NACIONALNI FORENZIČNI LABORATORIJ
NATIONAL FORENSIC LABORATORY

Vodovodna 95
1000 Ljubljana
SLOVENIJA

T: +386 (0)1 428 44 93
E: nfl@policija.si
www.policija.si

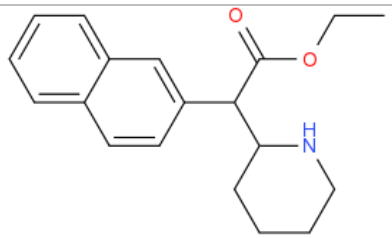
ANALYTICAL REPORT[1]

HDEP-28 (C19H23NO2)

ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl)acetate

Remark – other NPS detected: none

Sample ID:	1244-15
Sample description:	powder - white
Sample type:	test purchase /RESPONSE -purchasing
Date of sample receipt (M/D/Y):	8/18/2015
Date of entry (M/D/Y) into NFL database:	8/19/2015
Report (updates) will be published here:	http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified-structure[2] (base form)	
Systematic name	ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl)acetate
Other names	ethylnaphtidate, HDEP-28, 2-(2-ethoxy-1-(naphthalen-2-yl)-2-oxoethyl)piperidin
Formula (per base form)	C19H23NO2
M_w (g/mol)	297,17
Salt form	HCl
StdInChIKey	OTQVTBPHZRARTL-UHFFFAOYSA-N
Compound Class	Piperidines & pyrrolidines
Other NPS detected	none
Add.info (purity..)	pure by HPLC-TOF and NMR; thermal decomposition can occour in GC

[1] This report has been produced with the financial support of the Prevention of and fight against crime Programme of the European Union (grant agreement number JUST/2013/ISEC/DRUGS/AG/6413). The contents of this report are the sole responsibility of the National Forensic Laboratory and can in no way be taken to reflect the views of the European Commission.

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

Report updates

date	comments (explanation)
4. 11. 2015	minor corrections of text have been done

Instrumental methods (if applied) in NFL

1. GC-MS (Agilent): GC-method is RT locked to tetracosane (RT=9.53 min). Injection volume 1 ml and split mode (1:50) . Injector temperature: 280 ^{0}C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickens 0.25 mm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 ^{0}C for 1 min, followed by heating up to 293 ^{0}C at a rate of 18 ^{0}C/min, hold for 6.1 min, than heating at 50 ^{0}C/min up to 325 ^{0}C and finally 2.8 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 (40) to 550 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 (40) to 550 amu.
IR (condesed 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	soluble
MeOH	soluble
H_2O	soluble

Analytical technique:	applied	remarks
GC-MS (EI ionization)	+	NFL GC-RT (min): BP(1): 141; BP(2): 84,BP(3) :214, RT = 22,84 nonderivatized (modified GC-temperature program);(**RT for HDEP-28-TFB derivative)**= 9.58 (ions see 7 Figure 7).
HPLC-TOF	+	Exact mass (theoretical): 297,1729; measured value Δppm:-0,15; formula:C19H23NO2
TOF direct measurement	+	sample is pure
FTIR-ATR	+	direct measurement
FTIR (condensed phase) always as base form	+	scanned only at our standard analytical conditions - results are not shown
IC (anions)	+	
NMR	+	
validation		
other		melting point measurement (see analytical results)

ANALYTICAL RESULTS WITH COMMENTS

Key words: thermal decomposition of HDEP-28 in GC; derivatization by MBTFA (N-methyl-bis-trifluoroacetamide**)**

The HPLC-TOF and NMR analyses (see the attached results) confirmed the substance as "pure" HDEP-28. Anyhow, the result obtained by GC-MS under our standard analytical conditions (see above point 1) was not in agreement with TOF and NMR.

GC chromatogram of the sample is shown in Figure 1. We observed two peaks: a narrow peak at 5.638 min followed by a broad peak (extended over almost 3 minutes interval). Based on the obtained chromatographic profile of the sample in combination with TOF and NMR data we supposed that the compound HDEP-28 most probably decomposed in GC.

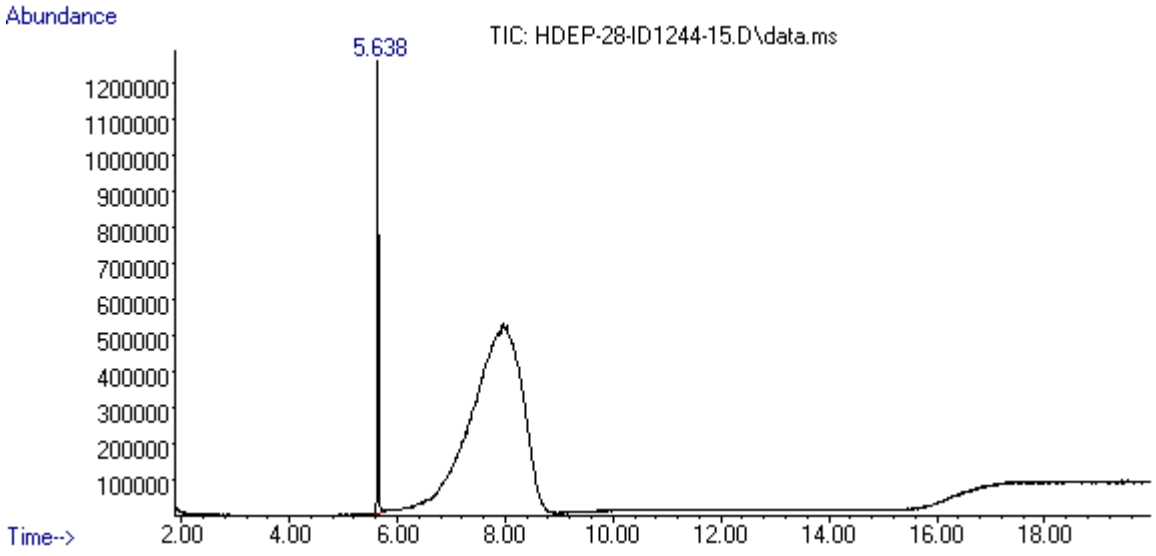


Figure 1: Chromatogram obtained at our standard analytical conditions

The study of mass spectral data of both chromatographic peaks (see Figure 2) showed that the fragmentation patterns of both peaks are closely related. Further interpretation of MS spectra revealed that spectra most likely corresponded to 2-naphtalenacetic acid, ethyl ester rather than to HDEP-28. Namely, both mass spectra shown on Figure 2 were missing ion m/z 84 (i. e. fragment of piperidine part of the HDEP-28 molecule).

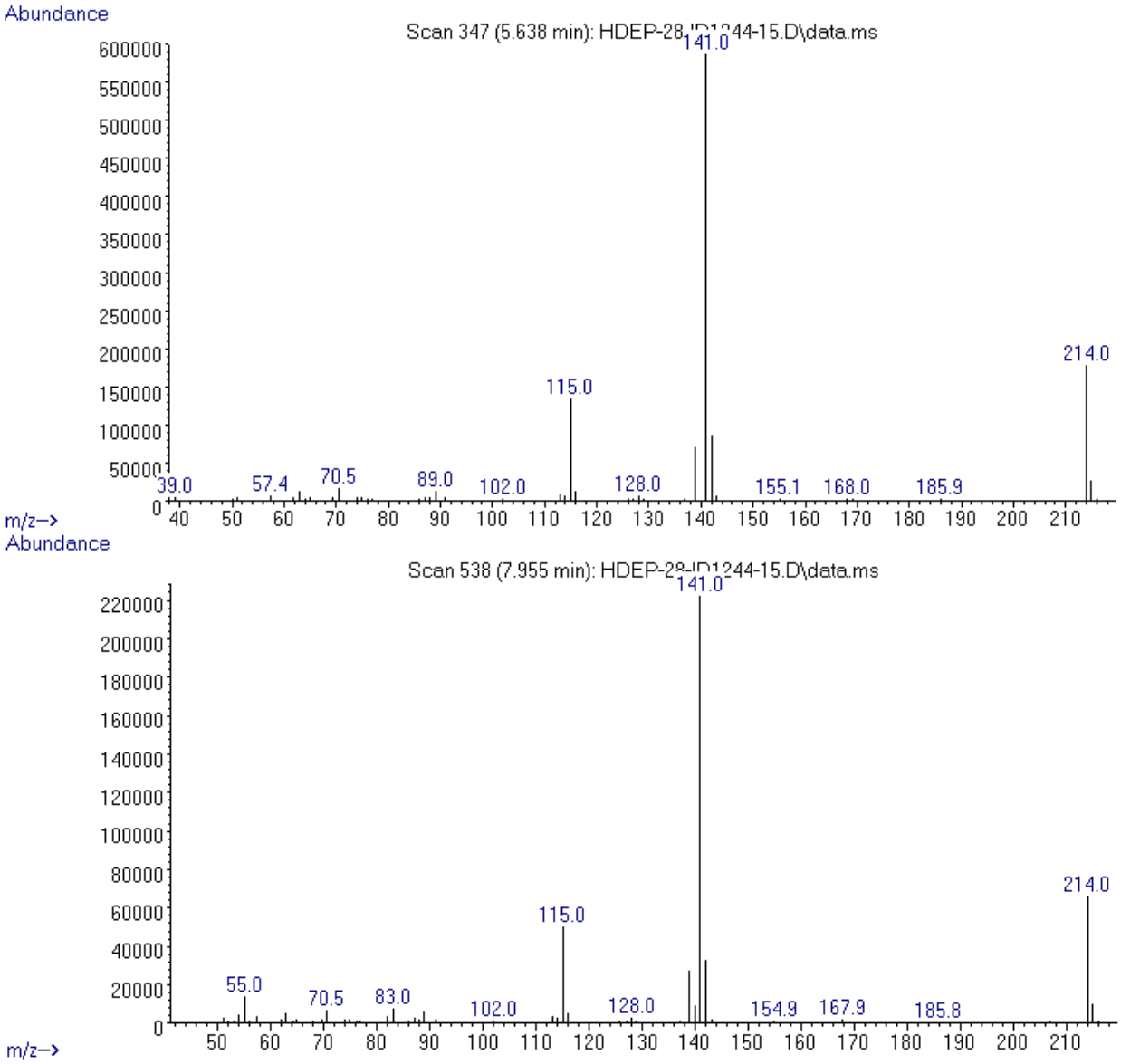


Figure 2: Mass spectra at 5.638 min and of broad peak (spectrum is shown for RT at 8 min) - the most intensive signals most likely correspond to 2-naphtalenacetic acid, ethyl ester.

We supposed that at our standard GC analytical conditions HDEP-28 decomposed into 2-naphtalenacetic acid, ethyl ester and most likely to 2,3,4,5-tetrahydropyridine (see Figure 3). Similar reaction was previously proposed by Flamm and Gal[3], who observed decomposition of

[3] Flamm, B. L., and J. Gal, The thermal decomposition of methylphenidate in the gas chromatograph mass spectrometer, Biological Mass Spectrometry Volume 2, Issue 5, pages 281–283, October 1975

methylphenidate, although we did not observed this effect for methylphenidate under our experimental conditions. In general, thermal decomposition of pyrrolidine/piperidine compounds is known[4] and used in chemical industry (for initiation of polymerization).

Along the chromatographic profile of HDEP-28 sample we think that decomposition occurs in GC inlet and additionally in column. Similar effect we observed previously also with 3,4-CTMP. The extent of decomposition is highly dependent on analytical parameters. We observed some misinterpretations of reported mass spectra of 3, 4-CTMP and HDMP-28, where mass spectra of degradation products were reported/interpreted as the spectra of non-decomposed compound.

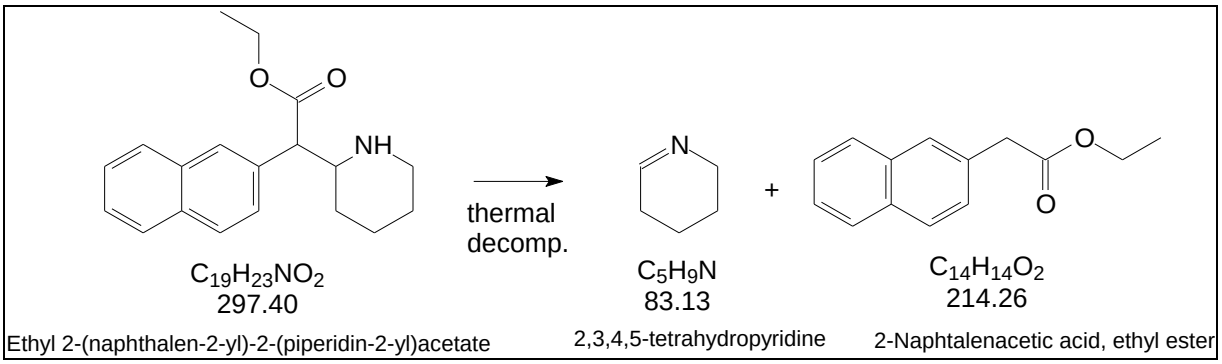


Figure 3: Thermal decomposition reaction - proposed mechanism

Decomposition of sample was confirmed also by melting point measurements (by Mettler Toledo MP90 Melting Point System) which showed a broad melting range of 206.6 to 213.4°C with decomposition (observed also visually).

[4] Pyrolysis of Organic Molecules: Applications to Health and Environmental Issues, 28th Volume, Serban Moldoveanu, RJ Reynolds Tobacco Co., Winston-Salem, NC, USA, 2010, Elsevier

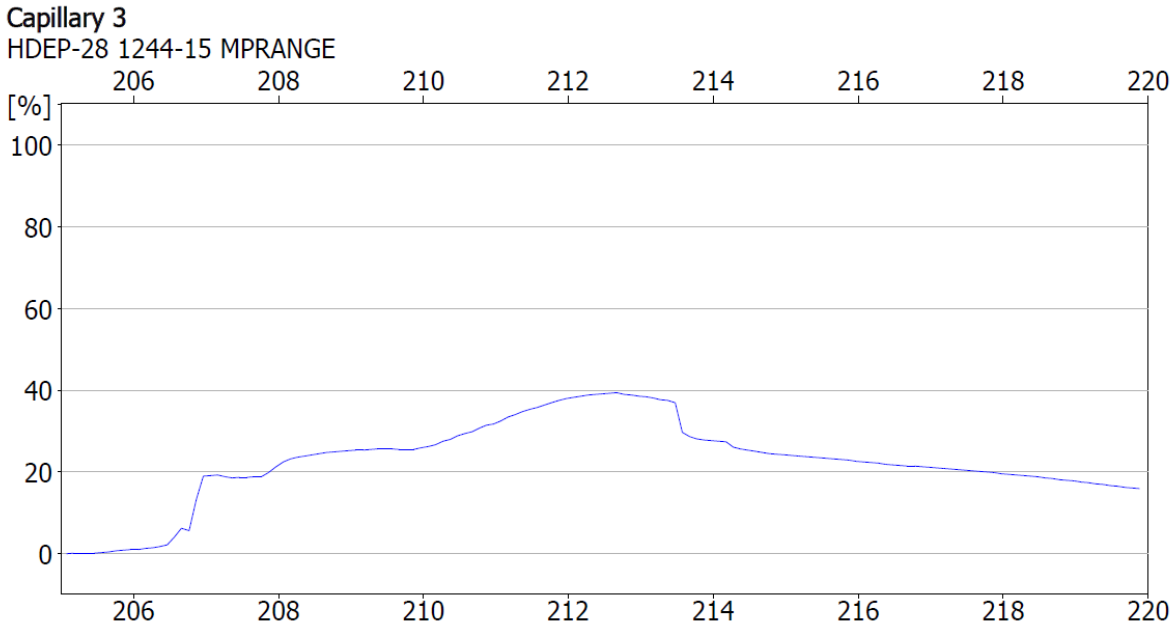


Figure 4: Melting curve for the sample purchased as HDEP-28.

We re-analyzed the sample under modified chromatographic conditions: injector temperature 150°C, detector interface 190°C and the oven temperature program as follows: initial temperature 100°C; hold for 20 min; ramp to 325°C with the rate of 30°C /min; final temperature 325°C.

Chromatogram is shown below (Figure 5) and mass spectrum at 22.8 min in Figure 6. In the mass spectrum we can see the fragment ion m/z 84, i. e. fragment of piperidine part of the HDEP-28 molecule. Molecular ion at *m/z* 297 was not detected. Spectrum of the broad peak in front of 22.840 min peak again corresponds to 2-naphtalenacetic acid, ethyl ester.

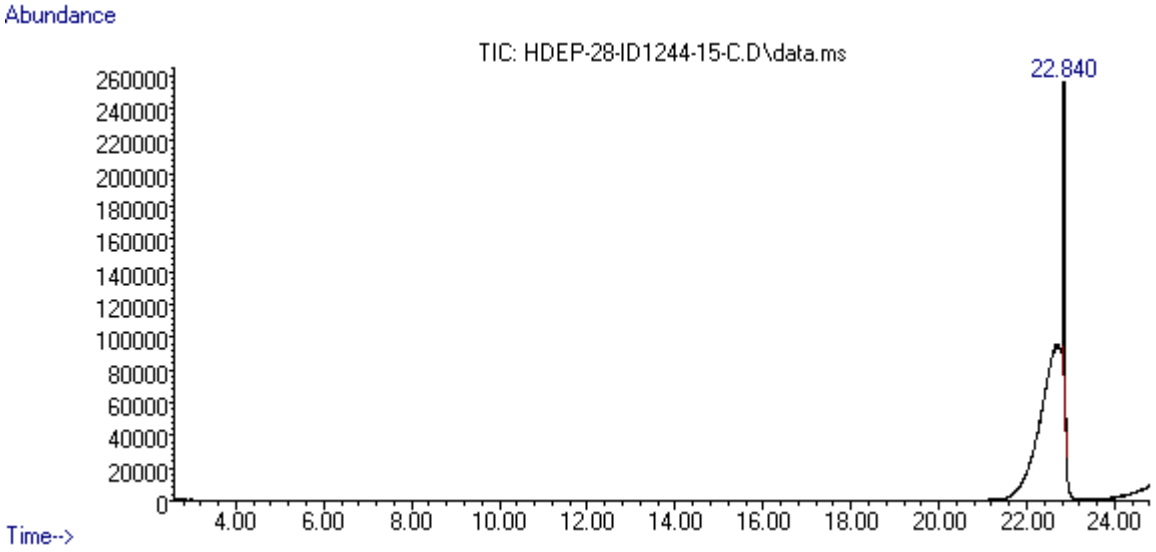


Figure 5: Chromatogram of the sample obtained under modified temperature conditions

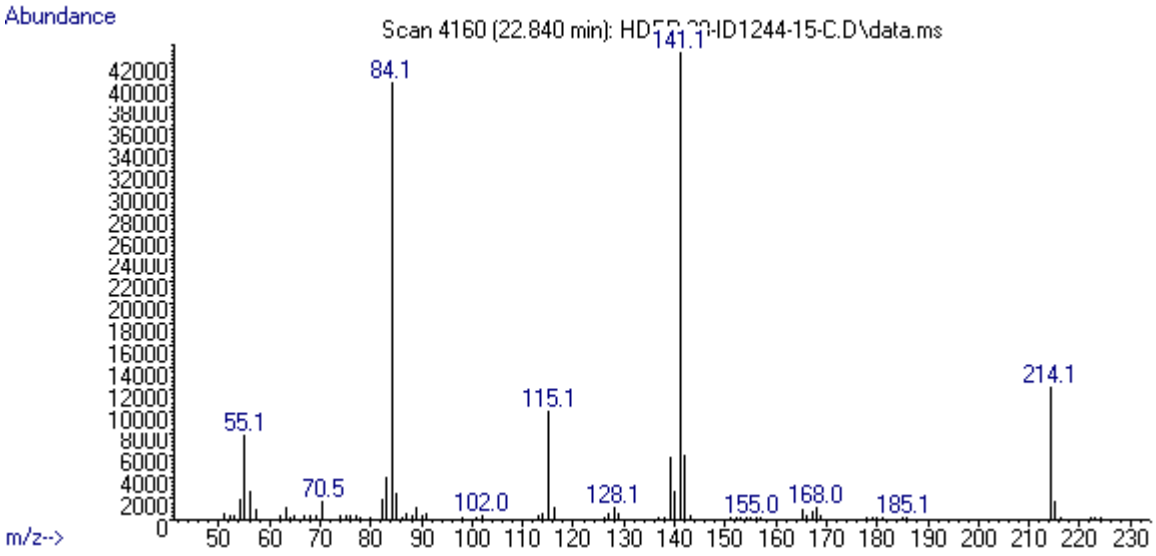


Figure 6: Mass spectrum of the compound at 22.84 min (with removed background-the of the broad peak in front of the peak at 22.840 min). Interpretation of the mass spectrum indicated on the compound ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl) acetate (HDEP-28). Ion of m/z 84 is clearly visible (fragment of piperidine part of the HDEP-28 molecule).

In the next experiment the sample, dissolved in methylenechloride, was treated with MBTFA (N-methyl-bis-trifluoroacetamide**)** for 30min at 80°C. The extract was analyzed by GC-MS at our standard analytical conditions. The mass spectrum of the peak at 9.583 min corresponded to trifluoroacetyl derivate of HDEP-28 with a molecular ion *m/z* 393 and a base peak at *m/z* 180 (Figure 7).

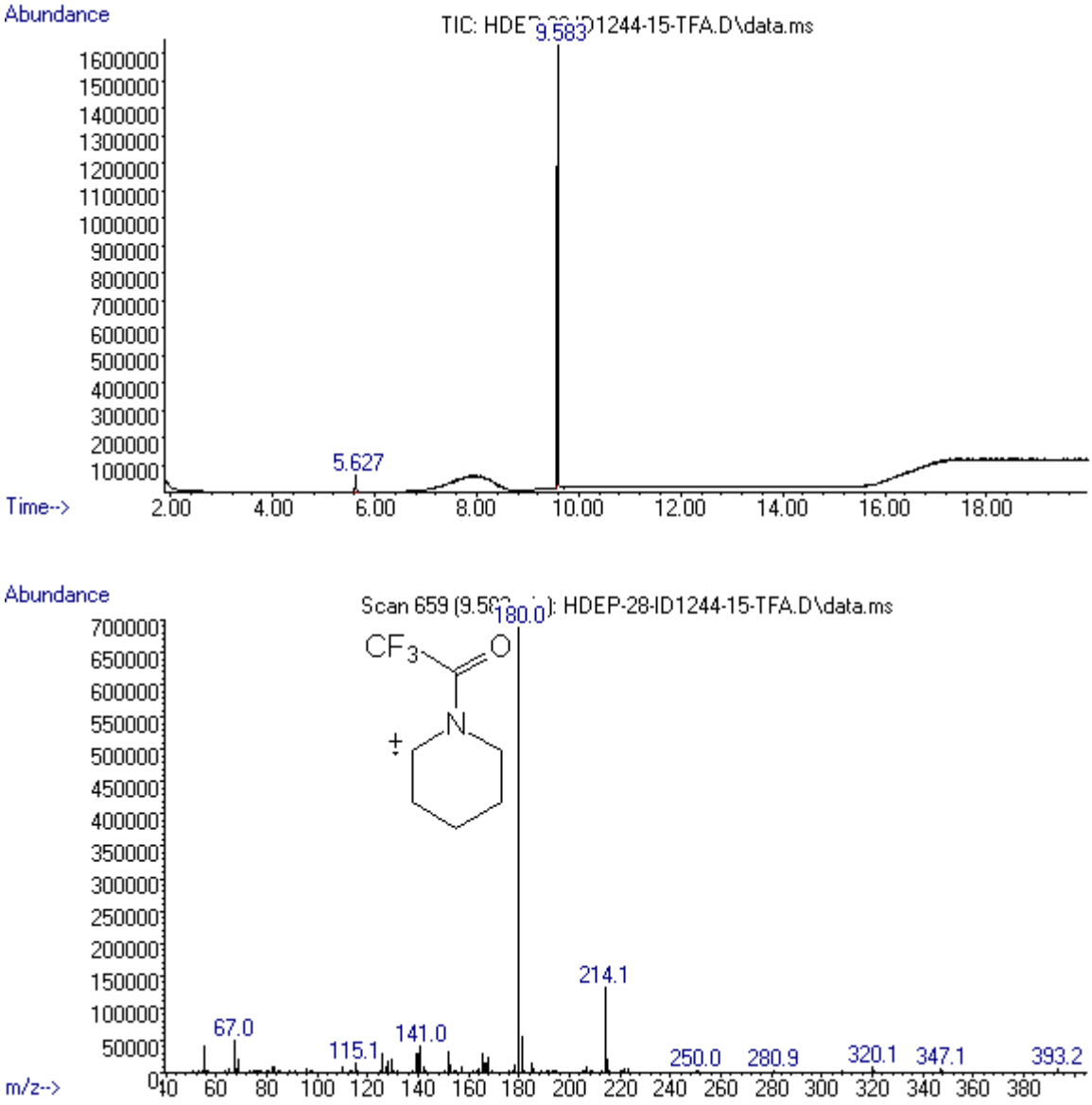


Figure 7: Chromatogram and mass spectrum of derivatized sample; the structure in the image implies to the base peak signal (*m/z* = 180).

FTIR-ATR (direct measurement)

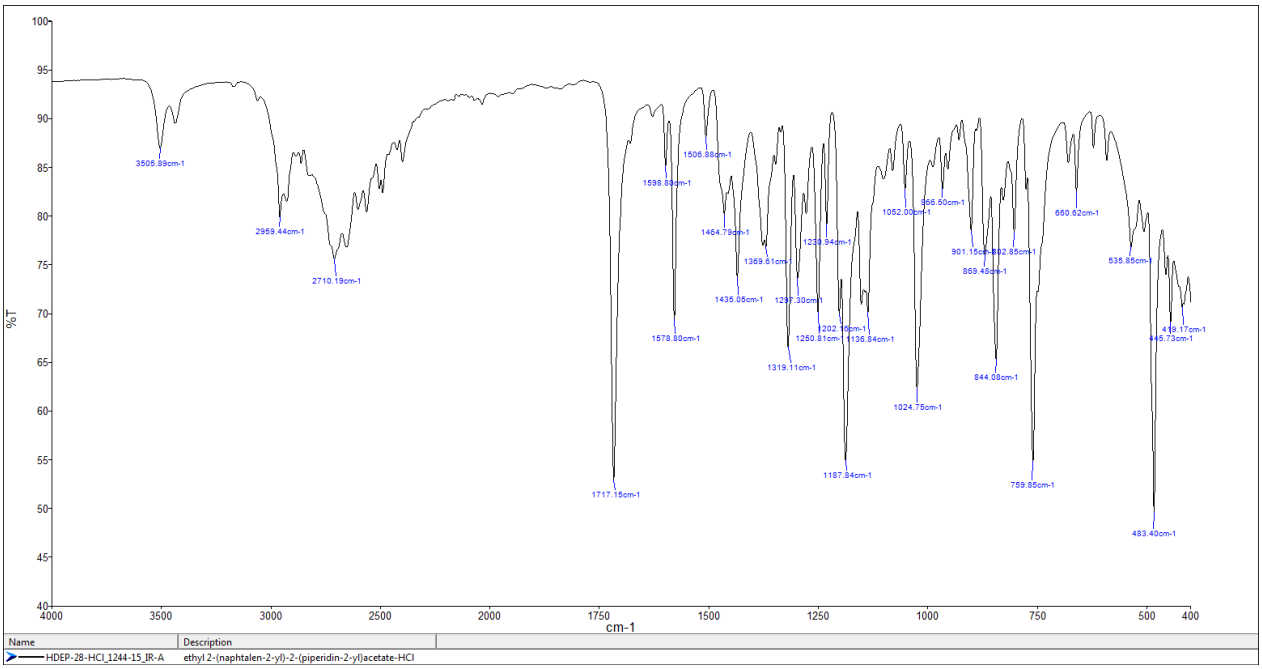


Figure 8: FTIR-ATR spectrum of sample – direct measurement

Target Compound Screening Report

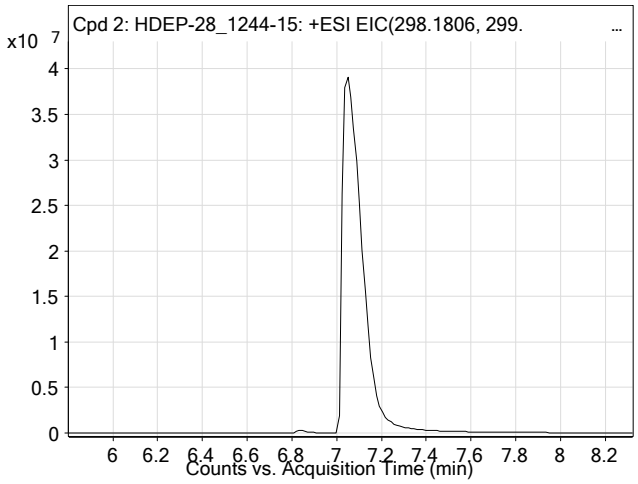
Data File	HDEP-28_1244-15_TOF.d	**Sample Name**	HDEP-28
Sample Type	Sample	**Position**	P2-E2
Instrument Name	6230B TOF LC-MS	**User Name**	TG
Acq Method	droge general-13-5-2015-XDB-C18-ESI-poz.m	**Acquired Time**	8/19/2015 12:43:21 PM
IRM Calibration Status	Success	**DA Method**	Droge_Default.m
Comment	extract in MeOH		

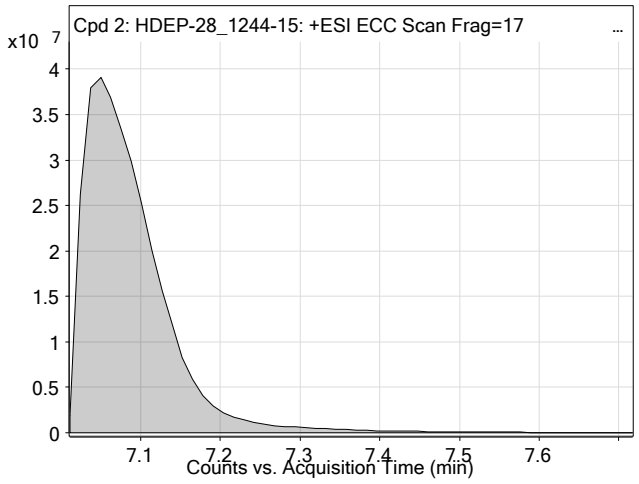
Compound Table

Label	Tgt Name	MFG Formula	Obs. RT	Obs. Mass
Cpd 2: HDEP-28_1244-15	HDEP-28_1244-15	C19 H23 N O2	7.061	297.1729

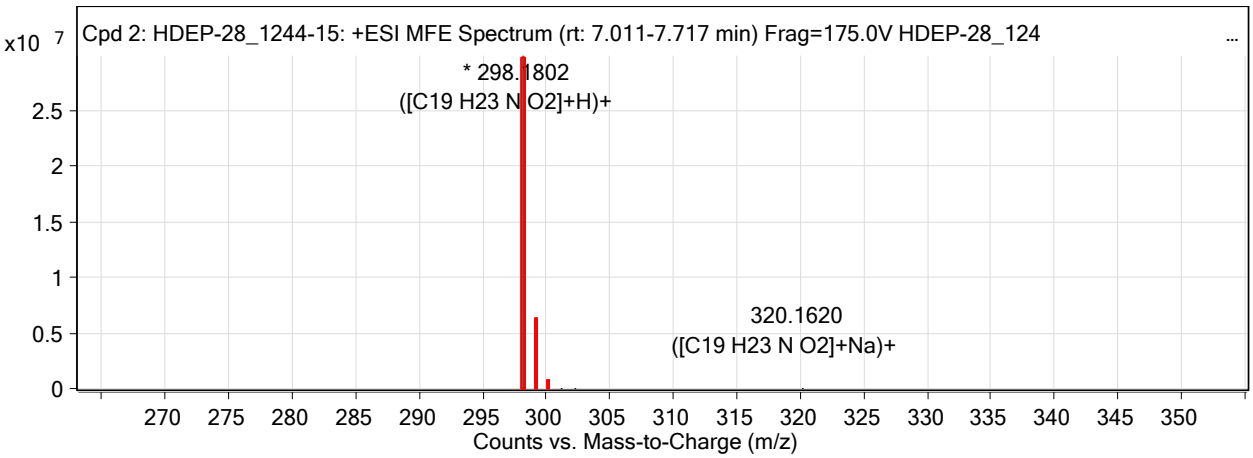
Name	Obs. *m/z*	Obs. RT	Obs. Mass	DB RT	DB Formula	DB Mass	DB Mass Error (ppm)	Find Cpds Algorithm
HDEP-28_1244-15	298.1802	7.061	297.1729	7.061	C19 H23 N O2	297.1729	-0.15	Find by Molecular Feature

Compound Chromatograms





MFE MS Zoomed Spectrum

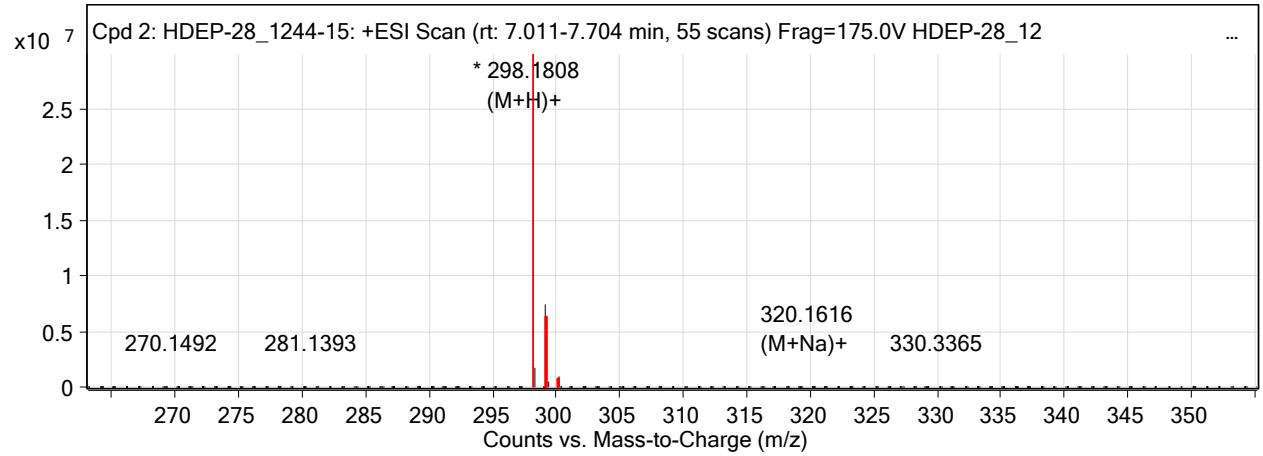


MS Spectrum Peak List

Obs. *m/z*	Charge	Abund	Formula	Ion/Isotope
298.1802	1	29867440	C19 H23 N O2	(M+H)+
299.1835	1	6344771	C19 H23 N O2	(M+H)+
300.1869	1	677073.05	C19 H23 N O2	(M+H)+
301.1896	1	63830.25	C19 H23 N O2	(M+H)+
302.1917	1	4951.23	C19 H23 N O2	(M+H)+
320.162	1	11912.82	C19 H23 N O2	(M+Na)+

Agilent Technologies

MS Zoomed Spectrum

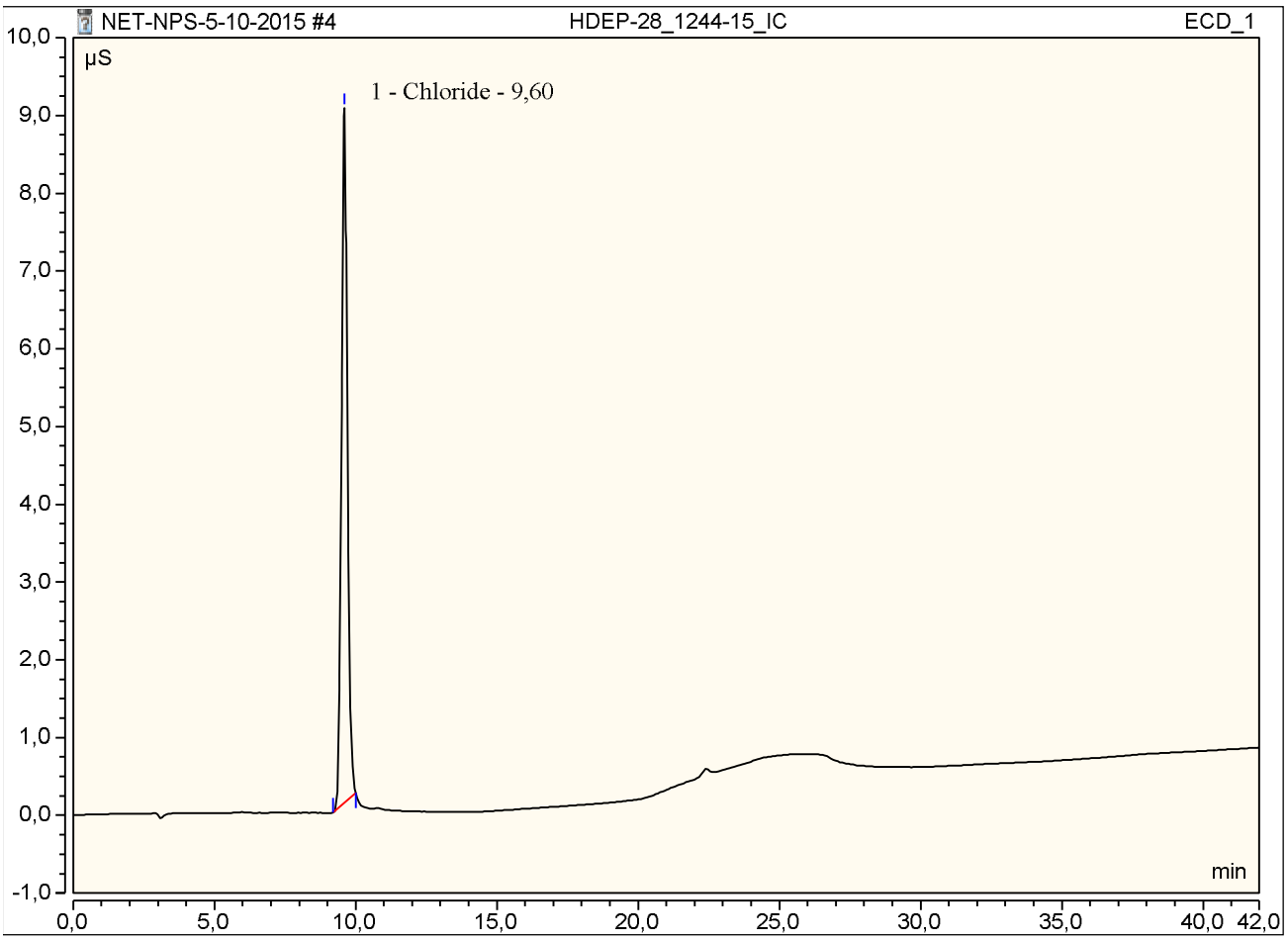


--- End Of Report ---

Peak Integration Report

Sample Name:	**HDEP-28_1244-15_IC**	*Inj. Vol.:*	**25,00**
Injection Type:	**Unknown**	*Dilution Factor:*	**1,0000**
Program:	**ANIONI**	*Operator:*	**kemija**
Inj. Date / Time:	**05-okt-2015 / 16:16**	*Run Time:*	**41,99**

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount mg/L
1,00	9,60	**Chloride**	BMB	2,16	8,94	n.a.
		TOTAL:		2,16	8,94	0,00



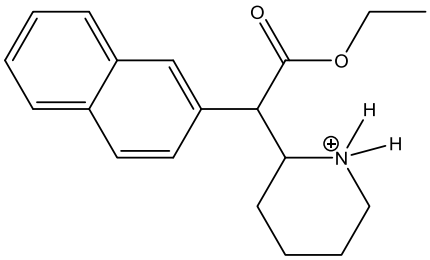
University
of Ljubljana

Faculty *of Chemistry and Chemical Technology*

Večna pot 113
P. O. Box 537
SI-1001 Ljubljana
Slovenia
Phone: +386 1 479 8558
janez.kosmrlj@fkkt.uni-lj.si

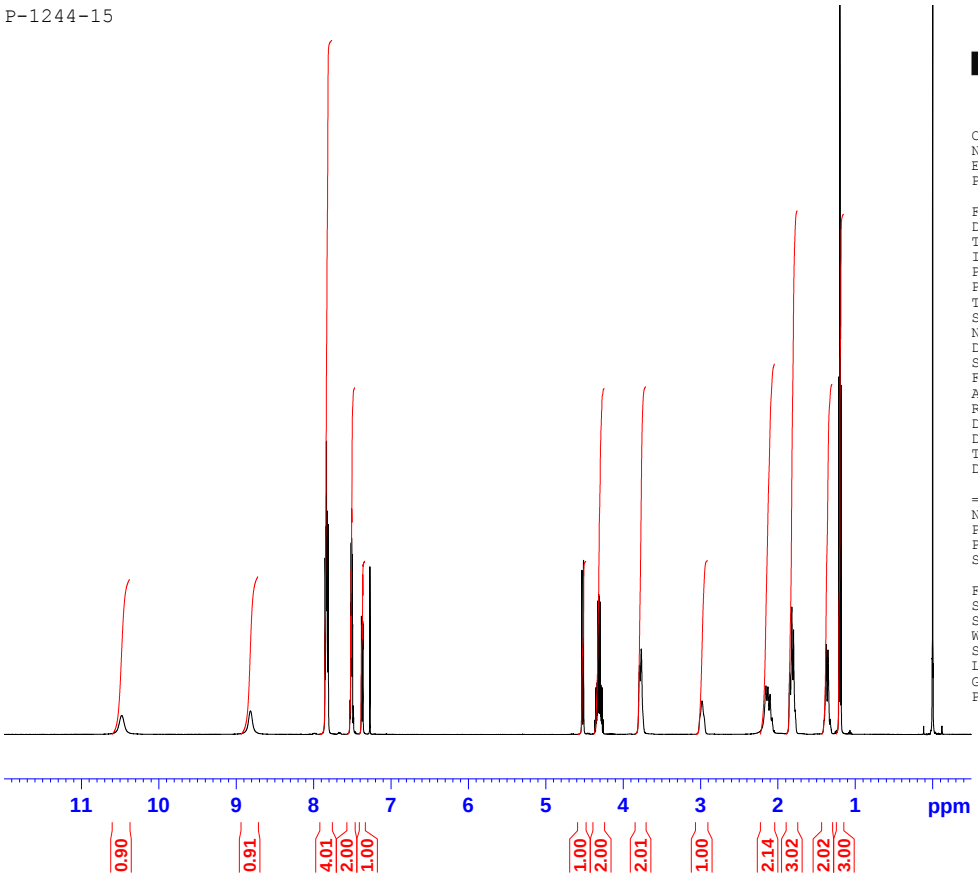
Co-funded by the Prevention of and Fight against Crime Programme of the European Union

REPORT

Sample ID:	**1244-15**
Our notebook code:	P-1244-15
NMR sample preparation:	15 mg dissolved in 0.7 mL $CDCl_3$
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–^{14}N *gs*-HMBC.
Proposed structure:	
Chemical name:	2-(2-ethoxy-1-(naphthalen-2-yl)-2-oxoethyl)piperidin-1-ium ion
Comments:	- Structure elucidation based on 1D and 2D NMR spectra - Compound is pure by NMR.
Supporting information:	Copies of ^{1}H and ^{13}C NMR spectra
Author:	Prof. Dr. Janez Košmrlj, Doc. Dr. Krištof Kranjc
Date of report:	October 17, 2015

This report has been produced with the financial support of the Prevention of and fight against crime Programme of the European Union (grant agreement number JUST/2013/ISEC/DRUGS/AG/6413). The contents of this publication are the sole responsibility of the Author and can in no way be taken to reflect the views of the European Commission.

P-1244-15

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PROCNO                1

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PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                71.8
DW               48.400 usec
DE                6.50 usec
TE               295.9 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 8.90 usec
PLW1         26.00000000 W
SFO1        500.1330885 MHz

F2 - Processing parameters
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WDW                  EM
SSB       0
LB                0.30 Hz
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PC                1.00
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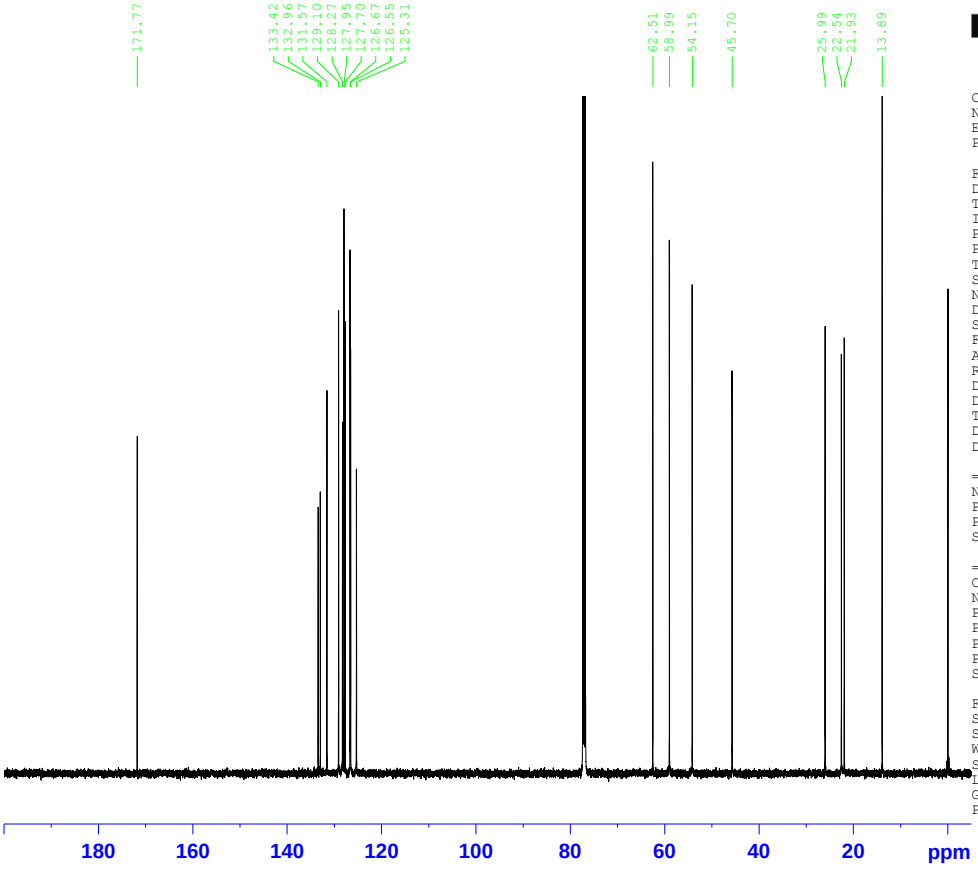
P-1244-15

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PROCNO                1

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PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 4096
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                2050
DW               16.800 usec
DE                6.50 usec
TE               296.4 K
D1           1.00000000 sec
D11          0.03000000 sec

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NUC1                 13C
P1                 9.00 usec
PLW1        122.00000000 W
SFO1         125.7703637 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             80.00 usec
PLW2         26.00000000 W
PLW12         0.32179001 W
PLW13         0.20595001 W
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                32768
SF          125.7577896 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
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