

**Adda Microcystins/Nodularins & BMAA Report***Project: Raw Living Spirulina*

Submitted to: Timothy White  
Organization: Raw Living Spirulina  
Address: 3375 66<sup>th</sup> Ave., Vero Beach, FL 32966  
Email: [info@rawlivingspirulina.com](mailto:info@rawlivingspirulina.com)  
Sample Receipt Date: 13 January 2022  
Sample Condition: 6.8 °C upon arrival  
Report#: 220112\_Raw\_Living\_Spirulina (Harvest)  
Date Report Prepared: 3 February 2022  
Prepared by: Mark Aubel

Table 1: Samples analyzed and collection date

<u>Sample ID</u>	<u>Site/Description</u>	<u>Collection Date</u>
Harvest	Kyanos Farms	12 January 2022

**Analytes:** Adda Microcystins/Nodularins (MCs/NODs),  $\beta$ -N-methylamino-L-alanine (BMAA)**Abbreviations**

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit
CCC	Continued Calibration Check	CV	Low-range calibration verification

## Sample Preparation

### *Extraction*

*MCs/NODs, BMAA (including BAMA, 2,4-DAB, AEG)*

The sample was extracted in 10 mg subsets with internal standards and pre-extraction LFSMs (Table 3). Extractant (75% acetonitrile in 0.1 M acetic acid) was added and solutions bath sonicated (10 min), filtered (0.2  $\mu\text{m}$ ), and a subset analyzed for BMAA. Aliquots (0.5 mL) were further clarified for MC/NOD analysis using Solid Phase Extraction (SPE). Preconditioned Strata X Polymeric SPE columns were loaded with sample (5 mg), rinsed with water, and eluted. Elutions were dried, reconstituted (deionized water), filtered (0.2  $\mu\text{m}$ ), and analyzed.

## Analytical Techniques

### *Enzyme-Linked Immunosorbent Assay (ELISA)*

*MCs/NODs*

A microcystins/nodularins Adda ELISA (Abraxis) was utilized for the quantitative and sensitive congener-independent detection of Adda MCs/NODs (US EPA Method 546 & Ohio EPA DES 701.0). The current method reporting limit is 0.30 ng/mL (ppb) for the Collected sample and 0.15  $\mu\text{g/g}$  (ppm) for the Harvest sample based on kit sensitivity and dilution factors.

### *Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)*

*BMAA (and isomers)*

HILIC LC-MS/MS was used in analysis of BMAA and isomers (BAMA, 2,4-DAB, AEG, 3,4-DAB). The  $[\text{M}+\text{H}]^+$  ion ( $m/z$  119) was fragmented and product ions ( $m/z$  73, 76, 88, 101, 102) monitored. The IS  $d_3$ -BMAA was also monitored ( $m/z$  122  $\rightarrow$  105). Differentiation of isomers was made by retention time, LFSMs, and relative abundance of product ions.

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

**Quality Control**

Table 2: QA/QC samples prepared for analyses. Additional Quality Control/Quality Assurance checks included method blanks, LFBs, and standard curves.

Analyte	Concentration	Sample ID	QC Type	%Return
MC-LR	1.0 µg/g	Harvest	LFSM	98%
MC-LR	1.0 µg/g	Harvest	LFSMD	107%
BMAA	5.0 µg/g	Harvest	LFSM	116%
BMAA	5.0 µg/g	Harvest	LFSMD	84%
BAMA	5.0 µg/g	Harvest	LFSM	95%
BAMA	5.0 µg/g	Harvest	LFSMD	75%
2,4-DAB	5.0 µg/g	Harvest	LFSM	90%
2,4-DAB	5.0 µg/g	Harvest	LFSMD	73%
AEG	5.0 µg/g	Harvest	LFSM	98%
AEG	5.0 µg/g	Harvest	LFSMD	70%
3,4-DAB	5.0 µg/g	Harvest	LFSM	72%
<i>d</i> <sub>3</sub> -BMAA	10 µg/g	all Harvest subsets	IS	42 ± 6% <sup>N</sup>

\*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Table 3: The percent reproducibility (%RPD) between lab duplicates or LFSM/LFSMDs prepared and analyzed.

QC Type	Sample ID	Analyte	Value 1	Value 2	% RPD	Pass/Fail (<40%)
LFSMs	Harvest	MCs/NODs	1.01	1.10	9%	Pass
LFSMs	Harvest	BMAA	5,790	4,201	32%	Pass
LFSMs	Harvest	BAMA	4,773	3,731	24%	Pass
LFSMs	Harvest	2,4-DAB	4,503	3,673	20%	Pass
LFSMs	Harvest	AEG	4,876	3,487	33%	Pass

%RPD is NA when values are below the method reporting/detection limit

Table 4: Adda MC-ELISA Quality Control Value Table

Date Analyzed:	21 January 2022	Requirement	Pass/Fail
<b>R<sup>2</sup> value:</b>	1.000	≥0.98	PASS
<b>%CV range STDs:</b>	0.4-3.7%	≤15%	PASS
<b>LFB (1 ppb) recovery:</b>	116%	±40% True Value	PASS
<b>%CV range LFB:</b>	4.0%	≤20%	PASS
<b>Low CCC (0.15 ppb) recovery:</b>	90%	±50% True Value	PASS
<b>LRB</b>	<0.08	<0.08	PASS

**Results**

Table 5: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	%CV	Concentration	Average
Harvest	MCs/NODs	1,000	0.03	23.2	< 0.15 µg/g	<b>ND</b>
		1,000	0.04		< 0.15 µg/g	
Harvest LFSM	MCs/NODs	1,000	1.02	1.3	1.02 µg/g	<b>1.01 µg/g</b>
		1,000	1.00		1.00 µg/g	
Harvest LFSMD	MCs/NODs	1,000	1.08	2.7	1.08 µg/g	<b>1.10 µg/g</b>
		1,000	1.12		1.12 µg/g	

**Summary of Results**


Table 6: Summary of results

<b>Sample ID</b>	<b>MCs/NODs (µg/g)</b>	<b>BMAA (µg/g)</b>
Harvest	ND	ND
<i>MRL (ng/mL):</i>	<i>0.15</i>	<i>0.5</i>
<i>Analyst Initials:</i>	<i>KC</i>	<i>KC</i>
<i>Date Analyzed:</i>	<i>1/21/2022</i>	<i>1/24/2022</i>

**Interpretations:**

Microcystins/nodularins were not detected in the Harvest sample above the method reporting limit of 0.15 µg/g.

Free BMAA and its isomers (2,4-DAB, 3,4-DAB, BAMA & AEG) were below the method reporting limits for the Harvest sample.

Submitted by:   
 Mark T. Aubel, Ph.D.  
 Lab Director

Date: February 3, 2022

*The results in this report relate only to the samples listed above.  
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