



Development of an LC-MS/MS Method for the Quantification of Microcystins in Blue-Green Algal Dietary Supplements

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Blue-Green Algal Dietary Supplements & Microcystins

• Problem:

The cyanobacterium *Aphanizomenon flos-aquae* (AFA), which is harvested from natural lakes and commercially distributed as blue-green algal (BGA) dietary supplements, may be contaminated with toxic microcystins produced by co-occurring *Microcystis aeruginosa*.

• Regulation:

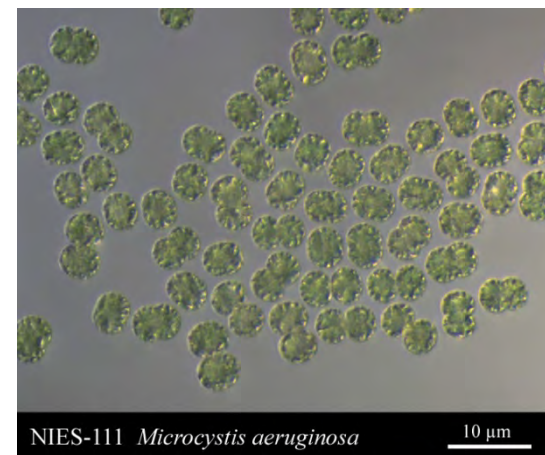
- No regulatory action level for microcystins (MC) in dietary supplements
- Oregon Health Division and Oregon Department of Agriculture: state guidance value of $1 \mu\text{g MC-LR}_{\text{eq}}/\text{g}$ for microcystins in BGA products

• Research Goal:

Develop and validate a selective LC-MS/MS method for the simultaneous detection and quantification of 7 MC congeners in AFA dietary supplements

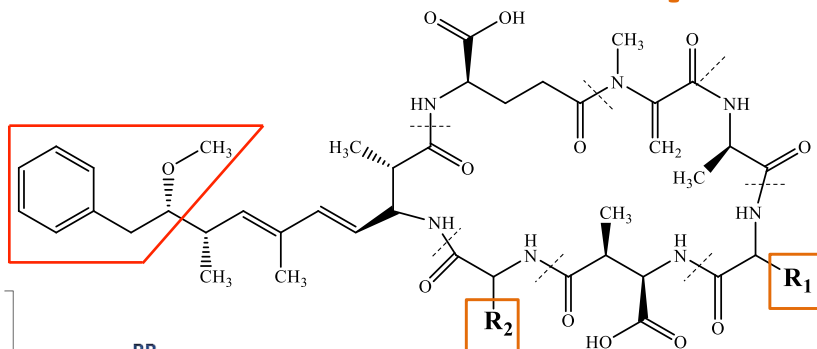


<http://www.oregonwild.org/waters/klamath/the-klamath-river/klamath-river-water-quality>

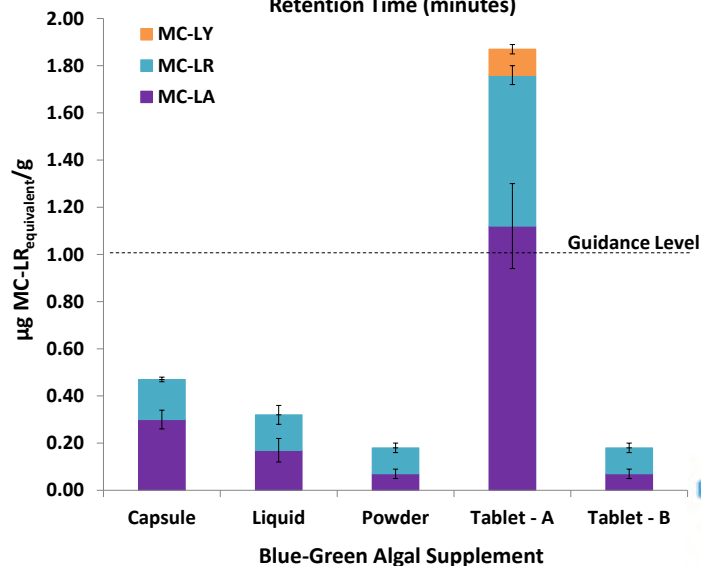
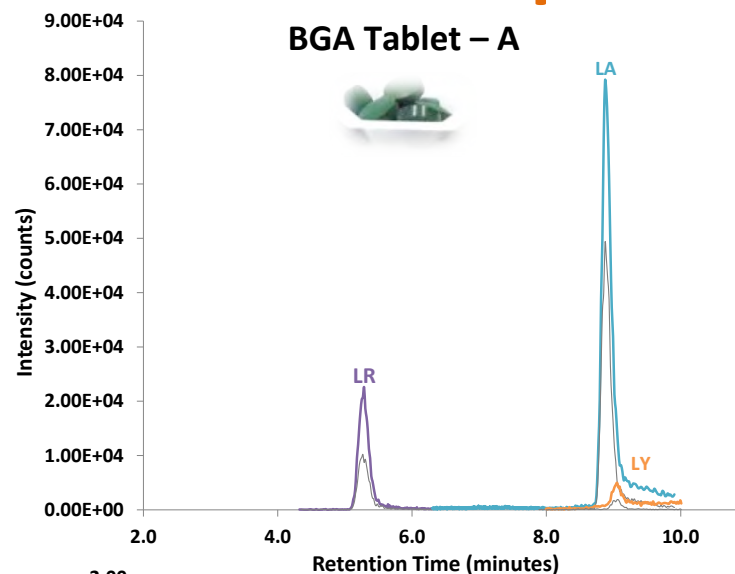
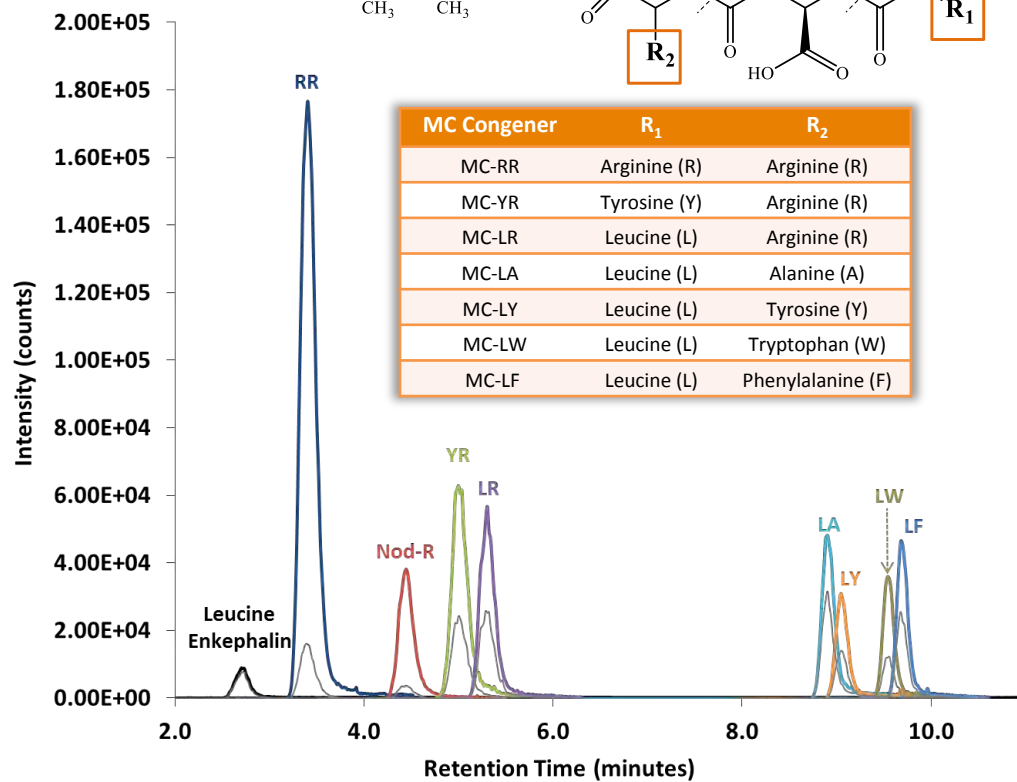


NIES-111 *Microcystis aeruginosa* 10 μm
<http://www.shigen.nig.ac.jp/algae/strainDetailAction.do?stockNo=NIES-111>

Quantitative LC-MS/MS Method Development



MC Congener	R ₁	R ₂
MC-RR	Arginine (R)	Arginine (R)
MC-YR	Tyrosine (Y)	Arginine (R)
MC-LR	Leucine (L)	Arginine (R)
MC-LA	Leucine (L)	Alanine (A)
MC-LY	Leucine (L)	Tyrosine (Y)
MC-LW	Leucine (L)	Tryptophan (W)
MC-LF	Leucine (L)	Phenylalanine (F)



The Challenge of Finding a Matrix Blank

• BGA supplements

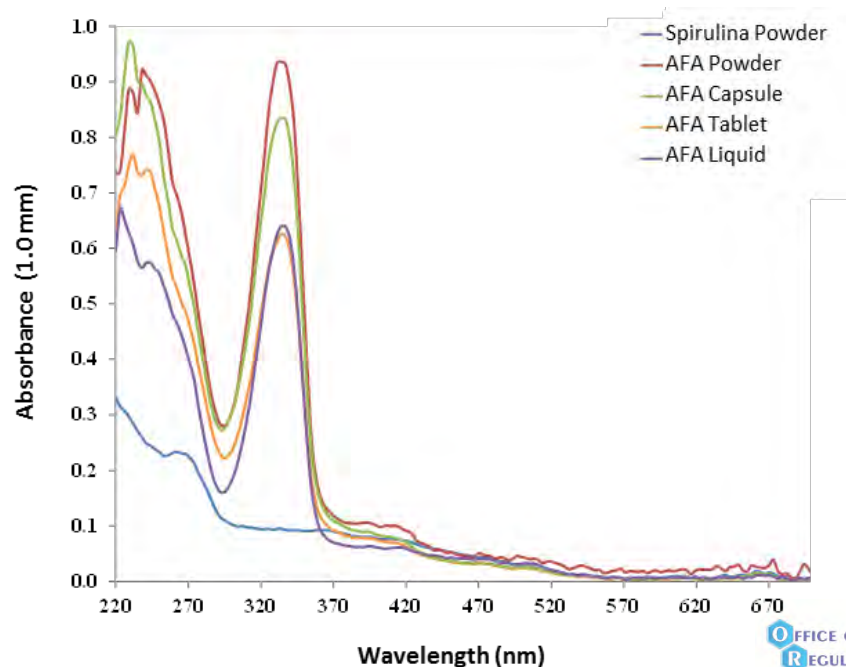
- AFA-based: detectable concentrations of MC in all supplements tested
- Spirulina-based: free of MC contamination, but not a suitable matrix blank

• Problems with Spirulina

- Lower sample processing recoveries
- Possibly due to the absence of planar Mycosporine-like amino acids (MAAs)— accessory pigment molecules produced in cyanobacteria under high UV radiation

Congener	z	% Recovery [†] AFA	% Recovery [†] Spirulina
MC-RR	2	65.1 ± 0.6	53.2 ± 1.0
Nod-R	1	72.2 ± 1.7	56.9 ± 4.0
MC-YR	1	54.9 ± 1.5	17.2 ± 0.4
MC-YR	2	59.8 ± 1.8	76.7 ± 2.7
MC-LR	1	67.7 ± 1.7	31.2 ± 1.6
MC-LR	2	71.7 ± 1.4	61.9 ± 1.1
MC-LA	1	77.2 ± 1.4	57.0 ± 2.2
MC-LY	1	63.3 ± 1.4	43.3 ± 1.5
MC-LW	1	50.0 ± 1.4	25.0 ± 1.4
MC-LF	1	60.1 ± 1.4	42.7 ± 2.7
Average		64.2 ± 8.3	46.5 ± 18.2
%RSD		13.0%	39.2%

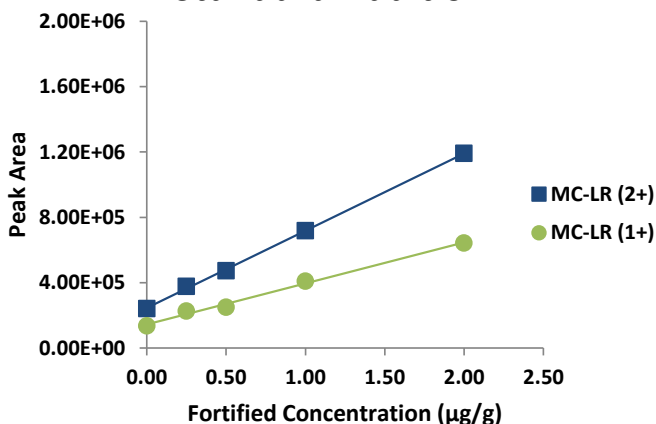
[†]Peak areas were compared for biological replicates of pre- and post-fortified sample extracts at a 1 µg/g spike concentration.



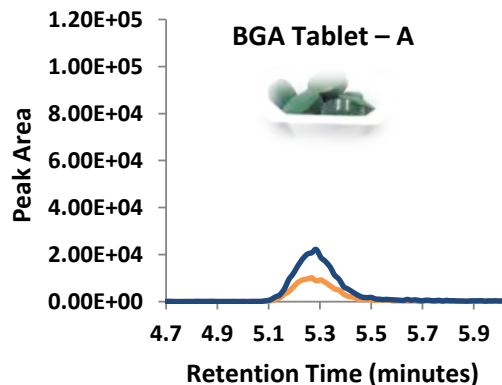
Solutions for Quantitation

Fortified Concentration (µg/g) MC-LR	Standard Addition % Recovery	Matrix-Corrected Neat Calibration %Recovery
2.00	100.1 ± 3.7	97.4 ± 3.6
1.00	100.1 ± 1.0	97.9 ± 1.4
0.50	95.9 ± 6.0	94.8 ± 6.1
0.25	111.3 ± 1.6	111.6 ± 4.3

Standard Addition

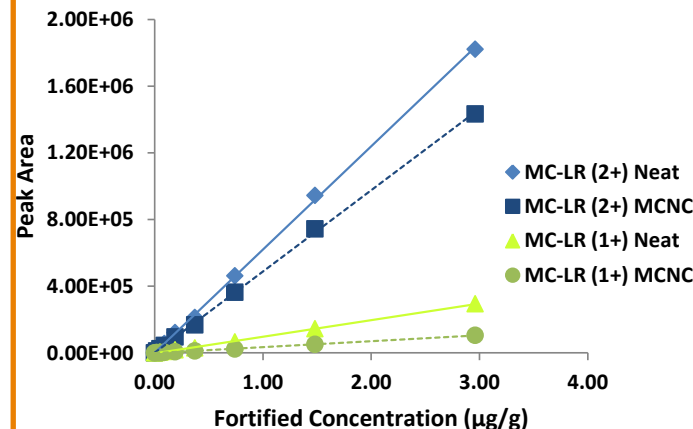


Endogenous MC-LR



Standard Addition	Matrix-Corrected Neat Calibration
0.52 ± 0.02 µg/g	0.49 ± 0.01 µg/g

Matrix-Corrected Neat Calibration



Conclusions & Ongoing Challenges

Microcystin Congener	AFA Capsule (µg/g)	AFA Liquid (µg/g)	AFA Powder (µg/g)	AFA Tablet Lot A (µg/g)	AFA Tablet Lot B (µg/g)
MC-LR	0.17 ± 0.01	0.15 ± 0.04	0.11 ± 0.02	0.64 ± 0.04	0.11 ± 0.02
MC-LA	0.30 ± 0.04	0.17 ± 0.05	0.07 ± 0.02	1.12 ± 0.18	0.07 ± 0.01
MC-LY	ND	ND	ND	0.11 ± 0.02	ND
Total µg MC-LR_{eq}/g	0.47 ± 0.04	0.32 ± 0.06	0.18 ± 0.03	1.87 ± 0.19	0.18 ± 0.02

ND = Not Detected

- **94 MC variants reported**
 - Toxicities for all MC variants have not been determined
 - Chronic effects of exposure are unknown
 - Risk assessment data is needed before a regulatory level(s?) can be established
- **Lot-to-lot variability poses a challenge for screening and regulation**
- **Isotopically labeled internal standards are not yet available, making accurate quantitation more challenging**

Experimental

- **Instrumentation:** AB Sciex QTrap 5500 equipped with a Turbo V ionization source and a Waters Acquity UPLC system

- **LC Parameters:**

Acquity UPLC Column	BEH C18 (1.7 μ m, 1.0 mm \times 150 mm)
Column Temperature	40 $^{\circ}$ C
Injection Volume	2 μ L

- **MS Parameters:**

Source Temperature	400 $^{\circ}$ C
IonSpray Voltage	5000 V
Curtain Gas	20 psi
Gas 1	40 psi
Gas 2	30 psi

