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Rapid, high throughput quantitation of cyanotoxins in natural water by UHPLC-MS-MS

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Tom Russell[1]; Deborah Schordock[2]
[1]Shimadzu Scientific Instruments, Columbia, MD;
[2]North East Ohio Regional Sewer District (NEORSD), Cuyahoga Heights, OH

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Introduction

Cyanotoxins are toxins produced by bacteria called cyanobacteria (also known as blue-green algae). Cyanobacteria are found in lakes and in the ocean where they can reproduce exponentially to form blooms. The most prevalent cyanotoxin is the hepatotoxin microcystin and it is a problem worldwide in freshwater ecosystems. The World Health Organization has set the guideline for permissible amount of the microcystin LR in drinking water at 1ug/L. The National Center for Environmental Assessment suggests a limit of 0.1 µg/L. Current EPA Method 544 requires solid phase extraction of 500 mL of sample, evaporation and reconstitution in 1 mL of methanol.

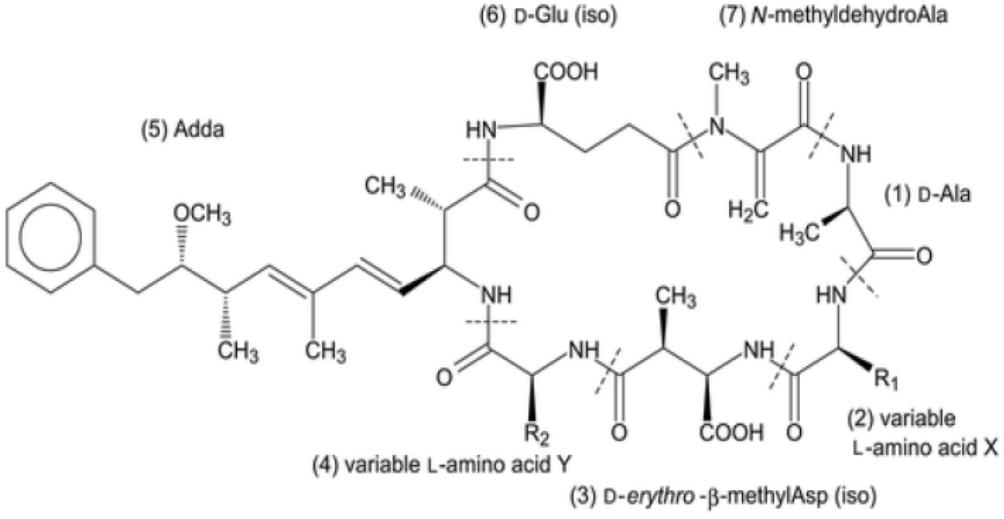
This work demonstrates a quick screening method without the use of sample fortification that greatly reduces sample prep time and meets detection requirements.

Method

- 2 MRM transitions were optimized each for seven microcystin compounds using flow injection analysis on the Shimadzu LCMS-8050.
- Chromatography and calibration curves were optimized using a C8 column with the Shimadzu Nexera UHPLC system coupled to the 8050.
- Seven-point calibration curves were generated with 7 µL injections of standards prepared in methanol/water. Total run time 8 minutes.
- Lake Erie samples were spiked at low and high concentrations, syringe filtered, and analyzed.
- All samples were spiked with a surrogate at 20 ng/mL.

General Structure of Microcystin

Microcystins are cyclic peptides containing 7 amino acids.
They are the most abundant cyanotoxin, comprising over 80 analogs.



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Example Chromatograms and Graphs

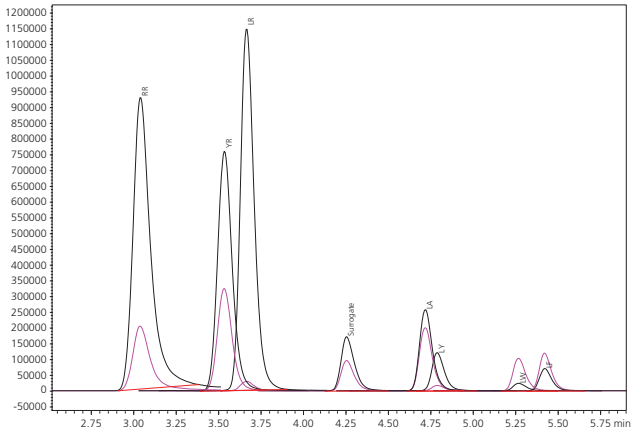

Figure 1. TIC of 100 ng/mL standard displaying target and reference ions for all compounds.

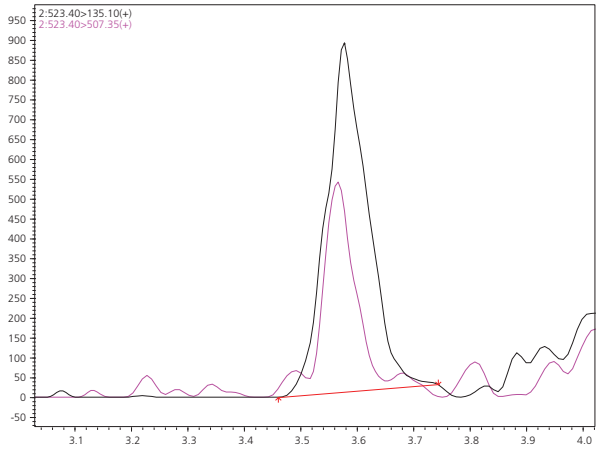

Figure 2. Quant ion chromatogram of MC-YR. 0.1 ng/mL

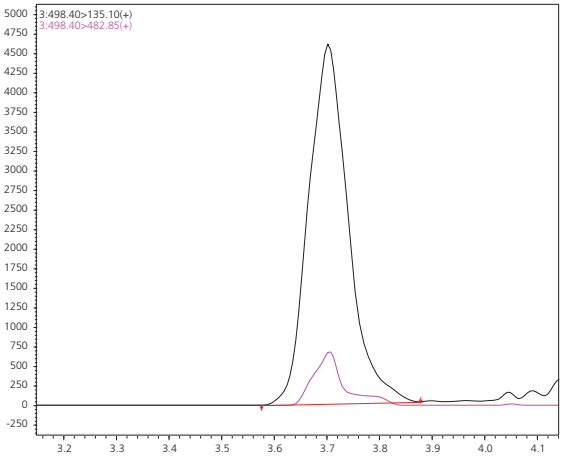

Figure 3. Quant ion chromatogram of MC-LR. 0.1 ng/mL

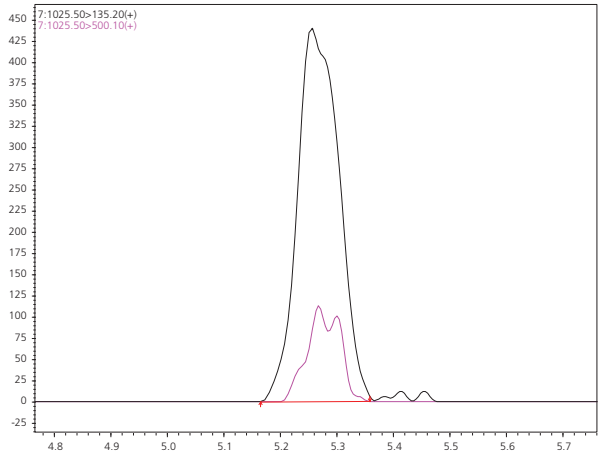

Figure 4. Quant ion chromatogram of MC-LW. 0.5 ng/mL

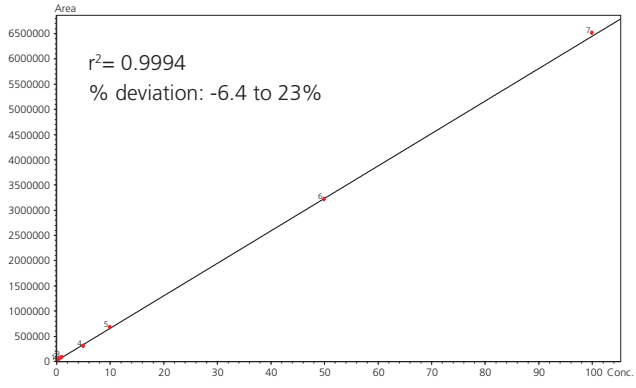

Figure 5. Calibration curve for MC-LR. 0.1 to 100 ng/mL

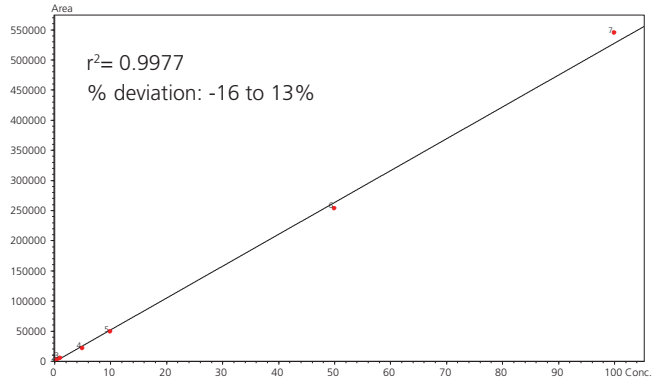

Figure 6. Calibration curve for MC-LW. 0.5 to 100 ng/mL

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Data Summary

Table 1. Data summary

Microcystin	Quant MRM	Cal range	r^2	Lake Erie Spl (ng/mL)		Lake Erie Spl (ng/mL)	
		ng/mL		Spike	Calc amt	Spike	Calc amt
RR	519.90>135.15	0.1 - 100	0.9915	1	0.937	50	49.3
YR	523.40>135.10	0.1 - 100	0.9993	1	1.012	50	48.2
LR	498.40>135.10	0.1 - 100	0.9994	1	0.993	50	48.3
LA	910.40>776.25	0.1 - 100	0.9977	1	0.951	50	45.6
LY	1002.50>135.25	0.5 - 100	0.9969	1	0.913	50	45.6
LW	1025.50>135.20	0.5 - 100	0.9979	1	0.894	50	45.4
LF	986.50>478.30	0.5 - 100	0.9985	1	0.943	50	45.4

Table 2. 20 ppb surrogate spike in Lake Erie samples. 12 replicates.

Surrogate	Quant MRM	Area Ct %RSD
MC-LR D5	514.90>135.25	2.49

Summary and Further Development

- The Nexera HPLC and LCMS-8050 achieved adequate sensitivity without sample enrichment. This screening method can be a valuable tool for quick turn-around data regarding the quality of drinking water sources.
- Optional column chemistry will be investigated to improve run time.
- Sensitivity may still be improved for some analytes. Microcystin ionization efficiency is greatly effected by sample solvent and mobile phase composition. Source and CE optimization could be profiled using mobile phase ratio at time of elution.

Acknowledgements

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