DEA TOX

DRUG ENFORCEMENT ADMINISTRATION

TOXICOLOGY TESTING PROGRAM

University of California San Francisco

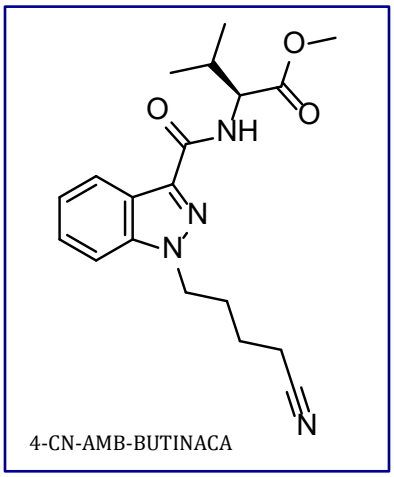
Announcement of a Newly Identified Synthetic Cannabinoid 4-CN-AMB-BUTINACA – December 17, 2020

The U.S. Drug Enforcement Administration (DEA) in collaboration with the University of California San Francisco Clinical Toxicology and Environmental Biomonitoring (CTEB) Laboratory has identified a new synthetic cannabinoid, 4-CN-AMB-BUTINACA (4-CYANO-AMB-BUTINACA), in urine samples submitted to our New Psychoactive Substances (NPS) surveillance program.

Cohort: In October 2020, prisoners under a work release program from Alabama who were suspected to be using tianeptine and the suspected synthetic cannabinoid-laced drug product, "No Show"

Drugs Detected: Tianeptine and the new synthetic cannabinoid, 4-CN-AMB-BUTINACA, were confirmed and quantified in a majority of the samples. Other substances detected in some of the samples include cathinones (NRG-3[1] and N-ethylbuphedrone (NEB)), and amphetamines (ethylamphetamine and PMMA[2]).

4-CN-AMB-BUTINACA: This new synthetic cannabinoid is related to 4-CN-CUMYL-BUTINACA (4-CN-CUMYL-BINACA), a synthetic cannabinoid placed in Schedule I by the DEA on July 13, 2020[3]. 4-CN-CUMYL-BUTINACA has been reported to be a potent cannabinoid receptor 1 (CB1) agonist (K_i= 2.6 nM; EC_{50}= 0.58 nM)[4] and is implicated in intoxication cases by the UCSF CTEB Lab[5] and other groups in Turkey and Europe.[6,7]



4-CN-AMB-BUTINACA

Analysis: 4-CN-AMB-BUTINACA was detected, confirmed and quantified in urine samples using liquid chromatography- quadrupole time-of-flight mass spectrometry. Details of the method used along with the chromatogram and mass spectra associated with the compound are presented in the attached supporting documents.

Reference Standard: As of the date of this release, there is currently no commercially available reference standard for 4-CN-AMB-BUTINACA. A reference standard of the compound was synthesized by our program collaborator at the University of Sydney, Dr. Samuel Banister. The compound is part of the "prophetic" cannabinoids library proactively synthesized to allow our surveillance to identify and confirm previously unreported NPS in real time. The chemical characterization of the reference standard was performed by Mr. Eric Sparkes and Dr. Adam Ametovski and is detailed in the attached supporting documents.

Pharmacological Data: Dr. Elizabeth Cairns and Ms. Charlotte Fletcher at the University of Sydney have collected preliminary pharmacological data on 4-CN-AMB-BUTINACA. Using a fluorescence-based imaging plate reader assay in AtT-20 cells stably transfected with human CB1 or CB2 receptors, 4-CN-AMB-BUTINACA (10 μM) elicited 99.7% ± 2.4% and 84.5% ± 3.6% of the maximal normalized response at human CB1 and CB2, respectively (n=3-4), confirming cannabinoid receptor agonist activity. A comprehensive pharmacological characterization of the compound is currently being conducted.

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[1] NRG-3 [2-(methylamino)-1-(napthalen-2-yl)pentan-1-one]

[2] PMMA [1-(4-methoxyphenyl)-*N*-methylpropan-2-amine; *para*-methoxymethamphetamine]

[3] Drug Enforcement Administration (DEA). Schedules of Controlled Substances: Placement of NM2201, 5F-AB-PINACA, 4-CN-CUMYL-BUTINACA, MMB-CHMICA and 5F-CUMYL-P7AICA in Schedule I. July 13, 2020. (Federal Register 85, Number 134)

[4] Kevin RC, Anderson L, McGregor IS, Boyd R, Manning JJ, Glass M, Connor M, Banister SD. 2019. Cumy-4-CN-BINACA is an efficacious and potent pro-convulsant synthetic cannabinoid receptor agonist. Front. Pharmacol. 10:595.

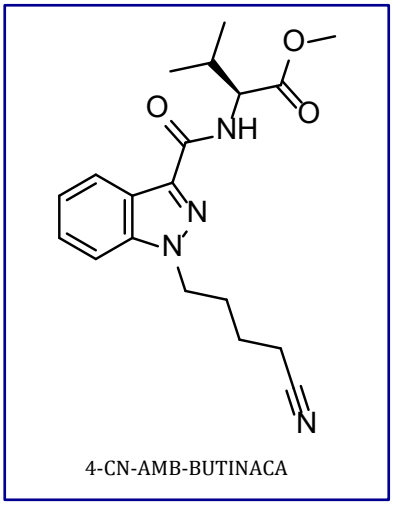
[5] El Zahran T, Gerona R, Morgan BQ, Pomerleau AC. 2019. A novel synthetic cannabinoid (Cumyl 4-CN-BINACA) resulting in hyperthermia, rhabdomyolysis, and renal failure in a 29-year-old patient: it's not meningitis. Clin. Toxicol. 57(5): 421-2.

[6] Ozturk YE. Yeter O, Ozturk S, Karakus G, Ates I, Buyuk K, Yurdun T. 2018. Detection of metabolites of the new synthetic cannabinoid Cumyl-4-CN-BINACA in authentic urine samples and human liver microsomes using high-resolution mass spectrometry. Drug Test. Anal. 10(3): 449-59.

[7] Astrand A, Vikingsson S, Lindstedt D, Thelander G, Green H, Kronstrand R, Wohlfarth A. 2018. Metabolism study for Cumyl-4-CN-BINACA in human hepatocytes and unthetic urine samples: free cyanide is formed during the main metabolic pathway. Drug Test. Anal. 2018 Mar 25. doi:10.1002/dta.2373

4-CN-AMB-BUTINACA

I. General Information



4-CN-AMB-BUTINACA

Synonyms: 4-CN-MMB-BUTINACA; AMB-4CN-BUTINACA; MMB-4CN-BUTINACA

IUPAC Name: methyl (*S*)-2-(1-(4-cyanobutyl)-1*H*-indazole-3-carboxamido)-3-methylbutanoate

InChi String: InChI=1S/C19H24N4O3/c1-13(2)16(19(25)26-3)21-18(24)17-14-9-5-6-10-15(14)23(22-17)12-8-4-7-11-20/h5-6,9-10,13,16H,4,7-8,12H2,1-3H3,(H,21,24)/t16-/m0/s1

InChi Key: WROCSMZNSLKYDI-INIZCTEOSA-N

SMILES:
O=C(N[C@@H](C(C)C)C(OC)=O)C1=NN(CCCCC#N)C2=C1C=CC=C2

CAS Number: Not available

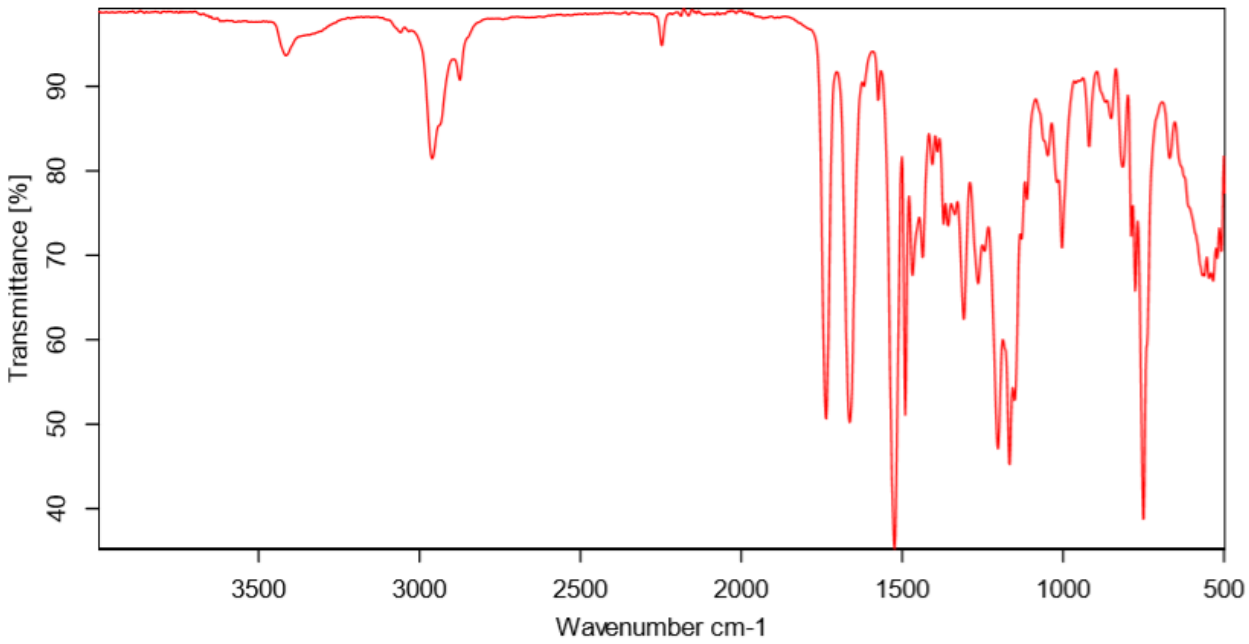
CFR: Not scheduled (12/2020)

II. Physical Properties

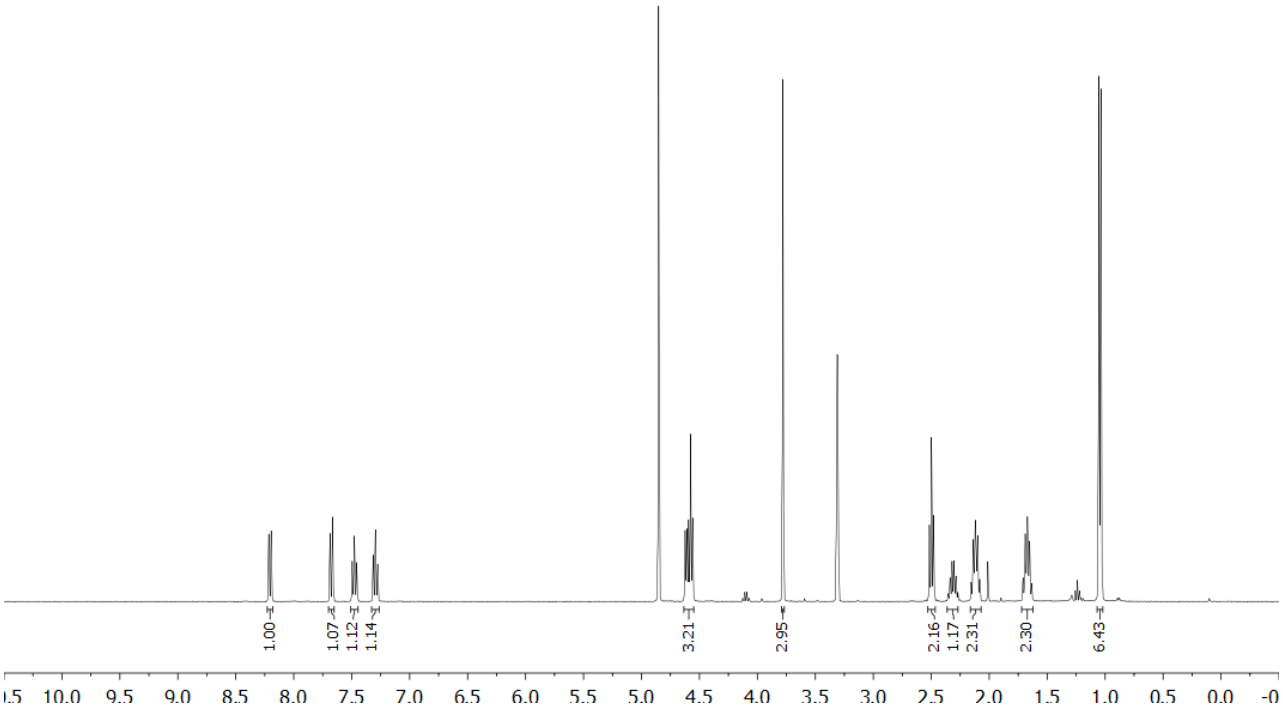
Melting Point: 141–143 °C

III. Chemical Characterization

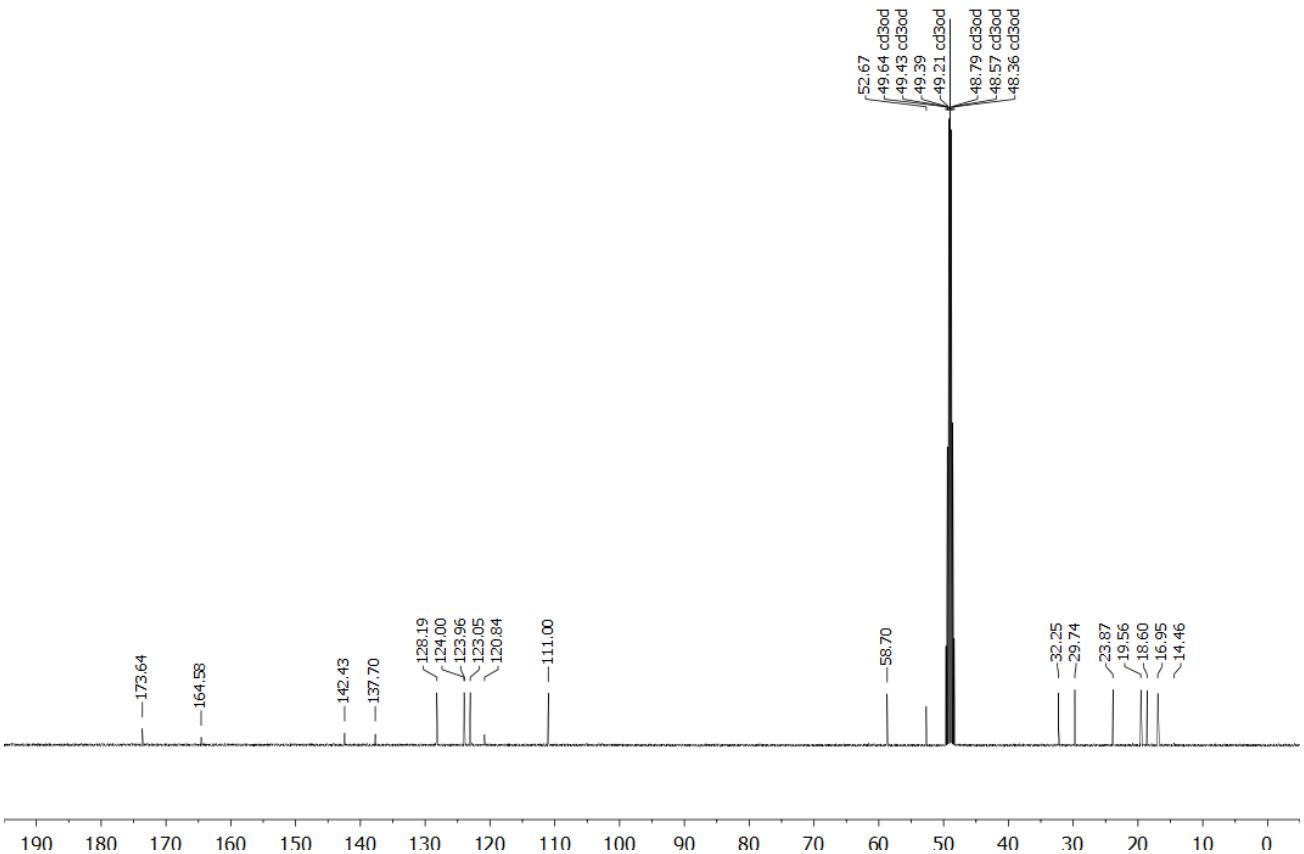
FTIR Spectrum: ν_{max} 2955, 1530, 1507, 1467, 1396, 1278, 1233, 1200, 1148, 1111, 992, 811, 770, 749, 599, 563, 506 cm^{-1}



^{1}H NMR Spectrum: (400 MHz, CD3OD) δ 8.22 (dd, J = 8.3, 1.3 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.53 – 7.46 (m, 1H), 7.34 – 7.28 (m, 1H), 4.66 – 4.56 (m, 3H), 3.80 (d, J = 1.3 Hz, 3H), 2.52 (t, J = 7.0 Hz, 2H), 2.33 (h, J = 6.7 Hz, 1H), 2.19 – 2.09 (m, 2H), 1.74 – 1.63 (m, 2H), 1.06 (dd, J = 6.9, 1.2 Hz, 6H), NH not observed (exchangeable).



^{13}C NMR Spectrum: (101 MHz, CD3OD) δ 173.6 (CO), 164.6 (CO), 142.4 (quat.), 137.7 (quat.), 128.2 (CH), 123.99 (CH), 123.96 (quat.), 123.1 (CH), 120.8 (CN), 111.0 (CH), 58.7 (CH), 52.7 (OCH3), 49.4 (NCH2), 32.3, 29.7, 23.9, 19.6 (CH3), 18.6 (CH3), 17.0 (CH2).



IV. LC-QTOF/MS Analysis

Instrument: Agilent 1260 Infinity, Agilent 6550 QTOF-MS/MS

Sample Preparation: Enzymatic deconjugation with *H pomatia* glucuronidase followed by dilution

Chromatography

***Column*:** Agilent Poroshell 120 EC-C18 (100 mm x 2.1 mm, 2.7 µm)

Column Temperature: 50 °C

Injection Volume: 2.5 µL

***Mobile Phase*:** A: Ammonium formate (5 mM) and Formic Acid (12.6 mM) in H_2O

B: Formic Acid (12.6mM) in acetonitrile

***Flow rate*:** 0.5 mL/min

***Elution Profile*:** Gradient- 95A:5B initially; 70A:30B from 0.5 to 1.5 min; 30A:70B from 1.5 to 4.5 min; 0A:100B from 4.5 to 7.5min; 95A:5B from 10.0 to 14.0 min

Run Time: 12 min

Mass Spectrometry

***Ion Source*:** Dual Jet Stream Electrospray Ionization

***Polarity*:** Positive

***TOF MS Scan Range*:** 75-1000 Da

***MS/MS Scan Range*:** 50-510 Da

Gas Temperature: 225 °C

Drying Gas Flow Rate: 14L/min

Sheath Gas Temperature: 350 °C

Sheath Gas Flow Rate: 11L/min

Nebulizer pressure: 14psi

Capillary Voltage: 3000 V

Nozzle Voltage: 500 V

Skimmer Voltage: 65 V

Octopole RF: 750 V

Fragmentor Voltage: 380 V

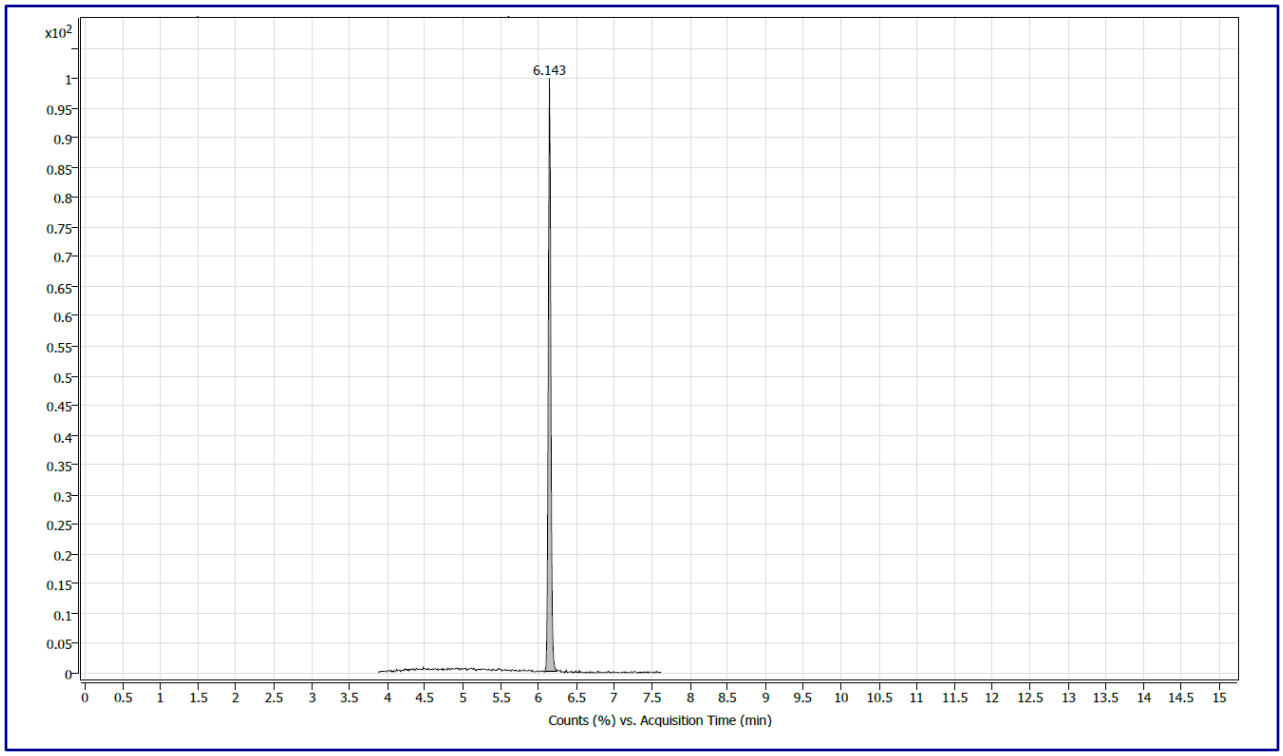
Internal Reference Masses: Purine at m/z 121.0509; HP-921 at m/z 922.0098

Data Acquisition: 2GHz, extended dynamic range

Fragmentation: Auto MS/MS, three maximum precursors (threshold: 500 counts) per cycle with active exclusion after 1 spectrum at a 30s release time

Extracted Ion Chromatogram

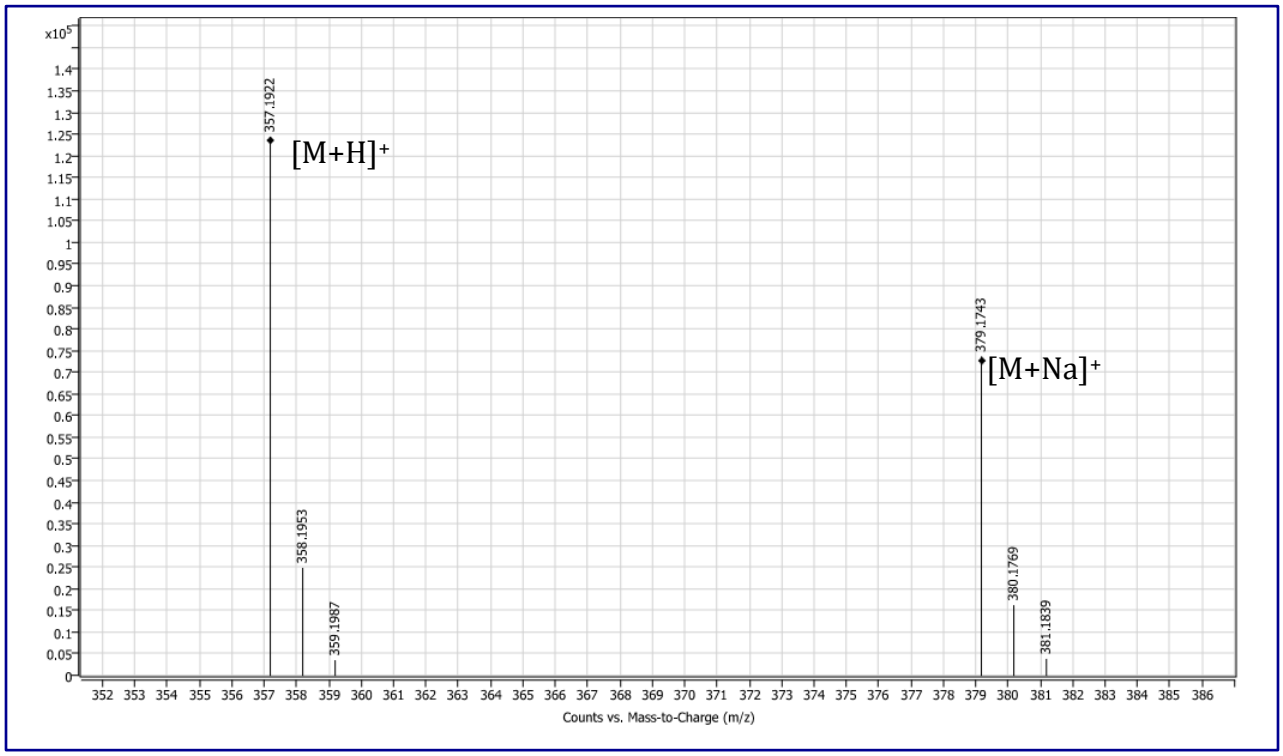
***Retention Time*:** 6.143 min



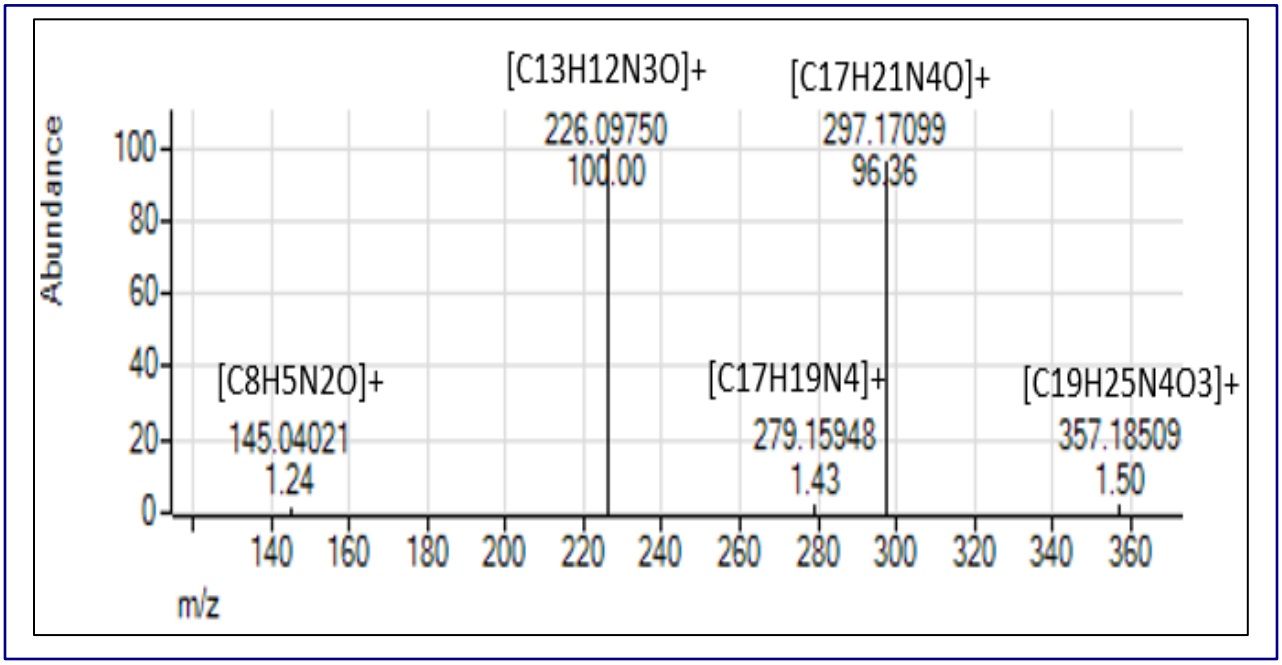
TOF-MS Spectrum

***Exact Mass*:** 356.1850

***Accurate Mass*:** $[M+H]^+$= 357.1992 (mass error=0.48ppm); $[M+Na]^+$= 379.1743 (mass error= 0.54ppm)



MS/MS Spectrum



U. S. Department of Justice
Drug Enforcement Administration
Diversion Control Division
Drug & Chemical Evaluation Section
Toxicology Testing Program
DEATOX@USDOJ.GOV

www.dea.gov

In response to the ongoing synthetic drug epidemic, the Drug Enforcement Administration (DEA) has initiated a contract with the University of California at San Francisco (UCSF) whereby biological samples generated from overdose victims of synthetic drugs can be further analyzed. In many cases, it can be difficult to ascertain the specific substance responsible for the overdose. We invite medical and law enforcement facilities to contact our program if you encounter an overdose of a suspected synthetic drug and desire to have any leftover biological samples (blood preferred) analyzed further for such synthetic substances.

- **Sample Qualifications:**
  - Patients thought to have ingested a synthetic drug, where the remainder of the drug screen has produced little or no viable options to explain the symptoms exhibited by the patient (alcohol and THC are exempted)
- **How to Contact Us and Send Your Samples:**
  - Once the above qualifications are satisfied:
    - Email **DEATOX@USDOJ.GOV** with a brief description of the case (including initial toxicology screen and history) and a request for testing.
    - If your request is approved by DEA, we will send instructions for packing and shipping your sample to UCSF.
      - The main reason for disapproval of a case would be the identification of substances including methamphetamine, heroin, fentanyl, cocaine, LSD, PCP etc. in a routine toxicology screening at your facility.
      - This program's goal is to connect symptom causation to abuse of newly emerging synthetic drugs (e.g. synthetic cannabinoids, synthetic cathinones, fentanyl-related substances, other hallucinogens etc.).
    - Ensure that you de-identify and label the sample with a numerical value, sex, date of birth or age, and the date and time the sample was collected in accordance with the labeling instructions (sent with shipping instructions).
    - Keep a master list of the patients and the numerical values you allocated to each sample at your institution.
- **Cost of sample analysis:**
  - DEA will cover the full cost of testing the patient samples.
  - The sender will only be responsible for paying for packing and shipping samples to UCSF.
- **Turn-around Time:**
  - Results are expected within three weeks of receipt of the sample at UCSF except in rare occurrences when a novel substance is identified.
- **For more information: https://www.deadiversion.usdoj.gov/dea_tox/index.html**