



## Wastewater ozonation and related formation of N-oxides

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### Abstract

The discharge of effluents from wastewater treatment plants is one of the main sources of contaminants in surface water. In order to decrease such contamination, many conventional wastewater treatment plants are being upgraded to perform ozonation. While ozone reacts with many chemicals and reduce their concentration, some transformation products are formed. Some studies have shown that compounds containing a tertiary amine react with ozone to form the corresponding N-oxide, which might still be biologically active. Therefore, this study applies high resolution mass spectrometry in order to assess the N-oxide formation potential during wastewater ozonation.

### 1. Introduction

Over the last two decades, the constant progress in analytical chemistry made possible the routine analysis of trace organic contaminants in a wide range of matrices, with detection limits in the ng/L range or even below. As a result, several reconnaissance studies in North America [1,2] and Europe [3] have shown the wide contamination of surface water with a large variety of chemicals and particularly with pharmaceuticals and personal care products (PPCPs). Nowadays, the discharge of treated wastewater effluent appears as the main source for the occurrence of such contaminants in the environment [4]. Therefore, upgrading conventional wastewater treatment plants in order to limit the amount of chemicals released in the environment is largely considered. Among the options available, ozonation of secondary wastewater effluent is of particular interest since the process is already widely applied for drinking water production and proved very effective in transforming trace organic contaminants. However, while ozone reacts with a wide range of wastewater contaminants [5], little is known regarding the transformation products (TPs). The complete mineralization of contaminants will likely not be achieved in a secondary wastewater effluent since the high concentration of natural organic matter would require unrealistic high ozone doses. Under such conditions, the expected degradation of contaminants will remain partial and TPs are formed, which might only differ from the parent compounds in the additional content of one or several atoms of oxygen. For instance, ozone is well-known to react with tertiary amine compounds to form the related N-oxides [6]. Hence tertiary amine PPCPs, including psychoactive drugs and antibiotics, which are extensively reported in wastewater effluents, might only be transformed into their respective N-oxide during ozonation. In fact, while some recent publications reported the occurrence of N-oxides of certain PPCPs [7,8], including venlafaxine and clarithromycin, in ozonated wastewaters, various others

already demonstrated that N-oxides can remain biologically active [9,10]. Therefore, this study aims to provide an overall assessment of potential N-oxide formation during wastewater ozonation. Liquid chromatography with high resolution mass spectrometry and non-target screening methods were used as analytical tools were used for this purpose.

### 2. Materials and methods

#### 2.1. Sample collection

This study was performed at a major wastewater treatment plant located in Germany. The secondary wastewater effluent was diverted to a pilot scale ozone generator. Ozone was applied at 6.3 mg/L, providing a 0.5 O<sub>3</sub>/DOC ratio with a 15 min contact time.

#### 2.2. Sample preparation and analysis

After centrifugation but without preconcentration, these samples were analyzed in triplicate with an Agilent 1260 Infinity liquid chromatography system coupled to an Agilent 6550 Q-TOF mass spectrometer. A sample volume of 100 µL was injected on a C18 column (Agilent Poroshell 120; 2.1 x 100 mm; 2.7 µm particle size), then compounds were eluted with a gradient of water and acetonitrile, both containing 0.1% formic acid. Analytes were subsequently detected through positive electrospray ionization while monitoring the range 50-1000 m/z at acquisition rate of 3 spectra per second.

#### 2.3. Data processing

Data interpretation was performed in several successive steps. First, a deconvolution was performed on each chromatogram with the Agilent algorithm "find compound by molecular feature". Such deconvolution provided a list of compounds occurring in the different samples and defined by three characteristics: accurate mass, retention time and abundance. Then, these compounds were subsequently processed with "Mass Profiler Professional" for statistical analysis. For instance a T-test (p-value < 0.05) was performed in order to characterize compounds with a statistically significant variation of concentration before and after ozonation. Compounds with decreasing concentration after ozonation were considered as precursors while compounds with increasing concentration were considered as TPs. Both types of compounds were then examined and paired according to their Kendrick Mass (KM) and their Kendrick Mass Defect (KMD) with a tolerance of 2 mDa. Indeed, when considering oxygen to define the Kendrick mass, any precursor will have the same KMD as its respective oxide but the KM of the oxide will increase by a multiple of 16 depending on the number of oxygen "added" by ozonation. While this approach does not discriminate different types of

oxygenated species, N-oxides were finally identified and characterized by their relative retention time and targeted MS/MS measurement.

### 3. Results

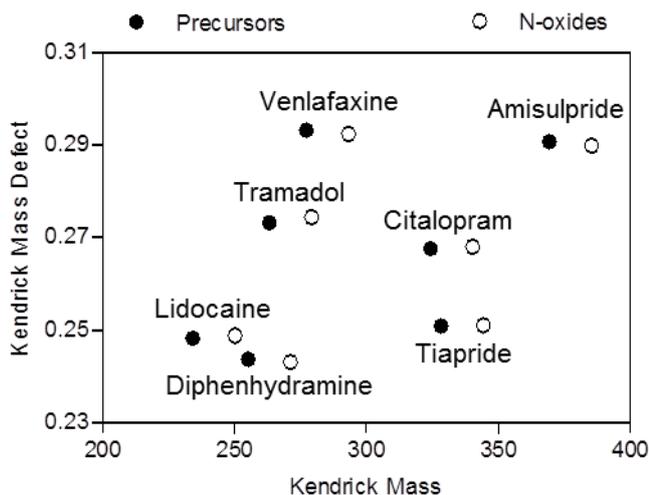
#### 3.1. Formation of oxygenated species

The analysis of secondary wastewater samples collected before and after pilot-scale ozonation revealed the occurrence of 1229 compounds. Among these compounds, the T-test showed that 853 were precursors attenuated by ozone and 165 were TPs resulting from ozonation. Pairing together these precursors and TPs according to their KM and KMD confirmed the partial degradation of numerous contaminants (99 compounds) into their potential oxygenated species.

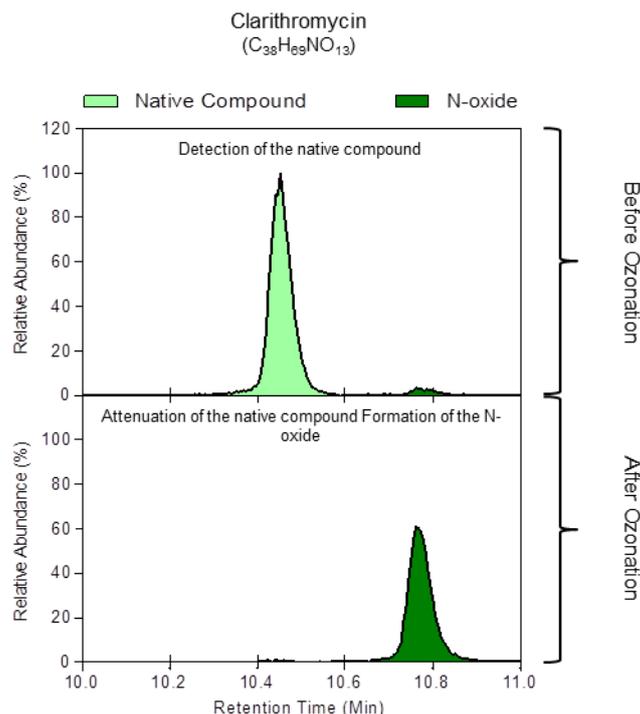
#### 3.2. Assessment of N-oxide formation

The pairs of precursors and oxygenated species previously isolated were further examined according to their relative retention time. Indeed, even though there might be a few exceptions, it is well documented that N-oxides would have a higher retention time than their precursors [11], while the fixation of oxygen on a carbon atom would rather result in a lower retention time. According to this criterion, 27 N-oxides are expected to be formed by addition of a single oxygen atom on the tertiary amine group of water contaminants.

Among these 27 potential N-oxides, 10 could be identified when matching high resolution tandem mass spectrometry measurements with library spectra. Some of them are presented on the KMD plot in Figure 1. Others correspond to sulphiride, diethyltoluamide, clarithromycin, clindamycin and clindamycin sulfoxide. For instance, the case of the antibiotic clarithromycin reacting with ozone to form clarithromycin N-oxide with a higher retention time is shown in Figure 2.



**Figure 1:** Kendrick Mass Defect (KMD) plot showing the precursors and the N-oxides (2 mDa tolerance) for some selected compounds.



**Figure 2:** Chromatograms showing the total transformation of clarithromycin during ozonation to form clarithromycin N-oxide.

The impossibility to assign an identity to the remaining potential N-oxides most likely indicate that half of the N-oxides are formed from unknown compounds, therefore indicating the need for larger but specific libraries for water contaminants.

### 4. Conclusion

This study shows the large potential for N-oxide formation during wastewater ozonation. Therefore, when implementing ozonation for advanced wastewater treatment to reduce the input of micropollutants into receiving water bodies, N-oxides form a class of TPs that should be particularly considered. Given their potential biological activity and their potential reduction to the precursor compound by N-oxide reductase enzymes, N-oxides would require further investigation regarding their toxicological properties and their fate in the environment.

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