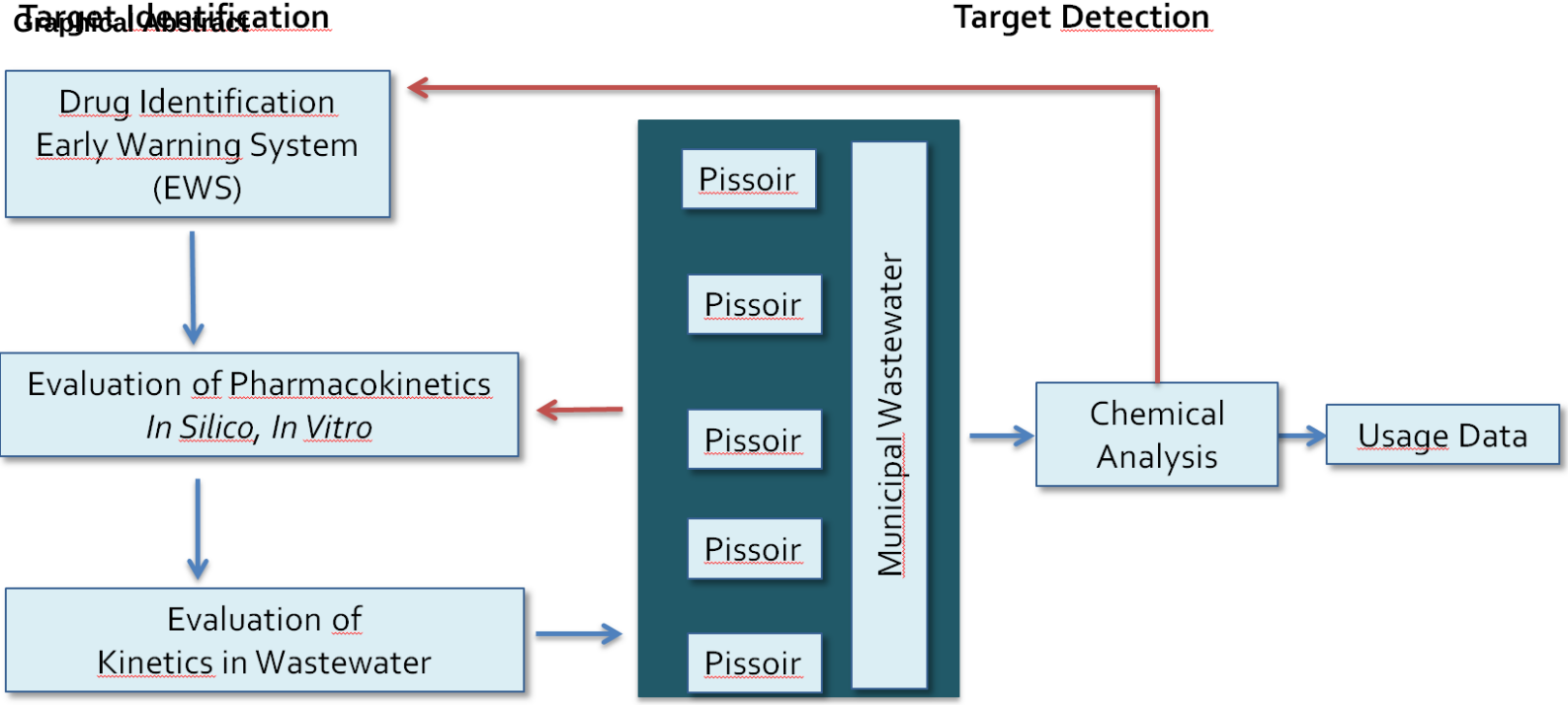
Graphical Abstract



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

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**Running Head** Measuring new psychoactive substances in wastewater

**Word Count** 3900

**Declaration of interest**

Conflict of interest: None.

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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 is 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.
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.

**Keywords** Drug epidemiology, sewage analysis, new psychoactive substances

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## 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 is a critical aspect of this work, and this is 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 is even more limited, and for the most part this data does not yet exist, so identification and selection of appropriate biomarkers requires 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 (Figure 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 resolution mass spectrometric (HRMS) data from wastewater samples (see section 3.2 below).

*2.3. Kinetics in wastewater (transformation, biotransformation and sorption)*

Any drug residue released into the sewer network will be subject to a number of different processes as it travels from the point of excretion to the point of sampling. In-sewer transformation and losses can have an influence on both quantitative and qualitative biomarker measurements. It is already known that certain commonly used illicit drugs (e.g. cocaine) are affected by in-sewer transformation that can lead to increased uncertainty around any quantitative measurements taken from sewers with long mean residence times (Baker and Kasprzyk-Hordern 2011; Castiglioni et al. 2013; Plósz et al. 2013). However, the absence of available data for NPS means that alternative approaches are required to allow for the rapid and effective assessment of in-sewer stability. The two-main processes that are likely to affect a biomarker during transit are transformation and sorption. Excessive in-sewer transformation can typically have two different negative effects on biomarker concentration. The simplest is the transformation of any chosen biomarker that results in reduced concentrations at the point of sampling, possibly to below the limits at which it can be detected. More complicated is the potential transformation of another compound which is also found in wastewater into the chosen biomarker. Since the microorganisms in wastewater also contain enzymes that are the same (or similar) to those found in the human body there is the potential that any residues of the parent drug that are excreted or disposed into the sewer network will be transformed into the very biomarker that is being measured. This will result in higher concentrations in the final sample, and although not a problem for qualitative analyses it, will result in unrealistically high concentrations of the biomarker for any quantitative estimation of community use.

As with PK prediction there is the potential to use a number of in-silico tools to aid in the acquisition of data and the subsequent decision process. For example there are models available that will predict the stability of a

compound in wastewater treatment processes. One such model is STPWIN™ that was originally developed by Mackay and coworkers at the University of Toronto and is freely available from United States Environmental Protection Agency as part of their Estimation Program Interface (EPI)Suite™. This model estimates the fate of a chemical present in sewage effluent as it becomes subject to transformation and sorption processes in a sewage treatment plant that uses activated sludge secondary treatment. We therefore evaluated the ability of STPWIN™ to estimate the stability of a number of common illicit drugs and newly identified psychoactive drugs (and some of their known metabolites) in order to see whether it could serve as part of a screening tool for identifying suitable biomarkers for sewage (Table 1). The most crucial variable in all such models is the rate of biodegradation and its dependence on the concentration of biomass. In the STPWIN™ model there are a number of options for setting the STP half-lives. Two options were evaluated. The first was the model default where 10,000 h was set as the half-life for the primary clarifier, aeration vessel and settling tank. This represents no biodegradation and is considered a worst-case scenario in terms of emission, so it is unlikely to be a useful tool in terms of evaluating in-sewer stability. The second option uses half-life estimates based upon the output of the BIOWIN™ component of the EPISuite™ model. BIOWIN™ is also a component of EPISuite™ and estimates the aerobic and anaerobic biodegradability of organic chemicals.

For traditional illicit drugs such as cocaine, amphetamines and THC (where published data are available on their stability in sewage) STPWIN™ generated a range of STP removal rates depending on whether the default (10,000 h) or BIOWIN™ predicted half-lives were used. With the default (10,000 h) half-life the model returns predicted STP removal rates that are simply a function of sorption so results are, not surprisingly, low when sorption is insignificant. More useful results are perhaps obtained when using BIOWIN™ predicted half-lives. Cocaine is known to be relatively unstable in sewage (van Nuijs et al. 2012; Plósz et al. 2013) and this was reflected by the model output. Stability estimations using predicted half-lives do however suggest a high rate of removal for benzoylecgonine, amphetamine and methamphetamine while published removal rates (Table 1) suggest that these compounds are relatively stable in sewage. For newer psychoactive substances there are very few data on in-sewer stability. The predicted stability estimations suggest some biodegradation of ketamine, mephedrone, para-methoxyamphetamine (PMA), para-methoxymethamphetamine (PMMA) and MDMA and this is reflected in what has been reported on their in-sewer stability (Table 1). For the cannabinoids, including THC and selected synthetic cannabinoids and their metabolites, STPWIN™ estimates a high level of removal that is in general a reflection of their sorption potential. Such information is valuable as

this may serve as a tool to quickly screen potential drug residues for their sorption potential in the absence of measured adsorption coefficients. The model does however struggle with JWH-018 N-(5-hydroxypentyl), which is known to be stable in sewage, unlike many of the other synthetic cannabinoids (Reid et al. 2013). This qualitative evaluation of STPWIN™ suggest that in the absence of any data the model using half-life generated by BIOWIN™ and not the default 10,000h, may provide an indication as to whether a group of NPS may be stable in-sewer and whether there may be any potential issues with adsorption. One challenge of using such a model is that many psychoactive compounds are very different in structure to the environmental contaminants that are typically used to develop and train the model. In addition, the model is based upon what occurs in a STP, whilst we are interested in the fate of NPS residues in the sewer. The model may however serve as a guide as to whether a selected drug residue may be stable or not, or whether it is likely to absorb to biomass.

In the absence of any stable drug residue it may be of interest to search for a transformation product that may provide evidence of the use of a drug by a community. A model for identifying the potential transformation products of new psychoactive substances is the University of Minnesota Pathway Prediction System (UM-PPS). The model is based upon biotransformation rules held in its database and has been widely used to evaluate the potential transformation products of environmental contaminants. In the context of identifying potential transformation of new psychoactive substances we have evaluated the model for MDMA, mephedrone, and two synthetic cannabinoids (MAM2201 and JWH-018) (Figure 2). The model predicts transformation of the synthetic cannabinoids via N-dealkylation and hydroxylation. Similarly both MDMA and mephedrone are also predicted to undergo N-dealkylation. For mephedrone, further transformation is predicted to result in conversion of the aromatic methyl group to a primary alcohol and subsequently to an aldehyde (Figure 2). Although not yet validated for use with NPS, the UM-PPS model offers a useful tool for predicting the potential transformation products of NPS in the absence of experimental data.

## 3. Detection of new drug targets

### *3.1. Pissoirs as a source of "pooled urine"*

Data obtained from the analysis of wastewater from large-scale sewage treatment plants has been used to study temporal and regional trends in the use of classical illicit drugs (such as cocaine, cannabis and amphetamines), and technological and methodological innovation over recent years has provided the

necessary increases in sensitivity which allow for the identification of new recreational drugs and their metabolites at very low concentrations (Reid et al. 2013). However, illicit drug (or metabolite) concentrations in waste water from large geographical areas are often below the limit of detection (Thomas et al. 2012). This problem is exacerbated by patterns of use with respect to NPS whereby the vast number of different compounds on offer and the sporadic nature of their availability mean that detecting one individual compound (or metabolite) in the vast volume of the combined sewage network may be problematic.

The collection and analysis of wastewater from pissoirs could however provide a good alternative for NPS detection, albeit limited to non-quantitative screening. Archer et al. recently undertook pilot studies analyzing urine from single stand-alone urinals in London. The results show a variety of drugs in differing locations such as nightclub environments (38 drugs and/or metabolites) (Archer et al. 2013a) and the center of London (109 parent drugs or metabolites of which, 7 was recreational drugs and 6 NPS) (Archer et al. 2013b). A similar pilot study on pooled wastewater from pissoirs in central Oslo also detected a vast array of differing compounds (Table 2). (See supplementary information for additional information on methodology). While amphetamine is always present in municipal wastewater samples from the Oslo region, it was notably absent from the pissoir samples. Conversely, MDMA is often below the lower limit of detection in the regional wastewater samples but is present at high levels in the wastewater from pissoirs. This analysis also provided the first positive identification of 1-(2-methoxyphenyl)piperazine (oMeOPP) in wastewater from the region. Such data highlights the difference between large-scale municipal wastewater analysis and the more “targeted” pissoir approach. Samples collected from pissoirs are likely to be from a more targeted population that may or may not be similar to the general population, so the types of drugs that are identified and the relative proportion of these to the total amount of drugs used may be different.

The results of these early studies show that it is possible to not only confirm the use of a range of NPS, but also provide a means of comparison between different populations in order to determine whether there are trends associated with, for example, types of festival and/or musical genre.

### *3.2. Analysis - The importance of non-targeted data acquisition techniques*

The dynamic nature of the NPS market means that identification and detection of the compounds (and/or metabolites) in bodily fluids is often complicated (at least initially) by the lack of standard reference materials. This dynamic nature also complicates detection because compounds may only be available on the market for

short periods of time before they are replaced by any number of different analogues. These challenges are best addressed via analysis of high-resolution mass spectrometric (HRMS) data of new samples, or retrospectively whereby old samples are reinvestigated and screened for the new psychoactive substances that have been identified (by the EWS or alike) after initial sample-collection (Bijlsma et al. 2011, 2013; Hernández et al. 2011; Mwenesongole et al. 2013). Non-targeted (unbiased) data acquisition via HRMS provides the ability to later process, detect and (tentatively) confirm the presence of a compound without the absolute need of a standard reference material. It should be noted however that whilst data-acquisition is best carried out in an unbiased manner in order to acquire and store as much information as possible, the data-processing and eventual screening is most effectively performed with reference to a set of compounds in a database (Hernández et al. 2011). Old samples can always be re-investigated as and when changes are made to the database so long as the initial data acquisition was unbiased.

The high mass accuracy and resolution of QTOF and Orbitrap instruments together with isotope abundance analysis aid in the elucidation of an empirical formula (Prasad et al. 2011). Structural information on the compound is then obtained via interpretation of the fragments and fragmentation pathways. The fact that many NPS share structural elements or moieties also allows for common-fragment searches and mass-defect filtering to be performed on HRMS data (Grabenauer et al. 2012). Common fragment searches are in essence the screening of complex chromatographic data for compounds with a common sub-structure and a subsequently common fragmentation pathway. Note that for this technique to be applied to new compounds in an expanding screening data-base it is highly dependent on the unbiased acquisition of parent and fragment ions without pre-selection such as by $MS^e$ (Waters Corporation, Milford MA) and All-Ions Technology (Agilent Technologies, Santa Clara CA) which are also referred to as “all-in-one” analysis techniques (Wrona et al. 2005). An example is given in Figure 3 which shows results of data acquired in $MS^e$ mode on a Xevo G2-S QTOF (Waters Corp., Milford MA). In Figure 3(B) we show the unsubstituted naphthal moiety common to numerous synthetic cannabinoids. This fragment is also common to any and all of the aliphatic hydroxy- or carboxy metabolites of these cannabinoids meaning that biomarkers of new synthetic analogues can be identified in complex matrices without the explicit need for information on biotransformation pathways. All that is needed is knowledge that the new synthetic analogue (NPS) has a known sub-structure that is shared by the previously identified analogues. Note however that in Figure 3 we see that searching for the common-fragment of the naphthal moiety has failed to detect the metabolites of RCS-4 and JWH-122 (Peaks (i) and (vii)) because RCS-4

does not have the moiety, and JWH-122 contains a methyl-substituted naphthal group. The napthal moiety is also not unique to synthetic cannabinoids so common-fragment searching could lead to false-positives if additional confirmation is not carried out. These factors show that while the number of potential targets can be significantly reduced by screening for common-fragments, this technique is not the perfect solution on its own.

Mass defect filtering works on the principle that the majority of metabolites of a compound will have a mass defect of within 50 mDa of that of the parent (Bateman et al. 2007; Zhang et al. 2009). This thereby focuses the search for unknown metabolites down to a very small mass window. The example in Figure 3(C) shows a Total Ion Chromatogram (TOF-MS 50 – 800 m/z) following mass-defect filtering on 0.15 Da with 50mDa tolerance. (Note that these filtering parameters were selected as a generic set applicable to a wide range of synthetic cannabinoids rather than to a specific parent compound). Mass spectral data is effectively stripped away leaving only the information that is most likely to be related to the compounds of interest, or in this case a series of structurally related NPS metabolites. Again, little or no information is required on the pharmacokinetics or biotransformation pathways of the parent NPS. Note however that one major draw-back with the use of mass-defect filtering on samples of wastewater is that many drugs have mass-defects in the 0.1 – 0.3 Da range which is also common to much of the endogenous matrix in urine samples (Zhang et al. 2009). Mass-defect filtering will therefore not eliminate all back-ground noise from chromatograms, but it does remain an excellent means of at least increasing the chances of success when attempting to detect and identify NPS in complex matrices such as wastewater and/or pooled urine from pissoirs (Grabenauer et al. 2012).

## 4. Conclusions

The key objective of work in this field is to detect, track and understand emerging drug trends. The EMCDDA aims to analyse the wealth of accumulated information by triangulation of data from a range of different sources including the internet, users, test purchasing, forensic toxicology, law enforcement and wastewater. The NPS market presents a unique set of challenges to all epidemiologists including those working with wastewater because this market-segment is extremely dynamic and new compounds are being identified at a rapid rate. The lack of experimental data on pharmacokinetics together with unanswered questions related to

biotransformation pathways severely impede the identification, detection and quantification of these new compounds in samples of wastewater.

A number of primarily in-silico-based tools are however available which can be used to predict these unknown parameters. Software such as SMARTCyp and the University of Minnesota Pathway Prediction System (UM-PPS) can provide a concise list of potential biomarker targets. It should be noted however that these models by no means guarantee the formation of a given metabolite or biotransformation product so extensive non-targeted screening by retrospective analysis of HRMS data will be required.

Screening via HRMS provides the ability to detect and (tentatively) confirm the presence of a compound without the absolute need of a standard reference material, and the fact that many NPS share structural elements also allows for common-fragment searches and mass-defect filtering to be performed. These techniques focus the search for unknown metabolites and strip-away spectral noise to leave only the information that is most likely to be related to a series of NPS biomarkers.

Illicit drug (or metabolite) concentrations in domestic wastewater networks from large geographical areas are however often below the lower limit of detection, so the collection and analysis of wastewater from pissoirs may have to be the primary alternative for NPS detection. Further, where clinical trials are lacking, the data from the analysis of pissoirs could potentially provide an additional source of primary technical data (such as spectra and chromatographic retention time) to the EWS and may also prove useful to toxicologists in clinical testing environments. Therefore, the combined collection of these tools and alternative data-sources provide an excellent framework which can be used to maximize the chances of success in identifying and detecting biomarkers of NPS in wastewater.

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**Figure 1.** Cytochrom P450 metabolism pathways for MDMA, mephedrone, and two synthetic cannabinoids (MAM-2201 and JWH-018) as predicted by SMARTCyp (University of Copenhagen).

**Figure 2.** Transformation pathways for MDMA, mephedrone, and two synthetic cannabinoids (MAM-2201 and JWH-018) as predicted by the University of Minnesota Pathway Prediction System (UM-PPS).

**Figure 3.** Chromatogram of a 5 ng/mL (25 pg on column) matrix-matched wastewater standard of; (i) RCS-4 N-(5-hydroxypentyl), (ii) JWH-073 N-butanoic acid, (iii) JWH-018 N-pentanoic acid, (iv) JWH-073 N-4-hydroxybutyl, (v) AM2201 N-4-hydroxypentyl, (vi) JWH-018 N-5-hydroxypentyl, and (vii) JWH 122 N-5-hydroxypentyl. Plot A is the Total Ion Chromatogram (TIC) of the positive electrospray TOF-MS scan (50 – 800 m/z). Plot B is the common-fragment scan of the naphthal moiety (155 m/z). Plot C shows the TIC (50 – 800 m/z) following mass-defect filtering (0.15 Da with 50mDa tolerance). All data from a single chromatophraphic run on a Waters Xevo G2-S QTOF acquiring in MSe mode.

**Table 1**
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**Table 1.** EPIWIN STP predicted removal of selected psychoactive substances

| Drug/metabolite | EPIWIN STP predicted removal (%) | | | | | | Published removal rates (%) | LogKoc |
|---|---|---|---|---|---|---|---|---|
| | Default[1] | | | BIOWIN™[2] | | | | |
| | Total | Biodeg | Ads | Total | Biodeg | Ads | | |
| **Cocaine** | 2.64 | 0.1 | 2.54 | 47.6 | 45.9 | 1.7 | -8 to -50[3] | 2.0 |
| **Benzoylecognine** | 1.85 | 0.1 | 1.75 | 75.1 | 74.4 | 0.6 | +6 to +14[3] | -0.7 |
| **Amphetamine** | 2.1 | 0.1 | 2.0 | 75.1 | 74.4 | 0.7 | -15 to +47[3] | 1.9 |
| **Methamphetamine** | 2.5 | 0.1 | 2.4 | 76.0 | 75.1 | 0.9 | 0 to +8[3] | 2.0 |
| **MDMA** | 2.4 | 0.1 | 2.3 | 23.3 | 21.3 | 2.0 | <15[5] | 2.2 |
| **PMA** | 2.1 | 0.1 | 2.0 | 22.5 | 20.9 | 1.6 | 16[4] | 1.9 |
| **PMMA** | 2.2 | 0.1 | 2.1 | 22.7 | 21.0 | 1.7 | 27[4] | 2.0 |
| **Cathinone** | 1.9 | 0.1 | 1.8 | 75.3 | 74.6 | 0.7 | No data | 1.9 |
| **Cathine** | 1.9 | 0.1 | 1.8 | 75.1 | 74.5 | 0.6 | No data | 1.0 |
| **Mephedrone** | 2.8 | 0.1 | 2.7 | 24.2 | 21.9 | 2.3 | 37[4] | 2.4 |
| **Ketamine** | 2.5 | 0.1 | 2.4 | 23.4 | 21.4 | 2.0 | <15 | 2.3 |
| **THC** | 93.8 | 0.8 | 93.0 | 99 | 37.3 | 61.7 | No data | 5.0 |
| **THC-COOH** | 93.2 | 0.8 | 93.1 | 98.2 | 37.5 | 60.7 | -8 to +2[3] | 3.9 |
| **JWH-018** | 93.8 | 0.8 | 93.0 | 99.0 | 37.3 | 61.7 | No data | 4.9 |
| **JWH-018 N-(5hydroxypentyl)** | 75.7 | 0.7 | 75.0 | 97.7 | 39.9 | 57.8 | 0[4] | 3.4 |
| **AM2201** | 93.8 | 0.8 | 93.0 | 99.0 | 37.1 | 61.9 | No data | 4.9 |
| **AM2201 N(4-hydroxypentyl)** | 85.2 | 0.7 | 84.5 | 97.2 | 46.7 | 56.5 | 87[4] | 3.6 |
| **MAM-2201** | 94.0 | 0.8 | 93.2 | 99.1 | 37.2 | 61.9 | No data | 5.2 |
| **MAM-2201 N(4-hydroxypentyl)** | 91.4 | 0.8 | 90.6 | 98.5 | 38.3 | 60.2 | No data | 3.9 |

[1]Default half-life of 10,000h. [2]Half-life based upon BIOWIN™ estimations. [3]Castiglioni et al., 2013. [4]Reid et al., 2013. [5]Mwenesongole et al., 2013.

**Table 2**

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**Table 2.** Positively identified drugs and pharmaceuticals resulting from the screening of pissoir-sourced wastewater that was collected in central Oslo during a music festival (2012). Analysis was carried out on a Waters Xevo G2-S QTOF and the resulting MSe data screened against a database of over 1000 drugs and metabolites with the Unifi Screening platform (Waters Corporation, Milford MA).

**Legal Substances**

| *Drug* | *Class* | *Drug* | *Class* |
|---|---|---|---|
| Acebutolol | Beta-blocker | Metoprolol | Beta-blocker |
| Amidopyrine | Analgesic | Mycophenolate | Immunosuppressant |
| Amisulpride | Antipsychotic | Nevirapine | Antiretroviral |
| Bisoprolol | Beta-blocker | Nicotine | Alkaloid |
| Bupropion | Antidepressant | Omeprazole | Proton Pump inhibitor |
| Caffeine | Alkaloid stimulant | Paracetamol | Analgesic |
| Carbamazepine | Anticonvulsant | Phenazone | Analgesic |
| Cetirizine | Antihistamine | Propranolol | Beta-blocker |
| Citalopram | Antidepressant | Ranitidine | Histamine H2-receptor antagonist |
| Fexofenadine | Antihistamine | Salbutamol | ß2-adrenergic receptor agonist |
| Flecainide | Antiarrhythmic | Sulfamethoxazol | Antibiotic |
| Fluconazole | Antifungal | Theobromine | Alkaloid, naturally occurring (coffee, chocolate) |
| Hordenine | Alkaloid, naturally occurring (barley) | Theophyline | Alkaloid, naturally occurring (coffee, tea) |
| Loperamide | Antidiarrhoeal | Trimethoprim | Antibiotic |
| Irbesartan | Angiotensin II receptor antagonist | Valsartan | Angiotensin II receptor antagonist |
| Lamotrigine | Antihistamine | Venlafaxine | Antidepressant |
| Metoclopramide | Antiemetic | Xylometazoline | Decongestant |

**Illegal and/or legal substances with significant misuse potential**

| *Drug* | *Class* |
|---|---|
| 1-(2-Methoxyphenyl)piperazine (oMeOPP) | Piperazine Stimulant |
| Cocaine | Alkaloid Stimulant |
| Cathinone | Alkaloid Stimulant |
| Codeine | Opiod |
| Dextromethorphan | Cough Suppressant (Not legally available in Norway) |
| Ethenzamide | Analgesic (Not legally available in Norway) |
| Ethylmorphine | Opiod |
| Lidocaine | Local Anesthetic, cocaine adulterant |
| MDMA | Phenylethylamine |
| Methadone | Opiod |
| Methamphetamine | Phenylethylamine |
| Oxazepam | Benzodiazepine |

| | |
|---|---|
| Stanozolol | Anabolic Steroid |
| Tramadol | Opiod |

**Figure 2**

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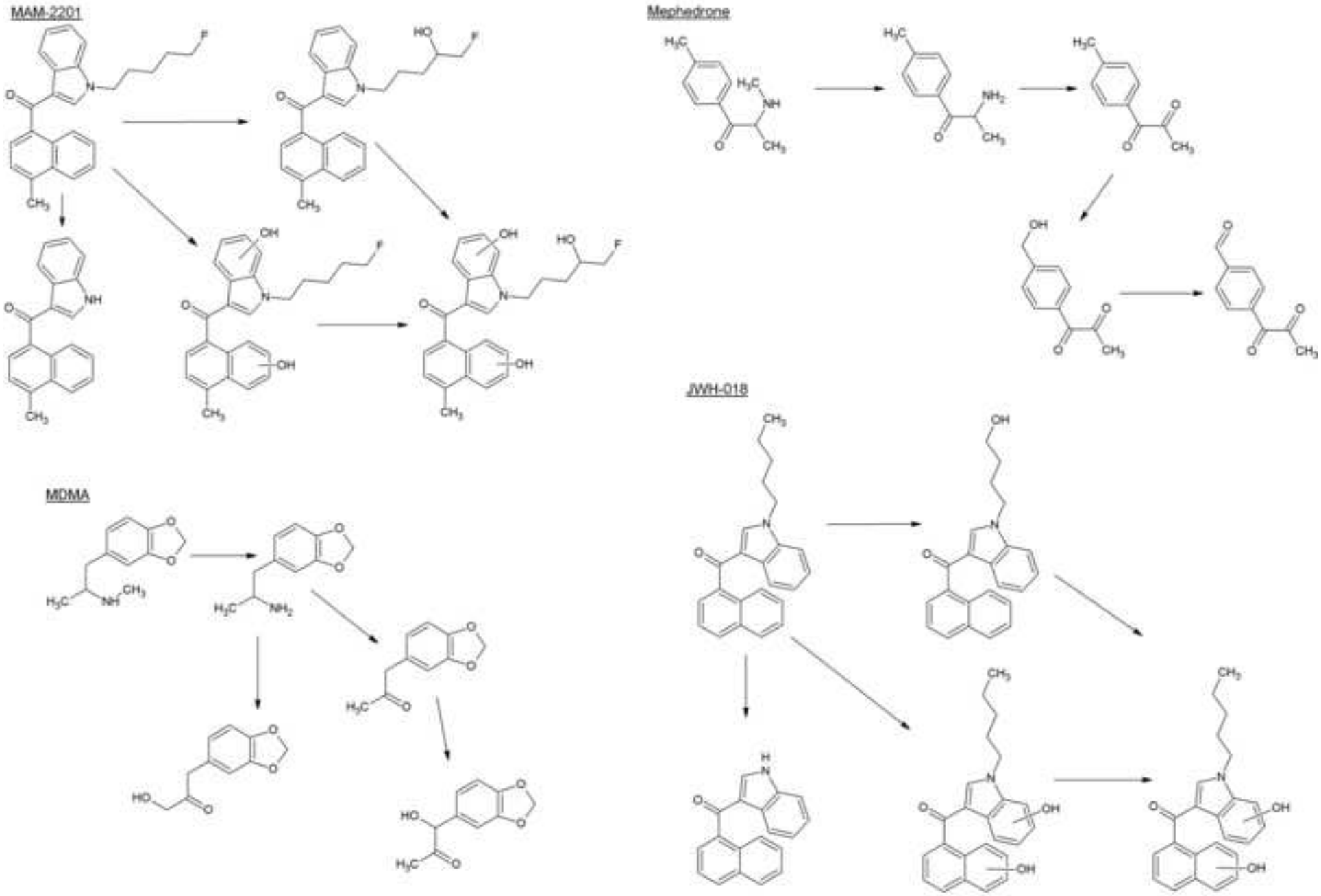

**Figure 3**

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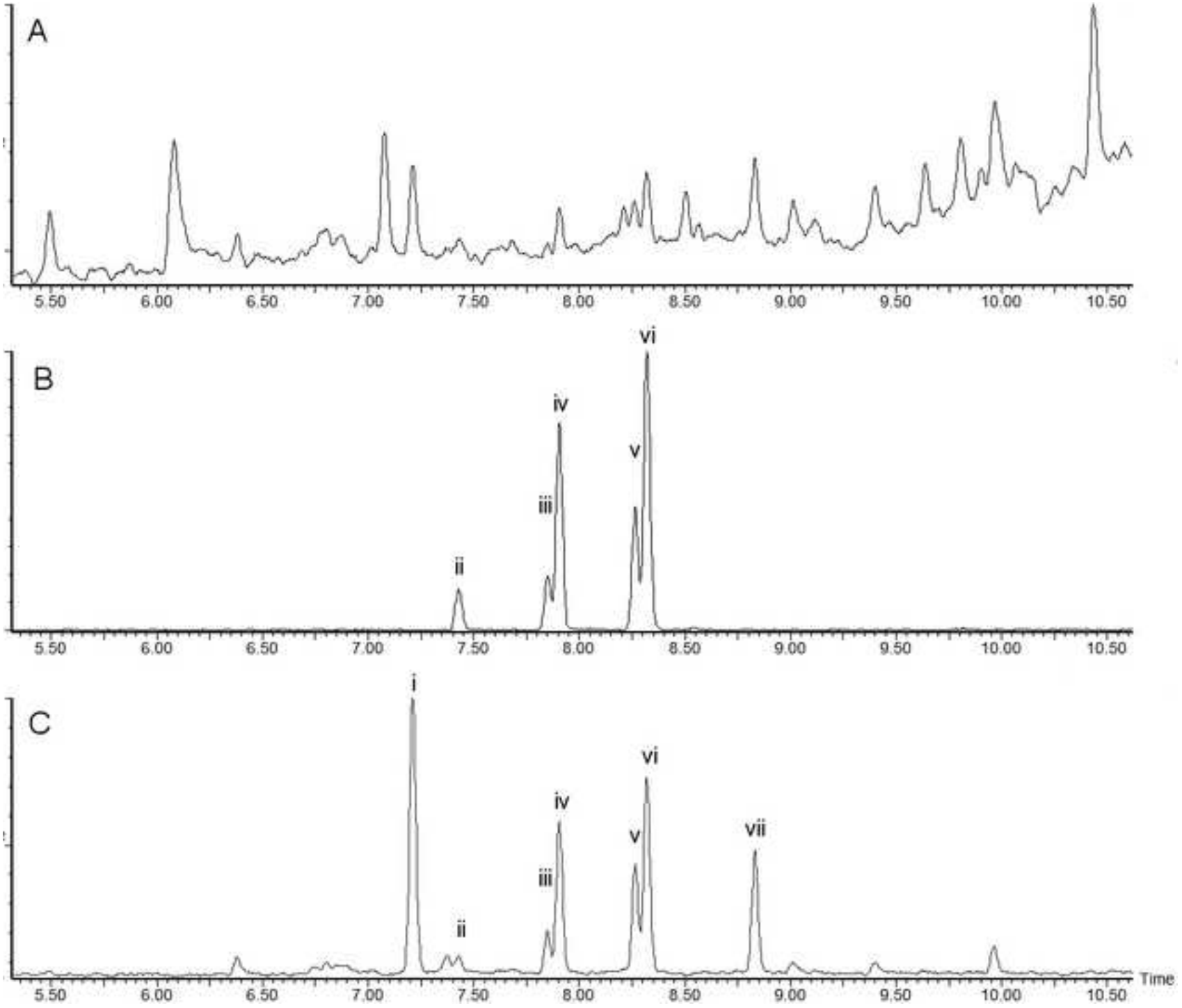

**Figure 1**

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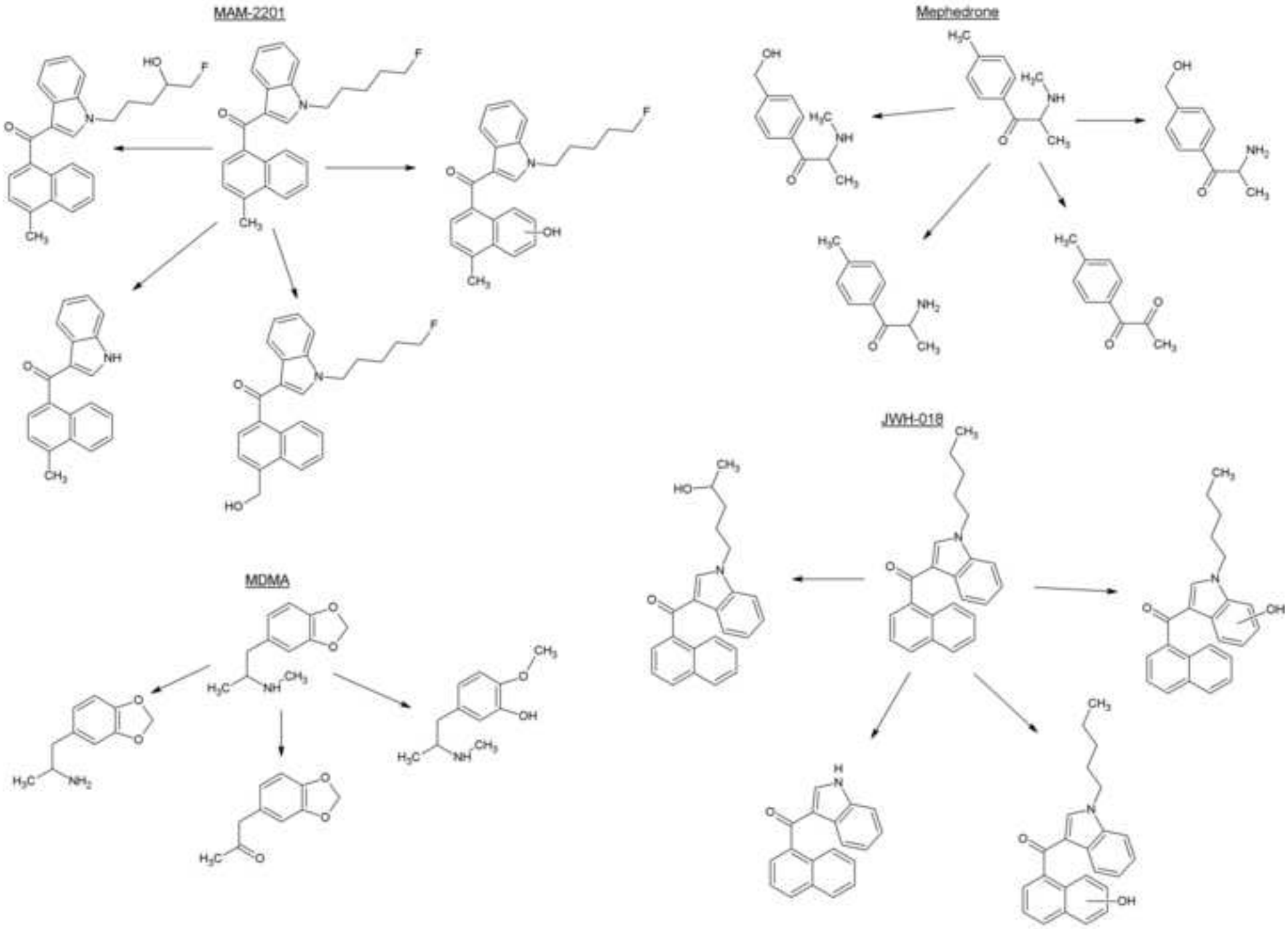

**Table S1**

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**Supplementary Analytical Details**

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**Figure S1**

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**Figure S2**

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