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Non-target screening of pharmaceuticals transformation products in urban wastewater and evaluation of their fate in constructed wetlands. A retrospective analysis

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1. Introduction

The path of the contaminant from its discharge in the environment to the complete degradation to inorganic salts, water, and carbon dioxide is rather long and complex. It has been stressed that one parent compound may generate several bio transformation products (TPs) due to incomplete mineralization [1]. Although the state of knowledge on this subject is still insufficient, it has been demonstrated that some TPs may be as harmful as their native forms, and might be much more harmful in an environmental situation, where they occur in mixtures [2].

Lack of TP reference standards and/or relatively high price make target screening challenging. Thus, the untargeted data acquisition modes of high-resolution mass spectrometry (HRMS) instruments are required, which can make the chemical profile of a sample accessible. In addition, conventional grab sampling methods might be inefficient for the identification of novel compounds, such as TPs. To overcome these limitations, e.g., the relatively low concentration of the analyte at the moment of sampling, passive samplers can provide new opportunities when it comes to the analysis of polar contaminants of concern in water [3].

Due to the aforementioned, this study combines passive sampling and HRMS for identification of pharmaceutical TPs in urban wastewater (WW) effluents. The final aim was to reveal the fate of these compounds during the treatment in constructed wetlands and to improve our understanding of their persistence.

2. Materials and methods

Polar organic chemical integrative samplers (POCIS) were placed in the maturation pond after the secondary treatment in the WW treatment facility in Murviel-les-Montpellier, France, and collected after 7 and 14 days. Grab samples of treated WW were collected as well and extracted using Oasis HLB SPE cartridges. More details about sample preparation and extraction can be found elsewhere [4].

Extracts were analyzed by liquid chromatography tandem high-resolution mass spectrometry using an Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, USA) at 75,000 resolving power. To obtain data dependent (DDA) and data independent analysis (DIA) higher-energy collision dissociation at normalized collision energies of 20 and 40 was performed. Chromatographic separation was done using an XBidge-BEH-C18 column (2.1 mm x 150 mm, 2.5 µm particle size) (Waters Corporation). The mobile phase consisted of a binary mixture of water + 0.1% formic acid and methanol + 0.1% formic acid. Non-target screening of TPs was performed using Compound Discoverer 3.2 software (Thermo Fisher Scientific, Waltham, MA, USA). A workflow, which included *in-silico* transformations prediction tool, generated a list of possible TPs of six previously detected parent compounds (i.e., clarithromycin, tramadol, venlafaxine, gabapentin, carbamazepine, and amisulpride). Finally, TP structures were confirmed with DDA.

3. Results and discussion

3.1. Identification of TPs

Obtained results demonstrated better suitability of POCIS in comparison with grab sampling technique for qualitative analysis of pharmaceutical TPs. First, fewer compounds were detected in grab samples and secondly, due to the higher pseudo-molecular ions abundance, passive sampling yielded better quality of MS² (DDA) spectrum of possible TPs, which were used for confirmation. In total 13 TPs were identified in the treated urban WW. Tramadol N-oxide, venlafaxine N-oxide, O-desmethylvenlafaxine N-oxide, and amisulpride N-oxide were confirmed with the reference standards, whereas additional 9 compounds (i.e., O-desmethylvenlafaxine, N-desmethylvenlafaxine, N,O-didesmethyltramadol, gabapentin lactam, O-

desmethyltramadol, clarithromycin N-oxide, hydroxyclearithromycin, hydroxycarbazepine, and carbamazepine-10,11-epoxide) were confirmed by matching their spectrum with library spectrum data (mz cloud).

3.2. Fate of TPs in constructed wetlands

A retrospective analysis of identified TPs and their parent compounds was applied on the data set obtained from the pilot-scale experiment where constructed wetlands were tested as tertiary treatment. For 11 compounds mean values of the removal efficiency were positive, whereas for 8 compounds negative removal efficiency was estimated based on the peak area (Figure 1). N-oxides and carbamazepine-10,11-epoxide appear to be the most recalcitrant, alongside amisulpride, gabapentin, and carbamazepine.

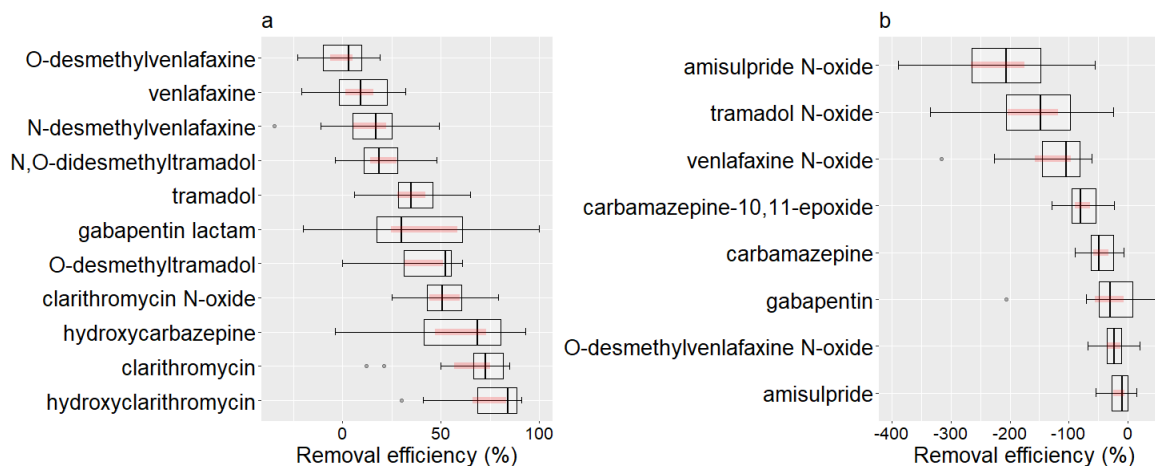


Figure 1: Positive (a) and negative (b) removal efficiency of detected compounds during tertiary treatment. (95% confidence interval of the mean values is marked with red color)

There are several concerns associated with poor or incomplete removal of these TPs. Since amidation and N-oxidation are reverse reactions, gabapentin lactam and detected N-oxides can transform to the parent compound recovering its full biological potential. Moreover, observed transformation reactions will not necessarily deactivate the molecule, rather render it suitable for biological activity. For instance, 14-hydroxyclearithromycin is still pharmacologically active, hence might contribute to the selective pressure on bacteria, carbamazepine-10,11-epoxide was proven to be more toxic than carbamazepine, and O-desmethylvenlafaxine was added to the 3rd Watch List due to its persistence and toxicity [2].

4. Conclusions

Non-targeted screening in combination with passive samplers proved to be a powerful methodology to identify pharmaceutical TPs in WW effluents. Moreover, retrospective analysis of the previously acquired data by non-target screening can be considered as a valid approach to reveal the fate of TPs during WW treatment. In such way, the performance of the current systems in the removal of novel compounds of interest can be evaluated and assist in the design of treatment systems.

5. References

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Short abstract

The path of the contaminant from its discharge in the environment to the complete degradation to inorganic salts, water and carbon dioxide is rather long and complex. Although the state of knowledge on this subject is still insufficient, it has been demonstrated that some transformation products (TPs) may be as harmful as their parent compounds, in addition, they may be much more harmful in an environmental situation, where they occur in mixtures. In this study, two sampling techniques, passive and grab sampling, were compared for qualitative analysis of pharmaceutical TPs in urban WW effluents. Results demonstrated better suitability of passive sampling mainly due to the better quality of MS2 spectrum. The non-target screening revealed the occurrence of 13 TPs in secondary treated effluents (i.e., tramadol N-oxide, N,O-didesmethyltramadol, amisulpride N-oxide, O-desmethylvenlafaxine N-oxide, O-desmethylvenlafaxine, N-desmethylvenlafaxine, venlafaxine N-oxide, gabapentin lactam, O-desmethyltramadol, clarithromycin N-oxide, hydroxycarithromycin, hydroxycarbazepine, and carbamazepine-10,11-epoxide). Retrospective analysis of the previously acquired data by high-resolution mass spectrometry was used to reveal the fate of these compounds during the treatment in constructed wetlands. Among identified compounds, N-oxides and carbamazepine-10,11-epoxide appeared to be the most recalcitrant, alongside amisulpride, gabapentin, and carbamazepine. The results obtained offer insight into the fate of pharmaceutical TPs and may serve as a guideline for the prioritization of contaminants TPs based on their persistence during the treatment and assist in the design of treatment systems.

Keywords: Non-target screening, transformation products, wastewater treatment,