Analysis of anonymous pooled urine from portable urinals in central London confirms the significant use of novel psychoactive substances

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Received 3 September 2012 and in revised form 6 October 2012

Summary

Aim: Analysis of urine samples collected across a city centre, for the detection of novel psychoactive substances (NPS).

Design: Cross-sectional study of anonymized urine samples used for the analysis of classical recreational drugs, NPS and metabolites.

Methods: Pooled urine samples collected from portable stand-alone four-person urinals across a city centre were analysed using full-scan accurate-mass high-resolution liquid chromatography coupled to tandem mass spectrometry. Data were processed against compound databases containing >1700 drug compounds and metabolites.

Results: Seven established recreational drugs (3,4-methylenedioxyamphetamine, cocaine, cannabis, ketamine, 3,4-methylenedioxyn-N-methylamphetamine, methamphetamine and amphetamine) and six potential NPS (hordenine (all 12 urinals), cathine (11), methylhexaneamine (9), 4-methylmethylaminone (6), methiopropamine and metabolites (2) and methoxetamine and metabolites (1)) were detected. Methylhexaneamine, methiopropamine and hordenine are currently uncontrolled in the UK, whereas methoxetamine is currently subject to a Temporary Class Drug Order. Metabolites of the anabolic steroid nandrolone were found in two urinals and trenbolone metabolites and clenbuterol in one urinal.

Conclusion: Analysis of pooled urine samples collected anonymously from stand-alone urinals in a large inner city can detect the use of recreational drugs, NPS and anabolic steroids. Metabolite detection indicates actual drug use, metabolism and elimination rather than simply discarded drugs in the urinals. This technique by confirming the actual drug(s) used has the potential to be additive to currently used datasets/key indicators providing more robust information for healthcare authorities, legislative and law enforcement on the drugs actually being used.

Introduction

Recreational drug use is common in the UK and the British Crime Survey for 2010/2011 estimates that 12 million people aged from 18 to 59 years have ever used illicit drugs in their lifetime, equating to one in three adults. Over recent years there has been a significant change in the range and type of drugs used with increasing use of novel psychoactive substances (NPS), often known as ‘legal highs’ in the UK, Europe and more widely. Information relating to the pattern of use of both classical recreational drugs and NPS is typically based on self-reported user surveys. Data on
Drug trends gained from these surveys need to be interpreted with care because a number of studies have shown that the content of both classical recreational drugs and NPS are variable and therefore users may not be aware of what they are using when completing self-reported use surveys.\textsuperscript{7–10} Analysis of biological samples such as urine can confirm the actual drug(s) being used; however, this requires individual consent.

Analysis of anonymized biological samples could potentially be useful in determining the trends in actual drug(s) being used. There is increasing interest in the use of wastewater (sewage) analysis, typically at the level of sewage treatment plants, to understand population/subpopulation patterns of drug use.\textsuperscript{11} Technological advances have increased the sensitivity of detection techniques (chromatography and mass spectrometry) making it possible to identify classical recreational drugs and their metabolites, even at very low concentrations in wastewater.\textsuperscript{11,12} However, analysis of wastewater samples for NPS is currently limited by the poor understanding of their metabolism and stability.\textsuperscript{11} Therefore, studies of water-water analysis predominately focus on classical recreational drugs such as 3,4-methylenedioxyn-methylamphetamine (MDMA), morphine and cocaine.\textsuperscript{13} In view of the limitations of the use of wastewater at sewage plants for NPS analysis, we recently undertook a pilot study collecting urine from a single stand-alone urinal in a nightclub environment.\textsuperscript{14}

In central London, as in many other large UK and European cities, stand-alone urinals (also known as pissoirs) are regularly used at weekends and during holiday periods to reduce street urination. Rather than emptying directly into sewers, they often incorporate a central holding area for urine. This study aimed to collect anonymous pooled urine samples from a number of urinals across a wide geographical area in central London, thereby focusing on detection of drug and NPS use within the wider night-time economy.

\section*{Methods}

\subsection*{Sample collection}

The study was conducted in the City of Westminster, a borough in central London, UK with a wide variety of night-time economy venues including bars, late night cafes, nightclubs/discotheques. Twelve four-bay stand-alone portable urinals that are used routinely during weekends in discrete locations were used in this study (each star in Figure 1 represents the location of each individual urinal). These urinals were available to be used by the general public over a 12-h period (1800-0600) on a Saturday night in March 2012. Use of these urinals was anonymous and no data on the number of people using the urinals or their identity were collected. The urinals are designed for use by men only. Urine is pooled in a central holding tank with a total capacity of 400 l. The urinals have no ‘flushing’ mechanism and so sample dilution did not occur. Following the collection period the volume in each urinal was measured. One-hundred millilitres of pooled urine from each urinal was taken using a single-use manual vacuum pump. Urine samples were stored at 4°C until analysis. The study had approval from Westminster City Council and was discussed with the local ethics committee (Institutional Review Board, IRB) and deemed not to require formal approval.

\subsection*{Sample analysis}

The samples were prepared for analysis using solid-phase and liquid–liquid extraction techniques. Three test extracts were prepared for each sample and these were analysed using full-scan accurate-mass high-resolution liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The acquired data were processed against compound databases containing in total, over 1700 drug compounds and metabolites.

\subsection*{Results}

After the 12-h collection period, the urinals contained a median of 65 l (interquartile range: 57–97) of urine. In total, 109 parent drugs or their metabolites were identified, these included: (i) classical recreational drugs and NPS, (ii) metabolites of both classical recreational drugs and NPS, (iii) potential drug adulterants found in both classical recreational drugs and NPS, (iv) anabolic steroids and (v) over-the-counter/prescription medications, which may have been used therapeutically or misused. All of the urinal samples were positive for nicotine and/or its major metabolite cotinine and capsaicin related to smoking and eating spicy foods, respectively.

\subsection*{Classical recreational drugs and potential NPS}

The frequency of detection of classical recreational drugs and NPS is illustrated in Figure 2. Hordenine was found with the highest frequency in all the urinal samples, with cocaine (and metabolites), cannabis metabolites, cathine and MDMA (and metabolites) found in 11 of the 12 urinals. The frequency
(number of urinals in which the target drug was detected) of the other recreational drugs detected was amphetamine (and metabolites) [10], methylhexaneamine (1,3-dimethylamylamine, DMAA) [9], MDA (3,4-methylenedioxyamphetamine [9], ketamine and metabolites [6], 4-methylmethcathinone (4-MMC, mephedrone) [6], methamphetamine [5], methiopropamine (MPA) and metabolite [2], and methoxetamine and metabolites [1]. No synthetic cannabinoid receptor agonists ('spice' products) were detected.

Detection of drug metabolites

Metabolites of a number of classical recreational drugs and NPS were detected. This indicated that these drugs had actually been used and metabolized by individuals using the urinals rather than the drugs being discarded into the urinal. The following parent drug–metabolite pairs were identified in the urinal samples: cocaine–benzoylecgonine/benzocaine; MDMA–HMMA (4-hydroxy-3-methoxymethamphetamine); amphetamine–hydroxymphetamine; ketamine–dehydro, dehydronor and hydroxynor metabolites; MPA–desmethyl metabolites; and methoxetamine–N-desethyl, O-desmethyl and the N-desethyl/O-desmethyl metabolites.

Potential drug adulterants

The presence of a number of drugs detected in the urine samples may be explained by adulteration of both classical recreational drugs and NPS. For cocaine, these included tetramisole in all 12 urinals (which may represent its leva-isomer, levamisole, being indistinguishable from tetramisole), benzocaine and lidocaine in 11 urinals. Caffeine was also found in all 12 urinals, which can be used as an adulterant of cocaine and is also mis-sold as or used as an adulterant in NPS. Finally quinine was detected in nine urinals; this is a potential adulterant of heroin. Alternatively caffeine and quinine may have been present due to the consumption of drinks such those containing caffeine (e.g. coca cola, energy drinks) or tonic water, respectively.

Anabolic steroids

Two urinal samples had a metabolite of the anabolic steroid nandrolone, one urinal had a metabolite of trenbolone and one had clenbuterol; no other
exogenous anabolic steroids (or their metabolites) were detected.

**Prescription and other-the-counter medications**

A number of prescription and/or over-the-counter medications were identified. These included antibiotics, anticonvulsants, antidepressants, antiemetics, anti-histamines (H1 and H2 inhibitors), anti-hypertensive, anti-platelet agents, antipsychotics, anti-virals, analgesics, beta-blockers, cough suppressants, decongestants, proton pump inhibitors and sildenafil.

A number of the prescription/over-the-counter medications detected can be used therapeutically or misused for recreational purposes. An example, methylphenidate (Ritalin) and its desmethyl metabolite were found in three urinal samples; this could be due to legitimate use for the treatment of attention-deficit–hyperactivity disorder (ADHD) or misuse due to its pharmacological similarities to cocaine and amphetamine.15

**Discussion**

In this study, we have demonstrated that analysis of pooled urine samples collected anonymously from stand-alone urinals in a large inner city can detect the use of both classical recreational drugs and potential NPS. The detection of metabolites validates that we were detecting actual drug use, metabolism and elimination rather than simple discarding of drugs into the urinals.

Mephedrone is a synthetic cathinone derivative that has significant acute sympathomimetic toxicity similar to classical stimulants such as cocaine and amphetamine.16 A number of recent studies have shown that it remains widely used despite its control under the UK Misuse of Drugs Act, 1971 in April 2010.1,6 However, the results of this study appear to suggest less widespread use of mephedrone as it was only detected in six urinals. Additionally, no other cathinone derivatives such as methylone, flephedrone and methylenedioxypyrovalerone (MPDV) were detected.

Methylhexaneamine (DMAA) was detected in the majority [9/12] of urinal samples. Metabolites for methylhexaneamine were not detected as the compound is excreted predominantly as the parent drug. Having been originally marketed in 1944 as a nasal decongestant, methylhexaneamine was reintroduced in 2006 as a dietary supplement and is also now found in many ‘fat burning’ nutritional supplements. More recently it has been banned by the World Anti-Doping Agency as a performance-enhancing substance. Methylhexaneamine containing supplements have been reported to be

![Figure 2. The different classical recreational drug (stripped) and NPS (solid black) identified in urine samples and their frequency of detection (number of urinals).](image_url)
associated with anxiety, hypertension, tachycardia and stress-induced cardiomyopathy.\textsuperscript{17–19} There have been two deaths reported associated with the use of methylhexaneamine containing supplements in US army soldiers.\textsuperscript{20} There is one published case report of cerebral haemorrhage following self-reported use of two methylhexaneamine containing capsules.\textsuperscript{21} However, there was no analysis of biological samples to confirm use, although analysis of similar capsules demonstrated that they contained methylhexaneamine.

Methoxetamine was found in 2 of the 12 urinal samples. This compound is a thiophene-based analogue of methamphetamine first reported in 1942.\textsuperscript{22} Since 2010, it has been marketed on a number of websites as a ‘legal high’ and has also been reported as a NPS by the European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA) Early Warning System.\textsuperscript{23} User reports are limited but that significant negative effects such as anxiety, difficulty passing urine, breathlessness, chest pain, tachycardia and a significant hangover effect may occur.\textsuperscript{24,25} There are no published analytically confirmed cases of MPA toxicity.

The fourth significant NPS detected was methoxetamine and its metabolites in one urinal sample. In March 2012, this compound was controlled under a Temporary Class Drug Order in the UK.\textsuperscript{26} Methoxetamine is an analogue of the Class C drug ketamine. Like ketamine, it has a similar pattern of toxicity including hallucinations, catatonia and dissociative effects. However, analytically confirmed case reports of methoxetamine toxicity have also identified additional effects including sympathomimetic effects (agitation and significant tachycardia/hypertension) and cerebellar toxicity.\textsuperscript{27,28}

Hordenine and cathine were detected in 12 and 11 of the urinals, respectively. Hordenine is a phenethylamine alkaloid which is naturally found in many plant varieties, particularly cacti e.g. san pedro (Echinopsis pachanoi), peyote (Lophophora williamsii) and peruvian torch (Echinopsis peruviana). It has been marketed as an appetite suppressant and many cactus preparations are also sold for their hallucinogenic effects. However, these preparations typically contain mescaline as the predominant hallucinogenic compound. Since mescaline was not detected in the urinal samples, we feel that that it is unlikely that the hordenine was derived from use of cactus preparations. It is more likely that it was present as a breakdown product of beer brewed from barley.\textsuperscript{29} Cathine (d-norpseudoephedrine) is a psychoactive substance found in the leaves of Catha edulis (Khat), a plant native to north east Africa, which is chewed for its stimulant and euphoric effects.\textsuperscript{30} Khat also contains the stimulant substance cathinone. Cathine is also the main metabolite of pseudoephedrine, a compound commonly found in over-the-counter nasal decongestants. We detected pseudoephedrine in 11 of the 12 urinals and no cathinone. Therefore, the cathine detected in this study is likely to be due to pseudoephedrine use and subsequent metabolism. This highlights the importance that those using this model to detect the use of NPS have a broad understanding of the potential alternative, more plausible, explanations for the detection of a compound.

Current methods for determining the use of classical recreational drugs and NPS rely on population and subpopulation self-reported use surveys. The main limitation of these studies is that individuals report what they believe they are using rather than what they are actually using. Collection of biological samples such as urine from individuals requires consent. We have shown in this study that collection and analysis of anonymous pooled urine samples from portable street urinals (pissoirs) is a novel technique that has the potential to detect the use of NPS and other recreational drugs. Compared to wastewater analysis, sampling occurs a step closer to the source and therefore allows for greater sensitivity at analysis and may help negate issues related to drug stability and metabolism. This technique can be used for repeat sampling in the same locations over time to establish time-trends in NPS use and use of new NPS. Additionally, sampling in different cities/countries has the potential to identify geographical trends in use. We feel that this information will be additive to and increase the robustness of current datasets and key indicators reported to national and international bodies such as the EMCDDA and United National Office on Drugs and Crime (UNODC).

\textbf{Conflict of interest:} None declared.

\textbf{References}


