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Monitoring psychoactive substance use at six European festivals through wastewater and pooled urine analysis

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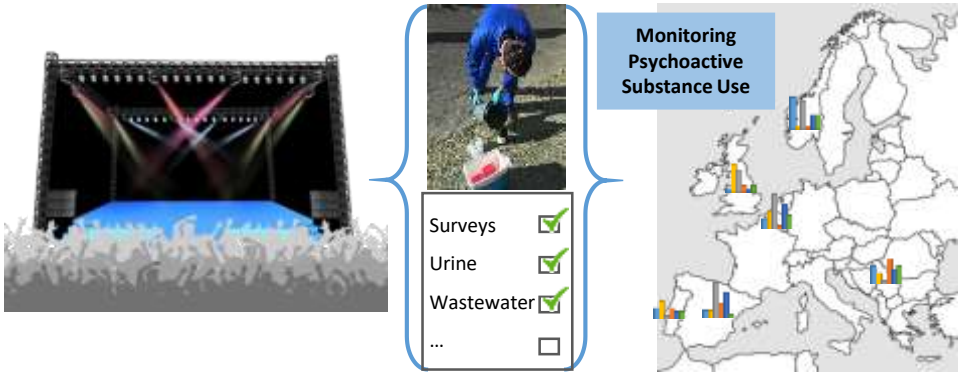
Graphical Abstract



Abstract

The consumption of psychoactive substances is considered a growing problem in many communities. Moreover, new psychoactive substances (NPS) designed as (legal) substitutes to traditional illicit drugs are relatively easily available to the public through e-commerce and retail shops, but there is little knowledge regarding the extent and actual use of these substances. This study aims to gain new and complementary information on NPS and traditional illicit drug use at six music festivals across Europe by investigating wastewater and pooled urine. Samples were collected, between 2015 – 2018, at six music festivals across Europe with approximately 465.000 attendees. Wastewater samples were also collected during a period not coinciding with festivals. A wide-scope screening for 197 NPS, six illicit drugs and known metabolites was applied using different chromatography-mass spectrometric strategies. Several illicit drugs and in total 21 different NPS, mainly synthetic cathinones, phenethylamines and tryptamines, were identified in the samples. Ketamine and the traditional illicit drugs, such as amphetamine-type stimulants, cannabis and cocaine were most abundant and/or frequently detected in the samples collected, suggesting a higher use compared to NPS.

The analyses of urine and wastewater is quick and a high number of attendees may be monitored anonymously by analysing only a few samples which allows identifying the local profiles of use of different drugs within a wide panel of psychoactive substances. This approach contributes to the development of an efficient surveillance system which can provide timely insight in the trends of NPS and illicit drugs use.

Keywords: Illicit drugs, New Psychoactive Substances, Wastewater-based epidemiology, Pooled urine, Wastewater, Music festivals.

1. Introduction

New psychoactive substances (NPS) are compounds designed to mimic effects of traditional internationally controlled drugs. Since 2005, around 700 NPS have been introduced into the European drug market (EMCDDA, 2015). This high number and the potential health and social risks these new drugs present are considered to be of alarming concern. NPS can be purchased through online vendors and smart shops, either individually or as mixtures, where they are frequently sold without or with misleading information about their effects and safety. Hence, users often do not know what they really consume. Also due to the high number and rapid transience of substances, it is difficult for healthcare professionals and toxicologists to assess the risks associated with consumption.

There is little knowledge regarding the extent and actual use of NPS (EMCDDA, 2018a). Data obtained from general population surveys on drug use, national Early Warning Systems (EWS) and searches on the open internet or dark web provide valuable but somewhat limited information. These data sources provide information on the dynamics of the NPS market, but it is difficult to derive any measurement of the amounts used. Understanding the actual use of each individual NPS is essential for correctly assessing the risks, and facilitate harm reduction, prevention and law enforcement activities. This highlights the need of applying alternative and complementary approaches to monitor NPS consumption such as targeted surveys or drug testing services at specific settings.

The analysis of biological samples from individuals is expensive, time consuming and requires consent. Wastewater and pooled urine analysis, however, can provide anonymised, but comprehensive information on community-wide use of NPS and illicit drugs (Archer et al., 2014a, 2013; Bade et al., 2017; González-Mariño et al., 2016; Ort et al., 2018). Pooled urine samples taken from portable urinals and/or toilets have the advantage over municipal wastewater in that sample collection is carried out closer to the point of actual excretion (Archer et al., 2014a), which reduces uncertainties associated with in-sewer stability (i.e., during transport in the sewer system) and dilution from water used in households, industry or surface runoff during wet weather. Data derived from the analysis of wastewater can provide quantitative information on substance use normalized to the population contributing to the sample (Ort et al., 2014;

Zuccato et al., 2008). This information can integrate the existing epidemiological data due to the unique ability to provide objective and updated information on the actual drugs having been consumed at specific events (EMCDDA, 2016a; Ort et al., 2018). This information is highly complementary with information on frequency of use, route of administration, the type of users or the purity of the drugs that can be provided only through other epidemiological drug use indicators (Lancaster et al., 2019). This approach, known as wastewater-based epidemiology (WBE), has been embraced as an additional drug use indicator by many scientists and organizations such as the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)(EMCDDA, 2018b, 2016a; Gracia-Lor et al., 2017; Ort et al., 2018, 2014).

Monitoring NPS in pooled urine or wastewater is challenging because prevalence of use is generally low and there is a lack of data on their biotransformation. Low use generally translates to low concentrations of the NPS (and/or metabolites) in the samples, which gives rise to analytical challenge. Advanced analytical instrumentation and updated methodologies can be efficiently applied for the screening of a large number of NPS in complex-matrix samples (Bijlsma et al., 2019; Hernandez et al., 2018), while targeting sample collection settings at user populations increase the success rate of identifying NPS and their metabolites. Studies focused on nightlife settings and festivities, such as music festivals, have reported higher rates of drug use (Bijlsma et al., 2014b; EMCDDA, 2018b; Hoegberg et al., 2018; Mohr et al., 2018; Riley et al., 2001). Hence, music festivals can be very suitable as targeted settings for the collection of pooled urine or wastewater to assess the use of NPS and illicit drugs.

In this study, a novel approach was applied to generate more knowledge on the actual use of psychoactive substances. By monitoring both NPS and traditional illicit drugs in pooled urine or wastewater a unique picture was obtained about which psychoactive substance were used during specific recreational occasions. This strategy has the potential to reveal possible large scale substitution of illicit drugs by specific NPS and act as a direct surveillance tool. To this aim, wastewater samples or pooled urine samples were collected from six music festivals in six countries across Europe. Data acquisition and processing was performed applying a strategic analytical workflow based on low- and high-resolution mass spectrometry coupled to liquid chromatography. The results

were compared, where possible, with information obtained from other sources e.g. surveys and literature, as well as the current legislation of some countries.

2. Material and methods

2.1. Chemicals and reagents

Thirty five psychoactive substances and 17 isotopically labelled analogues were used for quantitative analysis. Reference standards were purchased from Cerilliant (Round Rock, TX, USA) and Cayman Chemical Co. (An Arbor, MI, USA). The compounds selected were: amphetamine, benzoylecgonine (BE, the main metabolite of cocaine), buphedrone, butylone, cocaine, ethylone, ketamine, mephedrone, methamphetamine, methcathinone, methedrone, methoxetamine, methylenedioxypyrovalerone (MDPV), methylone, N-ethylcathinone, naphyrone, ephenidine (NEDPA), 3,4-methylenedioxymethamphetamine (MDMA), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH, the main metabolite of cannabis) 3,4-methylenedioxy-N,N-dimethylcathinone (bk-MDDMA), 4-bromo-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine (25-B-NBOMe), 4-chloro-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine (25-C-NBOMe), 4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-I-NBOMe), 4-isopropyl-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-iP-NBOMe), 4-methyl-α-pyrrolydinopropiophenone (4-MePPP), α-pyrrolidinopentiophenone (α-PVP), dimethylpentylone (bk-DMBDP), ρ-methoxymethamphetamine (PMMA), 2-phenethylamine, 3,4-dimethoxy-α-pyrrolidinopentiophenone (3,4-DiMeO-α-PVP), 3,4-dimethylmethcathinone (3,4-DMMC), 4,4'-dimethylaminorex (4-4'-DMAR), 4-chloro-α-pyrrolydinopropiophenone (4-chloro-α-PPP), 4-fluoromethcathinone (4-FMC) and 4-methylethcathinone (4-MEC). More specific information on the isotopically labelled analogues and chemicals used can be found in the supplementary information (SI).

2.2. Sample collection

Wastewater samples or pooled urine samples were collected at music festivals across Europe. Collecting samples from festivals is challenging and requires a good communication between operators. The success usually depends on the willingness of organizers, volunteers and wastewater treatment operators to provide samples, therefore no restrictions were set to the type of sample which could be collected. As agreed with the organizers and considering basic ethical principles (Hall et al., 2012; Prichard et al., 2014), data on the name and location of the festivals was anonymised.

Only their more relevant characteristics e.g. country, music genre and number of attendees are reported (Table 1). Disclosure at a research ethics committee and the Data Protection Agency was not required, since the study included anonymous data only.

2.2.1. Urine samples

Pooled urine samples were taken from portable urinals and/or toilets at three music festivals in UK, Belgium and Norway during 2015, 2016 or 2017 (**Table 1**). Grab samples were collected at several locations and time points (days or hours) throughout the festivals. Samples were drawn within 12 h from internal storage tanks, connected to male urinals or portable toilets with a 50 mL syringe. Samples were immediately placed on ice, to minimize possible degradation, and were transported within 12 h to the laboratory where they were stored in the dark at -20 °C. In total, 56 pooled urine samples were collected and analysed.

2.2.2. Wastewater samples

Wastewater samples were collected from the urban sewer network during three music festivals in Portugal, Serbia and Spain during 2017 or 2018 (**Table 1**). 24-h composite wastewater samples were collected using a time-proportional sampling mode (1 L, every hour). In addition, daily samples were collected during a one week control-period not coinciding with the festivals. All samples were collected at refrigerated conditions (4 °C), transported to the laboratory immediately, and stored in the dark at -20 °C. In total, 36 wastewater samples were collected and analysed.

2.3. Sample pre-treatment

The procedure used for urine samples was adapted from the literature (Matabosch et al., 2014). Briefly, 1 mL of pooled urine was spiked with a mixed surrogate internal standards and hydrolysed with 16 µL of β-glucuronidase from *E. Coli* K12 (140 Units / mL at 37 °C), buffering the sample with 400 µL of phosphate buffer adjusted to pH = 7. After incubating for 1 h at 55 °C with constant stirring, samples were frozen for 3 h in order to remove proteins and lipids by precipitation. Finally, samples were centrifuged at 12000 rpm for 10 min and the supernatant was injected into the LC-MS systems.

All wastewater samples were pre-concentrated by performing Solid Phase Extraction (SPE) using two types of cartridges (Oasis HLB and Oasis MCX), which resulted in two

extracts. This allowed widening the number of substances investigated with different physicochemical (acid, neutral or basic) properties *i.e.* NPS, traditional drugs and/or potential metabolites. Briefly, 100 mL of influent wastewater sample was loaded on the cartridges, previously spiked with a mixed surrogate internal standards and subsequently centrifuged for 5 min at 6000 rpm. Oasis HLB cartridges were conditioned with 6 mL of methanol and 6 mL of Milli-Q water, vacuum-dried for 10 min after sample percolation, and eluted with 5 mL of methanol. For the extraction with Oasis MCX, samples were acidified at pH 2. MCX cartridges were conditioned with 6 mL methanol, 3 mL Milli-Q water, and 3 mL acidified water (pH 2), and after percolation washed with acidified methanol (pH2) and vacuum-dried for 10 min. The analytes were eluted with 5 mL of methanol (2% ammonia). Both HLB and MCX eluates were evaporated to dryness under a gentle nitrogen stream and reconstituted to 1 mL with methanol:water (10:90 v/v). Finally, the two extracts were injected into the LC-MS systems for both qualitative and quantitative analysis. More details on sample pre-treatment of wastewater can be found elsewhere (Bade et al., 2017; Bijlsma et al., 2014a).

2.4. Chemical analysis

In this work, a notable number of samples from different festivals and countries were analysed. In order to homogenize analytical procedures as much as possible, all analyses were centralized in two laboratories, Mario Negri Institute (MNI) Milan, Italy and the University Jaume I (UJI) Castellon, Spain. Both laboratories performed a qualitative screening based on liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS), using a Q-Orbitrap (MNI) or QTOF (UJI) mass analyser, as well as a quantitative target analysis, based on LC coupled to tandem mass spectrometry (MS/MS) with triple quadrupole (QqQ) mass analyser. The UJI performed analysis of all pooled urine samples from the UK, Belgium and Norway, as well as wastewater from Portugal and Spain. MNI analysed pooled urine from Norway, as well as wastewater from Portugal and Serbia. Thus, the samples from Norway and Portugal were analysed by both laboratories, and the rest of the samples just by one laboratory.

Qualitative analyses of pooled urine and wastewater samples (i.e. two SPE extracts, HLB and MCX, in the case of wastewater) were performed using LC-HRMS with a Q-Orbitrap or QTOF mass analyser. In the latter, ion mobility spectrometry (IMS) was also

incorporated to improve the performance of the instrument. 197 NPS were screened using an *in-house* database (**Table S1**). Information was collected by reviewing the EWS reports most recently published from EMCDDA (EMCDDA, 2018c), the United Nations Office on Drugs and Crime (UNODC)(UNODC, 2017), and the scientific literature. More details on the analytical strategy, identification criteria, the database and a list of references consulted can be found in the SI and online (NPS-Euronet, 2018).

Quantitative analysis of the classical drugs and a selection of NPS (mostly synthetic cathinones) was performed using LC-MS/MS. **Table S2** show the limits of detection (LOD) and limits of quantification (LOQ) for quantitative analysis of up to 35 psychoactive substances and metabolites in wastewater and pooled urine. The quantitative procedures were adapted for the analysis of pooled urine, as the original methods were developed for wastewater analysis. Specific information on analytical methods can be found elsewhere: at UJI the analyses of drugs and NPS were performed using the methodologies described by (Bade et al., 2017; Bijlsma et al., 2014a; Celma et al., 2019) whereas at MNI the analyses of drugs and NPS were done using the methodologies by (González-Mariño et al., 2016; Zuccato et al., 2016).

Quality of the data generated was supported by the analysis of internal quality controls (samples spiked at different analyte concentrations) in each sequence. In the particular case of illicit drugs in wastewater (amphetamine, methamphetamine, MDMA, BE, THC-COOH), the laboratories participated and passed successfully the annual inter-laboratory comparison exercises coordinated by SCORE (van Nuijs et al., 2018).

2.5. Wastewater data treatment

WBE consists of several consecutive steps that allow the quantification of drug biomarkers in wastewater and the back-calculation of the amount of the corresponding drugs consumed by the population served by a wastewater treatment plant (Castiglioni et al., 2014; EMCDDA, 2016a; Zuccato et al., 2008). In the present study the amount of each substance measured (either parent drug or metabolite) was used to assess the consumption. These amounts of drug use (mass loads) normalized to the population were calculated as follows:

239 $$Normalized\ mass\ loads = \frac{\mathrm{C\ x\ F}}{\mathrm{p}}$$

240 Normalized mass loads (mg/day/1000 population) = mass loads (g/day) normalized to the
241 population
242 C = concentration of each drug in wastewater sample (ng/L)
243 F = measured flow rates of wastewater (m^3/day)
244 p = population contributing to the sample
245

246 Mass loads were normalised to the population using census data of the number of
247 inhabitants and census data on inhabitants plus festival attendees. The best practice
248 protocol available to perform WBE studies was used in order to keep results uncertainty
249 low and ensure reliability and comparability of results (Castiglioni et al., 2014; EMCDDA,
250 2016a; Gracia-Lor et al., 2017).

3. Results

3.1. Psychoactive substances in pooled urine

Chemical analysis of urine samples was performed by using a quantitative LC-MS/MS method, which included 35 compounds as target analytes. In addition, a complementary set of data could be obtained by performing a qualitative screening of the samples for around 200 NPS making use of HRMS. Although, it was not possible to correlate concentrations with the number of attendees, calculate daily loads, or even establishing unequivocal trends in use, due to the difficulties to know the total volume collected in urinals/toilets and the number of persons contributing to the samples, interesting information could be derived from the quantitative data obtained especially from individual samples. Yet, this data should be considered as indicative only.

Figure 1 shows the concentrations measured for each drug and the sum of all NPS. Detailed concentrations of each drug and NPS measured in the individual pooled urine samples are reported in **Tables S3-S6.** It is noteworthy, that urine samples from Norway were collected from portable toilets used by both male and female visitors, whereas urine samples from the UK and Belgium were collected from urinals designed for men only, and thus does not represent all festival attendees.

The traditional drugs cocaine and its metabolite BE, MDMA (ecstasy) and the cannabis metabolite THC-COOH were quantified in all pooled urine samples. In the samples collected in the UK, high concentrations of cocaine were found showing some unexpectedly high cocaine:BE ratios in several samples collected on 2015, which might indicate direct disposal of cocaine (Bijlsma et al., 2012; Postigo et al., 2010; Van Nuijs et al., 2009). MDMA was measured at highest concentrations in the UK and Belgium compared to the other traditional drugs, such as cocaine and cannabis, whereas in Norway, MDMA was found at lower concentrations compared to cocaine. Amphetamine and methamphetamine were detected in all pooled urine samples, but their levels were only sufficiently high in Norway to allow for accurate quantification, indicating a possible different pattern of use for these substances in this country.

Concentrations of NPS in pooled urine were in general lower than those found for traditional drugs. In the samples collected in the UK, no large differences were observed between concentrations of NPS measured in 2015 and 2016, but in terms of number of compounds and frequency of detection, more NPS were found in 2015 (**Table 2** and

Tables S3-S4). Furthermore, although quantitative information from pooled urine samples should be interpreted cautiously as indicated above, it seems interesting to mention the increase of ketamine concentrations in 2016, a controlled drug, which is reported together with the NPS in this study, as we wanted to distinguish it from the traditional illicit drugs. In addition, methoxetamine, a ketamine analogue was also detected in 2016. Overall, considerably fewer NPS were found in Belgium and Norway (**Tables S5-S6**) compared to the UK (**Tables S3-S4**). This fact can also be easily deduced from **Table 2** by comparing the green boxes for the three countries.

3.2. Psychoactive substances in wastewater

Figure 2 shows the loads of the most prevalent substances measured over one week during the festival week (solid line) and a normal week (dotted line). **Tables S7-S12** report the mass loads (g/day) of each drug and NPS measured in the individual wastewater samples.

In Portugal (**Figure 2A, Tables S7 and S8**) the increase in mass loads of THC-COOH, the main metabolite of cannabis, during the festival was most notable (approximately 10 times). Furthermore, several NPS (3,4-DMMC; α-methyltryptamine; buphedrone, mephedrone, methcathinone and ketamine) were identified in wastewater and another four substances (2-phenethylamine; 25-E-NBoMe; 4-chloro-α-PPP; DOiP) were detected but their identity could not be confirmed due to the absence of a reference standard or the low concentrations present in the samples (**Table 2**). Only four out of the six compounds confirmed could be quantified, i.e. their concentrations were above the LOQ of the method. (**Table S7**). It is noteworthy that NPS were found in wastewater only during the music festival. Thus, the wastewater samples collected during six days that did not coincide with a festival or other special event did not contain any of the NPS investigated. (**Table S8**).

In Serbia (**Figure 2B, Tables S9 and S10**) MDMA is clearly the most prevalent substance during the festival, whereas mass loads of amphetamine and cocaine were in the same order of magnitude as during the "normal" week. The highest mass loads of MDMA were actually determined on Sunday, two days after the festival. This is rather unexpected, taking into account its relatively short half-life of MDMA. A possible explanation for the high mass loads of MDMA in the weekend just after the festival could be that festival

attendees stayed over for the weekend and organized after-parties or entered night life together with the local residents. However, there is no evidence to support this hypothesis. Furthermore, MDA was also found. MDA is available on the illicit market and could be present due to the consumption of this drug. However, it is also a known minor metabolite (7% of a dose) of MDMA (Castiglioni et al., 2008) and was therefore also related to increased consumption of MDMA during another festival (Bijlsma et al., 2014b). Unfortunately, THC-COOH was not analysed in these samples, and despite the Serbian festival being the largest festival sampled within this study, only a few NPS were found and at low concentrations (resulting in low loads).

In Spain (**Figure 2C, Tables S11 and S12**), the use of MDMA, cocaine and ketamine considerably increased during the festival compared to the "normal" week. High weekly loads, especially for MDMA and cocaine, were also observed in wastewater during another Spanish music festival back in 2008 (Bijlsma et al., 2014b). This might indicate little or no shift in the types of drugs consumed over the years.

3.3. Overall number of NPS detected

The presence of 197 NPS and metabolites (**Table S1**) were screened by HRMS in the pooled urine samples and wastewater samples collected. In addition, several other NPS and metabolites *i.e.* ethylone, 5-APB, dehydronorketamine, hydroxynorketamine, dihydromephedrone and 2 metabolites of α-PVP (M-264 and M-234) were investigated retrospectively. Retrospective analysis consists into reprocessing the accurate-mass full-spectrum acquisition data obtained by HRMS to search for new substances, without the need to carry out new analysis, but with the assumption that substances were recovered during sample treatment, at least partially. This permits the screening to be further widened when new information is available or in order to gain more confidence of the presence of certain compounds e.g. by screening for their metabolites (Bijlsma et al., 2013). In this way, ethylone was retrospectively detected by UHPLC-IMS -QTOF. It could also be confirmed, since a reference standard was obtained, but not quantified because at the time of analysis the quantitative method was not fully validated for this NPS.

When applying wide-scope screening methodologies, false negatives cannot be discarded, as the method cannot be validated/tested for all compounds monitored. On the contrary, the value of the accurate-mass full-spectrum for the reliable identification

of the compounds detected in samples notably decreases the chance to report false positives. **Table 2** gives a complete overview of the NPS identified in the samples. Most of the NPS found in pooled urine and wastewater were already included in the target LC-MS/MS methods applied, and therefore their reference standards were available in our laboratories. In this way, the confirmation of the identity of the compounds found was feasible for the great majority of them (green boxes). In some cases, however, the low analyte concentration prevented the confirmation because only one of the MS/MS transitions, commonly the most abundant (Q, quantification transition), was observed (blue boxes) (see **Table S2** for LODs and LOQs of the quantitative methods applied). The in-parallel application of HRMS screening to the same samples did allow to confirm most of findings reported by the LC-MS/MS methodology, and additionally to identify other NPS not included in the target quantitative analysis such as 25-E-NBoMe, α-methyltryptamine and DOiP.

4. Discussion

4.1. Main findings

Wastewater and pooled urine analysis permitted the investigation of NPS and traditional illicit drugs use during music festivals across Europe. The most prevalent substances were cocaine and especially MDMA, which was measured at high concentrations in pooled urine from the UK and Belgium and in wastewater from Serbia and Spain. Specific local patterns of use were observed in Norway, where amphetamine and methamphetamine were found at the highest concentrations. In fact, they were the only pooled urine samples where these two drugs could be quantified. Results from wastewater analysis showed a notable increase in mass loads of the cannabis biomarker during the festival week in Portugal (around 10 times higher than the week that did not coincide with the festival; see **Tables S7 and S8** for comparison). A similar behaviour was observed in Spain, although the increase in loads was not so evident (around 3 times higher, see **Tables S11 and S12**). An increase in ketamine loads was also observed in Spain during the festival. Mass loads of the cannabis and cocaine biomarker remained high well after the festival, specifically in Portugal. This may due to the delay in excretion or a longer in-sewer residence time of the sample.

NPS concentration levels were far lower than cocaine and MDMA levels in all the samples. In pooled urine, most NPS were detected in the UK. Thus, several compounds such as 4-FMC, 4-MEC, α-PVP, butylone, ethylone and MDPV were only found in these samples. In wastewater, NPS were mainly found during the days of festivals indicating a recreational use that increases during specific events. Yet, the use of NPS seemed less widespread, this may be due to the high number of substances sold on the recreational market and the difficulty to identify them, but can also highlight the actual use of specific substances in cases where consumers are not perfectly aware of what they are taking as known for festivals goers. The combination of different drug-use indicators in future investigations would be very useful to clarify this issue.

4.2. Comparison with other sources

Relatively high concentrations of MDMA (ecstasy) were measured in pooled urine, especially in the UK and Belgium, compared to biomarkers of other drugs such as cocaine and cannabis. This indicates a higher consumption of this ‘party’ drug at these

recreational settings, which is in line with the high concentrations of MDMA in urine samples observed during a large dance festival in Belgium in 2015 (Gremeaux and Plettinckx, 2017). The relatively high mass loads of MDMA could be, in part, also explained by the high purity of seized ecstasy tablets (Gremeaux and Plettinckx, 2017). Furthermore, higher MDMA consumption was also linked to a big dance party in the Netherlands (van der Aa et al., 2013).

The relatively high mass loads found for ketamine in wastewater from Spain during the festival week is worth to notice. This compound was also detected in wastewater from Portugal, although the low analyte concentrations did not allow its quantification. Ketamine is getting more popular in special events, and its presence was reported during a street festival in the Netherlands (Causanilles et al., 2017) and in Italy, where mass loads in Milan increased from 1 to 3.6 g/day in 2008 - 2014 (Castiglioni et al., 2015). Furthermore, it was found in almost all pooled urine samples analysed in this work. The high prevalence in Spain, the larger proportions found in Belgium by Gremeaux and Plettinckx (Gremeaux and Plettinckx, 2017) and the increased concentrations found in pooled urine from the UK, might indicate an upcoming trend of this drug, especially in these recreational settings.

In Portugal, the increase in mass loads of cannabis biomarker (approximately 10 times) in wastewater coincided with information obtained from a survey undertaken during the same festival. Among the interviewed attendees (n = 887), 8.5% admitted to have consumed cannabis within the last 48h, whereas only 4% and 3% admitted having consumed cocaine and MDMA, respectively (Calado et al., 2017). The prevalence of NPS use was less than 1% i.e. 3 persons claimed to have taken synthetic cannabinoids and only 1 person reported the use of piperazine in the last 48h. There was no self-reported use of phenethylamines or synthetic cathinones, yet up to 10 NPS were identified in wastewater, of which 6 NPS could be confirmed and 4 quantified (i.e. 3,4-DMMC, buphedrone, methcathinone and mephedrone). This suggests a certain prevalence in the consumption of these substances.

Several NPS were detected in pooled urine samples collected from portable urinals. However, methiopropamine (MPA) and 5-(2-aminopropyl)benzofuran (5-APB) were not found in this study, whereas they were consistently identified in other pooled urine samples from the UK, including a music festival in 2014 (Archer et al., 2014b; Kinyua et

al., 2016). This might be related to the legal response to NPS in the UK, where a special Psychoactive Substances Act was established in 2016 (EMCDDA, 2016b) and might also explain the absence of the cathinones 4-FMC, 4-MEC, ethylone and MDPV in 2016. However, NPS are quickly replaced by new substances, which might not have been included in our database and consequently missed in the screening. Furthermore, mephedrone has been detected in this study, although the EMCDDA reports that its use in the UK had been decreasing for some years prior to this legal response (EMCDDA, 2018d, 2016b). Overall, considerably fewer NPS were found in pooled urine from Belgium and Norway compared to the UK. This information could indicate lower use of NPS, which is in line with reports of the Global Drug Survey (GDS, 2016). Belgium implemented in 2014 lists of tightly defined 'generic' groups of substances, rather than individual drugs, to broaden the coverage of their existing drug laws (EMCDDA, 2016b). Moreover, data on the prevalence of NPS use in Belgium and Norway, coming from a drug survey, also confirms the residual levels of use (EMCDDA, 2018d).

In general, NPS were detected at much lower concentrations compared to traditional drugs, and mainly cathinones, phenethylamines and tryptamines were found. Other studies on NPS use during music festivals also reported the detection of synthetic cathinones and phenethylamines as the most commonly consumed NPS categories (Archer et al., 2014b; Kinyua et al., 2016). Synthetic cannabinoids, despite being the largest class of NPS reported (EMCDDA, 2018d), and synthetic opioids, such as fentanyl derivatives, were not found. For synthetic cannabinoids, this may be due to the extensive metabolism in the human body and the limited knowledge of the metabolites excreted with urine (Erratico et al., 2015; Shevyrin et al., 2014; Wintermeyer et al., 2010; Znaleziona et al., 2015). Also, less frequently consumed NPS or those used at very low dosages, such as synthetic opioids, tryptamines and other hallucinogenics, are less easily detected resulting in reporting possible false negatives. Moreover, considering the changing nature of the NPS, it is possible that new substances are missed during the chemical analysis, a fact that would affect all the types of matrices, e.g. wastewater, urine, blood and oral fluids.

4.3. Strengths and limitations

Advanced analytical instrumentation can be efficiently applied for the screening of a large number of psychoactive substances in complex-matrix samples i.e. wastewater and pooled urine. However, different analytical challenges such as the generally low concentrations present in the samples and the quick replacement by new substances, makes it pivotal to have sensitive and updated methodologies (Bijlsma et al., 2019; Hernandez et al., 2018).

Interpretation of data among the different festivals or countries requires caution, because of the different nature of the festivals, variations in population, years, matrices and sampling. A drawback of pooled urine data is that it mainly gives a snapshot of the substances consumed. Although quantitative analysis is feasible, the number of individuals contributing to the samples cannot be predicted and concentrations alone is less informative. It principally gives an indication of the extent of use for a drug compared to the other substances quantified in the same sample. Moreover, an evident bias of data reported may occur when the urinals are differentiated by gender (e.g. the data obtained from the UK and Belgium in this work are only representative of use by male populations). Furthermore, a critical question may rise on the representativeness of grab samples. In this context it should be emphasized that possibly only a fraction of a drug dose is collected, since substances have different excretion rates, for example cannabis has a much longer excretion rates compared to substances like MDMA. The true value and advantage of pooled urine samples is, therefore, that they are less diluted compared to wastewater resulting in higher concentrations, which facilitates drug detection. This is especially interesting for substances with low prevalence of use such as NPS, or for drugs that are extensively metabolized and therefore the amount of biomarker excreted in urine is very low. The strength of wastewater on the other hand is the representativeness of 24-h composite samples, and which analysis provides population-normalized quantitative information on NPS and illicit drugs use. This allows comparing the pattern of use during the festival period and during a “normal” week in order to better reveal the contribution of the event.

The analysis of pooled urine and wastewater samples is thus complementary to obtain comprehensive information on community-wide use of NPS and illicit drugs. Ideally, both type of samples should be collected from the festivals at the same time. However,

485 collecting samples from festivals is challenging and depends on the willingness of
486 organizers, local authorities and wastewater treatment operators to offer collaboration.
487 This was particularly difficult in the present study because it was performed
488 internationally including different festivals in different country with their own rules and
489 bureaucracy. Thus, sampling both pooled urine and wastewater during the same festival
490 was not feasible here. Despite this limitation, the information provided separately by
491 either pooled urine or wastewater, is still very useful. The analyses of urine and
492 wastewater offer timely information and a high number of attendees are monitored
493 anonymously by analysing only a few samples which allows identifying the local profiles
494 of use of different drugs within a wide panel of psychoactive substances. In future
495 studies, an effort will be made to collect simultaneously both pooled urine and
496 wastewater to compare directly results.

497 **4.4. Future applications**

498 The main outcome of this study is not just providing a list of the identified substances
499 consumed, but also suggesting a comprehensive strategy for continuous surveillance of
500 the appearing NPS. The lack of self-report on the use of NPS in Portugal, despite
501 contradictory evidence from wastewater analysis, indicates that either there was
502 inaccurate self-reporting, or the NPS were consumed outside the festival terrain but
503 within the wastewater catchment area. In the case of inaccurate self-reported drug use,
504 this can stem from a too low participation rate (< 2% of the festival attendees), active
505 denial, or indeed from the user being unaware of the exact drug they have used.
506 Consumer awareness is important for ensuring safe user habits.
507 Future applications of the presented strategy, especially to night time economy settings
508 (nightclubs, city centres or entertainment districts) and music events (concerts,
509 festivals) may contribute to develop an efficient surveillance system to gain more insight
510 in the prevalence of use and to better understand the diffusion of NPS and illicit drugs.
511 Based on wide-scope HRMS monitoring, new drugs can also be identified alerting on
512 new trends or substances appearing in the market.
513 Data triangulation with traditional indicators, such as targeted surveys, online forums
514 (e.g., Reddit), data of drug testing services, hospitals and police data, is pivotal and will
515 ensure a more accurate picture of drug use in these recreational settings. In this context,

516 the potential of and complementary information provided by WBE has previously
517 demonstrated its value (Bade et al., 2018; Been et al., 2016; Zuccato et al., 2016). The
518 information obtained from different sources, including wastewater and pooled urine
519 analysis, may help to orientate and evaluate prevention strategies at future events and
520 to ensure an effective public health response.

Supplementary Information

In this section, information can be found related to the chemicals and reagents used, psychoactive substances selected for quantitative analysis, database built for screening (including the references consulted), instrumentation used for qualitative suspect screening and the analytical strategy and identification criteria applied. Furthermore, 12 tables, S1: NPS and metabolites included in the in-house database, S2: LODs and LOQ for quantitative analysis of wastewater and pooled urine, S3-S6: Concentration data (µg/L) of drugs and NPS measured in pooled urine samples of UK 2015, UK 2016, Belgium 2017 and Norway 2016, respectively, S7-S8: Loads (g/day) of drugs and NPS measured in wastewater samples of Portugal (2017), S9-S10: Loads (g/day) of drugs and NPS measured in wastewater samples of Serbia (2017), S11-S12: Loads (g/day) of drugs and NPS measured in wastewater samples of Spain (2018), are included to have supportive visual information on the written text. Supplementary information can be found in the online version to this article.

Contributors

LB, FH, SC and EZ planned and designed the study with contributions from MR, MD, AL, JM, JR, TS and AvN. LB, SC, JB, AL, LP, MT, TS and AvN organized the collection of the wastewater samples which were analysed by AC and NS. LBI performed synthesis and characterization of α-methyltryptamine. LB, AC and NS performed data analysis and interpreted the results with substantial contribution from all co-authors. LB, with contributions from SC, drafted the manuscript, which was critically revised by all co-authors. All authors are aware of the content, and accept responsibility, for the manuscript.

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784

785 **Table 1**: Characteristics of the music festivals investigated.

Festival No.	**1**	**2**	**3**	**4**	**5**	**6**
Country	**UK**	**Belgium**	**Norway**	**Portugal**	**Serbia**	**Spain**
Year	2015 and 2016	2017	2016	2017	2017	2018
Type of music	Electronic	Electronic	Pop/rock	Pop/rock	Pop/Rock	Electronic
No. of attendees	70.000	80.000	20.000	50.000	215.000	30.000
No. people connected to the wastewater treatment plant	-	-	-	425.000	300.000	54.000
Type of sample	Pooled urine (male urinals)	Pooled urine (male urinals)	Pooled urine (male/female portable toilets)	Wastewater (24h composite)	Wastewater (24h composite)	Wastewater (24h composite)
No. of samples	20 and 24	9	3	7 + 6 of not festival week	7 + 2 pooled samples of not festival week	7 + 7 of not festival week
Locations	10 and 12	1	1	1	1	1
Days (time frames; hours)	2	3 (16h, 20h, 24h)	3	13 / 3 festival days	9 / 3 festival days	14 / 4 festival days

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788 **Table 2**: Overview of NPS detected in samples collected during the festivals (number in cells is the % of positive samples)

NPS	Pooled Urine				Wastewater		
	UK 2015 (n=20)	UK 2016 (n=24)	Belgium 2017 (n=9)	Norway 2016 (n=3)	Portugal 2017 (n=7)	Serbia 2017 (n=7)	Spain 2018 (n=7)
2-Phenethylamine				67	100		
25-E-NBoMe					14		
25-iP-NBoMe						14	
3,4-DMMC					14		
4-4'-DMAR						28	
4-chloro-α-PPP				67	71		
4-FMC	20						
4-MEC	45						
α-methyltryptamine					14	14	
α-PVP	100	96					
Buphedrone					57		
Butylone	10	4					
DOiP					14	28	
Ethylone	65						
Ketamine	100	100	100	67	57		86
MDPV	5						
Methcathinone				100	57	57	
Mephedrone	25	17	11		100		
Methoxetamine		13		33			
Methylone	20	8	56				28
NEDPA						14	

789

Confirmed
Detected
Not detected

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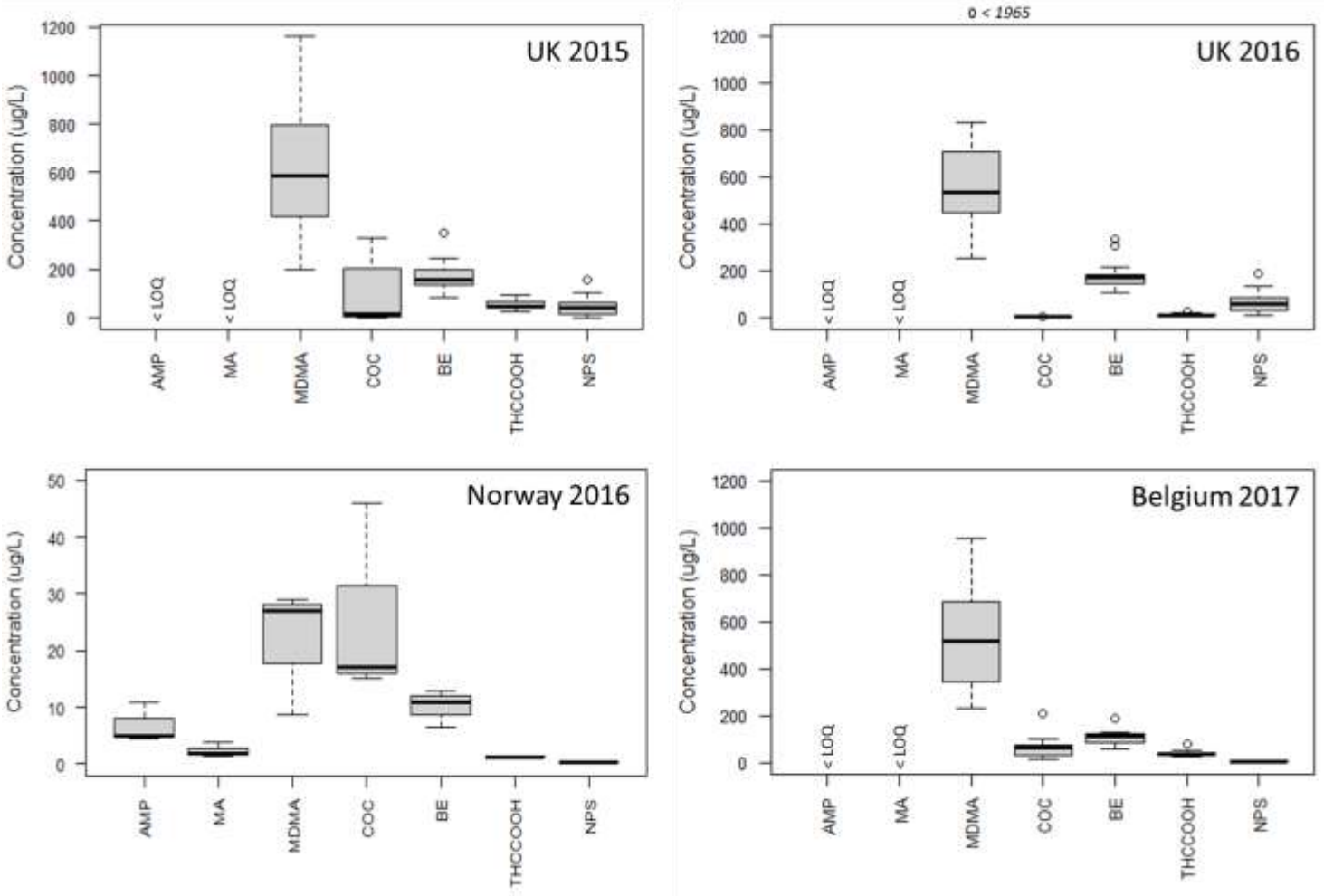
Figure captions

Figure 1: Boxplots representing concentrations (µg/L) of psychoactive substances measured in pooled urine samples collected at music festivals in the UK 2015 and 2016, Norway 2016 and Belgium 2017. Note that y-axis of Norway 2016 is adapted.

Figure 2: Population-normalized loads of psychoactive substances in wastewater samples collected in Portugal (A), Serbia (B) and Spain (C). TOP: represent the substances with highest loads. Samples were collected during one week which coincided with music festivals (continuous line) and on week without any festivities (dotted line). For Serbia (B) only two pooled wastewater samples were analysed during the week without festivities, i.e. weekdays (pool of Tuesday, Wednesday, Thursday) and weekend days (pool of Saturday, Sunday, Monday).



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807 **Figure 1**

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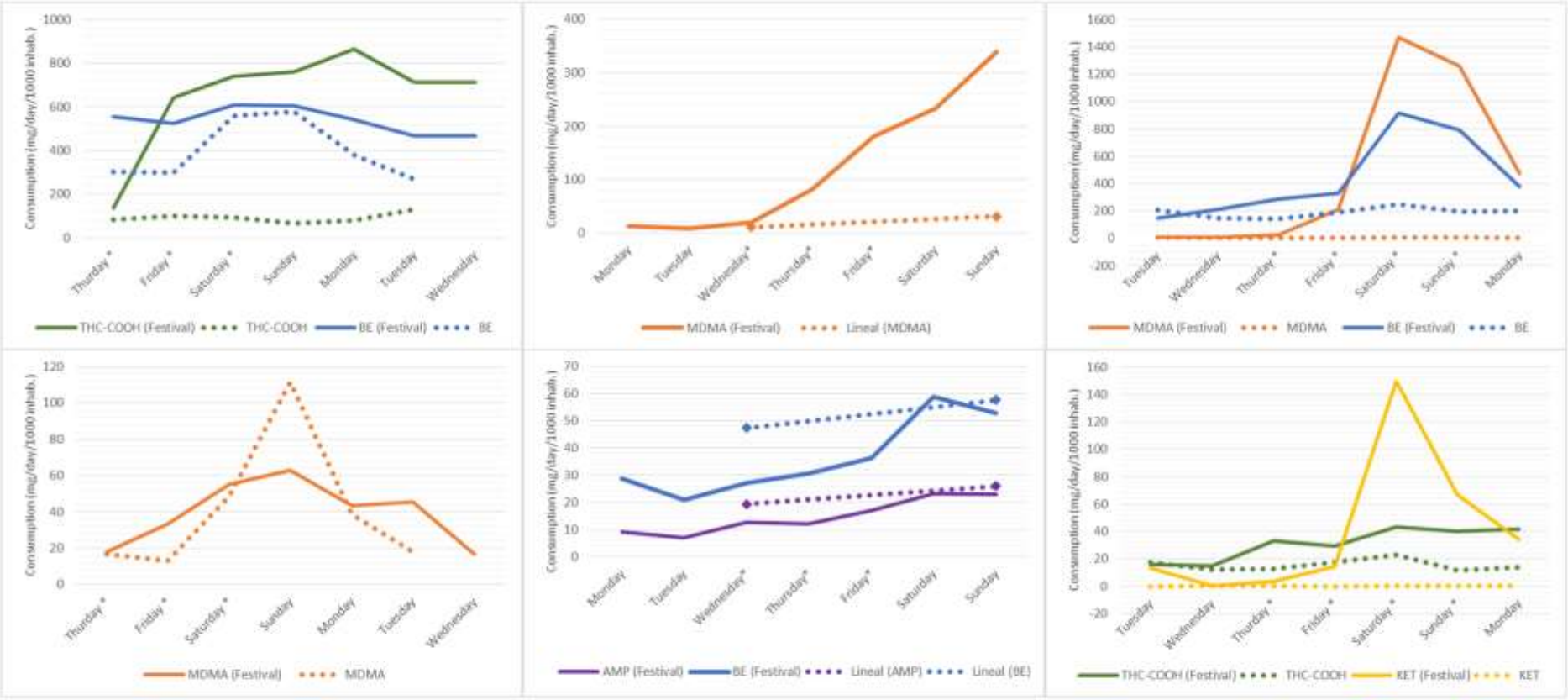

A B C

809

810 **Figure 2**

Supplementary Information

Monitoring psychoactive substance use at six European festivals through wastewater and pooled urine analysis

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Chemicals and reagents

HPLC-grade water was obtained by purifying demineralized water in a Mili-Q plus system from Millipore (Bedford, MA, USA). LC-MS grade acetonitrile (ACN), methanol (MeOH), ammonium acetate (NH_4Ac) and formic acid (HCOOH, 98 - 100 %) were acquired from Scharlab S.L. (Barcelona, Spain). β-glucuronidase from E.Coli K12 (140 Units / mL at 37 °C) was purchased from Roche Diagnostics GmbH (Mannheim, Germany) and Leucine-enkephalin was purchased from Sigma-Aldrich (Augsburg, Germany). SPE cartridges, generic Oasis HLB (3 cm^3; 60 mg) built of hydrophilic and lipophilic monomers, and Oasis MCX (3 cm^3; 60 mg) with strong cation-exchange properties, were purchased from Waters (Milford, MA, USA).

Psychoactive substances selected for quantitative analysis.

In total 35 drugs and NPS and 17 isotopically labelled analogues were purchased from Cerilliant (Round Rock, TX, USA) and Cayman Chemical Co. (An Arbor, MI, USA). The compounds selected were: amphetamine, benzoylecgonine (the main metabolite of cocaine), buphedrone, butylone, cocaine, ethylone, ketamine, mephedrone, methamphetamine, methcathinone, methedrone, methoxetamine, methylenedioxypyrovalerone (MDPV), methylone, N-ethylcathinone, naphyrone, ephenidine (NEDPA), 3,4-methylenedioxymethamphetamine (MDMA), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH, the main metabolite of cannabis) 3,4-methylenedioxy-N,N-dimethylcathinone (bk-MDDMA), 4-bromo-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine (25-B-NBOMe), 4-chloro-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine (25-C-NBOMe), 4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-I-NBOMe), 4-isopropyl-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-iP-NBOMe), 4-methyl-α-pyrrolydinopropiophenone (4-MePPP), α-pyrrolidinopentiophenone (α-PVP), dimethylpentylone (bk-DMBDP), ρ-methoxymethamphetamine (PMMA), 2-phenethylamine, 3,4-dimethoxy-α-pyrrolidinopentiophenone (3,4-DiMeO-α-PVP), 3,4-dimethylmethcathinone (3,4-DMMC), 4,4'-dimethylaminorex (4-4'-DMAR), 4-chloro-α-pyrrolydinopropiophenone (4-chloro-α-PPP), 4-fluoromethcathinone (4-FMC) and 4-methylethcathinone (4-MEC). Isotopically labelled analogues used were: amphetamine-d_6, benzoylecgonine-d_3, butylone-d_3, cocaine-d_3, ketamine-d_4, mephedrone-d_3, methamphetamine-d_5, methoxetamine-d_3, MDPV-d_8, methylone-

d_3, MDMA-d_5, naphryone-d_5, 25-B-NBOMe-d_3, 25-C-NBOMe-d_3, 25-I-NBOMe-d_3, α-PVP-d_8 and PMMA-d_3.

Database for screening

In total, 197 NPS were screened using an *in-house* database (**Table S1**). Information was collected by reviewing the EWS reports most recently published from EMCDDA, the United Nations Office on Drugs and Crime (UNODC), and the scientific literatur. The complete database is available on the NPS-Euronet website (Priority NPS Database; http://www.npseuronet.eu/results/2018) and include information on chemical family, communication source, metabolism (when available) information necessary to perform the chemical analysis (molecular formula, exact mass, chemical structure, mass spectrometric fragmentation data, and availability of reference standards) and the references consulted (see also special reference section in this SI).

Instrumentation qualitative suspect screening

A Waters Acquity I-Class UPLC system (Waters, Milford, MA, USA) was interfaced to a VION IMS-QTOF mass spectrometer, using an electrospray ionization (ESI) interface operating in positive mode.

The chromatographic separation was performed using a CORTECS® C18 2.1 x 100 mm, 2.7 µm fused core column (Waters) at a flow rate of 300 µL/min. Gradient elution was performed using mobile phases of A = H_2O and B = MeOH, both with 0.01% HCOOH. The initial percentage of B was 10%, which was immediately linearly increased to 90% for 14 min, followed by a 2 min isocratic period, then, returned to initial conditions (at 16.1 min) with 2 min equilibration of the column. The total run time was 18 min. Nitrogen was used as the drying gas and nebulizing gas. The injected volume was 3 µL for both pooled urine and wastewater extracts.

A capillary voltage of 0.8 kV and cone voltage of 20 V were used. The desolvation temperature was set to 550 °C, and the source temperature to 120 °C. The cone gas flow was 250 L/h and desolvation gas flow of 1000 L/h. The column temperature was set to 40 °C and sample temperature at 10 °C. MS data was acquired using the VION in HDMS^e mode, in the range 50-1000 m/z, with N_2 as the drift gas, an IMS wave velocity of 250 m/s and wave height ramp of 20-

50 V. Leucine enkephalin (*m/z* 556.27765) was used for mass correction. Two independent scans with different collision energies were acquired during the run: a collision energy of 6 eV for low energy (LE) and a ramp of 28-56 eV for high energy (HE). The LE and HE functions settings were for both a scan time of 0.3 s. Nitrogen (≥99.999%) was used as collision-induced dissociation (CID) gas. All data was examined using an in-house built accurate mass screening workflow within UNIFI informatics platform from Waters Corporation.

In addition, an Agilent HP-1200 Series LC system (Agilent Technologies, Santa Clara, CA) was coupled to a Q-ExactiveTM Hybrid Quadrupole-OrbitrapTM mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an ESI source. The chromatographic separation was performed at a flow rate of 200 µL min-1 using a XBrigde® C18 (2.1x100mm, 3.5 µm) column (Waters) and a mobile phase consisting of (A) 0.1 % formic acid in MilliQ water and (B) ACN. The gradient was as follows: 0 min (10 % B), 20 min (60 % B), 25 min (99 % B), 30 min (99 % B) and 31 min (10 % B); the initial conditions were finally kept for 6 min in order to re-equilibrate the column (total run time 38 min). The volume of injection was 8 µL both for pooled urine and wastewater extracts.

HRMS analyses were done in positive mode under the following working conditions: sheath gas pressure 45 bar, auxiliary gas pressure 5 bar, ion spray voltage 3.5 kV, heated capillary temperature 320 °C, S-lens RF 60. MS2 experiments were carried out using the collision-induced dissociation (CID) mode and applying two fixed collision energy (CE) 35 and 50 V in the quadrupole to a precursor ion selected with an isolation window of 3 m/z. Data processing was done with the Thermo XcaliburTM 2.3 software (Thermo Scientific).

Analytical strategy and identification criteria

Current analytical instruments provide the sensitivity, selectivity, and identification requirements to determine drugs, NPS and their metabolites in pooled urine and wastewater at low concentration levels. Accurate-mass full-spectrum measurements from HRMS are of great value for elucidation purposes and allow searching for a large number of compounds without the immediate need for reference standards. This is important since reference standards of NPS and their metabolites are not always commercially available. Moreover, purchasing of NPS reference

standards is time-consuming and expensive, not only the initial acquisition also its maintenance (e.g. considering stability and expiration of standards). Furthermore, the presence of newly reported NPS and metabolites, initially not considered in the suspect list, could be investigated at any time from data acquired in a retrospective way without the need for additional analysis (Bijlsma et al., 2013; Hernandez et al., 2018). Finally, ion mobility spectrometry in QTOF instruments adds a new dimension to the chromatographic and HRMS separations, which notably facilitates the identification process, which is particularly important in complex-matrix samples.

We reported compounds based on the identification levels for small molecules described by Schymanski *et al.* 2014 (Schymanski et al., 2014). A mass accuracy of < 5 ppm and at least 1 matched fragment was utilized in order to tentatively identify a suspect analyte. Obviously, reference standards are required for unambiguous confirmation, by matching MS and MS/MS spectra, retention time (and Collisional cross section (CCS) in ion mobility systems), but they need to be acquired only in a final stage, when well-founded evidence exists on the presence of the substance in the sample (Ibáñez et al., 2014). Hence, we endeavoured to purchase (if available) or synthesize reference standards of the substances tentatively identified. As was the case for α-methyltryptamine (AMT), which was synthetized and subsequently characterized using NMR and UHPLC-HRMS.

After screening pooled urine extracts and wastewater extracts (for wastewater, after SPE with HLB and MCX) by LC-HRMS, samples were also analysed for quantification and additional confirmation of the substances using a more sensitive target method based on LC-MS/MS. Quantification and confirmation was feasible by selecting three transitions for each compound. Furthermore, isotope-labelled internal standards were used for all drugs and most NPS detected to correct for potential losses during sample treatment and to compensate for matrix effects. Specific information on analytical methods can be found elsewhere (Bade et al., 2017; Bijlsma et al., 2014; González-Mariño et al., 2016; Zuccato et al., 2016).

Table S1: 197 NPS and metabolites included in the *in-house* database, together with the IUPAC name and chemical family

Compound	IUPAC NAME	Chemical family
25B-NBOMe	(2-(4-bromo-2,5-dimethoxyphenyl)-N,N-bis(2-methoxybenzyl)ethanamine)	phenethylamine
25C-NBOMe (2C-C-NBOMe)	2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine	phenethylamine
25E-NBOMe	2-(4-Ethyl-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine	phenethylamine
25H-NBOMe	2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine	phenethylamine
25I-NB34MD	(N-(1,3-Benzodioxol-5-ylmethyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethan-1-amine)	phenethylamine
25I-NBMD	2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2,3-methyldioxyphenyl)methyl]ethanamine	phenethylamine
25I-NBOMe	4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine	phenethylamine
25iP-NBOMe	2-[2,5-Dimethoxy-4-(propan-2-yl)phenyl]-N-(2-methoxybenzyl)ethanamine	phenethylamine
2C-B	4-bromo-2,5-dimethoxyphenethylamine	phenethylamine
2C-E	2,5-dimethoxy-4-ethylphenethylamine	phenethylamine
2-Cloro-4,5-MDMA	1-(6-chloro-1,3-benzodioxol-5-yl)-N-methylpropan-2-amine	phenethylamine
2-methoxyamphetamine	1-(2-methoxyphenyl)propan-2-amine	phenethylamine
2-PEA (phenetylamine)	2-phenethylamine	phenethylamine
3,4-DMA-NBOMe	1-(3,4-Dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]propan-2-amine	phenethylamine
3,4-MDPA	1-(1,3-benzodioxol-5-yl)-N-propylpropan-2-amine	phenethylamine
4-EA-NBOMe	1-(4-Ethylphenyl)-N-[(2-methoxyphenyl)methyl]propan-2-amine	phenethylamine
4-FA (4-fluoroamphetamine)	1-(4-Fluorophenyl)propan-2-amine	phenethylamine
4-FMA (4-fluorometamphetamine)	1-(4-Fluorophenyl)-N-methylpropan-2-amine	phenethylamine
4-MMA (4-methylmethamphetamine)	(N-methyl-1-(4-methylphenyl)propan-2-amine)	phenethylamine
4-MMA-NBOMe	N-[(2-Methoxyphenyl)methyl]-N-methyl-1-(p-tolyl)propan-2-amine	phenethylamine
5-APB-NBOMe	1-(Benzofuran-5-yl)-N-[(2-methoxyphenyl)methyl]propan-2-amine	phenethylamine
5-EAPB	1-(1-benzofuran-5-yl)-N-ethylpropan-2-amine	phenethylamine
6-APB [6-(2-Aminopropil)benzofurano]	1-(1-Benzofuran-6-yl)propan-2-amine	phenethylamine
6-APDB	1-(2,3-Dihydro-1-benzofuran-6-yl)propan-2-amine	phenethylamine
6-Bromo-MDMA	6-bromo-3,4-methylenedioxy-N-methylamphetamine	phenethylamine
6-EAPB	(1-(1-benzofuran-6-il)-N-etilpropan-2-amina)	phenethylamine
bk-2C-B	(2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethanone)	phenethylamine

DOC	1-(4-Chloro-2,5-dimethoxyphenyl)propan-2-amine	phenethylamine
DOIP	1-[2,5-Dimethoxy-4-(propan-2-yl)phenyl]propan-2-amine	phenethylamine
DOM	1-(2,5-Dimethoxy-4-methylphenyl)propan-2-amine	phenethylamine
MDAI	6,7-Dihydro-5H-cyclopenta[f][1,3]benzodioxol-6-amine	phenethylamine
MPA (Methylthienylpropamine)	N-methyl-1-(thiophen-2-yl)propan-2-amine	phenethylamine
NEDPA	(N-iso-propil-1,2-difeniletilamina)	phenethylamine
N-methyl-2AI	(N-methyl-2,3-dihydro-1H-inden-2-amine)	phenethylamine
N-methyl-2C-B	2-(4-Bromo-2,5-dimethoxyphenyl)-N-methylethanamine	phenethylamine
PMA	para-Methoxyamphetamine	phenethylamine
PMMA	para-Methoxy-N-methylamphetamine	phenethylamine
Deschloroketamine	(2-(Methylamino)-2-phenyl-cyclohexan-1-one)	arilcicloexilamine - ketamine anal.
Methoxetamine bromo derivative	2-(2-bromo,5-methoxyphenyl)-2-(ethylamino)cyclohexanone	arilcicloexilamine - ketamine anal.
Methoxethamine (MXE / 3-MeO-2'-Oxo-PCE)	2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone	arilcicloexilamine - ketamine anal.
2-MeO-diphenidine (MXP / 2-MXP)	1-(1-(2-methoxyphenyl)-2-phenylethyl)piperidine	piperidine
3-MeO-PCP	1-[1-(3-methoxyphenyl)cyclohexyl]-piperidine	piperidine
Diphenidine	1-(1,2-diphenylethyl)piperidine	piperidine
Isopropylphenidate	(Propan-2-yl 2-phenyl-2-piperidin-2-yl acetate)	piperidine
HDEP-28 (Ethylnaphthidate)	(Ethyl 2-(naphthalen-2-yl)-2-(piperidin-2-yl)acetate)	piperidine
HDMP-28 (methylnaphthidate)	(Methyl (2R)-2-naphthyl[(2R)-2-piperidinyl]acetate	piperidine
5-MeO-DALT	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-prop-2-enylprop-2-en-1-amine	tryptamine
5-MeO-EIPT	N-ethyl-N-(2-(5-methoxy-1H-indol-3-yl)ethyl)propan-2-amine	tryptamine
5-MeO-MIPT	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-methylpropan-2-amine	tryptamine
5-MeO-NiPT	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]propan-2-amine	tryptamine
AMT (α-methyltryptamine)	1-(1H-Indol-3-yl)propan-2-amine	tryptamine
DALT	N-[2-(1H-indol-3-yl)ethyl]-N-prop-2-enylprop-2-en-1-amine	tryptamine
MET (N-methyl-N-ethyltryptamine)	N-Ethyl-2-(1H-indol-3-yl)-N-methylethanamine	tryptamine
2-FMC (2-fluoromethcathinone)	(1-(2-fluorophenyl)-2-(methylamino)propan-1-one)	cathinone
2-methylmethcathinone	(1-(2-methylphenyl)-2-(methylamino)propane-1-one)	cathinone
3,4-dimethoxy-alpha-PHP (3,4-DMeO-α-PHP)	1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)octan-1-one	cathinone
3,4-Dimethylethcathinone (3,4-DMEC)	1-(3,4-dimethylphenyl)-2-(ethylamino)propan-1-one	cathinone

3,4-DMeO-α-PVP	1-(3,4-dimethoxyphenyl)-2-(pyrrolidin-1-yl)pentan-1-one	cathinone
3-CMC	1-(3-Chlorophenyl)-2-(methylamino)propan-1-one	cathinone
3-methoxymethcathinone	1-(3-methoxyphenyl)-2-(methylamino)propane-1-one	cathinone
3-methylmethcathinone (3-MMC)	2-(Methylamino)-1-(3-methylphenyl)-1-propanone	cathinone
3-methylethcathinone (3-MEC)	2-(ethylamino)-1-(3-methylphenyl)propan-1-one	cathinone
3,4-dimethylmethcathinone (3,4-DMMC)	1-(3,4-dimethylphenyl)-2-(methylamino)propan-1-one	cathinone
4'-chloro-α-PPP	1-(4-chlorophenyl)-2-pyrrolidin-1-ylpropan-1-one	cathinone
4-bromoamphetamine (4-BA)	(1-(4-Bromophenyl)propan-2-amine)	cathinone
4-Bromoethcathinone (4-BEC)	1-(4-bromophenyl)-2-(ethylamino)propan-1-one	cathinone
4Br-α-PVP	(1-(4-Bromophenyl)-2-(1-pyrrolidinyl)-1-pentanone)	cathinone
4-EEC (Ethylethcathinone)	2-(Ethylamino)-1-(4-ethylphenyl)propan-1-one	cathinone
4-FEC	(2-(Ethylamino)-1-(4-fluorophenyl)propan-1-one)	cathinone
4-fluoromethcathinone (4-FMC)	RS)-1-(4-Fluorophenyl)-2-methylaminopropan-1-one	cathinone
4-fluoro-N-isopropilnorpentedrone	1-(4-fluorophenyl)-2-(1-methylethylamino)pentan-1-one	cathinone
4-fluoropentedrone	(1-(4-fluorofenil)-2-(metilamino)pentan-1-one)	cathinone
4F-PBP	(1-(4-Fluorophenyl)-2-(1-pyrrolidinyl)-1-butanone)	cathinone
4F-PE	1-(4-Fluorophenyl)-2-(pyrrolidin-1-yl)heptan-1-one	cathinone
4F-α-POP	1-(4-fluorophenyl)-2-(pyrrolidin-1-yl)octan-1-one	cathinone
4F-α-PVP	(1-(4-fluorofenil)-2-(pirrolidin-1-il)pentan-1-one)	cathinone
4-MEC (4-Methylethcathinone)	2-(ethylamino)-1-(4-methylphenyl)propan-1-one	cathinone
4-MeO-α-PBP	1-(4-methoxyphenyl)-2-(pyrrolidin-1-yl)butan-1-one	cathinone
4-MeO-α-PEP or 4-MeO-α-PV8	1-(4-methoxyphenyl)-2-pyrrolidin-1-yl-heptan-1-one	cathinone
4-MeO-α-PV9	Methyl 2-{[1-(cyclohexylmethyl)-1H-indazole-3-carbonyl]amino}-3-methylbutanoate	cathinone
4-methyl-N-ethylpentedrone	2-(Ethylamino)-1-(4-methylphenyl)pentan-1-one	cathinone
4-methylpentedrone	(2-(metilamino)-1-(p-tolil)pentan-1-one (4-metilpentedrone)	cathinone
4-Methyl-N,N-diethylcathinone	2-Diethylamino-1-(4-methylphenyl)propan-1-one	cathinone
5-BPDi	1-(2,3-Dihydro-1H-inden-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one	cathinone
5-DBFPV	(1-(2,3-dihydro-1-benzofuran-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one)	cathinone
Bk-IVP	1-(2,3-dihydro-1H-inden-5-yl)-2-(ethylamino)pentan-1-one	cathinone

bk-MDMA (3,4-methylenedioxy-N-methylcathinone/ Methylone)	1-(1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one	cathinone
bk-PMMA (Methedrone)	1-(4-methoxyphenyl)-2-(methylamino)propan-1-one	cathinone
Clephedrone	1-(4-chlorophenyl)-2-(methylamino)propan-1-one	cathinone
bk-DMDBP (Dipentylone)	1-(1,3-benzodioxol-5-yl)-2-(dimethylamino)-pentan-1-one	cathinone
Eutylone	1-(1,3-benzodioxol-5-yl)-2-(ethylamino)butan-1-one	cathinone
MDPHP	1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one	cathinone
MDPV (3,4-Methylenedioxypyrovalerone)	1-(Benzo[*d*] [1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one	cathinone
Mephedrone (4-MMC)	(RS)-2-methylamino-1-(4-methylphenyl)propan-1-one	cathinone
MTTA (mephtetramine)	2-((metilamino)metil)-3,4-diidronaftalen-1(2H)-one)	cathinone
N-Methyl-bk-MMDA-2	1-(6-methoxy-1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one	cathinone
Nor-mephedrone	(2-Amino-1-(4-methylphenyl)propan-1-one)	cathinone
NPDPA	(1-(1,3-benzodiossol-5-il)-2-(dimetilamino)-pentan-1-one)	cathinone
α-ethylaminopentiophenone	2-(ethylamino)-1-phenylpentan-1-one	cathinone
α-PBT	2-(Pyrrolidin-1-yl)-1-(thiophen-2-yl)butan-1-one	cathinone
Pentedrone (α-methylamino-valerophenone/ β-ethyl-methcathinone)	1-phenyl-2-(methylamino)pentan-1-one	cathinone
α-PVP (α-Pyrrolidinopentiophenone / α-pyrrolidinovalerophenone)	1-Phenyl-2-(1-pyrrolidinyl)-1-pentanone	cathinone
α-PVT (α-Pyrrolidinopentiothiophenone)	2-(pyrrolidin-1-yl)-1-(thiophen-2-yl)pentan-1-one	cathinone
α-PHP (α-pyrrolidinohexanophenone)	2-(pyrrolidin-1-yl)-1-(phenyl)hexan-1-one	cathinone
α-POP (α-Pyrrolidinooctanophenone)	1-Phenyl-2-(pyrrolidin-1-yl)octan-1-one	cathinone
β-propylmethcathinone (Hexedrone / "hexa")	2-(methylamine)-1-(phenyl)hexan-1-one	cathinone
2NE1 (APICA/ JWH-018 adamantil carbossammide/ SDB-001)	N-[(3s,5s,7s)-Adamantan-1-iy]-1-pentyl-1H-indole-3-carboxamide	Synthetic cannabinoid
5F-APICA (STS-135)	N-(Adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide	Synthetic cannabinoid
5C-AKB48	(N-(Adamantyl)-1-(5-chloropentyl)-1H-indazole-3-carboxamide)	Synthetic cannabinoid
5F-AB-FUPPYCA	(N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxamide)	Synthetic cannabinoid
5F-ADBICA/ 5F-ADBICA-144/ 5F-AMBICA / 5-FADB/ 5F-ADBINACA	N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1Hindol-3-carboxamide	Synthetic cannabinoid
5F-ADB-PINACA	(N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide)	Synthetic cannabinoid

5F-AMB-PICA / AMB-PICA / MMB2201 / MMB-2201 / I-AMB	Methyl 2-({[1-(3-fluoropropyl)-1H-indol-3-yl]carbonyl}amino)-3-methylbutanoate	Synthetic cannabinoid
5F-AMB	Methyl (2S)-2-{[1-(5-fluoropentyl)-1H-indazol-3-yl]formamido}-3-methylbutanoate	Synthetic cannabinoid
5F-APINACA / AKB-48F / 5F-AKB48	N-(1-adamantyl)-1-(5-fluoropentyl)-1Hindazole-3-carboxamide	Synthetic cannabinoid
5F-APP-PICA	N-(2-amino-1-benzyl-2-oxo-ethyl)-1-(5-fluoropentyl)indazole-3-carboxamide	Synthetic cannabinoid
AB-PINACA	N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-pentyl-1H-indazole-3-carboxamide	Synthetic cannabinoid
5F-AB-PINACA	N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)indazole-3-carboxamide	Synthetic cannabinoid
5F-APP-PINACA / PX-2 / PX 2 / 5-fluoro APP PINACA / FU-PX	N-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide	Synthetic cannabinoid
5F-EMB-PINACA / 5F-AEB	(Ethyl 2-(1-[5-fluoropentyl]-1H-indazole-3-carboxamido)-3-methylbutanoate)	Synthetic cannabinoid
5-Fluoropentyl-3-pyridinoylindole	(1-(5-Fluoropentyl)-1H-indol-3-yl](3-pyridinyl)methanone)	Synthetic cannabinoid
5F-MDMB-PINACA / 5F-Methyl-AMB / 5-fluor-MAMB / 5-fluor ADB / 5F-ADB	Methyl (S)-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate	Synthetic cannabinoid
PB-22	1-Pentyl-1H-indole-3-carboxylic acid 8-quinolinyl ester	Synthetic cannabinoid
5F-PB-22	8-quinolinyl ester-1-(5-fluoropentyl)-1H-indole-3-carboxylic acid	Synthetic cannabinoid
5F-NPB-22	1-(5-Fluoropentyl)-8-quinolinyl ester-1H-indazole-3-carboxylic acid	Synthetic cannabinoid
5F-PB-22 indazole analogue	Quinolin-8-yl 1-(5-fluoropentyl)-1H-indazole-3-carboxylate	Synthetic cannabinoid
5F-PY-PICA	(1-(5-Fluoropentyl)-3-(pyrrolidine-1carbonyl)-1-H-indole)	Synthetic cannabinoid
5F-PY-PINACA	((1-(5-Fluoropentyl)-1H-indazole-3-yl)(pyrrolidine-1-yl)methanone)	Synthetic cannabinoid
AB-CHMINACA	N-[(2S)-1-amino-3-methyl-1-oxo-2-butanyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
AB-FUBINACA	(S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
ADAMANTYL-THPINACA	N-(1-adamantyl)-1-(tetrahydropyran-4-ylmethyl)indazole-3-carboxamide	Synthetic cannabinoid
ADB-CHMINACA / MAB-CHMINACA	N-[1-(Aminocarbonyl)-2,2-dimethylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
ADB-FUBINACA	(N-[(1S)-1-(amminocarbonil)-2-metilpropil]-1-[(4-fluorofenil)metil]-1H-indazolo-3-carbossamide)	Synthetic cannabinoid
ADB-PINACA	(N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide)	Synthetic cannabinoid
AKB-48 (APINACA)	N-(adamantan-1-yl)-1-pentyl-1H-indazole-3-carboxamide	Synthetic cannabinoid
AM-2201	[1-(5-Fluoropentyl)-1H-indol-3-yl](naphthalen-1-yl)methanone	Synthetic cannabinoid
AM-6527 5-fluoropentyl derivative / 5-Fluor-NNEI / 5F-NNEI / 5F-MN24	(1-(5-fluoropentil)-N-(naftalen-2-il)-1H-indolo-3-cabossamide)	Synthetic cannabinoid
AMB-CHMINACA / MA-CHMINACA	2-(1-(cicloesilmetil)-1H-indazolo-3-carbossammide)-3-metilbutanoato	Synthetic cannabinoid

AMB-FUBINACA	Methyl 2-({[1-(4-fluorobenzyl)-1H-indazol-3-yl]carbonyl}amino)-3-methylbutanoate	Synthetic cannabinoid
APP-FUBINACA	N-(1-amino-3-phenyl-1-oxopropan-2-yl)-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide	Synthetic cannabinoid
BB-22	8-Quinolinyl 1-(cyclohexylmethyl)-1H-indole-3-carboxylate	Synthetic cannabinoid
CBL-018	(Naphthalen-1-yl-1-pentyl-1H-indole-3-carboxylate)	Synthetic cannabinoid
CUMYL-5FPICA	1-(5-Fluoropentyl)-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide	Synthetic cannabinoid
CUMYL-5F-PINACA (SGT-25)	1-(5-Fluoropentyl)-N-(1-methyl-1-phenylethyl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
CUMYL-BICA (SGT-55)	1-Butyl-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide	Synthetic cannabinoid
CUMYL-PICA (SGT-56)	1-Pentyl-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide	Synthetic cannabinoid
CUMYL-PINACA	1-Pentyl-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
CUMYL-THPINACA (SGT-42)	N-(2-phenylpropan-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1-H-indazole-3-carboxamide	Synthetic cannabinoid
DB-MDBP	(1-((2,2-difluorbenzo [D] [1,3] dioxol-5-yl) methyl) piperazine)	Synthetic cannabinoid
EMB-FUBINACA	(Ethyl 2-(1-[4-fluorobenzyl]-1H-indazole-3-carboxamido)-3-methylbutanoate)	Synthetic cannabinoid
FDU-PB-22	Naphthalen-1-yl-1-(4-fluorobenzyl)-1H-indole-3-carboxylate	Synthetic cannabinoid
FUB-144 / FUB-UR-144	([1-(4-Fluorobenzyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone)	Synthetic cannabinoid
FUB-AKB48 / AKB48 N-(4-fluorobenzyl) analogue	N-((3s,5s,7s)-adamantan-1-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide	Synthetic cannabinoid
FUB-JWH-018	(1-(4-Fluorobenzyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone)	Synthetic cannabinoid
FUB-PB-22	(Quinolin-8-yl-1-(4-fluorobenzyl)-1H-indole-3-carboxylate)	Synthetic cannabinoid
JWH-018 indazole analogue (THJ-018)	Naphthalen-1-yl(1-pentyl-1H-indazol-3-yl)methanone	Synthetic cannabinoid
THJ-2201	[1-(5-Fluoropentyl)-1H-indazol-3-yl](1-naphthyl)methanone	Synthetic cannabinoid
EG-018	1-Naphthyl(9-pentyl-9H-carbazol-3-yl)methanone	Synthetic cannabinoid
BZ-2201	(1-(5-fluoropentyl)-1H-benzo[d]imidazol-2-yl)(naphthalen-1-yl)methanone	Synthetic cannabinoid
JWH-071	(1-etil-1H-indol-3-il)-1-naftalenil-metanone	Synthetic cannabinoid
JWH-122 (1-pentyl-3-(1-(4-methyl)naphthoyl)indol)	(4-methylnaphthalen-1-yl)-(1-pentylindol-3-yl)methanone	Synthetic cannabinoid
JWH-210	1-Pentyl-3-(4-ethyl-1-naphthoyl)indole	Synthetic cannabinoid
JWH-412 5-fluoropentyl derivative	(4-fluoronaphthalen-1-yl)[1-(5-fluoropentyl)-1H-indol-3-yl]methanone	Synthetic cannabinoid
M-CHMIC	(Methyl-1-(cyclohexylmethyl)-1H-indole-3-carboxylate)	Synthetic cannabinoid
MDMB(N)BZ-F' / MDMB-FUBINACA	metil-2-[1-(4-fluorobenzil)-1-H-indazol-3-carbossamide]-3,3-dimetilbutanoato	Synthetic cannabinoid
MDMB-CHMICA	Methyl 3,3-dimethyl-2-{[(1-(cyclohexylmethyl)-1H-indol-3-yl)carbonyl]amino}butanoate	Synthetic cannabinoid

MDMB-CHMICA /MMB-CHMINACA	(metil-2-(1-(cicloesilmetil)-1-H-indol-3-ilcarbonilamino)-3,3-dimetilbutanoato)	Synthetic cannabinoid
MDMB-FUBICA	Methyl 2-(1-(4-fluorobenzil)-1H-indol-3-carbossammide)-3,3- dimetilbutanoate	Synthetic cannabinoid
MDMB-CHMCZCA	methyl 2-(9-(cyclohexylmethyl)-9H-carbazole-3-carboxamido)-3,3-dimethylbutanoate	Synthetic cannabinoid
Mepirapim	(4-methylpiperazin-1-yl)(1-pentyl-1H-indol-3-yl)methanone	Synthetic cannabinoid
MN-18	N-(naphthalen-1-yl)-1-pentyl-1H-indazole-3-carboxamide	Synthetic cannabinoid
5F-MN18	1-(5-fluoropentyl-N-1-naphtalenyl-1H-indazole-3-carboxamide	Synthetic cannabinoid
NM-2201 / CBL-2201	Naphthalen-1-yl-1-(5-fluoropentyl)-1H-indol-3-carboxylate	Synthetic cannabinoid
PB-22 indazole analogue	Quinolin-8-yl 1-pentyl-1H-indazole-3-carboxylate	Synthetic cannabinoid
RCS-4	4-methoxyphenyl-(1-pentyl-1H-indol-3-yl)methanone	Synthtic cannabinoid
RH-34	(3-[2-[(2-methoxyphenyl)methylamino]ethyl]-1H-quinazoline-2,4-dione)	Synthtic cannabinoid
SDB-005	(Naphthalen-1-yl-1-pentyl-1H-indazole-3-carboxylate)	Synthetic cannabinoid
SDB-006	(N-benzyl-1-pentyl-1H-indole-3-carboxamide)	Synthetic cannabinoid
4-fluoro-butyrfentanyl	N-(4-fluorophenyl)-N-[(1-(2-phenylethyl)-4-piperidinyl)]butanamide	Synthetic oppioid
Acetylfentanyl	N-Phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide	Synthetic oppioid
AH-7921	3,4-dichloro-N-[(1-(dimethylamino)cyclohexyl)methyl]benzamide	Synthetic opioid
Butyrfentanyl	N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-butanamide	Synthetic opioid
Despropionyl-2-fluoro fentanyl	(N-(2-Fluorophenyl)-1-(2-phenylethyl)piperidin-4-amine)	Synthetic opioid
MT-45	1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine	Synthetic opioid
Ocfentanyl (A-3217)	(N-(2-fluorophenyl)-2-methoxy-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide)	synthetic opioid
U-47700	Trans 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide	synthetic opioid
W-18	4-Chloro-N-(1-[2-(4-nitrophenyl)ethyl]-piperidin-2-ylidene)benzenesulfonamide	Synthtic opioid
Para methyl-4-methylaminorex (4-4'-DMAR)	4-Methyl-5-(4-methylphenyl)-4,5-dihydro-1,3-oxazol-2-amine	aminorex derivate
4-Methylmethylphenidate	Methyl 2-(1-(4-fluorobenzyl)-1H-indol-3-carboxamido)-3,3-dimethylbutanoate	aminorex derivate
N-Methyl aminorex	(3-Methyl-5-phenyl-1,3-oxazolidin-2-imine)	aminorex derivate
Ibogaine	(-)-12-Methoxyibogamine	natural substance
Methallylescaline	(2-[3,5-dimethoxy-4-(2-methylprop-2-enoxy)phenyl]ethanamine)	natural substance
Mitragyna (kratom)	(αE,2S,3S,7aS,12bS)-3-ethyl-1,2,3,4,6,7,7a,12b-octahydro-7a-hydroxy-8-methoxy-α-(methoxymethylene)-indolo[2,3-a]quinolizine-2-acetic acid methyl ester	natural substance
Mesembrine	(3aS,7aS)-3a-(3,4-dimethoxyphenyl)-1-methyl-2,3,4,5,7,7a-hexahydroindol-6-one	natural substance
Clonazolam	6-(2-chlorophenyl)-1-methyl-8-nitro-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	benzodiazepine

Phenazepam	7-Bromo-5-(2-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	benzodiazepine
Deschloroetizolam / ETZ-2 / Etizolam-2	2-ethyl-9-methyl-4-phenyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine	benzodiazepine
Pyrazolam	8-Bromo-1-methyl-6-pyridin-2-yl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	benzodiazepine

Table S2: Limits of detection (LOD) and limits of quantification (LOQ) for quantitative analysis of wastewater and pooled urine.

	Influent WW		Pooled Urine	
	LOD (ng L^{-1})	LOQ (ng L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})
Amphetamine	30	100	1.06	3.54
Benzoylecgonine	0.6	2	0.02	0.07
Buphedrone	0.2	0.7	0.31	1.02
Butylone	1.5	5	0.21	0.71
Cocaine	1.5	5	0.05	0.18
Ethylone	50	167	0.25	0.84
Ketamine	6	19	0.20	0.67
Mephedrone	1.5	5	0.21	0.71
Methamphetamine	25	82	0.87	2.90
Methcathinone	8	27	0.28	0.95
Methedrone	1.5	5	0.21	0.71
Methoxetamine	2	5	0.05	0.18
MDPV	0.1	1	0.04	0.14
Methylone	1.5	5	0.21	0.71
N-ethylcathinone	1.5	5	0.21	0.71
Naphyrone	0.3	1	0.04	0.14
Ephenidine (NEDPA)	0.1	0.2	0.10	0.34
MDMA	9	30	0.32	1.06
THC-COOH	18	60	0.64	2.12
bk-MDDMA	6	20	0.21	0.71
25-B-NBOMe	0.3	1	0.04	0.14
25-C-NBOMe	1.5	5	0.21	0.71
25-I-NBOMe	1.5	5	0.21	0.71
25-iP-NBOMe	0.1	0.3	0.11	0.35
4-methyl-α-pyrrolydinopropiophenone (4-MePPP)	5	18	0.19	0.64
α-pyrrolidinopentiophenone (α-PVP)	13	43	0.46	1.52
Dimethylpentylone (bk-DMBDP)	2	6	0.06	0.21
ρ-methoxymethamphetamine (PMMA)	3	10	0.11	0.35
2-phenethylamine	36	120	1.27	4.25
3,4-DiMeO-α-PVP	3	9	0.10	0.32

3,4-dimethylmethcathinone (3,4-DMMC)	25	83	0.14	0.45
4,4'-dimethylaminorex (4-4'-DMAR)	0.1	0.2	0.08	0.27
4-chloro-α-PPP	5	17	0.18	0.60
4-fluoromethcathinone (4-FMC)	25	83	0.27	0.89
4-methylethcathinone (4-MEC)	8	27	0.27	0.91

Table S3: Concentration (µg/L) of drugs and NPS measured in pooled urine samples of UK 2015.

	Day 1										Day 2									
Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Amphetamine	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
Methamphetamine	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
MDMA	519	504	389	594	692	629	619	578	227	196	792	415	247	925	419	579	1166	1068	802	1044
Cocaine	10	35	3.9	207	203	43	218	216	0.8	d	24	1.1	0.7	1.8	0.9	2.1	329	254	7.4	94
Benzoylecgonine [a]	166	134	104	188	197	131	209	197	135	84	176	146	94	136	152	120	352	243	158	230
THC-COOH [b]	60	478	89	54	43	39	43	42	23	31	48	43	25	44	72	71	74	64	51	93
4-FMC	-	-	-	-	1.3	-	1.5	23	-	-	-	-	-	-	-	-	-	-	-	2.1
4-MEC	-	25	-	0.3	-	-	0.7	1.4	-	-	2.6	-	-	0.6	-	-	0.7	57	-	13
α-PVP	2.5	2.3	1.8	9.5	4.1	1.6	26	12	1.9	d	10	63	36	65	5.9	4.8	20	29	1.1	16
Butylone	-	-	-	-	-	-	-	-	-	-	-	0.7	0.5	-	-	-	-	-	-	-
Ethylone [c]	d	d	-	d	d	d	d	d	-	-	d	-	-	-	-	d	d	d	d	d
Ketamine	28	14	14	13	38	1.9	45	32	4.4	1.1	23	24	17	37	2.8	11	28	54	30	27
MDPV	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mephedrone	-	-	-	4.6	-	-	4.0	-	-	-	4.5	-	-	-	-	-	4.3	13	-	-
Methylone	-	-	-	1.7	0.4	-	-	-	-	-	-	-	-	-	-	-	0.7	1.0	-	-
SUM TOTAL NPS	31	41	16	29	44	3.5	77	68	6.3	1.1	40	88	54	103	8.7	16	54	154	31	58

d = detected, concentrations below limit of quantification (< LOQ)

[a] Benzoylecgonine is the main metabolite of cocaine

[b] 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) is the main metabolite of cannabis

[c] Ethylone was retrospectively detected by UHPLC-IMS -QTOF. It could be confirmed, but not quantified as at the time of analysis the quantitative method was not fully validated for this compound

Table S4: Concentration (µg/L) of drugs and NPS measured in pooled urine samples of UK 2016.

	Day 1												Day 2											
Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Amphetamine	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
Methamphetamine	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
MDMA	350	253	478	760	706	532	428	800	421	362	497	539	610	467	569	710	760	688	833	1965	401	497	487	689
Cocaine	2.6	0.9	3.9	1.7	1.4	d	3.2	0.8	1.3	1.2	5.5	1.2	0.7	-	1.8	3.3	d	1.0	0.9	d	d	1.0	1.7	0.9
Benzoylecgonine	213	124	333	181	185	199	308	174	126	107	179	126	126	171	157	178	174	162	177	132	176	169	166	173
THC-COOH	5.9	26	7.1	6.9	8.5	12	2.6	14	6.3	18	4.6	7.2	8.4	6.8	7.7	15	9.9	19	13	11	12	5.2	14	14
α-PVP	1.4	1.7	2.3	1.0	d	d	d	1.1	2.4	1.8	d	d	1.0	-	2.2	1.7	2.6	1.1	4.7	3.5	1.1	1.7	6.4	2.3
Butylone	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-
Ketamine	35	28	55	83	89	70	86	61	36	27	60	18	29	51	63	54	118	70	130	178	18	8.2	41	84
Mephedrone	-	-	-	-	d	-	-	-	-	-	-	-	-	d	-	d	-	-	-	-	-	-	-	d
Methoxetamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	2.8	-	-	-	1.7
Methylone	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	2.1	-	-	-	-
SUM TOTAL NPS	36	30	57	84	89	70	86	62	38	29	60	18	30	51	65	56	121	71	135	186	19	9.9	47	88

d = detected, concentrations below limit of quantification (< LOQ)

Table S5: Concentration (µg/L) of drugs and NPS measured in pooled urine samples of Belgium.

	Day 1			Day 2			Day 3		
Hour (h)	**16**	**20**	**24**	**16**	**20**	**24**	**16**	**20**	**24**
Amphetamine	d	d	d	d	d	d	d	d	d
Methamphetamine	d	d	d	d	d	d	d	d	d
MDMA	232	281	683	344	518	949	529	516	955
Cocaine	15	30	75	210	59	62	28	98	69
Benzoylecgonine	78	58	126	189	110	122	97	87	119
THC-COOH	43	78	50	34	27	41	34	32	32
Ketamine	0.5	0.5	7.3	d	2.1	6.2	d	6.9	8.0
Mephedrone	-	1.5	-	-	-	-	-	-	-
Methylone	1.6	-	-	0.4	1.0	-	-	0.8	0.7
SUM TOTAL NPS	2.1	2	7.3	0.4	3.1	6.2	d	7.7	8.7

d = detected, concentrations below limit of quantification (< LOQ)

Table S6: Concentration (µg/L) of drugs and NPS measured in pooled urine samples of Norway.

Day	**1**	**2**	**3**
Amphetamine	11	4.9	4.5
Methamphetamine	1.4	3.8	1.9
MDMA	27	29	8.7
Cocaine	15	46	17
Benzoylecgonine	6.6	13	11
THC-COOH	1.4	1.3	0.9
2-Phenethylamine	-	d	d
4-chloro-α-PPP	d	d	-
Ketamine	0.05	0.07	-
Methcathinone	0.3	0.3	0.3
Methoxetamine	d	-	-
SUM TOTAL NPS	0.35	0.37	0.3

d = detected, concentrations below limit of quantification (< LOQ)

Table S7: Loads (g/day) of drugs and NPS measured in wastewater samples of Portugal (2017) during one week, which coincided with a festival.

Day	1* THU	2* FRI	3* SAT	4 SUN	5 MON	6 TUE	7 WED
Amphetamine	-	-	-	-	-	-	-
Methamphetamine	-	d	d	d	d	-	-
MDMA	8.5	15.9	26.3	30.0	20.8	21.6	8.0
Cocaine	4.9	29.4	32.3	25.2	30.8	18.6	11.6
Benzoylecgonine	264	251	290	288	258	223	222
THC-COOH	65	305	353	362	413	340	338
2-Phenethylamine	d	d	d	d	d	d	d
25-E-NBoMe	d	-	-	-	-	-	-
3,4-DMMC	-	-	-	0.3	-	-	-
4-chloro-α-PPP	d	d	d	d	d	-	-
α-methyltryptamine	d	-	-	-	-	-	-
Buphedrone	-	-	0.5	0.3	0.2	-	0.2
DOiP	-	-	d	-	-	-	-
Ketamine	-	-	d	d	d	d	-
Methcathinone	-	0.2	0.1	0.1	0.1	-	-
Mephedrone	0.2	1.6	1.4	0.9	0.5	0.3	0.2
SUM TOTAL NPS	0.2	1.8	2.0	1.6	0.8	0.3	0.4

*Festival days; d = detected, concentrations below limit of quantification (< LOQ)

Table S8: Loads (g/day) of drugs and NPS measured in wastewater samples of Portugal (2017) during six consecutive days, which did not coincide with a festival or special event.

Day	1 WED	2 THU	3 FRI	4 SAT	5 SUN	6 MON
Amphetamine	-	-	-	-	-	-
Methamphetamine	-	-	-	-	-	-
MDMA	7.7	7.1	5.6	20.8	47.6	16.5
Cocaine	20.4	24.5	27.2	38.3	30.2	13.3
Benzoylecgonine	114	129	127	238	247	162
THC-COOH	56	35	42	39	28	34
SUM TOTAL NPS	-	-	-	-	-	-

d = detected, concentrations below limit of quantification (< LOQ)

Table S9: Loads (g/day) of drugs and NPS measured in wastewater samples of Serbia (2017) during one week, which coincided with a festival.

Day	1 MON	2 TUE	3* WED	4* THU	5* FRI	6 SAT	7 SUN
Amphetamine	4.7	3.6	6.5	6.2	8.7	11.9	11.8
Methamphetamine	-	-	d	d	0.1	0.2	0.1
MDMA	6.2	4.4	9.9	41.9	93.3	120	175
MDA	-	-	0.7	3.0	7.4	8.2	12.9
Cocaine	2.5	1.9	2.9	2.9	3.9	4.9	5.0
Benzoylecgonine	14.8	10.7	13.9	15.7	18.8	30.3	27.2
THC-COOH	na	na	na	na	na	na	na
25-iP-NBoMe	-	0.3	-	-	-	-	-
4,4-DMAR	-	-	0.08	0.03	-	-	-
α-methyltryptamine	-	-	-	-	d	-	-
Methcathinone	-	-	-	0.04	0.04	0.05	0.04
NEDPA	-	0.3	-	-	-	-	-
SUM TOTAL NPS	-	0.6	0.08	0.07	0.04	0.05	0.04

*Festival days; d = detected, concentrations below limit of quantification (< LOQ); na = not analysed. These samples were analysed using the methodology developed by Zuccato et al. 2016 for drugs and González-Mariño et al., 2016 for NPS.

Table S10: Loads (g/day) of drugs and NPS measured in pooled wastewater samples (week and weekend) of Serbia (2017), which did not coincide with a festival or special event.

	1	**2**
Day	**Week days**	**Weekend days**
Amphetamine	5.8	7.8
Methamphetamine	-	-
MDMA	3.2	9.2
MDA	-	0.8
Cocaine	3.1	3.4
Benzoylecgonine	14.2	17.3
THC-COOH	na	na
4,4-DMAR	-	0.11
α-methyltryptamine	-	d
Methcathinone	0.05	0.05
SUM TOTAL NPS	0.05	0.16

d = detected, concentrations below limit of quantification (< LOQ); na = not analysed. These samples were analysed using the methodology developed by Zuccato et al. 2016 for drugs and González-Mariño et al., 2016 for NPS.

Table S11: Loads (g/day) of drugs and NPS measured in wastewater samples of Spain (2018) during seven consecutive days, which coincided with a festival.

	1	2	3*	4*	5*	6*	7
Day	**TUE**	**WED**	**THU**	**FRI**	**SAT**	**SUN**	**MON**
Amphetamine	-	d	1.1	2.8	12.7	11.4	5.6
Methamphetamine	-	-	-	d	d	0.5	d
MDMA	0.5	0.7	1.8	17.8	123	106	39.7
Cocaine	3.0	4.5	6.0	5.7	20.0	17.4	10.4
Benzoylecgonine	12.3	17.5	24.2	27.9	76.9	66.8	31.9
THC-COOH	1.4	1.3	2.8	2.4	3.6	3.4	3.5
Ketamine	1.1	d	d	1.2	12.6	5.6	2.9
Methylone	-	-	-	-	-	d	d
SUM TOTAL NPS	1.1	d	d	1.2	12.6	5.6	2.9

*Festival days; d = detected, concentrations below limit of quantification (< LOQ)

Table S12: Loads (g/day) of drugs and NPS measured in wastewater samples of Spain (2018) during seven consecutive days, which did not coincide with a festival or special event.

	1	2	3	4	5	6	7
Day	**MON**	**TUE**	**WED**	**THU**	**FRI**	**SAT**	**SUN**
Amphetamine	-	-	-	-	-	-	-
Methamphetamine	-	-	-	-	-	-	-
MDMA	d	d	d	d	d	0.2	0.3
Cocaine	4.3	5.4	3.5	3.2	4.9	5.2	4.4
Benzoylecgonine	11.0	11.3	8.0	7.7	10.3	13.5	10.5
THC-COOH	0.7	0.9	0.8	0.7	0.9	1.2	0.6
Ketamine	-	-	d	-	-	d	d
Methylone	-	-	-	-	-	-	-
SUM TOTAL NPS	-	-	d	-	-	d	d

d = detected, concentrations below limit of quantification (< LOQ)

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