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アプリケーションノート

Targeted and Untargeted Screening of Microcystins in Lake Water Samples Using High Resolution Mass Spectrometry

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Abstract

In this application note, we describe a method that utilizes a combination of LC and high resolution mass spectrometry (HRMS) to perform targeted screening of microcystins in lake water samples.

Benefits

- Screening of water samples for targeted microcystins below regulatory limits.
- Perform qualitative and quantitative analysis using a single data set.
- Easily generate comprehensive HRMS library.
- Historical data review.

Introduction

Lake closures due to harmful algae blooms have become a regular occurrence during the summer months. Recent data show that harmful algae blooms have been implicated in human and animal illnesses and death in at least 43 states in the U.S.[1] In August 2016, at least 19 U.S. states reported public health advisories due to CyanoHABS.[1] These algae blooms are fueled by phosphorus and nitrogen runoff from fertilizers, animal feedlots, and leaky septic systems. The algal population explosions occur due to higher summer temperatures believed to be caused by global warming. Blue-green algae generate microcystins which are cyclic heptatpeptide hepatotoxins produced by certain species of cyanobacteria found in freshwater environments. The structure of the most common microcystin, Microcystin-LR, is shown in Figure 1. These secondary metabolites are toxic to higher organisms, causing human sickness or even death in some cases.[2] As they are produced in fresh and brackish waters, they can contaminate drinking water supplies. These public health advisories can cause panic and negatively impact state and municipal economies due to lost income from tourism, as was the case in Toledo Ohio in 2014, and the beach closures in Florida in July of 2016.

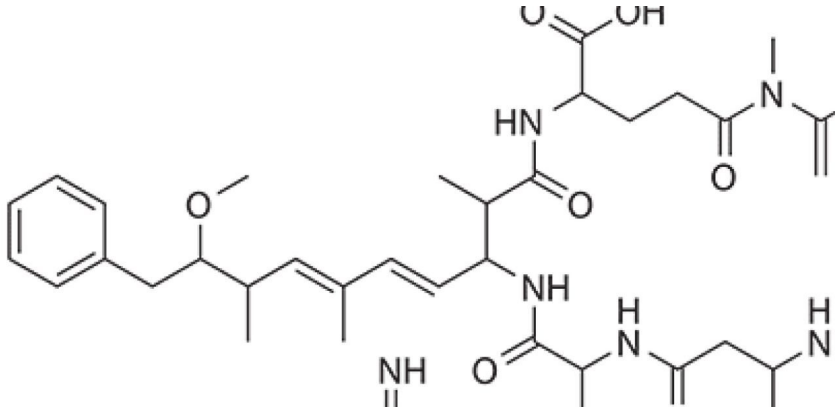


Figure 1. Chemical structure of microcystin-LR.

The WHO guideline limit for microcystin-LR is used in many countries. It includes a provisional value of 1 µg/L in drinking-water, and 10 µg/L for recreational exposure for total microcystin-LR (free plus cell-bound).[3] Some countries have set their own limits for microcystin-LR in drinking water (e.g. Australia and Canada have 1.3 µg/L and 1.5 µg/L respectively). There are only a handful of microcystin standards available on the market, while approximately 100 different microcystins variants have been reported in literature. These variants are produced by the substitution of the seven amino acids. Figure 2 shows the possible substitutions of a microcystin. As regulations are constantly changing and the fact that other microcystins may have a similar toxic effect as the regulated LR, it is important to develop targeted and untargeted methods for the analysis of these compounds.

AA1	AA2	AA3	AA4	AA5	AA6	AA7
Ala	$(H_4)Y$	MeAsp	Aba	Adda	Glu	Mdha
Gly	Aha	Asp	Ala	(6Z)Adda	Glu(OMe)	Dha
Leu	Ala		Apa	ADMAdda	$Glu(C_3H_7O)$	Dhb
Ser	Arg		Arg	DMAdda		dihydro
	Glu		Butyrine	EtAdda		MeAla
	Glu(OMe)		Glu(OMe)			MeLan
	Hil		Har			MeSer
	Hph		His			Ser
	hR		hR			
	Hty		Hty			
	Leu		Leu			
	M(O)		M(O)			
	Phe		Met			
	Trp		Phe			
	Tyr		Trp			
	Val		Tyr			
			Tyr(OMe)			
			Val			

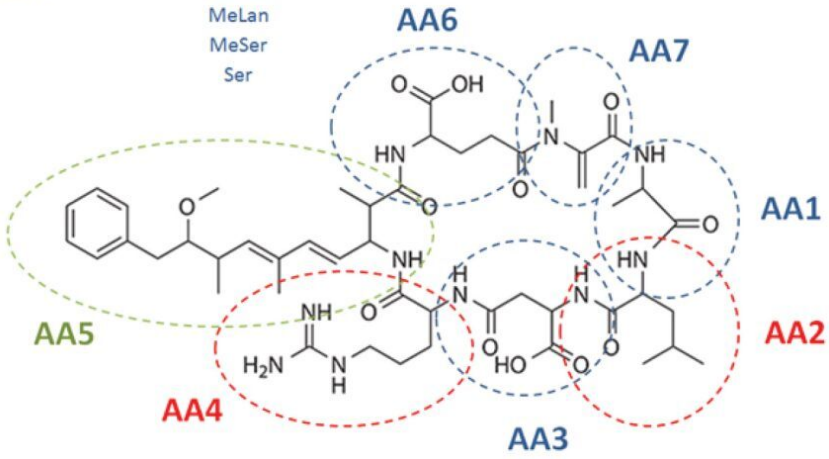


Figure 2. Illustration of the possible different combinations of seven amino acids that can produce over 100 different microcystin variants.

In this application note, we describe a method that utilizes a combination of LC and high resolution mass spectrometry (HRMS) to perform targeted screening of microcystins in lake water samples. A standard containing a mixture of 11 microcystins plus anatoxin A was used as a reference for positive identifications. Alongside the samples, a calibration curve of microcystin-LR was acquired to perform quantitation. The combination of accurate mass data for both precursor and fragment ions in a single analysis, combined with high quality UPLC separation was used to identify targeted compounds. As the data were acquired using a data-independent approach, additional compounds that were not included at the time of the initial analysis could be investigated.

Experimental

Water samples from lakes in the U.S. were screened for 12 targeted compounds in the UNIFI Scientific Library. The library was generated by running a standard mix and includes structural information, molecular formula, and retention time for each of the targets. A calibration curve of microcystin-LR standard in HPLC water was also run between 0.1 to 50 µg/L in order to quantitate the amount in the samples. Data were acquired using full spectral acquisition and alternating high- and low-collision energy states (MS^E). This allowed us to use the structural information to confirm the presence of targeted

compounds.

Sample description

Samples were obtained from U.S. lakes that reported harmful algae blooms in 2016. A lake water sample, a dock side sample, and scum layer sample were analyzed. The samples were lysed (freeze/thaw), filtered, and diluted before analysis. Prior to injection the samples were diluted 1 in 10 with water.

UPLC conditions*

UPLC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS T3 1.8 µm, 2.1 x 100 mm
Column temp.:	35 °C
Sample temp.:	8 °C
Flow rate:	0.450 mL/min
Injection volume:	1, 5, and 10 µL
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Total run time:	12 min

**This UPLC method was established and previously published by Waters.[4]*

Gradient:

Min.	Flow rate (mL/min)	%A	%B
Initial	0.45	98	2
0.80	0.45	98	2

Min.	Flow rate (mL/min)	%A	%B
9.00	0.45	30	70
9.05	0.45	10	90
9.90	0.45	10	90
9.91	0.45	98	2
11.50	0.45	98	2

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI+
Collision energy (LE):	4 eV
Collision energy (HE ramp):	25 to 80 eV
Scan time:	0.25 sec
Acquisition range:	50 to 1200 *m/z*
Capillary:	1.5 kV
Sampling cone:	36 V
Source temp.:	120 °C
Source offset:	50
Desolvation temp.:	500 °C
Cone gas flow:	150 L/Hr

Desolvation gas flow:	1000 L/Hr
Lockmass:	Leucine enkephaline (556.2766 *m/z*)

Data management

MassLynx v4.1 MS Software and the UNIFI Scientific Information System

Results and Discussion

Identification results in U.S. lake water samples

A standard at 10 µg/L of all 12 standards in HPLC water was run in order to establish retention times for the UPLC method. These retention times were added along with molecular formula and available structural information to the UNIFI Scientific Library. The UNIFI library was used to interrogate the highly complex data set for the 12 target compounds. In order to ensure the system was performing as expected, the above standard mix was acquired along with the samples of interest. Figure 3A shows the results from the standard injections. Figure 3B overlay shows the extracted ion chromatograms for each of the 12 compounds found in the standard. The standard data shows that the retention time delta is very low and the mass error for each compound of interest is within 5 ppm.

3A

Component Summary

	Label	Component name	Formula	Identification status	Expected RT (min)	Retention Time Error (min)	Mass error (ppm)	Isotope Match Mz RMS PPM	Response	A
1		Anatoxin	C10H15NO	Identified	2.18	0.00	0.43	0.58	19249	+
2		Microcystin dmLR	C48H72N10O12	Identified	6.08	0.00	2.16	1.93	147499	+
3		Microcystin dmRR	C48H73N13O12	Identified	5.22	0.01	-3.59	3.51	127269	2
4		Microcystin HilR	C50H76N10O12	Identified	6.24	0.00	-0.02	2.87	53746	+
5		Microcystin HtyR	C53H74N10O13	Identified	6.00	0.01	0.32	0.63	98596	+
6		Microcystin LA	C46H67N7O12	Identified	7.44	0.00	0.85	0.88	82915	+
7		Microcystin LF	C52H71N7O12	Identified	8.45	0.00	0.16	0.31	98869	+
8		MIcrocystin LR	C49H74N10O12	Identified	6.08	0.00	1.79	1.74	142885	+
9		Microcystin LW	C54H72N8O12	Identified	8.27	-0.01	1.21	1.81	82075	+
10		Microcystin LY	C52H71N7O13	Identified	7.60	0.00	0.91	0.76	72582	+
11		Microcystin WR	C54H73N11O12	Identified	6.30	0.01	-0.07	0.35	82608	+
12		Microcystin YR	C52H72N10O13	Identified	5.96	0.01	-0.04	0.33	105923	+

3B

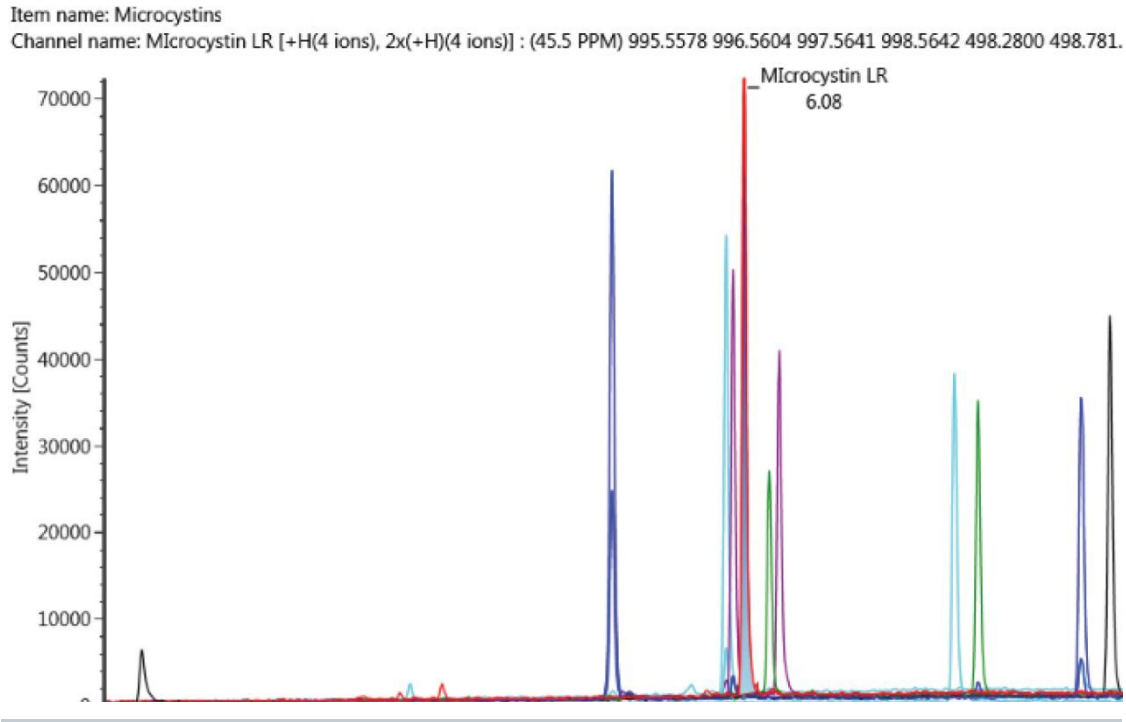


Figure 3A. Standard in HPLC water at 10 µg/L showing identification of all 12 Targets within 5 ppm; 3B. Overlaid chromatogram of microcystins standard at 10 µg/L.

Once it was established that the system was performing as expected, the four samples were run. A 1-µL aliquot from each sample was injected. The raw data was componentized and processed once by UNIFI Software.[6] In order to review the data of interest, a user-defined filter was applied (Figure 4). This filter was defined by the quality of the standard data and only showed the identified compounds that were within 5 ppm mass error, 0.1 minute retention time error, and above a minimal response. These user-defined filters can be combined with pieces of data the analyst wants to view, as well as previously saved data so that the same workflow can be followed for further data review. UNIFI' s filters, views, and workflows allow

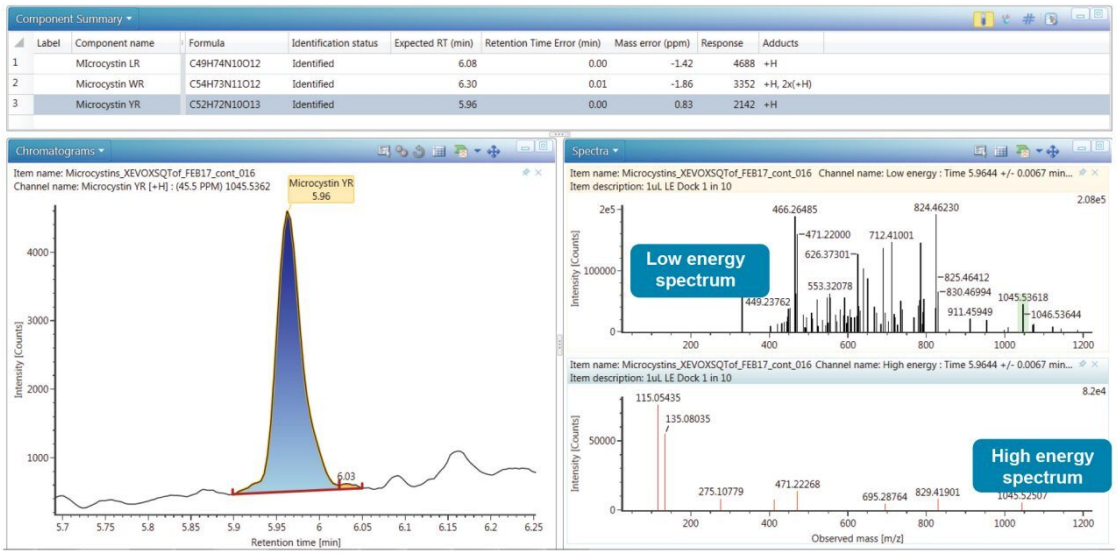
analysts to follow their own protocol for interrogating data, and it can help standardize how the data is reviewed.

Figure 4. Example of filter criteria used within UNIFI to show the data of interest. In this case only identified mass accurate, retention time consistent, and larger than 100 counts microcystins are displayed.

The results from each sample are shown in Figures 5a, 5b, and 5c. An example of one of the identifications depicted in these figures shows how an analyst can visualize the results. One of the advantages about using untargeted data acquisition is the ability to determine the presence different charge species of the target compounds. Microcystins can often form multiply charged species that can be potentially missed if the method is predefined by only one species. In this case both the single and double charged species were detected. By acquiring high and low energy data in one run the confirmation of the target compounds can be easily made. The high energy data is automatically used by the software to perform structural matching of the fragments to the compound of interest. Figure 6 shows the high and low energy spectra for the identification of microcystin-LR in the lake sample and in silico fragmentation using the compound’s structure.

5A

5B



5C

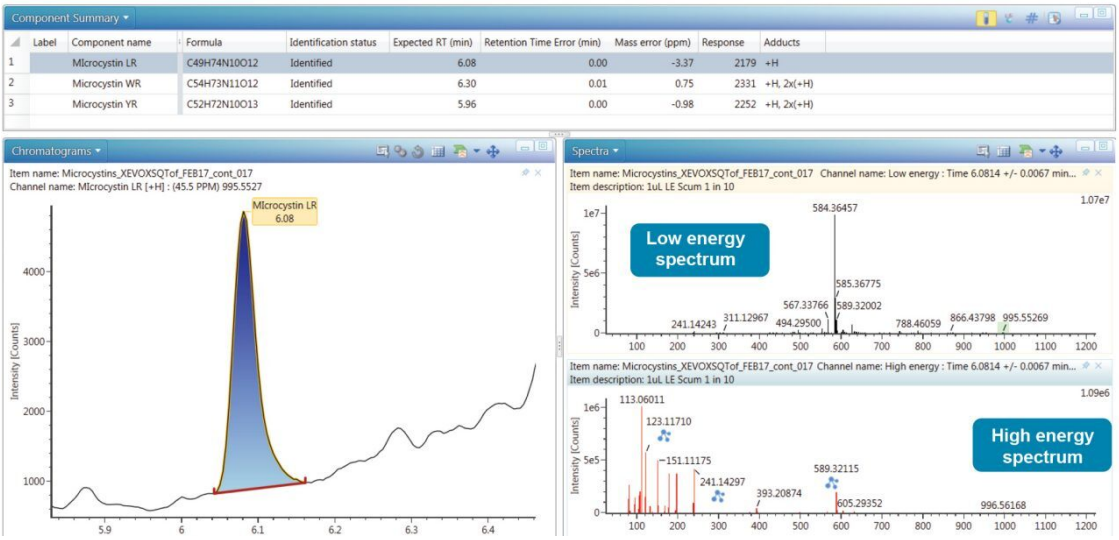


Figure 5A.Summary of results for the lake sample; 5B.Summary of results for the dock sample; 5C. Su

Quantification of Microcystin-LR

Alongside the samples, a calibration curve for microcystin-LR was acquired in order to determine the conc

- Detection and quantification performed using HRMS demonstrated excellent sensitivity, even with a small volume injection and single dimensional chromatography.
- The use of the ACQUITY UPLC I-Class System and the Xevo G2-XS QTof with UNIFI Scientific Software successfully met the regulatory requirements for screening microcystins.
- Historical data review allowed for another identification to be made in the same data set which is an advantage of HRMS over tandem quadrupole analysis.

References

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