Environmental Profiling of River Water Using Q-TOF LC/MS and Mass Profiler Software

Application Note

Environmental

Authors

Dr. Christian Zwiener
University of Tübingen
Tübingen, Germany

Tiffany Payne
Agilent Technologies, Inc.
Santa Clara, California

Abstract

The demand for the detection and identification of emerging contaminants in the environment requires a new approach to environmental screening that employs accurate mass LC/MS analysis and rigorous data analysis with a statistical software package. This method uses an Agilent 6550 iFunnel Q-TOF LC/MS with Agilent MassHunter Profiler software to investigate river water samples taken upstream and downstream from a water treatment plant. The presence of contaminants in the downstream versus the upstream sample indicates that contamination is taking place. These contaminants were extracted from the downstream water sample data and submitted to an accurate mass database and library for identification. Ultimately, 890 compounds were detected in the downstream sample, and 21 were successfully identified.

Introduction

Emerging contaminants in the environment have been of increasing concern to environmental scientists and the public in many parts of the world. Pesticides, herbicides, pharmaceuticals, corrosion inhibitors, and flame retardants are contaminants that pose a threat to the quality of surface, groundwater, and drinking water. They also adversely affect aquatic ecosystems and public health[1,2]. However, less than 0.1 % of oxidative stress on cells from water samples could be linked to the effects of known contaminants[3]. This clearly demonstrates the demand for further screening approaches.

The detection and quantitation of known contaminants in the environment using mass spectrometry is well established and understood, but these techniques fail to detect unexpected contaminants. As a result, new tools and techniques have been developed for a truly untargeted approach using high resolution mass spectrometry and chemometric (statistical) analysis.

Agilent Technologies

The full approach uses an Agilent 6550 iFunnel Q-TOF LC/MS system, Agilent Mass Profiler software, and Agilent MassHunter Qualitative analysis software to analyze, extract, and identify unanticipated contaminants in river water samples.

Experimental

Water samples were collected from the Ammer River, Germany, in locations upstream and downstream from a wastewater treatment plant. Enrichment was performed using solid phase extraction (HLB and ENBV+) at a preconcentration factor of 500.

Separation was carried out using an Agilent 1260 Infinity HPLC system consisting of an Agilent 1260 Infinity Binary Pump (G4220A), an Agilent 1260 Infinity High Performance Autosampler (G4226A), and an Agilent 1260 Infinity Thermostatted Column compartment (G1316C).

An Agilent 6550 iFunnel Q-TOF was operated with MassHunter Acquisition rev. B.05.01 in 2 GHz extended dynamic range mode with an acquisition rate of 1 scan/sec in MS mode and 3 scans/sec in MS/MS mode with mass-dependent collision energy setting for a targeted MS/MS inclusion list.

Experimental parameters		
UHPLC column	Phenomenex Synergi Polar-RP, 150 × 3 mm, 4 µm at 35 °C	
Mobile phase	A) water + 0.1% formic acid B) methanol + 0.1% formic acid	
Gradient program	Min	% B
	0	5
	1	5
	10	95
	17	95
Stop time	17 min	
Flow rate	0.3 mL/min	

Results and Discussion

The data were analyzed in MassHunter Qualitative Analysis rev. B.06.00 using the Molecular Feature Extractor (MFE) algorithm. MFE links MS data derived from a particular molecule of interest, and compiles the result into a chromatogram. Once the molecules (features) in a particular sample are extracted, the data are submitted to the Mass Profiler (MP) for statistical analysis.

In the water sample taken downstream from the water treatment plant and extracted in triplicate, 1,564 features were detected. Most of these were unique to the downstream sample (1,043 features), revealing the considerable impact of wastewater treatment plant effluents on surface water quality. Data reduction included alignment of retention time and mass data, blank subtraction, and the occurrence in all three replicates. The remaining 1,043 features attributed to wastewater effluents were further examined for potential identification. The accurate mass for the features of interest were used by Molecular Formula Generator (MFG) software to calculate and propose molecular formulae and were assessed for accuracy using a score derived from the isotopic pattern, isotopic spacing, and the difference between the theoretical exact mass of the assigned formula and the acquired accurate mass for the feature. For example, *m/z* 264.1967 was given a score of 97.1 for $C_{16}H_{25}NO_2$ based on combined scores of 94.5 for the mass difference, 99.7 for the isotopic pattern, and 99.4 for the isotopic spacing. A total of 894 sum formulae could be assigned to the measured accurate masses using the elements C, H, N, O, S, P, and Cl at a mass accuracy of 5 ppm.

The preliminary identification of the significant features in the downstream water samples by searching the sum formulae in databases yielded 18 hits with a homemade database of more than 450 contaminants relevant for the aquatic environment, 144 hits with an Agilent Forensic Toxicology Personal Compound Database, and 26 with an Agilent Pesticide Personal Compound Database. Relevant compounds were selected for

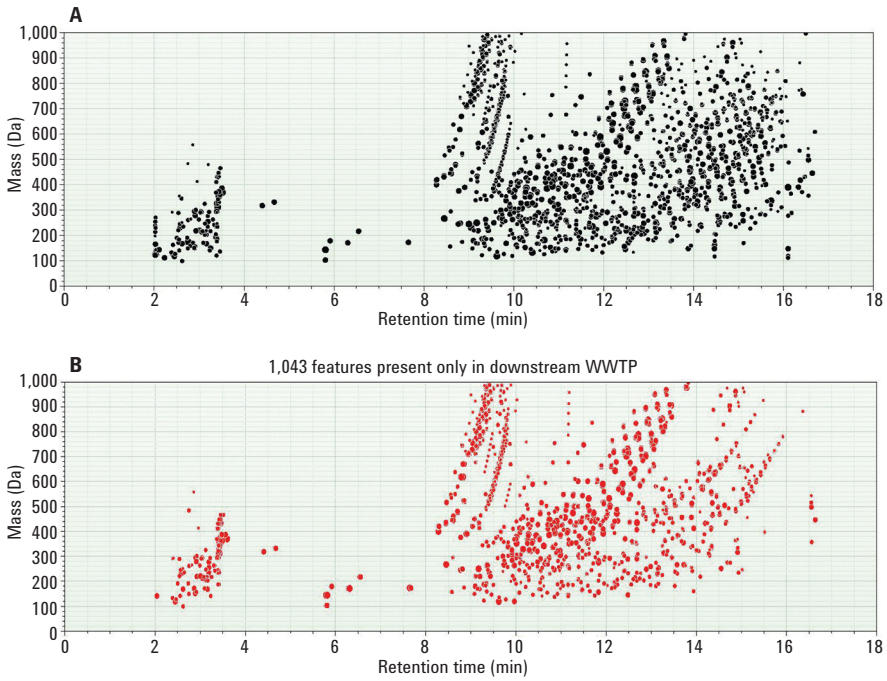

Figure 1. Features found by MFE in river water samples downstream of a WWTP. A) all features, B) features unique to the input of a WWTP.

further investigation and identification. These compounds were compiled into a compound list, where precursor ions were automatically transferred to a list for MS/MS analysis (MS/MS inclusion list) in a further Q-TOF LC/MS measurement.

The resulting accurate MS/MS data were then submitted to an Agilent Forensic Toxicology Personal Compound Database and Library for identification. Figure 2 shows an example of an identification of carbamazepine, an anticonvulsive and mood-stabilizing drug, by perfect matching of the acquired and library mass spectra. This result reveals the importance of the availability of comprehensive mass spectral libraries with accurate mass fragmentation information[4].

For compounds not found in the accurate mass databases or libraries, a search of the sum formulae in general chemical databases such as ChemSpider and PubChem was performed. These searches resulted in several hundreds of hits for possible structures. To narrow down and rank the positive hits for one sum formula, *in silico* fragmentation was performed using Agilent Molecular Structure Correlator (MSC) software. The input data are the molecular formula and the measured accurate mass MS/MS data for the unknown compound. The MSC software then submitted the sum formula to a ChemSpider search and ranked the positive hits based on matching calculated with measured mass fragments. The most probable chemical structures were selected from the high ranked hits of MSC, as the example of iopamidol reveals (Figure 3). The ultimate unequivocal identification should be validated by an authentic standard.

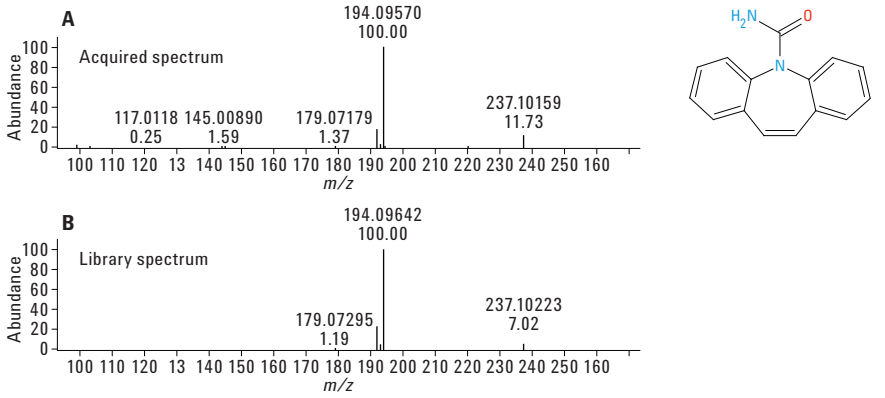

Figure 2. Structure, acquired spectrum (A) and library spectrum (B) for carbamazepine, a drug detected in the downstream water sample.

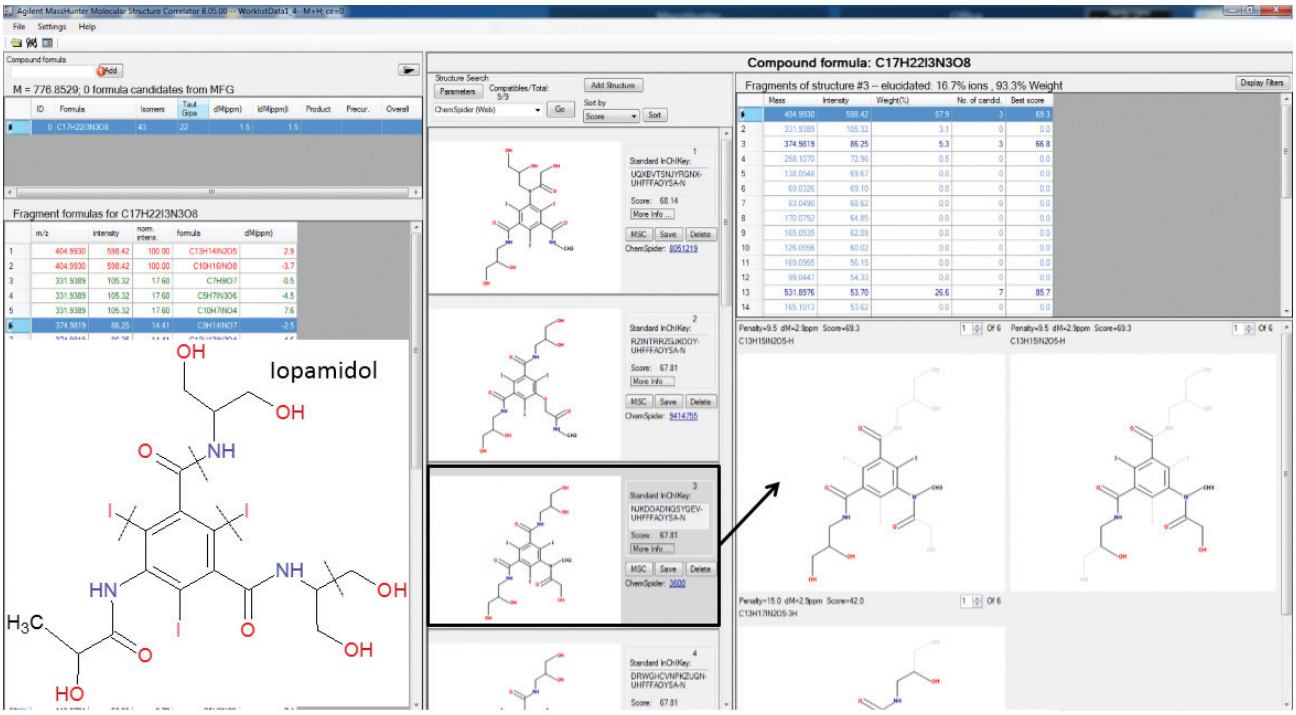

Figure 3. Identification of iopamidol using accurate mass data for precursor and products ions, Agilent MassHunter Molecular Structure Correlator (MSC) software, and ChemSpider.

Conclusions

River water samples taken upstream and downstream from a water treatment plant were analyzed using an Agilent 6550 iFunnel Q-TOF LC/MS and a suite of Agilent MassHunter statistical and qualitative analysis software packages. The result was an untargeted screening method that detected more than 890 unknown compounds. Compounds of interest were submitted for identification using accurate mass databases resulting in 150 suggested contaminants. MS/MS analysis with library matching identified 21 contaminants, and 32 additional contaminants were confirmed with authentic standards. For the compounds which were not present in the database or library, *in silico* fragmentation was used, along with a ChemSpider search. These results will be used to further expand compounds present in the accurate mass database and library for future studies.

References

1. M. Gust, *et al.* "Effects of Short-Term Exposure to Environmentally Relevant Concentrations of Different Pharmaceutical Mixtures on the Immune Response of the Pond Snail Lymnaea Stagnalis" *Science of the Total Environment* 445–446(0): 210-218 (**2013**).

2. R.H. Triebskorn, *et al.* "Ultrastructural Effects of Pharmaceuticals (Carbamazepine, Clofibric Acid, Metoprolol, Diclofenac) in Rainbow Trout (Oncorhynchus Mykiss) and Common Carp (Cyprinus Carpio)" *Analytical and Bioanalytical Chemistry* 387(4): 1405-1416 (**2007**).

3. B.I. Escher, *et al.* "Most Oxidative Stress Response in Water Samples Comes from Unknown Chemicals: The Need for Effect-Based Water Quality Trigger Values" *Environmental Science & Technology* (**2013**).

4. M. Zedda and C. Zwiener "Is Nontarget Screening of Emerging Contaminants by Lc-Hrms Successful? A Plea for Compound Libraries and Computer Tools." *Analytical and Bioanalytical Chemistry* 403(9): 2493-2502 (**2012**).

www.agilent.com/chem

Published in the USA, February 27, 2014
5991-3967EN

Agilent Technologies