Extended suspect screening to identify organic micropollutants and their transformation products as potential markers of wastewater contamination in riverine and coastal ecosystems

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Abstract
In this study, a comprehensive suspect screening of organic micropollutants (MPs), and some of their transformation products (TPs) and metabolites, was performed in waste, river and coastal waters from the Ebro Delta region (Catalonia, Spain). For this purpose, an automated suspect screening workflow was developed using two analytical steps: (i) identification of suspected compounds using on-line databases; and (ii) semi-quantification of identified compounds by using isotopic labelled standards. Using this strategy, several pollutants were identified comprising pharmaceuticals, pesticides, abused substances, personal care products, industrial chemicals and surfactants of major relevance in Catalonia. Additionally, their occurrence was evaluated along the wastewater-recipient water chain until they reach estuaries and the Mediterranean Sea. The most ecologically relevant compounds detected, that could be considered as suitable markers of wastewater contamination in freshwater and coastal ecosystems, were highlighted. Results reveal that some suspected TPs and metabolites were more ubiquitous than their parent compound and found at similar concentration levels. These results evidence that suspect screening methodologies can be a useful tool for the identification of relevant markers of wastewater contamination.
Keywords: Suspect screening; Micropollutants; High resolution mass spectrometry;

1. Introduction

It is already known that conventional wastewater treatment plants (WWTPs) are not particularly designed for the removal of MPs, and therefore, these compounds are released into the receiving aquatic environment. In this sense, an increasing number of emerging and unregulated MPs as well as their TPs and metabolites have been detected in the different aquatic compartments (Agüera, Martínez Bueno and Fernández-Alba, 2013). To evaluate their occurrence and distribution, the existing target analysis methodologies can only provide an overview of limited number of compounds, for which analytical standards are commercially available. However, the implementation of advanced liquid chromatography systems coupled to high-resolution mass spectrometers (LC-HRMS) allows the detection of a large number of unknown and suspected pollutants without requiring reference standards (a priori) and comprising reliable confidence on their identification. The applications using suspect screening methodologies in environmental analysis is currently increasing, due to the large amount of information that they can provide. Nevertheless, there is still the need to develop systematic and more automatized strategies for the rapid identification of relevant suspected compounds. Therefore, systematic and more automatized strategies are required for the rapid identification of relevant suspected compounds in environmental samples.
The aim of the present study was to develop a suspect screening approach for the rapid identification of MPs, TPs and metabolites along the Ebro Delta River. The suggested workflow was performed by using Compound Discoverer 2.1 software and on-line databases for compounds identification. Semi-quantification of the identified compounds was carried out using isotope-labeled internal standards.

2. Materials and Methods

2.1. Sampling and sample preparation

Samples were collected in 27 sampling points in reaches of the Ebro River Delta located upstream and downstream from two WWTPs located in the most populated areas, irrigation and drainage channels, the associated receiving estuaries and the Mediterranean Sea. Samples were treated following the methodology described elsewhere (Gago-Ferrero *et al.*, 2015). Briefly, samples were filtered through 1 µm glass fiber filters followed by 0.45 µm PVDF membrane filters. pH was adjusted to 6.5 with formic acid or ammonia and loaded into manually packed mixed-mode cartridges comprising 200 mg of Strata-X and 350 mg of mixture

Strata-X-AW: Strata-X-CW: Isolute ENV+ (1:1:1.5). After enrichment and drying of the cartridges, samples were eluted with a 4 mL basic (2% ammonia) followed by a 2 mL acid mixture (1.7% formic acid) of ethyl acetate/methanol (50:50, v/v). The extracts were combined and concentrated to dryness under a gentle nitrogen stream and diluted to 0.5 mL with methanol/HPLC-grade water (50:50, v/v).

2.2. LC-HRMS analysis and compound identification

The extracts were analyzed using a liquid chromatography system coupled to a LTQ-OrbitrapVelos as reported previously (Jaén-Gil *et al.*, 2019). The chromatographic separation was performed in a ZORBAX Eclipse XDB-C18 (150 mm × 4.6 mm, 5 µm) using a sample injection volume of 10 µL and a flow rate of 0.5 mL/min. The mobile phases selected were: ultra-pure water containing 10 mM ammonium formate (A) and acetonitrile (B) in positive electrospray ionization mode; and ultra-pure water containing 5mM ammonium acetate (A) and acetonitrile (B) in negative mode. All samples were acquired in data dependent acquisition (DDA). An in-house exact mass database from the Catalan Water Agency (ACA) was used for identification of MPs and TPs comprising more than 400 prioritized compounds and many relevant TPs and metabolites (~100). Data processing was performed using the Compound Discoverer 2.1 Software (Thermo Scientific).

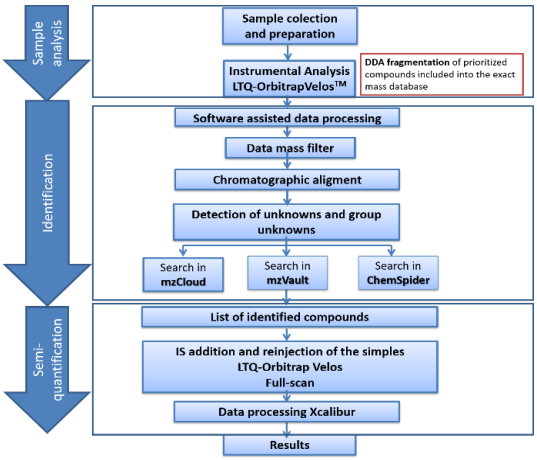

Figure 1. *Suspect screening workflow*

3. Results and Discussion

The suggested suspect screening workflow for the tentative identification of environmental micropollutants in the Ebro Delta is presented in Fig.1.

3.1. Compound identification

Firstly, the selected database containing ionized exact masses was loaded into the LTQ-Orbitrap system for DDA analysis. Then, fragmentation of suspected compounds was performed for the three most intense ions detected in the MS full scan and included in the selected database. Raw data files were loaded into Compound Discoverer 2.1 Software for further automatic data processing. Mass filtering and chromatographic alignment was carried out from m/z 100 to 1000 range and a retention time shift of ± 0.3 min. Mass filtering and chromatographic alignment was performed for all masses and peaks detected in MS spectra. Then, all the features containing MS/MS fragmentation spectra were matched with on-line databases for compound identification. To verify the occurrence of the identified MPs and TPs, mzCloud, mzVault and ChemSpider databases were used. All exact masses and MS/MS fragmentation spectra were identified using a mass tolerance error of ± 5 ppm. Finally, a ready-made list containing 54 suspected features was obtained, including pharmaceuticals, pesticides, abused substances, personal care products, industrial chemicals, surfactants and TPs.

3.2. Semi-quantification of suspected compounds

After data reduction and compound identification, samples were re-injected with addition of selected isotopically labeled standards for semi-quantification. Additionally, recovery evaluation of spiked isotope-labeled IS was performed for each aqueous sample type. The occurrence of the suspected compounds was tentatively identified along the Ebro Delta River.

4. Conclusions

This study demonstrates that suspect screening methodologies based on HRMS are required to identify all those compounds non-detected using conventional target screening methodologies, which can be considered as relevant markers of wastewater contamination. The suspect screening approach allowed the rapid and automatized detection of 54 suspected compounds, which are not usually included in target methods by using on-line databases and attaining reliable confidence on their identification. Additionally, the semi-quantification using isotopic reference standards allowed to tentatively evaluate their occurrence along the Ebro Delta River.

References

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