A WORKFLOW FOR THE ORTHOGONAL IDENTIFICATION OF BIOTRANSFORMATION PRODUCTS BY HILIC-QTOFMS

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The microbial degradation and formation of transformation products (TPs) under aerobic conditions is the fundamental process for the elimination of pharmaceuticals during biological wastewater treatment. It is of paramount importance to understand the microbial metabolic pathways; firstly, to obtain knowledge of how fast micropollutants degrade/transformed and, ultimately, to assess the exposure of aquatic biota to their potential TPs, as they can be more polar and consequently environmentally (pseudo)persistent [1].

In this study, batch reactors seeded with activated sludge from the WWTP of Athens were set up to assess biotic losses of selected pharmaceuticals that have been recently reported in influent and effluent wastewaters from the WWTPs of Greece [2, 3] and Europe [4, 5]. Different pharmaceutical classes and polarities were covered representatively by lidocaine, citalopram, ranitidine, metformin and atorvastatin. Biotransformation and transformation products were identified using reverse phase liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QToF-MS). Hydrophilic-lipophilic interactions liquid chromatography (HILIC) was used complementarily as an additional confirmatory technique for the identified TPs, instead of using another spectroscopic technique (e.g. NMR). A workflow for suspect and non-target screening was developed. A suspect list with possible metabolites was compiled by combining information from literature, online pathway prediction system (EAWAG-BBD PPS) and Metabolite Predict (Bruker). The structure elucidation of the candidate transformation products was based on accurate mass and isotopic pattern measurements by HRMS and tentative interpretation of MS/MS spectra, using *in silico* fragmentation tools.

The dominant mechanisms of biotransformation were found to be N-dealkylation, N-oxidation, hydrolysis and hydroxylation. Twenty eight TPs were identified in total. Nine of them were fully identified and confirmed by reference standard (monoethylglycinexylidide, lidocaine-N-oxide, desmethyl citalopram, citalopram amide, citalopram carboxylic acid, 3-oxo-citalopram, ranitidine-S-oxide, ranitidine-N-oxide, and guanylurea). For eleven more TPs, a probable structure was proposed based on MS/MS spectra evidence and retention time prediction. For the rest (eight TPs), tentative candidates were proposed. HILIC-HRMS analyses proved a powerful orthogonal tool for identification because many polar TPs were detected with higher sensitivity and hence clearer MS/MS spectra, facilitating the identification workflow substantially.

Keywords: transformation products; HRMS; liquid chromatography/quadrupole-time-of-flight mass spectrometry; activated sludge; pharmaceuticals

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