

## INTRODUCTION

- Sulfonamides are common chemotherapeutic agents used in veterinary and human medicine to treat bacterial and protozoa infections as well as used at subtherapeutic concentrations to promote animal growth.
- Because of their widespread usage, low soil sorption, and slow microbial and chemical degradation, sulfonamides are some of the most prevalent pharmaceuticals found in waterways.
- In this study, we utilize the sulfonamide enzyme immunoassay to measure sulfonamide levels in water samples obtained from surface water and wastewater treatment plants.
- The enzyme immunoassay results were confirmed with results obtained from q-TOF-LC-MS/MS.

## METHODS

### ELISA Procedure

- Direct competitive format using 96 well ELISA plates.
- Standard curve: 0, 0.025, 0.05, 0.1, 0.25, and 0.1 ppb.
- Filtered water from different sources was assayed directly.
- Dilute sample: (2-20x) when needed.

### Sample collection

Samples from two different wastewater treatment plants (using an activated sludge or a trickling system) and river water were collected, centrifuged, and filtered.

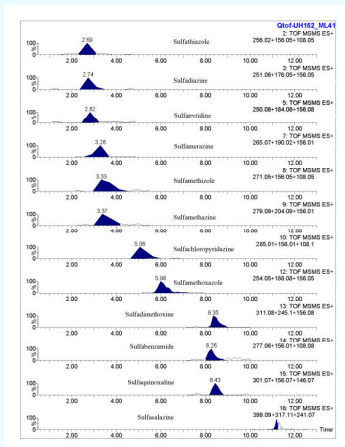
### Sample cleanup for LC-MS/MS

Oasis MCX solid phase cartridges were used (20 mL of wastewater; 100 mL of river water), eluted with 2.5% ammonium/MeOH, recovery checks were done at 10 ng or 100 ng in 100 mL H<sub>2</sub>O.

### LC-MS/MS parameter

| HPLC   | MS/MS                           |
|--|---------------------------------|
| Column: Waters Atlantis dC18<br>2.1 x 100 mm, 3 μm | Instrument: Waters Q-ToF API-ES |
| Detector: Waters PDA 2996                          | Ionization ES+                  |
|  | Scan type MRM                   |
|  | Software UH-152 MassLynx 4.1    |

Figure 1. LC-MS/MS quantitation (1 precursor ion and 2 product ions) corresponding to sulfonamides mixture (100 pg injected, 13 compounds showing here).



## ACKNOWLEDGMENTS

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## RESULTS AND DISCUSSION

- The ELISA gave good recoveries of sulfamethoxazole from spiked water samples and showed a relatively small variance (Table 1).

Table 1. Recovery and precision of sulfamethoxazole from spiked water.

| Spiked (ppb) | n | Found (ppt)   | % recovery | % CV |
|--------------|---|---------------|------------|------|
| 0.05         | 4 | 0.048 ± 0.010 | 96         | 20   |
| 0.10         | 4 | 0.092 ± 0.009 | 92         | 10   |
| 0.25         | 4 | 0.248 ± 0.015 | 99         | 6    |
| 0.50         | 4 | 0.500 ± 0.031 | 100        | 6.2  |

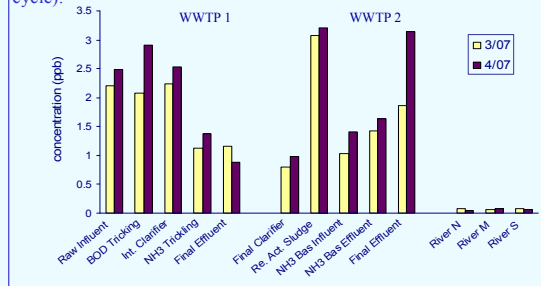
- The antibody is very sensitive and can detect sulfamethoxazole at 15 ppt (Table 2).
- A high degree of cross-reactivity toward sulfamethoxy-pyridazine, sulfachloropyridazine, and sulfadimethoxine has been observed (Table 2).
- Sulfadiazine, sulfacetamide, sulfamerazine, sulfaganidine, sulfabenzamide, and sulfamethazine have 50% B/B<sub>0</sub> > 100 ppb (Table 2).

Table 2. Sensitivity and cross-reactivity of sulfamethoxazole antibody.

| Compound                | 95% B/B <sub>0</sub> (ppb) | 50% B/B <sub>0</sub> (ppb) |
|-------------------------|----------------------------|----------------------------|
| Sulfamethoxazole        | 0.015                      | 0.255                      |
| Sulfamethoxy-pyridazine | 0.020                      | 0.146                      |
| Sulfachloropyridazine   | 0.019                      | 0.180                      |
| Sulfadimethoxine        | 0.016                      | 0.42                       |
| Sulfamethizole          | 0.116                      | 2.5                        |
| Sulfasalazine           | 0.450                      | 7.9                        |
| Sulfapyridine           | 0.365                      | 7.6                        |
| Sulfamer                | 0.068                      | 12.0                       |
| Sulfaquinoxaline        | 0.130                      | 26.5                       |

- The sulfamethoxazole equivalence levels (ppb) obtained from ELISA are in general agreement between the two collection periods (Figure 2).
- Water treatment plants were not able to process sulfonamides efficiently (Figure 2).
- Surface water has low but measurable amounts of sulfonamides (Figure 2).

Figure 2. Sulfamethoxazole equivalency in wastewater treatment plants (WWTP) and river water (mean of 2 repeats in each survey cycle).



- Sulfonamide levels from ELISA results were comparable with those found in reports from Europe as well as USGS surveys (Rev. Environ. Contam. Toxicol. 187:67-101, 2006).
- LC-MS/MS results show recoveries for the sulfonamides; in general are > 50%.
- LC-MS/MS confirms the presence of sulfomethoxazole and sulfapyridine in wastewaters.
- ELISA can serve as a cost-effective, portable, and high throughput analytical method for sulfonamide analysis. LC-MS/MS will provide detailed composition of sulfonamides in water.