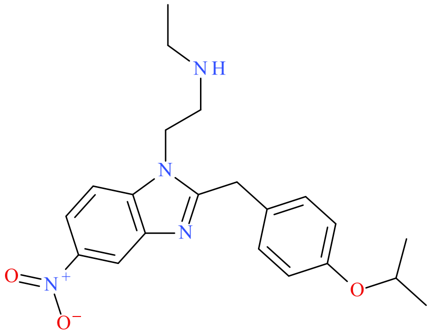
Isotonitazene



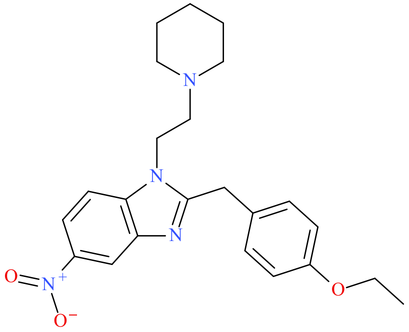
Metodesnitazene



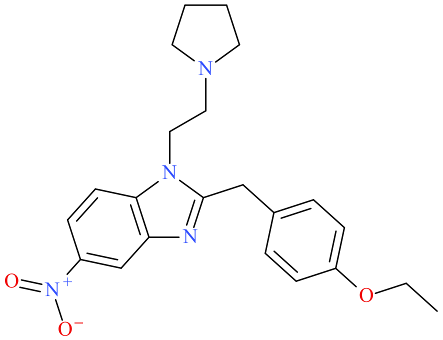
Metonitazene



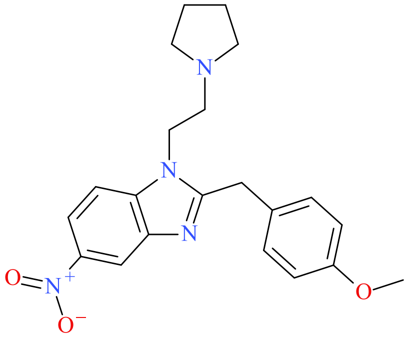
N-Desethyl Isotonitazene



N-Piperidinyl Etonitazene

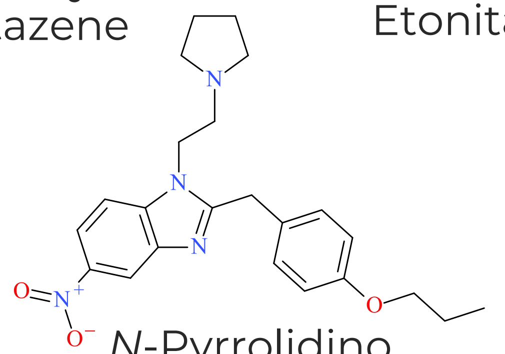


N-Pyrrolidino Etonitazene

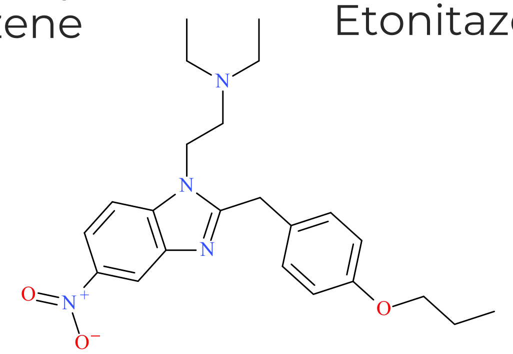


N-Pyrrolidino Metonitazene

Nitazene Analogues Reported by NPS Discovery

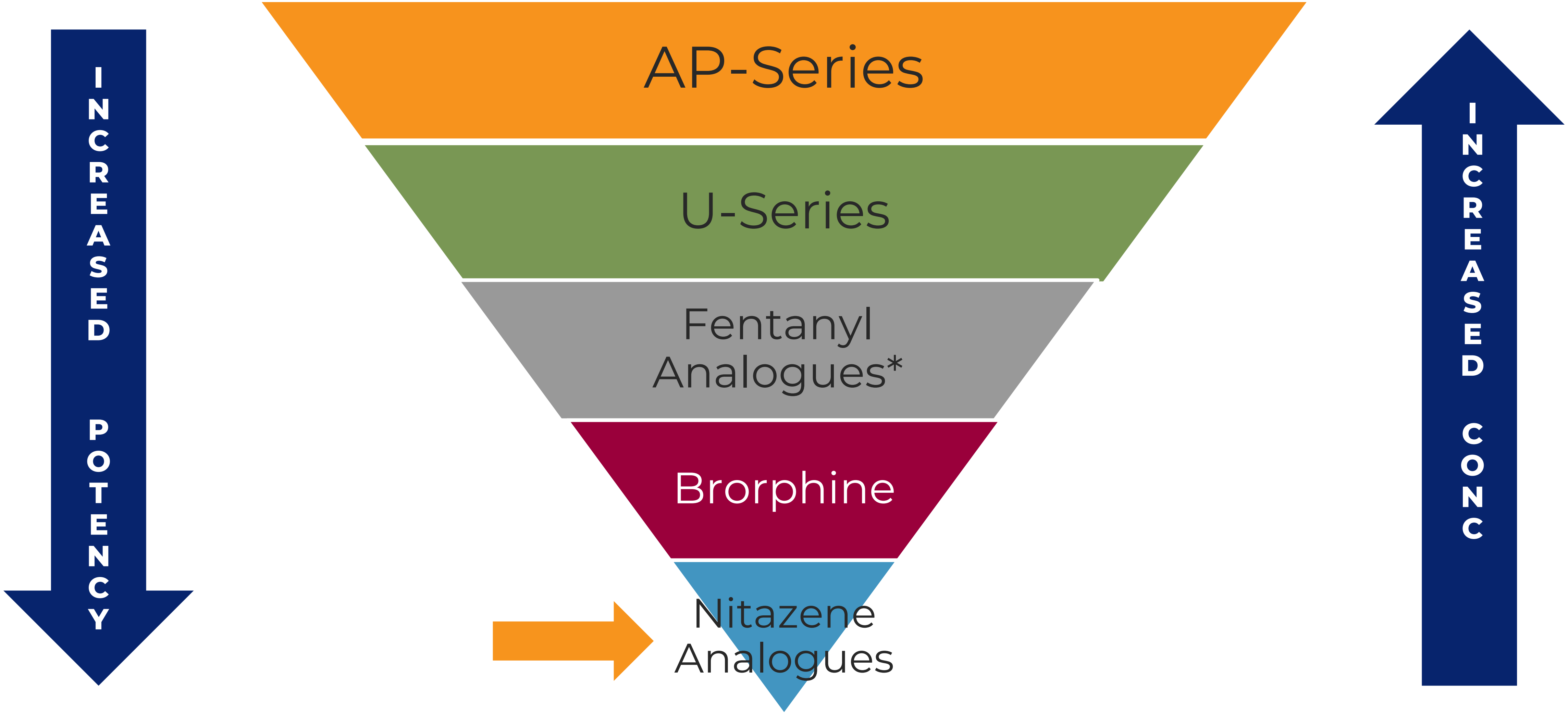


N-Pyrrolidino Protonitazene



Protonitazene

General Concentration Ranges



Published Potency Data – Vandeputte *et al.* (2020)

	MOR-βarr2		
	EC_{50} (nM)	E_{max} (% of fentanyl)	E_{max} (% of HM)
1. isotonitazene	1.63 (1.17–2.28)	110 (105–115)	179 (171–187)
2. *N*-desethylisotonitazene	0.614 (0.377–0.985)	140 (131–149)	229 (214–243)
3. 4′-OH-nitazene	176 (124–250)	81.9 (76.4–87.5)	133 (125–143)
4. 5-aminoisotonitazene	383 (263–554)	115 (108–123)	188 (176–201)
5. metonitazene	8.14 (5.12–12.8)	113 (106–121)	184 (172–197)
6. etonitazene	0.661 (0.338–1.26)	134 (122–146)	219 (199–238)
7. *N*-desethyletonitazene	1.81 (1.14–2.94)	101 (94.7–107)	164 (154–175)
8. protonitazene	3.95 (2.78–5.60)	107 (102–111)	174 (165–182)
9. butonitazene	36.2 (20.2–63.9)	103 (92.8–113)	167 (151–184)
10. clonitazene	140 (93.6–210)	106 (98.0–114)	173 (160–187)
11. flunitazene	377 (295–481)	118 (113–124)	192 (183–202)
12. isotodesnitazene	34.8 (22.1–54.4)	94.9 (88.1–102)	155 (144–166)
13. metodesnitazene	548 (365–811)	91.2 (85.1–97.5)	149 (139–159)
14. etodesnitazene	54.9 (36.1–82.0)	96.8 (90.2–103)	158 (147–169)
morphine	338 (239–478)	71.9 (68.3–75.4)	117 (111–123)
fentanyl	14.4 (11.5–18.0)	100 (96.5–103)	163 (157–169)
hydromorphone	36.2 (27.9–47.0)	61.3 (58.9–63.8)	100 (95.9–104)

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Waves of Nitazene Analogues

CFSRE First Identifications

2019
Isotonitazene

2020
Metonitazene

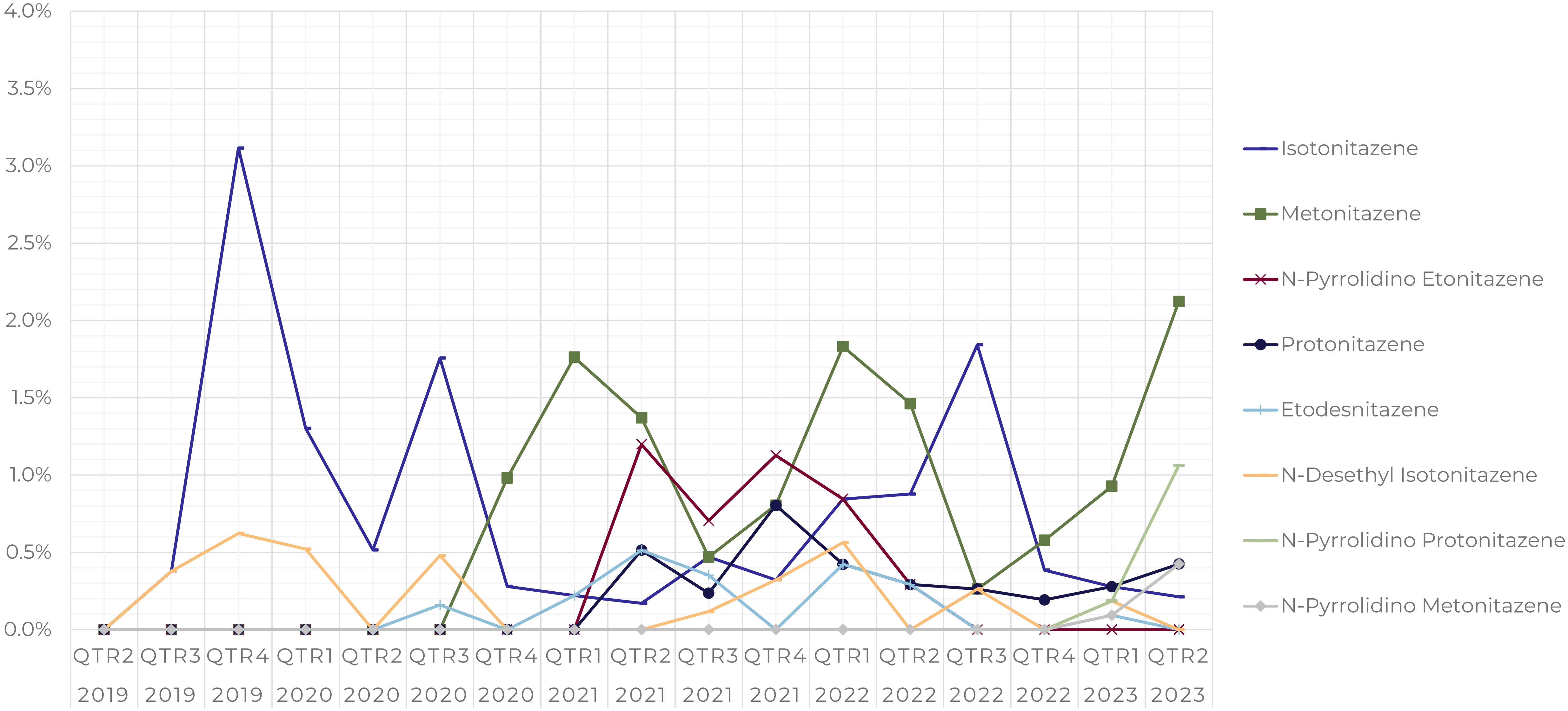
2021
Butonitazene
Etodesnitazene
Metodesnitazene
Flunitazene
N-Pyrrolidino Etonitazene
Protonitazene
N-Piperidinyl Etonitazene

2022
N-Desethyl Isotonitazene

2023
N-Pyrrolidino Metonitazene
N-Pyrrolidino Protonitazene

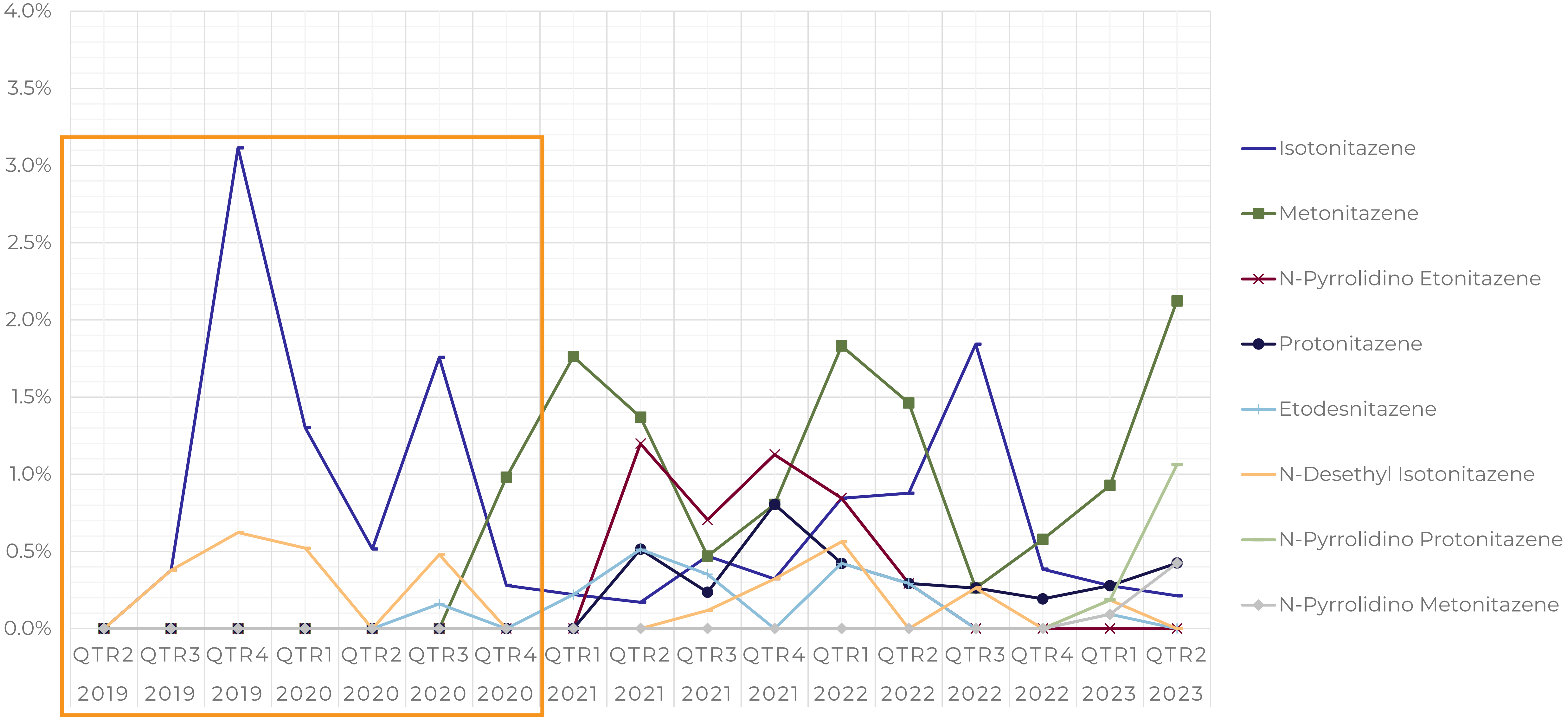
Nitazene Analogue Trends: 2019 – 2023

*CFSRE LC-QTOF-MS Data to Q1 2023



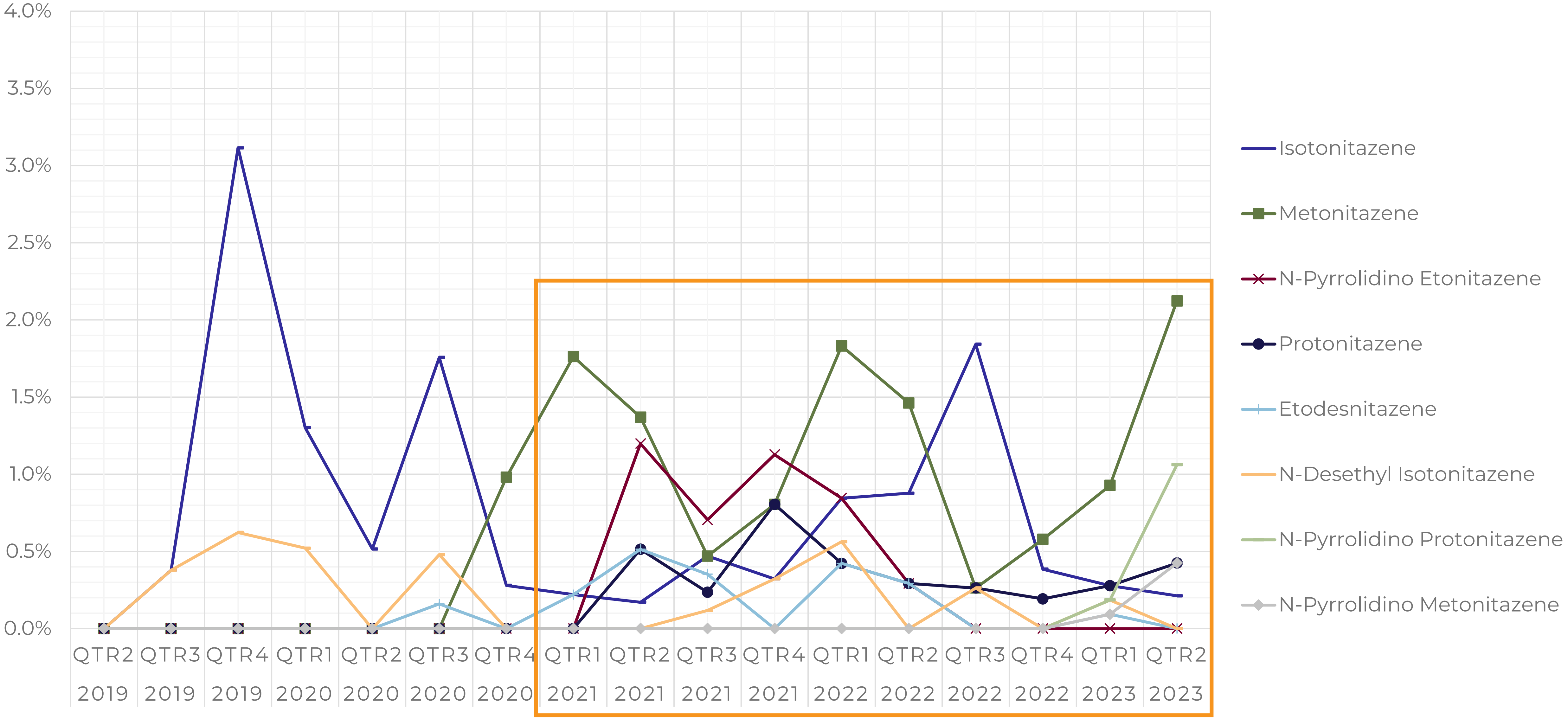
Nitazene Analogue Trends: 2019 – 2020

*CFSRE LC-QTOF-MS Data to Q1 2023



Nitazene Analogue Trends: 2021 – 2023

*CFSRE LC-QTOF-MS Data to Q1 2023



Nitazene Analogues Prevalent vs Infrequent

- Prevalent
  - Metonitazene: 74
  - Isotonitazene: 63
  - *N*-Pyrrolidino Etonitazene: 27
  - Protonitazene: 21
  - *N*-Desethyl Isotonitazene: 18
  - Etodesnitazene: 13

- Infrequent
  - Flunitazene: 1
  - Butonitazene: 2
  - *N*-Piperidinyl Etonitazene: 2
  - Metodesnitazene: 1

Up-and-coming

N-Pyrrolidino Protonitazene: 7

N-Pyrrolidino Metonitazene: 3

Nitazene Analogues Legal Actions in the U.S.

- Etonitazene & clonitazene placed under **Schedule I**
  - 1961
- Isotonitazene placed under **Schedule I**
  - August 2020
- Butonitazene, etodesnitazene, flunitazene, metodesnitazene, metonitazene, *N*-pyrrolidino etonitazene, protonitazene placed under **Schedule I**
  - December 2021

Schedules of Controlled Substances: Placement of Isotonitazene in Schedule I

A Rule by the Drug Enforcement Administration on 11/(

Schedules of Controlled Substances: Temporary Placement of Butonitazene, Etodesnitazene, Flunitazene, Metodesnitazene, Metonitazene, N-pyrrolidino etonitazene, and Protonitazene in Schedule I

A Proposed Rule by the Drug Enforcement Administration on 12/07/2021

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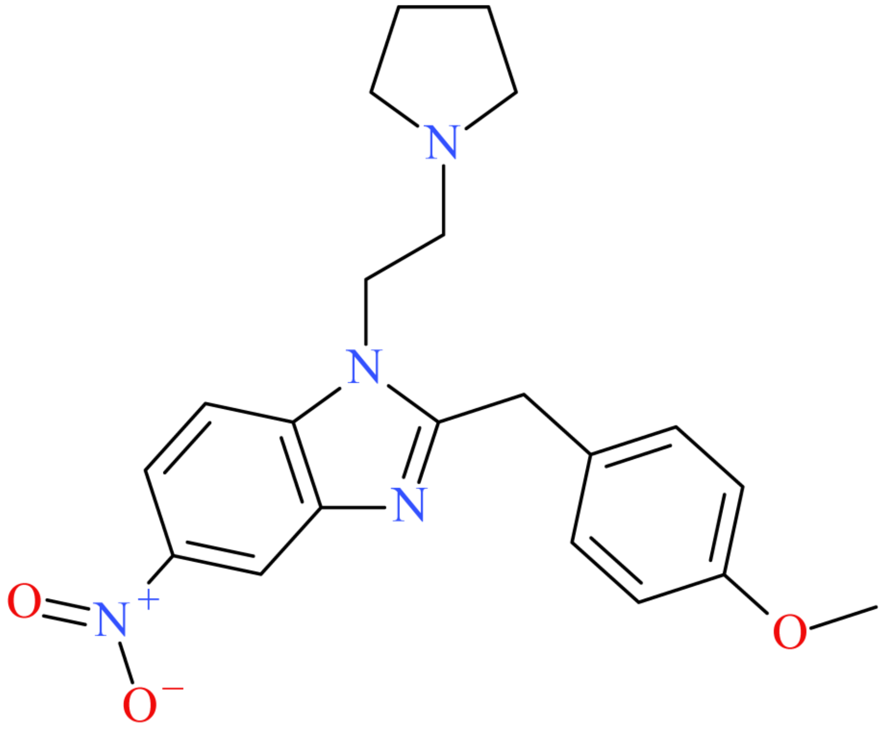
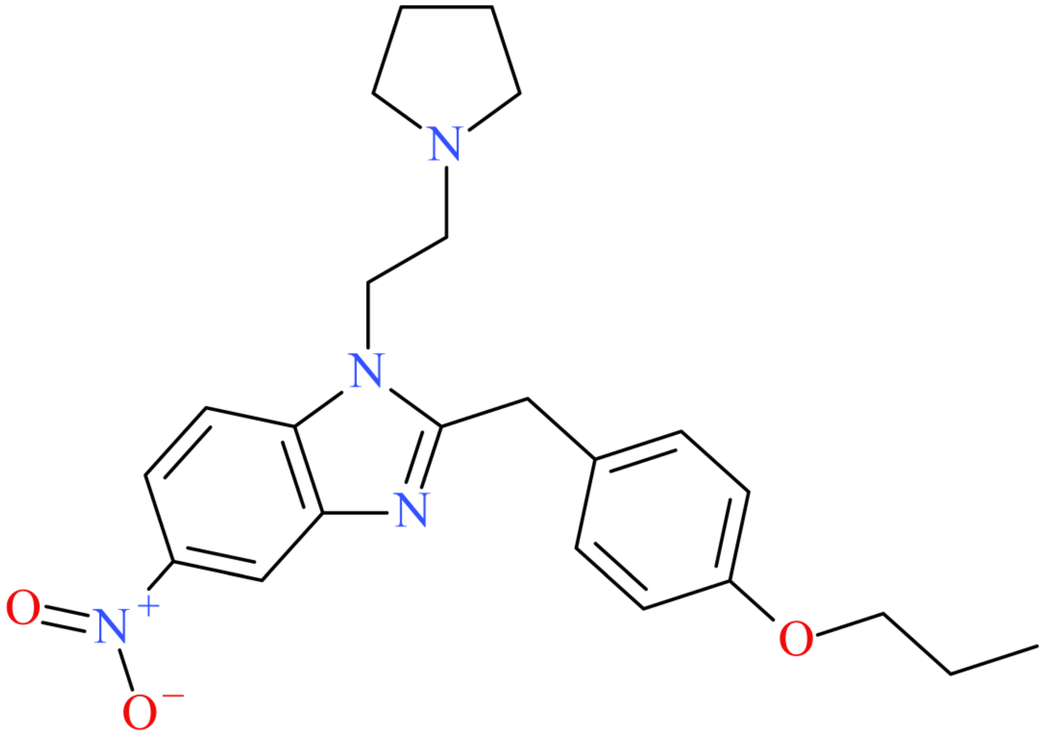
Newest Additions to the Drug Market

“Pyrrolidino” or “Ring” Nitazenes

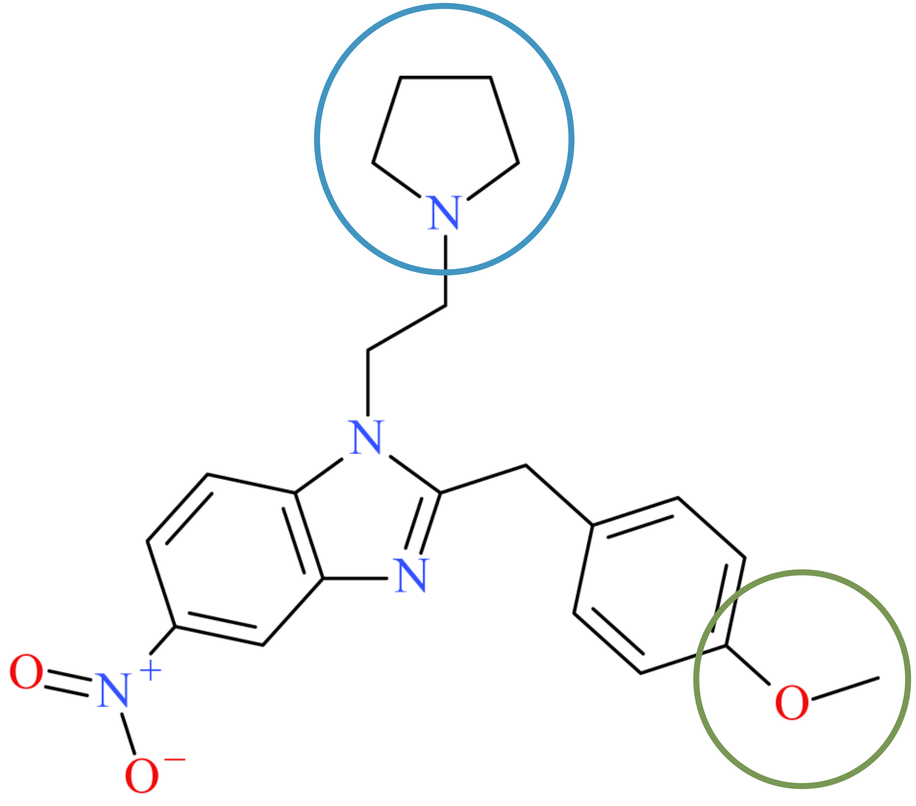


N-Pyrrolidino Metonitazene

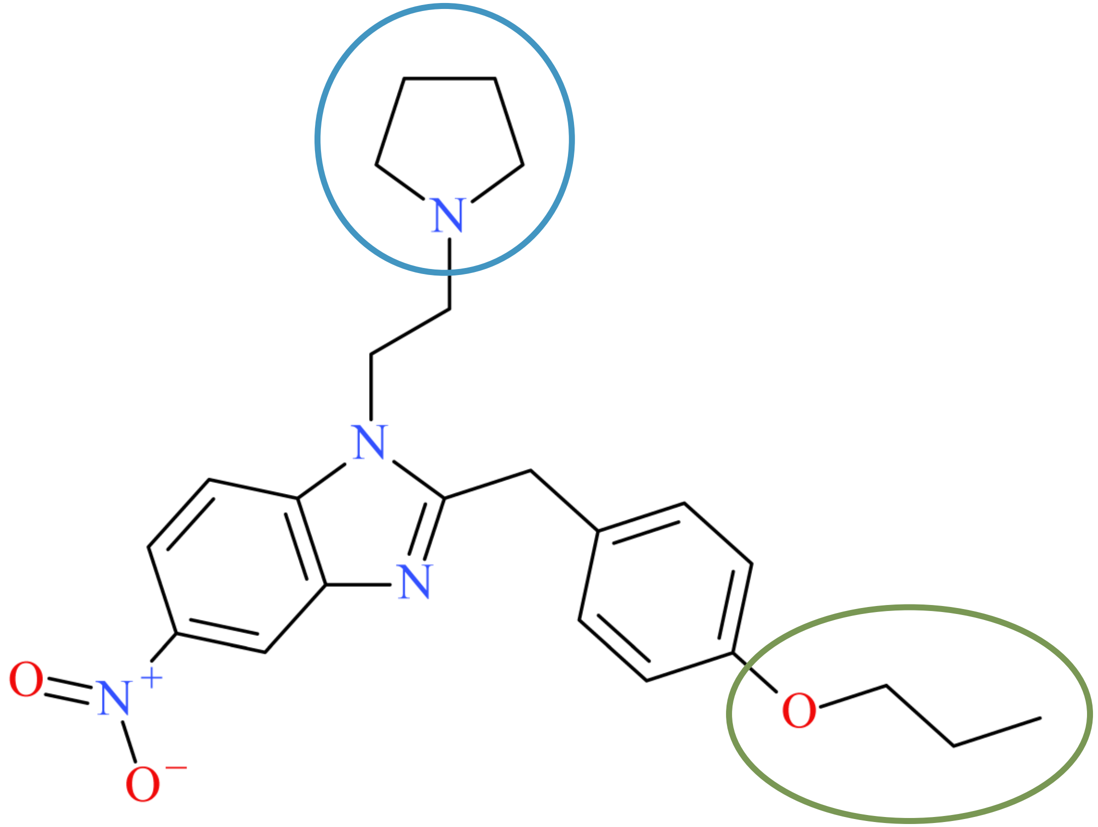


N-Pyrrolidino Protonitazene

“Pyrrolidino” or “Ring” Nitazenes



N-Pyrrolidino Metonitazene



N-Pyrrolidino Protonitazene

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CFSRE New Drug Monographs

www.npsdiscovery.org

NPS Discovery — New Drug Monograph 2023

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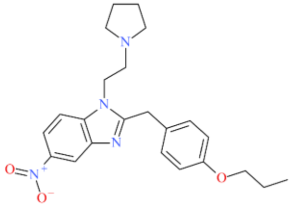
N-Pyrrolidino Protonitazene



NPS SUBCLASS	Opioid
REPORT DATE	June 22, 2023
SAMPLE RECEIVED	January 10, 2023
SAMPLE TYPE	Drug Material

Preferred Name	N-Pyrrolidino Protonitazene
Synonyms	Protonitazepyne
Formal Name	5-nitro-2-[(4-propoxyphenyl)methyl]-1-(2-pyrrolidin-1-ylethyl)benzimidazole
InChI Key	KCRWXNIIXGBPID-UHFFFAOYSA-N
CAS Number	Not Available
Chemical Formula	$C_{23}H_{28}N_4O_3$
Molecular Weight	408.49
Molecular Ion [M+]	408
Exact Mass [M+H]+	409.2234

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N-Pyrrolidino Protonitazene 2023

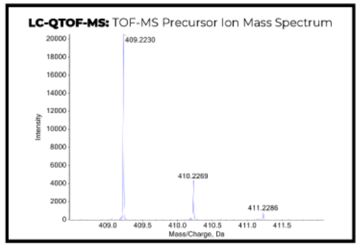
Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)

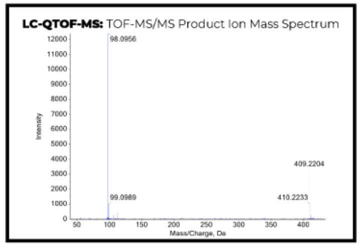
Laboratory: Center for Forensic Science Research and Education (CFSRE, Willow Grove, PA, USA)

Instrument: Sciex TripleTOF® 5600+ LC-QTOF-MS

Methods: www.cfsre.org/nps-discovery/monographs

Sample Preparation: Acid / base extraction





Confirmation Using Drug Standard: *Reference material (Batch: 0654417-3) was purchased from Cayman Chemical (Ann Arbor, MI, USA). The analyte was confirmed to be N-pyrrolidino protonitazene based on retention time (sample: 7.05 min vs. standard: 6.97 min) and mass spectral data comparisons. [Note: The retention time for N-pyrrolidino isotonitazene (Batch: 0624243-13, Cayman Chemical) was 6.84 min.]*

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NPS Discovery — New Drug Monograph 2023

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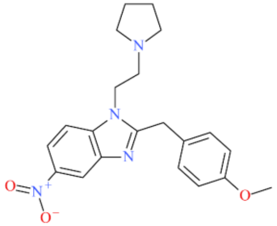
N-Pyrrolidino Metonitazene



NPS SUBCLASS	Opioid
REPORT DATE	June 23, 2023
SAMPLE RECEIVED	February 3, 2023
SAMPLE TYPE	Drug Material

Preferred Name	N-Pyrrolidino Metonitazene
Synonyms	Metonitazepyne
Formal Name	2-[(4-methoxyphenyl)methyl]-5-nitro-1-(2-pyrrolidin-1-ylethyl)benzimidazole
InChI Key	JTTDZHBAEQTIPA-UHFFFAOYSA-N
CAS Number	Not Available
Chemical Formula	$C_{21}H_{24}N_4O_3$
Molecular Weight	380.44
Molecular Ion [M+]	380
Exact Mass [M+H]+	381.1921

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N-Pyrrolidino Metonitazene 2023

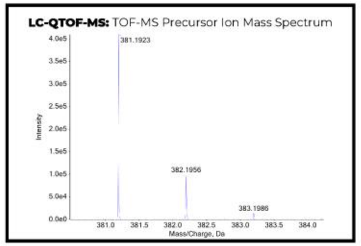
Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)

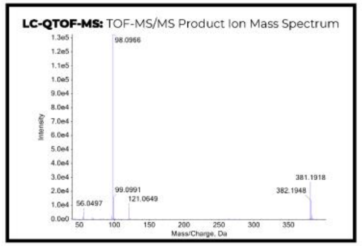
Laboratory: Center for Forensic Science Research and Education (CFSRE, Willow Grove, PA, USA)

Instrument: Sciex TripleTOF® 5600+ LC-QTOF-MS

Methods: www.cfsre.org/nps-discovery/monographs

Sample Preparation: Dilution in methanol





Confirmation Using Drug Standard: *Reference material (Batch: 0659685-13) was purchased from Cayman Chemical (Ann Arbor, MI, USA). The analyte was confirmed to be N-pyrrolidino metonitazene based on retention time (sample: 5.96 min vs. standard: 5.85 min) and mass spectral data comparisons.*

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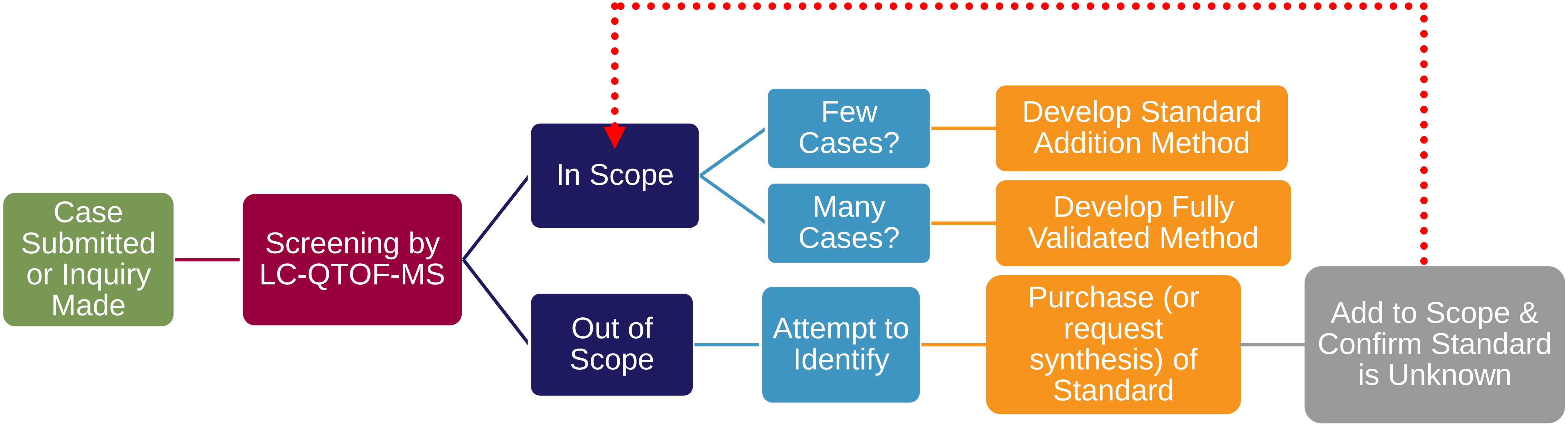
Methods

CFSRE Postmortem Workflow



Standard Addition Method

Sample Preparation

- Standard Addition
  - Four total aliquots
  - Up-spikes at 0.2, 2, 20 ng/mL
- Internal Standard
  - Isotonitazene-D7
- Basic Liquid-Liquid Extraction
  - 0.1 M Borax Buffer, pH 10.4
  - 70:30 *N*-butyl chloride:ethyl acetate

Instrumental Analysis

- Waters Xevo TQ-S Micro LC-QQQ-MS
- Mobile phase compositions:
  - 0.1% Formic Acid in Water
  - 0.1% Formic Acid in Methanol
- Analytical Column
  - Agilent InfinityLab Poroshell 120 EC-C18 3.0 x 100mm, 2.7 μm

Time (min)	Flow (mL/min)	%A	%B
Initial	0.4	60	40
3.5	0.4	40	60
4.0	0.4	10	90
4.5	0.4	10	90
4.6	0.4	60	40
5.0	0.4	60	40

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Case Study & Concentrations

Postmortem Case

- 43 y/o male
- West Virginia
- Case history: history of substance abuse
  - Received femoral blood
- Polydrug use:
  - Synthetic opioids
  - Designer benzodiazepines
  - Stimulants
- Found without other nitazene analogues, fentanyl, or any other opioids

Results:

- *N*-Pyrrolidino protonitazene: 1.1 ng/mL
- Other results:
  - Bromazolam: 36 ng/mL
  - Desalkylflurazepam: 11 ng/mL
  - Methamphetamine: 480 ng/mL
  - Amphetamine: 59 ng/mL

Comparing Concentrations in Death Cases

**Order similar to reported in vitro potency*

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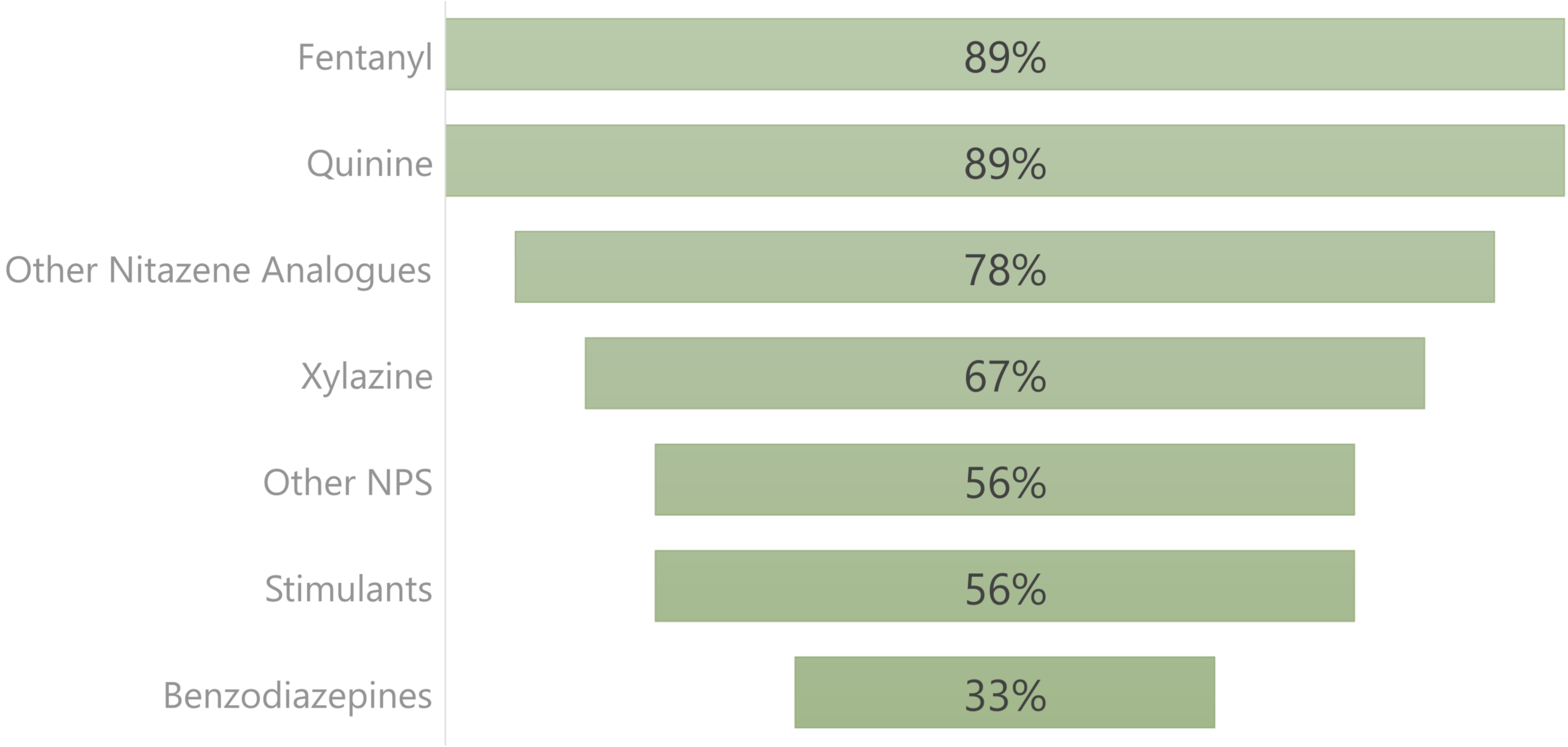
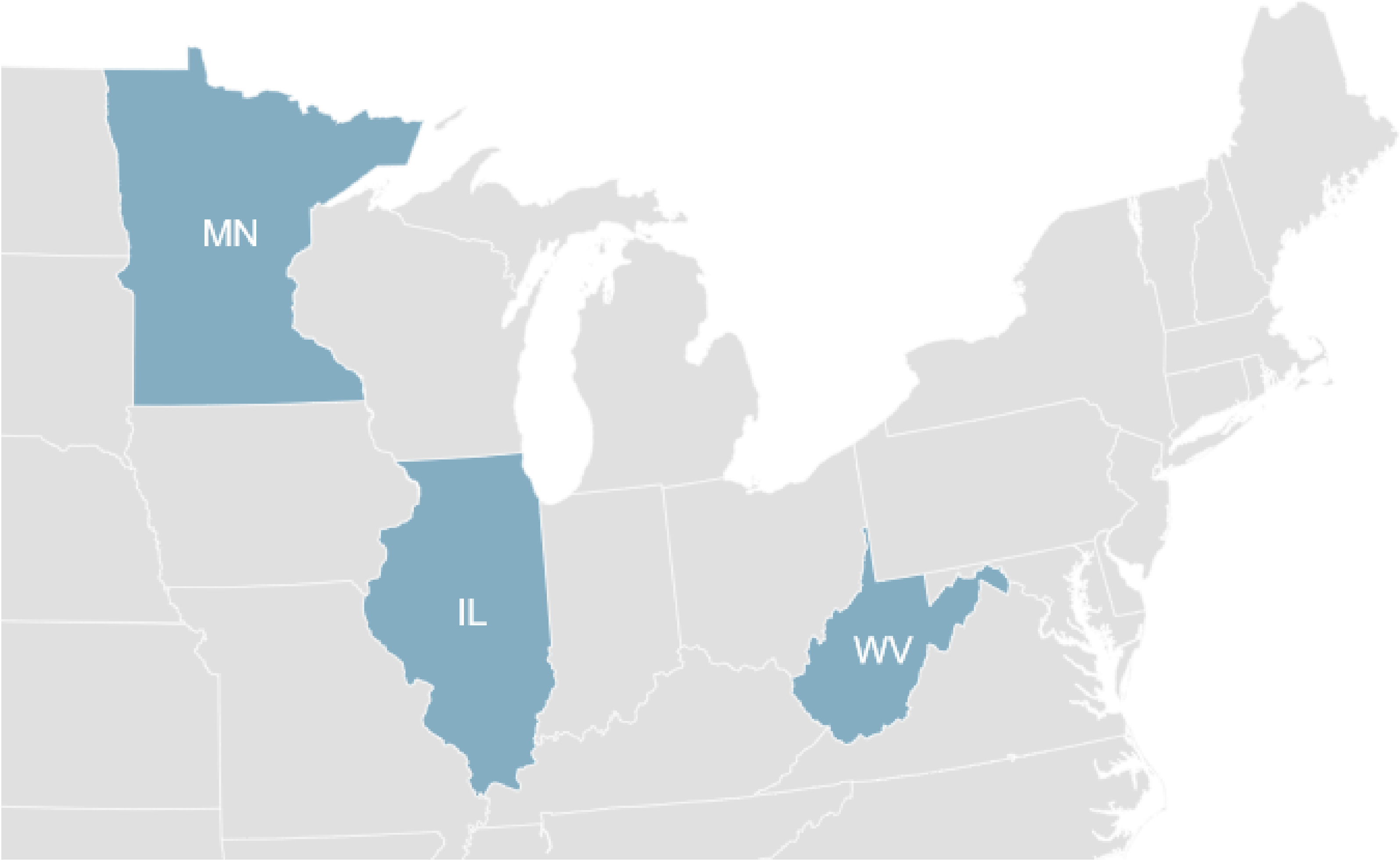
Drug	N	Mean (±SD) (ng/mL)	Median (ng/mL)	Range (ng/mL)	Potency Compared to Fentanyl
N-Pyrrolidino Etonitazene*	15	3.9 ± 5.9	2.4	0.3 - 25	43x more
N*-Pyrrolidino Protonitazene	**9**	**0.90 ± 0.43**	**1.0**	**0.1-1.5**	**25x more**
Isotonitazene*	69	1.59 ± 1.81	1.0	0.5 - 9	9x more
Protonitazene*	3	11 ± 9.9	5	3.1 – 25	4x more
Metonitazene	18	6.3 ± 7.5	3.8	0.5 - 33	2x more
***N*-Pyrrolidino Metonitazene**	**5**	**0.46 ± 0.14**	**0.49**	**0.25-0.63**	**2x more**
Butonitazene	1	3.2	*N/A*	*N/A*	2x less
Etodesnitazene	15	40 ± 61	5.2	0.53 - 230	4x less

Qualitative Trends (*N*-Pyrro. Meto and *N*-Pyrro. Proto)



Distribution of “Ring” Nitazenes in U.S.



Discussion & Conclusion

Discussion & Conclusion

- Nitazene analogues continue to proliferate in the United States
  - Scheduled nitazene analogues still appear in casework
- New "pyrrolidino" nitazene analogues are identified in samples at sub-ng/mL concentrations
  - Sensitive instrumentation is needed
- "Pyrrolidino" nitazenes are most often found with
  - Other nitazene analogues
    - Example: *N*-pyrrolidino metonitazene with metonitazene
  - Designer benzodiazepines
  - Fentanyl
- Importance of up-to-date scope of testing
  - NPS Scope Recommendations (SOFT & CFSRE)

Recommended Scope for NPS Testing in the United States | NPS SCOPE | Q2 2023

PURPOSE: The objective of this report is to provide updated guidance in developing an appropriate analytical scope of testing for novel psychoactive substances (NPS) in the United States (and around the world) based on current trends and intelligence. *This report is based on information available in Q1 2023 and early Q2 2023 and is subject to change along with the drug market.*

SUMMARY: The NPS landscape is changing rapidly, requiring laboratories to constantly remain abreast of new and emerging drugs locally, nationally, and internationally. To meet individualized needs, laboratories amend existing methods or develop new ones for detection and confirmation of NPS. This can be challenging for scientists as information about NPS detections can be regionalized and/or out-of-date, making it difficult to determine which drugs should be prioritized at a given time. **CFSRE's NPS Discovery** and the **SOFT NPS Committee** have established the below recommendations for NPS scope based on information from extensive collaborations, partnerships, and initiatives which yield national and international perspectives. Suggested cut-off concentrations or reporting limits (in ng/mL) are listed for each NPS. These values are categorized (i.e., <1, 1-10, and >10 ng/mL) and determined based on currently available quantitative data and/or comparison to structurally similar NPS within the given sub-class.

BENZODIAZEPINES		OPIOIDS		STIMULANTS & HALLUCINOGENS		SYNTHETIC CANNABINOIDS	
TIER ONE *(STRONGLY RECOMMEND)*							
Bromazolam	1-10	*Protonitazene	<1	*N,N*-Dimethylpentylone	>10	MDMB-4en-PINACA	<1
Etizolam†	1-10	Metonitazene	<1	Pentylone	>10	ADB-BINACA (-BUTINACA)	<1
Flualprazolam	1-10	**N*-Pyrrolidino Protonitazene	<1	Eutylone	>10	ADB-5'Br-BINACA	<1
Flubromazepam	1-10	*N*-Desethyl Isotonitazene	<1	*N*-Propyl Butylone	>10	CH-PIATA	<1
Clonazolam‡	<1	*o/m/p*-Fluorofentanyl	1-10	alpha-PHP / alpha-PiHP	>10	ADB-FUBIATA	<1
TIER TWO *(RECOMMEND)*							
8-Aminoclonazolam‡	1-10	*Isotonitazene	<1	Fluoroexetamine	1-10	5F-MDMB-PICA	<1
Flubromazolam	1-10	*N*-Pyrrolidino Metonitazene	<1	*N*-Cyclohexyl Butylone	>10	4F-MDMB-BINACA	<1
Desalkylgidazepam†	1-10	*N*-Pyrrolidino Etonitazene	<1	*N*-Cyclohexyl Methylone	>10	ADB-5'Br-PINACA	<1
4'Cl-Deschloroalprazolam	1-10	*Carfentanil	<1	2F-Deschloroketamine	<1	*4F-ABINACA (-ABUTINACA)	<1
TIER THREE *(CONSIDER)*							
Desalkylflurazepam†	1-10	Brorphine	<1	2,3,4-Methylmethcathinone	1-10	*CH-FUBIATA	<1
Deschloroetizolam	1-10	Etodesnitazene	1-10	3-HO-PCP / 4-HO-PCP	<1	5F-BZO-POXIZID	<1
Pyrazolam	1-10	*Ethyleneoxynitazene	1-10	3-MeO-PCP / 4-MeO-PCP	<1	ADB-4en-PINACA	<1
*Bromazepam†	1-10	**N*-Piperidinyl Etonitazene	<1	MDPHP	>10	*MDMB-BINACA (-BUTINACA)	<1

Note: This may not be an all-inclusive list. Laboratories should consider additional NPS for inclusion (or exclusion) based on local, national, and/or international trends.

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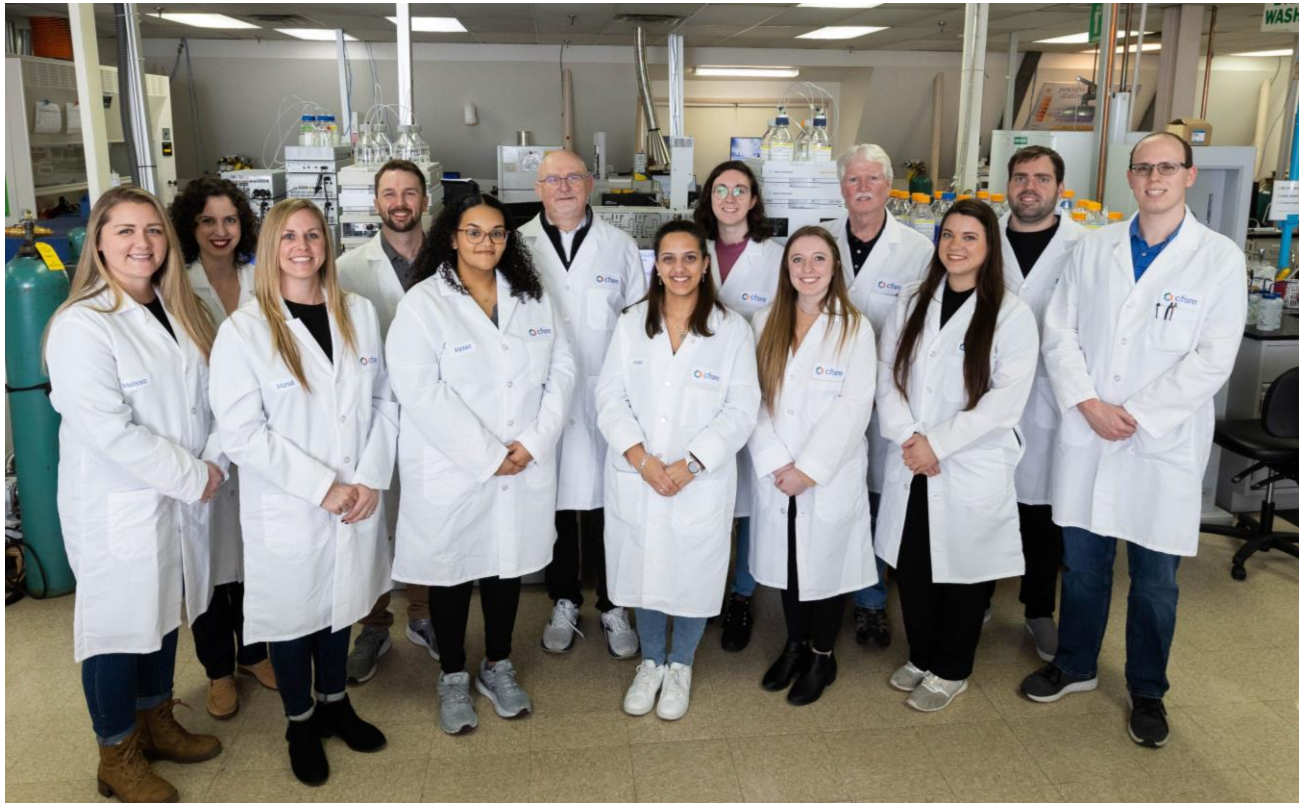
Acknowledgements



- Our many collaborations
  - Medical examiners & coroners
  - Toxicology laboratories
  - Hospitals
  - Police departments
  - Public health & safety
- CFSRE Staff
- NMS Labs
  - Donna Papsun

NMS LABS

When you need to know.™

Thank you!   Questions?

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