



Analysis of Microcystins in Drinking Water by LC/MS/MS and ELISA

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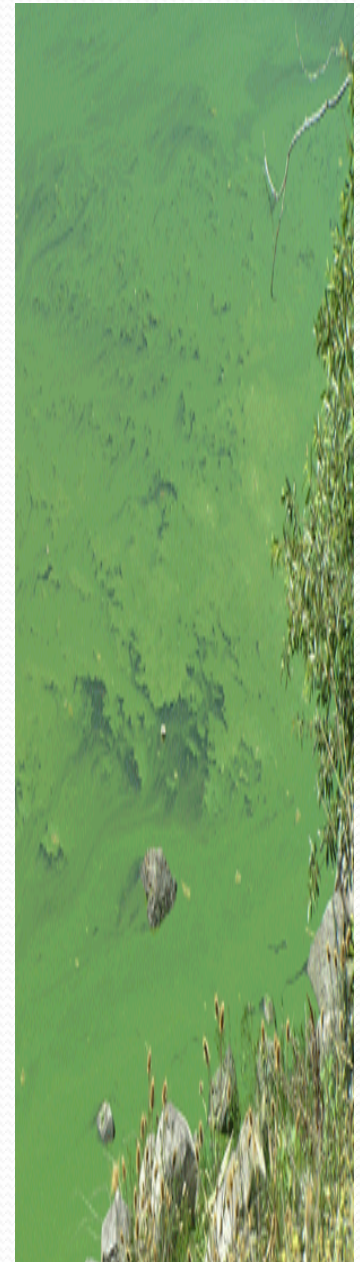


Presentation Outline

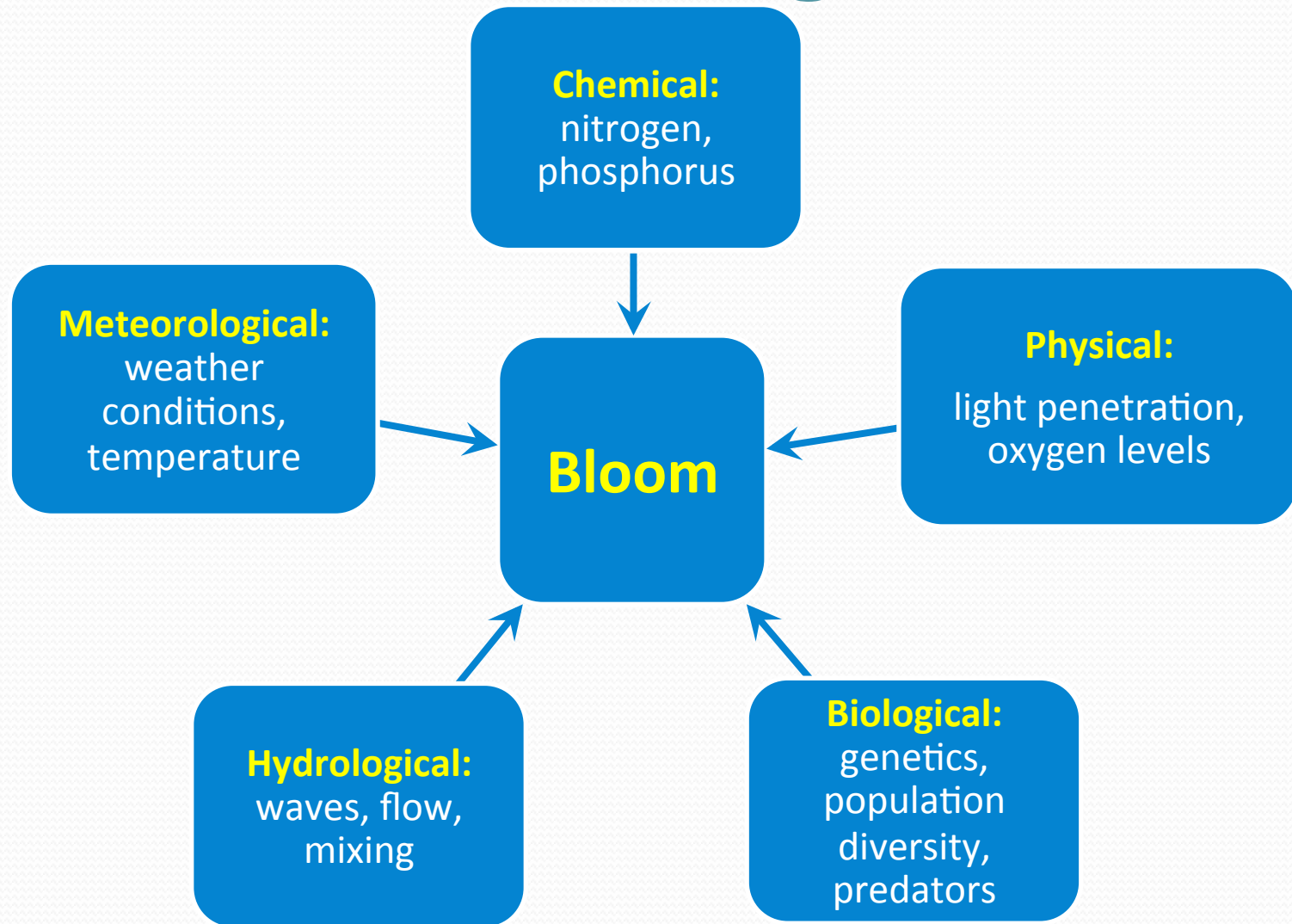
- Background
- Analytical methods
 - Enzyme-linked immunosorbent assay (ELISA)
 - Liquid chromatography/tandem mass spectrometry (LC/MS/MS)
- Comparison of the two methods
- Conclusions

Cyanotoxins (Algal Toxins)

- Produced by cyanobacteria (blue-green algae)
- Worldwide occurrence
- USGS, raw water
 - Iowa, 2006 – Microcystins 19,000 $\mu\text{g/L}$
 - Kansas, 2011 - Microcystins > 10,000 $\mu\text{g/L}$
- Toledo, Ohio, 2014, drinking water
 - Microcystins > 2 $\mu\text{g/L}$
 - Not to drink or bathe warning
- USEPA Draft Contaminant Candidate List 4



Factors Contributing to Blooms



When are Blooms a Problem?

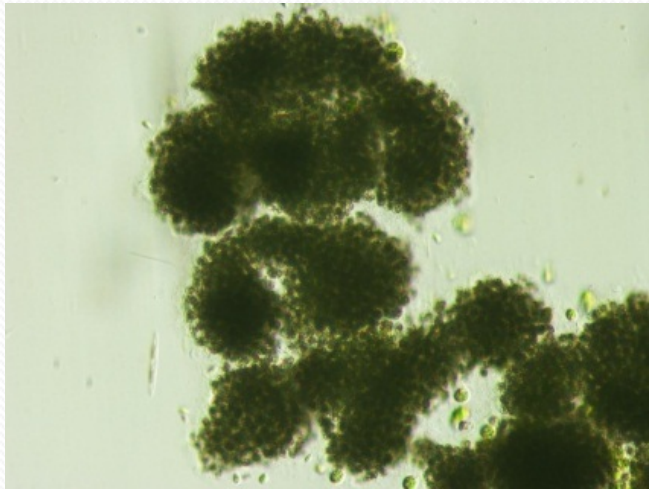
- Blooms occur naturally when the conditions are right
- Some, but not all, cyanobacteria can produce taste and odor compounds
- Some, but not all, cyanobacteria can produce cyanotoxins
 - Not all blooms are toxic
 - 45 - 75% probability of individual blooms of *Anabaena*, *Microcystis* and/or *Aphanizomenon* will be toxic

Health Related Guidelines

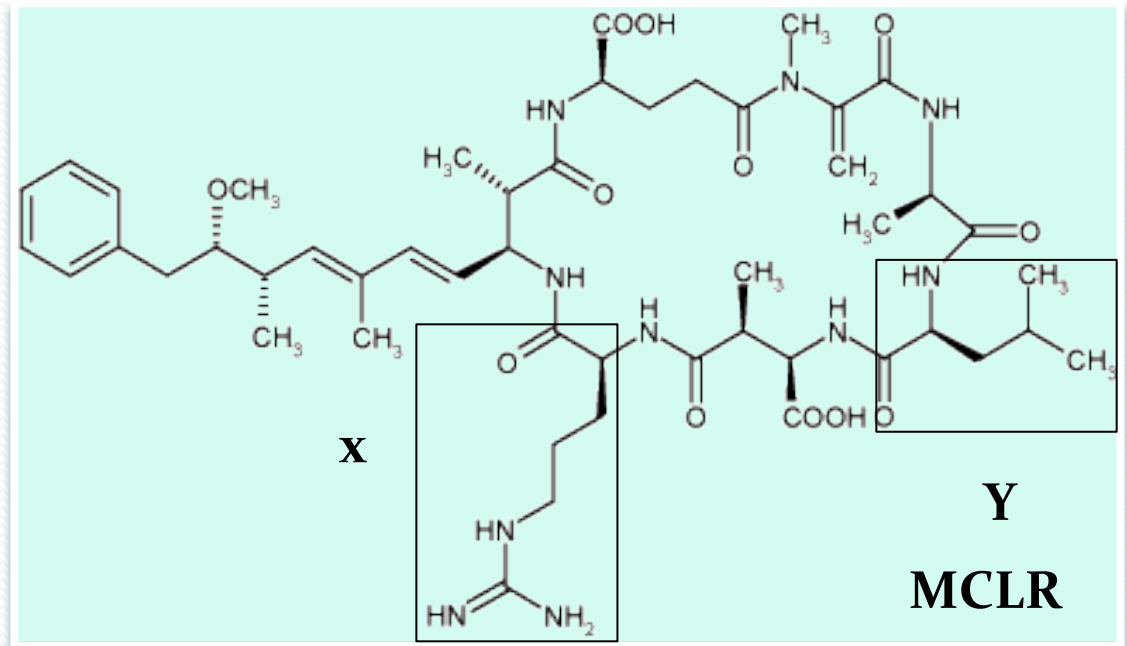
Cyanotoxin	Type of Toxin	Guidelines (Organization)
Microcystins	Hepatotoxin	<ul style="list-style-type: none">• 1.0 µg/L for MCLR in drinking water (WHO*)• 10-day Health Advisory (EPA)<ul style="list-style-type: none">○ 0.3 µg/L for children○ 1.6 µg/L for all others
Anatoxin-a	Neurotoxin	3.7 µg/L in drinking water (Quebec, Canada)
Cylindrospermopsin	Hepatotoxin	<ul style="list-style-type: none">• 1.0 µg/L in drinking water (WHO, suggested value)• 10-day Health Advisory (EPA)<ul style="list-style-type: none">○ 0.7 µg/L for children○ 3 µg/L for all others

*WHO: World Health Organization

Microcystins



Microcystis



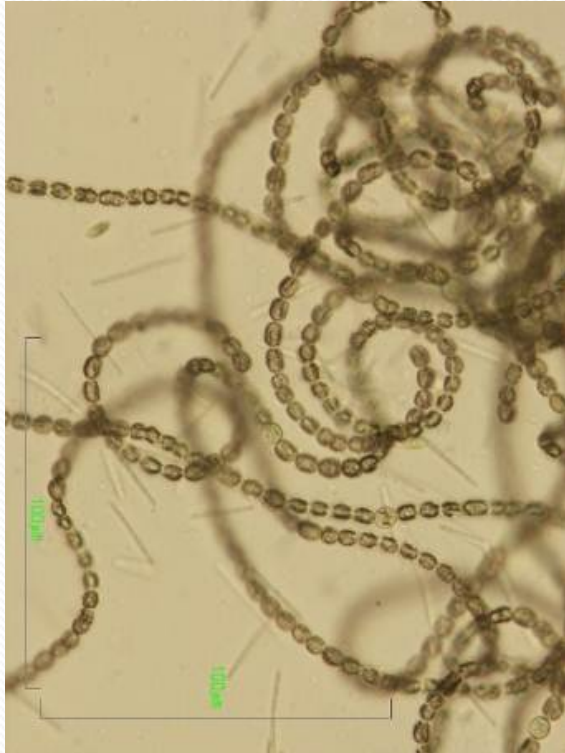
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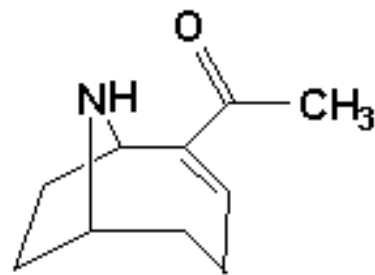
More than 90 microcystin variants have been isolated and characterized. Some of the most common variants are shown here.

LR	Arginine	Leucine
RR	Arginine	Arginine
YR	Arginine	Tyrosine
LA	Alanine	Leucine

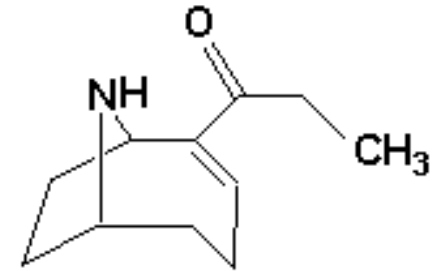
Anatoxin-a



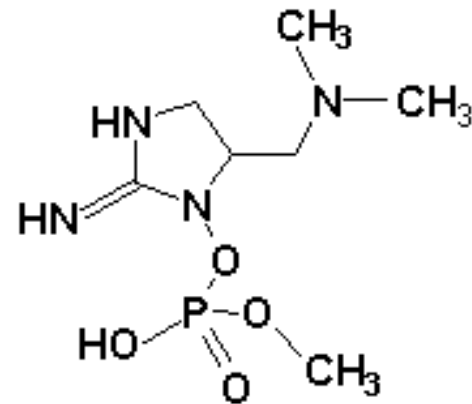
Anabaena



ANATOXIN-a

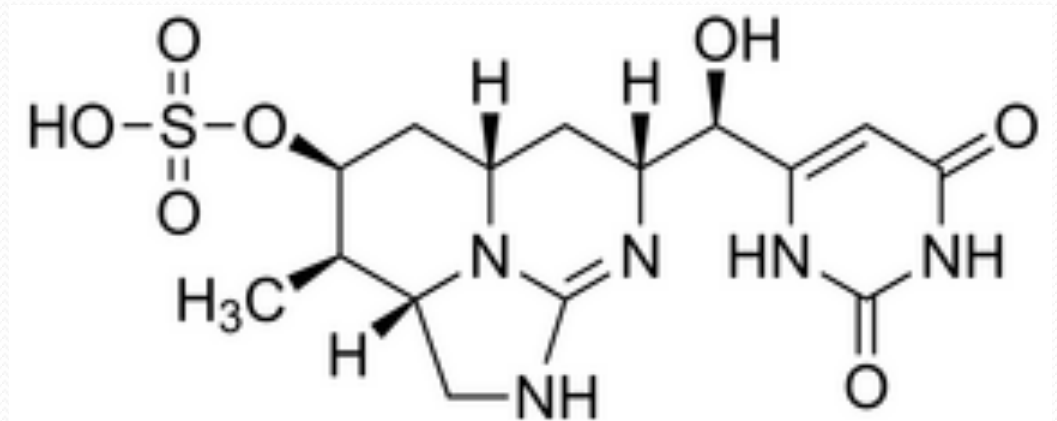


HOMOANATOXIN-a



ANATOXIN-a(s)

Cylindrospermopsin



Cylindrospermopsis

Analysis of Cyanotoxins

	Microcystins	Anatoxin-a	Cylindrospermopsin
ELISA ¹	✓		✓
PPIA ²	✓		
Mouse bioassay	✓	✓	✓
Receptor-binding assay		✓	
HPLC - DAD ³	✓	✓	✓
GC/MS ⁴		✓	
LC/MS ⁵	✓	✓	✓
LC/MS/MS ⁶			

¹Enzyme-linked immunosorbent assay

²Protein-phosphatase inhibition assay

³High Pressure Liquid Chromatography-Diode Array Detector

⁴Gas chromatography/mass spectrometry

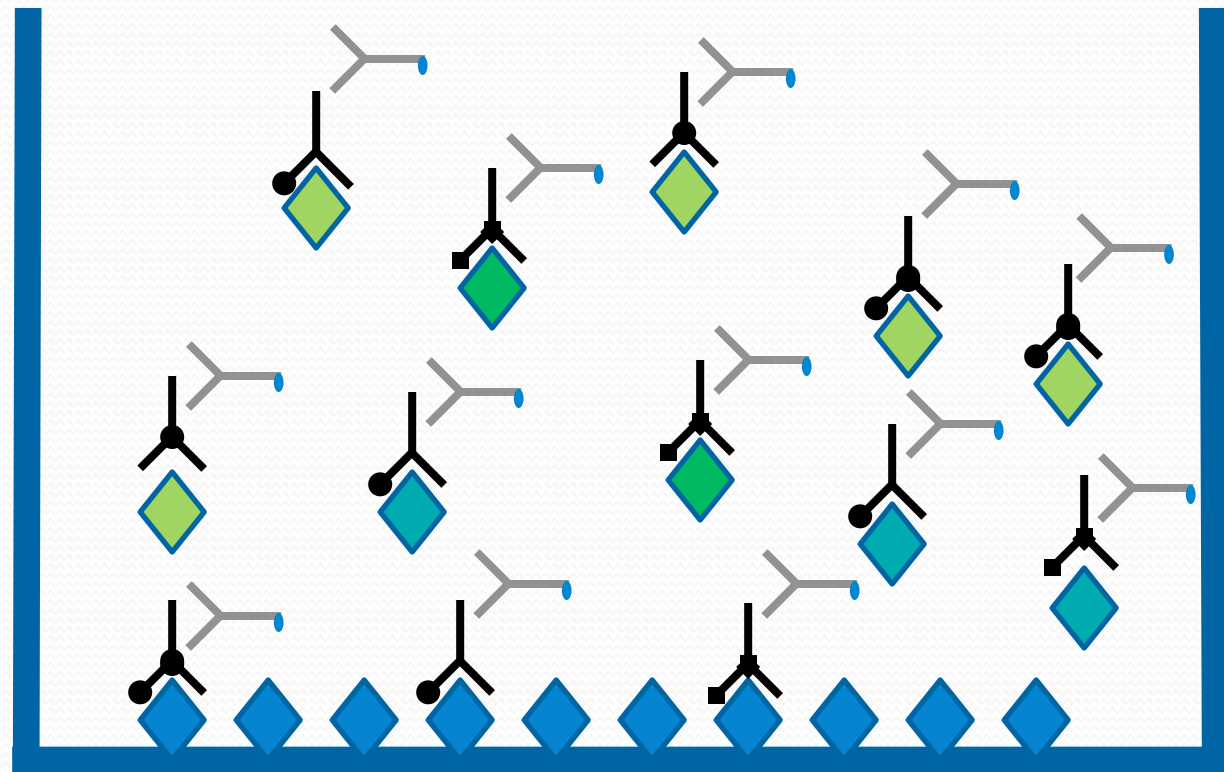
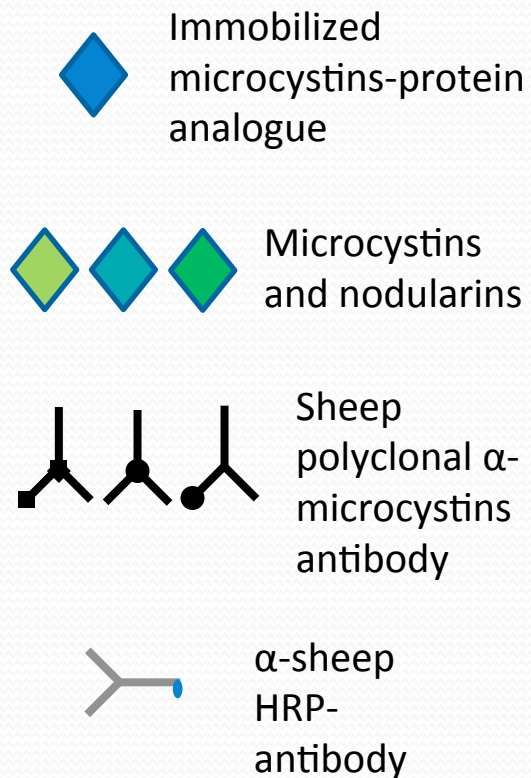
⁵Liquid chromatography/mass spectrometry

⁶Liquid chromatography/tandem mass spectrometry

Sample Handling

- **Collection, preservation and storage**
 - Glass bottles preferred
 - Freeze or 4 °C
- **Filtering – extracellular concentration**
 - Some filters showed absorption (e.g. Nylon filters)
- **Cell lysis – total concentration**
 - Freeze-thaw cycles
 - Sonication
 - QuikLyse™

Analysis by ELISA – An Example



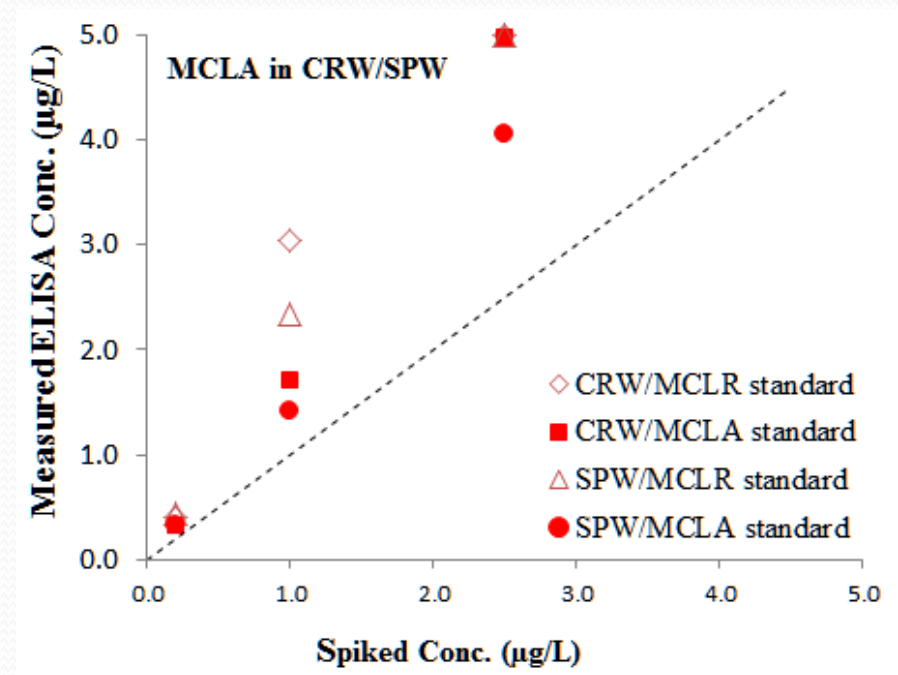
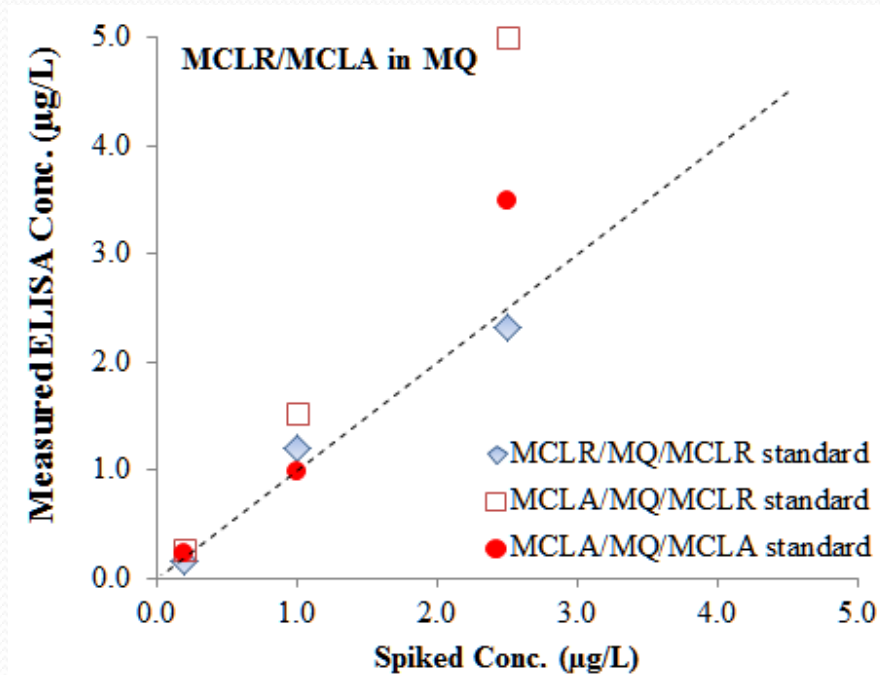
- **Indirect Competitive ELISA:** Immobilized analogue and targets in solution compete for primary antibody; HRP-secondary analogue is added afterwards.
- **Color is inversely proportional to target.**

ELISA (cont'd)

- Based on antibody response – total toxins, not individual variants
- Available for those with commercial kits
- Sensitive
 - Reporting limit: 0.15 µg/L (Abraxis microcystins ADDA-specific kit)
- Cost estimates
 - Microplate reader (\$8,000 to \$40,000)
 - ELISA kits (\$440 per 96 wells)
 - Commercial laboratory: ~ \$ 100 per sample



ELISA* - Calibration Standards



*Abraxis microcystins ADDA-specific kits

MQ: Milli-Q reagent water

CRW: Colorado River Water

SPW: State Project Water

Analytical Methods: LC/MS/MS

■ Direct injection

- Eight microcystins and nodularin
- Minimum reporting levels (MRLs): 0.1 – 0.2 µg/L
- Identifies individual toxin variants

■ Instrument Conditions

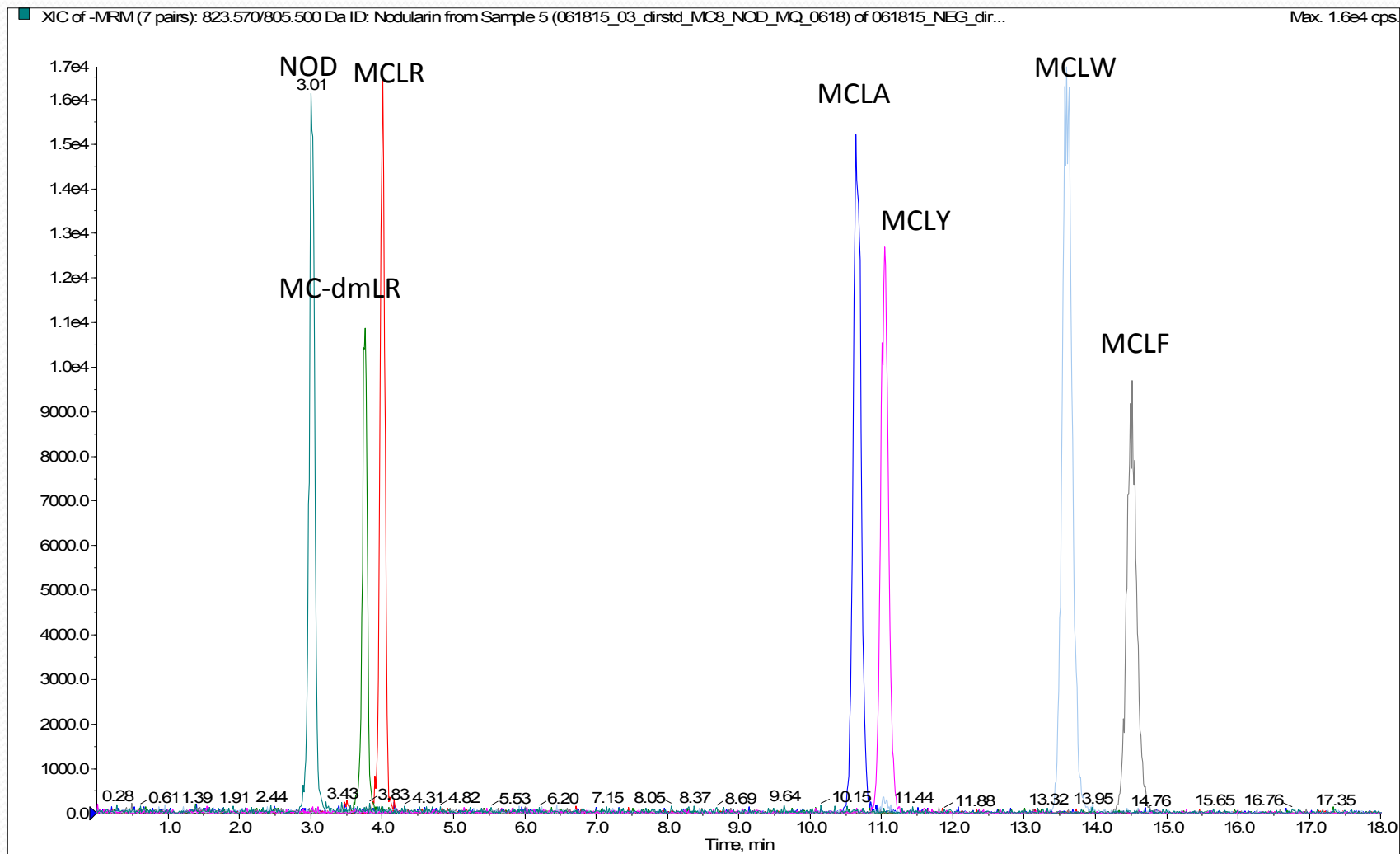
- API 4000 system: ESI + and ESI -
- Column: Phenomenex Luna C18(2), 5 µm, 4.6 x150 mm

■ Cost estimates

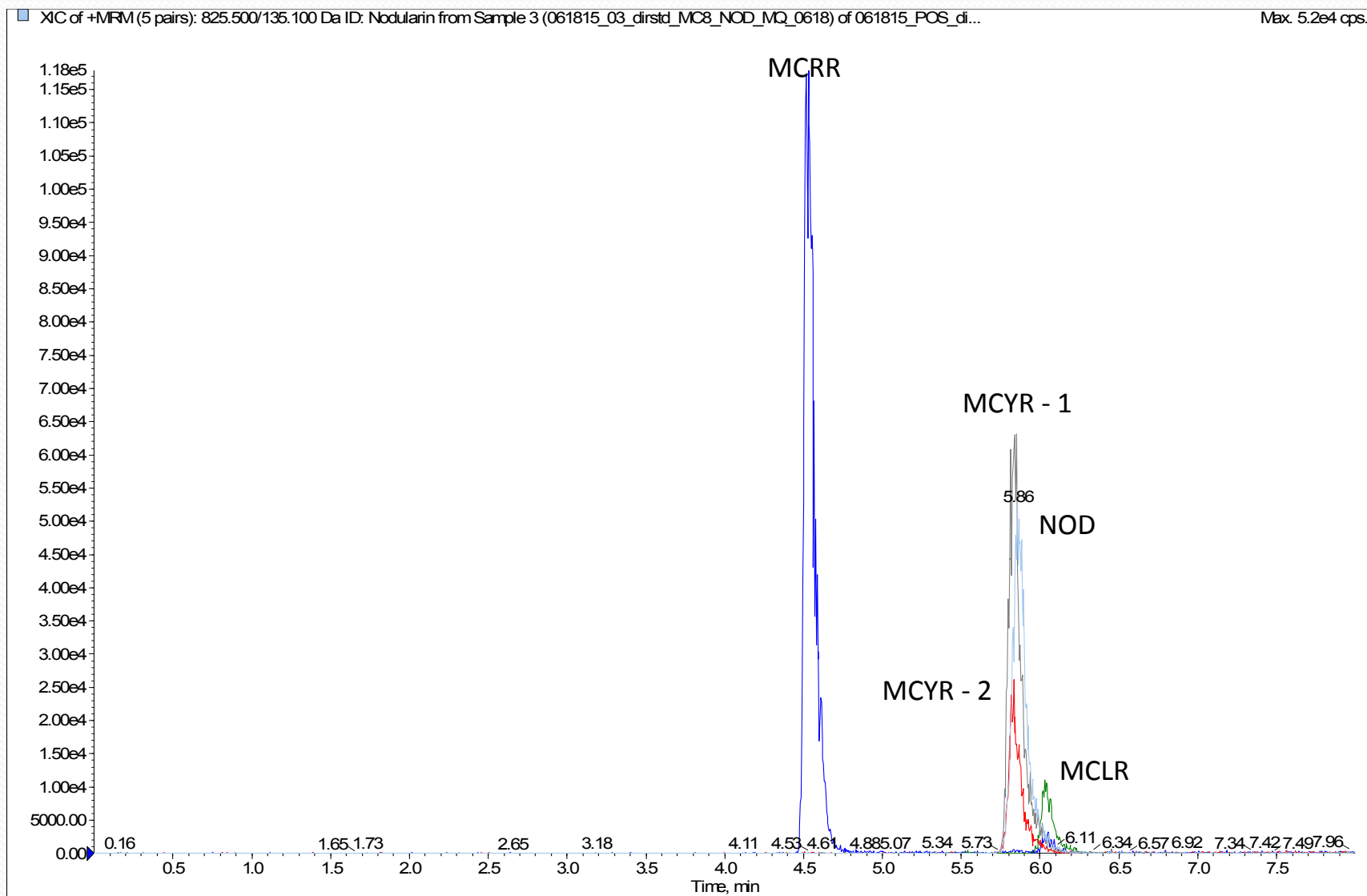
- LC/MS/MS (\$250,000 - \$600,000)
- Expensive standards
- Commercial laboratory: ~\$200-\$300 per sample



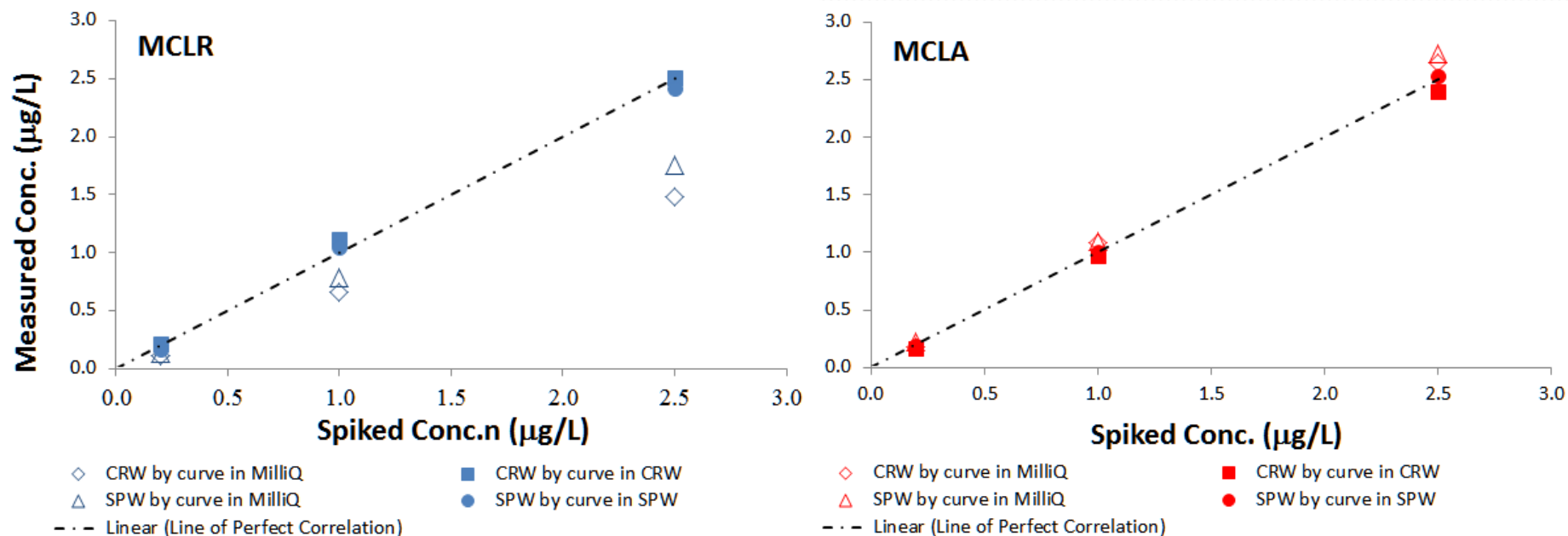
ESI- Mode



ESI+ Mode



Matrix Effects



CRW: Colorado River Water
SPW: State Project Water

EPA Method 544

- Published in February 2015
- For drinking water only
- Six microcystins and nodularin
 - Minimum reporting levels: 2.9-22 ng/L
- Other key points of the method
 - MeOH and freezing for lysing
 - Solid phase extraction - 500 mL sample volume
 - Phenomenex Kinetex C8 column, 2.6 μm , 2.1 x 100mm
 - C_2D_5 -MCLR as the internal standard

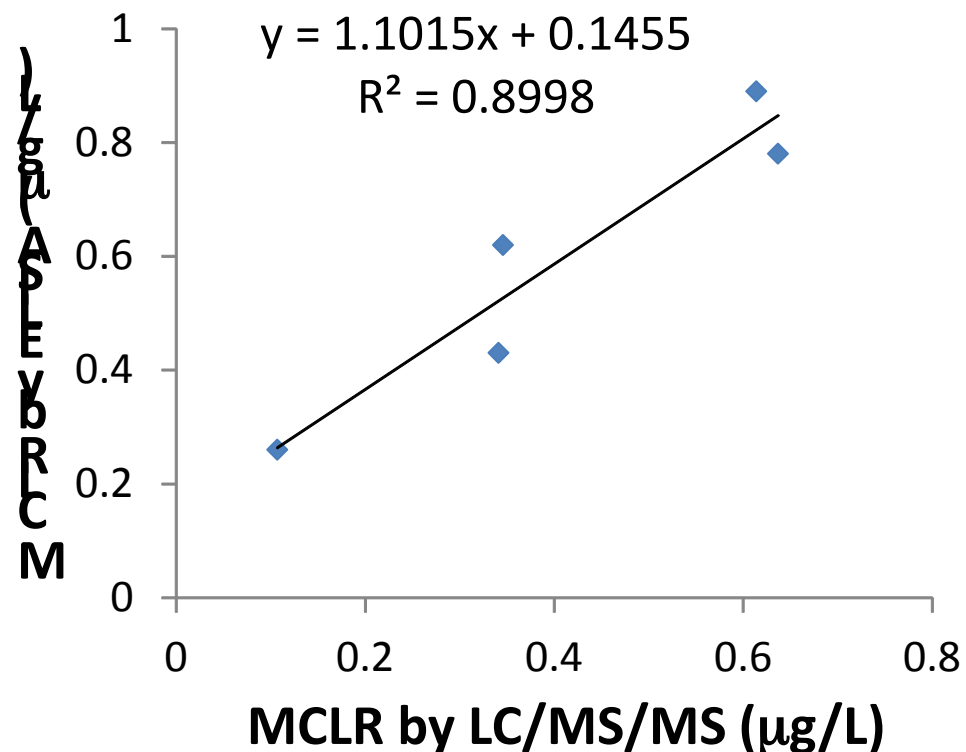
Ozonated Samples: ELISA vs. LC/MS/MS

CRW; MCLA spiked conc. = 85 µg/L; O₃ contact time = 1 min

O ₃ Applied Dose (mg/L)	LC/MS/MS Results (µg/L)	ELISA Results by MCLR Standards (µg/L)	ELISA Results by MCLA Standards (µg/L)
0.10	3.3	10.0	4.2
0.10	7.8	24.3	8.6
0.20	<0.1	2.5	1.1
0.25	<0.1	2.1	1.1
0.30	<0.1	1.4	0.8
0.40	<0.1	1.5	0.8
Control	81.3	>250	75.5

Chlorinated Samples: ELISA vs. LC/MS/MS

Cl ₂ Applied Dose (mg/L)	ELISA Results (µg/L)	LC/MS/MS Results (µg/ L)
1.0	0.78	0.64
1.0	0.89	0.61
1.5	0.43	0.34
1.5	0.62	0.35
2.5	0.26	0.11



- Abraxis microcystins ADDA-specific kits
- CRW
- MCLR spiked conc. = 5 µg/L
- Contact time = 60 min

ELISA vs. LC/MS/MS

	ELISA (ADDA)*	LC/MS/MS
Characteristic	Total toxins	Individual variants
Quantification	Semi-quantitative	Quantitative
Sample volume	<5 mL	<5 mL
MRLs	0.15 µg/L	0.1 – 0.2 µg/L
Turnaround time	Fast	Slower
Initial investment	Affordable	Expensive
Personnel training	Less	More
Other important aspects to consider	<ul style="list-style-type: none"> • Different vendors/kits • Needs correct calibration standards • Possible interference from treatment byproducts 	<ul style="list-style-type: none"> • Possible matrix effects • 12-14 microcystin variant standards available

*Abraxis microcystins ADDA-specific kit.

Conclusions

- **ELISA is an effective rapid screening tool**
 - Reporting limit = 0.15 µg/L (Abraxis microcystins ADDA kits)
 - Measures total microcystins
- **LC/MS/MS is quantitative**
 - Reporting limit = 0.1 -0.2 µg/L
 - Identifies individual variants

Conclusions (cont'd)

- ELISA and LC/MS/MS results may not correspond to each other
 - Microcystin variants may have different ELISA responses
 - Quantitation dependent on using correct variant(s) - need variant-specific standards for calibration
 - ELISA may respond to oxidation by-products
 - LC/MS/MS may experience matrix effects
 - Limited standard availability
 - Isotopically labeled standards as internal standards



Acknowledgements

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Questions?

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