

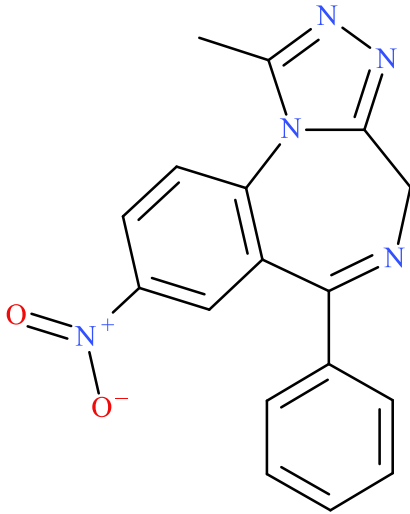
## Nitrazolam

Sample Type: **Seized Material**

Latest Revision: **December 19, 2018**

Date Received: **July 31, 2018**

Date of Report: **December 19, 2018**



### 1. GENERAL INFORMATION

<b>IUPAC Name:</b>	1-methyl-8-nitro-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
<b>InChI String:</b>	InChI=1S/C17H13N5O2/c1-11-19-20-16-10-18-17(12-5-3-2-4-6-12)14-9-13(22(23)24)7-8-15(14)21(11)16/h2-9H,10H2,1H3
<b>CFR:</b>	Not Scheduled (12/2018)
<b>CAS#</b>	Not Available
<b>Synonyms:</b>	Not Available
<b>Source:</b>	Department of Homeland Security
<b>Appearance:</b>	Yellow Solid Material

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

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## 2. CHEMICAL AND PHYSICAL DATA

### 2.1 CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Molecular Ion [M <sup>+</sup> ]	Exact Mass [M+H] <sup>+</sup>
Base	C <sub>17</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	319.3	319	320.1142

### 3. BRIEF DESCRIPTION

Nitrazolam is classified as a novel benzodiazepine, although its synthesis and activity have been previously described in the literature. Benzodiazepines are central nervous system depressants. Novel benzodiazepines, often pirated from early drug discovery or pharmaceutical studies, have appeared on the novel and illicit drug markets in recent years. These substances have caused adverse events, including deaths, as described in the literature. Nitrazolam is structurally similar to traditional benzodiazepines nitrazepam and alprazolam, Schedule IV substances in the United States, as well as the novel benzodiazepines flunitrazolam and clonazepam.

### 4. ADDITIONAL RESOURCES

Hester, JB. (19 October 1976). "Patent US3987052 - 6-Phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepines." <https://patents.google.com/patent/US3987052A/en>

Hester, JB; Rudzik, AD; Kamdar, BV. (1971) 6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepines which have central nervous system depressant activity. *J Med Chem.* 14 (11): 1078-81. <https://www.ncbi.nlm.nih.gov/pubmed/5165540>

Moosmann, B; Bisel, P; Franz, F; Huppertz, LM; Auwärter, V. (2016) Characterization and in vitro phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam, and nitrazolam. *Journal of Mass Spectrometry.* 51: 1080–1089. <https://www.ncbi.nlm.nih.gov/pubmed/27535017>

[https://www.policija.si/apps/nfl\\_response\\_web/0\\_Analytical\\_Reports\\_final/Nitrazolam-ID-1452-16\\_rpt100816.pdf](https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/Nitrazolam-ID-1452-16_rpt100816.pdf)

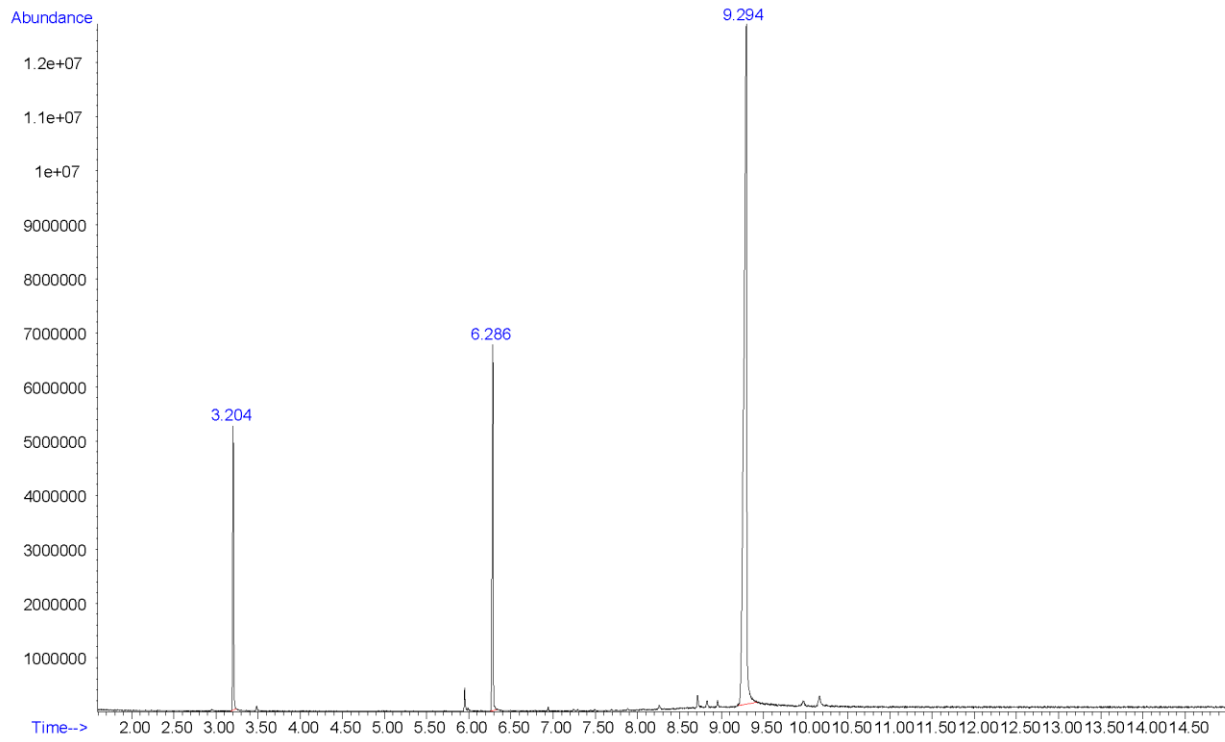
<https://www.sigmaaldrich.com/catalog/product/aldrich/ph004350?lang=en&region=US>

## 5. QUALITATIVE DATA

### 5.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

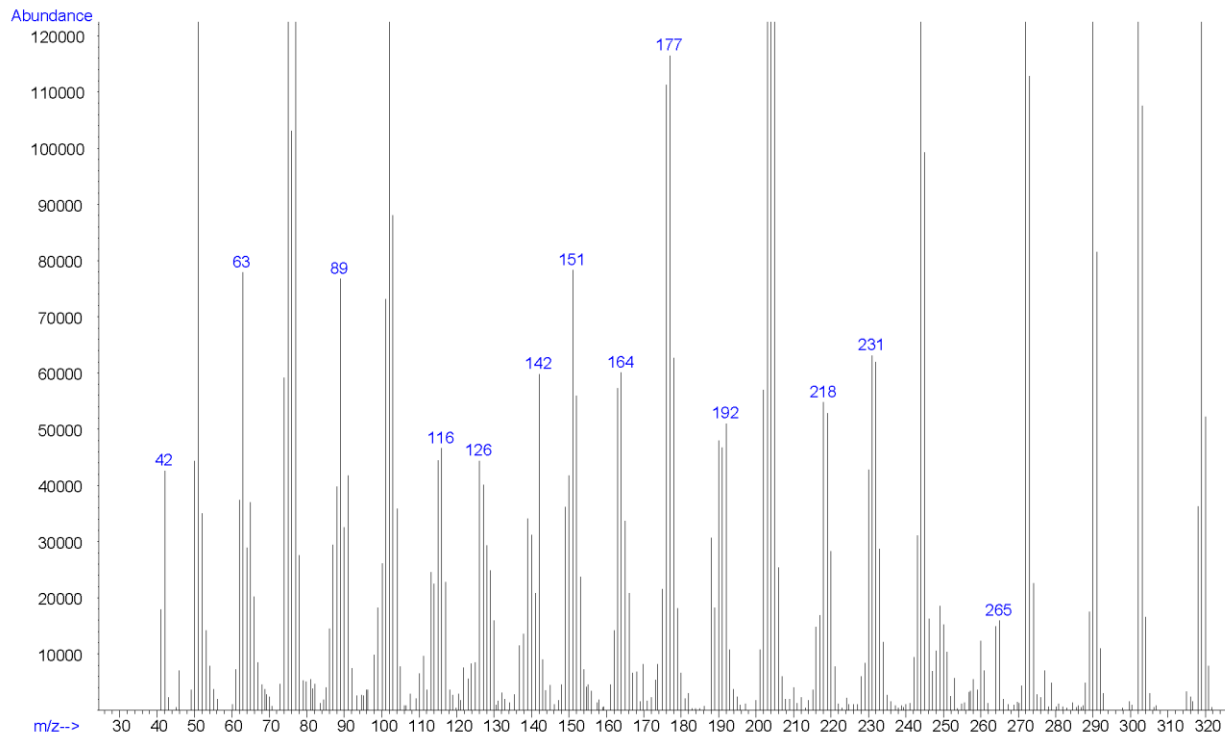
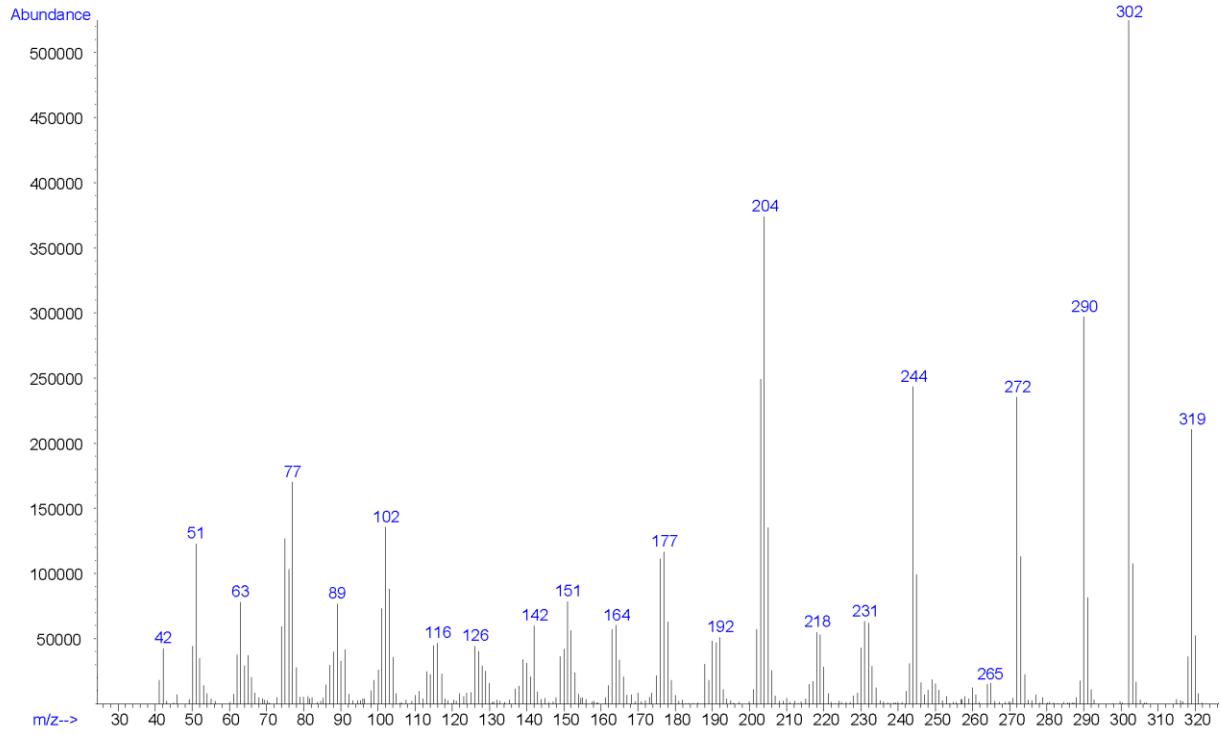
<b>Testing Performed At:</b>	NMS Labs (Willow Grove, PA)
<b>Sample Preparation:</b>	Acid/base extraction
<b>Instrument:</b>	Agilent 5975 Series GC/MSD System
<b>Column:</b>	Zebron™ Inferno™ ZB-35HT (15 m x 250 μm x 0.25 μm)
<b>Carrier Gas:</b>	Helium (Flow: 1 mL/min)
<b>Temperatures:</b>	Injection Port: 265 °C Transfer Line: 300 °C MS Source: 230 °C MS Quad: 150 °C Oven Program: 60 °C for 0.5 min, 35 °C/min to 340 °C for 6.5 min
<b>Injection Parameters:</b>	Injection Type: Splitless Injection Volume: 1 μL
<b>MS Parameters:</b>	Mass Scan Range: 40-550 m/z Threshold: 250
<b>Retention Time:</b>	9.294 min
<b>Standard Comparison:</b>	Reference material for Nitrazolam (Lot: B02521289) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Analysis of this standard resulted in positive identification of the analyte in the exhibit as Nitrazolam, based on retention time (9.229 min) and mass spectral data.

## Chromatogram: Nitrazolam



*Additional peaks present in chromatogram: internal standards (3.204 min and 6.286 min)*

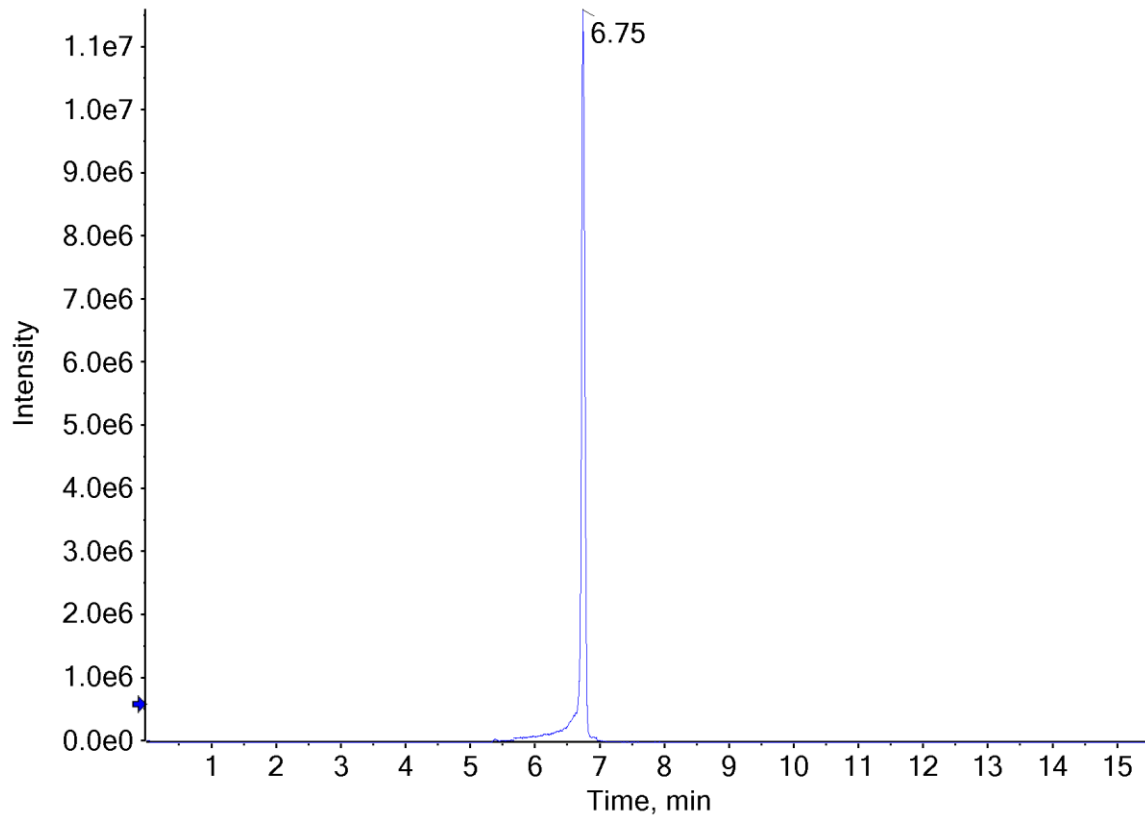
# EI (70 eV) Mass Spectrum (Top) and 10x (Bottom): Nitrazolam



## 5.2 LIQUID CHROMATOGRAPHY QUADRUPOLE TIME OF FLIGHT MASS SPECTROMETRY (LC-QTOF)

<b>Testing Performed At:</b>	The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)
<b>Sample Preparation:</b>	1:100 dilution of acid/base extraction in mobile phase
<b>Instrument:</b>	Sciex TripleTOF® 5600+, Shimadzu Nexera XR UHPLC
<b>Column:</b>	Phenomenex® Kinetex C18 (50 mm x 3.0 mm, 2.6 µm)
<b>Mobile Phase:</b>	A: Ammonium formate (10 mM, pH 3.0) B: Methanol/acetonitrile (50:50) Flow rate: 0.4 mL/min
<b>Gradient:</b>	Initial: 95A:5B; 5A:95B over 13 min; 95A:5B at 15.5 min
<b>Temperatures:</b>	Autosampler: 15 °C Column Oven: 30 °C Source Heater: 600 °C
<b>Injection Parameters:</b>	Injection Volume: 10 µL
<b>QTOF Parameters:</b>	TOF MS Scan Range: 100-510 Da Precursor Isolation: SWATH® acquisition (27 windows) Fragmentation: Collision Energy Spread (35±15 eV) MS/MS Scan Range: 50-510 Da
<b>Retention Time:</b>	6.75 min
<b>Standard Comparison:</b>	Reference material for Nitrazolam (Lot: B02521289) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Analysis of this standard resulted in positive identification of the analyte in the exhibit as Nitrazolam, based on retention time (6.53 min) and mass spectral data.

### Extracted Ion Chromatogram: Nitrazolam



### TOF MS (Top) and MS/MS (Bottom) Spectra: Nitrazolam

