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Determination of Microcystins in Drinking Water by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

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1. Introduction

Microcystins (MCs) are natural toxins produced by certain general cyanobacteria, which has a strong carcinogenic effect. MCs are seriously harmful to the residents, because they couldn't be degraded and removed. Therefore, the quantity of MCs in drinking-water is regulated by the WHO. This poster employed a ultra high performance liquid chromatography/electrospray ionization tandem mass spectrometry (UHPLC/ESI-MS/MS) method to determinate 10 MCs in drinking-water. The water samples were prepared without any pretreatment before determination. The method is simple, rapid and highly sensitive, which can meet the requirements for the analysis of MCs in drinking-water.

2. Methods and Materials

Sample Preparation

Drinking water samples were directly filtrated and MCs were determined using an UHPLC-MS/MS instrument.

UHPLC/MS/MS Analysis

UHPLC

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A$_5$ degasser, and an SIL-30AC autosampler. The separation was carried out on a Shimadzu XR-ODSIII column (50 mmL. × 2.0 mmi.d., 1.6 µm) with the column temperature at 40ºC.

The mobile phase consisted of (A) 0.1% formic acid water solution and (B) 0.1% formic acid acetonitrile solution using a gradient elution of 30%B (0 min) -80%B (1.5 min) -80%B (4.0 min) -30%B (4.1 min) -30%B (5.50 min). The flow rate was 0.4 µL/min. The injection volume was 20 µL.

MS/MS

A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was connected to the Nexera UHPLC instrument via an ESI interface. The mass spectra were acquired in positive ion mode. The DL temperature was set at 250 °C, heat block temperature at 400 °C, nebulizing gas at 3 L/min and drying gas at 15 L/min. The dwell time was 50 ms and pause time was 3 ms. The MRM parameters were shown in Table 1.

Standard MCs

Ten MCs (MC-RR, Demethyl-RR, LR, LY, LW, LA, YR, WR, CF and NOD) were dissolved in water. MC standards were purchased from Enzo Life Sciences.

3. Results and Discussion

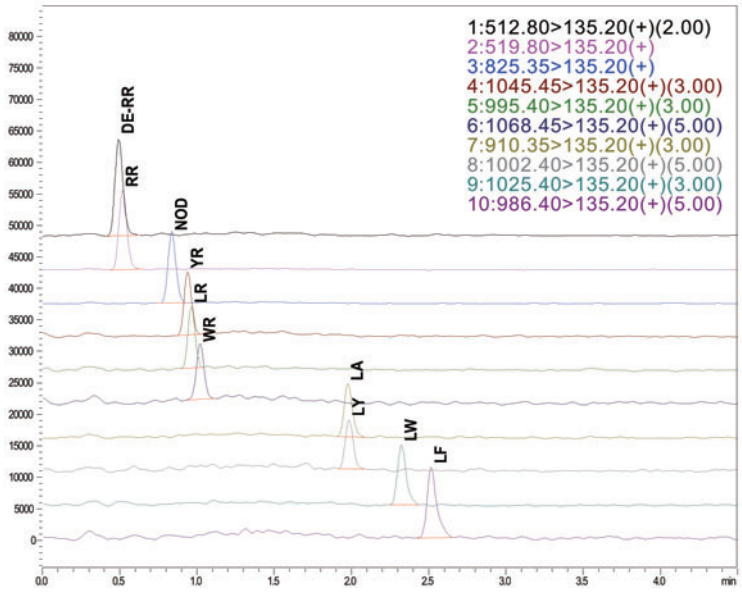

Fig. 1 MRM chromatograms of 10 MCs (1 µg/L)

Table 1 MRM parameters of 10 MCs

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
DE-RR	512.8	135.20*	-26.0	-34.0	-24.0
		103.05	-26.0	-55.0	-18.0
RR	519.8	135.20*	-26.0	-36.0	-23.0
		105.00	-26.0	-49.0	-19.0
NOD	825.35	135.20*	-32.0	-60.0	-25.0
		227.25	-32.0	-55.0	-24.0
YR	1045.45	135.20*	-32.0	-60.0	-25.0
		227.25	-32.0	-55.0	-24.0
LR	995.40	135.20*	-38.0	-65.0	-25.0
		213.10	-38.0	-64.0	-21.0
WR	1068.45	135.20*	-42.0	-65.0	-24.0
		213.10	-42.0	-64.0	-21.0
LA	910.35	135.20*	-34.0	-60.0	-24.0
		213.10	-34.0	-52.0	-22.0
LY	1002.40	135.20*	-38.0	-59.0	-24.0
		446.15	-38.0	-39.0	-30.0
LW	1025.40	135.20*	-40.0	-62.0	-25.0
		213.10	-40.0	-59.0	-22.0
LF	986.40	135.20*	-38.0	-60.0	-24.0
		375.20	-38.0	-40.0	-26.0

*: quantitative transition

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Ten microcystins were quickly seperated and analyzed in 5.5 min. The MRM chromatograms of 10 MCs in positive ion mode were shown in Fig. 1. A linear relationship was found between peak area and different concentrations of 10 MCs within 0.02-50 µg/L. The calibration curves of 10 MCs were constructed with correlation coefficients (r) more than 0.999 as shown in Table 2.

Table 2 The calibration curve of 10 MCs

Compound	Calibration curve	r	Linear range (µg/L)
DE-RR	Y = 23620.7X - 713.367	0.9999	0.02-50
RR	Y = 7876.04X - 306.677	0.9999	0.02-50
NOD	Y = 2554.31X + 139.085	0.9999	0.1-50
LR	Y = 1130.75X + 0.304781	0.9999	0.1-50
YR	Y = 1045.52X - 179.194	0.9999	0.5-50
WR	Y = 583.910X - 77.2702	0.9999	0.5-50
LY	Y = 1555.45X - 106.796	0.9999	0.1-50
LA	Y = 1688.33X + 70.7246	0.9999	0.1-50
LW	Y = 2787.07X - 231.222	0.9999	0.1-50
LF	Y = 1767.59X + 165.616	0.9999	0.5-50

In this study, the repeatability of 10 MCs in different concentrations (1 and 10 µg/L) was investigated. The RSD% of retention time were from 0.077 to 0.369 and RSD% of peak area were from 0.843 to 9.672 (Table 3).

Table 3 Repeatability of 10 MCs in different concentrations (n=6)

Compound	%RSD (1 µg/L)		%RSD (10 µg/L)	
	Area	R.T.	Area	R.T.
DE-RR	1.599	0.369	0.843	0.183
RR	3.965	0.307	1.627	0.215
NOD	4.735	0.134	1.387	0.120
YR	4.882	0.262	4.548	0.147
LR	4.811	0.132	4.315	0.162
WR	9.672	0.196	4.947	0.156
LA	4.310	0.098	2.911	0.091
LY	2.810	0.145	1.055	0.125
LW	4.586	0.077	2.764	0.091
LF	4.417	0.245	4.987	0.079

The mixed standard sample was spiked into the blank water at levels of 0.02 µg/L or 0.1 µg/L to show the method LOQ. All the analyses were performed using above analytical conditions. The chromatograms of 10 MCs were shown in Fig. 2. The results showed that there was no detection in tap water samples.

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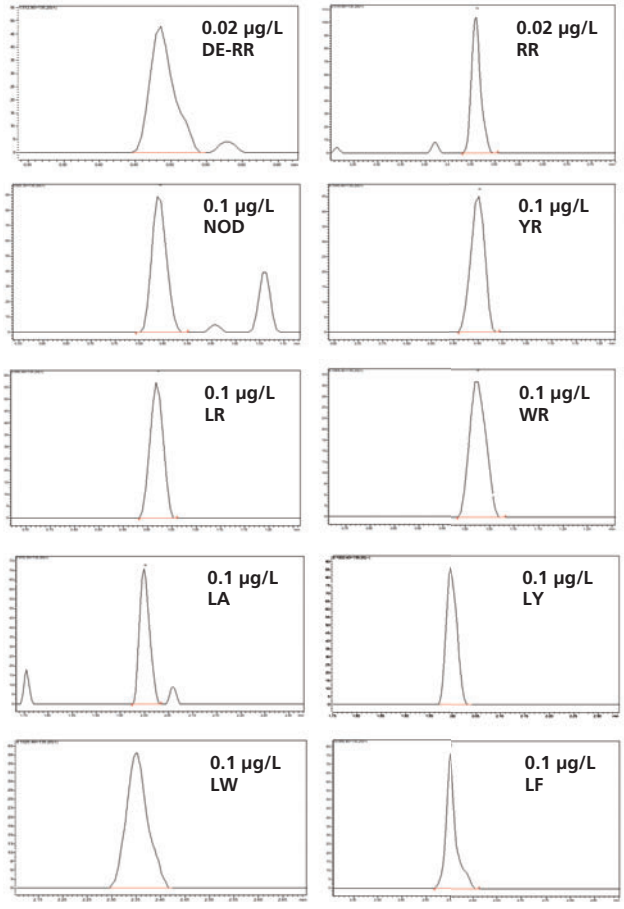

Fig. 2 MRM chromatograms of MCs spiked in tap water at 0.02 µg/L or 0.1 µg/L

4. Conclusions

A UHPLC/MS/MS method has been developed for 10 MCs in water. All of the target compounds were separated in 5.5 minutes, and analyzed in ESI positive mode. The calibration curves of 10 MCs were constructed over a concentration range of 0.02-50 µg/L with correlation coefficients (r) more than 0.999. Good repeatability on both retention time and peak area was obtained. The limits of quantitation (LOQs) for 10 MCs were within 0.02-0.5 µg/L. A reliable method was established for fast quantitative determination of 10 MCs in water.

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