Microcystins Analysis Methods

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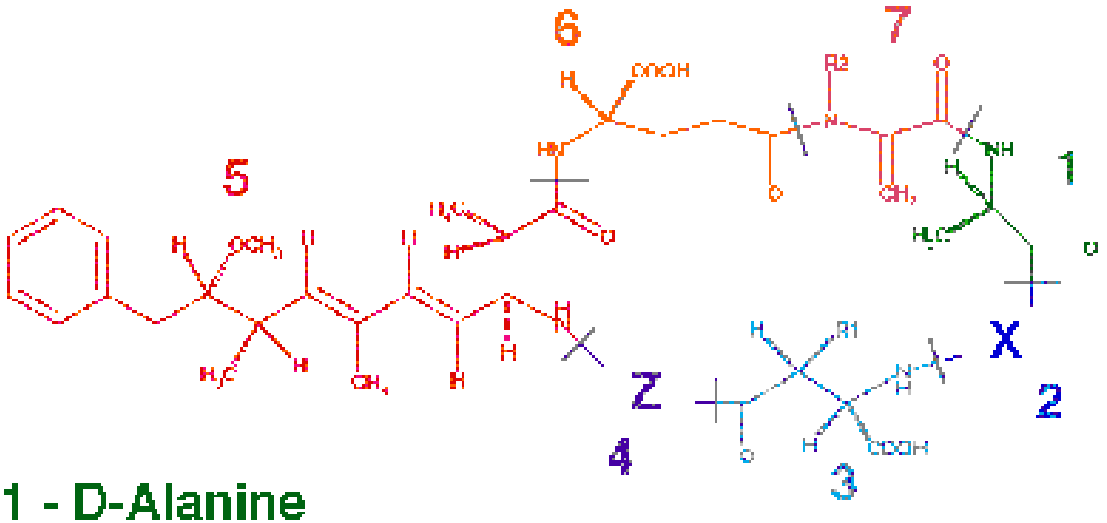
Heather Raymond
Ohio Harmful Algal Bloom Coordinator

Microcystins Testing

No "Perfect" Analytical Method for Detecting TOTAL Microcystins



1 - D-Alanine
2 - Variable L-amino acid
3 - D-Methylaspartic acid
4 - Variable L-amino acid
5 - 3-amino-9-methoxy-2,6,8-trimethyl
-10-phenyldeca-4,6-dienoic acid (Adda)
6 - D-Glutamic acid
7 - N-Methyldehydroalanine

- Over 140 Microcystin Variants
- Standards Not Available for Majority

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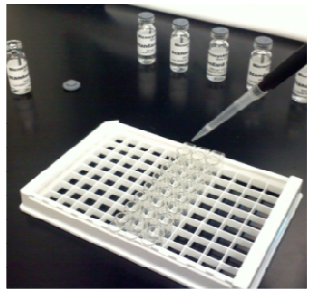
Microcystins Testing - ELISA

- **Enzyme-Linked ImmunoSorbent Assay (ELISA) Microcystin-ADDA Method** (detection of antigen using an antibody)
  - Measures Total Microcystins (all variants/congeners, based on ADDA)
  - Highly Selective/Specific (for ADDA)
  - Certified by USEPA (ETV Program)
  - Moderately sensitive (RL: 0.30ug/L)
  - Suitable for raw & finished water (complex matrices)
  - Quick (four hours), useful for operational adjustments
  - Relatively inexpensive
  - Does not require high end equipment or expertise to run (can be used in water system lab)
  - Does not require pre-concentration solid phase extraction (SPE) step



  - Does not provide concentrations of specific microcystin variants
  - Is an indirect measure of the toxin

Ohio EPA DES Method 701.0
Total Microcystins – ADDA by ELISA

- Developed in consultation with USEPA, PWSs, and National Experts.
- Helps ensure consistent sample handling, preparation, and application of analytical method.
  - Finished water samples and treatment train samples that are subjected to an oxidant must be quenched upon collection with 10 mg of sodium thiosulfate per 100 ml of sample.
  - At Lab, sample pH must be adjusted within the range of 5-11.
  - At Lab, all samples are subjected to three freeze/thaw cycles to lyse (break apart) cyanobacteria cells and release toxins.
- Labs must demonstrate they can achieve an acceptable level of precision and accuracy.
- Ohio EPA conducts site visits at labs preforming analysis; provides acceptance letters and certification
- Compliance microcystins monitoring under 3745-90-03 must use this method or “another method accepted by the director in writing.”

A Standard Method Increases Consistency & Confidence in Results

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High Performance Liquid Chromatography (HPLC) – Ultraviolet (UV) or Photodiode Array (PDA)

- HPLC-UV/PDA
  - Liquid Chromatography separates components
  - Microcystins have UV absorption maxima at 238 nm
  - Non-selective detector; co-eluting interferents prevent accurate identification of components and quantitation (potential for false positives and false negatives)
  - Less expensive than mass spectrometry.
  - Less sensitive than mass spec (average LOQ ~ 0.3 µg/L)

Adapted From USEPA

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Liquid Chromatography(LC) –Tandem Mass Spectrometry (MS/MS)

- LC/MS/MS
  - Highly specific identification of components (based on standards)
  - MS can identify a component in the presence of co-eluting interferents but quantitation may be compromised
    - Presence of co-eluting interferents can act to suppress or enhance response resulting in analytical bias
    - Sensitive (LOQ ~ 0.02 µg/L)
  - Expensive and requires highly skilled analysts
  - USEPA Method 544
    - Standard Method- includes QA/QC protocols and reduces variability in results between labs
    - Limited to 6 microcystin variants and finished water only

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Adapted From USEPA

LC/MS/MS
Additional Considerations

- **Microcystins**
  - "Weak" product ion abundance limits sensitivity
  - Limited based on available standards
  - Requires preconcentration with SPE to augment sensitivity (LOQs $\leq$ 0.02 µg/L)
    - Preconcentrates NOM too
- **Anatoxin-a and Cylindrospermopsin**
  - Abundant product ion responses
  - Direct injection (no SPE required to improve sensitivity – LOQs: anatoxin-a ~ 0.02 µg/L; cylindrospermopsin ~ 0.06 µg/L)

Source: USEPA

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LC-MS/MS MMPB Method

- MMPB (2-methyl-3(methoxy)-4-phenylbutyic acid) method analyzes the chemically cleaved Adda group common to all microcystin variants
- Measures total microcystins (all variants)
- Quick (~2 hours, does not require freeze/thaw or sonication)
- Sensitive (0.05 ug/L)
- Suitable for raw and finished water
- Does not require standards for individual variants
- Utilizes 4 PB internal standard

- Does not provide data on individual congeners
- Requires oxidation step
- Potential for detection of microcystins disinfection byproducts

Toxicon 104 (2015) 91-101 (Foss & Aubel): Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS)

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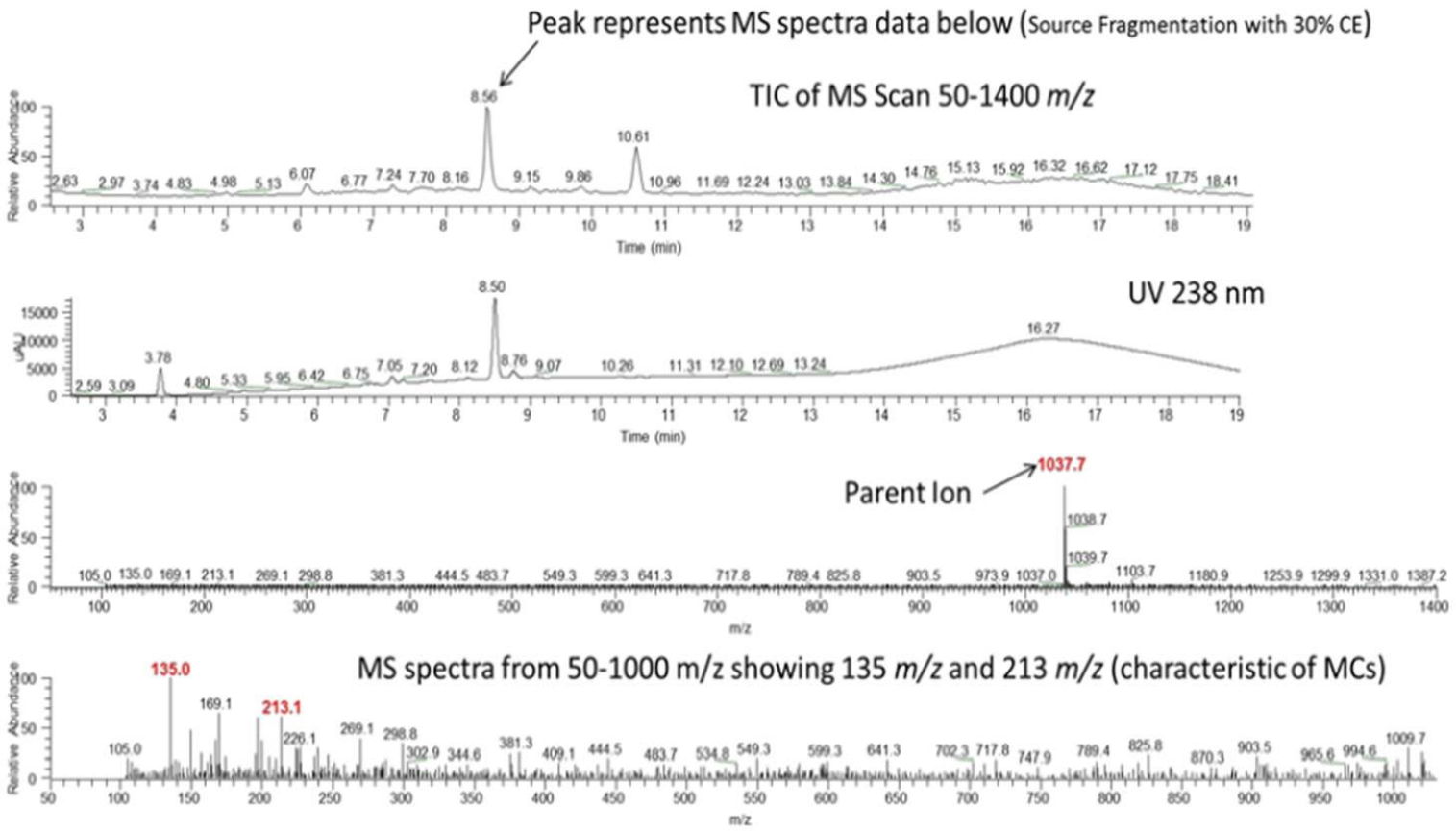
LC-UV/PDA & LC-MS Scan

- Uses two LC-based methods in tandem to independently confirm presence of microcystins
  - Can detect microcystin variants without standards
  - No standard methods, expensive, requires complex data-interpretation, time-consuming



Analytical Method Comparison & Microcystin Variant Evaluation

- 11 Sites: 4 Up-ground Reservoirs, 2 In-stream Reservoirs, 2 Lake Erie locations, 2 Canal-feeder Lakes, and 1 River Source.
- 22 Samples from 2014 Selected to Help Evaluate Spatial and Temporal Variability Within Source Waters
- Variety of Cyanobacteria Genera Represented
- Each Sample Analyzed Using 5 Separate Analytical Methods

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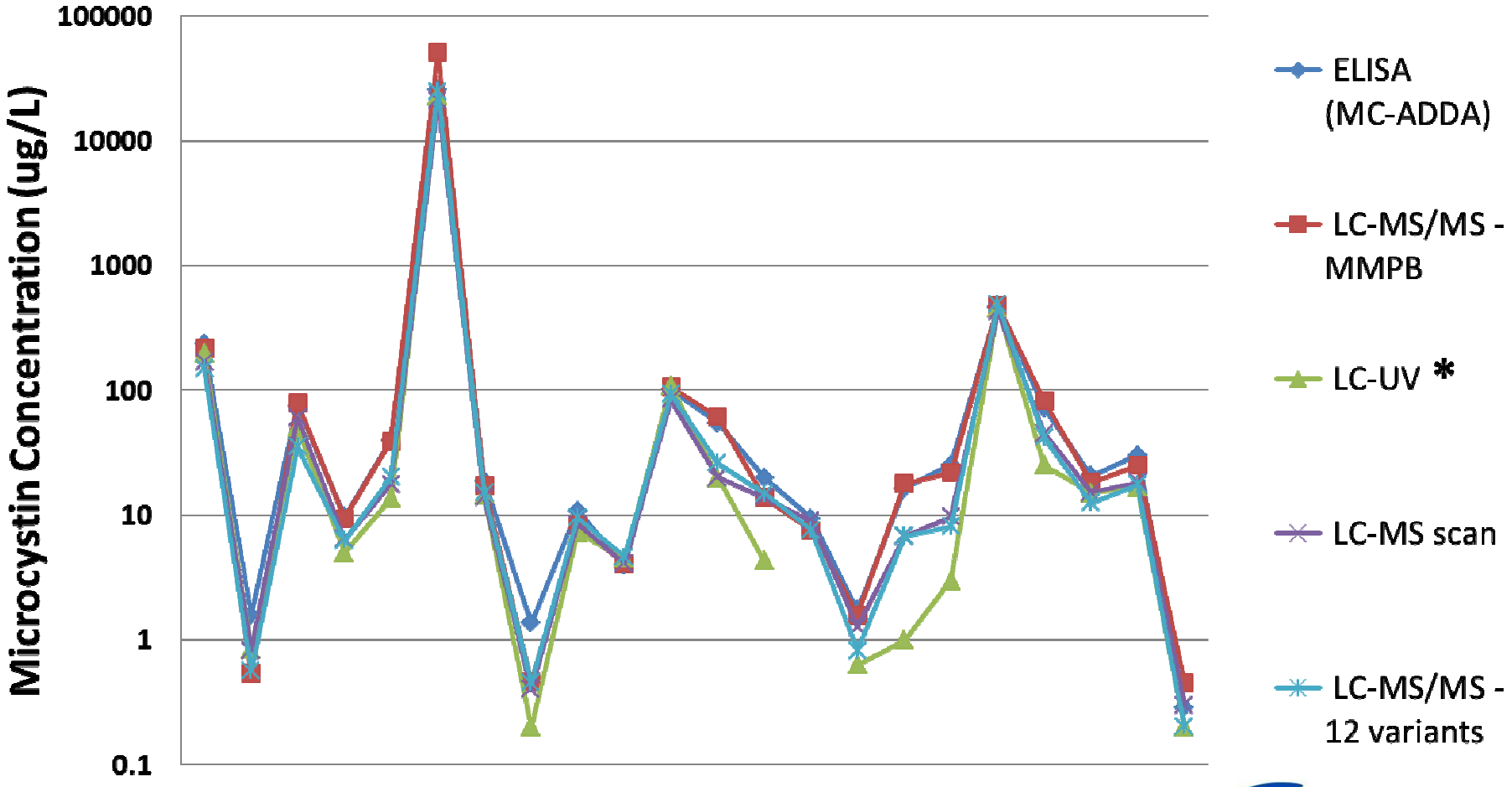
Results of Method Comparison



* LC-UV data presented does not include false-positives that were eliminated from total (Based on lack of confirmation with LC-MS methods).
Sample # 14 was non-detect using LC-UV.

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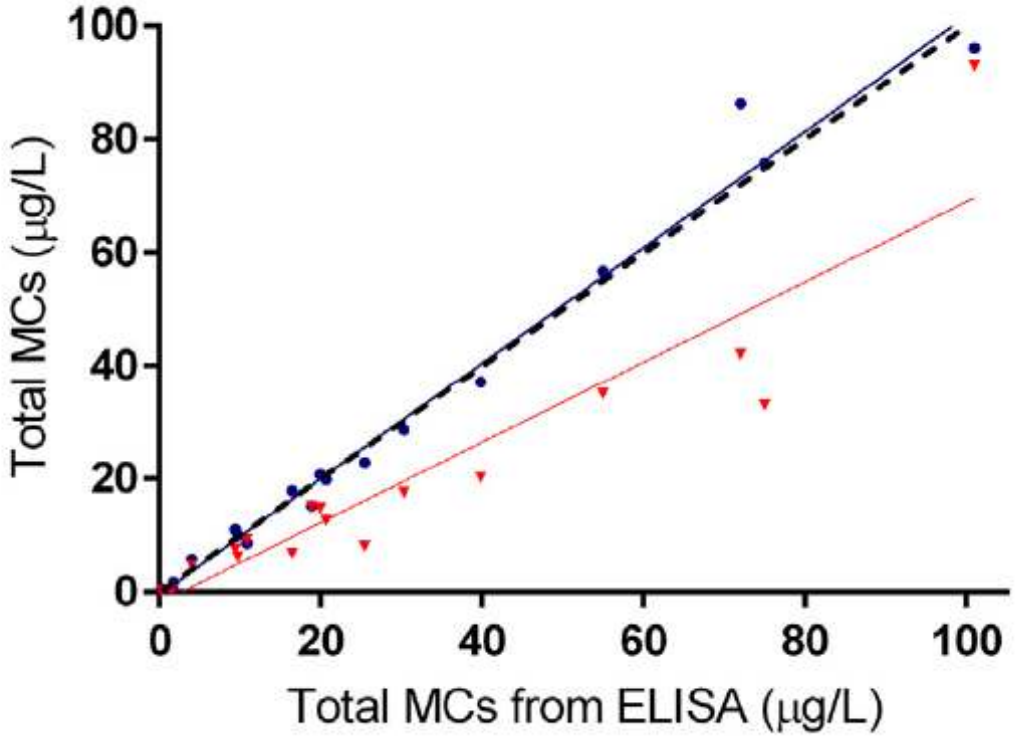
Results of LC-MS/MS MMPB and Individual Variant Analysis Compared to ELISA



MMPB

Y = 1.022*X - 0.4314

R^2=0.9830

Individual Variant Analysis

Y = 0.7095*X - 1.907

R^2=0.8724

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Spatial and Temporal Variability in Microcystin Variants

Lake Erie Microcystin Variants					
MC-Variant	Site 1 8/25/14	Site 2 8/4/14	Site 2 8/18/14	Site 2 9/29/14	Site 2 10/14/14
MC-RR	2.1	20	10	5.5	8.5
MC-YR	0.6	6	5	1.2	2.5
MC-LR	2.9	16	10	5.5	6.1
MC-WR	0.6		3-9	0.2-0.6	0.2-0.6
MC-LY			2-6		
8.7 min 1043.5 m/z			10-30	3.6	

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Inland Lake Microcystin Variants (Planktothrix)			
MC-Variant	Site 1 6/16/14	Site 2 6/16/14	Site 2 9/2/14
[DAsp3] MC-RR	5.3	6.1	17.5
[Dha7] MC-LR	1.1	1.4	1.5
MC-YR	0.2-0.6	0.2-0.6	1.2
MC-RR		0.1-0.3	

Inland Lake Microcystin Variants (Mixed Bloom)				
MC-Variant	Site 1 6/18/14	Site 2 6/18/14	Site 2 7/9/14	Site 3 6/30/14
[Dha7] MC-RR	2.9	3-9	1.0	0.08
MC-RR	1.4	39	1.0	0.01-0.03
MC-YR	1.1	15	1.0	
MC-LR	4.0	67	2.4	0.55
[DAsp3] MC-LR	0.6	18	0.4	0.03
[Dha7] MC-LR	3.6		1.0	0.05
MC-WR	0.2-0.6		0.2-0.6	
MC-LA	0.2-0.6			
MC-LY	0.2-0.6	6	0.2-0.6	0.10

Key Findings

- LC-based Methods Confirmed ELISA Results
- 16 Different MC-variants were detected
- MC-LR was only detected at 5 of 11 sites (45%)
- Most common variants were: MC-YR, [Dha7] MC-LR and [DAsp3] MC-RR
- HPLC-PDA Methods Prone to Interference, Especially at Lower Concentrations
- 91% of samples had MC-variants not detectable by USEPA Method 544 (including dominant MC-variant in some samples)
- LC-MS/MS individual variant analysis under-reported total microcystins, based on MMPB and LC-UV/MS Scan Data
- Generally, the Dominant/Co-Dominant MC-Variants Did Not Vary Spatially nor Temporally
- Secondary & Minor MC-Variants Did Vary.

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ELISA MC-ADDA Matrix Interference Studies

Treatment Chemical	Microcystins – ADDA ELISA Assay Tolerance (< / =)
Sodium Carbonate (Soda Ash)	≤25 gpg
Sodium Hexametaphosphate	≤250 ppm
Sodium Silicofluoride	≤10 ppm
Aluminum Sulfate[1]	≤100 gpg (with pH adjustment within assay tolerance)
Calcium Oxide (Lime)[1]	≤2000 gpg (with pH adjustment to within assay tolerance)
Potassium Permanganate[2]	≤10 ppm (with quenching using 1 mg sodium thiosulfate per 1 ml sample)
Sodium Chlorite[2]	≤10 ppm (with quenching using 1 mg sodium thiosulfate per 1 ml sample)
Carbon[3]	≤2 ppm with filtering at time of sampling

[1] Natural pH of solution outside assay tolerance, Chemical tolerance levels determined after pH adjustment.
[2] Oxidizers degrade microcystins, tolerance determined after quenching.
[3] Tolerance level due to effect of carbon on toxin, not assay performance.

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ELISA MC-ADDA Matrix Interference Studies

Lisa Kamp, et. at, 2016. *The effects of water sample treatment, preparation, and storage prior to cyanotoxin analysis for cylindrospermopsin, microcystin and Saxitoxin.* Chemico-Biological Interactions.

Studies by USEPA as part of ELISA MC-ADDA Method Development for UCMR 4:

- Storage Stability – Holding Times
- Sample Preservation and Container Studies
- Matrix Interference Studies
  - -Microcystins Variant Fortified Sample Studies (finished water, raw water, reagent water with chemical addition, etc.)
  - -Dilution Experiments (real world raw/finished water samples)
- USEPA Method Validation & Interlab Validation

LC-MS/MS MMPB Method Evaluation

- Potential concern regarding detection of microcystins disinfection byproducts
- ELISA MC-ADDA does not detect microcystins disinfection byproducts

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Ohio EPA Method Comparison Study-Round 2

- 17 Raw and treatment train samples from 2015 submitted for method comparison
- Additional MC variant standards available
- Preliminary LC-MS/MS results indicate MC-HtYR, MC-HilR, MC-LF, MC-LW, MC-RR, MC-YR, MC-LR, MC-WR, and MC-LY present in Lake Erie Samples
- 100% of samples had MC variants not included in USEPA Method 544
- Next Steps: Split sample interlab method comparison study

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2015 PWS HAB Response Strategy

Analytical Methods

	Microcystins (µg/L)	Cylindro-spermopsin (µg/L)	Saxitoxins (µg/L)	Anatoxin-a (µg/L)
Surveillance sampling	ELISA (MC-ADDA)	ELISA	ELISA	LC-MS/MS
Repeat sampling in response to a finished water detection	ELISA (MC-ADDA)	LC-MS/MS	LC-MS/MS	LC-MS/MS

ELISA: Enzyme-Linked Immunosorbent Assay

LC-MS/MS: Liquid Chromatography followed by tandem Mass Spectrometry

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Data Reporting Considerations

- Specify Analysis Method
- If reporting “total microcystins” using LC-MS/MS, include number of variants analyzing for in comment field
- For ELISA results greater than calibration curve, dilute and reanalyze sample or qualify result as estimate (or report using “>” symbol)

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Questions?
Heather.Raymond@epa.ohio.gov
(614) 644-2752
http://epa.ohio.gov/ddagw/HAB.aspx
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