

UPLC™/MS/MS Analysis of Benthic cyanobacterial blooms in stormwater and retention ponds

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- Extensive cyanobacteria-dominated mats were observed during late summer of 2021 within a stormwater retention pond network in northwest Florida.
- Common in residential communities across the region, such ponds may be routinely visited by humans, domestic and wild animals, and often contain a variety of aquatic life.
 - we investigated the temporal heterogeneity of potentially toxic cyanobacteria, associated toxins, and ‘under the radar’ bioactive peptides throughout the fall at several sites within the network.
 - Multiple toxin classes were detected in whole water samples using targeted LC/MS/MS analysis including levels that far exceeded national recreational guidelines.
 - Non-targeted analysis also revealed a collection of potentially unknown cyanobacteria-associated compounds that, along with known toxins, oscillated with periods of heavy rainfall and temperature variation.
- Furthermore, these retention ponds may serve as a consistent source for cyanobacteria biomass accumulation that is transported downstream in the form of cells for colonization and cyanotoxins to estuarine waters.
- Data from these and other ponds within the US will be discussed showing the wide variety of toxins that can be present in freshwater systems.

Algal Toxins

- These toxins are typically grouped by their main mode of action, such as:

- Hepatotoxins (toxins that damage the liver)
- Dermatotoxins (toxins that damage the skin)
- Neurotoxins (toxins that damage the nerve cells)



- Some toxins can even be harmful in more than one way, such as cylindrospermopsin, which not only have the ability to harm the liver, but are also harmful to kidneys and may even cause cancer

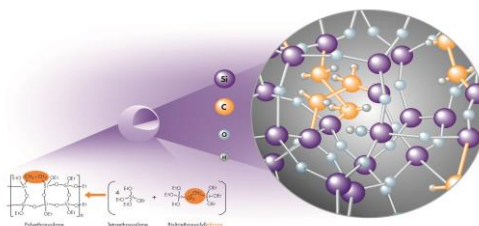
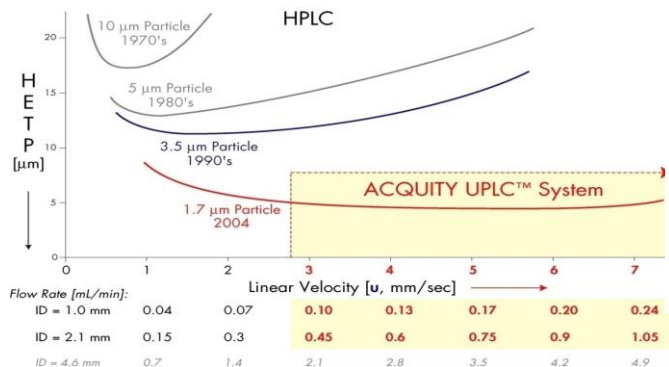
<https://www.greenwaterlab.com/what-are-algal-toxins/>

Area Studied



System Used...UltraPerformance LC/MS/MS

- Acquity HClass UPLC and Xevo™ TQ-S micro
- HSS T3 2.1x100mm Column (1.8µm)
 - Higher separation power
 - Higher tensile strength
- Aqueous Formic/ACN Gradient

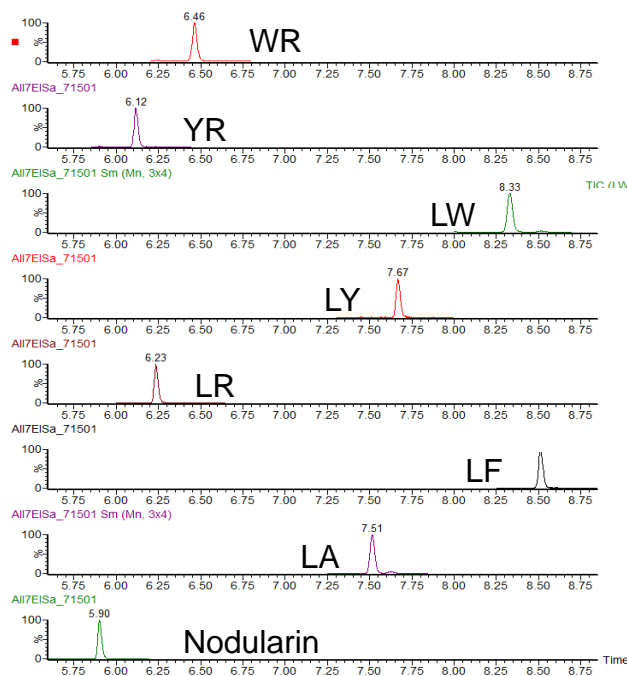
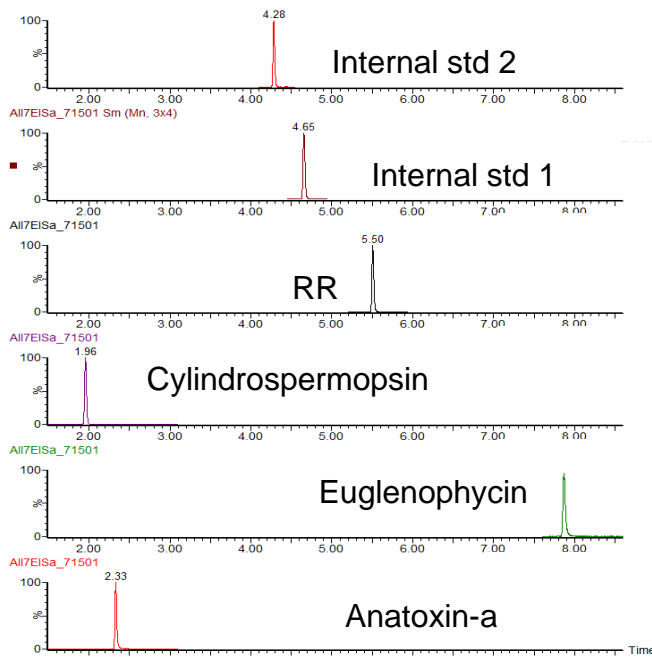


Xevo™ TQ-S micro

Acquity™
Ultra Performance LC

Analysis done using UPLC/MS/MS method (Expanded!)

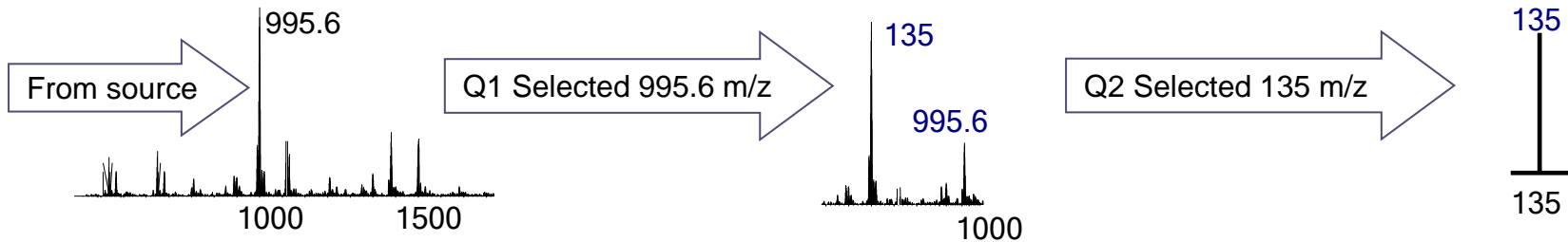
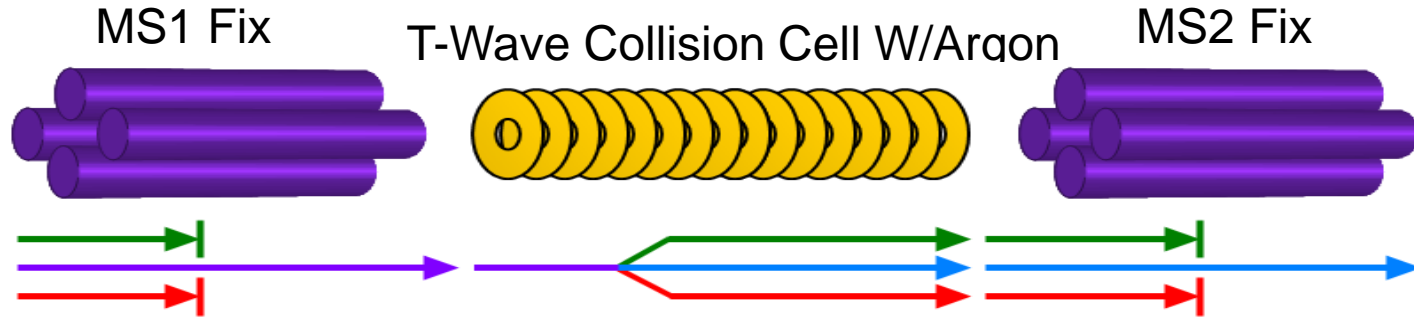
- Allows for screening of microcystins, anatoxin, euglenophycin, nodularin and cylindrospermopsin in a <14 minute run



Expanded List

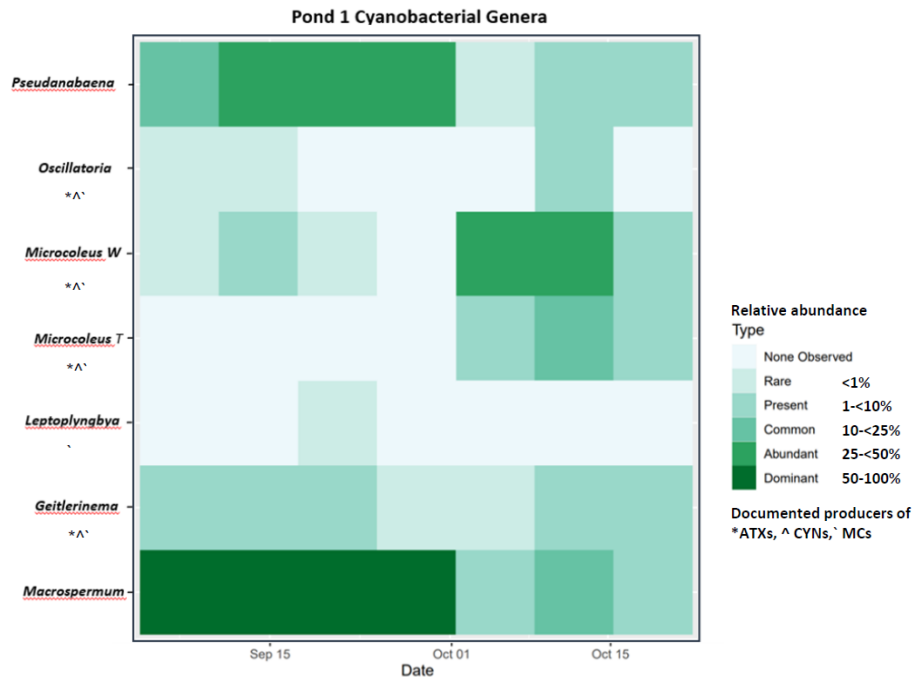
LR	Phe-Ala
RR	Anabaenopeptin A
YR	Anabaenopeptin B
LA	Cylindrospermopsin (CYN)
LF	Anatoxin
LY	Nodularin
LW	Euglenophycin
WR	Ethylated MC-LR (d5)(IS)
HtYR	PI-Cylco (IS)
D-Asp3-RR	Leu-Enk (IS)
D-Asp3-LR	Micropeptin 1106
D-Asp3-Dhb7-MC-Htyr	Aeroginosamide B
Homo-Anatoxin	7-epi-CYN
Deoxy-CYN	Anabaenopeptin E/F

Multiple Reaction Monitoring (MRM)

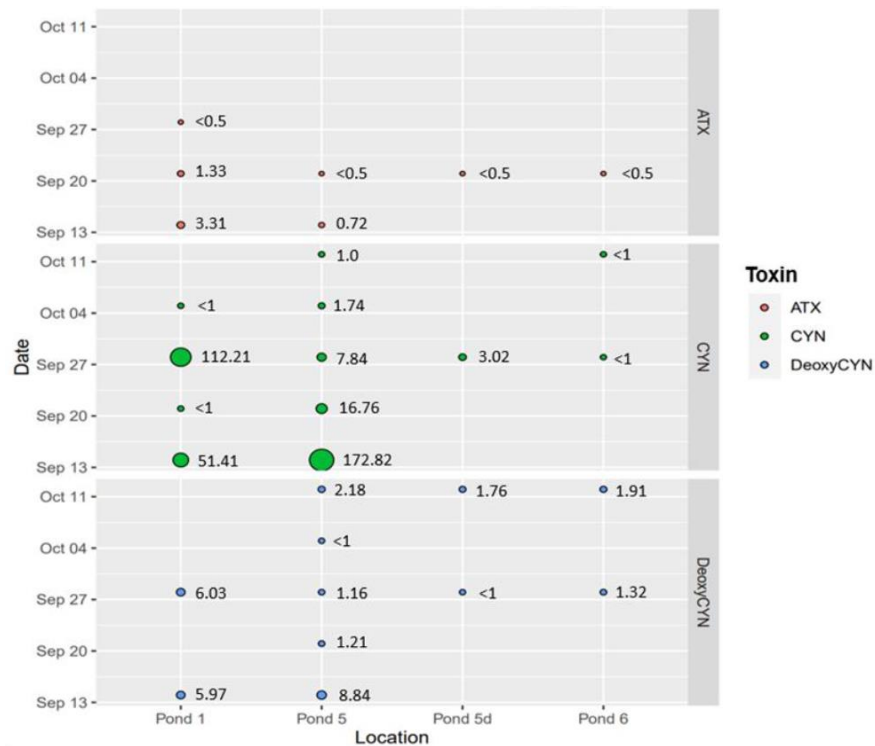


- The system is set up for selectivity, allowing only a selected product ion to be fragmented and one fragment ion to be detected.
- Multiple MRM's can also be use, as well as several fragments from a specified product ion for confirmation purposes.

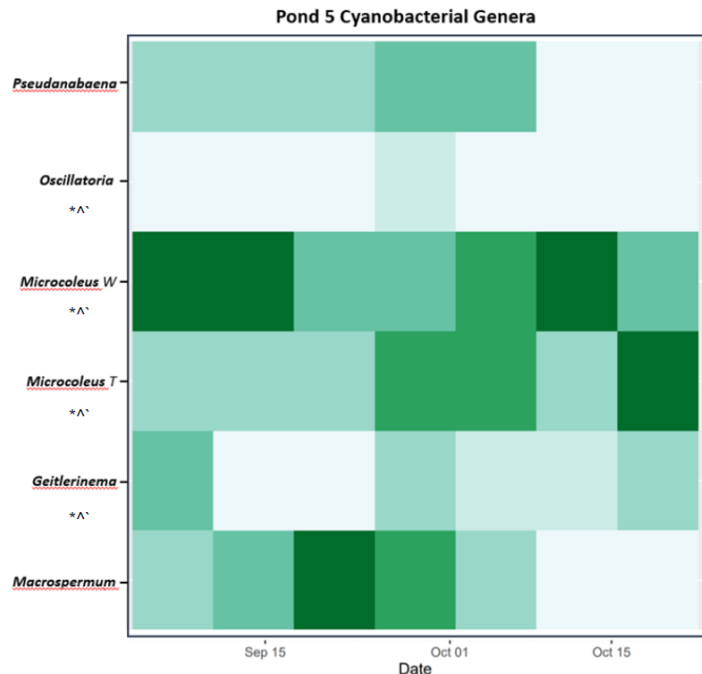
Sample Results



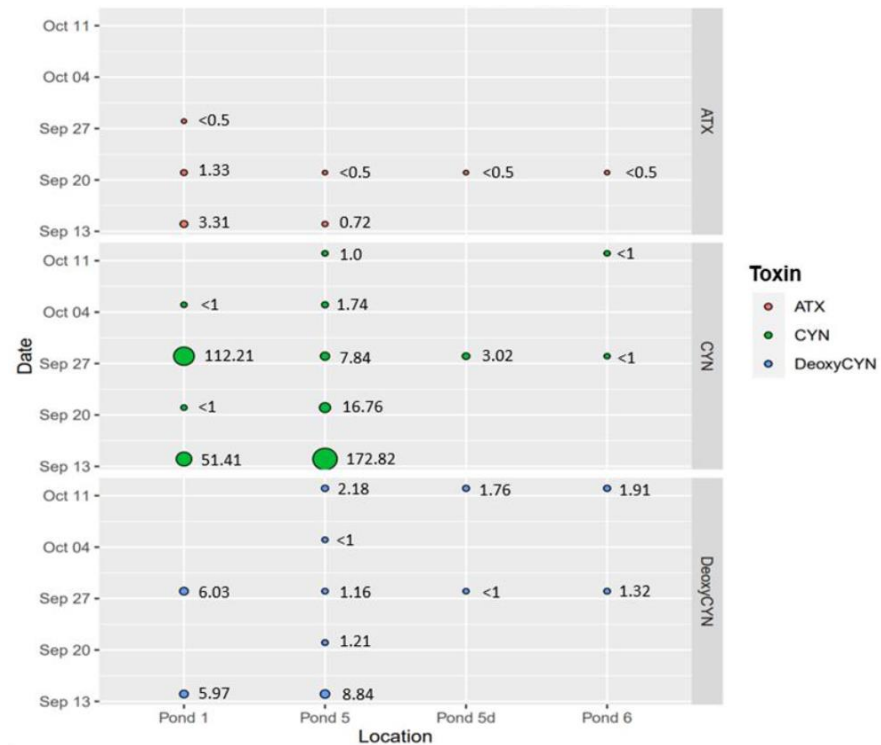
LC-MS/MS Results (µg/L)(ppb)



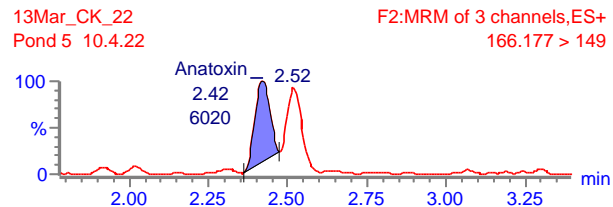
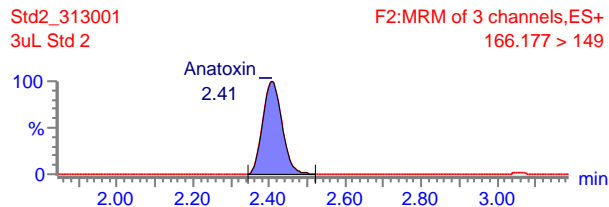
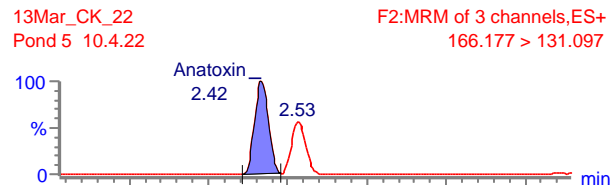
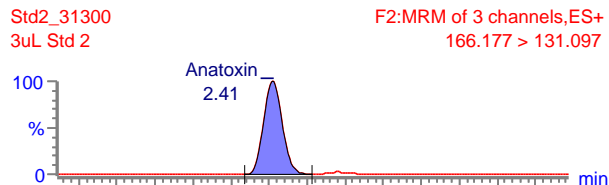
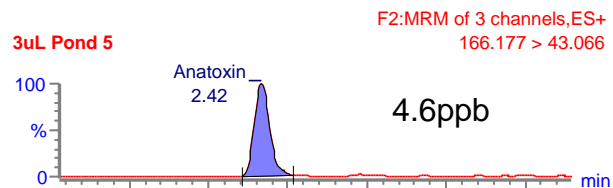
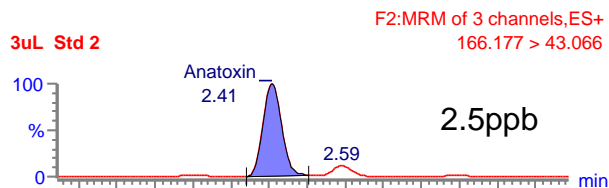
Sample Results



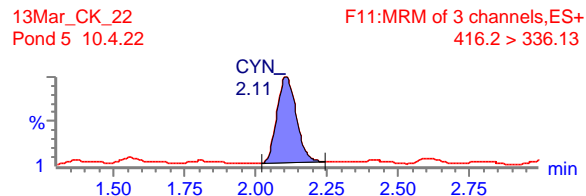
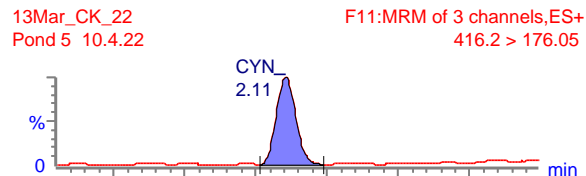
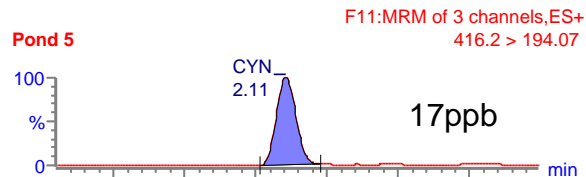
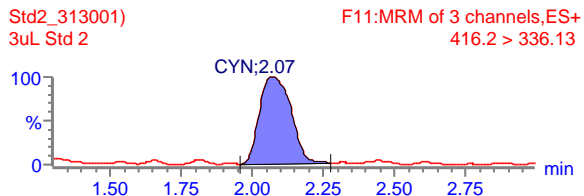
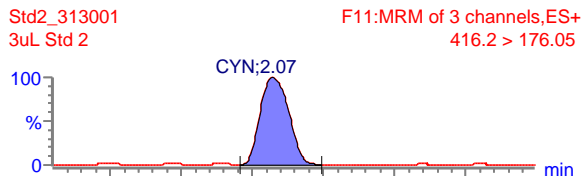
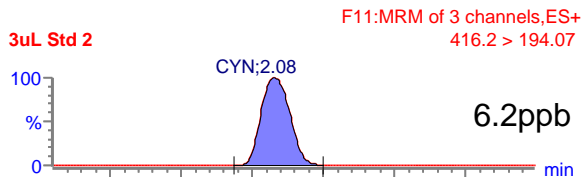
LC-MS/MS Results (µg/L)(ppb)



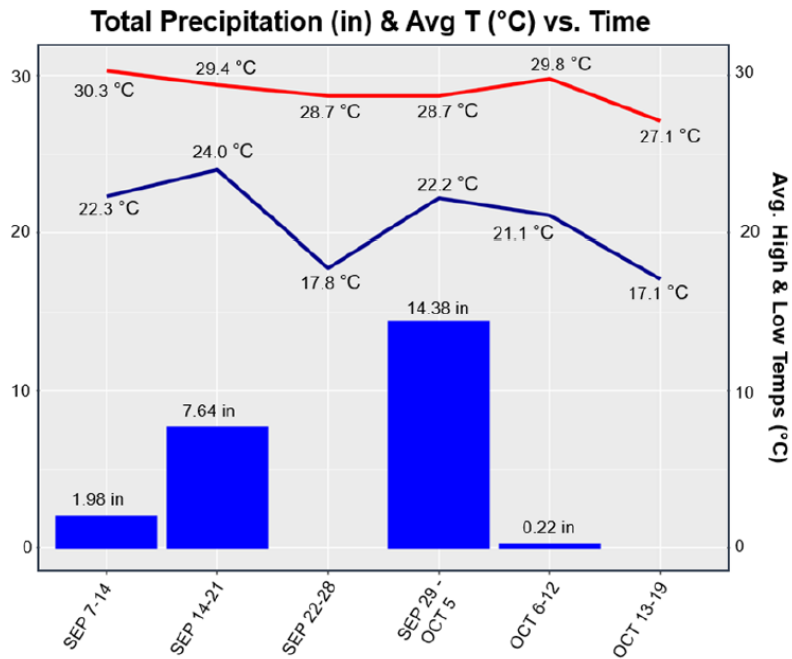
- Anatoxin and Cylindrospermopsin were the main toxins detected



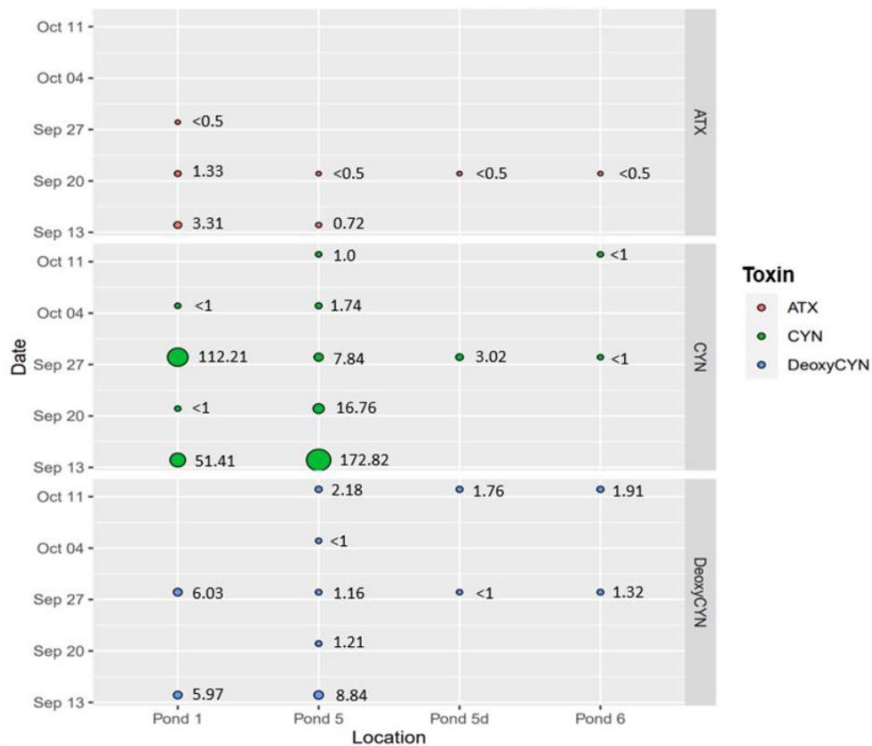
- Anatoxin and Cylindrospermopsin were the main toxins detected



Sample Results



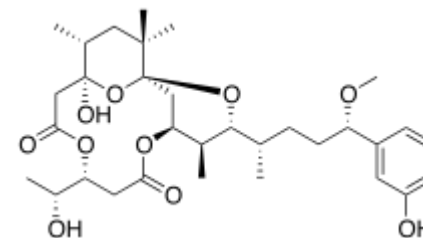
LC-MS/MS Results (µg/L)(ppb)



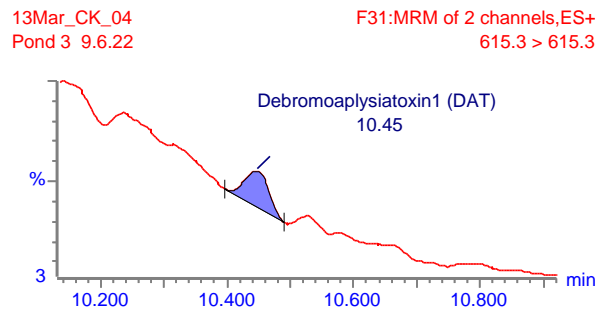
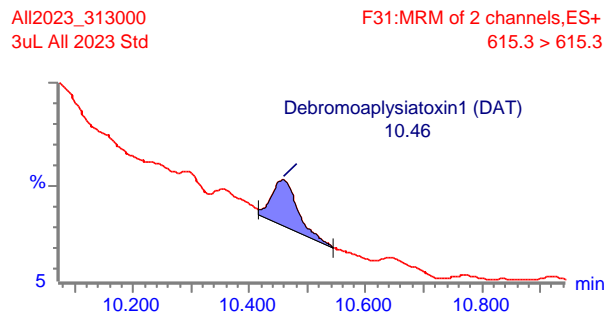
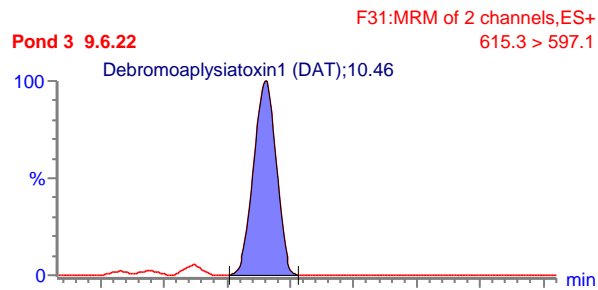
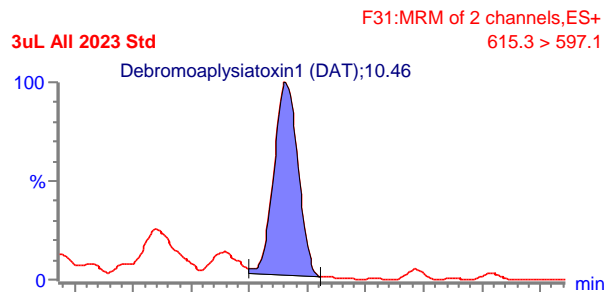
Additional Toxins? Dermatotoxins

- Dermatotoxins (toxins that damage the skin)
- Examples.
 - Debromoaplysiatoxin (DAT)
 - Aplysiatoxin-1 (AT)
 - Lyngbyatoxin-a

- Debromoaplysiatoxin (DAT)



Additional Toxins—Dermatoxins (DAT)

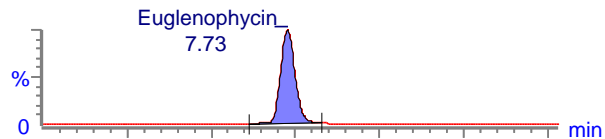


Additional Sampling later in the year (Mat samples)

Additional toxin found (Euglenophycin)

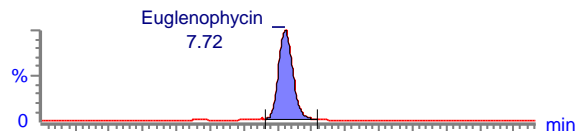
All2023_613001
3uL All 2023 Std

F9:MRM of 3 channels,ES+
288.2 > 97.056

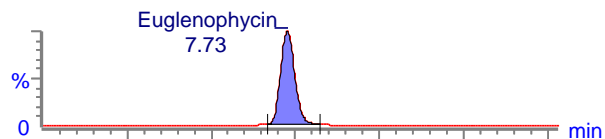


CK_29_M1
Pond 1

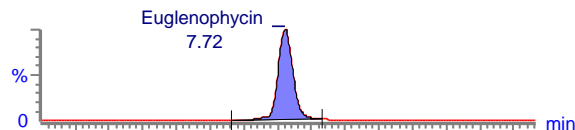
F9:MRM of 3 channels,ES+
288.2 > 97.056



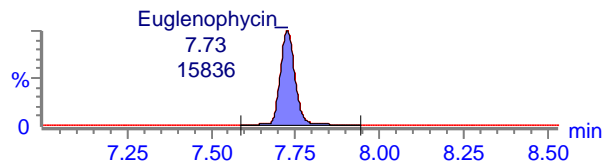
F9:MRM of 3 channels,ES+
288.2 > 110.1



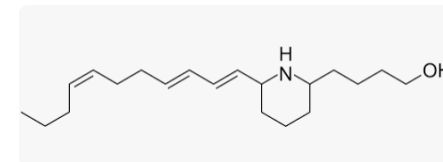
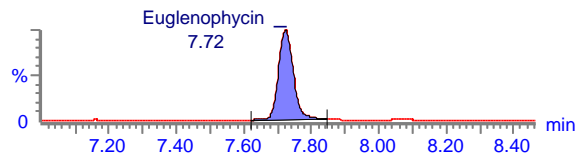
F9:MRM of 3 channels,ES+
288.2 > 110.1



F9:MRM of 3 channels,ES+
288.2 > 136.1

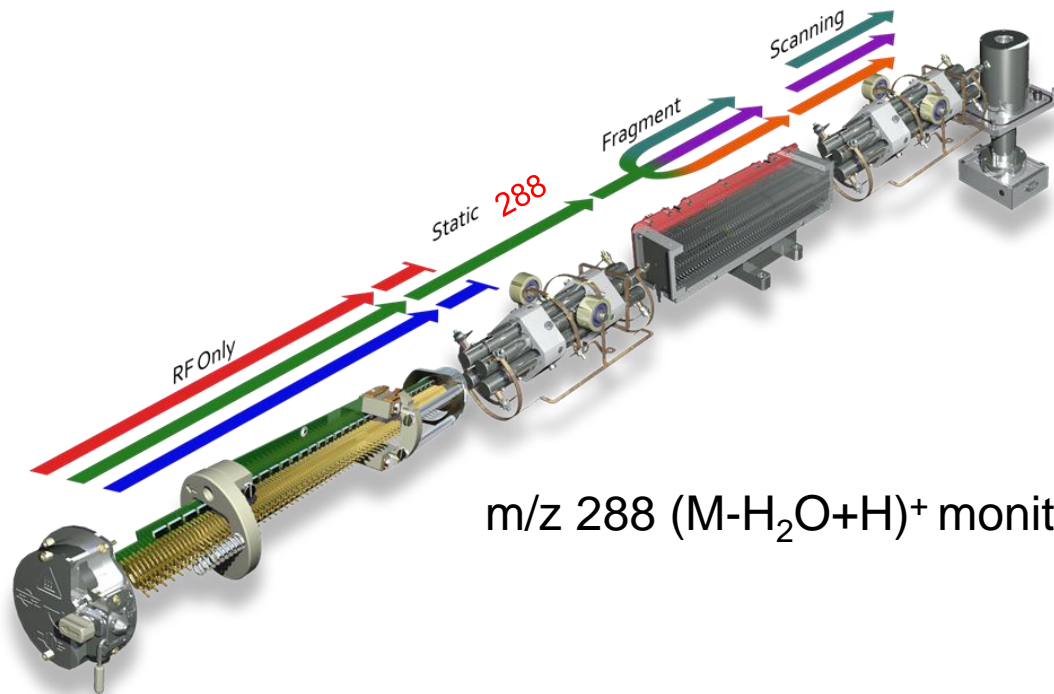
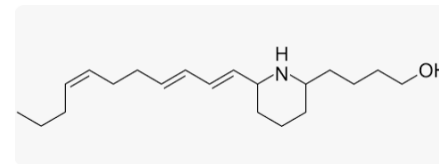


F9:MRM of 3 channels,ES+
288.2 > 136.1



Product Ion Scanning

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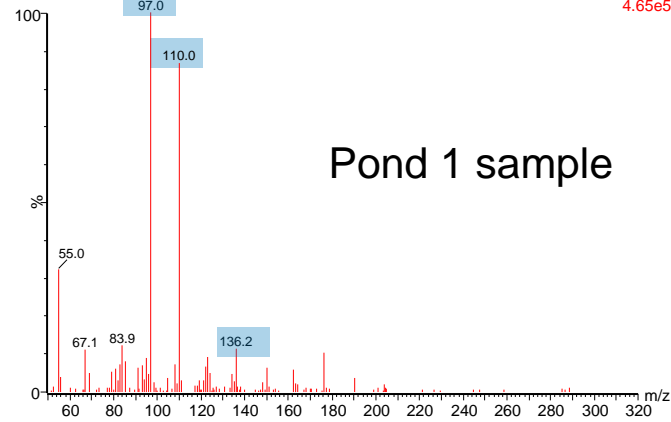
m/z 288 ($M-H_2O+H$)⁺ monitored and fragmented

Confirmed fragment ions for Euglenophycin

3uL All 2023 Std

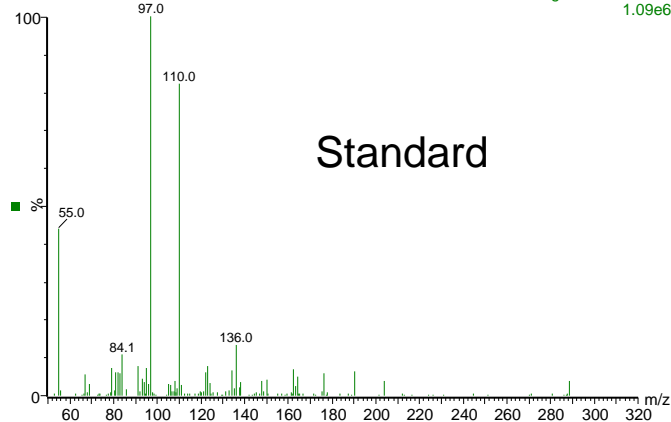
CK_29_M1d2

1: Daughters of 288ES+
4.65e5



All2023_613001d2

1: Daughters of 288ES+
1.09e6



- Same fragment ions for both
- Match literature references for Euglenophycin

Paul V. Zimba, I-Shuo Huang, Danielle Gutierrez, Woongghi Shin, Matthew S. Bennett, Richard E. Triemer, Harmful Algae, Volume 63, 2017, Pages 79-84, ISSN 1568-9883, <https://doi.org/10.1016/j.hal.2017.01.010>

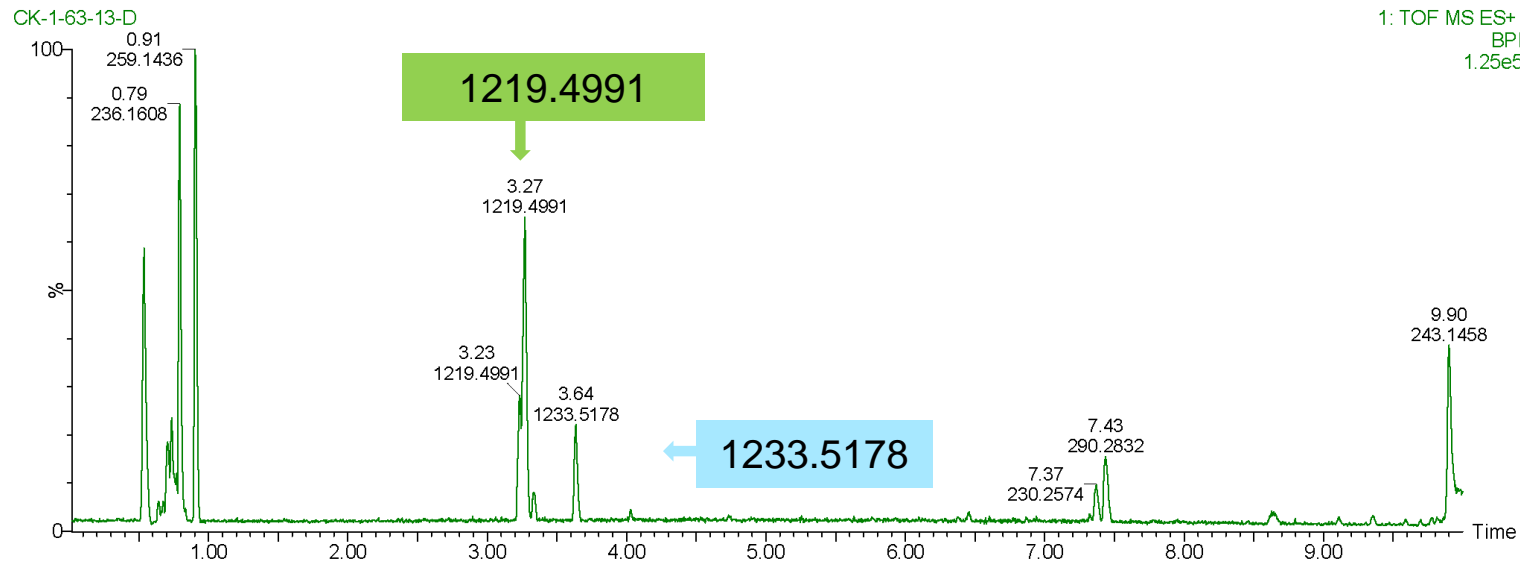
**HOT OFF
THE
PRESS**

Waters™

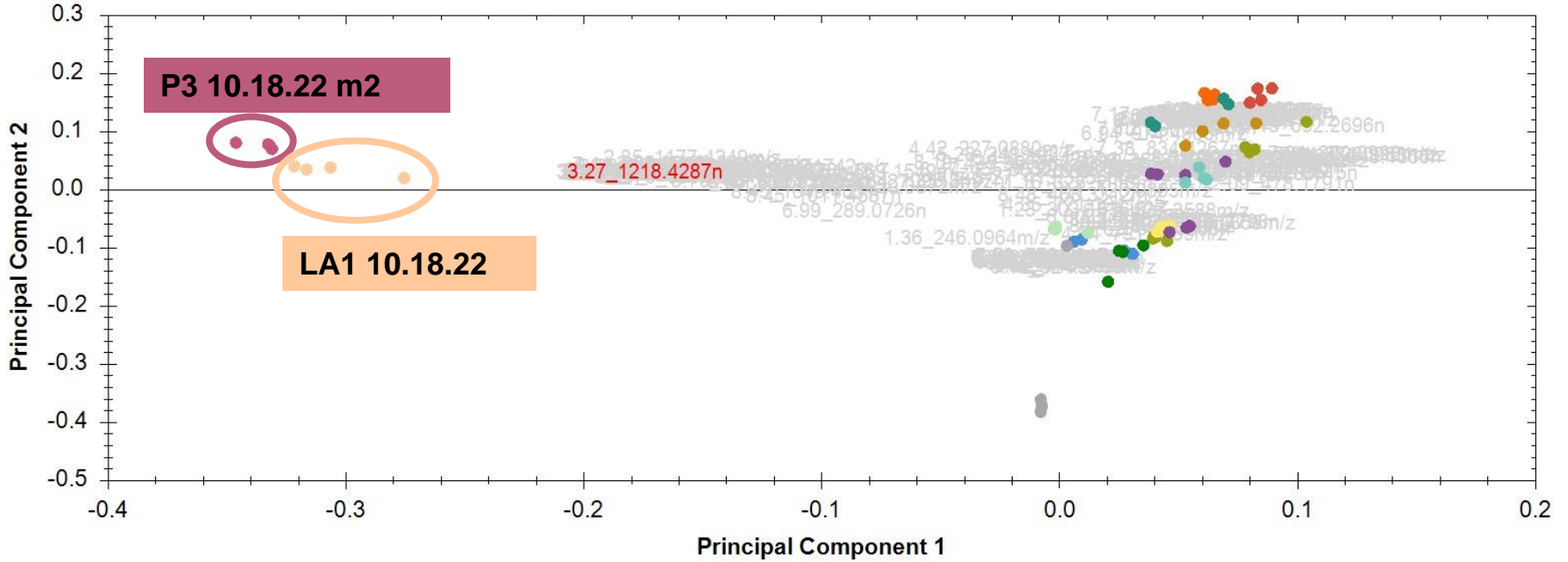
Preliminary Un-Targeted (TOF) Analysis



P3 10.18.22 Mat 2 Chromatogram



Principal Components Analysis



KEY

P3 10.25.22	P1 10.25.22	P3 9.20.22	P1 10.4.22	P3 11.1.22	P1 10.11.22 m2	P1 10.11.22 m1
LA1 10.18.22	P3 10.11.22 m1	P3 10.11.22 m2	P3 10.18.22 m2	P1 9.20.22	P3 11.1.22	P5 10.25.22

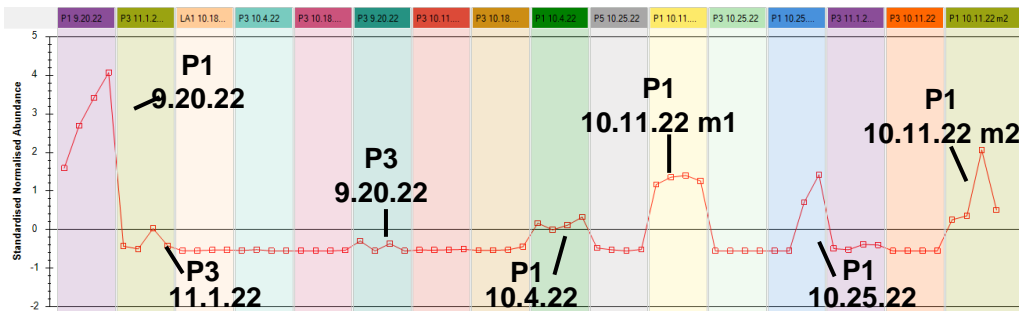
Major Unknown Compounds of Interest

Location/Date	Neutral Mass	Retention Time (min)	Max Abundance
P3 10.18.22	1232.4447	3.64	11428
P3 10.18.22 M2	1218.4287	3.27	37915
P3 10.11.22 m2	962.4999	6.94	53295
P3 9.20.22	692.2696	5.15	6954
P3 11.1.22 M2	672.3335	8.30	1518
P1 9.20.22	670.3177	7.81	4803

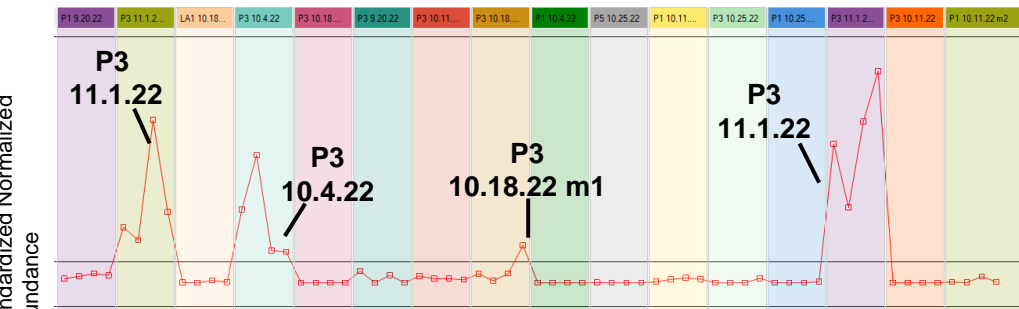
Normalized Abundance Profiles Across Mat samples

Waters™

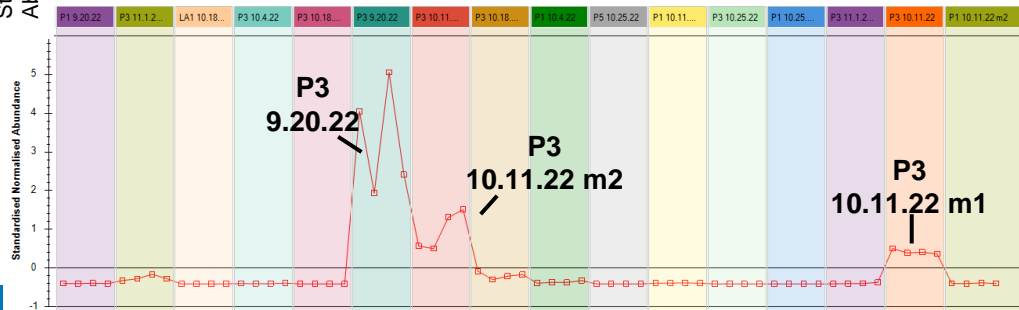
670.3177



672.3335



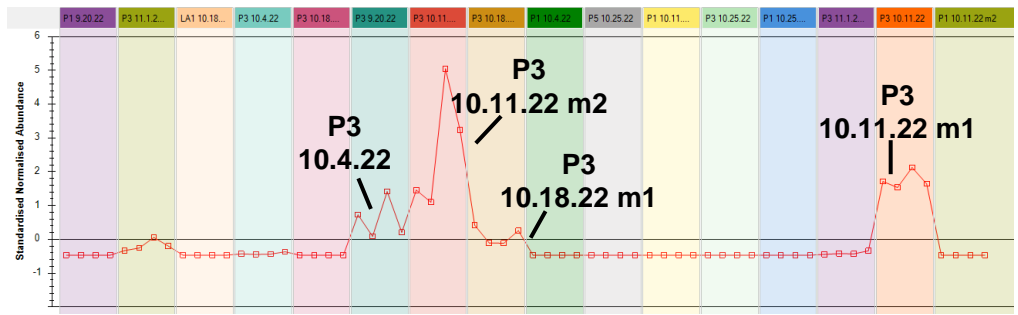
692.2696



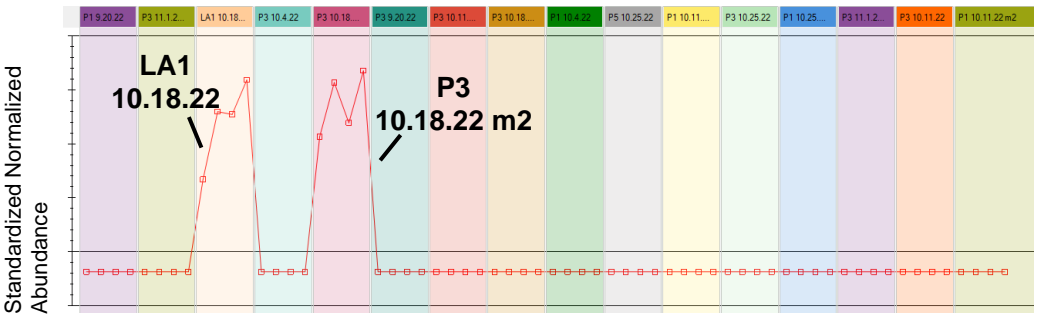
KEY
P3 10.25.22
P1 10.25.22
P3 9.20.22
P1 10.4.22
P3 11.1.22
P1 10.11.22 m2
P1 10.11.22 m1
LA1 10.18.22
P3 10.11.22 m1
P3 10.11.22 m2
P3 10.18.22 m2
P1 9.20.22
P3 11.1.22
P5 10.25.22
P3 10.18.22

Standardized Normalized Abundance Profiles

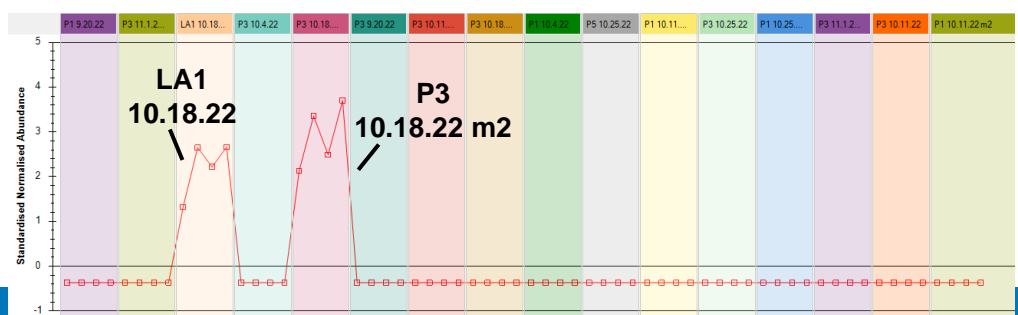
964.4999



1218.4287



1233.4447



KEY	
	P3 10.25.22
	P1 10.25.22
	P3 9.20.22
	P1 10.4.22
	P3 11.1.22
	P1 10.11.22 m2
	P1 10.11.22 m1
	LA1 10.18.22
	P3 10.11.22 m1
	P3 10.11.22 m2
	P3 10.18.22 m2
	P1 9.20.22
	P3 11.1.22
	P5 10.25.22
	P3 10.18.22

Summary

- Mass Spectrometry offers a sensitive and selective method to detect various toxins.
- UPLC offers high resolution and fast analysis times with additional toxin analysis easily added to the current method
- MS/MS technology can also be used for other critical/emerging water assays (pesticides and Persistent organic pollutants (POP's) for example).
- Further work being done using untargeted TOF instrument
- Questions? Interesting samples? Stuart_Oehrle@waters.com

Acknowledgements

- Waters Corporation
- Northern Kentucky University
- University of North Caroline-Wilmington

Thank You for Attending

