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Using biomarkers in wastewater to monitor community drug use: A conceptual approach for dealing with new psychoactive substances ☆

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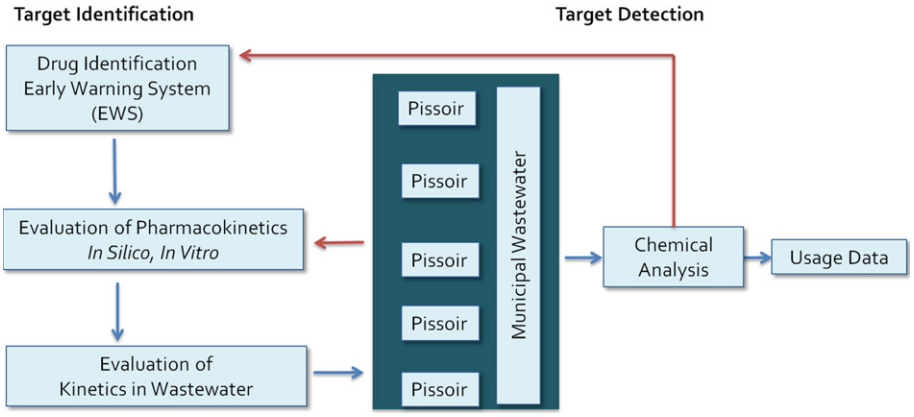
HIGHLIGHTS

- New psychoactive substances present unique challenges to epidemiologists working on wastewater
- There is limited data on the fate of the compounds via excretion or biotransformation in wastewater
- In-silico tools can be used to predict the fate of these compounds in wastewater
- Common-fragment and mass-defect filtering of HRMS data can aid in the identification of NPS biomarkers

GRAPHICAL ABSTRACT



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ABSTRACT

Data obtained from the analysis of wastewater from large-scale sewage treatment plants has been successfully applied to study trends in the use of classical illicit drugs such as cocaine, but the dynamic nature of the new psychoactive substances (NPS) market presents a unique set of challenges to epidemiologists. In an attempt to overcome some of the challenges, this paper presents a framework whereby a collection of tools and alternative data-sources can be used to support the design and implementation of wastewater-based studies on NPS use. Within this framework the most likely and most suitable biomarkers for a given NPS are predicted via in-silico metabolism, biotransformation and sorption models. Subsequent detection and confirmation of the biomarkers in samples of wastewater are addressed via high-resolution mass spectrometry (HRMS).

The proposed framework is applied to a set of test substances including synthetic cannabinoids and cathinones. In general, the in-silico models predict that transformation via N-dealkylation and hydroxylation is likely for these compounds, and that adsorption is expected to be significant for cannabinoids in wastewater. Screening via HRMS is discussed with examples from the literature, and common-fragment searching and mass-defect filtering are successfully performed on test samples such that spectral noise is removed to leave only the information that is most likely to be related to the NPS biomarkers. HRMS screening is also applied to a set of pissoir-sourced wastewater samples and a total of 48 pharmaceuticals and drugs including 1-(2-methoxyphenyl)piperazine (oMeOPP) are identified.

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The framework outlined in this paper can provide an excellent means of maximizing the chances of success when identifying and detecting biomarkers of NPS in wastewater.

1. Introduction

Drug epidemiology involves the study of factors which impact the frequency and distribution of drug use and the associated outcomes on health, education and crime. Detection, tracking and the attempted understanding of emerging drug trends are critical aspects of this work, and these are achieved with the aid of a range of different data-sources including the internet, users (interviews and surveys), test purchasing, forensic toxicology, law enforcement and wastewater (European Monitoring Centre for Drugs and Drug Addiction, 2007).

Seventy three new psychoactive substances (NPS) were observed in the European market for the first time in 2012, following on from the 49 NPS identified in 2011 and 41 in 2010 (European Monitoring Centre for Drugs and Drug Addiction, 2013). While data obtained from the analysis of wastewater from large-scale sewage treatment plants has been successfully applied to study temporal and regional trends in the use of classical illicit drugs such as cocaine and amphetamines (Reid et al., 2011; van Nuijs et al., 2011; Thomas et al., 2012), the extremely dynamic nature of the NPS market presents a unique set of challenges to epidemiologists working with wastewater. Most critically, the lack of standard reference materials severely impedes the detection, identification and quantification of these compounds in samples of wastewater. Further, a compound is only suitable as a drug biomarker when this compound has the following attributes:

- It must be a specific marker of the factor under investigation (i.e. be produced exclusively by the drug) and not formed exogenously by, for example, microorganisms in the sewer system
- It must be stable within the sewer system
- It must be present in sewage at sufficiently high concentrations to be accurately measured
- The compound must be excreted at sufficiently high levels to allow observation of significant differences between 'normal' and 'stressed' communities
- The compound must be excreted in urine and not extensively partitioned onto solids.

This implies that a significant amount of information is required on the fate of the compounds with respect to pharmacokinetics (metabolism and excretion) and within the wastewater system itself (biotransformation and partitioning) before a wastewater study can be initiated successfully.

In light of the limited information available on many NPS, this paper therefore presents a conceptual framework whereby biomarkers for NPS can be identified and subsequently tested for their suitability (according to the attributes above) in order to maximize the chances of successful identification in wastewater. We also discuss the suitability of pooled urine analysis on samples from pissoirs and how this information may support large-scale wastewater studies and potentially act as an additional source of primary technical data to the Early Warning System (EWS) and clinical toxicologists.

2. Identification of new drug targets and selection of appropriate analytes/biomarkers

2.1. Early Warning System (EWS) and the European Database on New Drugs (EDND)

New psychoactive substances (NPS) are typically identified (at a national level) by healthcare services (such as treatment centers, hospital emergency rooms, poisoning centers and psychiatric departments), law enforcement agencies (including customs authorities) and national medicines agencies. In Europe this information is centralized and collated and disseminated by EMCDDA and Europol under the EWS (European Monitoring Centre for Drugs and Drug Addiction, 2007). A key output of the EWS is the European database on new drugs (EDND) which presents dynamic information on the occurrence of new psychoactive substances in the EU. This database would therefore act as central source of drug targets that can potentially be analysed and/or detected in wastewater.

2.2. Pharmacokinetic properties and metabolite prediction

The monitoring of drug use via analysis of wastewater is highly dependent on the identification and quantification of specific drug residues, or biomarkers, that confirm the consumption of the particular drug(s). A thorough review of the pharmacokinetics (PK) of the drug (including absorption, distribution, metabolism and excretion) is therefore necessary in order to identify these compounds.

Urinary excretion of drug residues is of paramount importance because the main concept of sewage biomarker analysis is that a representative sample of wastewater serves as proxy pooled urine sample from the combined population (Daughton, 2001). Therefore the identity and kinetics of the urinary metabolites including excretion rate and the relative proportions of the differing metabolites to the parent drug have to be taken into account. This technique has been successfully applied to pharmaceuticals and the classic illicit drugs (cocaine, cannabis and amphetamines etc.) because data from clinical trials in humans is available (Castiglioni et al., 2011; Khan and Nicell, 2011, 2012). It should be noted however that PK information on the classic illicit drugs is somewhat minimal and it can be argued that clinical environments do not accurately represent the real-world administration of drugs where poly-drug use and underlying health problems can alter PK significantly (Rook et al., 2006; Parker and Casey Laizure, 2010). Unfortunately the amount and quality of PK data on NPS are even more limited, and for the most part this data does not yet exist, so identification and selection of appropriate biomarkers require alternative data-sources.

In the absence of clinical PK data in-vitro models can be used to predict the metabolic pathways of drugs in humans. Parent drugs are incubated with liver enzymes which metabolize the compounds, and the subsequent samples are analysed to identify and quantify the metabolites that are formed (Hengstler et al., 2000). Such experiments are an excellent source of PK data, but they are entirely dependent on the availability of sufficient quantities of the NPS which may not yet be readily available if the particular compound is only newly identified by the EWS. Another alternative is computer-based PK prediction or so-called in-silico modeling. Software such as Meteor (Lhasa Ltd., Leeds UK) or SMARTCyp (University of Copenhagen) can be used to overcome shortfalls in clinical data when little or no standard reference material is available. Such software provides an important insight into the metabolic pathway of new drugs as they predict metabolism of a given compound and rank the probability of different metabolites. We applied the SMARTCyp tool (which predicts the path of Cytochrom P450 metabolism) to a set of test substances including MDMA, mephedrone, JWH-018 and MAM-2201 and found that results (Fig. 1) had good agreement with the published literature (Abraham et al., 2009; Meyer et al., 2010; Grigoryev et al., 2011). It should be highlighted however that these models by no means guarantee the formation of a given metabolite or that such a metabolite will be excreted in urine, but they do provide a concise list of targets that can be screened for by analysis of high

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