Rapid and Accurate Analysis of Microcystins Using UPLC/MS/MS

Yongtao (Bruce) Li, Ph.D.
Joshua S. Whitaker

Eurofins Eaton Analytical, Inc.

NEMC, Chicago, IL
July 15, 2015

Presentation Outline

- **Introduction**
  - Harmful algal blooms
  - Drinking water guidelines for microcystins
  - Analytical techniques & monitoring
- **UPLC/MS/MS Method Development**
  - Demonstration of capability
  - Sample preservation
  - Matrix studies
- **Conclusions**

Harmful Algal Blooms(HABs)

- In 2010, EPA's First National Lakes Assessment reported that microcystins were found to be present in ~ 1/3 of lakes and at levels of concern in 1% of lakes.
- In 2014, HABs left nearly a half million people without drinking water in the Toledo area, Ohio.
- Responding to emergency of HABs requires timely and reliable results.
  - Algae identification and enumeration
  - Toxin screening and quantitation
  - Taste and odor (geosmin & MIB) problems

International Drinking Water Guidelines for Microcystins

Authority/Country	Microcystins
WHO (1998 provisional) - adopted by many countries.	1 µg/L MC-LR
Health Canada - proposing a change (June, 2015)	1.5 µg/L total
Australia	1.3 µg/L total
EPA Health Advisories - based on 10-day exposure (May, 2015)	0.3 µg/L infants and pre-school children 1.6 µg/L school-age children and adults

Analytical Techniques & Monitoring

- Microcystin-LR, cylindrospermospin, and anatoxin-a are included in EPA's drinking water contaminant candidate list 4 (CCL 4) – draft.
  - Potentially included in the UCMR 4, 2017-2019.
- LC/MS/MS is widely used for determination of algal toxins.
  - EPA 544: Determination of six microcystins & nodularin (combined intracellular and extracellular) in drinking water, combined with SPE.
  - EPA 545: Determination of cylindrospermospin and anatoxin-a in drinking water.
- ELISA (enzyme-linked immunosorbent assay) is widely used for screening analyses of algal toxins.

Study Objective

- Develop a fast and reliable analytical method for monitoring cyanobacterial blooms and for timely responding to emergency of microcystin contamination events.
  - High sensitivity - Meets EPA Health Advisories values.
  - High accuracy and precision
  - Results available within minutes

Method Summary

- Waters Acquity UPLC-H Class & Xevo TQ-S
  - Direct aqueous injection (DAI), 20 µL
  - Positive ESI
  - BEH C18, 2.1x100 mm, 1.7 µm
  - 0.45 mL/min flowrate
  - Gradient 20 mM ammonium formate/acetonitrile

- EPA Method 544 Preservatives & Refrigeration
  - Trizma – 7.75 g/L buffering reagent
  - 2-Chloroacetamide – 2 g/L antimicrobial agent
  - Ascorbic acid – 0.1 g/L dechlorinating agent
  - EDTA – 0.35 g/L inhibit binding of the targets to metals

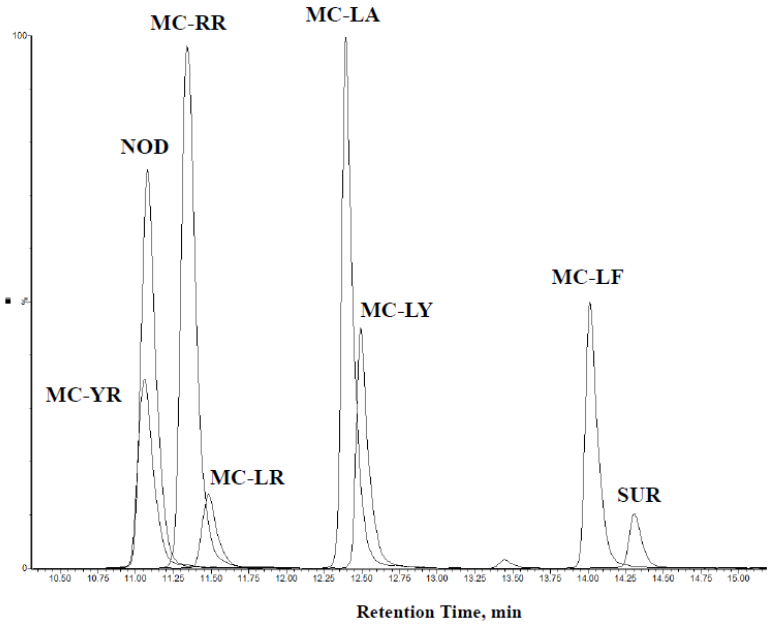
Quantitation Ions & Calibrations
(Correlation Coefficient ≥ 0.99)

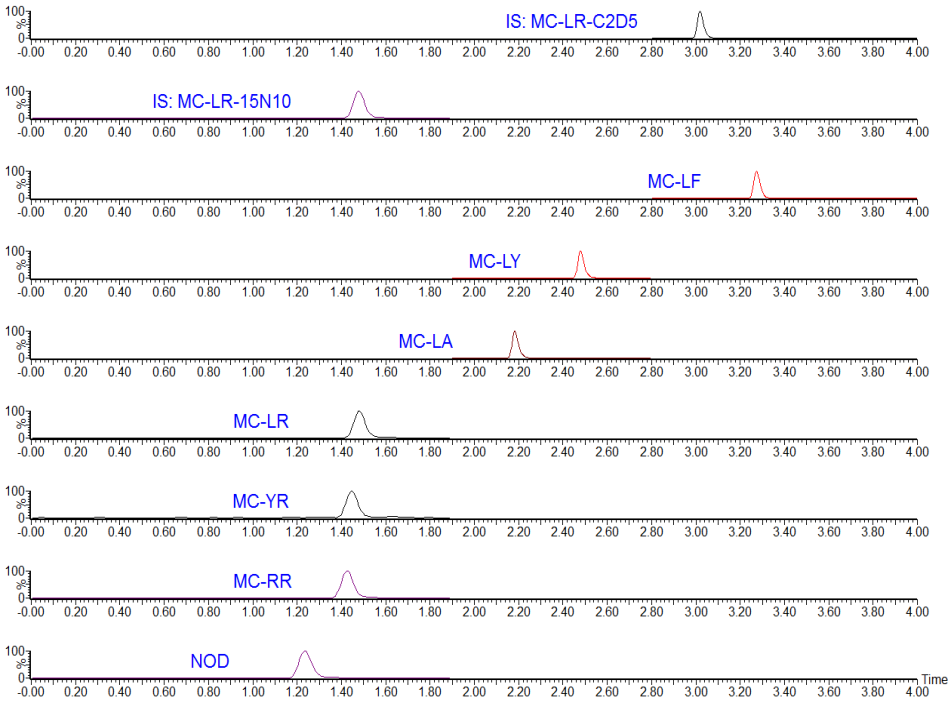
Analyte	Mass	Ext. Std. Cal.	Int. Std. Cal.
MC-LA	910.5>776.4	✓	MC-LR-C2D5
MC-LF	986.5>134.9	✓	MC-LR-C2D5
MC-LR	995.4>134.9	✓	MC-LR-15N10
MC-LY	1002.5>134.9	✓	MC-LR-C2D5
MC-RR	519.9>134.9	✓	MC-LR-15N10
MC-YR	523.4>134.9	✓	MC-LR-15N10
NOD	825.5>134.9	✓	MC-LR-15N10
MC-LR-15N10	1005.7>135.2	✓	
MC-LR-C2D5	1028.6>134.9	✓	

HPLC/MS/MS vs. UPLC/MS/MS

EPA 544
SPE-HPLC/MS/MS
500 mL sample to 1 mL extract
~ 26 min

DAI-UPLC/MS/MS
1 mL sample
~ 6 min





UPLC/MS/MS

Demonstration of Capability

UPLC/MS/MS
Sensitivity – MDLs (n = 7, RW)

Analyte	EPA 544 Ext. Std. Cal. (ng/L)	UPLC/MS/MS Ext. Std. Cal. (ng/L)	UPLC/MS/MS Int. Std. Cal. (ng/L)
MC-LA	4.0	35	31
MC-LF	3.4	19	13
MC-LR	4.3	52	41
MC-LY	2.2	18	13
MC-RR	1.2	12	20
MC-YR	4.6	46	26
NOD	1.9	8.8	11

UPLC/MS/MS
Sensitivity – LCMRLs

Analyte	EPA 544 Ext. Std. Cal. (ng/L)	UPLC/MS/MS Ext. Std. Cal. (ng/L)	UPLC/MS/MS Int. Std. Cal. (ng/L)
MC-LA	2.9	33	22
MC-LF	3.5	27	23
MC-LR	6.6	130	68
MC-LY	4.6	40	7.3
MC-RR	5.6	210	82
MC-YR	22	130	**200**
NOD	7.3	9.8	11

UPLC/MS/MS

Accuracy & Precision (n = 4, RW)

Analyte	Spiking Conc. (µg/L)	ES Cal. Recovery (%)	ES Cal. RSD (%)	IS Cal. Recovery (%)	IS Cal. RSD (%)
MC-LA	1.0	108	1.2	97	1.5
MC-LF	1.0	107	4.5	96	3.5
MC-LR	1.0	111	5.8	106	5.8
MC-LY	1.0	108	3.6	99	3.7
MC-RR	0.5	93	16	101	8.2
MC-YR	1.0	97	13	103	8.7
NOD	0.5	105	2.0	100	2.1

UPLC/MS/MS

Accuracy & Precision (n = 4, local DW)

Analyte	Spiking Conc. (µg/L)	ES Cal. Recovery (%)	ES Cal. RSD (%)	IS Cal. Recovery (%)	IS Cal. RSD (%)
MC-LA	1.0	96	3.4	95	4.5
MC-LF	1.0	111	1.9	108	2.6
MC-LR	1.0	101	2.9	100	5.5
MC-LY	1.0	104	2.2	102	1.1
MC-RR	0.5	83	7.3	81	10
MC-YR	1.0	99	11	101	11
NOD	0.5	91	2.5	89	5.2

UPLC/MS/MS

Accuracy & Precision (n = 4, Maumee River)

Analyte	Spiking Conc. (µg/L)	ES Cal. Recovery (%)	ES Cal. RSD (%)	IS Cal. Recovery (%)	IS Cal. RSD (%)
MC-LA	1.0	98	2.8	95	2.0
MC-LF	1.0	107	1.1	104	1.7
MC-LR	1.0	97	8.2	104	6.6
MC-LY	1.0	111	1.9	108	2.5
MC-RR	0.5	83	18	83	16
MC-YR	1.0	92	5.7	101	3.3
NOD	0.5	91	2.8	98	3.4

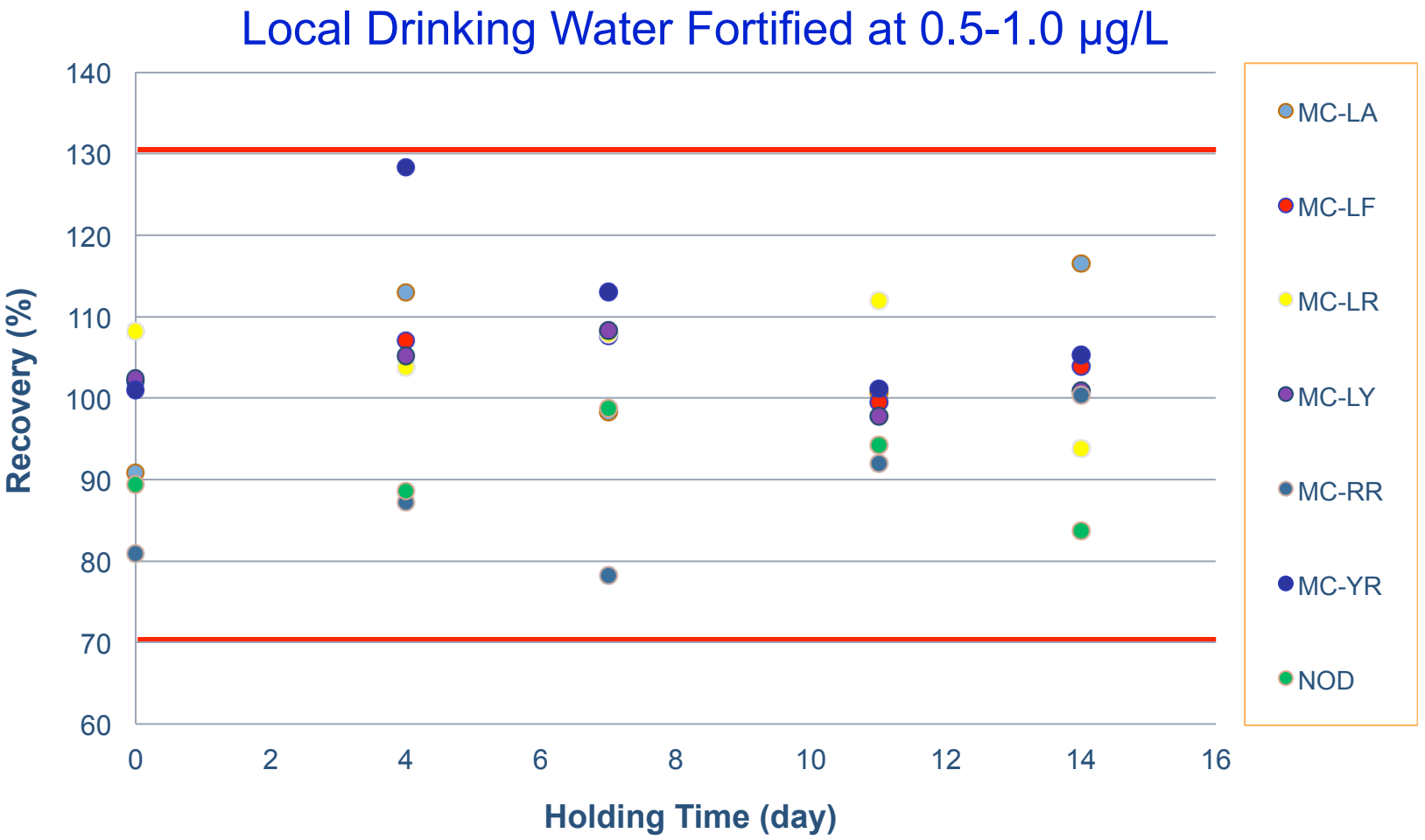
UPLC/MS/MS

Accuracy & Precision (n = 4, local well water)

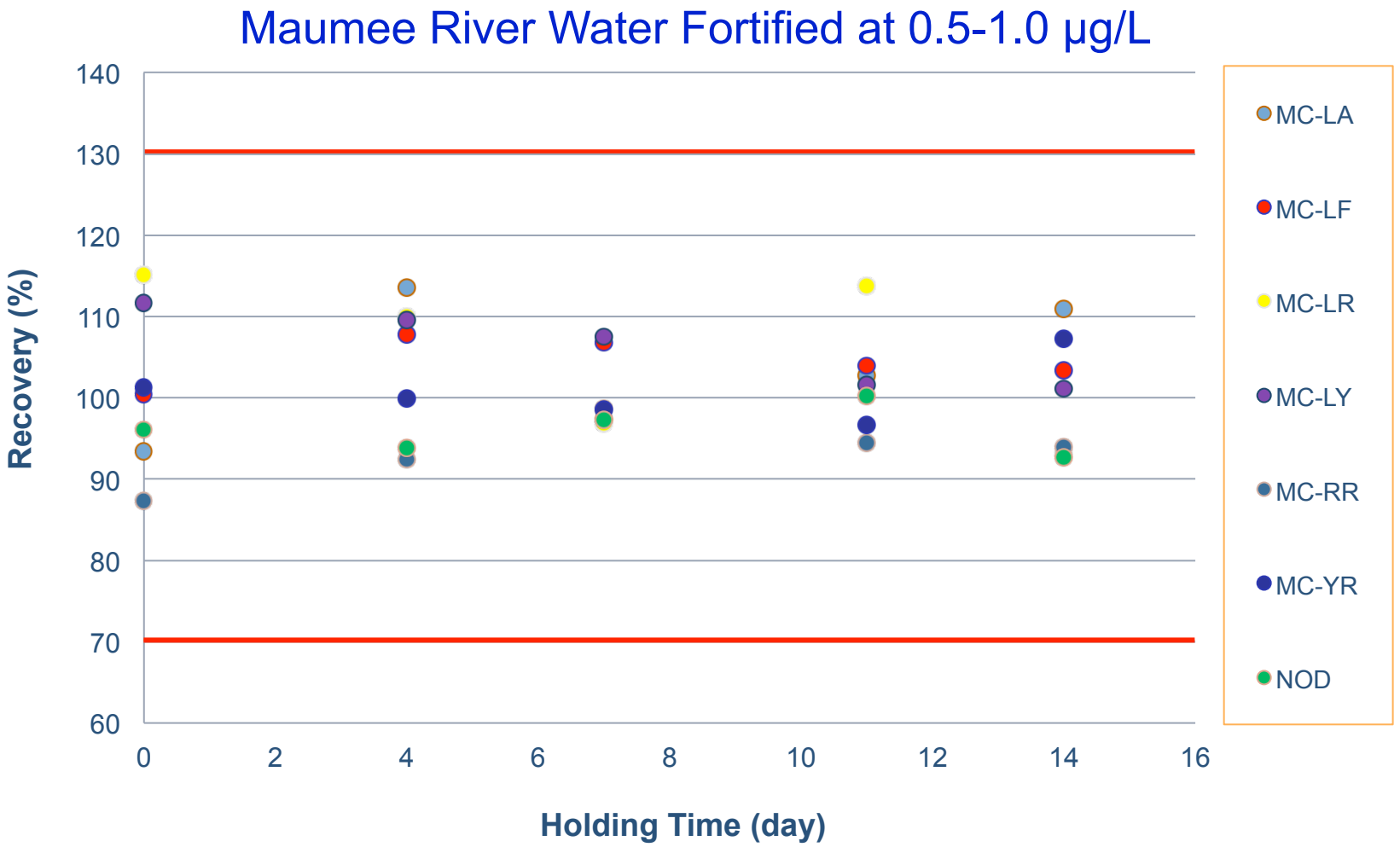
Analyte	Spiking Conc. (µg/L)	ES Cal. Recovery (%)	ES Cal. RSD (%)	IS Cal. Recovery (%)	IS Cal. RSD (%)
MC-LA	1.0	102	1.5	95	2.8
MC-LF	1.0	110	1.8	103	1.9
MC-LR	1.0	105	2.9	100	5.1
MC-LY	1.0	106	3.4	100	3.9
MC-RR	0.5	91	8.2	85	10
MC-YR	1.0	101	3.7	104	1.1
NOD	0.5	105	2.0	99	2.1

Sample Preservation & Stability

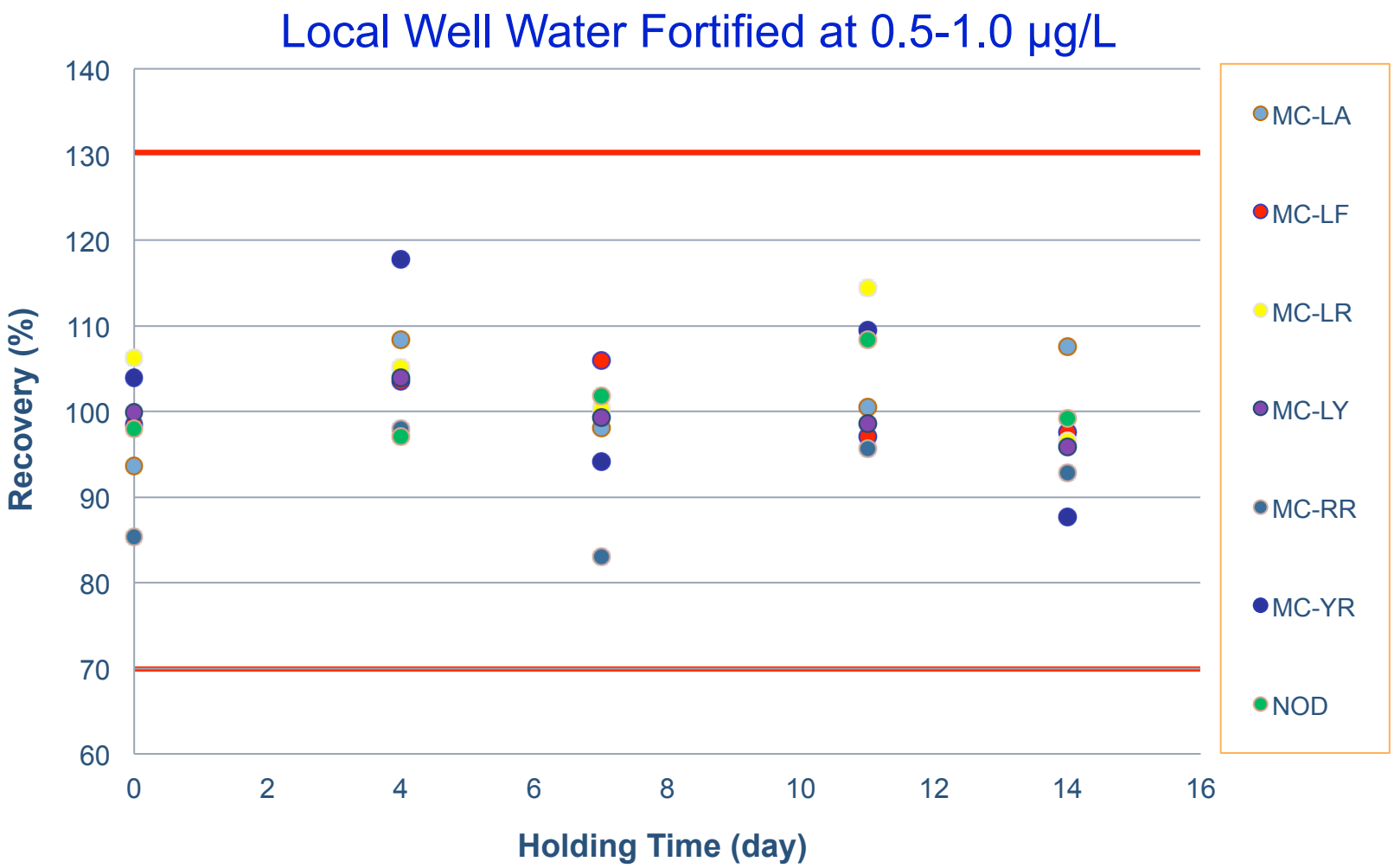
UPLC/MS/MS
Sample Stability



UPLC/MS/MS
Sample Stability



UPLC/MS/MS
Sample Stability

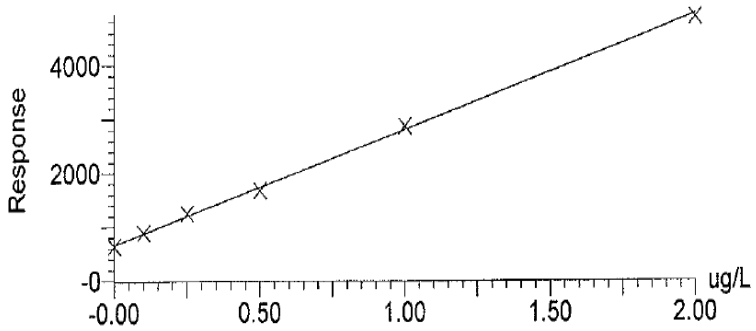


Standard Addition Calibrations

UPLC/MS/MS
Standard Addition Calibrations

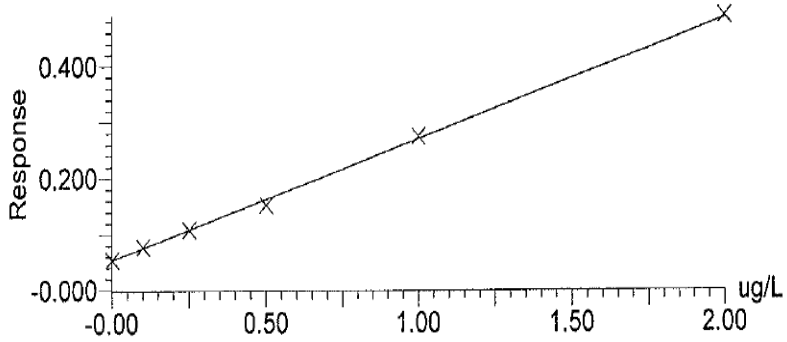
External Standard Calibration

Compound name: Microcystin-LR
Correlation coefficient: r = 0.998798, r^2 = 0.997598
Calibration curve: 2136.83 * x + 663.46
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Internal Standard Calibration

Compound name: Microcystin-LR
Correlation coefficient: r = 0.998977, r^2 = 0.997954
Calibration curve: 0.215972 * x + 0.0542226
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



UPLC/MS/MS

Standard Addition Results (local DW)

Analyte	Spiking Conc. (µg/L)	Ext. Std. Cal. Recovery (%)	Int. Std. Cal. Recovery (%)
MC-LA	0.25	97	84
MC-LF	0.25	119	105
MC-LR	0.25	96	83
MC-LY	0.25	101	88
MC-RR	0.125	136	113
MC-YR	0.25	107	100
NOD	0.125	94	81

UPLC/MS/MS

Standard Addition Results (Maumee River)

Analyte	Spiking Conc. (µg/L)	Ext. Std. Cal. Recovery (%)	Int. Std. Cal. Recovery (%)
MC-LA	0.25	145	118
MC-LF	0.25	110	89
MC-LR	0.25	124	99
MC-LY	0.25	106	85
MC-RR	0.125	121	104
MC-YR	0.25	111	94
NOD	0.125	111	88

UPLC/MS/MS

Standard Addition Results (local well water)

Analyte	Spiking Conc. (µg/L)	Ext. Std. Cal. Recovery (%)	Int. Std. Cal. Recovery (%)
MC-LA	0.25	111	94
MC-LF	0.25	112	96
MC-LR	0.25	142	130
MC-LY	0.25	122	104
MC-RR	0.125	111	97
MC-YR	0.25	108	88
NOD	0.125	116	106

Conclusions

- DAI-UPLC/MS/MS is a rapid & accurate quantitative technique for responding to cyanobacterial blooms.
  - Measure individual microcystins.
  - Report results to 0.1 µg/L or less
  - Demonstrate good percent recoveries and RSDs.
  - Report results within minutes.
  - Allow standard addition calibrations & maximize the compensation for matrix interference.
- EPA Method 544 preservatives were effective to stabilize microcystins in raw source water.
  - ≥ 14 days

Conclusions

- Labeled MC-LR-C2D5 and MC-LR-15N10 used the internal standards improved sensitivity, accuracy, and precision.
- Moving forward:
  - Resolve MC-RR, MC-YR, and MC-LR co-elution to improve sensitivity, recoveries, and RSDs.
  - Validate more new labeled algal toxin standards.
  - Combine microcystins, nodularin, cylindrospermopsin, and anatoxin-a into one DAI-UPLC/MS/MS method.

Questions?

Yongtao (Bruce) Li, Ph.D.

Technical Director

YongtaoLi@eurofinsUS.com

574-472-5562 or 574-707-5026

Eurofins Eaton Analytical, Inc.

110 South Hill Street

South Bend, IN 46617