ADEBAR
Aufbau analytischer Datenbanken, Erhebung und bundesweite Bereitstellung von analytischen Daten und Referenzmaterialien im Bereich neuer psychoaktiver Stoffe

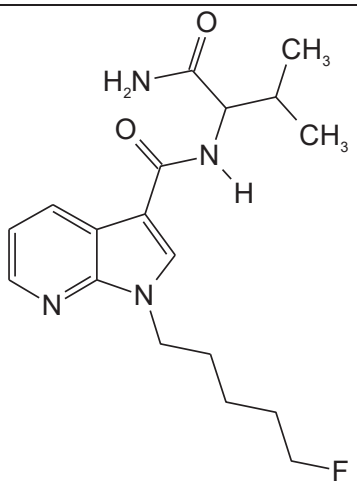
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).

Analytical Report[1]

5F-AB-P7AICA

N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-pyrrolo-[2,3-b]pyridine-3-carboxamide

Sample ID:	18/ADB-076
Sample description:	solid, white
Date of sample receipt (D.M.Y):	23.07.2018

Substance identified structure	
Systematic name	*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoro-pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide
Other names	
Formula	$C_{18}H_{25}FN_4O_2$
M_w (g/mol)	348
Compound Class	Cannabinoids
Salt form	free base
Additional information	

[1] The report has been produced with the financial support of the Internal Security Fund of the European Union (grant agreement number IZ25-5793-2016-27).

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Instrumental methods

1. Gas chromatography-mass spectrometry (GC-MS)

1a. Gas chromatography-electron ionization-mass spectrometry (GC-EI-MS)

Finnigan TSQ 8000 triple stage quadrupole mass spectrometer coupled to a gas chromatograph (Trace GC Ultra, Thermo Electron, Dreieich, Germany). Sample introduction: using a CTC CombiPAL (CTC Analytics, Zwingen, Switzerland) autosampler. Software: Xcalibur 4.0.
GC parameters: injection volume: 1 µL (approximately 2 mg compound in 2 mL appropriate solvent), splitless; injector temperature: 280 °C; carrier gas: helium; flow rate: 1.2 mL/min.
MS parameters: ionization mode: EI = 70 eV; emission current: 200 µA; ion source temperature: 175 °C; scan time: 1 s; scan range: m/z = 29 – 600.

1a.1 Temperature program 280 °C (final temperature)

Chromatographic conditions: fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program: 80 °C, held for 1 min, followed by a ramp to 280 °C at 15 °C/min, held for 21 min; GC-MS transfer line: 280 °C.

Kovats retention indices (RI) were calculated from measurement of an *n*-alkane mixture analyzed with the above-mentioned temperature programs. Marked: RI: xxx (DB-1)

1a.2 Temperature program 310 °C (final temperature)

Chromatographic conditions: fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program: 80 °C, held for 2 min, followed by a ramp to 310 °C at 20 °C/min, held for 23 min; GC-MS transfer line: 280 °C.

Kovats retention indices (RI) were calculated from measurement of an *n*-alkane mixture analyzed with the above-mentioned temperature programs. Marked: RI: xxx (DB-1, 310)

1b. Gas chromatography-chemical ionization-mass spectrometry (GC-CI-MS)

Chemical ionization (CI) mass spectra: using a Finnigan TSQ 7000 triple stage quadrupole mass spectrometer coupled to a gas chromatograph (Trace GC Ultra, Thermo Electron, Dreieich, Germany). Sample introduction: using a CTC CombiPAL (CTC Analytics, Zwingen, Switzerland) autosampler. Software: Xcalibur 4.0.
GC parameters: injection volume: 1 µL (approximately 2 mg compound in 2 mL appropriate solvent), splitless; injector temperature: 220 °C; carrier gas: helium; flow rate: 1.2 mL/min.
MS parameters: emission current: 200 µA, ion source temperature: 175 °C; collision gas: methane at 1.5 mmTorr; scan time: 1 s; scan range: m/z = 50 – 600.
Kovats retention indices (RI) were calculated from measurement of an *n*-alkane mixture analyzed with the above-mentioned temperature programs.

1b.1 Temperature program 280 °C (final temperature)

Chromatographic conditions: fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program: 80 °C, held for 1 min, followed by a ramp to 280 °C at 15 °C/min, held for 21 min; GC-MS transfer line: 280 °C.

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1b.2 Temperature program 310 °C (final temperature)

Chromatographic conditions: fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program: 80 °C, held for 2 min, followed by a ramp to 310 °C at 20 °C/min, held for 23 min; GC-MS transfer line: 280 °C.

2. Liquid chromatography-mass spectrometry (LC-MS)

2a. LC-ESI-Q-TOF-MS/MS

The samples were separated using an Agilent 1260 HPLC-System (Agilent technology, Waldbronn, Germany). HPLC parameters are shown in Table 1.

Table 1: HPLC Parameters

Column	Kinetex C_8 column (2.1 x 100 mm, 1,7 µm); Phenomenex, Aschaffenburg, Germany
Mobile phases	A: aqueous buffer (10 mM NH_4HCO_2, 0.1 % formic acid) B: methanol
Gradient	10 % B hold for 1.5 min, than in 7.5 min to 50 % B and in next 9 min to 95 % B followed by a flushing step and reconditioning (11 min)
Flow rate	0.275 mL/min
Column temperature	45 °C
Injection volume	1 µL

Mass spectrometry was performed using an Agilent 6530 Q-TOF equipped with an Agilent Jet Stream electrospray source and controlled by Agilent MassHunter Acquisition software. Q-TOF Mass spectrometer parameters are shown in table 2.

Table 2: Agilent 6530 QTOF Mass Spectrometer Parameters

Ionization mode	positive ion electrospray with Agilent Jet Stream technology
Mass range	100-1100 m/z
Collision gas (CID)	Nitrogen
Drying gas (N_2)	320 °C at 8 L/min
Sheat gas	350 °C at 11 L/min
Nebulizer	35 psi
Capillary	3000 V
Fragmentor	150 V
Nozzle	500 V
Skimmer	65 V
Collision Energy levels	5-40 V (5 V steps)

Accurate mass measurements were obtained through reference correction using protonated purine (m/z 121.0509) and protonated hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine (HP-921; m/z 922.0098).
Data processing was done using Agilent Masshunter Qualitative Software, Agilent PCDL-Manager and NIST MS Search. In MS/MS-mode small ions (<1.5 % of basepeak) will not be recorded.

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2b. LC-ESI-linear ion trap-MS/MS

Thermo Accela 1250 HPLC chromatograph coupled to Thermo Velos Pro (linear trap) spectrometer with electrospray ionization; software: XCalibur 4.0

Table 3: Recording of the collision spectra in 5 V steps with syringe pump

mobile phases	A: water with 0.0025 % formic acid B: methanol with 0.0025 % formic acid 98 % A and 2 % B, isocratic
solvent	methanol
concentration	10 µg/mL
flow rate pump	100 µL/min
flow rate syringe	depends on signal intensity (3 µL/min or 10 µL/min)
mass range	depends on molecular weight (automatic dynamic range)
iso width	1.2 (m/z)
collision gas	helium
collision energy	20 V - 60 V

Recording of the collision spectra was done without and with wideband. In each case the spectrum was chosen which shows the molecular ion with nearly 10% of the base peak intensity.

Table 4: Determination of RRT against fluorescein as internal standard

column	Aqua C18 (3 µm, 150 x 3 mm, 125 Å)
mobile phases	A: water with 0.0025 % formic acid B: methanol with 0.0025 % formic acid
gradient	100 % A for 3 min, than in 14 min to 98 % B, hold for 32 min, than to 100 % A for 10 min
flow rate	100 µL
injection volume	1 µL
column temperature	24 °C

2c. HR-LC-ESI-Q-TOF-MS

Bruker compact Q-TOF
Q-TOF calibrating substance: sodium formate

Table 5: Parameters

Column	Thermo Hypersil GOLD column (2.1 x 50 mm, 1.9 µm);
Mobile phases	A: aqueous buffer (10 mM NH_4HCO_2, 0.12 % formic acid) B: acetonitrile
Gradient	10 % B hold for 2 min, than in 31 min to 90 % B and in next 11 min to 10 % B
Flow rate	200 µL/min
Column temperature	30 °C
Injection volume	1 - 5 µL (depending on compound class and signal height for MS/MS fragmentation)
Ionization mode	positive ion electrospray
Mass range	50-1070 m/z
Gas (N_2)	190 °C at 6 L/min

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Nebulizer	0.9 bar
Capillary	4000 V

3. Infrared spectroscopy (IR)

3a. Attenuated total reflection-infrared spectroscopy (ATR-IR)

Nicolet 380 FT-IR spectrometer with Smart Golden Gate Diamond ATR. Software: OMNIC, Ver. 7.4.127 (Thermo Electron Corporation, Dreieich, Germany).
Wavelength resolution: 4 cm^{-1}; scan range: 650-4000 cm^{-1}; 32 scans/spectrum.
IR spectra were recorded from salts and from free bases as neat film after following sample preparation procedure: For generation of the free bases, 2-5 mg of the salt were dissolved in demineralized water and were alkalized with one drop of NaOH (5 % w/w). The solution was extracted with 1 mL diethylether, the ethereal phase was transferred in a new vial and the solvent was evaporated under a gentle nitrogen flow until the volume reached approximately 100 µL. The remaining fluid was aspirated with a glass pipette and transferred directly on the ATR crystal where the remaining diethylether was continuously evaporated.

3b. Gas chromatography solid-state infrared spectroscopy (GC-sIR)

GC-solid phase-IR-system consisting of an Agilent GC 7890B (Waldbronn, Germany) with probe sampler Agilent G4567A and a DiscovIR-GC™ (Spectra Analysis, Marlborough, MA, USA). The column eluent was cryogenically accumulated on a spirally rotating ZnSe disk cooled by liquid nitrogen. IR spectra were recorded through the IR-transparent ZnSe disk using a nitrogen-cooled MCT detector.
GC parameters: injection: 1 µL (approximately 2 mg compound in 2 mL appropriate solvent), splitless mode; injection port temperature: 240 °C; carrier gas: helium; flow rate: 2.5 mL/min.
Chromatographic conditions: fused silica capillary DB-1column (30 m x 0.32 mm i.d., 0.25 µm film thickness); oven temperature program: 80 °C for 2 min, ramped to 290 °C at 20 °C/min, and held at for 20 min; transfer line: 280 °C.
Infrared conditions: oven temperature: 280 °C; restrictor temperature: 280 °C; disc temperature: -40 °C; dewar cap temperatures: 35 °C; vacuum: 0.2 mTorr; disc speed: 3 mm/s; spiral separation: 1 mm; wavelength resolution: 4 cm^{-1}; IR range: 650-4000 cm^{-1}; acquisition time: 0.6 s/file; 64 scans/spectrum.
Data were processed using GRAMS/AI Ver. 9.1 (Grams Spectroscopy Software Suite, Thermo Fischer Scientific, Dreieich, Germany) followed by implementation of the OMNIC Software, Ver. 7.4.127 (Thermo Electron Corporation, Dreieich, Germany).

3c. Fourier-Transform Near-infrared spectroscopy (FT-NIR)

Perkin Elmer 100 N FT-NIR spectrometer.
Spectra were recorded at ambient temperature through a glass vial.
Wavelength resolution 4 cm^{-1}; scan range 4000-10000 cm^{-1}; 36 scans/spectrum.

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4. Raman spectroscopy

4a. Raman spectroscopy at 785 nm

B&W TEK Inc. i-Raman® Plus system: laser wavelength: 785 nm with BWS465-785S spectrometer: scan range: 174 - 3200 cm^{-1}; resolution: < 4.5 cm^{-1} @ 912 nm and with BAC151B Raman Video Microsampling System: objective lens magnification: 20x; camera: active pixels: 1280 x 1024
Software: BWSpec® 4.03_23_C
Integration time (in ms) was chosen and adjusted in that way that a relative intensity preferably above 45000 for the most intensive peak was reached. Additional information on parameters in spectrum title: sample_mc (for microscope)_wavelength_laser power level_integration time_average number of recorded spectra
For the analysis of powders: the powder material was measured directly through a grip-bag or on a cap with the Video Microsampling System.

4b. Raman spectroscopy at 1064 nm

B&W TEK Inc. i-Raman® EX system: laser wavelength: 1064 nm with BWS485-1064S-05 spectrometer: scan range: 170 - 2502 cm^{-1}; resolution: ~ 9.5 cm^{-1} @ 1296 nm and with BAC151B Raman Video Microsampling System: objective lens magnification: 20x; camera: active pixels: 1280 x 1024
Software: BWSpec® 4.03_23_C
Integration time (in ms) was chosen and adjusted in that way that a relative intensity preferably above 45000 for the most intensive peak was reached. Additional information on parameters in spectrum title: sample_mc (for microscope)_wavelength_laser power level_integration time_average number of recorded spectra
For the analysis of powders: the powder material was measured directly through a grip-bag or on a cap with the Video Microsampling System.

5. Nuclear Magnetic Resonance Spectroscopy (NMR)

5a. NMR Bruker Fourier 300

Spectrometer: Bruker Fourier 300
Sample preparation: approx. 10 mg of the sample were dissolved in deuterated solvents directly in the NMR tube.
Measurement: All measurements were performed without sample spinning
Typical set of experiments used for structure elucidation:
1D-^{1}H: 300 MHz, pulse program: zg, number of scans: 4, 90° pulse, spectral width: 17 ppm, transmitter offset: 5,5 ppm, time domain: 128 k, spectrum size: 128 k, exponential multiplication with line broadening 0.2 Hz
1D-^{13}C: 75 MHz, pulse program: jmod (APT), number of scans: 512 or more
Assignments are supported by COSY, HSQC and HMBC.

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5b. NMR Bruker AVANCE III HD 500

Spectrometer: Bruker AVANCE III HD 500
Probe: Bruker 5 mm broad band inverse with z-gradient (BBIGR)
Sample preparation: approx. 10 mg of the sample were dissolved in deuterated solvents directly in the NMR tube.
Measurement: All measurements were performed without sample spinning
Typical set of experiments used for structure elucidation:
1D-^{1}H: 500 MHz, pulse program: zg, number of scans: 4, 90° pulse, spectral width: 17 ppm, transmitter offset: 5,5 ppm, time domain: 128 k, spectrum size: 128 k, exponential multiplication with line broadening 0.2 Hz
1D-^{13}C: 125 MHz, pulse program: jmod (APT), number of scans: 512 or more
Assignments are supported by COSY, HSQC and HMBC.

Supporting information

Analytical technique		applied	remarks
GC-MS	EI [1a]	+	
	CI [1b]		
LC-MS	Q-TOF [2a]		
	linear ion trap [2b]	+	
HR-LC-MS	Q-TOF [2c]	+	
IR	ATR-IR solid [3a]	+	
	ATR-IR base neat [3a]	+	
	GC-sIR [3b]	+	
	FT-NIR solid [3c]		
Raman	Raman at λ = 785 nm [4a]	+	
	Raman at λ = 1064 nm [4b]	+	
NMR	^{1}H, ^{13}C [5b]	+	

HR-LC-MS of C18H25FN4O2

Calculated: $[M+H]^{+}$ = 349.2034
Found: [M+H]+ = 349.2034
Δ: 0.0 mDa

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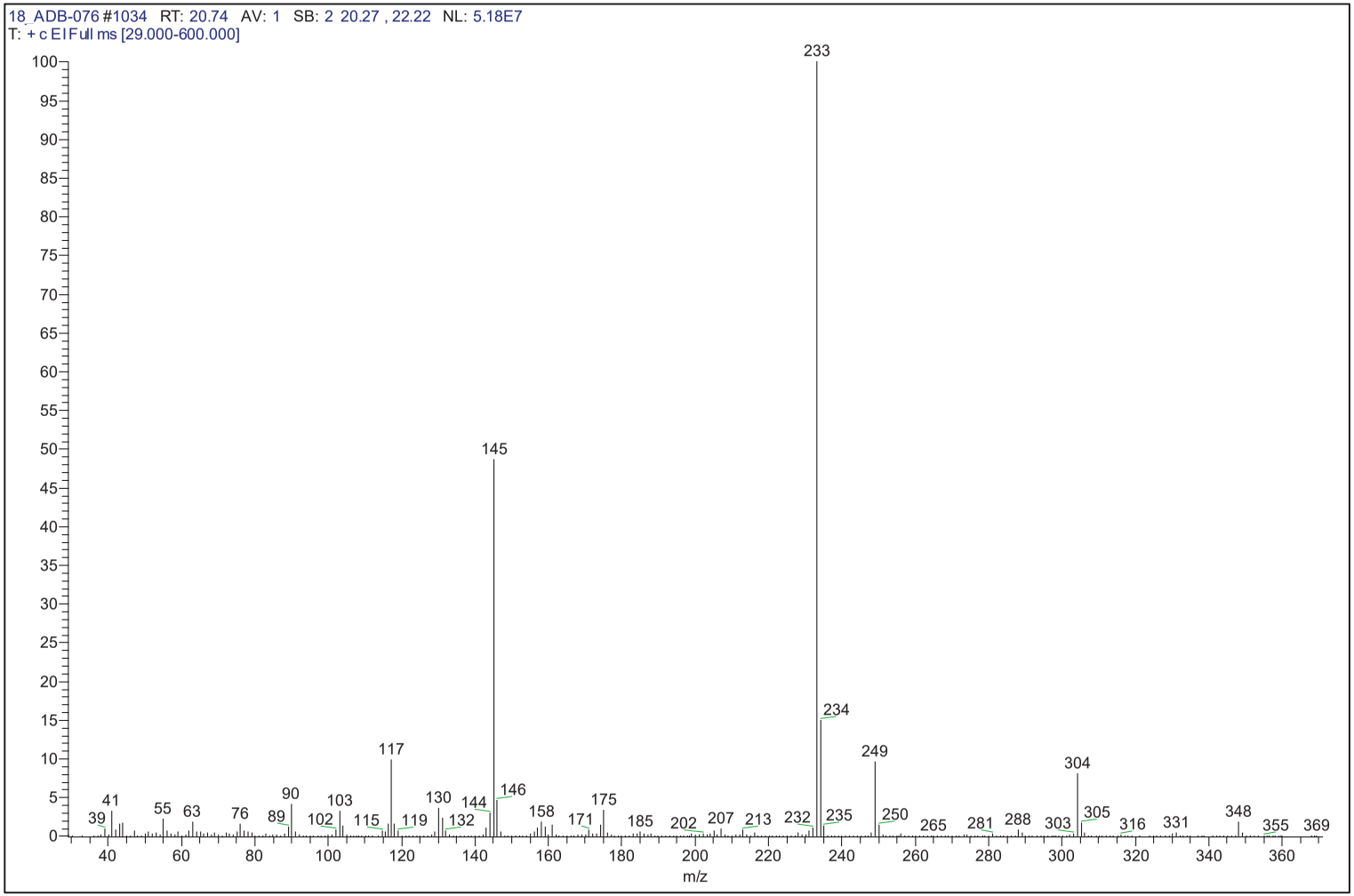

GC-EI-MS: 5F-AB-P7AICA ($CHCl_3$), RI: 2922 (DB-1)

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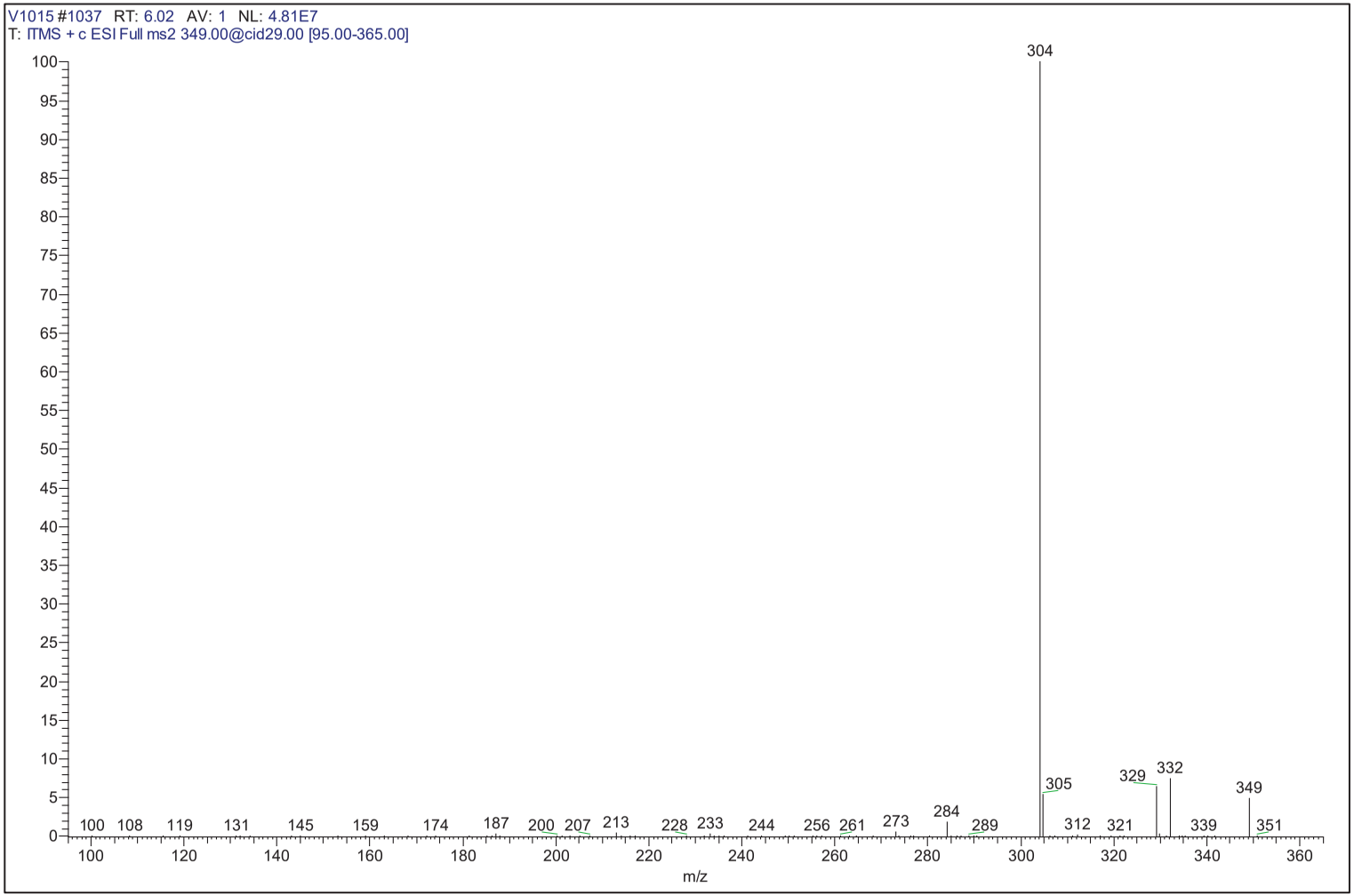

ESI-ion trap-MS: 5F-AB-P7AICA (MeOH) collision energy 29 V, RRT: 1.020

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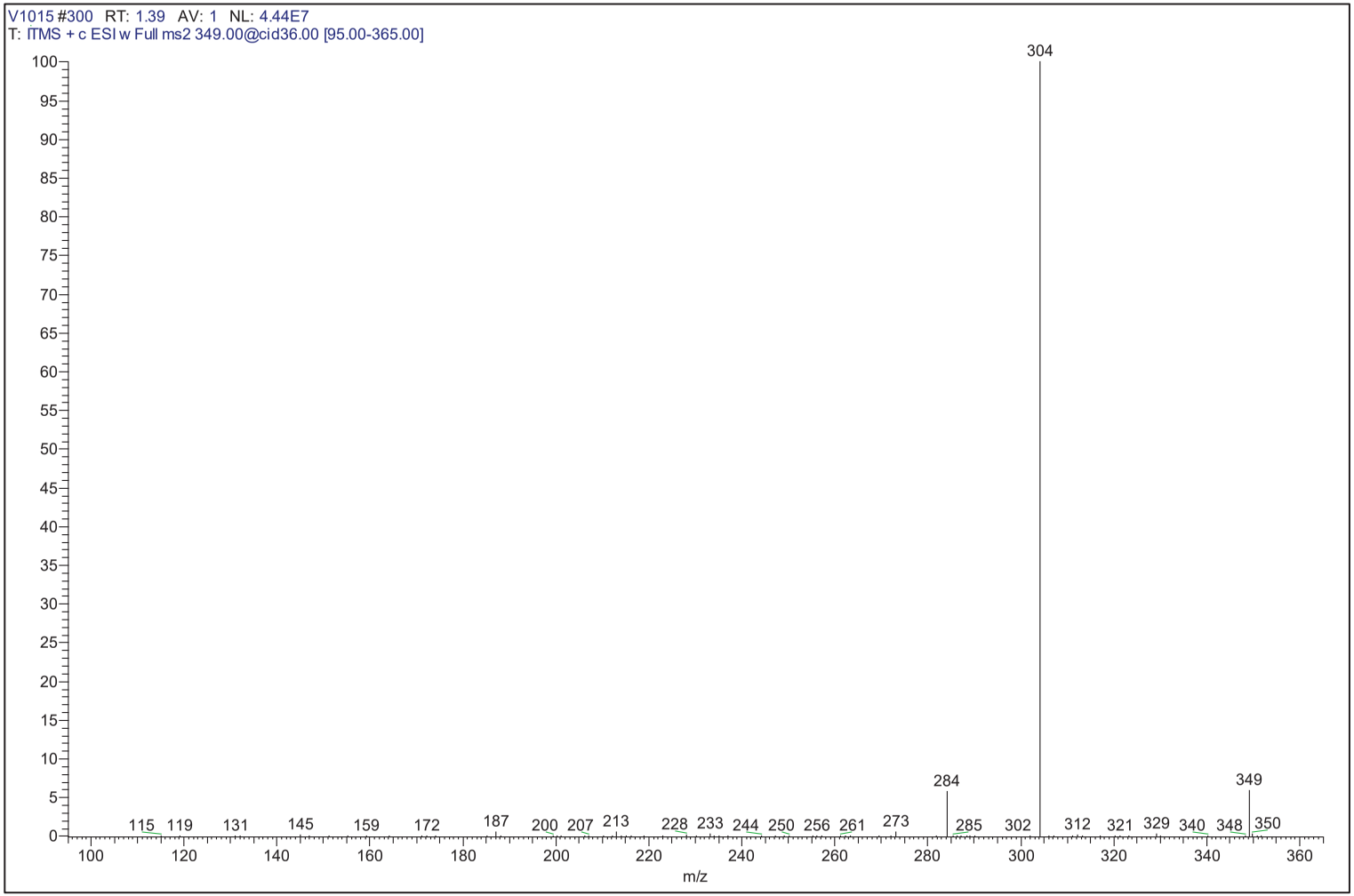

ESI-ion trap-MS: 5F-AB-P7AICA (MeOH) collision energy 36 V, wideband, RRT: 1.020

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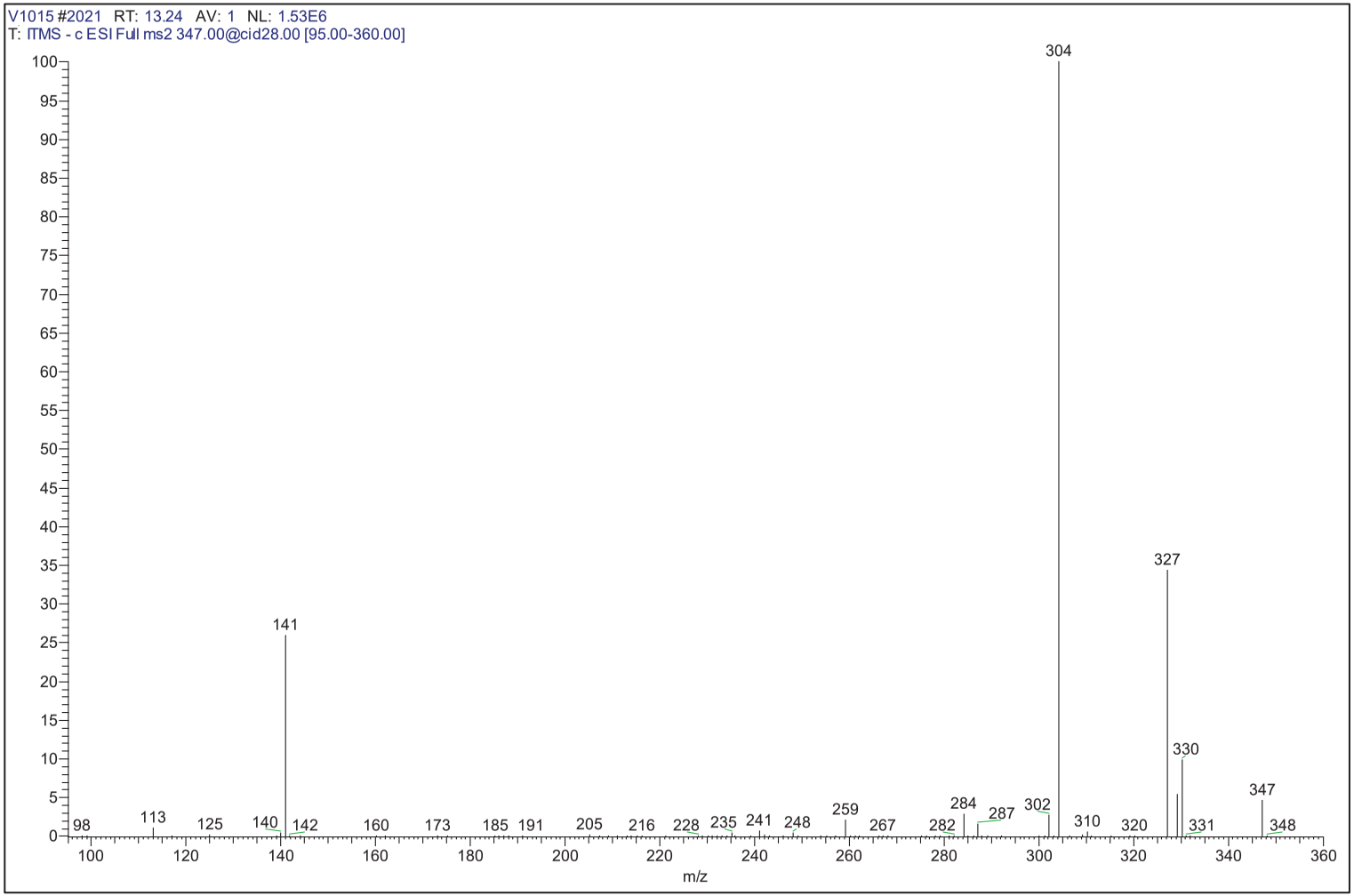

Negative ESI-ion trap-MS: 5F-AB-P7AICA (MeOH) collision energy 28 V, RRT: 1.020

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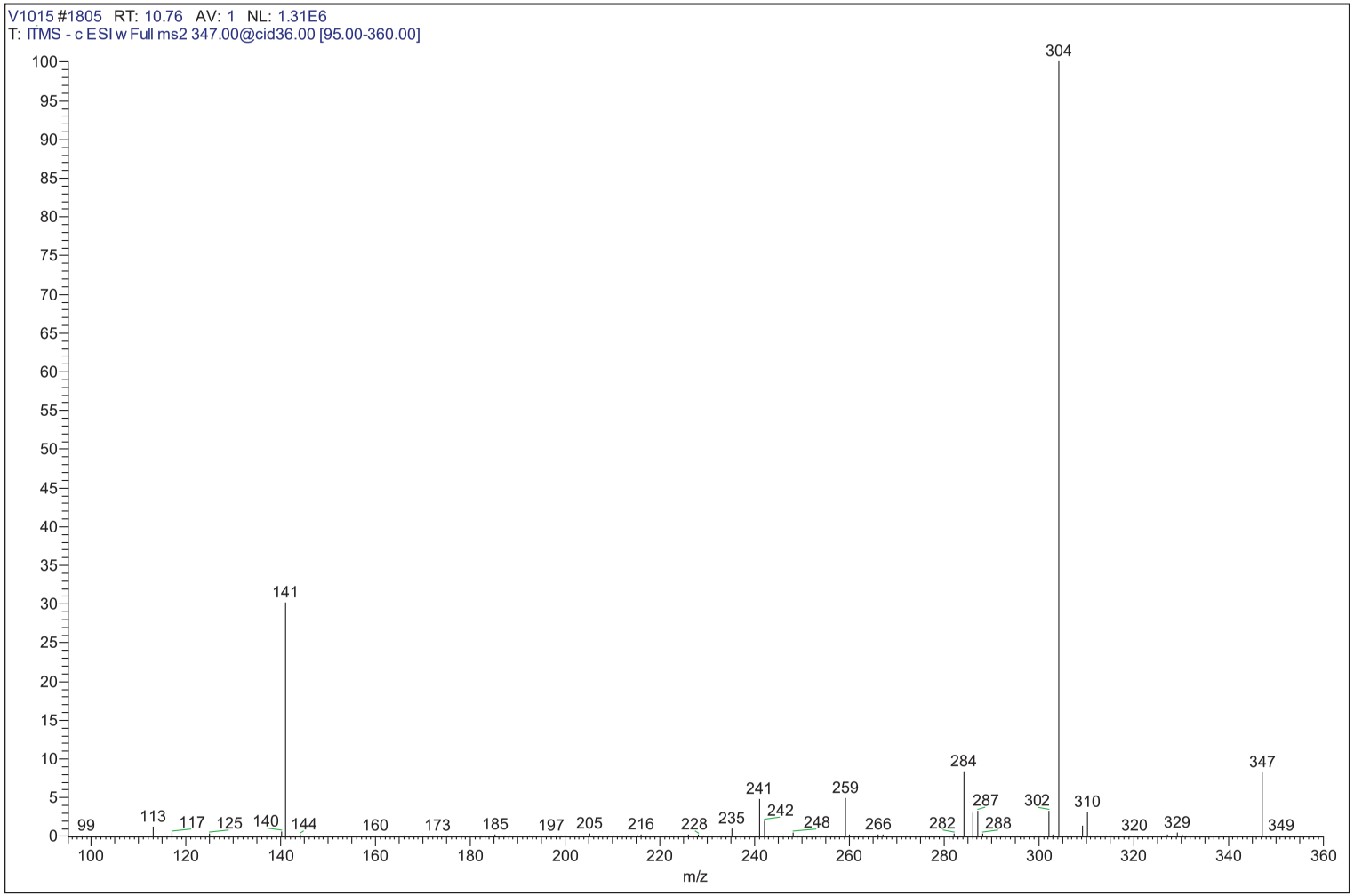

Negative ESI-ion trap-MS: 5F-AB-P7AICA (MeOH) collision energy 36 V, wideband, RRT: 1.020

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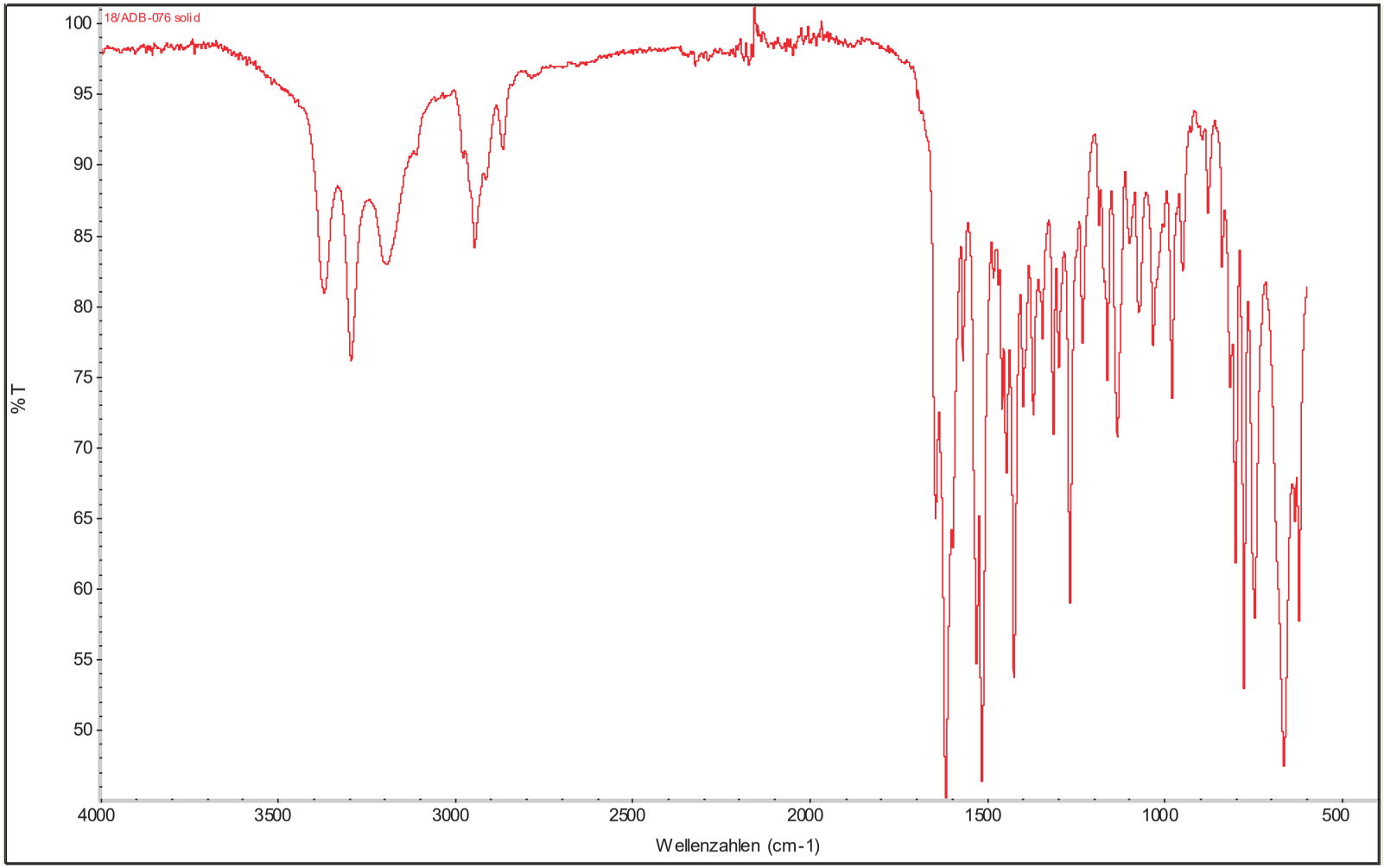

ATR-IR: 5F-AB-P7AICA solid (entire scan range)

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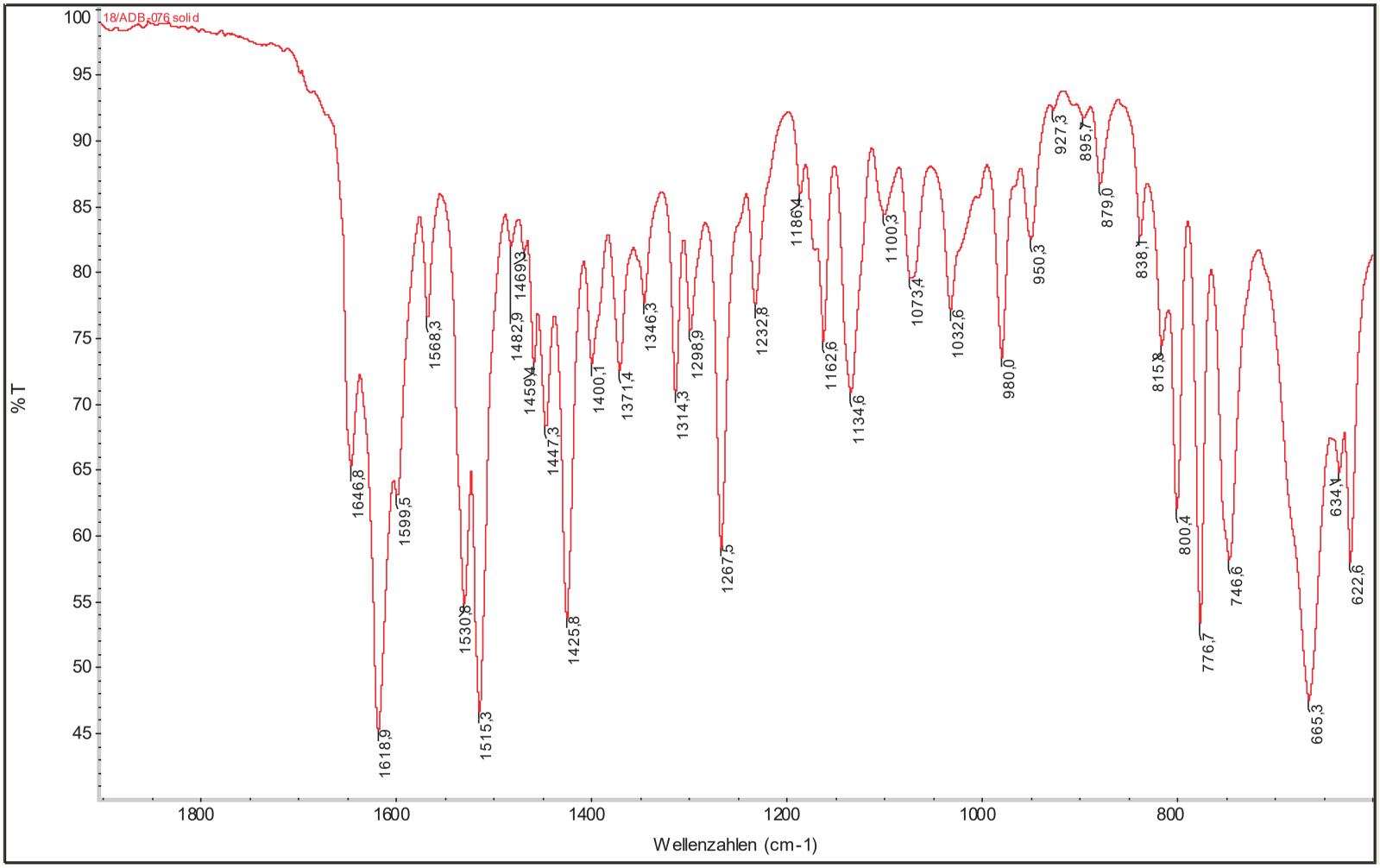

ATR-IR: 5F-AB-P7AICA solid (partial scan range)

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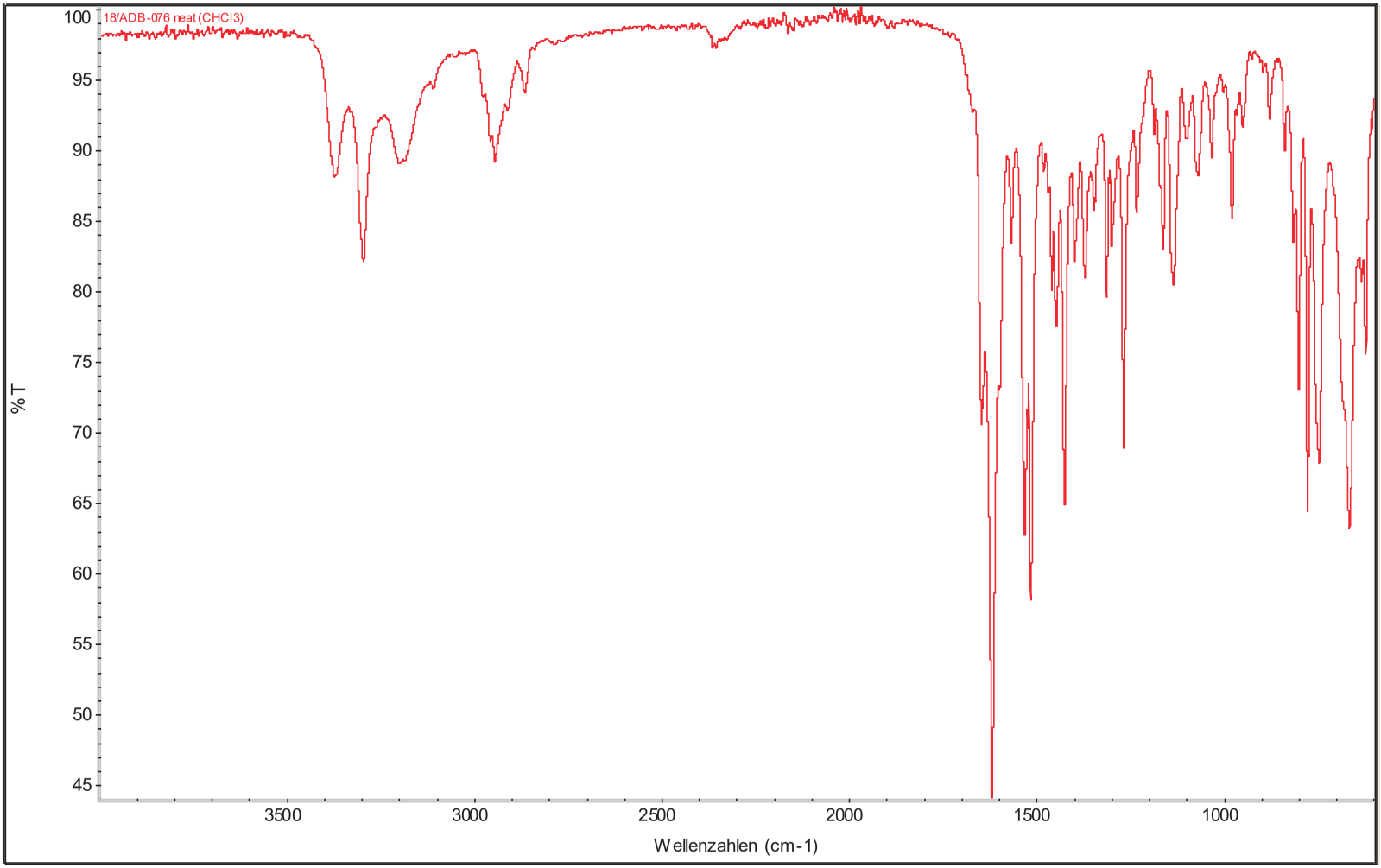

ATR-IR: 5F-AB-P7AICA neat (entire scan range)

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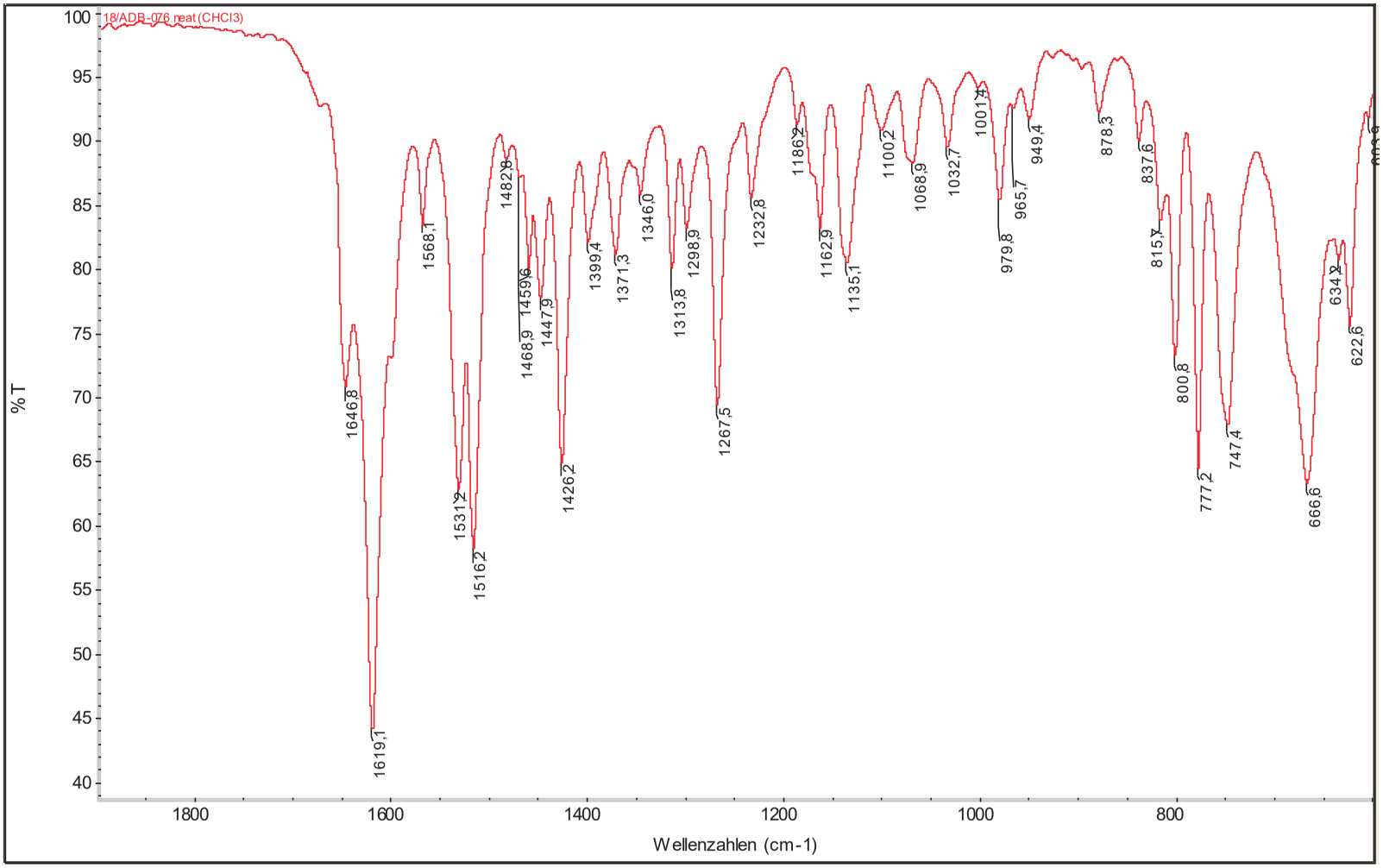

ATR-IR: 5F-AB-P7AICA neat (partial scan range)

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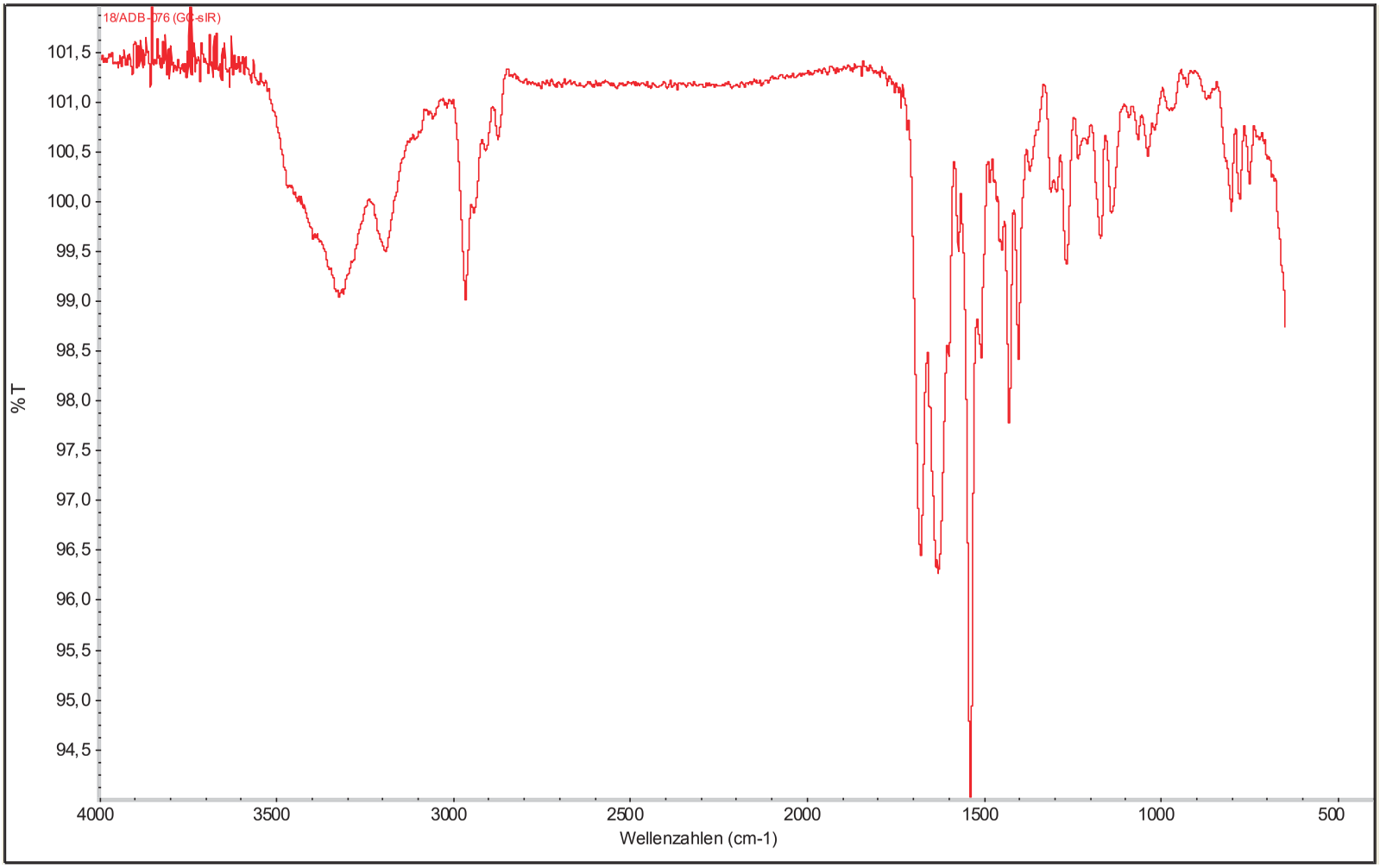

ATR-IR: 5F-AB-P7AICA ($CHCl_3$) GC-sIR (entire scan range)

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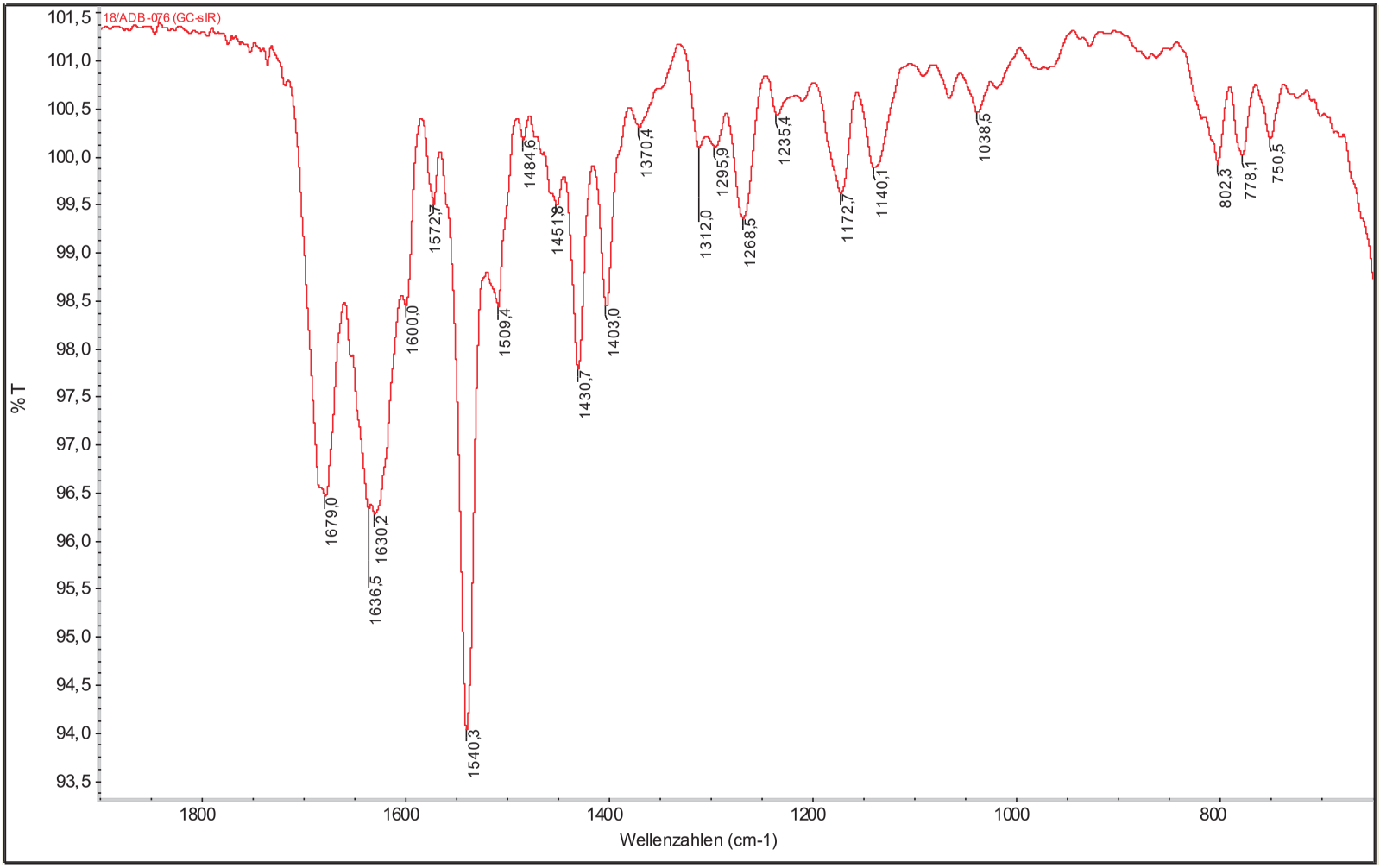

ATR-IR: 5F-AB-P7AICA ($CHCl_3$) GC-sIR (partial scan range)

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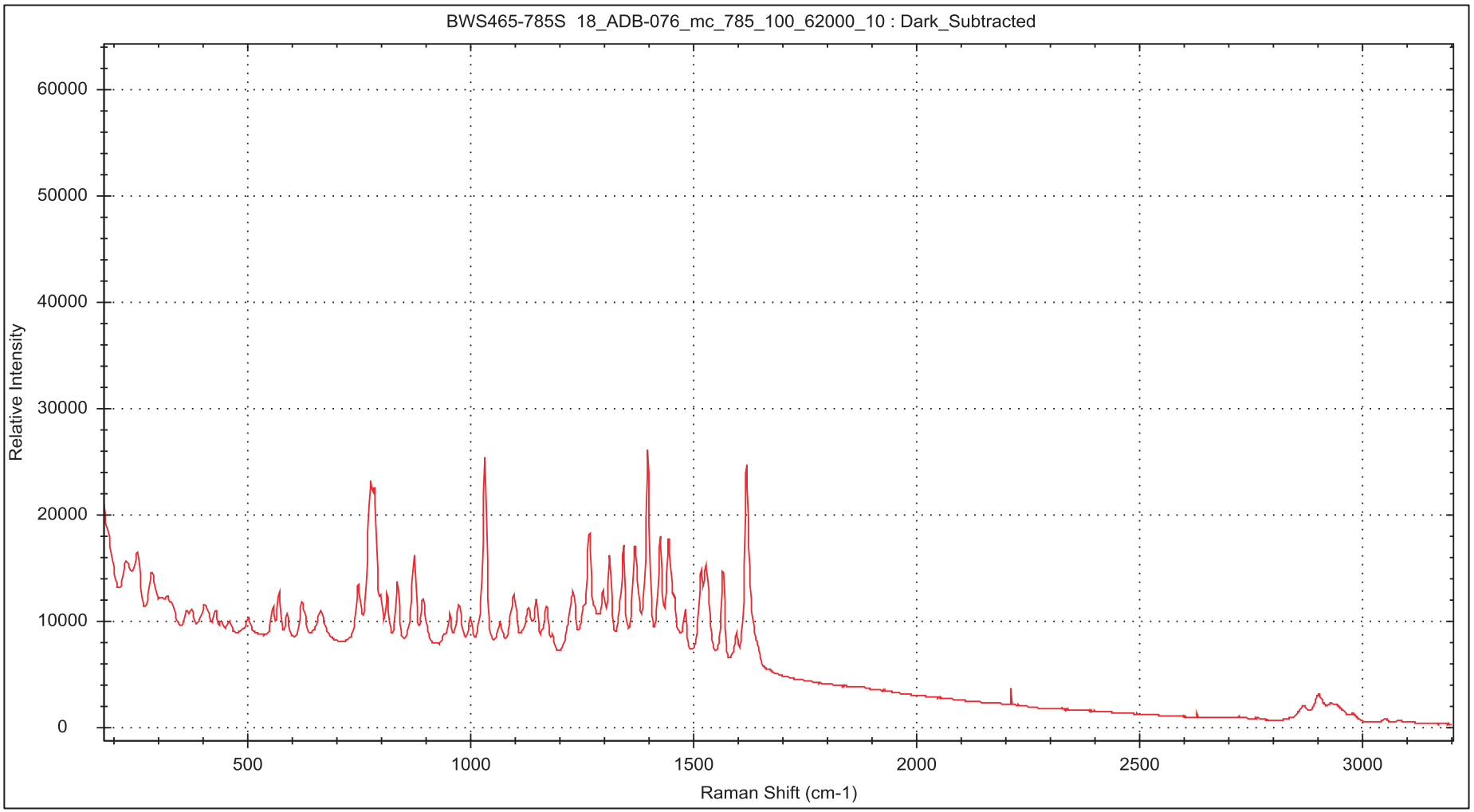

Raman: 5F-AB-P7AICA solid at λ = 785 nm (entire scan range)

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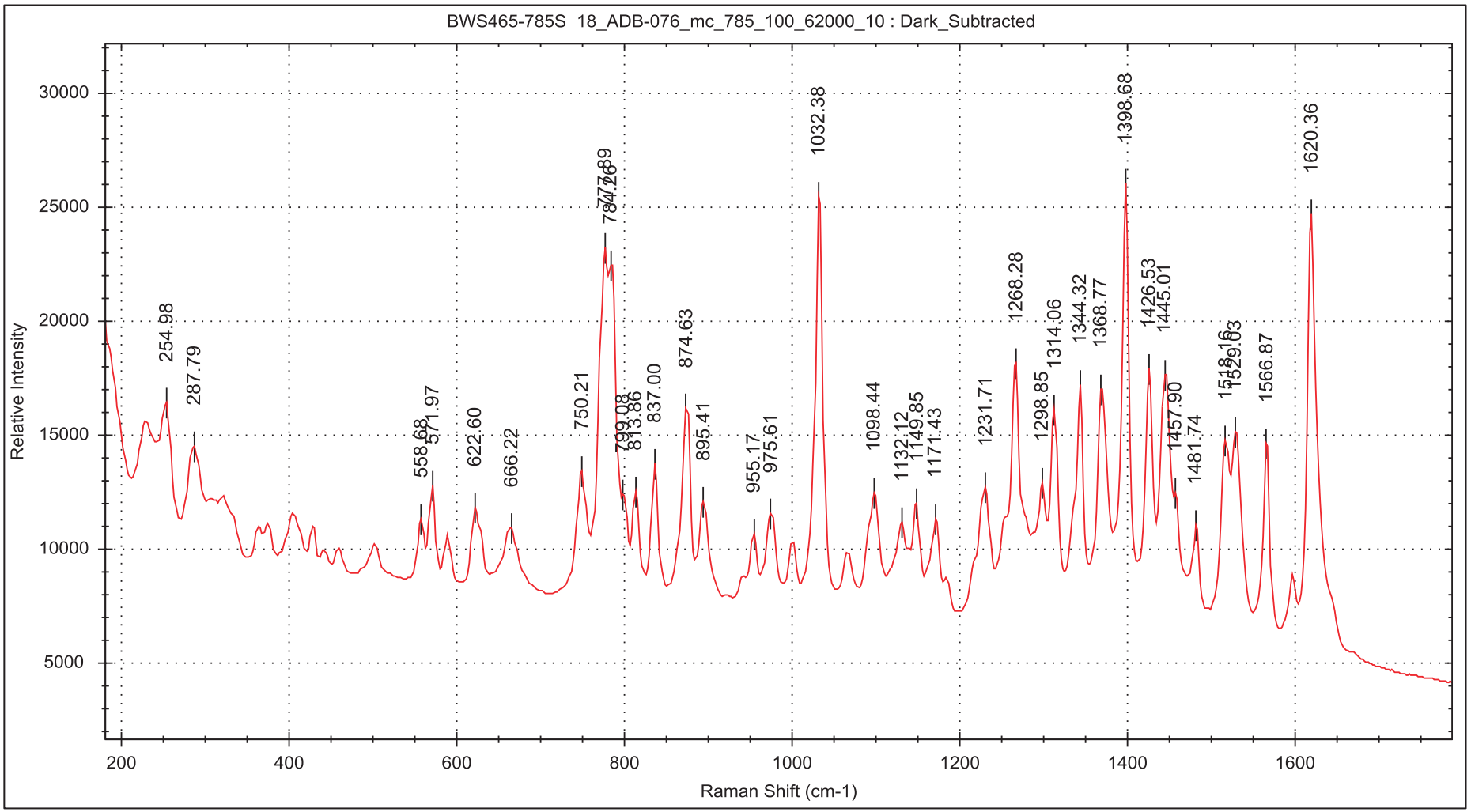

Raman: 5F-AB-P7AICA solid at λ = 785 nm (partial scan range)

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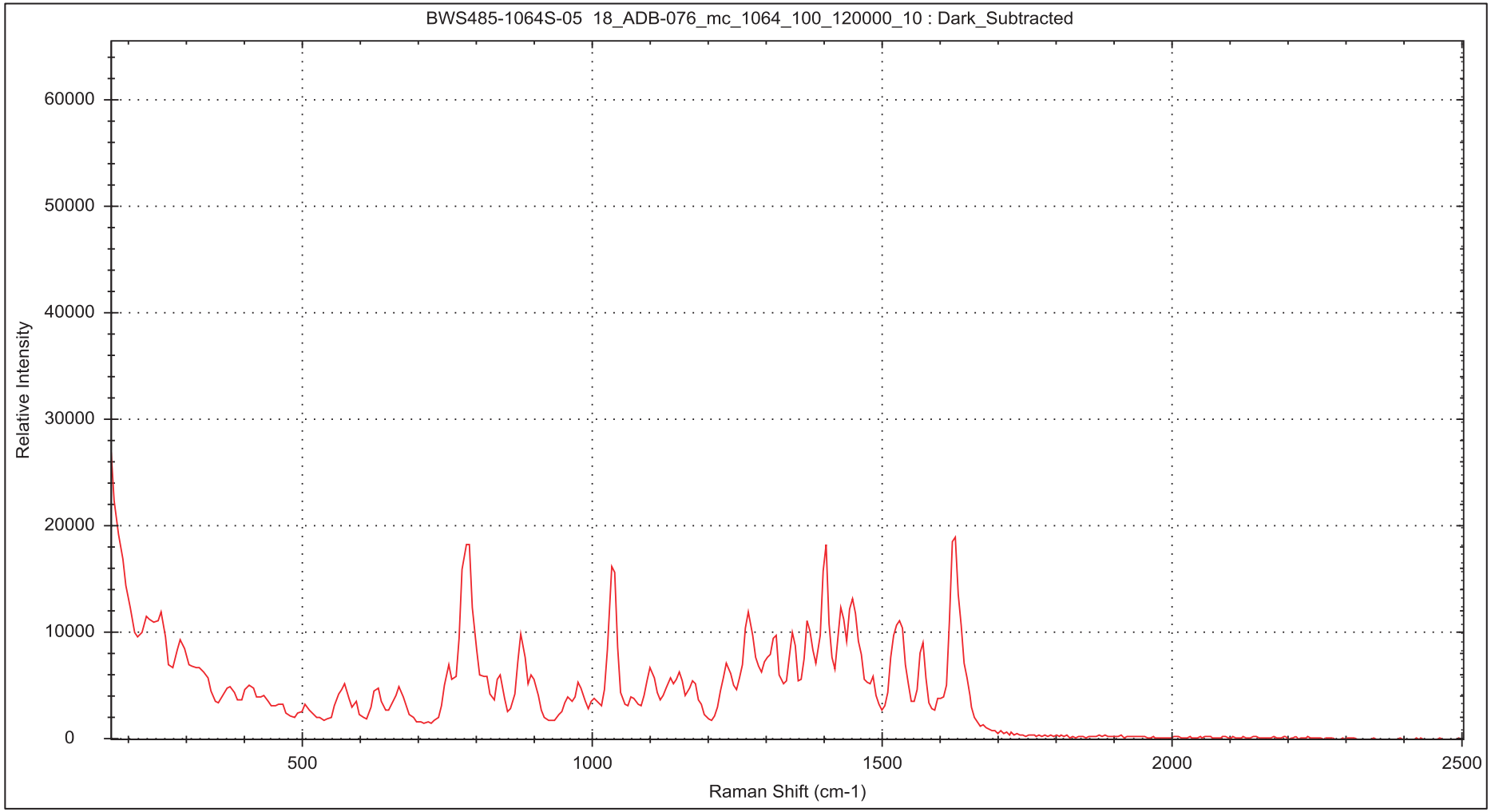

Raman: 5F-AB-P7AICA solid at λ = 1064 nm (entire scan range)

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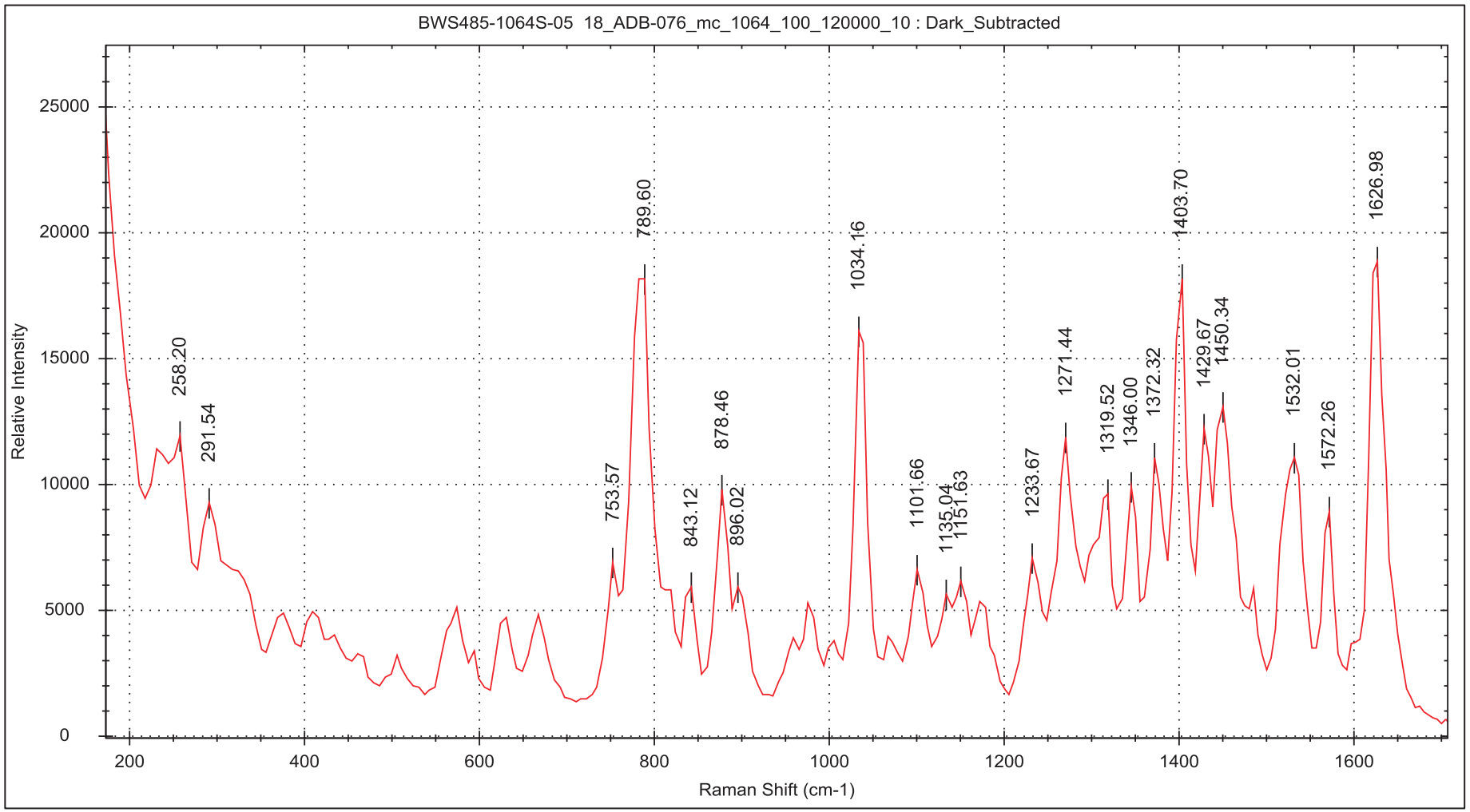

Raman: 5F-AB-P7AICA solid at λ = 1064 nm (partial scan range)

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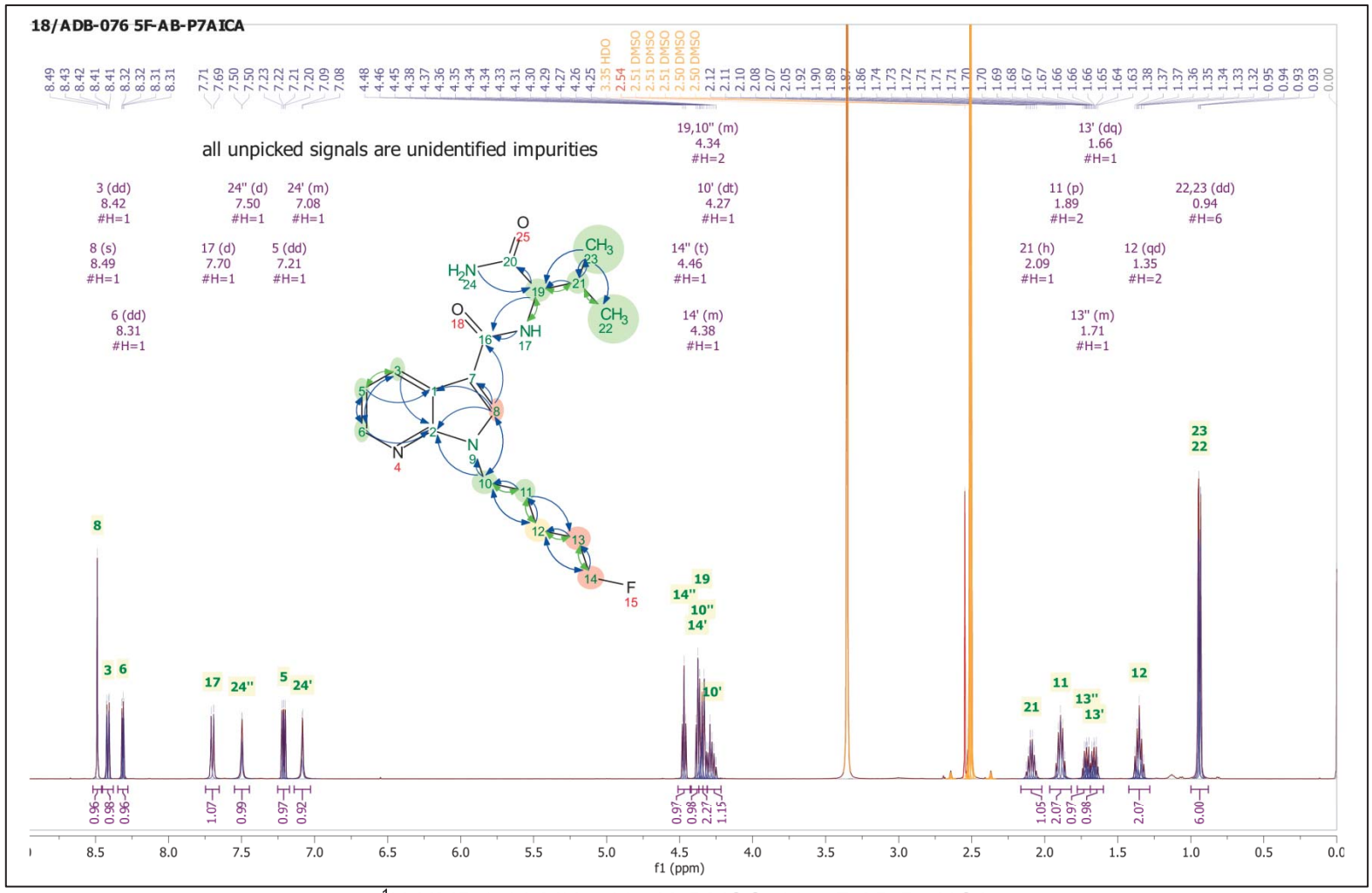

^{1}H-NMR (500 MHz, 297 K, DMSO-d_6): 5F-AB-P7AICA

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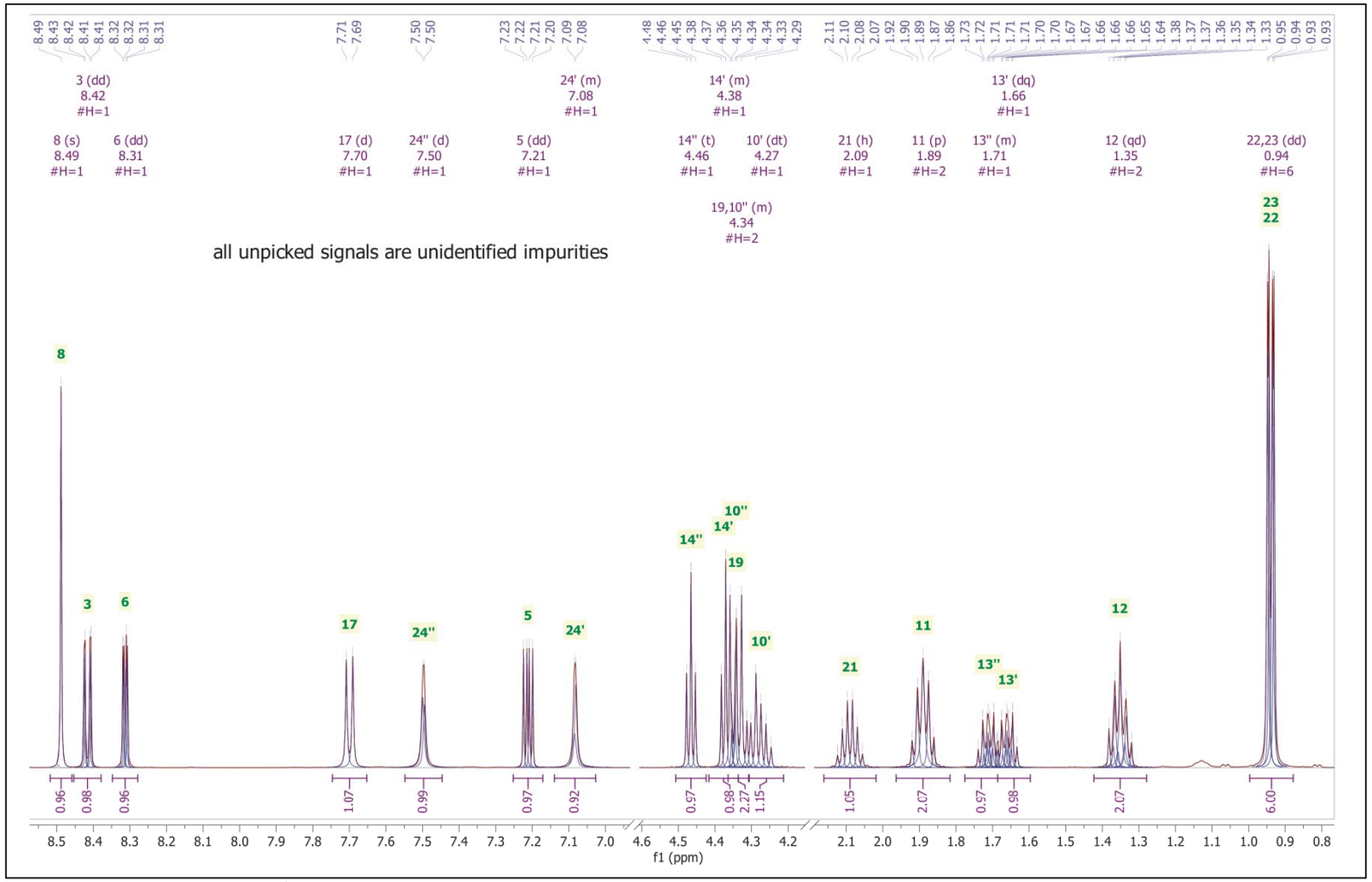

^{1}H-NMR (500 MHz, 297 K, DMSO-d_6): 5F-AB-P7AICA (enlarged signals)

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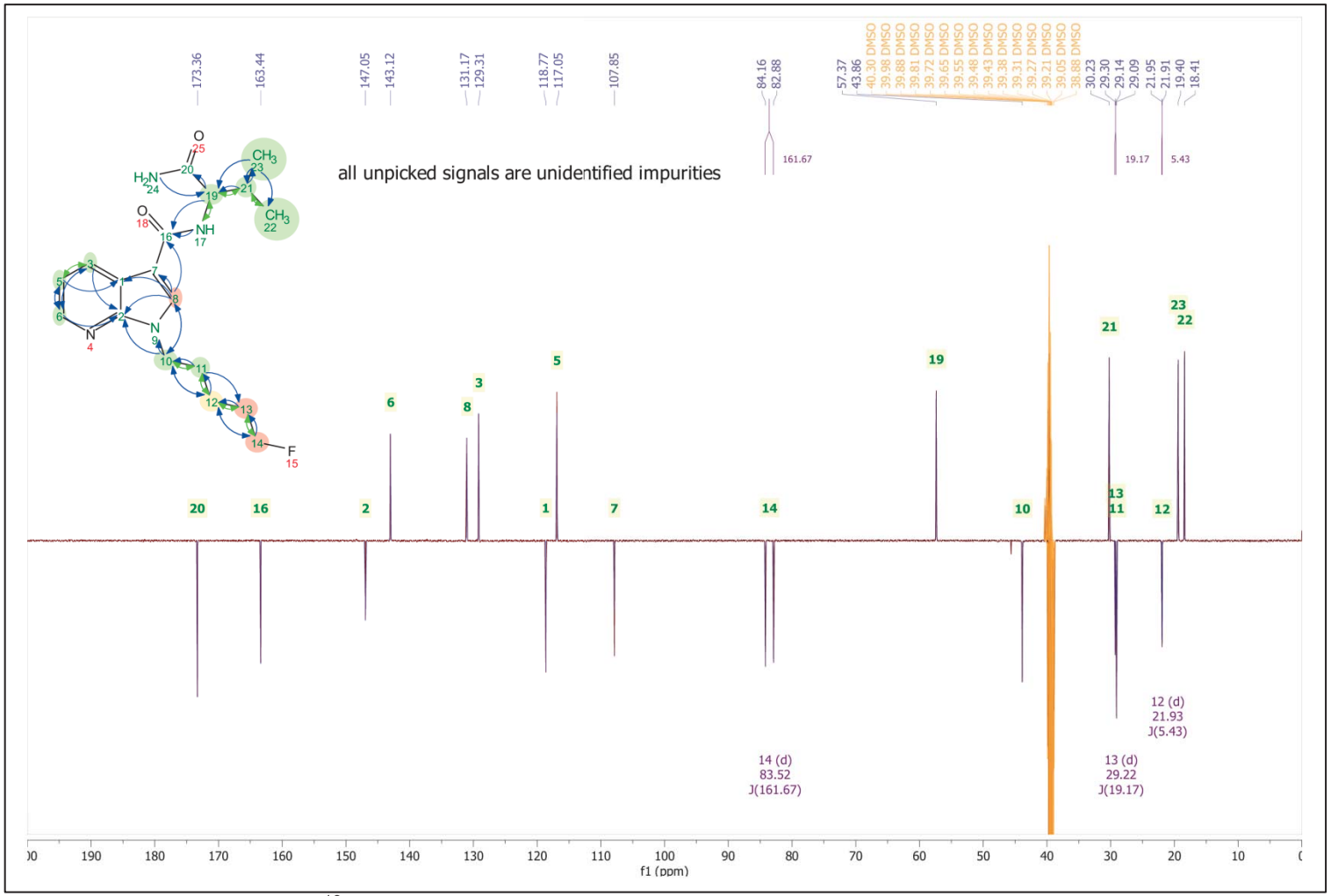
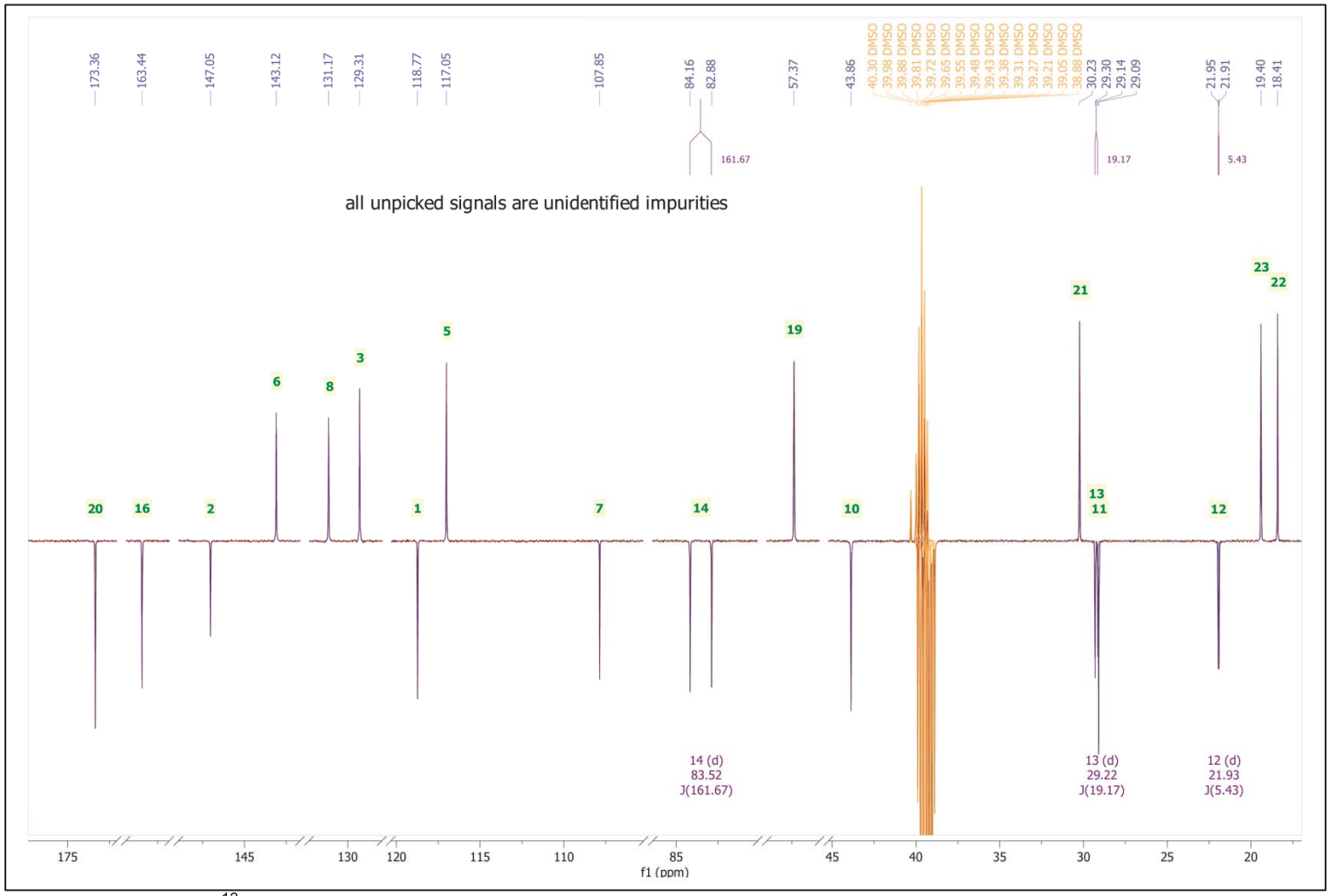


^{13}C-APT-NMR (125 MHz, 297 K, DMSO-d_6): 5F-AB-P7AICA

The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



^{13}C-APT-NMR (125 MHz, 297 K, DMSO-d_6): 5F-AB-P7AICA (enlarged signals)

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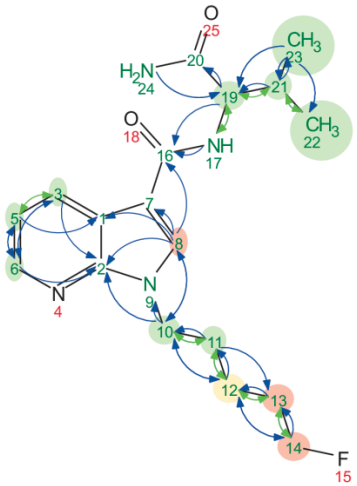

Mol Formula: $C_{18}H_{25}FN_4O_2$
Av Mass: 348.415
Mono Mass: 348.196
Molecule Name: 5F-AB-P7AICA
Molecule Label: 5F-AB-P7AICA_DMSO
Assignments Comment: complete, more assigned than in CDCl3
FS Class: Cannabinoid
FS Application Details: 18/ADB-076
Solubility Limit: DMSO:s ;CDCl3:s; Acetone:ns
IUPAC: N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide

		Assignments						Assignments					
Atom	Chemical Shift	Predicted Shift	Quality	COSY	HSQC	HMBC	Atom	Chemical Shift	Predicted Shift	Quality	COSY	HSQC	HMBC
1 C	118.77	121.15				5, 8	14 C	83.52	82.77			14', 14''	12, 13', 13''
2 C	147.05	149.85				3, 6, 8, 10', 10''	H'	4.37	4.46, 4.35	-0.35	13', 13''	14	12, 13
3 C	129.27	128.53			3	6	H''	4.46	4.46, 4.35	-0.35	13', 13''	14	12, 13
H	8.42	8.43	0.71	5	3	2, 6	15 F						
4 N		257.70					16 C	163.44	165.26				8, 17, 19
5 C	117.02	120.11			5	6	17 N	111.80	108.86				
H	7.22	7.39	0.71	3	5	1, 6	H	7.70			19		16, 19
6 C	143.09	145.04			6	3, 5	18 O						
H	8.32	8.33	0.62		6	2, 3, 5	19 C	57.34	58.44				17, 21, 23, 24', 24''
7 C	107.85	111.06				8	H	4.34	4.26	0.71	17, 21		16, 20
8 C	131.09	128.95			8	10', 10''	20 C	173.36	172.55				19
H	8.49	7.16	-0.26		8	1, 2, 7, 10, 16	21 C	30.18	31.07			21	23
9 N	148.30	141.23					H	2.09	2.14	0.71	19, 22	21	19, 22, 23
10 C	43.83	46.04			10', 10''	8, 11, 12	22 C	18.37	18.83			22	21, 23
H'	4.29	4.16, 4.11	0.71	11	10	2, 8, 11, 12	H3	0.94	0.94	0.71	21	22	
H''	4.34	4.16, 4.11	0.26	11	10	2, 8, 11, 12	23 C	19.37	18.83			23	21
11 C	29.06	28.58			11	10', 10'', 12	H3	0.95	0.99	0.71		23	19, 21, 22
H2	1.89	1.78, 1.76	0.61	10', 10'', 12	11	10, 12, 13	24 N		106.53				
12 C	21.93	23.83			12	10', 10'', 11, 13', 13'', 14', 14''	H'	7.08					19
							H''	7.50					19
H2	1.35	1.46, 1.39	0.09	11, 13', 13''	12	10, 11, 14	25 O						
13 C	29.22	29.94				11, 14', 14''							
H'	1.66	1.70, 1.61	-0.28	12, 14', 14''		12, 14							
H''	1.71	1.70, 1.61	-0.28	12, 14', 14''		12, 14							

Peak list and assignments of 5F-AB-P7AICA signals.