

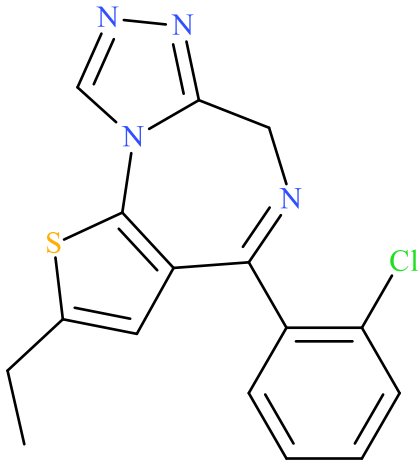
Metizolam

Sample Type: **Seized Material**

Latest Revision: **July 6, 2020**

Date Received: **March 3, 2020**

Date of Report: **July 6, 2020**



1. GENERAL INFORMATION

IUPAC Name:	4-(2-Chlorophenyl)-2-ethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine
InChI String:	InChI=1S/C16H13ClN4S/c1-2-10-7-12-15(11-5-3-4-6-13(11)17)18-8-14-20-19-9-21(14)16(12)22-10/h3-7,9H,2,8H2,1H3
CFR:	Not Scheduled (05/2020)
CAS#	40054-68-0
Synonyms:	Desmethyletizolam
Source:	Department of Homeland Security
Appearance:	White Solid Material

Important Note: All identifications were made based on evaluation of analytical data (GC-MS, LC-QTOF-MS, and NMR).

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2. CHEMICAL AND PHYSICAL DATA

2.1 CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Molecular Ion [M ⁺]	Exact Mass [M+H] ⁺
Base	C ₁₆ H ₁₃ ClN ₄ S	328.8	328	329.0622

3. BRIEF DESCRIPTION

Metizolam is classified as a novel benzodiazepine. Benzodiazepines are central nervous system depressants. Novel benzodiazepines, typically defined as emergent benzodiazepines not used medicinally, are often pirated from early drug discovery or pharmaceutical studies. Novel benzodiazepines have appeared on illicit drug markets in recent years and have caused adverse events, as described in the literature; fatalities linked to novel benzodiazepine use have occurred, commonly when used in combination with other depressants (e.g. opioids and alcohol). The synthesis of metizolam dates back to at least the 1970's based on previously published literature and patents.^{1,2,3} Metizolam was first identified among the illicit drug supply in 2015 by scientists in Germany.⁴ Metizolam is structurally similar to etizolam. Neither substance is federally controlled in the United States, however, some states have moved to control etizolam.

4. ADDITIONAL RESOURCES

1. Tahara, T.; Mikashima, H.; Terasawa, M; Maruyama, Y. (1987) PAF-antagonistic activity of some thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepines. *Chem. Pharm. Bull.*, **35**, 2119.
2. Nakanishi, M.; Tahara, T.; Araki, K.; Shiroki, M. (1975). Patent US 3904641 - Triazolothienodiazepine compounds. <https://patents.google.com/patent/US3904641>
3. Kitajima, H.; Ehara, S.; Sato, H.; Moriwaki, M.; Onishi, K. (1997) Patent EP 0776892 - Thienylazole compound and thienotriazolodiazepine compound. <https://patents.google.com/patent/EP0776892A1>
4. European Monitoring Centre for Drugs and Drug Addiction. (2016) EMCDDA–Europol 2015 Annual Report on the implementation of Council Decision 2005/387/JHA. *Implementation reports, Publications Office of the European Union, Luxembourg.* <http://www.emcdda.europa.eu/system/files/publications/2880/TDAS16001ENN.pdf>
5. Kintz, P.; Richeval, C.; Jamey, C.; Ameline, A.; Allorge, D.; Gaulier, J.; Raul, J. (2016) Detection of the designer benzodiazepine metizolam, in urine and preliminary data on its metabolism. *Drug Testing and Analysis.* **9** (7): 1026–1033.

4. ADDITIONAL RESOURCES (CONTINUED)

https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/Metizolam-ID-1576_16-report-250616.pdf

https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/Metizolam-ID-1562-16-report_080716.pdf

<https://en.wikipedia.org/wiki/Metizolam>

5. QUALITATIVE DATA

5.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

Testing Performed At: NMS Labs (Willow Grove, PA)

Sample Preparation: Acid/Base extraction

Instrument: Agilent 5975 Series GC/MSD System

Column: Zebtron™ Inferno™ ZB-35HT (15 m x 250 µm x 0.25 µm)

Carrier Gas: Helium (Flow: 1 mL/min)

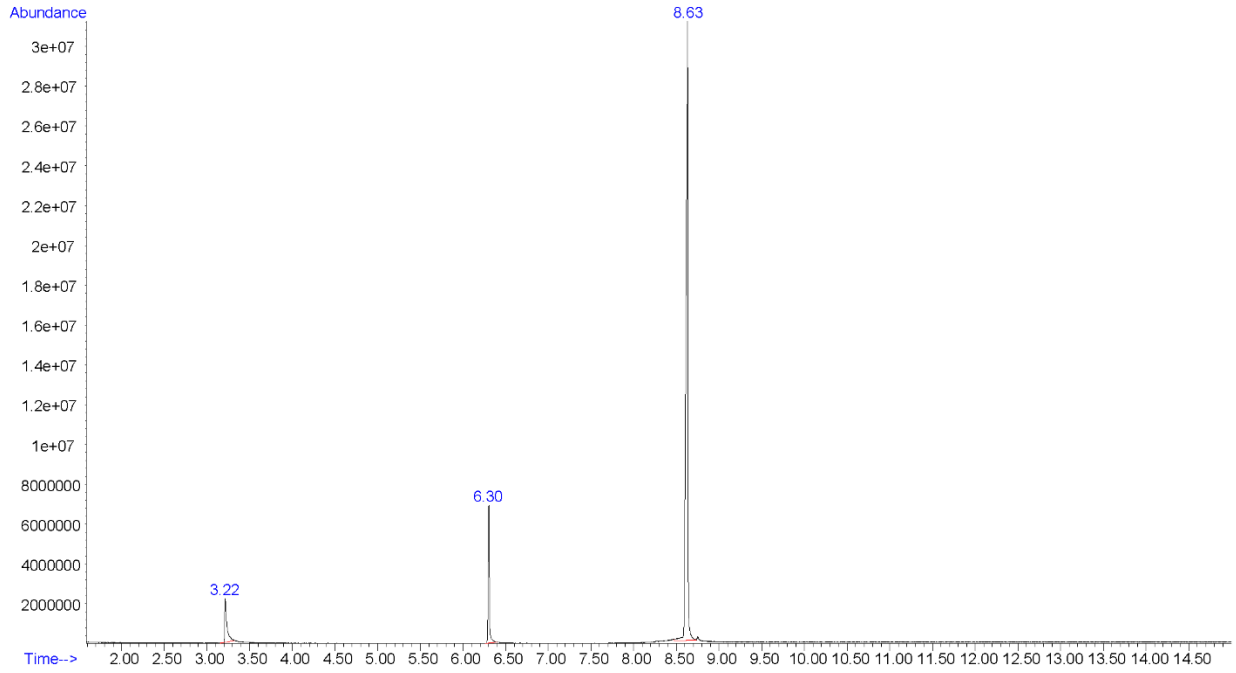
Temperatures: Injection Port: 265 °C
Transfer Line: 300 °C
MS Source: 230 °C
MS Quad: 150 °C
Oven Program: 60 °C for 0.5 min, 35 °C/min to 340 °C for 6.5 min

Injection Parameters: Injection Type: Splitless
Injection Volume: 1 µL

MS Parameters: Mass Scan Range: 40-550 m/z
Threshold: 250

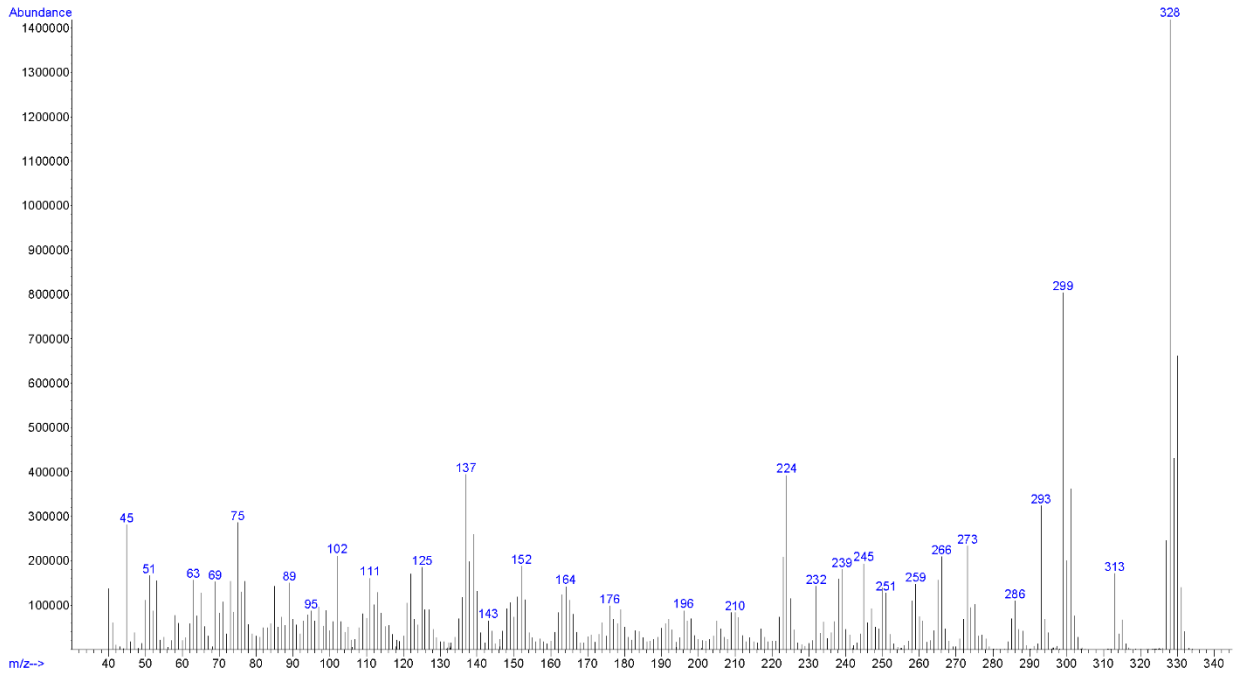
Retention Time: 8.63 min

Chromatogram: Metizolam

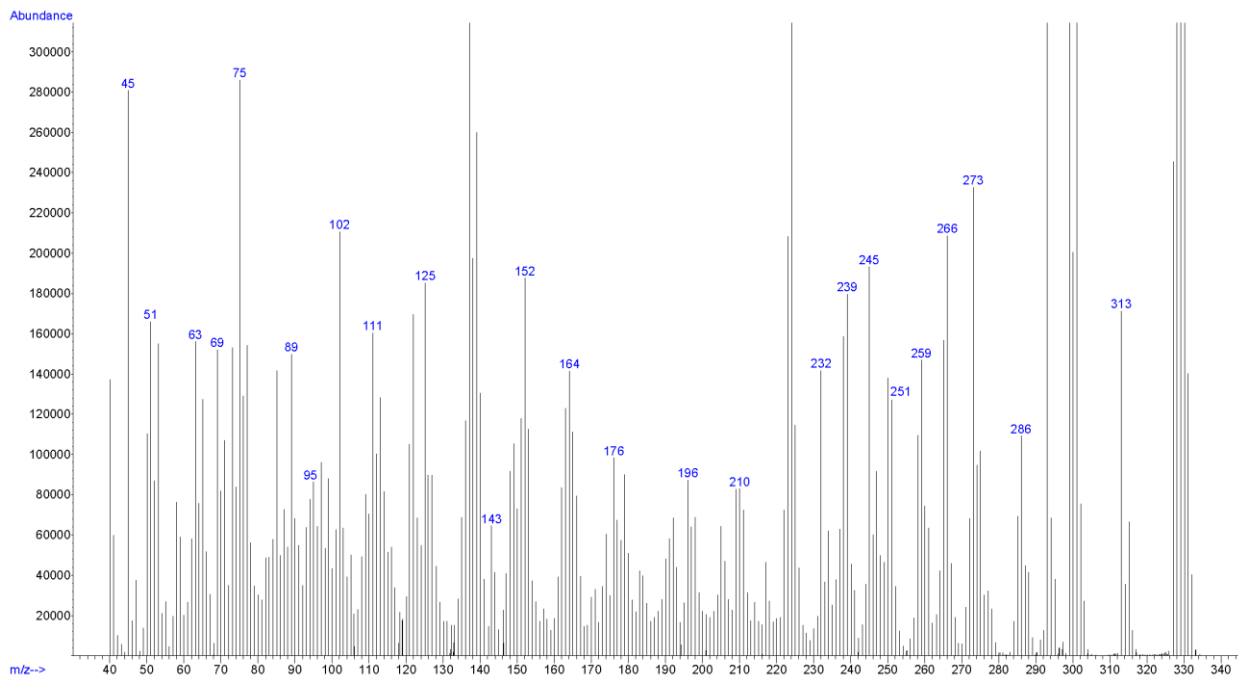


*Additional peaks present in chromatogram: internal standard (3.22 min)
and internal standard (6.30 min)*

EI (70 eV) Mass Spectrum: Metizolam



EI (70 eV) Mass Spectrum 10x Zoom: Metizolam



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

Testing Performed At: The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)

Sample Preparation: 1:100 dilution of acid/base extract in mobile phase

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

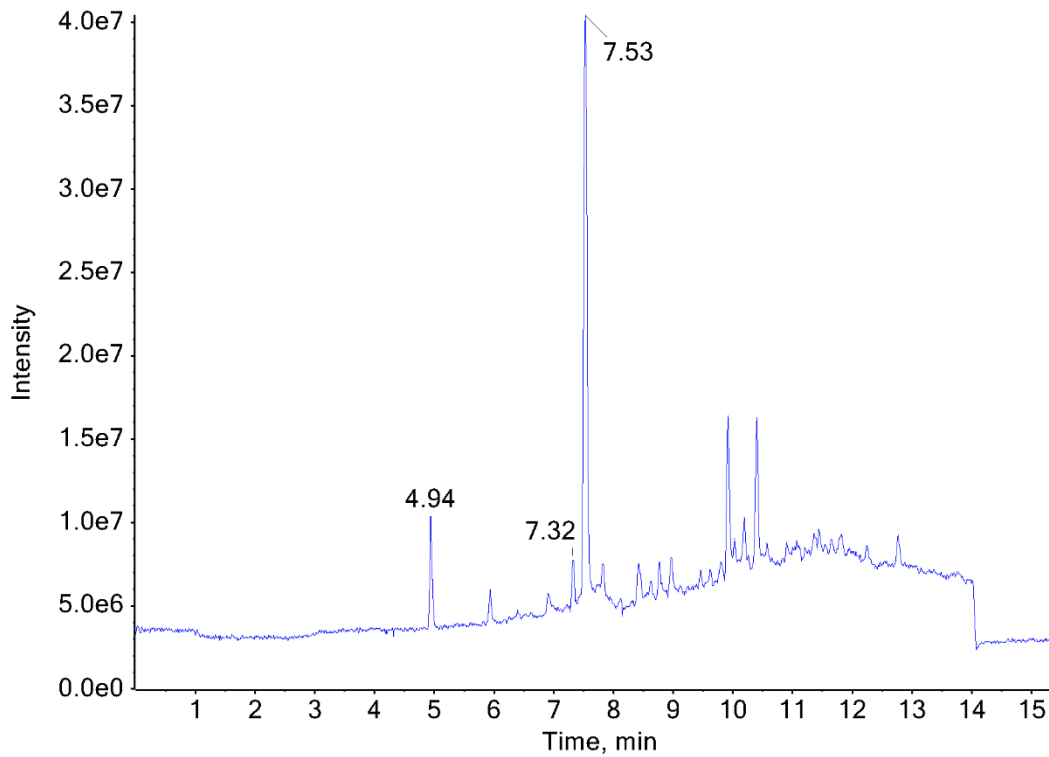
Precursor Isolation: SWATH® acquisition (27 windows)

Fragmentation: Collision Energy Spread (35±15 eV)

MS/MS Scan Range: 50-510 Da

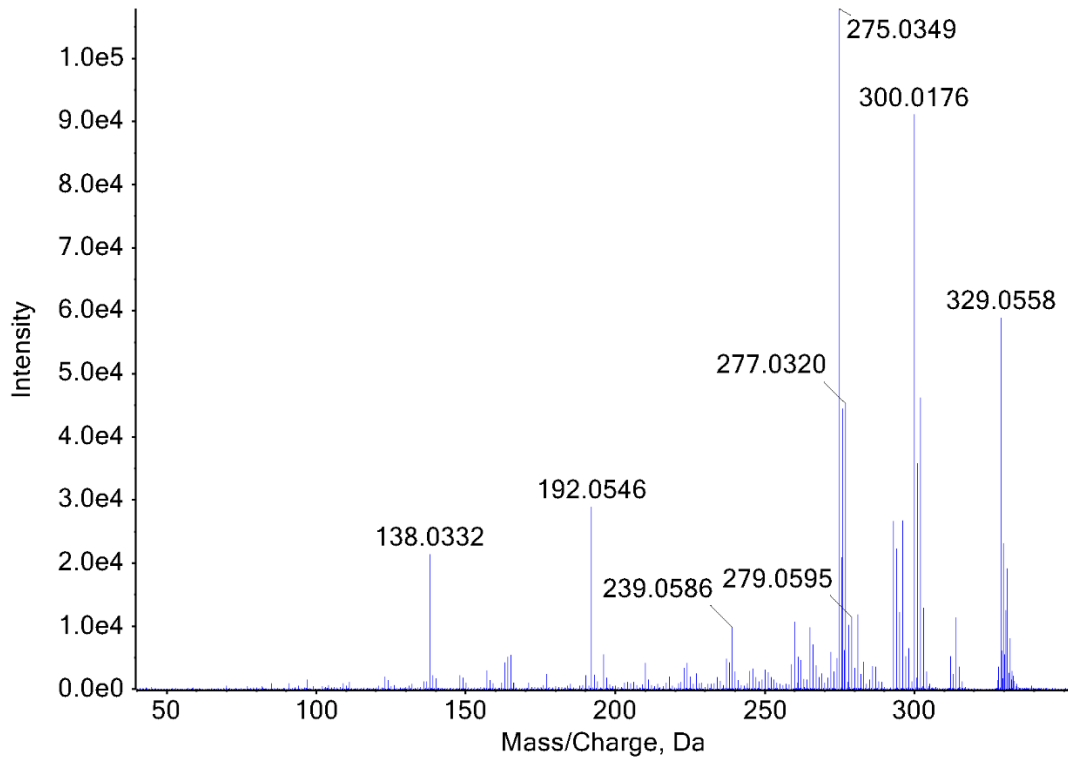
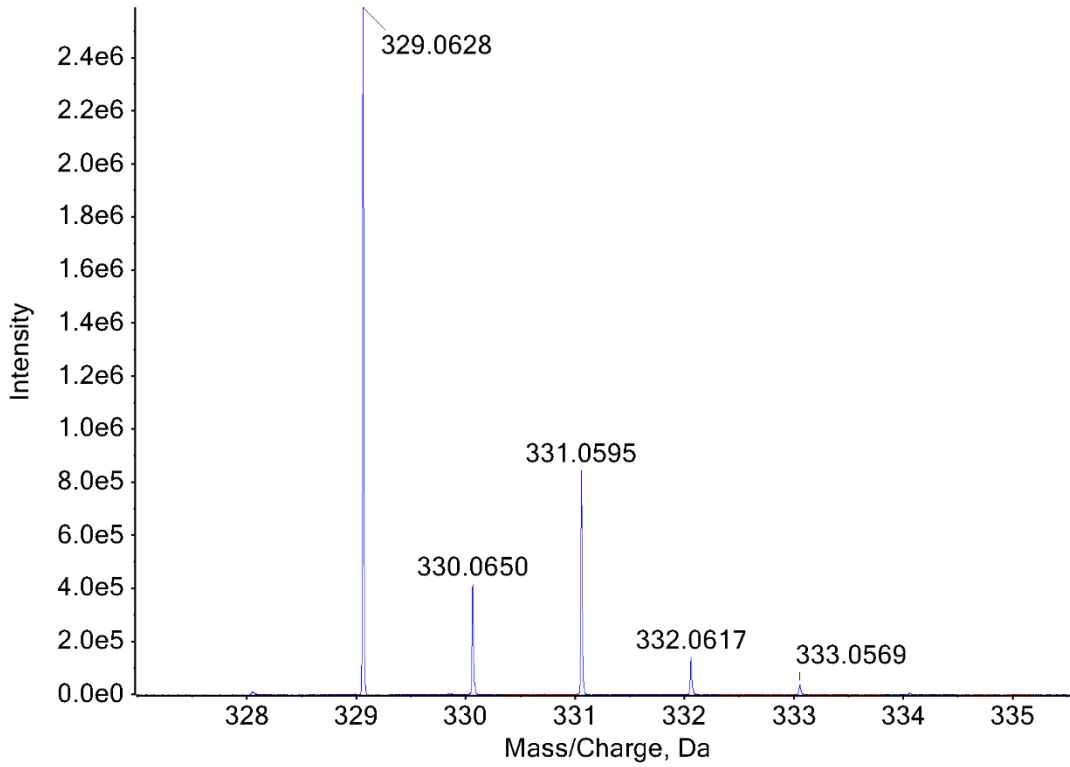
Retention Time: 7.53 min

Chromatogram: Metizolam

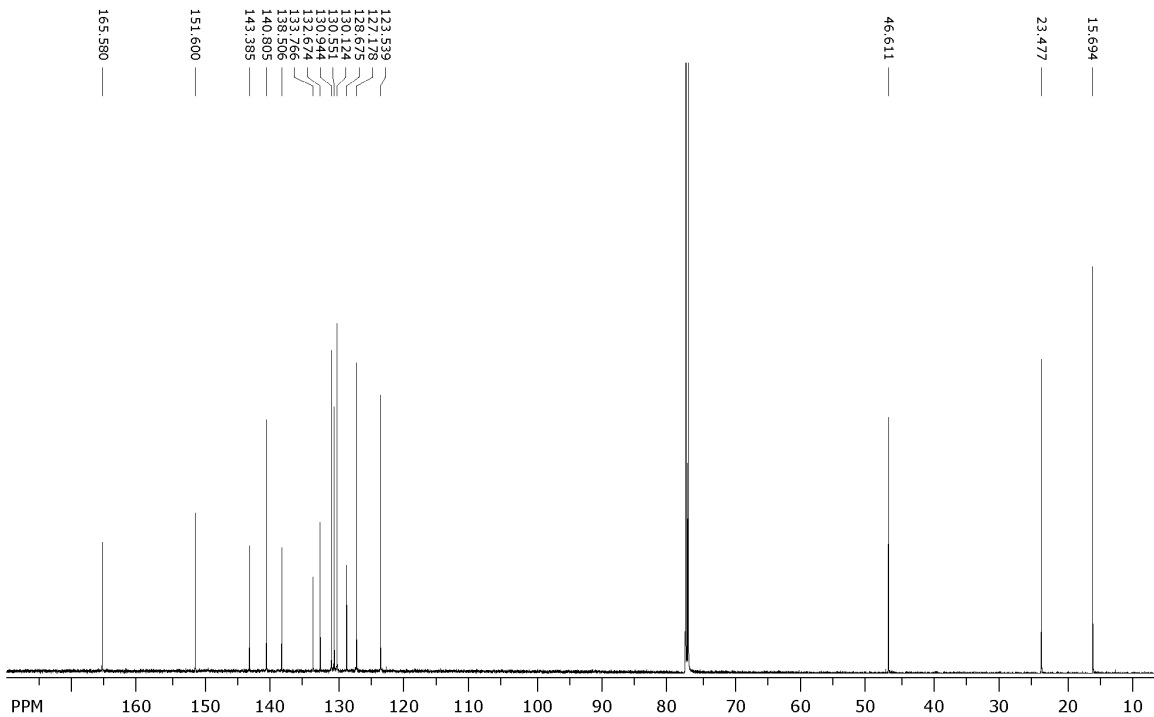


Additional peaks present in chromatogram: internal standards (4.94 min and 7.32 min)

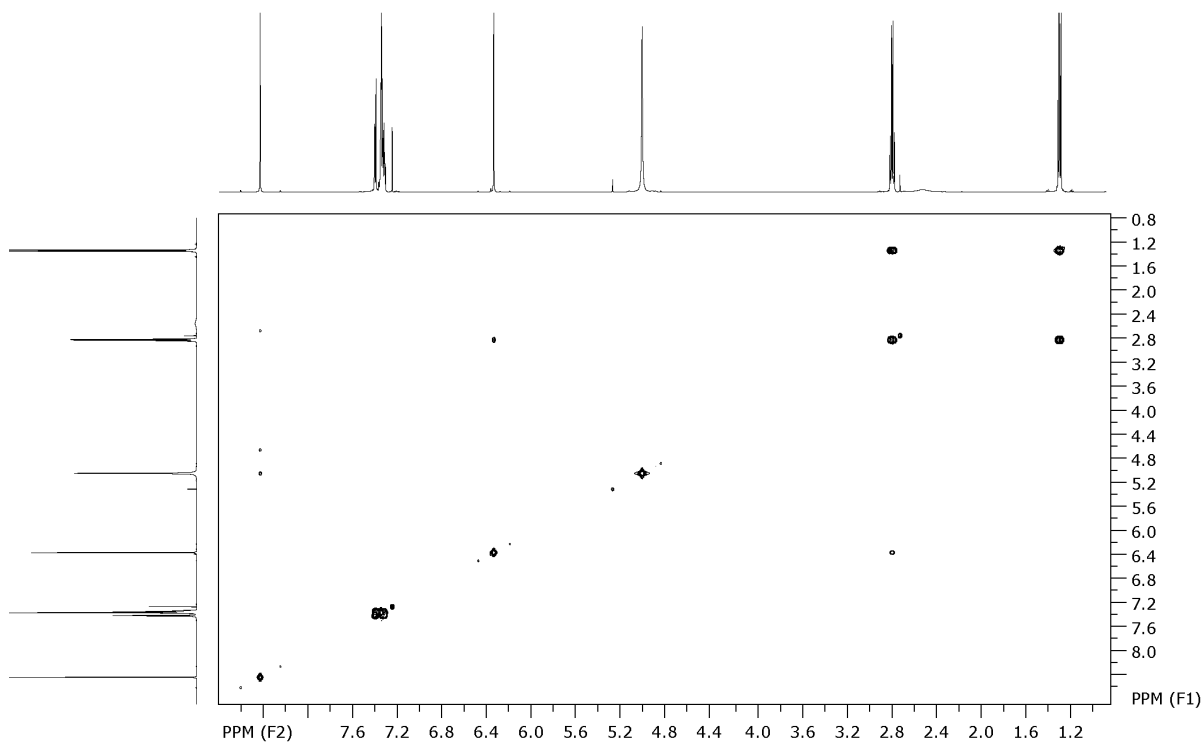
TOF MS (Top) and MS/MS (Bottom) Spectra: Metizolam



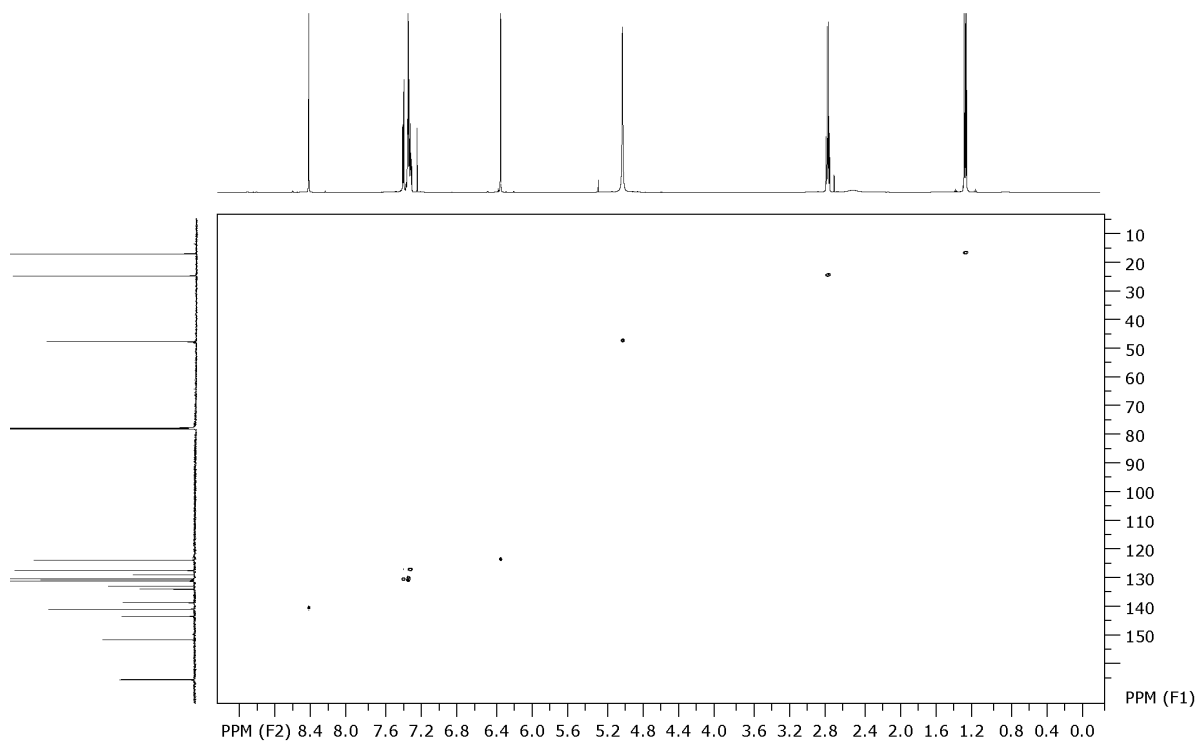
¹³C NMR: Metizolam



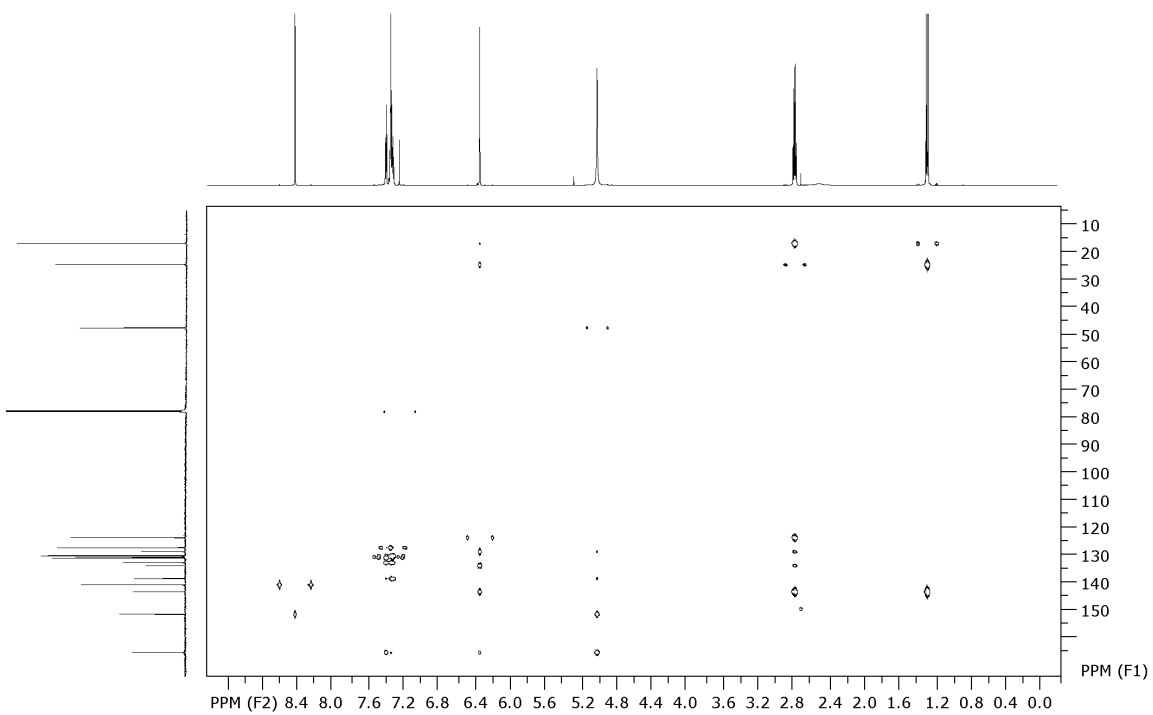
COSY NMR: Metizolam



HSQC NMR: Metizolam



HMBC NMR: Metizolam



HMBC NMR (Zoom): Metizolam

