Adda Microcystins/Nodularins & BMAA Report

Project: Raw Living Spirulina

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Sample Receipt Date:	20 May 20
Sample Condition:	11.3 ºC upon arrival
Report#	200519_Raw Living Spirulina
Date Prepared:	9 June 20
Prepared by:	Kamil Cieslik

Table 1: Samples analyzed

Site/Description	Collection Date	Collection Time
Kyanos Farms	19 May 20	0830

Analytes: Adda Microcystins/Nodularins (MCs/NODs)

Sample Preparation

Extraction(s)

MCs/NODs

The sample was extracted in 100 mg subsets with a lab duplicate. Pre-extraction LFSMs were prepared with the remaining subset (Table 2). Extraction was conducted using a 75% acetonitrile in 0.1 M acetic acid solution and sonication via water bath for 25 minutes. The samples were centrifuged at 3,000 RPM for 10 minutes with supernatants retained. The pellets were rinsed with extractant and the supernatants were pooled. An aliquot of each extract (10 mg) was diluted with deionized water and clarified using Solid Phase Extraction (SPE). An aliquot (0.5 mL) of each extract was diluted 2-fold, filtered, and analyzed for BMAA at a sample concentration of 10 mg/mL.

Solid Phase Extraction (SPE)

MCs/NODs

Preconditioned Strata X Polymeric SPE (200 mg) was loaded with sample (10 mg), rinsed with 5% MeOH, and eluted with 90% acetonitrile. Elutions were blown to dryness (N_2 at 60°C) and reconstituted in deionized water (1 mL) for a sample concentration of 10 mg sample per mL solution. A 10-fold dilution further was applied prior to analysis via ELISA.

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 0.15 µg/g (ppm) based on kit sensitivity and dilution factor (DF = 1,000).

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

BMAA

A SeQuant ZIC-HILIC 3.5µm 2.1 x 150 mm HILIC column was used in separation of BMAA, BAMA, DAB and AEG with mobile phases acetonitrile and water containing 2 mM formic acid and 3.6 mM ammonium formate. The $[M+H]^+$ ion for BMAA and its isomers (*m/z* 119) were fragmented and the product ions (*m/z* 46, 73, 76, 88, 101, 102) were monitored. Differentiation of isomers was made by retention time, LFSMs, and relative abundance of product ions. The internal standard method was used to determine LFSM returns.

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 (µg/g)	Sample ID	QC Type	Return
MC-LR	1.0	Kyanos Farms	LFSM	61%
MC-LR	1.0	Kyanos Farms	LFSMD	73%
BMAA	5.0	Kyanos Farms	LFSM	108%
BAMA	5.0	Kyanos Farms	LFSM	124%
2,4-DAB	5.0	Kyanos Farms	LFSM	133%
AEG	5.0	Kyanos Farms	LFSM	90%
BMAA	5.0	Kyanos Farms	LFSMD	109%
BAMA	5.0	Kyanos Farms	LFSMD	119%
2,4-DAB	5.0	Kyanos Farms	LFSMD	122%
AEG	5.0	Kyanos Farms	LFSMD	86%
d3-BMAA	10	Kyanos Farms	IS	124 ± 5%
Analyte	Concentration (µg/g)	Sample ID	QC Type	%RPD
MC-LR	0.64, 0.76	Kyanos Farms	LFSM/LFSMD	17%
BMAA	5.4, 5.5	Kyanos Farms	LFSM/LFSMD	1%
BAMA	6.2, 5.9	Kyanos Farms	LFSM/LFSMD	3%
2,4-DAB	6.6, 6.1	Kyanos Farms	LFSM/LFSMD	6%
AEG	4.5, 4.3	Kyanos Farms	LFSM/LFSMD	3%

Table 3: Adda MC-ELISA Quality Control Value Table

Date Analyzed:	**4 June 20**	**Requirement**	**Pass/Fail**
R^2 value:	0.999	≥0.98	PASS
%CV range STDs:	0.2-6.1%	≤15%	PASS
LFB (1 ppb) recovery:	94%	±40% True Value	PASS
%CV range LFB:	7.8%	<20%	PASS
Low CV (0.15 ppb) recovery:	93%	±50% True Value	PASS
LRB	<0.08	<0.08	PASS

Summary of Results

Table 4: Summary of results in µg/g, reported with average of lab duplicate data and the standard deviation (where applicable).

Sample ID	MCs/NODs (µg/g)	BMAA (µg/g)	BAMA (µg/g)	2,4-DAB (µg/g)	AEG (µg/g)
Kyanos Farms	ND	ND	ND	ND	ND
MRL (µg/g)	*0.15*	0.5	0.5	1.0	0.5
Analyst Initials	*KC*	*KC*	*KC*	*KC*	*KC*
Date Analyzed	*6/4/20*	*6/8/20*	*6/8/20*	*6/8/20*	*6/8/20*

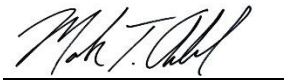
Abbreviations

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

Interpretations:
Adda microcystins/nodularins were not detected in the submitted sample above the method reporting limit (0.15 µg/g). The sample does not exceed the Oregon Department of Agriculture regulatory limit of 1.0 µg/g microcystin in BGA containing products intended for human consumption (Oregon Admin. R. 603-025-0190(2)).

Additionally, free BMAA and its isomers (DAB, BAMA, & AEG) were below method reporting limits.

The LFSM recoveries were within control limits (50-150%), indicative of minimal matrix effects.

Submitted by: 
Mark T. Aubel, Ph.D.
Lab Director
Date: June 9, 2020

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