Quantitative Analysis of Microcystin Cyclopeptides in Natural Water Using Ultra-Fast Triple Quadrupole LCMS-8050

Rachel Lieberman[1], William Lipps[1], Jeremy Post[1], Tom Russell[1], Deborah Schordock[2]

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

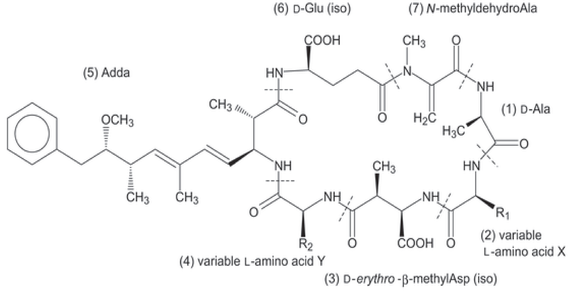
Introduction

Cyanobacteria found in eutrophic or hypereutrophic freshwater may accumulate in surface water supplies as “blooms” or “scums”. Some species of cyanobacteria produce toxins, one of which are hepatoxins called microcystins. At least 50 various types of microcystins are known, and several of these may be produced during a bloom. The chemical structure of microcystins includes two variable amino acids and an unusual aromatic amino acid, ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenylideca-4,6-diienoic acid). Different microcystins have different lipophilicities and polarities, which could affect their toxicity. The World Health Organization has set the guideline for permissible amount of the microcystin LR in drinking water at 1 µg/mL. The National Center for Environmental Assessment suggests a limit of 0.1 µg/L. Accurate, sensitive characterization and quantitation of seven different microcystin cyclopeptides is demonstrated. In this anaylsis, microcystins-RR, YR, LR, LA, LY. LW and LF were measured using ultra high pressure liquid chromatography (UHPLC) with an LCMS-8050 triple quadrupole mass spectrometry detector.

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 7uL 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 20ng/mL.

General structure of microcystin



Microcystins are cyclic peptides containing 7 amino acids.

They are the most abundant cyanotoxin, comprising over 80 analogs.

Example chromatograms and graphs

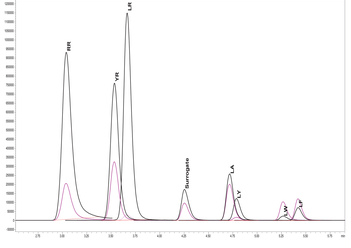


Figure 1. TIC of 100ng/mL standard displaying target and reference ions for all compounds.

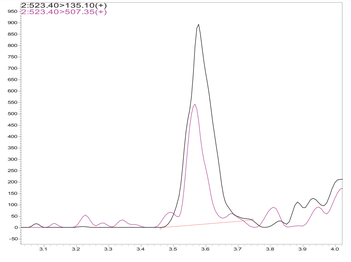


Figure 2. Quant ion chromatogram of MC-YR. 0.1ng/mL

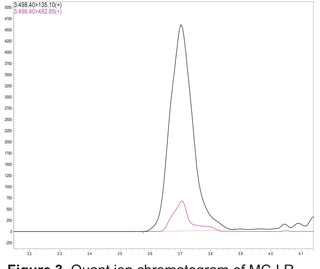


Figure 3. Quant ion chromatogram of MC-LR. 0.1ng/mL

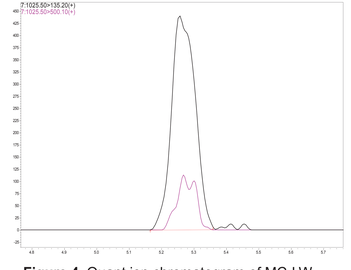


Figure 4. Quant ion chromatogram of MC-LW. 0.5ng/mL

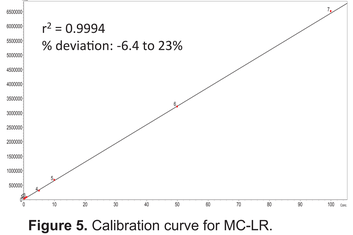


Figure 5. Calibration curve for MC-LR. 0.1 to 100ng/mL

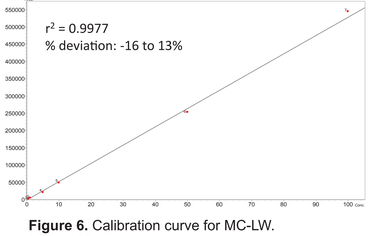


Figure 6. Calibration curve for MC-LW. 0.5 to 100ng/mL

Data summary

Microcystin	Quant MRM	Cal range ng/mL	r^2	Lake Erie Spl (ng/mL)		Lake Erie Spl (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 1. Data summary

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

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

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

Shimadzu would like to thank NEORSD for their time and effort in obtaining this data at their laboratory.