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Analysis of Pharmaceuticals and Personal Care Products in River Water Samples by UHPLC-TOF

Introduction

Identifying the presence of emerging pollutants in surface water samples is a growing area of concern in the environmental field.[1,2] Many of these pollutants are introduced into the surface waters anthropogenically through municipal waste water. Among the emerging pollutants, pharmaceuticals and personal care products (PPCPs) have been detected at parts per million and parts per trillion concentrations in surface waters. The presence of PPCPs suggests inefficient removal of these compounds by current sewage treatment processes.

We present a study of PPCPs in river water samples from the northeastern United States using UHPLC-TOF-MS for both targeted and non-targeted analytes. Unlike a triple quadrupole, which is operated in multiple reaction monitoring mode for screening only predefined targeted analytes, the time-of-flight (TOF) mass spectrometer provides full spectrum accurate mass data that can be used to analyze and identify an unlimited number of compounds, without prior knowledge of target analytes or when reference standards are not available. In this study we show how high mass accuracy information provided by the PerkinElmer AxION 2® TOF along with the priopriatory AxION EC ID software can be used to identify unknown analytes in surface river waters.

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Experimental Conditions

Sample collection and preparation:

River samples (400 mL) were collected in 1 L amber bottles. Samples were filtered through Whatman™ glass fiber filters (GF/C, 1.2 µm) and stored at 4 °C until analysis. Prior to analysis, the samples were extracted through C-18 solid phase extraction (SPE) cartridges.

SPE extraction:

Phenomenex Strata™-X SPE cartridges (500 mg/6 mL) were used for extraction. The cartridges were initially conditioned with methanol (5 mL) followed by water (5 mL). The filtered river sample was loaded on the cartridges with or without a spike of standard PPCPs (100 ng each) and extracted through the cartridge at 5-6 mL/min. The cartridge was dried under vacuum for ~20 mins prior to eluting with methanol (4 mL) and acidified methanol (containing 2% formic acid, 4 mL). The eluate was dried under nitrogen to ~0.3 mL and diluted to 0.5 mL with water.

Liquid chromatography conditions:

Pump: PerkinElmer Flexar™ FX-10 pump

Mobile phase A: Water containing 0.1% formic acid

Mobile phase B: Acetonitrile containing 0.1% formic acid

Gradient conditions: 10% B to 90% B in 5 min

Injection volume: 3 µL in partial fill mode

Column used: PerkinElmer Brownlee™ SPP C-18, 2X50 mm, 2.7 µm

Mass spectrometry conditions:

Mass spectrometer: PerkinElmer AxION™ 2 TOF

Ionization source: PerkinElmer Ultraspray™ 2 (Dual ESI source)

Ionization mode: positive

Pulse mode: 100-800 m/z

Spectral acquisition rate: 1.5 spectra/sec

Capillary exit voltage: 100 V

Trap mode: 100-800 m/z (D7:42, D8:63)

Internal calibration: Internal calibration was done using two ions m/z 118.08625 and 622.02896 as lock mass ions.

Results

In the present study the AxION 2 TOF MS was used to analyze nine targeted PPCPs and several unknown compounds present in the river water samples. The separation of the nine target analytes was achieved under 3 mins on column (Figure 1). The SPE extraction procedure resulted in an estimated 75% or greater recovery of the analytes (based on 100 ppb standard spiked in MilliQ water). Using the AxION 2 TOF MS in the higher sensitivity TrapPulse™ mode, showed excellent sub ppb instrument detection limits for most analytes (detection limits for caffeine and theobromine were 4.0 and 8.0 ppb, respectively) and linearity over a very wide concentration range (0.5 to 500 ng/mL).
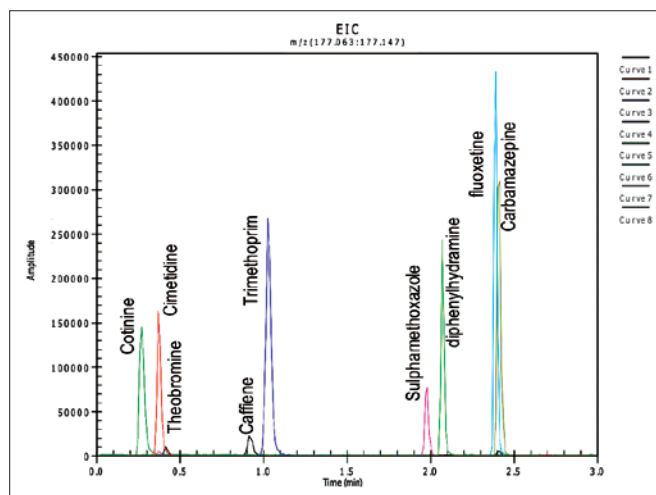

Figure 1. Shows the separation of PPCPs in under 2.5 min.
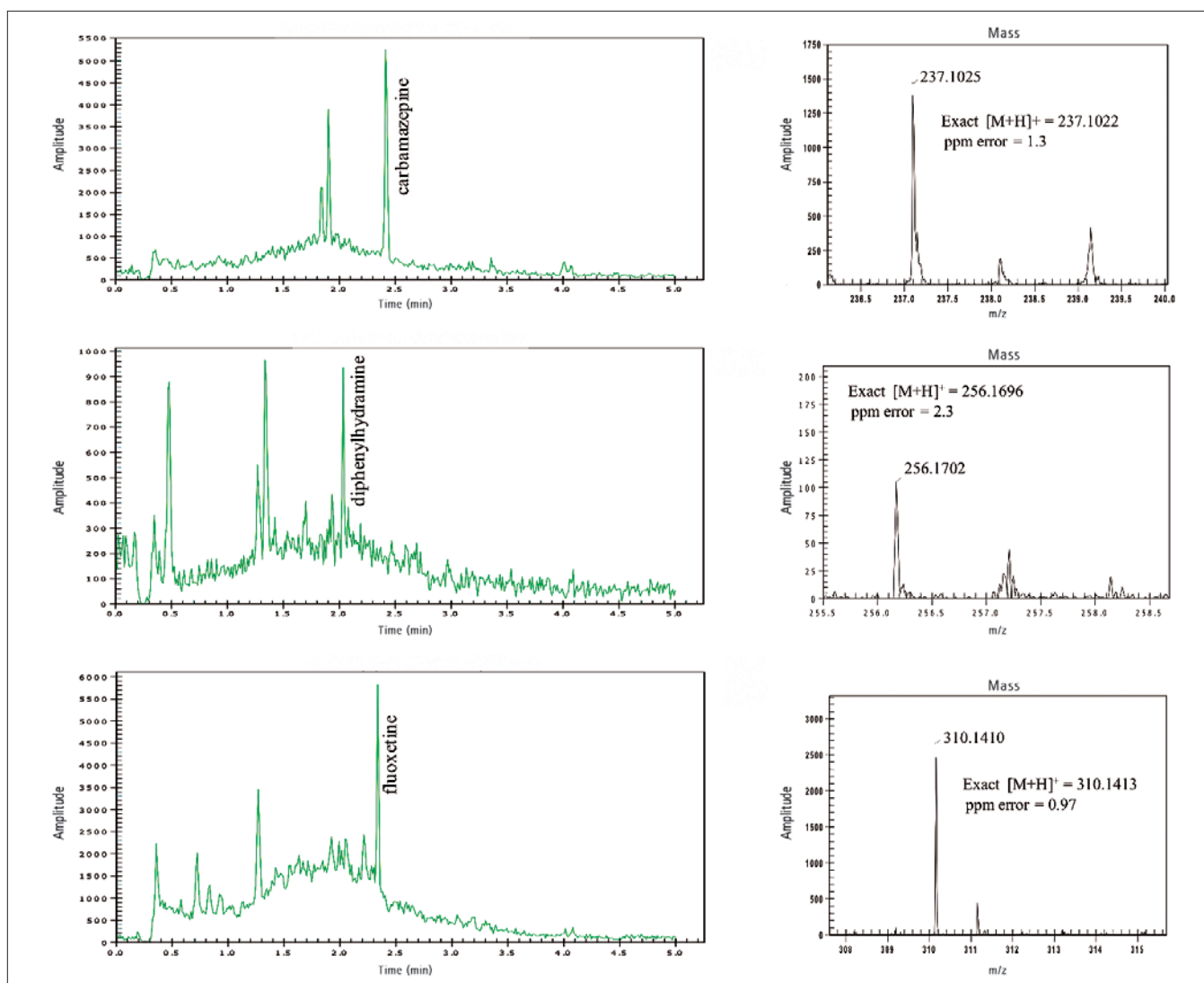
Figure 2. Identification of carbamazepine, diphenylhydramine, sulfamethoxazole and fluoxetine in river sample using accurate mass and retention time matching with standards.

Identification of target and non-target analytes in river water

The surface river water samples were collected downstream from a sewage treatment plant and were processed and analyzed as described above. This analysis revealed the presence of carbamazepine, diphenylhydramine, sulfamethoxazole and fluoxetine in the river water samples (Figure 2). The presence of these compounds was confirmed by accurate mass and retention time matching with standards.
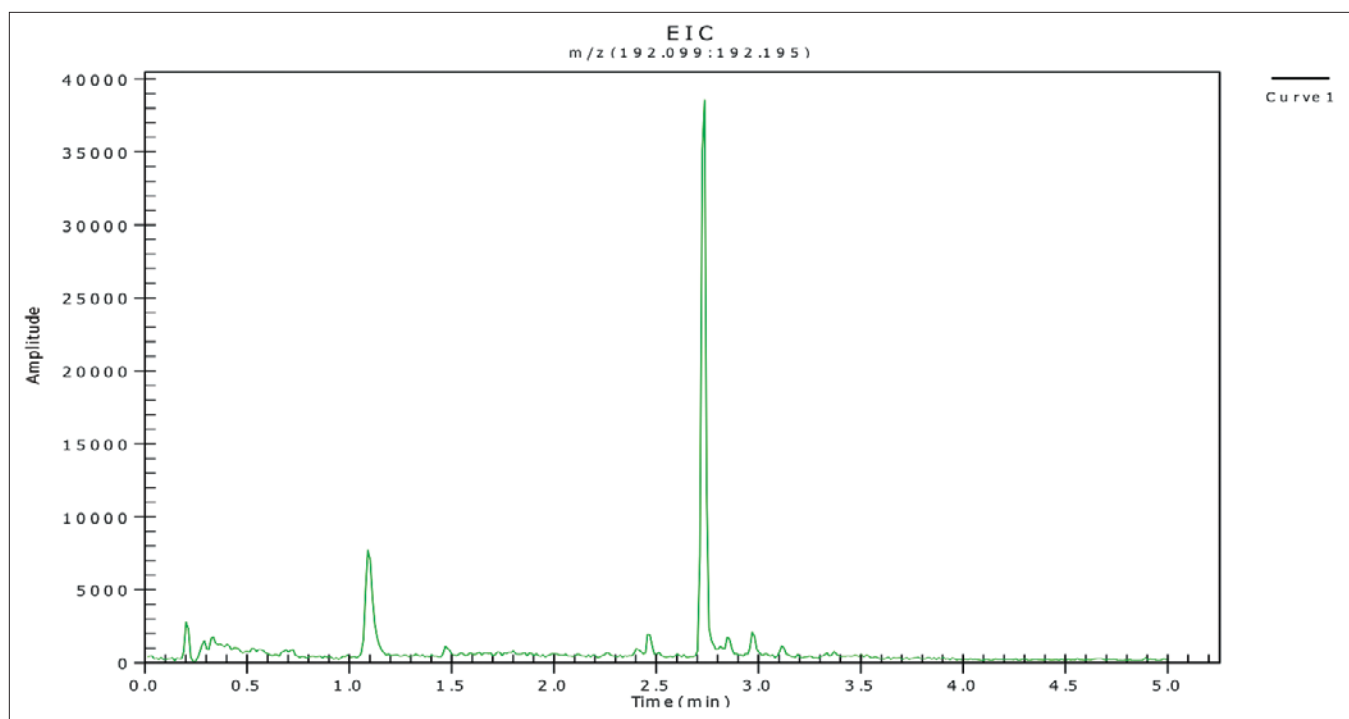
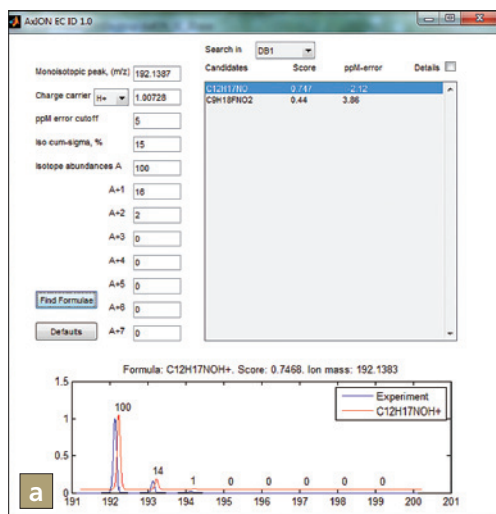*Figure 3.* Shows a major unknown target analyte eluting at ~2.7 mins.

Figure 4 a and b. Accurate mass and isotope ratio of unknown analyte was entered into AxION EC ID software to determine elemental composition. The AxION EC ID software searches against the PubChem database and by sorting for decreasing AIDs (chemical listed for most number of assays), the first possible candidate for the given elemental composition was given as N, N-Diethyl-m-toluamide (DEET), a common ingredient in insect repellents.
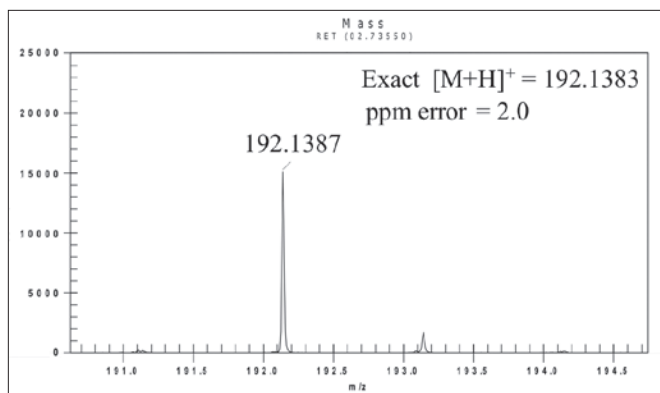
Figure 5. Presence of DEET was confirmed by accurate mass data

The river sample data was further examined for unknown non-target analytes. A major chromatographic peak eluting at ~2.7 min (Figure 3) was analyzed using AxION EC ID software. Accurate monoisotopic mass and isotope ratio information is used by AxION EC ID to search the PubChem database (or other data bases) for potential molecular formula matches and provide a ranked summary of the potential matches as well as suggestions for possible compound structures for a given elemental composition. In this example, the elemental composition, $C_{12}H_{17}NO$ was listed with highest score (Figure 4a) and the top candidate was identified as N, N-Diethyl-m-toluamide, (DEET) based on the number of active AIDS (Assay IDs) reported (Figure 4b). DEET is a common ingredient in insect repellent and the accurate mass of the unknown compound was verified to be within 2 ppm mass error of the expected mass of DEET (Figure 5). The presence of DEET in the river sample was further confirmed by retention time matching with the standard. Using a similar approach, acetaminophen, a commonly used analgesic drug was also identified in the river water (data not shown).

Conclusions

The PerkinElmer AxION 2 TOF operated in the higher sensitivity TrapPulse mode resulted in instrument detection limits of <1 ppb for majority of the PPCPs. The surface river water sample was screened for both target and non-target PPCPs. The accurate mass and isotope ratio provided by the TOF confirmed presence of several of the target PPCPs including antidepressant drugs in the surface waters. The non-target PPCPs in the sample were identified using accurate mass information along with powerful database search tools such as the AxION EC ID software.

References

1. Daughton, C.G., Jones-Lepp, T.L., Pharmaceuticals and personal care products (PPCPs) in the environment. Scientific and Regulatory Issues, ACS Symp. Ser. 791, Oxford University Press, Washington, U.S. (2001).

2. Daughton, C.G., Environ. Impact Assess. Rev. 24 (2004) 711.

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