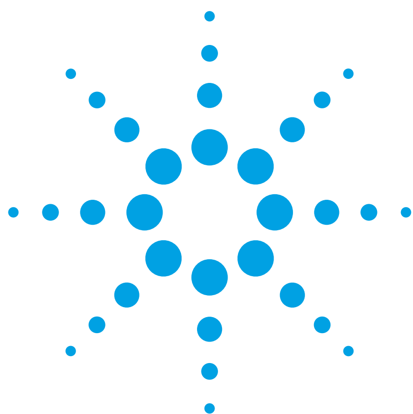

LC/MS/MS Analysis of Cylindrospermopsin and Anatoxin-a in Drinking Water Using US EPA Method 545

Application Note

Environmental

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Abstract

Analysis of drinking water samples according to EPA Method 545 on the Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole LC/MS System results in precision and accuracy values that are well within the method requirements and Lowest Calculated Minimum Reporting Limits (LCMRLs) that meet those cited in the method.

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Introduction

Cyanotoxins are produced by cyanobacteria (blue-green algae), which can form expansive blooms in lakes and oceans under the right conditions. These toxins can reach concentrations that can poison and even kill animals and humans. Two of the cyantoxins produced by blue-green algae are alkaloids known as cylindrospermopsin and anatoxin-a.

Cylindrospermopsin is toxic to liver and kidney tissue, and toxic blooms producing cylindrospermopsin are usually found in tropical, subtropical, and arid zone waters.

Anatoxin-a is a neurotoxin that produces very rapid toxic effects, including loss of coordination, twitching, convulsions, and rapid death by respiratory paralysis. Because of the dangers they present to recreational and drinking waters, these cyanotoxins are the subject of monitoring and regulation efforts in several countries, including the US.

The Safe Drinking Water Act (SDWA) requires the US EPA to publish a list of unregulated contaminants that are known or expected to occur in public water systems in the U.S., known as the Contaminant Candidate List (CCL). Cyanotoxins appear on the three drinking water CCLs, and the EPA has focused on three cyanotoxins for further research activities: microcystins, anatoxin-a, and cylindrospermopsin [1]. The EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) program to collect data on these cyanotoxins, and EPA Method 545 has been developed to measure anatoxin-a and cylindrospermopsin levels in drinking water for that purpose [2].

This application note describes the use of the Agilent 1200 Infinity Series LC and the Agilent 6460A Triple Quadrupole LC/MS System with Agilent Jet Stream technology to meet the stringent quality control requirements of Method 545. This analysis platform provided Lowest Calculated Minimum Reporting Limits (LCMRLs) that met or exceeded EPA requirements. In addition, recoveries and precision were well within the requirements of the method. Only 20-µL injections were required to satisfy the sensitivity requirements of the method, rather than the 50-µL injections recommended in the EPA method, enabling a reduction in maintenance requirements for the MS source.

Experimental

Reagents and materials

Anatoxin-a, cylindrospermopsin, and the internal standards were supplied by the EPA in Cincinnati, OH. An Agilent Polaris C18-Ether, 3 × 150 mm, 3 µm column (p/n A2021150X030) was used for the HPLC separations.

Internal standard	CASRN[a]	Catalog no.
Uracil-d_4, neat material	24897-55-0	C/D/N Isotopes D-5135
L-phenylalanine-d_5, neat material	56253-90-8	Cambridge Isotopes Labs DLM-1258-1

Instruments

The system was set up using Agilent 1200 Infinity Series LC modules coupled to an Agilent 6460A Triple Quadrupole LC/MS System, using electrospray positive ionization with Agilent Jet Stream technology. The LC system used the Agilent 1290 Infinity Binary Pump (G4220A), Agilent 1290 Infinity Autosampler (G4226A), and Agilent 1290 Infinity Column Compartment (G1316C). The LC/MS run conditions are shown in Table 1.

Table 1. HPLC and MS Conditions

HPLC	
Analytical column	Agilent Polaris C18-Ether, 3 × 150 mm, 3 µm column (p/n A2021150X030)
Column temperature	30 °C
Injection volume	20 µL
Mobile phase	A) 0.15 % acetic acid in water B) 0.15 % acetic acid in methanol
Flow rate	0.3 mL/min
Gradient	Time (min) Mobile phase (% B) 0 3 1 20 5 50 6 60 8 60
Post time	6 minutes
Run time	15 minutes, injection to injection
MS	
Acquisition parameters	ESI mode, positive ionization, MRM
Sheath gas temperature	350 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	350 °C
Drying gas flow rate	9 L/min
Nebulizer pressure	35 psig
Nozzle voltage	1,000 V
Vcap	4,000 V positive

Sample preparation

Sample preparation is very straightforward, involving collection of 10 mL samples in bottles containing preservatives, adding internal standards, and filtering 1 mL of each sample into a vial using 0.2-µm PVDF filters and disposable syringes. Ascorbic acid (0.1 g/L as reducing agent for chlorine) and sodium bisulfate (1 g/L as microbial inhibitor) were used as preservatives per EPA Method 545.

Analysis parameters

The multiple reaction monitoring (MRM) transitions used for cylindrospermopsin, anatoxin-a, and the two internal standards are shown in Table 2.

Results and Discussion

EPA Method 545 requirements

EPA Method 545 calls for seven Laboratory Field Blanks (LFBs) and seven tap water samples, spiked near mid-range of the calibration curve for each analyte. The resulting precision expressed as percent relative standard deviations (RSDs) must be ≤ 20 % and accuracy expressed as recovery must be between 70 and 130 %. A Laboratory Reagent Blank (LRB) must be analyzed directly following analysis of the highest calibration standard concentration for each analyte. Resulting peaks in the LRB must have areas < 1/3 of the lowest calibrator concentration. Finally, the goal of this implementation of Method 545 would be to generate LCMRLs equal to or lower than those reported in the EPA method.

Table 2. Multiple Reaction Monitoring (MRM) Analysis Parameters

Compound	Precursor ion	Product ion	Fragmentor voltage	Collision energy (V)	Dwell (msec)	Polarity	Type
Anatoxin-a	166.1	149.1*	95	12	150	Positive	Target
		131.1	95	12	150	Positive	Target
Cylindrospermopsin	416.1	194.1*	125	20	150	Positive	Target
		336.1	125	32	150	Positive	Target
Uracil-d4 †	115.1	98.0*	95	12	150	Positive	ISTD
Phenylalanine-d5 †	171.1	125.0*	95	8	150	Positive	ISTD

† Internal standard
*Transition used for quantitation

Method performance

The extracted ion chromatogram (EIC) in Figure 1 illustrates complete resolution of the two cyanotoxins as well as the internal standards. Analysis of the LFB directly following the analysis of the highest calibration standard concentration (0.30 µg/L) revealed no carryover for either anatoxin-a or cylindrospermopsin (Figure 2).

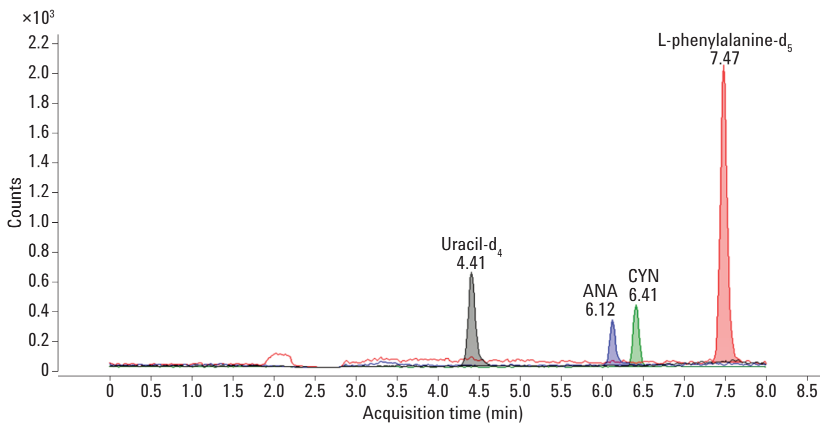

Figure 1. Extracted ion chromatogram (EIC) illustrating complete resolution of anatoxin-a (ANA) and cylindrospermopsin (CYN) as well as the two internal standards.

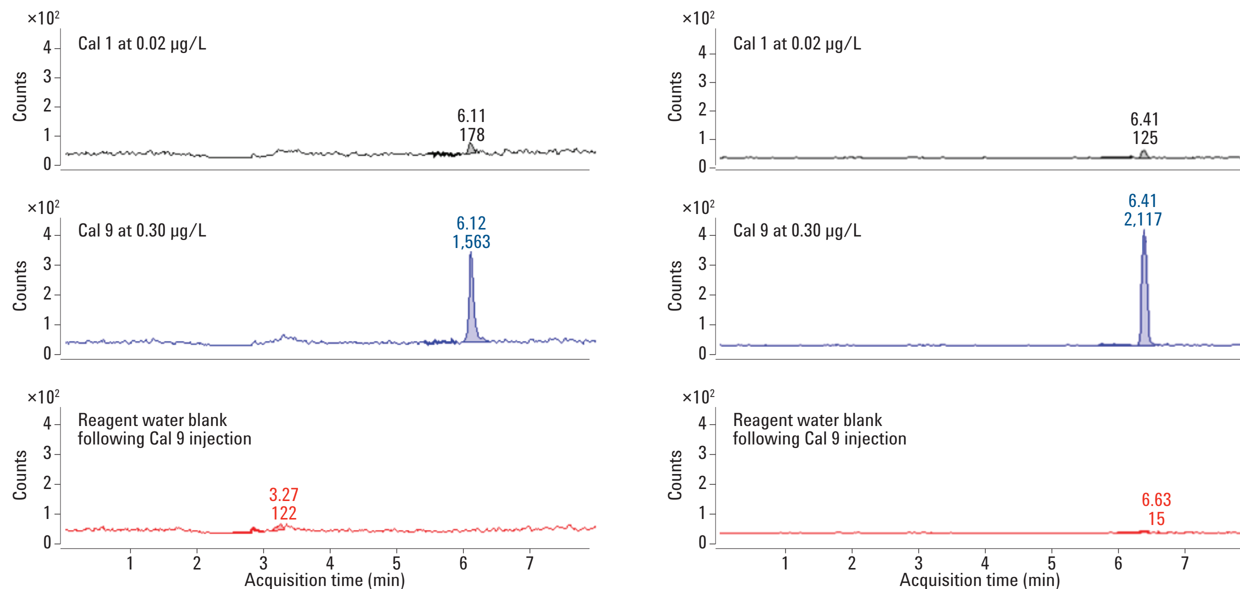

Figure 2. EICs of reagent water blanks for anatoxin–a and cylindrospermopsin run immediately following analysis of the highest calibrator concentration (Cal 9, 0.30 µg/L). No carryover was detected for either cyanotoxin.

The initial calibration curves were run from 0.030 to 0.300 µg/L, but these proved to be too high for the subsequent calculation of the LCMRLs. Therefore, a second set of calibration curves was run, from 0.005 to 0.050 µg/L. Both ranges yielded highly linear calibration, with all calibration coefficients (R^2) ≥ 0.996 (Figure 3).

As required by Method 545, seven replicates of LFBs and chlorinated tap water were spiked at 0.100 µg/L and preservatives were added. Recovery (accuracy) and precision were then determined for each set of samples (Table 3). Accuracy and precision were well under the ± 30 % recovery and 20 % RSD limits set by Method 545 for these values, in both reagent and tap water.

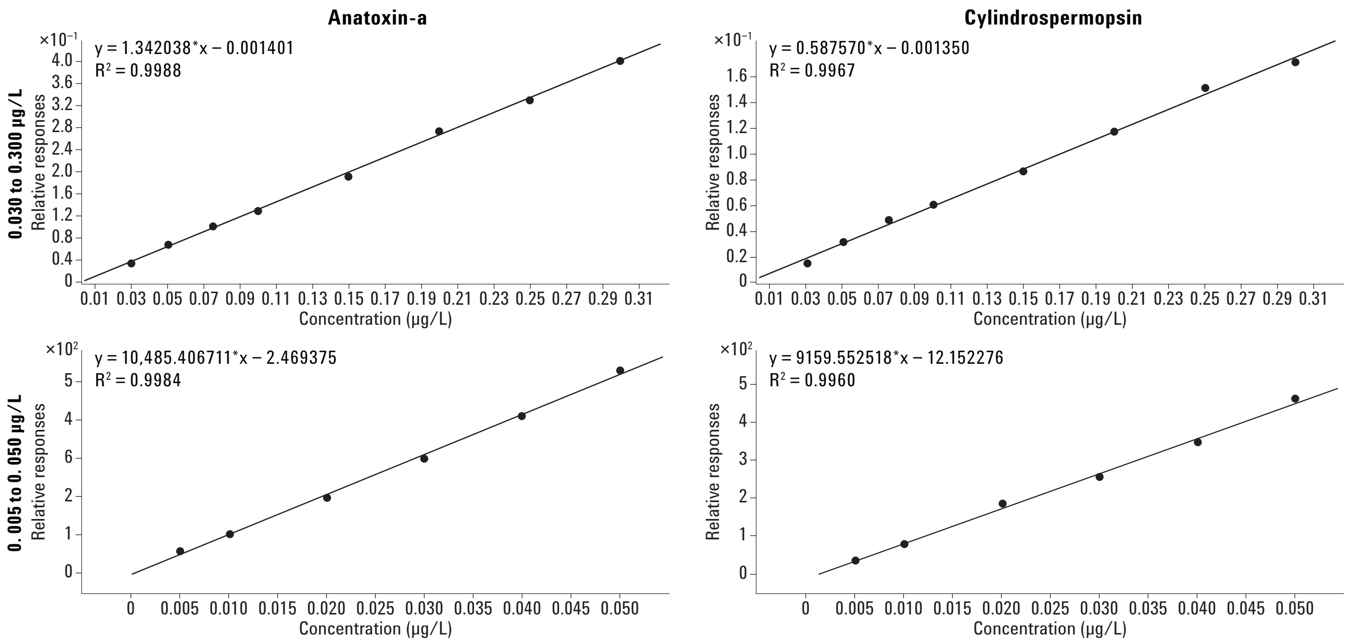

Figure 3. Linear calibration curves for two concentration ranges of the two cyanotoxins. The curve from 0.030 to 0.300 µg/L gave R^2 values of 0.9988 and 0.9967 for anatoxin-a (ANA) and cylindrospermopsin (CYN), respectively. The second calibration range (0.005–0.050 µg/L) gave R^2 values of 0.9984 and 0.9960 for ANA and CYN, respectively.

Table 3. Accuracy and Precision for Anatoxin-a (ANA) and Cylindrospermopsin (CYN) Analyses at 0.1 µg/L

	Reagent water		Tap water	
Replicate no.	**ANA***	**CYN***	**ANA***	**CYN***
1	0.115	0.108	0.089	0.104
2	0.100	0.097	0.083	0.106
3	0.096	0.097	0.086	0.107
4	0.092	0.106	0.090	0.110
5	0.087	0.100	0.097	0.105
6	0.096	0.096	0.086	0.115
7	0.087	0.102	0.099	0.094
Accuracy	96.1%	100.9%	90.0%	105.9%
RSD†	10.0%	4.7%	6.6%	6.1%

* Recovery values, µg/L

† Precision expressed as percent relative standard deviation (RSD)

LCMRL calculations

Method 545 requires the calculation of the LCMRL, which is accomplished by entering values in an EPA-supplied LCMRL Calculator [3]. The LCMRL is the lowest spiking concentration at which recovery of between 50 and 150 % is expected 99 % of the time by a single analyst. It requires a minimum of four replicates at each of seven fortification levels, plus four Laboratory Reagent Blanks (LRBs). The LCMRL Calculator constructs mean and variance models of measurement as a function of spiking level, taking into account both precision and accuracy. The first LCMRL calculations were done with a sample set ranging from 0.030–0.250 µg/L, but this range was too high to determine an LCMRL for anatoxin-a.

An additional two sets of replicates were run for both cyanotoxins, at 0.010 and 0.020 µg/L, using a calibration curve constructed from 0.005–0.050 µg/L. All nine fortification levels were then input into the calculator for LCMRL determinations, for both compounds. Figure 4 shows the LCMRL results, which were equal to or lower (0.018 µg/L for ANA and 0.038 µg/L for CYN) than those cited in Method 545 (0.018 µg/L for ANA and 0.063 µg/L for CYN).

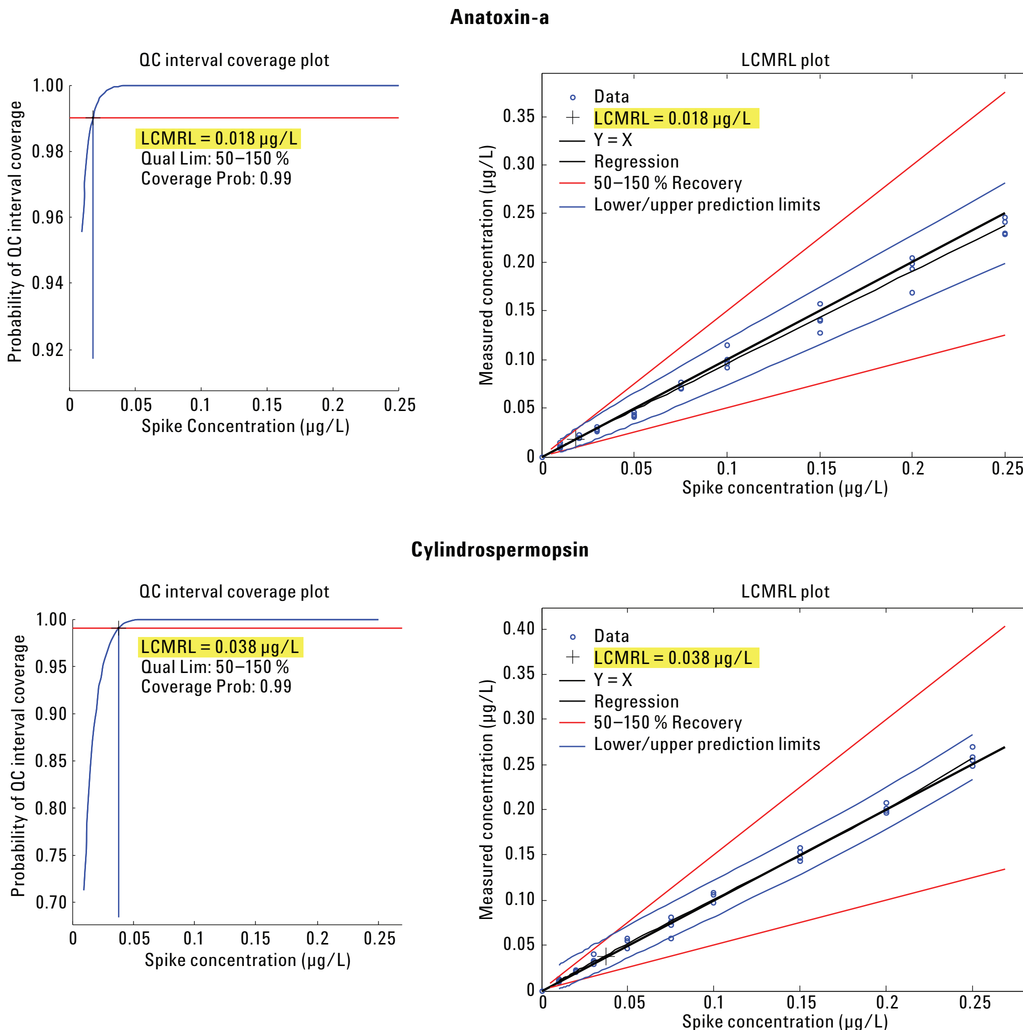

Figure 4. LCMRL results for anatoxin–a and cylindrospermopsin (0.018 and 0.038 µg/L respectively), which are equal to or lower than those reported in the EPA Method 545 (0.018 and 0.063 µg/L respectively).

Conclusions

Using an Agilent 1290 Infinity LC coupled to the Agilent 6460 Triple Quadrupole LC/MS System with Agilent Jet Stream technology for the analysis of algal toxins in drinking water can enable laboratories to meet the stringent QC requirements of EPA Method 545. Recoveries were between 90 and 106 %, well within the ± 30 % required by EPA. Precision ranged from 4.7–10 % in reagent water and chlorinated tap water, also well below the EPA limit of 20 %. In addition, the relative retention of Uracil-d4 on the Agilent Polaris C18-Ether column is greater than the retention on the column recommended in Method 545. The use of this column can help reduce potential ion suppression effects from preservatives and other non-retained compounds that may be present in drinking waters. LCMRLs that were equal to or below those found in the EPA method were obtained with only 20-µL injections, instead of the 50-µL injections recommended in Method 545. Injecting smaller sample amounts can help keep the MS source cleaner during routine use, reducing maintenance requirements.

Acknowledgements

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2. EPA Method 545 – Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS); August 2013.
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