APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Identification of Microcystins as Markers of Algal Bloom Contamination in Surface Waters by UHPLC/ESI/TOF MS

Introduction

Contamination of surface waters by algal blooms has emerged as a public health issue. Among the types of algae that can form algal blooms, photosynthetic cyanobacteria (also known as blue-green algae) can be particularly toxic, and the level of toxicity can change very quickly. Issues with taste and odor with potable water can also occur[1].

The dynamic status of blooms and their concomitant public health issues imply that their monitoring requires analytical methodologies to determine levels of chemical markers[3]. This application note describes the use of ultra-high performance liquid chromatography (UHPLC) coupled to Time-Of-Flight mass spectrometry (TOF/MS) to monitor the chemical markers of these particular algal blooms, known as microcystins. TOF/MS is well-suited to this task, because of its ability to perform targeted and non-targeted analysis in the same experiment, which allows measurement of expected contaminant compounds, and the detection of previously unsuspected relevant chemical species[2,4].

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The general structure of a representative microcystin is shown in Figure 1, while Table 1 lists the formulae and calculated ion mass values for the microcystins studied in this report.

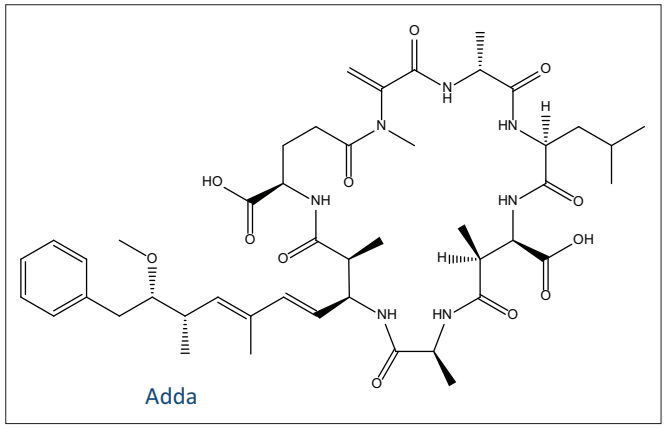

Figure 1. Example microcystin structure (LA) with Adda moiety labeled.

Table 1. Formula and calculated *m/z* for microcystins examined in this study.

Microcystin	Formula	Calculated *m/z*
LR	$C_{49}H_{74}N_{10}O_{12}$	995.5560
RR	$C_{49}H_{75}N_{13}O_{12}$	519.7902 *
YR	$C_{52}H_{72}N_{10}O_{13}$	1045.5353
LW	$C_{54}H_{72}N_8O_{12}$	1025.5342
LF	$C_{52}H_{71}N_7O_{12}$	986.5233
LA	$C_{46}H_{67}N_7O_{12}$	910.4920

*RR detected as $[M + 2H]^{2+}$ ion, all other analytes detected as $[M + H]^+$.

These peptide-derived structures are amenable to electrospray mass spectrometry, which provides a convenient tool for their measurement when coupled with UHPLC.

Experimental

Sample Collection. Two sources of contaminated public surface waters were collected as "grab samples" and shipped to Wayne State University (Detroit, MI) for processing. Both samples were drawn in the U.S.; one from the West and the other from the Midwest.

Samples were stored in 2 Liter containers in a laboratory freezer. These containers were subject to three freeze-thaw cycles prior to analysis. 10 mL of surface water samples were transferred to 15 mL centrifuge tubes and centrifuged at 3,000 rpm for 20 min. The supernatant was filtered with 0.45 µm cellulose acetate membrane filters. 1 mL of the filtrate was shipped to PerkinElmer (now in Downers Grove, IL) for analysis. Mixed analyte standard solutions were provided by Wayne State University and Lake Superior State University. Samples were tested immediately upon receipt.

Chromatography. For all chromatographic separations, a PerkinElmer Flexar™ FX-15 UHPLC pumping system was used. Column equilibration time was three minutes. The system configuration and operational parameters are shown in Table 2.

Table 2. UHPLC Parameters.

Column	Brownlee™ SPP C18 2.1 x 100 mm, 2.7 µM (Part # N9308410)
Mobile Phase	Solvent A: 0.1% formic acid in water Solvent B: 0.1% formic acid in acetonitrile
Oven Temp	40 °C
Injection Vol.	5 µL (partial loop mode, 20 µL loop)

Gradient Program

Step	Time (min.)	Flow (mL/min.)	%B	Curve
0	3	0.5	15	
1	6.5	0.5	90	1
2	9.5	0.5	15	1

Mass Spectrometry. A PerkinElmer AxION® 2 TOF mass spectrometer was used with a lockmass solution introduced using a Flexar isocratic pump via a separate ion source nebulizer with a 20:1 flow split to minimize suppression of the analytes of interest. This addition ensures accurate mass calibration during data acquisition. Data were processed using Chromera® software and results were verified by comparison with results obtained in an independent analysis performed at Wayne State University. MS instrument configuration and parameter details are shown in Table 3.

Table 3. Mass spectrometry parameters.

Parameter	Value
Spectra per second	1
Acquisition	Pulse
Low *(m/z)*	100
High *(m/z)*	1600
Ion polarity	Positive
Cylinder (V)	-4000
Endplate (V)	-5000
Endplate heater	Medium
Capillary Entrance (V)	-6000
Drying Gas Flow (L/min.)	13
Drying Gas Heater	350 °C
Right Nebulizer Gas	80 PSI
Left Nebulizer Gas	80 PSI
Divert valve	Load
Calibration vial	Off
Lockmass ions *(m/z)*	622.029, 1521.9715
Lockmass flow rate	1 mL/min., split 20:1

Results and Discussion

Figure 2 shows a typical chromatogram obtained from a 50 parts-per-billion (50 ng/mL) concentration standard solution of six microcystins.

The chromatographic resolution and mass accuracy of TOF/MS allowed this method to be applied to the analysis of surface water samples. These data, shown in Figures 3 and 4, show distinct microcystin profiles between the samples. The sample collected in the Midwest (Figure 3) shows a high abundance of the RR and LR species as well as detectable levels of YR at the expected retention times. The measured mass errors for the detected ions were low (-0.6, -0.6, and 0.4 ppm for RR, LR, and YR respectively) indicating good confidence in identification. The water sample collected on the West Coast (Figure 4) shows a predominance of the microcystin-LA. The extracted ion chromatogram for LA showed an additional peak (Figure 4, LA*) near the expected retention time. The measured mass errors for the two peaks were -0.2 ppm for the first peak at 2.88 min. and 0.3 ppm for the second peak at 2.95 min. In addition, the isotopic pattern for both peaks matched closely with the expected pattern for LA (data not shown). These results suggest the presence of LA structural isomers. This is consistent with isomerization of the Adda moiety of microcystins (Figure 1) which has been demonstrated in response to irradiation by sunlight. This isomer produces a later-eluting peak in reversed-phase chromatography[5]. Interestingly, the structural isomers have been shown to have reduced hepatotoxicity and tumor-promoting activity compared to unreacted microcystins[5].

A split sample inter-lab comparison determined that, for two surface water samples, TOF/MS provided the same qualitative identification as MS/MS (data not shown). Additionally, the TOF/MS approach allows for non-targeted analysis, i.e. the ability to re-analyze the data post-acquisition to interrogate previously unexpected signals, thus avoiding sample re-injection.

The data sets for the two samples were reprocessed using the AxION Solo Multi-Quant software for multi-target analysis. Although this result requires confirmation with authentic standards, this type of analysis demonstrates the potential for rapid screening for multiple analytes post-acquisition.

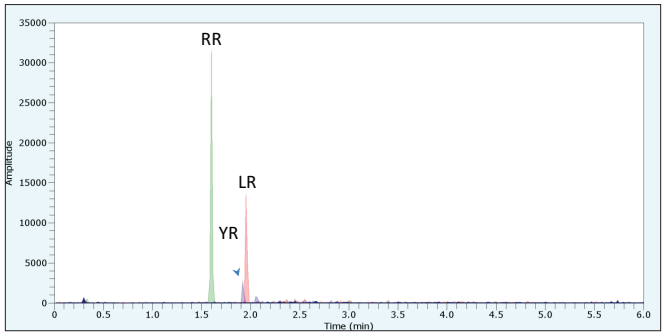

Figure 3. Extracted ion chromatogram of surface water sample from the U.S. Midwest.

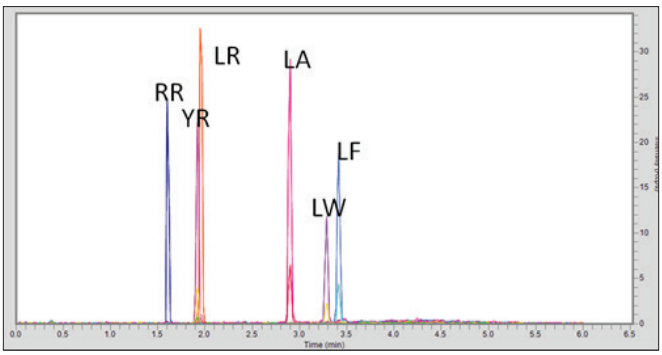

Figure 2. Extracted ion chromatogram of neat solution analyte standards.

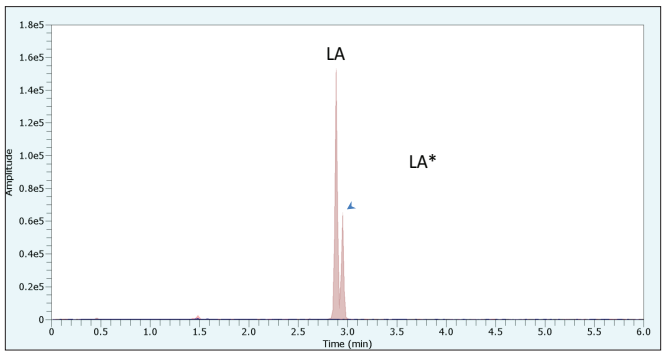

Figure 4. Extracted ion chromatogram of surface water from the U.S. west coast.

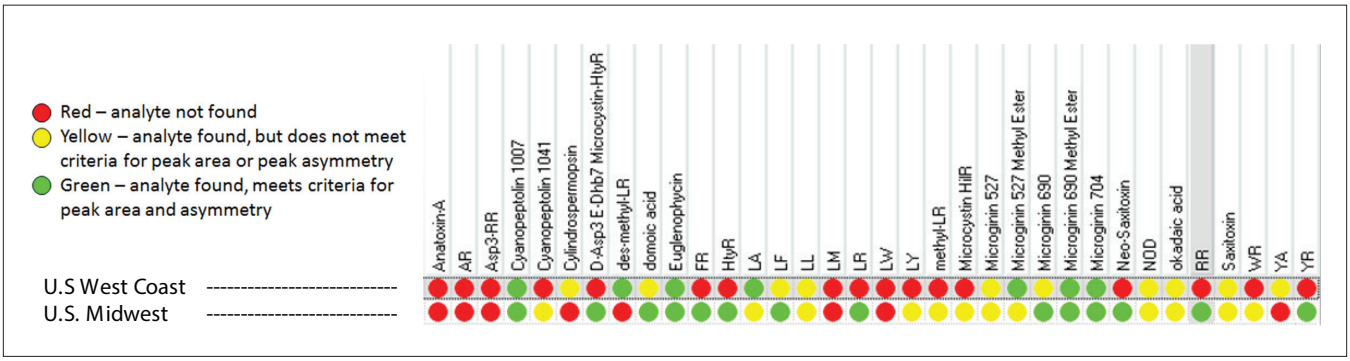

Figure 5. Example of data processed with AxION Solo Multi-Quant software.

Conclusion

This work demonstrates the applicability of UHPLC/TOF/MS analysis for qualitative profiling in water samples collected from sites with surface waters potentially contaminated with toxins during algal blooms.

References

1. http://blooms.uwcfl.org/what-are-habs/ accessed 9/8/2015.
2. Lenca N, Kero F, Weisenseel J, Southwell B, Bogdanov B, Young C, Westrick J, Method optimization strategies for the determination of microcystins as markers of algae bloom contamination in surface waters by UHPLC-ESI-TOF-MS, The Proceedings of the 52nd annual North American Chemical Residue Workshop (NACRW), St Pete's Beach, FL July 19-22, 2015.
3. J. Puddick et al., High Levels of Structural Diversity Observed in Microcystins from Microcystis CAWBG11 and Characterization of Six New Microcystin Congeners, Marine Drugs 2014, 12(11), 5372-5395.
4. Lenca N, White N, Kero F, Weisenseel J, Southwell B, Bogdanov B, Young C, Westrick J, Demonstration of scalable analytical methods for the screening of algae bloom contaminated surface waters by UHPLC-TOF MS equipped with a novel and automated analyte search algorithm, SCIX 2015, Rhode Island, RI, September 27-OCT 2, 2015.
5. Tsuji, K et al., Stability of Microcystins from Cyanobacteria: Effect of Light on Decomposition and Isomerization, Environ. Sci. Technol., 1994, 28(1), 173-177.

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