EPA Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Aqueous Direct Injection and LC/MS/MS

UCT Part Numbers:
SLAQ100ID21-3UM - Selectra® Aqueous C18, 100 x 2.1mm, 3µm
SLAQGDC20-3UM - Selectra® Aqueous C18, Guard column, 10 x 2.0mm, 3µm
SLGRDHLDR - Guard Cartridge Holder

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Summary:

Cyanotoxins are toxins that are produced by some species of photosynthetic cyanobacteria (also known as blue-green algae) blooming under certain conditions [1], such as stagnant or slow moving warm water with high-level nutrients like phosphates and nitrogen. Cyanotoxins are dangerous to humans and wildlife, affecting their livers (hepatotoxic), nervous systems (neurotoxic), and skin (acutely dermatotoxic). Human exposure to cyanotoxins can occur through ingestion of either the contaminated drinking water or exposed fish and shellfish, in addition to inhalation or dermal contact with the contaminated recreational water [2].

This application note describes a direct aqueous injection and liquid chromatography tandem mass spectrometry (DAI-LC/MS/MS) method for the determination of cylindrospermopsin (hepatotoxin) and anatoxin-a (neurotoxin) in drinking water under EPA Method 545 [3]. These two target analytes are also included in the UCMR4 screening compound list that will be monitored by public drinking water systems soon.

UCT's Aqueous C18 HPLC column was utilized for analyte retention and separation, which had demonstrated excellent consistency in peak area and retention times. 7-point calibration curves were constructed for analyte quantification. The responses were linear ($R^2 \geq 0.9982$) over the analytical range from 0.1 to 10 µg/L. Excellent accuracy (93.6 – 110.3%) and precision (RSD% < 10%, n=7) were achieved in fortified reagent water and tap water samples.

Procedure:

1. Preserve water samples with 1 g/L of sodium bisulfate (antimicrobial) and 0.1 g/L of ascorbic acid (dechlorination).
2. Add 10 µL of 0.5-2 ng/µL internal standard mixture to 2-mL vials, and appropriate amounts of spiking solutions for fortified samples, and bring the final volume to 1 mL with the preserved water samples.
3. Vortex the samples for 30 sec and analyze by LC-MS/MS equipped with an Aqueous C18 HPLC column.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System		
Column: UCT, Selectra®, aQ C18, 100 x 2.1 mm, 3 µm		
Guard column: UCT, Selectra®, aQ C18, 10 x 2.0 mm, 3 µm		
Column temperature: 30 °C		
Column flow rate: 0.300 mL/min		
Auto-sampler temperature: 10 °C		
Injection volume: 50 µL		
Gradient program:		
Time (min)	**A%** (50 mM acetic acid in DI water)	**B%** (MeOH)
0	100	0
1.5	100	0
4.5	70	30
6	70	30
6.1	10	90
7.5	10	90
7.6	100	0
13.5	100	0

Divert mobile phase to waste from 0 – 1.8 and 7 – 13.5 min to prevent ion source contamination.

MS parameters	
Instrumentation	Thermo Scientific TSQ Vantage tandem MS
Polarity	ESI +
Spray voltage	4000 V
Vaporizer temperature	400 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	25 arbitrary units
Q1 and Q3 peak width (FWHM)	0.2 and 0.7 Da
Collision gas and pressure	Ar at 1.5 mTorr
Cycle time	0.6 sec
Acquisition method	EZ Method (scheduled SRM)

Retention Times and SRM Transitions							
Compound	**Rt (min)**	**Precursor**	**Product 1**	**CE 1**	**Product 2**	**CE 2**	**S-Lens RF**
Uracil-d4	2.05	115.1	98.1	16	72.1	14	45
L-phenylalanine-d5	4.58	171.1	125.2	14	106.1	28	47
Cylindrospermopsin	5.40	416.1	194.1	31	176.1	31	106
Anatoxin-a	5.74	166.1	149.1	12	131.0	15	62

Results:

Relative Standard Deviation (RSD) of Peak Area and Retention Times by aQ C18 HPLC Column

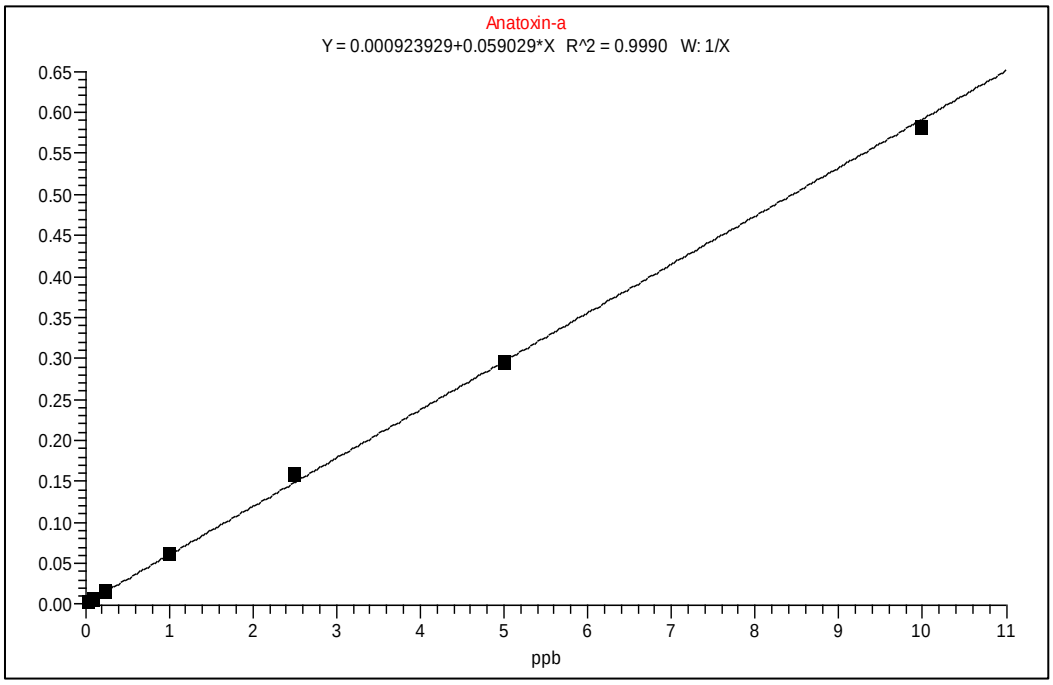
Compound	**RSD% (n=44)**	
	Peak Area	**Retention Time**
Uracil-d4	3.7	0.1
L-phenylalanine-d5	2.5	0.4
Cylindrospermopsin	ND	0.1
Anatoxin-a	ND	0.3

ND: not determined.

Analytical Range and Linearity Data

Compound	Analytical range (µg/L)	Linearity (R^2)	
		Reagent water	Tap water
Cylindrospermopsin	0.1 - 10	0.9989	0.9992
Anatoxin-a	0.1 - 10	0.9990	0.9982

Calibration Curve of Anatoxin-a in Reagent Water



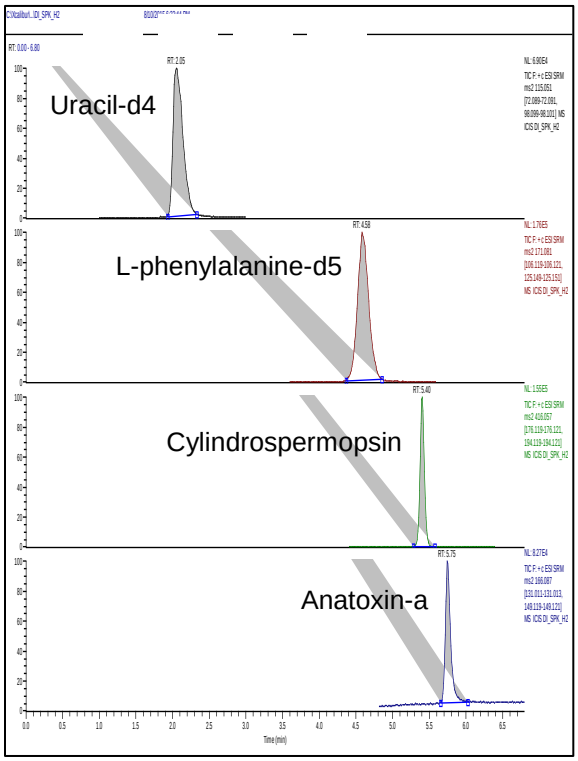
Accuracy and Precision in Fortified Reagent Water (n=7)

Compound	Spiked at 0.1 µg/L		Spiked at 2.5 µg/L	
	Recovery%	RSD%	Recovery%	RSD%
Cylindrospermopsin	**99.8**	4.5	**105.8**	2.3
Anatoxin-a	**101.5**	8.6	**103.1**	2.0

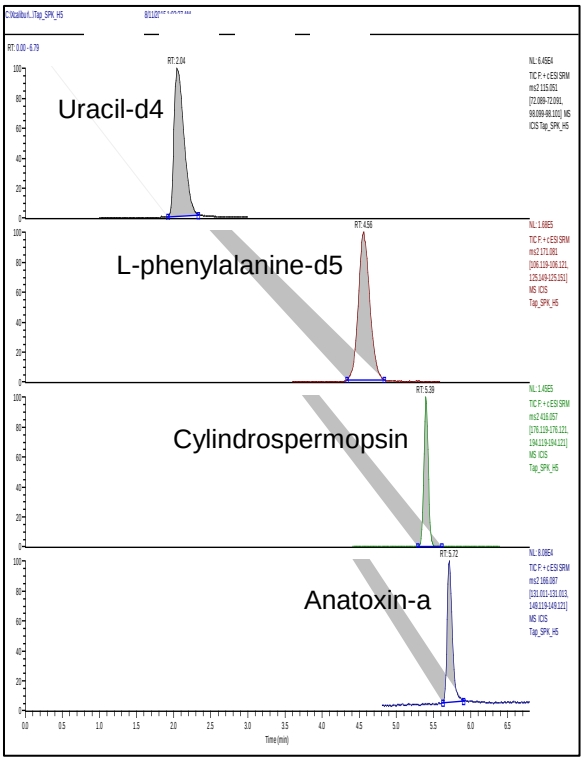
Accuracy and Precision in Fortified Tap Water (n=7)

Compound	Spiked at 0.1 µg/L		Spiked at 2.5 µg/L	
	Recovery%	RSD%	Recovery%	RSD%
Cylindrospermopsin	**100.4**	6.4	**110.3**	0.7
Anatoxin-a	**93.6**	5.2	**106.8**	0.6

Chromatograms of Fortified Water Samples



Reagent Water at 2.5 µg/L



Tap Water at 2.5 µg/L

References:

[1] https://en.wikipedia.org/wiki/Cyanotoxin

[2] http://www.waterrf.org/PublicReportLibrary/4548a.pdf

[3] http://water.epa.gov/scitech/drinkingwater/labcert/upload/epa815r15009.pdf

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