Method Development and Validation for Simultaneous Quantification of Microcystin Congeners in Water

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Research

Keywords: Antibiotic resistance genes, Drinking water reservoir, Temporal and spatial variations

Posted Date: June 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-630260/v1

Version of Record: A version of this preprint was published at Environmental Sciences Europe on November 12th, 2021. See the published version at https://doi.org/10.1186/s12302-021-00556-1.

Abstract

Background: Microcystins, as secondary metabolites of cyanobacteria, are hepatotoxic to humans through the ingestion of cyanobacteria-contaminated water. Microcystins with diverse congeners in water can be precisely quantified using online solid phase extraction-ultra performance liquid chromatography-tandem mass spectrometry (online-SPE UPLC-MS/MS). A method was developed and validated to simultaneously quantify eight microcystin congeners 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. Linearity, accuracy, and precision were validated on matrix-mixed water with the leucine enkephalin internal standard. The limit of detection calculated using the signal-to-noise ratio of 3 passed the surface water daily inspection for microcystins. Except for the lower recovery of individual substances at individual concentrations, the recoveries of the remaining microcystin congeners ranged from 70 to 130%, and the relative standard deviation was less than 10%.

Conclusion: The method was used to analyze microcystins in 12 water samples collected from Chaohu Lake. The sum of all microcystin congeners ranged from 101 to 585 ng L^{-1} in water (<WHO drinking water safe limit of 1 μg L^{-1} for microcystin-LR).

Background

Microcystins (MCs) are cyanotoxins produced by many species of cyanobacteria[1]. Chemically, microcystins are monocyclic heptapeptides with a general structure of cyclo-(-D-Ala^1-L-X^2-DisoMeAsp^3-L-Z^4-Adda^5-D-isoGlu^6-Mdha^7), where D-MeAsp^3 is D-erythro-b-methylaspartic acid, Mdha^7 is N-methyldehydroalanine, and Adda^5 is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4, 6-dienoic acid. X^2 and Z^4 are multiple substituted amino acids that result in at least 279 variants of microcystins in nature[2]. Microcystins are highly toxic because they are a specific inhibitor of serine/threonine protein phosphatases that induce the breakdown of the cellular cytoskeleton and promote the growth of tumors. The greatest environmental risk associated with microcystins is from microcystins-contaminated water, which causes animal poisoning and hepatotoxicity in humans. Previous epidemiological studies correlated the high incidence of developing primary liver cancer in southern China with the intake of microcystin-contaminated drinking water [3, 4].

Globally, among all of these MC congeners, MC-LR is the most common and most studied variant in water samples, and it is currently considered to be the reference compound because of its high and acute toxicity and frequent occurrence. The guideline value for MC-LR in drinking water (1.0 μg L^{-1}) has been established by the World Health Organization [5]. 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. Recent research concerning the bioaccumulation and toxicity of cylindrospermopsin, an inhibitor of protein synthesis, has indicated that it should also be classified as a potential carcinogen due

to its proved cytotoxicity and genotoxicity. However, blooms frequently contain different MC variants at the same time. Graham et al. detected different cyanotoxin types in 48% of cyanobacterial blooms, 95% of which had multiple MC variants [6]. A greater variety of MC congeners were detected in China where most of the studies were carried out[6 ~ 10]. From August 2011 to July 2012, a survey of drinking water sources in the eastern part of Chaohu Lake showed that the total concentration of microcystins reached 8.86 μg L^{-1} [11].

The overall structure of MCs is relatively hydrophilic (polar), which comes from carboxylic acids at positions 3 and 6 and frequent occurrence of arginine at positions 2 and 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 about the occurrence and toxicity of all these MCs other than MC-LR are limited[14, 15]. The increasing number of structural toxin congeners also 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] ;. ELISA kits used to determine algal toxins are relatively inexpensive, simple, and rapid, but they are less sensitive and selective for target algal toxins [17, 20, 21]. The method based on direct injection coupled with liquid chromatography with tandem mass spectrometry (LC-MS/MS) for the detection of six MC congeners (MC-LA, MC-LF, MC-LR, MC-LY, MC-RR, and MC-YR) in drinking water samples is recommended by the United States Environmental Protection Agency [22], but poor sensitivity of this method is one of the limitations. Besides, the ability to characterize the 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, specificity, and interferences from complex matrices [23 ~ 26]. To enhance the sensitivity and specificity of the analytical methods for the detection and quantification of MCs in water samples and complex matrices, ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) has been developed. Note that the pretreatment process of water samples prior to using UPLC–MS/MS is complicated and prone to errors.

Microcystins, as secondary metabolites of cyanobacteria, are hepatotoxic to humans through the ingestion of cyanobacteria-contaminated water. Domestic detection methods for MCs are generally limited to one or two, therefore, it is urgent to study a rapid and accurate detection method to ensure the safety of drinking water for residents. The principal objectives were as follows: 1) selected the best SPE column between two columns, mobile phase in six conditions, and best gradient elution procedures to establish a sensitive and accurate analytical method for quantifying eight MCs (MC-LA, MC-LF, MC-LR, MC-WR, MC-LW, MC-LY, MC-RR, and MC-YR) in freshwater systems using online solid phase extraction (SPE) coupled with UPLC-MS/MS; 2) tested the recoveries of these compounds from water and analytical merits such as accuracy and precision ; 3) applied the method to analyze microcystins in 12 freshwater samples collected in Chaohu Lake, China.

Materials And Methods

Chemicals and standards

Methanol, acetonitrile, and deionized water 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, Fig. 1) were obtained from BePure (China). The internal standard leucine enkephalin was purchased from Zhenzhun Biologicals, China. Stock standard solutions of microcystins were prepared in methanol in an amber glass container and stored at − 20°C in the dark for a maximum of 24 months. The 1 mg microcystins/L working solution from the stock solutions was prepared daily in methanol.

Eight MCs stock solutions were prepared by mixing MC standards with methanol. MC solutions were prepared in different concentrations by adding desired volumes of stock solution to methanol. The prepared standard solutions with a concentration of 1.0 mg L^{-1} were stored and removed in the dark at − 20°C. The leucine-enkephalin internal standard solution (50 μg L^{-1}) was prepared in methanol/water (1:1, v/v) and stored at − 3°C.

Optimization of chromatographic conditions

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

Solid phase extraction (SPE) column

The separation efficiency of online SPE columns depends on their retainability to microcystins. Microcystins are polar substances, and chromatographic columns with strong color fillers are optimal for the separation of macromolecular substances. Therefore, we evaluated the separation efficiency of XBridge® C8 and Oasis® HLB C18 by comparing the chromatographic resolution of eight microcystin congeners (20 ng L^{-1}). Compared with the commonly used Oasis® HLB Direct Connect HP (20 μm, 2.1 mm×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 is XBridge® C8 Direct Connect HP (10 μm, 2.1mm ×30 mm) to retain the strong polar substances of eight MCs eluted in the void volume of the Oasis® HLB C18 column.

Chromatographic resolution (R) is used to characterize the degree of separation of two adjacent chromatographic peaks, which equals 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) (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 is the retention times of first peak, t_2 is the retention times of second peak, w_1 *is* the width of the first peak, w_2 is the second peak.

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%. Usually R = 1.5 is used as a sign that two adjacent components have been completely separated.

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 loss of analyte during sample pretreatment[27, 28]. Leucine enkephalin is considered an internal standard for microcystin determination. Xu et al. showed that microcystins and leucine enkephalin can be separated well and their recovery rates are similar owing to their similar structure [29].

Pure 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 absorb partial MCs through hydrogen bonds. Therefore, the recovery of three different membranes were evaluated in this study: polyethersulfone (PES), polytetrafluoroethylene (PTFE), and mixed cellulose ester (MCE) filters.

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 intensity of eight microcystin congeners with the column maintained at 35°C.

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, 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, 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 followed by another decrease to 5% over 3 min, washed for 3 min at 95% before the next injection; (4) the water phase was held at 95% for 6 min, followed by a decrease to 5% over 3 min, washed for 2 min at 95% before the next injection.

Operating conditions of UPLC-MS/MS

The sample was introduced into the injection loop and transferred to the 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 by the UPLC mobile phase and separated by the chromatographic column prior to MS/MS detection. The mobile phase of the binary pump was applied as described in Sect. 2.2.3.

Analysis of eight MCs was performed using an ACQUITY UPLC system coupled to a Xevo TQ-MS (triple quadrupole MS/MS) mass spectrometer (Waters, Manchester, UK). The analytical column and solid phase extraction column were ACQUITY UPLC® BEH C18 (1.6 μm, 2.1 mm × 50 mm) and XBridge®C8 Direct Connect HP (10 μm, 2.1 mm × 30 mm), respectively. The system was operated in the electrospray positive mode (ESI+), with the capillary voltage of 3.70 kV, source and desolvation temperatures of 150 and 500°C, respectively, desolvation gas flow 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 of 0.06 mL min^{-1}, optimized to produce the best sensitivity across all analytes. Detection and quantification were achieved using targeted analysis via positive ion scanning and multiple reaction monitoring. Other mass spectrometer parameters are presented in Table 1.

The flow rate was set at 0.4 mL min^{-1} with the organic phase held at 2% for 8.1 min, followed by an increase to 75% over 2.1 min, washed for 0.8 min, and returned to 2%. The mobile phases of the quaternary pump included pure water (mobile phase A) and acetonitrile (mobile phase C). The flow rate was kept at 2.0 mL min^{-1} with the water phase held at 100% for 4.1 min, followed by a decrease to 0 over 2.9 min, washed for 4 min at 100% before the next injection. The injection volume was set at 5 mL.

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

Compound	Precursor ion (*m/z*)	Quantification fragment (*m/z*)	Confirmation fragment (*m/z*)	Cone energy (V)	Collision energy (eV)
MC-LR	995.4	135	213	85	75
MC-YR	1045.6	135	213	85	85
MC-RR	519.7	135	440.4	42	32
MC-WR	1068.5	135	213	60	95
MC-LA	910.5	135	776	40	80
MC-LF	986.3	135	213	42	68
MC-LY	1002.6	135	985	45	80
MC-LW	1025.53	135	213	45	65
* Quantitative ion					

Method validation

To validate the method, a seven-point matrix of standards ranging from 0.01 to 1.00 ng mL^{-1} was configured to match the calibration curve. The microcystin standard stock solution was serially diluted step by step to 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, and 1.00 ng mL^{-1}. Sequential determination was performed under chromatographic analysis conditions. Linear regression was applied with the injection concentration (x) corresponding to the corresponding peak area (y). The internal standard of leucine enkephalin was added to 0.01 ng mL^{-1}, and the authenticity and absolute recovery of the analyte were calculated.

Low-, medium-, and high-concentration standard samples were measured six times in parallel according to the above method, and the average recovery rate and relative standard deviation (RSD) were calculated. The limit of detection (LOD, ng g^{-1}) and limit of quantification (LOQ, ng g^{-1}) were calculated using the signal-to-noise ratios of 3 and 10 based on the lower-end calibration curve levels. These values were calculated for 2 mL injection, which is equivalent to 2 pg injected on the column.

Six samples were combined into one batch; three batches of standards were added and recovery experiments were conducted using purified water; blank samples were added for quality control. The specific operation method described in Sect. 2.5 sets three concentrations of 0.05, 0.10, and 0.50 ng mL^{-1} to test and calculate the recovery rate. The precision and recovery results are presented in Table 3.

Water samples for method development

We used pure water to optimize the chromatographic conditions (herein referred to as water MO), and 12 water samples (CH1, CH2, …CH12) collected from Chaohu Lake (Anhui Province, China) in August 2020 were used to validate the method. We chosed at least 12 zones in Chaohu Lake, used a water harvester to remove the surface scum, and collected ~ 500 mL water sample in bottles from each zone from the 0–50 cm depth. Next, ~ 100 mL of each water sample was in-situ filtered using a 0.45 μm cellulose acetate filter membrane (JiuDing, China) to a 120 mL polypropylene bottle. The samples were then placed in a cooler with ice packs and transferred to the laboratory for further treatment. Twelve sampling points are shown in Fig. 1.

After 20 mL of water MO was filtered using a disposable medical syringe coupled with a 0.22 μm filter in the injection vials, 10 ng L^{-1} internal standard was spiked to each sample prior to injection. To quantify MCs, a seven-point mixed standards calibration curve in the range of 0.01– 1.00 ng mL^{-1} was created based on the initial sample size of 20 mL.

Results And Discussion

Online SPE column selection

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

The comparison of the separation effect of two chromatographic columns showed that MC-LA, LY, LW, and LF (4/8) were not successfully eluted from the Oasis® HLB C18 column because these four substances are more polarized than the others, which makes it more difficult to elute them when they are adsorbed on the chromatographic column; thus, there is not a completely separated chromatographic peak. However, XBridge® C8 separated all the congeners well. The resolutions between the chromatographic peaks were as follows: 2.47, 0.73, 1.13, 5.43, 1.11, 4.36, 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, which indicates that they separate well. The Oasis® HLB C18 column cannot effectively elute and separate the four MCs with similar polarities. Due to the differences in the internal structure of the column packing, the C18 column has 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 smaller molecular weights. Although the Oasis® HLB C18 column did not separate four MCs efficiently in this study, the separation time (8.4 min) was 4 min shorter than those achieved using the previously developed methods [10, 30, 31,].

Selection of membranes

Filter membranes removed eight microcystin congeners efficiently from water with recovery rates of 79–108% (Fig. 3). For reference, the U.S. EPA criterion is 70–130% [22, 32]. The chromatograms under different filter membrane conditions are shown in Fig. 4. The MCE filter retained the smallest amount of MCs than PES and PTFE filters because microcystin recovery rates were greater than 90%. The similar

pattern of recoveries also indicated that MCE is stable for microcystin congeners. Thus, the MCE filter membrane was selected as the optimal one for this study. Note that the recovery of MC-RR is lower than the other congeners because MC-RR differs from other congeners due to its two arginine molecules. This increases the formation of cation bridges with the protonated arginine molecule, a well-known mechanism for binding onto the surface of the organic phase. 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, pure acetonitrile and pure water were selected as the mobile phases of the online solid phase extraction system, and acetonitrile with formic acid and water with formic acid were selected as the mobile phases of the UPLC system. The chromatograms of the mobile phase obtained under six different conditions are shown in Fig. 5.

The chromatographic peak separation of eight MCs was observed under six different conditions. Acetonitrile as the organic phase can simultaneously separate eight substances better than methanol as the organic phase with a higher response value. The comparison of the chromatograms obtained with different concentrations of formic acid in acetonitrile showed that the effect is 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 in the chromatogram. The (1) procedure described in Sect. 2.2.4 had the strongest peaking effect, which resulted in the best chromatogram resolution and material peak intensity. Thus, this condition was selected for the experiment. Under these conditions, the analysis spectrum of eight MCs is shown in Fig. 6.

Recovery of microcystins from pure water

The average recovery rate was expressed in terms of the spiked concentration (0.01 ng mL^{-1}). The absolute recovery rate of the analyte was 40.89–135.85%. The two microcystins with low recovery rates were MC-RR (recovery rate of 58.88–74.13%) and MC-WR (recovery rate of 40.89–78.41%). Except for the recovery rate of the individual analytes, the remainder of the parameters met the standard. As shown in Table 2, RSD of the measured value of low-, medium-, and high-concentration standard samples ranged from 0.88–9.83%, from 6.43–9.72%, and from 2.10–9.83%, respectively, meeting the daily measurement requirements.

Table 2
Relative standard deviation (n = 6, %).

Compound	50 ng L^{-1}		100 ng L^{-1}		500 ng L^{-1}	
	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
MC-LR	6.26	96.23	8.49	94.21	7.08	80.28
MC-YR	0.88	81.72	6.43	92.24	4.87	85.83
MC-RR	5.61	74.13	7.38	64.75	2.10	58.88
MC-WR	8.86	78.41	6.49	45.07	8.22	40.89
MC-LA	9.83	79.47	7.26	85.68	9.39	135.85
MC-LF	8.21	67.61	9.67	134.87	9.83	106.46
MC-LY	7.49	87.80	6.46	93.10	9.38	78.16
MC-LW	7.04	71.35	9.72	103.59	7.21	104.41

Analytical method validation

The seven-point calibration curves ranging from 0.01 to 1 ng mL^{-1} were fitted via linear regression with large coefficients of determination (R^2 = 0.990–0.995) over a linear range that spanned 2–3 orders of magnitude (Table 3). The LODs for the optimized chromatographic conditions were 0.02–0.37 ng L^{-1}, while LOQs ranged from 0.07 to 1.24 ng L^{-1}. The accuracy of the quality control samples was 86.35%. This falls within the acceptable range of accuracy based on the U.S. EPA Method 544. The precision of the samples was 7.26%.

Table 3

Retention time (min), coefficient of determination (R^2), linear range (ng L^{-1}), signal-to-noise ratio(S/N), limits of detection (LODs), limits of quantification (LOQs), accuracy (%), and precision (%) for microcystins under the optimized chromatographic conditions and quantified by online SPE-UHPLC-MS/MS.

Compound	Retention time (min)	Linear range (ng L^{-1})	R^2	S/N	LOQ (ng L^{-1})	LOD (ng L^{-1})	Accuracy (%)	Precision (%)
MC-LR	7.20	10–1000	0.991	422.84	0.24	0.07	90.24	7.28
MC-YR	7.16	10–1000	0.993	509.81	0.20	0.06	86.60	4.06
MC-RR	6.95	5–500	0.995	753.14	0.07	0.02	65.92	5.03
MC-WR	7.29	50–1000	0.992	404.7	1.24	0.37	54.78	7.86
MC-LA	7.67	10–1000	0.991	458.43	0.22	0.07	100.33	8.83
MC-LF	8.01	10–1000	0.995	365.49	0.27	0.08	102.98	9.24
MC-LY	7.72	10–1000	0.990	201.66	0.50	0.15	86.35	7.78
MC-LW	7.96	10–1000	0.994	530.65	0.19	0.06	119.61	7.99

Application of the optimized method to quantify microcystins in lake water

The mean concentrations of total microcystin ranged from 0.101 to 0.590 μg L^{-1}, whereas the total microcystin level was below the WHO limit of 1.0 μg L^{-1} for MC-LR (Table 4 and Fig. 7). Out of the 12 lake water samples, 11 (91.7%) contained MC-WR and MC-LA, and 10 (83.3%) contained MC-LF. The most common MCs in the lake water were MC-LR, MC-YR, and MC-RR (12/12, 100%), and the least common ones were MC-LY and MC-LW (9/12, 75%). The number of detected species is greater than the previous three species, but the concentration of total microcystins decreased significantly.

Table 4
Concentrations (ng L^{-1}) of microcystins in water from Chaohu Lake, China.

Compound	Mean value (ng L^{-1})	Concentration range (ng L^{-1})
MC-LR	83	19–119
MC-YR	21	15–40
MC-RR	55	37–169
MC-WR	24	23–29
MC-LA	60	5.8–6.4
MC-LF	7	7–8
MC-LY	10	8–21
MC-LW	9	8.5–8.7

Conclusions

Automated sample preparation can greatly reduce the material and labor required to produce a sample for analysis. On-line enrichment and high signal-to-noise ratios significantly reduced the amount of labor and sample size required for the analysis compared to offline sample enrichment protocols. For all analytes, an excellent linearity was observed for 10 to 1000 ng L^{-1} ($r^2 \geq 0.99$). The %RSD values were always lower than 10%.

The method of determination after optimizing the chromatographic conditions of online SPE UPLC-MS/MS was robust and reliable for eight microcystin congeners. This method showed high sensitivity and high resolution in the separation of eight MCs 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 microcystin control standards[5]. This UPLC–MS/MS method is fast and sensitive because of the simple preprocessing steps and reduces the interferences in the surrounding environment effectively.

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

Abbreviations

online solid phase extraction-ultra performance liquid chromatography-tandem mass spectrometry (online-SPE UPLC-MS/MS);

Microcystins (MCs);

International Agency for Research on Cancer (IARC);

liquid chromatography with tandem mass spectrometry (LC-MS/MS);

ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS);

solid phase extraction (SPE);

polyethersulfone (PES);

polytetrafluoroethylene (PTFE);

mixed cellulose ester (MCE) filters;

electrospray positive mode (ESI+);

relative standard deviation (RSD);

limit of detection (LOD);

limit of quantification (LOQ).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Special Research Funding for the Public Benefits sponsored by the Major Science and Technology Program for Water Pollution Control and Treatment (No. 2017ZX07401004).

Authors' contributions

Conceptualization, X.Q., S.G., Y.L., X. L.; methodology, S.G., C.L.,R.Z.; formal analysis, X.Q., S.G., Y.L, X.L. and R.Z.; investigation, X.Q., S.G.，L. J., R.Z.,T.Q., Y.L.; resources, Y.L., R.Z.; data curation, S.G., R.Z., T.Q.,Y.L.; writing—original draft preparation, X.Q., S.G.; writing—review and editing, Y.L., X.Q., and R.Z.; visualization, S.G.; supervision, Y.L.; project administration, Y.L.; funding acquisition, Y.L..

Acknowledgements

None applicable.

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Figures

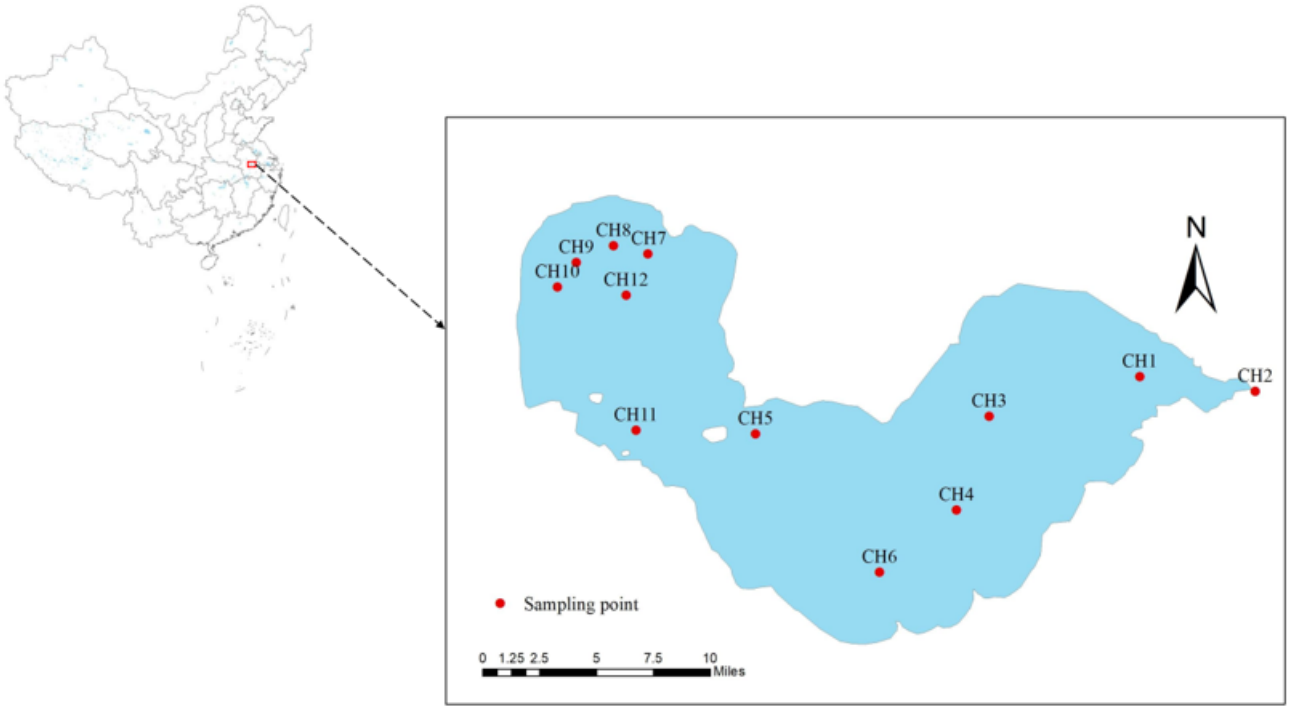

Figure 1

Sampling distribution in Chaohu Lake.

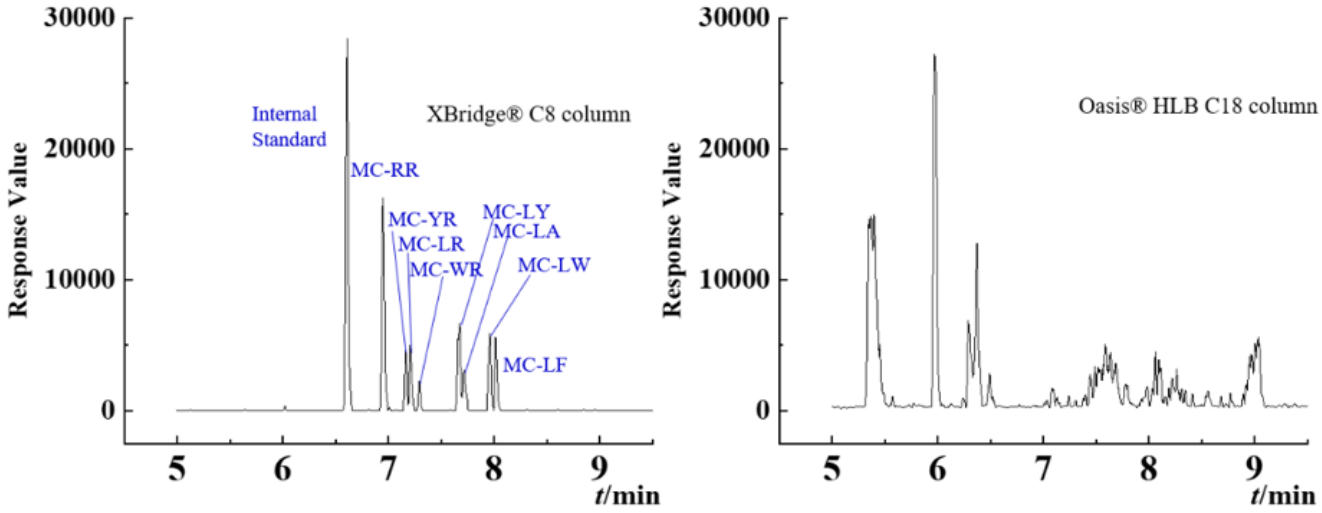

Figure 2

Chromatograms of mixed standard solution using two different columns.

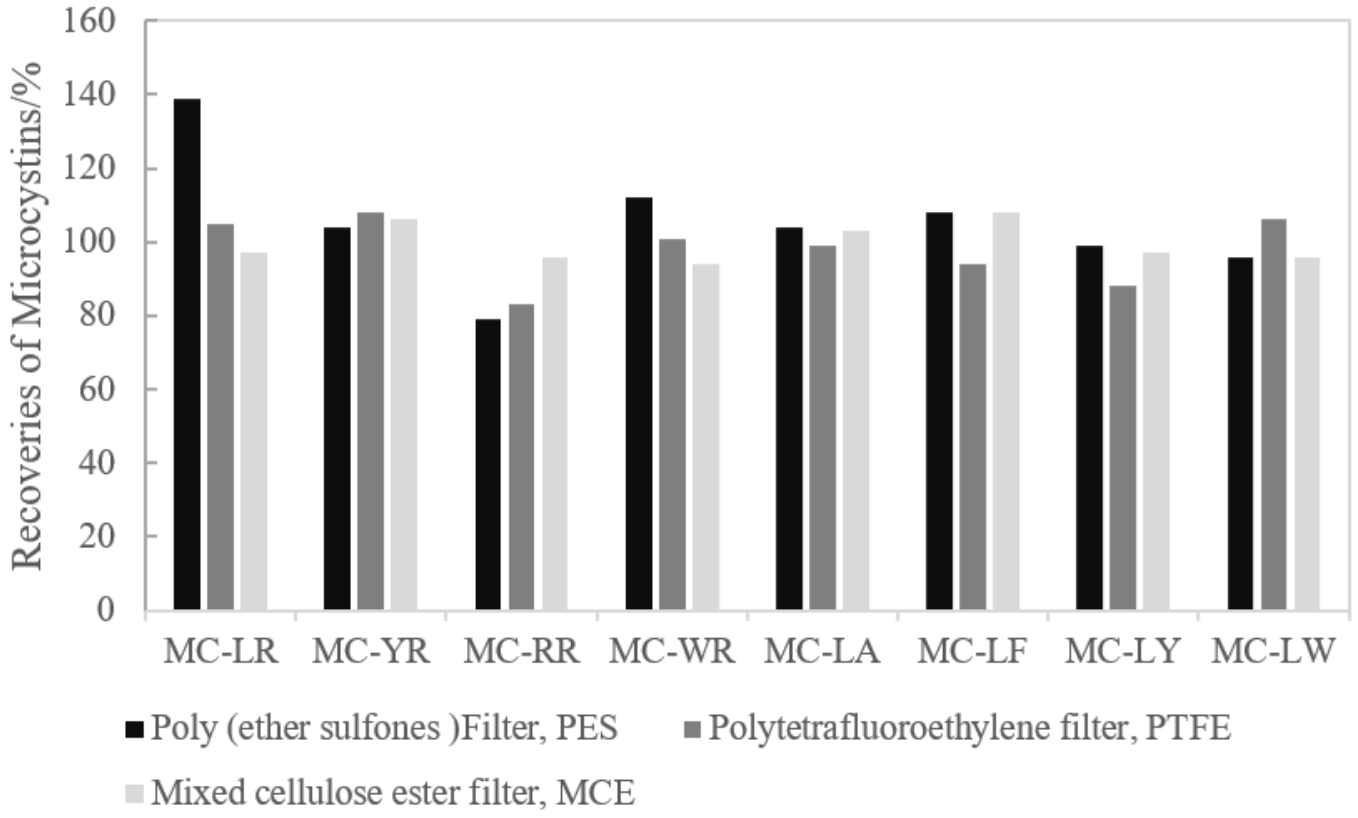

Figure 3

Recovery (%) of microcystins through different membranes (pore size 0.22 μm).

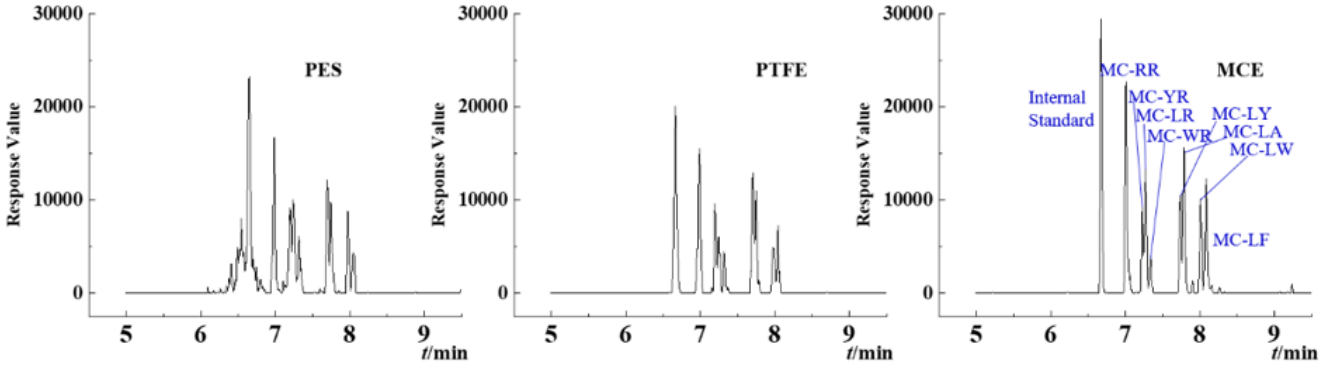

Figure 4

Chromatograms under three different filter membranes (pore size 0.22 μm).

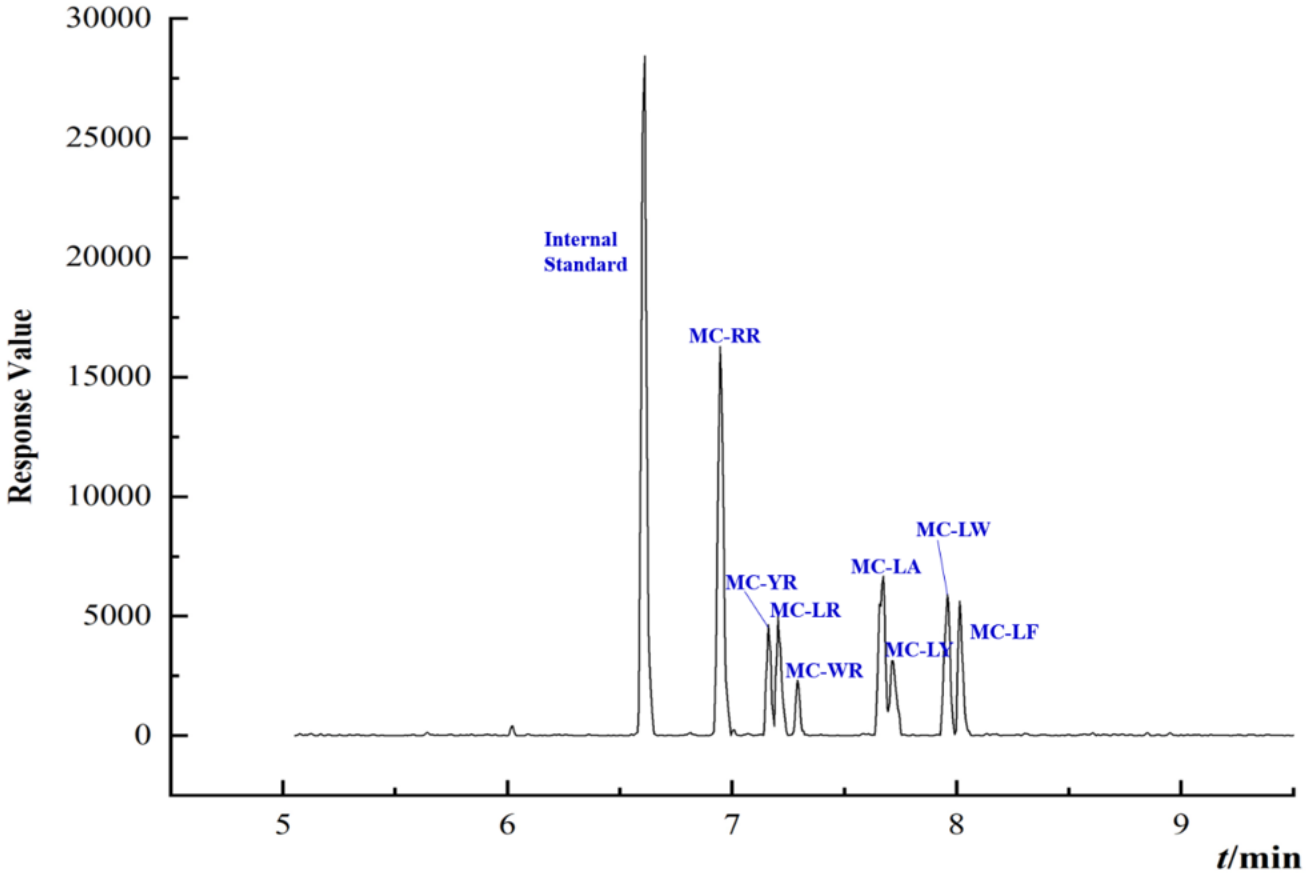

Figure 5

Chromatogram of 20 ng L-1 eight mixed MCs standard solutions with mobile phase of (4): Acetonitrile with 0.1% (v/v) formic acid + Water with 0.1 % (v/v) formic acid.

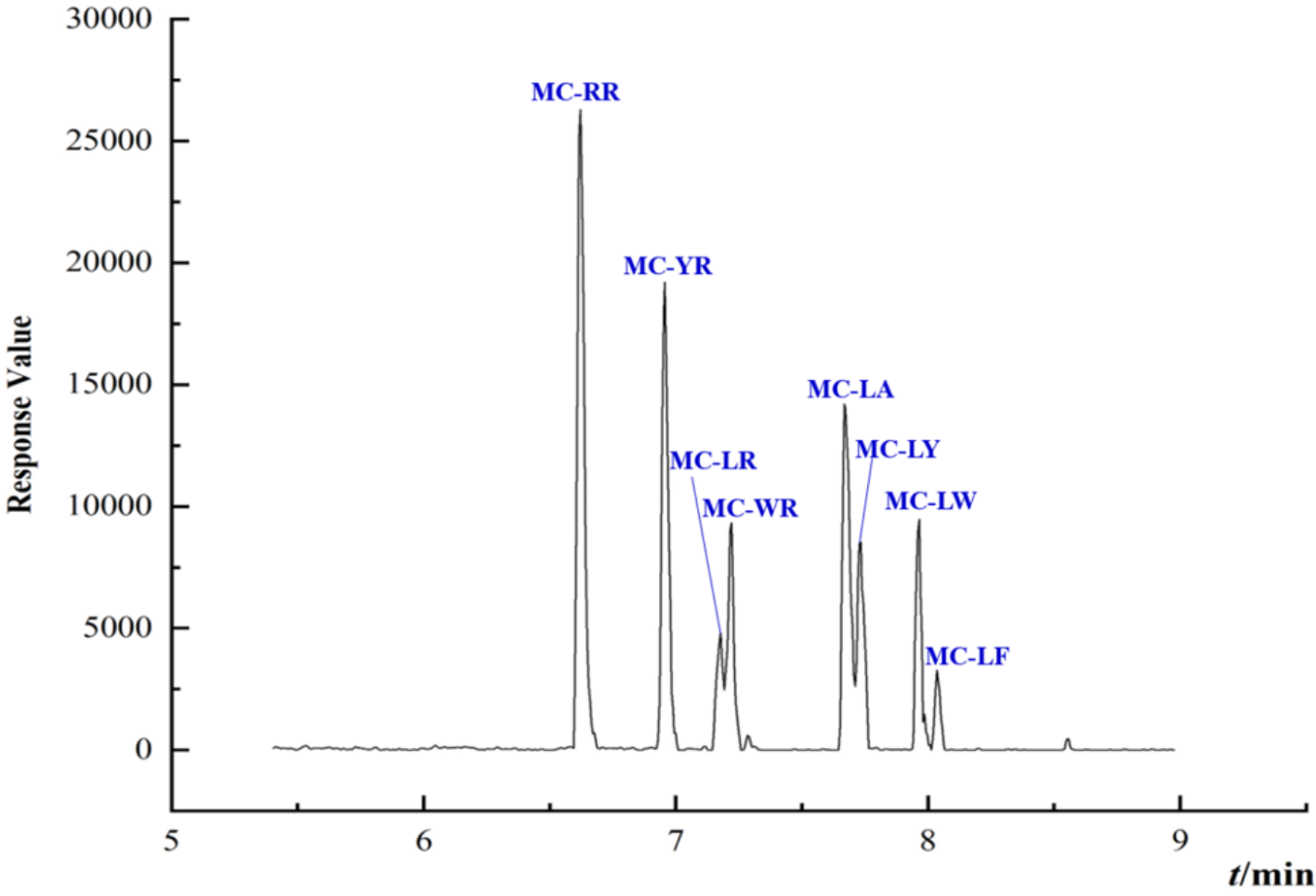

Figure 6

Chromatograms under gradient elution procedure (1) in Section 2.2.4.

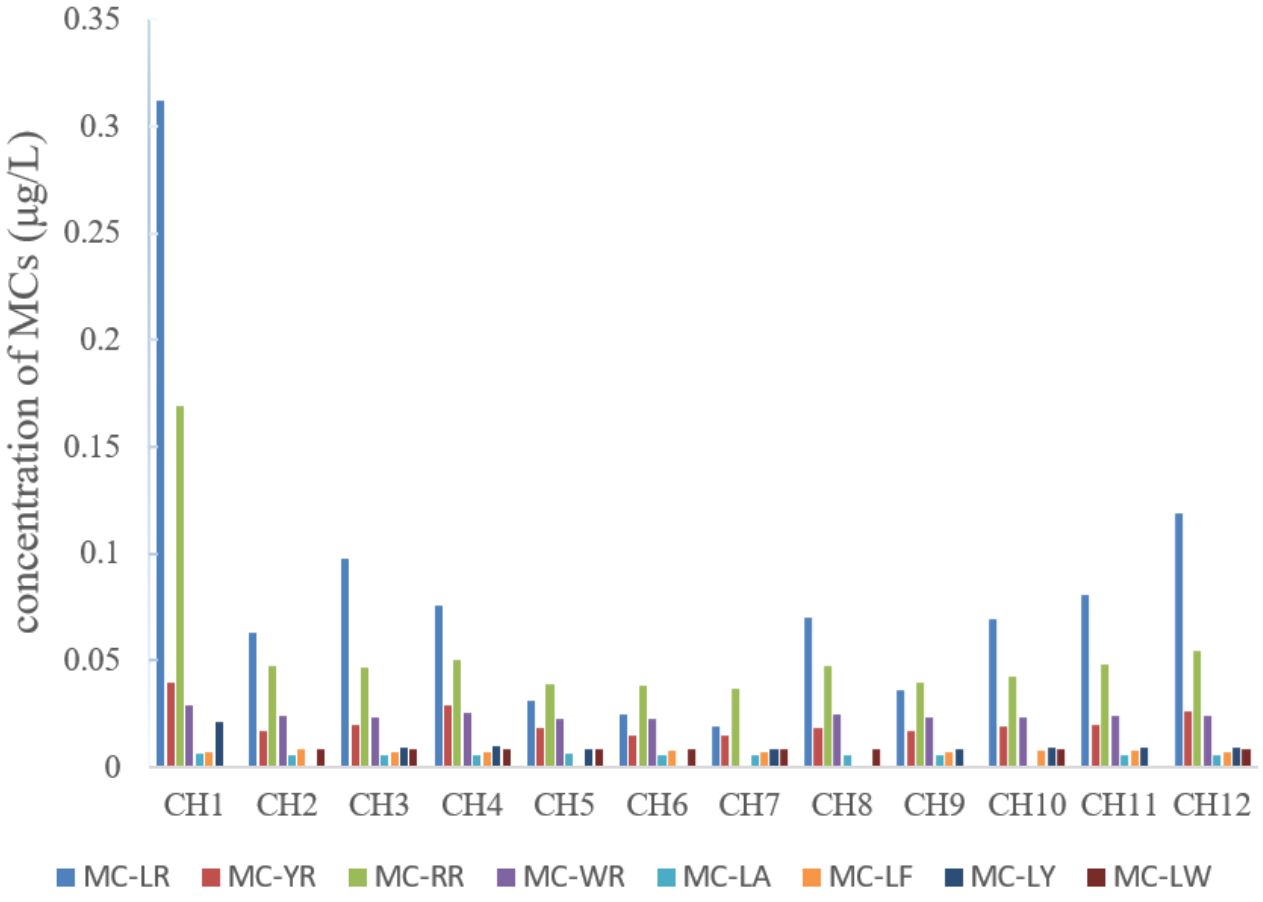

Figure 7

Concentrations of MCs at 12 sampling points in Chaohu Lake.