Comparative Analysis of ELISA & MALDI-TOF Mass Spectrometry Methods for Microcystins in Freshwater Samples

Kimberly Kadlec[1], Andrew Turczyn[1], Jennifer E. Grant[2], Ph.D.

1. The Applied Science Program, University of Wisconsin at Stout, Menomonie, WI 54751 2. The Biology Department, University of Wisconsin at Stout, Menomonie WI 54751

Introduction

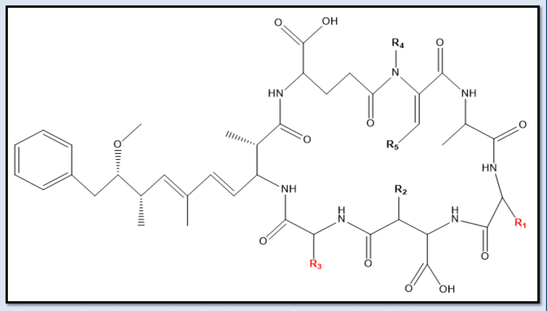

Figure 1. *Chemical drawing of microcystin. Congener identification and toxicity is based on the amino acids at the R_1 and R_3 sites (in red).*

The eutrophication of cyanobacterial blooms in freshwater and marine systems releases a collection of microcystin congeners – monocyclic heptapeptides that pose a serious health threat in both animals and humans, known to cause acute and chronic hepatotoxicity upon ingestion[1].

Congeners differ in toxicity levels based on the amino acids present at two locations of the shared structure, R_1 and R_3 (***Figure 1***). The side chain off the large cyclic structure – the ADDA complex, is present not only in microcystins, but also in other known cyanobacterial organisms such as nodularins[2].

In this study, two methods of microcystin (MC) analysis – Enzyme-Linked Immunosorbant Assay (ELISA) and Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF MS), are compared to assess the benefits and drawbacks of each for the routine screening of soluble MC levels in the local watershed. Primary concerns are rapid sample preparation and results procured in a cost-effective manner. ELISA results indicate the presence of MC at the height of cyanobacterial blooms, but in greater concentrations than indicated by MALDI-TOF MS – which also indicated specific MC congeners at their individual specific concentrations.

The study demonstrates that ELISA is a simple analysis method producing a rough estimate of MC concentration to identify if the MC levels are within World Health Organization guidelines (1.0 µg/L)[3], whereas MALDI-TOF provides greater detail through congener identification in a specific system, but requires a trained analyst to obtain the spectra.

Materials & Methods

Near-shore lake water samples (50 mL) were collected from two separate locations on Lake Menomin – Lakeside Park (LS) and Wolske Bay (WB) during the first week of June, July and August for three consecutive years (2012-2014). The samples were centrifuged (10,000 g, 5-20 min) to remove insoluble material, separated into 1 mL aliquots, concentrated using vacuum evaporation to 10.0 µL and stored at -20 °C for future analysis.

ELISA was performed using Microcystins (ADDA)-DM ELISA Microtiter Plate, following the manufacturer's protocol (Abraxis Inc.)[4]. Colormetric analysis for absorbance was measured at 450 nm on a BioTek Epoch Microplate spectrophotometer.

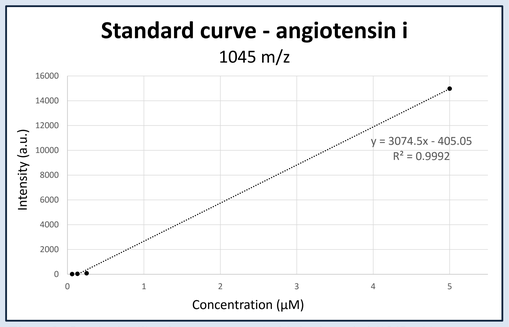

Figure 2. *Standard calibration curve created with Angiotensin i (1045 m/z) at concentrations 0.06 µM, 0.13 µM, 0.25 µM, and 5.0 µM.*

Samples were directly applied to a MALDI-TOF stainless steel plate using the dried-droplet method[5], followed by a matrix comprised of a saturated solution of α-cyano-4-hydroxycinnamic acid in 70% acetonitrile, including 0.1% TFA. Mass spectra were collected using a Bruker Microflex Linear Time of Flight Mass Spectrometer over a range of 300 to 2000 Daltons (Da).

A standard calibration curve was created using a peptide of known mass (angiotensin I - ***Figure 2***), using concentrations ranging from 0.06 to 5.0 µM range. Within the linear region, this standard curve had an R^2 value of 0.999

ELISA Results

With a detection limit of 0.1 µg/L, MC was detected in the months of July and August, as well as June 2013, all three years in both locations (***Figure 3***). The ELISA kit did not measure MC for the month of May, in either location for any of the three years. Several samples provided concentrations higher than 5.0 µg/L, greater than the highest standard (5.0 µg/L) – all concentration values above that are rough estimates. The Wolske Bay location routinely showed higher concentrations of MC than the Lakeside Park location.

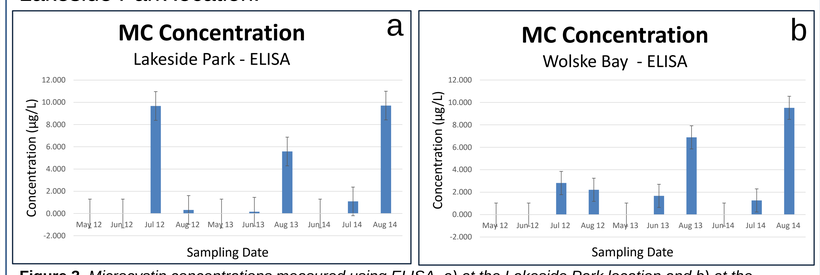

Figure 3. *Microcystin concentrations measured using ELISA, a) at the Lakeside Park location and b) at the Wolske Bay location.*

MALDI-TOF MS Results

Individual spectra were collected for each sample. A total of 14 MC congeners were visualized: MC-Laba, -LR, [D-Asp3,ADMAdda5]MCYST-LR, [Dha7]MCYST-FR, [L-Ser] MC-HtyR, L-Ser] MC-E(OMe)E(OMe), [D-Asp] MC-RR, -WR, -LW, -FR, -RR, [D-Ser, ADMAdda] MC-LR, [L-Ser] MC-RR, MC-(H4)YR. The most frequently observed congeners was MC-Laba (*12 out of 20 times*). MC-LR was observed three times: July 2012 (LS) at 0.34 µg/L, August 2014 (LS) at 0.85 µg/L and August 2014 (WB) at 1.08 µg/L. MC concentrations ranged from 0.22-1.34 µg/L for any one congener.

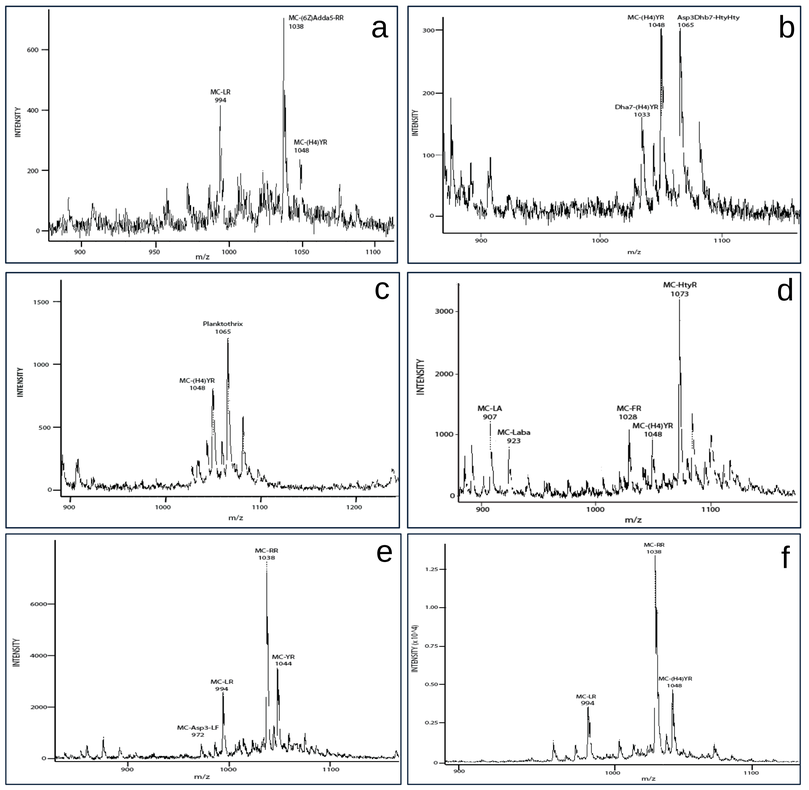

Figure 4. *MALDI-TOF mass spectrum of microcystin congeners, comparing the Lakeside Park and Wolske Bay locations. a.) Lakeside Park, Jul 2012 indicating MC-LR at 994 m/z, -(6Z)Adda5-RR at 1038 m/z and –(H4)YR at 1048 m/z, b.) Lakeside, Aug 2012 indicating MC-Dha7-(H4)YR at 1033 m/z, -(H4)YR at 1048 m/z and Asp3Dhb7-HtyHty at 1065 m/z, c.) Lakeside Park, Aug 2013 indicating MC-(H4)YR at 1048 and Planktothrix at 1065 m/z, d.) Wolske Bay, Aug 2013 indicating MC-LA at 907 m/z, -Laba at 923 m/z, -FR at 1028 m/z, -(H4)YR at 1048 m/z and –HtyR at 1073 m/z, e.) Lakeside Park, Aug 2014 indicating MC-Asp3-LF at 972 m/z, -LR at 994 m/z, -RR at 1038 m/z and –YR at 1044 m/z, f.) Wolske Bay, Aug 2014 indicating MC-LR at 944 m/z, -RR at 1038 and –(H4)YR at 1048 m/z.*

Conclusions

The results between the two forms of analysis show general agreement in the MC concentration levels (***Figure 5***).

ELISA: Samples analyzed with ELISA primarily fell within the range of the provided standards, though four samples indicated MC concentration levels greater than the highest standard value (5.0 µg/L). More often than not, ELISA indicated higher concentrations of MC than the MALDI-TOF MS cumulative MC concentrations (*5 out of 7 times*). Several times ELISA indicated MC concentration at < 0.10 µg/L, with the MALDI-TOF MC concentrations (cumulative) indicated > 0.10 µg/L (*8 out of 20 times*). The large disparity between high levels of MC with ELISA and lower levels of MC with MALDI-TOF MS can possibly be attributed to nodularins, another cyanobacterial species also possessing the –ADDA complex. It can be argued that the elevated ELISA measurements could be due to nodularins, providing a misleading result.

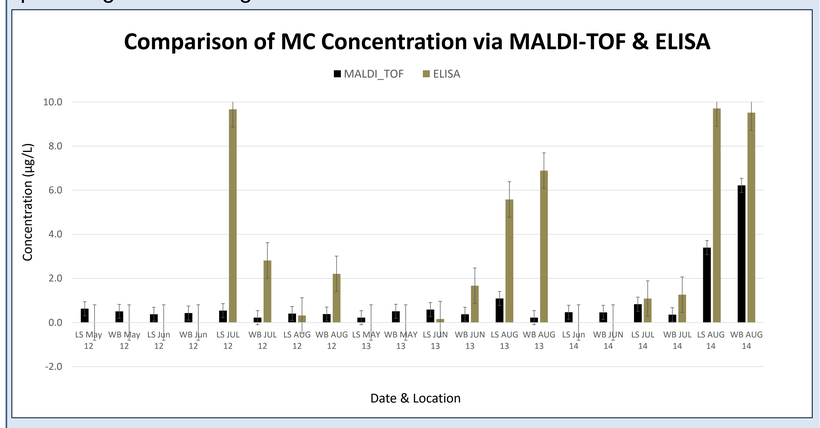

Figure 5. *A comparison of microcystin concentrations between ELISA and MALDI-TOF MS analysis*

MALDI-TOF MS:

MALDI-TOF MS analysis allows visualization of individual MCs. However, this is also a disadvantage since individual MC congener measurements must be combined to compute a total MC content value.

The World Health Organization has set an acceptable concentration limit for MC-LR at 1.0 µg/L, which was not reached in all but one instance. However, as each congener possesses a different toxicity level – the combined MC concentration is only an estimation of total toxicity rather than a definitive value, except in instances where MC-LR is specifically, and solely, being targeted for analysis.

Each of these methods of analysis possess inherent strengths and weaknesses which must be understood and considered in order to be correctly applied and the results properly disseminated.

Future Directions

- Continued comparison of analysis to include with HPLC-MS
- Continued annual catalogue of indigenous microcystin congeners in Lake Menomin.

Acknowledgments

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