

# Identification of biotransformation products of p-methoxy methamphetamine and dihydro-mephedrone formed in activated sludge by LC-QToF-MS

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## Extended abstract

Para-methoxy-methamphetamine (PMMA) is a new psychoactive substance (NPS). As a structurally related analog of methamphetamine, PMMA is presented mimic effects to ecstasy (3,4-methylenedioxymethamphetamine, MDMA) and through metabolic paths is transformed to para-methoxyamphetamine (PMA) (WHO, 2015). In addition, dihydro mephedrone (DHMEPH) is the major phase I human metabolite of mephedrone, a synthetic stimulant drug of the class of cathinones (WHO, 2014). These parent compounds and their metabolites are excreted in the aquatic environment due to incomplete removal by the sewage treatment or/and by biotransformation process occurring at the wastewater treatment plants (WWTPs) (Kiyua *et al.*, 2015)

In the aeration tanks of the WWTPs, microbial degradation processes are taking place, resulting in the elimination of the organic load during biological wastewater treatment, by formation of transformation products (TPs). The key-role to assess the risk to the exposure of aquatic biota to the formed TPs is; a) to obtain knowledge of how fast the parent compounds degrade/transformed and b) to identify the formed TPs as they can be more polar and thus environmentally (pseudo)persistent (Clarke and Smith, 2011).

Little is known about the behavior and transformation products of PMMA and DHMEPH during wastewater treatment. Herein, batch reactors seeded with activated sludge from the WWTP of Athens were set up to assess biotic, abiotic and sorption losses of PMMA and DHMEPH. The main objectives of the study were: (a) the identification of the formed TPs by applying suspect and non-target screening strategies based on liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QToF-MS) and (b) the structure elucidation of the candidate TPs, based on accurate mass and isotopic pattern measurements by HRMS and tentative interpretation of MS/MS spectra, using *in silico* fragmentation tools. The complementary use of reversed phase liquid chromatography (RPLC) and hydrophilic

interaction liquid chromatography (HILIC) for the identification of polar TPs, and the application of in-house developed quantitative structure-retention relationship (QSRR) prediction models, in addition to the comprehensive evaluation of the obtained MS/MS spectra, provided valuable information to support the identification (Beretsou *et al.*, 2016).

O-demethylation and N-demethylation were found among the dominant biotransformation mechanisms. Other main observed reactions were hydroxylation, oxidation and N-methylation. Mephedrone was found to be formed back from its human metabolite DHMEPH. For all the TPs, tentative candidates were proposed. HILIC-HRMS analyses proved a powerful orthogonal tool for the identification, because many polar TPs presented higher sensitivity and clearer MS/MS spectra.

**Keywords:** transformation products; HRMS; activated sludge; PMMA; dihydro-mephedrone.

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