SCREENING OF RECENTLY SEIZED NEW PSYCHOACTIVE SUBSTANCES IN URINE OF POTENTIAL CONSUMERS AND URBAN WASTEWATER

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Alberto Celma[1*], Lubertus Bijlsma[1], Juan V. Sancho[1], Félix Hernández[1], Mario Joao Dias[2], Susana Simões[2], Noelia Salgueiro[3], Sara Castiglioni[3], Ettore Zuccato[3]

[1] Research Institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain. *E-mail: alberto.celma@uji.es*.
[2] Instituto Nacional de Medicina Legal e Ciencias Forenses (INMLCF), Lisbon, Portugal.
[3] IRCCS-Istituto di Recerche Farmacologiche "Mario Negri", Department of Environmental Health Sciences, Milan, Italy.

INTRODUCTION

New Psychoactive Substances (NPS) are constantly being developed as legal substitution of traditional drugs of abuse. Although the traditional drugs maintain popular, new drugs are regularly introduced, changing the drug market ceaselessly [1]. Monitoring NPS and its consumption is challenging as these compounds are normally missed in routine drug analysis, users do often not exactly know what they consume and information available is limited of what is being sold. Furthermore, very little information exists on metabolism of these newly introduced NPS [2]. This highlights the need of applying a strategic workflow making use of modern analytical techniques to face this novel public health safety challenge. The workflow presented in this work consists of three stages: i) The creation of a database with around 200 NPS, including NPS recently reported to the National Early Warning Systems (EWS) of Spain and Italy and the EWS of the European Monitoring Center for Drugs and Drug Addiction (EMCDDA). ii) The collection of urine samples of individuals suspected of drug consumption, pooled urine samples from festivals and urban wastewater samples. iii) The screening of NPS using ultra-high performance liquid chromatography coupled to high resolution mass spectrometry with a hybrid quadrupole time-of-flight mass analyzer.

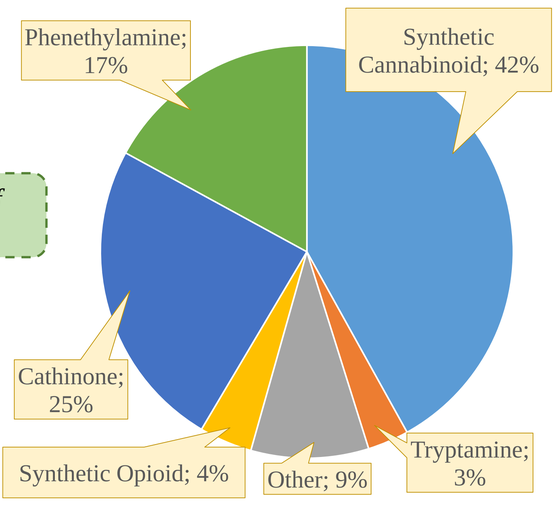
Although the database is under constant development, including NPS reported to be present in wastewaters around Europe, new compounds appearing in the market, as well as metabolites reported in the literature, the workflow applied allowed the detection of several unchanged NPS in urine and wastewater samples, which indicates that consumption of some of these compounds is a reality. The absence of reference standards for several NPS found, as well as for some NPS metabolites, made it unfeasible the full confirmation of some compounds in the urine/wastewater samples analyzed. However, accurate-mass full-spectrum data provided by HRMS allowed their detection / identification with high degree of reliability. The subsequent acquisition of reference standards, when available, will allow the unequivocal confirmation of their identity.

1) Database creation

Seizures
Notifications
Clinical & Forensic cases

EMCDDA
UNODC
EWS from Spain and Italy

Database of ***217 NPS***



2) Sample collection

- ✓ Collection of **Urine samples** of potential consumers:
  - Emergency Rooms and Drug Addiction Treatment Departments: individual urine samples from suspect consumers
  - Music festivals:
    - Pooled urine samples from volunteers
    - Pooled urine samples from portable toilets
- ✓ Collection of **Urban Wastewater**:
  - Pooled Urban Wastewater collected during local festivities


> 900 samples from 6 hospitals


37 samples from 4 music festivals


9 wastewater samples

3) Screening of NPS

SAMPLE TREATMENT

Individual urines from Hospitals	Pooled urines from Music Festivals	Urban Wastewater
❑ Hydrolysis	❑ Hydrolysis ❑ HLB SPE ❑ MCX SPE	❑ HLB SPE ❑ MCX SPE

1.25 mL urine sample → **Deglucuronidation** (**β-glucuronidase** from E.Coli) pH 7, 55ºC for 1 hour → **LC-QTOF MS**

25 mL sample +75mL Milli-Q H_2O → **Oasis HLB** (generic cartridge) *Elution*: 5ml MeOH → **LC-QTOF MS**

100 mL sample (pH 2) → **Oasis MCX** (cationic exchange cartridge) *El.*: 5ml MeOH (2% NH_3) → **LC-QTOF MS**

INSTRUMENTATION (UHPLC- QTOF MS)

UHPLC (Acquity UHPLC, Waters)
Column: Cortecs® C18 2.7µm 2.1x100 mm
Mobile phase: A: H_2O 0.01 % HCOOH
B: MeOH 0.01% HCOOH
Flow: 0.3 mL min^{-1}
Volume injection: 20 µL

Gradient	
0 min	10 % B
14-16 min	90 % B
16.1 min	10 % B
18 min	10 % B

QTOF MS (Xevo G2 QTOF, Waters)
ESI+ mode
Acquisition Mass range: 50 to 1000 *m/z* units
Sampling cone: 25 V
MS^E Low Energy Function (LE): 4 eV
High Energy Function (HE): 10-40 eV
Capillary voltage: 0.7 kV
Source Temperature: 130 ºC

SCREENING CRITERIA

Screening parameters [3]:
Retention time (Rt) – Accurate m/z ions – Mass errors – Isotope pattern (Cl, Br…)

Standard available

Detection
OPTION 1
- Rt agreement
- One ion (Q)
- Mass error < 5 ppm
- Isotope pattern

or

OPTION 2
- Rt agreement
- Two ions (Q and q)
- Mass error > 5 ppm
- Isotope pattern

Identification
- Rt agreement
- Two ions (Q and q)
- Q mass error < 5 ppm
- q mass error < 5 ppm
- Isotope pattern

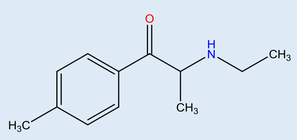
Standard unavailable

Tentative identification
- Expected ion (Q) present with mass error < 5 ppm
- Compatible isotopic pattern (Cl, Br…)
- One or more fragment ions (q),
  - in agreement with data reported
  - compatible with the chemical structure of the candidate (mass error < 5 ppm)

Confirmation required with reference standard

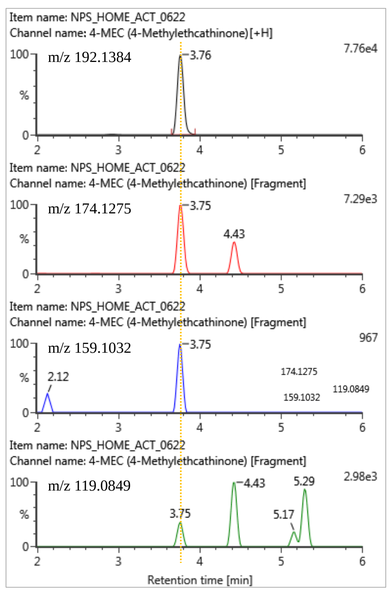
SCREENING ANALYSIS

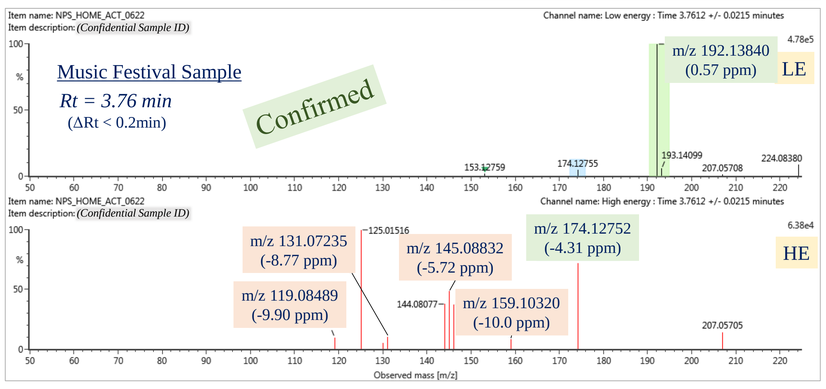
4-Methylethcathinone (4-MEC)

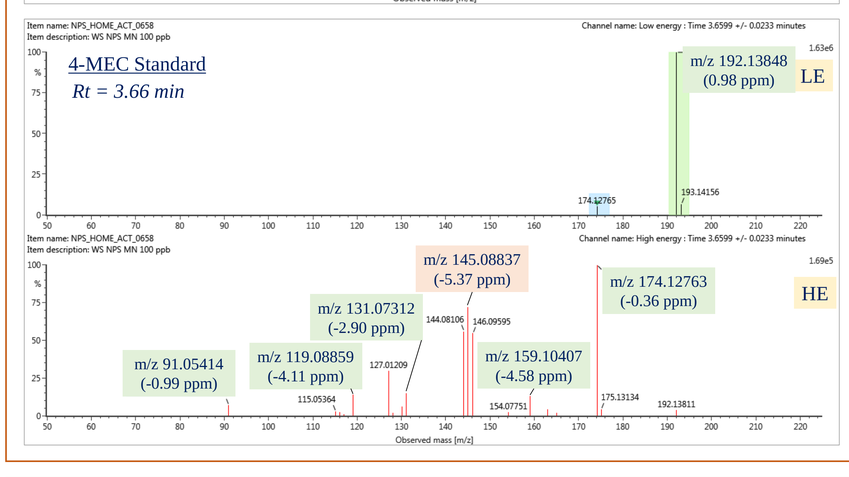


- ✓ Recreational psychostimulant
- ✓ Alert released in March 2010 by EMCDDA [4]
- ✓ Submitted to control and criminal penalties in EU in December 2010 [4]

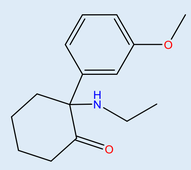
✓ Found in Pooled Urine Sample from Music Festivals





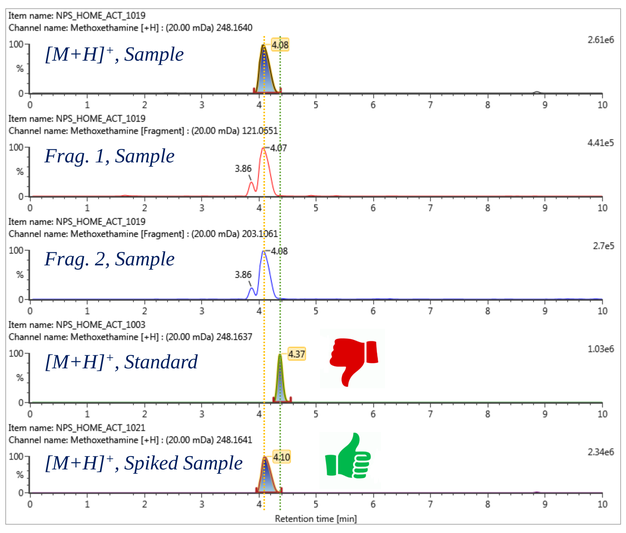


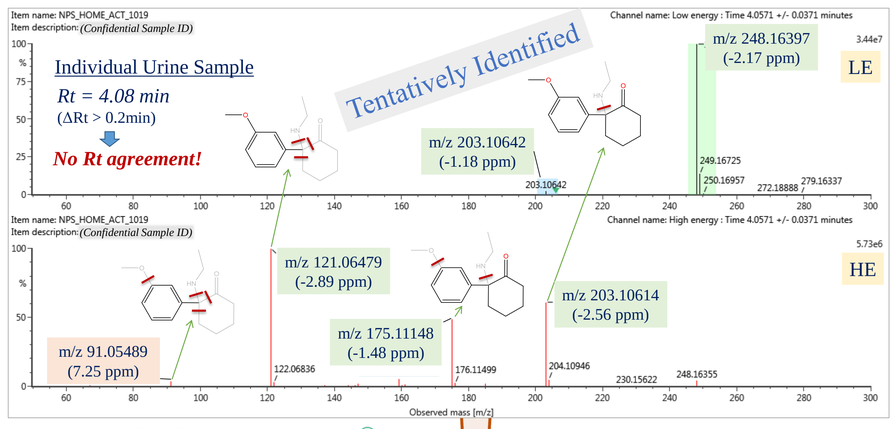
Methoxetamine (MXE)



- ✓ Ketamine analogue
- ✓ Health and Social risks assessed by EMCDDA in April 2014 [5]
- ✓ Several intoxications across EU reported in 2014 [5]

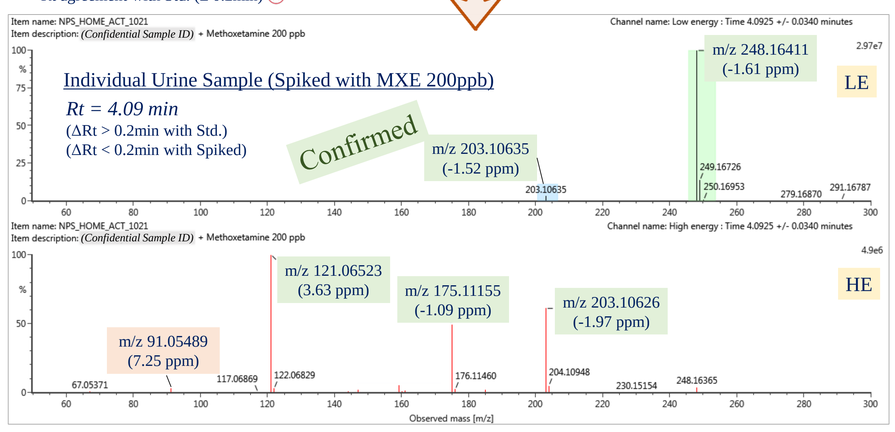
✓ Found in Individual Urine Sample from Hospital



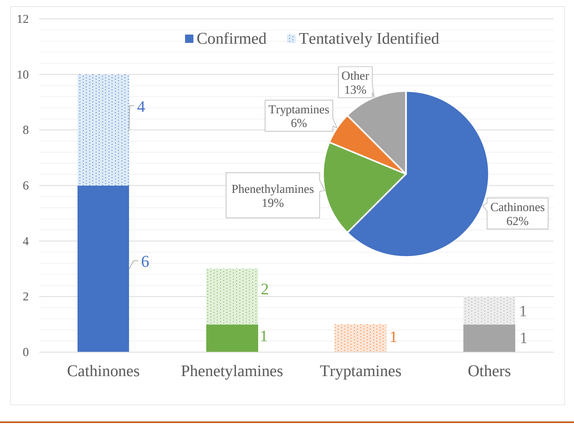


- Expected ion ([M+H]+) with mass error < 5ppm
- Fragments according with structure (and Std.) with < 5 ppm
- Isotope pattern
- Rt agreement with Std. (± 0.2min)

Spiked sample required for confirmation



RESULTS



	Individual Urines from Hospitals	Pooled Urines from Music Festivals	Urban Wastewater
Confirmed	• Methoxetamine (MXE)	• 3-methylmethcathinone (3-MMC) • 4-fluoromethcathinone (4-FMC) • 4-methylethcathinone (4-MEC)	• Mephedrone (4-MMC) • Methylone • Butylone
Tentatively Identified	• 3,4-dimethoxy-α-PVP • 4-methylmethylphenidate • α-methyltryptamine	• 3,4-dimethoxy-α-PVP • 3-methoxymethcatinone (3-MeOMC) • *bk*-DMDBP (Dipentylone) • α-methyltryptamine • α-PVP • 4'-chloro-α-PPP	• *p*-methoxymethamphetamine (PMMA) • 2-phenethylamine

CONCLUDING REMARKS

- ✓ A database containing more than 200 recently seized compounds was created based on the information provided by different national and international authorities.
- ✓ Approximately 1000 samples were collected with an strategic sampling process in locations with high probability of NPS consumption.
- ✓ A total of 19 NPS were found in urine samples from hospitals and music festivals and in urban wastewater.
- ✓ The majority of NPS detected were cathinones (62%) which agrees with reports of EMCDDA on NPS consumption.
- ✓ No synthetic cannabinoids were found. Probably, this was due to the fact that these type of compounds are highly metabolised and therefore no 'original' compound is excreted.

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