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Application News

No. C204

Liquid Chromatography Mass Spectrometry

Analysis of Microcystin in Drinking Water and Environmental Water Using Triple Quadrupole LC/MS/MS

Water bloom or algal bloom is a phenomenon that occurs when certain types of phytoplankton spread widely in ponds, lakes and marshes, and standing water. Some species of phytoplankton which form algae blooms, can produce toxic substances. Microcystin is one of hepatotoxin which is contained in phytoplankton.

Guidelines for Drinking-water Quality (GDWQ) [1] published by the World Health Organization (WHO) set a regulatory value of 1 µg/L or less for microcystin. Many analogues of microcystin are already known. In Japan, microcystin LR is a designated study item in water quality standards for drinking water, and a target value of 0.8 µg/L has been established for drinking water.

Currently, its quantification is carried out with concentration and cleanup techniques, like solid phase extractions. This article introduces an example in which microcystin LR, RR, and YR in drinking water sample were measured with high sensitivity without complex pretreatment.

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Sample Preparation

Standards of microcystin LR, RR, and YR were dissolved and mixed with water/methanol = 8/2 (v/v), to obtain a concentration of 10 mg/L. Calibration standards were prepared by serial dilution with ultrapure water.

Samples were prepared by adding the microcystin analogues to three types of water, i.e., mineral water, tap water, and pond water. The pond where "pond water" was sampled was a manmade reservoir for agricultural water located in an urban park.

To remove solid matter, these samples were subjected to centrifugal separation followed by filtration with disposable filters. The microcystin analogues were added to these samples so as to obtain the WHO regulatory value of 1 µg/L, and these samples were analyzed.

Analysis Conditions

Table 1 shows the analysis conditions.

Table 1 Analysis Conditions

[HPLC conditions] (Nexera™ X2)		
Column	:	Shim-pack Scepter™ C18-120 (2.1×100, 3 µm)
Mobile phases	:	A) 0.1% formic acid in H_2O B) 0.1% formic acid in Acetonitrile
Gradient Program	:	B 5% (0-3 min) – B 55% (4 min) – B 95% (6-7 min) – B 5% (7.01-10 min)
Flow rate	:	0.35 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 µL
Rinse type	:	Internal and External
[MS conditions] (LCMS™-8060)		
Ionization	:	ESI (Positive mode)
Probe Voltage	:	+5.0 kV
Mode	:	MRM
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL Temp.	:	150 °C
Heat Block Temp.	:	200 °C
Interface Temp.	:	350 °C
Probe position	:	+1.0 mm

[MS/MS parameters]

Compound	MRM transition (*m/z*)	DL/Qarray Bias (V)	Collision energy (V)
Microcystin YR	1045.20>135.10 1045.20>112.05	20	-70.0 -67.0
Microcystin LR	498.30>135.10 498.30>90.95	20	-13.0 -40.0
Microcystin RR	519.80>135.10 519.80>103.05	20	-33.0 -61.0

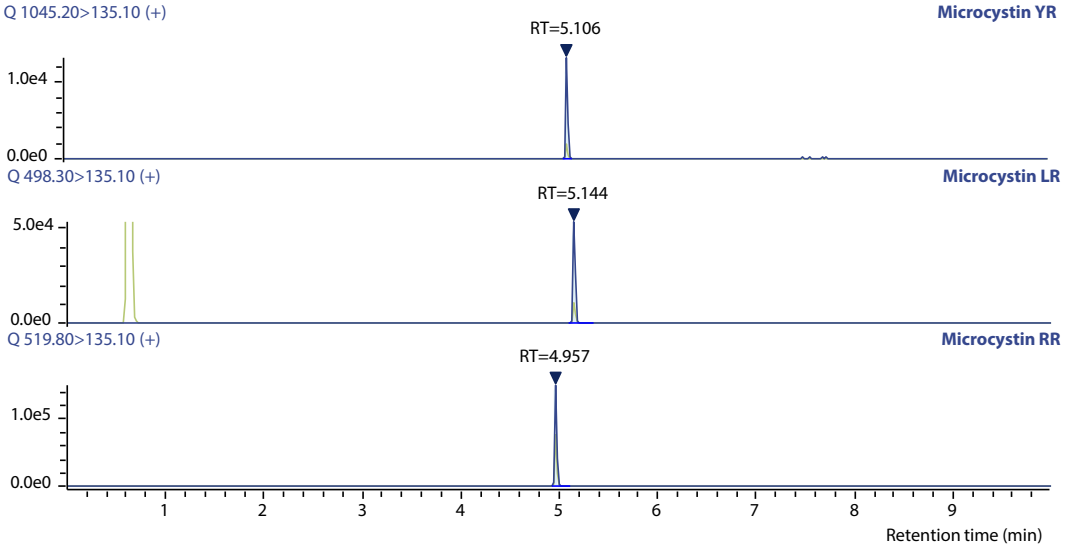

Fig. 1 Chromatogram of 1 µg/L Standard Samples

Results of Analysis of Standards

Fig. 2 shows the calibration curves prepared for the microcystin concentration range of 0.1 to 10 µg/L. Although the regulatory value of 1 µg/L, the calibration curves showed a good linearity in the range including 0.1 µg/L,1/10 of the WHO value.

To confirm repeatability, analyses were repeated 6 times at 0.08 µg/L, 1/10 of the target value of the Japanese water quality standard. These results are shown in Table 2 and Fig. 3. Satisfactory repeatability was obtained, as the relative standard deviation (%RSD) of the peak area value was 10% or less for all components.

Table 2 Repeatability of 0.08 µg/L Standard Samples in Repeated Analysis (n = 6)

Compound	Area%RSD	Rt%RSD
Microcystin YR	5.98%	0.04%
Microcystin LR	4.31%	0.04%
Microcystin RR	2.29%	0.02%

Conclusion

- An analysis method using simple pretreatment requiring only centrifugal separation and filtration, without solid phase extraction pretreatment, was studied.
- This method showed satisfactory recovery with mineral water, tap water, and pond water.
- Quick and accurate measurement of microcystin was developed to save time and limit manual preparation.

Table 3 Recovery and Area % RSD for Water Samples

	Compound	Mineral water	Tap water	Pond water
Recovery	Microcystin YR	92.4%	78.7%	106.8%
	Microcystin LR	86.8%	83.5%	90.5%
	Microcystin RR	102.5%	94.9%	97.9%
Area%RSD	Microcystin YR	1.2%	2.3%	4.6%
	Microcystin LR	1.2%	1.9%	2.7%
	Microcystin RR	1.2%	2.3%	4.6%

Results of Analysis of Water Samples

Recovery was calculated from the quantitative results of spiked samples. Table 3 shows the results. Satisfactory recovery within 70 to 130% were obtained for all samples.

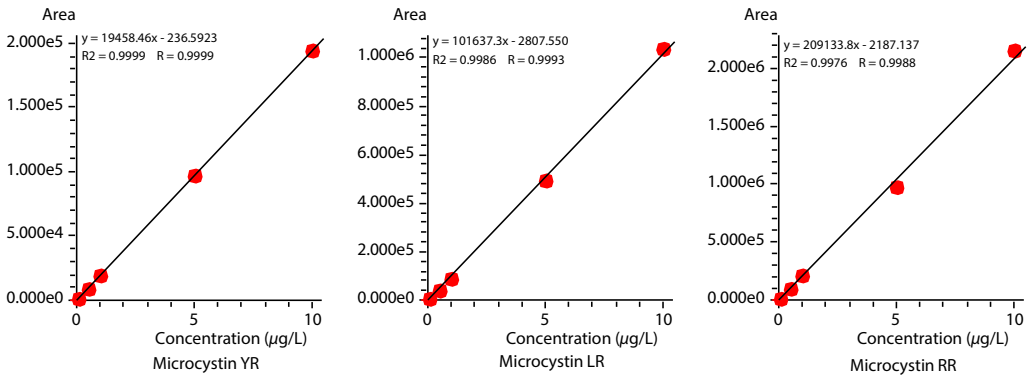

Fig. 2 Calibration Curves of Microcystin

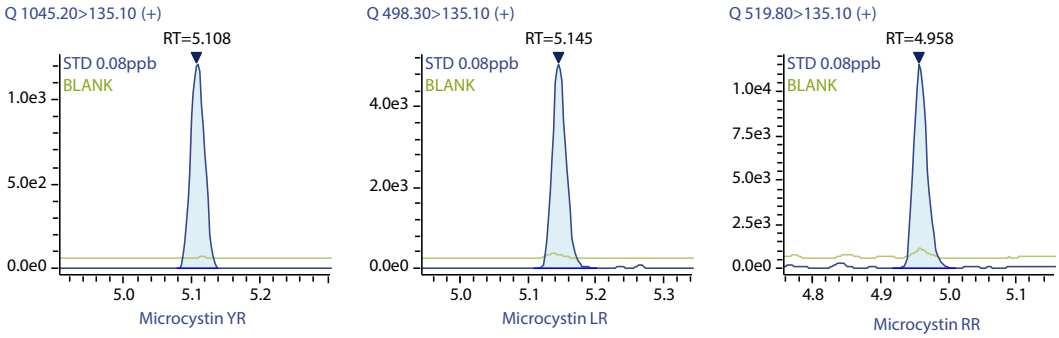

Fig. 3 Chromatograms of 0.08 µg/L Standard Samples

<Reference>
(1) WHO (2003) Cyanobacterial toxins: Microcystin-LR in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/03.04/57).

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