Adventures in Targeted Toxin Analysis of various Water sources..the more you look the more you find

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Abstract

- Toxin analysis of various water sources (recreational and intake for drinking) is critical from a safety standpoint. Targeted LC/MS/MS analysis is a particularly useful technique to monitor for various algal toxins produced by various algae species. While hundred of variants exist, very few are available as standards or reference material. Fortunately, the common toxins are available however many “others” are beginning to also appear in blooms. Backward (precursor) monitoring can provide a useful screening tool for the detection of any unusual toxins. This talk will cover several examples of toxin analysis of waters from a variety of locations to show how you can “see” (detect) additional compounds and how you can “search” for others with some basic knowledge.

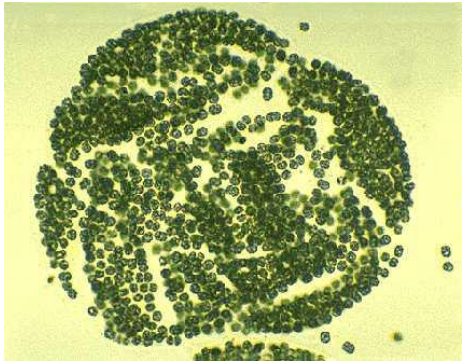
Algal Toxins

- These toxins are typically grouped by their main mode of action, such as:
  - Hepatotoxins (toxins that damage the liver)
  - Dermatoxins (toxins that damage the skin)
  - Neurotoxins (toxins that damage the nerve cells)

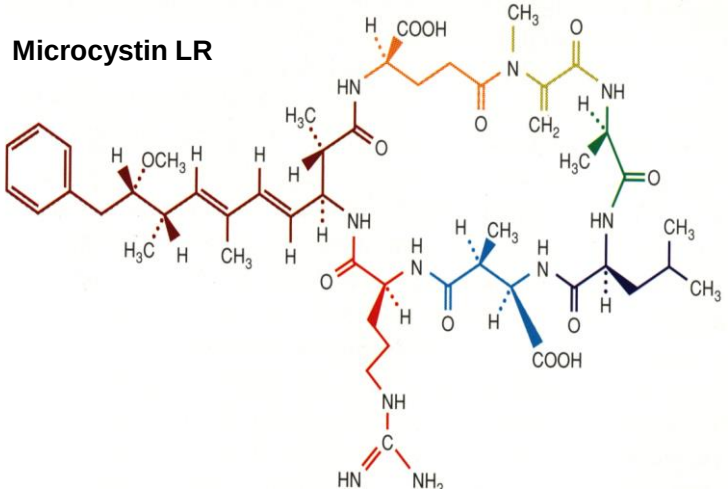
- Some toxins can even be harmful in more than one way, such as cylindrospermopsin, which not only have the ability to harm the liver, but are also harmful to kidneys and may even cause cancer

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

Microcystis

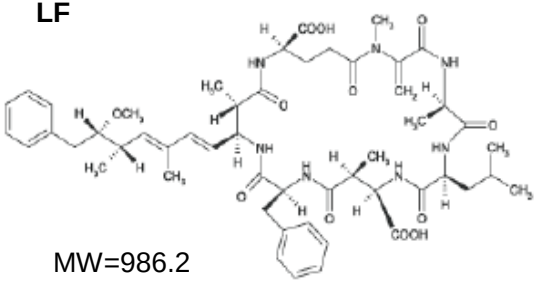


Microcystin LR



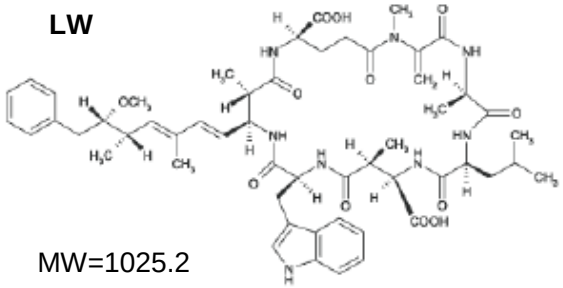
Various Microcystins

LF



MW=986.2

LW



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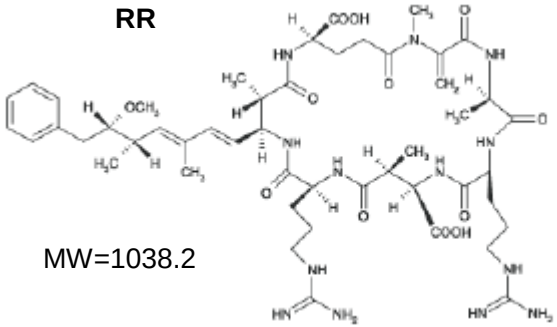
RR



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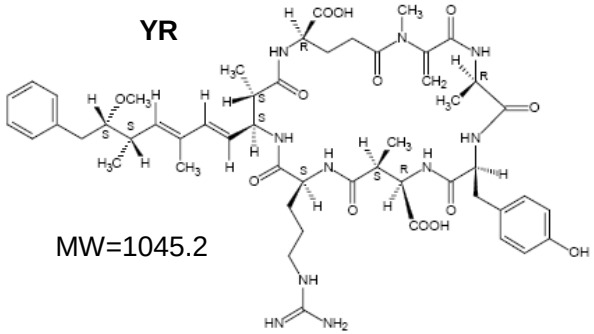
YR



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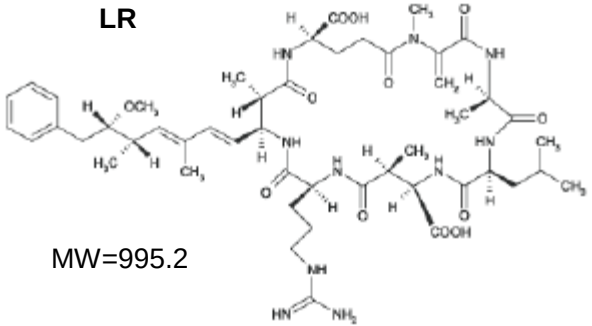
Various Microcystins and others

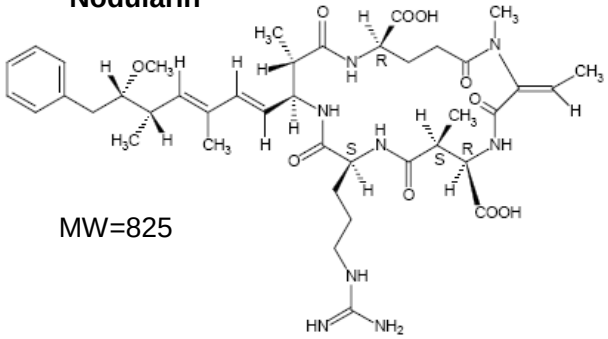
Waters™

LR



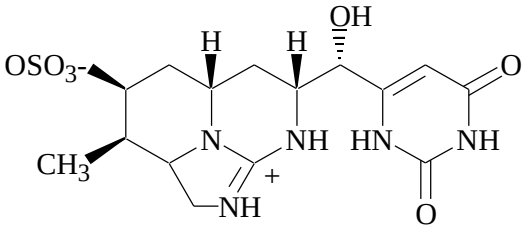
MW=995.2

Nodularin



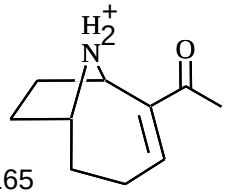
MW=825

Cylindrospermopsin



MW=415.4

Anatoxin



MW=165

Pathway/Route of Exposure

- Recreational waters - dermal, inhalation, and ingestion
- Drinking water - ingestion, dermal, ingestion.
- Dietary Supplements – ingestion
- Vegetables and Fruits - ingestion

This is a "Global Challenge"

Microcystin Detection Assay

Waters™

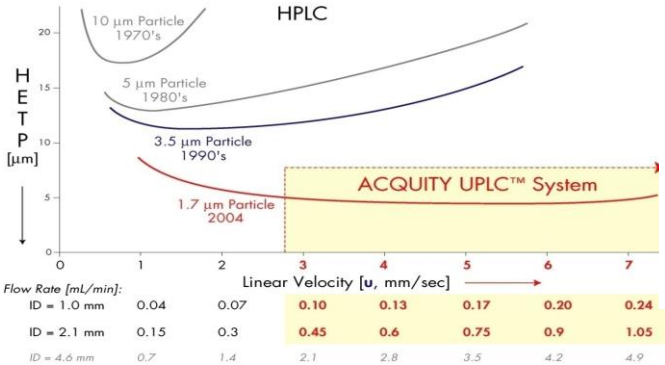
- Enzyme-linked Immunosorbent Assay (ELISA)
  - Uses polyclonal antibodies against different microcystin variants.
  - Samples are read spectrophotometrically to determine microcystin concentration.
  - Detection limit is low ppb
  - Cloudy or Murky samples can pose a challenge
  - Measures total toxins (not specific ones)

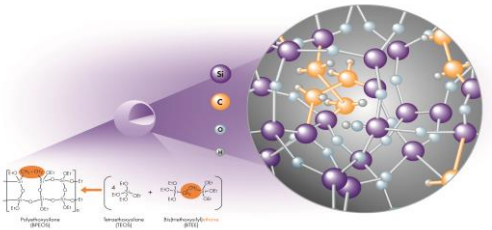
- High-Performance LC
  - Powerful separation capability
  - UV detection (not sensitive w/o SPE)

- LC and Mass Spectrometry
  - Offers specificity and sensitivity

System Used…UltraPerfomance LC/MS/MS

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





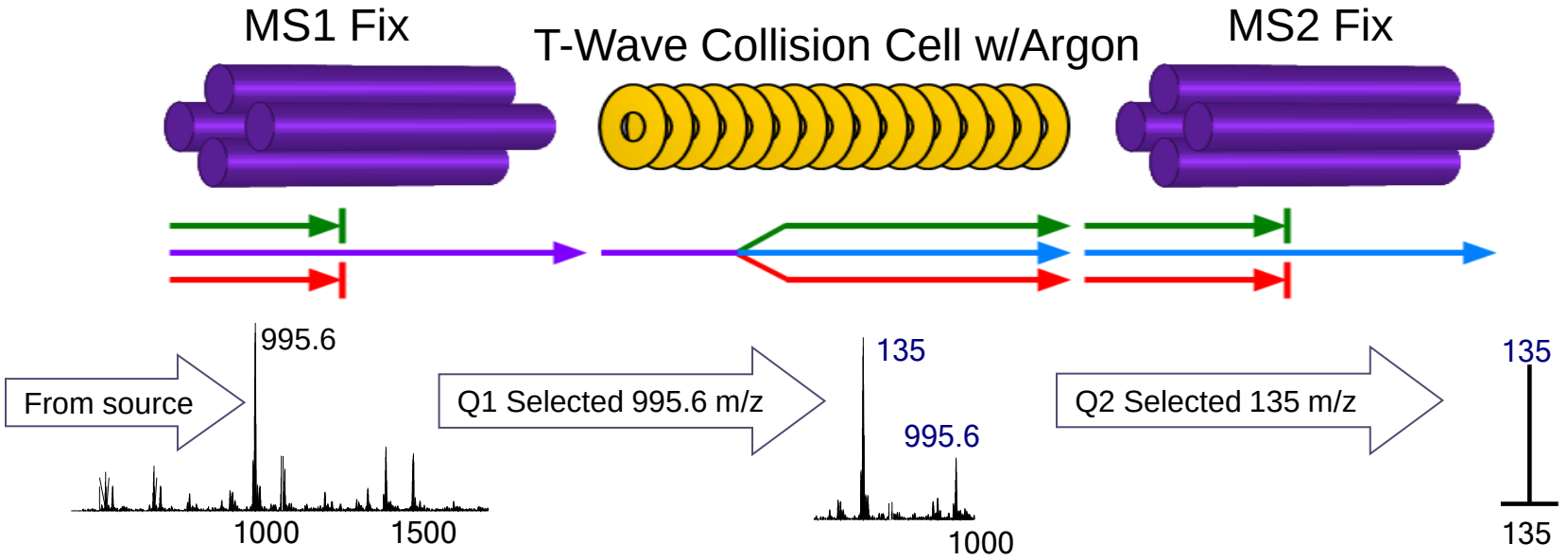
Xevo™ TQ-S micro

Acquity™ Ultra Performance LC

Tandem Experiments done by MRM

Waters™

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.

UPLC/MS/MS Analysis

- UPLC/MS/MS system used.
  - H-Class UPLC and Xevo TQSmicro mass spectrometer
  - Targeted mrm analysis
  - UPLC method used formic acid/ACN gradient based on previous work.
- Current targeted List**

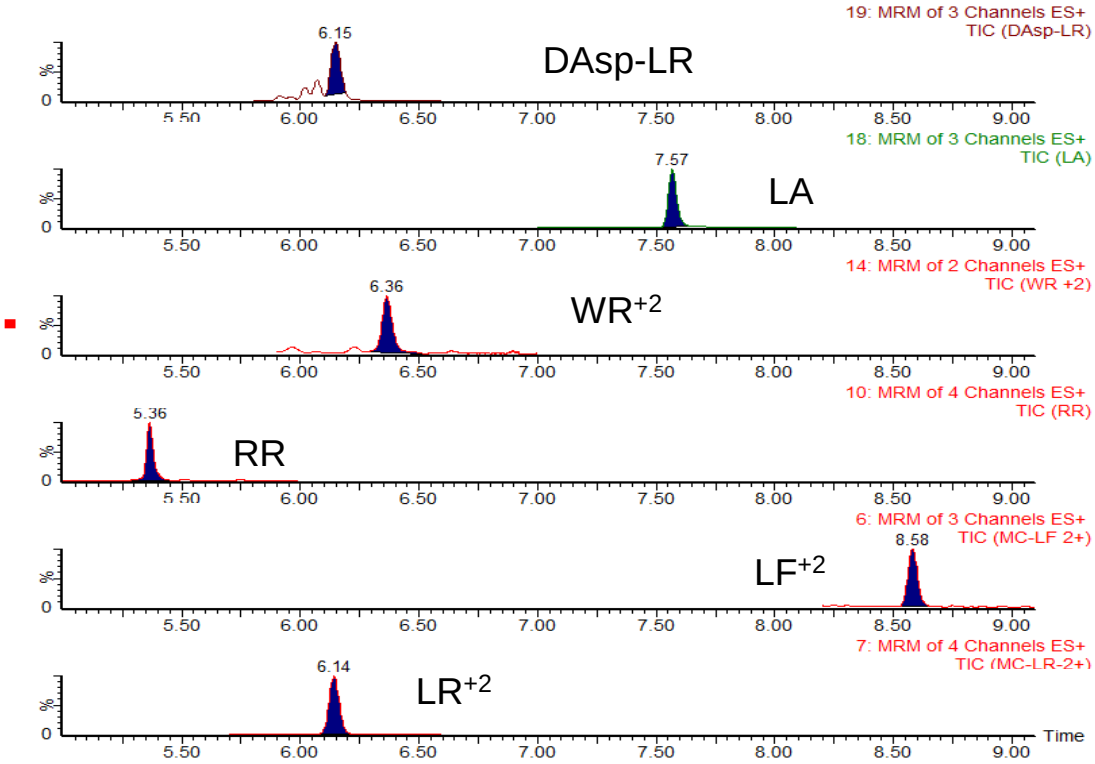
LR	Phe-Ala
RR	Anabaenopeptin A
YR	Anabaenopeptin B
LA	Cylindrospermopsin (CYN)
LF	Anatoxin
LY	Homo-Anatoxin
LW	Dihydro-Anatoxin
WR	Ethylated MC-LR (d5)(IS)
HtYR	PI-Cylco (IS)
D-Asp3-RR	Leu-Enk (IS)
D-Asp3-LR	Micropeptin 1106
D-Asp3-Dhb7-MC-Htyr	Aeroginosamide B
HilR	7-epi-CYN
SPX1	GYM-B
Deoxy-CYN	Anabaenopeptin E/F
Nodularin	Euglenophycin
Debromoaplysiatoxin1 (DAT)	Lyngbyatoxin-a (LA)

**In addition, I do screen for additional toxins epoxy-anatoxin, dihydrohomoanatoxin and guanitoxin based on published mrm transitions

Regulated Methods

- Method 544: Determination of Microcystins and Nodularin in Drinking Water
- Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water
- Method 546: Determination of Total Microcystins and Nodularins in Drinking and Ambient Waters
- ISO 22104:2021: Water quality — Determination of microcystins — Method using liquid chromatography and tandem mass spectrometry (LC-MS/MS)
- Guidelines for Canadian Recreational Water Quality - Cyanobacteria and their Toxins

Example Chromatogram--Florida

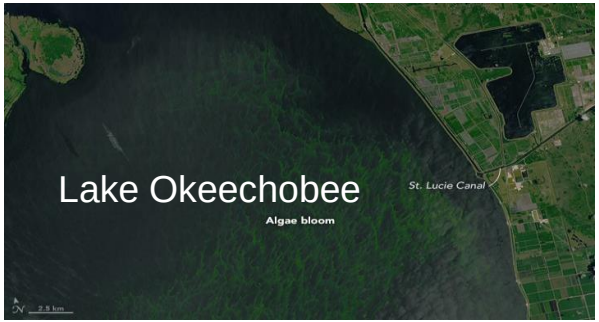


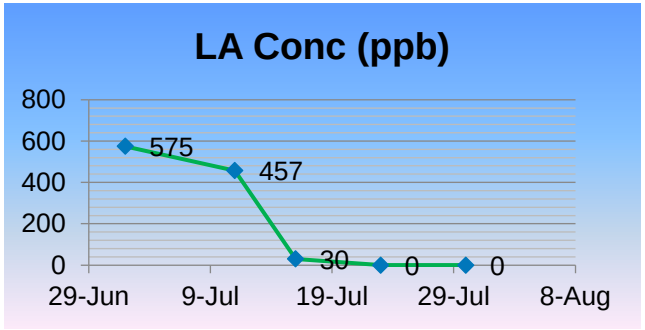
	ppb
LR	2600
RR	6
WR	7
DAsp-LR	120
LF	5
LA	632

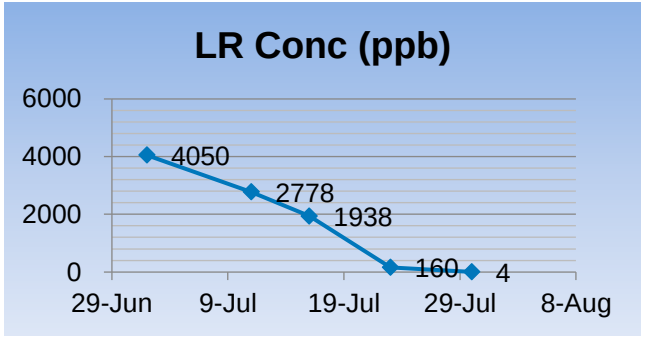
Microcystis aeruginosa

Toxin Incidents

- Florida…Martin County, Florida*







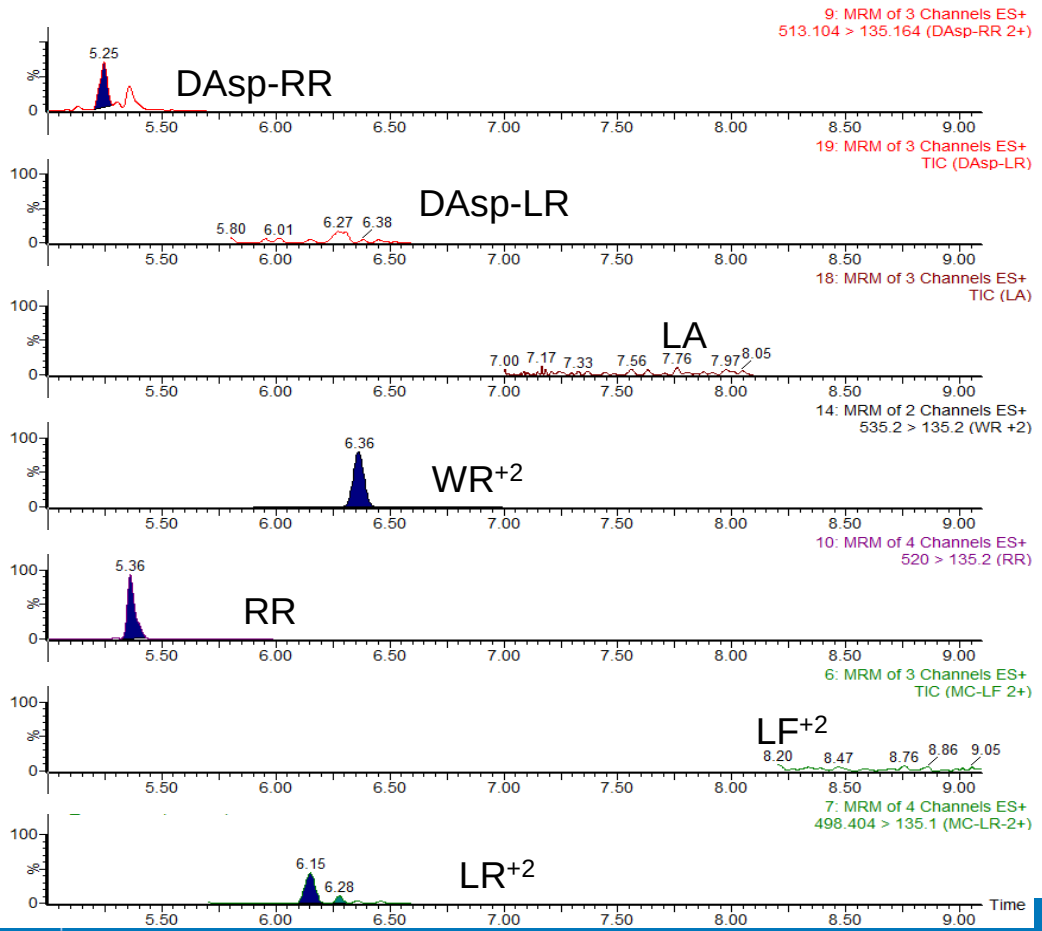
***"Toxin composition of the 2016 *Microcystis aeruginosa* bloom in the St. Lucie Estuary, Florida"**
Oehrle S, Rodriguez-Matos M, Cartamil M, Zavala C, Rein KS.
Toxicon. 2017 Nov; vol.138: p.169-172.

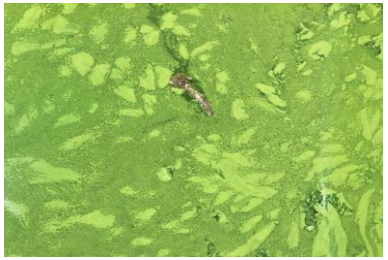
Additional Toxin Incidents

- California…Lake Elsinore

https://www.esa.int/Applications/Observing_the_Earth/Copernicus/The_Sentinel_missions

Example Chromatogram—Lake Elsinore

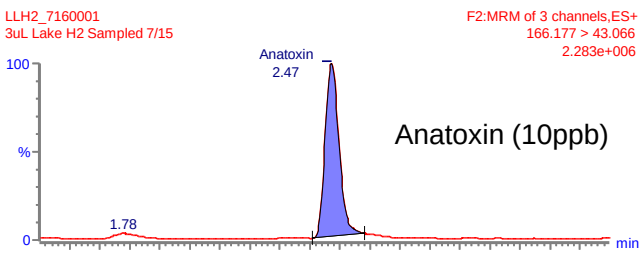




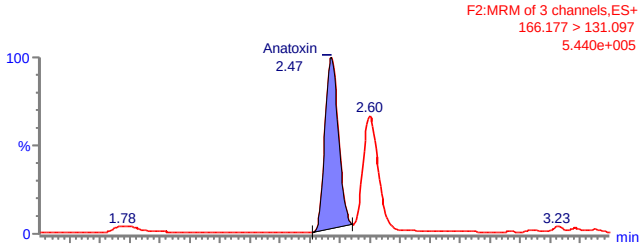
Microcystis flos-aquae

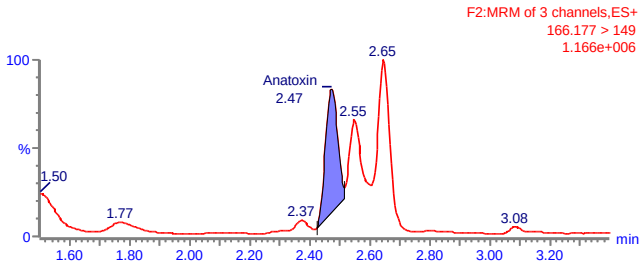
	ppb
LR	6
RR	7671
WR	2628
DAsp-LR	ND
LF	ND
LA	ND
DAsp-RR	142

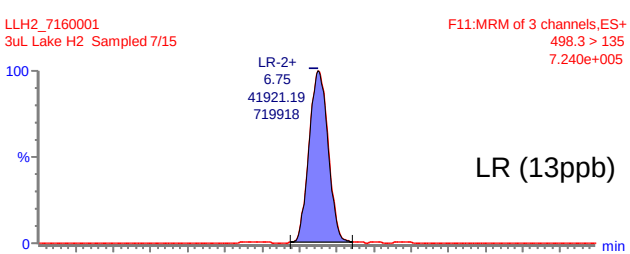
Ohio Lake (2021)



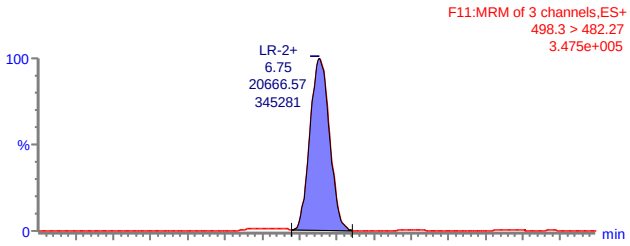
Anatoxin (10ppb)

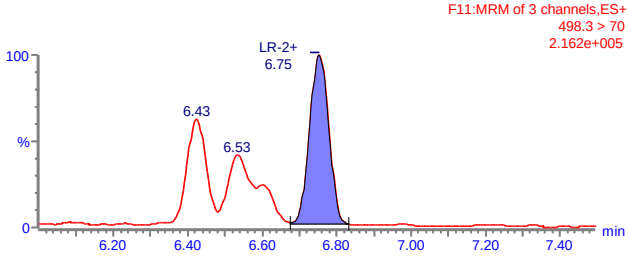


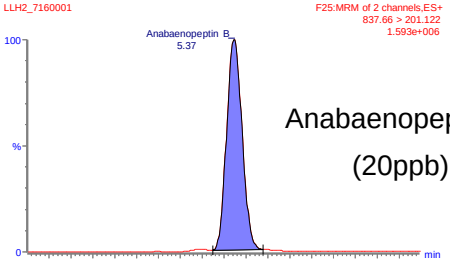




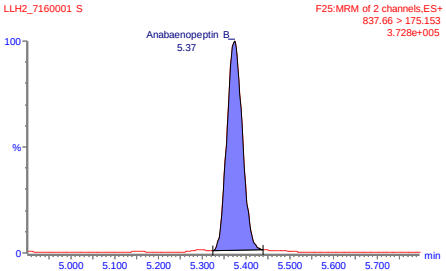
LR (13ppb)







Anabaenopeptin B (20ppb)



Using the Added Capabilities of the Tandem

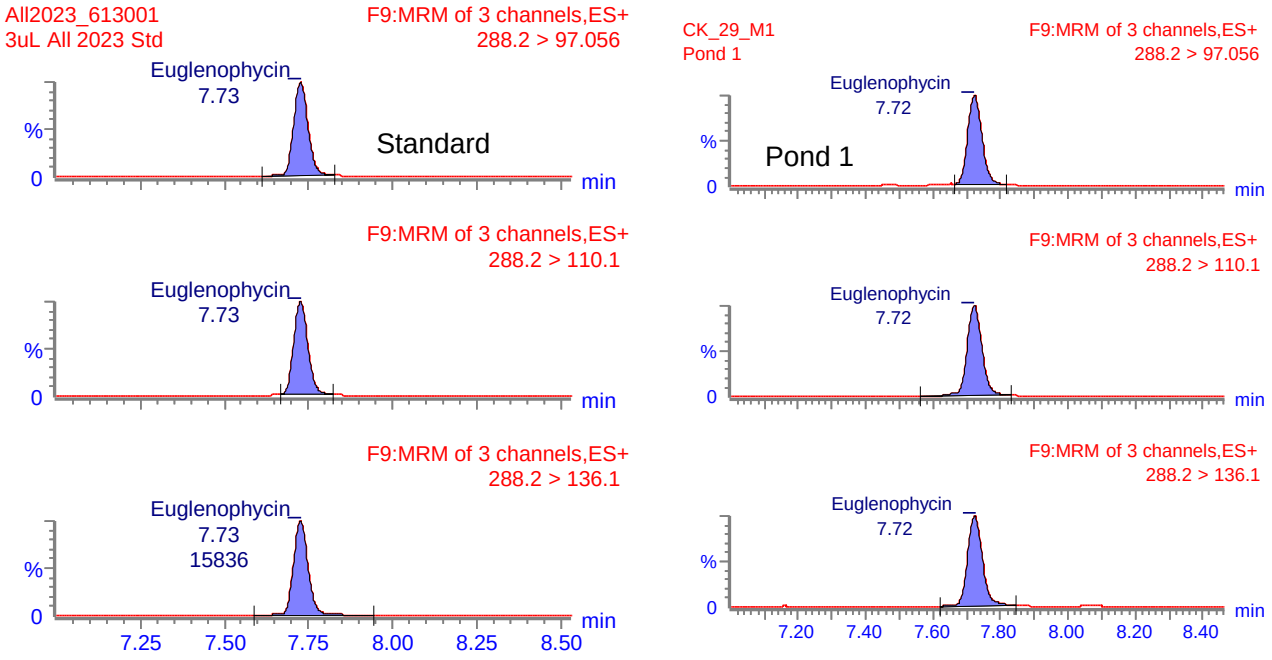
-Precursor (Parent) Ion Scans

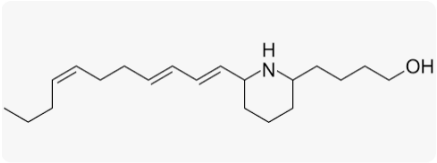
-Product (Daughter) Ion Scans

-RADAR™

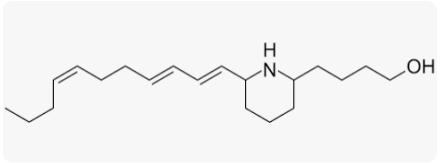
Mat samples

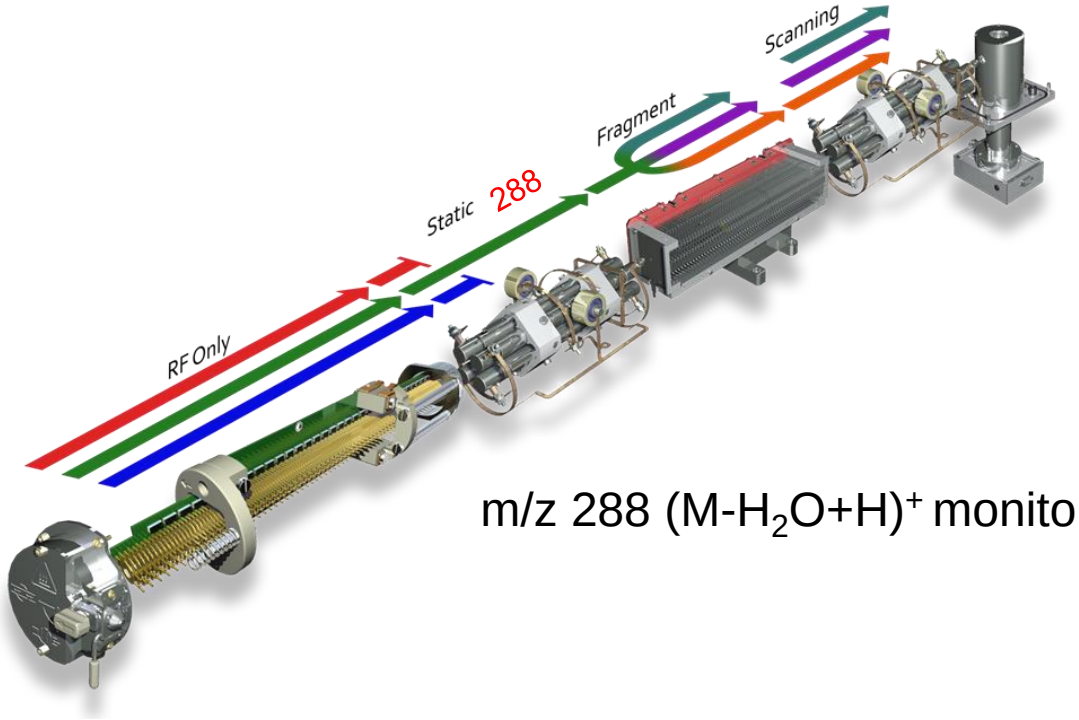
- Additional toxin found (Euglenophycin)





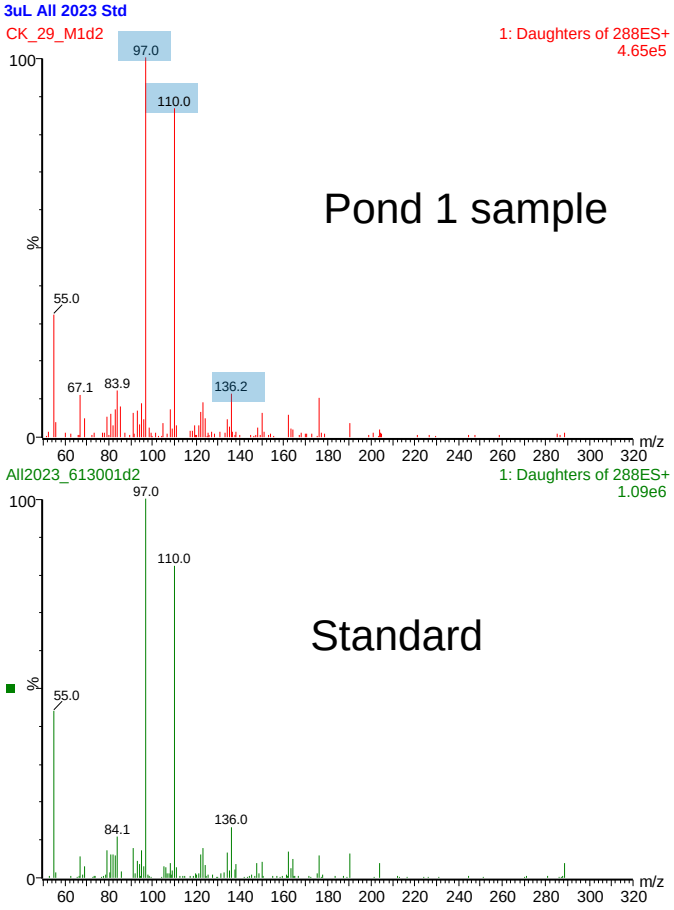
Product (Daughter) Ion Scanning





m/z 288 $(M\text{-}H_2O+H)^+$ monitored and fragmented

Confirmed fragment ions for Euglenophycin



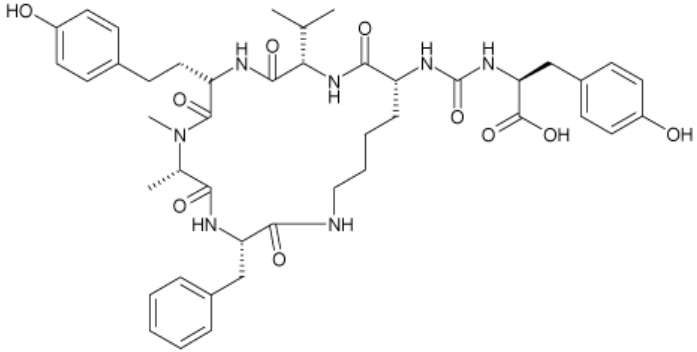
- Same fragment ions for both
- Match literature references for Euglenophycin

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

Anabaenopeptins

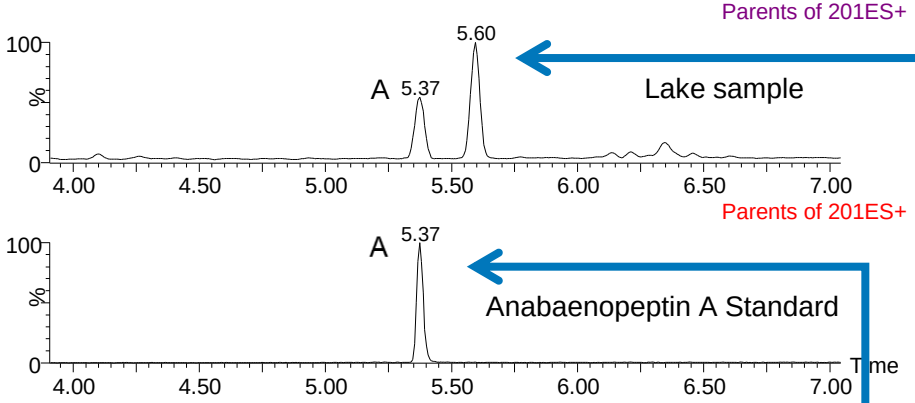
- Anabaenopeptins are a highly diverse group of bioactive peptides
- Produced by several genera of cyanobacteria such as *Anabaena, Planktothrix, Microcystis and Nodularia.*
- These peptides are commonly detected in cyanobacterial blooms along with the well known microcystins.

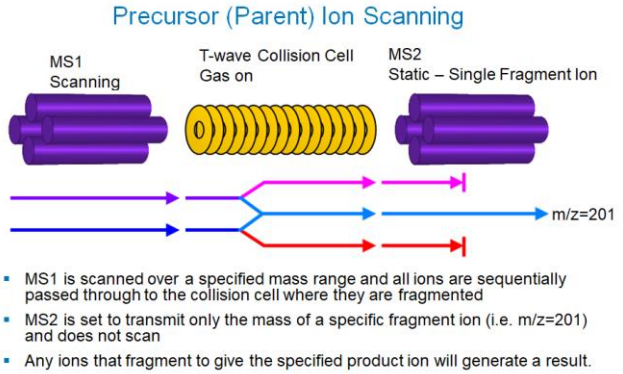
- Anabaenopeptin A

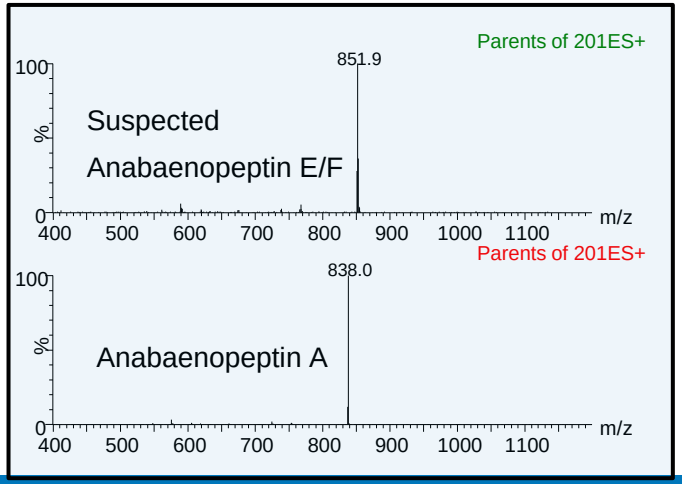


Finding more

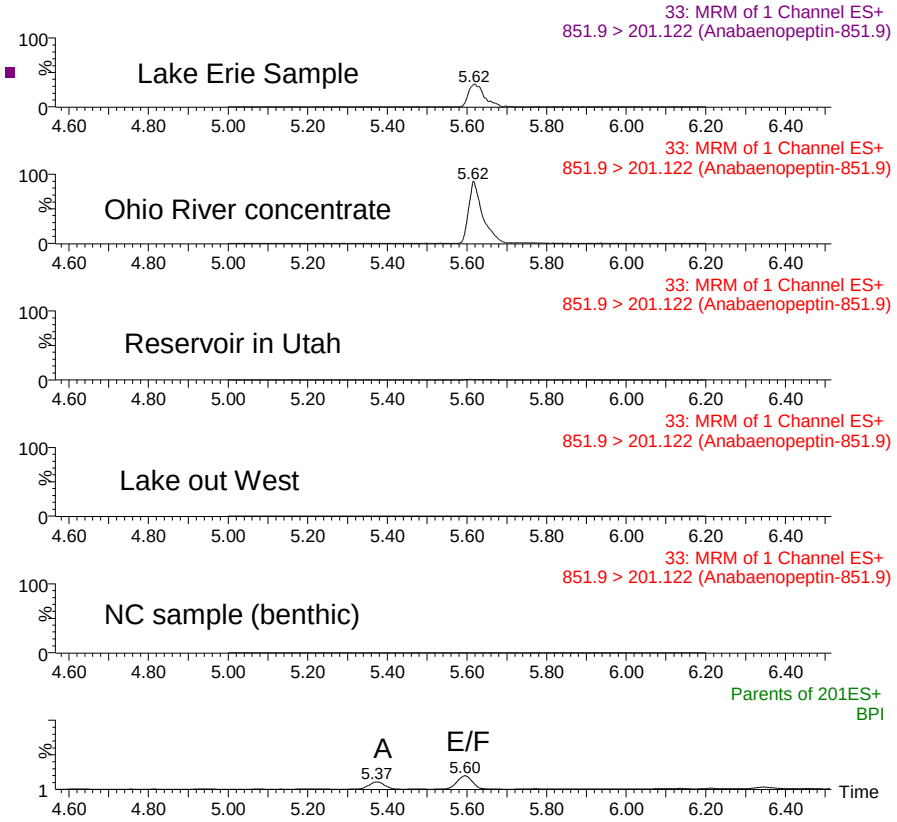
- Looking for other anabaenopeptins
  - A and B are available as standards.
  - Common fragment ion is m/z 201
    - $(CO\text{-}Arg)^{+1}$
  - Can scan for precursor (parent) mass



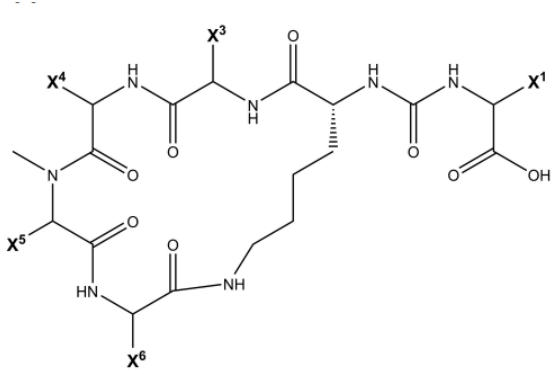




Monitoring for new compound



- Monitor for anabaenopeptin E/F



August 2019

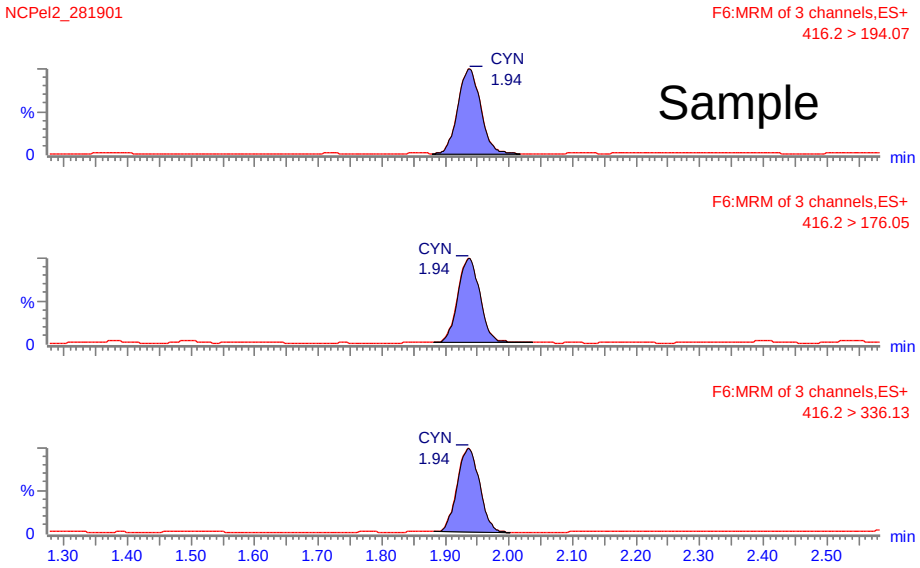
- Dog Deaths in Wilmington, NC pond

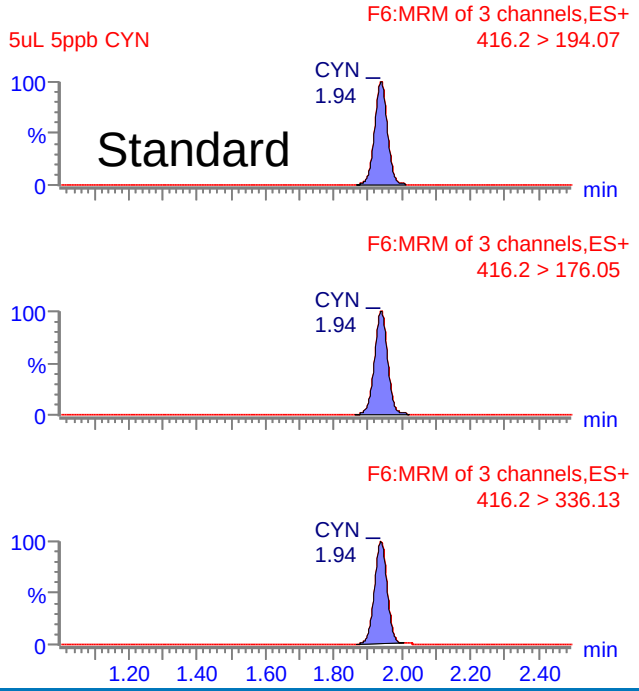
Benthic Algae produced

Primary strain *Oscillatoria*

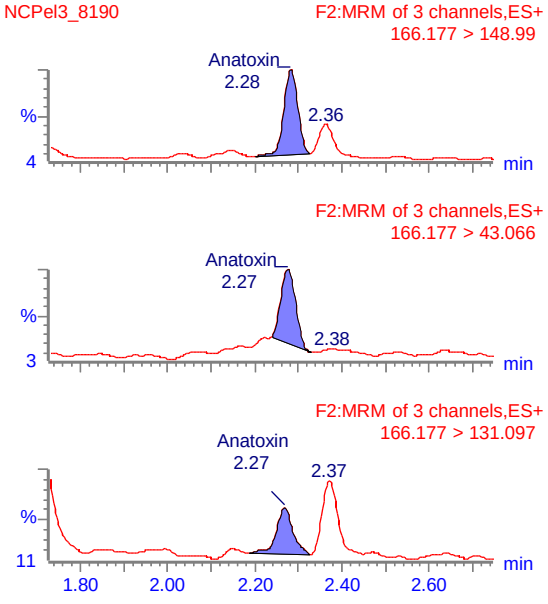
LC/MS/MS Analysis

- Samples Frozen and shipped
- 2 water samples and a concentrate (pellet)



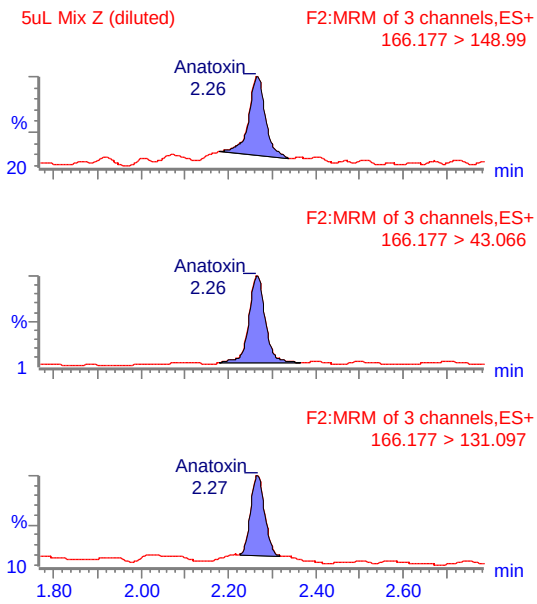


NC Pellet



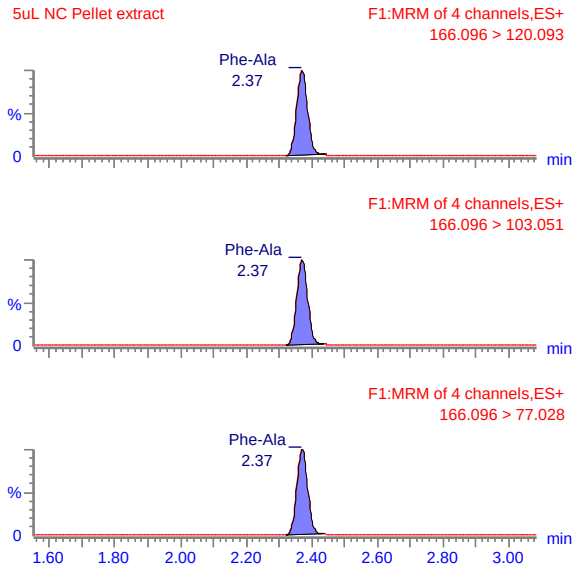
Anatoxin

Standard



Anatoxin

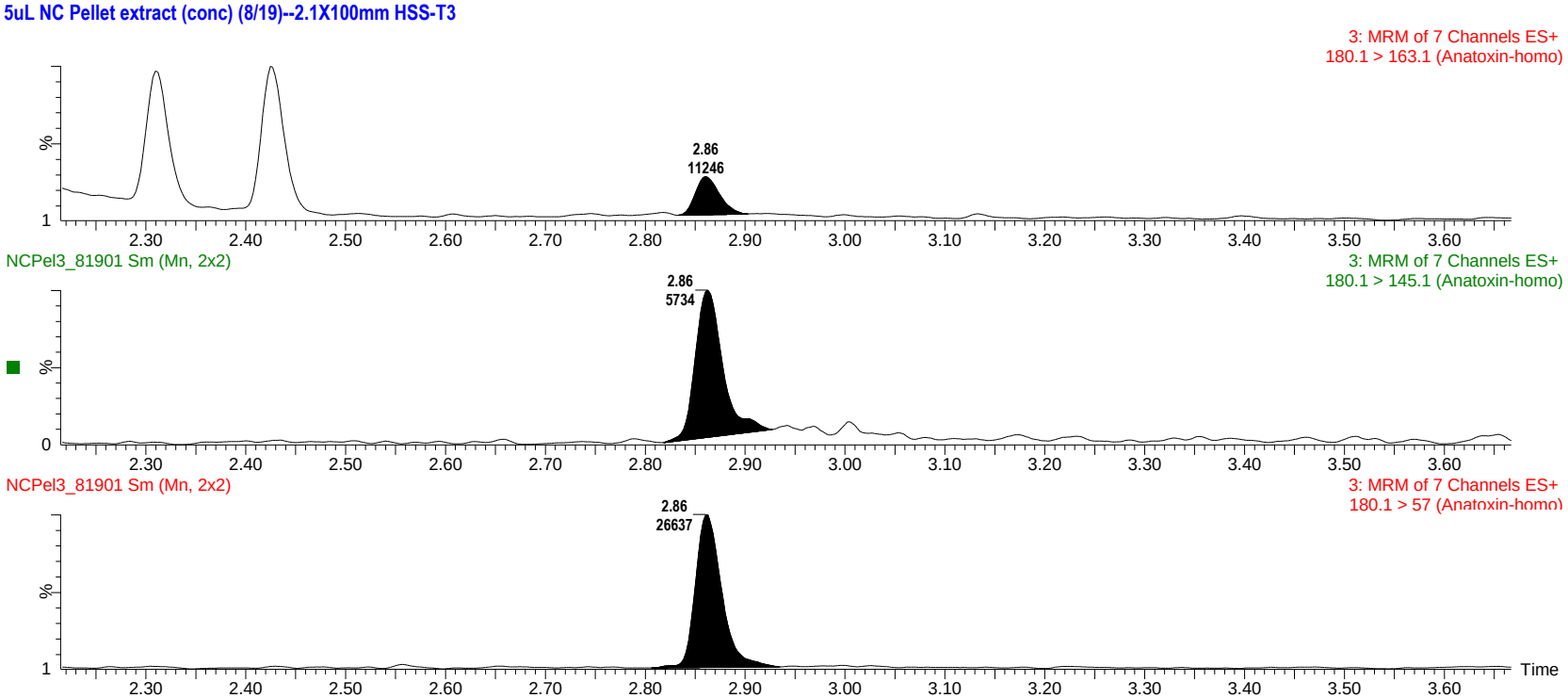
NC Pellet



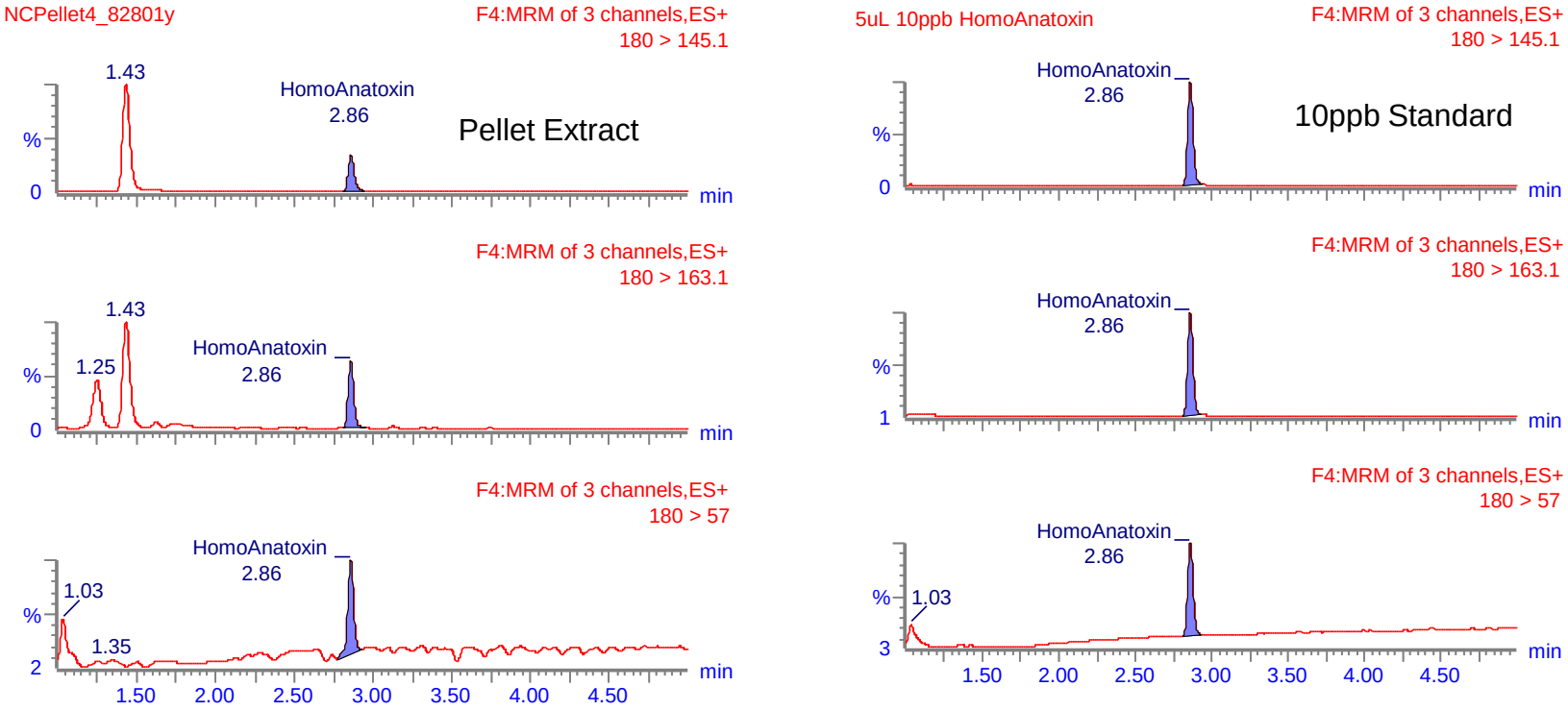
Phe-Ala

Initial screen-Homoanatoxin

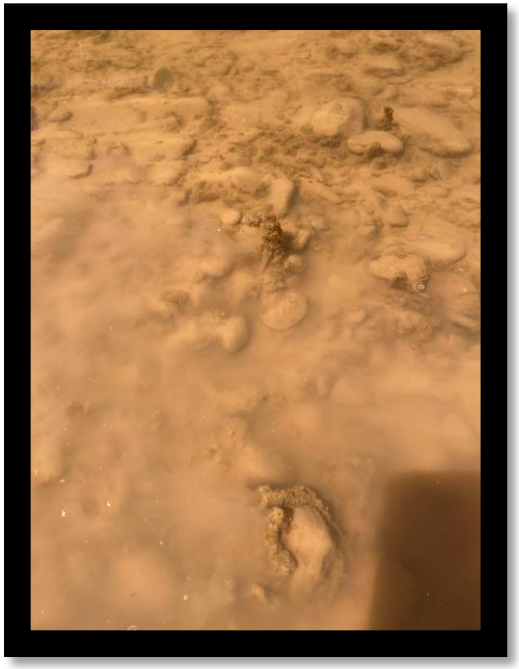
Waters™



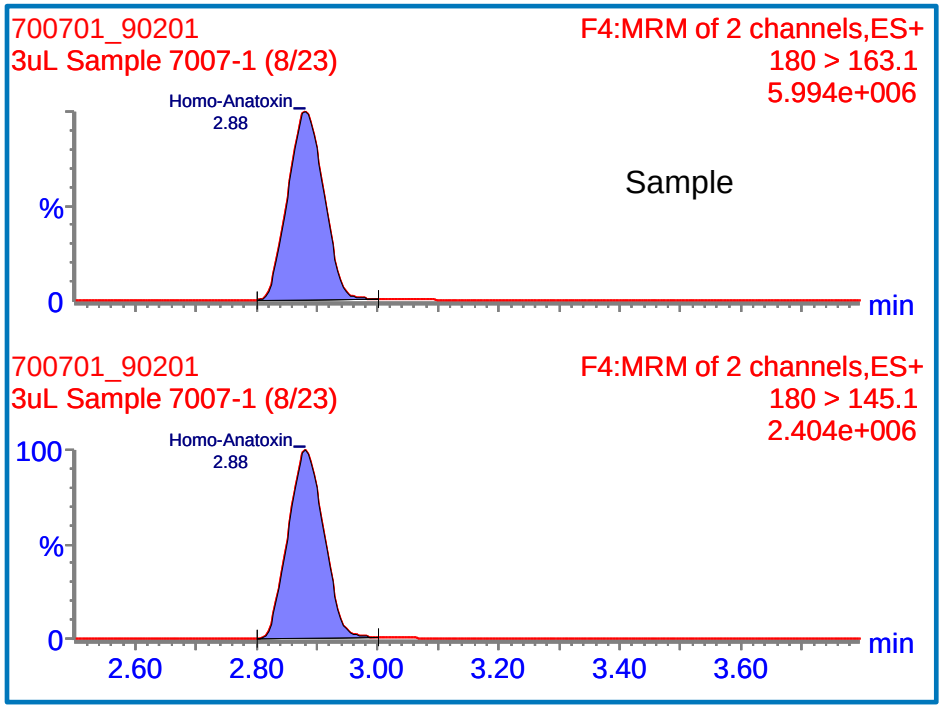
Sample and Standard (Homoanatoxin)

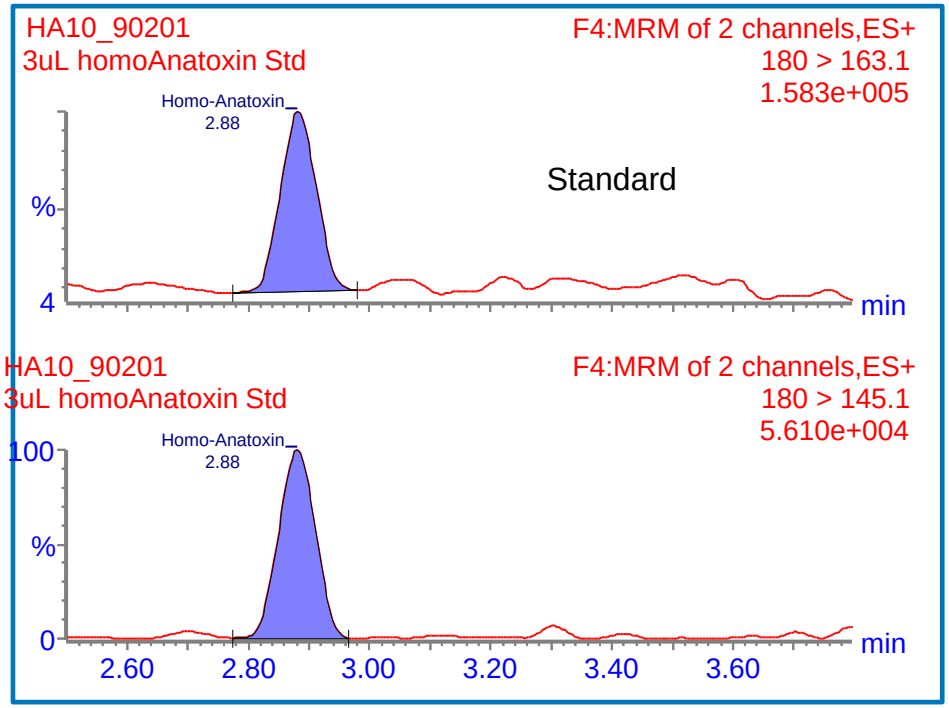


Samples suspected in Dog Deaths



Sample Results





RADAR (Simultaneous MRM and Scanning)

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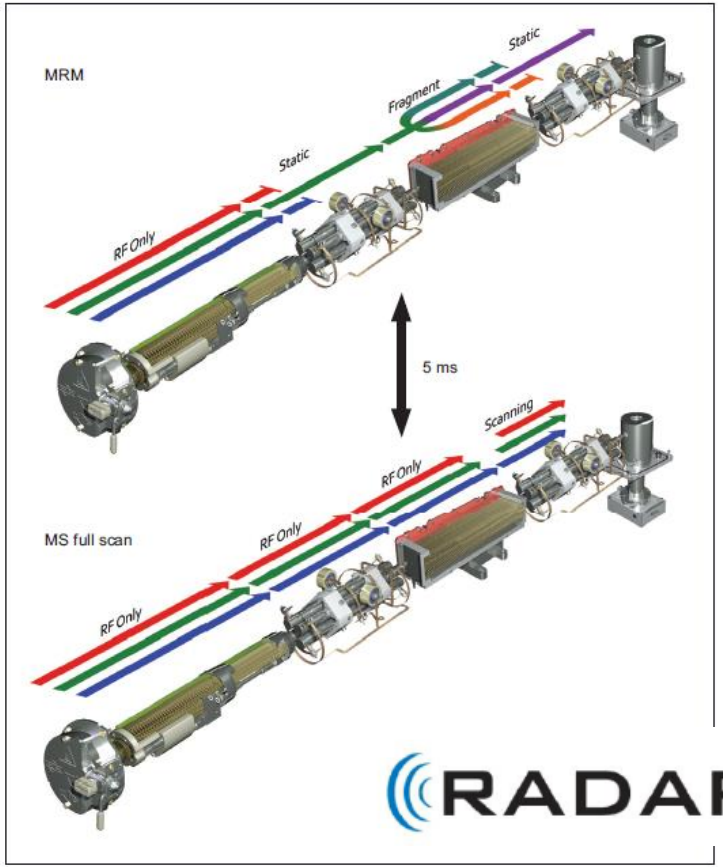


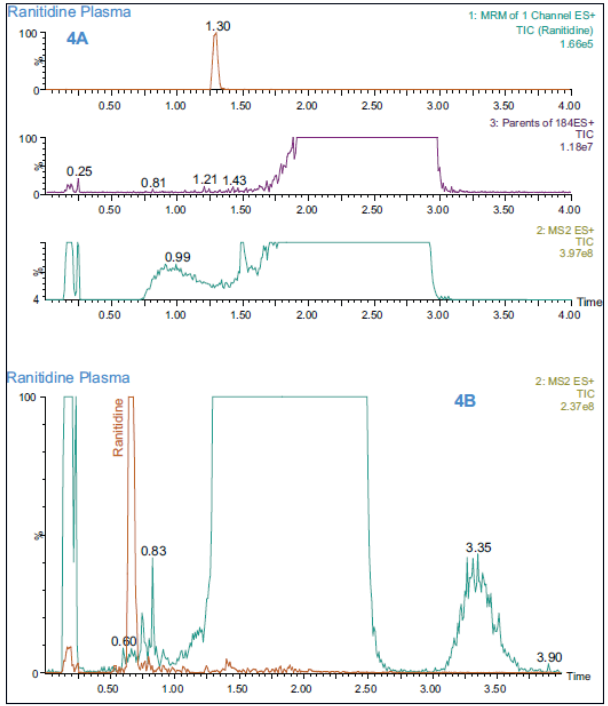
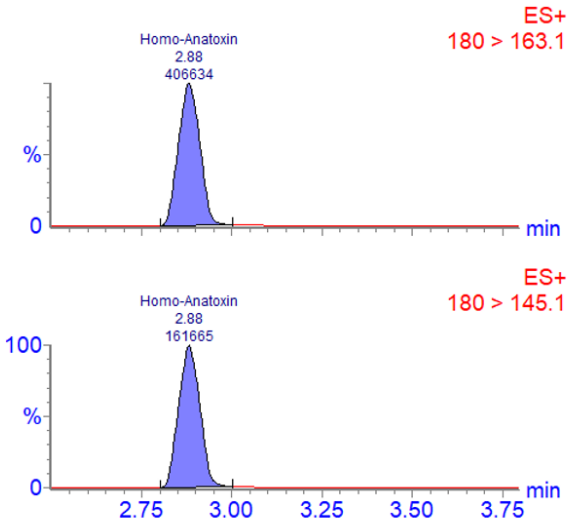
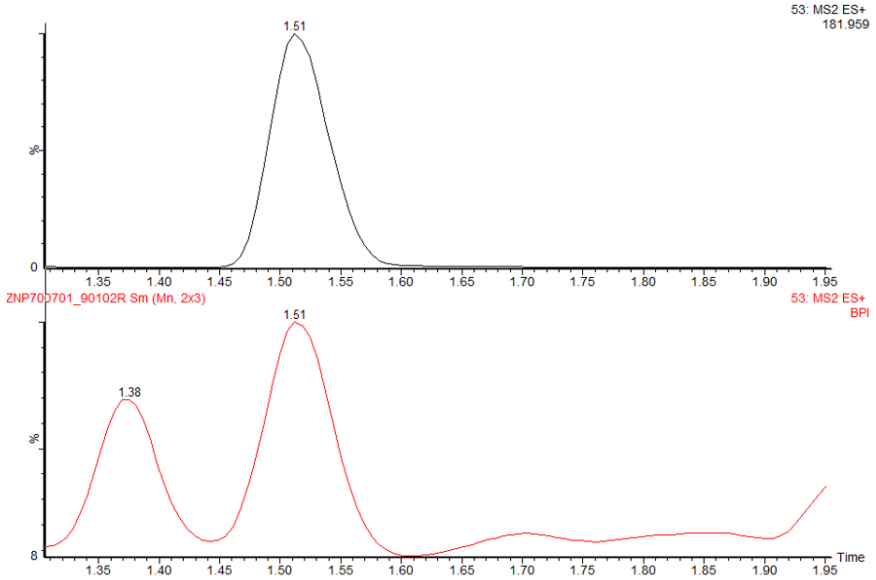


Figure 4: LC/MS/MS analysis of ranitidine hydrochloride in rat plasma using aqueous basic methanol gradients. 4A shows the initial method and ranitidine coeluting with matrix components in the full scan TIC trace. The second trace shows parents of m/z 184 ions which are characteristic of phospholipids. 4B shows the final method resulting in ranitidine being resolved from the matrix components.

Water sample from dog death

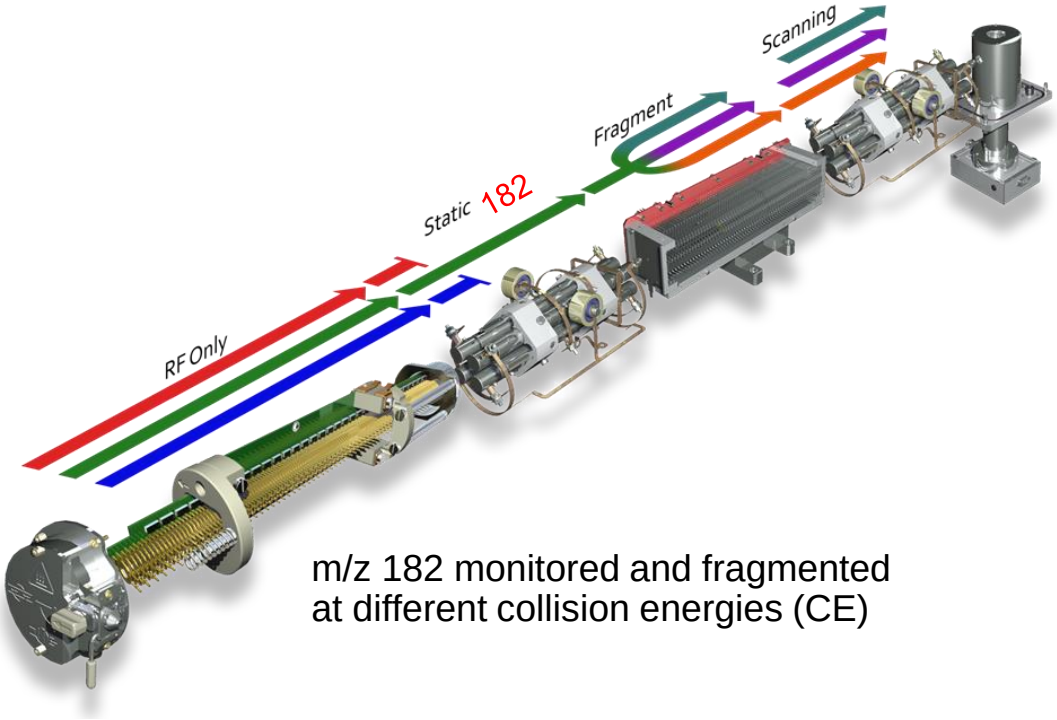


mrm channel showing positive for homo-anatoxin

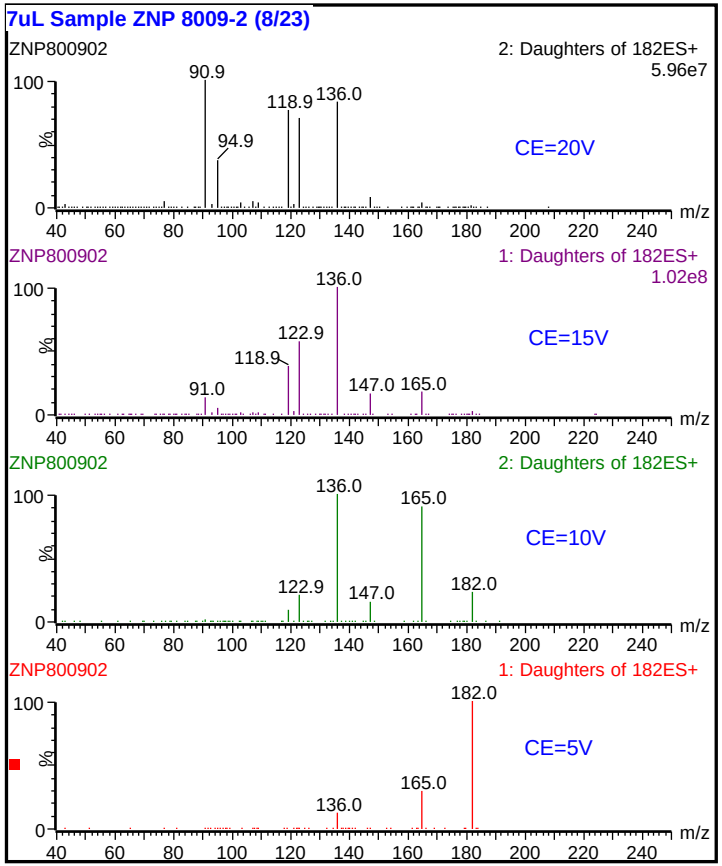


RADAR scanning showed large peak at mass 182 (possible ion for dihydro-homoanatoxin)

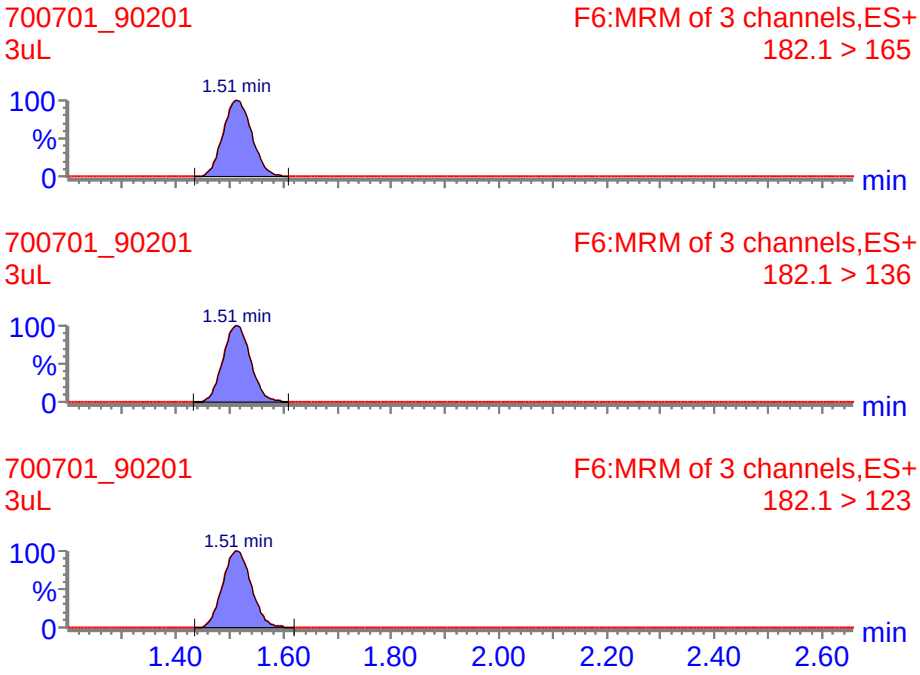
Product Ion Scanning



m/z 182 monitored and fragmented at different collision energies (CE)



Sample Results—7007-1



Mass 182 detected

Dihydrohomoanatoxin?

Epoxy/hydroxyanatoxin?

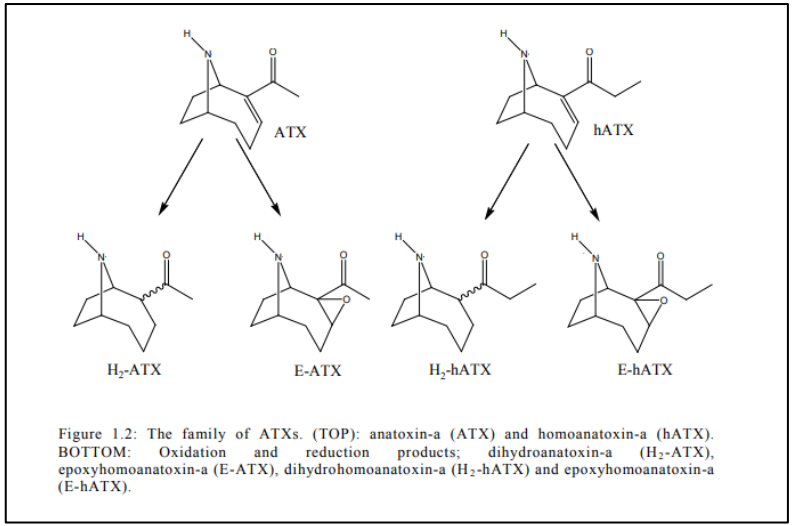


Figure 1.2: The family of ATXs. (TOP): anatoxin-a (ATX) and homoanatoxin-a (hATX). BOTTOM: Oxidation and reduction products; dihydroanatoxin-a (H_2-ATX), epoxyhomoanatoxin-a (E-ATX), dihydrohomoanatoxin-a (H_2-hATX) and epoxyhomoanatoxin-a (E-hATX).

Interesting Screen (Precursor Scan (135 (ADDA))

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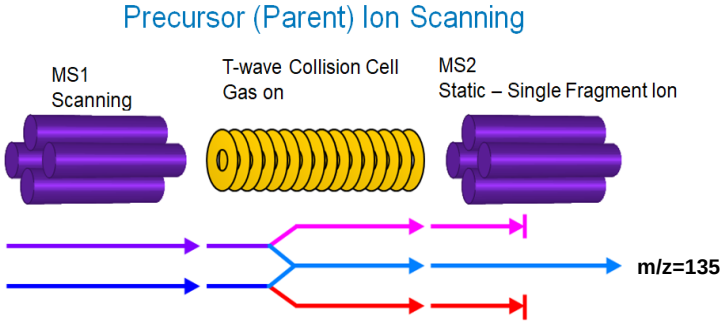
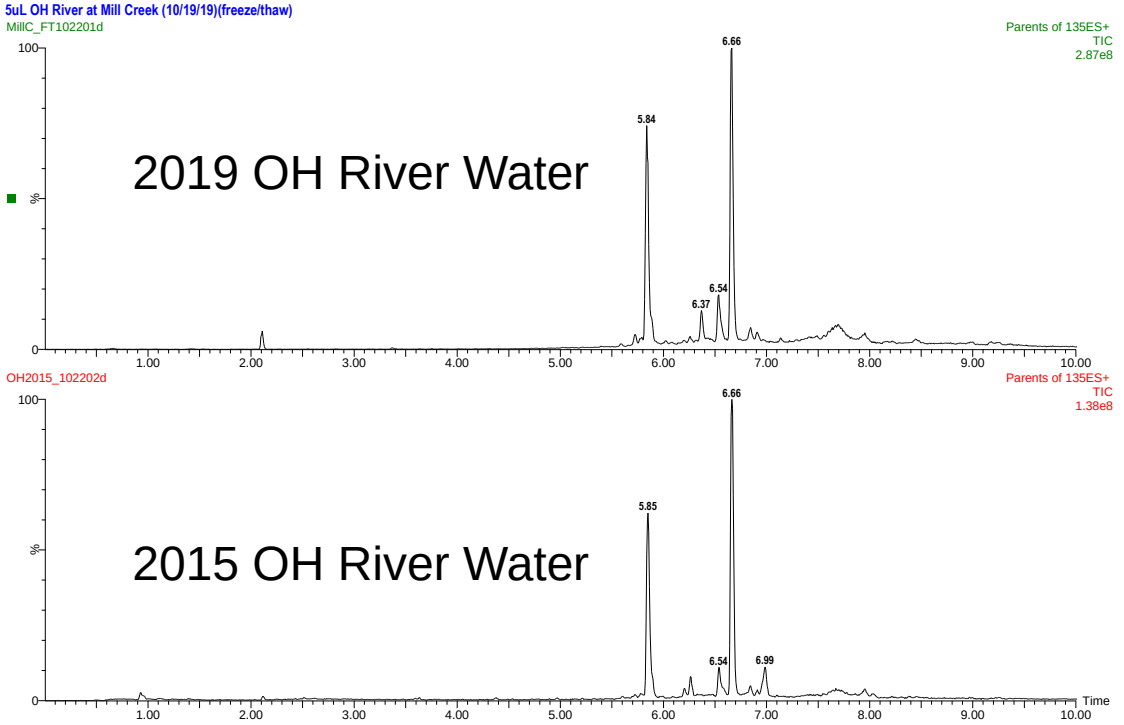
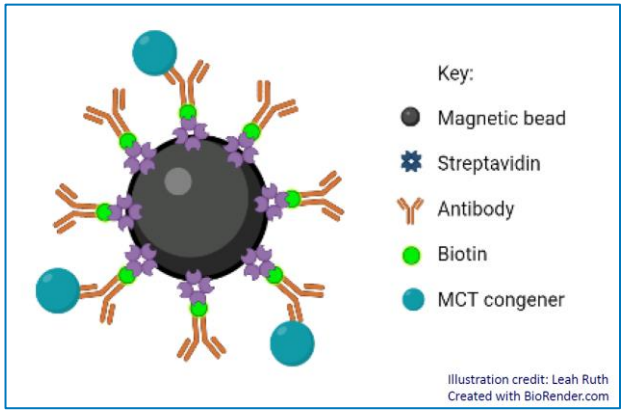


- MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented
- MS2 is set to transmit only the mass of a specific fragment ion (i.e. m/z=201) and does not scan
- Any ions that fragment to give the specified product ion will generate a result.



“Other” Crazy stuff working on

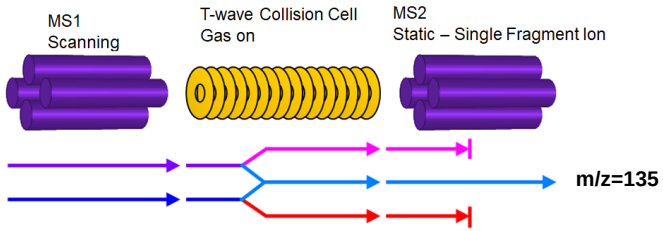
- Microcystins in urine
  - Commercial testing kit using Immuno-affinity Magnetic Beads



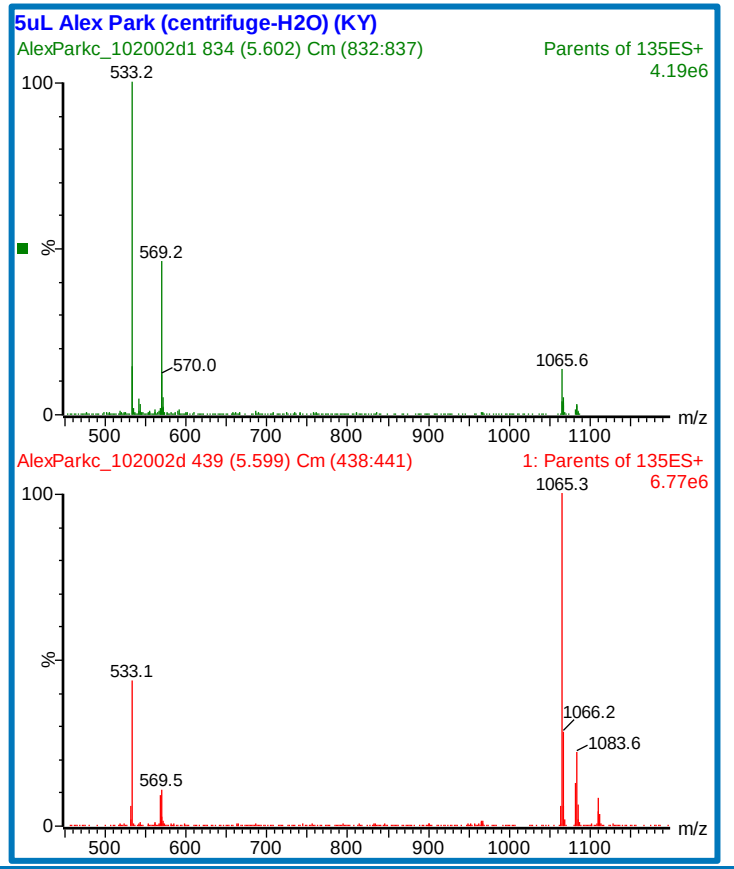
“Other” Crazy stuff working on

- “weird” toxins in blooms
  - October bloom of *Woronichinia naegeliana*

Precursor (Parent) Ion Scanning

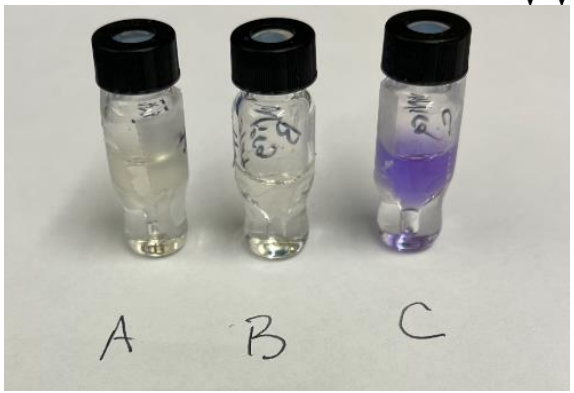


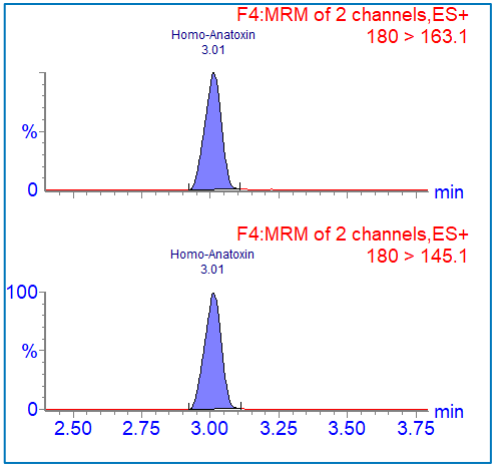
- MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented
- MS2 is set to transmit only the mass of a specific fragment ion (i.e. m/z=201) and does not scan
- Any ions that fragment to give the specified product ion will generate a result.

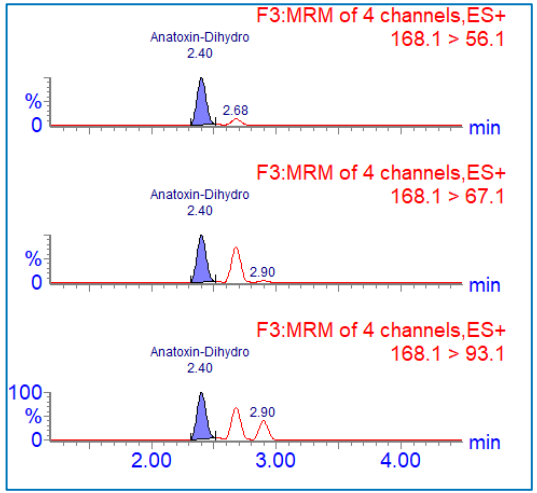


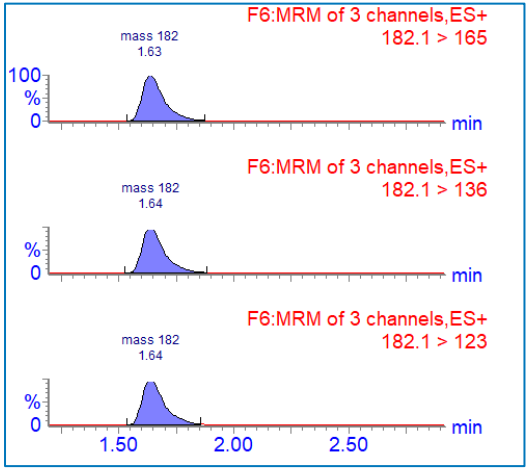
“Other” Crazy stuff working on

- Benthic blooms producing anatoxin and analogs
  - Homo-anatoxin, dihydroanatoxin and
  - “possibly” dihydrohomoanaoxin









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

Acknowledgements

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

GOT TOXINS?

Questions? Interesting samples?

Oehrle@nku.edu

Waters™