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On-site identification of psychoactive drugs by portable Raman spectroscopy during drug-checking service in electronic music events

ENRICO GERACE[1], FABRIZIO SEGANTI[1], CLEMENTE LUCIANO[1], TONIA LOMBARDO[1], DANIELE DI CORCIA[1], HEINO TEIFEL[2], MARCO VINCENTI[1,3] & ALBERTO SALOMONE[1,3]

[1]*Regional Antidoping and Toxicology Center "A. Bertinaria", Turin, Italy,* [2]*Thermo Fisher Scientific, Munich, Germany, and* [3]*Department of Chemistry, Universiy of Turin, Turin, Italy*

Abstract

Introduction and Aims. *Hundreds of new psychoactive substances (NPS) have burst into the marketplace, making both the scientific community and people who use drugs lacking of adequate information about their diffusion and effects. In this scenario, drug-checking services have been recently proposed to assist harm reduction policies and provide a global description of the circulating drugs.* ***Design and Methods.*** *The results obtained by a portable Raman spectroscopy device on 472 alleged drugs within the first formal implementation of drug checking in Italy, are reported. The testing was made through a plastic bag held by the applicant and containing the alleged drug. The substance identification was executed by comparison with a spectral library.* ***Results.*** *Illicit substances were detected in 304 samples. Findings included MDMA (106 samples), ketamine (87 samples), cocaine (51 samples), amphetamine (47 samples), methamphetamine (two samples), heroin (two samples) and NPS (nine samples). Two samples were identified as precursors of psychoactive substances. Identification of a non-controlled substance occurred in 38 samples. Output of inconclusive result was recorded from 128 samples tested on-site, from which the applicant allowed us to collect a small portion in 68 cases, for a delayed laboratory analysis by GC–MS or LC–MS/MS.* ***Discussion and Conclusions.*** *Drug checking by Raman spectroscopy proved effective to identify psychoactive drugs including NPS and track the drug distribution in various recreational settings. The field testing activity revealed the presence of several NPS in the nightlife scenario, often in replacement of traditional illicit drugs, thus posing a high overdose risk and a life-threatening situation.* [Gerace E, Seganti F, Luciano C, Lombardo T, Di Corcia D, Teifel H, Vincenti M, Salomone A. On-site identification of psychoactive drugs by portable Raman spectroscopy during drug-checking service in electronic music events. *Drug Alcohol Rev* 2019]

Key words: drug checking, Raman, NPS, harm reduction.

Introduction

Nightlife plays an essential role in the personal growth of youth and allows easy social interactions for people of any age around the world. Although nightlife is commonly associated with celebration, festivals and a sense of group identity, it also provides the setting for risk taking and experimentation, especially regarding the consumption of alcohol and drugs [1]. While the drug scenario has remained basically unchanged through the 20th century, the first two decades of the new millennium are facing the emergence of a new phenomenon, identified with the "NPS" acronym worldwide. As a matter of fact, hundreds of new psychoactive substances (NPS) have burst into the marketplace in recent years, making both the scientific community and users lacking in adequate information about the effects of these new drugs. Synthetic cathinones (i.e. 'bath salts') raise particular concern because some of these drugs (e.g. mephedrone, methylone) are trafficked as replacements for ecstasy [2,3], but they entail unpredictable and often unknown adverse effects. Likewise, it occurs that other classes of 'traditional drugs', for example, hallucinogens or heroin, are also replaced or added with new designer compounds, making many drug users unintentionally or unknowingly using synthetic NPS. Even when NPS are intentionally purchased online, substantial risk exists that they are mislabeled, either because they contain chemical analogues of the ordered drug

Enrico Gerace PhD, Researcher, Fabrizio Seganti Researcher, Clemente Luciano Researcher, Tonia Lombardo Technician, Daniele Di Corcia Researcher, Heino Teifel Sales Manager Safety and Security EMEIA, Marco Vincenti Full Professor, Alberto Salomone PhD, Associate Professor. Correspondence to Alberto Salomone, Centro Regionale Antidoping "A. Bertinaria", Regione Gonzole 10/1, 10043 Orbassano, Torino, Italy. Tel: +3901190224232; Fax: +3901190224244;
E-mail: alberto.salomone@antidoping.piemonte.it

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(e.g. 25B/C-NBOMe instead of 25I-NBOMe, pentedrone instead of 3,4-DMMC), or because the active ingredient differs from what was advertised on the website [4].

As an intervention of harm reduction within this context of drug use, a pill testing/drug checking service has been used in the nightlife context for the last 20 years. For many people who use drugs, drug checking is often the first point of contact with the social support system. Drug checking can be completed in a drug counselling centre and also on-site, for example, at parties, raves and festivals [5]. Pioneer reports of drug-, pill- and substance-testing have been published in recent years, describing the identification of the active ingredient, particularly NPS, in different contexts [6–11]. Even though some limitations of drug checking have been recently highlighted [12], it is hardly disputable that testing drugs before they are consumed involves three primary advantages: (i) adverse effects (including overdose) can be avoided by the consumer; (ii) institutions in charge of the problem (such as hospitals and testing laboratories) and public health authorities are made aware when a new substance breaks into the market; and (iii) a global picture of the circulating drugs is generated, with respect to the appearance time and the different geographical areas [13].

The most common analytical techniques used for drug checking include thin layer chromatography, gas chromatography–mass spectrometry (GC–MS), liquid chromatography (LC), Raman spectroscopy, colorimetric tests, infrared spectroscopy and nuclear magnetic resonance. Most of these analytical techniques offer adequate performances: high specificity, good sensitivity, versatility with different matrices, quantitative analysis and comprehensive libraries [5]. Unfortunately, most of them are not portable and require extensive operators' training. Moreover, they involve some handling of the analysed material and its destruction, with consequent legal hurdles to overcome.

The aim of our study was to identify the drugs purchased and commonly used by partygoers and music festivals attendants, using a Raman-based portable instrument. Raman spectroscopy is commonly used in chemistry to provide a structural fingerprint by which molecules can be identified [14]. In most cases, sample preparation is minimal or unnecessary, allowing for the non-destructive *in situ* analysis of tablets, powders and liquids. The aforementioned features are particularly important with regard to the speed of analysis, prevention of sample contamination and preservation of evidential material [15]. Moreover, the analysis can be performed through the drug-containing envelope, avoiding any contact with the operator.

Over the past decade, there have been numerous reports detailing the use of Raman spectroscopy to screen for drugs [16–20], and more recently a very few exploratory studies investigated the identification of NPS [21–25]. The identification of NPS aimed at risk assessment and drug control poses a great analytical challenge, due to the wide number of NPS already identified, the presence of adulterants in them, or the presence of NPS themselves as adulterants added to traditional drugs (e.g. MDMA), and the continued emergence of new and unknown chemical substances [25].

In this paper, we report the results obtained by the analysis of 472 alleged drugs, tested within the first formal implementation of drug checking in Italy by means of a portable Raman device. All samples were tested during 27 night events, including electronic dance music festivals, rave parties, Goa parties and street parades in the Italian territory, during 2016 and 2017. The hazard caused by the unaware intake of drugs will be also highlighted.

Experimental

Drug checking procedure

The drug-checking protocol consisted of the following steps: (i) the substance to be tested is voluntarily taken by the user (or by someone on his/her behalf) to the drug-checking service, where he/she is informed about the procedure. The alleged drug is not actually handled by any social workers or technicians. The person requesting drug checking inserts a small amount of the compound in a plastic bag and he/she gets it back after the analysis is completed, with no need of disposal; (ii) a picture of the substance is taken; (iii) rapid analysis (less than 2 min) with the portable Raman instrument is performed; (iv) if the active ingredient or the main component is identified, the user is immediately informed about the result; (v) in case a NPS is identified, a general warning accessible to all the participants at the event is released; (vi) if the analysis result is displayed as 'inconclusive', there no evidence that a prohibited substance is present in the sample, making it manageable by the operator; (viii) thus, a small sample aliquot is collected, anonymised and transferred to the lab for deferred GC–MS or LC–MS analysis; viii) a final report is published on a specific website; and (ix) in case a NPS is identified, a report is prepared and sent to the National Early Warning System.

Raman instrumentation

On-site drug checking was performed using a ThermoScientific TruNarc® (Munich, Germany) portable Raman

analyser, running library version v1.6, equipped with a 785-nm Class IIIB laser at 250 mW. Raman spectra in the interval 300–1800 cm^{-1} were recorded. The identification of the substances was performed by comparing the spectrum of the unknown compound with those present in a proprietary library containing traditional drugs, NPS, cutting agents and precursors. When the compound is identified, the result is directly shown on the instrument display, with no need of a connected PC. The results are color-coded to highlight four circumstances: (i) the instrument identifies one or more controlled substances (alarm result, red colour); (ii) the instrument identifies one precursor used in the manufacturing of illegal drugs (warning result, orange colour); (iii) the instrument does not identify any controlled substances, but recognises a legal cutting agent present in its library (clear result, green colour); (iv) the instrument does not identify any of the controlled substances, precursors or cutting agents present in its library (inconclusive result, grey colour). The terminology used to categorise the results (alarm, warning, clear) is not referred to a possible health hazard but rather meant to inform the typical user of the instrument (e.g. law enforcement, customs) about the legal state of the identified compound.

GC–MS and LC–MS

Sample preparation and screening analysis for unknown substances were performed using a previously published method [26]. Briefly, a 6890 N GC apparatus (Agilent Technologies, Milan, Italy) equipped with a 17-m fused-silica capillary column (J&W Scientific HP-5), of 0.2-mm inner diameter and 0.33-μm film thickness. Helium was employed as the carrier gas at a constant pressure of 23.24 psi. The GC oven temperature was set at 90°C for 1 min and then raised to 180°C with a 30°C/min heating rate. The oven temperature was maintained at 180°C for 7 min and then raised to 315°C with a 15°C/min heating rate. The GC injector and transfer line were maintained at 280°C. Full scan spectra in the interval 40–650 amu were acquired using a 5975 inert mass-selective detector (Agilent Technologies, Milan, Italy) operating in the EI mode at 70 eV. The qualitative identification of underivatised compounds was initially performed by comparing the full scan spectra obtained with those recorded in updated spectra libraries (PMWTox2, SWGDRUG version 2.5, AAFS2012, CaymanSpectraLib), and then by comparing the retention times and relative abundances of the diagnostic ions obtained from a reference standard.

Targeted analysis for selected NPS was performed using a previously published method used for hair analysis [27]. Briefly, the analyses were performed using an Agilent 1290 Infinity LC system (Agilent, Palo Alto, CA, USA), interfaced to a QTRAP® 4500 mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with an electrospray Turbo Ion source operated in the positive ion mode. A Zorbax Eclipse Plus C18 RRHD column (100 mm × 2.1 mm, 1.8 μm), protected by a C18 pre-column, was used for the separation of the target analytes. The column oven was maintained at 45°C and the elution solvents were water/formic acid 5 mM (solvent A) and acetonitrile/methanol 80:20 plus formic acid 5 mM (solvent B). After an initial isocratic elution at 95% A for 0.5 min, the mobile phase composition was varied by a linear gradient (A:B; v/v) from 95:5 to 45:55 in 2.5 min; then isocratic elution at 55% B was maintained for 0.5 min. The flow rate was 0.5 mL/min and the total run time was 5.5 min including re-equilibration at the initial conditions before each injection. MS/MS detection was executed in the selected reaction monitoring mode.

Results and Discussion

Altogether, 472 samples were analysed using the ThermoScientific TruNarc portable Raman analyser, mostly powders, crystals and pills. A summarised description of all results is presented in Figure 1. Taking into account all the conclusive results (alarm, clear and warning coded), a total of 344 samples (72.9%) were identified by the TruNarc instrument, generally in less than 2 min per sample. In about three cases out of four, the offered drug-checking service proved to yield prompt answer to the subject willing to test the alleged drugs in his/her possession.

Detection of illicit substances (code: alarm result). Illicit substances were detected in 304 samples (64.4%). Positive findings included MDMA (106 samples), ketamine (87 samples), cocaine (51 samples), amphetamine (47 samples), methamphetamine (two samples), heroin (two samples) and NPS (nine samples; 3%). Among NPS, the instrument identified mephedrone (two samples), methylone, 4-fluoroamphetamine (4-FA), 2,5-dimethoxy-4-chloroamphetamine (DOC), 4-methylethcathinone (4-MEC), mexedrone, methoxyphenidine and a mixture 4-FA/methylone.

Detection of precursors of psychoactive substances (code: warning result). Two samples were identified as precursors of psychoactive substances. Specifically, one was recognised as norephedrine and one as pseudoephedrine. For all traditional drugs, the result matched the

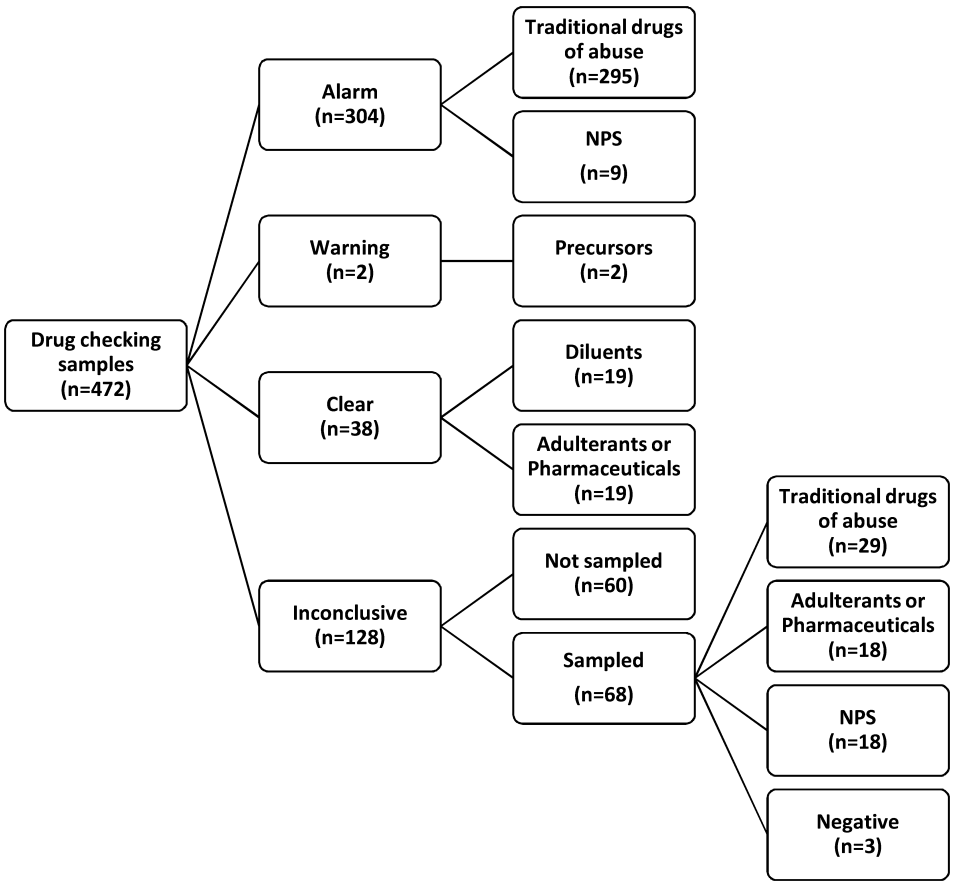

Figure 1. *Summary of findings during the drug-checking activity.*

expected results anticipated by the subject who volunteered to drug-check the substance he/she was about to use. Even though a correlation with other independent methods of analysis for all samples would have been beneficial, we decided to not transfer the identified illicit drug to the laboratory.

Detection of non-controlled substances (code: clear result). Thirty-eight samples (8.0% of the total) were recognised as non-controlled substances. The identified compounds included caffeine (10 samples), dipyrone (three samples), lidocaine, procaine, baking soda, calcium carbonate, cellulose, corn starch, lactose, epsom salt, polyethylene, mannitol and sodium sulphate. When caffeine or a cocaine cutting agent (e.g. mannitol) was identified, two analogous scenarios were plausible: either a fake drug was packaged, or the active substance (cocaine or speed, i.e. amphetamine) was diluted in a predominant amount of cutting agent, so that the illicit substance was masked in the analysis. On the other hand, the identification of licit drugs with psychoactive effects (e.g. dipyrone, which was sold as MDMA during an event), still poses a significant health concern, because the user is not aware of the real composition of the substance he or she is taking.

Inconclusive results. One hundred and twenty-eight tested samples (27.1% of the total) produced an inconclusive result as none of the controlled substances, precursors or cutting agents present in the TruNarc library matched the experimental spectrum. In 68 cases, the user allowed us to sample a small part of the dose to perform a delayed laboratory analysis by GC–MS or LC–MS/MS. Notably, no law infringement was committed in the sampling, as no occurrence of illicit drugs had been evidenced. A summary of the results is presented in Table 1.

In 29 cases (43%), a traditional drug was identified, including amphetamine, MDMA, heroin or LSD. It is likely that these drugs were not previously identified by the Raman instrument because of their low concentration. In 18 cases (26%), adulterants/diluents or licit pharmaceuticals drugs were detected, including caffeine, acetaminophen, dipyrone, modafinil, lidocaine, metronidazole and oxycodone. In three samples, no active ingredient or pharmaceutical drug was identified. The remaining 18 samples were found to contain a NPS. The active ingredients found were 5-MeO-MiPT (5-Methoxy-N-methyl-N-isopropyltryptamine; four cases), 2-CI (2,5-Dimethoxy-4-iodophenethylamine;

Table 1. *GC–MS and LC–MS analysis for samples with inconclusive result after drug checking by TruNarc®*

Substance	Number of cases	Notes
Traditional drugs		
Amphetamine	11	
MDMA	9	
Heroin	4	
LSD	3	Too fluorescent for Raman detection
Opium	1	Too fluorescent for Raman detection
Cocaine	1	Found in traces
Total	**29 (42.6%)**	
Adulterants/diluents pharmaceuticals		
Caffeine	8	Found alone or in combination with traces of AMP
Lidocaine	2	
Modafinil	2	Not present in TruNarc library
Acetaminophen	1	
Dipyrone	1	
Metronidazole	1	Not present in TruNarc library
Levomepromazine	1	Not present in TruNarc library
Buprenorphine	1	In combination with lidocaine
Oxycodone	1	
Total	**18 (26.5%)**	
NPS		
5-MeO-MiPT	4	Not present in TruNarc library
2C-I	2	Blot samples
MXE	2	
25B-NBOMe	2	Blot samples
25I-NBOMe	1	
4-AcO-MET	1	Not present in TruNarc library
4-FA	1	
2C-B	1	
DOC	1	Blot sample
DOM	1	Blot sample
DMT	1	Dry herb
Pentylone	1	
Total	**18 (26.5%)**	
Negative samples	**3 (4.4%)**	
Total samples	**68 (100%)**	

GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; NPS, new psychoactive substances.

two cases), MXE (Methoxetamine; two cases), 25B-NBOMe (two cases), 2-CB (2,5-dimethoxy-4-bromophenethylamine), 4-AcO-MET (4-Acetoxy-N-ethyl-N-methyltryptamine), 4-FA, 25I-NBOMe, DOC, DOM (2,5-Dimethoxy-4-methylamphetamine) and DMT (N,N-Dimethyltryptamine). Overall, 27 samples out of 472 (5.7%) proved to contain at least one NPS.

The possible reasons why the TruNarc was unable to identify the NPS in 18 cases out of 27 were either: (i) their low amount/concentration; (ii) the high fluorescence of the main component, corresponding to the active ingredient itself or some excipient/additive/filler; or (iii) the lack of their Raman spectrum in the current library. While the third issue can be easily handled by updating the library periodically, to keep pace with the introduction of NPS into the black market (or at least their analytical characterisation), the first two limitations appear not to be easily overcome, since they require further hardware or software improvement. In particular, two important challenges when using Raman spectroscopy are: (i) the presence of more than one active ingredient, because the Raman spectroscopy will only report the most abundant chemical in a mixture; and (ii) the interference of fluorescence, which can eventually mask the Raman signal completely and result in a significant amount of background noise. Fluorescence is encountered particularly with plant-based narcotics, and substances that are pigmented with an array of colours, insomuch that drugs like heroin and illicit tablets that contain pigments and binders are challenging, and results from plant material like marijuana are impossible to generate [28]. Changing the operating wavelength is surely a promising future development [24,28], basically designing systems at higher excitation wavelengths (e.g. from 785 to 1064 nm), in order to minimise fluorescence. However, more Raman scattering will occur with the more energetic excitation wavelength (i.e. at 785 nm) [29].

Finally, we observed that the presence of NPS in the tested samples was often in disagreement with the user's expected result (see Table 2). The replacement of traditional drugs with NPS is a well-known problem of the illegal market context. In previous studies [30–32], we combined surveys and hair analysis to demonstrate that people who are using MDMA are often taking NPS without being aware. Quite often, alleged ecstasy crystals or pills, believed to contain only MDMA, were composed either entirely or partially by some NPS. In the present study, 15 samples out of 121 (i.e. 12.4%) expected to be MDMA turned out to be an unexpected NPS. While some were simply 'fake drugs' with no active ingredient in it and consequently represent a minor hazard, others were containing some NPS. In this study, we found that also alleged hallucinogens are not containing LSD, that is, the substance expected by the applicant. As a matter of fact, it is common belief of most 'hallucinogen' buyers to acquire a traditional 'acid', whereas it is actually very likely that a compound of the NBOMe series is spotted on the blot, in place of LSD, with highly magnified effects. This scenario, in which an unexpected drug is used without knowing its real effects, represents a

Table 2. *Cases of discordant result between user declaration and instrumental analysis outcome*

Expected drug	Found drug	Samples (*n*)
MDMA	Amphetamine	3
MDMA	Heroin	1
MDMA	Buprenorphine + Lidocaine	1
MDMA	Metronidazole	1
MDMA	Levomepromazine	1
MDMA	Lidocaine	2
MDMA	Baking soda	1
MDMA	Dipyrone	1
MDMA	5-MeO-MiPT	2
MDMA	Methylone	1
MDMA	None	1
Amphetamine	Caffeine	7
Amphetamine	Methylone +4FA	1
Amphetamine	Modafinil	1
Amphetamine + Mescaline	5-MeO-MiPT	1
Amphetamine	MDMA	1
Ketamine	Cocaine	1
Ketamine	Pseudoephedrine	1
LSD	25I-NBOMe	1
LSA	25B-NBOMe	1
Mescaline +2C-B (aka 'Solaris')	25I-NBOMe	1
Psilocybin	DOC	1
Tryptamine	4-AcO-MET	1
PMA	Ketamine	1
Mescaline	2C-B	1
Unknown (generic 'Legal High')	4-FA	1
Bk-2CB	Pentylone	1
Generic NBOMe	2C-I	2
DMT	Dipyrone	1
	TOTAL	**40**

further risk to the health of people who use illicit substances. Undoubtedly, the pharmacological effects of NPS are frequently much more potent than the drugs they are intended to mimic and this increases the risk of overdose and death [33,34].

Conclusions

The present study has a double value, one of which is merely technical and the other essentially social. In a pioneering activity, the portable Raman-based ThermoScientific TruNarc instrument was used for an extended on-site drug-checking investigation, that is, without prosecuting commitment. Drug checking by Raman spectroscopy assisted to explore the drug distribution found in various recreational settings at different times and proved effective in the identification of several psychoactive drugs, including NPS. In particular, portable Raman instrumentation demonstrated several advantages within these contexts, because it allows the direct sample analysis through water, glass and plastic bags, avoiding direct contact with the substance, and it is non-destructive, non-invasive and fast. Moreover, it does not require specific facilities and power supply. All these features proved essential for the fulfilment of our project's goal, which aimed to NPS qualitative identification. For the future, the regular update of the instrument's library with spectra collected from the most recent NPS remains an essential requirement. Other analytical limitations of the RAMAN instrument were related to its limited sensitivity and occasional background fluorescence, especially for heroin. Future development of research should be aimed to: (i) estimate the limit of detection for different drugs in different matrices, especially when a mixture of active ingredients is present; and (ii) verify the detectability of other classes of narcotics. In particular, the presence of novel synthetic opioids as possible adulterants of heroin looks of utmost importance in the harm reduction projects.

This project also represented the first formal implementation of a drug-checking activity in the Italian territory. The field testing activity revealed the presence of several NPS in the nightlife scenario, often in replacement of the traditional illicit drugs. Since several of these substances are potentially more toxic than the usual recreational drugs, their intake poses a high overdose risk and a life-threatening situation, especially for unaware users. With this said, the identification of a particular substance (either suspected or unsuspected) does not necessarily equate to harm, given that drug use and individual health risks are also influenced by various other factors, such as social contexts, route of administration and dose. Therefore, drug checking is a service that has to be integrated in outreach interventions or in services that offer drug prevention or harm reduction advices and counselling. In conclusion, in order to pursue a real harm reduction policy in different nightlife contexts, we envision that national governments would authorise and co-ordinate the work of local organisations able to offer efficient and comprehensive drug-checking services with the medical staff appointed to provide counselling and emergency intervention.

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Conflict of Interest

The authors have no conflicts of interest.

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