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Method development and validation for simultaneous quantification of microcystin congeners in water

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Abstract

Background: Microcystins (MCs) are secondary metabolites of cyanobacteria that are hepatotoxic to humans through the ingestion of cyanobacteria-contaminated water and accidental inhalation from lake activities. MCs with diverse congeners in water can be precisely quantified using online solid-phase extraction-ultra performance liquid chromatography coupled with tandem mass spectrometry (online-SPE UPLC–MS/MS). A method was developed and validated to simultaneously quantify eight different MCs (microcystin-RR, -LR, -YR, -WR, -LA, -LF, -LY, and -LW) in water using online-SPE UPLC–MS/MS.

Results: The method achieved the highest efficiency and sensitivity by selecting acetonitrile with 0.1% formic acid and water with 0.1% formic acid as the best mobile phase conditions. The linearity, accuracy, and precision were validated using matrix-mixed water with a leucine enkephalin internal standard. The limit of detection (LOD) was calculated using the signal-to-noise ratio of three passes of the daily water-surface inspection for MCs. This method showed both high sensitivity and high resolution for the separation of eight MC congeners with LODs ranging from 0.020 to 0.371 ng L⁻¹ and limits of quantitation ranging from 0.066 to 1.24 ng L⁻¹. The detection time was reduced to 11 min. Except for MC-RR (58.8% recovery at high concentration) and MC-WR (45.1% and 40.9% recoveries at medium and high concentrations, respectively), the recoveries of the other MCs ranged from 70 to 135%, and the relative standard deviation was less than 10%.

Conclusion: Eight different MCs in 12 water samples collected from Chaohu Lake, China, were analyzed. The sum of all MC congeners was calculated to range from 101 to 585 ng L⁻¹ (less than the World Health Organization's safe drinking water limit of 1 µg L⁻¹ for MC-LR).

Keywords: Microcystin congeners, Filter membranes, Online solid-phase extraction, Detection method development, Lake eutrophication

Background

Microcystins (MCs) are cyanotoxins produced by many cyanobacteria species [1]. Chemically, MCs are monocyclic heptapeptides with a general structure of cyclo-(D-Ala¹-L-X²-DisoMeAsp³-L-Z⁴-Adda⁵-D-isoGlu⁶-Mdha⁷),

where D-MeAsp³ is D-erythro-β-methylaspartic acid, Mdha⁷ is N-methyldehydroalanine, and Adda⁵ is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. X² and Z⁴ are variable amino acid positions that result in at least 279 variants of MCs in nature [2]. Microcystins are highly toxic because they are specific inhibitors of serine/threonine protein phosphatases that induce the breakdown of the cellular cytoskeleton and promote tumor growth. The greatest environmental risk associated with MCs is from microcystin-contaminated water, which causes animal poisoning and hepatotoxicity

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in humans. Among all the MC congeners, MC-LR is the most common and most studied variant in water samples; thus, it is currently considered to be the reference compound because of its high, acute toxicity and frequent occurrence. The guideline for MC-LR concentration in drinking water ($<1.0 \mu\text{g L}^{-1}$) was established by the World Health Organization (WHO) [3]. In 2010, the International Agency for Research on Cancer (IARC) characterized MC-LR as a Group 2B carcinogen with strong evidence that it supports tumor promotion.

Water blooms usually result in the presence of different MCs simultaneously. Graham et al. detected different cyanotoxin types in 48% of cyanobacterial blooms, 95% of which had multiple MC variants [4]. A greater variety of MC congeners was detected in China, where most of the studies were conducted [4–8]. From August 2011 to July 2012, a survey of drinking water sources in the eastern part of Chaohu Lake showed that the total MC concentration reached $8.86 \mu\text{g L}^{-1}$ [9]. In previous epidemiological studies, the high incidence of developing primary liver cancer in southern China has been correlated with the intake of MC-contaminated drinking water [10, 11]. Therefore, identifying the concentrations of various MCs is critical.

The overall structure of MCs is relatively hydrophilic (polar), which comes from carboxylic acids at positions 3 and 6 and the frequent occurrence of arginine at positions 2 and/or 4 [12]. However, among the wide range of MC variants, there are more lipophilic compounds than MC-LR, which implies that some congeners can be even more toxic than MC-LR [13]. The data available in the scientific literature regarding the occurrence and toxicity of MCs other than MC-LR are limited [14, 15]. The increasing number of toxic structural congeners complicates the identification of MCs. Many techniques have been utilized to analyze MCs, such as immunological and biological assays, UV absorbance, and mass spectrometry [16–19]; enzyme-linked immunosorbent assay (ELISA) kits used to determine MCs are relatively inexpensive, simple, and rapid, but they are less sensitive and selective for target MCs [17, 20, 21]. A method based on direct injection combined with liquid chromatography tandem mass spectrometry (LC–MS/MS) is recommended by the United States Environmental Protection Agency for the detection of six MC congeners (MC-LA, MC-LF, MC-LR, MC-LY, MC-RR, and MC-YR) in drinking water samples [22]. However, the poor sensitivity of this method is a limitation. In addition, the ability to characterize individual congeners present in a given sample is limited by the availability of analytical standards. Other detection methods have been utilized for analyte separation, with UV absorbance commonly employed, but limitations include low sensitivity (with an LOD of $70\text{--}300 \mu\text{g L}^{-1}$),

specificity, and interference from complex matrices [23–26]. Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) has been developed to enhance the sensitivity and specificity of the analytical methods for the detection and quantification of MCs in water samples and complex matrices.

MCs are a potential threat to aquatic animals, plants, and human health, and many types of MCs have been detected at concentrations ranging from several ng L^{-1} to several $\mu\text{g L}^{-1}$. Accurately determining the concentration of MCs is of dire significance for protecting human health and studying the mechanism of bloom outbreaks. The existing methods have limitations depending on the detection type, such as varying detection limits and complex operation; thus, for practicality, a set of scientific and efficient methods for the analysis and detection of MCs in water must be established. The effects of the online-SPE column, membranes, mobile phase, and gradient-elution procedures were fully considered in our study, and water samples from typical areas of Chaohu Lake were also collected to (1) establish a sensitive and accurate analytical method for quantifying eight different MCs (MC-LA, MC-LF, MC-LR, MC-WR, MC-LW, MC-LY, MC-RR, and MC-YR) in freshwater systems using online-SPE coupled with UPLC–MS/MS; and (2) verify the feasibility of the detection method in typical areas.

Materials and methods

Chemicals and standards

Methanol and acetonitrile were purchased from J.T. Baker (USA). All standards of MC-LA, MC-LF, MC-LR, MC-WR, MC-LW, MC-LY, MC-RR, and MC-YR (95% purity) were obtained from BePure, China. The internal standard leucine enkephalin was purchased from Zhenzhun Biologicals, China. Formic acid was obtained from Waters, USA. Ultrapure water was produced using a Milli-Q water purification system (USA).

MC standard solutions were diluted to 1 mg L^{-1} with methanol, placed in a brown glass bottle, and stored at -20°C . The 1 mg L^{-1} MC solution was diluted to the required concentration before each experiment. The leucine enkephalin internal standard solution was diluted to $50 \mu\text{g L}^{-1}$ with methanol/water (1:1, v/v) and stored at -3°C .

Mixed calibration standard solutions were serially diluted to 1000, 500, 200, 100, 50, 20, and 10 ng L^{-1} with methanol before use, and then stored at -20°C .

Operating conditions of UPLC–MS/MS

The sample was introduced into the injection loop and transferred to an online-SPE column for analyte preconcentration. The online aqueous mobile phase continued to flow after sample loading to ensure matrix and salt

removal. The analyte was eluted by back-flushing the online-SPE column using the UPLC mobile phase and separated by the chromatographic column prior to MS/MS detection.

Eight different MCs were analyzed using an ACQUITY UPLC system coupled to a Xevo TQ-MS (triple-quadrupole MS/MS) mass spectrometer (Waters, Manchester, UK). The analytical column and SPE column were ACQUITY UPLC BEH C18 (1.6 μm , 2.1 mm \times 50 mm) and XBridgeC8 Direct Connect HP (10 μm , 2.1 mm \times 30 mm), respectively. The system was operated in electrospray positive mode (ESI+), with a capillary voltage of 3.70 kV, source and desolvation temperatures of 150°C and 500 °C, respectively, desolvation gas flow rate of 1000 L h⁻¹, cone hole backflush gas pressure of 30 V, cone hole backflush gas flow rate of 50 L h⁻¹, and collision gas flow rate of 0.06 mL min⁻¹. All parameters were optimized to provide the best sensitivity for all analytes. Detection and quantification were achieved using targeted analysis via positive ion scanning and multiple-reaction monitoring. The other mass spectrometer parameters are listed in Table 1.

Optimization of chromatographic conditions

Several gradients were investigated to optimize the peak resolution and sensitivity and to minimize the run time, which involved altering the flow rate, gradient, and concentration of acetonitrile. The MC congeners were detected on a triple-quadrupole mass spectrometer using multiple-reaction monitoring with transitions optimized manually to achieve maximum sensitivity.

SPE column

The separation efficiency of online-SPE columns depends on their retention of MCs. The fillers used in the online-SPE column are usually silica gel as the matrix and a bonded phase with relatively weakly polar functional groups. The more polar components of the sample elute

from the online-SPE column first, whereas the less polar components are retained on the column. Therefore, the online-SPE column with reverse-phase bonded, weakly polar silica gel adsorbent is optimal for the separation of MCs. Therefore, we evaluated the separation efficiency of XBridge C8 and Oasis HLB C18 by comparing the chromatographic resolution of eight different MCs (20 ng L⁻¹). Compared with the commonly used Oasis HLB Direct Connect HP (20 μm , 2.1 mm \times 30 mm), XBridge C8 is used for reversed-phase extraction, which is more suitable for nonpolar to medium-polarity target compounds. The online-SPE column used in this study was the XBridge C8 Direct Connect HP (10 μm , 2.1 mm \times 30 mm) to retain the strongly polar substances of eight different MCs eluted in the void volume of the Oasis HLB C18 column.

Water spiking with internal standard and filter membranes

An appropriate internal standard can balance the change in the signal response intensity of the analyte caused by matrix interference under certain conditions and reduce the interference of the analyte loss during sample pretreatment [27, 28]. Leucine enkephalin is used as the internal standard for the determination of MC. Xu et al. showed that MCs and leucine enkephalin can be separated well, and their recovery rates are similar owing to their similar structures [29].

Ultrapure water samples need to be filtered before analysis to protect the instruments and reduce matrix effects. However, MCs can be lost because hydrophilic filter membranes can partially absorb MCs through hydrogen bonds. Therefore, the recovery of three different membranes was evaluated in this study: polyethersulfone (PES), polytetrafluoroethylene (PTFE), and mixed cellulose ester (MCE). During the membrane evaluation experiment, the MC concentration was 50 ng L⁻¹, and each membrane was evaluated four times in parallel.

Table 1 Compound-dependent MS/MS parameters of eight MC congeners

Compound	Precursor ion (<i>m/z</i>)	Qualitative ion (<i>m/z</i>)	Quantitative ion (<i>m/z</i>)	Cone energy (V)	Collision energy (eV)
MC-LR	995	135	213	85	75
MC-YR	1046	135	213	85	85
MC-RR	520	135	440	42	32
MC-WR	1069	135	213	60	95
MC-LA	911	135	776	40	80
MC-LF	986	135	213	42	68
MC-LY	1003	135	985	45	80
MC-LW	1026	135	213	45	65

Mobile phases

The two components of the mobile phase were termed as “A + B” in this study. Six different mobile phases were evaluated: (1) methanol + water; (2) acetonitrile + water; (3) methanol with 0.1% (v/v) formic acid + water with 0.1% (v/v) formic acid; (4) acetonitrile with 0.1% (v/v) formic acid + water with 0.1% (v/v) formic acid; (5) acetonitrile with 0.25% (v/v) formic acid + water with 0.1% (v/v) formic acid; (6) acetonitrile with 0.5% (v/v) formic acid + water with 0.1% (v/v) formic acid. The separation efficiency was evaluated by comparing the peak intensities of the eight different MCs with the column maintained at 35 °C.

Chromatographic resolution (R) was used to characterize the degree of separation of two adjacent chromatographic peaks, which is equal to the ratio of the difference between the retention times of adjacent chromatographic peaks (t_1 and t_2) and the average peak width of the two chromatographic peaks (w_1 and w_2), as shown in Eq. (1):

$$R = \frac{t_2 - t_1}{\frac{1}{2}(w_1 + w_2)} = \frac{2(t_2 - t_1)}{w_1 + w_2}, \quad (1)$$

where t_1 and t_2 are the retention times of the first and second peaks, respectively, and w_1 and w_2 are the widths of the first and second peaks, respectively.

When $R < 1$, the two peaks overlap; when $R = 1$, the resolution can reach 98%, and when $R = 1.5$, the resolution can reach 99.7%. When $R = 1.5$, two adjacent components are usually considered to be completely separated.

Gradient elution procedures

Four gradient-elution procedures were employed: (1) the water phase was held at 100% for 4.1 min, followed by a decrease to 0% over 2.9 min, and then washed for 4 min at 100% before the next injection; (2) the water phase was held at 98% for 4.6 min, followed by a decrease to 25% over 5.4 min, and then washed for 2 min at 98% before the next injection; (3) the water phase was held at 95% for 4.1 min, followed by a decrease to 60% over 1.9 min and another decrease to 5% over 3 min, and then washed for 3 min at 95% before the next injection; and (4) the water phase was held at 95% for 6 min, followed by a decrease to 5% over 3 min, and then washed for 2 min at 95% before the next injection.

Method validation

Seven concentration sequences (10, 20, 50, 100, 200, 500, and 1000 ng L⁻¹) of the standard MC solution were detected according to the finalized method. Leucine enkephalin (10 ng L⁻¹) was added as the internal standard, and the authenticity and absolute recovery of the analyte were calculated. A linear regression and standard

curve were applied with the injection concentration (x) corresponding to the peak area (y).

Three mixed standard solutions with high (500 ng L⁻¹), medium (100 ng L⁻¹) and low (20 ng L⁻¹) concentrations were added to the blank water samples. After filtration by using a 0.22-μm MCE membrane, the concentrations of the target substances in the water samples were determined by online-SPE UPLC–MS/MS. Six samples of each concentration were taken for parallel experiments, and the recovery rate and relative standard deviation (RSD) were calculated. The LOD (ng L⁻¹) and LOQ (ng L⁻¹) were calculated using signal-to-noise ratios (SNRs) of 3 and 10 based on the lower-end calibration curve levels. The accuracy values were calculated as the average value of the recovery for concentrations of 20, 100, and 500 ng L⁻¹, and the precision values were calculated as the average RSD for concentrations of 20, 100, and 500 ng L⁻¹.

Water samples for method development

Twelve water samples (CH1, CH2, ...CH12) collected from Chaohu Lake (Anhui Province, China) in August 2020 were used to validate the method. At least 12 zones in Chaohu Lake were chosen for water sample collection, and 500 mL of water was collected from depths of 0–50 cm in each zone after the surface scum was removed. Then, 100 mL of each water sample was filtered in situ using a 0.45-μm cellulose acetate filter membrane (JiuDing, China) in a 120-mL polypropylene bottle. The samples were then placed in a cooler with ice packs and transferred to the laboratory for further treatment. The 12 sampling points are shown in Fig. 1.

Each water sample of 20 mL was filtered using a disposable medical syringe coupled with a 0.22-μm filter in the injection vials; each sample was spiked with 10 ng L⁻¹ of internal standard prior to injection. To quantify the MCs, a seven-point mixed standard calibration curve in the range of 10–1000 ng L⁻¹ was created based on an initial sample size of 20 mL.

Results and discussion

Online-SPE column selection

Chromatograms of the mixed standard solutions obtained using the Oasis HLB C18 column and XBridge C8 column are shown in Fig. 2.

A comparison of the separation effect of the two chromatographic columns revealed that MC-LA, LY, LW, and LF (4/8) were unsuccessfully eluted from the Oasis HLB C18 column because these four substances are more polar than the others, which makes their elution from the column more difficult, as they are more strongly adsorbed; thus, the chromatographic peak is not completely separated. However, The XBridge C8 column separated all congeners well. The resolutions of the

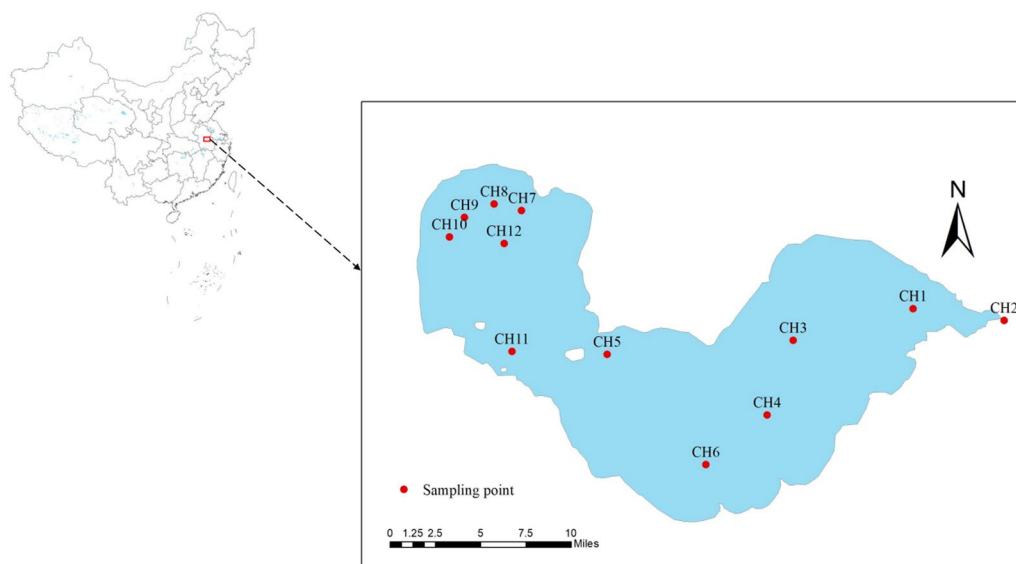


Fig. 1 Sampling distribution in Chaohu Lake

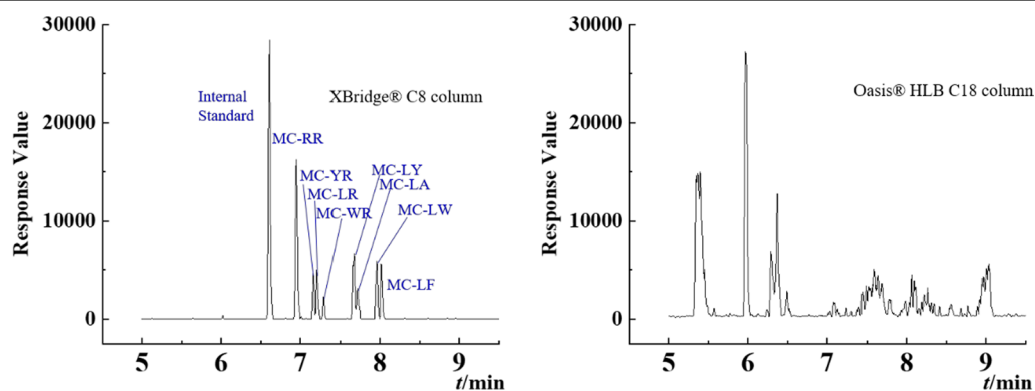


Fig. 2 Chromatograms of mixed standard solution using two different columns

chromatographic peaks were as follows: 2.47, 0.73, 1.13, 5.43, 1.11, 4.36, and 0.83. The two peaks of MC-YR & MC-LR and MC-LF & MC-LW overlapped slightly. The resolution between the peaks of the other adjacent substances was >1 , indicating good separation. The Oasis HLB C18 column cannot effectively elute and separate the four MCs with similar polarities. Owing to the differences in the internal structure of the column packing, the C18 column had better retention characteristics than the C8 column. Therefore, the C8 column is more suitable for the analysis of macromolecular substances and often used to analyze and separate substances with lower molecular weights. Although the Oasis HLB C18 column did not separate the four MCs efficiently in this study, the separation time (8.4 min) was 4 min shorter than those

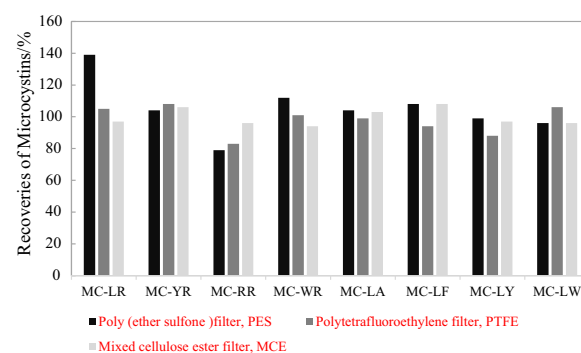


Fig. 3 Recovery (%) of MCs using different membranes (pore size: 0.22 μm)

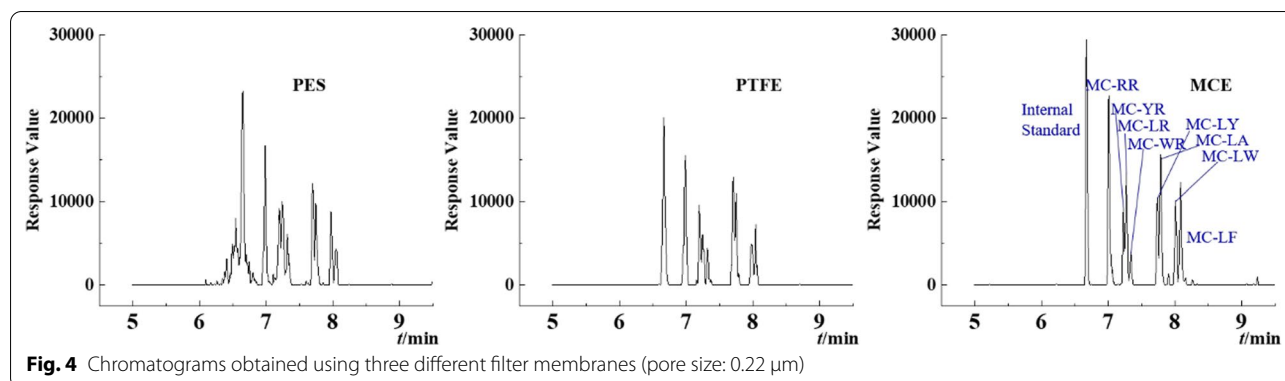


Fig. 4 Chromatograms obtained using three different filter membranes (pore size: 0.22 μm)

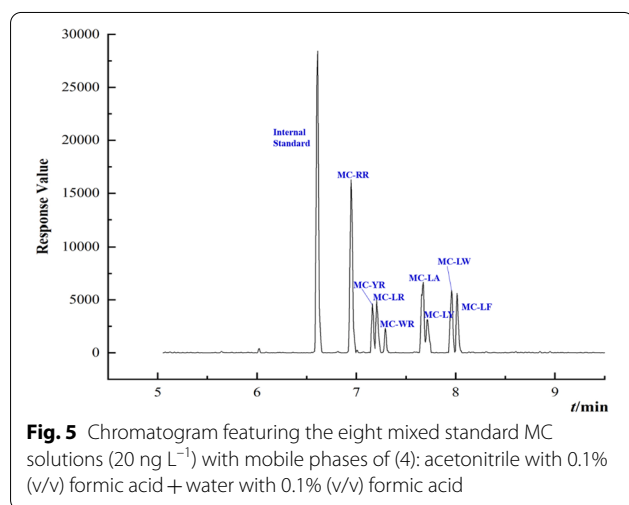


Fig. 5 Chromatogram featuring the eight mixed standard MC solutions (20 ng L^{-1}) with mobile phases of (4): acetonitrile with 0.1% (v/v) formic acid + water with 0.1% (v/v) formic acid

achieved using previously developed methods [10, 30, 31].

Selection of membranes

The filter membranes removed eight different MCs efficiently from water with recovery rates of 79–108% (Fig. 3). For reference, the US EPA criterion is 70–130% [22, 32]. The chromatograms obtained under different filter membrane conditions are shown in Fig. 4. The MCE filter retained the lowest amount of MCs compared to the PES and PTFE filters because the MCs recovery rates were greater than 90%. A similar pattern of recovery also indicated that the MCs were stable on the MCE. Thus, the MCE filter membrane was selected as the optimal membrane for this study. Note that the recovery of MC-RR is lower than that of the other congeners because MC-RR contains two arginine molecules, unlike the other congeners. This increases the formation of cation bridges with the protonated arginine molecule, which is a well-known mechanism for binding onto the surface of the organic phase [33]. Moreover, protonated arginine

can form additional hydrogen bonds with the surface of the MCE membrane. Consequently, MCE retains MC-RR during filtration, which also explains the lower recovery rate of MC-RR in this study.

Determination of mobile phase

In this study, acetonitrile and ultrapure water were selected as the mobile phases of the online-SPE system, and acetonitrile with formic acid, along with water with formic acid were selected as the mobile phases of the UPLC system. The chromatograms of the mobile phase obtained under the six different conditions are shown in Fig. 5.

The chromatographic peak separation of the eight different MCs was observed under six different conditions. Acetonitrile, as the organic phase can simultaneously separate eight substances better than methanol with a higher response value. A comparison of the chromatograms obtained with different concentrations of formic acid in acetonitrile showed that the effect was the strongest at 0.1% formic acid. The Oasis HLB C18 column cannot effectively elute and separate the four MCs with similar polarities because the addition of formic acid in the mobile phase facilitates the protonation of positive ions and maintains the ionization state of the sample in the mobile phase. Therefore, we chose 0.1% formic acid + acetonitrile as the organic phase in the mobile phase.

Determination of gradient-elution procedures

The gradient-elution procedure of the mobile phase affects the retention time, peak shape, and peak resolution of the chromatogram. Procedure (1) which was previously described, was determined to be the most suitable, resulting in the best chromatographic resolution and material peak intensity. Therefore, this procedure was selected for the experiments. The chromatogram of the eight different MCs under this condition is shown in Fig. 6.

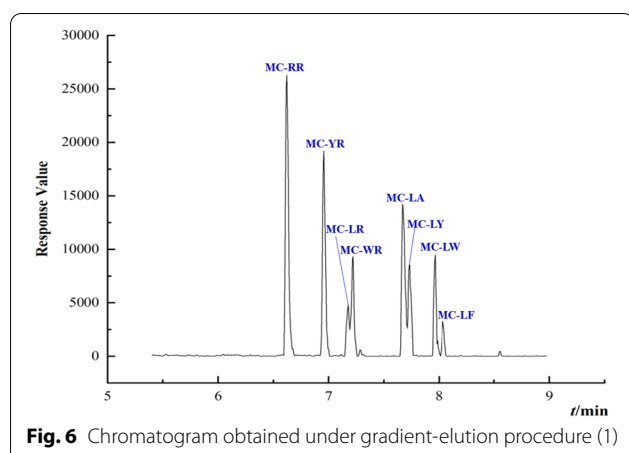


Fig. 6 Chromatogram obtained under gradient-elution procedure (1)

Recovery of MCs from ultrapure water

For the eight different MCs, the absolute recovery rates ranged from 40.9 to 135%. The recoveries of MC-RR and MC-WR were relatively low, with recovery rates ranging from 58.9 to 74.1% and 40.9% to 78.4%, respectively. These lower recovery rates may have been caused by

the adsorption of the MCE membrane. By contrast, the recovery rates of MC-LA and MC-LF at medium, and high concentrations were relatively high; this may be due to the influence of the matrix during testing. Except for MC-RR and MC-WR, the recovery rates of all other MCs meet the standard. As shown in Table 2, the RSDs of the measured values of the low-, medium-, and high-concentration standard samples are in the range of 0.88–9.83%, 6.43–9.72%, and 2.10–9.83%, respectively, meeting the daily measurement requirements.

Analytical method validation

The seven-point calibration curves ranging from 10 to 1000 ng L⁻¹ was fitted by using linear regression with large coefficients of determination ($R^2=0.990$ – 0.995) over a linear range spanning 2–3 orders of magnitude (Table 3). The LODs for the optimized chromatographic conditions were 0.02–0.37 ng L⁻¹, whereas the LOQs ranged from 0.07 to 1.24 ng L⁻¹. The accuracy of the MC standard solution was 86.4%, which falls within the acceptable range of accuracy based on the US EPA Method 544. The precision of the samples was 7.26%.

Table 2 Experimental recovery parameters ($n=6$)

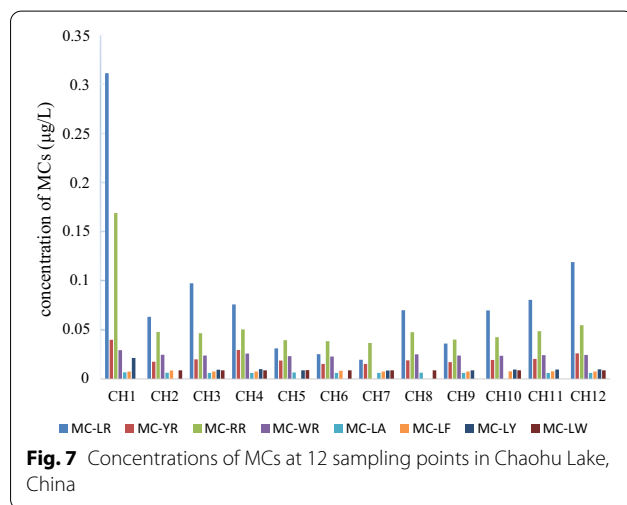
Compound	20 ng L ⁻¹		100 ng L ⁻¹		500 ng L ⁻¹	
	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
MC-LR	6.26	96.2	8.49	94.2	7.08	80.3
MC-YR	0.88	81.7	6.43	92.2	4.87	85.8
MC-RR	5.61	74.1	7.38	64.8	2.10	58.9
MC-WR	8.86	78.4	6.49	45.1	8.22	40.9
MC-LA	9.83	79.5	7.26	85.7	9.39	135.9
MC-LF	8.21	67.6	9.67	134.9	9.83	106.5
MC-LY	7.49	87.8	6.46	93.1	9.38	78.2
MC-LW	7.04	71.4	9.72	103.6	7.21	104.4

Table 3 Method parameters for MCs under the optimized chromatographic conditions and quantified by online-SPE UHPLC–MS/MS

Compound	Retention time (min)	Linear range (ng L ⁻¹)	R ²	SNR	LOQ (ng L ⁻¹)	LOD (ng L ⁻¹)	Accuracy (%)	Precision (%)
MC-LR	7.20	10–1000	0.991	423	0.24	0.07	90.2	7.28
MC-YR	7.16	10–1000	0.993	510	0.20	0.06	86.6	4.06
MC-RR	6.95	5–500	0.995	753	0.07	0.02	65.9	5.03
MC-WR	7.29	50–1000	0.992	405	1.24	0.37	54.8	7.86
MC-LA	7.67	10–1000	0.991	458	0.22	0.07	100	8.83
MC-LF	8.01	10–1000	0.995	365	0.27	0.08	103	9.24
MC-LY	7.72	10–1000	0.990	202	0.50	0.15	86.4	7.78
MC-LW	7.96	10–1000	0.994	530	0.19	0.06	120	7.99

Table 4 Concentrations (ng L⁻¹) of MCs in water from Chaohu Lake, China

Compound	Mean value (ng L ⁻¹)	Concentration range (ng L ⁻¹)	Detected frequency (%)
MC-LR	83	19–119	100
MC-YR	21	15–40	100
MC-RR	55	37–169	100
MC-WR	24	23–29	91.7
MC-LA	6	5.8–6.4	91.7
MC-LF	7	7–8	83.3
MC-LY	10	8–21	75
MC-LW	9	8.5–8.7	75

**Fig. 7** Concentrations of MCs at 12 sampling points in Chaohu Lake, China

Application of the optimized method to quantify MCs in lake water

The optimized method was used to analyze MCs in 12 water samples collected from Chaohu Lake, China; eight different MCs were detected. Among the 12 lake water samples, the detected frequency (DF) of MC-LR, MC-YR, and MC-RR were the highest, with a value of 100%; MC-WR and MC-LA were detected both in 11 samples (with a DF value of 91.7%). MC-LF exhibited the third-highest DF of 83.3%; and the DF values of MC-LY and MC-LW were both 75%. The mean concentrations of the total MCs ranged from 101 to 590 ng L⁻¹, whereas the total concentration level was below the WHO limit of 1000 ng L⁻¹ for MC-LR (Table 4 and Fig. 7). For the single index, the top three substances with the highest mean concentrations were MC-LR (83 ng L⁻¹), MC-RR (55 ng L⁻¹), and MC-WR (24 ng L⁻¹), respectively.

Conclusions

Automated sample preparation can considerably reduce the material and labor required to produce a sample for analysis. Online-SPE and high SNR significantly reduced the amount of labor and sample size required for the analysis compared to offline sample enrichment protocols. For all analytes, excellent linearity was observed for samples with concentrations of 10–1000 ng L⁻¹ ($r^2 \geq 0.99$). The RSD values were all lower than 10%.

The method of determination after optimizing the chromatographic conditions of online-SPE UPLC–MS/MS was robust and reliable for eight different MCs. This method showed high sensitivity and high resolution for the separation of eight MC congeners with LODs ranging from 0.020 to 0.371 ng L⁻¹ and LOQs ranging from 0.066 to 1.235 ng L⁻¹, which meet the requirements of the WHO control standards for MCs. This UPLC–MS/MS method is fast and sensitive because of the simple preprocessing steps and effectively reduces interference in the surrounding environment.

The developed method has the characteristics of high pretreatment efficiency, strong selectivity; and high sensitivity; meets the requirements for the detection of eight different MCs in surface water; provides a reference for early-warning forecasts of MCs; and is a key to ensuring the safety of drinking water sources.

Abbreviations

Online-SPE UPLC–MS/MS: Online solid-phase extraction-ultra performance liquid chromatography coupled with tandem mass spectrometry; MCs: Microcystins; WHO: World Health Organization; IARC: International Agency for Research on Cancer; ELISA: Enzyme-linked immunosorbent assay; LC–MS/MS: Liquid chromatography with tandem mass spectrometry; UPLC–MS/MS: Ultra-performance liquid chromatography coupled with tandem mass spectrometry; SPE: Solid-phase extraction; PES: Polyethersulfone; PTFE: Polytetrafluoroethylene; MCE: Mixed cellulose ester filters; ESI+: Electrospray positive mode; RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification; SNR: Signal-to-noise ratios.

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Authors' contributions

Conceptualization, XQ, SG, YL, XL; methodology, SG, CL, XZ; formal analysis, XQ, SG, YL, XL and XZ; investigation, XQ, SG, LJ, RZ, TQ, YL; resources, YL, XZ; data curation, SG, RZ, TQ, YL; writing—original draft preparation, XQ, SG; writing—review and editing, YL, XQ, and XZ; visualization, SG; supervision, YL; project administration, YL; funding acquisition, YL. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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