ANALYTICAL REPORT\(^1\)

**HDEP-28 (C19H23NO2)**

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

Remark – other NPS detected: none

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>1244-15</th>
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<tbody>
<tr>
<td>Sample description:</td>
<td>powder - white</td>
</tr>
<tr>
<td>Sample type:</td>
<td>test purchase /RESPONSE -purchasing</td>
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<tr>
<td>Date of sample receipt (M/D/Y):</td>
<td>8/18/2015</td>
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<tr>
<td>Date of entry (M/D/Y) into NFL database:</td>
<td>8/19/2015</td>
</tr>
<tr>
<td>Report (updates) will be published here:</td>
<td><a href="http://www.policija.si/apps/nfl_response_web/seznam.php">link</a></td>
</tr>
</tbody>
</table>

Substance identified-structure\(^2\) (base form)

![Structure](image)

Systematic name

\[
\text{ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl)acetate}
\]

Other names

\[
\text{ethylnaphtidate, HDEP-28, 2-(2-ethoxy-1-(naphthalen-2-yl)-2-oxoethyl)piperidin}
\]

Formula (per base form)

\[
\text{C19H23NO2}
\]

\(M_w\) (g/mol)

297,17

Salt form

HCl

StdInChIKey

OTQVTBPHZRRTL-UHFFFAOYSA-N

Compound Class

Piperidines & pyrrolidines

Other NPS detected

none

Add.info (purity..)

pure by HPLC-TOF and NMR; thermal decomposition can occur in GC

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\(^1\) 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/](http://opsin.ch.cam.ac.uk/) DOI: 10.1021/ci100384d
## Report updates

<table>
<thead>
<tr>
<th>date</th>
<th>comments (explanation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. 11. 2015</td>
<td>minor corrections of text have been done</td>
</tr>
</tbody>
</table>

## 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 °C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickness 0.25 mm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170 °C for 1 min, followed by heating up to 293 °C at a rate of 18 °C/min, hold for 6.1 min, than heating at 50 °C/min up to 325 °C and finally 2.8 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235°C, source and quadropole temperatures 280°C and 180°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)


   MSD source EI = 70 eV. GC-MS transfer line T= 235°C, source and quadropole temperatures 280°C and 180°C, respectively. Scan range m/z scan range: from 50 (40) to 550 amu.

   IR (condensed 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

<table>
<thead>
<tr>
<th>Solubility in</th>
<th>result/remark</th>
</tr>
</thead>
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<tr>
<td>CH$_2$Cl$_2$</td>
<td>soluble</td>
</tr>
<tr>
<td>MeOH</td>
<td>soluble</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>soluble</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical technique:</th>
<th>applied</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS (EI ionization)</td>
<td>+</td>
<td>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).</td>
</tr>
<tr>
<td>HPLC-TOF</td>
<td>+</td>
<td>Exact mass (theoretical): 297.1729; measured value Δppm:-0.15; formula: C$<em>{19}$H$</em>{23}$NO$_2$</td>
</tr>
<tr>
<td>TOF direct measurement</td>
<td>+</td>
<td>sample is pure</td>
</tr>
<tr>
<td>FTIR-ATR</td>
<td>+</td>
<td>direct measurement</td>
</tr>
<tr>
<td>FTIR (condensed phase) always as base form</td>
<td>+</td>
<td>scanned only at our standard analytical conditions - results are not shown</td>
</tr>
<tr>
<td>IC (anions)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NMR validation</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td></td>
<td>melting point measurement (see analytical results)</td>
</tr>
</tbody>
</table>
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-naphthalenacetic acid, ethyl ester.

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

![Thermal decomposition reaction - proposed mechanism](image)

**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).

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

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<tr>
<th>Label</th>
<th>Tgt Name</th>
<th>MFG Formula</th>
<th>Obs. RT</th>
<th>Obs. Mass</th>
</tr>
</thead>
</table>

### Compound Chromatograms

![Cpd 2: HDEP-28_1244-15: +ESI EIC(298.1806, 299.1835)](image1)

![Cpd 2: HDEP-28_1244-15: +ESI ECC Scan Frag=17](image2)

### MFE MS Zoomed Spectrum

![Cpd 2: HDEP-28_1244-15: +ESI MFE Spectrum (rt: 7.011-7.717 min) Frag=175.0V](image3)

### MS Spectrum Peak List

<table>
<thead>
<tr>
<th>Obs. m/z</th>
<th>Charge</th>
<th>Abund</th>
<th>Formula</th>
<th>Ion/Isotope</th>
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<tbody>
<tr>
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<td>1</td>
<td>29867440</td>
<td>C19 H23 N O2</td>
<td>X+H+</td>
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<tr>
<td>299.1835</td>
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<td>6344771</td>
<td>C19 H23 N O2</td>
<td>X+H+</td>
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<tr>
<td>300.1869</td>
<td>1</td>
<td>677073.05</td>
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<tr>
<td>301.1896</td>
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<td>302.1917</td>
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<td>X+H+</td>
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<td>320.1621</td>
<td>1</td>
<td>11912.82</td>
<td>C19 H23 N O2</td>
<td>X+Na+</td>
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</tbody>
</table>

Printed at 3:10 PM on 19-Aug-2015
2
Page 1 of 2

Agilent Technologies
Cpd 2: HDEP-28_1244-15: +ESI Scan (rt: 7.011-7.704 min, 55 scans) Frag=175.0V HDEP-28_12...

* 298.1808 (M+H)+
281.1393 270.1492
320.1616 (M+Na)+ 330.3365
# Peak Integration Report

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

<table>
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<th>No.</th>
<th>Time min</th>
<th>Peak Name</th>
<th>Peak Type</th>
<th>Area µS*min</th>
<th>Height µS</th>
<th>Amount mg/L</th>
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<tbody>
<tr>
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<td>9,60</td>
<td>Chloride</td>
<td>BMB</td>
<td>2,16</td>
<td>8,94</td>
<td>n.a.</td>
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**TOTAL:** 2,16  
8,94  
0,00

---

**Graph:**

- **X-axis:** min  
- **Y-axis:** µS  
- **Peaks:** 1 - Chloride - 9,60
## REPORT

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>1244-15</th>
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<tbody>
<tr>
<td>Our notebook code:</td>
<td>P-1244-15</td>
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<tr>
<td>NMR sample preparation:</td>
<td>15 mg dissolved in 0.7 mL CDCl₃</td>
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<tr>
<td>NMR experiments:</td>
<td>¹H, ¹³C, ¹H–¹H gs-COSY, ¹H–¹³C gs-HSQC, ¹H–¹³C gs-HMBC, ¹H–¹⁴N gs-HMBC.</td>
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<td>Proposed structure:</td>
<td><img src="image" alt="Chemical Structure" /></td>
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<td>Chemical name:</td>
<td>2-(2-ethoxy-1-(naphthalen-2-yl)-2-oxoethyl)piperidin-1-iyum ion</td>
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<tr>
<td>Comments:</td>
<td>- Structure elucidation based on 1D and 2D NMR spectra</td>
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<tr>
<td></td>
<td>- Compound is pure by NMR.</td>
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<tr>
<td>Supporting information:</td>
<td>Copies of ¹H and ¹³C NMR spectra</td>
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<tr>
<td>Author:</td>
<td>Prof. Dr. Janez Košmrlj, Doc. Dr. Krištof Kranjc</td>
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*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.*
Current Data Parameters
NAME          P-1244-15
EXPNO                 1
PROCNO                1
F2 - Acquisition Parameters
Date_          20151008
Time               22.15
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SNH           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
SI                65536
SF          500.1300076 MHz
WDW                  EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00

Current Data Parameters
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EXPNO                 3
PROCNO                1
F2 - Acquisition Parameters
Date_          20151009
Time               0.54
INSTRUM           spect
PROBHD   5 mm PABBO BB-
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
======== CHANNEL f1 ========
NUC1                13C
P1                 9.00 usec
PLW1       122.00000000 W
SFO1        125.7703637 MHz
======== CHANNEL f2 ========
CPWOG2         wals16
NUC2                1H
PCWOG2            80.00 usec
PLM2        122.00000000 W
PLM12       0.32179991 W
PLM13       0.20595001 W
SFO2        500.1320005 MHz
F2 - Processing parameters
SI             32768
SF         125.7703637 MHz
WDW                  EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40