Automated detection of suspected and non-targeted metabolites in sewage water after biological and chemical treatment

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Overview

Purpose

Run a General Unknown Screening approach in an automated fashion.

Methods

Waste water samples form the city of Duisburg, Germany were analyzed in full scan / AIF mode with a Thermo Scientific™ Exactive Plus™ mass spectrometer and analyzed in a widely automated workflow using Thermo Scientific TraceFinder™, Thermo Scientific SIEVE™ and Thermo Scientific Compound Discoverer™ software.

Results

Differences in the load of contaminants could be easily determined in the different samples; Easy detection and identification of a significant number of contaminants could be achieved.

Introduction

The emission of a broad variety of anthropogenic pollutants as industry chemicals, pesticides, pharmaceuticals and personal care products as well as their degradation products into the aquatic environment is a constantly growing issue. Besides the issue if direct discharge of these substances, the efficiency of biological and chemical degradation of these substances in municipal waste water treatment plants (WWTPs) is coming into the focus of research. Modern high resolution accurate mass (HRAM) mass spectrometry instrumentation with its highly sensitive full scan detection methodology opens the doors for generic broadband screening approaches for suspected and unknown components and their degradation products. Here we show how an intelligent software approach can lead to the quick and easy detection of known and suspected transformation products together with putative unknown compounds in different samples from a municipal WWTP in western Germany.

Methods

Three water samples from the municipal WWTP of Duisburg-Vierlinden, Germany, were taken and analyzed with a generic method on a Thermo Fisher Exactive Plus™ bench top Orbitrap™ mass spectrometry system (Thermo Fisher Scientific, Bremen, Germany) coupled to an ultra high pressure LC system (Aria Transcend™, Thermo Fisher Scientific, Franklin, MA, USA). Data processing was carried out using Compound Discoverer™ 1.0 Preview software in combination with TraceFinder™ 3.1 and Sieve™ 2.1 software (all Thermo Fisher Scientific)

Sample Preparation

Samples were taken from the WWTP influx (before mechanical and biological cleanup), before ozonation and after ozonation. The samples were filtered and extracted by solid phase extraction

Liquid Chromatography

For chromatographic separation a Thermo Scientific Aria Transcend™ system was used. A sample volume of 60 µL was injected onto a Thermo Scientific Hypersil Gold™ aQ 100x2.1 mm analytic column. A 7 minute solvent gradient was applied resulting in a total cycle time of 15 minutes for chromatographic separation.

Mass Spectrometry

For mass spectrometric detection a Thermo Scientific Exactive Plus mass spectrometer was used and run in full scan / all ion fragmentation (AIF) mode. In this mode full scans are permanently alternated with AIF fragmentation scans. A resolution setting of 70,000 (FWHM @ *m/z* 200) was used (see Fig. 2). A mass range of *m/z* 100 to 1500 was applied (resp. *m/z* 7050 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. Further optimization of the instrument (compound tuning) was not required.

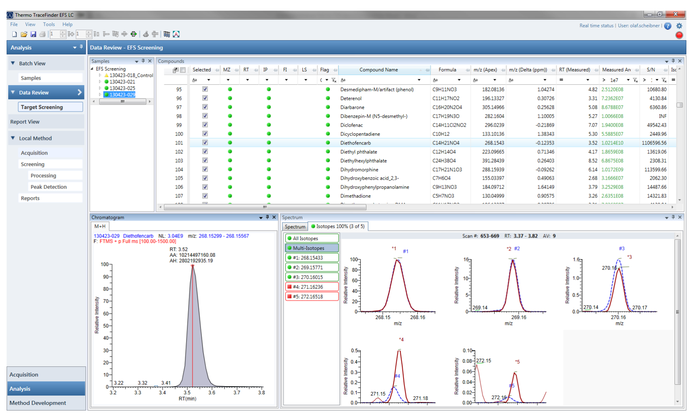
FIGURE 1. Thermo Scientific™ Exactive Plus™ system with Transcend™ UHPLC system.

Results

Suspect Screening

As a first approach, a suspect target screening was run on the samples to get an overview over the content of contaminants in the different samples and get a semi quantitative overview of the most prominent contaminants. For this the samples were processed with TraceFinder software in a suspect screening, using a built in database of 2900 compounds most commonly found in the environment (Fig. 2). A first confirmation of the results was achieved by automated isotopic pattern matching, using the elemental compositions given ion the data base used for the suspect screening.

FIGURE 2. Suspect target screening result view.

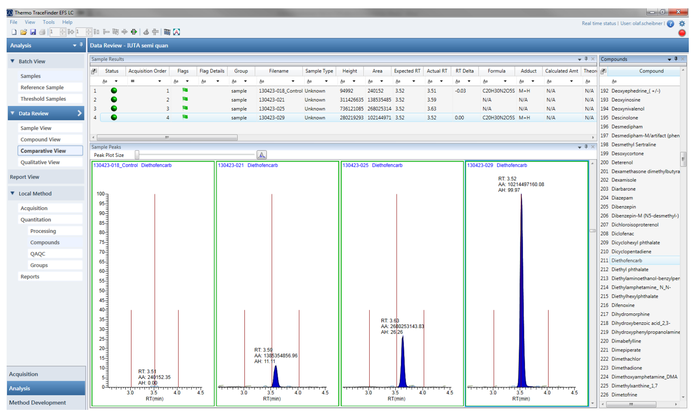


As an additional quality criterion the signal S/N ratio of the chromatographic peaks was used to filter the results for significance.

Semi-quantitative data processing

The suspects confirmed by isotopic pattern match were used for a semi-quantitative comparison. For many components a clear signal intensity trend from WWTP intake to efflux of the ozonation step was visible, giving an impression of the degradation efficiency of the overall treatment as well as for the single steps. Relative signal intensity in relation to the highest concentration was given by the application for easy review (Fig. 3).

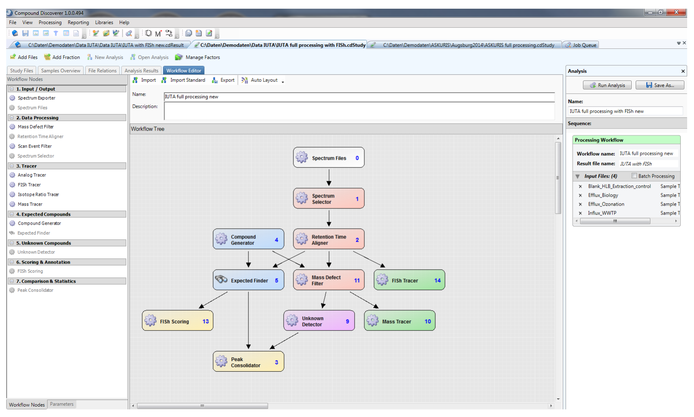
FIGURE 3. Semi-quantitative comparison.



Search for degradation products

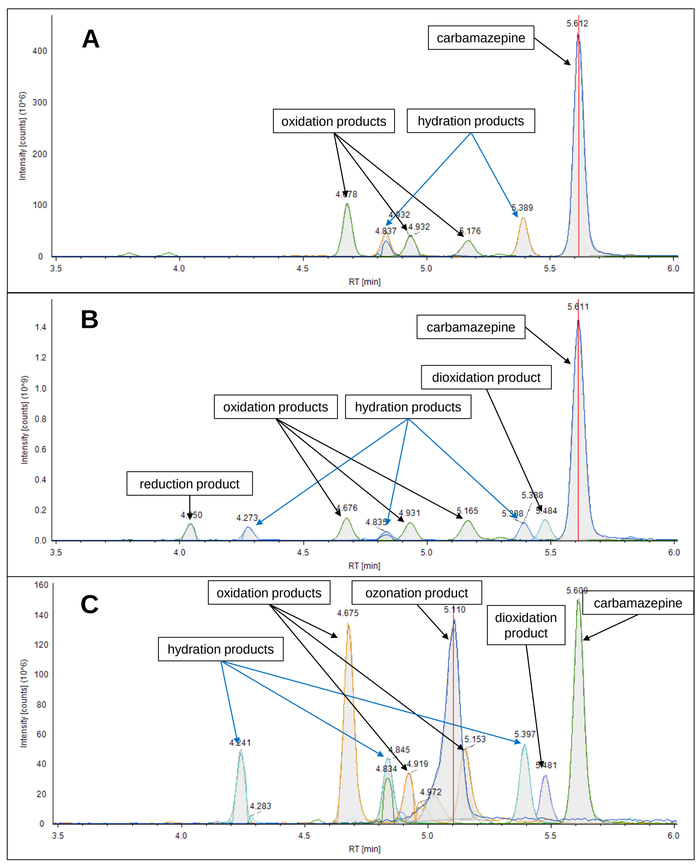
For further analysis, the information on the most prominent contaminants was passed on to Compound Discoverer software for an automated search for degradation products. The software allows for a flexible experiment setup so customized processing workflows can be set up in advance and used to purpose later on accordingly. The workflow complexity can range between a simple screen for known transformation steps up to a suspect and unknown screening including multiple mass defect filtering. Figure 4 shows the workflow used in this case, which includes suspect screening, unknown screening for additional transformation products, filtered by multiple mass defect filtering around the parent molecule and their known transformation products.

FIGURE 4. Workflow and method setup on Compound Discoverer software.



The workflow includes parallel processing of several parent molecules. It contains libraries of known biological transformation reactions (phase I and phase II). In addition, 29 known and suspected ozonation reactions were added to the transformation data base. Figure 5 shows a comparison of the major transformation products of carbamazepine as an example.

FIGURE 5. Comparison of degradation product occurrence in the different samples; A: WWTP intake, B: before ozonation, C: after ozonation

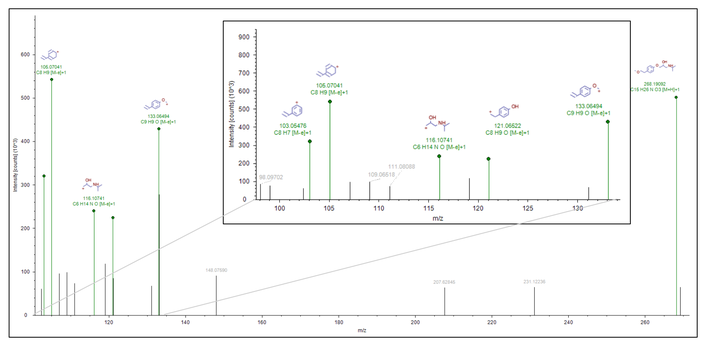


The comparison of the three different samples shows a clear difference in the occurrence of different transformation products. For better visibility the annotation of the graphs doesn‘t show the more detailed information on the transformation products available in the software and the according reports.

Unknown Screening

For unknown screening, elimination of background signals and insignificant peaks is a major issue. To achieve this, in Compound Discoverer a multiple mass defect filtering step is run before the component 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™.

FIGURE 6. FISh scoring for detected compound with fragment annotation.



FISh processing

A dditionally, FISh processing (**F**ragment **I**on **S**earc**h**) can be applied for known and suspected compounds and transformation products. The FISh process 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. Figure 6 shows such an annotated fragment spectrum of metoprolol. Besides isotopic pattern matching for all found hits the FISh processing serves for an additional confirmation of the obtained results.

Conclusion

High resolution accurate mass data from an Orbitrap™ system, acquired with a generic acquisition method combining full scan data with all ion fragmentation data can be used for an in depth investigation of samples from a waste water treatment plant. Starting with a screening for anthropogenic pollutants, subsequent analysis of their degradation efficiency as well as detection of known and unknown degradation products and their identification is easily achievable using TraceFinder and Compound Discoverer software.

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