

A Novel Screening Method for Anthropogenic Sewage Pollutants in Waste Water, Ground Water and Drinking Water Samples by LC-HRAM Analysis

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Overview

For the present study, the Thermo Scientific™ Q Exactive™ mass spectrometer was coupled with an Thermo Scientific Accela™ Open Autosampler to do fully automated high-volume injection and sample enrichment of up to 5 mL sample volumes. Samples from waste water to drinking water were investigated for anthropogenic compounds in the water cycle down to low ppt concentration levels without time consuming solid phase extraction for screening of all kinds of water matrices.

Introduction

In the last decade, there has been growing public concern about potential contamination of water and the environment with anthropogenic compounds and their degradation products and possible negative impacts on nature and public health. As a response, there is an increased interest in more efficient screening techniques for larger numbers of possible pollutants compared to those traditionally carried out by triple quadrupole mass spectrometers. Full scan MS with high-resolution and accurate-mass does not require optimization of compound-specific parameters and has the ability to properly separate matrix interferences from compounds of interest. A combination of software suites covering the workflow make it possible to acquire and process data on the fly. Data mining in several fields of investigation side by side can help get the full picture about contaminants.

Methods

Sample Preparation

Water samples of each location were filtered through 0.25 micrometer PTFE syringe filters prior to analysis.

Liquid Chromatography (or more generically Separations)

Chromatographic analysis was performed using the Thermo Scientific Accela Open U-HPLC system.

Chromatographic conditions were as follows:

Column: Thermo Scientific™ Hypersil™ GOLD aQ C18 column, 100 x 2.1 mm, 3.0 μ m particle size.

Mobile Phases: A (Water), B (Methanol) ; both buffered with 5 mM ammonium formate + 0.05 % formic acid. Sample Injection Volume: 0.25 – 5 mL; Column Temp.: 25 °C

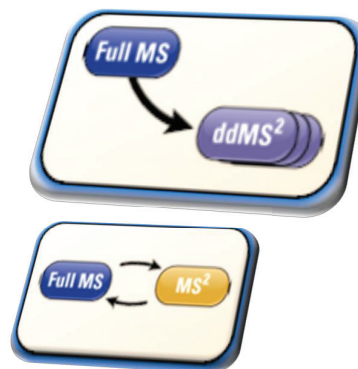
Gradient

Pump General		Gradient Program				
Pump 1						
	Time	A%	B%	C%	D%	μ /min
0	0.00	100.0	0.0	0.0	0.0	500.0
1	10.00	100.0	0.0	0.0	0.0	500.0
2	11.00	80.0	20.0	0.0	0.0	500.0
3	18.00	0.0	100.0	0.0	0.0	500.0
4	25.00	0.0	100.0	0.0	0.0	500.0
5	25.10	100.0	0.0	0.0	0.0	500.0
6	30.00	100.0	0.0	0.0	0.0	500.0
7		100.0	0.0	0.0	0.0	500.0



Mass Spectrometry and Source Conditions

HR/MS Scan Range: 120 to 1200 (m/z)
Polarity switching: off
Resolution: 70k (Full scan), 35k (ddMS2)
HDC Fragmentation: Collision Energy 35 eV
Heated Electrospray Ion Source
Spray Voltage (pos/neg): 4800 V / 3800 V
Capillary Temp 300 °C
Sheath Gas: 60, Aux Gas: 15 (Ion Sweep Gas: 1)
Vaporizer Temperature: 350 °C



Data Processing Tools

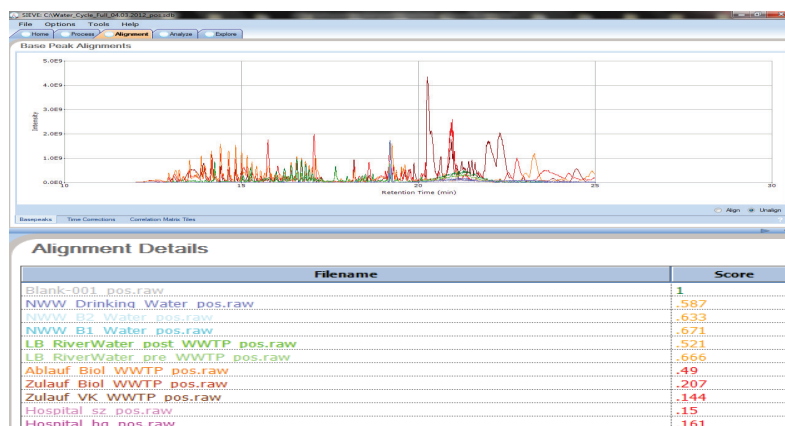
Thermo Scientific™ ExactFinder™ software was used for fully automated data processing of generated Full Scan MS + ddMS2 experiments for targeted screening approaches with a simple four-click procedure. Additional features like Isotopic Pattern Match, customized compound data stores and spectral libraries have been applied to find compounds of interest. High-resolution, accurate-mass data files are open to retrospective data analysis also by other software such as Thermo Scientific™ SIEVE™, Thermo Scientific™ MetWorks™, Thermo Scientific™ Mass Frontier™ softwares as well as a ChemSpider™ (ChemZoo Inc, Wake Forest North Carolina) search based on elemental composition proposals from present accurate-mass signals. With these software tools, it was possible to drive investigation in different directions simultaneously with a single injection of a sample set. Therefore, it is recommended to have a second data set ready. This can be generated by using a combination Full Scan MS + All Ion Fragmentation MS2 experiment to have full information for possible fragments as well.

Results

Mapping the Water Cycle in SIEVE Software

The sampling area comprised several locations within a few square miles around a waste water plant (WWTP): Water works, drinking water drainage area, WWTP with its catchment area, and a receiving water course in an area where hospitals are located. To show the impact of a WWTP to downstream waterways including waterworks, SIEVE software has been used to monitor 70 compounds of interest, which are known pollutants to drinking water sources like Carbamazepine, Phenazone, Simazine, and Tramadol. These are known to be persistent in the environment and could be used as tracer substances.

FIGURE 1. Sample Characteristics Comparison



By applying differential analysis to samples from the different locations (Figure 1) we get first information about the sample's characteristics when we set the blank as a reference. In terms of having similar score levels the locations are well aligned. Trend comparison of the compounds of interest within the different locations has been generated and a visual display of the samples is shown as overlapped chromatograms. SIEVE performs background subtraction and framing before identification of the compounds in the different samples.

Figure 2 shows the heavy pollution impact of the waste water treatment plant effluent to its receiving water course. Carbamazepine passed through the WWTP into the receiving water course towards the water works. In Figure 3, the performance of SIEVE and its automatic background subtraction is shown in detail. This feature is helpful in terms of showing the impact of the WWTP effluent to its receiving water course by having instrument background noise eliminated as well.

FIGURE 2. Location Survey of Carbamazepine

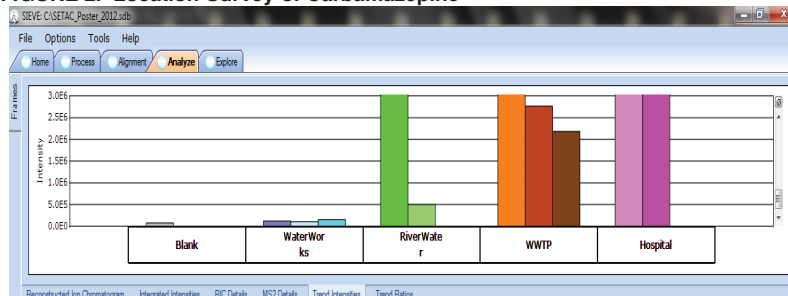
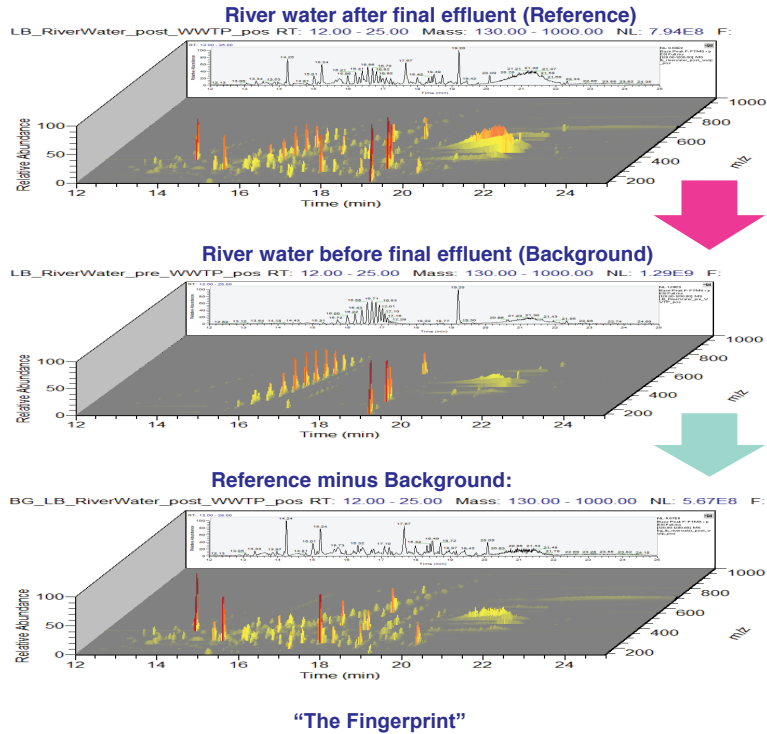


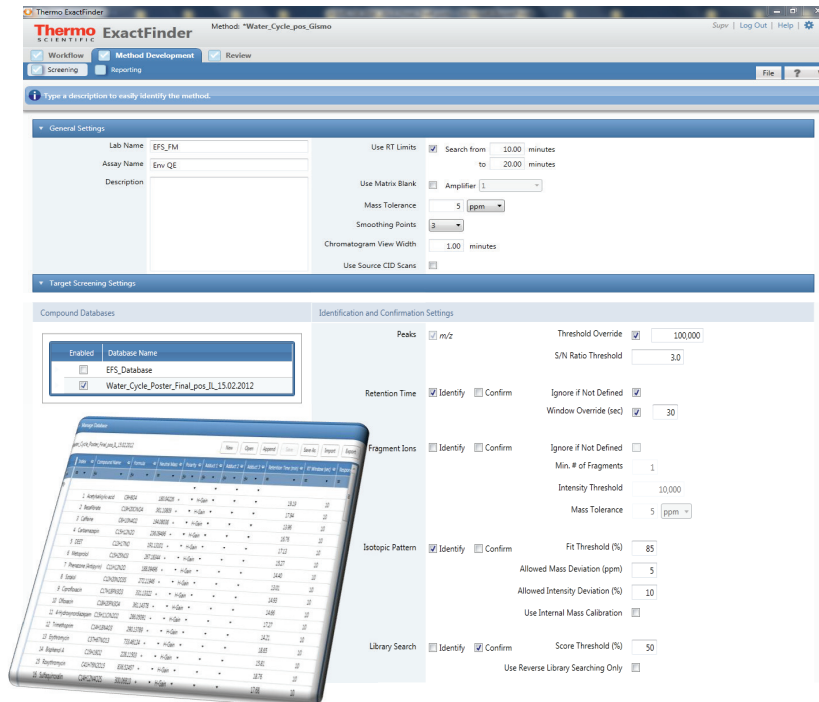
FIGURE 3. Data processing receiving water sample (background subtraction)



Identify, Confirm & Quantitate in ExactFinder Software

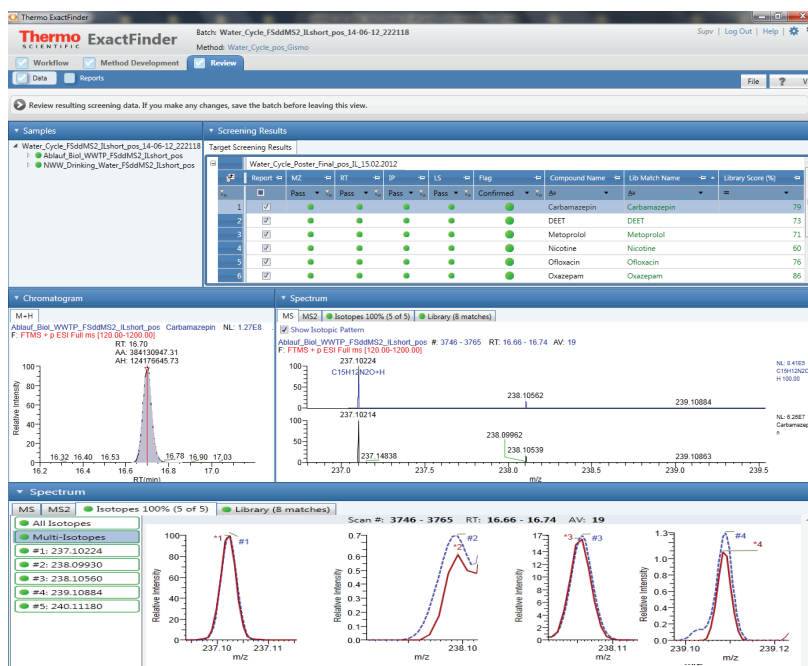
ExactFinder software was used to extract from the raw data information for both confirmation and quantitation in a single processing step. The processing method has set retention time and exact mass and isotopic pattern as identification criteria and spectral library matching using ddMS2 spectra as confirmation criteria. An example of the method setup is shown in Figure 4.

FIGURE 4. ExactFinder Method Setup



In Figure 5, the extracted information for identification of Carbamazepine is shown as an XIC (5 ppm). Isotopic pattern match in the new visualization mode was used as an identification criteria in addition to accurate mass and retention time checked against standard samples.

FIGURE 5. Targeted Screening & Isotopic Pattern Match with ExactFinder



The quantitation table in Figure 6 gives an overview about present concentration levels of Carbamazepine within the investigated water cycle from the hospital, WWTP, downstream water ways and drinking water facilities. In drinking water samples concentrations of 54 and 59 ppt has been calculated based on Full Scan MS experiment data. The calibration curve shows linear regression from 10 to 1000 ppt.

Figure 7 shows the confirmation of Carbamazepine by comparison to the Spectral Library.

FIGURE 6. Quantitation of Carbamazepine from the Full Scan MS experiment

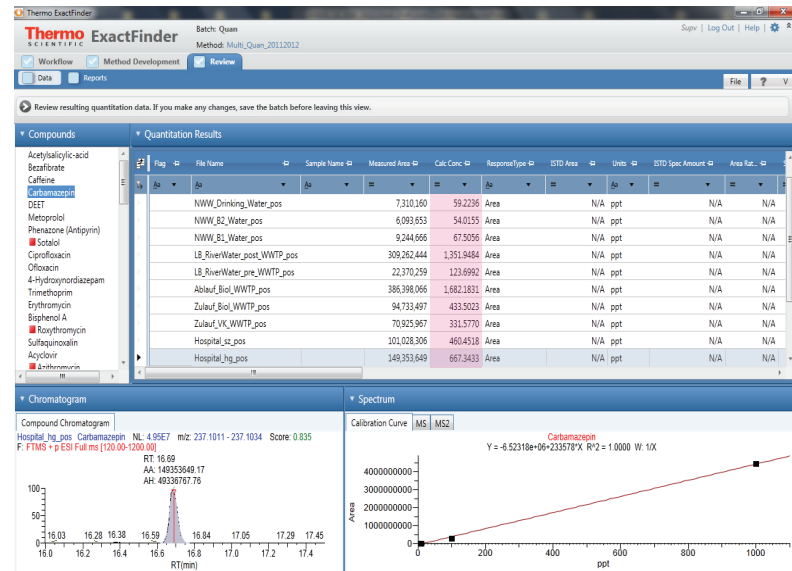
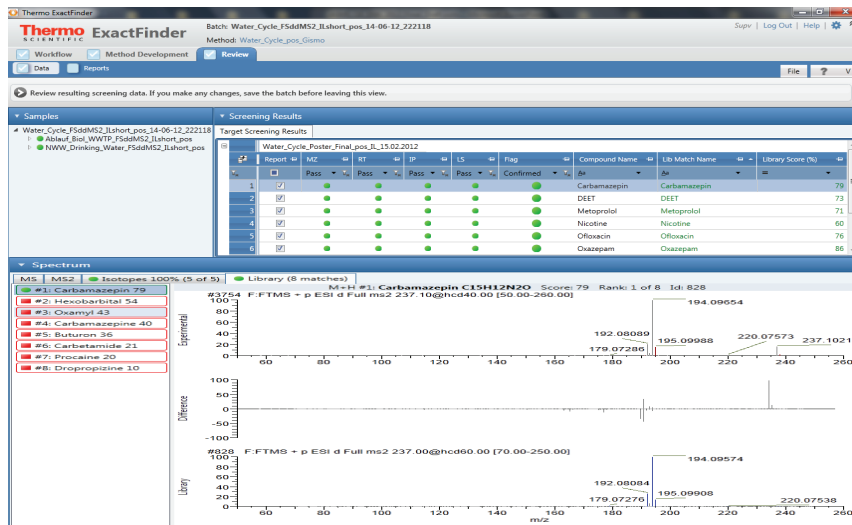
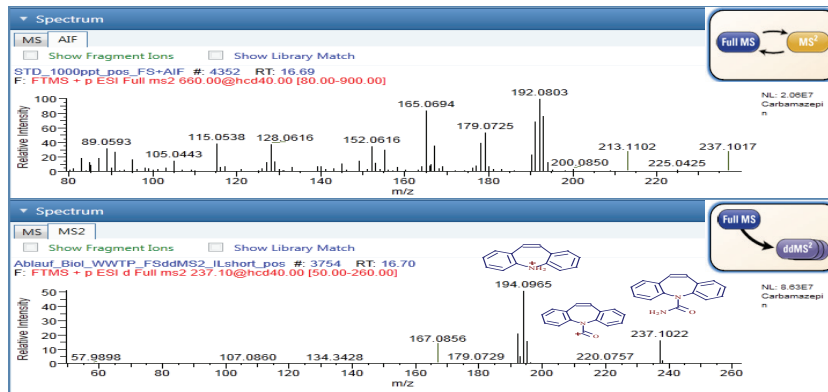


FIGURE 7. Spectral Library Confirmation with ExactFinder



In Figure 8, the ddMS2 spectra is shown compared to an All Ion Fragmentation MS2 spectra. Both scans were generated from the same sample vial. Where the quadrupole filters the precursor ion before fragmentation, it delivers cleaner spectra for specific confirmation against spectral libraries. Suspected or targeted screening tasks can be applied based on this.

FIGURE 8. Cleaner Spectra through ddMS2 experiments on Q Exactive MS



Conclusion

The presented data was based on Q Exactive Full Scan MS in combination with ddMS2 experiments for targeted screening purposes only. For unknown screening tasks it is recommended to generate Full Scan MS + All Ion Fragmentation MS2 data. This gives access to fragment information over the full chromatographic time scale. Because, ddMS2 experiments are strictly based on preset RT windows and precursor masses which have to be investigated and uploaded to inclusion list in advance. Q Exactive inclusion list files can be generated from a compound data base file by a single mouse click.

ExactFinder software coupled with the Q Exactive provided easy access to full quantitative, confirmation and screening data in one package including retrospective data analysis. New features like isotopic pattern multi view and improved spectral library comparison algorithm supports quicker investigation and extraction of information of interest.

By using SIEVE, we could generate location surveys for further monitoring investigation and mass balance calculations in ExactFinder. Exported data is fully compatible.

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