APPLICATION NOTE

Liquid Chromatography

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Analysis of Cyanotoxins in Drinking Water Using UHPLC/TOF

Introduction

Toxin-producing bacteria are a growing concern for water utilities that use surface water supplies. Cyanobacteria, also known as blue-green algae, are photosynthetic bacteria that live in many bodies of water used for recreation and drinking water; therefore, an overgrowth can cause both ecological and public health concerns.

Rapid, excessive cyanobacteria growth is commonly referred to as a "bloom," identified as a foamy, blue-green or brown, mat or scum covering the water surface. During summer months, this layer can be inches thick, especially when located near the nutrient rich shorelines of lakes and reservoirs. A single bloom may contain multiple types of cyanobacteria and produce several toxins, having multiple toxicological impacts on the liver, nervous system, skin, and gastrointestinal system of animals and humans. Cyanotoxin-producing blooms have been identified in recreational waters more frequently in recent years. Due to this trend, recreational activities in rivers and lakes have been increasingly restricted out of concern for people and animals.

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Cyanobacteria and cyanotoxins produced in these blooms impact drinking water utility operations. As of early 2015, there are no federal regulatory standards or guidelines for cyanotoxins in drinking water, although the Safe Drinking Water Act (SDWA) requires the United States Environmental Protection Agency (USEPA) to publish a list of substances that could potentially be of concern and warrant further study. USEPA's research is expected to focus on anatoxin-a, microcystin-LR, and cylindrospermopsin, of which only two US states currently have set drinking water advisory thresholds. These two states, Ohio and Oregon, have established advisory limits for microcystin-LR and cylindrospermopsin at 1 ppb, while the anatoxin-a advisory threshold is set at 20 ppb in Ohio and 3 ppb in Oregon.[1] Globally, the World Health Organization (WHO) has set a provisional cyanotoxin limit of 1ppb.[2]

In this application, we describe a UHPLC method for monitoring anatoxin-a, cylindrospermopsin and microcystin-LR (structures shown in Figure 1) at ppb levels in drinking water. This method requires no pre-concentration of drinking water, and uses a TOF MS for detection.

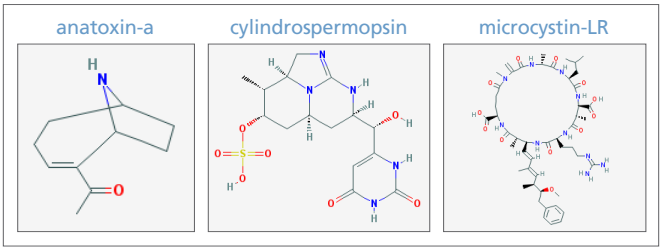

Figure 1. Structures of anatoxin-a, cylindrospermopsin and microcystin-LR cyanotoxins.

Experimental

Hardware/Software

A PerkinElmer Altus™ UPLC® system was used, which included the A-30 Sampling and Solvent Delivery Modules (quaternary pump) and Column Heater. An AxION® 2 TOF MS (PerkinElmer, Shelton, CT, USA) was used as the detector. A PerkinElmer Brownlee™ HRes DB AQ C18 1.9 µm, 2.1x100-mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). Instrument control, analysis, and data processing was performed via Mass IQ™/ e-Cipher™ Control/Processing Software (PerkinElmer, Shelton, CT, USA)

Method Parameters

The LC and TOF MS method parameters are shown in Tables 1 and 2, respectively.

Solvents, Standards and Samples

All solvents were LC/MS grade, including water, which was used for all dilutions.

10-ppm (10-µg/mL) cylindrospermopsin and microcystin-LR stock solutions in methanol were obtained from Abraxis, LLC (Warminster, PA). Anatoxin-a fumarate was obtained from R&D Systems®, Inc. (Minneapolis, MN) and was prepared by dissolving 1 mg in 1 mL of water. 10 µL of this solution was diluted to 10 ppm by adding 990 µL of water.

Table 1. LC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee HRes DB Aqueous C18 1.9 µm, 2.1 x 100-mm (Part# N9303919)
Mobile Phase:	Solvent A: 0.05% formic acid in water Solvent B: 0.05% formic acid in acetonitrile

	Time (min.)	Flow Rate (mL/min.)	%A	%B	Curve
1	Initial	0.4	100.0	0.0	
2	10.00	0.4	20.0	80.0	6
3	12.00	0.4	20.0	80.0	6
4	12.05	0.4	10.0	90.0	11
5	13.00	0.4	10.0	90.0	6
6	14.00	0.4	100.0	0.0	6

Analysis Time:	12.0 min.; wash/equilibration time = 3.0 min.
Flow Rate:	0.4 mL/min. (~8000 psi pressure)
Injection Volume:	10 µL
Sampling (Data) Rate:	10 pts./sec
Diluent:	LC/MS-grade water

Table 2. TOF Method Parameters.

TOF MS Conditions	
Ionization Source:	Ultraspray™ 2 (Dual ESI Source)
Ionization Mode:	Positive
Capillary Exit Voltage:	150 V
TrapPulse™ Mode:	75-1000 m/z (IG Exit Low D7:57, Trap/Pulse Delay D8:68)
Real-time mass calibrated extracted ion signals (lock mass) was performed using m/z 149.0233 and 992.0098 by infusion	

The 10-ppm stock solutions were used to prepare a standard solution in LC/MS grade water, as well as for spiked drinking water samples, collected from Washington, CT and Shelton, CT. 20-ppb working standards and spiked samples were prepared in duplicate by adding 20 µL of the 10-ppm stock solutions to 10-mL of water/sample.

The spiked LC/MS water working standard and spiked drinking water samples were further diluted to 2 ppb by adding 1 mL of the 20-ppb dilution to 9 mL of water/sample. Finally, 1-ppb solutions were prepared by adding 1 mL of water to 1 mL of the 2-ppb solutions. All injections were made in triplicate and recoveries calculated against the response of the working standards.

Results and Discussion

Figure 2 shows the extracted ion chromatogram (EIC) of the 100-ppb cyanotoxin standard mixture containing cylindrospermopsin, anatoxin-a, and microcystin-LR. Separation of the three cyanotoxins was achieved within 12 minutes. Figure 3 shows the mass spectra of each cyanotoxin at 100 ppb.

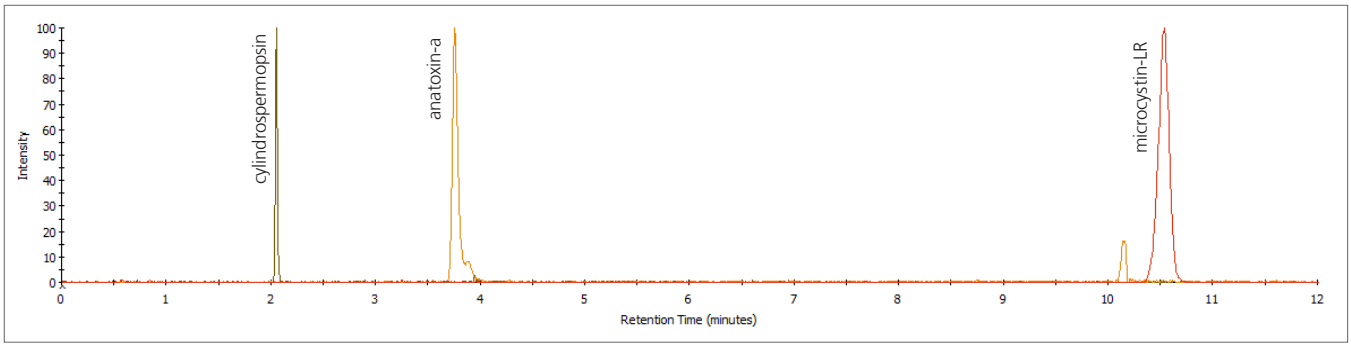

Figure 2. EIC chromatogram showing the 100-ppb standard solution of cylindrospermopsin ($[M+H]^+$ = 416.1234 Da) anatoxin-a ($[M+H]^+$ = 166.1226 Da) and microcystin-LR ($[M+H]^+$ = 995.5560 Da). The peak at 10.1 minutes was attributed to the background matrix.

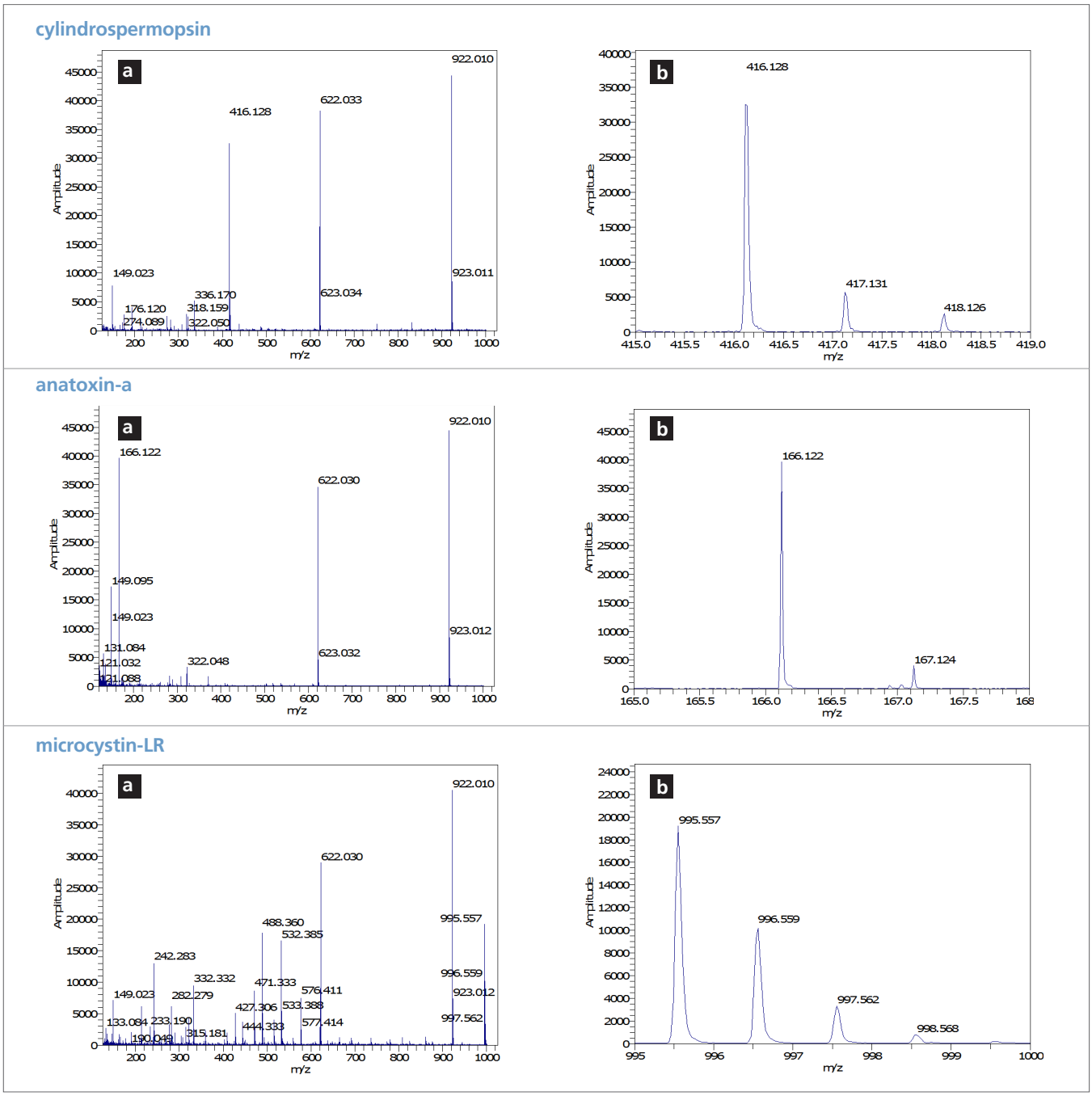

Figure 3. Mass spectra showing the a) full analyte peak spectra and b) $[M+H]^+$ ions for the cyanotoxins.

As shown in Figure 4, calibration curves for cylindrospermopsin, anatoxin-a, and microcystin-LR demonstrated excellent linearity ($R^2 \geq 0.999$), with triplicate injections at each level. This was over a 2-ppb to 100-ppb range for cylindrospermopsin and aflatoxin-a, and a 1-ppb to 100-ppb range for microcystin-LR.

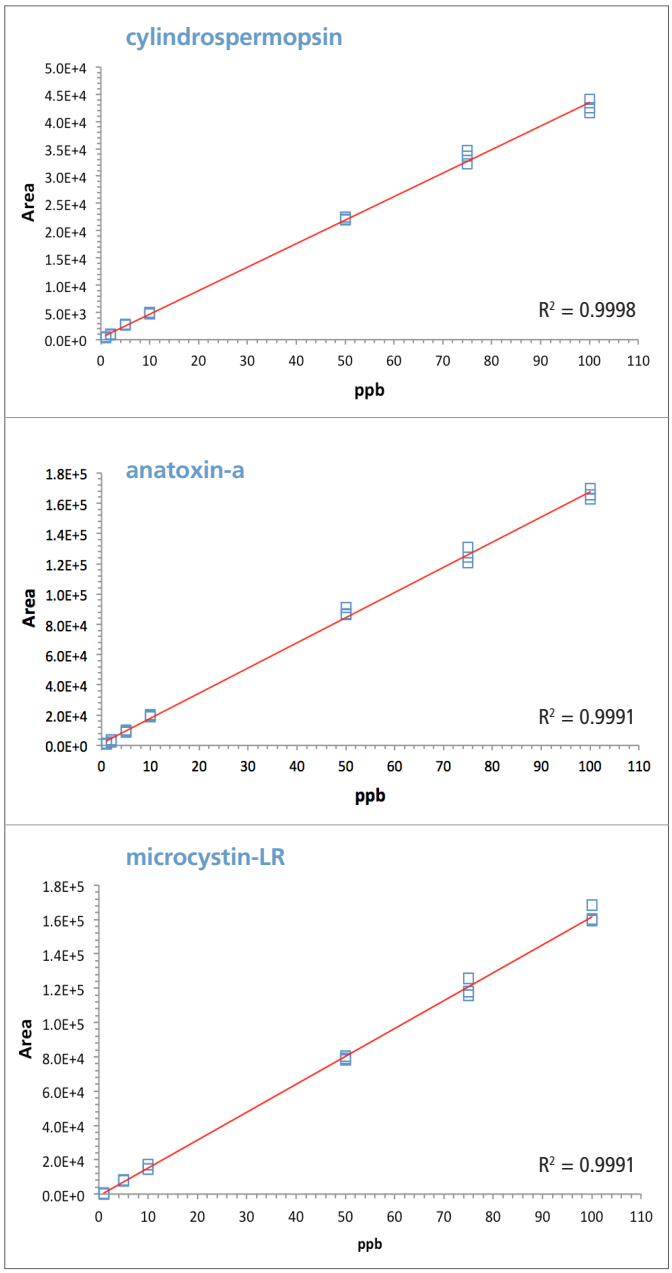

Figure 4. Linearity plot for cylindrospermopsin, anatoxin-a, and microcystin-LR.

The AxION 2 TOF was operated in the proprietary TrapPulse mode, increasing the duty cycle of the TOF, resulting in significant signal improvement. Using this mode, we were able to achieve detection limits down to less than 1 ppb for each of the cyanotoxins. Table 4 shows the calculated LOQ and LOD levels for each analyte. These levels were calculated based upon s/n of >10/1 for LOQ and >3/1 for LOD.

The cyanotoxin recoveries for the 20-ppb and 2-ppb spiked samples ranged from 74-103%, compared against LC/MS water standards (Table 5). A 1-ppb spiked cyanotoxin level was also tested. At this level, as the peaks for the individual analytes were slightly below the LOQ, recoveries could not be calculated; however, all analytes were above the LOD.

Table 4. LOQ and LOD values for cylindrospermopsin, anatoxin-a and microcystin-LR.

Cyanotoxin	LOQ (ppb)	LOD (ppb)
Cylindrospermopsin	1.9	0.6
Anatoxin-a	1.4	0.5
Microcystin-LR	1.3	0.4

Table 5. Cyanotoxin recovery results for spiked drinking water (n=3 injections).

Average recovery results for 20-ppb spiked water samples			
Drinking Water Source	**cylindrospermopsin (%)**	**anatoxin-a (%)**	**microcystin-LR (%)**
Shelton, CT	74.3	98.9	103.3
Washington, CT	84.9	99.1	93.7
Average recovery results for 2-ppb spiked water samples			
Drinking Water Source	**cylindrospermopsin (%)**	**anatoxin-a (%)**	**microcystin-LR (%)**
Shelton, CT	91.0	107.9	97.6
Washington, CT	85.6	93.5	74.3

Figure 5 demonstrates the analyte response of a 2-ppb cyanotoxin-spiked drinking water sample obtained from Shelton, CT. The spiked water sample from Washington, CT (not shown) looked very similar. No quanitifiable cyanotoxins were observed in the un-spiked drinking water from either Washington, CT or Shelton, CT.

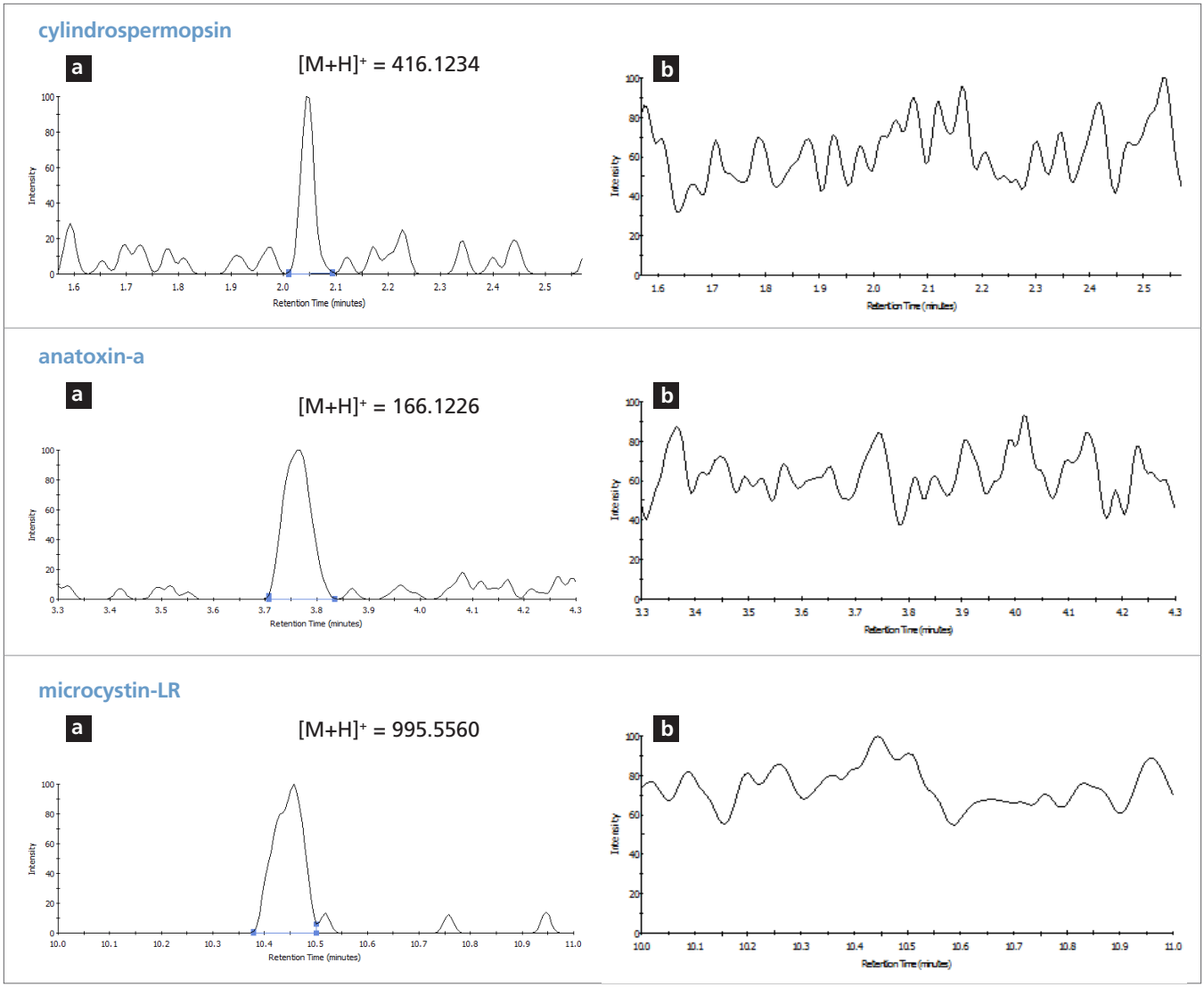

Figure 5. EICs of a) 2-ppb spiked drinking water and b) un-spiked drinking water obtained from Shelton, CT.

Conclusion

This work demonstrated the effective chromatographic separation and quantitation of three highly toxic cyanotoxins (cylindrospermopsin, anatoxin-a, and microcystin-LR), using a PerkinElmer Altus UPLC® system with the AxION 2 TOF MS detector. The results exhibited exceptional linearity for each cyanotoxin at very low ppb levels. With this method, the WHO's provisional drinking water cyanotoxin guideline limit of 1 ppb was detectable, and levels of 2 ppb and above were quantifiable in drinking water.

Although none of the un-spiked drinking water samples showed any detectable amount of cyanotoxin, the spike recovery analysis demonstrated the ability of the AxION 2 TOF MS detector to detect the analytes at very low ppb levels without the need for water sample pre-concentration.

Reference

1. "A Water Managers Guide to Cyanotoxins", American Water Works Association and Water Research Foundation, 2015 http://www.waterrf.org/PublicReportLibrary/4548a.pdf
2. WHO Guidelines for Drinking-water Quality, 3rd Ed., Vol. 1, Recommendations; WHO, Geneva, 2004

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