Monitoring New Psychoactive Substances use through wastewater analysis: current situation, challenges and limitations

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Abstract

New Psychoactive Substances (NPS) are compounds that produce similar effects to those induced by illicit drugs (ID), such as cocaine, cannabis and amphetamines, but are not strictly regulated by international conventions. The consumption of NPS is a growing public health problem in many communities. However, there is little knowledge regarding the extent and actual use of these new substances. Monitoring NPS use is arduous and, therefore, different sources of information need to be used to get more insight of the prevalence and diffusion of NPS use. Analysis of pooled urine (PU) and wastewater (WW) shows strong potential, giving a different and complementary light on this issue, although presents some limitations and challenges that must be taken into account. Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) is one of the most powerful approaches for screening a large number of NPS because of the accurate-mass full-spectrum acquisition measurements. By using a comprehensive and updated NPS database, LC-HRMS is flexible enough to confront the ever-changing NPS market. In this "current opinion", we give our point of view on the usefulness of PU and WW analysis, and on the potential application of wastewater-based epidemiology as source of information for NPS use, explaining the main bottlenecks and future perspectives in this emerging research field.

Keywords New Psychoactive Substances, pooled urine, urban wastewater, wastewater-based epidemiology, mass spectrometry

Introduction

New Psychoactive Substances (NPS) can be defined as substances that produce similar effects to those induced by illicit drugs (ID) such as cocaine, cannabis and amphetamines, but are not strictly regulated by international conventions [1]. Although many NPS are synthesized introducing only minor modifications to the chemical structures of controlled substances, the term 'new' does not directly refer to 'newly developed' chemicals, but to 'newly misused' substances [2]. The NPS market is, therefore, very dynamic creating, quickly, new alternative substitutes. Hence, the Early Warning System (EWS) of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported more than 670 different NPS between 2005 and 2017 [3]. NPS can be classified in different categories depending on their structural back-bone. Cathinones and synthetic cannabinoids are most often reported, but also benzodiazepines, arylcyclohexylamines, phenethylamines and synthetic opioids were found (**Figure 1**) [3]. These new drugs have become easily available to the general public mainly through e-commerce, and are considered a growing problem in many communities since they are responsible for numerous fatal intoxications [4–6]. Although several countries have suffered the emergence of NPS *i.e.* use and harms, not all governments have been able to act upon all of them in an effective way in terms of penalizing its supply and use [7,8].

Understanding the extent and actual use of NPS is important for healthcare professionals and toxicologists to assess the risks associated, but also for policy makers to help orient prevention and define law enforcement activities. Different sources of information, such as general population surveys [9–11], EWS [3], internet [12], seizure data [13–16] and the analysis of biological samples (urine of users from hospital emergency rooms, post-mortem fluids...) [17–20], can be consulted to get insight of the prevalence and diffusion of NPS use.

A recent approach that shed a different light on this issue is the analysis of pooled urine (PU) and urban wastewater (WW) samples. PU and WW analysis can provide anonymized, but comprehensive and objective information, on community-wide use of NPS [21–24]. The wastewater-based epidemiology (WBE) approach relies on the fact that traces of almost everything humans consume are excreted, unaltered or as metabolites, via urine or feces [25]. Thus, the determination of appropriate urinary excretion products (biomarkers) and subsequent concentration data in WW can be used to estimate illicit and licit drug use by a population [25,26]. The Sewage analysis CORe group Europe (SCORE) has promoted and coordinated WBE campaigns for the worldwide monitoring of ID consumption since 2011 [27–29] reporting the results to the EMCDDA, who considers WBE as a complementary source of information to the conventional indicators on drug use. In addition, the Australian Criminal Intelligence Commission (as part of their drug monitoring program:

https://www.acic.gov.au/publications/intelligence-products/national-wastewater-drug-monitoring-program-report [30]), as well as New Zealand and China have set up strategies to implement such studies in their countries. For the proper application of WBE, however, several key aspects such as the selection of suitable and unique biomarkers and excretion rates need to be taken into account in order to obtain population-normalized quantitative data *i.e.* information on amounts consumed [31–34]. WBE has been successfully applied to the monitoring of tobacco [35,36], alcohol [37] and ID use [27,28,33,38], and has the potential to detect and discover newly consumed NPS [25,26,39,40].

In this review, we give our viewpoint on the monitoring of NPS in PU and WW, and the potential application of WBE in this field, with special emphasis on challenges and limitations. Finally, future perspectives are briefly presented. The analysis of PU has been included in this paper due to the very few studies available of NPS in WW (in comparison to conventional ID) and the challenges to obtain information of NPS use from WW, as explained later in the manuscript. In addition, searching for NPS in PU can provide useful and complementary information on this topic.

Analytical challenges for monitoring NPS

The ever-changing nature of NPS poses a challenge for analytical forensic laboratories. The NPS market is very dynamic and the rapid introduction of new substances makes it highly difficult to keep the analytical methodologies up to date. The detection, identification and quantification of NPS is time-consuming, complex and expensive. However, identifying the new substances that are appearing in the market is the first necessary step in assessing the risks associated with these substances and in controlling potentially dangerous new drugs. Under these circumstances, the analysis of commercially available products (sometimes known as 'legal highs') provides updated information of the compounds possibly consumed. A combination of several techniques, such as NMR, HRMS, GC-MS, X-ray crystallography, FTIR, ultraviolet and circular dichroism, is often needed for a full characterization and true confirmation of the identity of unknown new drugs [41–46].

The continuous appearance of new substances joined to the limited availability of reference standards and difficulties to purchase them make the development of quantitative target methods somehow a limited approach and non-affordable task when monitoring hundreds of changing NPS. Therefore, there is an increasing interest in developing qualitative screening methodologies able to detect and identify a large number of compounds. The hyphenation of liquid chromatography (LC) with high resolution mass spectrometry (HRMS) is one of the most powerful approaches to this aim [47–49]. LC-HRMS appears as the technique of choice due to the polar character of most NPS, especially of metabolites, and the useful information contained in accurate-mass full-spectrum acquisition data.

The main reason for the shift toward qualitative, suspect screening methodologies based on LC-HRMS, is that there is, in principle, no need of reference standards for tentative identifications and the list of compounds that can be searched is only limited by the suspect screening database [39,47,48,50,51]. To help in the identification of NPS, a new web-based database (NPS Data Hub) has been developed with the aim to elicit data from the forensic laboratories to facilitate identification of unknown substances [52]. In this way, the time for valuable data to be accessible to analytical laboratories for identification of newly emerging compounds is notably reduced. Analytical data of any type can be added for a given compound, but the mostly applied techniques are NMR, (HR)MS and IR/Raman. The combination of a compound database and HRMS spectral library represents a useful tool for the identification of NPS in forensic HRMS-based screening applications [53].

If the identification of NPS in commercially available products (herbal blends, powder, pilots, crystals, etc.) is complex, the detection and identification of NPS residues in urine samples is even more challenging. Unfortunately, most of the techniques mentioned above are not useful in this type of analysis due to the low analyte concentrations in the samples, and the complex nature of the urine matrix with endogenous components being at concentrations much higher than the NPS potentially consumed. In addition, the low rate of positive findings when analyzing individual urine samples complicates even more the monitoring of NPS. To this aim, the analysis of pooled urine samples from hundreds (or thousands) of individuals at specific settings with higher probability of NPS consumption is preferred. Nightlife areas, music festivals or local festivities are strategic locations for the collection of PU samples from the inner container of pissoirs or portable toilets. The likelihood of having NPS consumers among all PU contributors increases the rate of success in identifying NPS consumed.

Additional difficulties appear in the investigation of NPS in wastewater, mainly because of the extremely low concentrations of NPS due to the lower consumption in comparison with popular, conventional ID, and to the high dilution factor in WW. The main drawback of LC-HRMS screening of NPS in PU or WW comes from its lower sensitivity compared to target quantitative methods (e.g. by LC-MS/MS QqQ), an aspect that is crucial in this field. In addition, strong ionization suppression commonly occurs on the analyte signal in these complex matrices. For this reason, the target quantitative methods (e.g. LC-MS/MS with triple quadrupole (QqQ)) are still valuable, although they are restricted to the limited target list of compounds included in the scope of the method, with the corresponding reference standards being required for method optimization, data acquisition and quantification [23,24,49,54–57].

Another relevant issue is NPS metabolism, which plays a key role for the selection of appropriate biomarkers (parent compound or metabolites) for monitoring NPS in PU or WW. Due to the general

lack of information on metabolic pathways for many NPS, there is a great interest in the scientific community to perform metabolic studies to identify compounds proposed as target compounds in urine or in WW [58–62]. However, even if information of the major metabolites is available from the literature, their analysis can be complicated due to the lack of reference standards, and therefore only tentative identifications may be possible using HRMS.

Investigation of NPS in pooled urine

The analysis of urine from intoxication cases or potential consumers seems, *a priori*, a suitable source of information for the monitoring of NPS [5,49]. However, it is not easy to obtain these samples, and the consent of the users or family members is required. The analysis of PU collected from places with higher probability of NPS consumption (e.g. discotheques, music festivals or nightlife areas) can give a more realistic picture of the NPS situation within a population. Besides, samples are anonymized and ethical issues are limited [63,64].

Table 1 summarizes the studies on PU analysis for NPS reported in the last five years. The vast majority of these studies applied the potential of LC-HRMS for qualitative identification of NPS using time-of-flight (TOF) [48,65–67] or Orbitrap [68] mass analyzers. A few studies focused on a limited list of target compounds, which were quantified using low resolution mass analyzers (LC-MS/MS QqQ) [21].

The selection of specific settings for the analysis of PU increases the degree of success in the detection of NPS. For this reason, 60% of the studies reported data from music festivals because of the higher probability of drugs or NPS consumption [48,65–67]. Samples were collected from urine containers of pissoirs, or from portable toilets, resulting in an anonymous mixture of urines from an undetermined numbers of contributors. It is remarkable that most studies collected samples from pissoirs, resulting in cleaner samples than those collected in portable toilets. The latter are contaminated with feces and disinfection chemicals, which may have an unknown effect on NPS stability. Furthermore, it must be taken into account that pissoirs are designed for men, and thus only represent a part of the setting.

In these works, the most commonly detected NPS categories were synthetic cathinones and phenethylamines. It seems logical that mainly invigorating drugs were found since music festivals and nightlife locations are more prone to the intake of stimulant compounds. Paying attention to the individual NPS consumed, mephedrone [21,66,68] and ketamine [22,66–68] were the most reported drugs in PU analysis.

Investigation of NPS in wastewater

The application of WBE for the estimation of psychoactive substances consumption is mainly focused on ID [25], and has been scarcely applied to NPS. As mentioned in previous sections, the investigation of NPS in WW is very complicated due to several factors that make the full application of WBE to NPS still quite limited. The lack of information on excretion rates and metabolic pathways of NPS, and the very low concentrations in WW, are the main drawbacks. The majority of the published studies on NPS in WW only dealt with detections and concentrations, without producing either mass loads (i.e. concentrations multiplied by flow rates of WW) or normalized data to the population within the WW catchment area.

Table 2 summarizes the main developments in the monitoring of NPS consumption through WW analysis. The vast majority of reported studies applied solid phase extraction (SPE) for the pre-concentration of target compounds followed by LC-MS/MS (QqQ) analysis because of the enhanced sensitivity of this type of mass analyzers [23,24,49,54–57,69–77]. However, there are also studies using LC-HRMS [47–49,51,70,78–82]. Although back calculations to estimate the consumption of NPS by a population is complicated and for now unrealistic, the quantification of NPS (as in most of LC-MS/MS methods) may give a better comprehension of the actual use when comparing with the mass loads found for conventional ID.

Several studies focus the collection of samples on weekends, festivities or festivals, when higher concentrations of NPS in WW are expected [24,47,56]. In general, 24-hours composite samples are collected at the entrance of a wastewater treatment plant (WWTP).

The NPS most found in WW are synthetic cathinones. Thus, 21 out of the 30 reviewed studies reported positive findings of at least one synthetic cathinone, of which methylone [23,48,49,54,55,77–79,83–85] and mephedrone [23,24,49,54,73,75–77,79,81,84] were most often reported. Despite the fact that these compounds are currently illegal in many countries, they seem to be well-established in the drug market showing a recurrent detection in WW. Other NPS, such as synthetic cannabinoids, were scarcely detected [47,56,57,78,83], which could be related to the fact that synthetic cannabinoids are highly and quickly metabolized by humans [86,87], and therefore should be mostly found as major metabolites in WW. The particular case of synthetic opioids is of major concern because of the epidemic increase of opioids consumption over the last years, especially in the US [88], with alarming news stories in the ordinary press [89–91]. Recently, first detection of fentanyl and metabolites was reported by different studies in Europe and the US [72,77,92].

Some compounds included in Tables 1 and 2 might not be considered as NPS, as it is very difficult to differentiate these compounds being used illicitly or legally. For example, hordenine is present in beer but some studies considered this substance as a 'potential NPS' [22,47,65,66]. Also, ketamine is used for certain applications as veterinary and medical drug, but is considered as a recreational substance by the EMCDDA. Besides, as stated above, this organism defines NPS as 'newly misused' substances, which embraces these cases of chemicals intended for other purposes than for which it is originally developed.

The most of the scientific production about determination of NPS in WW is done over 2016 [48,51,76,77,81,82,85], 2017 [23,47,69,79,80,83] and 2018 [49,70–74,92,93], with Europe being the most productive region [23,24,47,48,51,54–57,71,72,74–76,79–82,93], followed by Australia [49,70,77,78,84,85]. Asia [69,94], US [92] and Africa [73] have barely applied strategies for NPS monitoring through WW analysis.

Future perspectives

Monitoring NPS use through PU and WW analysis is a challenge due to several factors: 1) their rapid transience on the drug market creates a scenario with constantly moving analytical targets; 2) the lack of data on NPS metabolism and pharmacokinetics i.e. for the selection of unique biomarkers and information on excretion rates; 3) the lack of data on stability of potential biomakers in urine and sewage; 4) the generally very low concentrations, because of the high choice for consumers in number of compounds, the low dose of some NPS and low prevalence in use, plus the elevated dilution factor of WW i.e. dilution of urine and feces with water used in households, industry, etc.; 5) the high sensitivity and selectivity required in the analytical methods, as a consequence of the low analyte concentrations and the complexity of the sample matrix.

Target quantitative methods based on LC-MS/MS QqQ, although limited by the target list of compounds, are useful because of the excellent sensitivity of this technique. However, LC-HRMS is the technique of choice for screening a large number of both NPS and metabolites. Hence, the maintenance of comprehensive and updated databases is essential. Data from surveys, police seizures, forensic analyses, as well as from EWS, and the scientific literature are necessary. The database should be fed with information from analysis of the products potentially consumed (e.g. herbal blends, crystals, pilots, powder purchased online or in smart shops), where non-targeted analytical strategies may be necessary to identify non-expected or unknown compounds, in order to include substances that are actually sold on the market. Furthermore, the inclusion of metabolites in

the database is pivotal for realistic studies, as it will allow focusing the analysis on those targets that are more likely present in urine and WW samples.

Figure 2 illustrates the different steps and topics that should be considered to get a comprehensive overview on NPS use, including analysis of WW and PU as one of the key issues.

As can be seen, the scenario around NPS use is rather complex. Lot of research is required in the next years to provide more information in different areas, with analytical chemistry playing a key role. Close collaboration is needed between different disciplines and actors that are relevant in the drugs scenario. This scenario includes not only collaboration between analytical chemists, but also toxicologists, health professionals, as well as police forces, national governments, national focal points and organizations like EMCDDA and UNODC.

Regarding WW analysis, more information is required for full application of WBE, such as excretion rates and stability of NPS in sewage, in order to obtain estimates of NPS consumed. Despite the limitations, data from screening WW (and PU) is highly valuable to understand the extent and actual use of NPS within certain populations, at least of those most widely consumed. In this context, HRMS screening of WW and PU collected from special settings (e.g. in festivals, near discotheques or nightclubs), where higher NPS consumption is expected, is a good strategy. The possibility to re-evaluate HRMS data in a retrospective way, without the need of additional analysis, is worth to noticing as it allows re-examine data previously obtained searching for new/additional compounds not considered in the initial analysis.

As illustrated in the workflow of **Figure 2**, different sources are needed to get a broad overview of NPS use. Data triangulation i.e. combining information obtained from PU and WW analysis with other sources, like survey data and forensic data, seems one of the best approaches nowadays to get a comprehensive insight on the NPS situation [49].

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542 **Table 1.** Summary of recently reported studies on NPS determination in Pooled Urine samples. (**4-chloro-α-PPP**: 4'-chloro-α-pyrrolidinopropiophenone **4-**
543 **FA**: 4-fluoroamphetamine; **5-APB**: 5-(2-aminopropyl)benzofuran; **α-PVP**: α-pyrrolidinovalerophenone; **BZP**: 1-benzylpiperazine; **M-234:** 1-phenyl-2-
544 (pyrrolidin-1-yl)pentan-1-ol; **M-264**: hydroxy-4-((1-oxo-1-phenylpentan-2-yl)amino)butanal; **TFMPP**: trifluoromethylphenylpiperazine)

Sampling area	Type of sample	Compounds [1]	NPS positive findings [1]	Analytical technique	Reference
United Kingdom (City of Westminster, London)	Pooled urine Weekend sampling Pissoir (male urinal)	1700 compounds (ID, NPS and *metabolites*)	ketamine, hordenine, d-norpseudoephedrine, methylhexanamine, 4-methylmethcathinone, methopropamine and *metabolites*, methoxetamine and *metabolites*	SPE, LLE LC-MS/MS [2] Qualitative	Archer, 2013 [22]
Norway (Oslo)	Pooled urine Sampling during festival Pissoir (male urinal)	ID, NPS	hordenine, 1-(2-methoxyphenylpiperazine), cathinone	UHPLC-QTOF Qualitative	Reid, 2014 [65]
United Kingdom (Night Club in London)	Pooled urine Weekend sampling Pissoir (male urinal)	900 compounds (ID, pharmaceuticals, steroids, NPS and *metabolites*)	mephedrone and *metabolites*, TFMPP and *metabolites*, 2-aminoindane	SPE, LLE, shoot techniques LC- MS/MS [2] Qualitative/Quantitative	Archer, 2014 [21]
United Kingdom (City of Westminster, London)	Pooled urine Weekend sampling Pissoir (male urinal)	ID, NPS	mephedrone, methylhexaneamine, methiopropamine, pipradol, cathinone, 5-APB, 4-methylethcathinone, TFMPP, 4-methylbuphedrone, methcathinone, ethylmethcathinone, d-norpseudoephedrine, ketamine, 1,4-methoxyphenylpiperazine, 4-fluoroephedrine	SPE UHPLC-LTQ Orbitrap Qualitative	Archer, 2014 [68]
United Kingdom (City center and festival) and Belgium (festival)	Pooled urine Weekend sampling in the city and during festivals Pissoir (male urinal)	1500 compounds (ID, NPS and *metabolites*)	MPA, methylone, ethylone, methedrone, mephedrone, *dyhidromephedrone*, *normephedrone*, 5-APB, ketamine, *norketamine*, *hydroxynorketamine*, *dehydronorketamine*, 4-FA, α-PVP, *M-264 and M-234 (α-PVP metabolites)*, hordenine, methoxetamine	UHPLC-QTOF Qualitative	Kinyua, 2016 [66]

Sampling area	Type of sample	Compounds [1]	NPS positive findings [1]	Analytical technique	Reference
Norway (Festivals)	Pooled urine Sampling during festivals Pissoir and portable toilets	Suspect screening: 1000 compounds (including ID, pharmaceuticals and 16 NPS) Target: 51 compounds (including synthetic cathinones, phenethylamines, ketamine and phencyclidine-type sustances)	methylphenidate, BZP	SPE UHPLC-QTOF Qualitative	Baz-Lomba, 2016 [48]
Denmark (Festival, Roskilde)	Pooled urine Sampling during festival Portable toilets	467 compounds (ID, NPS and *metabolites*)	ketamine, methylphenidate	SPE UHPLC-QTOF Qualitative	Hoegberg, 2018 [67]

[1] NPS metabolites highlighted in italic letters.

[2] No information available about the specified analytical technique used

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Table 2. Summary of recently reported studies on NPS determination in WW samples. (**2C-B**: 4-bromo-2,5-dimethoxyphenethylamine; **25H-NBOMe:** 2,5-dimethoxyphenethylamine; **3,4-DMMC**: 3,4-dimethylmethylcathinone; **4-FMC**: 4-fluoromehcathinone; **4-MEC**: 4-methylcathinone; **4'MePHP**: 4' -methyl-α-pyrrolidinohexanophenone; **5F-APINACA:** N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide; **α-PVP**: α-pyrrolidinovalerophenone; **MA-2201**: 1-(5-fluoropentyl)-3-(naphthalen-1-oyl)indole; **BZP**: 1-benzylpiperazine; **CP47,497**: 2-[(1S,3R)-3-Hydroxycyclohexyl]-5-(2-me thyl-2-octanyl) phenol; **JWH-018**: 1-Naphthyl (1-pentyl-1H- indol-3-yl) methanone; **JWH-073**: 1-naphthyl (1-butyl-1H-indol-3-yl) methanone; **JWH-122**: 4-Methyl-1-naphthyl) (1-pentyl-1H-indol-3-yl) methanone; **JWH-210**: (4-Ethyl-1-naphthyl)(1- pentyl-1H-indol-3-yl) methanone; **L-759,633**: (6aR,10aR)-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; **mCPP**: 1-(3-chlorophenyl)piperazine; **MDA**: 3,4-methylenedioxyamphetamine; **MDEA**: 3,4-methylenedioxyethylamphetamine; **MDPV**: methylenedioxypyrovalerone; **MPA**: methiopropamine; **PMA**: 4-methoxyamphetamine; **PMMA**: 4-methoxymethamphetanime; **TFMPP**: trifluoromethylphenylpiperazine; **U-47700:** 3,4-Dichloro-N-[(1R,2R)-2-(dimethylamino)cyclohexyl]-N-methylbenzamide; **UR-144**: (1-pentylindol-3-yl)-(2,2,3,3-tetramet hylcyclopropyl)methanone)

Sampling area	Type of sample	Compounds [1]	Positive NPS findings [1]	Analytical technique	Reference
Australia (Adelaide)	24 h composite	MDMA, methcathinone, mephedrone, methylone, MDPV, BZP, TFMPP	methcathinone, mephedrone, methylone, MDPV, BZP, TFMPP	SPE UHPLC-Qtrap Quantitative	Chen, 2013 [84]
Norway (Oslo, Bergen, Harmar)	72 h composite Weekend sampling	14 NPS (synthetic cathinones, *metabolites* of synthetic cannabinoids and phenethylamines)	d-norpseudoephedrine, pseudoephedrine, *JWH-018 N-5-hydroxypentyl*	SPE UHPLC-QqQ Quantitative	Reid, 2014 [56]
Belgium (Antwerp, Boechout, Ninove, Ruisbroek, Zele) and Switzerland (Zurich)	24 h composite	methoxetamine, butylone, ethylone, methylone, MPA, PMMA, PMA	methoxetamine, butylone, ethylone, methylone, PMMA	SPE LC-QqQ Quantitative	Kinyua, 2015 [55]
South Korea (Busan, Ulsan, Changwon, Kimhae, Milyang)	24 h composite	17 compounds (ID, ketamine, *norketamine*, mephedrone and methylone)	None	SPE UHPLC-Qtrap Quantitative	Kim, 2015 [94]
Greece (Santorini Island)	Grab	10 NPS (synthetic cannabinoids, cathinones, piperazines and pyrrolidophenones)	JWH-210, JWH-122, α-PVP, CP47,497	SPE UHPLC-QqQ Quantitative	Borova, 2015 [57]

Sampling area	Type of sample	Compounds [1]	Positive NPS findings [1]	Analytical technique	Reference
Croatia (Zagreb, Vinkovci, Velika Gorika)	24 h composite and grab	25 NPS (mainly synthetic cathinones and other substituted phenylalkylamines)	flephedrone, methylone, methedrone, mephedrone, ketamine, *norketamine*	SPE LC-QqQ Quantitative	Senta, 2015 [54]
Italy (from 17 cities)	24 h composite	ketamine, mephedrone	ketamine, mephedrone	SPE UHPLC-QqQ Quantitative	Castiglioni, 2015 [75]
Spain (Valencia)	24 h composite	Target: 42 compounds (21 emerging psychoactive substances) Suspect screening: 2000 compounds (pharmaceuticals, pesticides, mycotoxins and psychoactive substances)	Target: ephedrine Suspect screening: ephedrine, ethylamphetamine, α-PVP,4'MePHP, ketamine, *methylephedrone*	SPE UHPLC-QTOF MS/MS Quantitative/Qualitative	Andrés-Costa, 2016 [82]
Italy (Milan, Bologna, Turin, Perugia)	24 h composite	52 NPS (synthetic cannabinoids, synthetic cathinones, ketamine derivatives, phenethylamines and others)	None	SPE UHPLC-LTQ Orbitrap Qualitative	González-Mariño, 2016 [51]
United Kingdom (Bath)	24 h composite	56 compounds (ID, pharmaceuticals, mephedrone, ketamine, benzylpiperazine, ephedrine, pseudoephedrine and PMA)	mephedrone, ketamine, benzylpiperazine, ephedrine	SPE UHPLC-QqQ Quantitative	Castrignano, 2016 [76]
Italy (Florence, Bologna, Turin, Perugia, Milan), Spain (Santiago de Compostela), Norway (Oslo) and United Kingdom (Southwest)	24 h composite Weekend sampling	18 synthetic cathinones	mephedrone, N,N-dimethylcathinone, methcathinone, 4-FMC, 4-MEC, MDPV, ethylone	SPE UHPLC-QqQ Quantitative	González-Mariño, 2016 [24]
Australia (South East Queensland)	24 h composite	methylone, mephredone	methylone	Direct injection LC-QqQ Quantitative	Thai, 2016 [85]

Sampling area	Type of sample	Compounds [1]	Positive NPS findings [1]	Analytical technique	Reference
Poland (Plaszow, Krakow)	24 h composite	MDMA, mephedrone, 4-MEC, MDPV, mCPP	mephedrone, 4-MEC	SPE LC-QTOF Quantitative	Styszko, 2016 [81]
Australia (Adelaide)	24 h composite	21 compounds (ID and 10 NPS)	methylone, methcathinone, MDPV, BZP, mephedrone, TFMPP, α-PVP	SPE LC-QqQ Quantitative	Tscharke, 2016 [77]
Norway (Oslo, Trondheim)	24 h composite	51 compounds (ID, pharmaceuticals and 16 NPS)	methylone, ketamine, methoxetamine	SPE (POCIS) UHPLC-QTOF Qualitative	Baz-Lomba, 2016 [48]
The Netherlands (Amsterdam)	24 h composite Sampling during festival	2000 compounds (including ID, pharmaceuticals and NPS)	PMMA, methylhexanamine, 4-fluoroamphetamine, MDEA, mCPP, 2C-B, fentanyl, L-759,633, ketamine, hordenine	SPE UHPLC-QTOF UHPLC-LTQ Orbitrap Qualitative	Causanilles, 2017 [47]
European cities (Zurich, Copenhagen, Oslo, Castellon, Milan, Brussels, Utrecht, Bristol)	24 h composite	10 NPS (cathinones and phenethylamines)	MDPV, mephedrone, methylone	SPE UHPLC-QqQ Quantitative	Bade, 2017 [23]
Spain (Tarragona, Reus)	24 h composite	10 compounds (ID, mephedrone, 4-methylephedrine and MDPV)	None	SPE UHPLC-Exactive Orbitrap Quantitative	Prosen, 2017 [80]
New Zeeland (Auckland)	24 h composite	17 compounds (ID, methylone, ketamine *norketamine*, mephedrone, JWH-073 and JWH-018)	methylone, JWH-018	Direct injection, SPE LC-QqQ Quantitative	Lai, 2017 [83]
China (18 major cities)	24 h composite	Mephedrone, MDPV, BZP, TFMPP, mCPP	MDPV, BZP	SPE UHPLC-QqQ Quantitative	Gao, 2017 [69]

Sampling area	Type of sample	Compounds [1]	Positive NPS findings [1]	Analytical technique	Reference
Spain (Tarragona)	24 h composite	12 synthetic cathinones and one metabolite	flephredone, methylone, buphedrone, 4-methylephedrine, butylone, mephedrone, pentedrone, 3,4-DMMC, α-PVP, MDPV	SPE UHPLC-Exactive Orbitrap Quantitative	Fontanals, 2017 [79]
South Australia	24 h composite	Qualitative: 346 compounds (ID, pharmaceuticals and NPS) Target: subset of these compounds	α-PVP, MDPV	SPE UHPLC-QqQ UHPLC-QTOF Quantitative/Qualitative	Bade, 2018 [70]
South Australia	24 h composite	187 NPS	Qualitative: α-PVP, ethylone, MDPV, mephedrone, methcathinone, methylone, BZP, TFMPP, pentylone, 25H-NBOMe, MDA Quantitative: butylone, ethylone, α-PVP, methcathinone, MDPV, pentylone, mephedrone	SPE UHPLC-QqQ UHPLC-QTOF Quantitative/Qualitative	Bade, 2018 [49]
Spain (Santiago de Compostela)	24 h composite	38 compounds (ID, pharmaceuticals, mephedrone, ketamine and mCPP)	None	SPE UHPLC-QqQ Quantitative	González-Mariño, 2018 [71]
Norway (Trondheim)	24 h composite	8 compounds (THC, 3 metabolites of THC and 4 metabolites of synthetic cannabinoids)	None	LLE UHPSFC-QqQ Quantitative	González-Mariño, 2018 [93]
Croatia (Zagreb, Split)	24 h composite	27 opioids and metabolites	Detection of fentanyl, *norfentanyl* and sufentanil	SPE UHPLC-QqQ Quantitative	Krizman-Matasic, 2018 [72]
USA (Southwestern university campus)	24 h composite	19 compounds (ID and metabolites, oxycodone, fentanyl, buprenorphine, methylphenidate, alprazolam)	fentanyl, *norfentanyl*	Isotope dilution (ID-LC-MS/MS) Quantitative	Gushgari, 2018 [92]

Sampling area	Type of sample	Compounds [1]	Positive NPS findings [1]	Analytical technique	Reference
South Africa (Johannesburg, Cape Town)	24 h composite	18 compounds (ID, mephedrone, ephedrine, pseudoephedrine, *norephedrine*)	mephedrone	SPE UHPLC-QqQ Quantitative	Archer, 2018 [73]
Spain (Barcelona)	24 h composite	37 compounds (ID, pharmaceuticals, ephedrine, mephedrone, ketamine, methoxetamine, MDPV)	None	On-line SPE UHPLC-QqQ Quantitative	López-García, 2018 [74]
Australia	24 h composite and grab	187 NPS	Confirmed: MDA, AM-2201, UR-144, 4-FMC, α-PVP, ethylone, methcathinone, methylone, pentedrone, methoxetamine Detected: 5F-APINACA, JWH-018, JWH-073, 4-MEC, butylone, mephedrone, pentylone, U-47700, methiopropamine	SPE UHPLC-QTOF Qualitative	Bade, 2019 [78]

[1] NPS metabolites highlighted in italic letters.

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Figure captions

Figure 1: Number and categories of new psychoactive substances notified to the EU Early Warning System for the first time within 2005-2017 (reproduced with authorization from the European Drug Report 2018 of the EMCDDA [3])

Figure 2: Sources of information, steps and topics required to build a comprehensive database for monitoring NPS use

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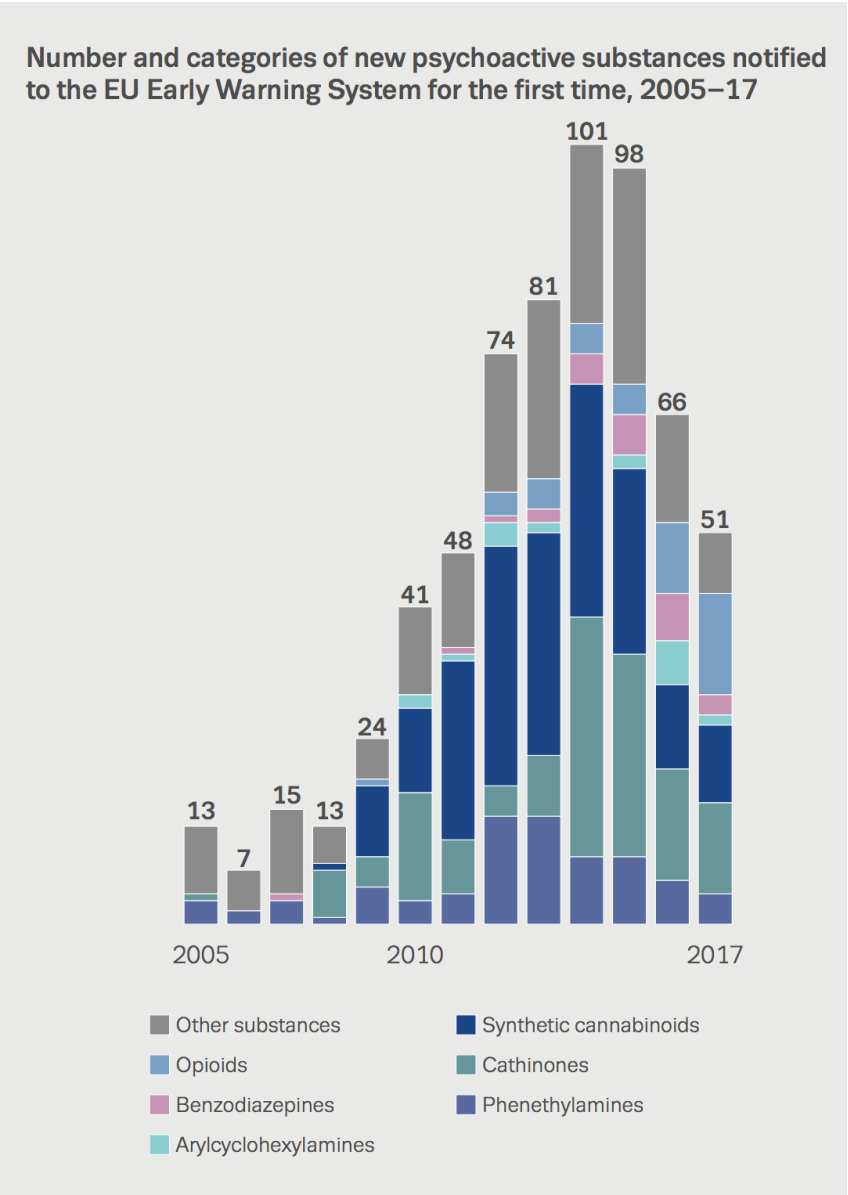

568

569 **Figure 1**

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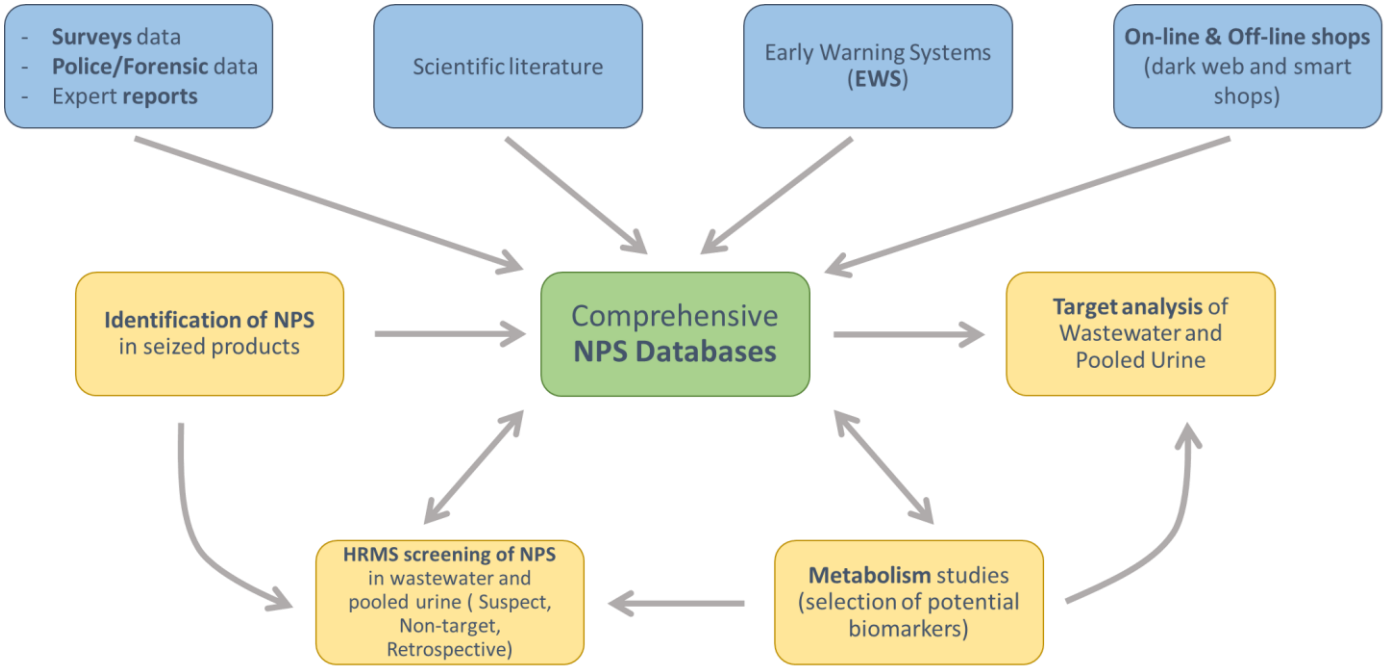

571

572 **Figure 2**