

Rapid Emerging Drug Deployment (REMEDY) Characterization Results

Item identifier: RP0001

Date of report: February 24, 2022

Analysts: Edward Sisco & Aaron Urbas

Summary Results

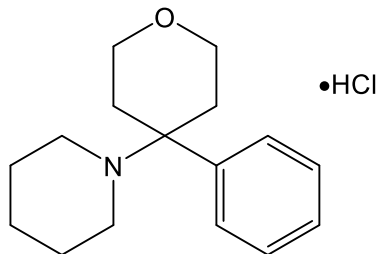
Qualitative identity of compound: 1-(4-phenyltetrahydro-2H-pyran-4-yl)piperidine hydrochloride

Synonyms (if known): POXP; 1-(4-phenyloxan-4-yl)piperidine hydrochloride

Chemical formula: C₁₆H₂₃NO

Monoisotopic Molecular Mass: 245.1774 Da

InChiKey (Neutral Molecule): MLIUPPBENLKXSU-UHFFFAOYSA-N



Structure:

Purity (if measured): Not measured

Sample characteristics: White Powder

Sample origin: Seized substance provided by collaborating laboratory

Analytical techniques used: Nuclear magnetic resonance spectroscopy (NMR), direct analysis in real time mass spectrometry (DART-MS), gas chromatography mass spectrometry (GC-MS), gas chromatography flame ionization detection (GC-FID), and liquid chromatography mass spectrometry (LC-MS)

Note: Supporting data and supplementary information can be found at the following link:
<https://doi.org/10.18434/mds2-2527>.

Disclaimer: Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

Analytical Results - NMR

Instrument and method used: Measurements were made using a Bruker Avance II 600 MHz NMR equipped with a broadband-inverse (BBI) probe. A single aliquot (approximately 10 mg) of the sample was used for all NMR analysis. Multiple 1D and 2D spectra were collected to characterize the sample including ^1H and ^{13}C , ^1H COSY (correlated spectroscopy), ^1H - ^{13}C HSQC (heteronuclear single quantum coherence), ^1H - ^{13}C HMBC (heteronuclear multiple bond correlation), and 1D ^1H NOE (nuclear Overhauser effect). Acquisition parameters for the experiments are given in Table 1. The residual solvent peak of CDCl_3 was used as the ^1H chemical shift reference and assigned a value of 7.260 ppm. The chemical shift axis scale of the remaining nuclei was established from this according to the IUPAC unified scale.

Table 1. Acquisition parameters for 1D and 2D NMR spectral data.

Parameter	^1H 1D	^{13}C 1D	HSQC-EDITED (^1H , ^{13}C)	HMBC (^1H , ^{13}C)	COSY (^1H , ^1H)	^1H 1D NOE
Pulse Sequence	zg (90 deg pulse)	zpgg (90 deg pulse)	hsqcetedtgpsisp2.3	hmhbcgplpndqf	cosygpppqf	selnogp
Number of Scans	32	1024	2	8	4	256
Relaxation Delay (s)	25	4	2	1.498	1.9947	2
Acquisition Time (s)	5.4526	0.9088	0.142	0.1331	0.1331	2.7263
Spectrometer Frequency (MHz)	600.13	150.92	(600.13, 150.91)	(600.13, 150.92)	(600.13, 600.13)	600.13
Spectral Width (Hz)	12019.2	36057.7	(7211.5, 24875.6)	(7692.3, 33557.0)	(7692.3, 7692.3)	12019.2
Lowest Frequency (Hz)	-2321.8	-2943.9	(-803.4, -1879.2)	(-15.5, -1717.6)	(28.3, 28.3)	-2321.8
Spectral Width (ppm)	20.03	238.92	(12.02, 164.83)	(12.82, 222.35)	(12.82, 12.82)	20.03
Acquired Size	65536	32768	(1024, 256)	(1024, 256)	(1024, 256)	32768

Form sample was analyzed in: A CDCl_3 (D, 99.96%) solution with an approximate concentration of 16 mg/mL.

Controls used: A sample of phencyclidine (PCP) hydrochloride (Cayman Chemical, #14276) as obtained and run in CDCl_3 for comparison.

Results: The sample aliquot was dissolved in $\approx 700 \mu\text{L}$ of CDCl_3 and found to be readily soluble. All 1D and 2D NMR spectra were acquired from this sample. After initial spectral processing the data set was analyzed for proton counts, proton and carbon peak locations, 1-bond ^1H - ^{13}C connectivity and ^1H - ^1H and ^1H - ^{13}C correlations. A broad proton signal was observed at 11.80 ppm with no corresponding ^1H - ^{13}C HSQC correlation that was attributed to a protonated amine in solution. This data and a molecular formula of $\text{C}_{16}\text{H}_{24}\text{NO}$ were used in the structure elucidation tool in MNova (14.2.2) to identify potential chemical

structures. A molecular formula of $C_{16}H_{23}NO$ was indicated from DART-MS data and an additional proton was added based on the protonated amine. The best structure identified in the structure elucidation tool corresponded to the structure describe below. Additional information about this analysis is provided in the appendix.

The confirmed structure, atom numbering used for NMR assignments and observed 1H - ^{13}C HMBC correlations are shown in Figure 1. The 1H spectrum, shown in Figure 2, exhibited 14 distinct proton signals, some overlapping, attributed to 24 hydrogens including 1 amine, 18 methylene and 5 methine protons. Several notable impurity peaks are labeled in this figure. Impurities were not identified. No counterion was observed in the 1H NMR spectrum indicating an inorganic salt form based on the protonated amine. The ^{13}C spectrum, shown in Figure 3, exhibited 10 distinct carbon peaks attributed to 16 carbon atoms. The 2D NMR data indicated phenyl, tetrahydropyran and piperidine rings with connectivity across the structure established largely through the 1H - ^{13}C HMBC spectrum. Table 2 is a summary of the NMR peak assignment data and observed unambiguous 2D correlations. All methylene groups in the molecule exhibited non-symmetric protons. The scarcity of correlations reported for the atoms in the phenyl ring is largely due to the narrow chemical shift range of both the protons and carbons, which resulted in difficulty resolving and assigning correlations. No through-bond correlations were observed between the piperidine ring and the remaining chemical structure. A 1D selective NOE spectrum with excitation of the amine proton (at $\delta = 11.80$ ppm) showed 1H correlations within the piperidine ring (on C10, C11, C14, and C15) as well as on carbons C12 and C13. The complete collection of 1D and 2D NMR associated with the structure elucidation are included in the appendix as well as a comparison of the ^{13}C NMR peak locations with a PCP (HCl) sample run $CDCl_3$.

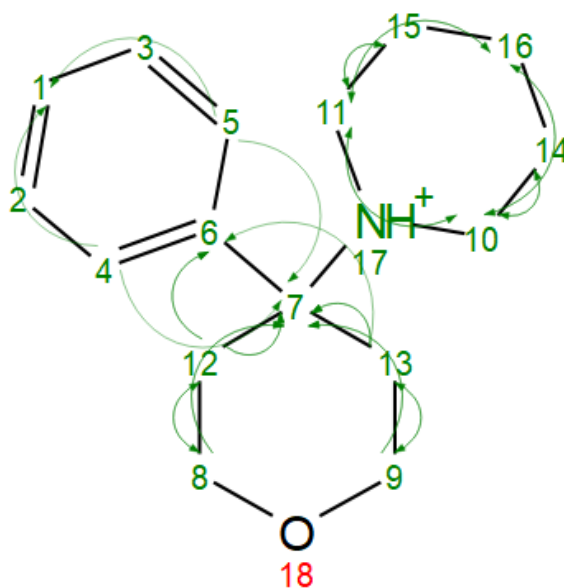


Figure 1. Confirmed structure with atom numbering used for NMR data peak assignments with observed 1H - ^{13}C HMBC indicated by arrows.

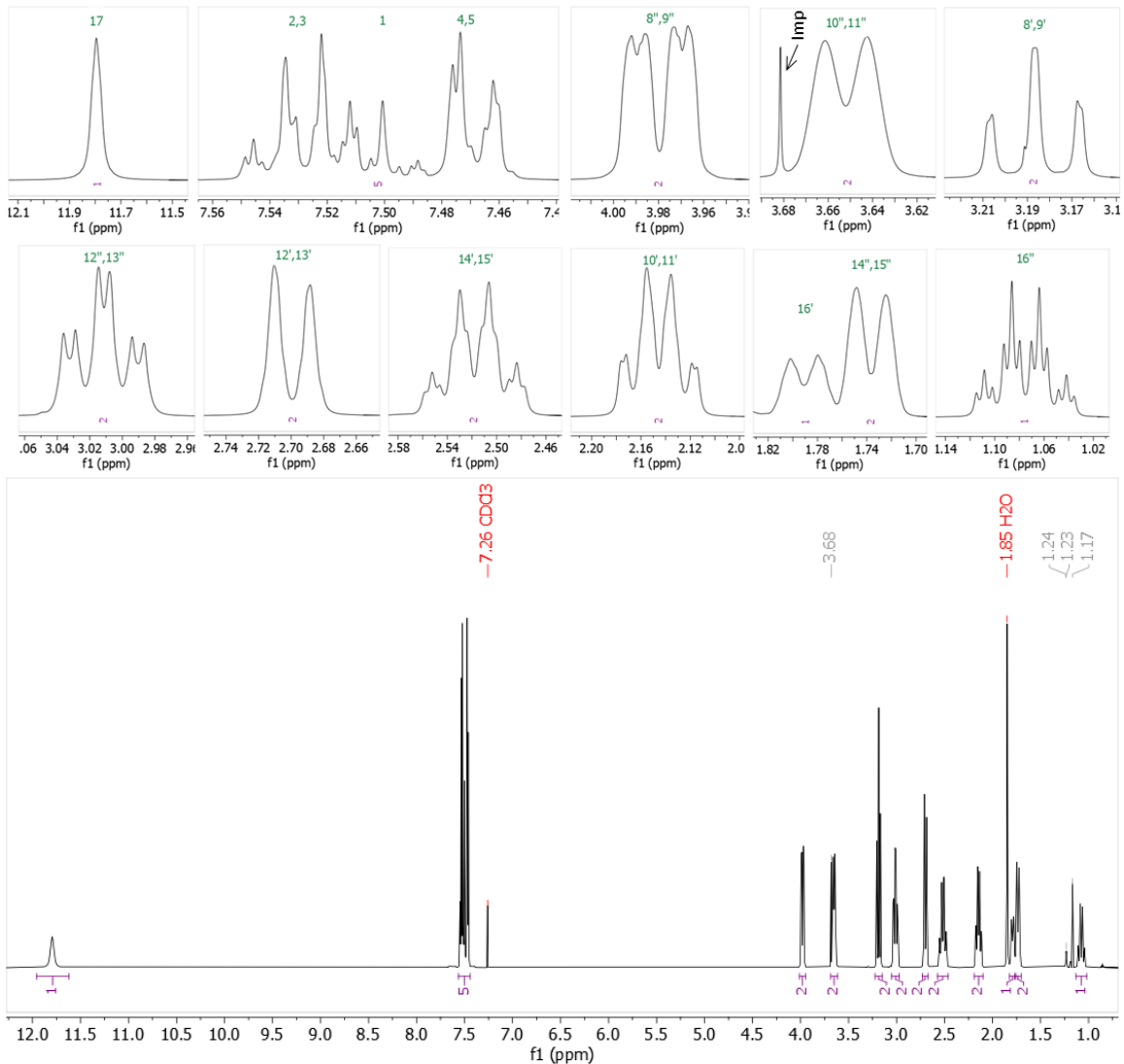


Figure 2. The ^1H NMR spectrum of RP0001 sample in CDCl_3 is shown in the bottom panel. Residual solvent and water peaks are red, while notable impurity peaks are grey. The top panels show expanded views of each proton signal with corresponding assignments. Nearby impurity peaks are labeled (Imp) when present. Relevant proton counts are shown beneath all curves.

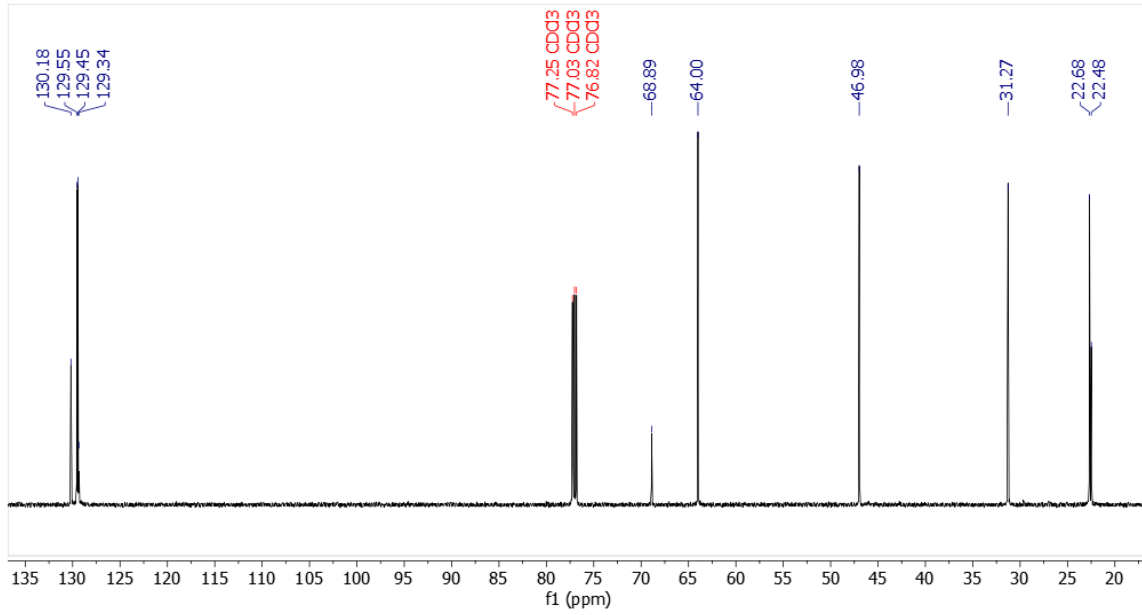


Figure 3. The ¹³C NMR spectrum of RP0001 in CDCl₃. Analyte and solvent peaks are labeled in blue and red, respectively.

Table 2. Summary of NMR peak locations, assignments and observed unambiguous 2D correlations.

Atom	δ (ppm)	Multiplicity	COSY	HSQC	HMBC
1 C	130.182	1		1	4, 5
H	7.501	1		1	
2 C	129.552	1		2	
H	7.53	1		2	
3 C	129.552	1		3	
H	7.53	1		3	
4 C	129.447	1		4	
H	7.471	1		4	1, 7
5 C	129.447	1		5	
H	7.471	1		5	1, 7
6 C	129.343	1			12', 12", 13'
7 C	68.894	1			4, 5, 8', 8", 9', 9", 12', 12", 13', 13"
8 C	64.001	1		8', 8"	12"
H'	3.187	1	12', 12"	8	7
H''	3.979	1	12', 12"	8	7, 12
9 C	64.001	1		9', 9"	13"
H'	3.187	1	13', 13"	9	7
H''	3.979	1	13', 13"	9	7, 13
10 C	46.978	1		10', 10"	11", 14', 16'
H'	2.145	1	14', 14", 17	10	14
H''	3.652	1	14'	10	11, 16
11 C	46.978	1		11', 11"	10", 15', 16'
H'	2.145	1	15', 15", 17	11	15
H''	3.652	1	15'	11	10, 16
12 C	31.274	1		12', 12"	8"
H'	2.701	1	8', 8"	12	6, 7
H''	3.011	1	8', 8"	12	6, 7, 8
13 C	31.274	1		13', 13"	9"
H'	2.701	1	9', 9"	13	6, 7
H''	3.011	1	9', 9"	13	7, 9
14 C	22.678	1		14', 14"	10'
H'	2.517	1	10', 10", 16"	14	10
H''	1.736	1	10', 16"	14	
15 C	22.678	1		15', 15"	11'
H'	2.517	1	11', 11", 16"	15	11
H''	1.736	1	11', 16"	15	
16 C	22.484	1		16', 16"	10", 11"
H'	1.79	1		16	10, 11
H''	1.075	1	14', 14", 15', 15"	16	
17 N	100	1			
H	11.796	1	8', 9'		
18 O	130.182	1			

Analytical Results – DART-MS

Instrument and method used: Measurements were made using an IonSense DART-SVP ion source coupled to a JEOL AccuTOF 4G LC-plus mass spectrometer. The sample was analyzed in both positive and negative ionization modes. For both analyses, helium (99.999 % purity) was used as the source gas with a gas stream temperature of 400 °C and a grid voltage of ± 150 V. For the positive mode analysis, a scan range of m/z 80 to m/z 800 was used along with an RF Guide voltage of +700 V, a ring lens voltage of +5 V, and an orifice 2 voltage of +5 V. The orifice 1 voltage was cycled (+30 V, +60 V, and +90 V) at 0.2 s cycle⁻¹. For negative mode analysis a scan range of m/z 30 to m/z 550 was used, at 0.2 s scan⁻¹ along with an RF Guide voltage of -250 V, an orifice 1 voltage of -30 V, a ring lens voltage of -5 V, and an orifice 2 voltage of -5 V.

Form sample was analyzed in: An acetonitrile solution with an approximate concentration of 1 mg mL⁻¹. Additionally, an aqueous solution with an approximate concentration of 1 mg mL⁻¹ was analyzed in negative ionization mode for salt form determination.

Controls used: Polyethylene glycol 600 was used an m/z calibration compound in both ionization modes. A 0.1 mg mL⁻¹ methanolic solution of cocaine was used a positive control in positive ionization mode. A ≈ 0.1 mg mL⁻¹ methanolic solution of AB-FUBINACA was used as a positive control in negative ionization mode. Acetonitrile was run as a negative control in both ionization modes.

Results: In the low fragmentation orifice 1 voltage (+30 V) spectrum of the sample dominant peaks at m/z 86.099, m/z 161.097, and m/z 246.186 were observed (Figure 4 and Table 3). These peaks were within mass tolerance of [C₅H₁₂N]⁺, [C₁₁H₁₃O]⁺, and [C₁₆H₂₄NO]⁺, which led to a presumed molecular formula of C₁₆H₂₃NO (assuming the ion was a protonated molecule). These ions were observed in the +60 V orifice 1 spectrum as well, along with several additional fragment ions (Figure 5 and Table 4). The +90 V orifice 1 spectra was dominated by the [C₇H₇]⁺ ion (m/z 91.057) (Figure 6 and Table 7). The negative mode spectrum of the acetonitrile solution produced no observable ions of interest (data not shown). The aqueous solution analyzed in negative ionization mode produced ions at m/z 34.967 and m/z 36.964, indicating the sample was a hydrochloride salt.

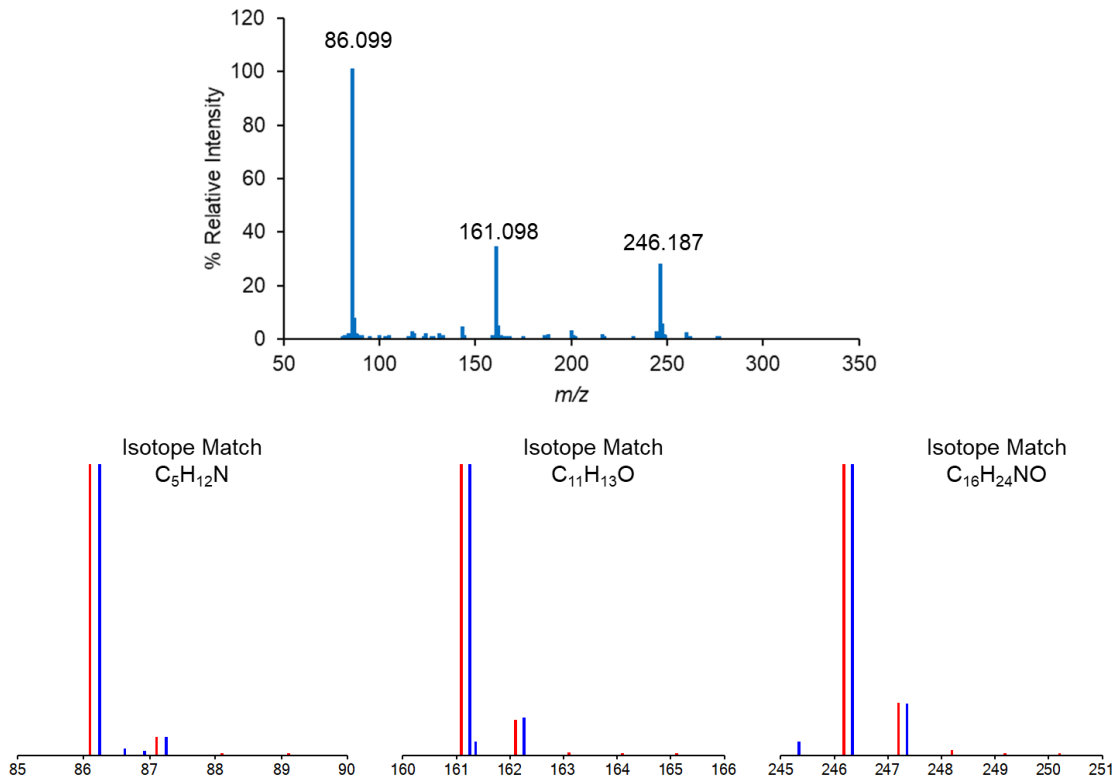


Figure 4. Low fragmentation orifice 1 voltage (+30 V) positive mode spectrum of the sample (top). Isotope matches (red is theoretical, blue is measured) for the m/z 86.099 ion to $[C_5H_{12}N]^+$ (bottom left), the m/z 161.098 ion to $[C_{11}H_{13}O]^+$ (bottom center) and the m/z 246.187 ion to $[C_{16}H_{24}NO]^+$ (bottom right) are also shown.

Table 3. Peak list for the low fragmentation orifice 1 voltage (+30 V) positive mode spectrum of the sample. Formulas and mass drifts (Δ_{mmu}) are also shown. Only peaks above 5 % relative intensity are provided. Presumed formulae were obtained using Mass Mountaineer[1] software. Isotopic peaks above 5 % relative intensity are not listed.

<i>m/z</i>	% Rel. Intensity	Presumed Formula	Δ_{mmu}
86.099	100.0	$[C_5H_{12}N]^+$	-1.89
161.098	33.6	$[C_{11}H_{13}O]^+$	-1.61
246.187	27.3	$[C_{16}H_{24}NO]^+$	-1.41

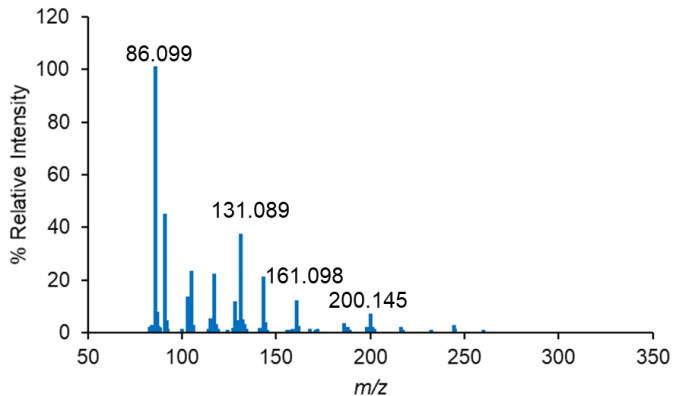


Figure 5. Mid-range fragmentation orifice 1 voltage (+60 V) positive mode spectrum of the sample. Select peaks of interest are identified.

Table 4. Peak list for the mid-range fragmentation orifice 1 voltage (+60 V) positive mode spectrum of the sample. Formulas and mass drifts (Δ_{mmu}) are also shown. Only peaks above 5 % relative intensity are provided. Presumed formulae were obtained using Mass Mountaineer software. Isotopic peaks above 5 % relative intensity are not listed.

<i>m/z</i>	% Rel. Intensity	Presumed Formula	Δ_{mmu}
86.099	100.0	$[C_5H_{12}N]^+$	-1.97
91.057	44.0	$[C_7H_7]^+$	-2.12
103.057	12.7	$[C_8H_7]^+$	-2.69
105.073	22.5	$[C_8H_9]^+$	-2.77
117.073	21.3	$[C_9H_9]^+$	-2.96
128.066	10.9	$[C_{10}H_8]^+$	-3.23
131.089	36.7	$[C_{10}H_{11}]^+$	-3.16
143.089	20.4	$[C_{11}H_{11}]^+$	-2.70
161.098	11.2	$[C_{11}H_{13}O]^+$	-2.27
200.145	6.2	$[C_{14}H_{18}N]^+$	-1.57

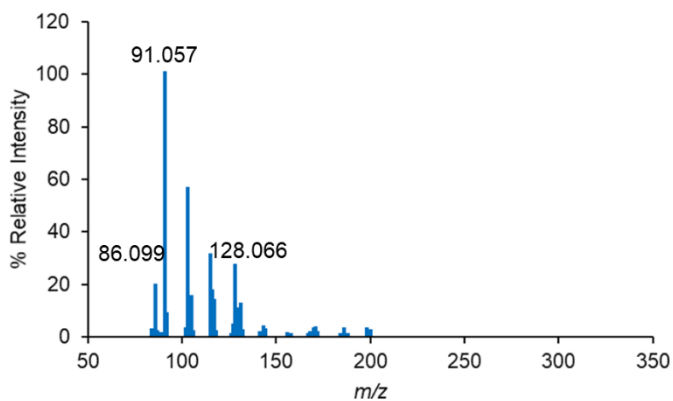


Figure 6. High fragmentation orifice 1 voltage (+90 V) positive mode spectrum of the sample. Select peaks of interest are identified.

Table 5. Peak list for the high fragmentation orifice 1 voltage (+90 V) positive mode spectrum of the sample. Formulas and mass drifts (Δ_{mmu}) are also shown. Only peaks above 5 % relative intensity are provided. Presumed formulae were obtained using Mass Mountaineer software. Isotopic peaks above 5 % relative intensity are not listed.

<i>m/z</i>	% Rel. Intensity	Presumed Formula	Δ_{mmu}
86.099	19.2	[C ₅ H ₁₂ N] ⁺	-2.09
91.057	100.0	[C ₇ H ₇] ⁺	-2.15
103.057	55.9	[C ₈ H ₇] ⁺	-2.70
105.073	14.9	[C ₈ H ₉] ⁺	-2.75
115.058	30.8	[C ₉ H ₇] ⁺	-2.83
116.065	16.9	[C ₉ H ₈] ⁺	-2.24
117.073	13.3	[C ₉ H ₉] ⁺	-2.58
128.066	26.9	[C ₁₀ H ₈] ⁺	-3.21
129.072	10.2	[C ₁₀ H ₉] ⁺	-2.13
131.086	11.9	[C ₁₀ H ₁₁] ⁺	-3.25

Analytical Results- GC-MS

Instrument and method used: A Thermo Trace 1310 gas chromatograph coupled with a TSQ8000evo mass spectrometer was used for this analysis. Helium (99.999 %) was used as the carrier gas along with an Agilent DB-35 column (30 m x 0.25 mm x 0.25 μ m). Relevant method parameters are provided in Table 6.

Table 6. GC-MS method parameters.

Temperature Program	1) 80 °C for 0.5 min 2) Ramp 15 °C min ⁻¹ to 290 °C 3) Hold 15 min
Flow Rate	1.8 mL min ⁻¹
Injection Volume	1.0 μ L
Inlet Temperature	250 °C
Split Ratio	8:1
Transfer Line	300 °C
Quad Temperature	150 °C
Source Temperature	280 °C
Tune Mode	EI Standard Tune
Solvent Delay	1.5 min
Mass Scan Range	<i>m/z</i> 40 – <i>m/z</i> 600
Threshold	None
Scan Speed	0.2 s scan ⁻¹

Form sample was analyzed in: An acetonitrile solution with an approximate concentration of 0.25 mg mL⁻¹.

Controls used: A \approx 0.1 mg mL⁻¹ methanolic solution of cocaine was used as a positive control. Acetonitrile was used as a negative control. An alkane ladder (C₇-C₄₀) was used for retention index calculations.

Results: The compound was found to have a retention time of 11.887 min (11 min 53 s) using the method specified and was the only peak above background (Figure 7, left). The corresponding mass spectrum (Figure 7, right and Table 7) was dominated by *m/z* 188, *m/z* 200, and *m/z* 216 ions. A presumed molecular ion at *m/z* 245 was observed. Using an alkane ladder, a retention index of 2263 a.u. was obtained.

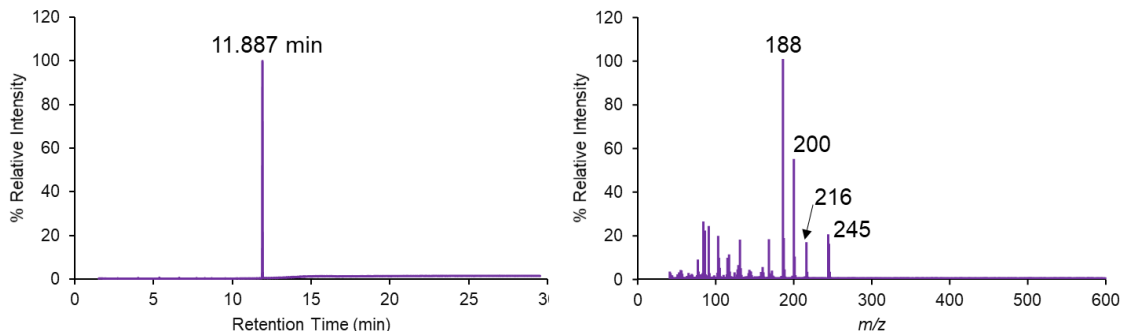


Figure 7. Representative GC-MS chromatogram (left) and mass spectrum (right) of the sample.

Table 7. Peak list for the mass spectrum obtained using GC-MS. Only peaks above 5 % relative intensity are provided. Presumed formulae were obtained using MS Interpreter software and the structure determined by NMR. Isotopic peaks below 5 % relative intensity are not listed. Formulae with an asterisk (*) were not explained using MS Interpreter.

<i>m/z</i>	% Rel. Intensity	Presumed Formula
77	8.1	[C ₆ H ₅] ⁺
84	25.6	[C ₅ H ₁₀ N] ⁺
86	21.4	[C ₅ H ₁₂ N] ⁺
91	23.5	[C ₇ H ₇] ⁺ *
103	19.0	[C ₈ H ₇] ⁺
104	9.0	[C ₈ H ₈] ⁺
115	8.9	[C ₉ H ₇] ⁺ *
116	5.8	[C ₉ H ₈] ⁺ *
117	10.4	[C ₉ H ₉] ⁺
129	5.7	[C ₁₀ H ₉] ⁺ *
131	17.2	[C ₁₀ H ₁₁] ⁺
168	17.5	[C ₁₀ H ₁₈ NO] ⁺
186	100	[C ₁₃ H ₁₆ N] ⁺
200	54.2	[C ₁₄ H ₁₈ N] ⁺
201	9.5	[C ₁₃ H ₁₅ NO] ⁺
216	16.2	[C ₁₄ H ₁₈ NO] ⁺
244	19.7	[C ₁₆ H ₂₂ NO] ⁺
245	15.3	[C ₁₆ H ₂₃ NO] ⁺

Comparison of the measured spectrum to the SWGDRUG 3.9[2] spectral library showed no reasonable matches to any of the library spectra. A comparison of the sample to phencyclidine using a hybrid similarity search with a precursor molecular weight of 245 Da is provided in Figure 8.

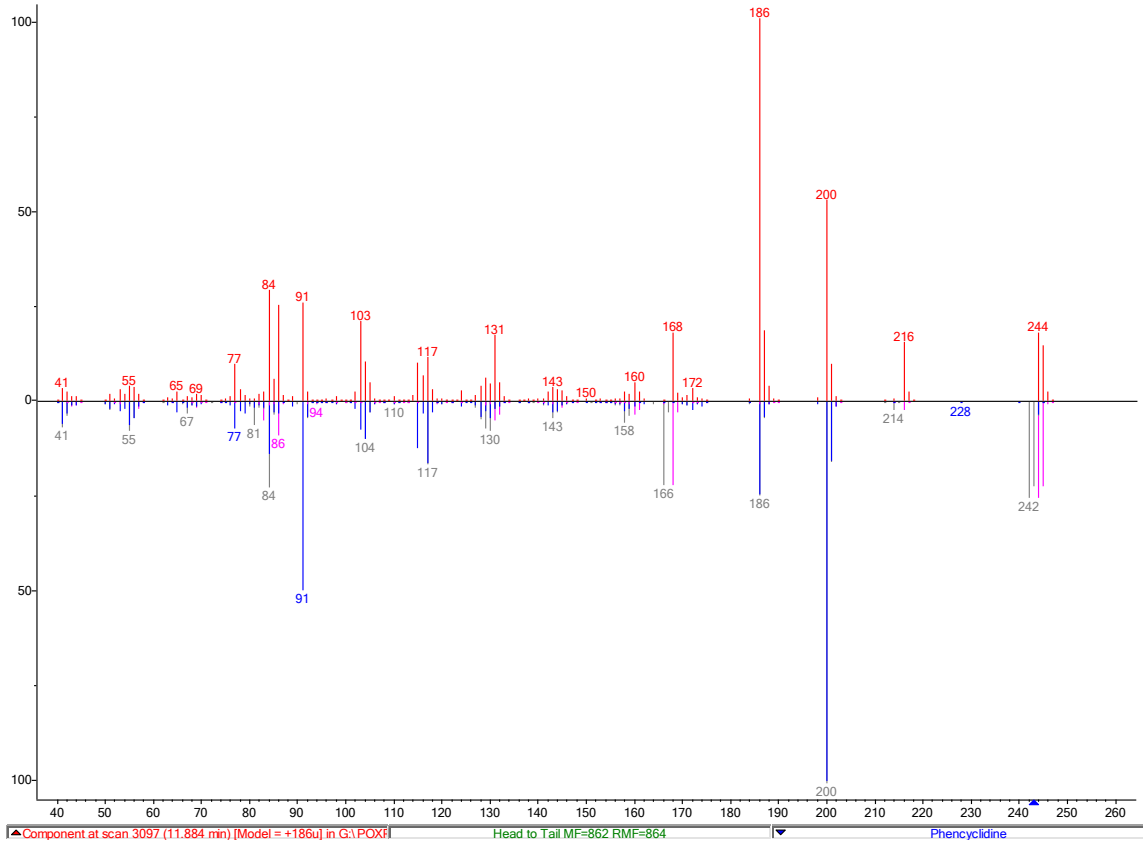


Figure 8. Comparison of the sample (top, red) to phencyclidine (bottom, blue) using a hybrid similarity search. Peaks labeled in pink show those from the phencyclidine mass spectrum in grey that were shifted to align the mass spectra of the two compounds.

Analytical Results – GC-FID

Instrument and method used: A Thermo Trace 1310 gas chromatograph was used for this analysis. Helium (99.999 %) was used as the carrier gas along with an Agilent DB-5 column (30 m x 0.25 mm x 0.25 μm). Relevant method parameters are provided in Table 8.

Table 8. GC-FID method parameters.

Temperature Program	1) 80 °C for 0.5 min 2) Ramp 15 °C min ⁻¹ to 290 °C 3) Hold 15 min
Flow Rate	1.8 mL min ⁻¹
Injection Volume	1.0 μL
Inlet Temperature	250 °C
Split Ratio	10:1
Solvent Delay	2.0 min
Data Collection Rate	5 Hz
Detector Temperature	300 °C
Detector Air Flow Rate	350 mL min ⁻¹
Detector N ₂ Flow Rate	5 mL min ⁻¹
Detector H ₂ Flow Rate	10 mL min ⁻¹

Form sample was analyzed in: An acetonitrile solution with an approximate concentration of 1.0 mg mL⁻¹.

Controls used: A 1.0 mg mL⁻¹ methanolic solution of cocaine was used as a positive control. Acetonitrile was used as a negative control. An alkane chain (C₇-C₄₀) was used for retention index calculations.

Results: The compound was found to have a retention time of 10.37 min (10 min 22 s) using the method specified and was the only peak above background that was observed (Figure 9). Using an even-numbered alkane ladder, a retention index of 2171 a.u. was obtained.

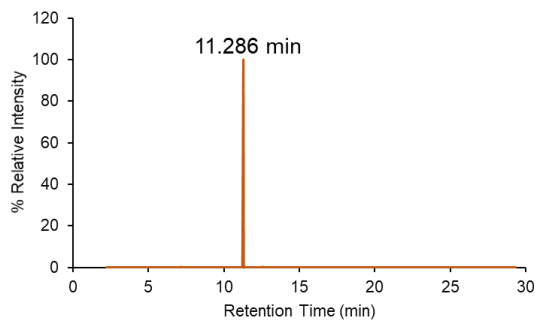


Figure 9. GC-FID chromatograph of the sample.

Analytical Results – LC-MS/MS

Instrument and method used: A Sciex QTrap 4000 mass spectrometer coupled with a Thermo UltiMate 3000 liquid chromatography system were used for analysis along with a Restek Raptor Biphenyl 2.7 μm , 150 x 4.6 mm column. Relevant method parameters are provided in Table 9.

Table 9. LC-MS/MS method parameters.

Run Time	18 min
Mobile Phases	A: Methanol with 0.1 % Formic Acid B: Water with 0.1 % Formic Acid
Mobile Phase Program	0 min: 95 % A / 5 % B 9 min: 0 % A / 100 % B 11 min: 0 % A / 100 % B 12 min: 95 % A / 5 % B
Injection Volume	10 μL
Column Oven Temperature	30 $^{\circ}\text{C}$
Curtain Gas	10 a.u.
IonSpray Voltage	5500 V
Source Temperature	550 $^{\circ}\text{C}$
Ion Source Gas 1	50 a.u.
Ion Source Gas 2	50 a.u.
Declustering Potential	50 V
Entrance Potential	10 V
Scan Range (Full Scan)	m/z 40 – m/z 600
Scan Rate (Full Scan)	0.25 s scan ⁻¹
Product Ion (Product Ion Scan)	m/z 246
Scan Range (Product Ion Scan)	m/z 30 – m/z 250
Scan Rate (Product Ion Scan)	0.1 s scan ⁻¹
Collision Energy (Product Ion Scan)	45 V
Collision Cell Exit Potential (Product Ion Scan)	10 V

Form sample was analyzed in: A ≈ 0.01 mg mL⁻¹ acetonitrile solution was used for analysis.

Controls used: A 5-component solution of ≈ 0.025 mg/mL cocaine-d3, fentanyl-d5 heroin-d9, methamphetamine-d3, and THC-d10 was used for a positive control. Pure acetonitrile was used a negative control.

Results: The sample was found to have a retention time of 7.08 min (7 min 5 s) on the method used. Two different mass spectral analyses were completed, on separate injections – a full scan method to identify major ions and potential impurities and a product ion scan. The full scan analysis produced a single peak at 7.08 min, with no remarkable additional

peaks. For the product ion scan a single peak at 7.08 min (7 min 5 s) was also observed (Figure 10, left). The fragment ion spectrum of m/z 246 (Figure 10, right) produced peaks consistent with the DART-MS data, with notable fragments at m/z 86, m/z 91, m/z 117, and m/z 161.

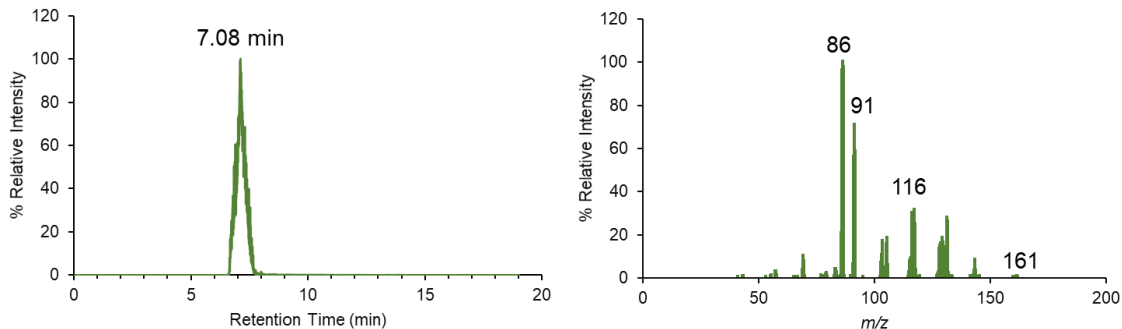


Figure 10. Representative LC-MS chromatogram (left) and mass spectrum (right) of the sample.

Software & Spectral Library References

- [1] Mass Mountaineer, <https://diabloanalytical.com/ms-software/mass-mountaineer/>.
- [2] SWGDRUG MS Library, United States Department of Justice Drug Enforcement Administration, <https://www.swgdrug.org/ms.htm>.