

# 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.

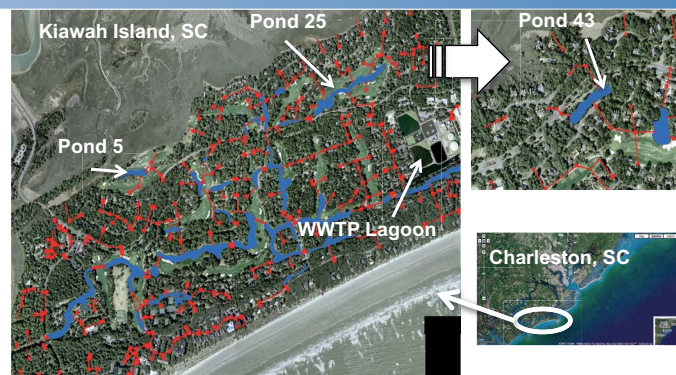


Figure 1. Aerial view of Kiawah Island, SC. Water collection ponds, shown in blue, are connected as indicated by the red lines.

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

### 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	UltiMate 3000 Binary Rapid Separation Pump 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.

**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.

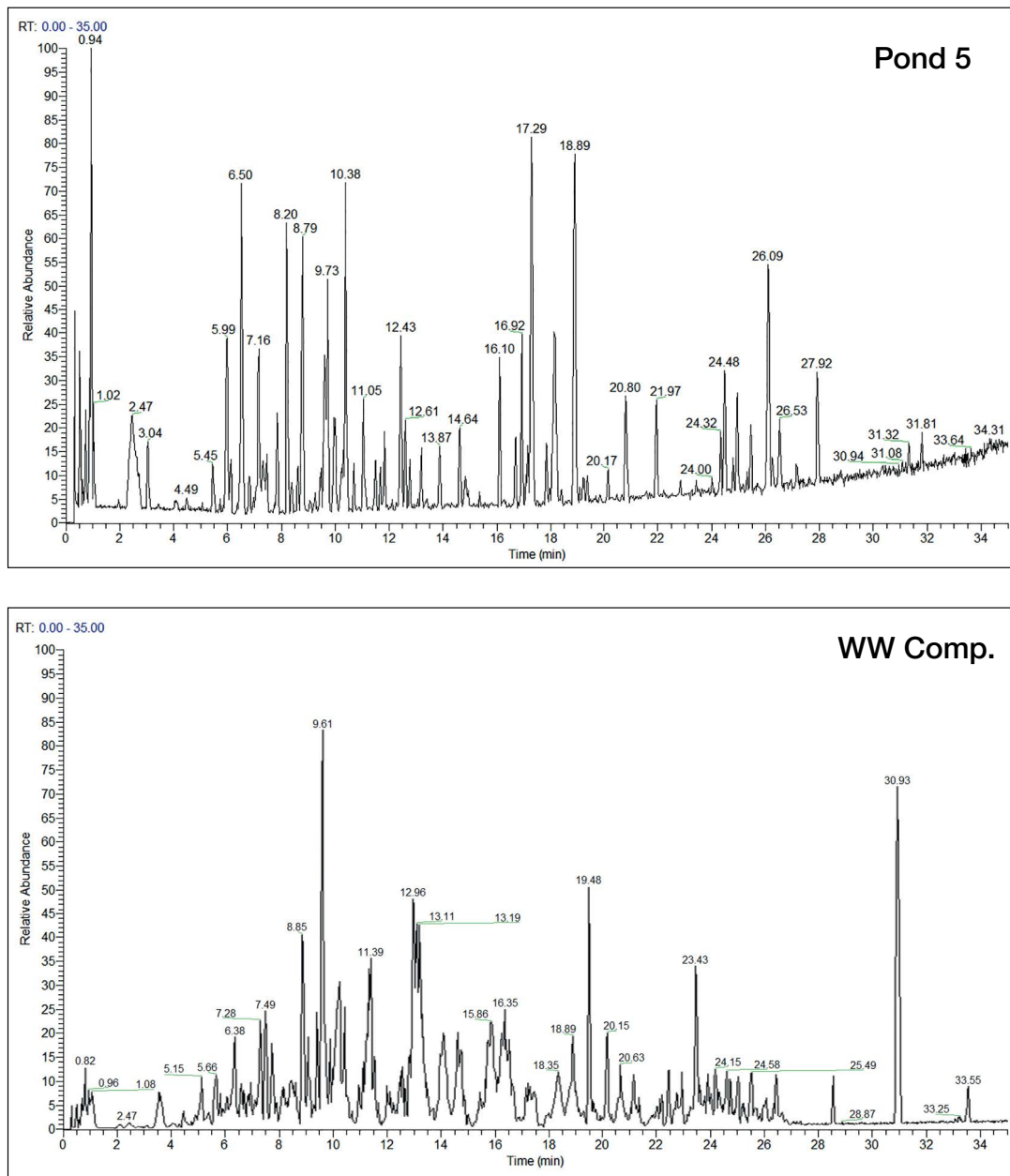


Figure 2. Representative HRAM chromatograms from non-targeted screening of SPE extracts from Pond 5 sample (top) and wastewater composite sample (bottom)

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<sup>2</sup> spectrum returned a match to the EFS library entry for fluridone with a score of 70%.

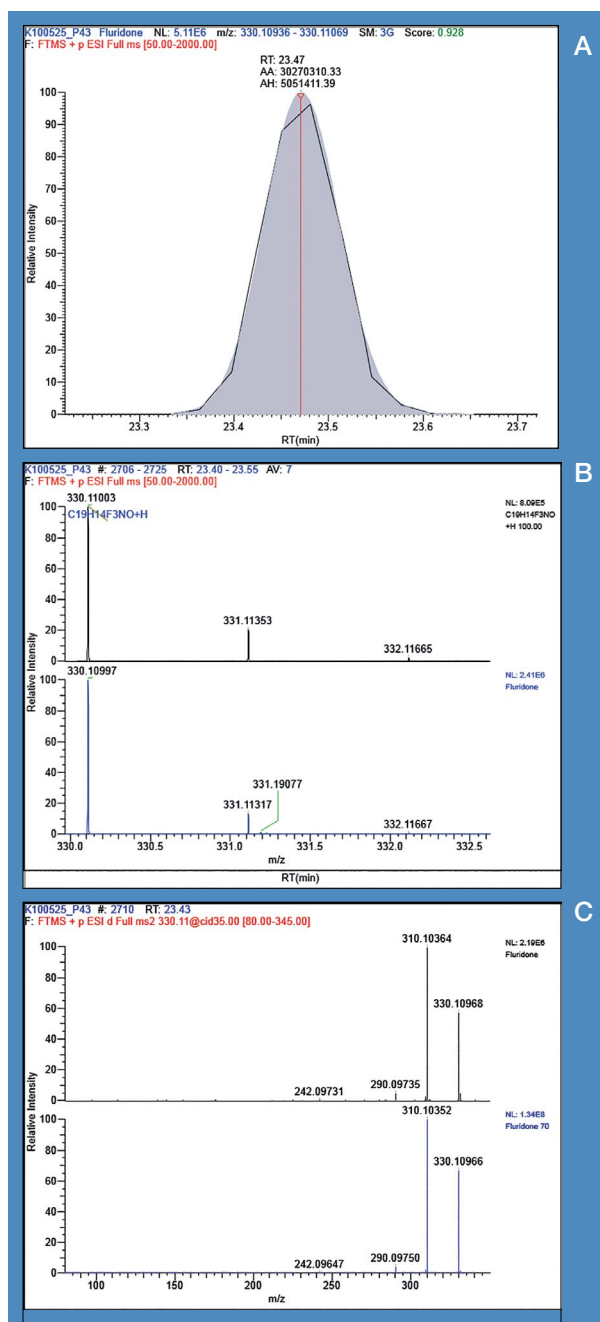


Figure 3. Non-targeted identification of fluridone in Pond 43. A) EFS database match for fluridone between a modeled chromatographic peak (gray area) and the observed peak (black trace). B) Comparison of a modeled mass spectrum for the proposed pseudomolecular ion  $[C_{19}H_{14}F_3NO]^m+H$  (blue) and averaged full-scan observed data (black). C) Library searching of the observed HRAM CID MS<sup>2</sup> 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.
Propranolol	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
Formsulfuron	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

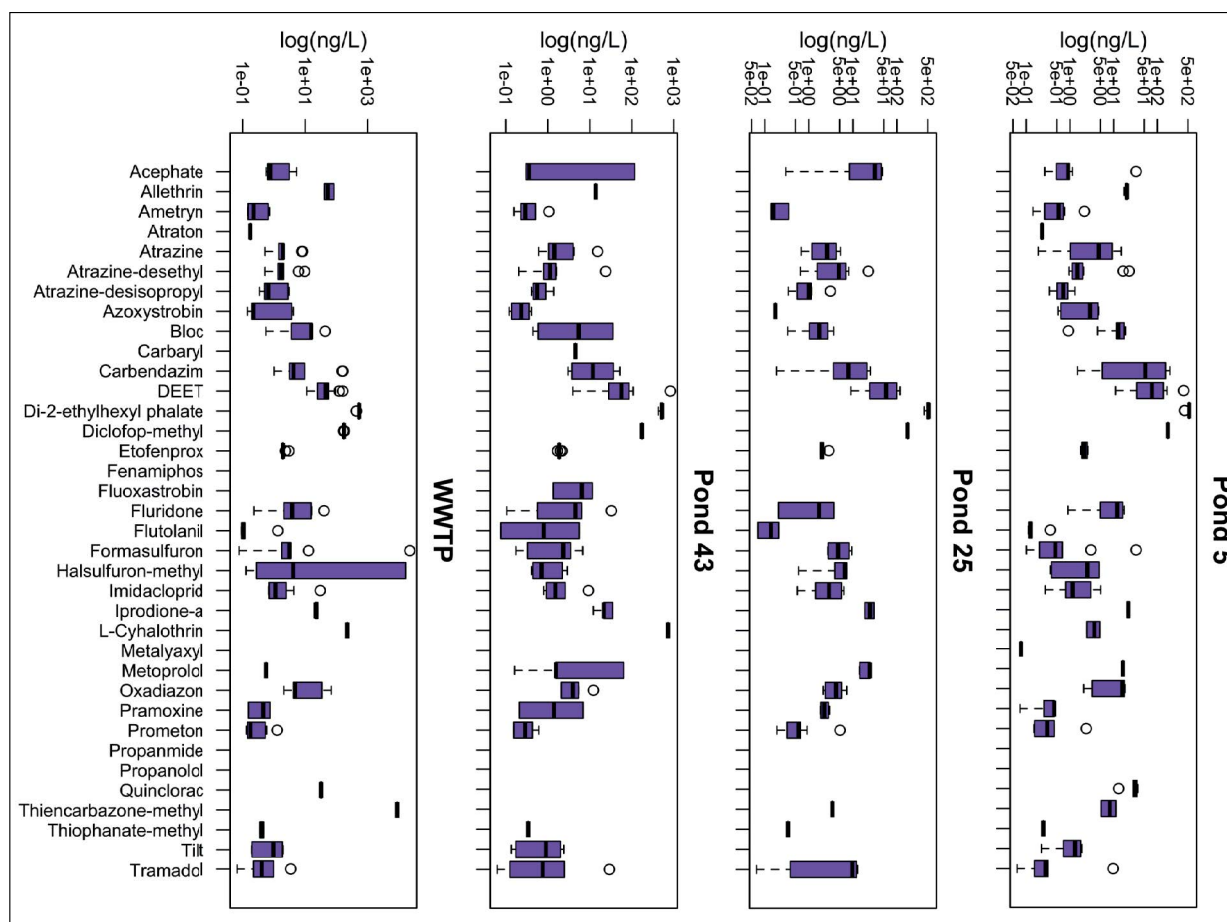


Figure 4. Boxplots depicting the measured contaminant concentrations in wastewater storage and storm water retention ponds on Kiawah Island. Purple boxes represent the interquartile range and the bar represents the median value. Hashed lines depict the range of the data and outliers are plotted as open 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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