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Investigating in-sewer transformation products formed from synthetic cathinones and phenethylamines using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

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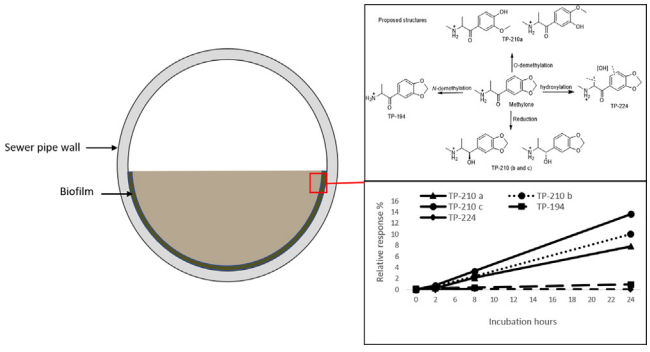
HIGHLIGHTS

- There is a paucity of biomarkers for NPS in wastewater-based epidemiology (WBE).
- Experiments conducted using in-sewer incubations of NPS in the presence of biofilm.
- We investigated formation of transformation products (TPs) using LC-QToFMS.
- 18 TPs were identified, 5 TPs unique to wastewater matrix interactions.
- In-sewer stability and transformations are important considerations in WBE.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent studies have demonstrated the role of biofilms on the stability of drug residues in wastewater. These factors are pertinent in wastewater-based epidemiology (WBE) when estimating community-level drug use. However, there is scarce information on the biotransformation of drug residues in the presence of biofilms and the potential use of transformation products (TPs) as biomarkers in WBE.

The purpose of this work was to investigate the formation of TPs in sewage reactors in the presence of biofilm mimicking conditions during in-sewer transport. Synthetic cathinones (methylenedioxypyrovalerone, methylone, mephedrone) and phenethylamines (4-methoxy-methamphetamine and 4-methoxyamphetamine) were incubated in individual reactors over a 24 h period. Analysis of parent species and TPs was carried out using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToFMS). Identification of TPs was done using suspect and non-target workflows.

In total, 18 TPs were detected and identified with reduction of β-keto group, demethylenation, demethylation, and hydroxylation reactions observed for the synthetic cathinones. For the phenethylamines, *N*- and *O*-demethylation reactions were identified. Overall, the experiments showed varying stability for the parent species in wastewater in the presence of biofilms. The newly identified isomeric forms of TPs particularly for methylone

List of Abbreviations: COC, Cocaine; CODA, COmponent Detection Algorithm; DO, Dissolved oxygen; EMCDDA, European Monitoring Centre for Drug and Drug Addiction; FbF, Find by Formula; HRMS, High-resolution mass-spectrometry; HRT, Hydraulic residence time; LC, Liquid chromatography; LC-QToFMS, Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry; *m/z*, Mass-to-charge ratio; MDPV, Methylenedioxypyrovalerone; MS, Mass-spectrometry; NPS, New psychoactive substances; OECD, Organisation for Economic Co-operation and Development; PCDL, Personal compound database and library; PMA, 4-methoxy-amphetamine; PMMA, 4-methoxy-methamphetamine; TP, Transformation product; t_r, Retention time; TSS, Total suspended solids; VSS, Volatile suspended solids; WBE, Wastewater-based epidemiology; WWTP, Wastewater treatment plant.

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and mephedrone can be used as potential target biomarkers for WBE studies due to their specificity and detectability within a 24 h residence time.

1. Introduction

The emerging field of wastewater-based epidemiology (WBE) has been instrumental in estimating illicit drug consumption in communities. WBE is now used as a complimentary approach by the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) to monitor spatio-temporal trends in the use of conventional illicit drugs like cocaine, methamphetamine, ecstasy, and amphetamine (European Monitoring Center for Drugs and Drug Addiction, 2016; Ort et al., 2014a). Synthetic cathinones and other phenethylamines have emerged over the last decade as major classes of new psychoactive substances (NPS) in many European countries (European Monitoring Center for Drugs and Drug Addiction, 2017). Structurally, synthetic cathinones are ring-substituted phenethylamines with the substitution of a ketone group at the β carbon position. These substances are often used as alternatives to amphetamine-type stimulants and cocaine because they possess similar psychoactive effects. Their use has been linked to numerous cases of acute and fatal intoxications (Murray et al., 2012; Adamowicz et al., 2013).

Monitoring NPS consumption using WBE has been a growing area of interest in recent years, where few studies (González-Mariño et al., 2016; Kankaanpää et al., 2014; Kinyua et al., 2015a; Castiglioni et al., 2015; Thai et al., 2016; Bade et al., 2017; Senta et al., 2015) have investigated the presence and amount of NPS in wastewater. However, only few NPS have been detected and levels were generally low in sewage. Several factors may contribute to this: firstly, the prevalence of use, when a NPS enters the drug scene its popularity is generally low until it becomes more recognized and thus the concentrations in sewage may be very low (Archer et al., 2013; Kinyua et al., 2016). Secondly, limited information exists about the metabolism and excretion of NPS, and therefore, target biomarkers remain largely unknown. Additionally, the focus on targeted analysis in WBE studies is likely to miss NPS not included in the targeted methods (Causanilles et al., 2017). Lastly, their stability and (bio)transformation in wastewater is complex and not (yet) fully understood (Ramin et al., 2016; McCall et al., 2016a).

For compounds with unknown or low stability during *in-sewer* transport, (in)stability is considered a major source of uncertainty in the estimation of drug use by WBE and can result in significant under- or overestimation (Lai et al., 2011; Nuijs et al., 2012). Most stability studies related to WBE have focused on *in-sample* stability (McCall et al., 2016b), which involves sample preparation, preservation and storage through the testing of different conditions: filtration of samples, storage at different temperatures, and addition of preservatives (Baker and Kasprzyk-Hordern, 2011). Few stability studies have considered environmental processes occurring in sewers that may affect the overall fate of target biomarkers (McCall et al., 2016a; Thai et al., 2014) and estimated the possible effect in entire catchments considering known and unknown variables (McCall et al., 2017).

Wastewater contains a large number of components and is subject to different environmental processes which are also influenced by the design of the sewer and operation modes (Thai et al., 2014; McCall et al., 2016b). Presence of biofilms on sewer walls (Thai et al., 2014; Hvitved-Jacobsen et al., 2013) and processes involving sorption to particulate matter (Ramin et al., 2016), sedimentation, uptake by organisms (McCall et al., 2016b) and those causing structural changes of compounds (Schwarzenbach et al., 2002) should also be considered. Some biomarker stability studies investigated the role of biofilm (Ramin et al., 2016; McCall et al., 2016a; Thai et al., 2014), and isolated microbial strains (Mardal et al., 2017) on pharmaceuticals and select drugs of abuse. Accounting for aerobic and anaerobic conditions these studies showed that degradation rates are significantly higher in the presence of biofilm and/or suspended solids. One study modeling *in-sewer* transformation for three catchments of different size showed that in small catchments target biomarkers were mostly affected by biofilm processes. Even though biomarkers in large(r) catchments have on average longer residence times in the sewer, the biomarkers have less contact with biofilm, consequently, their stability is mainly affected by abiotic processes (McCall et al., 2017).

Subsequently, parent compounds can undergo further transformation in the sewage environment, leading to transformation products (TPs) other than those normally observed in biological matrices, such as urine. The ideal WBE biomarker would be one that is stable in wastewater during *in-sewer* transport, specific, and detectable. In many cases, TPs are more stable than the parent compound (Ramin et al., 2016), therefore it would be worthwhile to identify TPs formed during *in-sewer* transport and assess them as potential biomarkers to be used in WBE studies.

Studies conducted to investigate TP formation during wastewater treatment plant (WWTP) processes like ozonation and chlorination were performed by spiking high levels of the compounds of interest individually in reactors (Bijlsma et al., 2013; Boix et al., 2014). Such studies have been instrumental in detecting TPs, which are used for the evaluation of removal efficiencies in WWTPs. Application of individual spike experiments and high-resolution mass-spectrometry (HRMS) techniques would be useful in determining potential biomarkers for NPS to be used in WBE studies.

The aims of this study were: (i) to conduct experiments with real wastewater and biofilm to investigate the formation of TPs of selected synthetic cathinones (methylenedioxypyrovalerone (MDPV), methylone, mephedrone) and selected phenethylamines (4-methoxy-methamphetamine (PMMA) and 4-methoxy-amphetamine (PMA)), (ii) to identify and characterize the TPs formed using liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QToFMS) using suspect and non-target screening approaches, and (iii) to recommend potential biomarkers for these NPS to be used in WBE studies.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals standards for cocaine (COC), mephedrone, MDPV, methylone, PMMA, and PMA were obtained from LGC Standards SARL (Molsheim, France) and Cerilliant (Round Rock, Texas, USA) at the concentration of 1 mg/mL or 100 μg/mL in methanol or acetonitrile. LC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Nanopure water was obtained by purifying demineralized water in an Elga LabWater Purelab Flex system (Veolia Water Solutions & Technologies Belgium, Tienen, Belgium). Formic acid (eluent additive for LC-MS, 98%) was obtained from Sigma-Aldrich (Steinheim, Germany). The internal standards ranitidine-D_6 and fluoxetine-D_5 (with purity >98%) were purchased from Cerilliant (Round Rock TX, USA) at concentrations of 1 mg/mL in methanol. Working solutions were prepared for concentrations ranging between 0.005 and 100 ng/μL in methanol.

2.2. Biotransformation reactor setup

The biotransformation experiments were conducted as previously optimized by McCall et al. (2016a). This protocol was adapted from