Forensic Science International 365 (2024) 112299

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Detected substance abuse among injecting drug users through analysis of used syringes in Tunisia

Bilel Moslah [a,*], Omar Smaoui [a], Thomas Néfau [b], Houyem Boukassoula [c], Nadia chaouali [a], Meriem Laaribi [a], Mohamed Anouar Nouioui [a], Dorra Amira [a], Abderrazek Hedhili [a]

[a] Laboratory of Toxicology, Research unit of Toxicology and Environment LR12SP07, Center Mahmoud Yaccoub for Urgent Medical Assistance, Tunis 1008, Tunisia
[b] European Union Drugs Agency (EUDA), Lisbon, Portugal
[c] Tunisian Association for Information and Guidance on AIDS and Drug Addiction (ATIOST), Tunis 1005, Tunisia

ARTICLE INFO

Keywords:
Syringes
Chemical analysis
Polydrug use
People who inject drugs (PWID)
New psychoactive substances
Liquid chromatography-tandem mass spectrometry

ABSTRACT

Background and aims: Injecting drug use poses significant public health risks due to unsafe practices such as syringe sharing, reuse, and risky sexual behaviors, which increase the transmission of bloodborne viruses. In Tunisia, limited data on injecting drug use hinders the development of informed health and harm reduction policies.

Methods: A syringe collection campaign was conducted in Tunis in November 2022. The used syringes were provided by the Tunisian Association for Information and Orientation on AIDS and Addiction (ATIOST), a harm-reduction service. These syringes had been distributed to people who inject drugs (PWID) as part of a mobile syringe exchange program. The objective of the study was to analyze the contents of the used syringes to gain further insights into drug use patterns among PWID. The residual substances in the syringes were examined using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), in accordance with the standardized protocol of the European Syringe Collection and Analysis Project Enterprise (ESCAPE).

Results: A total of 261 syringes from five collection sites were analyzed. Among these, 87 % contained at least one psychoactive substance, while 32 % contained more than two psychoactive substances. The most frequently identified psychoactive substances were buprenorphine (50.28 %), amphetamine (11.65 %) and tramadol (9.66 %). No substances were detected in 34 syringes.

Conclusion: This method provides rapid data on drug use trends in specific regions and timeframes, revealing differences that can inform tailored prevention and harm reduction strategies. Such analyses are valuable for comparative studies across countries in the European Neighbourhood Policy (ENP-South) region.

1. Introduction

Traditional techniques for tracking patterns of abuse (and addiction) have been to collect data in a very random way from surveys, crime reports, forensic toxicology investigations and rapid blood, urine or saliva screening tests [1–3]. All these contributions have misled us into believing for several years that drug use in Tunisia is not on the upsurge, i.e., that a large proportion of illicit drug use goes unnoticed. In fact, this indiscriminate approach leads to an underestimation of abuse, to incomplete results and unrepresentative data. New approaches based on alternative tools, have been adopted by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) to provide community local information on substances used and to track changes in drug use patterns over time and as they occur, by the analysis of wastewater and the analysis of used syringes among intravenous drug users [4–6]. For example, limited information available in Tunisia on injecting drugs to inform health-related and harm reduction policy responses. A few studies like sero-behavioural surveys among PWID were conducted to analyse their vulnerability to HIV and HCV. Four Integrated Biological and Behavioural Surveys (IBBS) were conducted in 2009, 2011, 2014 and 2017 by ATIOST (Tunisian Association for Information and Orientation on Aids and Addiction) [7,8]. HIV prevalence among PWIDs remained stable in 2009 (2,7 %), 2011 (2,4 %) and 2014 (3,9 %), respectively, before increasing to 6 % in 2017. On the other hand, 21.3 % reported having had sexually transmitted infections in the last 12 months.

* Corresponding author.
E-mail address: bilel.moslah@rns.tn (B. Moslah).

https://doi.org/10.1016/j.forsciint.2024.112299
Received 26 February 2024; Received in revised form 31 October 2024; Accepted 13 November 2024

Self-reported data on injecting may be underestimated or biased, cultural backgrounds and sensitivities are likely to contribute to under reporting of drug use among young people [9] or PWID may be unaware of the substances or adulterants they inject, making it impossible to estimate the prevalence of injecting drug use.

Given the challenges in obtaining timely and geographically specific data on injecting drug use, this study aims to bridge this gap by analyzing the residual contents of used syringes to deliver real-time insights into local drug use patterns in Tunisia. Analytical chemistry offers reliable and effective data on injecting drug use, addressing the urgent need for accurate monitoring in this area. The present study, conducted in the five most popular districts of the Tunis area, is part of the European Syringe Collection and Analysis Project Enterprise (ESCAPE). This initiative aims to enhance existing data sources by providing timely, local information derived from the analysis of the residual content of used syringes. Specifically, this study seeks to assess the prevalence and types of substances used within these districts, offering insights that are crucial for public health interventions and policy formulation. This study serves as a comparative case study to Europe, conducted under the EU4 Monitoring Drugs project funded by the European Union to help countries in the European Neighbourhood Policy area to monitor the drugs problem [10]. It highlights Tunisia's current drug use trends and emphasizes the potential of syringe residue analysis as a valuable tool for real-time drug monitoring.

2. Material and methods

2.1. Chemicals and reagents

2.1.1. Psychoactive substances and metabolites

Morphine (MOR), Cocaine (COC), 6-Monoacetylmorphine (6-MAM), ketamine (KET), Methylenedioxymethamphetamine (MDMA), codeine (COD), tramadol (TRA), Heroine (HER), buprenorphine (BUP), benzoyl ecgonine (BZE), amphetamine (AMP) and methamphetamine (MET) standards were purchased from LGC Standards GmbH (Luckenwalde, Germany). Δ-9-tetrahydrocannabinol (THC) standards were purchased from Cerilliant (Round Rock, TX, USA).

2.1.2. Adulterants

Paracetamol (PAR), theophylline (THEO), procaine (PROC), strychnine (ST), lidocaine (LDC), levamisole (LEV), atropine (ATR), caffeine (CAF), bupivacaine (BVC), phenacetin (PHE), hydroxyzine (HDR) and griseofulvin (GR) were purchased from LGC Standards GmbH (Luckenwalde, Germany).

All solvents used in sample preparation and chromatographic separation were LCMS-grade. Methanol was supplied by VWR Chemicals (Netherlands). Water was purified using a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). Formic acid OPTIMA LC/MS Grade (99.5 %) was supplied by Fisher Scientific SAS (Strasbourg, France). Nitrogen Generator MAESTRO 8050 - LCMS for drying > 99 % of purity was from DGS SAS (Evry, France).

2.2. Collecting sites

The syringe collection campaign took place during the last two weeks of November 2024 in the area around the capital, Tunis, in north-eastern Tunisia (Fig. 1). Used syringes were collected and provided by ATIOST in five sites (site 1: Helal, site 2: Zahrouni, site 3: El Mellassine, site 4: El Kabaria and site 5: Mohamedia). The entire syringe collection campaign was organized with the assistance of ATIOST, which contributed by selecting and coordinating the sites and provided guidance documentation on each site's context, such as area descriptions and user demographics. The role of ATIOST was to organize and oversee the collection process through their needle exchange program, which provides users with clean syringes while facilitating the safe return of used syringes. The collection sites have been selected with the aim to cover five of the most popular districts in the area around the capital, Tunis. This provides a comprehensive picture through the provision of timely and localised information on substances injected. The profile of users visiting the website is predominantly male, between the ages of 25–35. Following collection, our team managed all laboratory analyses, including syringe handling, storage, and compound analysis. Syringes were immediately transferred to the laboratory under refrigerated conditions (+4 °C) and stored in the dark at —20 °C. In summary, ATIOST managed the site selection and syringe collection, while our

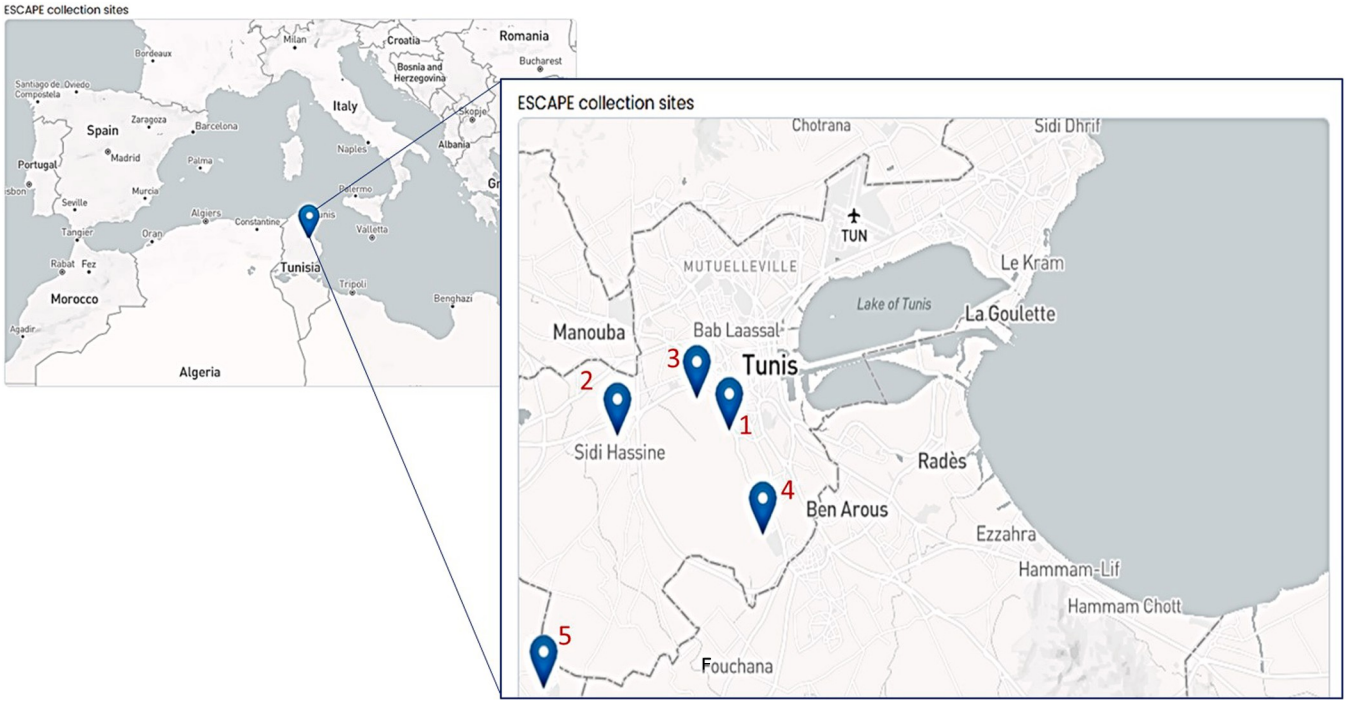

Fig. 1. The collection sites of used syringes in the capital Tunis area, located in the North East of Tunisia.

research team conducted all laboratory analyses and data interpretation. The whole procedure as well as syringe safety protocols, were conducted following the generic guidelines established by the ESCAPE network [11], ensuring consistent methodology across studies in this research area. The number of syringes collected per site is presented in Table 1. To ensure the safety of the staff and to prevent compound degradation during storage, syringes were collected with protective gloves in medical waste disposal (MWD) boxes.

2.3. Sample preparation

Residual drug still present in the used syringes was extracted by rinsing the syringe body pump with high purity methanol. Methanolic solutions were filtered and then transferred into appropriate vials for UPLC-MS/MS analysis. More detailed information on the sample preparation has been described elsewhere [12].

2.4. Analytical procedure

Analysis was performed using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to analyse the residual contents of used syringes. One for classical drugs of abuse (DAs) and adulterants and another qualitative method for new psychoactive substances (NPS), benzodiazepines and other psychoactives drugs. Both target methods incorporated a Nexera X2 ultra-high performance liquid chromatography (UHPLC) coupled to an LCMS-8050 triple quadrupole (QqQ) system (Shimadzu Corporation, Japan) equipped with an electrospray ionization (ESI) source. LabSolutions software was used for data acquisition. The QqQ mass spectrometer was operated in electrospray positive ionization mode with two selected reaction monitoring transitions. Multiple Reaction Monitoring (MRM) was used as the acquisition mode. Two transitions were selected for each compound, corresponding to the two most abundant fragmentation products of the protonated pseudo-molecular ions of each analyte. In this study, we employed two separate analytical approaches tailored to the specific requirements of each compound class. For classical drugs of abuse, metabolites and common adulterants, we developed and optimized a custom LC-MS/MS method to enhance sensitivity and specificity for these target compounds. This approach involved fine-tuning the chromatographic and mass spectrometric conditions, including adjustments to mobile phase composition, gradient elution, and ionization parameters, to achieve robust and reproducible identification and separation allowing a comprehensive understanding of our tailored analytical strategy.

For new psychoactive substances (NPS), we utilized the Shimadzu MRM Forensic Toxicology method package within LabSolutions™ LCMS software. This approach facilitated a broad screening for NPS using predefined settings optimized by the manufacturer, which was particularly advantageous given the limited availability of certified reference standards for these substances.

2.4.1. Classical drugs of abuse (DAs), metabolites and adulterants

Qualitative LC-MS/MS method was optimized for the identification of traditional DAs and adulterants. Chromatographic separation was achieved with a SunShell PFP column (2.1x100mm, particle size 2.6 µm), supplied by ChromaNick Technologies Inc. The total sample run time was 15 min and the high-pressure gradient for the analytical separation was achieved by changing the ratio of the elution pump. Mobile phases were eluent A (Water 0.1 % HCOOH) and eluent B (Methanol 0.1 % HCOOH). The elution gradient was set at a flow rate of 0.3 mL/min. MS parameters were optimized and performed by direct flow injection (FIA) analysis for each compound. Data generated by the automatic optimization are listed in Table 2 and Table 3.

Table 1
Distribution of collected syringes by collection site.

	Frequency	Percentage (%)
1. Cit´e Helal	9	3,4
2. El Mellassine	75	28,7
3. Zahrouni	55	21,1
4. El Kabaria	62	23,8
5. Mohammadia	60	23,0
Total	261	100,0

2.4.2. New psychoactive substances (NPS), benzodiazepines and other drugs

The screening of NPS is challenging due to the large number of existing NPS and their constant introduction into the drug market. Therefore, due to the lack of standards, only a tentative identification of NPS may be possible [13]. Tentative identification for NPS and benzodiazepines was carried out using an MRM Forensic Toxicology method package developed by Shimadzu for LabSolutions™ LCMS software. Information on 1250 drugs of abuse, including NPS, is included in this method package (Table 4). A Kinetex 2.6 µm XB-C_{18} (2.1 mm×100 mm) column supplied by Phenomenex was used for the chromatographic separation. The mobile phases were eluant A (10 mmol/L ammonium formate + 0.1 % formic acid - water) and eluant B (10 mmol/L ammonium formate + 0.1 % formic acid - methanol), used in a gradient mode as follows 5 % B (0 min) - 95 % B (7.5–10 min), 5 % B (10.01–15 min). All laboratory analysis methods are described in detail elsewhere [14]. The minimum criteria for a tentative identification are that the peak signal-to-noise ratio should be greater than 3 (S/N>3), the measured retention time should agree with the expected retention time given in the method package (±0.4 min), and there should be at least two MRM product ions and an acceptable relative ion ratio tolerance (±30 %). If a specific compound was tentatively identified, the identification could be further confirmed by subsequent purchase of the certified reference standards [15].

3. Results and Discussion

3.1. Psychoactive drugs detected

A total of 261 syringes from 5 collection sites were analysed. The analysis shows a high proportion of syringes (87 %) containing at least one psychoactive substance, with no residue found in 13 % of the syringes. Buprenorphine is the most commonly detected psychoactive substance, found in 117 (50.28 %) of the syringes containing at least one psychoactive substance (Fig. 2). This is followed by amphetamine and tramadol, found in 41 (11,65 %) and 34 (9,66 %) syringes, respectively. Buprenorphine and tramadol remain the most commonly detected substances, with a cumulative rate of 60 %. The emergence of diverted use of pharmaceutical opioids [16], may be explained by the fact that they are considered to be among the most accessible drugs of choice for Tunisian adolescents and students in popular neighbourhoods, where they are bought at low prices. For example, cocaine was found in 5 syringes in this study, representing only 1,42 % of the total number of syringes collected. Indeed, the purchase of cocaine or heroin, the traditional opioid of choice, is a matter of cost. This may also explain the observed regional shifts observed in drug injecting trends and patterns compared to northern countries, where heroin and cocaine remain the most commonly used substances observed in needle exchange services, according to a study conducted by the ESCAPE network in 7 cities in Europe in 2017 and 2018 [17]. Therefore, regular implementation of this methodology across different continental regions can provide specific insights into localised trends and potential health threats as they emerge.

On the other hand, the results of this present study showed a slight similarity to those found in Europe with a relatively high proportion of syringes (13 %) containing traditional amphetamine (11,65 %) and methamphetamine (1,42 %) stimulants among PWID [18,19].

Table 2
MS/MS parameters for the analysis of target analytes by MRM positive ionization mode.

Target Compounds	Retention Time [min]	Precursor ion [*m/z*]	Product ions: Q transition q_1 transition	Dwell time [ms]	Q1 Pre-bias [V]	Collision cell energy (CE) [V]	Q3 Pre-bias [V]
Morphine (MOR)	2.98	286.1 $[M+H]^+$	286.1> 165.1	42	−14	−44	−12
			286.1> 153.1	42	−14	−45	−29
Amphetamine (AMP)	3.43	136.1 $[M+H]^+$	136.1> 91.05	42	−14	−17	−19
			136.1>119.05	42	−14	−15	−25
Tramadol (TRA)	3.80	264.10 $[M+H]^+$	264.10> 58.20	15	−18	−24	−25
Methamphetamine (MET)	3.95	150.01 $[M+H]^+$	136.1> 91.05	42	−10	−19	−18
			136.1>119.05	42	−10	−10	24
Codeine (COD)	4.15	300.1$[M+H]^+$	300.1> 165.1	42	−15	−41	−19
			300.1> 44.1	42	−15	−30	−17
6-Monoacetylmorphine (6-MAM)	4.26	328.1$[M+H]^+$	328.1>165.1	42	−16	−41	−19
			328.1>43.1	42	−16	−50	−16
Benzoylecgonine (BZE)	4.52	290.1 $[M+H]^+$	290.1 >168.1	42	−14	−19	−13
			290.1 >105.1	42	−14	−30	−21
Methylenedioxymethamphetamine (MDMA)	5.02	194.1 $[M+H]^+$	194.1 >163	47	−10	−13	−12
			194.1 >105.1	47	−10	−25	−22
Heroin (HER)	5.26	370.15$[M+H]^+$	370.15>58.15	33	−10	−30	−23
			370.15>44.10	33	−10	−40	−17
Ketamine (KET)	5.71	238.1 $[M+H]^+$	238.1 >125	42	−12	−26	−26
			238.1 >207	42	−12	−15	−15
Cocaine (COC)	6.39	304.15 $[M+H]^+$	304.15>182.25	35	−11	−21	−14
			304.15>82.10	35	−11	−35	−17
Buprenorphine (BUP)	7.55	468.3 $[M+H]^+$	468.3 >55	80	−24	−50	−21
			468.3 >396.3	80	−24	−41	−28
Δ—9-te´trahydrocannabinol (THC)	7.85	315.2 $[M+H]^+$	315.2 >123	47	−16	−33	−25
			315.2 >193.2	47	−16	−30	−24

Q: quantification; q: confirmation

The most striking finding observed in this study, was the presence of new psychoactive substances such as synthetic cathinones like MDA, pentylone, mephedrone (4-MMC) and buphedrone, even at low frequencies: (5.97 %), (2.56 %), (0.57 %) and (0.57 %), respectively.

Health experts in Ireland have been reporting since 2015 that injecting stimulants such as amphetamines, cocaine and synthetic cathinones is associated with an increased risk of HIV and hepatitis C virus (HCV) transmission through more frequent use and sharing of injecting equipment [18,20,21].

Fig. 3 shows the distribution of detected psychoactive substances detected by collection sites. While similar trends were observed across the five collection sites, this study found a local distinction related to the area of El-Mallassine (site 3) with a lower rate of buprenorphine detection compared with the other sites. However, this region accounts for almost all tramadol detection (8.23 %) compared with total tramadol detection (9.66 %). The second notable specificity strongly affecting this area is the dominance of fentanyl use.

Adulterants are added to drugs for a variety of reasons, not always intended by the manufacturer. Adulterants may be incorporated to bulk up, dilute, or to enhance the effects of the psychoactive drugs. The effects of adding adulterants are varied, but in some cases may result in additional and unknown health risks for PWID [22,23]. Fig. 4 shows the frequency of adulteration associated with each detected psychoactive substance detected in all syringes (N=261). Of the 12 adulterants examined, 9 were found 127 times in association with the different psychoactive substances identified. Procaine, hydroxyzine and griseofulvin were not found in association with any psychoactive substance. Caffeine, levamisole, paracetamol and bupivacaine were the most

Table 3
MS/MS parameters for the analysis of target analytes by MRM positive ionization mode.

Target Compounds	Retention Time [min]	Precursor ion [*m/z*]	Product ions: Q transition q_1 transition	Dwell time [ms]	Q1 Pre-bias [V]	Collision cell energy (CE) [V]	Q3 Pre-bias [V]
Paracetamol (PAR)	1.07	152.0[M+H]$^+$	152.0> 110.0	34	−10	−18	−23
			152.0> 65.05	34	−10	−30	−13
Theophylline (THEO)	1.20	181.05[M+H]$^+$	181.05> 124.15	36	−20	−19	−22
			181.05>96.15	36	−20	−24	−20
Procaine (PROC)	2.52	237.15[M+H]$^+$	237.15> 100.10	36	−27	−15	−18
			237.15>120.0	36	−27	−21	−23
Strychnine (ST)	3.18	335.10[M+H]$^+$	335.10> 184.10	36	−24	−38	−18
			335.10> 156.15	36	−23	−45	−16
Lidocaine (LDC)	3.20	235.0 [M+H]$^+$	235.0>86.10	36	−26	−19	−18
			235.0 >58.10	36	−26	−45	−12
Levamisole (LEV)	3.28	205.10[M+H]$^+$	205.10>91.10	36	−10	−39	−16
			205.10>178.15	36	−18	−21	−18
Atropine (ATR)	3.60	290.15[M+H]$^+$	290.15>124.15	36	−14	−25	−25
			290.15>93.20	36	−14	−30	−19
Caffeine (CAF)	3.95	195.05[M+H]$^+$	195.05>138.05	36	−21	−20	−28
			195.05>42.12	36	−21	−40	−17
Bupivacaine (BVC)	4.59	289.15[M+H]$^+$	289.15>140.15	36	−30	−20	−30
			289.15>84.25	36	−30	−45	−17
Phenacetin (PHE)	5.03	180.10 [M+H]$^+$	180.10>110.20	44	−19	−21	−22
			180.10>138.20	44	−19	−18	−13
Hydroxyzine (HDR)	6.01	375.20[M+H]$^+$	375.20 >201.0	36	−20	−20	−20
			375.20>165.05	36	−26	−60	−21
Griseofulvin (GR)	8.27	353.1 [M+H]$^+$	353.1>165.10	36	−18	−20	−16
			353.1>215.10	36	−10	−19	−22

Q: quantification; q: confirmation

commonly detected adulterants. Buprenorphine, tramadol and amphetamine were the most commonly adulterated substances. This finding should be treated with caution. In fact, the presence of caffeine, for example, in traces of blood originally found in some used syringes, can be related to the use of many other legal drugs. Thus, the presence of caffeine is not necessarily due to its use as a cutting agent for buprenorphine, amphetamine or cocaine. It should also be noted that several syringes were found to contain more than one psychoactive substance; at this stage we cannot confirm whether the presence of levamisole, for example, is due to adulteration, substance contamination or polydrug use. In order to gain a better understanding of the content of drugs at the point of sale, further studies are being carried out in our forensic laboratory to determine the profile of adulterants commonly found in drug seizures in Tunisia, to determine the purity of drugs of abuse.

3.2. Psychoactive drugs detected in conjunction

Across the five sites studied, the analysis shows a high proportion of syringes (87 %) containing at least one psychoactive substance and 32 % containing more than two psychoactive substances (Fig. 5). In line with other European syringe analysis studies, the Tunisian findings suggest that polydrug use is common among PWID [17]. According to the latest available results from the 2020–2021 ESCAPE syringe collection, which analyzed 1392 used syringes collected in 8 European cities, one-third of all syringes contained two or more drugs. This finding indicates potential polydrug use or syringe reuse. The detection of multiple substances in a single syringe could suggest behaviors like syringe sharing or the injection of drug mixtures, both of which carry a heightened risk of bloodborne virus transmission, such as HIV or hepatitis. Although this study focuses on substance analysis rather than serological testing, the potential health implications highlight the importance of harm reduction measures. Caution is warranted in interpreting these results, as traces of blood from prior non-injectable drug use may also be drawn into the syringe during injection [19].

The joint detection of psychoactive substances by collection site is summarised in Fig. 6. While comparable trends were observed across the five collection sites, this study found a regional difference in relation to the area of El-Mallassine (site 3), which has the highest frequency of polysubstance use compared with other sites. This is the same region that showed a disparity in the type of drug injection (see Fig. 3). A notable specificity that strongly affecting this region is the dominance of fentanyl use. There is a greater risk of exposure to fentanyl mixed with benzodiazepines (lorazepam) [24]. Overall, there were slight differences between inner cities, probably reflecting the profiles of PWID in different areas of the city. Indeed, several factors may have an impact on substance use and abuse, such as geographical location (proximity to a port, borders, etc.) and the socioeconomic characteristics of a geographical area (unemployment rate, proportion of people covered by universal health insurance, etc.) [25].

3.3. Limitations

This approach does not measure the prevalence of injecting, nor does it necessarily indicate the relative prevalence of use of different substances among IDUs, as the number of syringes collected and analysed cannot be converted into the number of individual users. In addition, drug residues may degrade over time to undetectable levels. Caution should therefore be exercised in interpreting these findings, as there are a number of possible selection biases. In cases where multiple substances are detected in the same syringe, it is difficult to know whether the second substance is the result of a deliberate injection of a mixture of substances, or whether the substance is present in the blood as a result of reuse of injection equipment or contamination by contact in the needle sharps container.

4. Conclusions

While this study has inherent limitations, it offers a practical approach for identifying substances present within specific periods and geographical areas through syringe residue analysis. This method does not strictly follow a formal epidemiological design, such as a cross-sectional or cohort study, given the unique nature of syringe residue analysis. Instead, our approach is exploratory, aiming to provide insights based on the substances detected in used syringes within a specific timeframe and location, rather than making population-based prevalence estimates. Our findings highlight the presence of fentanyl and

Table 4
MS/MS parameters for the analysis of NPS, benzodiazepines and other drugs by MRM positive ionization mode.

	Expected Rt [min]	Precursor ion $[M+H]^+$	MRM Product ions	Q1 Pre-bias [V]	Collision cell energy (CE) [V]	Q3 Pre-bias [V]
Naloxone	0.75	328.1	328.1000>310.0500	−24	−21	−15
			328.1000>212.0500	−24	−38	−23
Methiopropamine	3.06	156.1	156.1000>97.1000	−29	−22	−20
			156.1000>58.3500	−11	−12	−24
4-Hydroxy—3-methoxymethamphetamine (HMMA)	3.20	196.1	196.1500>165.1000	−10	−10	−12
			196.1500>105.1000	−10	−25	−21
Cathinone	3.33	150.1	150.1000>132.0500	−15	−16	−27
			150.1000>117.0500	−15	−25	−24
Acetyl norfentanyl	3.45	219.1	219.1000>84.1500	−30	−18	−16
			219.1000>55.2000	−30	−35	−22
Methcathinone	3.49	164.1	164.1000>146.1000	−17	−15	−17
			164.1000>131.0500	−17	−20	−15
Ephedrine	3.56	166.1	166.1000>148.05	−11	−14	−17
			166.1000>117.05	−11	−20	−24
4-Methoxymethcathinone	3.63	194.2	194.2000>145.0500	−23	−19	−26
			194.2000>146.3000	−14	−30	−16
Oxycodone	3.63	316.1	316.1500>298.1500	−16	−19	−22
			316.1500>241.1000	−16	−30	−18
Methylone	3.70	208.1	208.1000>160.0000	−10	−18	−18
			208.1000>132.1000	−10	−25	−10
Buphedrone	3.71	178.0	178.1000>160.0000	−30	−14	−17
			178.1000>131.0000	−30	−23	−24
4-Methylmethcathinone (Mephedrone)	3.90	178.1	178.1000>160.15	−12	−15	−12
			178.1000>145.05	−12	−20	−17
Ethylone	4.03	222.1	222.1000>174.05	−30	−18	−13
			222.1000>204.05	−30	−15	−15
3,4-Me´thyle`nedioxyamphe´tamine (MDA)	4.10	180.1	180.1000>105.1000	−12	−23	−12
			180.1000>77.0000	−12	−40	−29
3',4′-Methylenedioxy-α-pyrrolidinobutiophenone (MDPBP)	4.13	262.2	262.2000>112.2000	−19	−24	−21
			262.2000>161.0000	−14	−23	−18
4-Fluoroamphetamine(4-FAP)	4.21	154.1	154.1000>109.0000	−10	−21	−22
			154.1000>137.1000	−10	−15	−15
3-Fluoromethamphetamine	4.29	168.1	168.1000>109.0000	−11	−18	−22
			168.1000>83.0500	−11	−45	−16
N-m´ethyldi´ethanolamine (MDEA)	4.45	208.1	208.1500>163.0500	−10	−14	−19
			208.1500>105.0500	−10	−25	−22
Pentedrone	4.46	192.2	192.2000>132.2000	−14	−15	−25
			192.2000>174.1500	−13	−13	−19
Pentylone	4.58	236.1	236.1000>188.2000	−12	−16	−21
			236.1000>131.3000	−12	−37	−25
α-Pyrrolidinopentiophenone (alpha-PVP)	4.61	232.1	232.1000>91.1500	−16	−24	−18
			232.1000>126.1000	−16	−26	−23
Norketamine	4.80	224.1	224.1000>125.0000	−11	−25	−25
			224.1000>207.0000	−11	−10	−15
Fentanyl-M nor	4.80	233.3	233.3500>84.0500	−12	−17	−17
			233.3500>55.0000	−12	−35	−17
Acetyl fentanyl	4.89	323.2	323.2000>105.1000	−23	−39	−20
			323.2000>188.0500	−12	−23	−20
α-Pyrrolidinohexiophenone (alpha-PHP)	5.24	246.2	246.2000>91.1000	−30	−25	−17
			246.2000>140.1000	−30	−27	−26
Methylenedioxypyrovalerone (MDPV)	5.26	276.1	276.1500>126.2000	−30	−28	−25
			276.1500>135.1000	−30	−30	−27
Zolpidem	5.39	308.1	308.1500>235.2000	−30	−30	−26
			308.1500>236.2000	−30	−29	−19
LSD	5.47	324.2	324.2000>223.1000	−16	−25	−17
			324.2000>208.0500	−16	−35	−25
4-Methylethcathinone	5.51	192.2	192.2000>69.0000	−19	−28	−15
			192.2000>130.1000	−10	−40	−23
Fentanyl-M despropionyl	5.66	281.4	281.4000>105.0500	−14	−30	−21
			281.4000>188.1000	−14	−20	−14
α-Pyrrolidinoheptaphenone (alpha-PHPP)	5.83	260.3	260.3000>91.1000	−30	−25	−17
			260.3000>77.1000	−30	−52	−30
Fentanyl	5.89	337.2	337.2000>105.1000	−10	−40	−22
			337.2000>188.1000	−10	−25	−14
Methadone metabolite iEDDP)	6.11	278.2	278.2000>234.1000	−30	−33	−18
			278.2000>249.1500	−30	−25	−19
Bromazepam	6.50	316	316.0000>182.1500	−12	−39	−11
			316.0000>209.0500	−12	−29	−24
Midazolam	6.55	326.1	326.1000>291.1000	−12	−28	−22
			326.1000>223.0000	−12	−38	−18

(*continued on next page*)

Table 4 (*continued*)

	Expected Rt [min]	Precursor ion $[M+H]^+$	MRM Product ions	Q1 Pre-bias [V]	Collision cell energy (CE) [V]	Q3 Pre-bias [V]
Clonazepam	6.69	316.1	316.1000>270.0000	−12	−28	−13
			316.1000>214.0000	−12	−40	−26
Methadone	6.91	310.2	310.2000>265.1500	−30	−16	−20
			310.2000>105.0500	−30	−30	−22
Clobazam	6.91	301	301.0500>259.0000	−11	−22	−22
			301.0500>224.0000	−11	−37	−29
Lorazepam	7.00	321.	321.0500>302.9500	−12	−10	−24
			321.0500>274.9000	−12	−22	−22
Alprazolam	7.07	309.1	309.1000>281.1000	−16	−26	−22
			309.1000>205.1000	−16	−40	−23
JWH398 (N-pentanoic acide metabolite)	7.21	406.1	406.1000>188.9000	−145	−26	−20
			406.1000>160.8500	−15	−50	−30
Diazepam	7.57	285.1	285.1000>154.1000	−11	−28	−18
			285.1000>193.0500	−11	−29	−23
Prazepam	8.17	325.1	325.1000>271.0500	−12	−20	−22
			325.1000>139.9500	−12	−39	−29
JWH—250 (1-pentyl—3-(2-methoxyphenylacetyl)indole)	8.26	336.2	336.2000>121.0500	−30	−23	−25
			336.2000>91.1000	−30	−45	−18
JWH—073 (Cannabinoid Receptor Agonist)	8.28	328.1	328.1500>155.0100	−16	−23	−18
			328.1500>127.0500	−16	−50	−25
JWH—018 (Cannabinoid Receptor Agonist)	8.82	342.2	342.2000>155.0500	−10	−23	−18
			342.2000>127.0500	−10	−50	−25
CP 47,497 (Cannabinoid Receptor Agonist)	9.22	319.2	319.2500>233.1500	−16	−15	−30
			319.2500>233.1501	−16	−20	−15
HU 210 (Cannabinoid Receptor Agonist)	9.30	387.3	387.3000>71.0500	−11	−29	−14
			387.3000>43.1500	−11	−45	−17

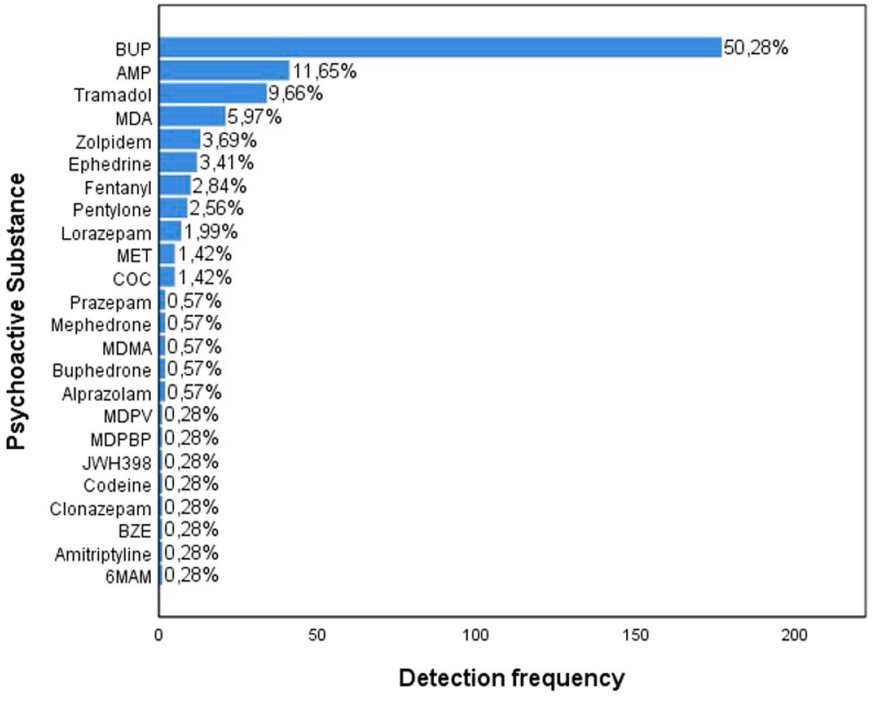

Fig. 2. The detection rate of detected psychoactive substances found in all syringes (N=261).

synthetic cathinones like pentylone, mephedrone (4-MMC), and buphedrone, giving a snapshot of substance use in the Tunis area. With planned follow-up studies in the same regions, syringe residue analysis has the potential to reveal emerging trends over time, serving as a complementary tool alongside other surveillance methods to inform harm reduction and public health responses. By situating Tunisia's data within a broader regional context, this approach supports the development of more targeted prevention strategies in response to shifting drug landscapes.

Funding

This work was funded by the European Union through the EU4MD project managed by the European Union Drugs Agency (EUDA) through a grant number n° CT.21.EU4MD.0044.1.0

CRediT authorship contribution statement

Thomas Ne´fau: Visualization, Validation, Project administration,

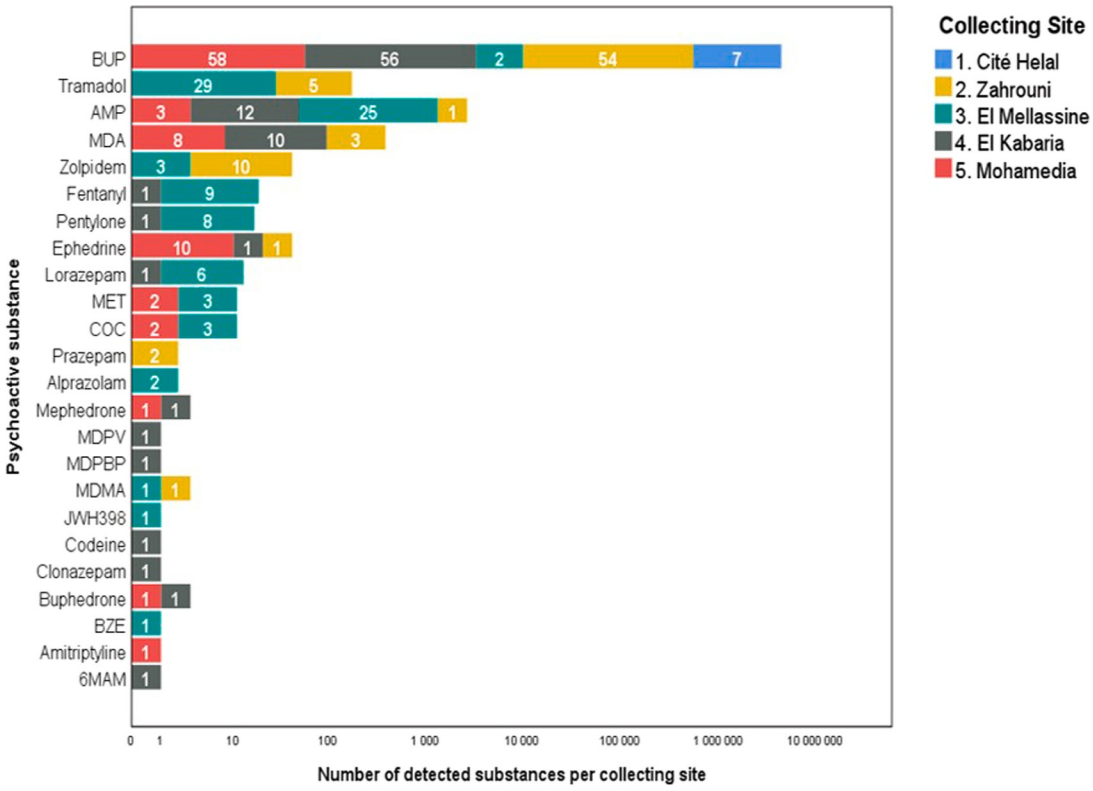

Fig. 3. The distribution of detected psychoactive substances according to the sites of collection.

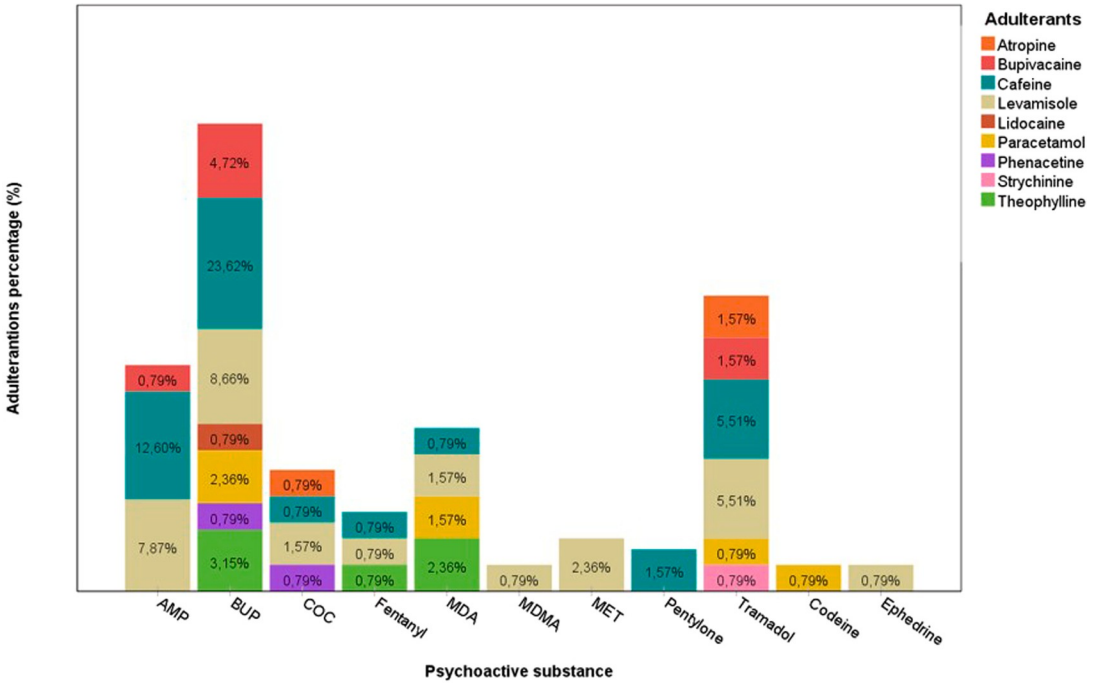

Fig. 4. The frequency of adulteration associated with each detected psychoactive substance found in all syringes (N=261).

Methodology, Funding acquisition, Data curation, Conceptualization. **Omar Smaoui:** Validation, Formal analysis, Data curation. **Bilel Moslah:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Abderrazek Hedhili:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Dorra Amira:** Writing – review & editing, Resources. **Mohamed Anouar Nouioui:** Visualization, Validation. **Meriem Laribi:** Formal analysis. **Nadia Chaouali:** Investigation, Formal analysis. **Houyem Boukassoula:** Methodology, Investigation, Conceptualization.

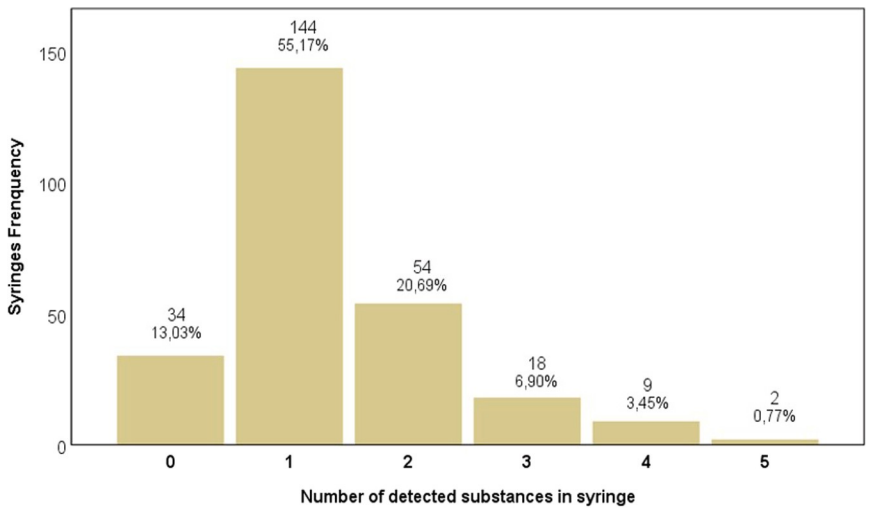

Fig. 5. Percentages of syringes in which none, one or more substances were (co-)detected.

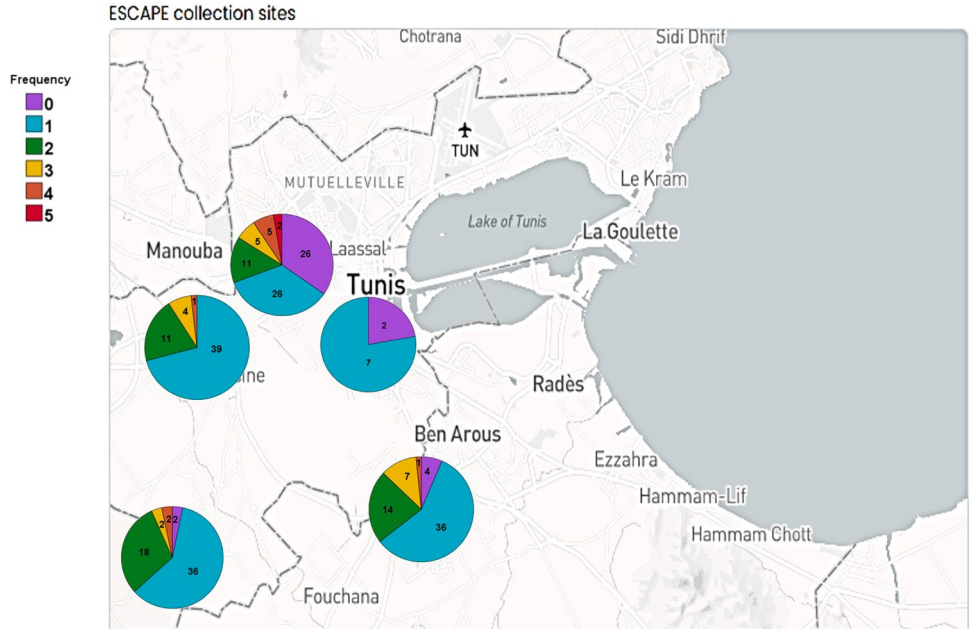

Fig. 6. Joint detection of psychoactive substances by collection site.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

All authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgements

This work was carried out as part of cooperation with the European Union Drugs Agency (EUDA) funded by the European Union through a grant number n° CT.21.EU4MD.0044.1.0 as part of the ESCAPE project (European Syringe Collection and Analysis Project Enterprise) through the EU4MD project in neighbouring countries of the European Union. The authors also acknowledge the Tunisian Association for Information and Guidance on AIDS and Drug Addiction (ATIOST) to provide us with the collected syringes.

References

[1] B. Moslah, M. Araoud, M.A. Nouioui, S. Najjar, D. Amira, N. Ben Salah, A. Hedhili, Fast screening tests for the simultaneous detection of 11 drugs of abuse in urine specimens. A forensic epidemiology study of 28,298 cases in Tunisia, Forensic Sci. Int 283 (2018) 35–40, https://doi.org/10.1016/j.forsciint.2017.12.004.

[2] N. Chaouali, B. Moslah, K.B. Salem, D. Amira, A. Hedhili, N.B. Salah, Illicit substances identified in the urine of 11.170 suspected drug users in North Tunisia, Pan Afr. Med J. 38 (2021) 20, https://doi.org/10.11604/pamj.2021.38.20.26781.

[3] H. Aounallah-skhiri, H. Zalila, T. Zid, H. Boukassoula, N. Ben Salah, Drug situation and policy, Ctry. Profile, Tunis. P-PG/MedNET 12 (2014).

[4] D. European Monitoring Centre for, D. Addiction, An analysis of drugs in used syringes from sentinel European cities – Results from the ESCAPE project, 2018 and 2019, Publications Office 2021. https://doi.org/doi/10.2810/100354.

[5] E.M.Cf Drugs, D. Addiction, S. Castiglioni, Assessing illicit drugs in wastewater – Advances in wastewater-based drug epidemiology, Publications Office2016. https://doi.org/doi/10.2810/6622.
[6] B. Moslah, E. Hapeshi, A. Jrad, D. Fatta-Kassinos, A. Hedhili, Pharmaceuticals and illicit drugs in wastewater samples in north-eastern Tunisia, Environ. Sci. Pollut. Res Int 25 (19) (2018) 18226–18241, https://doi.org/10.1007/s11356-017-8902-z.
[7] MedSPADII, The Mediterranean School Survey Project on Alcohol and Other Drugs, 2017,
[8] S. Bouarrouj, HIV/AIDS survey of injectable drug users in Tunisia 2014, 2015,
[9] N. Al-Hemiery, R. Dabbagh, M.T. Hashim, S. Al-Hasnawi, A. Abutiheen, E. A. Abdulghani, J.K. Al-Diwan, N. Kak, H. Al Mossawi, J.C. Maxwell, M.L. Brecht, V. Antonini, A. Hasson, R.A. Rawson, Self-reported substance use in Iraq: findings from the Iraqi National Household Survey of Alcohol and Drug Use, 2014, Addiction 112 (8) (2017) 1470–1479, https://doi.org/10.1111/add.13800.
[10] E.M.Cf Drugs, D. Addiction, Overview of drug markets in the European Neighbourhood Policy-South countries – Regional report 2022, Publications Office of the European Union 2022. https://doi.org/doi/10.2810/765393.
[11] E.M.Cf Drugs, D. Addiction, European syringe collection and analysis enterprise – Generic protocol, Publications Office2021. https://doi.org/doi/10.2810/665510.
[12] T. Nefau, E. Charpentier, N. Elyasmino, C. Duplessy-Garson, Y. Levi, S. Karolak, Drug analysis of residual content of used syringes: a new approach for improving knowledge of injected drugs and drug user practices, Int J. Drug Policy 26 (4) (2015) 412–419, https://doi.org/10.1016/j.drugpo.2014.09.010.
[13] L. Bijlsma, A. Celma, F.J. Lo´pez, F. Herna´ndez, Monitoring new psychoactive substances use through wastewater analysis: current situation, challenges and limitations, Curr. Opin. Environ. Sci. Health 9 (2019) 1–12, https://doi.org/10.1016/j.coesh.2019.03.002.
[14] B. Moslah, O. Smaoui, M.A. Nouioui, M. Araoud, N. Chaouali, M. Laribi, D. Amira, N. Ben Salah, A. Hedhili, Sewage analysis as an alternative tool for assessing drug of abuse and new psychoactive substances in Tunisia, Forensic Sci. Int 347 (2023) 111672, https://doi.org/10.1016/j.forsciint.2023.111672.
[15] R. Bade, J.M. White, J. Chen, J.A. Baz-Lomba, F. Been, L. Bijlsma, D.A. Burgard, S. Castiglioni, N. Salgueiro-Gonzalez, A. Celma, A. Chappell, E. Emke, R. Steenbeek, D. Wang, E. Zuccato, C. Gerber, International snapshot of new psychoactive substance use: Case study of eight countries over the 2019/2020 new year period, Water Res 193 (2021) 116891, https://doi.org/10.1016/j.watres.2021.116891.
[16] MedSPADIII, The Mediterranean School Survey Project on Alcohol and Other Drugs, 2021,
[17] T.M. Brunt, E. Lefrancois, T. Gunnar, A. Arponen, T. Seyler, A.E. Goudriaan, A. McAuley, D.A. McKeown, V. Detrez, J. Csorba, D. Deimel, V. Auwarter, J. Kempf, S. Karolak, T. Nefau, Substances detected in used syringes of injecting drug users across 7 cities in Europe in 2017 and 2018: The European Syringe Collection and Analysis Project Enterprise (ESCAPE), Int J. Drug Policy 95 (2021) 103130, https://doi.org/10.1016/j.drugpo.2021.103130.
[18] E.M.Cf Drugs, D. Addiction, Stimulants – Health and social responses, European Monitoring Centre for Drugs and Drug Addiction 2021.
[19] E.M.Cf Drugs, D. Addiction, European drug report 2022 – Trends and developments, Publications Office of the European Union 2022. https://doi.org/doi/10.2810/75644.
[20] C. Giese, D. Igoe, Z. Gibbons, C. Hurley, S. Stokes, S. McNamara, O. Ennis, K. O'Donnell, E. Keenan, C. De Gascun, F. Lyons, M. Ward, K. Danis, R. Glynn, A. Waters, M. Fitzgerald, t. outbreak control, Injection of new psychoactive substance snow blow associated with recently acquired HIV infections among homeless people who inject drugs in Dublin, Ireland, 2015, Eur. Surveill. 20 (40) (2015) 30036, https://doi.org/10.2807/1560-7917.ES.2015.20.40.30036.
[21] N.K. Sin´ead McNamara, Siobhan Stokes and Eamon Keenan, Irish Syringe Analysis Pilot Project: The identification of current injecting trends in the Dublin and Midland Region through the application of syringe analysis methodology., 2022,
[22] P. Mj, R. S, S. Htd, M. F, The Cathinone Hydra: Increased Cathinone and caffeine adulteration in the English MDMA market after Brexit and COVID-19 lockdowns, 20503245221099209, Drug Sci., Policy Law 8 (2022), https://doi.org/10.1177/20503245221099209.
[23] C. Cole, L. Jones, J. McVeigh, A. Kicman, Q. Syed, M. Bellis, CUT: A Guide to Adulterants, Bulking Agents and Other Contaminants Found in Illicit Drug, 2010.
[24] B. Pardo, Insights Into Mixing Fentanyl and Benzodiazepines From Canadian Drug Seizures, JAMA Psychiatry 79 (1) (2022) 81–83, https://doi.org/10.1001/jamapsychiatry.2021.3292.
[25] S. Nordmann, V. Pradel, M. Lapeyre-Mestre, E. Frauger, V. Pauly, X. Thirion, M. Mallaret, E. Jouanjus, J. Micallef, Doctor shopping reveals geographical variations in opioid abuse, Pain. Physician 16 (1) (2013) 89–100, https://doi.org/10.36076/ppj.2013/16/89.