

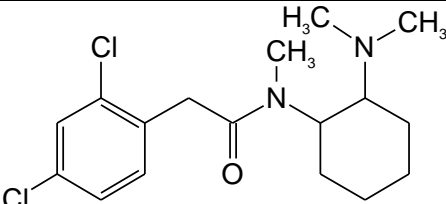


Analytical Report¹

U-48800

2-(2,4-Dichlorophenyl)-N-(2-(dimethylamino)cyclohexyl)-N-methylacetamide

Sample ID:	17/ADB-042
Sample description:	solid, white
Date of sample receipt (D.M.Y):	09.10.2017

Substance identified structure	
Systematic name	2-(2,4-Dichlorophenyl)-N-(2-(dimethylamino)cyclohexyl)-N-methylacetamide
Other names	U-48,800
Formula	C ₁₇ H ₂₄ Cl ₂ N ₂ O
M_w (g/mol)	343
Compound Class	Opioids
Salt form	chloride
Additional information	

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.

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.

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).



MS parameters: ionization mode: EI = 70 eV; emission current: 200 μ A; ion source temperature: 175 °C; scan time: 1 s; scan range: m/z = 29 – 600.

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

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 μ L (approximately 2 mg compound in 2 mL appropriate solvent), splitless; injector temperature: 220 °C; carrier gas: helium; flow rate: 1.2 mL/min.

Chromatographic conditions: fused silica capillary DB-1 column (30 m x 0.25 mm, film thickness 0.25 μ m); 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.

MS parameters: emission current: 200 μ A, 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.

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

2a. LC-ESI-QTOF-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 μ m); Phenomenex, Aschaffenburg, Germany
Mobile phases	A: aqueous Buffer (10 mM NH ₄ COO, 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 μ L

Mass spectrometry was performed using an Agilent 6530 QTOF equipped with an Agilent Jet Stream electrospray source and controlled by Agilent MassHunter Acquisition software. QTOF 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.

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: MeOH 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: MeOH 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



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 alkalinized 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-GCTM (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.

4. Raman spectroscopy

B&W TEK Inc. i-Raman® Plus system: laser wavelength: 785 nm with BWS465-785S spectrometer: scan range: 150 - 3350 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.

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-¹H: 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-¹³C: pulse program: jmod (APT), number of scans: 512 or more

Assignments are supported by COSY, HSQC and HMBC.

5b. NMR Bruker AVANCE III HD 500

Spectrometer: Bruker AVANCE III HD 500

Probe: Bruker 5mm 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-¹H: 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-¹³C: 125 MHz, pulse program: jmod (APT), number of scans: 512 or more

Assignments are supported by COSY, HSQC and HMBC.



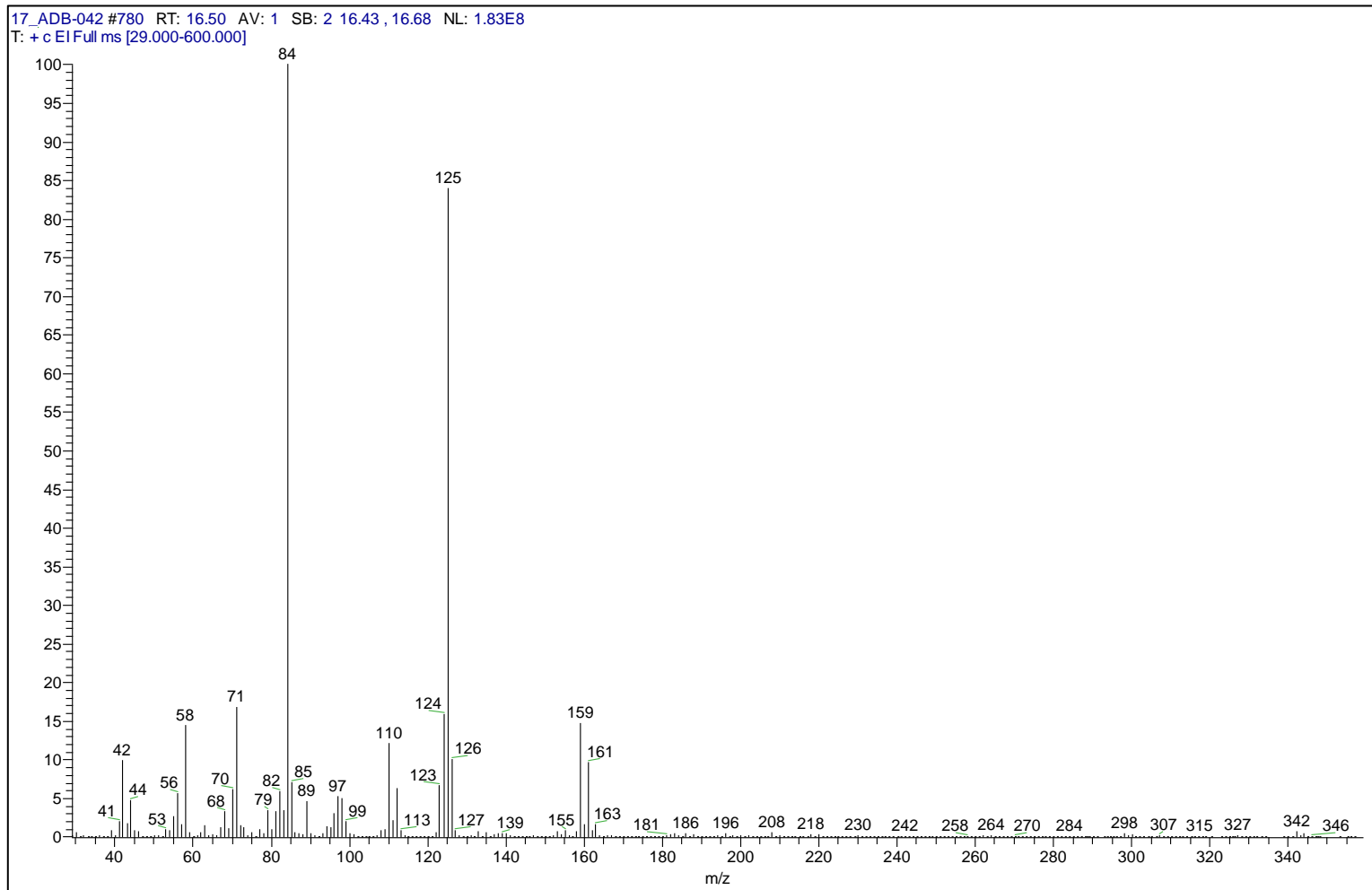
Supporting information

Analytical technique		applied	remarks
GC-MS	EI ^{1a}	+	
	CI ^{1b}	+	
LC-MS	QTOF ^{2a}		
	linear ion trap ^{2b}	+	
IR	ATR-IR solid ^{3a}	+	
	ATR-IR base neat ^{3a}	+	
	GC-sIR ^{3b}	+	
	FT-NIR solid ^{3c}	+	
Raman	Raman at $\lambda = 785 \text{ nm}$ ⁴		
NMR	¹ H, ¹³ C ^{5a}	+	

Analytical results

Gas chromatography-mass spectrometry

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

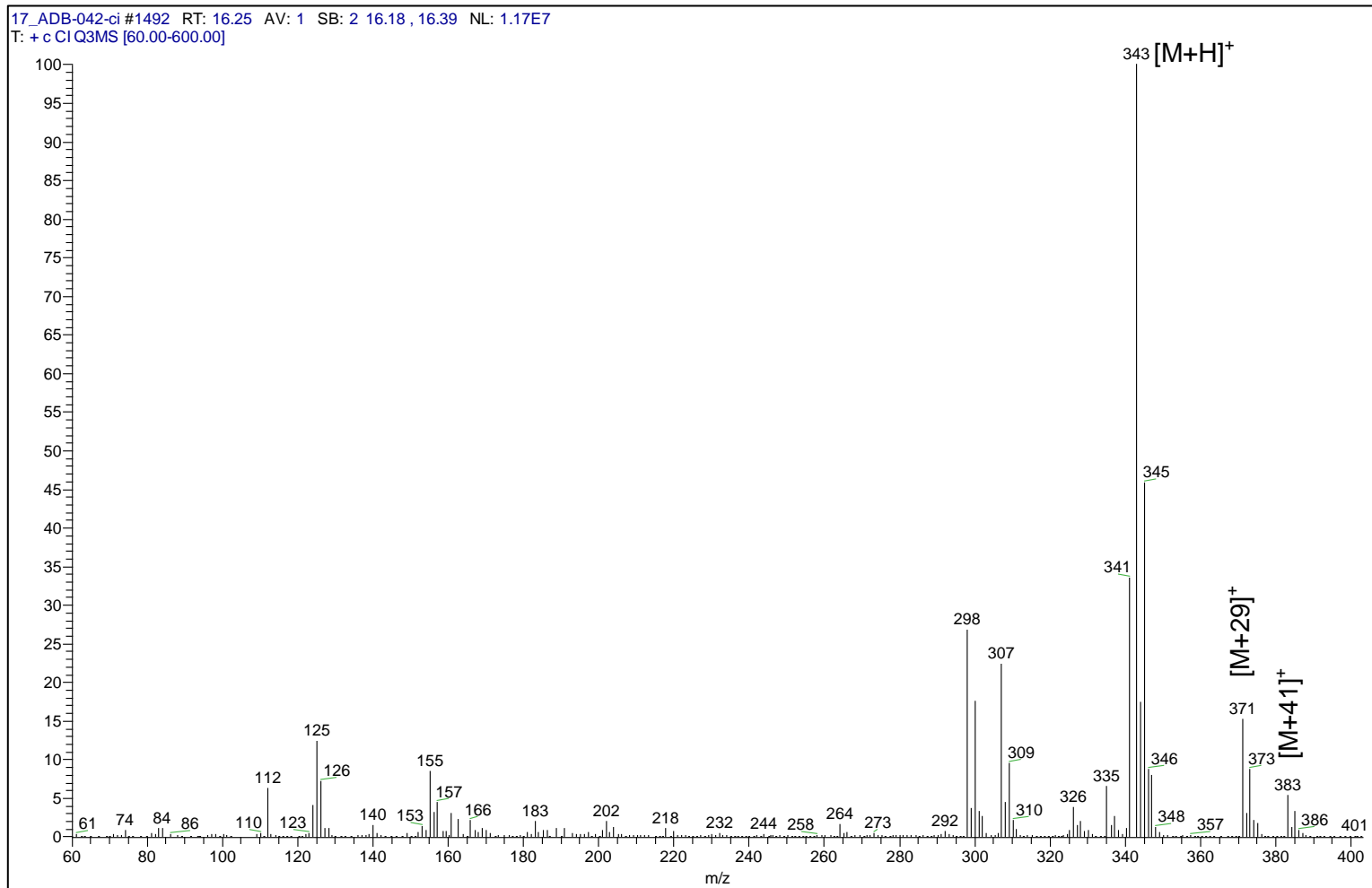


GC-EI-MS: U-48800 (alkaline diethyl ether extract), RI: 2534 (DB-1)

Analytical results

Gas chromatography-mass spectrometry

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

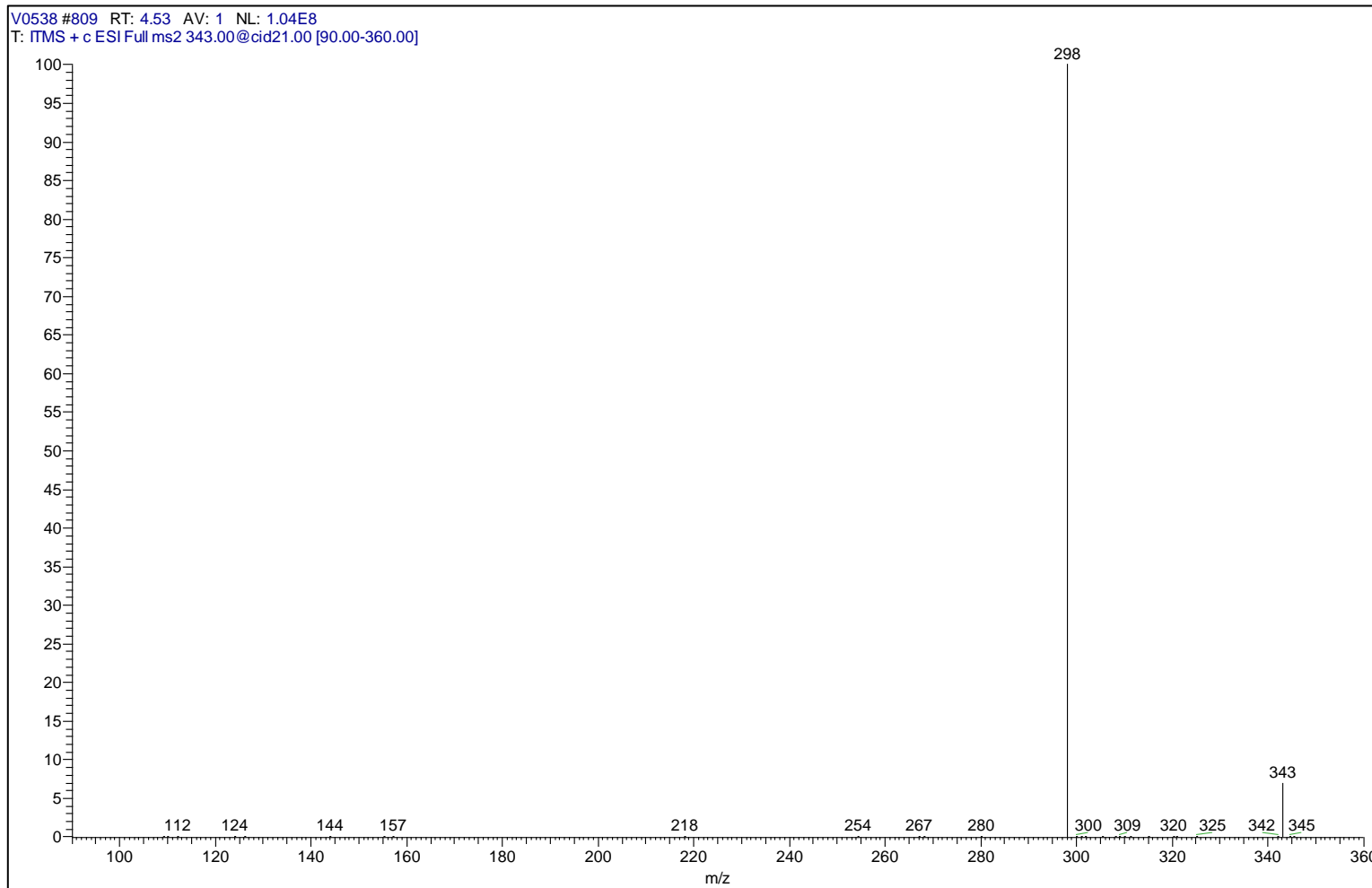


GC-Cl-MS (methane): U-48800 (alkaline diethyl ether extract), RI: 2534 (DB-1)

Analytical results

Liquid chromatography-mass spectrometry

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

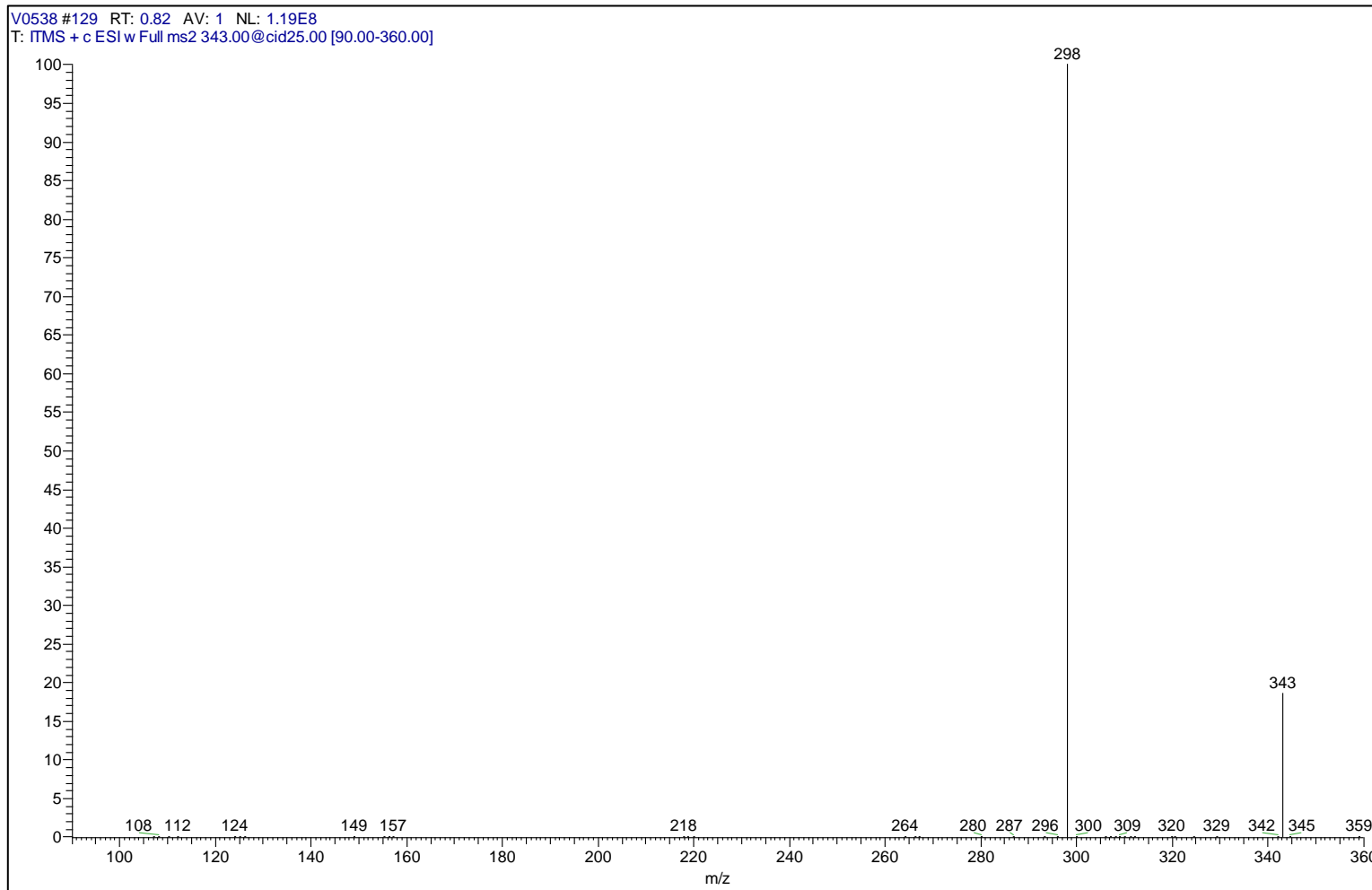


ESI-ion trap-MS: U-48800 (MeOH) collision energy 21 V, RRT: 0.829

Analytical results

Liquid chromatography-mass spectrometry

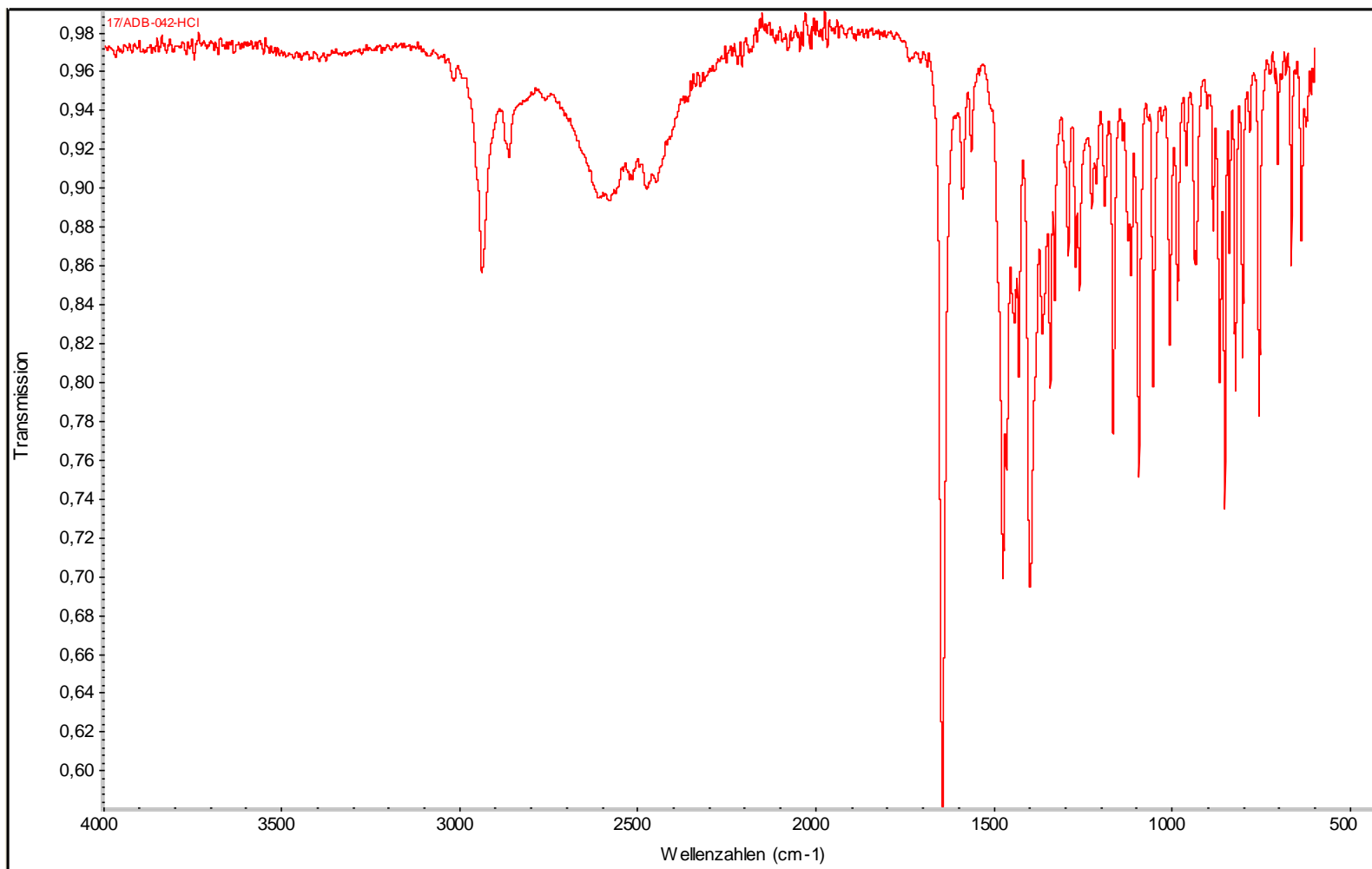
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



ESI-ion trap-MS: U-48800 (MeOH) collision energy 25 V, wideband, RRT: 0.829

Analytical results
Infrared spectroscopy

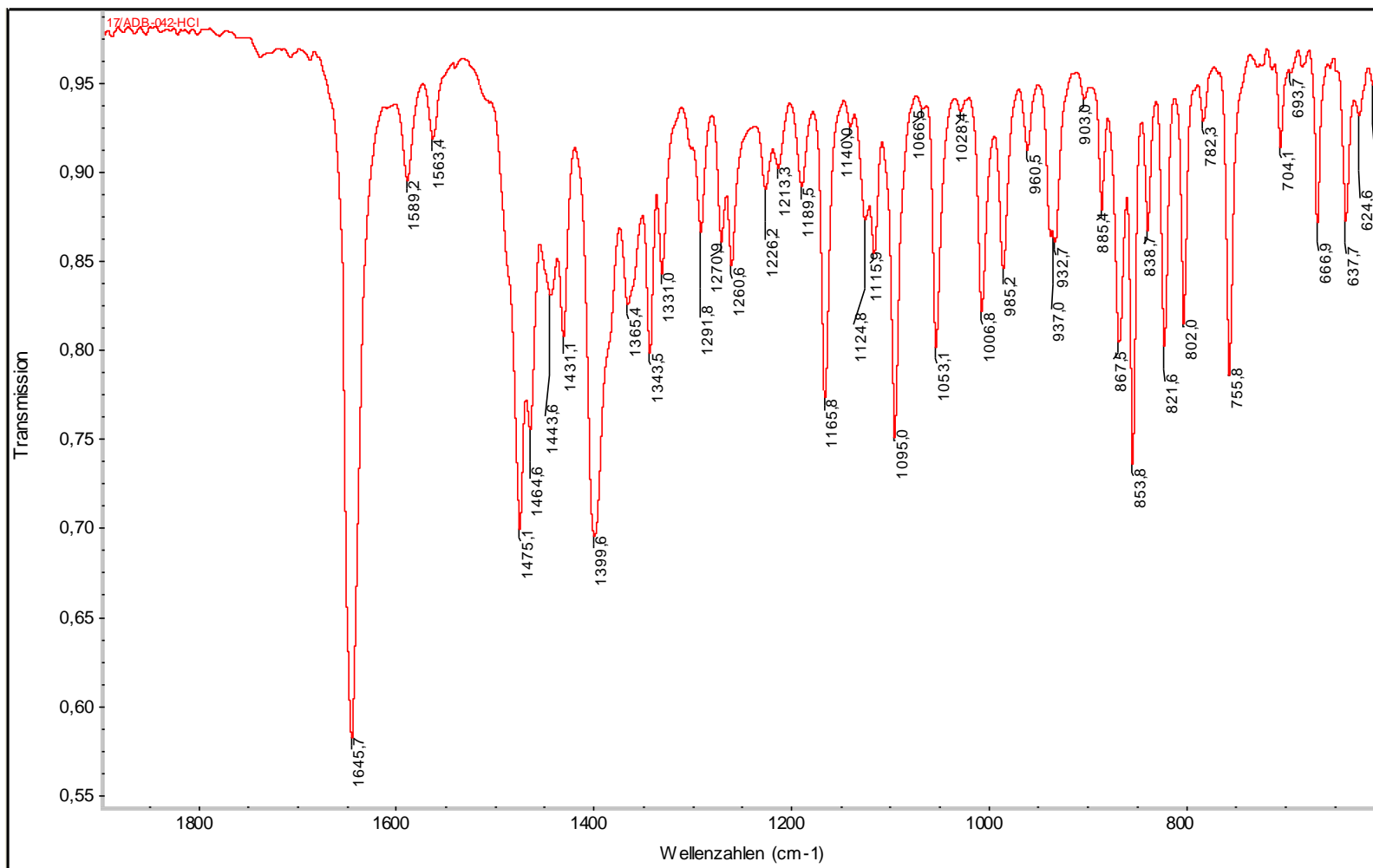
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



ATR-IR: U-48800-HCl (entire scan range)

Analytical results
Infrared spectroscopy

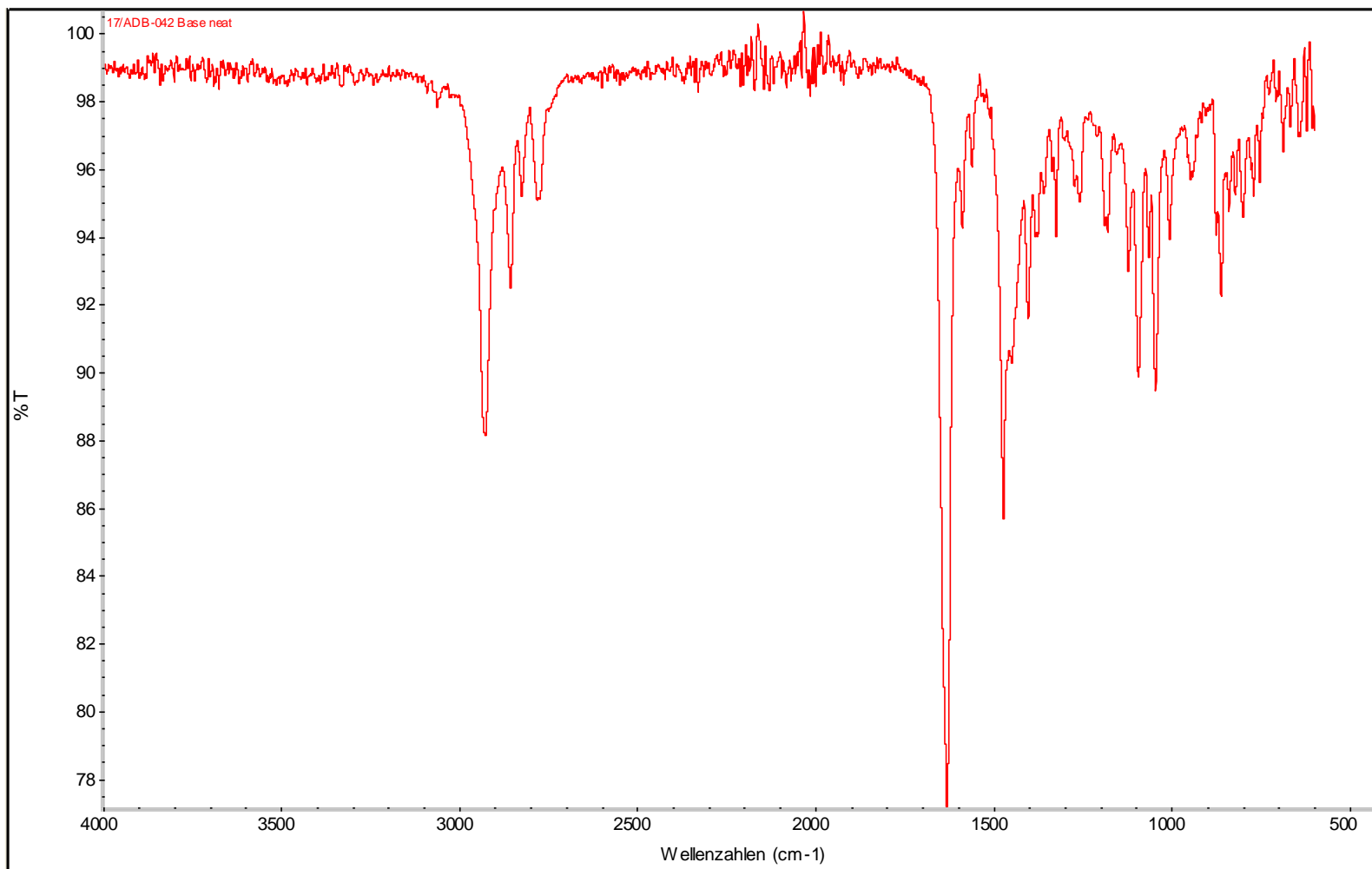
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



ATR-IR: U-48800-HCl (partial scan range)

Analytical results
Infrared spectroscopy

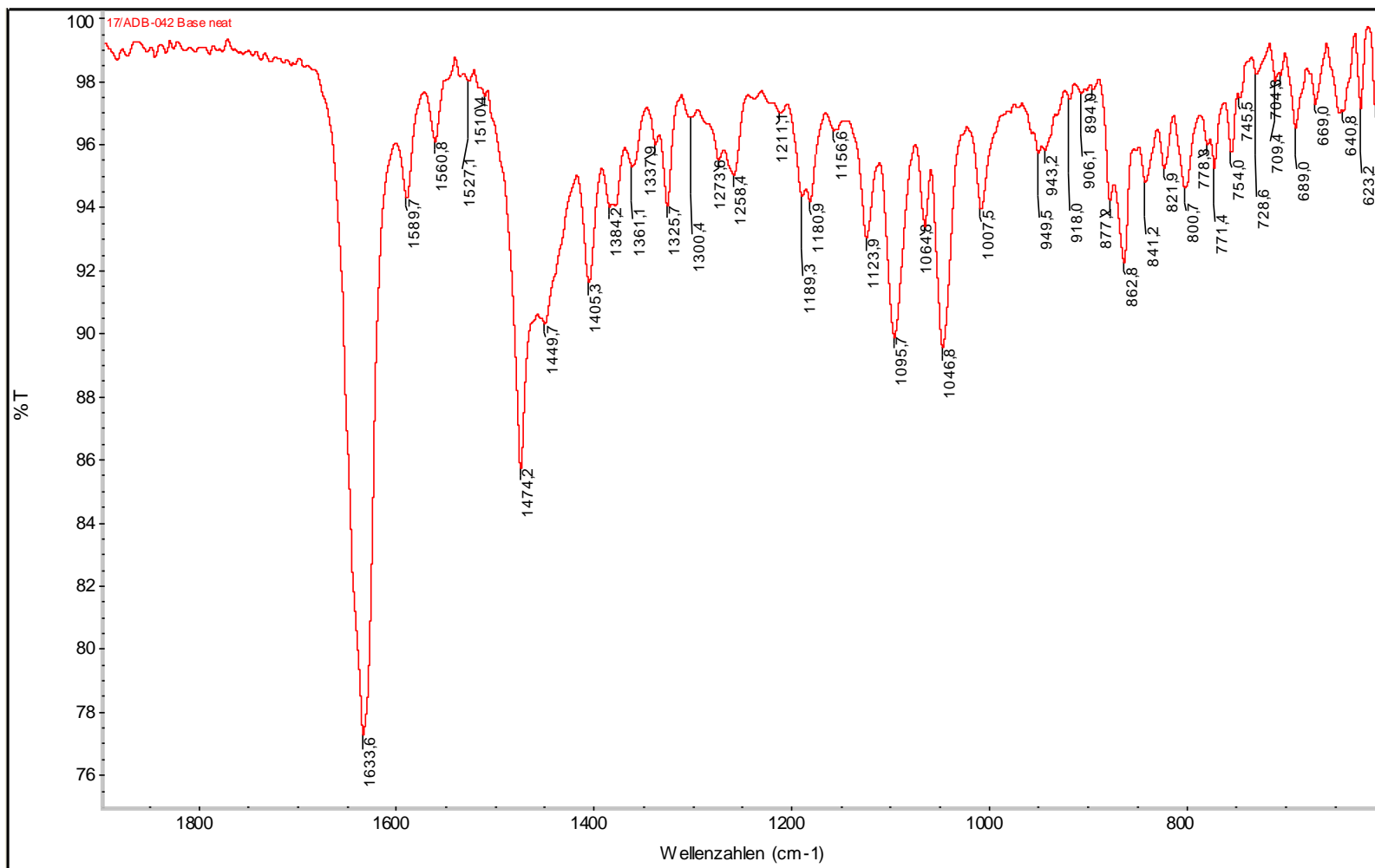
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



ATR-IR: U-48800 base neat (entire scan range)

Analytical results
Infrared spectroscopy

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

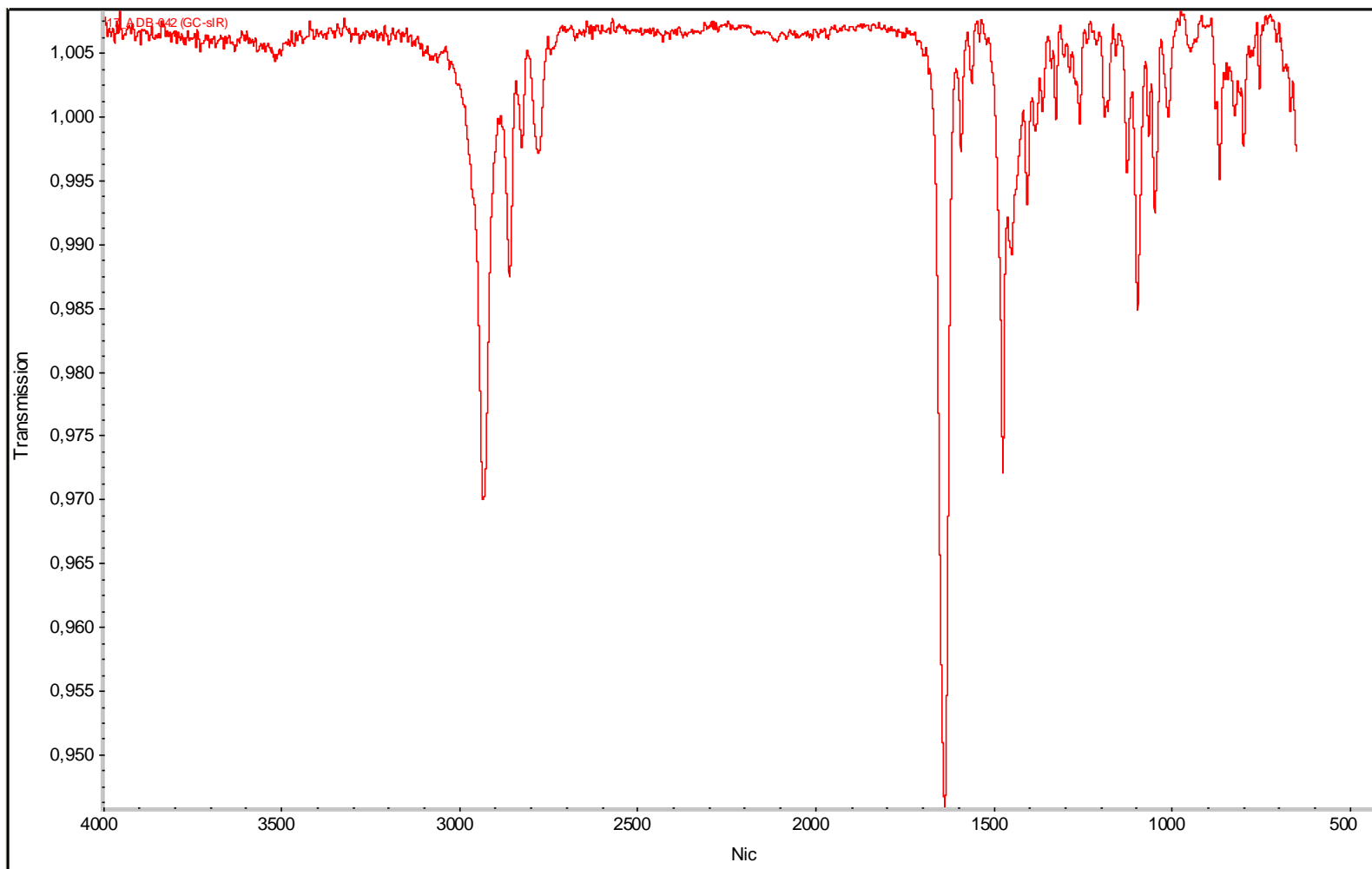


ATR-IR: U-48800 base neat (partial scan range)

Analytical results

Infrared spectroscopy

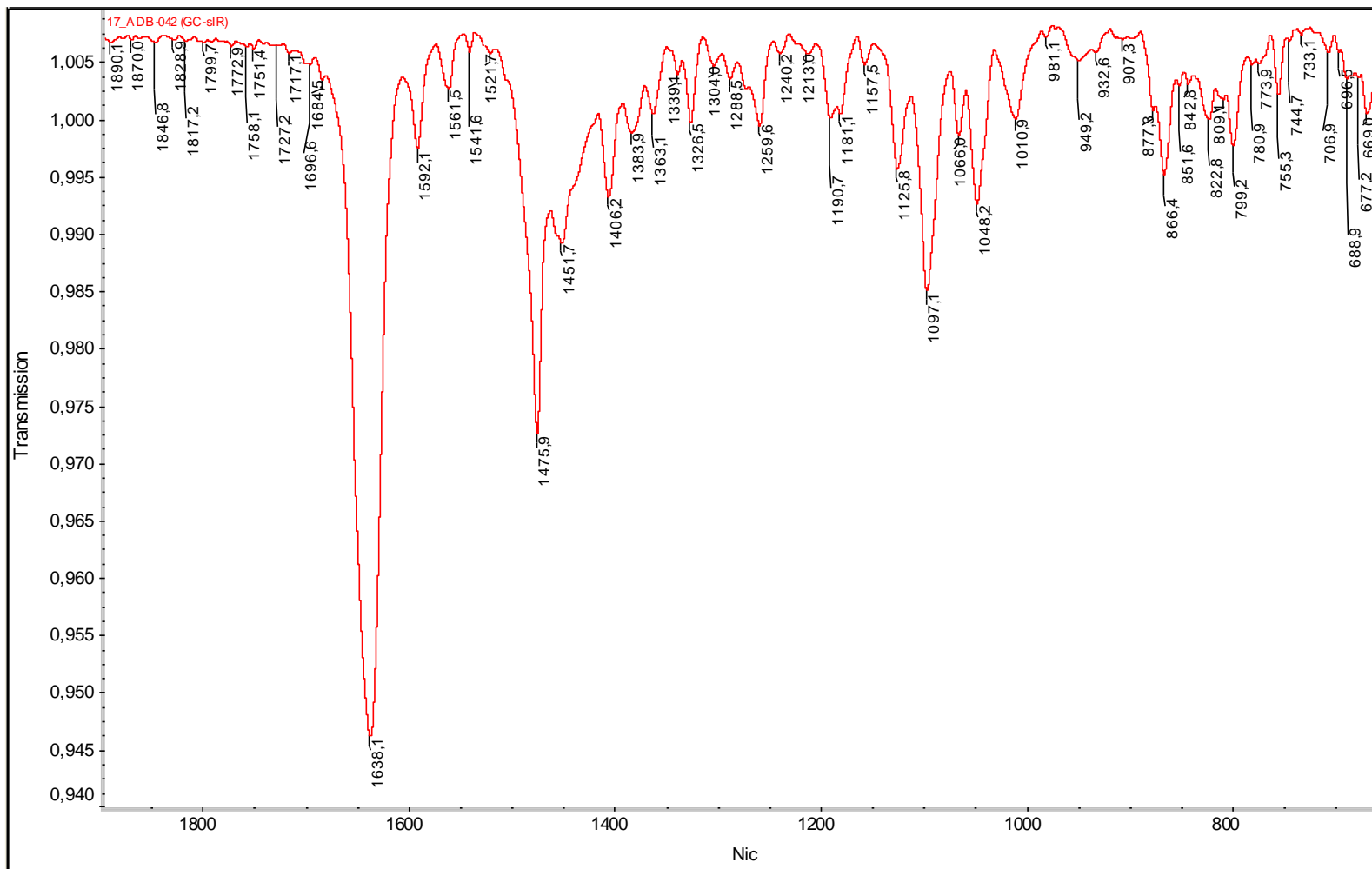
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



ATR-IR: U-48800 (alkaline diethyl ether extract) GC-sIR (entire scan range)

Analytical results
Infrared spectroscopy

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

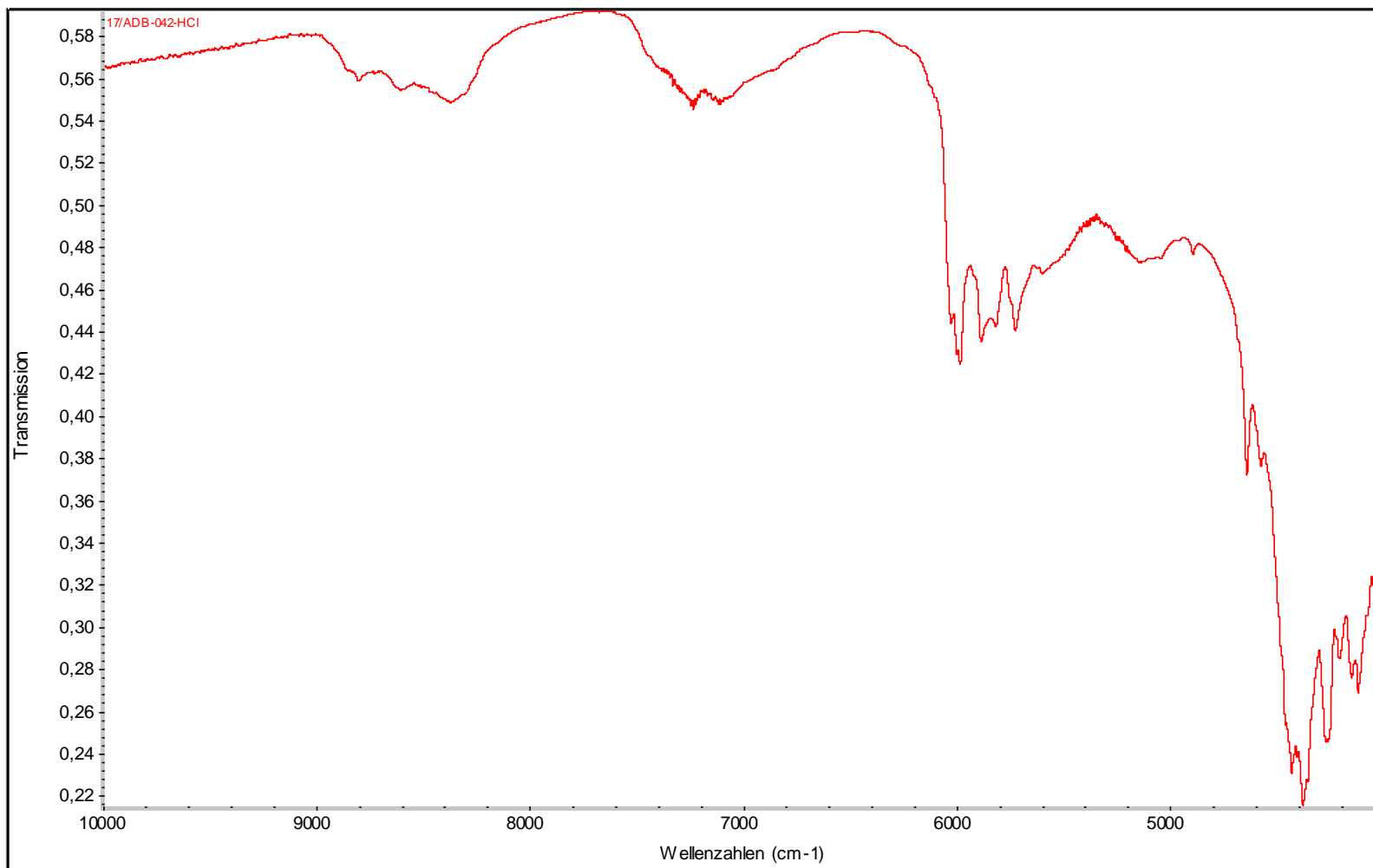


ATR-IR: U-48800 (alkaline diethyl ether extract) GC-sIR (partial scan range)

Analytical results

Infrared spectroscopy

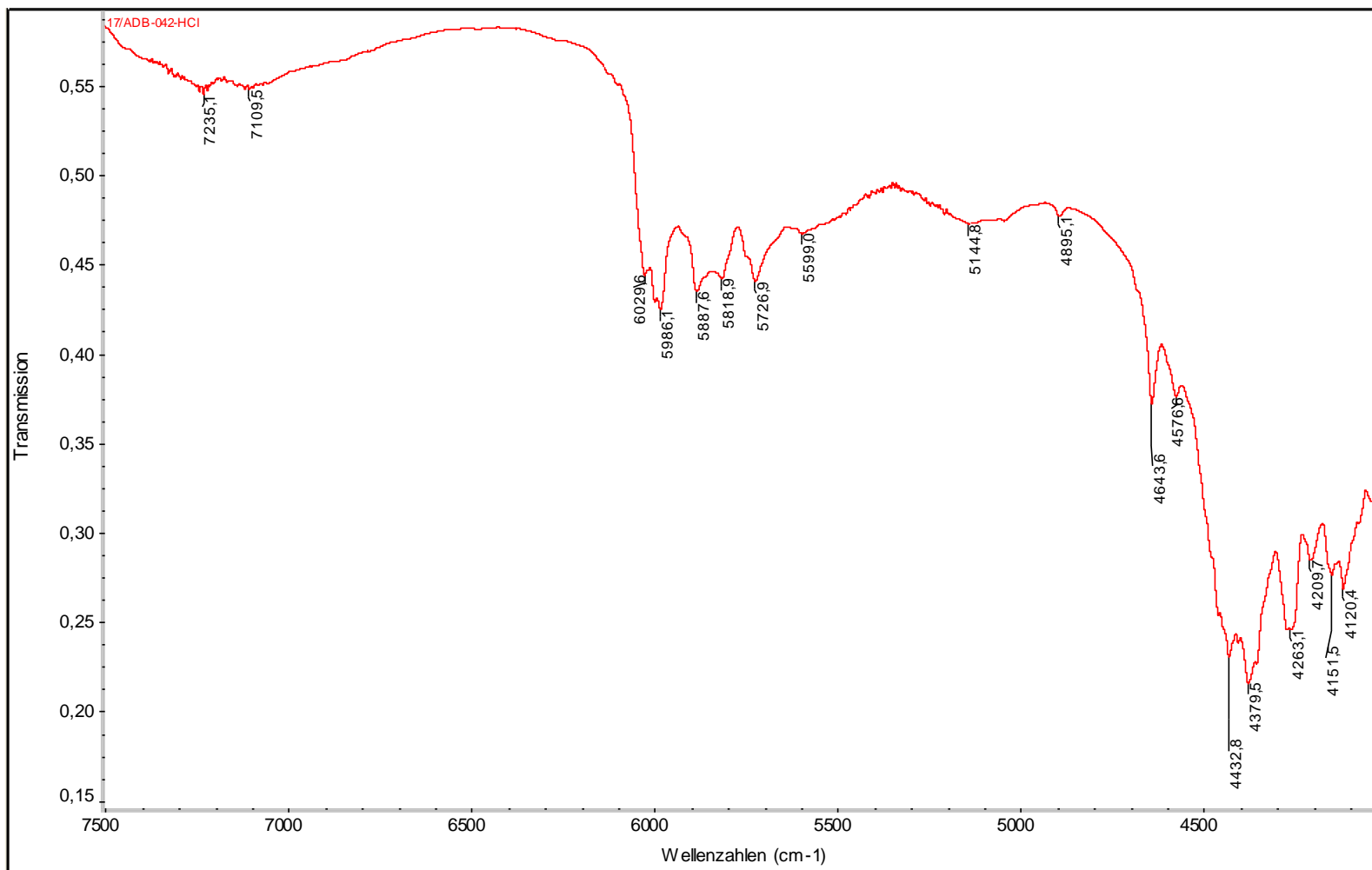
The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).



FT-NIR: U-48800-HCl (entire scan range)

Analytical results
Infrared spectroscopy

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

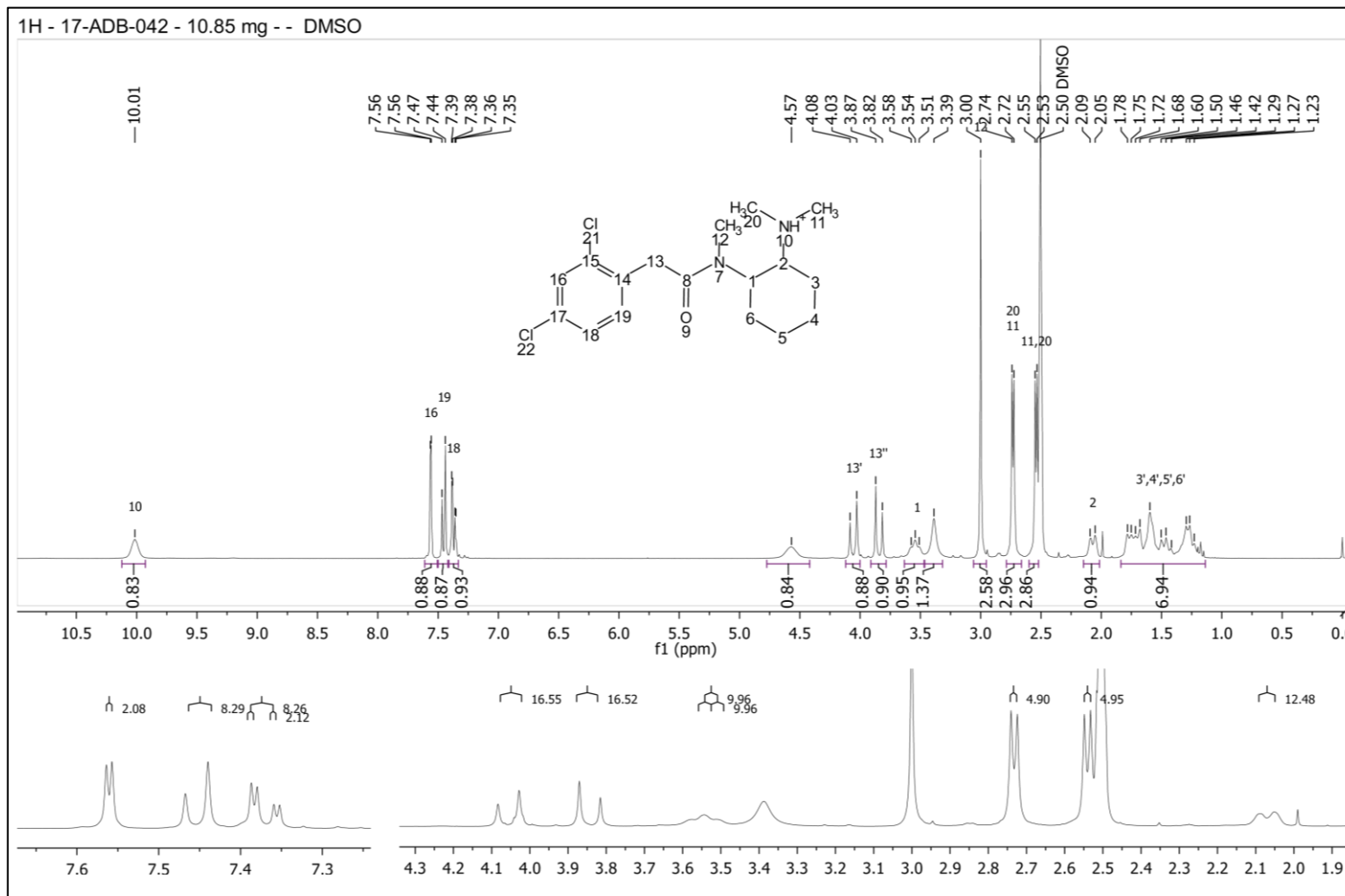


FT-NIR: U-48800-HCl (partial scan range)

Analytical results

NMR

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

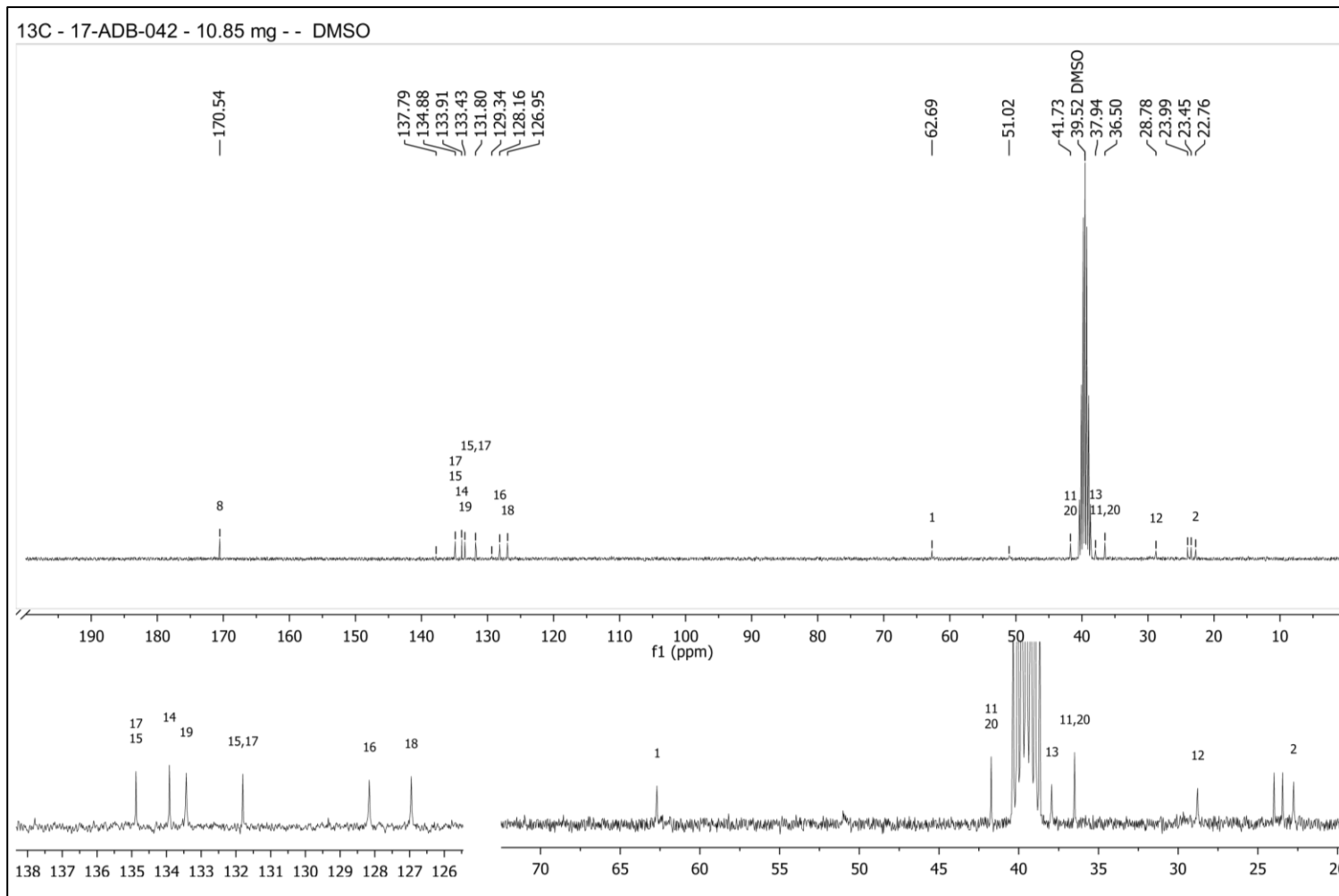


¹H-NMR (300 MHz, 297 K, DMSO-d₆): U-48800 (bottom: enlarged signals)

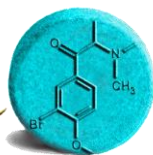
Analytical results

NMR

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



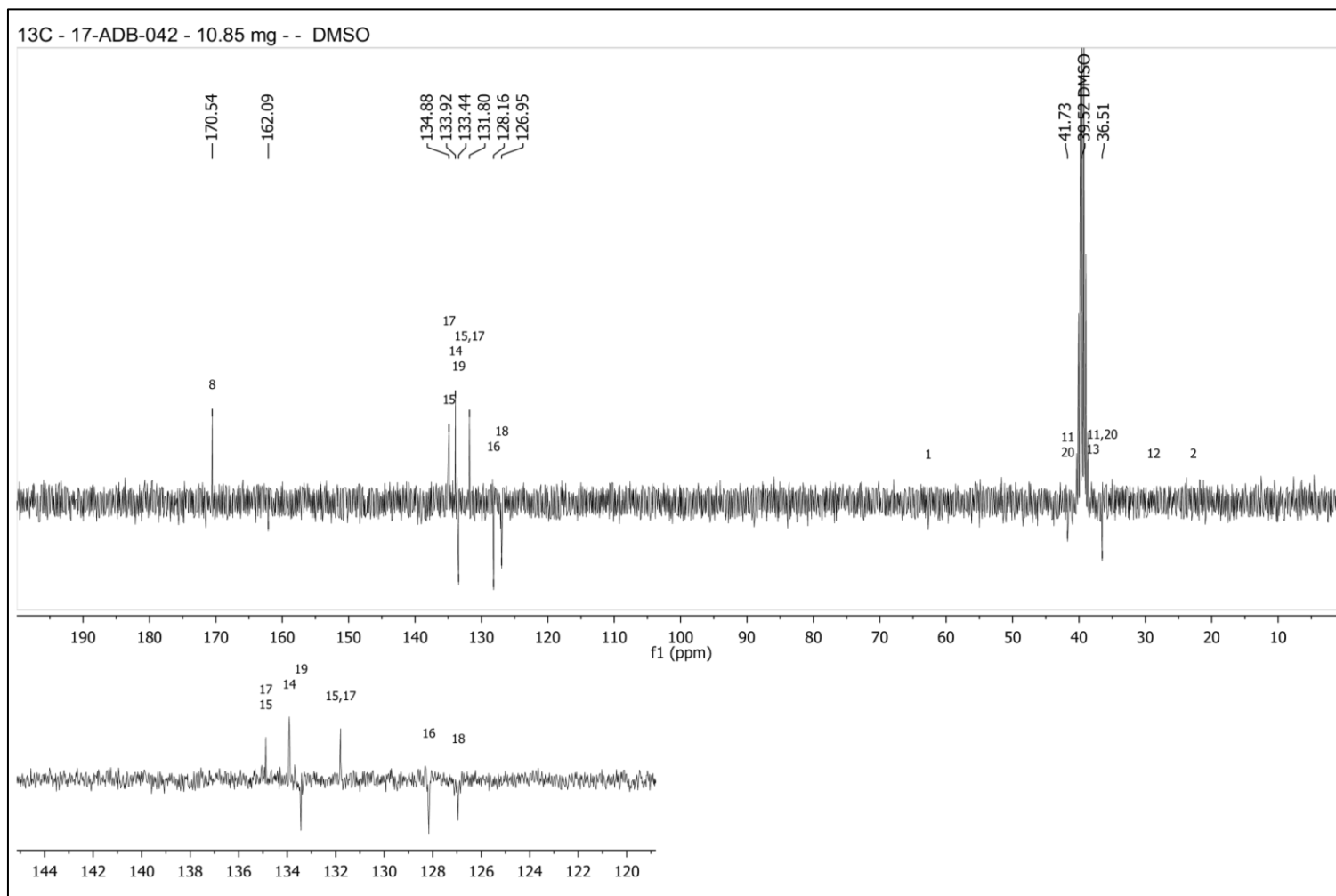
¹³C-NMR (75 MHz, 297 K, DMSO-d₆): U-48800 (bottom: enlarged signals)



Analytical results

NMR

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



¹³C-APT-NMR (75 MHz, 297 K, DMSO-d₆): U-48800 (bottom: enlarged signals)

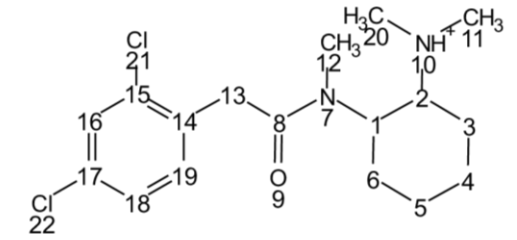
Analytical results

NMR

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



Assignments		Assignments	
Atom	Chemical Shift	Atom	Chemical Shift
1 C	62.69	11 C	36.50, 41.73
H	3.52	H3	2.54, 2.73
2 C	22.76	12 C	28.78
H	2.07	H3	3.00
3 C		13 C	37.94
H'	1.51	H'	4.05
H''		H''	3.85
4 C		14 C	133.91
H'	1.51	15 C	131.80, 134.88
H''		16 C	128.16
5 C		H	7.58
H'	1.51	17 C	131.80, 134.88
H''		18 C	126.95
6 C		H	7.37
H'	1.51	19 C	133.43
H''		H	7.45
7 N		20 C	36.50, 41.73
8 C	170.54	H3	2.54, 2.73
9 O		21 Cl	
10 N		22 Cl	
H	10.01		



Peak list and assignments of U-48800 signals.