

Anatoxin-a, Cylindrospermopsin, Microcystin & Saxitoxin Report
Project: Spicer Group

<u>Sample ID</u>	<u>Site</u>	<u>Collected</u>
AG1	P. P. Lake	8/30/16
AG2	P. P. Lake	8/30/16
AG3	P. P. Lake	8/30/16
AG4	P. P. Lake	8/30/16
AG5	P. P. Lake	8/30/16

Toxins – Anatoxin-a (ANTX-A), cylindrospermopsin (CYN), microcystin (MC), saxitoxin (STX)

Sample Prep

The samples were ultra-sonicated to lyse cells and release toxins. Duplicate sub-samples (Lab Fortified Matrix, LFM) were spiked at 0.1 µg/L of ANTX-A, 1.0 µg/L of CYN, 1.0 µg/L MCLR and 0.2 µg/L STX.

Analytical Methodology

Liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (166 m/z) was fragmented and the product ions (56.0, 91.1, 107.0, 131.1 & 149.6 m/z) were monitored. The current method detection limit is 0.05 µg/L for ANTX-A.

A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive to down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR was 97%.

A cylindrospermopsin enzyme linked immunosorbent assay (ELISA) was also utilized for the quantitative detection of CYN. The current assay is sensitive down to a LOD/LOQ of 0.1 µg/L for CYN. A lab fortified blank (LFB) spiked with 1.0 µg/L CYN was recovered at 116%.

A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a LOD/LOQ of 0.05 µg/L saxitoxin. The LFB (0.2 µg/L STX spike) recovery was 105%.

Summary of Results

<u>Sample</u>	<u>ANTX-A</u> ($\mu\text{g/L}$)	<u>CYN</u> ($\mu\text{g/L}$)	<u>MC</u> ($\mu\text{g/L}$)	<u>STX</u> ($\mu\text{g/L}$)
AG1	ND	ND	ND	ND
AG2	ND	ND	ND	ND
AG3	ND	ND	ND	ND
AG4	ND	ND	ND	ND
AG5	ND	ND	ND	ND
<i>Limit of Detection ($\mu\text{g/L}$)</i>	0.05	0.10	0.15	0.05

ND=Not detected above detection limits

Submitted by:



Mark T. Aubel, Ph.D.

Date:

9/16/16

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**Spicer Group****MICROCYSTIN RESULTS**

Tested on: 9/9/2016
 Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
 Analyte: Microcystins/Nodularins
 Analyzed by: Kamil Cieslik

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery	Avg. LFM Recovery	Final Concentration (ug/L)	Average (ug/L)
AG1 8/30/2016	1x	none none	0.04 0.05	1 1	97%	89%	<0.15 <0.15	ND
AG2 8/30/2016	1x	none none	0.08 0.06	1 1	97%	---	<0.15 <0.15	ND
AG3 8/30/2016	1x	none none	0.04 0.02	1 1	97%	---	<0.15 <0.15	ND
AG4 8/30/2016	1x	none none	0.05 0.04	1 1	97%	---	<0.15 <0.15	ND
AG5 8/30/2016	1x	none none	0.10 0.08	1 1	97%	---	<0.15 <0.15	ND

ND = Not detected above LOD/LOQ
 LOD/LOQ = 0.15 µg/L
 LFB = 1.0 µg/L MCLR
 LFM = 1.0 µg/L MCLR

Submitted by:

Amanda Foss
 Amanda Foss, M.S.
 9/14/2016

Date:

Submitted to:

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**Spicer Group****CYLINDROSPERMOPSIN RESULTS**

Tested on: 9/8/2016
 Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
 Analyte: Cylindrospermopsin
 Analyzed by: Kamil Cieslik

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery	Avg. LFM Recovery	Final Concentration (ug/L)	Average (ug/L)
AG1 8/30/2016	1x	none none	0.00 0.01	1 1	116%	---	<0.10 <0.10	ND
AG2 8/30/2016	1x	none none	0.02 0.03	1 1	116%	80%	<0.10 <0.10	ND
AG3 8/30/2016	1x	none none	0.01 0.00	1 1	116%	---	<0.10 <0.10	ND
AG4 8/30/2016	1x	none none	0.01 0.02	1 1	116%	---	<0.10 <0.10	ND
AG5 8/30/2016	1x	none none	0.00 0.02	1 1	116%	---	<0.10 <0.10	ND

ND = Not detected above LOD/LOQ
 LOD/LOQ = 0.10 µg/L
 LFB = 1.0 µg/L CYN
 LFM = 1.0 µg/L CYN

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 9/14/2016

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**Spicer Group****PARALYTIC SHELLFISH TOXINS / SAXITOXIN RESULTS**

Tested on: 9/8/2016
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: PSTs (saxitoxins)
Analyzed by: Kamil Cieslik

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery	Avg. LFM Recovery	Final Concentration (ug/L)	Average (ug/L)
AG1 8/30/2016	1x	none none	0.00 0.00	1 1	105%	---	<0.05 <0.05	ND
AG2 8/30/2016	1x	none none	0.00 0.00	1 1	105%	---	<0.05 <0.05	ND
AG3 8/30/2016	1x	none none	0.00 0.00	1 1	105%	90%	<0.05 <0.05	ND
AG4 8/30/2016	1x	none none	0.00 0.00	1 1	105%	---	<0.05 <0.05	ND
AG5 8/30/2016	1x	none none	0.00 0.00	1 1	105%	---	<0.05 <0.05	ND

ND = Not detected above LOD/LOQ
 LOD/LOQ = 0.05 µg/L
 LFB = 0.2 µg/L STX
 LFM = 0.2 µg/L STX

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