Purification and Characterization of 3-Methyl-6-[3-(trifluoromethyl)-phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872)

Edward P. Erisman* and Luke M Augustine
U.S. Department of Justice
Drug Enforcement Administration
North Central Laboratory
536 S. Clark Street, Room 800
Chicago, IL 60605
[edward.p.erisman@usdoj.gov]

ABSTRACT: The purification, identification, and characterization of 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]-pyridazine (CL 218872) is presented. Analytical data (mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy) is included.

KEYWORDS: 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]pyridazine, CL 218872, synthetic cannabinoid, GC/MS, FTIR, NMR, forensic chemistry

In June 2012, three exhibits of adulterated plant material were analyzed at the DEA North Central Laboratory and were found to contain 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872) in a mixture of synthetic cannabinoids. Two of the exhibits also contained 1-(5-fluoropentyl)-3-(2,2,3,3-tetramethylcyclopropoyl)indole (XLR-11) and 1-pentyl-N-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-1H-indazole-3-carboxamide (AKB48). The other exhibit also contained XLR-11 and suspected 1-(5-fluoropentyl)-N-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-1H-indole-3-carboxamide (STS-135). To the best of our knowledge CL 218872 had not been previously identified in synthetic cannabinoid exhibits, although its presence was suspected in numerous exhibits analyzed by other laboratories in the United States [1]. First synthesized by Albright et al. in the 1970s, CL 218872 is a benzodiazepine agonist that does not appear to have been developed into a commercial pharmaceutical product [2] and is currently not scheduled in the United States. The analytical profile for CL 218872 is presented herein.

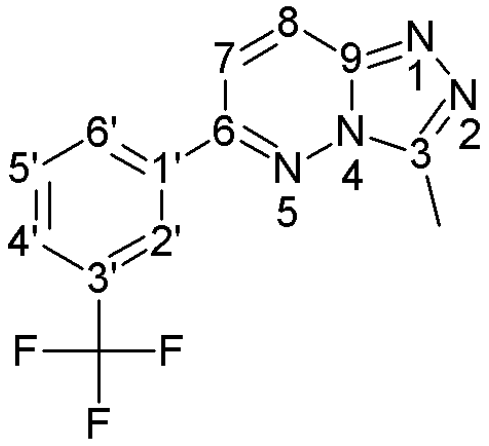

Figure 1 - Structure of 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872)
Chemical Formula: $C_{13}H_9F_3N_4$
Molecular Formula: 278.24 amu
Exact Mass: 278.0779 amu
CAS Number: 66548-69-4

Experimental

Chemicals, Reagents, and Materials

Methanol was obtained from EMD (Billerica, MA) and Fisher Scientific (Pittsburg, PA). Deuterochlroform was obtained from Aldrich Chemical (Milwaukee, WI). 18MΩ water was obtained from a Millipore filtration system. A CL 218872 reference standard was obtained from Tocris Biosciences (Bristol, United Kingdom).

Gas Chromatography/Mass Spectrometry (GC/MS)

The mass spectrum of CL 218872 was acquired using an Agilent Model 5975C quadrapole mass-selective detector (MSD) interfaced with an Agilent 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV and in scan mode with a mass range of 35-500 amu. Samples were extracted with methanol and 1.0 µL was injected with a split ration of 50:1 onto a 30 m x 250 µm I.D. x 0.25 µm 5% phenyl 95% dimethylpolysiloxane (HP-5MS) column. The oven temperature was held at an initial temperature of 80°C for 1.5 minutes, ramped at 30°C/minute to 320°C, and held for 10 minutes.

Purification by High Performance Liquid Chromatography (HPLC)

The sample was purified in order to obtain FTIR and NMR data. Purification was carried out on an Agilent 1200 series HPLC equipped with a quaternary pump, autosampler, and diode array detector. A solvent system consisting of 85:15 MeOH:H_2O, with a flow rate of 1.5 mL/min was used with a Supelco-ODS column (15 cm x 4.6 mm, 3 µm particle size), and an injection volume of 100 µL. Multiple fractions were manually collected, combined, and evaporated on a 75°C hot plate under an air stream. This method allowed for purification of the suspected CL 218872, which had the shortest retention time of the three major sample components, but gave poor chromatography due to the high sample loading.

Fourier Transform Infrared Spectroscopy (FTIR)

The spectra were collected using a diamond attenuated total reflectance (ATR) accessory on a Nicolet 6700 FTIR (Thermo

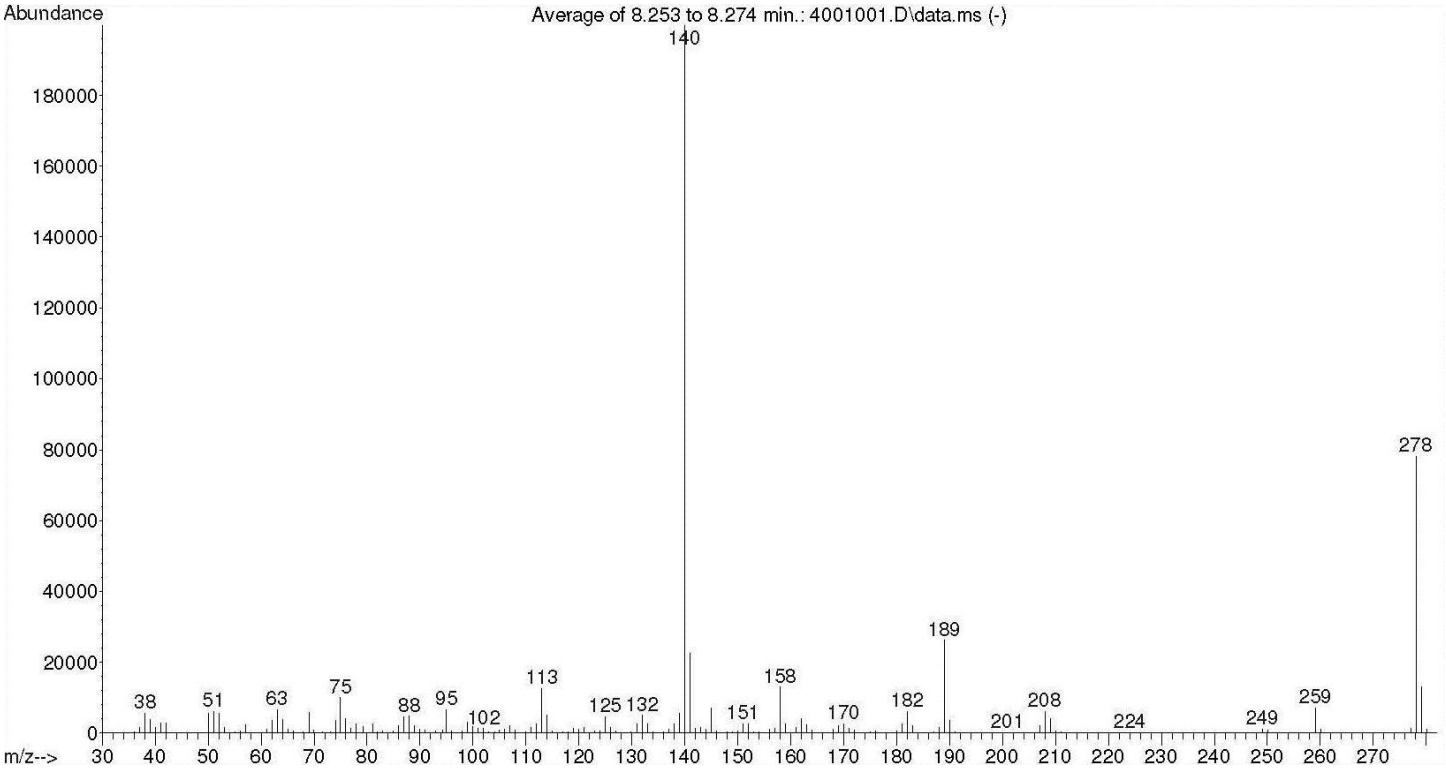

Figure 2 - EI mass spectrum of CL 218872 reference standard.

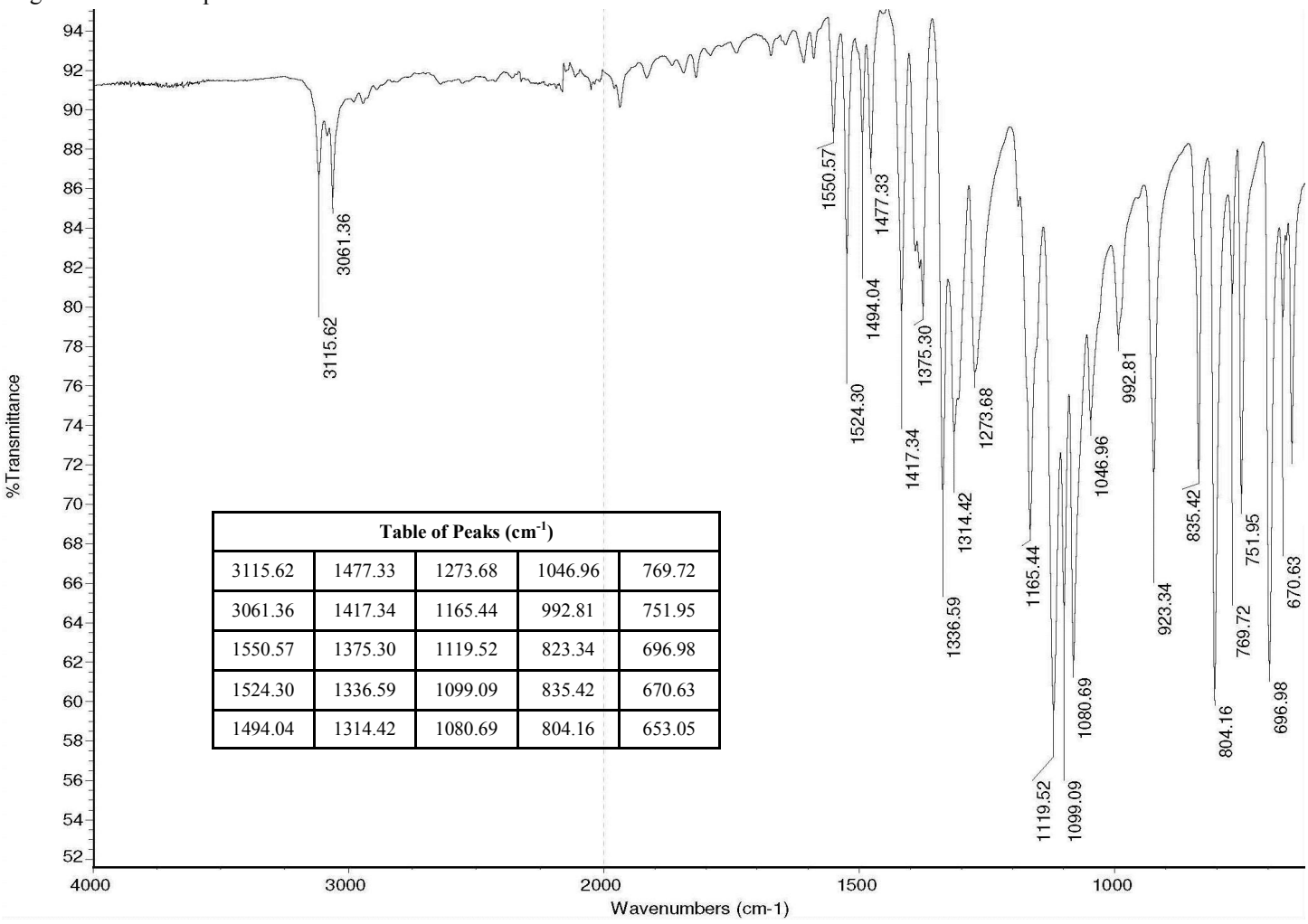

Table of Peaks (cm^{-1})				
3115.62	1477.33	1273.68	1046.96	769.72
3061.36	1417.34	1165.44	992.81	751.95
1550.57	1375.30	1119.52	823.34	696.98
1524.30	1336.59	1099.09	835.42	670.63
1494.04	1314.42	1080.69	804.16	653.05

Figure 3 - FTIR spectrum of CL 218872 reference standard.

Scientific). The spectrum was collected with a resolution of 4 cm^{-1}, spectral range of 525-4000 cm^{-1}, optical velocity of 0.3165 cm/s, an aperture of 80, and 8 scans/sample.

Nuclear Magnetic Resonance (NMR)

The purified sample and the standard were dissolved in CDCl3 and spectra were acquired with standard Varian pulse sequences [3] on a Varian Model 400-MR with Varian AutoX Indirect Detection Pulse Field Gradient probe. Spectra for proton, carbon, and 2-D NMR (HSQC, HMBC, 15N CIGAR) were collected.

Results and Discussion

Gas Chromatography/Mass Spectrometry (GC/MS)

The mass spectrum of CL 218872 (Figure 2) is dissimilar to known synthetic cannabinoids [4] and to other compounds present in several mass spectral libraries routinely used for comparison purposes at the North Central Laboratory. CL 218872 has a base peak of 140 *m/z* and molecular ion of 278 *m/z*. In the mass spectrum, multiple losses of 19 *m/z* and a loss of 70 *m/z* indicated the possibility of one or more fluorine atoms which were possibly from a CF_3 group [5]. The DEA Northeast Laboratory (New York, New York) analyzed an exhibit presumably containing the same compound in which electrospray ionization mass spectroscopy (ESI-MS) data and accurate mass data was collected. The mass $[M+H]^+$ was determined to be 279.0852 amu, which correlated well with a proposed molecular formula of $C_{13}H_9N_4F_3$ [6].

Fourier Transform Infrared Spectroscopy (FTIR)

The spectrum of the purified component of the sample (Figure 3) contained neither ketone band nor any readily discernible bands for common functional groups, but did indicate that the compound was aromatic [7]. The best match from a search of several spectral libraries of interest was a poor quality match for fenfluramine.

Nuclear Magnetic Resonance (NMR)

Integration of the proton NMR (Figure 4) for the purified unknown showed a total of nine protons- one methyl singlet (3H) and six protons in the aromatic region- one triplet (1H), four doublets (4H) , and one singlet (1H). The carbon NMR

Position	Shift (ppm)	H's	Type	J (Hz)
CH3	2.91	3	s	-
7	7.58	1	d	9.78
5'	7.71	1	t	7.80
4'	7.83	1	d	7.83
8	8.20	1	d	9.80
6'	8.20	1	d	7.80
2'	8.26	1	s	-

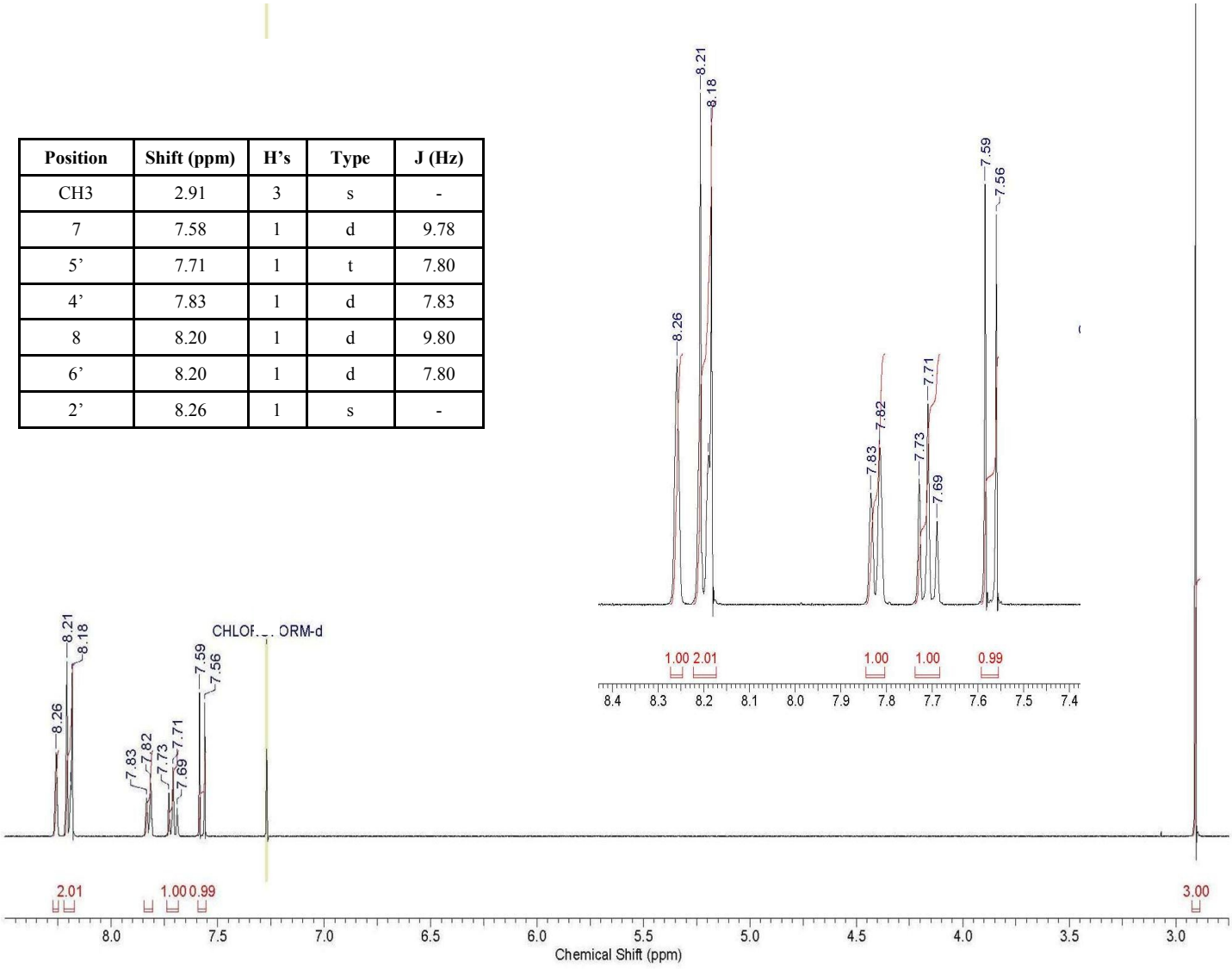

Figure 4 - 400 MHz proton NMR spectrum of CL 218872 reference standard.

Position	Shift (ppm)	Type	J_{CF} (Hz)
CH3	9.88	s	-
7	118.30	s	-
CF3	123.73	q	272.50
2’	124.12	q	3.90
8	125.52	s	-
4’	127.47	q	3.90
5’	129.85	s	-
6’	130.46	s	-
3’	131.84	q	33.00
1’	135.31	s	-
9	143.27	s	-
3	147.66	s	-
6	152.03	s	-

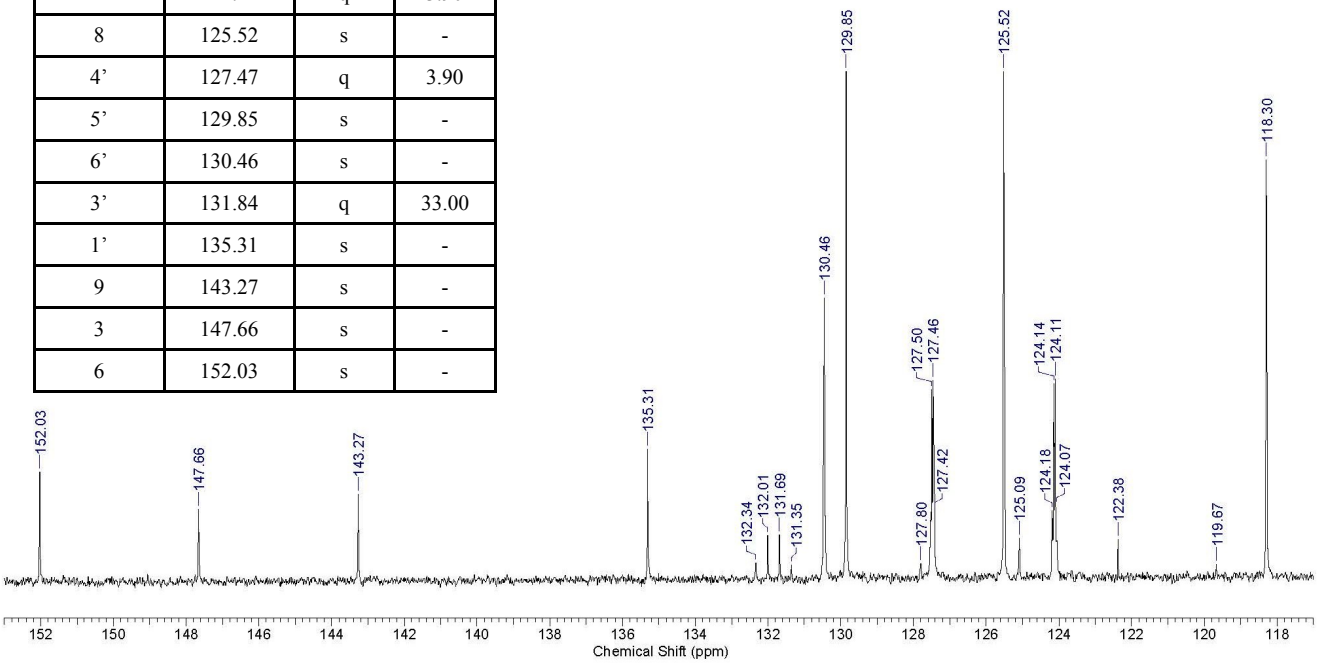

Figure 5 - 100 MHz carbon spectrum of CL 218872 reference standard.

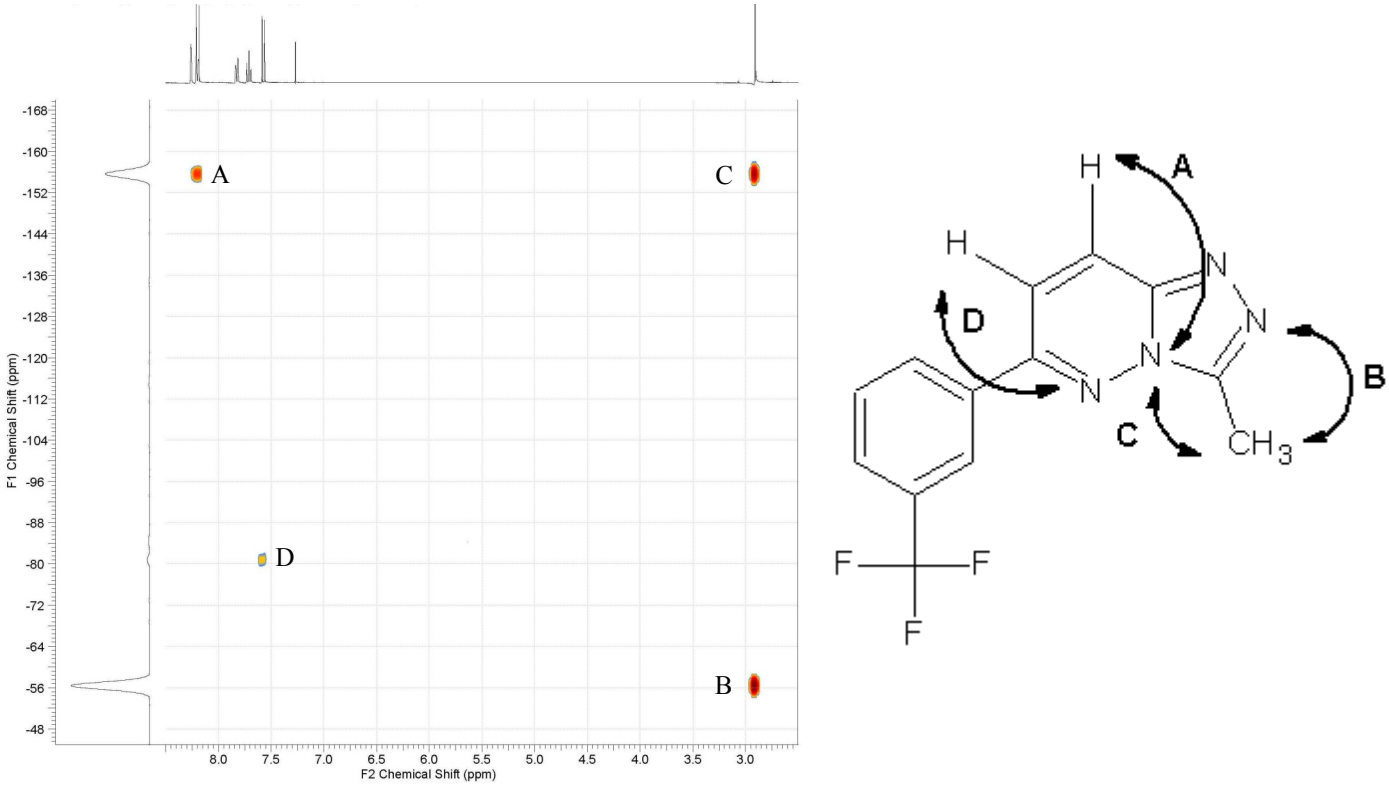

Figure 6 - ^{15}N-CIGAR NMR of the CL 218872 reference standard showing N-H couplings of 2-3 bond lengths and proposed couplings. (Note: No chemical shift reference used in the nitrogen dimension (F1))

(Figure 5) for the purified unknown was weak making an accurate counting of the carbon atoms difficult and was exacerbated by the fluorine splitting of the carbon signals. The peak at 131.84 ppm was split into a quartet (J_{CF} = 33.0 Hz) and the peaks at 124.12 ppm and 127.47 ppm appeared to be split into doublets or quartets (depending on the line broadening used) (J_{CF} = 3.90 Hz), suggesting a CF_3 group. The data from the reference material showed the wide coupling characteristic of a CF_3 group (J_{CF} = 272.50 Hz) making it initially difficult to discern if there was a carbon multiplet or multiple carbons signals. The assignments of all protons and carbons are listed in tables in Figures 4 and 5, respectively. The 15N CIGAR experiment (2-3 bond length N-H hetero-nuclear interactions) provided data supporting the assignments for the H-7 and H-8 protons. Proposed nitrogen assignments are in figure 7, but further understanding of the 15N CIGAR experiment is needed to make a definitive assignment of the nitrogen signals.

References

1. http://forendexforum.southernforensic.org/viewtopic.php?f=4&t=145. original post 4/6/2012.
2. JD Albright, et. al. Synthesis and Anxiolytic Activity of 6-(substituted-phenyl)-1,2,4-triazolo[4,3-b]pyridazines. J. Med. Chem. 1980;24,592-600.
3. Agilent VnmrJ 3.2 Experiment Guide, Agilent Technologies, Revision A, 2011.
4. Advisory Council on the Misuse of Drugs, *Consideration of the cannabinoid agonists*, London, 2009.
5. McLafferty F. *Interpretation of Mass Spectra*, 4th ed. University Science Books, Sausalito, CA, 1993.
6. Personal communication with Yuriy Uvaydov.
7. Silverstein R. *Spectrometric Identification of Organic Compounds*, 7th ed. John Wiley & Sons, Inc., Hoboken, NJ, 2005.