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# Chemical characterizations and reporting of new psychoactive substances – RESPONSE project methodologies

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*The contents of this publication are the sole responsibility of the authors and can in no way be taken to reflect the views of the European Commission.*

Staff involved in chemical characterizations and reporting did a great job and is kindly acknowledged for their hard work and several thousand of characterizations and interpretations!

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## 1. Introduction

In recent years “internet-NPS/ legal highs” which market is expanding rapidly represent a “new” threat. Forensic and customs laboratories detect such new drugs when analyzing materials with GC-MS and/or other techniques, GC-IRD, LC-MS and FT-IR. The difficulty lies in their identification. In order to identify a new substance, either a reference material (CRM) is needed or modern laboratory but expensive techniques such as NMR and high resolution mass spectrometry (HRMS) are needed to perform a fast unambiguous identification of »unknown« compound. Lacking both (CRM and modern techniques) a majority of laboratories have to rely on comparison of spectra to an existing libraries (spectra repositories) – databases. Therefore, there is a clear need for more novel NPS spectra from different techniques to make identifying new drugs possible for all labs. CRMs at mg-level scale are expensive and analyses in each single laboratory are time consuming. With state-of-the-art methodology of forensic analyses the project aimed to identify numerous novel NPS, build a knowledge base and spectral databases and enhance NPS identifiaction capabilities in forensic, toxicological, customs and other research orientated laboratories. One should have in mind that: only when a substance is identified a risk assessment can be made and further actions taken by national and international stakeholders.

Another problem which is often underestimated is a rapid delivery, i. e. sharing of chemical data (spectra and other chem-informations) and/or substances to interested comunities. Therefore, chemical characterizations shall be followed by appropriate dissemination strategies.

In this document overview of the applied laboratory techniques is given and overall identifications and dissemination methodologies implemented in the RESPONSE project are highlighted.

For more information on the project deliverables and outputs please visit the RESPONSE project web site[1,2] and explore resources there:

http://www.policija.si/eng/index.php/generalpolicedirectorate/1669-nfl-page-response

## 2. Analytical Methods applied in the National forensic laboratory (Slovenia)

### 2.1 Gas chromatography coupled by mass spectrometry (GC-MS)

GC-MS (EI+ ionisation) is a routine technique in many laboratories. Identification is based on two parameters (retention time and mass spectrum). GC is effective way of separation of compounds in mixtures, while mass spectrometry and specific fragmentation patterns provide some structural elucidations clues, especially if good match is obtained in existing MS libraries. Anyhow, for novel NPS (where reference materials and MS spectra are not yet available) some additional techniques shall be implemented to elucidate/confirm the structure of compound in question.

In general, GC-MS is applicable for detection and identification of organic compounds if they are soluble/ extractable in appropriate solvent or solvent system. At applied instrumental parameters are evaporable in the GC-injection port (for some types of compounds evaporability can be improved by derivatization), thermally stabile (for some compounds better stability can be achieved by derivatization), chromatographable, which means that chromatographic peak will be observed at particular retention time, when compound elutes from column. Compounds should be MS detectable and give specific mass fragmentation pattern, after the molecules interact with electrons.

GC-MS have some limitations one shall keep in mind: it is not applicable for the compound form (salt/base) determination; it may be difficult to make the inferences (elucidation) about the isomeric forms of substances, when diastereomers[1] (e.g. ephedrine/pseudoephedrine) or structural isomers (positional isomers e.g. o-, m-, p- positions on benzene; cis-, trans- isomers; or chain isomers e.g. normal or isoalkanes) are in question, if under the applied chromatographic conditions, isomers are not chromatographically resolvable (i.e. identification without RT data). Enantiomers[2] cannot be chromatographically resolved (except on specially designed “chiral columns” and chiral derivatisation reagents) and cannot be differentiated by MS. The possibility for identification of some solvents, highly volatile or low molecular mass compounds is highly dependent on the chromatographic parameters (the injection port and temperature programming).

[1] diastereomers as well as structural isomers have different physical-chemical characteristic, therefore under appropriate chromatographic conditions they may be separated chromatographically. MS differentiation capability is dependent on particular molecular structure and also on the availability of »background studies« and special knowledge of the »expert«.

[2] enantiomers – have identical chemical and physical properties except for their ability to rotate plane-polarized light (+/-))

National forensic laboratory applied ISO-17023 flexible scope accredited methods (KEM-053: Identification of general unknown drugs, by products and admixes; and related methods) for NPS sample preparations, GC-MS analyses and reporting criteria.

Figure 1: Laboratory for the samples preparation

Figure 2: GC-MS laboratory (equipped with six GC-MS units with auto samplers (all Agilent). Four of them were applied for screening of NPS.


Figure 3: GC-MS unit with special sampling device (cofounded from RESPONSE project budget – applied for drugs profiling purposes only)

## 2.2 Fourier Transform Infrared with Attenuated Total Reflection sampling unit (FTIR-ATR)

FTIR-ATR is routinely utilized to harvest the information-rich mid-IR region (4000 to 400 cm–1) of the electromagnetic spectrum. The FT-IR ATR has become widespread in the recent years because of its simplicity and minimum sample preparation requirements. Solid and liquid samples can be analyses. When organic molecules are irradiated with light within this region, the radiation is absorbed and converted into molecular vibrations. Certain groups of atoms (functional groups) give characteristic peaks that occur at or near the same frequency regardless of the structure of the rest of the molecule. FTIR-ATR enables differentiation of salt and base forms of compounds, the positional-isomers of substances can be distinguished. Technique is sensitive to polymorph modifications of the same compound.

Spectra of unknown samples can be identified by searching a spectrum of interest against spectral libraries. The problem for identification of novel NPS is that the FTIR spectra are mostly not available yet. On many occasions samples of forensic interest are not pure, which can further complicate identifications or made them impossible.

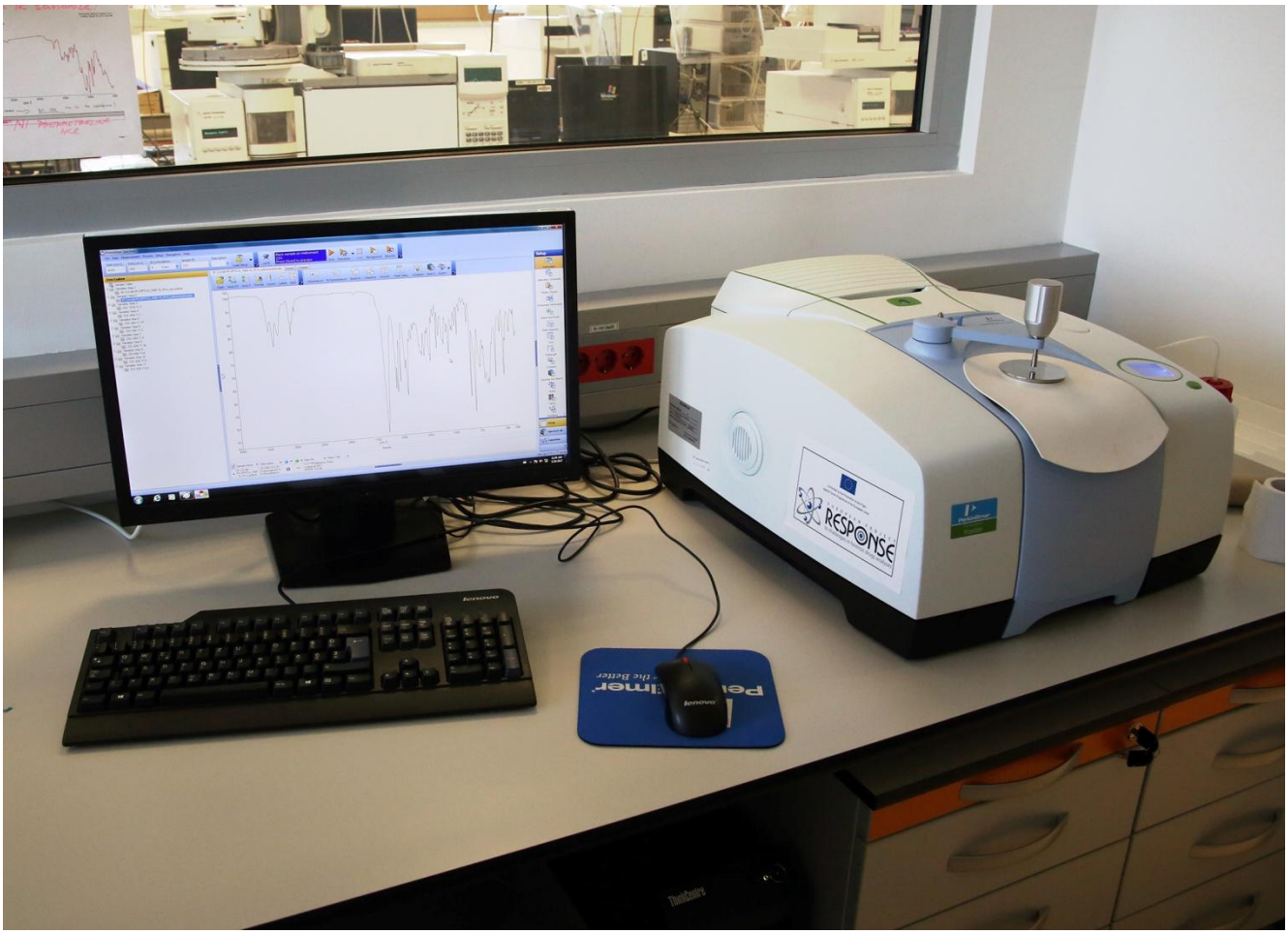


Figure 4: FTIR-ATR Perkin Elmer instrument (co-funded from the RESPONSE project budget – applied for profiling purposes)

## 2.3 Gas chromatography coupled by mass spectrometry and IR (solid phase) detector (GC-(MS)-IRD

A gas chromatograph (GC) coupled with an infrared detector (IRD) and also by mass spectrometer (MS) is additional solution to the problematic identification of compounds in complex mixtures. In GC different components of the sample are separated. A part of eluent goes to MS where mass spectrum is scanned and a second part of GC eluent is transferred to IR detector chamber (operating under vacuum) where eluent condenses (solid material is deposited) onto a slowly moving ZnSe disc cooled to $-30^0$ to $-40^0$C. ZnSe is an IR transparent material which enables generation of solid phase transmission spectra on material deposited. The IR light is directed through the disk just after deposition of the solid phase. The measurement range in NFL was 4000 $cm^{-1}$ to 650 $cm^{-1}$.

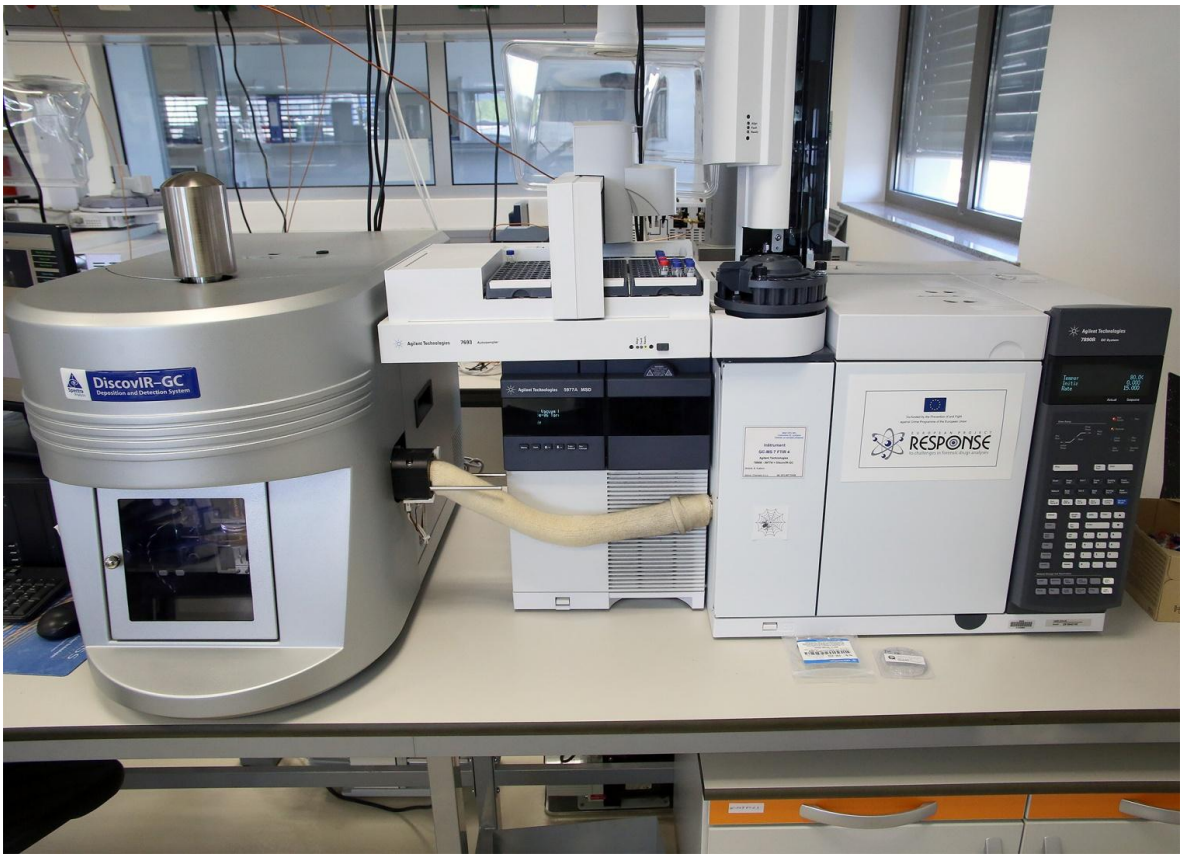


Figure 5: GC-MS-IRD instrument (co-funded from the RESPONSE project budget – applied for collecting of FTIR-condensed phase IR spectra after the chromatographic separation. MS spectra are scanned simultaneously)

## 2.4 High performance Liquid Chromatography coupled by Time of Flight Mass spectrometer (HPLC-TOF)

High performance liquid chromatography is a powerful tool in forensic chemistry. It has the ability to separate, the compounds that are present in any sample that can be dissolved in a liquid. By coupling HPLC and Time of Flight mass Spectrometer (TOF) with ESI (electro spray ionisation) technique the accurate molecular mass (monoisotopic mass) and empirical formula of investigated compound can be determined.

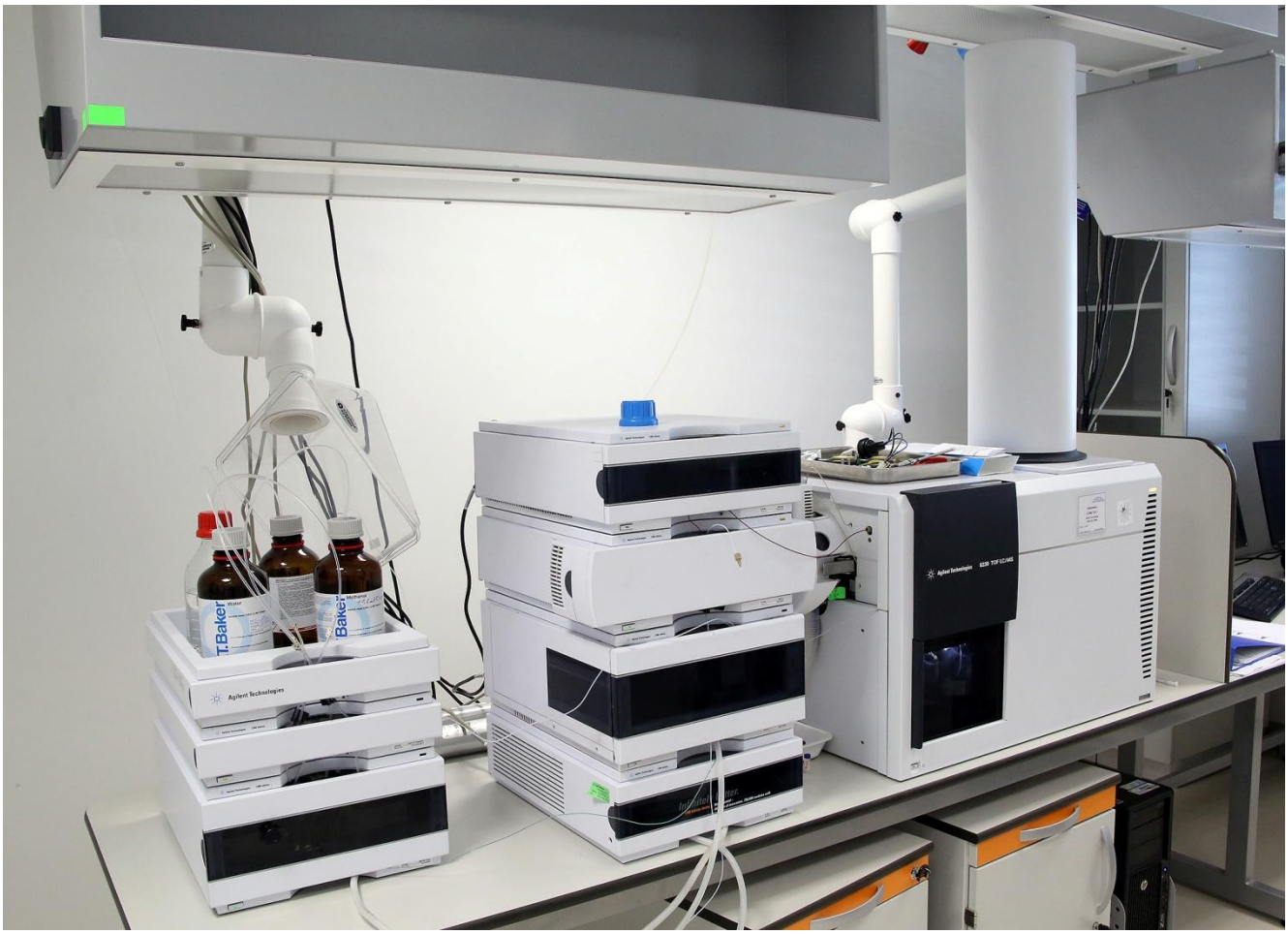


Figure 6: HPLC coupled by TOF-MS

## 2.5 Ion chromatography – anions (IC anions)

Ion chromatography (IC) is used for determination of anions (which can infer on salt form or traces of impurities from synthesis). The anions of interest are separated on the anion exchanger (separator) column and measured by conductivity. They are identified on the basis of retention time as compared to standards. The quantification is possible as well and was applied on few samples. The IC method covers the determination of inorganic anions (e.g. chloride, sulphate, phosphate, iodide, bromide...) as well as organic anions such us succinate, oxalate, tartrate, fumarate, etc…

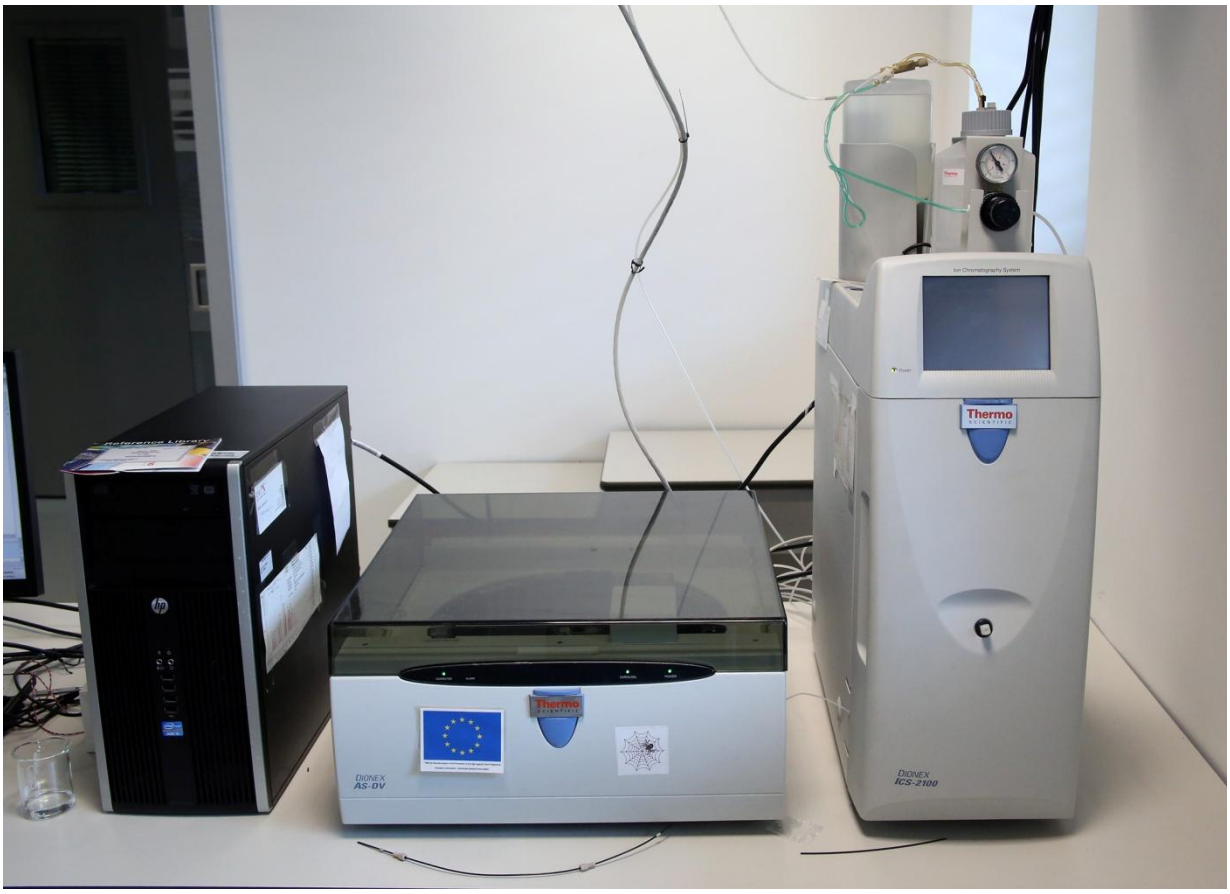


Figure 7: IC system (determination of anions)

## 2.6 Melting point measurements

Mettler Toledo MP 90 Melting Point System device was applied on as needed basis for accurate melting point and melting range measurements. Data can infer on purity and thermal stability of sample.


Figure 8: Mettler Toledo melting point measurement device

## 3. Nuclear Magnetic Resonance spectroscopy (NMR) at the Faculty of Chemistry and Chemical Technology, University of Ljubljana

A strategy for structural elucidation/confirmation of compounds by using modern NMR spectroscopy is divided into a couple of phases. Although an inspection of 1D 1H and 13C NMR spectra reveals most of the structural features, unequivocal determination of skeletal connectivity is deduced by combining data from both homonuclear (COSY) and heteronuclear (HSQC and HMBC) correlation spectroscopy. A combination of 1H and COSY (correlation spectroscopy) spectra assists in deducing structural fragments of the unknown compound. Spatial structure including stereochemistry of the molecule can be deduced by 1D NOE (nuclear Overhauser effect) or NOESY (nuclear Overhauser effect spectroscopy) spectrum where cross-peaks indicate through-space correlations between protons. The HSQC (heteronuclear single quantum coherence) spectrum assigns each proton to its directly attached carbon and can in principle eliminate the need for 1D 13C NMR edited experiments such as APT (attached proton test) or DEPT (distortionless enhancement by polarization transfer). Once structural fragments have been identified, they are combined to establish the overall skeletal connectivity by using the HMBC (heteronuclear multiple-bond correlation) experiment, which detects heteronuclear correlations over longer ranges of two to four bonds. The correlations can be transmitted through heteroatoms and quaternary carbon atoms. In addition to 1H–13C HMBC, 1H–15N HMBC is a powerful source of information for nitrogen containing compounds.

## 4. Chemical characterizations – methodlogy summarized

The main goal of the project was to identify numerous compounds and provide MS and FTIR spectral data on newly appearing or recently reported NPS and to report the findings to the customer, national and international stakeholders and other interested communities (focused on chemists). Several sources of materials have been applied (see Table 1). The selection of characterization methods depends on the sample type and information available about the sample (see Table 1). Chemical characterization strategy is schematically depicted on Figure 9. A part of the project was devoted also to presumptive tastings of NPSs which are described elsewhere.

Table 1: Types of material and analytical methods

| Type of the material | Analytical methods applied |
|---|---|
| Reference materials (RM)<br>(Different vendors ) | GC-MS, HPLC-TOF FTIR-ATR, GC-MS-FTIR-(condensed phase)<br>exceptionally: IC, NMR, melting point measurements |
| Test purchases<br>(from internet based vendors) | GC-MS, HPLC-TOF, FTIR-ATR, GC-(MS)-FTIR-(condensed phase), IC and NMR for "unknowns", i.e. novel NPS<br><br>exceptionally: melting point measurements to evaluate thermal stability and purity of compounds, head space sampling for determination of volatile ingredients |
| Seized samples<br>(Police/ Customs) | |
| Collected samples<br>(NGO – anonymous users, project partners, other contributors) | |

Sample preparation shall follow requirements of the methods applied taking into account specific sample characteristics (solubility in different solvents was studied, different extraction and/or derivatization procedures have been applied on as needed basis, etc..).

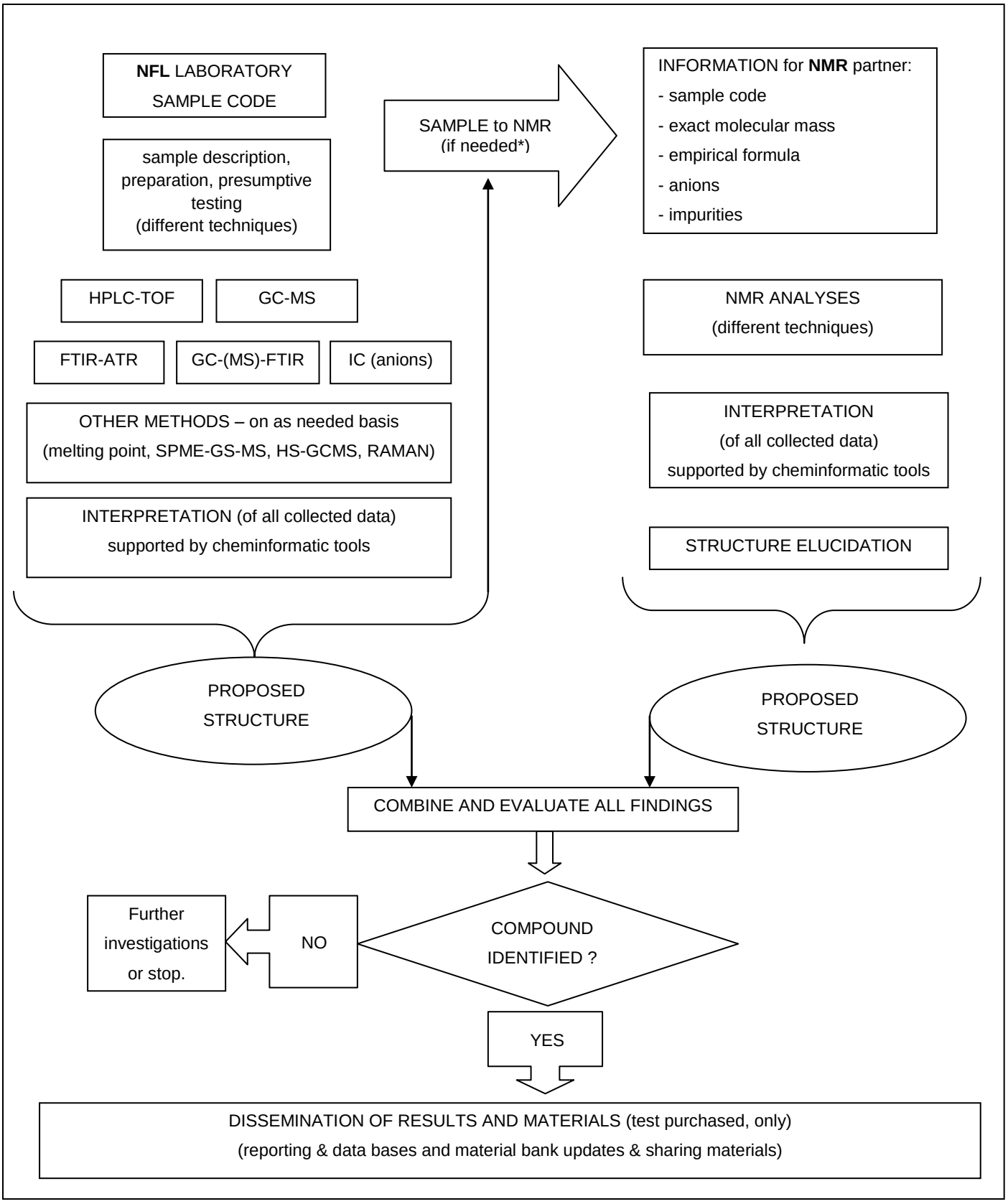


Figure 9: Schematic presentation of chemical characterizations strategy and outputs - *NMR was only applied for “unknowns” (seized, test purchased and some collected samples)

The instrumental characterizations usually started by GC-MS and HPLC-TOF followed by FTIR-ATR and GC-(MS)-FTIR. For pills, blotters and some complex mixtures we mostly only applied IR after chromatographic separation. Ion chromatography was mainly applied on sized, collected and test purchased samples. The goal was to define the anion part of sample (which is important for IR interpretations and can also affect NMR interpretations).

For most of the test purchased, seized and collected novel NPS samples the structure was confirmed or elucidated by NMR. The NMR partner only received the following data from NFL: sample code, exact mono-isotopic mass and proposed empirical formula, anions and info if impurities were detected in NFL.

For novel NPSs (unknowns) the interpretations of NFL and FKKT results were done by at minimum two experts, independently. Afterwards NFL and FKKT results were combined and compared. If all findings leaded to the same final decision on structure the identity of the compound was confirmed, otherwise some more research work followed. In few occasions the identity could not be confirmed.

In few occasions we detected errors in the reference materials identity or salt form data. Therefore, it is important that experts do not trust the reference materials vendors blindly!

### 4.1 Cheminformatic tools applied (short overview)

Several cheminformatic tools (in addition to those included into original instrumental software) were explored and applied at different stages of data interpretation. Some of them are listed below:

- MS spectra and FTIR interpretation and validation
  - Mass Frontier[3] version 7.0 (mass spectra fragmentation interpretation tool - for the given structure)
  - MS fragmentation tools and FTIR tools of KnowIAll software[4] (http://www.bio-rad.com/en-uk/category/products/spectroscopy-software

- Structure and structure descriptors generation and validation[5]
  - Marwin Sketch (free tool from ChemAxon)[6]; generates IUPAC name from structure and oposite, structure in .mol format file

  - OPSIN[7] (Open Parser for Systematic IUPAC nomenclature) – validation of generated structure from IUPAC name and InChI (IUPAC International Chemical Identifier) Key and string as well as SMILES (Simplified molecular-input line-entry system)
  - InChI Trust (InChI key and string)[8], generation and validation of InChI Key from .mol file of structure
- NMR predictors: as for example (http://www.nmrdb.org/about/

## 5. Dissemination of results and materials - methodology

Numerous new compounds have been characterized by means of several analytical methods listed previously. Procedures and some findings have been presented at conferences (public open presentations are available on the project website)[1]. Collected data (spectra and accompanying documentation) have been evaluated, organized systematically and relevant data shared with forensic community in EU and globally trough different communication channels.

The main communication channels and tools applied in the framework of the RESPONSE project are schematically explained on figure below (Figure 10).

One of the biggest challenges was how to share gathered knowledge and information effectively, i.e. in real time when possible. For this purposes three public open databases, managed directly by Chemistry department of NFL have been developed.

a) Drugs Monographs (NPS and related compounds) database[9]. Database description and gudelines for use are published.[10] (updated in real time)
b) Analyses of anonymously collected samples in Slovenia[11] (updated in real time)
c) Presumptive color tests[12] database (in future it will be updated only periodically- one or two times per year, if applicable).

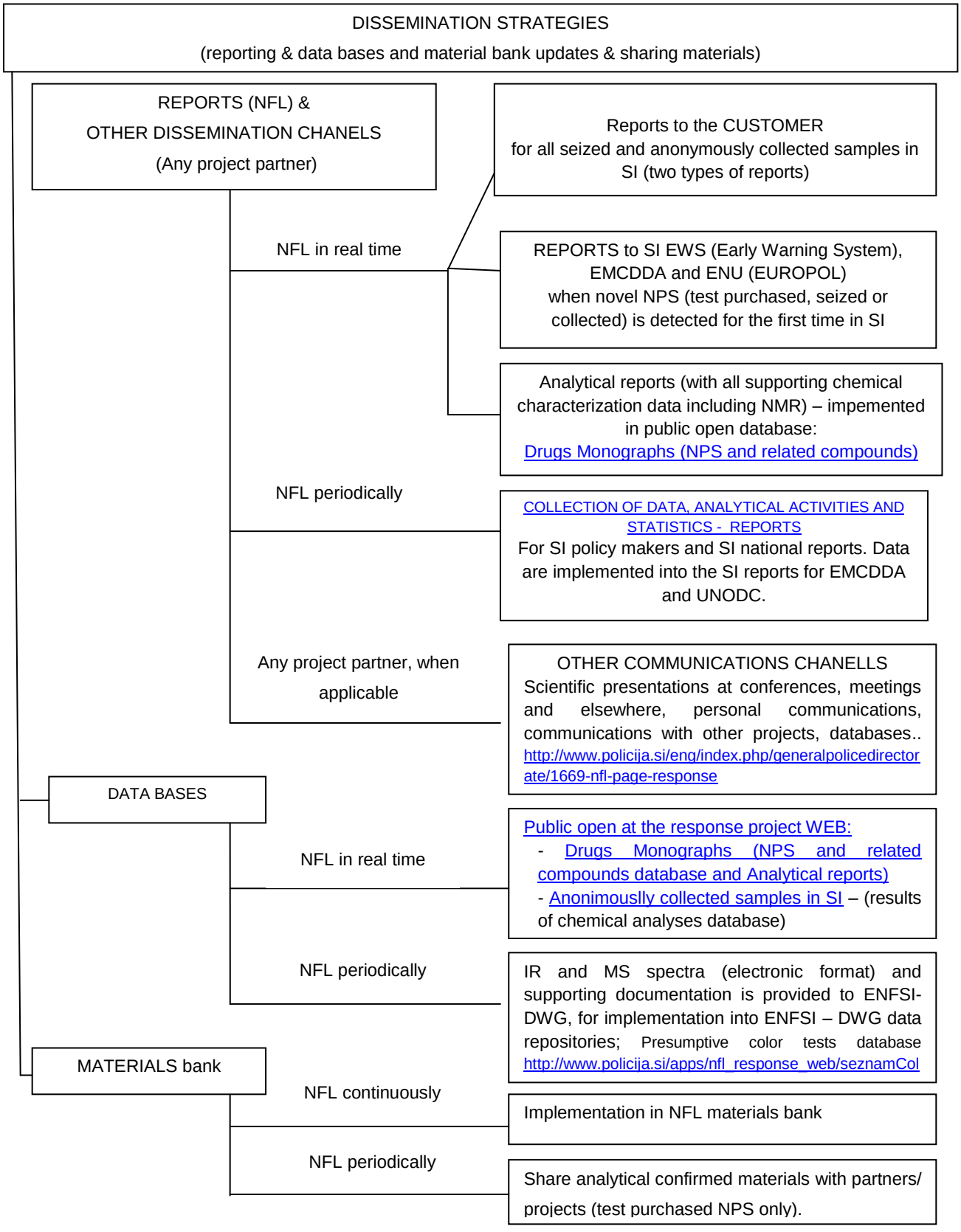


Figure 10: RESPONSE project dissemination channels and strategies

## 5.1 Some statistical figures of dissemination (period from 5th January 2015 - June 4th 2017)

More than 530 records were implemented into the RESPONSE project database until June 2017. To the great majority of records "Analytical report" [see APENDIX 1] is enclosed. Several contributors supported the project by reports or contributed some interesting NPS samples. Please see information in the database. We kindly acknowledge their support.

Around 400 MS spectra (raw data) from the project with supporting documentation and electronic structure data files were provided to ENFSI-DWG MS library manager until April 2017.

More than 400 FTIR-ATR and more than 300 IRD (condensed phase) spectra (raw data files) from the project with all supporting documentation were sent to ENFSI DWG IR library managers until April 2017.

More than 160 standard and several special reports to EMCDDA (see APPENDIX 2) and to SI EWS NFP (Slovenian Early Warning System – National Focal Point) and ENU (Europol National Unit) contact persons were issued. In addition materials for several special reports were prepared on request of EMCDDA and/or EUROPOL which NFL receives National units. Altogether RESPONSE project (NFL data) reported approximately 50 new (seized, collected or test purchased) NPS including those from special reports not opened to public. In addition many novel NPS have been reported until the project end.

In the first 5 months of 2017 NFL issued around 90 reports on anonymously collected samples (example in APPENDIX 3].

Around 300 records (and reports) on reference materials analyses have been implemented into database until end of the May 2017.

Until the end of 2016 analytical amounts of 142 different compounds (identification reference materials – from test purchased samples) with supporting analytical data were offered and distributed to 9 interested laboratories (altogether around 1000 sample unites were prepared and dispatched from NFLs Chemistry department).

## 6. Appendixes – examples of most common types of reports on single compound

Apendix 1: Analytical report on single NPS – chemical characterization data and supporting information - included into RESPONSE public open database “Drugs-monographs”

Apendix 2: Report to EMCDDA and SI EWS (first identification of novel NPS in SI and worldwide)

Appendix 3: Report on Anonymously collected samples (report to the request sender – some personal information of reporting officers is hidden);

Remark: Reports on seized NPS samples are not open to public.

## 7. References

[1] RESPONSE projec web site (English): http://www.policija.si/eng/index.php/generalpolicedirectorate/1669-nfl-page-response

[2] RESPONSE projec web site (Slovenian): http://www.policija.si/index.php/component/content/article/174-splono/77783-response

[3] https://www.thermofisher.com/order/catalog/product/IQLAAEGABOFAGUMZZZ

[4] http://www.bio-rad.com/en-uk/category/products/spectroscopy-software

[5] S. Klemenc, Substance descriptors: structure, common name, systematic name, systematic chemical identifiers and their consistency, (oral presentation: Presented by D. Saboti at ENFSI-DWG meeting, May **2017**, Linkoping, Sweden;http://www.policija.si/eng/images/stories/GPUNFL/PDF/2017/ENFSI-DWG_Meeting2017_SubstancDescriptor.pdf

[6] https://www.chemaxon.com/

[7] (http://opsin.ch.cam.ac.uk/)

[8] http://www.inchi-trust.org/

[9] RESPONSE project Drugs Monographs (NPS and related compounds) database: http://www.policija.si/apps/nfl_response_web/seznam.php

[10] RESPONSE PROJECT DATABASE (NPS AND RELATED COMPOUNDS); DESCRIPTION AND GUIDELINES FOR USE: http://www.policija.si/eng/images/stories/GPUNFL/PDF/DrugsMonographsDatabase_DescriptionAndGuidelines.pdf

[11] Analize anonimno zbranih vzorce v Sloveniji (Slovenian: http://www.policija.si/apps/nfl_response_web/seznamVzorci.php

[12] Presumptive Color Tests: http://www.policija.si/apps/nfl_response_web/seznamColors.php?lang=eng

# APPENDIXES ATTACHED

Apendix 1: Analytical report on single NPS – chemical characterization data and supporting information - included into RESPONSE public open database “Drugs-monographs”

Apendix 2: Report to EMCDDA and SI EWS (first identification of novel NPS in SI and worldwide)

Appendix 3: Report on Anonymously collected samples (report to the request sender – some personal information of reporting officers is hidden)

EUROPEAN PROJECT RESPONSE to challenges in forensic drugs analyses

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

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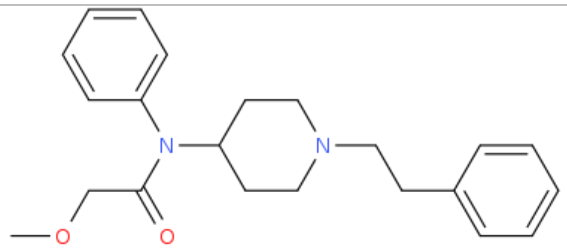
# ANALYTICAL REPORT[1]

## Methoxyacetyl-F (C22H28N2O2)

**2-methoxy-N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide**

Remark – other NPS detected: none

| | |
|---|---|
| Sample ID: | 1733-16 |
| Sample description: | powder - brown |
| Sample type: | test purchase /RESPONSE -purchasing |
| Date of sample receipt (M/D/Y): | 11/14/2016 |
| Date of entry (M/D/Y) into NFL database: | 12/2/2016 |
| Report updates (if any) will be published here: | http://www.policija.si/apps/nfl_response_web/seznam.php |

| | |
|---|---|
| Substance identified - structure[2] (base form) |  |
| Systematic name | 2-methoxy-N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide |
| Other names | Methoxy-AcF, Methoxyacetyl fentanyl |
| Formula (per base form) | C22H28N2O2 |
| $M_w$ (g/mol) | 352.48 |
| Salt form/anions detected | HCl |
| StdInChIKey | SADNVKRDSWWFTK-UHFFFAOYSA-N |
| Compound Class | Opioids |
| Other NPS detected | none |
| Add.info (purity..) | pure by HPLC-TOF, some minor impurities by NMR,GC-MS ans GCMS-IR (possibly partial thermal degradation) |

[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) |
|---|---|
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## **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 6.1 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

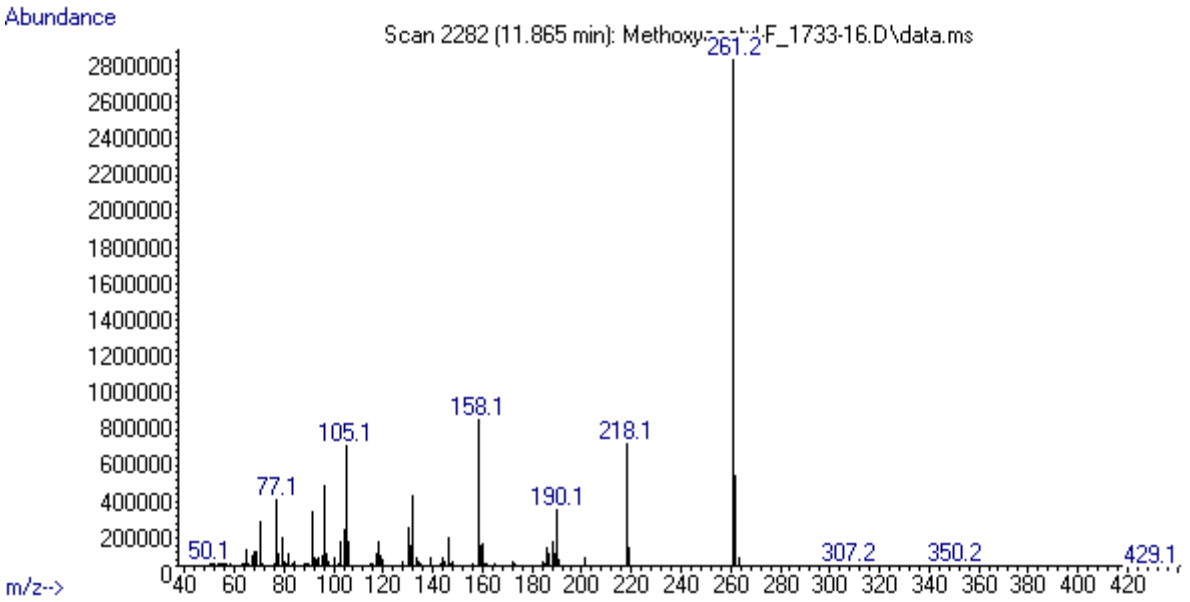
## Supporting information

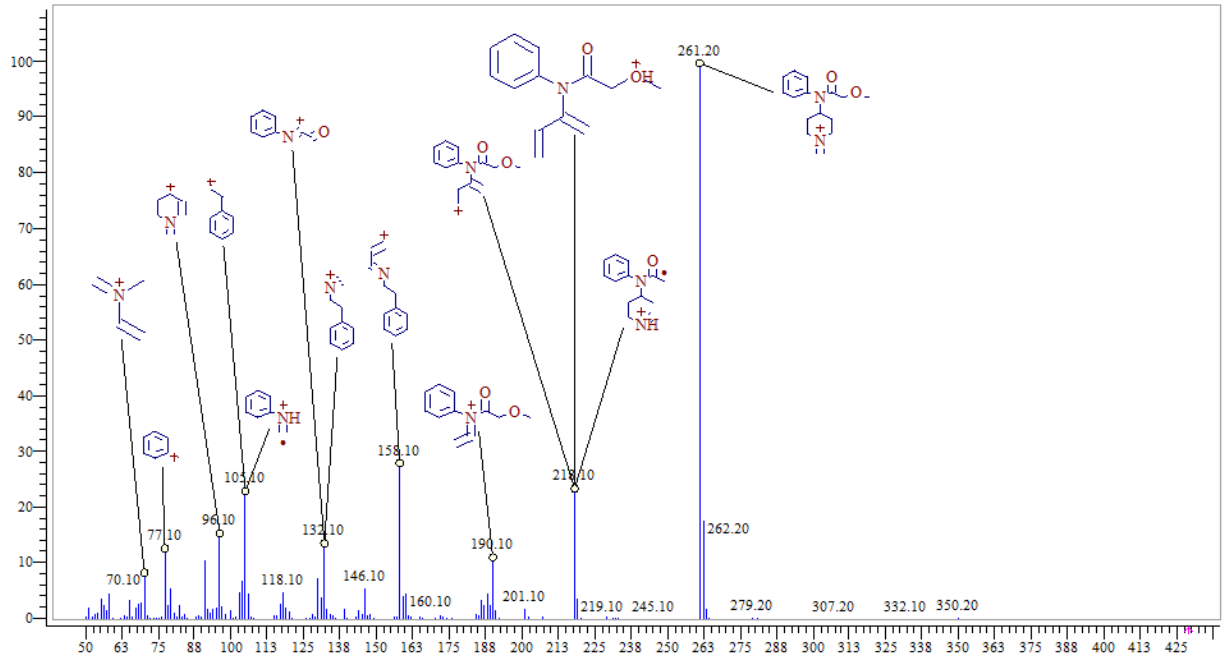
| Solubility in | result/remark |
|---|---|
| $CH_2Cl_2$ | soluble |
| MeOH | soluble |
| $H_2O$ | soluble |

| Analytical technique: | applied | remarks |
|---|---|---|
| GC-MS (EI ionization) | + | NFL GC-RT (min): 11.87<br>BP(1): 261; BP(2): 158,BP(3) :218, |
| HPLC-TOF | + | Exact mass (theoretical): 352.2151;<br>measured value Δppm:-0.08;<br>formula:C22H28N2O2 |
| FTIR-ATR | + | direct measurement (sample as received) |
| FTIR (condensed phase) always as base form | + | |
| IC (anions) | + | |
| NMR (in FKKT) | + | |
| validation | | |
| other | | |

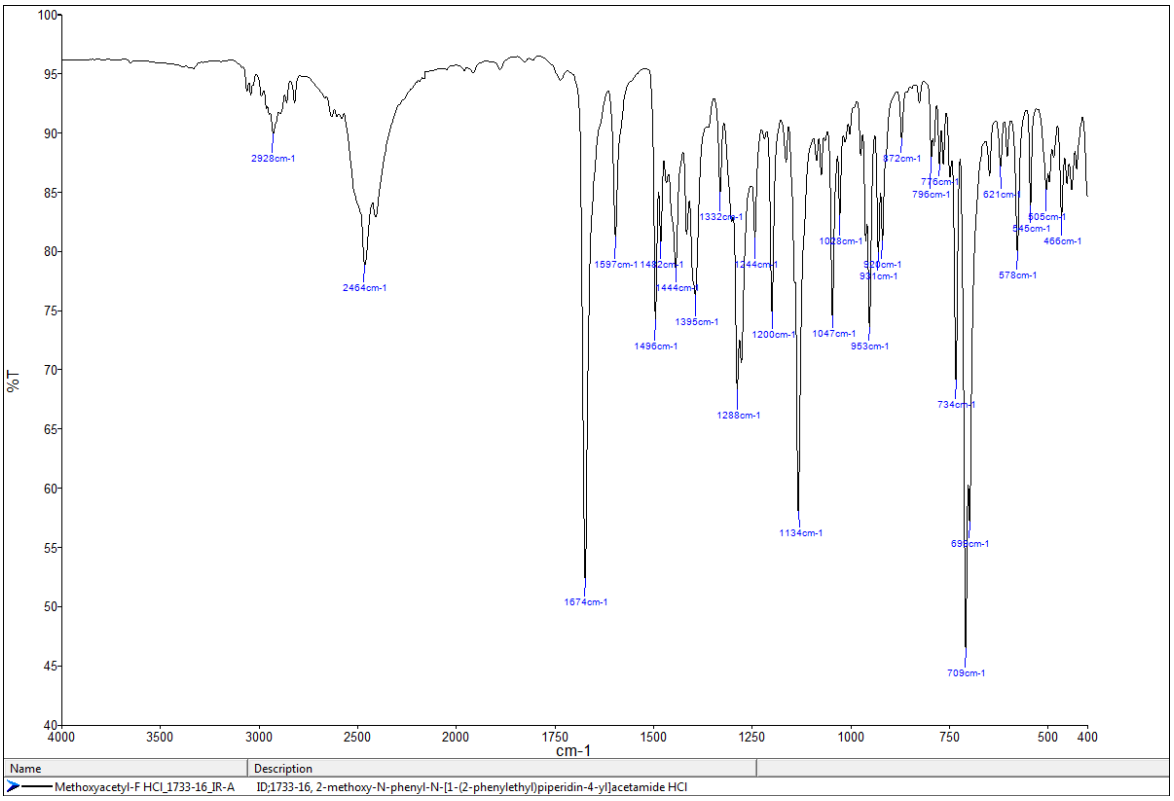
## ANALYTICAL RESULTS

MS (EI)

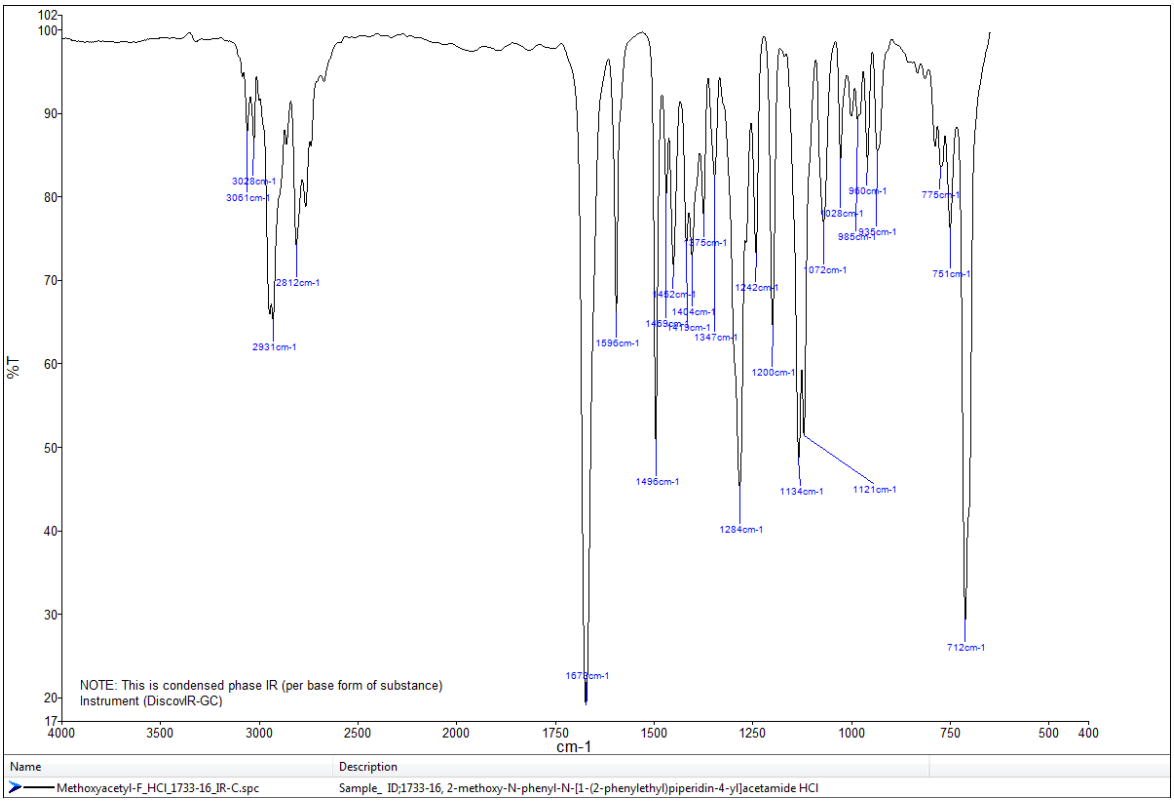


FTIR-ATR - direct measurement (sample as received)




IR (condensed phase – after chromatographic separation)



# TOF REPORT

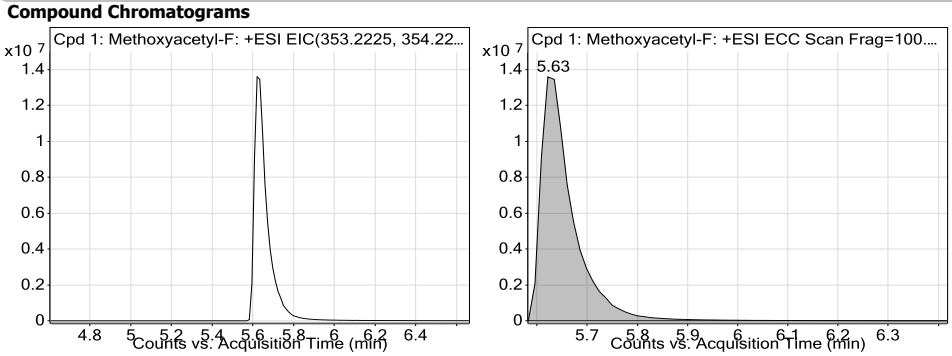
| | | | |
|---|---|---|---|
| **Data File** | Methxyacetyl-F_1733-16.d | **Sample Name** | ID_1733-16 |
| **Sample Type** | Sample | **Position** | P1-C4 |
| **Instrument Name** | 6230B TOF LC-MS | **User Name** | TG |
| **Acq Method** | general-10_10_2016-XDB-C18-ESI-poz-soft.m | **Acquired Time** | 11/16/2016 1:01:33 PM |
| **IRM Calibration Status** | Success | **DA Method** | Drugs_NFL.m |
| **Comment** | extract in MeOH | | |

**Compound Table**

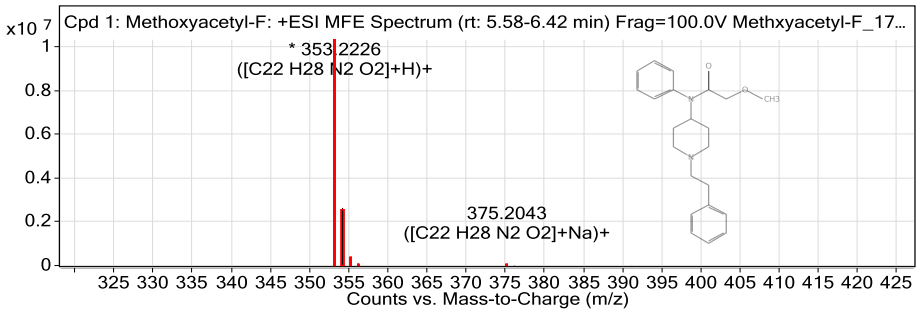
| Label | Compound Name | MFG Formula | Obs. RT | Obs. Mass |
|---|---|---|---|---|
| Cpd 1: Methoxyacetyl-F | Methoxyacetyl-F | C22 H28 N2 O2 | 5.63 | 352.2151 |

| Name | *Obs. m/z* | Obs. RT | Obs. Mass | DB RT | DB Formula | DB Mass | DB Mass Error (ppm) |
|---|---|---|---|---|---|---|---|
| **Methoxyacetyl-F** | 353.2226 | 5.63 | 352.2151 | 5.63 | C22 H28 N2 O2 | 352.2151 | -0.08 |

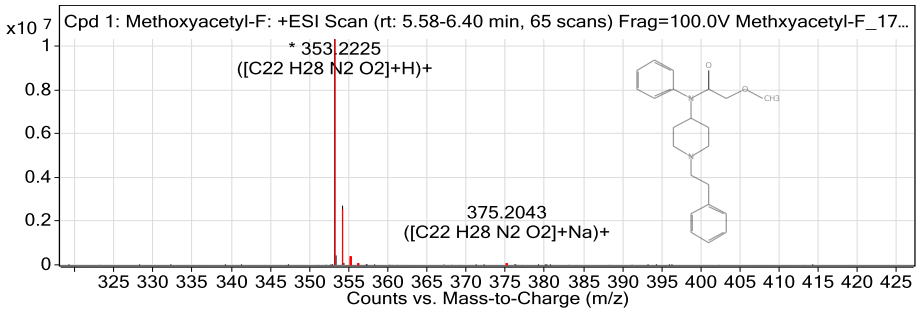
**Compound Chromatograms**




MFE MS Zoomed Spectrum




MS Zoomed Spectrum




**MS Spectrum Peak List**

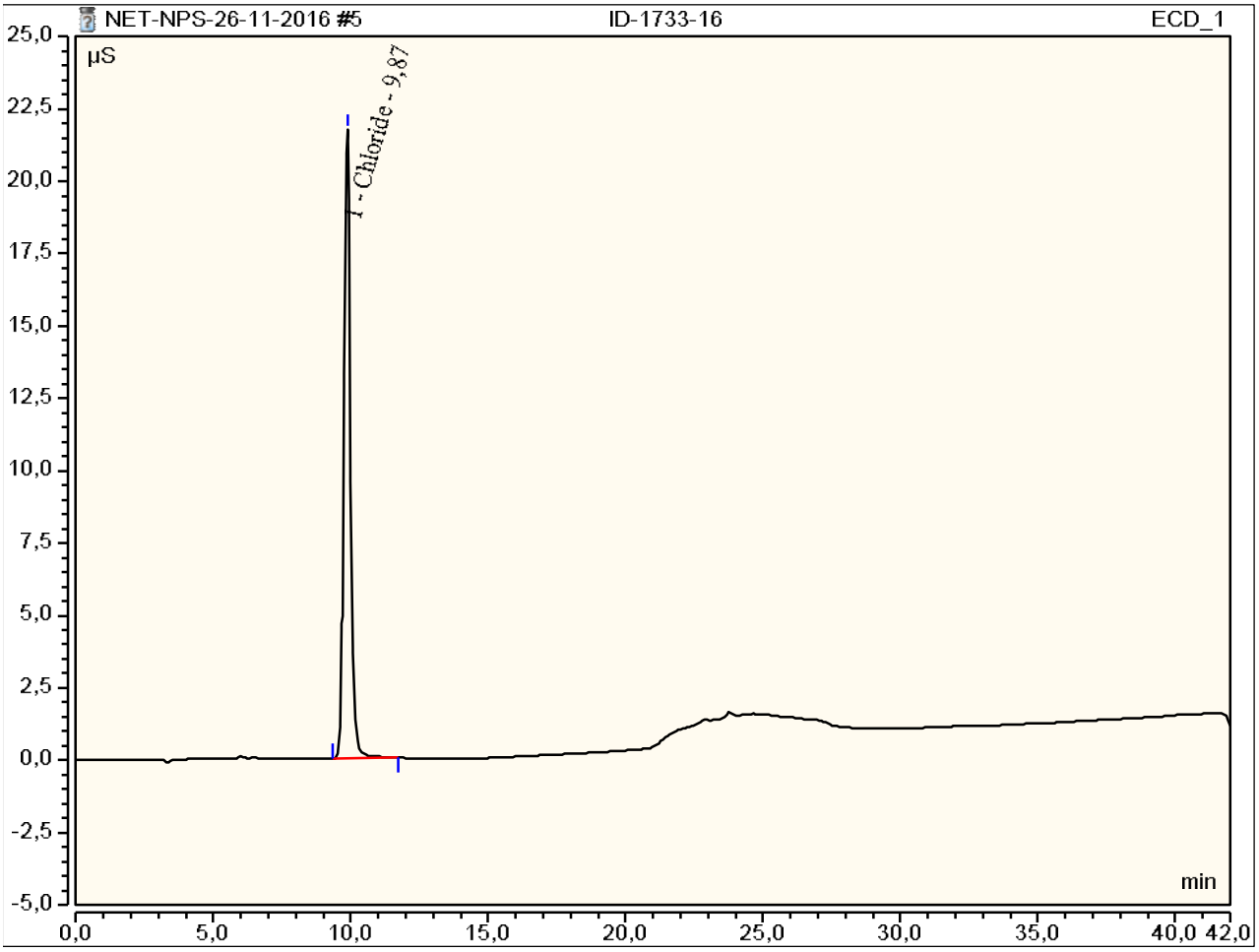
| *Obs. m/z* | Charge | Abund | Formula | Ion/Isotope |
|---|---|---|---|---|
| 353.2226 | 1 | 10316578 | C22 H28 N2 O2 | (M+H)+ |
| 354.2259 | 1 | 2630628.69 | C22 H28 N2 O2 | (M+H)+ |
| 355.2292 | 1 | 319413.95 | C22 H28 N2 O2 | (M+H)+ |
| 356.2315 | 1 | 30690.91 | C22 H28 N2 O2 | (M+H)+ |
| 357.2338 | 1 | 2761.22 | C22 H28 N2 O2 | (M+H)+ |
| 375.2043 | 1 | 64698.14 | C22 H28 N2 O2 | (M+Na)+ |
| 376.2075 | 1 | 15631.84 | C22 H28 N2 O2 | (M+Na)+ |
| 377.2101 | 1 | 2181.4 | C22 H28 N2 O2 | (M+Na)+ |
| 391.1788 | 1 | 4936.35 | C22 H28 N2 O2 | (M+K)+ |
| 392.1832 | 1 | 1349.18 | C22 H28 N2 O2 | (M+K)+ |

--- End Of Report ---

# Peak Integration Report

| | | | |
|---|---|---|---|
| *Sample Name:* | **ID-1733-16** | *Inj. Vol.:* | **25,00** |
| *Injection Type:* | **Unknown** | *Dilution Factor:* | **1,0000** |
| *Program:* | **ANIONI** | *Operator:* | **kemija** |
| *Inj. Date / Time:* | **16-nov-2016 / 15:34** | *Run Time:* | **42,00** |

| No. | Time min | Peak Name | Peak Type | Area µS*min | Height µS | Amount mg/L |
|---|---|---|---|---|---|---|
| 1,00 | 9,87 | **Chloride** | BMB | 6,07 | 21,75 | n.a. |
| | | **TOTAL:** | | 6,07 | 21,75 | 0,00 |



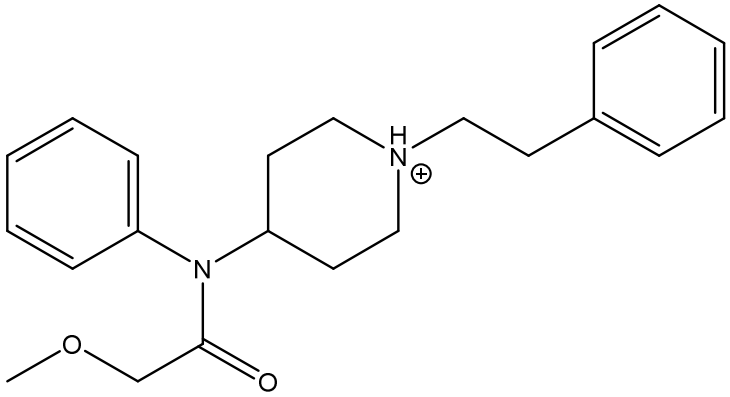
University
*of Ljubljana*

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*

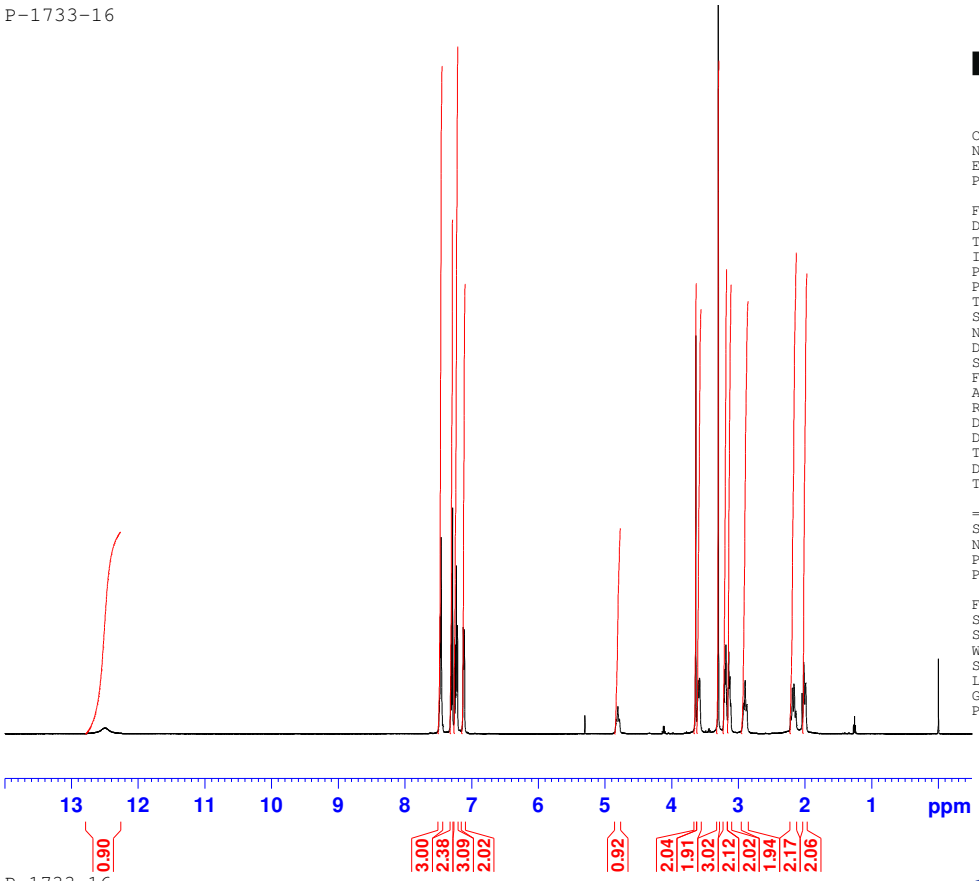
Co-funded by the Prevention of and Fight against Crime Programme of the European Union

# REPORT

| | |
|---|---|
| Sample ID: | **1733-16** |
| Our notebook code: | P-1733-16 |
| 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–$^{15}$N *gs*-HMBC. |
| Proposed structure: |  |
| Chemical name: | 4-(2-methoxy-*N*-phenylacetamido)-1-phenethylpiperidin-1-ium cation |
| Comments: | - Structure elucidation based on 1D and 2D NMR spectra<br>- Sample contains some minor impurities as evident 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: | November 29, 2016 |

*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-1733-16



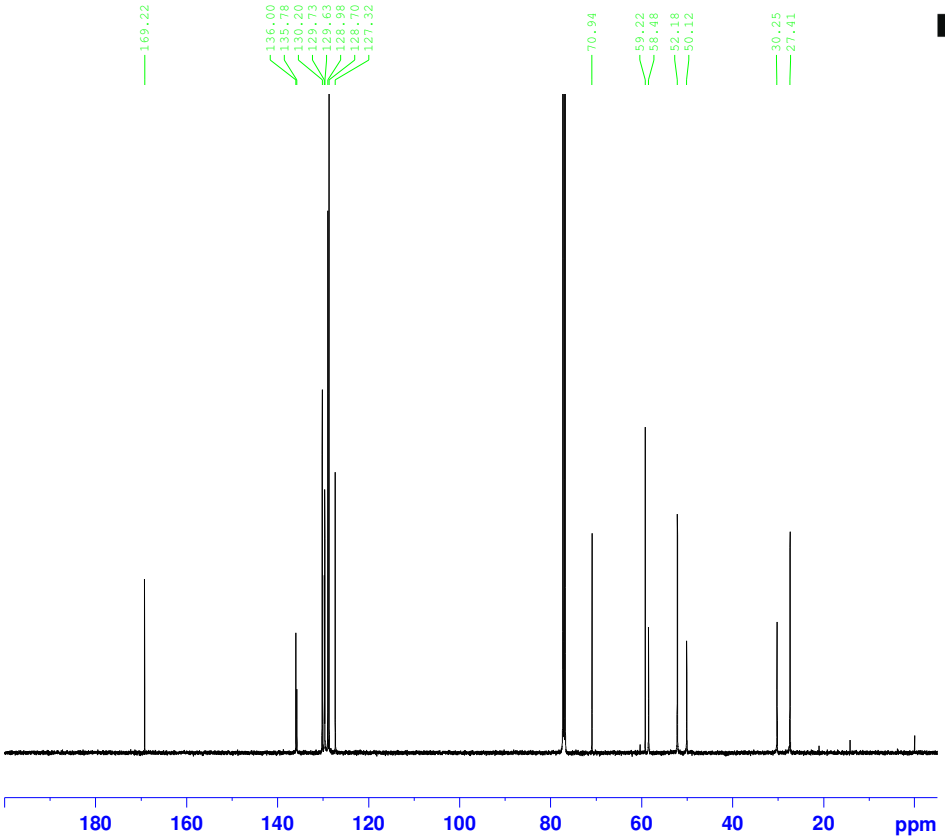

Current Data Parameters
NAME P-1733-16
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20161126
Time 17.33
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2768500 sec
RG 71.8
DW 50.000 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 8.60 usec
PLW1 26.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

P-1733-16




Current Data Parameters
NAME P-1733-16
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20161126
Time 20.13
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 1.1010048 sec
RG 2050
DW 16.800 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 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 125.7577887 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

**REPORTING FORM ON NEW PSYCHOACTIVE DRUG**

In accordance with Council Decision 2005/387/JHA of 10 May 2005 on information exchange, risk assessment and control of new psychoactive substances.

This section should be filled in by Europol or EMCDDA

Transmitted by Europol ☐ Transmitted by EMCDDA ☐
Ref. nº: Date of transmission:

The following sections should be filled by the Europol National Units (ENU) or REITOX National Focal Points (NFP) based on the information available and their respective competences

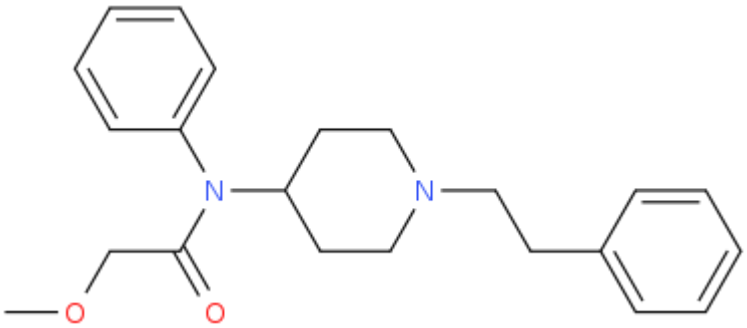
1. Member State: SI

Ref. n°:**315-24/2009/248 (ID 1733-16)**
Date: 2. 12. 2016

Reporting authority:

ENU ☐ REITOX ☒

2. Chemical name: 2-methoxy-N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide




Mw (g/mol) per base form: 352,478
Formula (per base form): C22H28N2O2
Other name(s): Methoxy-AF, Methoxyacetyl-F

Street name(s):

3. Source of information (fill one or more as appropriate)

Seizure(s) ☐ Specify amount (weight, number of tablets, etc.):

Seizing authority:

Date: Place:

Biological sample(s)[1] ☐ Specify type:

Identifying authority:

Date: Place:

[1] Biological (human) samples e.g. body fluids (urine, blood), tissues, hair, etc.

Collected sample(s)[2] ☒ Specify amount (weight, number of tablets, etc): 5 g

Collecting authority: National Forensic Laboratory – test purchase performed in the frame of EU co-funded project RESPONSE ( JUST/2013/ISEC/DRUGS/AG/6413)

Sample was shipped from China.

Date: 14/11/2016 Place: Ljubljana

Other substances present (if more than one case, specify for which one):

Psychoactive ingredients:

Other ingredients:

4. Physical description (in case of seizure/collection)

Form: powder ☒ tablet ☐ capsule ☐ liquid ☐ other (specify):

Colour: brown

For dosage unit: weight: diameter: shape: logo/markings:

5. Circumstances: production ☐ trafficking ☐ distribution ☐ use ☐

6. Price: retail (per dosage unit): wholesale:

7. Chemical precursors:

8. Patterns of use:

9. Other possible uses[3]:

10. Effects in man

Objectively observed:

Subjective (described by users):

11. Context of use

User group(s):

Setting(s):

Availability at consumer level:

12. Indication on possible risks

Health (individual):

Public health:

[2] Actively collected by drug monitoring systems for monitoring or research purposes
[3] For example, for medical, industrial, ritual, cosmetic, etc., purposes

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Social: | | | | | | | |
| 13. | In case of production: large-scale ☐ | | small scale | ☐ | unknown | ☐ | |
| | Has any form of organised crime been detected: | | yes | ☐ | no | ☐ | |
| 14. | In case of trafficking: | large scale ☐ | small scale | ☐ | unknown | ☐ | |
| | | national ☐ | international | ☐ | | | |
| | Has any form of organised crime been detected: | | yes | ☐ | no | ☐ | |
| 15. | In case of distribution: large-scale ☐ | | small scale | ☐ | unknown | ☐ | |
| | Has any form of organised crime been detected: | | yes | ☐ | no | ☐ | |

Sample was identified in SI National Forensic Laboratory (GC-MS, HPLC-TOF, FTIR-ATR, FTIR-condensed phase and Ion Chromatography). Structure was confirmed by NMR at the Faculty of Chemistry and Chemical technology.

Analytical results are published here:

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

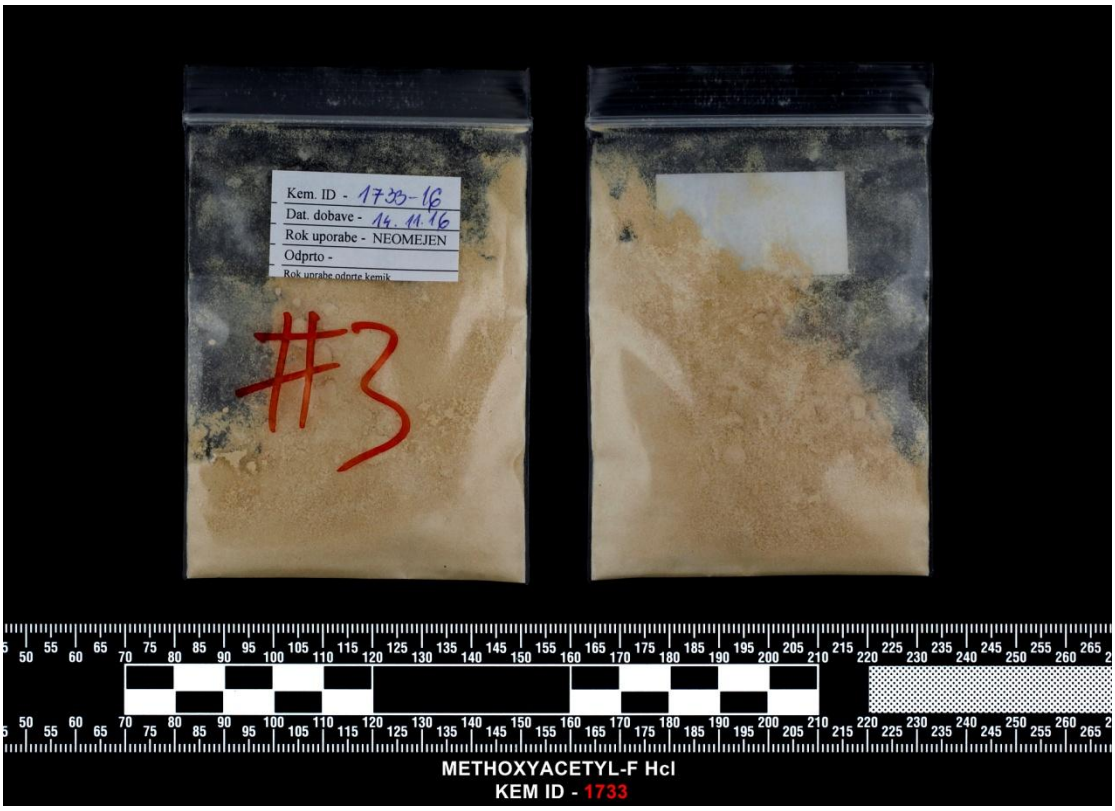



Photo: National Forensic Laboratory

REPUBLIKA SLOVENIJA
MINISTRSTVO ZA NOTRANJE ZADEVE
POLICIJA
Generalna policijska uprava
Nacionalni forenzični laboratorij
Vodovodna 95, 1000 Ljubljana

Co-funded by the Prevention of and Fight against Crime Programme of the European Union
Grant agreement no: JUST/2013/ISEC/DRUGS/AG/6413

text delited

(2201-04)

Številka: 233-2102/2017 /2 (2P502-12)
Datum: 11/05/2017

T: 01 428 44 93
F: 01 428 49 86
E: nfl@policija.si

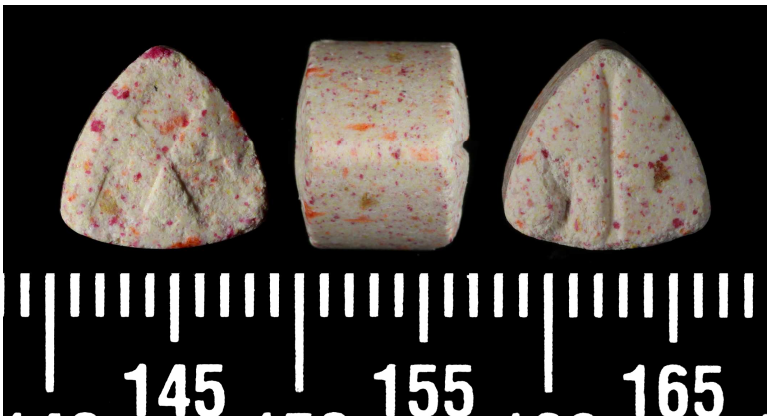
**ZADEVA: Anonimno testiranje vzorca – analizno poročilo**

Zveza: dopis št. 2312-19/2017/132 (2201-04) z dne 04/05/2017

| | |
|---|---|
| Datum prejema v NFL | 08/05/2017 |
| Vrečka kontrolna št. | 10204 |
| Vzorec - oznaka | 62 |

| **VZOREC - OPIS** | |
|---|---|
| osnovni opis vzorca | tableta |
| dodatni opis | umazano bela trikotna bikonveksna tableta z logom Mitsubishi in z razdelilno zarezo, s stranico 8,0 mm in debeline 6,8 mm (Slika 1) |
| količina<br>masa/ volumen/ število | 319 mg |

| **REZULTATI ANALIZ** | spojina | opomba |
|---|---|---|
| glavna aktivna sestavina (NFL) | etilon | |
| spremljajoče aktivne sestavine (NFL) | kofein, lidokain | |
| druge sestavine | nd | |
| komentar (NFL) | prevladujoča komponenta je kofein | |




**Slika 1: Slika tablete iz treh strani, sestavljena iz treh fotografij**

Vzorce smo:

poslali v hrambo ☐; porabili ☐ in uničili vrečko ZM; obdržali v zbirki NFL ☒, vrečko ZM pa uničili

Poročilo podal/a: signature delited

signature delited

prejemniki:
- naslovnik