Targeted and Non-Targeted Analysis of Contaminants in Storm Water Retention Ponds Using LC-HRMS and Online Solid Phase Extraction

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Separations are often demonstrated using clean solutions, and as a result do not always reflect real world samples which come with many challenges associated with them, either due to the complex matrix components, or where some form of pre-concentration is required to allow for detection of the analyte at very low levels. When analysing environmental water samples both of these challenges are present and so the use of hyphenated technology to allow the online solid phase extraction coupled to HPLC-HRMS (High Resolution Mass Spectrometry) allows for both selectivity and sensitivity, with enhanced selectivity being provided by the use of HRMS.

The coupling of mass spectrometry to LC is now a routine operation but in the early days, the hyphenating of these two technologies was not considered a simple process, with many different approaches attempted with little success. John Fenn [1], who subsequently won a Nobel prize for his work in this area, found a universally accepted approach to the removal of liquid and ionisation of the analyte molecules. The introduction of the electrospray revolutionised mass spectrometry and has lead to this technology becoming routine in many laboratories across the world [2-6].

It has also led to an increase in the development of mass spectrometry and in particular in the field of high resolution where the introduction of the Orbitrap™ technology has allowed for incredible resolution of nominally isobaric compounds without the requirement of a FT-ICRMS technology [7]. It has also led to the possibility of performing a screening method for a very wide range of compounds, without the limitations that face more specific technology such as tandem mass spectrometry where a compromise has to be maintained between the number of compounds and the sensitivity of the instrument to those compounds.

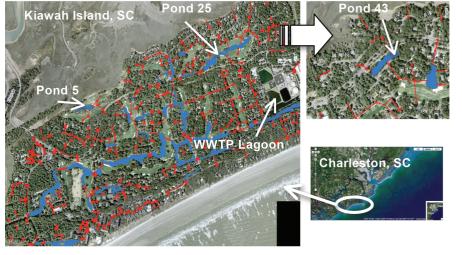Within environmental monitoring, one area of concern is the overuse of agrochemicals,

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

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

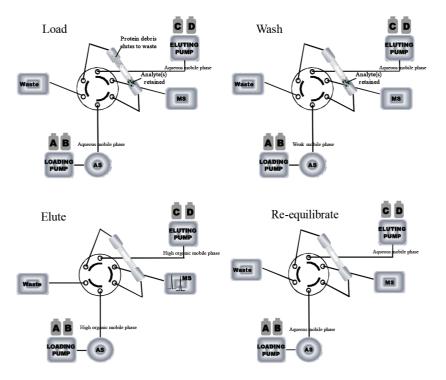| Sample Site	Inputs
Pond 5	Golf course runoff
Pond 25	Golf course runoff
Pond 43	Residential storm water
Wastewater treatment plant lagoon (WWTP)	Treated municipal wastewater
Wastewater composite (WW Comp.)	24 hr composite effluent
Well 1	Infiltration from pond 25
Well 7	Infiltration from pond 5

Figure 2. Diagram of single valve system using two pumps, reprinted with kind permission from ref 10.

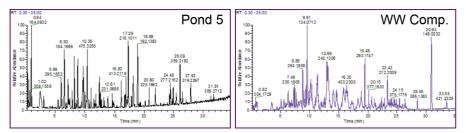*Figure 3. Representative TIC from non-targeted screening of SPE extracts from Pond 5 sample (left) and wastewater composite sample (right)*

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
Carbamazepine	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.
Temazepam	WW Comp.
Trimethoprim	WWTP, WW Comp.

WWTP = Wastewater treatment plant lagoon

WW Comp = Wastewater composite

which has resulted in run-off into local aquatic ecosystems [8,9]. This can have a dramatic effect on this particular ecosystem and thus, monitoring of the water around these sites will help to ensure that the over usage of agrochemicals does not occur. It is often the case that these chemicals are used too widely throughout the year with little benefit to the crop. Careful management of the use of the agrochemicals will ensure that both a minimal effect on the environment as well as ensuring an effective crop protection regime is employed.

It is, therefore, necessary to develop analytical technologies which can identify and quantify a wide range of aquatic pollutants and as this will allow the determination of the environmental fate of these contaminants, and thus reduce pollution and aid the usage of agrochemicals. A 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 will be presented, which couples high-resolution, accurate-mass mass spectrometry and highly sensitive online solid phase extraction (SPE) coupled to liquid chromatography analysis, ensuring complete characterisation of organic pollutants in aquatic environments.

In this work, water samples were collected from a coastal golf course community and screened for the presence of trace organic contaminants by initially using a non-targeted HPLC-HRMS workflow. The occurrence of identified and confirmed contaminants was then quantitatively assessed by a more targeted high-throughput online SPE LC/MS method. The coupling of SPE with LC allows for a greater robustness of the assay and also allows for sample pre-concentration which will allow for the improvements in the sensitivity of the assay. This will aid the development of mathematical models to describe the fate of micropollutants within an aquatic environment.

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 was collected in ponds, which are connected in series and communicate tidally with a neighbouring estuary.
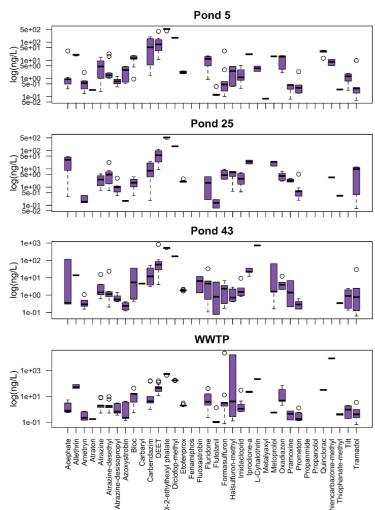
Figure 4. Non-targeted identification of fluridone in Pond 43. A database match for fluridone between a modelled chromatographic peak (gray area) and the observed peak (black trace). B. Comparison of a modelled mass spectrum for the proposed pseudomolecular ion $[C_{19}H_{14}F_3NO]^{M+H}$ (blue) and 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%.

Figure 5. Boxplots depicting the measured contaminant concentrations in storm- and wastewater retention ponds on Kiawah Island. Purple boxes represent the interquartile range and the bar represents the median value. Hashed lines depcit the range of the data and outliers are plotted as open circles.

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 samples were collected in May 2011 for quantitative analysis. 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.

Broad-Spectrum HPLC-HRMS Screening

Initially a scoping gradient was run to determine which compounds may be present. This was done using coupled to a Thermo Scientific™ LTQ Orbitrap Velos™ MS, with the data analysis being performed using Thermo Scientific™ ExactFinder™, which

found approximately 1000 known compounds based on the available databases. The use of a high resolution mass spectrometer meant that a high degree of confidence could be associated with the identification of these compounds.

Targeted Quantification

Once the screen process had been performed a targeted quantification was performed using the EQuan MAX Plus™ online SPE and HPLC system (Thermo Scientific™, San Jose USA).

There are many different valve configurations that can be used for coupling of SPE to LC. In this scenario Figure 2 shows the configuration of a six port vale that is utilised to allow the hyphenation of SPE to LC, where two pumps are used, one is referred to the loading pump where the pump is

used to move the sample onto the SPE column, and the eluting pump which is used to elute the compound from the column to the detector. Specifically there are four steps when utilising this configuration; load, wash, elute and re-equilibration of the column. These steps are very comparable to the four basic steps involved in off-line solid phase extraction (condition, load, wash, elute) and serve comparable purposes. In most cases the loading and washing steps take place simultaneously.

The sample is loaded onto the extraction column in a weak solvent to ensure that retention of the compound is achieved. A wash step uses the same valve position and allows for a stronger wash solvent to be employed compared to the loading solvent is used in some cases. In both the load and the wash configurations, the valve is positioned such that all the eluent goes to the waste

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

Compound	Retention Time (min)	Precursor Mass	Product Mass 1	Product Mass 2	LOD (ng/L)
Acephate	4.36	184	143	95	0.24
Allethrin	12.43	303.2	135	220	7.8
Ametryn	9.55	228.1	186	96	0.12
Atraton	8.22	212.2	170	100	0.12
Atrazine	9.72	216.1	174	104	0.12
Atrazine Desethyl	7.61	188.1	146	104	0.12
Atrazine-desisopropyl	6.52	174.1	132	104	0.24
Azoxystrobin	10.38	404.1	372	329	0.12
Benzotriazole	6.6	120.1	65	92	7.8
Bioresmethrin	13.24	339.2	171	293	62.5
Bloc (Fenarimol)	10.3	331.2	268	311	0.24
Carbaryl	9.32	202	145	127	0.12
Carbendazim	6.01	192.1	160	132	0.12
DEET	9.79	192.1	119	91	0.98
Etofenprox	13.55	394	177	135	3.9
Fenamiphos	11.25	304.1	217	234	0.12
Fluoxastrobin	10.95	459.1	427	188	0.5
Fluridone	10.31	330.1	309	310	0.12
Flutolanil	10.75	324	262	242	0.06
Formasulfuron	9.41	453.1	183	272	0.12
Halosulfuron-methyl	11.23	435.1	182	139	0.12
Imidacloprid	6.89	256	209	175	0.06
Iprodione_a	11.26	330	245	-	15.63
Iprodione_b	11.26	332	247	-	31.25
Metalaxyl	9.81	280.2	220	160	0.06
Metoprolol	7.34	268.2	116	191	0.24
Oxadiazon	12.44	345.1	303	220	3.9
Pramoxine	9.65	294.2	128	100	0.12
Prometron	9.11	226.1	142	170	0.12
Propanmide	10.81	256	173	209	0.12
Quinclorac	8.33	242	161	224	7.8
Thiencarbazone-methyl	8.67	391	359	230	3.9
Thiophanate-methyl	8.88	343	151	311	0.24
Tramadol	7.25	264.2	58	246	0.06

stream. Since the matrix consists of a broad spectrum of physiochemical properties, some components of the matrix will not be retained during this stage and will go directly to waste, effectively cleaning the sample or extracting it from the bulk matrix.

The next stage is the elution step where the components retained after the initial loading step, are eluted from the column with a high elutropic mobile phase. At this point the valve is switched so that the eluent stream now goes to the detector rather than to waste, either via an analytical column or directly. The use of a second column allows for either greater separation of the analyte(s)

and any remaining matrix components. The loading pump is used in this step to flush out the autosampler and associated tubing reducing the re-equilibration time and also reducing carryover.

The final phase is to re-equilibrate the system to be ready for the next sample injection. The valve is re-positioned to its original position, with the mobile phase composition being reset to its starting conditions.

On the actual system the valving looks a little more complicated due to the use of two injection ports to allow the user to swap between LC and SPE-LC, with the LC only injector valve also being used by the SPE-LC

configuration.

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 (100 x 2.1 mm, 2.6 µm particle size) by gradient elution with methanol/water mobile phase. The MS data was acquired in selected reaction monitoring (SRM) mode on a Thermo Scientific™ TSQ Quantiva™ MS equipped with an H-ESI interface. Quantitative analysis was performed using Thermo Scientific™ TraceFinder™ software version 3.1.

Results and Discussion

HRMS Screening and Non-Targeted Identification

Representative HRMS 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 3.

The non-targeted identification of fluridone in Pond 43 by EFS database screening and spectral library searching in ExactFinder software is demonstrated in Figure 4. Panel A shows an EFS database match for fluridone with a goodness of fit score of 0.93 between a modelled chromatographic peak and the observed peak. Panel B compares a modelled mass spectrum for the proposed pseudomolecular ion $[C_{19}H_{14}F_3NO]^{M+H}$ 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 MS2 spectrum returns a match to the EFS library entry for fluridone with a score of 70%. Although in the examples shown there is a good match, it should be noted that in general EI and CI generated MS spectra tend to have more consistency, and thus databases generated using these sources are better.

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

Targeted Quantification 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 (pg/mL) level.

Figure 5 displays the measured contaminant concentrations in representative storm and wastewater retention ponds.

Conclusion

A multifaceted approach to identifying and quantifying non-targeted emerging compounds in environmental surface and ground water samples impacted by treated wastewater has been demonstrated, which utilises hyphenated technology coupled to liquid chromatography.

- HRMS can be used to identify environmental compounds in wastewater treatment plant impacted environments.

- Online SPE coupled with a triple quadrupole MS can be used to quantitate 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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