Application Note

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Analysis of microcystins and nodularin in drinking water using an Agilent Ultivo triple quadrupole LC/MS

Figure 1. Agilent Ultivo Integrated into LC Stack.

Authors

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Abstract

Microcystins and nodularin are potent hepatotoxins produced by various species of cyanobacteria. During algal blooms, large quantities of microcystins and nodularin can occur in freshwater systems, threatening livestock and human drinking water sources. Ingesting these compounds carries health risks; therefore, regulatory agencies such as the US EPA and the World Health Organization (WHO) recommend limiting microcystins in drinking water to sub-ppb levels. This study extracted and prepared 500-mL water samples according to the US EPA method 544 for microcystins and nodularin in drinking water. This Application Note demonstrates a sensitive and robust analytical method for analyzing six microcystins and nodularin in drinking water using the innovative Agilent Ultivo triple quadrupole LC/MS.

Introduction

Cyanobacteria, also known as blue-green algae, are a group of common environmental bacteria occurring in different types of ecosystems. When conditions are favorable for cyanobacteria growth, their occurrence in slow-moving or standing freshwater systems can spiral out of control in an event called a bloom[1]. These blooms can be encouraged by warm temperatures and excess nutrients in the surface runoff from agricultural and residential soils by precipitation events[2]. In addition to other negative effects of algal blooms on the natural environment, certain species of cyanobacteria can produce high levels of microcystins and nodularin, which are toxic to many organisms, and can threaten drinking water supplies[1].

The US EPA has established a health advisory for maximum levels of microcystins in drinking water at 0.3 µg/L for infants and young children, and at 1.6 µg/L for school-aged children and adults[3]. Some individual US states regulate certain microcystins at levels as low as 0.1 µg/L. Consequently, these microcystins and nodularin have been included in the US EPA's unregulated contaminant monitoring rule (UCMR). This requires routine monitoring of these compounds in drinking water supplies in the US from 2018–2020. The World Health Organization (WHO) recommends keeping microcystin levels below 1 µg/L in drinking water[4]. US EPA method 544 analyzes six microcystins and nodularin in drinking water using a 500-mL sample that undergoes solid phase extraction (SPE) and LC/MS/MS analysis.

This method was performed on the Agilent Ultivo triple quadrupole LC/MS system (Figure 1). The space-saving and intuitive design of Ultivo addresses many of the challenges faced by labs performing high-throughput environmental analyses. Ultivo innovations, such as the Cyclone Ion Guide, Vortex Collision Cell, and the Hyperbolic Quads, maximize quantitative performance. They allow the Ultivo to have a small size, and enhance instrument sensitivity, reliability, and robustness. Ultivo VacShield technology and easy-change detector reduce the time required for maintenance, making it attractive for large-volume laboratories. The Agilent MassHunter software suite simplifies data acquisition, method setup, data analysis, and reporting. This leads to faster acquisition-to-reporting time, and confidence in results.

This Application Note demonstrates the sensitive and precise quantification of six microcystins and nodularin (Table 1) included in EPA method 544, using the space-saving and innovative Ultivo triple quadrupole LC/MS system.

Table 1. Selected transitions for analyte detection in dMRM mode.

Compound	ISTD	Precursor ion (*m/z*)	Product ion (*m/z*)	Retention time (min)	Fragmentor (V)	Collision energy (V)
MC-YR	No	1045.5	135.2	6.299	150	70
C2D5-LR	Yes	1028.6	135.2	7.255	150	80
MC-LY	No	1002.5	135.2	7.277	150	80
MC-LR	No	995.6	135.2	6.482	150	80
MC-LF	No	986.5	135.2	7.871	150	70
MC-LA	No	910.5	135.2	7.174	150	70
Nodularin	No	825.5	135.2	6.041	150	70
MC-RR	No	520	135.2	5.813	150	30

Experimental

Reagents and chemicals

All reagents used were HPLC or LC/MS grade. Acetonitrile, isopropanol, and methanol were purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral system with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid (FA) and ammonium formate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA), and ammonium fluoride was purchased from Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA). Chemical standards were purchased from Abraxis Inc. (Warminster, PA).

Sample preparation

Drinking water samples were collected in California, USA. A 500 mL amount of sample was extracted and prepared in accordance with the procedure outlined in US EPA method no. 544[5]. After filtration and SPE steps, extracts were concentrated to dryness and reconstituted to a 1 mL volume with 1:1 ultrapure water/methanol for analysis.

Instrumentation

Agilent 1290 Infinity II UHPLC:

- Agilent 1290 Infinity high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler with cooler (G7167B)
- Agilent 1290 Infinity II MCT (G7116B)

Agilent Ultivo Triple Quadrupole LC/MS system:

- Agilent Jet Stream Electrospray ionization source

Method

Table 2 summarizes the Agilent 1290 Infinity II UHPLC conditions. Table 3 summarizes Ultivo triple quadrupole parameters and Agilent Jet Stream (AJS) ESI source parameters, while Table 1 presents the individual parameters for each transition. Analysis was carried out with positive ionization and dynamic multiple reaction monitoring (dMRM). Data were evaluated using the MassHunter Quantitative Analysis Software B.09 with the Quant-My-Way feature. Figure 2 shows chromatograms for all six microcystins, nodularin, and the internal standard.

Table 2. Agilent 1290 Infinity II UHPLC parameters.

Parameter	Value
Column	Agilent InfinityLab Poroshell SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)
Column temperature	50 °C
Injection volume	10 µL
Mobile phase	A) 0.1 % FA + 5 mM ammonium formate + 2 mM ammonium fluoride in water B) 80:20 Acetonitrile/isopropanol
Flow rate	0.600 mL/min
Gradient	Time (min) %B 0 20 4.0 30 11.0 100

Table 3. Agilent Ultivo triple quadrupole and AJS source parameters.

Parameter	Value
Gas temperature	350 °C
Gas flow	12 L/min
Nebulizer	40 psi
Capillary	5,500 V
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Nozzle voltage	0 V

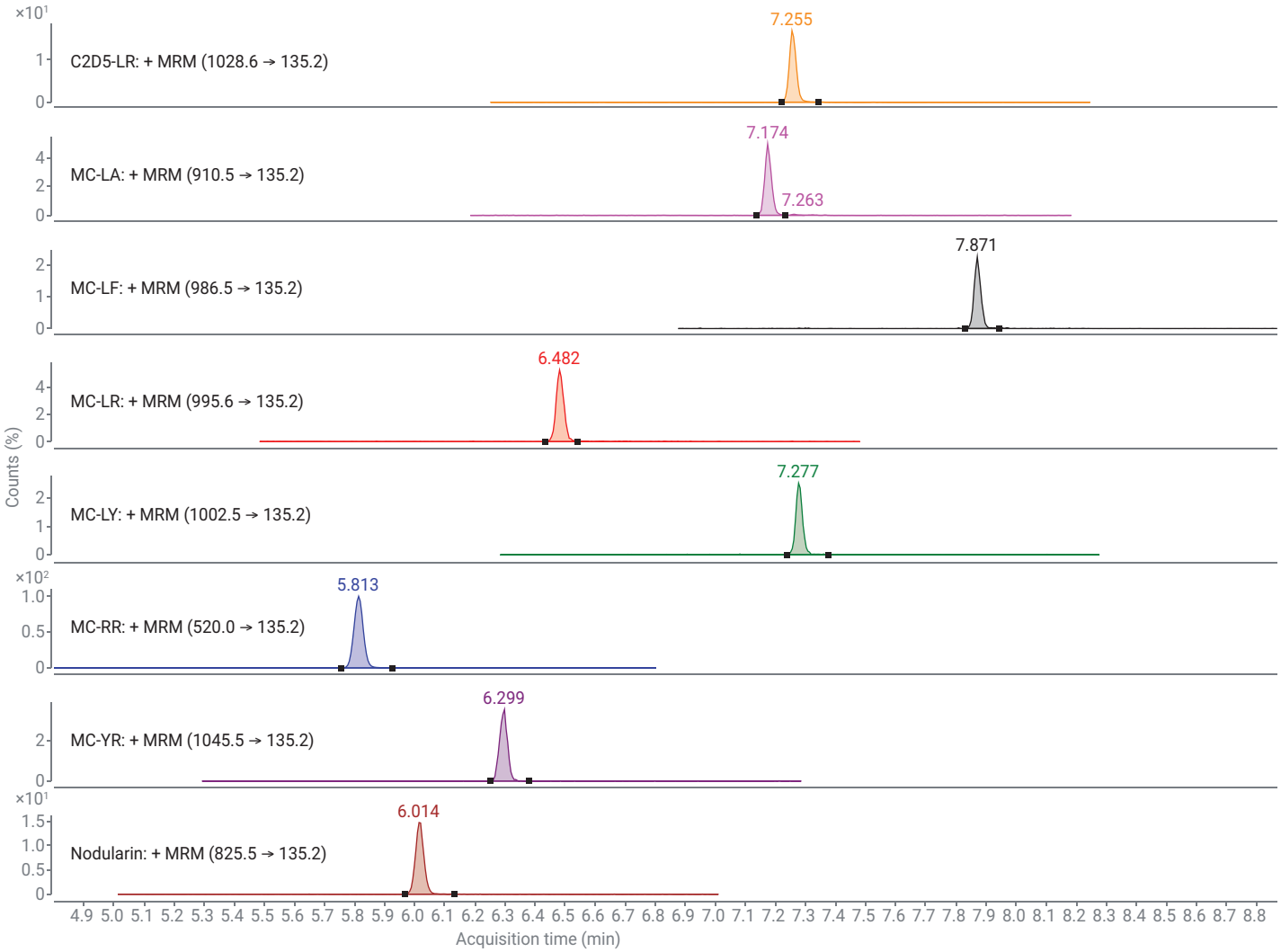

Figure 2. Chromatogram of microcystin and nodularin analytes included in this study at 10 µg/L (Table 4).

Table 4. Concentration (µg/L) of samples in the calibration curve. Values refer to final extract concentration.

Target	Cal 0 (Blank)	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6
MC-LA	0	4	8	20	40	80	160
MC-LF	0	3	6	15	30	60	120
MC-LR	0	10	20	50	100	200	400
MC-LY	0	4.5	9	22.5	45	90	180
MC-RR	0	3	6	15	30	60	120
MC-YR	0	10	20	50	100	200	400
NOD-R	0	2.5	5	12.5	25	50	100

Results and Discussion

Method recovery and sensitivity

Recovery of the microcystins and nodularin ranged 82–115 % for both mid level (Cal 3 level spike) and low level (Cal 1 level spike), demonstrating the exceptional efficiency of the extraction method as well as the exceptional sensitivity of Ultivo (Figure 3). The internal standard (MC-LR C2D5) recovery was between 70–130 %, which is within EPA guidelines. The mid level spike is at least 10 times lower than the WHO guidelines for microcystins and nodularin in drinking water. The low level spike is at least 50 times lower than the WHO guidelines, well below any individual US state advisory limits for microcystins and nodularin. This demonstrates that the Ultivo triple quadrupole LC/MS coupled to the 1290 Infinity II HPLC is an excellent tool for this application.

Method precision and linearity

Table 4 lists the calibration levels for each compound in the final extract, and the calibration samples were prepared in 1:1 ultrapure water/methanol. Exceptional linearity was achieved for the standard curves run on Ultivo. There were R^2 values greater than 0.99 for all compounds, as displayed for selected compounds in Figure 4, and all compounds in Table 5.

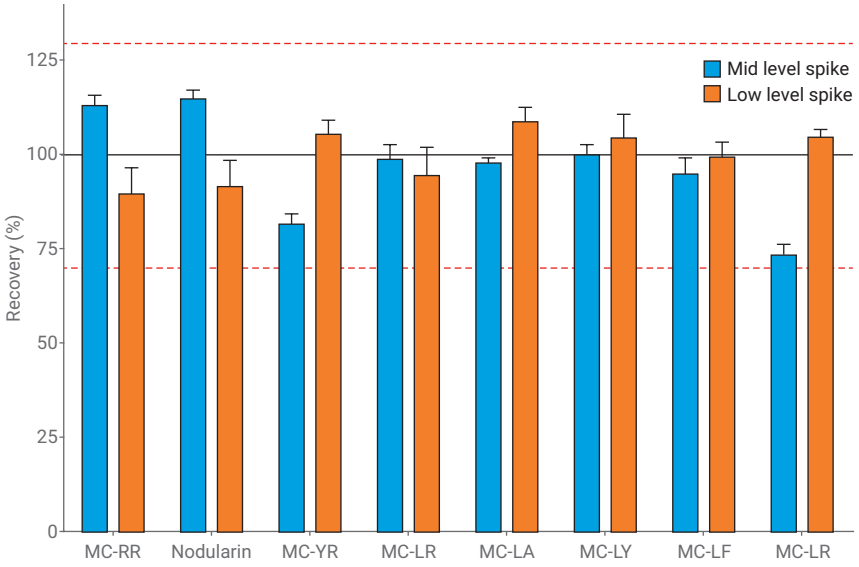

Figure 3. Recovery of microcystins and nodularin in drinking water at Cal 1 level for the low level spike, and Cal 3 level for the mid level spike in water samples (calibration levels listed in Table 4).

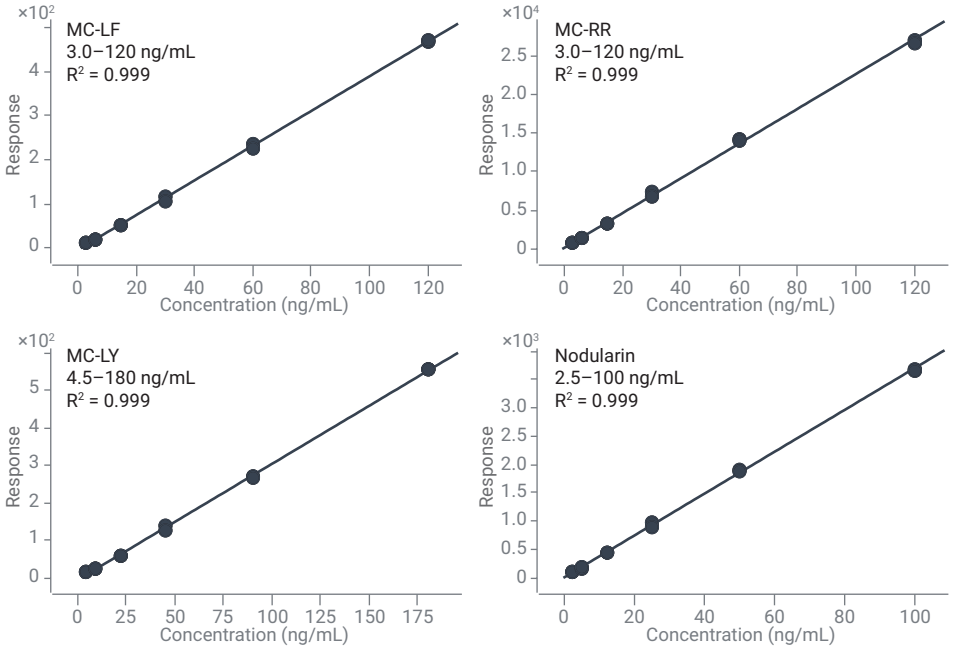

Figure 4. Calibration curves for MC-LF, MC-RR, MC-LY, and nodularin, prepared in post spiked drinking water extract. Linear fit, no weighting.

Excellent precision was achieved when a matrix spike was run over a 17-hour period. The matrix was analyzed 18 times, providing a measure of robustness for the method. The matrix spike for the robustness and precision study was spiked to the Cal 3 level (Table 4). Figure 5 illustrates how dirty the matrix spike sample extracts were, further proving the outstanding robustness of this method. Relative standard deviation (RSD%) for peak area responses were less than 10 % for all compounds in the method. The retention times for each compound had an RSD% of less than 1.7 %, highlighting the precision of the LC method developed for this application.

Table 5. The R^2 values, peak area precision, and retention time precision of each compound included in the calibration curve, n= 18 over 17 hours.

Compound	R^2	Matrix spike area RSD (%)	Matrix spike RT RSD (%)
MC-RR	0.9989	7.4	1.6
Nodularin	0.9994	1.1	0.65
MC-YR	0.9963	7.2	1.7
MC-LR	0.9931	4.6	0.16
MC-LA	0.9978	5.8	0.11
C2D5-LR	-	5.9	0.08
MC-LY	0.9992	7.1	0.06
MC-LF	0.9994	9.7	0.09

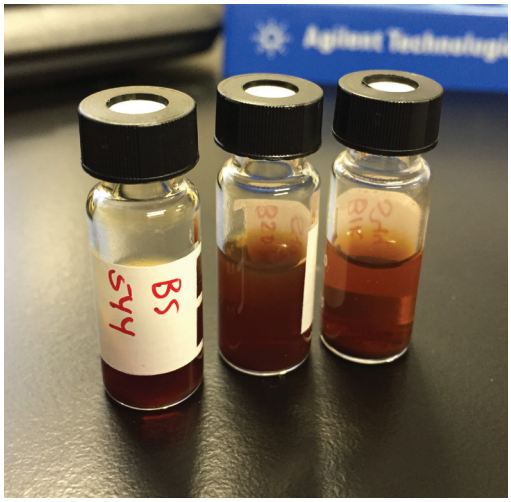

Figure 5. Drinking water extracts from the EPA 544 method, which were run on an Agilent Ultivo LC/MS for this study.

Conclusions

The Agilent Ultivo triple quadrupole LC/MS and the Agilent 1290 Infinity II LC are the perfect combination for detecting microcystins and nodularin in drinking water far below regulatory levels using EPA method 544. The exceptional precision, linearity, and innovative space-saving design of Ultivo, plus the intuitive Agilent MassHunter software package, make it an ideal instrument for the high-throughput laboratory.

References

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