Determination of psychotropic and abuse substances: a new cooperation between University of Milano-Bicocca and Prefecture of Milano

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Introduction

According to the European Drug Report 2019, the consumption of illicit drugs in Italy is on the rise: about 30% of the Italian population (aged 15-64 years) has used illicit drugs at least once in their life and 10% in the last year [1]. Focusing the attention on the Lombardy region, 59% of the anti-drug operations were registered in the area of Milano. In 2018, 2557 people were reported for drug trafficking and 131 for association. The area of Milano recorded the second peak (after that of 2009) of drugs seized in the last decade, equal to 58% of those seized in the whole regional territory, for a total of kg 3706.5 [2]. This involves a high workload for the laboratories of the Gabinetto Regionale di Polizia Scientifica per la Lombardia and of the Comando Provinciale dell' Arma dei Carabinieri di Milano. With the aim of reducing the response time of analyses and supporting the law enforcement, in 2021 an agreement was signed between the University of Milano-Bicocca and the Prefecture of Milano for the determination of psychotropic and substances of abuse. For this purpose, a procedure for the quantification of the most used illicit substances (cocaine, heroin, 6-MAM, morphine, amphetamine, methamphetamine, MDMA, ketamine, GHB, GBL, LSD, trans-Δ9-THC and THCA), derived from street seizures by the law-enforcement officers were developed. The procedure comprises the registration step of samples through a home-made software, their preparation and analysis by a sensitive UHPLC-MS/MS method developed and validated at the analytical laboratories of the University of Milano-Bicocca (**Figure 1**). Generally, the analytical report is relayed to the Prefecture in 3-4 days, except for urgent requests that are processed in 24 hours.

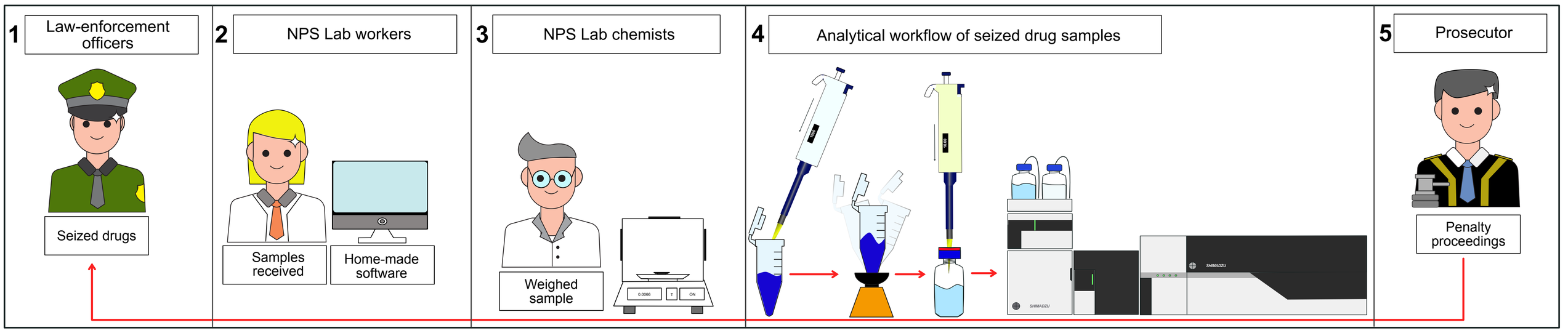


Figure 1. *Registration and sampling workflow of the entire process.*

Methods

1. Registration and sampling processes. The home-made software (**Figure 2a**) allows NPS workers to record all seized drugs easily, including a detailed description of the samples (origin, penalty proceeding, gross and net weight, morphological characterization, supposed substance). Information was collected and stored in a home-made database (**Figure 2b**). Then, all samples were accurately weighed (approx. 10 mg) and stored in a conical tube before analysis.

2. Extraction of drug samples and UHPLC/MS-MS analysis. Ten mg of seized drugs were diluted in 5.0 mL of methanol (Carlo Erba, Italy) and vortexed for 30 s. Then, the samples were sonicated for 5 min. 5.0 µL of each sample were added to further 5.0 mL of methanol, previously spiked with a mixture of deuterated internal standards (IS) for each targeted compound (**Figure 2c**) at a concentration of 100 ng/mL. One mL of spiked sample was filtered with 0.22 µm PTFE filter and 100 µL of this solution were added to 900 µL of a mixture of 98:2 (v/v) water:methanol both acidified with 0.02% of acetic acid (LC-MS purity grade, Carlo Erba, Italy).

Chromatographic conditions: An UHPLC Shimadzu Nexera X2 was used in gradient elution mode using a mobile phase consisting of: A) 98:2 (v:v) of water:methanol acidified with 0.02% of acetic acid; B) methanol acidified with 0.02% of acetic acid with a flow rate of 450 µL/min starting from a percentage of methanol of 5%. The chromatographic separation was carried out using a Raptor biphenyl column (100 mm x 2.1 mm, 1.8 µm particle size, Restek, Italy).

Mass spectrometric conditions: An LC-MS 8060 Shimadzu triple quadrupole mass spectrometer equipped with an ESI source was set using the following parameters: interface T: 300 °C; DL T: 250 °C; heat block T: 400 °C; nebulizing gas 3 mL/min; heating gas flow: 10 L/min. Instrumental parameters reporting name of compounds, relative IS standards, MRM transitions, and collision energies are shown in **Figure 2c**.

3. Report generation. The two-way connection of the home-made database (blue and green arrows) allows an easy access to the acquired data in the sample registration step from the analytical instrumentation software (**Figure 2d**). It leads to an accurate and very rapid report generation (**Figure 2e**), minimizing erroneous results.

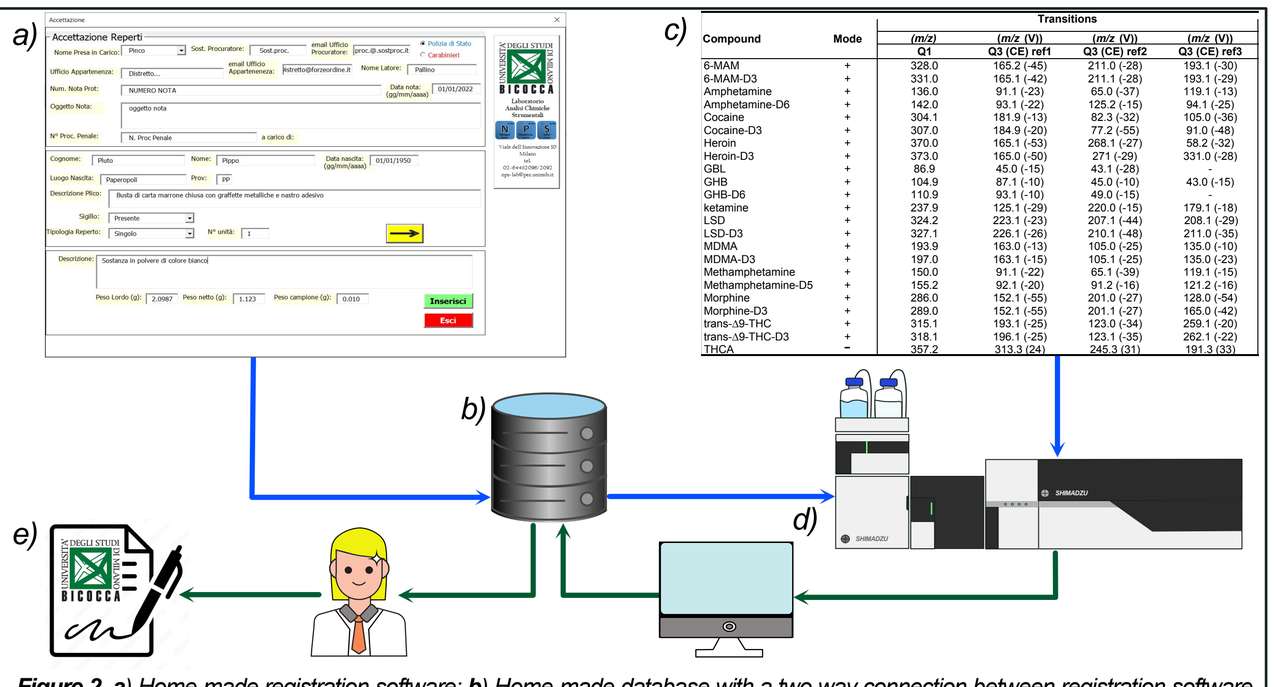


Figure 2*. **a)** Home-made registration software; **b)** Home-made database with a two way connection between registration software and UHPLC-MS/MS instrumentation software; **c)** ESI-MS/MS instrumental parameters; **d)** UHPLC-MS/MS instrumentation; **e)** Report generated and relayed to prosecutor .*

Analytical results

The method was validated considering LODs, LOQs, linearity range, repeatability, reproducibility, recovery and matrix effects. A chromatographic separation of selected compounds and relative IS is illustrated in **Figure 3a**. It is important to note the excellent profile of each analyte in a very short time of analysis (lower than 5 min). LODs were at low-ppb level, as shown in Figure **3b**. The calibration curves for each analyte showed excellent R^2 in the considered concentration range (LOQ - 250 ng/mL). Recovery values obtained for each analyte spanning from 96% and 106% demonstrating the suitability of the developed extraction procedure even in the presence of complex matrices such as marijuana and hashish seized samples. Analytical results showed that cocaine and trans-Δ9-THC are the most detected psychoactive substances, as shown in **Figure 4a**. **Figure 4b** and **Figure 4c** illustrate the relationship between the percentage of psychoactive ingredient versus analyzed seizures of cocaine and trans-Δ9-THC, respectively.

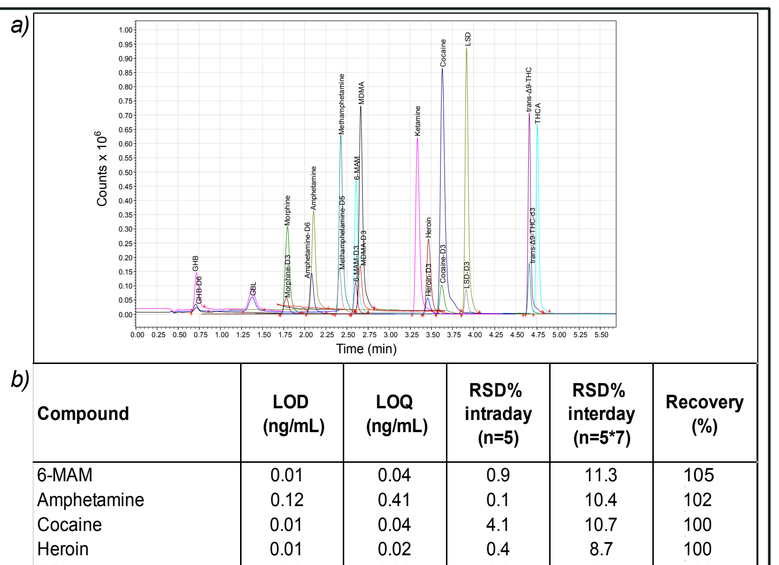
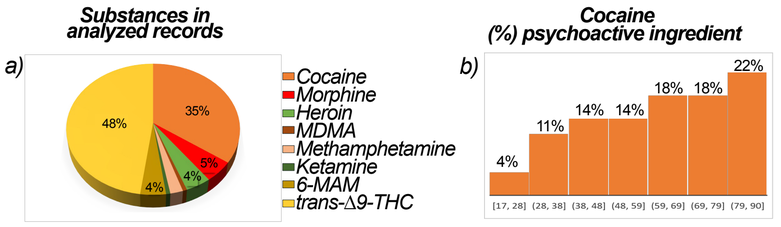


Figure 3 a*) Chromatographic separation of targeted compounds and their IS in MRM acquisition mode. **b)** Method validation parameters of developed procedure.*

Multivariate statistical analysis

One hundred twenty-four records of seized drugs, registered from September 2021 to February 2022, were described considering the following information extracted from the home-made database: 1) time (min) spent to prepare the registration report, weighing the seizures, sampling, and returning the material; 2) number of different types of seizures; 3) total number of seizures; 4) total net mass of the seizures; 5) percentage of phsycoactive ingredient for the 13 analyzed substances. These data were analyzed using the classical methods of multivariate exploratory analysis [3]. In particular, a similarity analysis was performed by calculating the Dehmer distance [4] between all of the pairs of records, from which it was possible to obtain a graphical tree representation of the similarity relationships between the records using the Minimum Spanning Tree (MST) method [5]. In the tree graphs of **Figure 5**, the vertices represent the records and the edges their similarity relationships. Therefore, the branches of the tree comprise groups of records with the same features. Those groups that required longer registration time were highlighted by red circles. From the comparison of trees **(a)**, **(b)** and **(c)**, it can be concluded that the registration time is mainly influenced by the total number of seizures to be weighed and sampled; moreover, longer registration times are observed for cocaine seized samples than those containing trans-Δ9-THC.



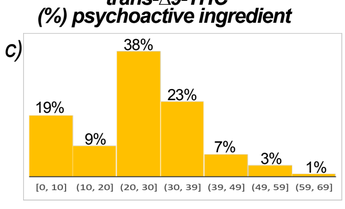


Figure 4. a) *Percentage of detected substances in the 124 records. **b)** Frequency distribution of % psychoactive ingredient (cocaine) in analyzed seizures. **c)** Frequency distribution of % psychoactive ingredient (trans-Δ9-THC) in analyzed seizures.*

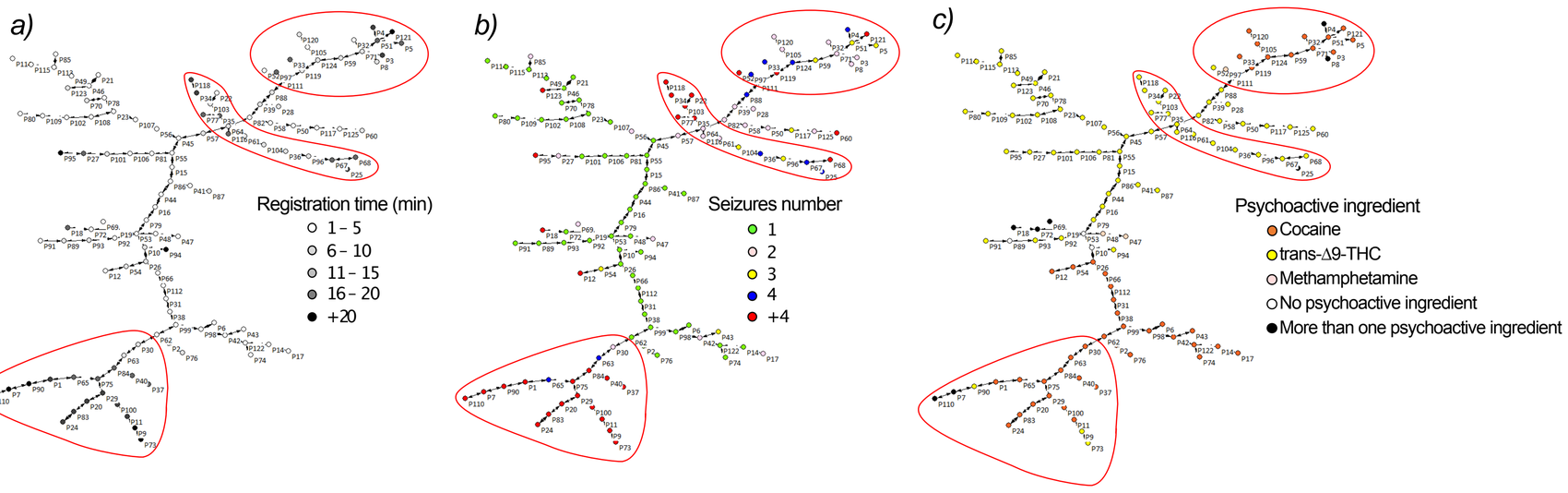


Figure 5. *Minimum Spanning Tree (MST) representation of records similarity. The vertices are colored in accordance with: **a)** registration time (longer the registration darker the color); **b)** total number of seizures in the records; **c)** types of psychoactive ingredient.*

Conclusions and Perspectives

The home-made software allows an easy and rapid access to the built database for rapid registration and report processes. The fully validated analytical method ensures a robust and efficient procedure for an accurate quantification of selected psychoactive substances even in the presence of complex matrices. As a future goal, the method will be gradually implemented with the increasing number of new psychoactive substances (NPS) present on the market, drugs that are not controlled by international drug convention yet, but which may pose a serious threat to public health.

References

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