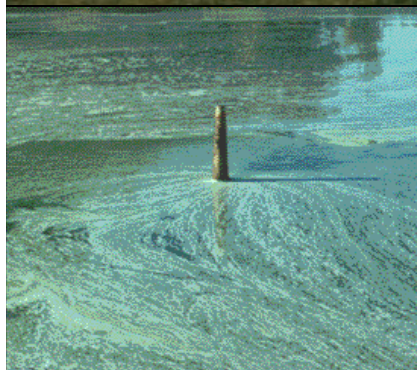
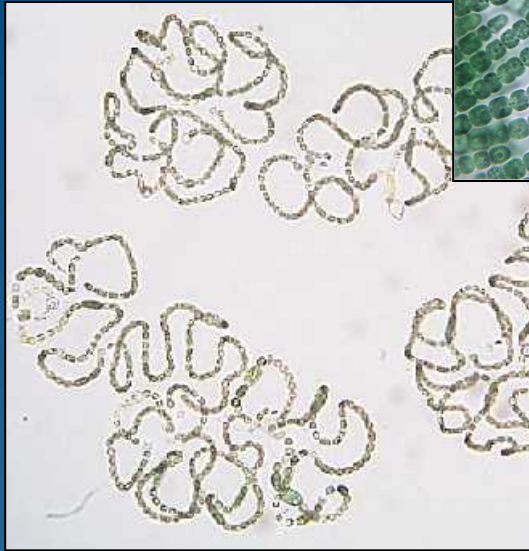


Analysis of Microcystins RR, LR and YR in Bottled, Tap and Surface Water by 2D LC-MS/MS

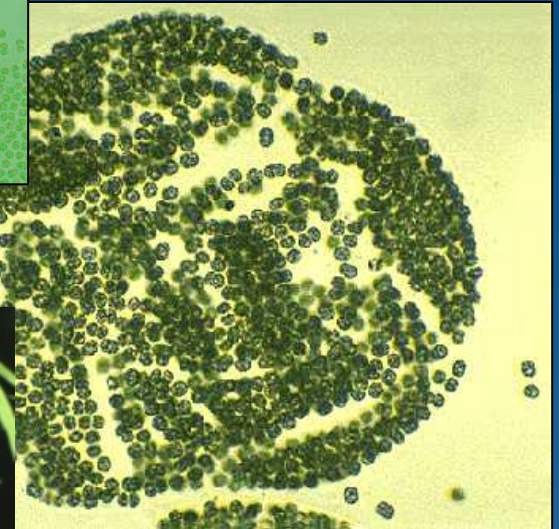
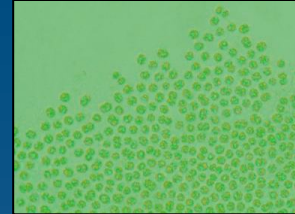
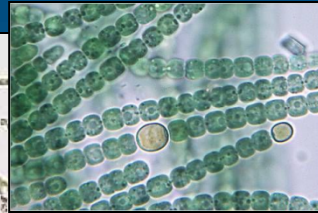
Juliette L. Smith, Virginia Institute of Marine Science
Claude Mallet, Workflow Integration Group, Waters Corporation



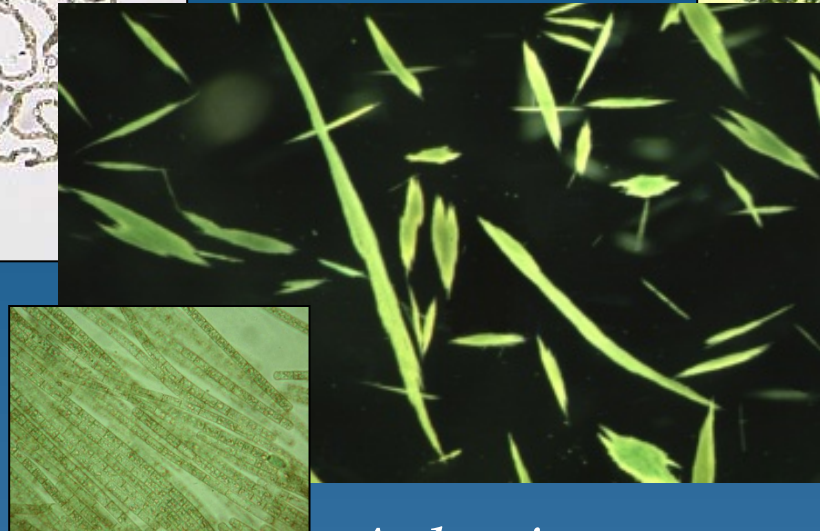
Toxic Cyanobacteria



Anabaena



Microcystis



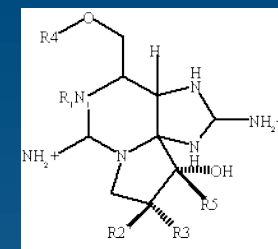
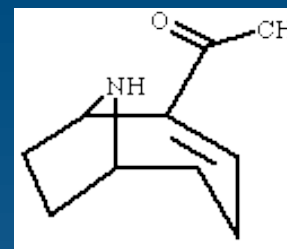
Aphanizomenon

- Common producers of cyanotoxins
- General characteristics
- *Microcystis* produces microcystins

Toxins of Cyanobacteria

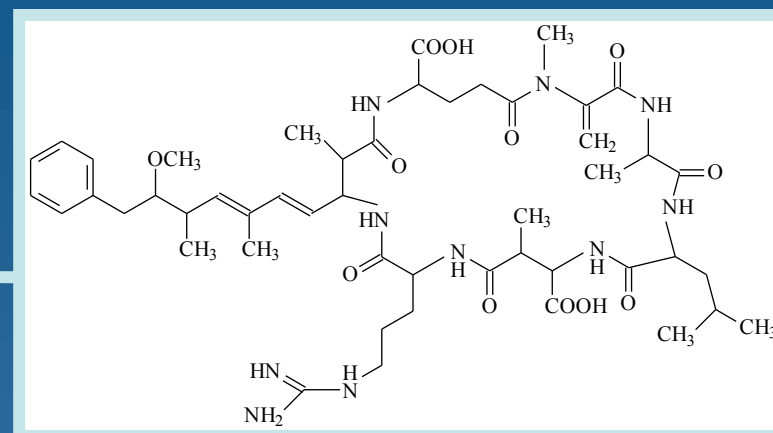
Neurotoxins

Saxitoxins
Anatoxins
Antillatoxins
Kalkitoxin
Jamaicamides
BMAA



Hepatotoxins

Microcystins
Nodularins

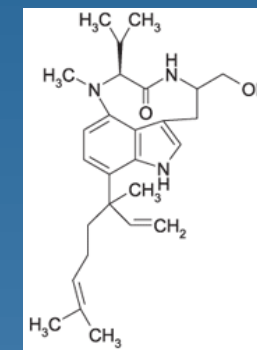
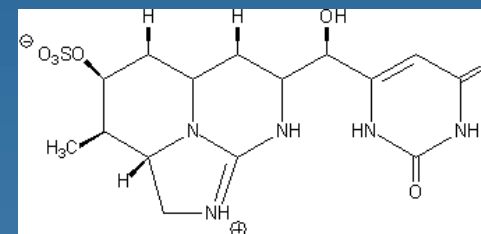


Cytotoxins

Cylindrospermopsins

Dermatotoxins

Lyngbyatoxins
Aplysiatoxins



- heptapeptides, 2 variable groups
- 100+ variants
- hepatotoxin, carcinogen



MCs – Human Health Issue

Microcystin-LR

- EPA Chemical Contaminant List 3, CCL3

Regulatory Guidelines – microcystin-LR

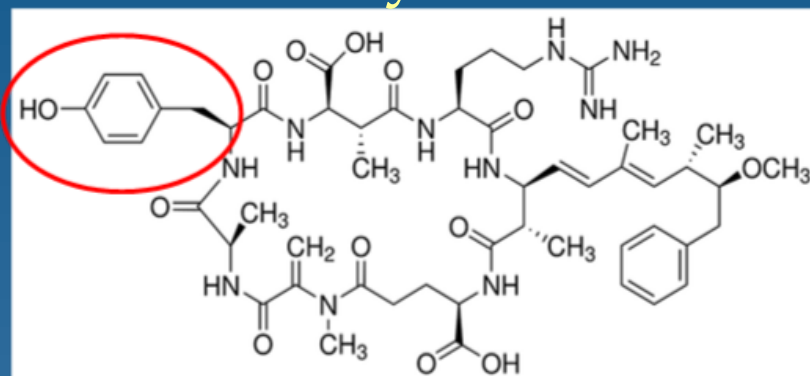
- WHO guideline: 1 $\mu\text{g/L}$ (1ppb)
- EPA Drinking water: 1.6 $\mu\text{g/L}$
- Recreational: 10 – 20 $\mu\text{g/L}$
- TDI: 0.04 $\mu\text{g} / \text{kg} / \text{d}$



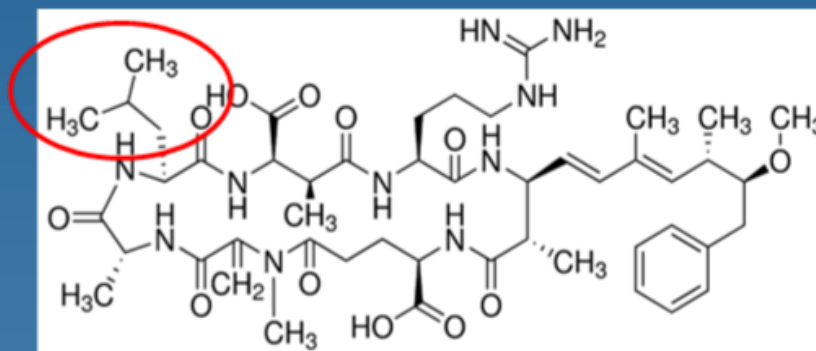
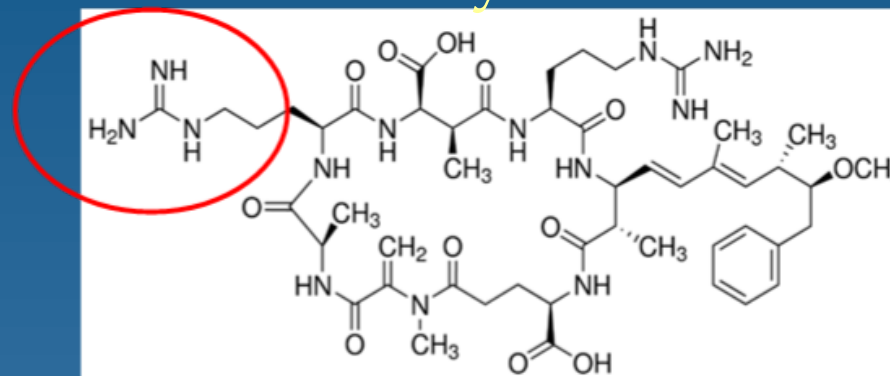
Objectives

Investigate analytical methods for the trace analysis of microcystins in surface and potable water.

Microcystin-YR

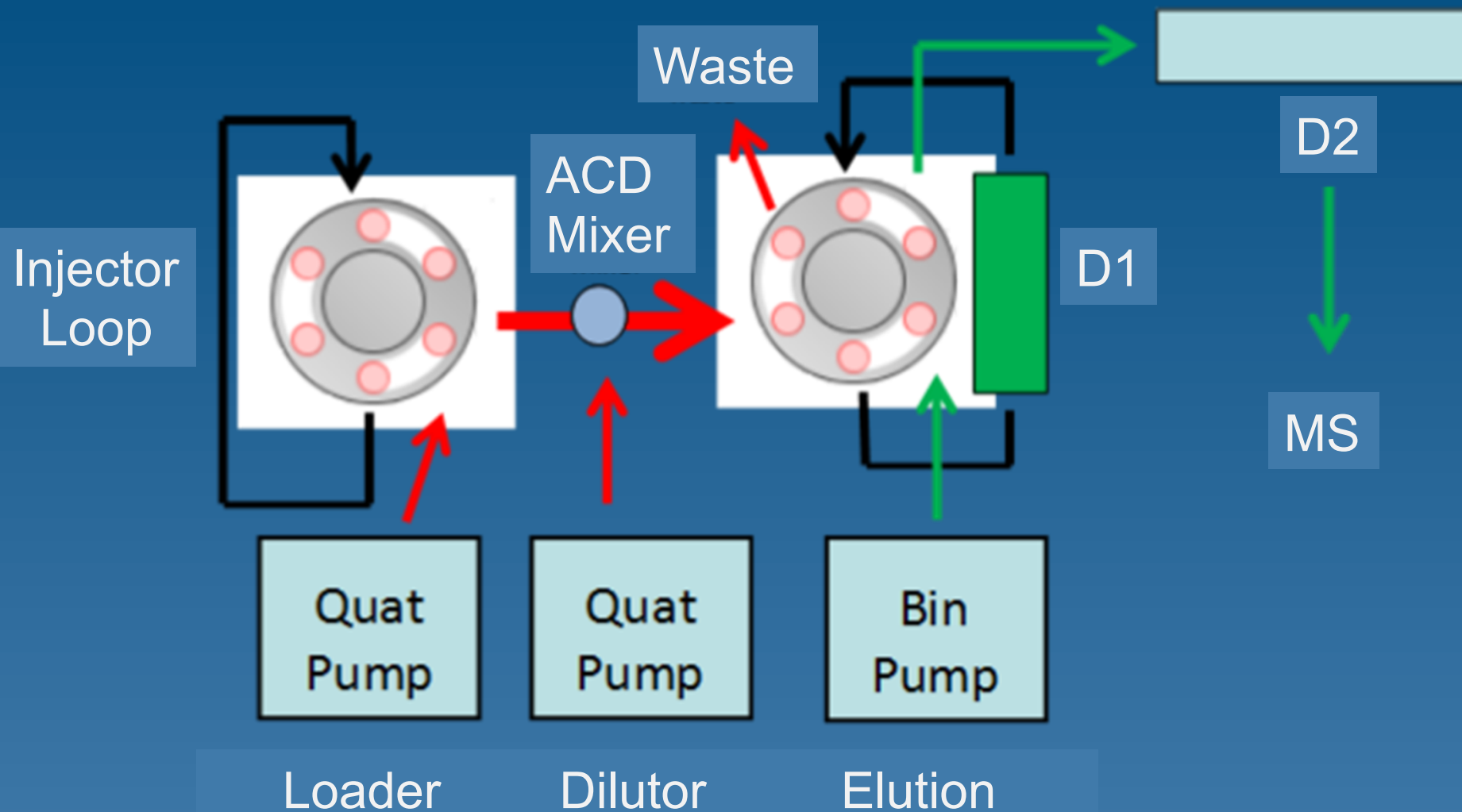


Microcystin-RR

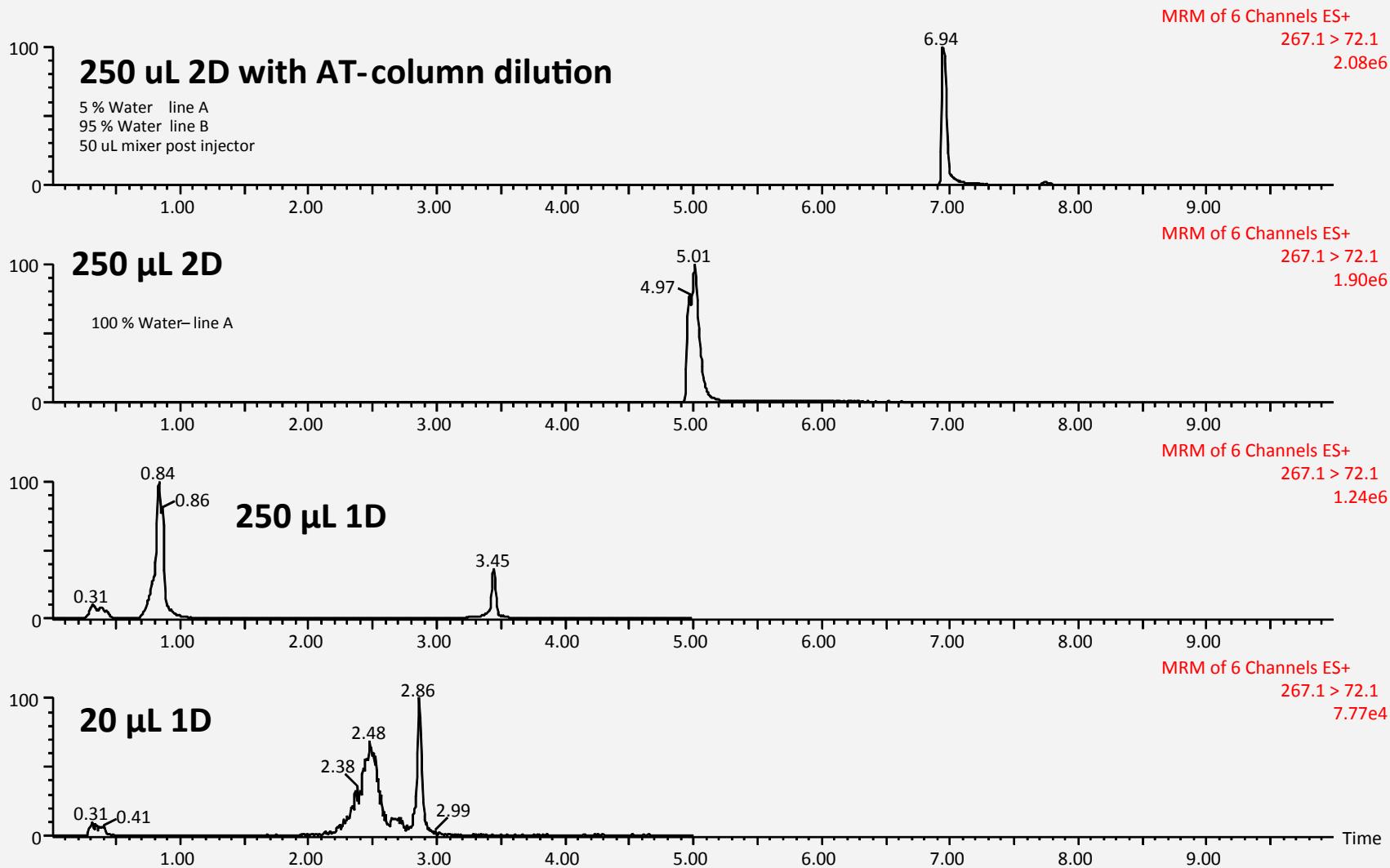


Microcystin-LR

2D UPLC with ACD



2D UPLC with ACD

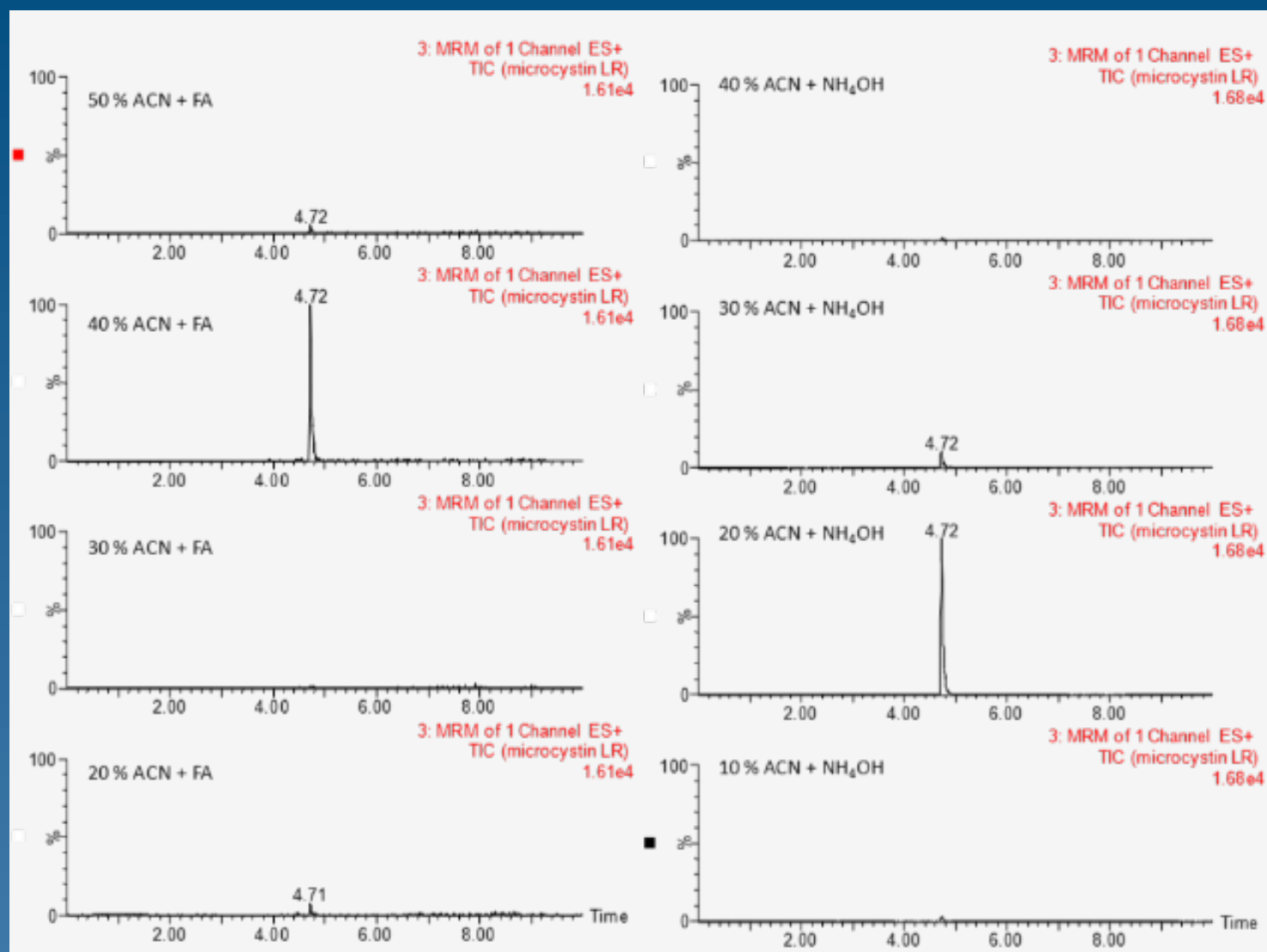


Micro-extraction

MC-LR

Formic acid, pH 3

NH₄OH, pH 10

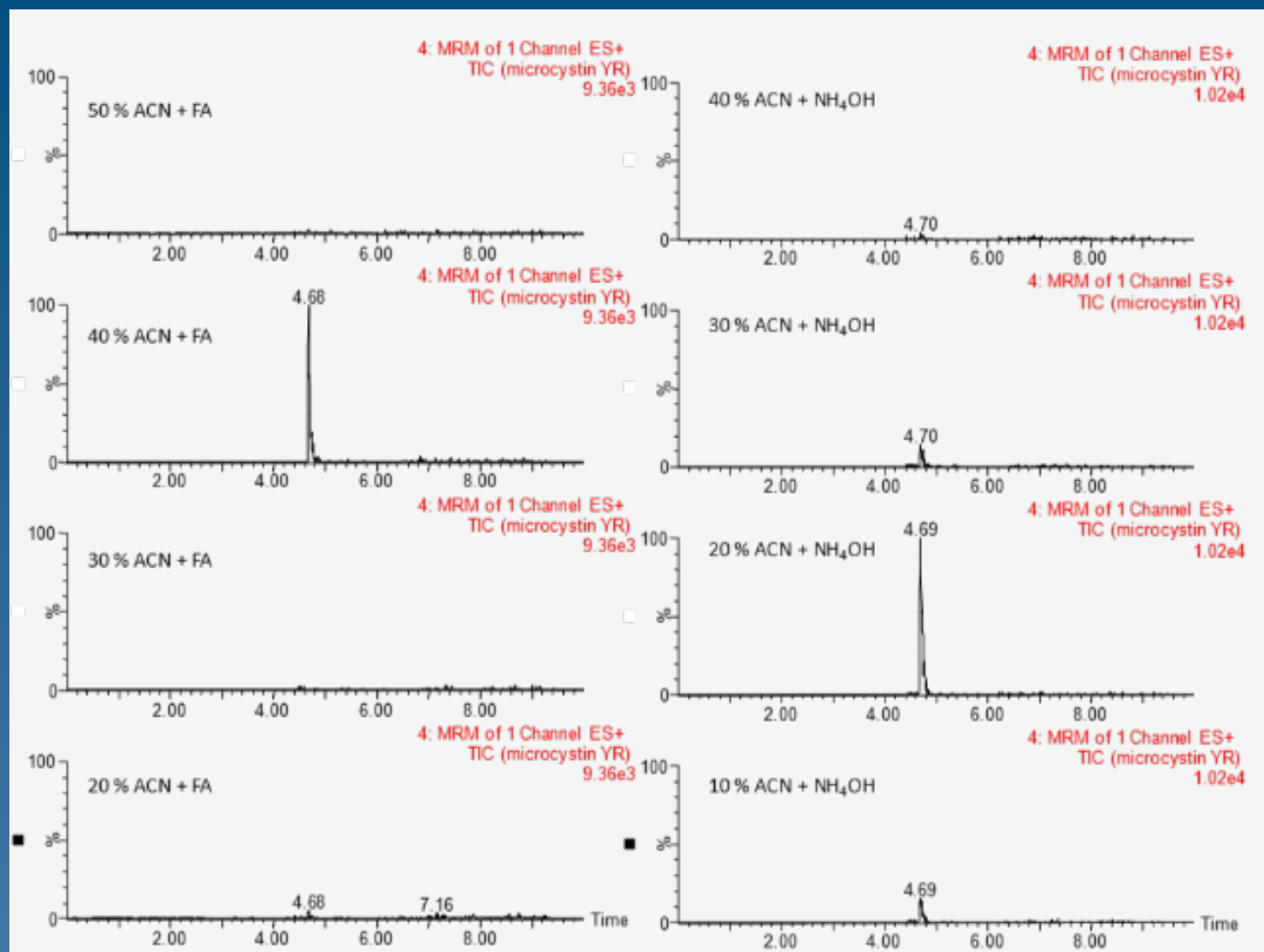


Micro-extraction

MC-YR

Formic acid, pH 3

NH₄OH, pH 10



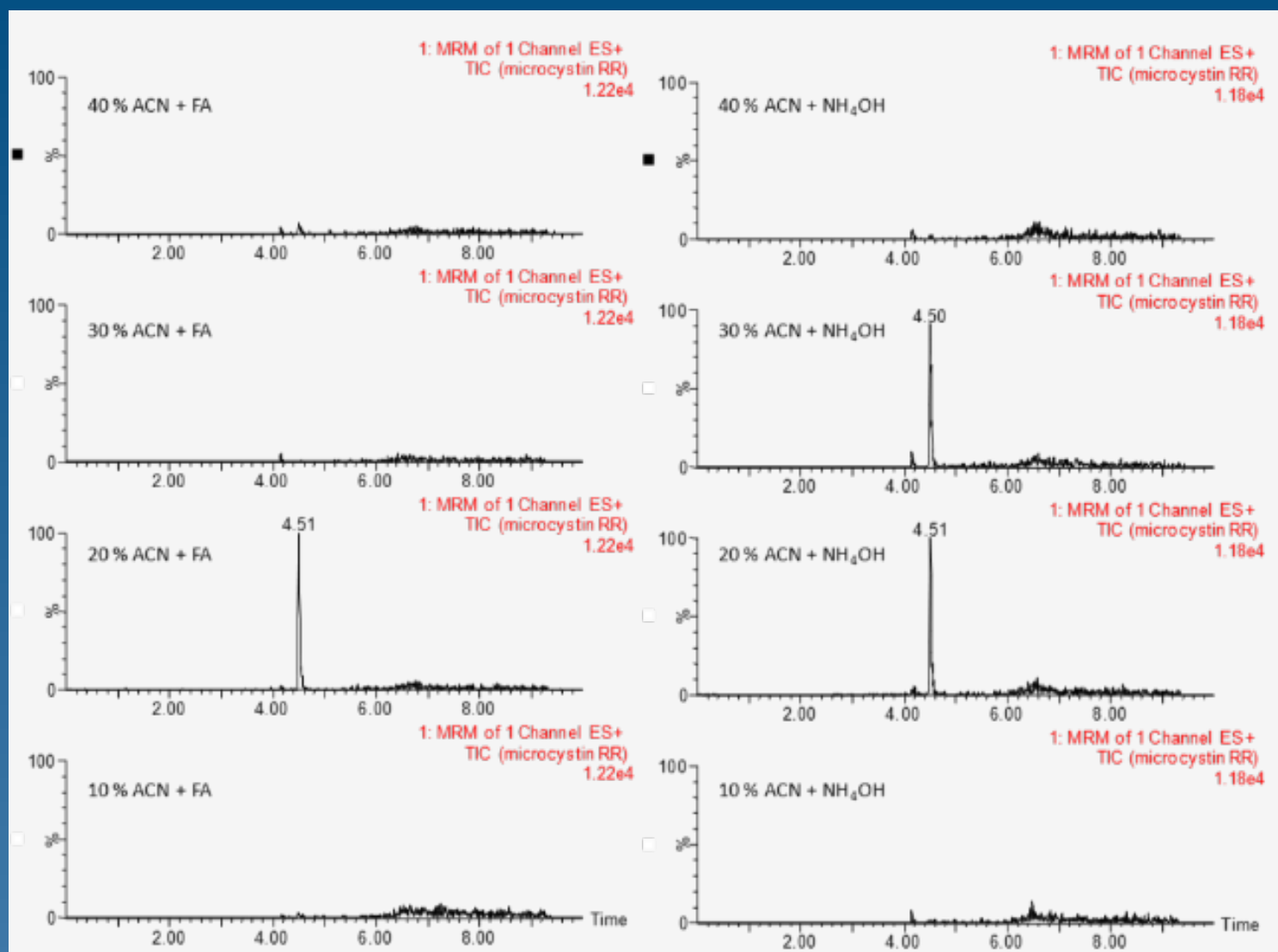
Micro-extraction

MC-RR

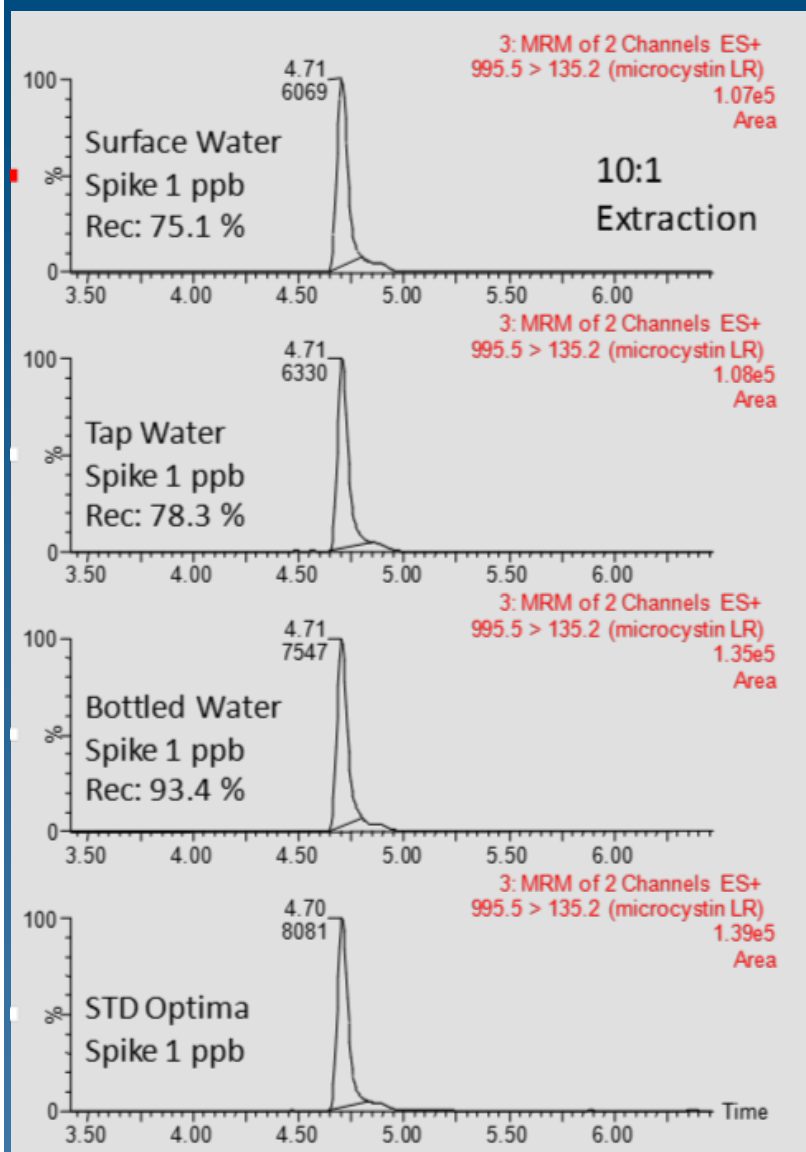


Formic acid, pH 3

NH₄OH, pH 10



Recovery of MCs



MC-LR: 75 – 93%

MC-YR: 75 – 91%

MC-RR: 77 – 103 %

Recoveries: >90% for
bottled water, >78% for
tap water, and >75% for
surface water

10:1 enrichment, 250 μ L injection

Detection Limits

Lower quantitation limits
(10:1) are estimated at:

MC-YR: 5 ppt

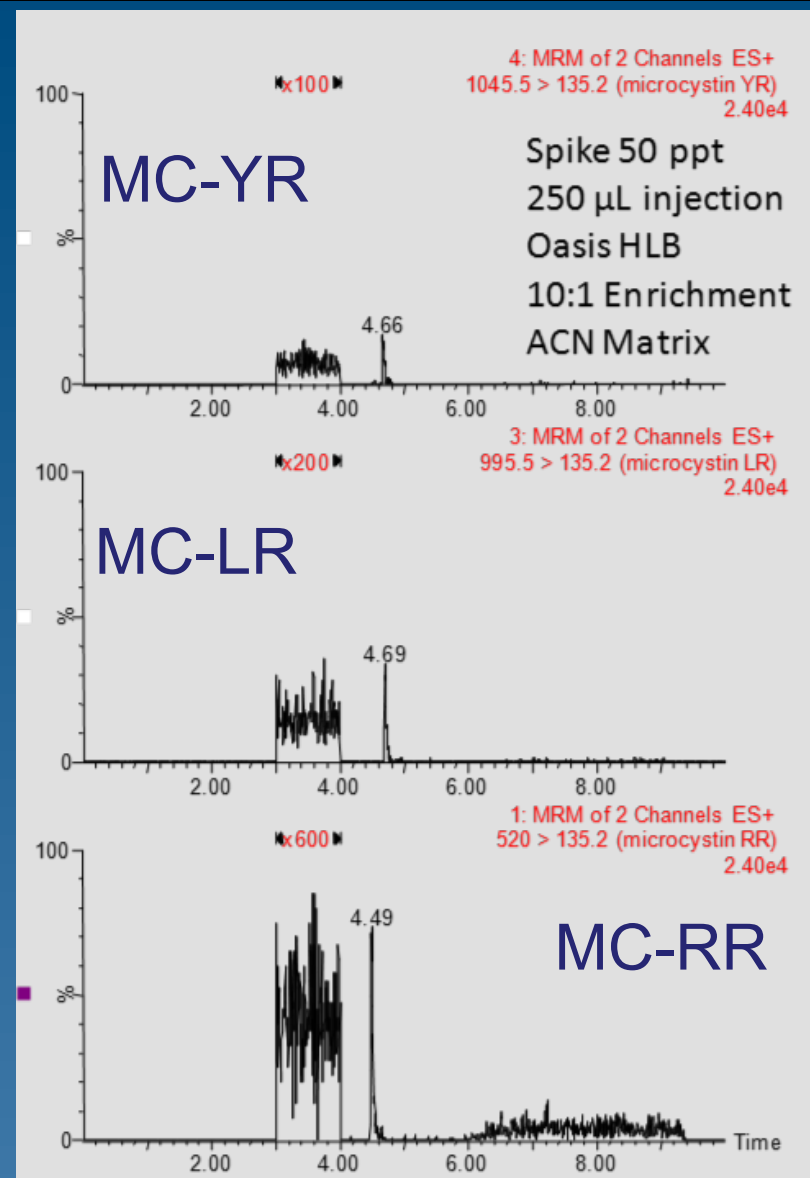
MC-LR: 2.5 ppt

MC-RR: 0.8 ppt

Guidelines (MC-LR):

EPA: 1.6 ppb

WHO: 1 ppb



Summary

- Fast extraction protocol (15 min) for 10:1 enrichment, with no interfering contaminants upon 2D LC-MS/MS analysis
- No evaporation to dryness / no reconstitution due to at-column dilution which allows for high volume injection (250 μ L) of organic extract
- Recoveries > 90% for bottled water, >78% for tap water, and >75% for surface water
- Trace level detection (0.8 - 50 ppt) is 3 orders of magnitude below EPA regulatory limit, 1.6 ppb, and the WHO recommendation level, 1 ppb.



WILLIAM
& MARY

VIRGINIA INSTITUTE OF MARINE SCIENCE



Waters

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Thank you!

Collaborators:

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Marta Sanderson (VIMS)

C. Taylor Armstrong (VIMS)