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Rapid and highly sensitive quantitation of Microcystins and Nodularin in water by modern LC-MS/MS

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1. Introduction

- Cyanobacteria, commonly known as blue-green algae, and harmful algae blooms are proliferating in water bodies more frequently because of warmer temperatures and nutrient-rich environments across the world.
- They possess the potential to significantly impact water quality because they can produce cyanotoxins, such as microcystins (MCs) and nodularin (NOD). Exposure to cyanotoxins can lead to a range of adverse health effects in human and ecosystems.
- The World Health Organization and the Environmental Protection Agency (EPA) advises maintaining MCs in water in the low µg/L range. [1]
- 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 on a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer. [2]

Fig. 1: LCMS-8060NX

2. Methods

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.

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 and conditions are shown in Table 1.

Table 1: Gradient and MS conditions

Gradient Program	: 15% B (0 – 0.5 min) -> 90% B (5 – 6.5 min) -> 15% B (6.51 - 8 min)		
Interface	: ESI	**Heating Gas Flow**	: 10.0 L/min
Mode	: MRM	**Interface Temperature**	: 300 ℃
No. of MRM Transitions for Each Compound	: 2	**DL Temperature**	: 250 ℃
Polarity	: Positive	**Heat Block Temperature**	: 400 ℃
Nebulizing Gas Flow	: 3.0 L/min	**Drying Gas Flow**	: 10.0 L/min

3. Results

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 2. The overlapping MC-LA and MC-LY signals have distinct MRM transitions which ensures they can be identified and quantified by mass spectrometry.

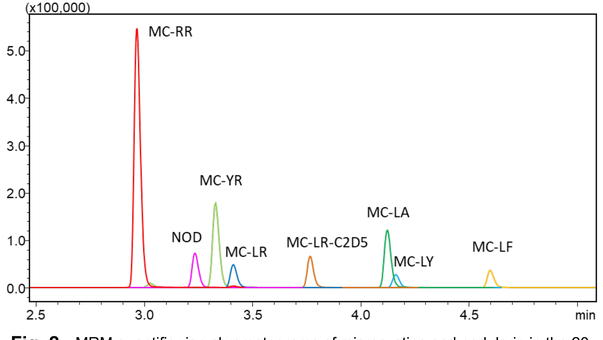

Fig. 2 MRM quantifier ion chromatograms of microcystins and nodularin in the 20 µg/L solvent calibration standard. The concentration of internal standard MC-LR-C2D5 is 25 µg/L.

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 3 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 (n=3) 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.

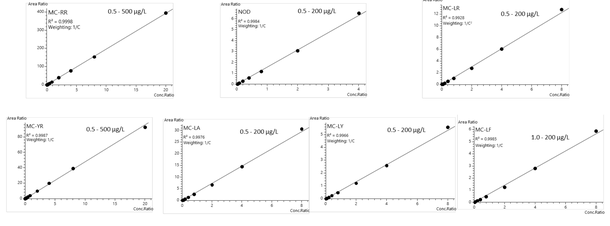

Fig. 3 Calibration curves for microcystins and nodularin.

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. Representative chromatograms of the quantifier ions in LOQ injections are shown in Figure 4.

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.

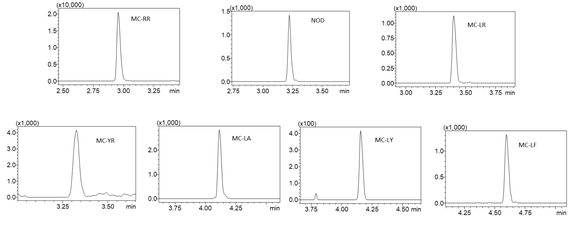

Fig. 4 Representative chromatograms of the quantifier ions in LOQ injections.

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. No carryover was observed in the blank injections during or at the end of the batch.

4. Conclusion

- A rapid 8-min LCMS method was successfully developed for the analysis of the six microcystins and nodularin in water, according to EPA 544.
- A strong linear correlation was established across a broad calibration range, achieving an R^2 value exceeding 0.99.
- 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.

Reference
1) Cyanobacterial toxins: microcystins. WHO/SDE/WSH/03.04/57
2) Method 544. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=306953

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