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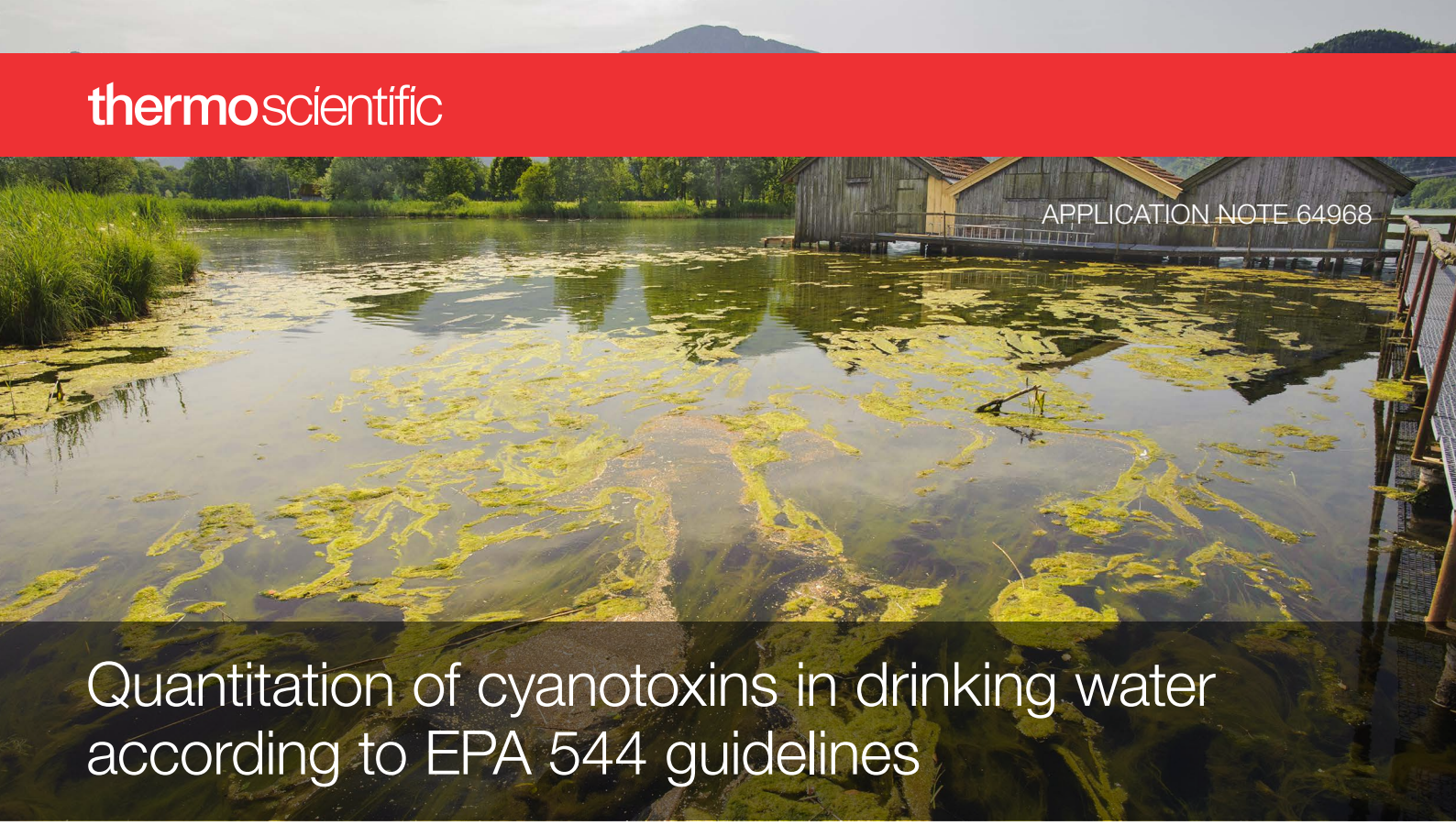

APPLICATION NOTE 64968

Quantitation of cyanotoxins in drinking water according to EPA 544 guidelines

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Keywords

Cyanotoxin, microcystins, EPA, TSQ Quantis MS

Goal

To demonstrate a sensitive, accurate, and reliable LC-MS/MS methodology in the quantitation of cyanotoxins in drinking water according to EPA 544 guidelines.

Introduction

Harmful algal blooms are a major environmental problem in the United States and in other nations. Known as red tides, blue-green algae, or cyanobacteria, harmful algal blooms have severe impacts on human health, aquatic ecosystems, and the economy. As a result, the United States Environmental Protection Agency (EPA) has developed EPA Method 544[1] for the Unregulated Contaminant Monitoring Rule 4 (UCMR 4) program, which collects data for contaminants suspected to be present in drinking water that lack health-based standard regulation under the Safe Drinking Water Act (SDWA).[2] The quantitative performance of the latest generation of triple quadrupole instruments enhances quantitation for these groups of compounds.

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This study demonstrates the performance of the new Thermo Scientific™ TSQ Quantis™ triple quadrupole MS platform via EPA Method 544: Determination of Microcystins in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization and Tandem Mass Spectrometry (LC-ESI-MS/MS).

Experimental

Sample preparation

The sample preparation was based on EPA Method 544. A 500 mL water sample (fortified with a surrogate) was filtered and both the filtrate and the filter were collected. The filter was placed in a solution of methanol containing 20% reagent water and held for at least one hour at -20 ºC to release the intracellular toxins from cyanobacteria cells captured on the filter. The liquid was drawn off the filter and added back to the 500 mL aqueous filtrate. The 500 mL sample (plus the intracellular toxin solution) was passed through an SPE cartridge to extract the method analytes and surrogate. The analytes were eluted from the solid phase with a small amount of methanol containing 10% reagent water. The extract was concentrated to dryness by evaporation with nitrogen in a heated water bath, and then adjusted to a 1 mL volume with methanol containing 10% reagent water.

Liquid chromatography

Chromatographic separation was performed using the Thermo Scientific™ Vanquish™ Flex HPLC system equipped with a Thermo Scientific™ Accucore™ C18 LC column (2.6 × 100 mm, 2.6 µm) maintained at 30 °C. Mobile phase A was 20 mM ammonium formate in water and mobile phase B was methanol. The injection volume was 5 µL.

Analytes were separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS/MS conditions. The concentration of each analyte was determined by external standard calibration.

Mass spectrometry

Compounds were detected on a TSQ Quantis triple quadrupole mass spectrometer equipped with a heated electrospray ionization source.

Ion Source Parameter	Value
Spray Voltage	3500 V
Sheath Gas	45 Arb
Aux Gas	10 Arb
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	325 °C
Vaporizer Temperature	275 °C

Instrument parameters are listed in Table 1.

Requirements

The EPA has strict requirements for the analysis of any sample, referred to as the Initial Demonstration of Capability (IDC). These requirements include the demonstration of low background noise, precision by analyzing four to seven extracted laboratory-fortified reagent water blanks (LFB) at mid-level, the demonstration of accuracy, and finally, the demonstration of capability necessary to meet the minimum reporting limit (MRL). The percent relative standard deviation (%RSD) of the results for replicate analyses must be ≤ 30%. The average percent recovery for each analyte must be within ± 30% of the true value.

Table 1. Instrument parameters.

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
MC-RR-$[M+2H]^{2+}$	Positive	519.9	135.0	27.40	178
MC-YR-$[M+2H]^{2+}$	Positive	523.4	135.1	10.23	130
Nodularin-R-$[M+H]^{+}$	Positive	825.4	135.1	54.89	299
MC-LA-$[M+H]^{+}$	Positive	910.4	776.3	17.73	264
MC-LF-$[M+H]^{+}$	Positive	986.4	852.3	19.17	299
MC-LR-$[M+H]^{+}$	Positive	995.5	135.1	54.47	299
MC-LY-$[M+H]^{+}$	Positive	1002.4	868.3	46.96	299
C2D5-MC-LR (SUR)	Positive	1028.5	135.1	49.24	299

Results and discussion

Excellent linearity was demonstrated for a range starting at UCMR 4 MRL up to 20-fold at the highest calibration standard (Figure 1). Table 2 displays low background noise evaluation with low detection in laboratory blanks. Tables 3, 4, and 5 summarize, respectively, precision and accuracy, minimum reporting limit confirmation, and matrix spikes method evaluations after the analysis of a spiked drinking water sample (reagent water spiked for fulfilling IDC).

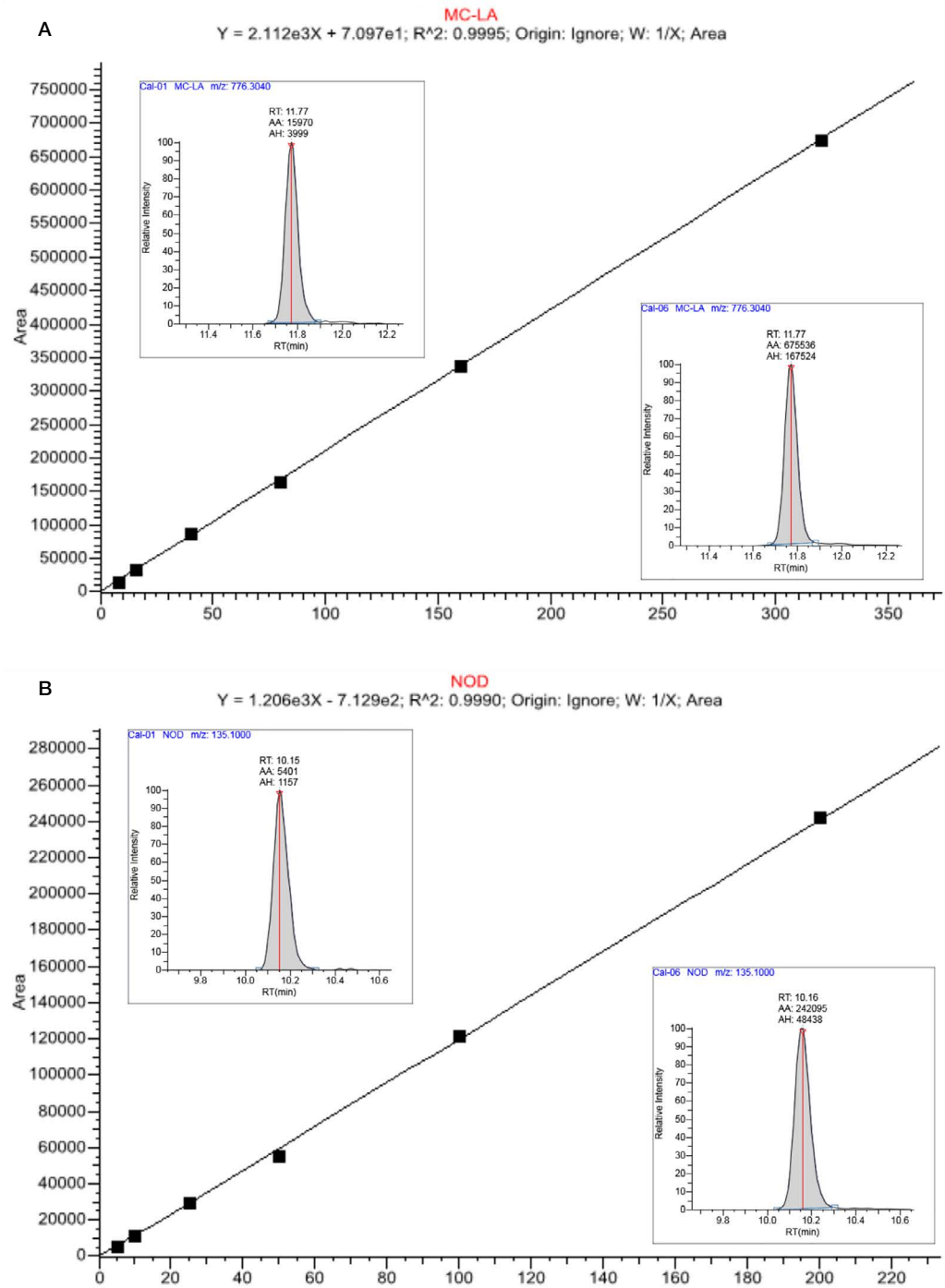

Figure 1. Calibration curves for (A) MC-LA and (B) nodularin. Chromatograms are shown representing the highest and the lowest calibration points.

Initial demonstration of capability

The more recent EPA methods for drinking water include a section for initial demonstration of capability requirements prior to sample analysis. The criteria for EPA 544 were tested and met as described below.

Low system background was measured. All method blanks exhibited less than 1/3 MRL contamination or carryover (Table 2).

The initial demonstration of precision and accuracy was met by analyzing four LFBs spiked at 3× the MRL with < 30% RSD and ± 30% difference achieved (Table 3).

Table 2. Low background noise for all EPA Method 544 analytes.

Analyte	MRL (ng/L)	1/3 MRL (ng/L)	Detectable at the Method Blank
MC-LA-$[M+H]^+$	8	2.7	0
MC-LF-$[M+H]^+$	6	2	0
MC-LR-$[M+H]^+$	20	6.7	1.4
MC-LY-$[M+H]^+$	9	3	1.6
MC-RR-$[M+2H]^{2+}$	6	2	0
MC-YR-$[M+2H]^{2+}$	20	6.7	4
Nodularin-R-$[M+H]^+$	5	1.7	0
C2D5-MC-LR (SUR)			108%

Table 3. Precision and accuracy at 3x MRL for all EPA Method 544 analytes.

Analyte	Actual (ng/L)	LFB1 (ng/L)	LFB2 (ng/L)	LFB3 (ng/L)	LFB4 (ng/L)	Average (ng/L)	%Rec	%RSD
MC-LA-$[M+H]^+$	40	45.692	47.658	47.87	48.949	47.54225	119%	3%
MC-LF-$[M+H]^+$	30	37.745	36.977	37.477	38.609	37.702	126%	2%
MC-LR-$[M+H]^+$	100	103.254	117.884	117.487	121.733	115.0895	115%	7%
MC-LY-$[M+H]^+$	45	54.685	54.204	56.539	57.402	55.7075	124%	3%
MC-RR-$[M+2H]^{2+}$	30	34.403	32.4	31.317	34.56	33.17	111%	5%
MC-YR-$[M+2H]^{2+}$	100	114.478	115.423	111.507	118.196	114.901	115%	2%
Nodularin-R-$[M+H]^+$	25	30.042	28.238	27.065	30.396	28.93525	116%	5%
C2D5-MC-LR (SUR)		118%	116%	109%	120%			

Minimum reporting limit (MRL) confirmation was evaluated by fortifying, extracting, and analyzing seven replicate LFBs at the proposed MRL concentration. The mean and the half range (HR) were then calculated. The Prediction Interval of Results (PIR) is defined as:

$PIR = Mean + HR_{PIR}$

where $HR_{PIR} = 3.963s$; s is the standard deviation and 3.963 is a constant value for seven replicates. The upper and lower limits for the PIR met the recovery criteria (upper PIR < 150% and lower PIR > 50%, Table 4).

Table 4. Minimum reporting limit confirmation for all EPA Method 544 analytes.

Analyte	Actual (ng/L)	MRL 1	MRL 2	MRL 3	MRL 4	MRL 5	MRL 6	MRL 7	Lower PIR >50%	Upper PIR <150%
MC-LA-$[M+H]^+$	8	8.4	8.8	8.2	8.0	8.4	8.6	8.1	91%	118%
MC-LF-$[M+H]^+$	6	6.9	6.4	5.8	6.0	6.1	6.2	6.5	81%	129%
MC-LR-$[M+H]^+$	20	25.5	23.2	23.1	23.7	25.0	20.6	22.4	84%	149%
MC-RR-$[M+2H]^{2+}$	6	7.0	7.0	7.3	7.2	7.4	7.3	7.2	111%	129%
MC-LY-$[M+H]^+$	9	10.3	10.5	10.4	9.7	9.9	10.4	10.2	101%	126%
MC-YR-$[M+2H]^{2+}$	20	27.7	27.3	27.2	27.8	27.6	27.6	27.6	134%	142%
Nodularin-R-$[M+H]^+$	5	5.5	5.2	6.2	5.5	6.0	6.2	6.1	86%	147%
C2D5-MC-LR (SUR)		118%	116%	109%	120%					

PIR stands for Prediction Interval of Results.

Monrovia, CA, tap water (comprised of ground and surface water) was extracted and analyzed using the methodology developed. Results are shown in Table 5.

Table 5. Monrovia, CA, water sample analyzed using the TSQ Quantis MS.

Analyte	Actual (ng/L)	FS	LFSM	LFSMD	Average	%Rec	Std Dev	%RSD
MC-LA-$[M+H]^+$	40	0.4	49	49	49	122%	0.19	0.4%
MC-LF-$[M+H]^+$	30	0	39	38	39	127%	0.38	1.0%
MC-LR-$[M+H]^+$	100	3.8	120	119	119	119%	0.69	0.6%
MC-LY-$[M+H]^+$	45	1.6	55	54	55	121%	0.15	0.3%
MC-RR-$[M+2H]^{2+}$	30	3.9	34	35	35	117%	0.96	2.7%
MC-YR-$[M+2H]^{2+}$	100	9.4	116	117	117	117%	0.63	0.5%
MC-YR-$[M+H]^+$	100	0	112	115	114	114%	2.15	1.9%
Nodularin-R-$[M+H]^+$	25	0	28	26	27	108%	1.51	5.6%
C2D5-MC-LR (SUR)	260	119%	125%	118%				
Surrogate	60–130%							
%Recovery	60–140%							
%RSD	<30%							

LFSM stands for Laboratory Fortified Sample Matrix. LFSMD stands for Laboratory Fortified Sample Matrix Duplicate. FS stands for Field Sample.

Conclusion

- The TSQ Quantis triple quadrupole MS proved to be sensitive, accurate, reproducible, and reliable in the quantitation of microcystins and nodularin in drinking water according to the EPA method requirements.
- Adequate sensitivity was obtained using a 5 µL injection volume.

References

1. Shoemaker, J., Dan Tettenhorst, and A. Delacruz. Method 544. Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Washington, DC, 2015. cfpub.epa.gov/si/si_public_record_report.cfm?direntryid=306953 (accessed April 20, 2017).
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