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

EU CO-FUNDED PROJECT: "RESPONSE TO NEW CHALLENGES IN FORENSIC DRUG ANALYSES" - OBJECTIVES AND SOME SELECTED RESULTS

EUROPEAN PROJECT RESPONSE
to challenges in forensic drugs analyses

Dr. Sonja Klemenc[a] and Prof. Dr. Janez Košmrlj[b];

[a]National Forensic Laboratory, Ljubljana, Slovenia and [b]University Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia

corresponding author: sonja.klemenc@policija.si

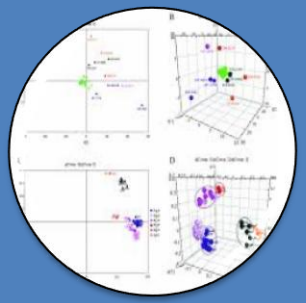
“RESPONSE” PROJECT IN BRIEF

PROJECT (http://www.policija.si/eng/index.php/generalpolicedirectorate/1669-nfl-page-response **)**
addresses two specific topics of forensic drugs investigations:

- **New psychoactive substances** (NPS) identification - challanges
  - unexpected growth in the number and type of NPSs [1], easy availability which are advertised and sold over the Internet at affordable prices
  - lack of the availability of reference materials (RM)
  - Non specific reliable spectra databases MS and FTIR are the main problem for accurate NPS identification
- **Drugs profiling** where the main problem is the recognized gap between customers (law enforcement, judicial system, EU policy makers) needs and ForL capacities, methodologies and staff competencies.

[1] New Psychoactive substances in Europe, EMCDDA, March 2015, (http://www.emcdda.europa.eu/publications/2015/new-psychoactive-substances)

RESPONSE PROJECT FORENSIC MODULES

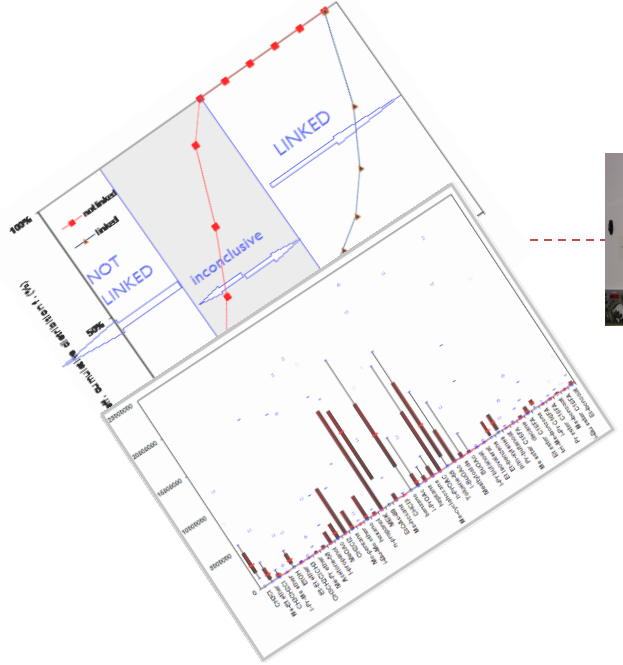


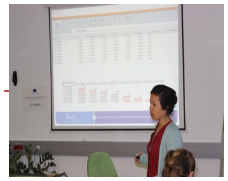
Drugs profiling

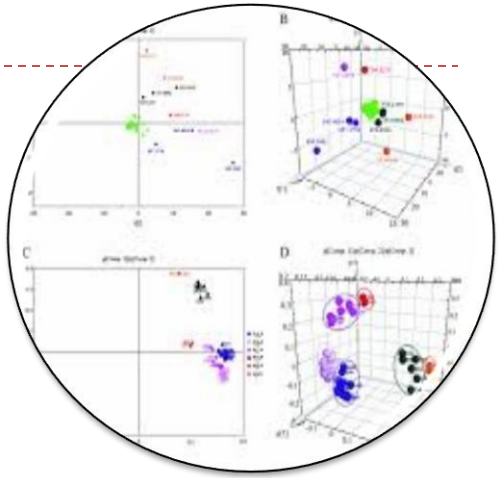
NPS identification – chemical characterisations

Data bases (PROFILING and NPS)

COLLECT, ANALYSE, ORGANIZE, EVALUATE, SHARE







PROFILING

Some “profiling modules” deliverables and achievements are presented on two posters and in DWG Subcommittees presentations of this meeting.

PROFILING OBJECTIVES and activities

Developement of profiling methodologies (3x) and databases in NFL

- MENTOR BASED APPROACH
- EXPERTS EXCHANGE (SI/FR)

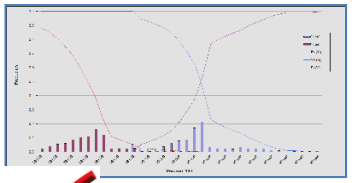
Rise the competencies and understanding of drugs profiling principles in forensic labs

- WORKSHOP ASSISTED LEARNING (2x)

Rise the knowledge about profiling and its evidential value among target users

- ONE DAY ONE TOPIC SEMINARs 2x - (Law enforcement, Prosecutors, judicial)

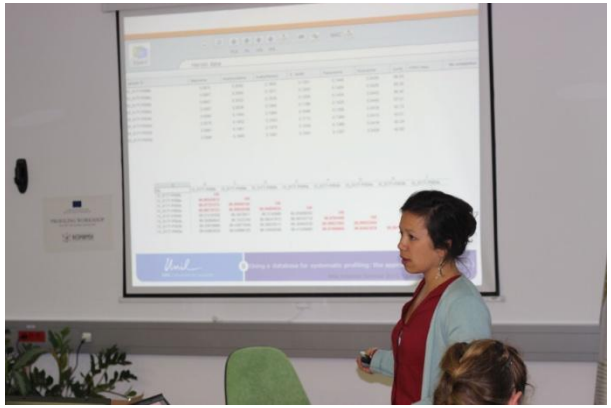
DELIVERABLES

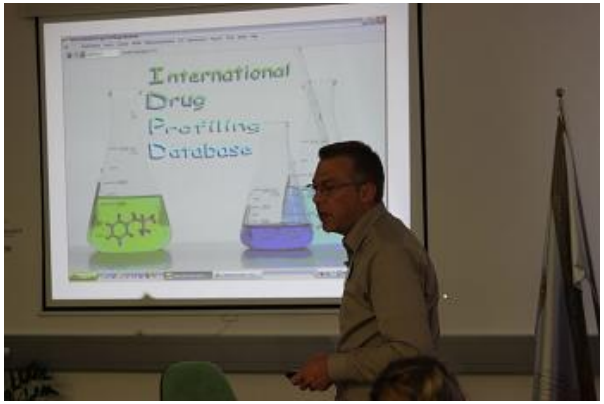


PROFILING (RESPONSE activities)

1. Mentor based approach of GC-MS methods development on the expert exchange basis (NFL SI/ INPS Lyon/France)
   - Experimental design and validation of analytical method (heroin, cocaine and amphetamine profiling)
   - Measurements (many sets of linked and unlinked samples shall be analysed) – to set up the decision thresholds
   - Data processing - chemometric evaluations (comparisons of chromatograms):
     - pre-processing of data (raw chromatographic results) – options: no pre-processing or use of normalization and/or scaling
     - choice of the appropriate metric /tool/ for the **intended purpose of use**
     - calculation of similarity
   - set up of the decision thresholds, i.e. criteria for reporting : linked or not linked samples or inconclusive findings
   - databases
2. Workshop assisted learning (2x) to rise the competencies and understanding of drugs profiling principles (how to evaluate multivariate data effectively (in real time) and on non-subjective basis) (workshops are mainly opened to ENFSI-DWG members- the task will be supported also by ENFSI-DWG.
3. One day one topic seminars 2x, for SI audience only (police, judicial)

PROFILING WORKSHOP June 2015 - mentors

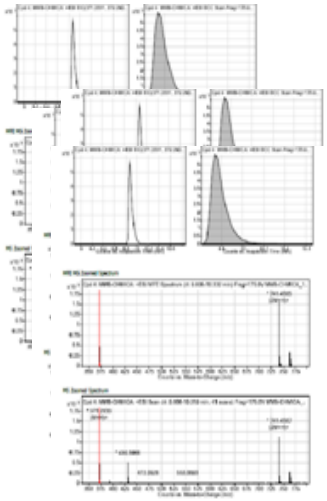


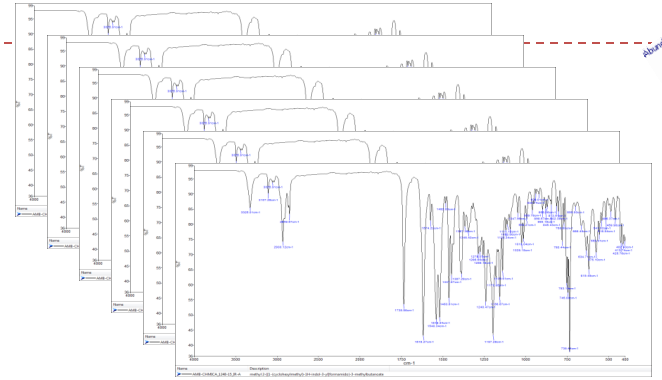


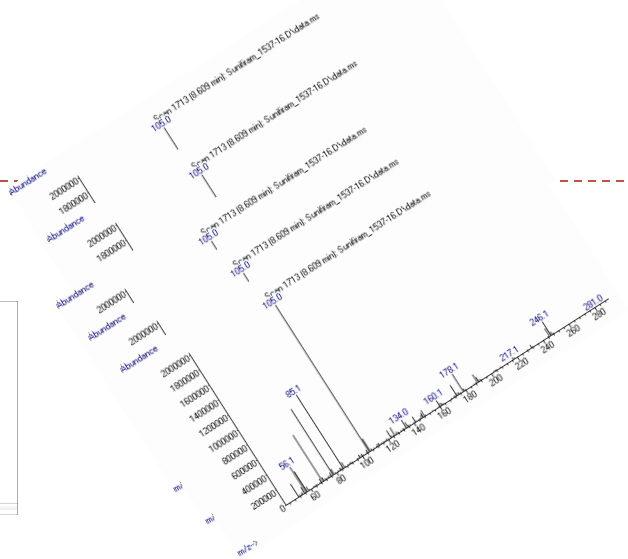
EUROPEAN PROJECT RESPONSE to challenges in forensic drugs analyses

ENFSI DWG

PROFILING THEORY IN PRACTICE (2015)

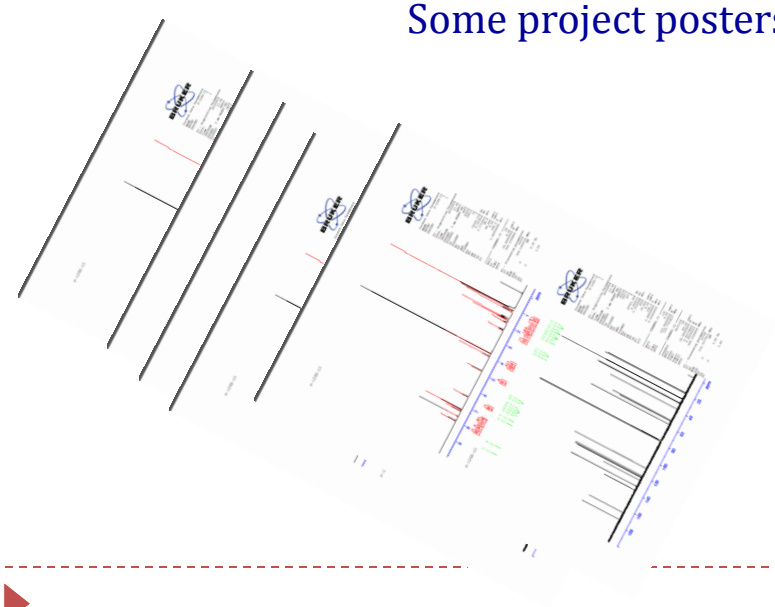


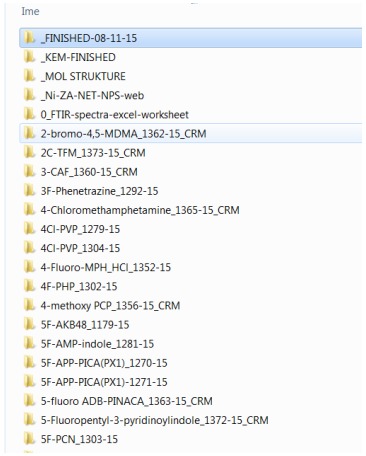


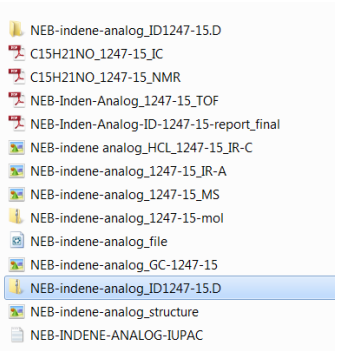


NEW PSYCHOACTIVE SUBSTANCES

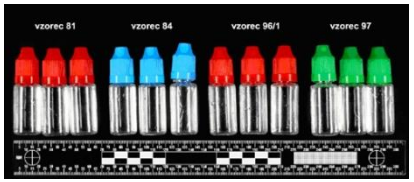
Some project posters are presented at this meeting as well







NPS OBJECTIVES



- to provide numerous spectral data MS, FTIR-ATR and FTIR-condensed phase on newly appearing NPS
- To create FTIR database (tool) and guidelines on FTIR spectra acquisition and interpretation (joined ENFSI DWG –RESPONSE documents)
- to implement spectra into electronic data repositories ENFSI - DWG.
- TO SHARE analytical data, knowledge and information **EFFICIENTLY** through different communication platforms (SI EWS, EMCDDA, EUROPOL, TAXUD (customs network), I-SEE project…)
- By internet purchasing of NPSs the project will aim at implementing a pro-active forensics respond to the NPS phenomena and form " NPS material bank" for share with project partners and wider

Sources of materials & Chemical characterizations

Type of material	Analytical methods
Reference materials (Different vendors)	GC-MS, FTIR-ATR, GC-MS-FTIR-(condensed phase) – optional, HPLC-TOF
Test purchases (Internet based vendors]	GC-MS, FTIR-ATR, GC-(MS)-FTIR-(condensed phase), HPLC-TOF, NMR, for "unknowns" combined with ion chromaand some supplementary tography for anions (IC)…
Seized samples (Police/ Customs)	
Collected samples (NGO – anonymous users, project partners, other)	

The compounds are identified in the Slovenian forensic laboratory (NFL) and by NMR in FKKT (SI) and spectra provided to EI-MS and IR libraries of ENFSI DWG

TEST PURCHASES OVER THE INTERNET

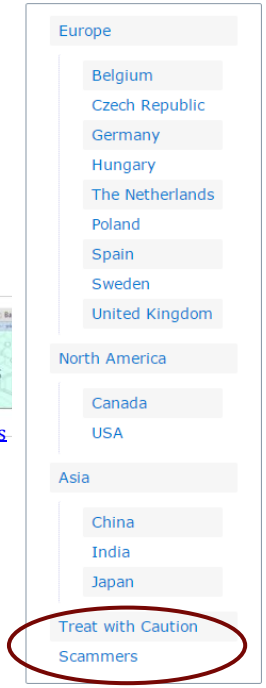
Detect what is new [L. Ask Reitzel et all, Systematical methodology for finding novel NPS (New Psychoactive Substances) over the Internet, EAFS-2015, Prague, 2015]

- use a Google engine and simple word search
- follow social networks related to recreational drugs (blogs/discussion forums/chats)

Find a internet vendor(s) and evaluate its reliability (NFL-SI)

https://www.reddit.com/r/RCSources/wiki/vendors

- use information (and updates) from the internet
- check the payment options
- check minimum required order (some only sell bulk quantities)
- test vendors by small orders (few samples) first

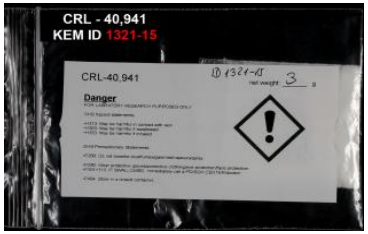


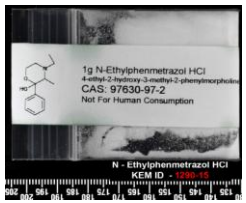
Purchasing procedure:

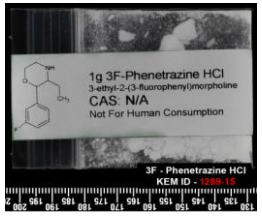
- shall follow institutional and national rules (quite complicated)
- information exchange system between national authorities concerning internet orders has been established. (Police (NFL), Ministry of Health (general approval was issued before the project started, Customs (NFL informs about every purchase in advance and afterwards when samples are received).

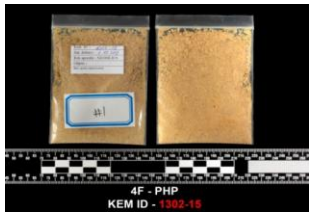
TEST PURCHASES - EXPERIENCES

- Around 150 samples were ordered and are available in NFL "NPS materials bank"/ so far, only 2 samples were not delivered
- Delivery time vary (from few days to two months)
- The rate of false advertised/ delivered compounds is around 20-30 %
- Labeling and packaging very different /depends on vendor

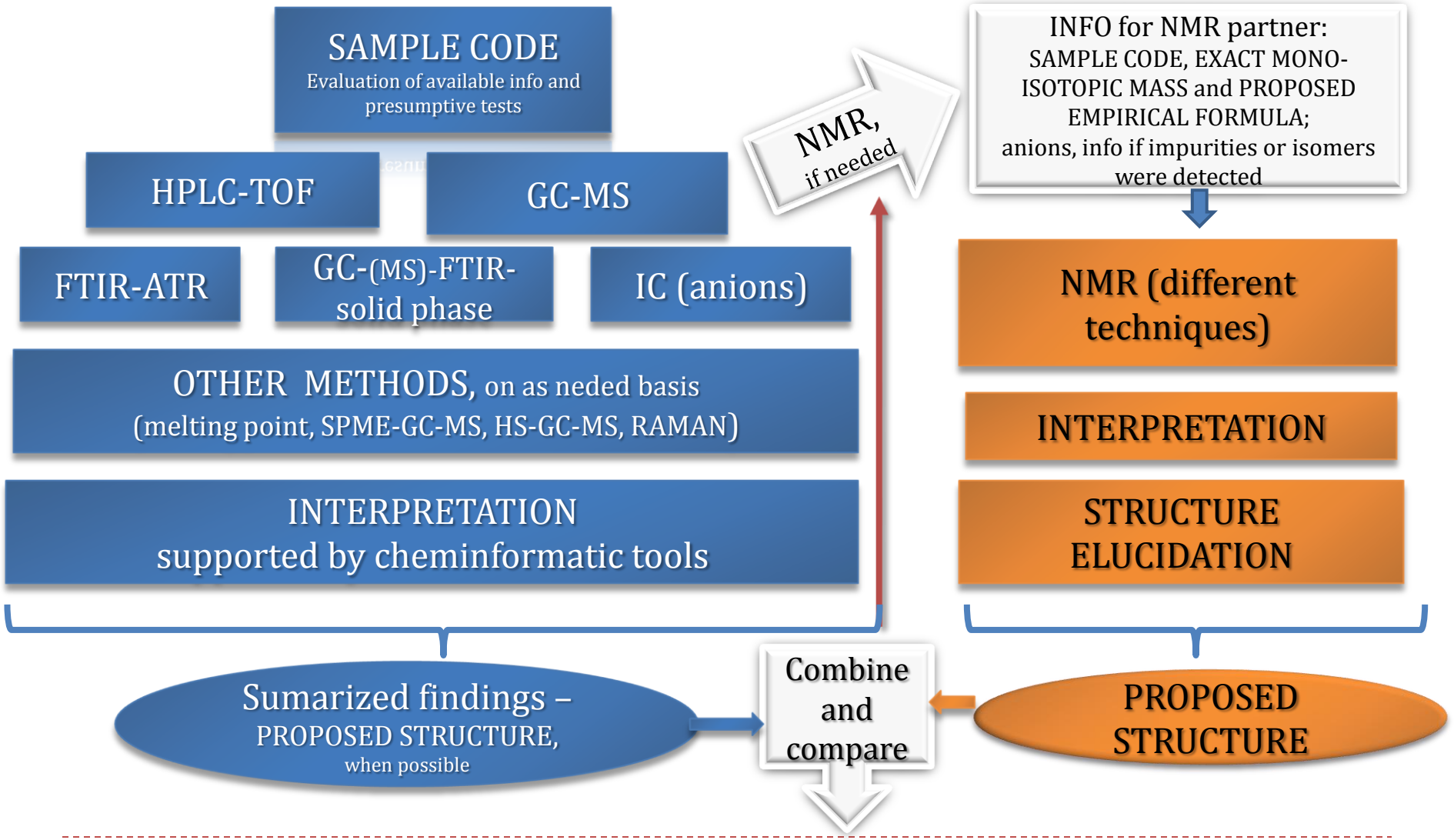




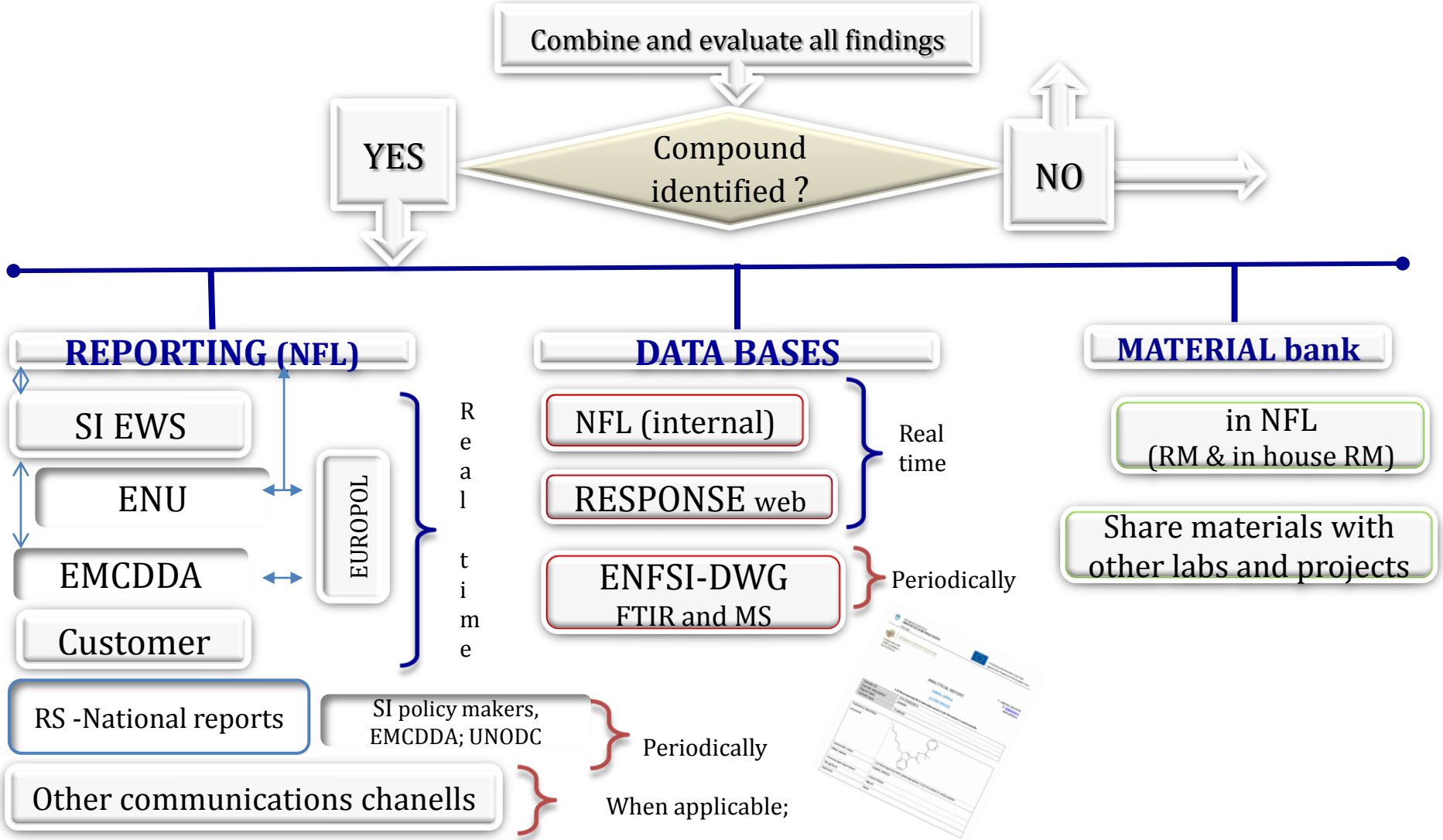




CHEMICAL CHARATCTERIZATIONS - Strategy

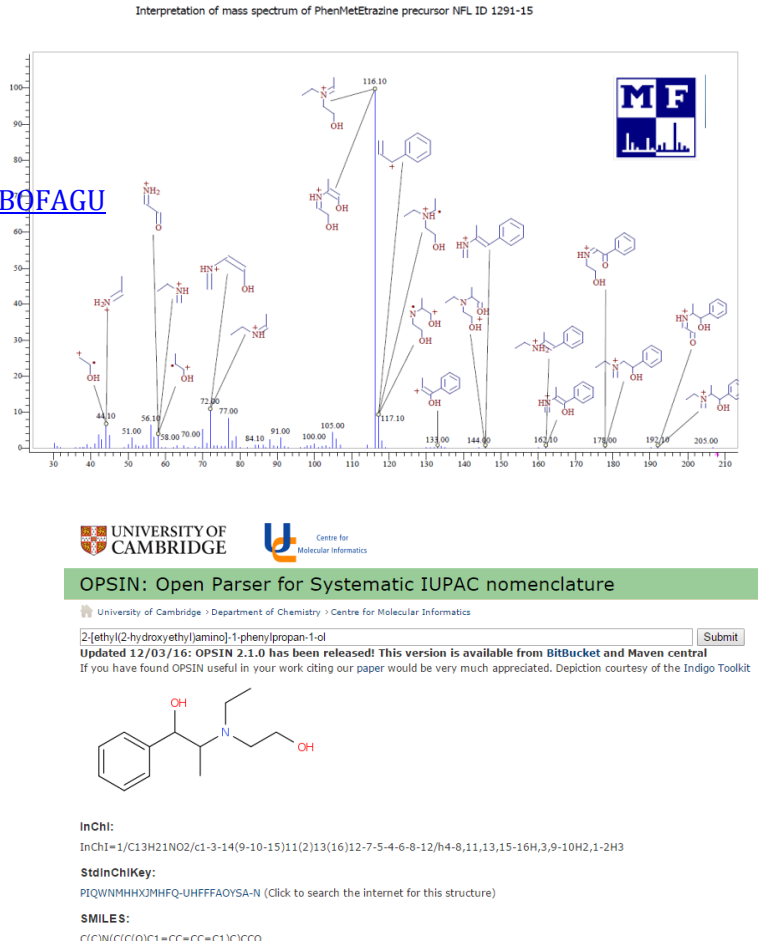


CHEMICAL CHARATCTERIZATIONS & REPORTING

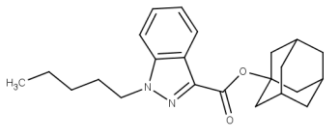


INTERPRETATION - SUPPORTING TOOLS

- MS spectra interpretation
  - MS tools of KnowIAll software (http://www.bio-rad.com/en-uk/category/products/spectroscopy-software
  - Mass Frontier https://www.thermofisher.com/order/catalog/product/IQLAAEGABOFAGUMZZZ
- FTIR (tools of KnowIAll software)
- STRUCTURE –NAME-STRUSTURE
  - ChemAxon (Marwin Sketch) https://www.chemaxon.com/
  - OPSIN (http://opsin.ch.cam.ac.uk/)
- Other free tools from web
  - for example -NMR predictor (http://www.nmrdb.org/about/



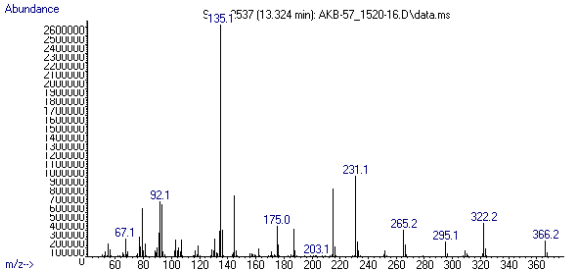
Characterization (NFL): AKB-57



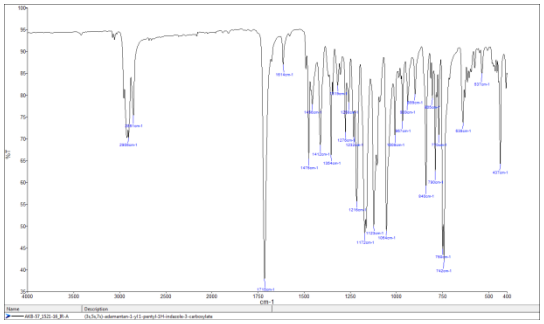
SAMPLE ID 1521-16

GC-MS

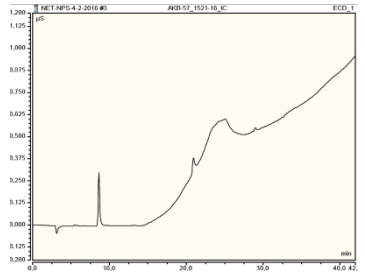
Single chromatographic peak was observed
(No hits)



FTIR-ATR (no hits)



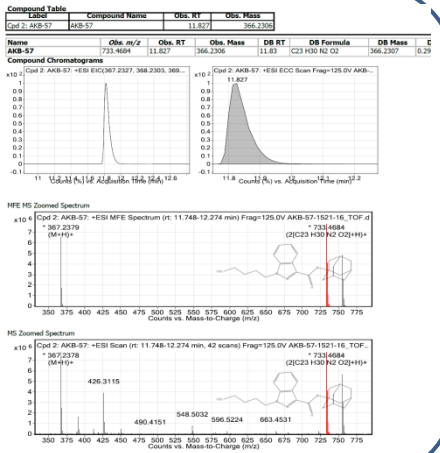
IC -anions



HPLC-TOF

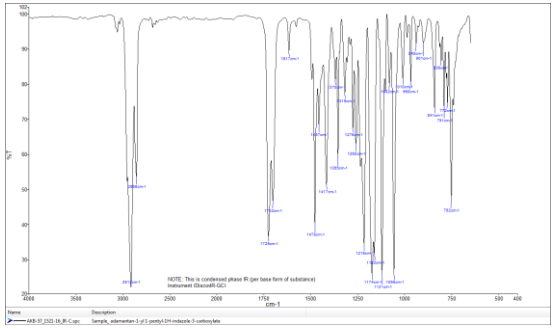
Single chromatographic peak

Mass: 366.31
Exact mass: 366.2307
measured value Δppm: 0,29
Formula: C23H30N2O2



GC-(MS)-IR-condensed phase

(no hits)



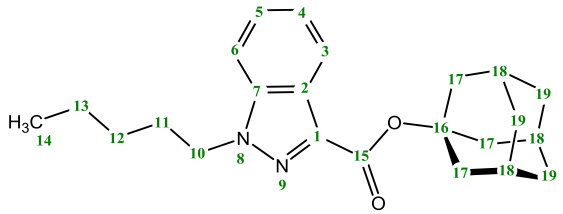
Solubility in	result/remark
CH_2Cl_2	soluble
MeOH	soluble
H_2O	low (bad)

DATA TO NMR

AKB-57 (NMR-FKKT)

Sample ID:	**1521-16**
Our notebook code:	P-1521-16
NMR sample preparation:	15 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.

RESULTS:



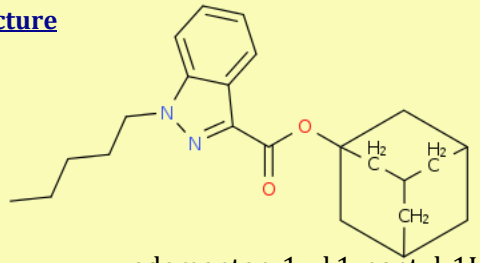
Atom	^{1}H NMR Exp. Shift (ppm)	Multiplet	^{13}C NMR Exp. Shift (ppm)	^{15}N NMR Exp. Shift (ppm)
1	–	–	135.4	–
2	–	–	123.4	–
3	8.03	d	122.0	–
4	7.30	dd	123.1	–
5	7.47	dd	127.0	–
6	7.80	d	111.1	–
7	–	–	140.8	–
8	–	–	–	197
9	–	–	–	331
10	4.50	t	49.4	–
11	1.85	tt	29.6	–
12	1.30	tt	28.8	–
13	1.22	m	22.2	–
14	0.83	t	14.3	–
15	–	–	161.2	–
16	–	–	81.3	–
17	2.28	brs	41.7	–
18	2.21	brs	30.8	–
19	1.69	m	36.1	–

Combine and evaluate all findings (NFL and FKKT)

COMPOUND IS IDENTIFIED ☺

Formula: C23H30N2O2; M_W: 366.31g/mol

Structure

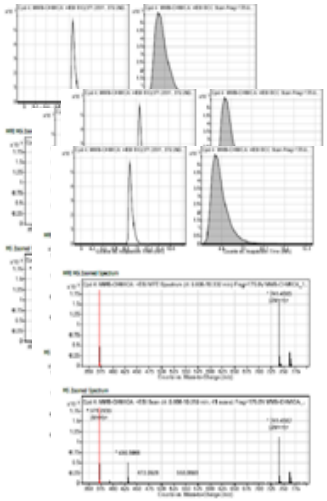


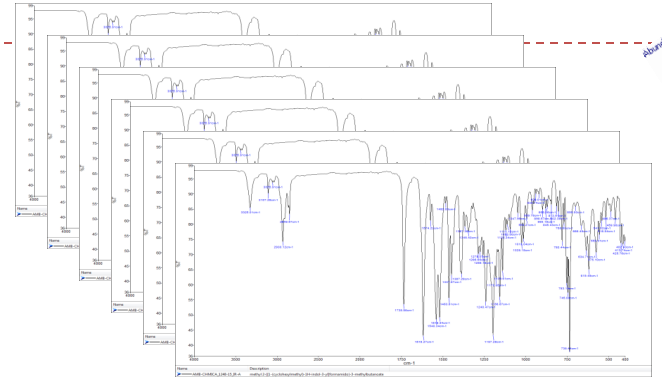
Chemical name: adamantan-1-yl 1-pentyl-1H-indazole-3-carboxylate

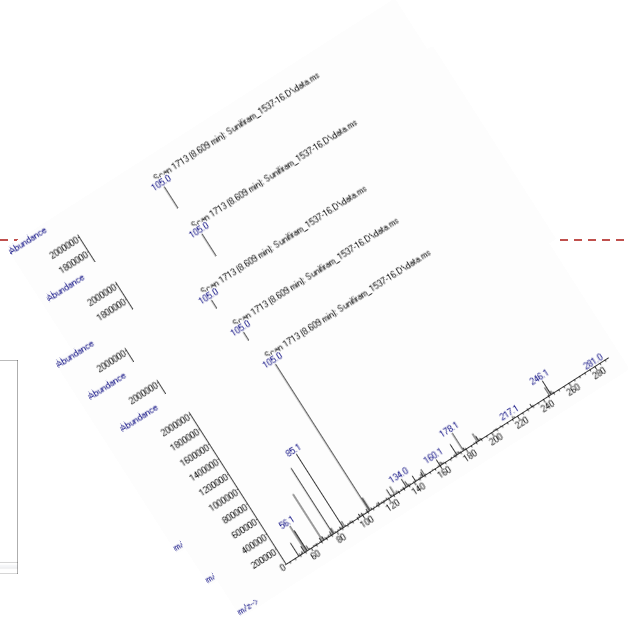
Nick name: AKB-57

StdInChIKey: KCCVWUAAHDXNNQ-UHFFFAOYSA-N

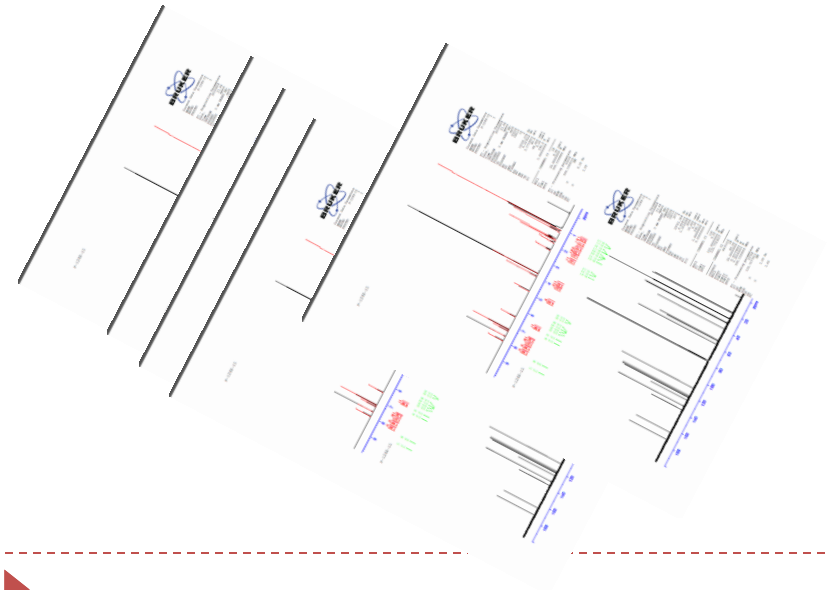
REPORTING

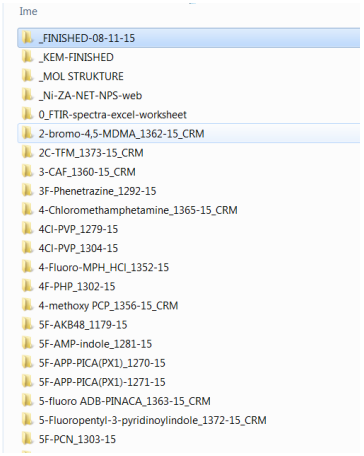


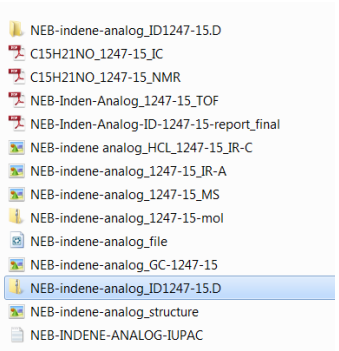




SOME INTERPRETATION PITFALS



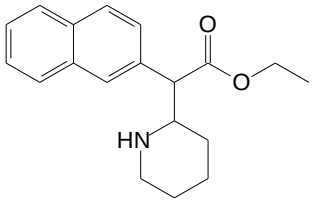




NMR CONFIRMED – are the MS spectrum and RT correct?

TEST PURCHASE

Sample ID:1244-15



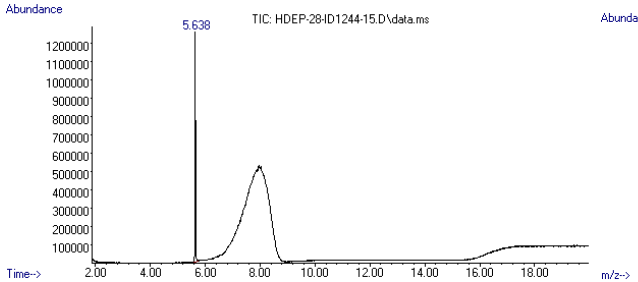
HDEP-28

ethylnaphtidate

HPLC-TOF
Impurities not detected
Formula: C19H23NO2
Exact mass: 297.1729
Mw: 297.398 g/mol
RESULTS: **AS EXPECTED**

NMR
Proposed structure **CONFIRMED**.
Compound is **pure** by NMR.

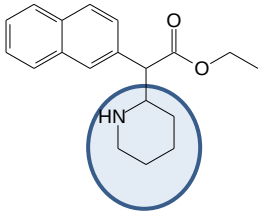
GC-MS (std. analytical conditions)



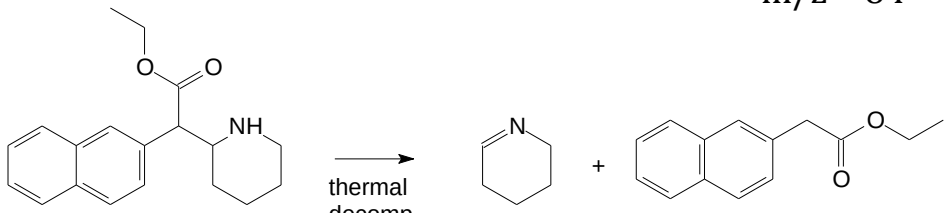
Chromatogram and MS obtained at our standard analytical conditions (inj. Port 280 st. C). Sharp and broad beak shows practically identical spectra. Both are missing of m/z = 84.

Fragmentation patteren does not fit!

We supposed that the compound HDEP-28 most probably decomposed in GC



m/z =84



$C_{19}H_{23}NO_2$
297.40
Ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl)acetate

C_5H_9N
83.13
2,3,4,5-tetrahydropyridine

$C_{14}H_{14}O_2$
214.26
2-Naphtalenacetic acid, ethyl ester

next step

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 st. C with decomposition (observed also visually).

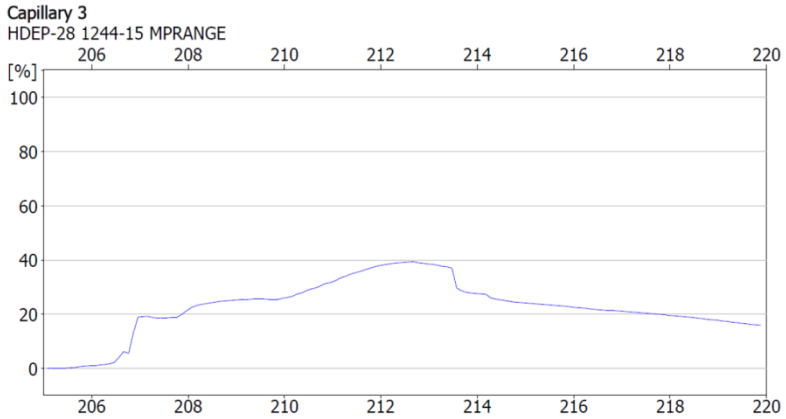
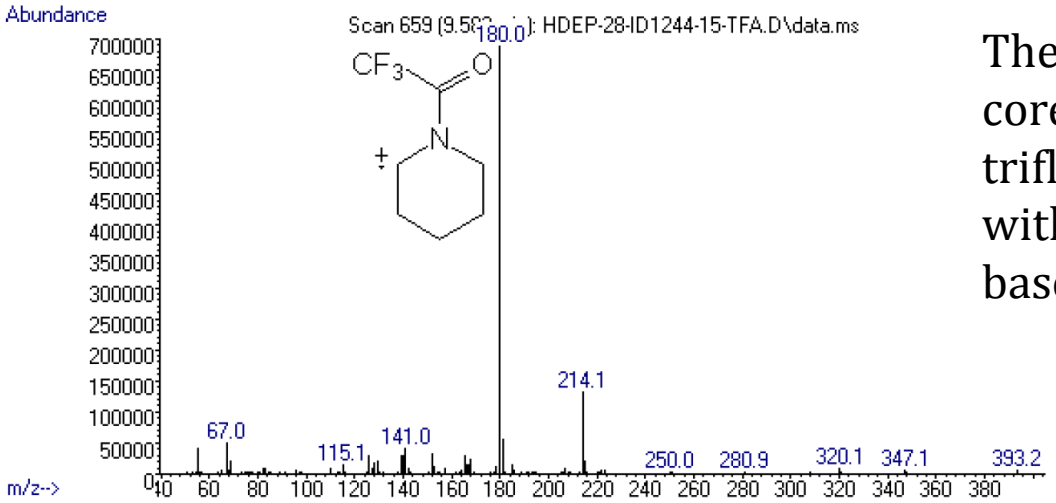


Figure 4: Melting curve for the sample purchased as HDEP-28.

Sample was derivatized by MBTFA (n-methyl-bis-trifluoroacetamide)

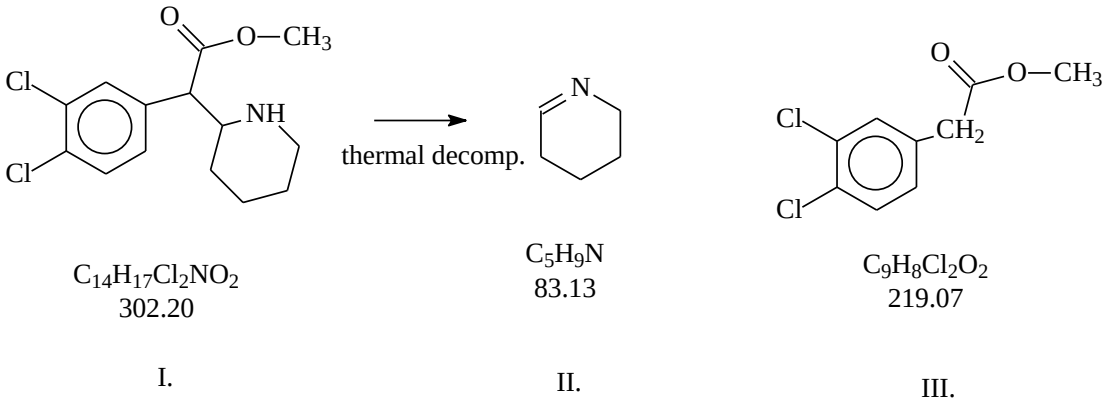


The obtained mass spectrum coresponds to trifluoroacetylderivative of HDEP-28, with molecular ion m/z 939 and a base peak at m/z 180.

MS spectra of thermal decomposition products are quite often reported as the spectra of non decomposed compounds (especially when substances were NMR confirmed).

FEW EXAMPLES:

a) 3, 4- CTMP (I) – the published EI-MS spectrum in Forensic Science International, 243 (2014), p 1-13, coresponds to thermal degradation product III



$C_{14}H_{17}Cl_2NO_2$
302.20

I.

C_5H_9N
83.13

II.

$C_9H_8Cl_2O_2$
219.07

III.

b) HDMP-28– the published EI-MS spectrum in EMCDDA EDND data base (2014) coresponds to thermal degradation product

c) 25C-NBOH – the publised EI MS in EMCDDA EDND data base (2016) corresponds to thermal degradation product 2C-C.

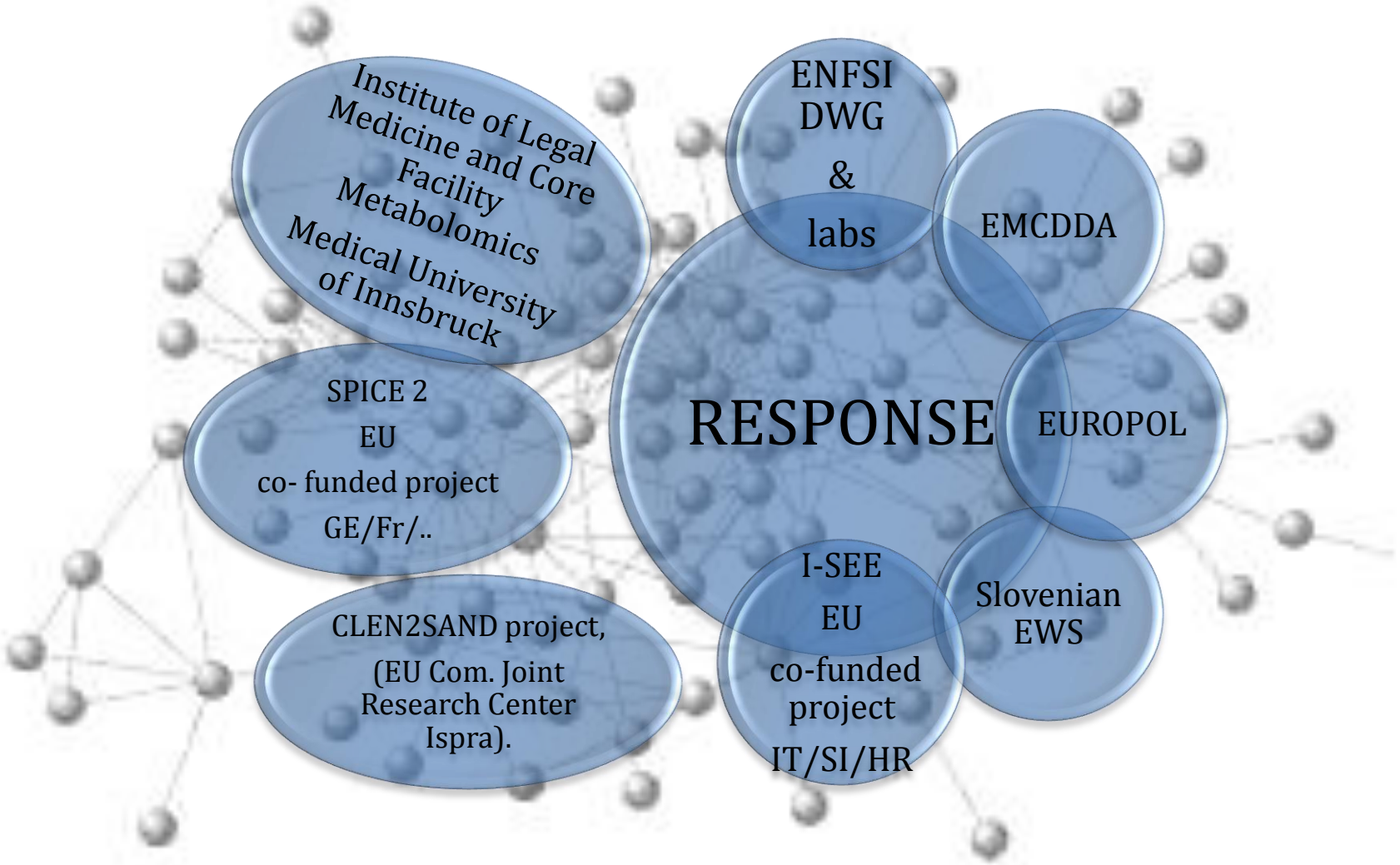
DATA BASES & "RESPONSE" CONTRIBUTIONs

- **RESPONSE PROJET WEB site (**info, tools, database and more)
  - **http://www.policija.si/eng/index.php/generalpolicedirectorate/1669-nfl-page-response**
- **RESPONSE project "Database", public open searchable along different parameters**

  http://www.policija.si/apps/nfl_response_web/seznam.php

- **FTIR database**(tool) in (close cooperation with ENFSI)
  - "Documents on FTIR spectra acquisition and interpretati" (joined ENFSI DWG –RESPONSE documents)
  - FTIR spectra from RESPONSE implemented
- **MS-ENFSI (**spectra from RESPONSE implemented)
- **EMCDDA - EDND** –reports & analytical data from RESPONSE - trough EWS

Networking


Institute of Legal Medicine and Core Facility Metabolomics Medical University of Innsbruck
ENFSI DWG & labs
EMCDDA
SPICE 2 EU co- funded project GE/Fr/..
RESPONSE
EUROPOL
CLEN2SAND project, (EU Com. Joint Research Center Ispra).
I-SEE EU co-funded project IT/SI/HR
Slovenian EWS

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Thank you!