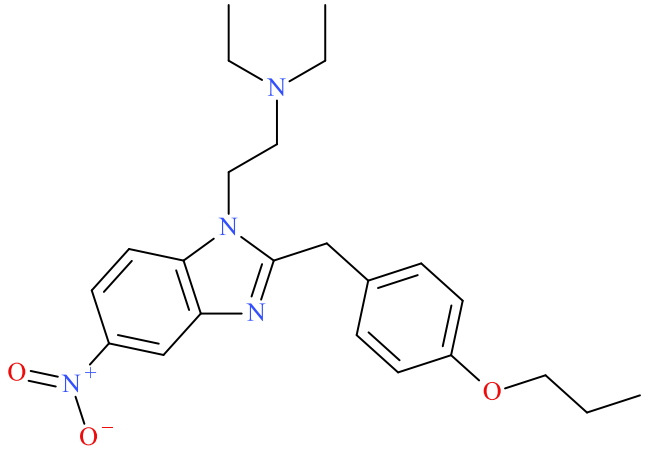
Protonitazene

Sample Type: **Biological Fluid**



Latest Revision: **May 26, 2021**

Date of Report: **May 26, 2021**

1. GENERAL INFORMATION

IUPAC Name: N,N-diethyl-2-[5-nitro-2-[(4-propoxyphenyl)methyl]benzimidazol-1-yl]ethanamine

InChI String: InChI=1S/C23H30N4O3/c1-4-15-30-20-10-7-18(8-11-20)16-23-24-21-17-19(27(28)29)9-12-22(21)26(23)14-13-25(5-2)6-3/h7-12,17H,4-6,13-16H2,1-3H3

CFR: Not Scheduled (05/2021)

CAS# 119276-01-6

Synonyms: Pronitazene, Propoxynitazene

Source: NMS Labs – Toxicology Department

***Important Notes**: All identifications were made based on evaluation of analytical data (LC-QTOF-MS) in comparison to analysis of acquired reference material.*

***Prepared By:** Alex J. Krotulski, PhD; Sara E. Walton, BS; Donna M. Papsun, MS, D-ABFT-FT; Melissa F. Fogarty, MSFS, D-ABFT-FT; and Barry K. Logan, PhD, F-ABFT*

2. CHEMICAL DATA

Analyte	Chemical Formula	Molecular Weight	Molecular Ion [M^+]	Exact Mass [$M+H]^+$
Protonitazene	$C_{23}H_{30}N_4O_3$	410.5	410	411.2391

3. SAMPLE HISTORY

To date, protonitazene was identified in one case in May 2021. The geographical and demographical breakdown is below:

Geographical Location: Iowa (n=1)

Biological Sample: Blood (n=1)

Date of First Receipt: April 2021

Other Notable Findings: Metonitazene (n=1), Etodesnitazene (n=1), Etizolam (n=1)

4. BRIEF DESCRIPTION

Protonitazene is classified as a novel opioid of the benzimidazole sub-class and is structurally dissimilar from fentanyl. Novel opioids have been reported to cause psychoactive effects similar to heroin, fentanyl, and other opioids. Novel opioids have also caused adverse events, including death, as described in the literature. Structurally similar compounds include etonitazene, metonitazene, and isotonitazene. Etonitazene and structurally related synthetic opioids were first synthesized and reported in the literature in the 1950s.[1] Data suggest that this group of analogues can have potency similar to or greater than fentanyl.[2] Recent *in vitro* data suggest that protonitazene is more potent than fentanyl.[3] Protonitazene is not explicitly scheduled in the United States; however, etonitazene and isotonitazene are Schedule I substances. Protonitazene and isotonitazene are isomeric analogues, sharing the same molecular formula and mass. Increased specificity is required to distinguish these two drugs during analytical testing (e.g., retention time, fragmentation pattern).

5. ADDITIONAL RESOURCES

1. Hunger, A; Kebrle, J; Rossi, A; Hoffmann, K. (1957) Synthesis of analgesically active benzimidazole derivatives with basic substitutions. *Experientia*, **13**, 400-401. https://link.springer.com/article/10.1007/BF02161116

2. Hoffmann, K; Hunger, A; Rossi, A. (3 May 1960). "Patent US2935514A – Benzimidazoles." https://patents.google.com/patent/US2935514A/en

3. Vandeputte, MM; Van Uytfanghe, K; Layle, NK; St. Germaine, DM; Iula, DM; Stove , CP. Synthesis, Chemical Characterization, and μ-Opioid Receptor Activity Assessment of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids. *ACS Chem. Neurosci*. 2021. https://pubs.acs.org/doi/10.1021/acschemneuro.1c00064

https://www.caymanchem.com/product/29381/protonitazene-(hydrochloride)

6. QUALITATIVE DATA

6.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

Testing Performed At:	The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)
Sample Preparation:	Standard diluted in methanol
Instrument:	Agilent 5975 Series GC/MSD System
Standard:	Reference material for protonitazene (Batch: 0575807-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). (https://www.caymanchem.com/product/29381/protonitazene-(hydrochloride))

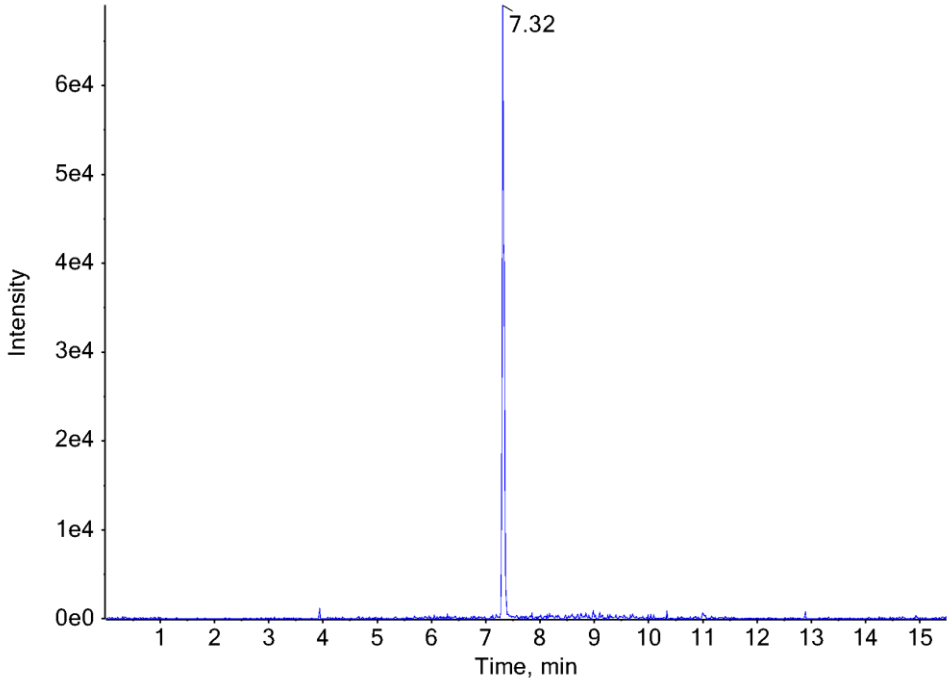
EI (70 eV) Mass Spectrum: Protonitazene (Standard)

6.2 LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (LC-QTOF-MS)

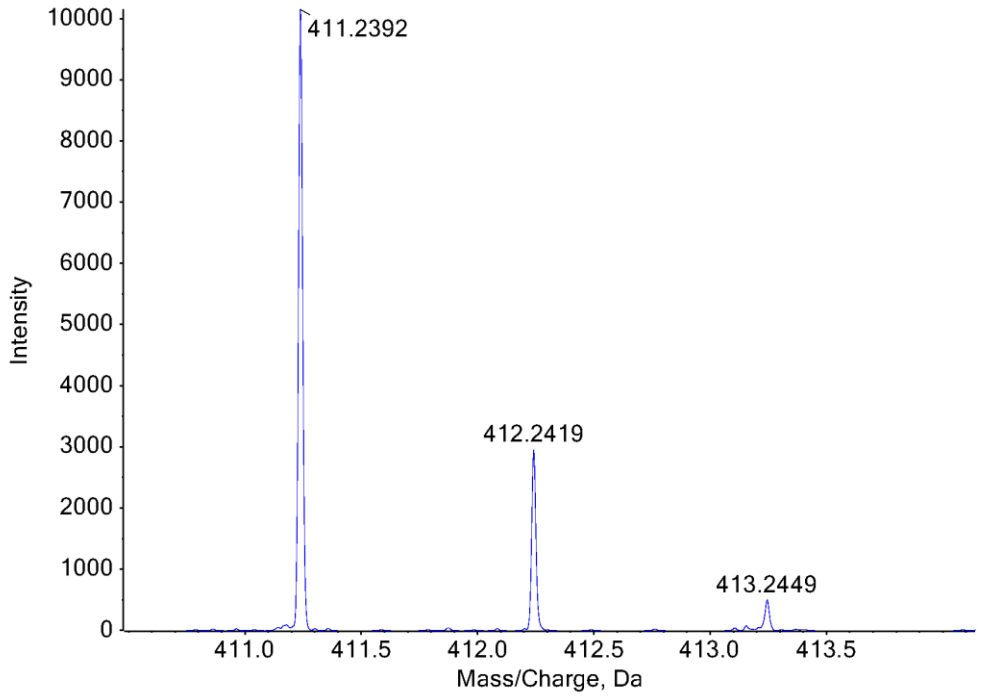
Testing Performed At:	The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)
Sample Preparation:	Liquid-liquid extraction (LLE)
Instrument:	Sciex TripleTOF® 5600+, Shimadzu Nexera XR UHPLC
Column:	Phenomenex® Kinetex C18 (50 mm x 3.0 mm, 2.6 µm)
Mobile Phase:	A: Ammonium formate (10 mM, pH 3.0)
	B: Methanol/acetonitrile (50:50)
	Flow rate: 0.4 mL/min
Gradient:	Initial: 95A:5B; 5A:95B over 13 min; 95A:5B at 15.5 min
Temperatures:	Autosampler: 15 °C

	Column Oven: 30 °C
	Source Heater: 600 °C
Injection Parameters:	Injection Volume: 10 µL
QTOF Parameters:	TOF MS Scan Range: 100-510 Da
	Precursor Isolation: SWATH® acquisition (27 windows)
	Fragmentation: Collison Energy Spread (35±15 eV)
	MS/MS Scan Range: 50-510 Da
Retention Time:	7.32 min
Standard Comparison:	Reference material for protonitazene (Batch: 0575807-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Analysis of this standard resulted in positive identification of the analyte in the extract as protonitazene, based on retention time (7.19 min) and mass spectral data. (https://www.caymanchem.com/product/29381/protonitazene-(hydrochloride))

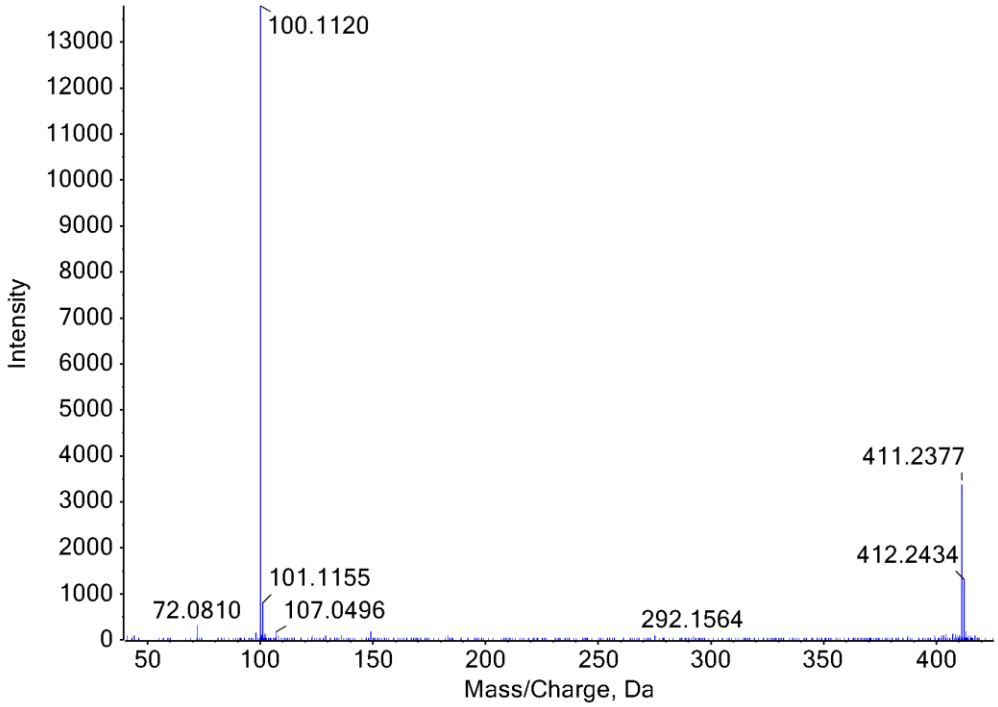
Extracted Ion Chromatogram: Protonitazene (Biological Sample)



TOF MS Spectra: Protonitazene (Biological Sample)



MS/MS Spectra: Protonitazene (Biological Sample)



7. FUNDING

Our program is supported in part by 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 author(s) and do not necessarily reflect those of the Department of Justice.