

# Supplementary Materials: Detection of Cyanotoxins in Algae Dietary Supplements

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## SI1. Chemicals, reagents and stock solutions

2-methyl-3-methoxy-4-phenylbutyric acid sodium salt (MMPB, purity  $\geq 94\%$ ) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-phenylbutyric acid (4-PB, purity  $\geq 99\%$ ), L-BMAA hydrochloride (BMAA, purity  $\geq 97\%$ ), DL-phenylalanine (PHE, purity  $\geq 99\%$ ) and nodularin (NOD,  $\geq 99\%$ ) were obtained from Sigma-Aldrich (Oakville, ON, Canada). All selected standards for microcystins (MCs-RR, -YR, -LR, -LY, -LW, -LF and -LA, purity  $\geq 99\%$ ), anatoxin-a (ANA-a, purity  $\geq 99\%$ ) and cylindrospermopsin (CYN, purity  $\geq 99\%$ ) were purchased from Abcam Biochemicals (Cambridge, MA, USA). 2,4 diaminobutyric acid-2,4,4-D<sub>3</sub> dihydrochloride (DAB-D<sub>3</sub>, 99 atom % D) and DL-phenylalanine ring-D<sub>5</sub> (PHE-D<sub>5</sub>, purity  $\geq 98\%$ ) were purchased from CDN isotopes (Pointe-Claire, QC, Canada). Ampoule of certified standard solutions of saxitoxin dihydrochloride (STX, 66.3  $\mu\text{M}$  in 3 mM hydrochloric acid) was obtained from the Certified Reference Materials Program (NRC, Halifax, NS, Canada). Potassium permanganate (KMnO<sub>4</sub>, purity  $\geq 99,0\%$ ), sodium (meta)periodate (NaIO<sub>4</sub>, purity  $\geq 99,0\%$ ), sodium bisulfate (purity  $\geq 95\%$ ), sodium hydroxide (NaOH, purity  $\geq 98\%$ ), sodium tetraborate (Borax, purity  $\geq 99\%$ ), dansyl chloride (DNS, purity  $\geq 99\%$ ), citric acid (purity  $\geq 99.5\%$ ), and formic acid (HCOOH, purity  $\geq 95.0\%$ ), hydrochloric acid (HCl, ACS reagent 37%) and sulfuric acid standard solution (1.000 M) were obtained from Sigma-Aldrich (Oakville, ON, Canada). All solvents used were of high performance liquid chromatography (HPLC) grade purity from Fisher Scientific (Whitby, ON, Canada). Deionized/distilled water (dd-H<sub>2</sub>O) was used recovery evaluation. Compressed air (Ultra Zero Certified grade;  $\leq 2$  ppm water) used as carrier gas for the LDTD desorption was purchased from MEGS Specialty Gases, Inc. (St-Laurent, QC, Canada).

**Table S1.** Recoveries from matrix effects of targeted cyanotoxins evaluated at two different concentrations levels ( $\mu\text{g g}^{-1}$ ) with standard deviation (STD,  $n = 3$ ).

Compounds <sup>a</sup>	Signal Recovery from Matrix Effect $\pm$ STD (%)		
	Low	High	
1	MMPB <sup>b,c</sup>	85 $\pm$ 11	87 $\pm$ 8
	ANA-a <sup>b</sup>	90 $\pm$ 10	92 $\pm$ 9
2	ANA-a	93 $\pm$ 5	95 $\pm$ 6
	MC-RR	91 $\pm$ 7	94 $\pm$ 5
	MC-YR	94 $\pm$ 4	92 $\pm$ 5
	MC-LR	98 $\pm$ 5	102 $\pm$ 7
	MC-LA	109 $\pm$ 8	105 $\pm$ 6
	MC-LY	95 $\pm$ 3	91 $\pm$ 6
	MC-LW	103 $\pm$ 6	108 $\pm$ 8
	MC-LF	112 $\pm$ 9	104 $\pm$ 8
3	CYN	108 $\pm$ 10	102 $\pm$ 7
	STX	94 $\pm$ 8	98 $\pm$ 9
	BMAA	105 $\pm$ 4	109 $\pm$ 6

<sup>a</sup> Validation results are divided between the three methods. Low concentration and High concentrations are respectively set as the follow: 1 and 10  $\mu\text{g g}^{-1}$  (1), 0.1 and 10  $\mu\text{g g}^{-1}$  (2) and 0.5 and 10  $\mu\text{g g}^{-1}$  (3). <sup>b</sup> Analysis using LDTD-APCI-HRMS detection. <sup>c</sup> Results reported as total MCs equivalent.

**Table S2.** Cyanotoxins detection in CB dietary supplements ( $\mu\text{g g}^{-1}$ ) with relative standard deviation (RSD-%).

No.	MCs tot <sub>a,b</sub>	ANA-a <sup>a</sup>	ANA-a	DH-ANA-a <sup>c</sup>	E-ANA-a <sup>c</sup>	MC-RR	MC-YR	MC-LR	MC-LA	MC-LY	MC-LW	MC-LF	MCs tot <sup>d</sup>	CYN	STX	BMAA
1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	<b>1.6 (12)</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	<b>0.21(11)</b>	ND	ND	<b>0.34 (12)</b>	ND	ND	ND	ND	<b>0.25 (9)</b>	ND	ND	ND	<b>0.25</b>	ND	ND	ND
9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	<b>0.84 (12)</b>	ND	ND	ND	<b>2.7 (11)</b>	ND	<b>0.53 (7)</b>	<b>0.02 (5)</b>	<b>0.08 (6)</b>	ND	ND	ND	<b>0.63</b>	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	<b>0.24 (8)</b>	ND	ND	ND	ND	ND	ND	<b>0.01 (11)</b>	ND	ND	ND	ND	<b>0.01</b>	ND	ND	ND
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	<b>8.2 (7)</b>	ND	<b>0.15 (7)</b>	<b>1.1 (13)</b>	<b>0.92 (10)</b>	<b>0.4 (9)</b>	ND	<b>4.3 (7)</b>	<b>1.1 (4)</b>	<b>0.02 (8)</b>	ND	ND	<b>5.8</b>	ND	ND	<b>0.04 (8)</b>
16	<b>1.5 (11)</b>	ND	ND	ND	<b>0.07 (9)</b>	ND	ND	<b>1.2 (5)</b>	<b>0.4 (6)</b>	ND	ND	ND	<b>1.6</b>	ND	ND	ND
17	<b>4.1 (9)</b>	<b>0.44 (9)</b>	<b>0.40 (9)</b>	<b>7.2 (12)</b>	ND	ND	<b>0.22 (6)</b>	<b>0.05 (9)</b>	<b>3.2 (4)</b>	ND	ND	ND	<b>3.4</b>	ND	ND	<b>0.55 (5)</b>
18	<b>0.8 (8)</b>	ND	ND	ND	ND	ND	ND	<b>0.52 (7)</b>	ND	ND	ND	ND	<b>0.52</b>	ND	ND	ND

ND – Not detected. <sup>a</sup> Analysis using LDTD-APCI-HRMS detection. <sup>b</sup> Total microcystins determined via MMPB reported as total MCs equivalent. <sup>c</sup> Concentrations are expressed as ANA-a equivalents.

**Table S3.** Parameters of HRMS detection of target cyanotoxins and internal standards.

Compounds	RT (min)	Experimental precursor mass (m/z)	Quantification fragment (m/z)	Confirmation fragment (m/z)	NCE <sup>b</sup> (%)
MMPB <sup>a</sup>	NA	207.1019	135.0808	91.0547	30
4-PB <sup>a</sup>	NA	163.0756	91.0546	NA	35
ANA-a <sup>a</sup>	NA	166.1231	149.0965	131.0859	30
PHE-D <sub>5</sub> <sup>a</sup>	NA	171.1181	154.0917	NA	30
ANA-a	0.96	166.1231	149.0965	131.0859	30
DH-ANA-a	1.01	168.1383	140.1069	164.1068	30
E-ANA-a	1.04	182.1176	133.1010	93.0696	30
MC-RR	2.42	519.7907	135.0804	103.0542	35
MC-YR	2.61	1045.5353	135.0804	213.0870	45
MC-LR	2.68	995.5560	135.0804	213.0870	40
MC-LA	3.43	910.4920	135.0804	213.0870	35
MC-LY	3.77	1002.5188	265.1587	135.0804	40
MC-LW	4.24	1025.5347	891.5199	213.0870	35
MC-LF	4.49	986.5239	213.0870	375.2391	35
NOD	2.45	825.4505	103.0542	NA	45
CYN	1.26	649.1744	194.1291	176.1184	35
STX	2.97	533.1925	204.0877	138.0665	35
BMAA	3.65	585.1836	277.1007	71.0131	30
DAB-D <sub>3</sub>	3.50	588.2024	279.1130	NA	25

NA – Not applicable. <sup>a</sup> Analysis using LDTD-APCI-HRMS detection. <sup>b</sup> Fragmentation energy for precursor ion in HCD cell.