Analysis of Algae Toxins by Liquid Chromatography Tandem Mass Spectrometry – Method Development and Demonstration

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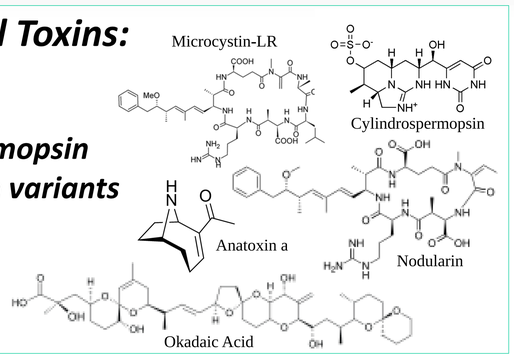
Introduction: Increasing eutrophication of surface water have resulted in an increased risk of algal blooms. In particular, harmful algal blooms (HABs) which are formed by cyanobacteria can produce extremely dangerous toxins and jeopardize drinking water sources. Several common classes of these compounds include neurotoxins such as anatoxin-a, cytotoxins such as cylindrospermopsin, and hepatotoxins such as microcystins. In May of 2015, the United States Environmental Protection Agency issued a health advisory recommending microcystins and cylindrospermopsin in drinking water for children younger than school age should not exceed 0.3 µg/L and 0.7 µg/L, respectively. Only a limited number of laboratories perform algae toxin testing. In addition, many of the standard methods developed only address one or two classes of toxins (i.e. the US EPA Method 544 for microcystins and nodularins). Therefore, in response to the need for this type of testing coupled with the need to encompass more classes of algae toxins in a single assay, ISTC has begun developing analytical methods to address these emerging contaminants of concern.

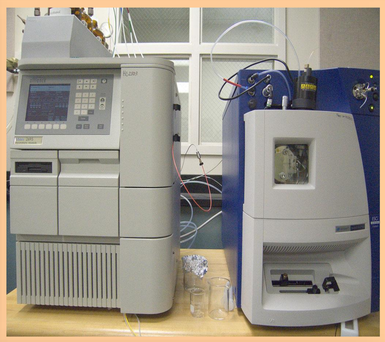
Target Algal Toxins:

- ***Anatoxin-a***
- ***Cylindrospermopsin***
- ***8 Microcystin variants***
- ***Nodularin***
- ***Okadaic Acid***



Liquid Chromatography Tandem Mass Spectrometry Analysis for Algae Toxins

- *Waters 2895 Alliance Coupled to Quattro Micro Mass Spec*
- *Column, Thermo Scientific Hypersil Gold (100 mm x 2.1 mm) 5µ part. size*



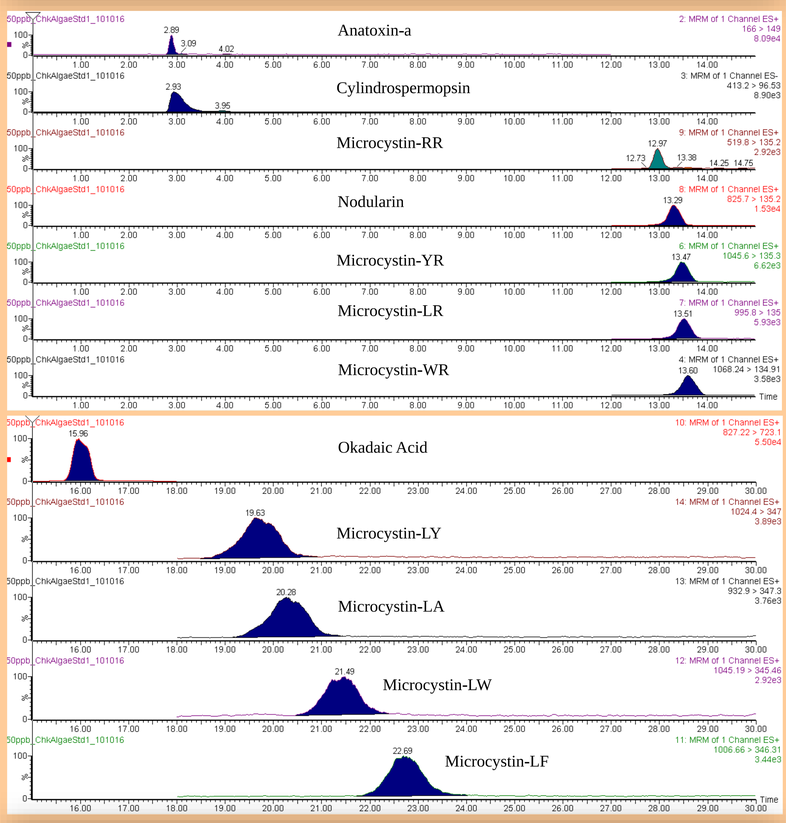
Separation Parameters:

Mobile Phase A – 0.1% Formic Acid in DI Water
Mobile Phase B – Methanol - Acetonitrile (4:6)
Gradient – 100% A for 2 minutes, 100% B from 2 minutes to 25 minutes, 100% A from 25 minutes to 30 minutes
Flow Rate – 0.1 ml/min
Injection volume – 30 µL

Table 1: Mass Spectrometry Parameters

Compound	Ion Mode	Parent Mass	Daughter Mass	Cone Voltage	Collision energy (eV)
Anatoxin-a	Positive	166	149	20	15
Cylindrospermopsin	Negative	413	96.5	40	40
Microcystin WR	Positive	1068	135	73	65
Microcystin YR	Positive	1046	135	50	70
Microcystin LR	Positive	996	135	55	60
Nodularin	Positive	826	135	50	55
Microcystin RR	Positive	520	135	30	30
Okadaic Acid	Positive	827	723	50	45
Microcystin LF	Positive	1007	346	49	60
Microcystin LW	Positive	1045	345	50	65
Microcystin LA	Positive	933	347	60	55
Microcystin LY	Positive	1024	347	70	70

Chromatogram of a 50 ng/ml Algae Toxin Mix



Detection Limits: The minimum instrumental signal of an analyte that can be measured with confidence can be determined with repeated injections of a blank. These data are then used to calculate the instrument detection limit (IDL) by the following expression:
IDL =average blank signal + 3 (standard deviation blank signal)
These detection limits were calculated from data by 6 blank injections. In addition, the calculated method detection limits (MDL) assume a 1L sample size and 0.5 ml final extraction volume.

Table 2: Instrument and Method Detection Limits

	IDL, µg/L	MDL, ng/L		IDL, µg/L	MDL, ng/L
Anatoxin-a	0.39	0.19	Microcystin LY	3.9	2.0
Cylindrospermopsin	0.60	0.30	Microcystin LA	0.49	0.25
Microcystin RR	0.7	0.36	Microcystin LW	3.7	1.9
Microcystin YR	2.7	1.37	Microcystin LF	4.0	2.0
Microcystin LR	1.2	0.58	Microcystin WR	3.4	1.7
Nodularin	0.15	0.077	Okadaic Acid	0.14	0.070

Instrument Method Reproducibility: To demonstrate the precision of the developed method a 50 ng/ml and 250 ng/ml algae toxin mixed standard was injected 7 times daily for four consecutive days. Precision was determined on an inter-day and intra-day basis by calculating a percent relative standard deviation (%RSD) from the multiple injections.

Inter-Day Precision – Inter-day precision expresses across-day variations.
Intra-Day Precision – Intra-day precision expresses within-day variations.

Table 3: Calculated Precision Parameters

	50 ng/ml Intra-day Precision (%RSD)				50 ng/ml Inter-day Precision (%RSD)
Analyte	Day 1	Day 2	Day 3	Day 4	Total over 4 days
Anatoxin-a	6.0	8.3	11	13	25
Cylindrospermopsin	1.5	1.4	2.2	2.1	7.5
Microcystin RR	1.9	5.7	4.7	3.2	26
Microcystin YR	2.0	0.82	3.7	2.2	13
Microcystin LR	2.0	1.5	3.5	2.1	14
Nodularin	1.6	1.4	3.2	0.9	16
Microcystin LY	1.2	2.7	3.1	1.7	14
Microcystin LA	2.1	4.4	3.0	1.7	14
Microcystin LW	2.6	5.4	4.1	3.5	12
Microcystin LF	2.7	6.7	3.0	3.2	14
Microcystin WR	3.0	1.1	4.1	3.2	15
Okadaic Acid	0.50	2.5	1.9	1.9	15

	250 ng/ml Intra-day Precision (%RSD)				250 ng/ml Inter-day Precision (%RSD)
Analyte	Day 1	Day 2	Day 3	Day 4	Total over 4 days
Anatoxin-a	4.0	10.1	13	4.0	31
Cylindrospermopsin	1.0	1.0	1.5	1.0	8.9
Microcystin RR	2.8	2.3	3.6	2.6	26
Microcystin YR	0.6	1.3	0.7	0.57	11
Microcystin LR	0.7	4.0	1.6	0.67	12
Nodularin	0.6	1.0	1.0	0.55	15
Microcystin LY	0.7	1.1	3.9	0.71	12
Microcystin LA	1.2	1.1	4.1	1.2	13
Microcystin LW	2.4	0.9	5.2	2.4	13
Microcystin LF	1.7	1.2	4.7	1.7	13
Microcystin WR	0.9	1.1	1.2	0.9	13
Okadaic Acid	0.61	0.68	1.9	0.61	13

Summary: A method to analyze 12 algae toxins has been developed at ISTC. The method has been demonstrated to be very sensitive with method detection limits below concentrations relevant to public health concerns. In addition, the method has been demonstrated to be reproducible over a 4 day time span.
Our next task is to develop a solid phase extraction technique to isolate and concentrate the target algae toxins. Preliminary experiments with standard methods have shown excellent recoveries for the microcystins, nodularin, and okadaic acid. However, the same experiment has shown low recoveries for the more polar algae toxin's anatoxin-a and cylindrospermopsin. Therefore, now we are performing experiments with mixed extraction materials to improve recoveries.

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