Investigating the Appearance of New Psychoactive Substances in South Australia using Wastewater and Forensic Data

Richard Bade, Peter Stockham, Ben Painter, Alberto Celma, Lubertus Bijlsma, Felix Hernandez, Jason M. White, Cobus Gerber

1 School of Pharmacy and Medical Sciences, University of South Australia, Adelaide 5001, Australia
2 Forensic Science SA, GPO Box 2790, Adelaide 5001, Australia
3 Flinders University, College of Science and Engineering, Flinders University, Bedford Park, South Australia
4 Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat s/n, E-12071 Castellon, Spain

§ Visiting researcher at University Jaume I

* Corresponding author: Cobus Gerber, School of Pharmacy and Medical Sciences, University of South Australia, GPO Box 2471, Adelaide, South Australia 5001, Australia
Abstract

New psychoactive substances (NPS) have increased in use and popularity worldwide. Wastewater analysis has been successfully applied to evaluate illicit drugs use within a population. However, for NPS, such approach may be limited due to low doses of NPS combined with their ever-changing composition and usage. The dynamic nature of the NPS market means use may be opportunistic, infrequent and with few users. Hence, the use of complementary information sources is recommended to improve the knowledge on NPS consumption. The aim of this study was to investigate the changing landscape of NPS use on a community scale by combining wastewater analysis and forensic toxicology. Forensic analysis provided specific information on NPS prevalence in post-mortem blood samples in Adelaide, South Australia over five years, while wastewater analysis showed community use over the same period. A qualitative liquid chromatography-high resolution mass spectrometry method was initially used to screen the wastewater samples. A total of 24 NPS were found: six in wastewater only, 13 in forensic post mortem toxicology samples only and five in both. As these results showed the presence of NPS, a targeted method was subsequently employed to quantify levels of these NPS in wastewater. Temporal trends were found in wastewater with distinct tendencies for synthetic cathinones visible over the period studied.

Keywords: Synthetic cathinones, High resolution mass spectrometry, Triple quadrupole, Wastewater, Forensic toxicology
1. Introduction

The use of new psychoactive substances (NPS) is an area of worldwide concern, with NPS gaining popularity, sometimes in place of more conventional illicit drugs. In Europe alone, more than 670 such compounds have been reported to date, with this number growing every year as producers and sellers attempt to avoid legislation. Existing means to monitor NPS use and exposure include drug seizures, police intelligence, media, surveys, forensic toxicology reports and hospital admissions. Nationwide seizure data can provide information on the most prevalent drugs entering the country or particular cities, but the effects on the drug-taking community of any large seizures could take months to be seen. Furthermore, effects may not be evident at a local level. Surveys may not reflect actual use due to unwitting consumption of adulterated drugs. On a community level, roadside drug testing and population surveys predominantly inform on the most common drugs such as MDMA, cannabis, methamphetamine and alcohol. However, both have their own bias in terms of “targeted policing” sampling and reporting. Mechanisms to report hospital admissions and forensic toxicology findings may not be publicly available. Wastewater analysis (WWA) has thus been proposed as a suitable complementary means to provide temporal and spatial trends in NPS use, because it can give information on the identity and amount of drugs being used at any given time.

The ever-changing nature of the NPS market means acquiring standards and developing quantitative analytical methods for compounds which may have a short commercial lifetime is unfeasible. Therefore, targeted, quantitative wastewater methods have limitations, due to the time and expense involved in acquiring standards and developing methods for compounds which may not have an extended lifetime. In this regard, there has been a shift toward qualitative, suspect compound screening methodologies using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). These do not initially require standards and the range of compounds that can be analysed is limited only by the suspect screening database.

The drawback of qualitative screening based on HRMS is its inherent lower sensitivity compared to targeted quantitative methods, e.g. based on LC-MS/MS with triple quadrupole (QqQ). In contrast to popular, conventional, illicit drugs, the use of NPS at any particular time is generally low. Added to that, the low doses and extensive metabolism of some NPS mean that excreted levels of drug residues in wastewater may be very low. Furthermore, their
detection by LC-HRMS could be affected by the complexity of the matrix. Thus, targeted, quantitative methods still have value, although they are limited to the 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 \(^{14-19}\).

In the forensic context, biological samples may be taken from members of the public for drug testing as part of investigations into traffic offences assaults and other criminal activity. Blood samples are taken routinely in post-mortem examinations. Therefore, forensic toxicology can be considered a frontline in the detection of the latest NPS. Toxicological analysis of post mortem cases can demonstrate the presence of particularly harmful substances in the community. The concentration of some NPS in acute intoxications may be relatively high, which may facilitate identification of hitherto unknown intoxicants through generation of molecular formula and interpretation of spectral information. However, in contrast to wastewater, forensic data is unlikely to be able to show changing temporal patterns of use. Thus, the comparison of forensic data with the results of WWA enables a better informed and targeted approach to investigate both which NPS are being used and the temporal changes in their NPS.

Our group has been analysing wastewater samples from South Australia since 2009, primarily to quantify conventional illicit drugs. \(^{20-22}\) Until this study, only the most popular NPS were included in the method due to difficulties in the selection of target compounds from the wide range of possible NPS candidates. In the present work, data from the analysis of 156 wastewater and over 3,500 forensic samples were combined to show the NPS prevalence in Adelaide, South Australia over a 5 year period. The aim of this study was to investigate the use of NPS on a community scale by combining wastewater analysis and forensic toxicology. The forensic data comprised the results of post-mortem investigations from Forensic Science South Australia (FSSA) in known or suspected drug related deaths over the period. After identification of NPS in wastewater by HRMS using a database of 186 compounds, quantitative analysis of those NPS identified in the samples was performed.
2. Materials and Methods

2.1 Chemicals and Reagents

A total of 85 NPS reference standards in the form of mixed standard solutions in methanol were made available for use by Forensic Science SA (FSSA) for the screening method (Table S1). The mixed solutions were supplied in accordance with the appropriate licencing conditions at both the FSSA and the University of South Australia sites. Butylone, mephedrone, methylenedioxyxypovalerone (MDPV), methedrone, methylone, naphyrone and N-ethylcathinone were analysed quantitatively as in our previous work, with pentylnone, ethylene, alpha-pyrrolidinopentiophenone (alpha-PVP), methcathinone, dimethylone, methoxetamine, 4-methylethcathinone, β-pentedrone, N,N-dimethylcathinone, 4-fluoromethcatinone, 3,4-dimethylmethcathinone, buphedrone and 1,3-benzodioxolyl-N-methylbutanamine (MBDB) additionally analysed. Only methylone-d₃ and MDPV-d₈ were added as internal standards as the deuterated analogues for all of the above NPS were not available at the time of the study. The standards and deuterated analogues were purchased from Cerilliant (Round Rock, TX, USA) and Cayman Chemicals (Ann Arbor, MI, USA).

Reagents for the work performed at the University of South Australia: Glacial acetic acid, sodium acetate, isopropanol, ammonia (28 %) and formic acid (99 %) were purchased from VWR Chemicals (Tingalpa, Queensland, Australia), while methanol, hydrochloric acid (37 %) and dichloromethane were purchased from Merck (Kilsyth, VIC, Australia), and sodium metabisulphate (Na₂S₂O₅) from Chem-Supply (Gillman, SA, Australia) Ultrapure water was prepared using an Arium® pro VF system (Sartorius Stedim biotech).

Reagents for the analysis performed at University Jaume I: HPLC-grade methanol, ammonium acetate, ammonia solution (25 %) and formic acid (98–100 %) were acquired from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA).

2.2 Samples

2.2.1 Wastewater samples

24-h (8 a.m.–8 a.m.) flow proportional composite influent wastewater (IWW) was collected bimonthly during the first week of February, April, June, August, October, December (or the second week to avoid public holidays) from June 2012 – June 2017 from two wastewater treatment plants (WWTPs) in South Australia. Samples from the two sites investigated,
hereafter called Site A (covering approximately 700,000 inhabitants) and Site B (covering
approximately 200,000 inhabitants), corresponded to at least one weekend sample (Saturday
or Sunday) and one weekday (Monday – Friday) sample. A total of 156 samples were
analysed for this study. South Australia has a population of approximately 1.6 million
inhabitants, so these two sites cover approximately 75% of the state. Specific information on
sample collection was reported previously.  

Immediately after collection, samples were stored at 4 °C in 2 g/L Na₂S₂O₅ for up to one
week prior to sample preparation. Sample extracts were stored at -20°C, prior to analysis.

A selection of the extracted samples, covering five time periods from February 2015 –
August 2017 were sent to the University Jaume I, Castellon (Spain) for the quantitative
analysis of various NPS.

2.2.2 Forensic samples

The Toxicology Group at Forensic Science SA conducts forensic examinations on biological
samples at the direction of the South Australian State Coroner, South Australia Police, and
other agencies. Approximately 900 South Australian post mortem toxicology cases are
analysed annually for a range of pharmaceutical and illicit substances on behalf of the South
Australian Coroner. All post mortem toxicology cases between August 2013 and June 2017.
Those in which one or more NPS were detected through the routine toxicological screening
methodology are included in this dataset (Table S2). Peripheral blood samples (femoral)
were the typical sample specimen type used for drug screening. Hospital ante-mortem or
other blood or tissue specimens may also have been examined as specific case circumstances
dictated. Permission was obtained from the South Australian Coroner to use de-identified
data relating to NPS detections in this dataset. The case types include those where the cause
of death was not drug related, as well as known overdose cases. Therefore the presence of
any drug must not be interpreted as being implicated in the cause of death.

2.3 Sample Treatment

2.3.1 Wastewater

Sample preparation and solid phase extraction (SPE) were performed as in our previous
work.  Briefly, samples were warmed to room temperature then filtered under vacuum
using glass microfibre filters GF/A 1.6 μm (Whatman, Kent, U.K.). The deuterated internal
standards (200 μL) were then spiked into 200 mL sample. 10% Acetic acid was added to
lower the pH (4.5-5) of the samples. The acidified samples were loaded onto mixed-mode SPE cartridges (UCT XRDAH (UCT Inc., Bristol, PA, USA); 500 mg/6 mL) which had been conditioned with methanol (6 mL) and sodium acetate buffer (20 mM pH 5, 6 mL). The cartridges were successively washed with sodium acetate buffer (6 mL), 0.1 M acetic acid (2 mL) and methanol (6 mL). Analytes were eluted with a mixture of dichloromethane:isopropanol:ammonia (80:16:4) and evaporated to 200 µL under nitrogen at 40°C, when 1% HCl in methanol was added, then evaporated to dryness. The dry residue was reconstituted with 0.1% formic acid in methanol (20 µL) and 0.1% formic acid in milliQ water (180 µL). Analyses were performed by injecting 10 µL in the LC-QTOF-MS and 3µL in the UHPLC-QqQ-MS. The pre-concentration factor along sample treatment was x1000 (200 mL sample to a final extract volume of 200 µL).

2.3.2 Post Mortem Blood Samples

Sample preparation was performed as previously described. Briefly, an aliquot of whole blood (500 µL) was mixed with distilled water (1.5 mL), mixed internal standard solution (25 µL), concentrated ammonia solution (250 µL) and butyl chloride (5 mL). The blood was agitated on a rotating extractor at 80 rpm for 10 min and centrifuged at 3,000 rpm for 15 min. The supernatant was decanted and evaporated to dryness in a centrifugal evaporator (GeneVac EZ2 plus, Scitek, Melbourne, Australia). The residue was reconstituted in 100 µL ethanol.

2.4 Instrumentation

All liquid chromatography-mass spectrometry parameters can be found in the supporting information.

2.5 Criteria for Qualitative and Quantitative Analysis

The criteria used for the identification of compounds in wastewater in this study were similar to those devised by Hernandez et al. and Schymanski et al.25,26 as outlined below. In the Forensic toxicology samples, mass spectrometry identification met criteria for Australian and New Zealand Forensic Toxicology laboratories. The identification of NPS in forensic samples was assisted by supporting intelligence from local and interstate NPS seizures, case notes, and the examination of drugs and paraphernalia from the scene of death.

2.5.1 QTOF Screening
Compounds in wastewater and post mortem blood samples were detected using one accurate mass ion (mass error ± 2 mDa) and retention time agreement with a reference standard (± 2%). Confirmation of the identity of the compound detected involved at least two accurate mass ions (± 2 mDa), with one of which preferably being the protonated molecule, and agreement of retention time and isotopic pattern with a reference standard (± 2%). Tentative identification was made in those cases when the reference standard was not available at the laboratory. It was based on the presence of at least two accurate mass ions (mass error ± 2 mDa), supported by literature mass data on the suspect compound.

2.5.2 QqQ Quantification

The wastewater sample treatment indicated in Section 2.3.1 was optimized and validated in 20. It was applied at the University of South Australia (Australia). The SPE eluates were shipped to Spain and analyzed at the University Jaume I of Castellon (Spain), applying the instrumental conditions reported in the supporting information and 19. At least two transitions were monitored for each compound, one quantification transitions (Q) and two confirmation transitions (q1 and q2), except for methedrone for which only two transitions could be selected. For positive confirmation, the following criteria were applied: retention time compatibility with the standard (± 2 %), and ion ratio (q/Q) deviation within ± 30% for at least one confirmation transition in comparison with the reference standard.

3. Results and Discussion

3.1 Database for Qualitative Screening Analysis

A database including 85 NPS reference standards of FSSA and exact mass information of an additional 101 NPS was used in this work and are shown in Table S1. The selection of NPS standards was based on detection by FSSA’s illicit drug laboratory, police and interstate forensic laboratories intelligence as well as media and literature (such as the EMCDDA early warning system and the Australian National Drug and Alcohol Research Centre bulletin of drugs and the internet 28). This was a compromise between exhaustive NPS coverage and finite resources, but, encapsulated a significant number of NPS likely to be encountered in South Australia. 24

3.2 Suspect Compound Screening of New Psychoactive Substances by QTOF-MS

HRMS suspect compound screening is becoming the technique of choice for forensic toxicology centres to detect and confirm NPS in various biological matrices, using databases
similar to that described above. The value of qualitative HRMS screening is supported by the fact that some forensic science experts have even questioned the value of quantitative analysis of NPS, since the toxicology and metabolism of many of the compounds are unknown. In this context, WWA is a complementary source of information on population-scale drug use.

Figures 1a and b show the qualitative temporal comparison between the toxicological data (post mortem blood samples) and wastewater data from June 2012 – June 2017. The colours represent the means of identification: wastewater analysis (blue), toxicological data (orange) and both (green). Confirmation of the identity was possible for all compounds shown in Figure 1, while 25H-NBOMe was detected, but not fully confirmed in wastewater according to the criteria outlined in Section 2.5.1, and pentylone could only be tentatively identified in wastewater due to the lack of a reference standard at the laboratory. In total, 18 NPS were found in the forensic samples, 11 in wastewater and five in both.

All wastewater samples were screened by applying a three-step workflow using MasterView. The first step assumed that no standard was available and contained just the exact mass of the NPS. On average, 140 compounds could be excluded from the initial database of 186 compounds. All substances found within the aforementioned mass threshold of 2 mDa were then screened employing the second step, which included retention times of all NPS for which reference standards were available to get a list of “detected” compounds (described in Section 2.5.1). This further reduced the number of compounds down to 11. Finally, step 3 involved confirmation of the identity of all “detected” compounds by using information of fragment ions (“confirmation” in section 2.5.1), to give the results shown in Figure 1. Therefore, this three-step workflow shows the risk of finding false positives in the absence of reference standards.

Since 2008, synthetic cathinones have accounted for the highest proportion of NPS seizures in Australia. The cathinones mephedrone and methylene have been monitored in Australia as part of the National Wastewater Drug Monitoring Program. Both were usually detected below the limit of reporting with detections decreasing over the monitoring program. They were also the most common family of NPS found in wastewater and early toxicological samples included in this study. Between August 2015 and December 2016, fentanyl derivatives were more commonly identified in toxicological samples. Alpha PVP was the compound most commonly found in both sources, in June 2014 and from February 2015 –
August 2015. Synthetic cathinones and piperazines were predominantly reported in wastewater samples, while only phenethylamines, cannabinoids and fentanyl derivatives were found in toxicological samples. These latter NPS families are typically very low dose compounds i.e. low µg, and are often difficult to detect even in blood samples. This rendered them unlikely to be detected in wastewater unless they had widespread use, while synthetic cannabinoids are notoriously difficult to find in wastewater due to their extensive metabolism and requiring the need for specific sample treatment.

From the wastewater data, a trend in the use of synthetic cathinones is visible. Methylone was prevalent from 2013-mid 2014, then disappeared. At this point, ethylone entered the scene until early 2017, with pentylone tentatively identified in more recent 2017 samples. The cathinones ethylone, alpha-PVP and mephedrone, as well as TFMPP were in common with the forensic toxicology samples. Since a number of NPS were found using the qualitative wastewater and forensic data, quantitative analysis of the relevant samples were conducted to determine their prevalence in wastewater.

3.3 Quantitative Results

Quantitative analysis in WWA can demonstrate the scale and prevalence of use. Based on a previously validated method, a selection of weekend samples from April 2015 – August 2017 were quantitatively analysed. A further 13 NPS were added to the method (Table S3), due to them being found in the QTOF screening method, with quantification based on the criteria outlined in Section 2.5.2. In total, the number of target analytes included in the LC-MS/MS method was 20. The quantitative method was not fully validated for the 13 additional NPS but was based on their validated structural analogues. Therefore, for these compounds concentration data should be considered as semi-quantitative. For quantitative analysis, a calibration standard curve (1 – 20 ng/L) was injected in duplicate. The limit of quantification (LOQ) and limit of detection (LOD) were estimated directly from positively detected samples where the compound had a signal to noise of >10 (LOQ) or >3 (LOD). Information on LOD and LOQ is presented in Table S4.

Seven NPS were detected and quantified in total across all samples. Using concentration data (ng/L), the daily mass loads were estimated making use of the wastewater flow rates and population (Table 1). Details of these parameters are given in Table S5 and calculations performed are the same as in 20. The population figure was kept constant in spite of the
different years of the samples as the greater Adelaide region has had a population growth of 1% per year from 2011-2017, which we deem minimal.

There are some distinct patterns in NPS use visible in Table 1. Methcathinone was detected at a relatively constant concentration in all samples. Alpha-PVP and mephedrone were only detected in the 2015 samples, which mirrors the screening data (Figures 1a and b). Ethylone was detected in every sample. However, it decreased in use from 2015-2017. It is interesting to note that butylone started to be detected as ethylone started to decline (Figure 2). As both compounds have the same transitions (222.1 > 174, 222.1 > 146 and 222.1 > 131.2), it was easy to monitor their changes from the common chromatograms, as shown in Figure 2. The 97% decline in ethylone use coincided with a 200% increase in butylone, based on peak area.

In a previous study, we showed that methylone disappeared from South Australia WWA in 2014.20 This is similar to what was seen in South East Queensland, where there was a peak in use of methylone in 2012-2013.33 It was thus interesting to note the rise in ethylone subsequent to 2014, which matched the observations in the qualitative method. Another synthetic cathinone, pentylone, was detected intermittently, but at a higher concentration in more recent samples. It will be of interest to see whether these cathinones will continue to be seen in future samples.

3.4 Complementarity of forensic and wastewater data

In this study, forensic data confirmed the presence of 18 NPS in post mortem specimens. Such deaths represent a small subset of the drug user population which provides a source of intelligence regarding the presence of NPS. The detection of NPS in the post-mortem samples is determined by several factors. These include the consumption of a substance leading up to the death when it is still detectable in blood, even if the compound was not the cause of death. When a NPS is taken by a sub-population, it may thus not appear in any forensic toxicology post mortems.

WWA is a complementary tool and can be considered as a diluted pooled urine sample, allowing the measurement and estimation of a drug that is consumed in a community. In this study, it was used to indicate the presence of 11 NPS. Due to dilution effects and low excretion rates, infrequently used NPS may not be found in wastewater. In addition, the metabolism of some NPS remains unknown and therefore a method targeting the parent drug may not find the drug in wastewater. As pharmacokinetic information becomes available, this limitation may be overcome. In addition to detecting community use of a NPS, WWA can
show the scale of use. Since there will always only be partial overlap between the two datasets, this work emphasises the complementarity of these sources.

**Conclusion**

A temporal investigation into NPS use in South Australia from 2012 – 2017 has been done utilising both post mortem forensic and wastewater data. A total of 24 NPS were found: six in wastewater only, 13 in forensic post mortem toxicology samples only and five in both. Synthetic cathinones were most prevalent, with an interesting temporal pattern of use. Methylone was used in the early years, followed by ethylone, while in more recent samples butylone and pentylone were found. The study showed that by combining forensic and wastewater data, it increased the likelihood of detecting NPS use in a community. This work highlights the value and complementary nature of WWA and forensic data in evaluating the total use of NPS.

**Acknowledgements**

The authors gratefully acknowledge SA Health, Generalitat Valenciana (Prometeo II 2014/023) and the Spanish Ministry of Economy and Competitiveness (Project ref CTQ2015-65603) for their financial support. Richard Bade acknowledges the financial support of the Thyne Reid Foundation and the University of South Australia Early Career Researcher International Travel Grant. We would also like to thank the staff at SA Water and Allwater for their assistance in sample collection and the South Australian Coroner for provision of the toxicological data.
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Figure Captions

**Figure 1a:** NPS found from QTOF screening of wastewater and forensic toxicological samples from June 2012 - December 2014. Dark blue indicates that the NPS was confirmed in wastewater, orange indicates the NPS was confirmed in forensic toxicological samples and green indicates the NPS was confirmed in both.

Abbreviations: Alpha-PVP (α-pyrrolidinopentiophenone); MDPV (methylenedioxyprovalerone); 5-APB (5-(2-aminopropyl)benzofuran); 5-EAFB (1-(benzofuran-5-yl)-N-ethylpropan-2-amine); BZP (benzylpiperazine); TFMPP (3-trifluoromethylphenylpiperazine); MDA (3,4-methylenedioxymphetamine)

**Figure 1b:** NPS found from QTOF screening of wastewater and forensic toxicological samples from February 2015 – June 2017. Dark blue indicates that the NPS was confirmed in wastewater, orange indicates the NPS was confirmed in forensic toxicological samples and green indicates the NPS was confirmed in both.

*compound only detected in wastewater; *tentatively identified as no reference standard was available.

Abbreviations: Alpha-PVP (α-pyrrolidinopentiophenone); MDPV (methylenedioxyprovalerone); 5-APB (5-(2-aminopropyl)benzofuran); 5-EAFB (1-(benzofuran-5-yl)-N-ethylpropan-2-amine); BZP (benzylpiperazine); TFMPP (3-trifluoromethylphenylpiperazine); MDA (3,4-methylenedioxymphetamine)

**Figure 2:** Detection of ethylone (2.54) and butylone (2.73) from April 2015, August 2016 and August 2017. Both ethylone and butylone have the same transitions: 222.1 > 164 (TOP), 222.1 > 146 (MIDDLE) and 222.1 > 131.2 (BOTTOM).
Tables

*Table 1:* Average weekend (i.e. Saturday and Sunday) excreted mass loads of NPS found using the quantitative method (mg/day/1000 people)

|        | Butylone | Ethylone | Alpha PVP | Methcathinone | MDPV | Pentylone | Mephedrone |
|--------|----------|----------|-----------|---------------|------|-----------|------------|
| Apr 15 A | 0.96     | 0.04     | 0.32      | D             | D    |           |            |
| Apr 15 B | 0.70     | 0.05     | 0.29      | D             |      |           |            |
| Aug 16 A | 0.65     |          | 0.43      |               |      |           |            |
| Aug 16 B | 0.22     | 0.05     | 0.67      | D             |      |           |            |
| Feb 17 A | 0.23     |          | 0.38      | D             |      |           |            |
| Feb 17 B | 0.13     |          | 0.32      |               |      |           |            |
| Jun 17 A | 0.35     |          | 0.21      | 0.12          |      |           |            |
| Jun 17 B | 0.29     |          | 0.30      | D             |      |           |            |
| Aug 17 A | D        | 0.13     | 0.51      | D             |      |           |            |
| Aug 17 B | D        | 0.12     | 0.46      |               |      |           |            |

D = below LOQ
| NPS family       | Compound               | Jun-12 | Aug-12 | Oct-12 | Dec-12 | Feb-13 | Apr-13 | Jun-13 | Aug-13 | Oct-13 | Dec-13 | Feb-14 | Apr-14 | Jun-14 | Aug-14 | Oct-14 | Dec-14 |
|------------------|------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| **Cannabinoids** | AB-CHMINACA            |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | NM-2201                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| **Cathinones**   | Alpha-PVP               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Ethylone                |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | MDPV                    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Mephedrone              |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Methcathinone           |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Methylene               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | N-ethylpentylone        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Pentyline               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| **Fentanyl derivatives** | Acetyl Fentanyl       |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Furanylfentanyl         |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Ocfentanil              |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | P-Fluorobutrylfentanyl  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| **Phenethylamine** | 25B-NBOME               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | 25H-NBOMe               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | 25I-NBOMe               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | 5-APB                   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | 5-EAPB                  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| **Piperazine**   | BZP                     |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | TFMPP                   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| **Other**        | MDA                     |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | U-47700                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|                  | Methylhexanamine        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |

*Figure 1a*
| NPS family   | Compound                  | Feb-15 | Apr-15 | Jun-15 | Aug-15 | Oct-15 | Dec-15 | Feb-16 | Apr-16 | Jun-16 | Aug-16 | Oct-16 | Dec-16 | Feb-17 | Apr-17 | Jun-17 |
|--------------|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Cannabinoids | AB-CHMINACA               |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | NM-2201                   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Cathinones   | Alpha PVP                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Ethylone                  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | MDPV                      |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Mephedrone                |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Methcathinone             |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Methylone                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | N-ethylpentylone          |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Pentyloone\(^a\)          |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Fentanyl derivatives | Acetyl Fentanyl      |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Furanylfentanyl           |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Ocfentanil                |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | P-Fluorobutrylfentanyl    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Phenethylamine | 25B-NBOMe                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | 25H-NBOMe\(^*\)           |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | 25I-NBOMe                 |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | 5-APB                     |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | 5-EAPB                    |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Piperazine   | BZP                       |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | TFMPP                     |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Other        | MDA                       |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | U-47700                   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|              | Methylhexanamine          |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |

\(^a\) Pentylone is specific for 2017.
Figure 2