# Targeted and Nontargeted MS Analysis of Contaminants in Storm Water Retention Ponds

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## Key Words

Environmental analysis, water analysis, wastewater, micropollutants, EQuan MAX Plus, TSQ Quantiva, LTQ Orbitrap Velos

## Goal

To demonstrate a data-driven environmental monitoring approach for examining the occurrence and distribution of wastewater-derived contaminants and turf-grass management organic compounds in storm water retention ponds.

## Introduction

Comprehensive assessment of the aquatic fate and effects of organic micropollutants is greatly hindered by the need to develop compound-specific methodologies prior to sampling and analysis. A data-driven workflow, coupling high-resolution, accurate-mass (HRAM) mass spectrometry and highly sensitive online solid phase extraction (SPE) analysis, ensures complete characterization of organic pollutants in aquatic environments. In this work, water samples collected from a coastal golf course community were screened for the presence of trace organic contaminants by a non-targeted HPLC–HRAM mass spectrometry workflow. The occurrence of identified and confirmed contaminants was then quantitatively assessed by a high-throughput online SPE LC-MS/MS method.

## Experimental

### Sample Collection

Surface water, groundwater, and wastewater effluent samples were collected from Kiawah Island, SC (Figure 1), a coastal golf course community where turf-grass management chemicals are extensively applied and reclaimed wastewater is used for irrigation. Golf course and storm water runoff are collected in ponds, which are interconnected through a series of culverts and communicate with the adjacent tidal estuary through managed outfalls.

Initial sampling for non-targeted screening consisted of 0.5 L grab samples collected and field extracted by SPE over two weeks in May 2010. Similarly, 10 mL grab samples were collected in May 2011 for quantitative analysis.
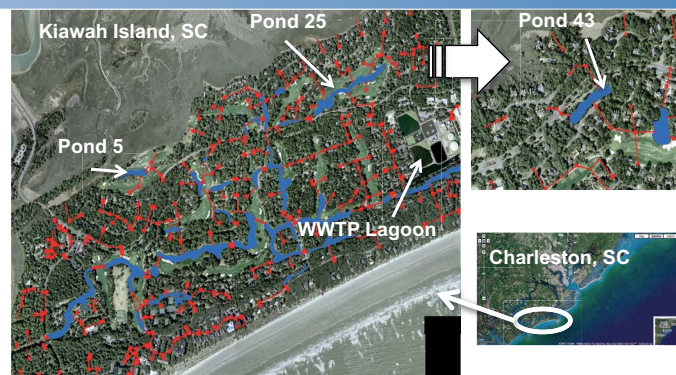


Figure 1. Aerial view of Kiawah Island, SC. Water collection ponds, shown in blue, are connected as indicated by the red lines.

Sample sites were chosen to represent various routes of micropollutant loading into the aquatic environment and potential routes of chemical exposure as detailed in Table 1. Golf course runoff consists of both turf-grass-management chemicals applied to the course and wastewater-derived contaminants introduced through irrigation.

Table 1. Sample sites and descriptions of potential sources of micropollutants to those site

| Sample Site | Inputs |
|---|---|
| Pond 5 | Golf course runoff |
| Pond 25 | Golf course runoff |
| Pond 43 | Residential storm water |
| Wastewater treatment plant lagoon (WWTP) | Treated municipal wastewater |
| Wastewater composite (WW Comp.) | 24 hr composite effluent |
| Well 1 | Infiltration from pond 25 |
| Well 7 | Infiltration from pond 5 |

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### Broad-Spectrum HPLC-HRAM MS Screening

To begin analysis, broad-spectrum MS screening was performed on a Thermo Scientific™ LTQ Orbitrap Velos™ hybrid ion trap-Orbitrap MS using heated electrospray ionization (HESI). The instrument was operated in positive full-scan ($m/z$ 100-1000) mode at a resolving power of 60,000 (FWHM) at $m/z$ 400. Data-Dependent Top 3 HRAM MS/MS experiments were performed with dynamic exclusion and peak apex detection.

### Non-Targeted Compound Identification

After broad-spectrum data acquisition, Thermo Scientific™ ExactFinder™ software version 2.5 was used for non-targeted compound identification. The HRAM data was screened for approximately 1000 known contaminants using the environmental and food safety (EFS) compound database and HRAM MS/MS spectral library. Automated feature scoring and filtering was based on chromatographic peak shape, mass error (ppm), and isotope pattern. Structures were tentatively assigned by library searching and later confirmed by analysis of authentic standards.

### Targeted Quantitation

Targeted quantitation was performed with the Thermo Scientific™ EQuan MAX Plus™ online SPE and HPLC system. A 1 mL injection was loaded onto a Thermo Scientific™ Hypersil GOLD aQ™ column (20 x 2.1 mm, 12 μm particle size) and separated on a Thermo Scientific™ Accucore™ aQ analytical column (50 x 2.1 mm, 2.6 μm particle size) by gradient elution with methanol/water mobile phase.

#### LC Conditions

| | | |
|---|---|---|
| Loading pump | Thermo Scientific™ Dionex™ UltiMate™ 3000 Quaternary Analytical Pump LPG-3400SD | |
| Flow rate | Isocratic 1 mL/min | |
| Solvent A (water) | 98% | |
| Solvent B (methanol) | 2% | |
| Total run time 1 | 8.4 min | |

| | | |
|---|---|---|
| Analytical pump Pump | UltiMate 3000 Binary Rapid Separation HPG-3200RS | |
| Solvent A (water) | 98% | |
| Solvent B (methanol) | 2% | |
| Gradient elution | 0.3 mL/min | |
| Gradient | Time | %A |
| | 0 | 98 |
| | 1.5 | 98 |
| | 12.0 | 2 |
| | 15.0 | 2 |
| | 15.1 | 98 |
| Total run time | 18.4 min | |

| | |
|---|---|
| Autosampler | Thermo Scientific™ Open Accela™ autosampler |
| Valve switching | At 1.5 min and 16.6 min |

The MS data was acquired in selected-reaction monitoring (SRM) mode on a Thermo Scientific™ TSQ Quantiva™ triple-stage quadrupole MS equipped with a HESI interface.

#### MS Conditions

| | |
|---|---|
| Ion mode | Positive HESI |
| Cycle time (s) | 0.75 |
| CID gas pressure (mTorr) | 1.5 |
| Spray voltage (V) | 3500 |
| Sheath gas (arb units) | 60 |
| Aux gas (arb units) | 20 |
| Sweep gas (arb units) | 2 |
| Ion transfer tube temp (°C) | 350 |
| Vaporizer temp (°C) | 350 |
| RF lens | Used calibrated RF lens values |

Data processing, calibration, and quality control were performed using Thermo Scientific™ TraceFinder™ software version 3.1.

## Results and Discussion
### HRAM Screening and Non-Targeted Identification

Representative HRAM chromatograms of SPE extracts subjected to non-targeted screening for the identification of organic pollutants and selection of target compounds for quantitative analysis are shown in Figure 2.
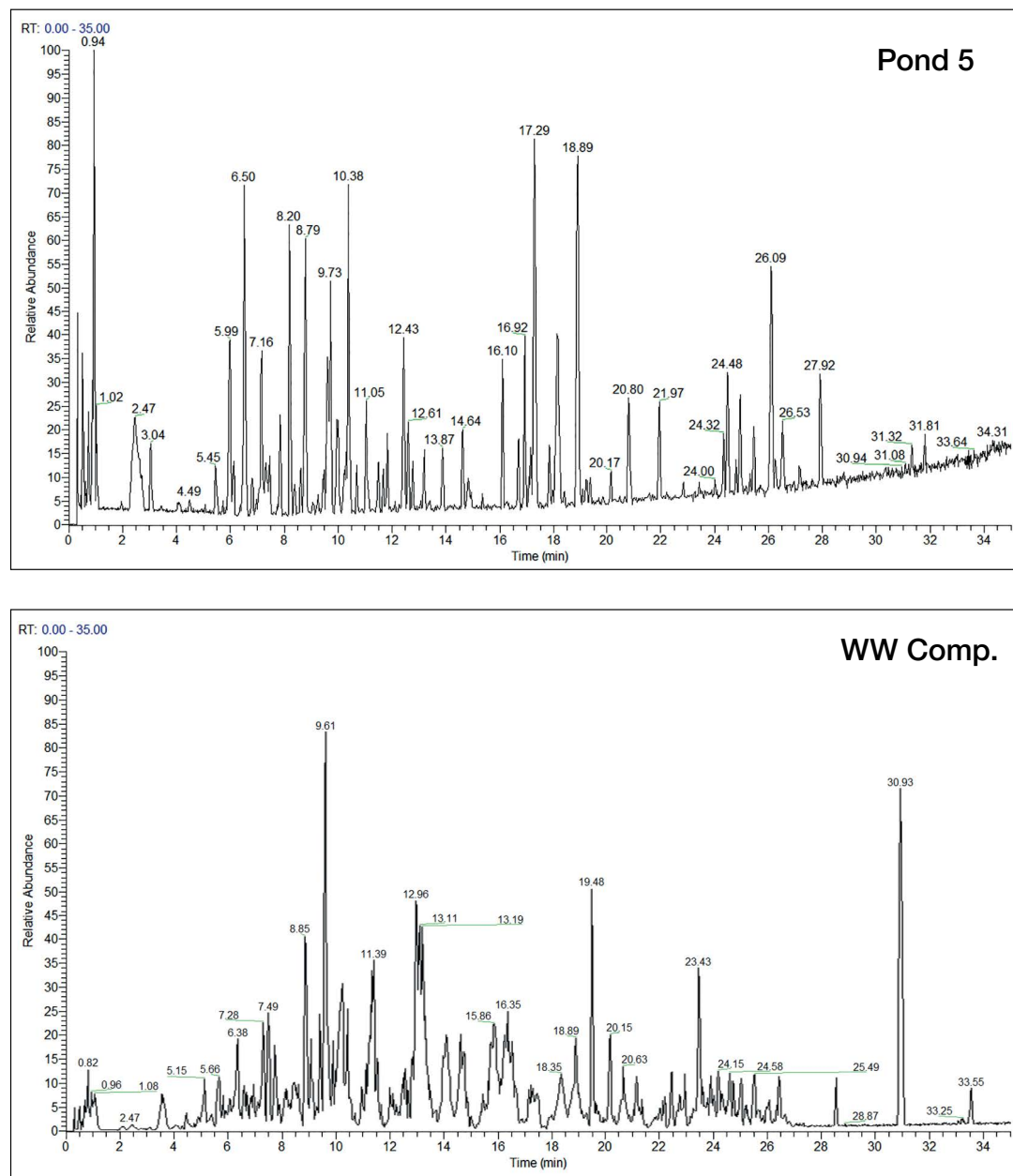


Figure 2. Representative HRAM chromatograms from non-targeted screening of SPE extracts from Pond 5 sample (top) and wastewater composite sample (bottom)

The non-targeted identification of fluridone in Pond 43 by EFS database screening and spectral library searching in ExactFinder software is demonstrated in Figure 3. Panel A shows an EFS database match for fluridone with a goodness of fit score of 0.93 between a modeled chromatographic peak and the observed peak. Panel B compares a modeled mass spectrum for the proposed pseudomolecular ion $[C_{19}H_{14}F_3NO+H]^+$ and the averaged full-scan observed data with excellent mass accuracy (-0.31 ppm) at the mono-isotopic peak and a 100% isotope pattern score. In Panel C, library searching of the observed HRAM CID $MS^2$ spectrum returned a match to the EFS library entry for fluridone with a score of 70%.
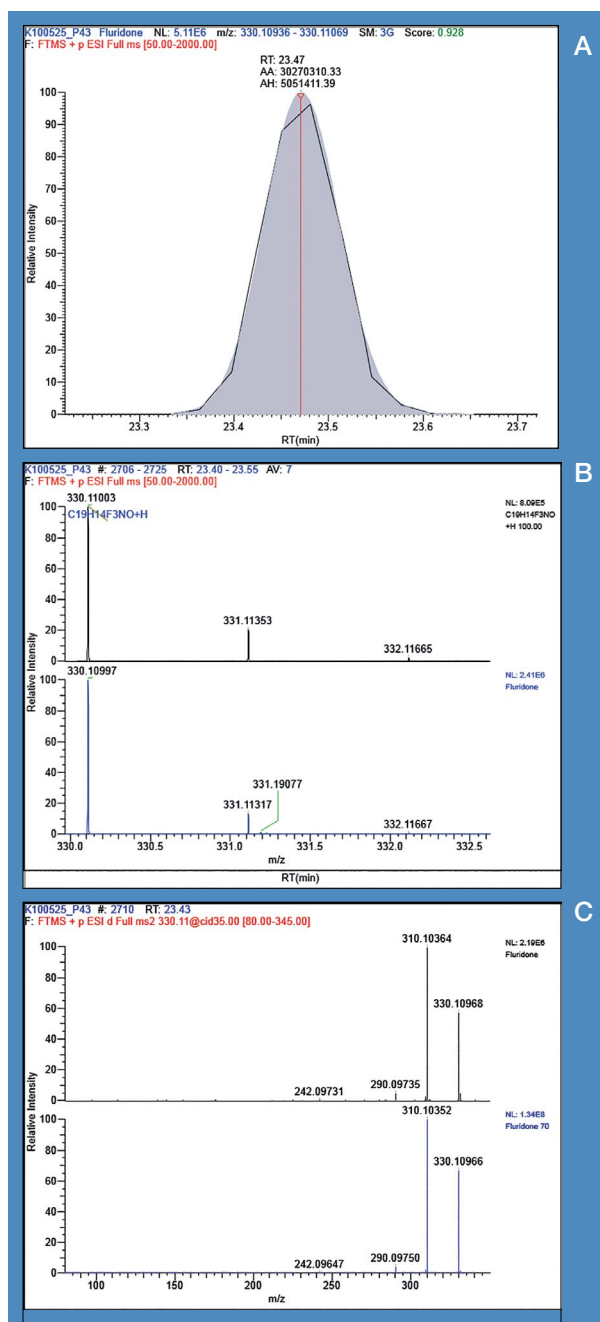


Figure 3. Non-targeted identification of fluridone in Pond 43. A) EFS database match for fluridone between a modeled chromatographic peak (gray area) and the observed peak (black trace). B) Comparison of a modeled mass spectrum for the proposed pseudomolecular ion $[C_{19}H_{14}F_3NO]^{M+H}$ (blue) and averaged full-scan observed data (black). C) Library searching of the observed HRAM CID $MS^2$ spectrum (black) returns a match to the EFS library entry for fluridone (blue) with a score of 70%.

A partial list of compounds identified by non-targeted screening and the samples in which they were found are listed in Table 2.

Table 2. Compounds identified by non-targeted screening

| Compound | Sample(s) |
|---|---|
| Atraton | Ponds 25, 43 |
| Atrazine | Ponds 5, 25, 43, WWTP, WW Comp. |
| Atrazine-2-hydroxy | Pond 25 |
| Carbamazepin | WWTP, WW Comp. |
| Carbendazim | WWTP |
| DEET | Ponds 5, 25, 43, WWTP, WW Comp. |
| Fluridone | Ponds 25, 43 |
| Hydrocortisone | WWTP, WW. Comp. |
| Mefluidide | Ponds 5, 25 |
| Metolcarb | WWTP |
| Metoprolol | WWTP, WW Comp. |
| Promecarb | WW Comp. |
| Propanolol | WWTP, WW Comp. |
| Pyroquilon | Ponds 5, 25, WWTP, WW Comp. |
| Sulfamethoxazole | WW Comp. |
| Temeazepam | WW Comp. |
| Trimethoprim | WWTP, WW Comp. |

WWTP = Wastewater treatment plant lagoon
WW Comp = Wastewater composite

## Targeted Quantitation by Online SPE LC/MS

Based on the results of the non-targeted screening, knowledge of chemical usage on the island, and readily available reference standards, an online SPE LC/MS method was developed to quantify the occurrence and distribution of wastewater- and turf-grass-management-derived organic pollutants on Kiawah Island.

Table 3 provides details of the online SPE LC/MS method, including the compounds monitored and the instrument limits of detection (LOD). Samples were quantitated down to the sub-ppt (ng/L) level.

Figure 4 displays the measured contaminant concentrations in representative storm and wastewater retention ponds.

Table 3. Compounds monitored by online SPE LC/MS, method parameters, and instrument limits of detection

| Compound | Retention Time (min) | Precursor Mass (*m/z*) | Product Mass 1 (*m/z*) | CE Mass 1 (V) | Product Mass 2 (*m/z*) | CE Mass 2 (V) | LOD (ng/L) |
|---|---|---|---|---|---|---|---|
| Acephate | 4.4 | 184.0 | 143 | 10 | 95 | 25 | 0.24 |
| Allethrin | 12.4 | 303.2 | 135 | 15 | 220 | 20 | 7.8 |
| Ametryn | 9.6 | 228.1 | 186 | 19 | 96 | 26 | 0.12 |
| Atraton | 8.2 | 212.2 | 170 | 19 | 100 | 29 | 0.12 |
| Atrazine | 9.7 | 216.1 | 174 | 16 | 104 | 29 | 0.12 |
| Atrazine Desethyl | 7.6 | 188.1 | 146 | 16 | 104 | 30 | 0.12 |
| Atrazine-desisopropyl | 6.5 | 174.1 | 132 | 17 | 104 | 28 | 0.24 |
| Azoxystrobin | 10.4 | 404.1 | 372 | 15 | 329 | 33 | 0.12 |
| Benzotriazole | 6.6 | 120.1 | 65 | 25 | 92 | 18 | 7.8 |
| Bioresmethrin | 13.2 | 339.2 | 171 | 14 | 293 | 15 | 62.5 |
| Bloc (Fenarimol) | 10.3 | 331.2 | 268 | 23 | 311 | 33 | 0.24 |
| Carbaryl | 9.3 | 202.0 | 145 | 12 | 127 | 30 | 0.12 |
| Carbendazim | 6.0 | 192.1 | 160 | 20 | 132 | 33 | 0.12 |
| DEET | 9.8 | 192.1 | 119 | 19 | 91 | 34 | 0.98 |
| Etofenprox | 13.6 | 394.0 | 177 | 14 | 135 | 26 | 3.9 |
| Fenamiphos | 11.2 | 304.1 | 217 | 25 | 234 | 17 | 0.12 |
| Fluoxastrobin | 11.0 | 459.1 | 427 | 18 | 188 | 38 | 0.5 |
| Fluridone | 10.3 | 330.1 | 309 | 37 | 310 | 29 | 0.12 |
| Flutolanil | 10.8 | 324.0 | 262 | 18 | 242 | 26 | 0.06 |
| Formasulfuron | 9.4 | 453.1 | 183 | 25 | 272 | 15 | 0.12 |
| Halosulfuron-methyl | 11.2 | 435.1 | 182 | 20 | 139 | 50 | 0.12 |
| Imidacloprid | 6.9 | 256.0 | 209 | 18 | 175 | 20 | 0.06 |
| Iprodione_a | 11.3 | 330.0 | 245 | 16 | - | - | 15.63 |
| Iprodione_b | 11.3 | 332.0 | 247 | 16 | - | - | 31.25 |
| Metalaxyl | 9.8 | 280.2 | 220 | 17 | 160 | 30 | 0.06 |
| Metoprolol | 7.3 | 268.2 | 116 | 17 | 191 | 20 | 0.24 |
| Oxadiazon | 12.4 | 345.1 | 303 | 15 | 220 | 20 | 3.9 |
| Pramoxine | 9.6 | 294.2 | 128 | 22 | 100 | 32 | 0.12 |
| Prometron | 9.1 | 226.1 | 142 | 24 | 170 | 19 | 0.12 |
| Propanmide | 10.8 | 256.0 | 173 | 25 | 209 | 20 | 0.12 |
| Quinclorac | 8.3 | 242.0 | 161 | 34 | 224 | 18 | 7.8 |
| Thiencarbazone-methyl | 8.7 | 391.0 | 359 | 10 | 230 | 20 | 3.9 |
| Thiophanate-methyl | 8.9 | 343.0 | 151 | 24 | 311 | 13 | 0.24 |
| Tramadol | 7.2 | 264.2 | 58 | 18 | 246 | 12 | 0.06 |
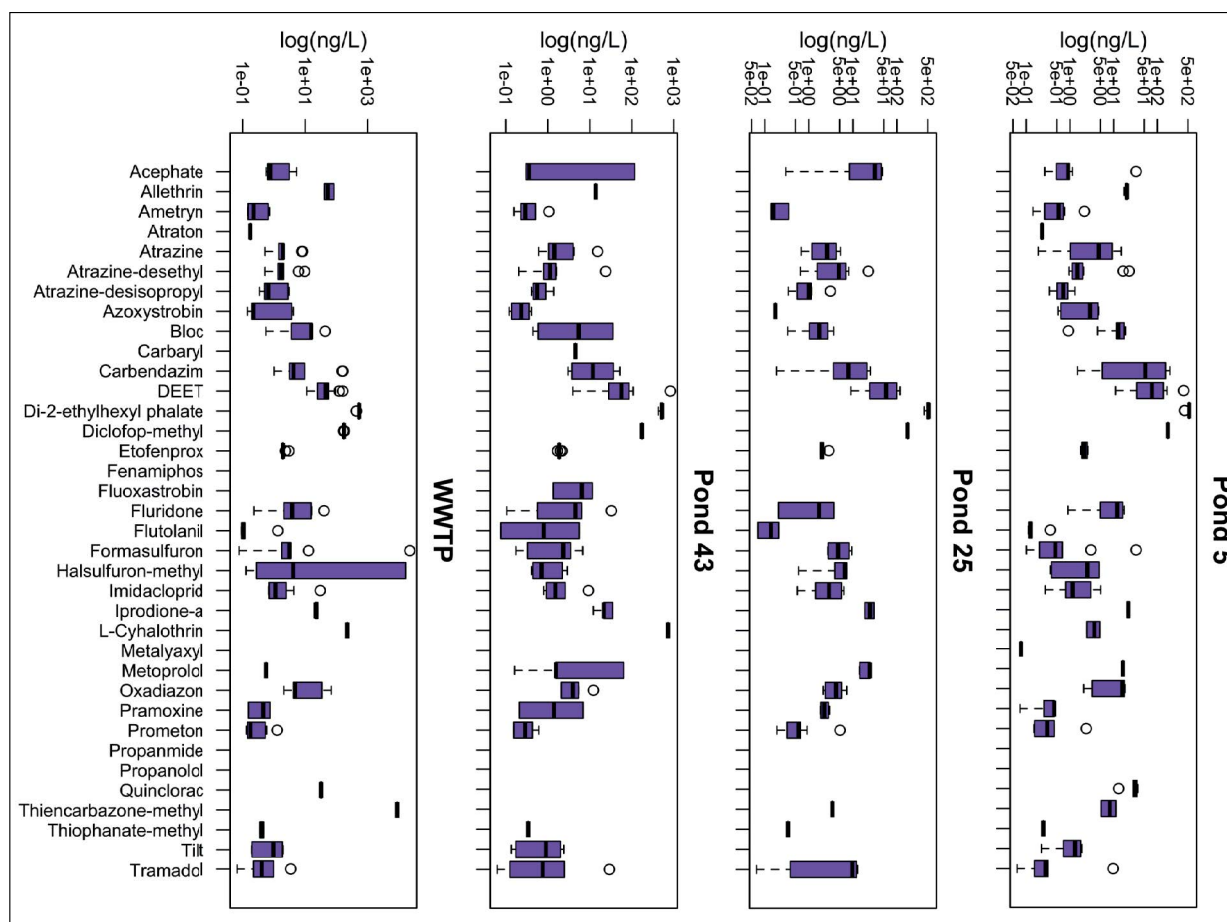
Figure 4. Boxplots depicting the measured contaminant concentrations in wastewater storage and storm water retention ponds on Kiawah Island. Purple boxes represent the interquartile range and the bar represents the median value. Hashed lines depict the range of the data and outliers are plotted as open circles.

## Conclusion

A multifaceted approach to identifying and quantifying non-targeted emerging compounds in environmental surface and ground water samples impacted by reclaimed water irrigation has been demonstrated.

- HRAM can be used to identify organic micropollutants in wastewater-impacted environments, golf course runoff, and storm water ponds.

- Online SPE coupled with a triple quadrupole MS can be used to quantitate micropollutants in water samples down to the sub-ppt (ng/L) level.

- Future work will include studying the toxicological impact of these compounds on aquatic species.

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