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Application News

LCMS-8060NX High Performance Liquid Chromatograph Mass Spectrometer

Highly Sensitive Quantitation of Microcystins and Nodularin in Water using the Triple Quad LCMS-8060NX in Accordance with EPA Method 544

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User Benefits

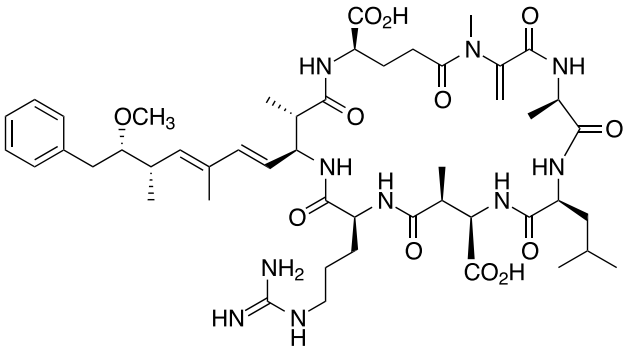
- Shimadzu LCMS-8060NX triple quadrupole mass spectrometer can easily achieve the detection limits reported in EPA Method 544 on microcystins and nodularin analysis.
- This method provides at least 10 times lower LOQs than those reported in EPA Method 544 (that requires sample preconcentration by SPE), demonstrating that direct injection analysis of the target compounds is possible in the same system.
- The run time is shortened to only 8 minutes while maintaining good chromatographic separation and analyte peak shape.

■ Background

Both freshwater and marine environments can be inhabited by photosynthetic organisms known as cyanobacteria, or blue-green algae. The frequency of algal blooms and location where they occur have risen over the years, with human-caused inputs of phosphorus and nitrogen into natural water systems being significant factors driving the global increase in harmful algal blooms. Microcystins and nodularin are highly potent hepatotoxins produced by different species of cyanobacteria. These toxins pose a significant threat to both livestock and human drinking water sources, particularly during algal blooms in freshwater systems, where large quantities of microcystins and nodularin can accumulate.

There are at least 80 known microcystins, among which Microcystin-LR (MC-LR) is widely recognized as one of the most toxic variants.[1] Over a dozen countries have established regulations or guidelines pertaining to microcystins in both drinking water and recreational waters. The majority of these guidelines for drinking water are derived from the provisional value established by the World Health Organization, which sets the limit for microcystin-LR at 1.0 µg/L.[2] Advisory published by US EPA, microcystins in drinking water should be less than 0.3 µg/L for children of pre-school age and younger (less than six years old) and 1.6 µg/L for school-age children and adults.[3]

This application demonstrates the accurate and sensitive quantification of six microcystins (MC-LR, MC-RR, MC-LA, MC-LF, MC-LY, MC-YR) and nodularin in accordance with EPA method 544[4] on a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer.



Microcystin-LR

■ Method

Six microcystins and nodularin were purchased from Enzo Life Sciences. The internal standard ethylated D5 microcystin-LR (MC-LR-C2D5) was obtained from Gold Standard Diagnostic. LC-MS grade solvents (formic acid, acetonitrile, and water) were sourced from Honeywell.

Standards preparation: Stock standards of six microcystins and nodularin were prepared by adding 1 mL of methanol directly to manufacturers' vials. After dilution, 100 µg/mL of analyte stock standards were obtained. The initial standard stock solutions were further diluted to 1 µg/mL with methanol/water (1:1). A series of calibration standards were prepared using methanol/water (1:1) as diluent to obtain the final concentrations of 0.5 - 500 µg/L for the various calibration levels. Calibration standards and samples were spiked with internal standard MC-LR-C2D5 at a final concentration of 25 µg/L.

Instrumentation parameters: A Shimadzu LCMS-8060NX triple quadrupole mass spectrometer was used to quantify microcystins and nodularin in water. The chromatographic separation of the analytes and internal standard was achieved in only 8 minutes using a Shim-pack Velox SP-C18 column (2.1 × 100 mm, 2.7 µm, PN: 227-32003-03).

The flow rate of the mobile phase was 0.3 mL/min, with an injection volume of 10 µL, and the column oven temperature was maintained at 40 ℃. Details of the gradient conditions are shown in **Table 1**.

The LCMS-8060NX was utilized in positive ion mode with electrospray ionization, operating in multiple reaction monitoring (MRM) mode. Quantitation and confirmation of the targeted analytes were performed through the monitoring of two selective MRM transitions. **Tables 2** and **3** provide details on the parameters used, including both source-specific and compound-specific information.

Data analysis: Data was acquired using LabSolutions software and analyzed using LabSolutions Insight™ LCMS. Insight features fast data processing and data review, allowing scientists to analyze data efficiently.

Table 1: Gradient time program of mobile phases

Time (min)	%A	%B
0	85	15
0.50	85	15
5.00	10	90
6.50	10	90
6.51	85	15
8.00	85	15

Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile

Table 2: MS conditions

Interface	: ESI
Mode	: MRM
Polarity	: Positive
Interface Voltage	: 1 kV
Focus Voltage	: 2 kV
Nebulizing Gas Flow	: 3.0 L/min
Heating Gas Flow	: 10.0 L/min
Interface Temperature	: 300 ℃
DL Temperature	: 250 ℃
Heat Block Temperature	: 400 ℃
Drying Gas Flow	: 10.0 L/min

Table 3: Detailed MRM settings for the analysis of microcystins and nodularin. For every analyte, the upper product ion signifies the quantifier fragment ion, while the lower product ion signifies the qualifier fragment ion.

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Dwell Time (ms)	Q1 (V)	Collision Energy (V)	Q3 (V)	Retention Time (min)
MC-RR	520.00	135.20 103.25	32	-26.0	-30.0 -55.0	-28.0 -20.0	2.951
NOD	826.00	135.30 825.50	26	-22.0	-53.0 -15.0	-27.0 -24.0	3.216
MC-LR	995.50	135.35 244.30	26	-28.0	-54.0 -48.0	-26.0 -17.0	3.396
MC-YR	523.40	135.30 103.30	26	-26.0	-15.0 -50.0	-15.0 -11.0	3.314
MC-LA	910.40	135.30 163.30	26	-26.0	-53.0 -48.0	-15.0 -11.0	4.111
MC-LY	1002.60	135.30 868.50	26	-38.0	-55.0 -21.0	-26.0 -20.0	4.147
MC-LF	986.60	135.30 213.30	40	-22.0	-54.0 -45.0	-23.0 -15.0	4.586
MC-LR-C2D5	1028.60	135.30 163.30	26	-38.0	-54.0 -50.0	-29.0 -18.0	3.746

■ Results and Discussion

Chromatographic separation: The use of the Shim-pack Velox SP-C18 column, combined with optimized gradient conditions, resulted in the effective retention of analytes and enabled baseline separation of most of the seven cyanotoxins and internal standard in 8 minutes, as shown in **Figure 1**. The overlapping MC-LA and MC-LY signals have distinct MRM transitions which ensures they can be identified and quantified by mass spectrometry.

Calibration and LOQ: Linear calibration curves for MC-LR, MC-LA, MC-LY and NOD were achieved in the concentration range of 0.5 - 200 µg/L, with a calibration range from 1.0 - 200 µg/L for MC-LF and 0.5 - 500 µg/L for MC-RR and MC- YR. **Figure 2** shows the linearity of the calibration curves. Excellent linearity, evidenced by R^2 values exceeding 0.99 for all seven analytes, was successfully attained across the broad calibration range. The calibration curve was generated by analyzing triplicate injections of each standard concentration, with the accuracy of all injections falling within the range of 70% to 130%. For all seven cyanotoxins, %RSD of concentration at each calibrator was less than 15% for all calibrators, which indicates the excellent robustness and reproducibility of the system.

Limits of quantitation (LOQs) of the method were 0.5 µg/L for all analytes, except for MC-LF (1.0 µg/L). LOQ was determined based on accuracy, reproducibility, and S/N ratio ≥10. The accuracy of the data points obtained at the LOQ was within 70 – 130% with the %RSD <10%, meeting the EPA Method 544 requirements. Representative chromatograms of the quantifier ions in LOQ injections are shown in **Figure 3**. The LOQs are more than 10 times lower than the LOQs reported in EPA method 544. If water samples are pre-concentrated by 500-fold through solid phase extraction (SPE) according to EPA's sample preparation process, our system can measure the concentration of 1 ng/L microcystins (2 ng/L for MC-LF) in water samples.

Spiked water sample analysis: To showcase the method's applicability, three water samples were prepared and processed to analyze the seven cyanotoxins. Accuracy and precision were evaluated by spiking three levels of the seven cyanotoxins (8, 25 and 80 µg/L) into LC-MS grade water Accuracies within a 20% range of the anticipated values, along with %RSD values below 10%, were noted for all seven cyanotoxins at the three spiking levels for all water samples (**Table 4**). No carryover was observed in the blank injections during or at the end of the batch.

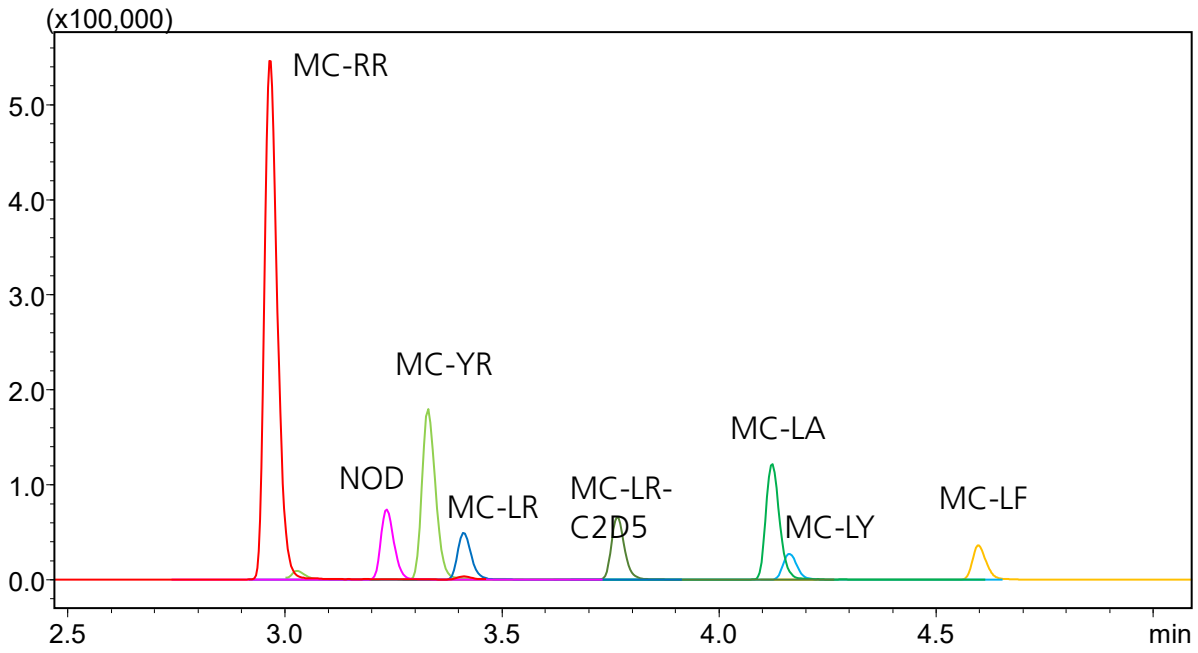


Figure 1: MRM quantifier ion chromatograms of microcystins and nodularin in the 20 µg/L solvent calibration standard. The concentration of internal standard MC-LR-C2D5 is 25 µg/L. The Shim-pack Velox SP-C18 column successfully retained compounds while demonstrating effective baseline chromatographic separation.

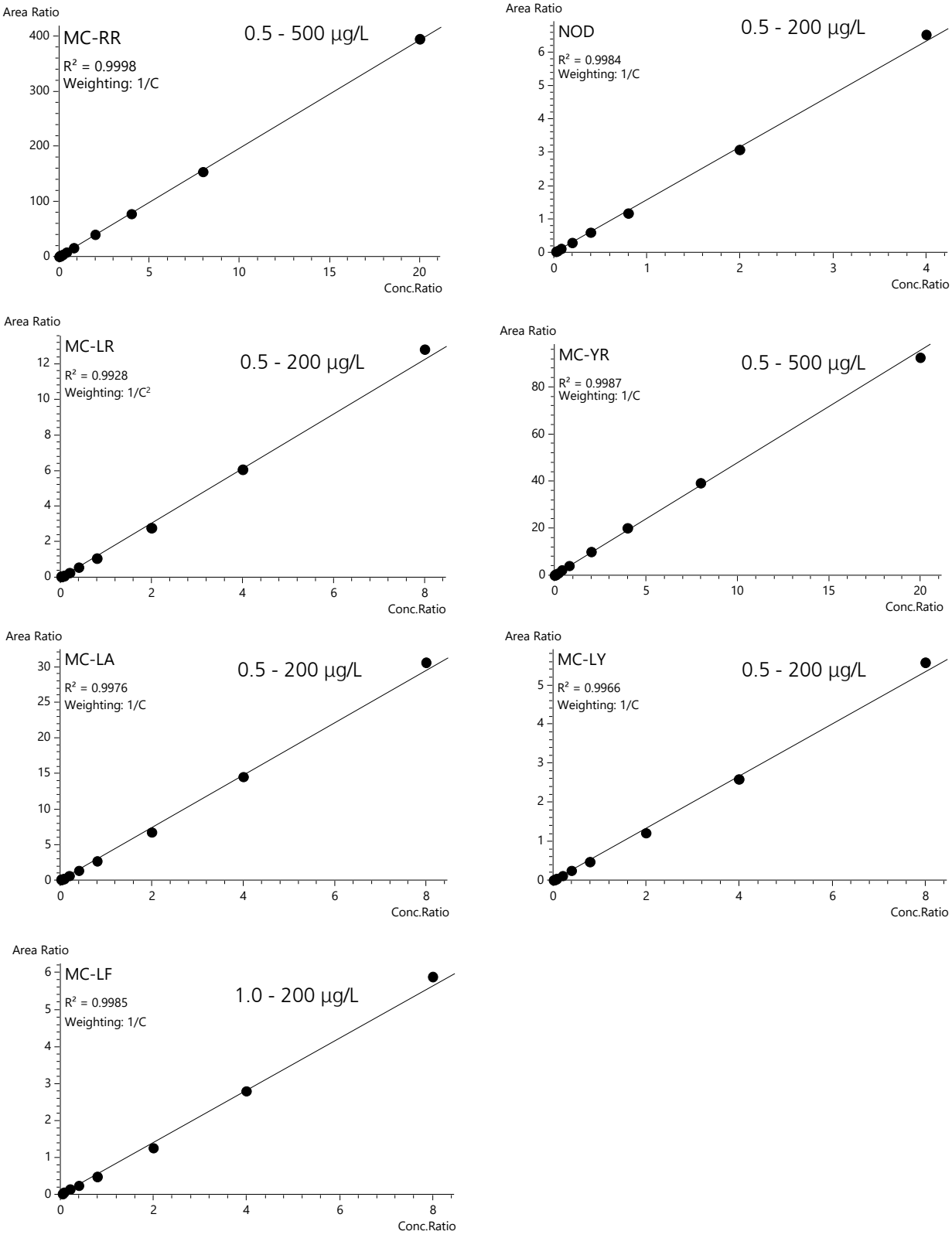


Figure 2: Calibration curves for microcystins and nodularin. Calibration range is 0.5 - 200 µg/L for MC-LR, MC-LA, MC-LY and NOD, 1.0 - 200 µg/L for MC-LF and 0.5 - 500 µg/L for MC-RR and MC-YR.

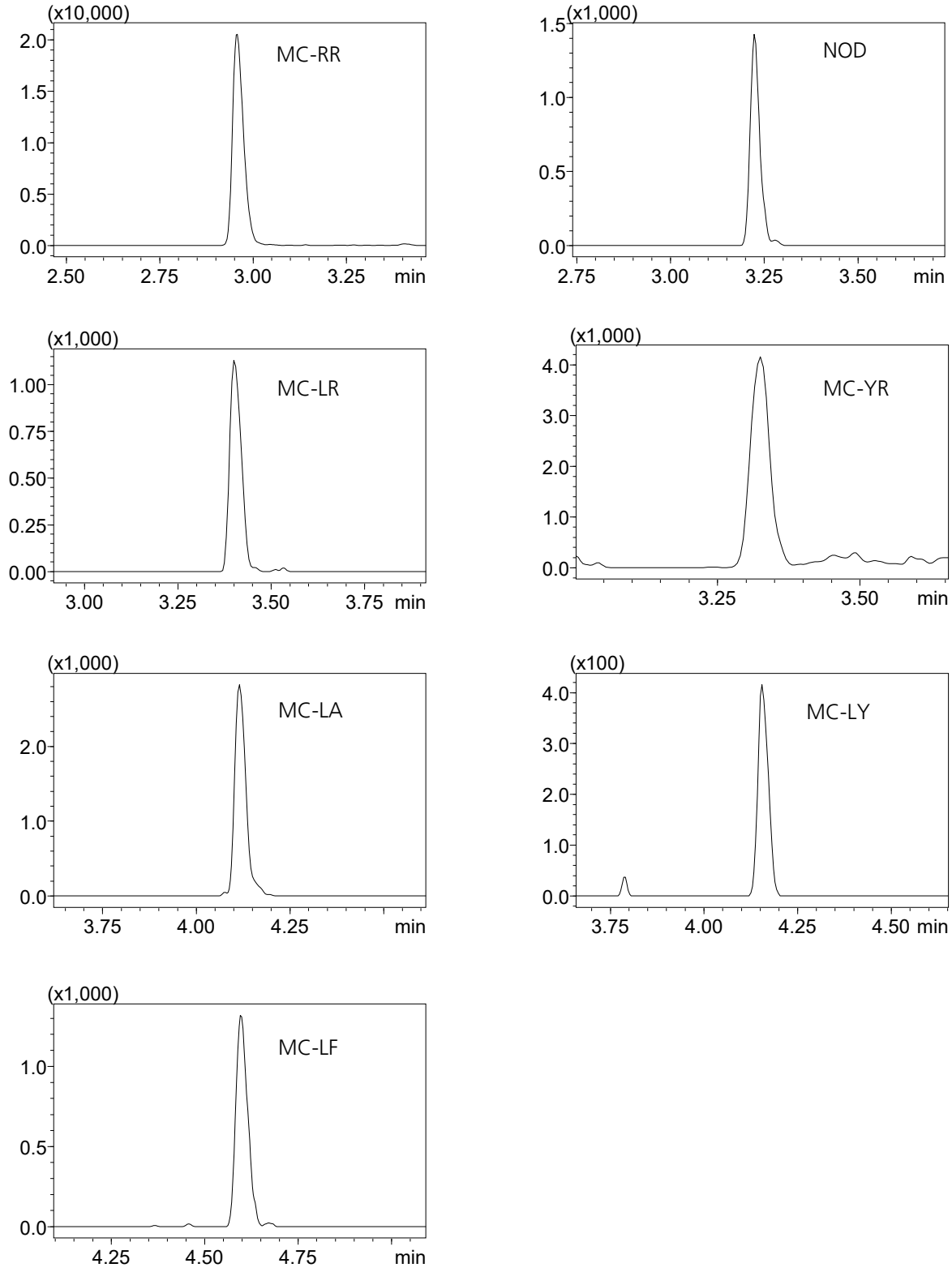


Figure 3: Representative chromatograms of the quantifier ions in LOQ injections.

Table 4: Precision and accuracy for the analysis of microcystins and nodularin in water samples

Replicate no.	Spiked concentration					
	8 µg/L		25 µg/L		80 µg/L	
	Conc. (µg/L)	Accuracy	Conc. (µg/L)	Accuracy	Conc. (µg/L)	Accuracy
			MC-RR			
1	8.3	103.4%	25.1	100.3%	83.5	104.4%
2	8.3	104.2%	23.5	94.1%	83.6	104.5%
3	7.7	95.8%	23.5	94.1%	78.2	97.7%
RSD% (Conc.)	4.6		3.7		5.6	
			NOD			
1	7.1	88.5%	23.3	93.4%	74.5	93.1%
2	7.4	93.1%	22.9	91.7%	75.9	94.9%
3	7.5	93.9%	23.8	95.2%	74.1	92.6%
RSD% (Conc.)	3.1		1.8		1.3	
			MC-LR			
1	7.5	94.0%	23.7	95.0%	77.4	96.8%
2	7.9	99.2%	23.9	95.6%	80.2	100.2%
3	7.3	91.1%	24.4	97.5%	80.1	100.1%
RSD% (Conc.)	4.4		1.4		2.0	
			MC-YR			
1	8.2	102.1%	26.4	105.8%	79.9	99.9%
2	8.5	106.1%	25.8	103.0%	81.1	101.3%
3	8.6	107.8%	26.5	106.0%	79.9	99.9%
RSD% (Conc.)	2.8		1.6		0.8	
			MC-LA			
1	7.5	93.8%	23.3818	93.5%	74.8	93.6%
2	7.2	90.4%	23.1467	92.6%	73.3	91.6%
3	7.6	94.7%	22.0432	88.2%	73.7	92.1%
RSD% (Conc.)	2.4		3.1		1.1	
			MC-LY			
1	7.2	90.6%	22.7	90.9%	70.0	87.5%
2	6.8	84.8%	21.9	87.6%	71.7	89.6%
3	7.3	91.0%	22.7	90.8%	74.2	92.7%
RSD% (Conc.)	3.9		2.1		2.9	
			MC-LF			
1	8.4	104.5%	25.7	102.9%	80.3	100.4%
2	8.3	103.1%	23.0	92.1%	81.4	101.8%
3	8.2	103.0%	25.2	100.8%	81.9	102.3%
RSD% (Conc.)	0.8		5.8		1.0	

■ Conclusion

In this application, a rapid LCMS method was successfully developed for the analysis of the six microcystins and nodularin in water, according to EPA 544. Chromatography of the seven cyanotoxins and internal standard was achieved in only 8 minutes using the Shim-pack Velox C18 column. A strong linear correlation was established across a broad calibration range, achieving an R^2 value exceeding 0.99. The applicability of the method was verified by spiking the six microcystins and nodularin into water samples at three spiking levels, and the results of accuracy were all within 80 - 120% with %RSD less than 10%.

This application demonstrates the sensitivity and reproducibility of the Shimadzu LCMS-8060NX in the analysis of microcystins and nodularin in water following EPA Method 544. Additionally, the demonstrated performance makes the LCMS-8060NX suitable for the analysis of microcystins and nodularin without the need of the sample preconcentration step required in EPA 544.

■ References

1. Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems [Online] https://www.epa.gov/sites/default/files/2014-08/documents/cyanobacteria_factsheet.pdf
2. Cyanobacterial toxins: Microcystins - Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. [Online] https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/microcystins-background-201223.pdf?sfvrsn=6d60aa6d_3
3. EPA Drinking Water Health Advisories for Cyanotoxins. [Online] https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins
4. Method 544. Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). [Online] https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=306953

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