Development of a non-target screening workflow for the evaluation of micropollutants fate during oxidation processes

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Abstract Non-target screening (NTS) is of great interest for environmental monitoring because it enables the screening of numerous pollutants, including unknown products. However, methods to identify unknowns are still time-consuming and only deal with a limited portion of the signal. The objective of this work was to develop a NTS workflow that enables an automated and comprehensive characterization of the evolution of a sample during water treatment, using high-resolution mass spectrometry (HRMS). Considering different conditions (matrix, micropollutant concentration), methods based on the obtention of raw formulas of detected compounds were evaluated. Oxidation processes (chlorination, UV/H_2O_2 or ozonation) were applied to wastewater samples to obtain contrasted HRMS fingerprints and to assess the relevance of the workflow to characterize transformation products (TPs). Batch experiments were conducted to confirm the ability of such a workflow to properly characterize the modification of the sample. Different extraction methods were also applied to enlarge the range of detected TPs. Preliminary results show that the workflow is able to rapidly compare the fingerprints of the samples (e.g., highlighting the presence of oxidation products characterized by the incorporation of oxygen atoms into compounds). The applied workflow also enables the tracking and identification of specific TPs formed by oxidation.

Keywords: Micropollutants, non-target screening, oxidation, transformation products, wastewater

1. Introduction

Non-target screening (NTS) has gained a lot of interest in the last decades for its ability to identify unknown contaminants, thanks to high-resolution mass spectrometry (HRMS) (Krauss et al., 2010; Schymanski et al., 2014). However, data analysis is time consuming, especially when dealing with contaminants or transformation products (TPs) that are scarcely documented or completely unknown. Therefore, the focus is often made on a limited list of unknown compounds prioritized based on their high signals or their availability in MS databases, while many other peaks remain unidentified.

The objective of this work was to evaluate a NTS workflow that does not rely on manual identifications of selected compounds but rather on automated steps to characterize the evolution of a sample during wastewater treatment using HRMS. Global methods, based on markers detected by HRMS, their raw formulas and the full fingerprint of every sample, were used to assess the evolution of the pool of organic contaminants present in wastewater effluents, as well as the formation of TPs. Oxidation processes (chlorination, advanced oxidation by UH/H_2O_2 or ozonation) were applied to wastewater samples to obtain contrasted HRMS fingerprints and to assess the relevance of the workflow. Batch experiments on model compounds in Milli-Q water, on wastewater effluent spiked with some micropollutants of interest and on non-spiked wastewater effluent were carried out to confirm the ability of the applied workflow to properly characterize the modification of the sample in various conditions and with different concentrations of pollutants.

2. Material and methods

2.1. Wastewater collection and sample treatment

Batch experiments kinetics were carried out with single compounds (atenolol, diclofenac, irbesartan and furosemide) at 1 mg/L in 10 mM phosphate buffered Milli-Q water solutions. Secondary wastewater effluent was collected at the *Seine Amont* wastewater treatment plant (Valenton, France). Spiked experiments were performed by adding 1 mg/L of a mixture of the compounds described above to a sample of secondary effluent.

Chlorination was performed using a commercial chlorine solution, spiked into wastewater effluent at 25 mgCl_2/L (i.e., 3 x dissolved organic carbon + 10 mg/L) to maximize the formation of TPs. UV/H_2O_2 oxidation was carried out at 3 mgH_2O_2/L, using a monochromatic UV lamp emitting at 254 nm with a power output of 0.34 mW.cm^{-2}, measured by actinometry on atrazine (Canonica et al., 2008). An ozone stock solution was produced by a corona discharge ozone generator, fed by oxygen (>98%), and bubbled into cold Milli-Q water through a porous glass disk. Ozone was spiked into wastewater effluent at 3.5 mg O_3/L, measured by indigo standard method (Bader and Hoigné, 1981).

2.2. *Non-target screening*

Single compound samples and spiked wastewater samples were filtered on 0.2 µm pore size filters, and directly injected on the analytical system, whereas non-spiked wastewater samples were first extracted on HLB cartridges and concentrated 500 times before injection. Non-target analyses were carried out with an UPLC-IMS-QTOF (Vion, Waters). Separation was performed by an UPLC-BEH-C18 chromatographic column (1.7 µm, 100 × 2.1 mm, Waters) and mass spectrometry was operated in both positive and negative ionization mode using an electrospray ion source. A suspect screening workflow was applied using UNIFI software (Waters). For the NTS workflow, raw data were first converted (to the open format *.mzML) for further analysis using RStudio with the patRoon package (Helmus et al., 2021) for data pre-treatment. Generation of raw formulas was carried out with Genform (Meringer et al., 2011) with C, H, N,O and Cl selected as possible atoms. Formulas were retained depending on the score calculated from isotopic fit and fragment correspondence. Global characterization was performed using in-house R scripts.

3. Results

3.1. *Global comparison of oxidation processes*

Oxidation processes were carried out with spiked and non-spiked wastewater effluent. Properties of detected markers, before and after oxidation, were first investigated through the number of markers detected, their average *m/z*, retention time and drift time (Table 1) to assess the influence of the process on the sample.

Table 1: Global characterization of the samples from positive ESI analysis

	Chlorination		**UV / H_2O_2**		**Ozonation**	
	Before	After	Before	After	Before	After
			Non-spiked wastewater effluent			
Number of markers	10 666	11 553	12 734	10 863	9 326	8 383
Total signal area^a	132×10^6	215×10^6	780×10^6	759×10^6	75×10^6	57×10^6
Average *m/z*^{b}	429.0933	494.6125	606.7418	598.5006	411.5678	396.5204
Average retention time (min)	9.64	11.27	16.39	16.71	9.80	9.69
Average drift time (ms)	6.02	6.57	7.16	7.07	5.03	4.96
			Spiked wastewater effluent			
Number of markers	2 451	2 731	2 446	2 464	1 852	1 610
Total signal area^a	5.7×10^6	4.5×10^6	5.7×10^6	5.2×10^6	6.0×10^6	4.9×10^6
Average *m/z*^{b}	431.9392	433.8393	433.3253	428.1877	439.1320	426.5768
Average retention time (min)	8.11	8.39	8.01	7.62	7.29	7.99
Average drift time (ms)	5.85	5.89	5.84	5.79	6.36	6.46

[a]: total signal area was calculated by summing the areas of all the detected markers
[b]: analysis were performed with a screening between 100 and 1000 *m/z*

Chlorination produced significantly more by-products with high area, which also exhibited higher molecular weight (MW, i.e., average *m/z*) than the initial compounds, which can be explained by the addition of chlorine atoms on molecules. They had higher retention times as well, indicating a higher hydrophobic property. On the contrary, UV/H_2O_2 and ozonation eliminated more compounds than it produced by-products, and the detected markers were generally more polar after oxidation and exhibited a lower MW. This is in accordance with a strong oxidation power of hydroxyl radicals produced by these processes, leading to the elimination of organic compounds through aromatic rings opening and breakage of molecular bonds to form smaller organic structures. Similar results were obtained for both non-spiked and spiked wastewater experiments.

To further investigate this behavior of organic compounds during the oxidation processes, markers were classified by their fold change value (Figure 1). Fold change value of each marker was calculated by dividing their area in the sample after oxidation by their area before oxidation. All markers were subsequently classified in the following 5 categories depending on their fold change value: 1) eliminated or 2) formed during oxidation, 3) exhibiting a significant (>50%) decrease or 4) increase in signal area, or 5) consistent during the process (Bader et al., 2016).

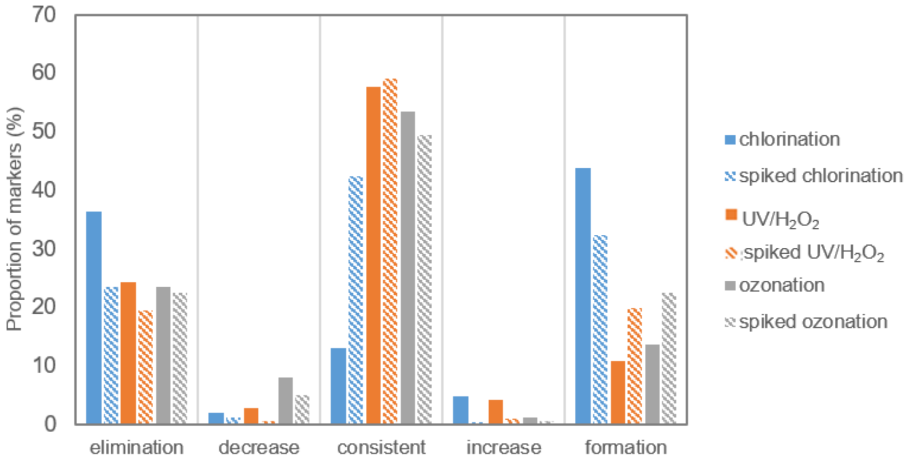

Figure 1: Classification of the detected markers as defined by their fold change values

This procedure confirmed that chlorination formed more markers than UV/H_2O_2 and ozonation. The proportion of eliminated markers was similar between UV/H_2O_2 oxidation and ozonation, but ozonation exerted a higher impact on the areas of markers that were still detected after oxidation. It should be however noted that chlorination only formed more markers that were amenable to detection in the analytical conditions employed, while the other oxidation processes could either form less oxidation byproducts or form byproducts that were not detected by UPLC-IMS-QTOF (e.g., volatile, very polar or very hydrophobic compounds).

3.2. Ability of the workflow to identify transformation products

Batch oxidation experiments were carried out with single molecules in order to identify specific TPs for each oxidation process. Tentative identifications were performed by searching for markers that were not present at the beginning of reaction, but appeared at the following reaction time, and then showed an increasing or constant area at the following reaction times. Among those markers, the most intense ones were selected for further investigation (Table 2).

Table 2: Examples of markers identified as TPs from chlorination (Cl), UV/H_2O_2 (UV) or ozonation (O_3) experiments

Model compound	TPs' m/z (processes)	Identification level (Schymanski et al., 2014)	Previously reported?
Atenolol	237.1365 (Cl)	3	(Quintana et al., 2012)
	267.1471 (Cl)	3	
	224.1161 (Cl)	3	
Diclofenac	214.0427 (UV)	4	-
	283.9790 (Cl)	3	(Soufan et al., 2012)
	329.9855 (Cl)	3	
Furosemide	329.3312 (UV, O_3)	1	-
	311.0346 (UV)	4	-
Irbesartan	447.2509 (O_3)	4	-

Experiments on spiked and non-treated wastewater effluent were investigated to see if the data treatment methods were able to track the TP previously identified. Markers classified as "formed" by their fold change value were first selected and a suspect screening (i.e. searching the corresponding *m/z*, retention time, ...) of the previously identified marker was performed. 83% and 66% of TPs were successfully found among the formed markers in spiked and non-spiked wastewater, respectively. For further characterization of the evolution of the whole pool of organic compounds in real wastewater, results obtained with raw formulas and the corresponding van Krevelen plots (Figure 2) were derived from the raw formulas generated from each markers.

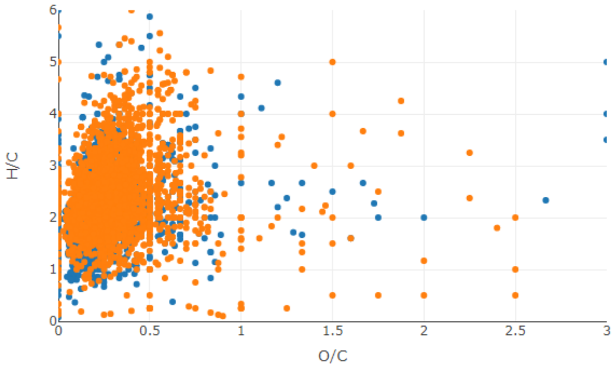

Figure 2: van Krevelen diagram for the comparison of wastewater effluent (blue) and chlorinated wastewater effluent (orange)

This approach provides additional information about the chemical functions and categories of pollutants formed during each treatment. Figure 2 shows taht chlorination seems to increase H/C and O/C which cas observed in a previous work with data obtained by FT-ICR-MS (Fourier-Transform Mass Spectrometry, providing very high-resolution results) on chlorination of natural organic matter (Lavonen et al., 2013). Such approach has scarcely been applied to UPLC-QTOFso forthcoming work will be dedicated to the comparison of the results described in this study with analyses performed with FT-ICR-MS on the same samples. The relevance of this workflow will also be evaluated in less contrasted situations (e.g. during conventional wastewater treatment processes).

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