CHEMICALS OF CONCERN ANALYZED IN WATER SOURCES USING ONLINE SPE-LC-MS/MS

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OVERVIEW

Chemicals of concern include pharmaceuticals, veterinary drugs, pesticides, nanomaterials, personal care products and household chemicals.

These may be found at trace levels in water sources and therefore need to monitored.

Automated sample preparation combined with LC/MS/MS plays a key role to

- **Decrease labor intensive steps**
- **Decrease sample turnaround time**
- **Reduce sample volume requirements**
- **Increase lab return on investment**

The utility of the online SPE/LC/MS/MS for the analysis of chemicals of concern in drinking and river water, is shown here.

INTRODUCTION

The analysis of chemicals of concern has become an area of significant interest in the field of environmental research.

In order to achieve trace level analysis, large sample volumes are usually extracted using various off-line extraction methods to remove background interferences and concentrate the analytes into a smaller volume. By combining the extraction column, analytical separation column, MS/MS and software, an online SPE/LC/MS/MS system can be created.

A major advantage of this integrated platform is that the most time consuming steps in the off-line method are reduced or eliminated altogether. By reducing the sample volume requirement from liters to milliliters, the sample loading time is markedly reduced. In addition, direct elution into the mass spectrometer means there is no requirement for evaporation and reconstitution steps.

The time required for the analysis can be reduced by up to 80%.

Here we show the use of online SPE/LC/MS/MS for the analysis of pesticides and pharmaceuticals in drinking and river water.

METHODS

Drinking water analysis

Selected pharmaceuticals and pesticides (Table 1) were spiked into drinking water samples and introduced to the online SPE LC/MS/MS system. After sample loading, the SPE column (Oasis HLB) was washed with 2% NH_4OH in 20 % methanol.
Analytes were eluted from the SPE column to an ACQUITY BEH C18 2.1 x 50 mm 1.8 mm column with a back flush gradient of 5% B to 95% B over 5 min. The gradient was held at 95% B for 3 min before the analytical column was re-equilibrated to initial conditions.
The pharmaceutical method used 20 mM NH_4HCO_2 , pH 3.2 for mobile phase A and a mixture of 50/50 methanol/acetone with 20 mM NH_4HCO_2 pH 3.2 for mobile phase B.
The pesticide method used 0.5 % formic acid for A and a mixture of 80/20 methanol/acetonitrile with 0.5 % formic acid for B.
The SPE column was reconditioned with 2 % NH_4OH in methanol.
A single MRM transition was selected for each of the analytes.
The sample turnaround time including SPE and analysis was 13 minutes.

Pharmaceuticals	Pesticides
Carbamazepine	Aldicarb
Cimetidine	Simazine
Diphenihydramine	Propuxur
Atenolol	Propaclor
Metoprolol	Simetryn
Chlorpheniramine	Atrazine
Tripolidine	Carbofuran
Trimethoprime	Methiocarb
Terbinafine	Propazine
Codeine	Terbuthylazine
Cocaine	Cyanazine
Clotrimazole	Prometryn
Miconazole	Metolachlor
Erythromycin	Tebuconazole
Azithromycin	Propiconazole

Table 1. List of pharmaceuticals and pesticides analyzed in drinking water.

River water analysis

River water samples were collected to screen for 20 pharmaceuticals shown in Table 2. The water samples did not require pre-filtering. Seven different locations of interest were selected as shown in Figure 4. These sites included tributaries to the main river, upstream and downstream sites of waste water treatment plants (WWTP) and a point source.
The samples were loaded, washed and eluted as described above for the pharmaceutical compounds.

Pharmaceuticals Monitored

Chlorpheniramine	Tripolidine
Carbamazepine	Dextromethorphan
Clotrimazole	Propranolol
Erythromycin	Terbinafine
Azithromycin	Atenolol
Ranitidine	Miconazole
Tramadol	Metoprolol
Trimethoprim	Cocaine
Codeine	Cimetidine
Diphenhydramine	Amphetamine

Table 2. List of pharmaceuticals analyzed in river water.

DRINKING WATER RESULTS

Figure 1 shows extracted ion chromatograms of two pesticides (atrazine and carbofuran) spiked into drinking water at 10ppt. Figure 2 shows chromatograms of two pharmaceuticals (clotrimazole and tripolidine) at the same level. The excellent peak shape and signal-to-noise ratio at these low levels was achieved with a 15mL injection of drinking water.
The robustness of the method is demonstrated in Figure 3 where the results after 250 and 500 injections are compared to the first injection. The retention time and peak shape are maintained for more than 500 analyses.
One major advantage of the online method is the reduction of on-site sampling volume from 1 liter to 20 mL. The extraction protocol was reduced from 3-5 hours to less than 5 minutes.

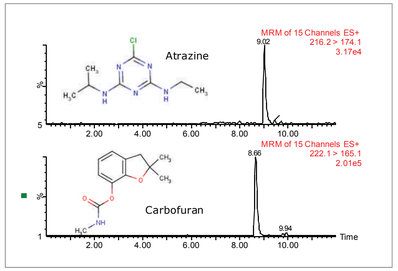

Figure 1. Extracted ion chromatograms of Atrazine and Carbofuran at 10 ppt in drinking water.

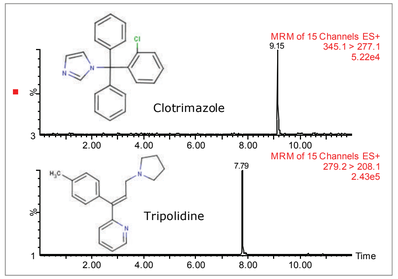

Figure 2. Extracted ion chromatograms of Clotrimazole and Tripolidine at 10 ppt in drinking water.

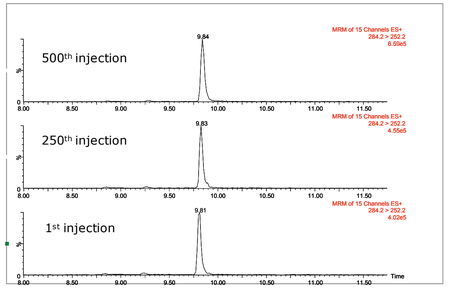

Figure 3. Extracted ion chromatograms of Metaclor (100 ppt) showing excellent peak quality after 500 injections .

RIVER WATER RESULTS

The seven sites selected for sampling included locations upstream and downstream from water treatment works as well as tributaries to the main river. Figure 4 shows the sampling sites selected. Some compounds were detected at multiple sampling sites, whereas others were not detected at all. Figure 5 summarizes the number of sites that each compound was detected over the LOQ. With the 1 000:1 enrichment factor, the limit of quantitation (LOQ) was 10 ppt. Atenolol and metoprolol, beta blockers for treating hypertension, and the antibiotic azythromycin were detected at 5 of the 7 sites.

Figure 6 displays the results for each of the sampling sites. The red boxes indicate the sites at which each compound was detected above the LOQ. Diphenhydramine (an antihistamine), atenolol (for hypertension) and azythromycin (an antibiotic) were detected at higher concentrations at site 5 compared to site 4 (data not shown), suggesting the water treatment works between those sites may have released those compounds into the river unchecked. However, this was a very small study and these results may warrant further investigation.

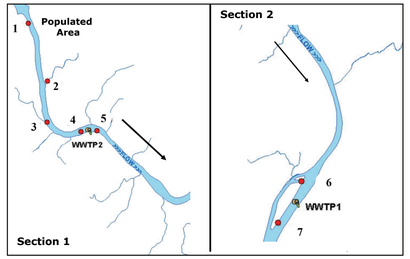

Figure 4. Sampling sites for river water.

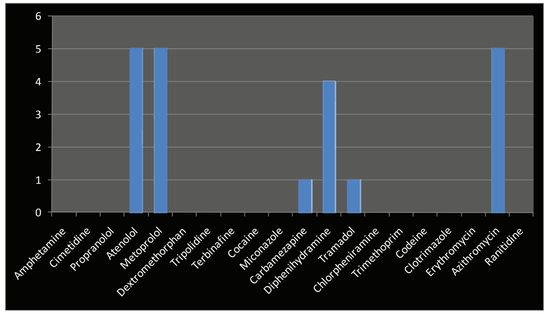

Figure 5. Number of sites at which compound was detected above the LOQ

River Water Sites	1	2	3	4	5	6	7
Amphetamine							
Cimetidine							
Propranolol							
Atenolol	■			■	■	■	■
Metoprolol	■			■	■	■	■
Dextromethorphan							
Tripolidine							
Terbinafine							
Cocaine							
Miconazole							
Carbamezapine					■		
Diphenhydramine	■				■	■	■
Tramadol					■		
Chlorpheniramine							
Trimethoprim							
Codeine							
Clotrimazole							
Erythromycin							
Azithromycin	■			■	■	■	■
Ranitidine							

Figure 6. Analytical results from river water sites. Red boxes indicate analyte was detected above the LOQ at that site.

CONCLUSIONS

- **Same on-column concentration can be achieved with a much smaller sample volume**
- **Reduction in manual sample handling resulting in excellent precision**
- **Unattended operation frees up analyst for other tasks**
- **Reduction in cost per test due to reduced labor costs**
- **Reduced turnaround time resulting in increased throughput**

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