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EUROPEAN PROJECT RESPONSE 2 to challenges in forensic drugs analyses
Co-funded by the Internal Security Found of the European Union

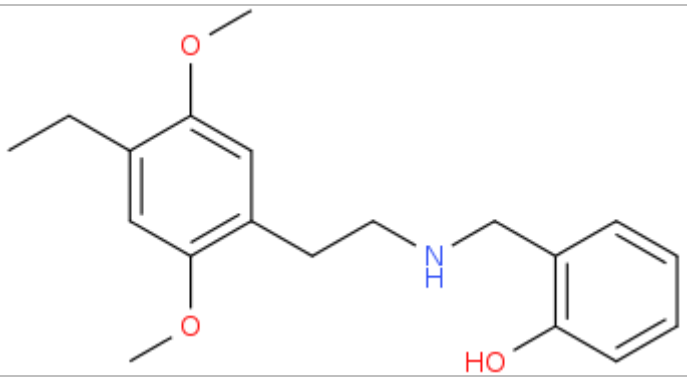
ANALYTICAL REPORT

25E-NBOH (C19H25NO3)

2-({[2-(4-ethyl-2,5-dimethoxyphenyl)ethyl]amino}methyl)phenol

Remark – other NPS detected:

Sample ID:	1901-18
Sample description:	powder
Sample type:	test purchase /ISF projekt (NFL-SI)
Date of sample receipt (M/D/Y):	1/3/2018
Date of entry (M/D/Y) into NFL database:	1/24/2018
Report updates (if any) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure[1] (base form)	
Systematic name	2-({[2-(4-ethyl-2,5-dimethoxyphenyl)ethyl]amino}methyl)phenol
Other names	NBOH-2C-E
Formula (per base form)	C19H25NO3
M_w (g/mol)	315,41
Salt form/anions detected	HCl
StdInChIKey (per base form)	SYBINTRPEZWFLZ-UHFFFAOYSA-N
Other NPS detected	
Additional info (purity..)	>96% purity by NMR

[1] Created by OPSIN free tool: http://opsin.ch.cam.ac.uk/ **DOI:** 10.1021/ci100384d

Report updates

date	comments (explanation)

Instrumental methods (if applied) in NFL

1. GC-MS (Agilent): GC-method is RT locked to tetracosane (9.258 min). Injection volume 1 ml and split mode (1:50). Injector temperature: 280 0C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickens 0.25 µm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 ^{0}C for 1 min, followed by heating up to 190 ^{0}C at rate 8 ^{0}C/min, then heating up to 293 0C at a rate of 18 ^{0}C/min, hold for 7.1 min, then heating at 50 ^{0}C/min up to 325 ^{0}C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300 until 6 min) amu.

2. HPLC-TOF (Agilent): 6230B TOF with Agilent 1260 Infinity HPLC with binary pump, column: Zorbax Eclipse XDB-C18, 50 x 4.6 mm, 1.8 micron. Mobile phases (A) 0.1% formic acid and 1mM ammonium formate in water; (B) 0.1% formic acid in methanol (B). Gradient: starting at 5% B, changing to 40% B over 4 min, then to 70% over 2 min and in 5 min to 100%, hold 1 min and back to 5%, equilibration for 1.7 min. The flow rate: 1.0 ml/min; Injection volume 1 µl. MS parameters: 2GHz, Extended Dynamic range mode to a maximum of 1700 amu, acquisition rate 1.30 spectra/sec. Sample ionisation: by Agilent Jet Stream technology (Dual AJS ESI). Ion source: positive ion scan mode with mass scanning from 82 to 1000 amu. Other TOF parameters: drying gas (N2) and sheath temperature 325 °C; drying gas flow rate 6 l/min; sheath gas flow rate 8 l/min; nebulizer 25 psig; Vcap. 4000 V; nozzle 2000 V; skimmer 65 V; fragmentor 175 V and Octopole RF 750 V.

3.FTIR-ATR (Perkin Elmer): scan range 4000-400 cm-1; resolution 4cm-1

4. GC- (MS)-IR condensed phase (GC-MS (Agilent) & IR (Spectra analyses-Danny)
GC-method: Injection volume 1 ml and split mode (1:5). Injector temperature 280 ^{0}C. Chromatographic separation as above **(1).**
Split MS : IR = 1: 9.
MSD source EI = 70 eV. GC-MS transfer line T= 235^{0}C, source and quadropole temperatures 280^{0}C and 180^{0}C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300) amu.
IR (condesed (solid) phase): IR scan range 4000 to 650, resolution 4 cm^{-1}.

5. **IC** (anions) (Thermo Scientific, Dionex ICS 2100), Column: IonPac AS19, 2 x 250mm; Eluent: 10mM from 0 to 10 min, 10-58 mM from 10 to 40min; Flow rate: 0.25 ml/min; Temperature: 30˚C; Suppressor: AERS 500 2mm, suppressor current 13mA; Inj. Volume: 25 µl

Supporting information

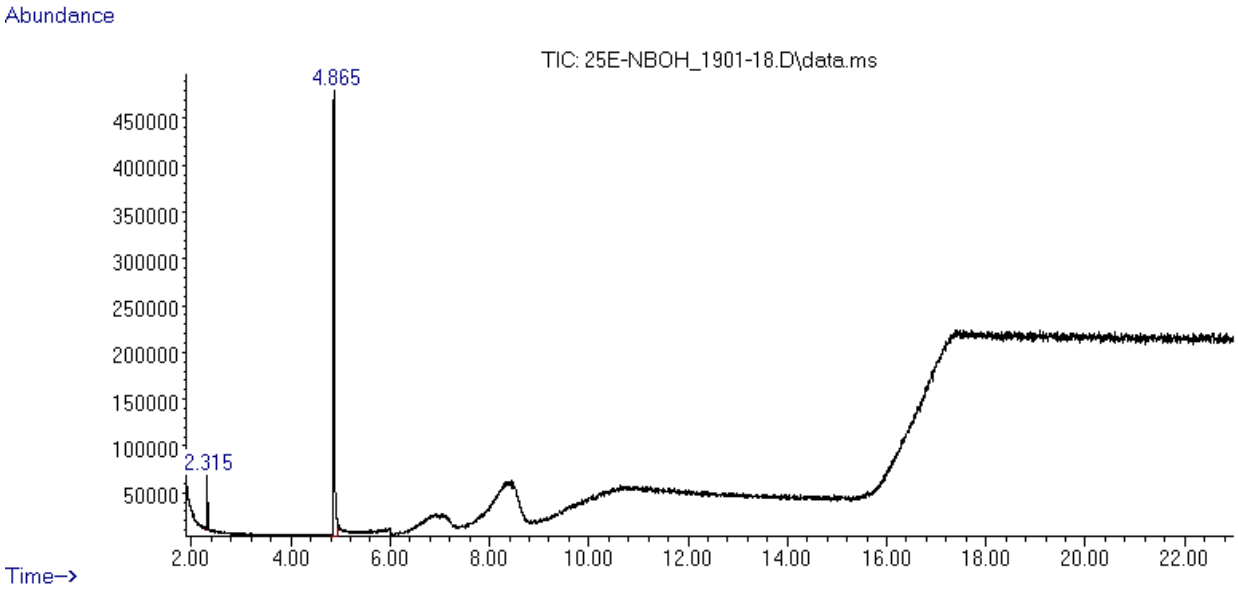
Solubility in	result/remark
CH_2Cl_2	partially
MeOH	soluble
H_2O	partially

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): 10,3 BP(1): 179; BP(2): 280,BP(3) :73, (RT and m/z refer to N,O-diTMS derivative)
HPLC-TOF	+	Exact mass (theoretical): 315,1834; measured value Δppm:1,27; formula:C19H25NO3
FTIR-ATR	+	direct measurement (sample as received)
FTIR (condensed phase) always as base form	+	IRs of O-TMS and O,N-diTMS derivative are shown)
IC (anions)	+	
NMR (in FKKT)	+	
validation		
other		

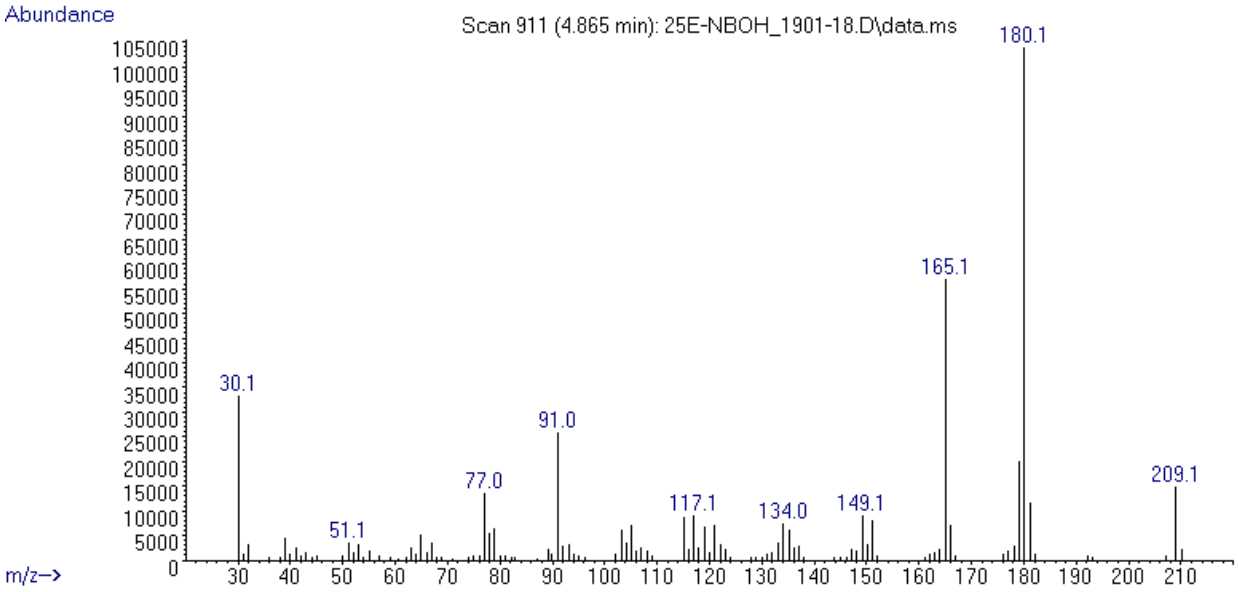
ANALYTICAL RESULTS

GC-MS:

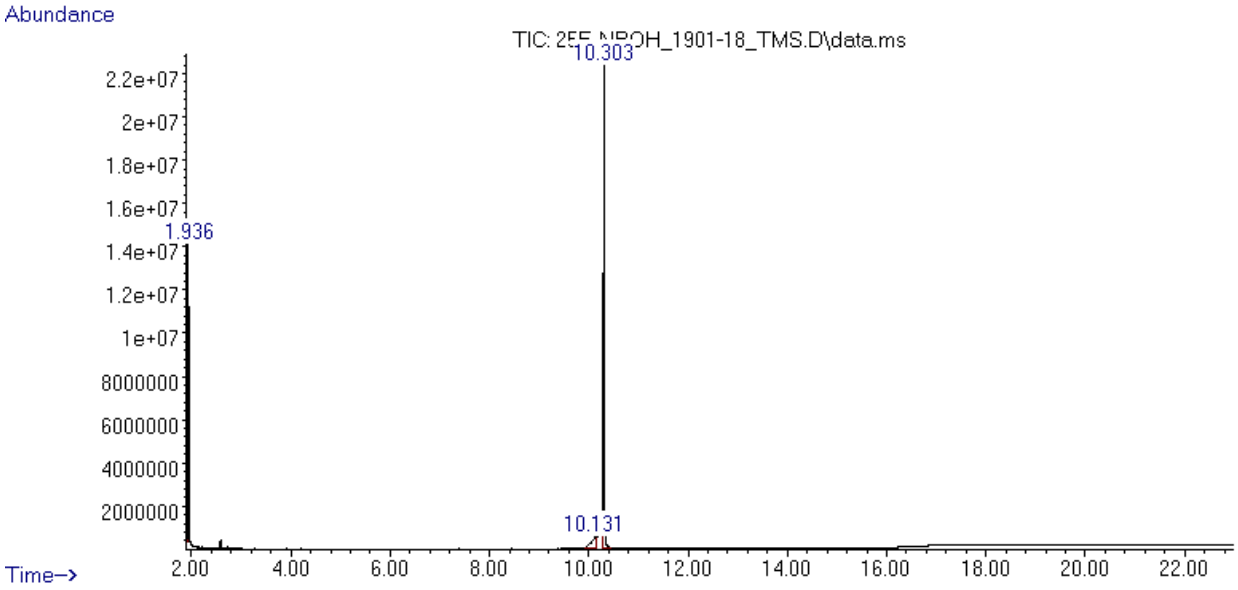
1. Non derivatized compound decomposes to 2C-E under our experimental GC conditions.



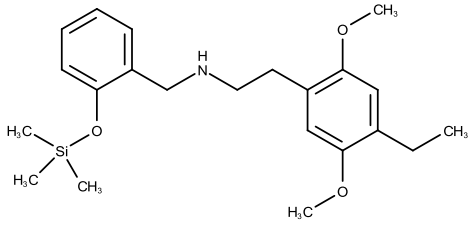
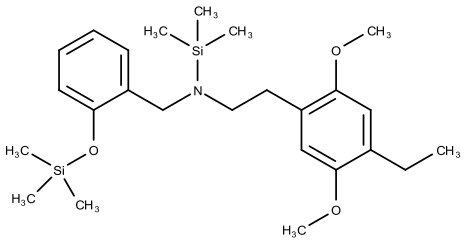
MS spectrum of decomposition product, i. e. 2C-E at 4.86 min.



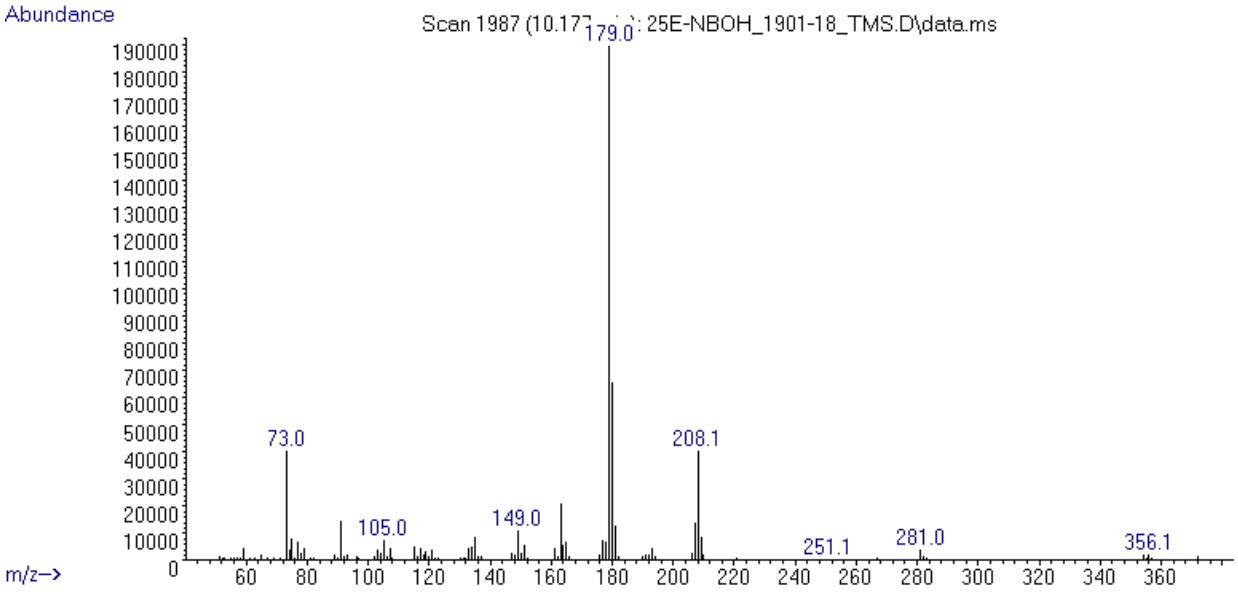
2. Derivatization of sample by MSTFA



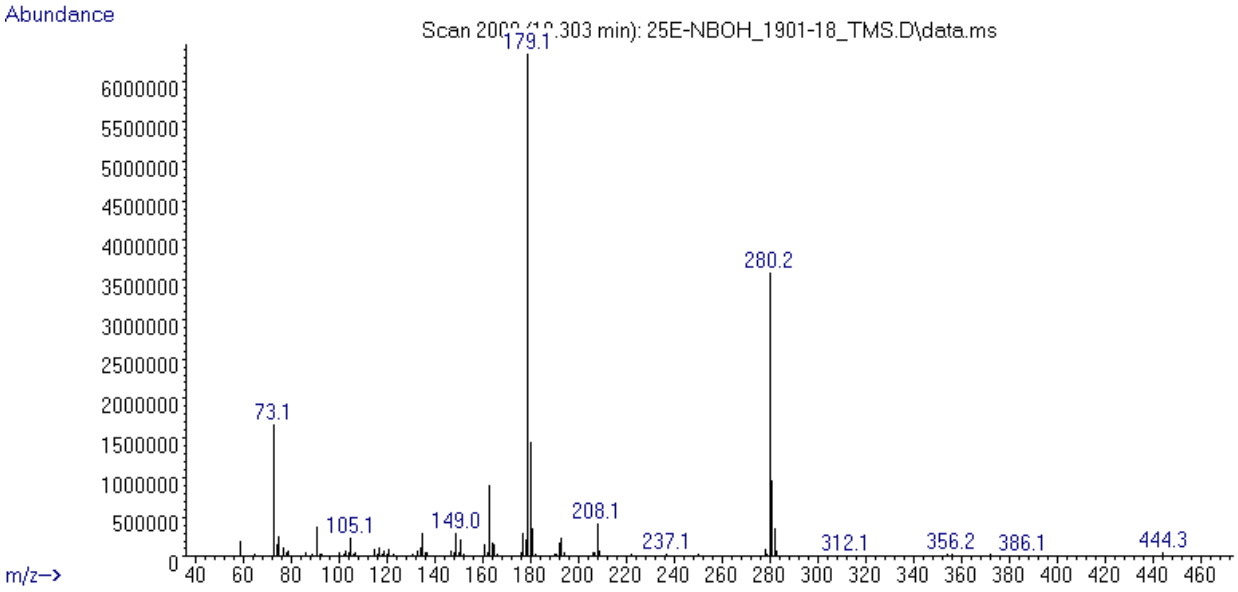
Chromatogram: mono-TMS derivative (O-TMS) at 10.13 and di-TMS derivative O,N-diTMS at 10.30 min.

O-TMS derivative	O,N-diTMS derivative
	

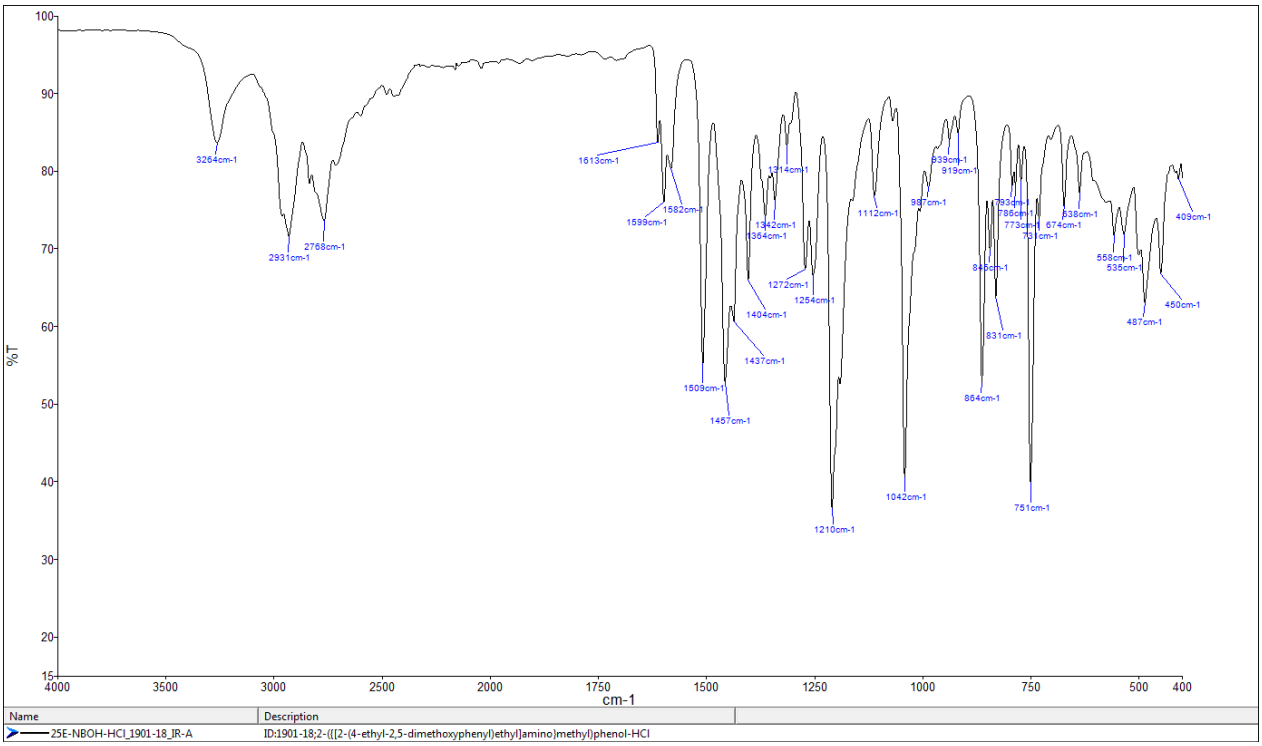
MS spectrum of O-TMS at 10.7 min



MS spectrum of O,N-diTMS at 10.30 min.

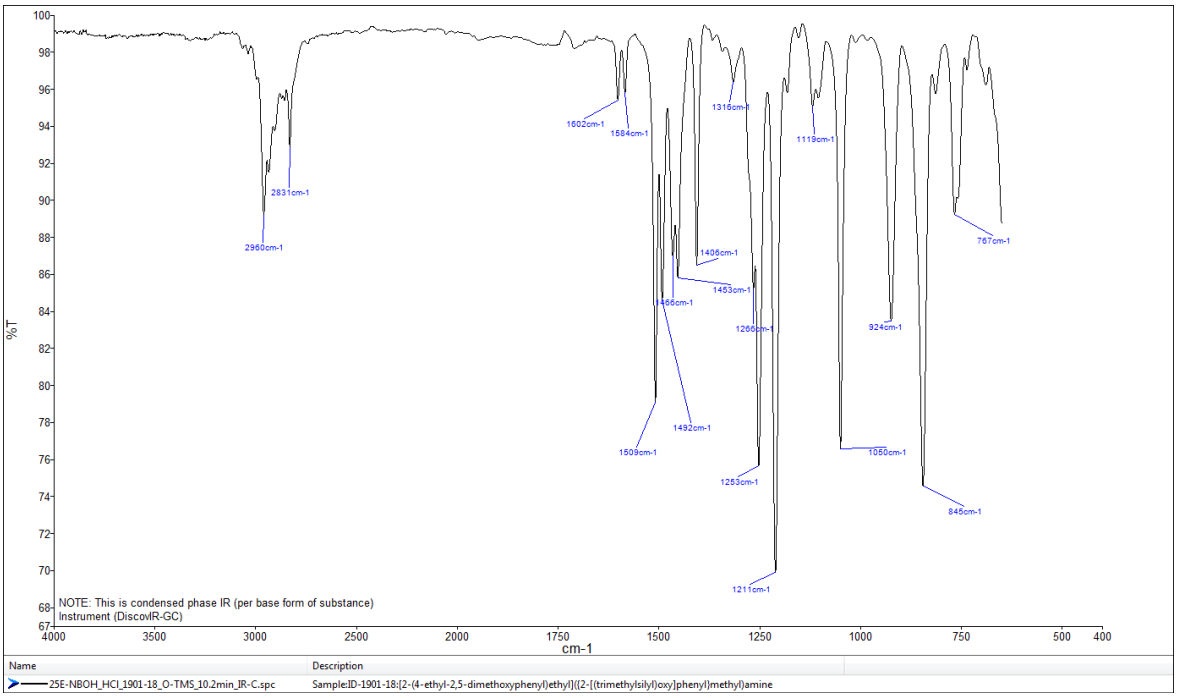


FTIR-ATR - direct measurement (sample as received)

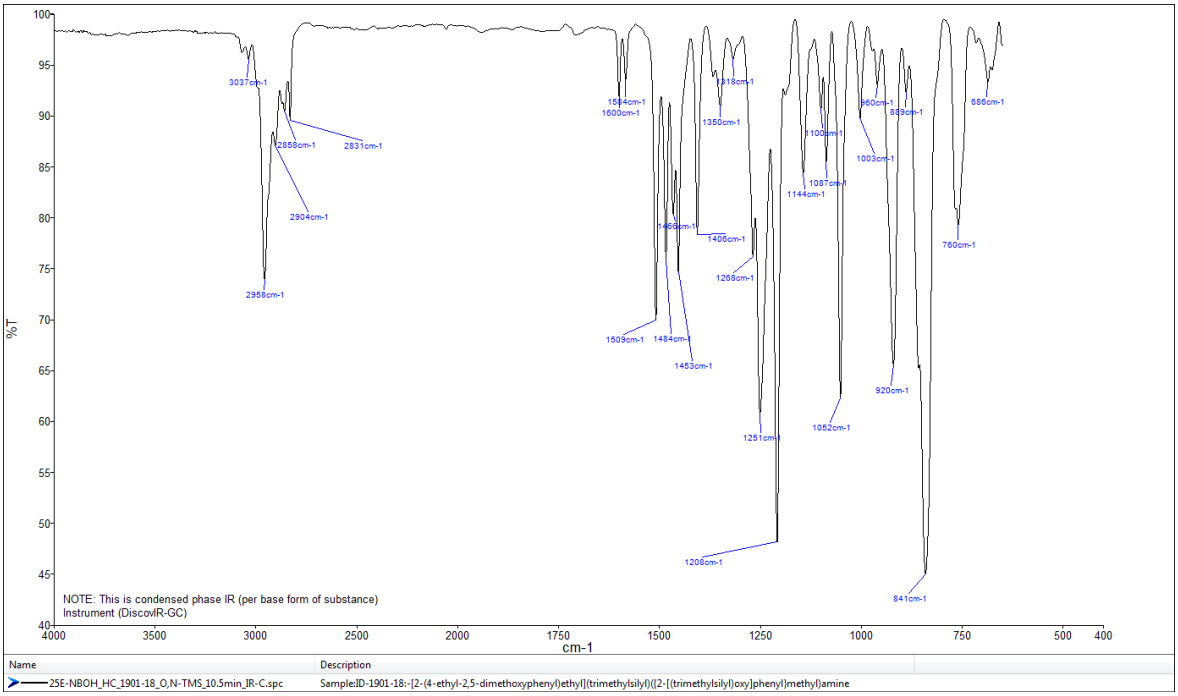


IR (SOLID PHASE – AFTER CHROMATOGRAPHIC SEPARATION OF TMS DERIVATIZED SAMPLE)

A) IR of mono-TMS derivative (O-TMS)



A) IR of di-TMS derivative (O, N-TMS)



TOF REPORT

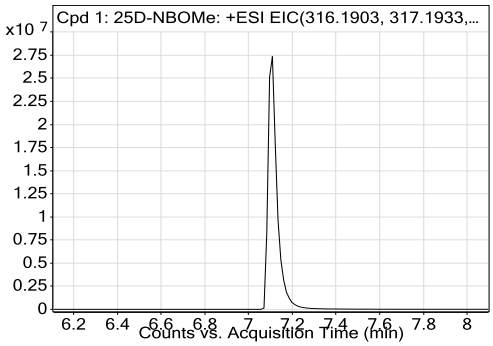
Data File	25E-NBOH_1901-18.d	**Sample Name**	1901-18
Sample Type	Sample	**Position**	P1-A4
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	general-04_12_2017-XDB-C18-ESI+.m	**Acquired Time**	1/4/2018 9:34:07 AM
IRM Calibration Status	Success	**DA Method**	Drugs_NFL.m
Comment	MeOH		

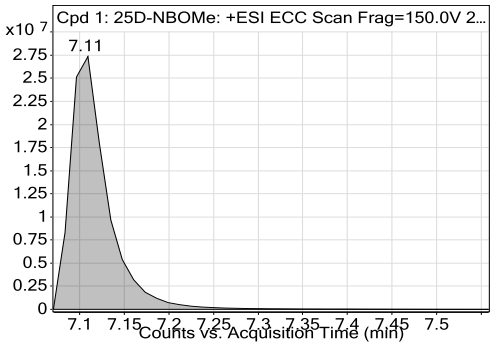
Compound Table

Label	Compound Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 1: 25D-NBOMe	25D-NBOMe	C19 H25 N O3	7.11	315.183

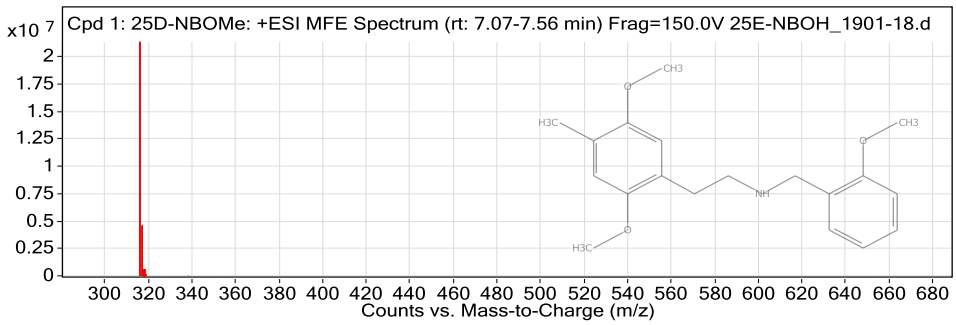
Name	*Obs. m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)
25D-NBOMe	316.1903	7.11	315.183	7.08	C19 H25 N O3	315.1834	1.27

Compound Chromatograms

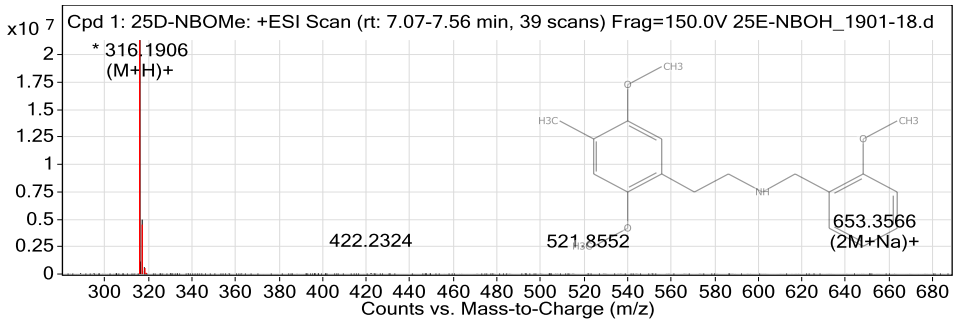




MFE MS Zoomed Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

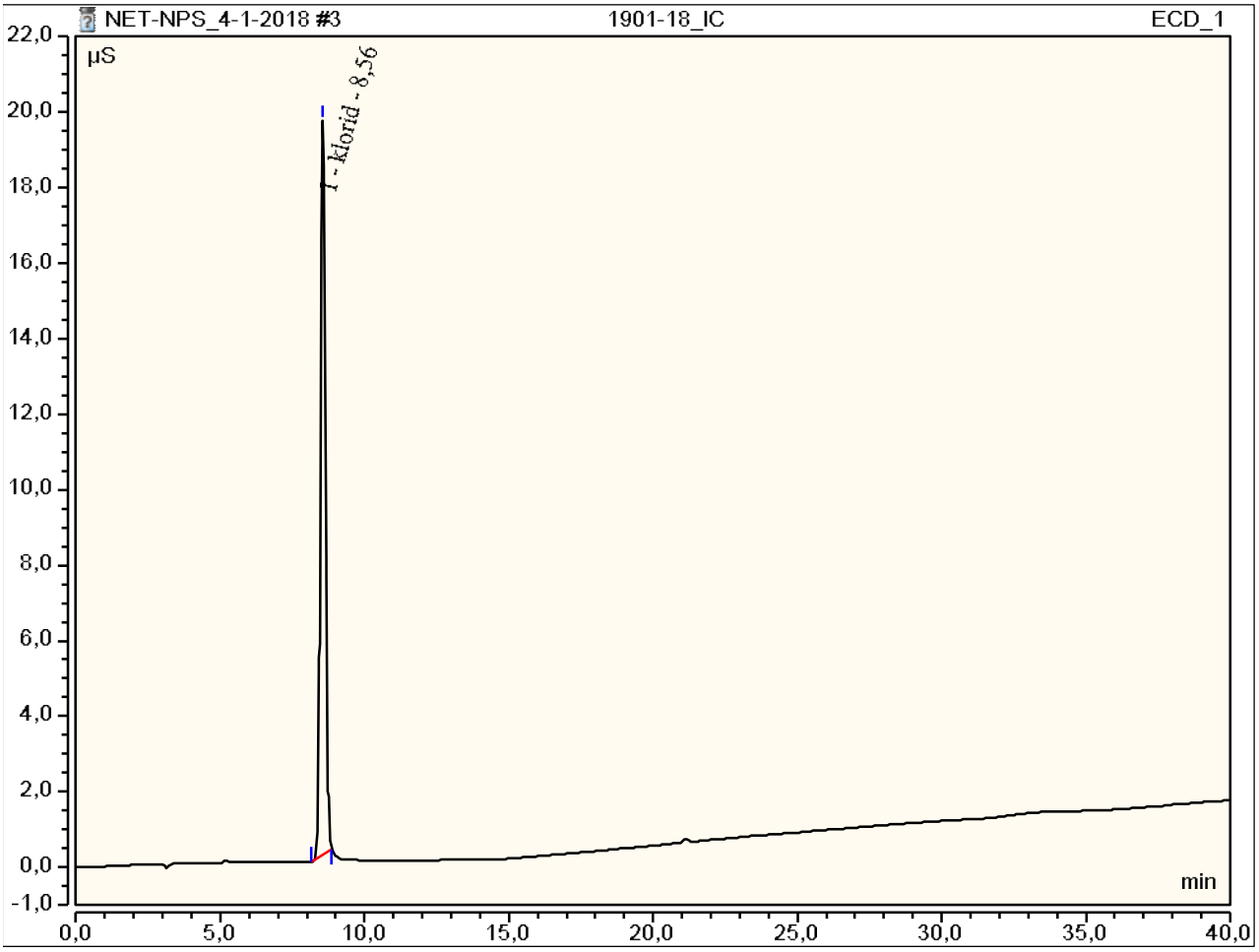
Obs. m/z	Charge	Abund	Formula	Ion/Isotope
316.1903	1	21347298	C19 H25 N O3	(M+H)+
317.1936	1	4660004.49	C19 H25 N O3	(M+H)+
318.1969	1	521573.36	C19 H25 N O3	(M+H)+
319.199	1	53071.32	C19 H25 N O3	(M+H)+
320.2016	1	4147.81	C19 H25 N O3	(M+H)+
338.172	1	31679.61	C19 H25 N O3	(M+Na)+
339.1748	1	6922.75	C19 H25 N O3	(M+Na)+
354.1467	1	2593.11	C19 H25 N O3	(M+K)+
653.3567	1	4387.42	C19 H25 N O3	(2M+Na)+

--- End Of Report ---

Peak Integration Report

Sample Name:	**1901-18_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**04-jan-2018 / 10:17**	*Run Time:*	**42,00**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount mg/L
1,00	8,56	**klorid**	BMB	3,80	19,44	n.a.
		TOTAL:		3,80	19,44	0,00



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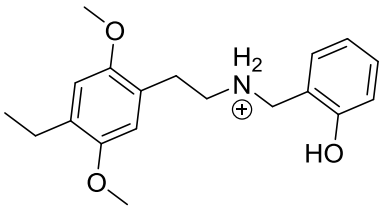
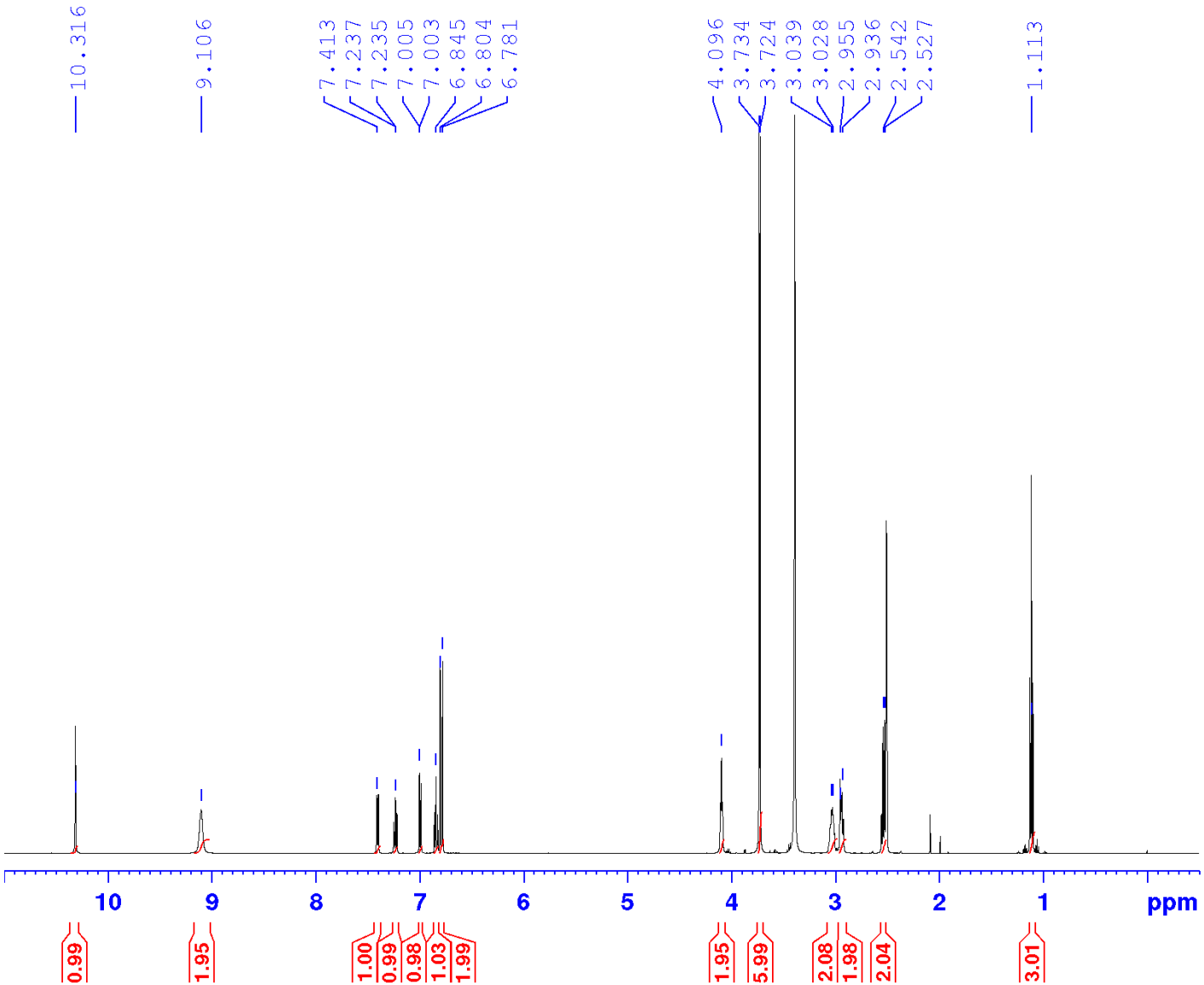
R E P O R T

Contract No.	C1714-17-460078 (Republic of Slovenia, Ministry of the Interior, POLICE)
Sample ID:	**1901-18**
Received date:	January 11, 2018
Our notebook code:	NFL-1901-18
NMR sample preparation:	18.0 mg dissolved in 0.7 mL DMSO-d_6
NMR experiments:	^{1}H, ^{13}C, ^{1}H–^{1}H *gs*-COSY, ^{1}H–^{13}C *gs*-HSQC, ^{1}H–^{13}C *gs*-HMBC, ^{1}H–^{15}N *gs*-HMBC
Proposed structure with formula, exact mass, molecular weight:	 Chemical Formula: $C_{19}H_{26}NO_3^+$ Exact Mass: 316,191 Molecular Weight: 316,420
Chemical name:	*N*-protonated 2-(((4-ethyl-2,5-dimethoxyphenethyl)amino)methyl)phenol
Comments:	- Structure elucidation based on 1D and 2D NMR spectra and HRMS. - >96% purity of a sample based on ^{1}H NMR spectrum
Supporting information:	Copies of ^{1}H and ^{13}C NMR spectra, ^{1}H and ^{13}C FIDs.
Principal investigator:	Prof. Dr. Janez Košmrlj
Date of report:	January 19, 2018

NFL-1901-18
1H



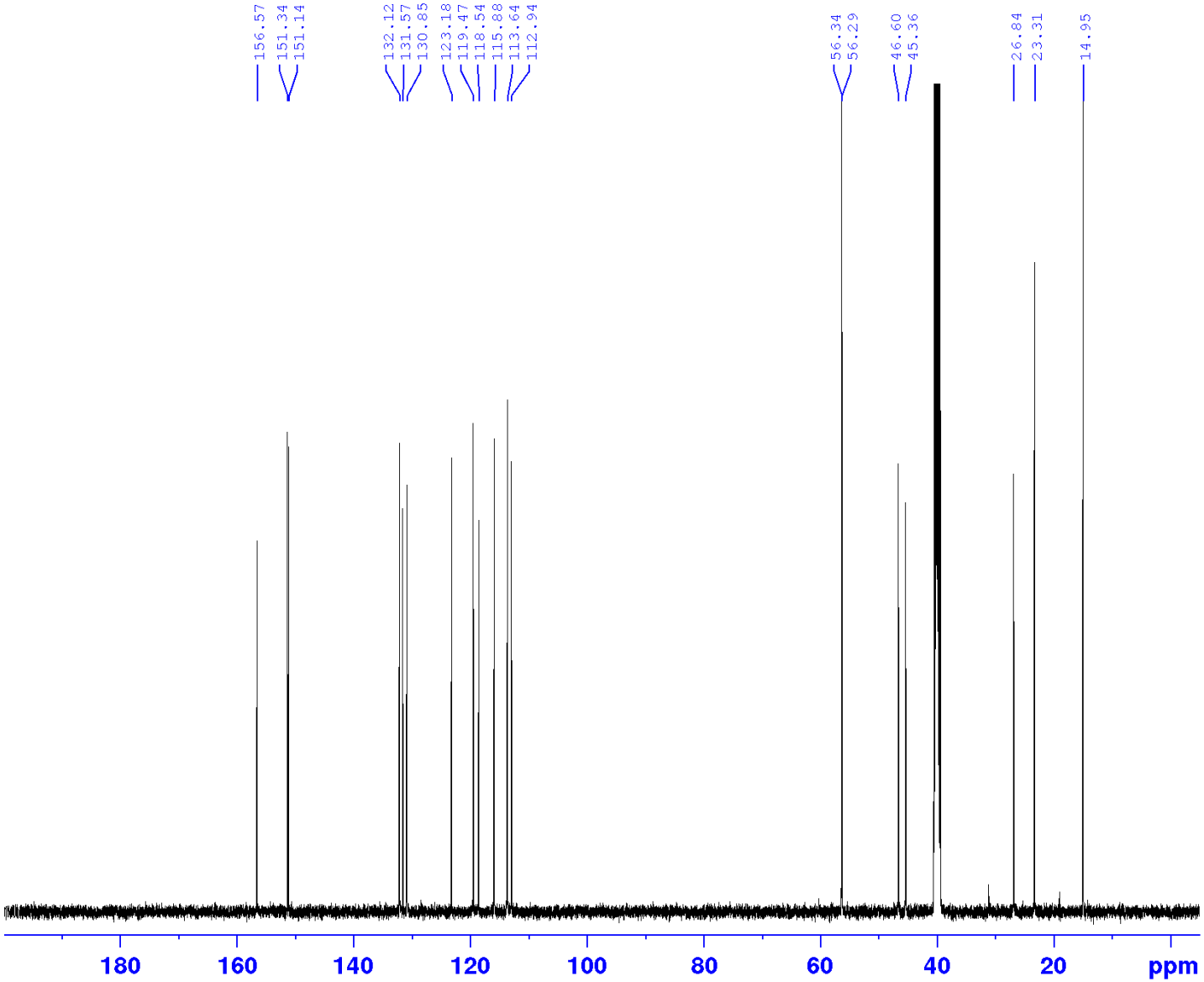

```
Current Data Parameters
NAME         NFL-1901-18
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180112
Time                0.43
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG                  71.8
DW               50.000 usec
DE                6.50 usec
TE                296.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.1330885 MHz
NUC1                   1H
P1                 8.70 usec
PLW1        26.00000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300002 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```

NFL-1901-18
13C

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Current Data Parameters
NAME         NFL-1901-18
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180112
Time                2.43
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  2048
DS                     4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010048 sec
RG                  2050
DW               16.800 usec
DE                6.50 usec
TE                296.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        125.7703637 MHz
NUC1                  13C
P1                 8.70 usec
PLW1       122.00000000 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         26.00000000 W
PLW12         0.30046001 W
PLW13         0.15113001 W

F2 - Processing parameters
SI                 32768
SF          125.7577885 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```