Microcystins/Nodularins & BMAA Report

Project: Raw Living Spirulina

Submitted to:	Timothy White
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Sample Receipt Date:	11 April 18
Sample Condition:	6.1 ºC upon arrival
Report#	180410_ Raw Living Spirulina
Date Prepared:	19 April 18
Prepared by:	Kamil Cieslik

Sample Identification	Description/Site	Sample Collection Date
RLS Supreme	Kyanos Farms	10 April 18

Analytes: Microcystins/Nodularins (MCs/NODs), β-*N*-methylamino-L-alanine (BMAA)

Sample Preparation

Extraction(s)

MCs/NODs, BMAA

Materials were extracted in 0.100 ± 0.005 gram subsets (wet weight). Pre-extraction LFSM and LFSMD were prepared (Table 1). Extractions were conducted using a 75% methanol in 0.1 M acetic acid solution and sonication via water bath for 25 minutes. Sample and spike were centrifuged at 3,000 RPM for 10 minutes with supernatants retained. The pellets were rinsed and the supernatants were pooled. Methanol was then removed from the samples using N_2 at 60°C, diluted with deionized water for MCs/NODs and 0.1 M trichloroacetic acid for BMAA, and clarified using Solid Phase Extraction (SPE).

Solid Phase Extraction (SPE)

MCs/NODs

Preconditioned Strata X Polymeric SPE (200 mg) was loaded with sample (0.1 g), 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 0.1 g/mL. The extracts were diluted to 0.001 g/mL for analysis by ELISA.

BMAA
Preconditioned Oasis MCX SPE (150 mg) was loaded with sample (0.1 g), rinsed with 0.1 M HCl (2 mL) followed with 100% MeOH (2 mL), and eluted with 1.65% of NH_4OH in MeOH. Elutions were blown to dryness (N_2 at 60°C) and reconstituted in 50% ACN in 1% acetic acid (1 mL) for a sample concentration of 0.1 g/mL. The extracts were diluted to 0.01 g/mL for analysis.

Quality Control

Table 1: LFSM/LFSMD and IS QC samples prepared for analyses pre-extraction.

Analyte	Concentration (µg/g)	Sample ID	QC Type	Return
MC-LR	1.0	RLS Supreme	LFSM	80%
MC-LR	1.0	RLS Supreme	LFSMD	69%[N]
BMAA	5.0	RLS Supreme	LFSM	90%
BAMA/DAB/AEG	5.0 / 5.0 / 5.0	RLS Supreme	LFSM	102% / 119% / 63%
d3-BMAA	10	RLS Supreme	IS	94% (avg)

Additional Quality Control/Quality Assurance checks included method blanks, standard checks, and external curves.

Qualifier	Flag
N	Spiked sample control was outside limits

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 MCs/NODs (US EPA Method 546 & Ohio EPA DES 701.0). The current assay is sensitive down to a quantification limit of and 0.15 µg/g (ppm) for MCs/NODs as determined from dilution factors, MC-LR response and kit sensitivity (0.15 ng/mL).

BMAA
HILIC-MS/MS analysis was utilized for BMAA, BAMA, DAB and AEG detection. The $[M+H]^+$ ion for BMAA and its isomers (119 *m/z*) was fragmented and the product ions (46, 73, 76, 88, 101 and 102 *m/z*) were monitored. Differentiation of isomers was made by retention time, LFSMs, and relative abundance of product ions. Method detection limits (MDLs) were determined with a pre-extraction matrix spike (LFSM).

Summary of Results

Sample ID	MCs/NODs (µg/g)	BMAA (µg/g)	BAMA (µg/g)	DAB (µg/g)	AEG (µg/g)
RLS Supreme	ND	ND	ND	ND	ND
MDL (ng/mL)	*0.15*	*0.03*	*0.04*	*0.23*	*0.05*
Analyst Initials	*AF*	*MA*	*MA*	*MA*	*MA*
Date Analyzed	*4/13/18*	*4/18/18*	*4/18/18*	*4/18/18*	*4/18/18*

Discussion

No toxins were detected above the method detection limits in the sample submitted. The MC-LR LFSMD recovery was just outside of control limits (70-130%), but indicative of minimal matrix effect.

The Oregon Department of Agriculture regulatory limit of for microcystins in BGA containing products intended for human consumption is 1.0 µg/g. (Oregon Admin. R. 603-025-0190(2)).

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	SUR	Surrogate
Blank	Regent Water free from interferences	–	Not Analyzed
LFB	Lab Fortified Blank		

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

Date: April 20, 2018

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