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Determination of Microcystin-LR in Municipal Water Using HPLC-UV/Vis

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Abstract

Algae is a natural and important component of freshwater ecosystems, but blue-green algae, also known as cyanobacteria, release toxins called microcystin (MC) when they die or are lysed, posing substantial health concerns. In recent years, these toxic algae blooms have formed on western Lake Erie and have threatened drinking water supplies. Globally there are multiple variants of MC found in drinking water; however, MC-LR, a cyclic heptapeptide defined by its leucine (L) and arginine (R) constituents, is the most harmful variant in regards to its acute toxicity. The World Health Organization established a maximum contaminant level of 1 microgram/liter for MC-LR in drinking water. Since local municipal water treatment facilities source water from Lake Erie, it is important to monitor MC-LR throughout the treatment process. The standard method for detecting MC-LR is the ELISA assay, which has been shown to have unreliable identification, often leading to false positives. This research aims to develop a highly sensitive protocol for our local municipal water provider which uses high performance liquid chromatography (HPLC) with ultra violet-visible spectroscopy (UV/Vis) to detect and quantify MC-LR. Water samples will be acquired from the water treatment facility at various stages of the purification process. These samples will be lysed in order to release all MC from the algal blooms, purified by solid-phase extraction, and detected using HPLC-UV/Vis. The results from this protocol will be compared to data acquired from the ELISA assay. The development of a rapid, reliable, and relatively inexpensive protocol for detecting MC-LR could be applied to multiple municipal water providers in communities whose water may be at risk of contamination from toxic algae blooms.

Keywords: microcystin-LR, HPLC, cyanobacteria

1. Introduction

Western Lake Erie (near Toledo, Ohio) is the site of an August 2014 blue-green algae (cyanobacteria) bloom. This bloom led to entry of the freshwater cyanobacteria-produced toxin microcystin (MC) into the municipal water supply[1,2] and institution of "do-not-drink" orders in response. Both the World Health Organization and United States Environmental Protection Agency identify microcystin variants as a serious health risk. MC is comprised of a cyclic heptapeptide ranging from 800-1,100 Da.[3] The MC-LR variant (Figure 1), defined by its leucine (L) and arginine amino acid constituents, is the most harmful variant.[4] The maximum allowable concentration of MC-LR in drinking water is 1 μg/L as established by the World Health Organization (WHO).[5] The principal route of acute poisoning is via ingestion; however, absorption through skin can also lead to health issues.[6] MC-LR inhibit enzymatic function of protein serine/threonine phosphatases, which can lead to skin irritation, blistering of the mouth, liver damage, and cancer.[4-7]

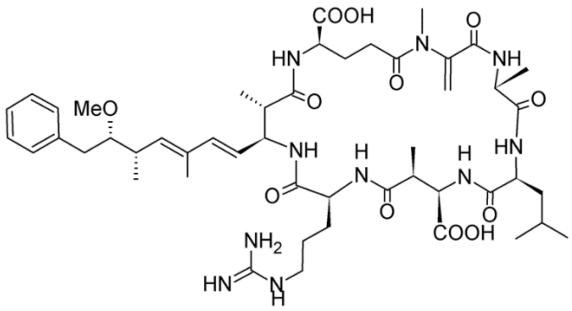

Figure 1. Chemical structure of the variant MC-LR

Municipalities using water sources susceptible to blue-green algae blooms risk having municipal drinking water with potentially harmful levels of cyanobacterial toxins (MC has been detected in bodies of water in nearly every state in the United States). Monitoring MC levels in municipal water at all stages of the treatment process is imperative to confirming that drinking water is safe to consume, and that the most effective means for degrading cyanotoxins are being employed.

Current MC research efforts have primarily focused on understanding how MC toxins impact human health[3,7,12-15] and the development of improved techniques for detecting MC in water sources.[18-11, 16-19] Recent work has also assessed the efficiency of MC degradation at each stage of water treatment to evaluate treatment strategies needed to minimize deleterious health effects associated with cyanobacterial toxins in treated water.[20-23]

This work aims to develop a relatively simple, sensitive, and economical analytical method for eventually confirming and quantifying MC in municipal water. This method uses high performance liquid chromatography (HPLC) with UV detector, which is more reliable than the commonly used enzyme-linked immunosorbent assay (ELISA) that has been shown to give unreliable identification, often leading to false positives.

2. Methodology

2.1 Materials

Solvents (acetonitrile, water, and trifluoroacetic acid) were HPLC grade and used as received from Sigma-Aldrich Company. MC-LR standard (10 μg/mL in methanol) was obtained from Abraxis, Inc. Dilutions (0.25, 0.5, 1, 2, 4, 6, and 8 μg/mL) were prepared by diluting the standard solution with distilled water.

2.2 Instrumentation

The HPLC-UV/Vis system used was a Waters 1525 Binary HPLC pump coupled with a Waters 2487 Dual λ Absorbance Detector set at 238 nm. Separations were obtained with an Acclaim™ Polar Advantage II Liquid Chromatography column (3 μm particle size, 3.0 mm × 150 mm dimensions) from ThermoFisher Scientific. Both the organic, acetonitrile, and aqueous, water, mobile phases contained 0.05% trifluoroacetic acid. The sample injection volume was 20 μL. The flow rate was 0.70 mL/min. The column temperature was maintained at 30°C. A 20% (v/v) acetonitrile to 60% (v/v) acetonitrile gradient was applied over six minutes.

3. Data

To obtain the best resolution of MC-LR using HPLC-UV/Vis, several different mobile phase combination were evaluated including with and without solvent gradient. A 20% (v/v) acetonitrile to 60% (v/v) acetonitrile gradient (with all solvent mixtures containing 0.05% trifluoroacetic acid) applied over six minutes gave the best resolution of MC-LR.

In the HPLC-UV/Vis method developed, MC-LR was detected in ~6 min (Figure 2 and Table 1). The method was found to be suitable for detecting MC-LR in aqueous samples.

A calibration curve for MC-LR was constructed in the range of 0.025 – 10 µg/mL. The peak areas from the chromatogram were measured (using the Waters Breeze software) and plotted *versus* concentration. Each standard solution was run in triplicate. Data were linear over the entire concentration range studied with a coefficient of determination (R^2) of 0.998. Figures 3 shows the calibration curve for MC-LR.

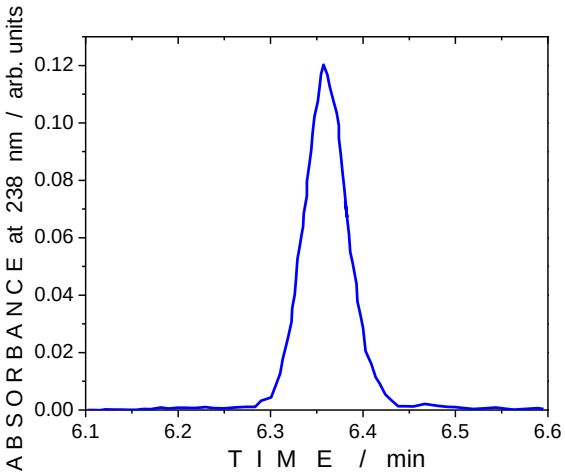

Figure 2. Representative HPLC chromatogram of MC-LR standard solution.

Table 1. Average Retention Times for Microcystin-LR

Concentration (µg/mL)	Retention Time (min)
0.25	6.238
0.5	6.384
1	5.995
2	6.225
4	5.570
6	5.473
8	6.117
10	6.315

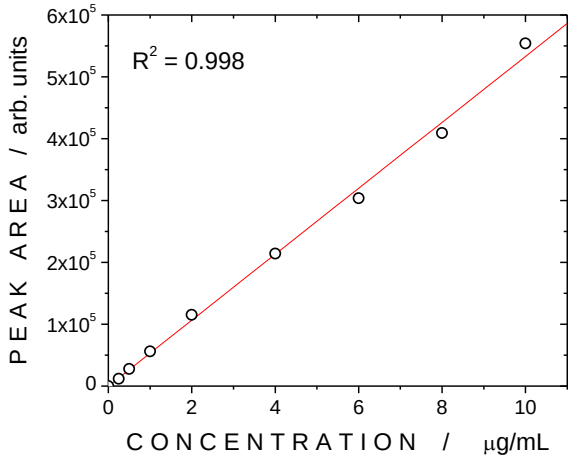

Figure 3. Calibration curve for MC-LR standards.

With the calibration curve for MC-LR generated, water samples acquired from a municipal water treatment facility can be analyzed (after appropriate processing) using the aforementioned HPLC-UV/Vis method to both detect and quantify MC-LR.

4. Conclusion

An HPLC-UV/Vis method has been developed that can quickly confirm (~6 min) the presence of MC-LR in aqueous samples. A linear calibration curve was obtained for MC-LR in the concentration range of 0.25 to 10 μg/L. Separation and suitable resolution in MC-LR analysis was achieved with the solvent combination described. The relatively fast, sensitive, and inexpensive analytical method for detecting MC-LR, described here, may be of interest to municipal water providers, concerned with and at risk of potential blue-green algae blooms.

5. Future Work

The HPLC-UV/Vis method developed in this current work will be repeated to develop calibration curves for others of the ~140 microcystin variants. A method for preparation of raw municipal water samples are needed to include lysing, to release MC from the blue-green algae in water samples, and purification, most likely by solid-phase extraction before analysis using the HPLC-UV/Vis method. Results from the method described here will be compared to data acquired from the ELISA assay to further confirm the suitability of the liquid chromatography method.

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7. References

1. Qian, S.S., et al., *Quantifying and Reducing Uncertainty in Estimated Microcystin Concentrations from the ELISA Method.* Environmental Science & Technology, 2015. **49**(24): p. 14221-14229.
2. Hu, C., et al., *Relative importance of Microcystis abundance and diversity in determining microcystin dynamics in Lake Erie coastal wetland and downstream beach water.* Journal of Applied Microbiology, 2016. **120**(1): p. 138-151.
3. Rastogi, R.P., R.P. Sinha, and A. Incharoensakdi, *The cyanotoxin-microcystins: current overview.* Reviews in Environmental Science and Bio-Technology, 2014. **13**(2): p. 215-249.
4. *Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins*. 2015, U.S. EPA (United States Environmental Protection Agency): Washington, DC.
5. Chorus, I. and J. Bartram, *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. 1999, London, United Kingdom: World Health Organization (WHO).
6. Pekar, H., et al., *Fast, rugged and sensitive ultra high pressure liquid chromatography tandem mass spectrometry method for analysis of cyanotoxins in raw water and drinking water-First findings of anatoxins, cylindrospermopsins and microcystin variants in Swedish source waters and infiltration ponds.* Journal of Chromatography A, 2016. **1429**: p. 265-276.
7. Maidana, M., et al., *Effects of microcystins over short- and long-term memory and oxidative stress generation in hippocampus of rats.* Chemico-Biological Interactions, 2006. **159**(3): p. 223-234.
8. Foss, A.J. and M.T. Aubel, *Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS).* Toxicon, 2015. **104**: p. 91-101.

9. Mekebri, A., G.J. Blondina, and D.B. Crane, *Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry.* Journal of Chromatography A, 2009. **1216**(15): p. 3147-3155.
10. Pacheco, A.B.F., I.A. Guedes, and S. Azevedo, *Is qPCR a Reliable Indicator of Cyanotoxin Risk in Freshwater?* Toxins, 2016. **8**(6): p. 26.
11. Vilarino, N., et al., *Innovative detection methods for aquatic algal toxins and their presence in the food chain.* Analytical and Bioanalytical Chemistry, 2013. **405**(24): p. 7719-7732.
12. Li, X.B., et al., *Alterations in neurobehaviors and inflammation in hippocampus of rats induced by oral administration of microcystin-LR.* Environmental Science and Pollution Research, 2014. **21**(21): p. 12419-12425.
13. Li, X.B., et al., *Maternal Repeated Oral Exposure to Microcystin-LR Affects Neurobehaaviors in Developing Rats.* Environmental Toxicology and Chemistry, 2015. **34**(1): p. 64-69.
14. Yuan, G.L., et al., *In vivo studies on the immunotoxic effects of microcystins on rabbit.* Environmental Toxicology, 2012. **27**(2): p. 83-89.
15. Zhou, L., H. Yu, and K. Chen, *Relationship between microcystin in drinking water and colorectal cancer.* Biomedical and Environmental Sciences, 2002. **15**(2): p. 166-171.
16. de Andrade, F.M., A.N. de Macedo, and E.M. Vieira, *Determination of Microcystin-LR in Cyanobacterial Blooms from the Mogi Guacu River (Brazil) by High-Performanc Liquid Chromatography.* Journal of Liquid Chromatography & Related Technologies, 2014. **37**(9): p. 1310-1319.
17. Marion, J.W., et al., *In Vivo Phycocyanin Flourometry as a Potential Rapid Screening Tool for Predicting Elevated Microcystin Concentrations at Eutrophic Lakes.* Environmental Science & Technology, 2012. **46**(8): p. 4523-4531.
18. Richardson, S.D. and S.Y. Kimura, *Water Analysis: Emerging Contaminants and Current Issues.* Analytical Chemistry, 2016. **88**(1): p. 546-582.
19. Jiang-qi, Q., et al., *Optimization of Microcystin Extraction for Their Subsequent Analysis by HPLC-MS/MS Method in Urban Lake Water.* International Journal of Environmental Science and Development, 2013. **4**(5): p. 600-603.
20. Ding, J., et al., *Release and Removal of Microcystins from Microcystis during Oxidative-, Physical-, and UV-Based Disinfection.* Journal of Environmental Engineering-Asce, 2010. **136**(1): p. 2-11.
21. Jurczak, T., et al., *Elimination of microcystins by water treatment processes - examples from Sulejow Reservoir, Poland.* Water Research, 2005. **39**(11): p. 2394-2406.
22. Pestana, C.J., et al., *Fate of cyanobacteria in drinking water treatment plant lagoon supernatant and sludge.* Science of the Total Environment, 2016. **565**: p. 1192-1200.
23. Mohamed, Z.A., et al., *Occurrence of cyanobacteria and microcystin toxins in raw and treated waters of the Nile River, Egypt: implication for water treatment and human health.* Environmental Science and Pollution Research, 2015. **22**(15): p. 11716-11727.