Investigation of Suspected and Unknown Micropollutants and Transformation Products from a Waste Water Treatment Plant with Full Scale Ozonation

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Overview

- Investigation of micropollutants and transformation products
- Waste water treatment with ozonation as final step for elimination
- Comparision of influent, effluent after conventional biological cleaning and effluent after full scale ozonation
- Combination of suspected and non target screening with LC-HRMS
- Identification of transformation products using *in silico* transformation and fragmentation

Introduction

Due to the extensive use of industrial chemicals, pesticides, personal care products and pharmaceuticals, many anthropogenic organic micropollutants and their biological metabolites have become ubiquitously detectable in the aquatic environment. The emission of these substances into the aquatic environment occurs through both direct and indirect discharge into the sewage in combination with insufficient biological or chemical degradation efficiency of conventional municipal waste water treatment plants (WWTPs). Against this background, samples from the influent, the effluent after the biological treatment and after the final ozonation from the WWTP in Duisburg-Vierlinden, Germany were picked up and analyzed using liquid chromatography high-resolution mass spectrometry (LC-HRMS). The data investigation was focused on occurrence of suspected target and non target micropollutants, biological metabolites and transformation resulting from the chemical treatment step.

Methods

Sample Preparation

Samples were taken from the WWTP influent, the effluent after the biological treatment and effluent after the ozonation. The samples were filtered and extracted by solid phase extraction (Oasis® HLB, Waters®).

Liquid Chromatography

For chromatographic separation an ultra high pressure system (Thermo Scientific Aria Transcend™) was used. A sample volume of 60 µL was injected onto a 100x2.1 mm analytic column (Thermo Scientific Hypersil Gold™ aQ). A 7 minute solvent gradient (Eluent A: Water + 0.1% formic acid, B: Methanol + 0.1% formic acid) form 5-99% B was applied resulting in a total cycle time of 15 minutes for chromatographic separation at a flow rate of 400µl/min. The column oven were set to 25 °C.

Mass Spectrometry

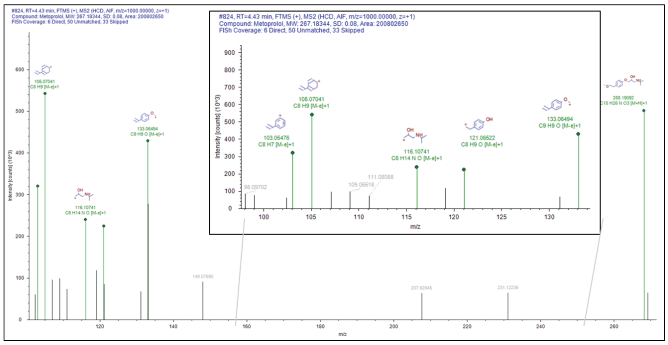
For mass spectrometric detection a high resolution mass spectrometry system (Thermo Scientific Exactive Plus™) with positive and negative electrospray ionization was used and run in permanently alternated mode of full scan and all ion fragmentation (AIF). A resolution setting of 70.000 (FWHM @ *m/z* 200) was used. A mass range of *m/z* 100 to 1500 was applied (resp. *m/z* 50 to 750 and resolution setting 70.000 FWHM for the AIF scans) to be prepared for all possible contaminants. The mass axis of the system was calibrated with the standard calibration mix once prior measurement.

Data Analysis

The data were analyzed in a widely automated workflow using Thermo Scientific TraceFinder™ 3.1, Thermo Scientific SIEVE™ 2.1 and Thermo Scientific Compound Discoverer™ 1.0 software.

Results

FISh scoring for Metoprolol with fragmentation

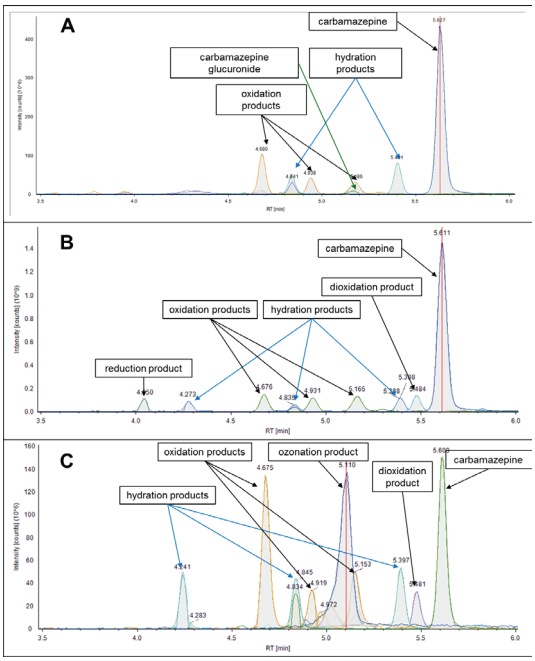


The FISh scoring takes into account all fragment information available in the raw data and combines it with *in silico* fragmentation data of the compounds used for screening. The figure shows such an annotated product ion spectrum of Metoprolol. Besides isotopic pattern matching for all found hits the FISh scoring serves for an additional confirmation of the obtained results.

Non target screening

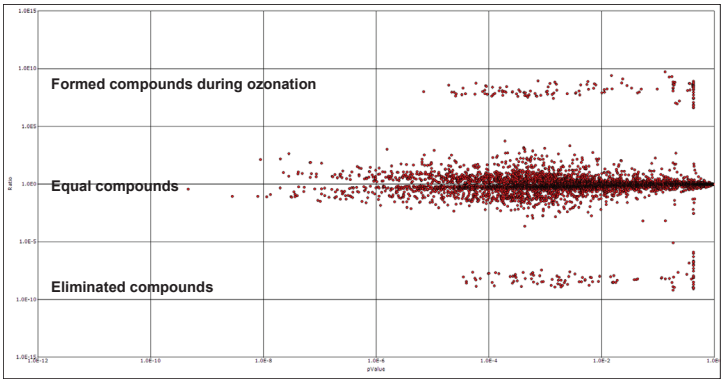
For unknown screening, elimination of background signals and unsignificant peaks is a major issue. To achieve this, a multiple mass defect filtering step runs before the componen extraction. The multiple mass defect filters are connected to the components used for suspect screening, so a large quantity of signals is eliminated which is not likely be related to the parent components of the study. The resulting masses can be used for further identification using online databases like ChemSpider™ or mzCloud™.

Comparison of transformation products of carbamazepine



The transformation products of carbamazepine in the influent (A), the effluent (B) and after the ozonantion indicates the complex reaction pathways during the WWTP process.

Identification of transformation products during ozonation



Volcano plot of the samples effluent after the biological treatment vs. after the ozonantion. The figure shows the compounds formed during the ozonation and the eliminated ones. Many peaks were found in both samples.

Database file name	Formula	*m/z (Measured)*	Mass error (ppm)	Fit isotopic pattern	Fit RT	Identified *in silico*
TP_379	C15H10N2O2	251.0814	-0.3328	Yes	Yes	Yes
TP_377	C15H12N2O3	269.0922	0.1911	Yes	n.a.	Yes
TP_386	C15H14N2O2	255.1127	-0.2695	Yes	Yes	Yes

Identified transformation products of carbamazepine in the effluent of the full scale ozonation for the suspected target screening using a custom made database with 354 transformation products of 32 common micropollutants based on a literature research and own experiments. All compounds could be verified be the *in silico* screening approach.

Conclusions

- **Successful elimination of micropollutants with ozonation as final waste water treatment step**
- **Identification of transformation products using a combination of suspected target and non target screening**
- **New screening approach for the investigation of transformation pathways during the biological waste water treatment process and ozonantion by *in silico* transformation and fragmentation**

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