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A fully automated system with on-line micro solid-phase extraction combined with capillary liquid chromatography–tandem mass spectrometry for high throughput analysis of microcystins and nodularin-R in tap water and lake water

Yuanhong Shan, Xianzhe Shi*, Abo Dou, Cunjie Zou, Hongbing He, Qin Yang, Sumin Zhao, Xin Lu, Guowang Xu

CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Zhongshan Road 457, Dalian 116023, China

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ABSTRACT

Microcystins and nodularins are cyclic peptide hepatotoxins and tumour promoters from cyanobacteria. The present study describes the development, validation and practical application of a fully automated analytical method based on on-line micro solid-phase extraction–capillary liquid chromatography–tandem mass spectrometry for the simultaneous determination of seven microcystins and nodularin-R in tap water and lake water. Aliquots of just 100 μL of water samples are sufficient for the detection and quantification of all eight toxins. Selected reaction monitoring was used to obtain the highest sensitivity. Good linear calibrations were obtained for microcystins (50–2000 ng/L) and nodularin-R (25–1000 ng/L) in spiked tap water and lake water samples. Excellent interday and intraday repeatability were achieved for eight toxins with relative standard deviation less than 15.7% in three different concentrations. Acceptable recoveries were achieved in the three concentrations with both tap water matrix and lake water matrix and no significant matrix effect was found in tap water and lake water except for microcystin-RR. The limits of detection (signal to noise ratio = 3) of toxins were lower than 56.6 ng/L which is far below the 1 μg/L defined by the World Health Organization provisional guideline for microcystin-LR. Finally, this method was successfully applied to lake water samples from Tai lake and proved to be useful for water quality monitoring.

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1. Introduction

Microcystins (MCs) and nodularins, cyclic peptides produced by blue-green algae, are increasingly of interest in water supplies and algae food supplements because of their toxicity to animals and plants, and the high risk to human health [1,2]. More than 80 structural variants of microcystins have been described which are all heptapeptides with a generalized structure of *cyclo*-(-D-Ala^1-L-X^2-D-isoMeAsp^3-L-Y^4-Adda^5-D-isoGlu^6-Mdha^7), where MeAsp stands for methylaspartic acid, Mdha for *N*-methyldehydroalanine, Adda for (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and X and Y for the variable amino acids which give rise to the naming system of the toxins. As to nodularins, less than 10 known variants are cyclic pentapeptides. The most common structure of nodularins, named nodularin-R, is *cyclo*-(D-isoMeAsp^1-L-Arg^2-Adda^3-D-isoGlu^4-Mdhb^5) where Mdhb stands for 2-(methyl-amino)-2-(Z)-dehydrobutyric acid [3]. The characteristic feature of both microcystins and nodularins is the presence of the unusual amino acid Adda which is important for recognition of binding sites responsible for hepatotoxic effect. The World Health Organization (WHO) has provided a provisional guideline value of 1 μg/L for microcystin-LR (MC-LR) in drinking water [4]. This low guideline limit was the impetus to develop a rapid and sensitive method for the detection of this toxin and other commonly occurring toxins in lake water and tap water.

High-performance liquid chromatography tandem mass spectrometry has been widely used to analyze these toxins for its good sensitivity and specificity [5–11]. The routine analysis of aqueous samples for microcystins requires a sample pretreatment. A variety of techniques have been reported for this purpose, including solvent extraction, solid-phase extraction (SPE) using immunosorbents [12,13] or reversed-phase sorbents [14] and solid-phase microextraction (SPME) [15,16]. Sample treatment using SPE is mainly an off-line procedure, which is tedious, time consuming and poorly reproducible at trace levels. Contrary to the off-line technique, on-line SPE-LC offers a fast and reliable approach to the monitoring of trace pollutants in water [17] and has also been applied to the preconcentration and matrix removal of microcystin from water samples prior to LC [18]. As to the importance of the

* Corresponding author. Tel.: +86 411 84379757; fax: +86 411 84379559.
E-mail address: shixianzhe@dicp.ac.cn (X. Shi).

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development of environmentally and biologically favorable analytical methods, miniaturization of the sample preparation and analysis with low sample requirements, and low solvent consumption has been regarded as one of the key developing trends. Compared to the on-line SPE-LC systems with conventional LC, on-line microSPE-μLC with 0.5–1.5 mm i.d. columns have economic and safety advantage with less sample and solvents needed and higher sensitivity. Rivasseau et al. developed an on-line microSPE-μLC UV/MS system to detect three kinds of microcystins in water samples [19]. In the procedure of on-line microSPE-μLC, four steps, i.e. condition, percolation, cleanup and desorption, were also needed just like in off-line SPE. It can provide ideal preconcentration and cleanup, but it was still complicated and hard to realize full automatic operation.

In capillary LC system with 0.15–0.5 mm i.d. columns, the on-line technique is more delicate to use, due to the risk of dead volume that would induce severely band broadening. Consequently, it has not been frequently employed until now. Famiglini et al. compared the performances of microSPE with traditional SPE followed by capillary LC-ESI/MS for the analysis of 4 pesticides from aqueous samples [20]. Better recoveries were obtained when microSPE traps were used. In our work, a fully automated on-line microSPE-capillary LC system which interfaces a 1.5 cm × 0.3 mm i.d. precolumn packed with octadecyl silica with a 10 cm × 0.3 mm i.d. analytical column packed with the same stationary phase was firstly developed and validated for the simultaneous determination of the eight toxins in water samples. In order to realize automation easily, SPE procedure was simplified into three steps of condition, preconcentration and desorption. Furthermore, the low flow rate used in capillary LC allows a direct coupling with mass spectrometry (MS). The developed method was applied to the analysis of tap water and lake water in order to obtain an accurate monitoring picture about the occurrence of microcystins and nodularin-R in a few selected locations.

2. Experimental

2.1. Materials

Microcystins (MCs) standards and nodularin-R (Nod-R) were purchased from Alexis Biochemicals (San Diego, CA, USA), including MC-RR, MC-LR, MC-LY, MC-LA, MC-LW, MC-LF and MC-YR. HPLC-grade acetonitrile was from Merck (Darmstadt, Germany), methanol and formic acid (96%) were from TEDIA (Fairfield, OH, USA). The pure water was purified by a Milli-Q water purification system (Millipore, Billerica, MA, USA) before used.

2.2. Sample and standard preparation

Stock standard solutions for each of the MCs and Nod-R were prepared in methanol and stored under light exclusion at −20 °C. Standard solutions of the mixtures of each MC at 2 μg/L and Nod-R at 1 μg/L were prepared by appropriate dilution of the stock solutions in a reconstitution solvent (methanol/pure water, 15/85, v/v). To prepare a standard addition calibration, reconstitution solvent (methanol/tap water, 15/85, v/v) and (methanol/lake water, 15/85, v/v) were used to dilute the standard solution. The blank tap water and lake water were filterd through 0.22 μm filters prior to on-line SPE analysis. Six different concentrations of mixtures of toxins with 50, 100, 200, 500, 1000, 2000 ng/L of MCs were used to obtain the calibration curves. The concentration of the Nod-R in the mixture was always half of that of MCs. To evaluate the matrix effect of tap water and lake water, three different concentrations of mixtures of toxins with solvent (methanol/pure water, 15/85, v/v) were also prepared.

2.3. Analysis parameters

2.3.1. Chromatography

The on-line microSPE-capillary LC system included two LC-20ADnano pumps, one LC-20AD pump, SIL-20A autosampler with 100 μL injection loop and a FCV nano Valve unit (Shimadzu, Kyoto, Japan). Valve-switching technique was used to realize the autochange of the preconcentration and elution to analytical separation. Preconcentration was carried out on a guard column (C18 1.5 cm × 0.3 mm i.d., 5 μm, Micro-Tech Scientific) as the microSPE column at a flow rate of 30 μL/min and analytical separations were performed on a 10 cm × 0.3 mm i.d. column packed with 5 μm octadecyl silica (Micro-Tech Scientific) at 5 μL/min.

100 μL samples were preconcentrated on the microSPE column with the methanol/pure water (5/95, v/v) at 30 μL/min for 6 min. After that, switching the valve to the elution position, gradient elution was performed using water containing 0.2% formic acid (mobile phase A) and acetonitrile containing 0.2% formic acid (mobile phase B). The gradient elution program was started with 30% B and raised to 80% B in 14 min; then the composition was rapidly raised to 100% B in 1 min and held for 2 min; then the valve was switched back to the injection position after 1 min; thereafter, the microSPE column and analytical column were reequilibrated respectively for the next analysis.

In the recovery test, a sample injector with 5 μL injection loop (Shimadzu, Kyoto, Japan) was used to replace the FCV nano Valve unit and microSPE column. Without the preconcentration procedure, 20 times of the concentration of the toxins mixture were used in the recovery test.

2.3.2. MS/MS-SRM parameters

An API QTRAP 2000 mass spectrometer (Applied Biosystems/MDS SCIX, USA) was used as the detector with an electrospray ion source (ESI). The ESI source was operated as follows: ion-spray voltage, 5000 V; source temperature, 80 °C; nebulizing gas, 30 psi; auxiliary gas, 10 psi; curtain gas, 25 psi. In order to obtain the highest sensitivity, selected reaction monitoring (SRM) as used in positive ion mode with low resolution and the dwell time was 150 ms. Instrument control and data acquisition are performed with the Analyst 1.4.2 software package from Applied Biosystems.

3. Results and discussion

3.1. Mass spectrometer parameters optimization

In this method, the flow rate of mobile phase for capillary liquid chromatography was just 5 μL/min. The rate is near to the lower limit of ESI source. In order to avoid the unstable or weak signal caused by the low flow rate, the parameters of ESI source were optimized carefully. Different source temperatures were tested and 80 °C was chosen finally to be the best. After that, the individual toxin standard solution was infused into the ESI source by using a syringe pump at a flow rate of 10 μL/min to obtain the ion transition and the optimized SRM parameters. With the addition of 0.2% formic acid, all toxins were ionized and the $[M+H]^+$ ions were the predominant ions except for MC-RR which was detected as doubly charged species $[M+2H]^{2+}$ with *m*/*z* 520.3. The charge resides preferentially on the Adda methoxy moiety which occurs in all MCs and Nod-R, so all of the eight toxins have the same product ions of *m*/*z* 135 with the highest relative intensity. To perform the determination and quantification in SRM mode with good sensitivity and selectivity, the most abundant Q1/Q3 ion pairs were chosen. The optimized SRM parameters of the MCs and Nod-R are given in Table 1.

Table 1
Optimized SRM parameters for toxins.

Toxins	Q1/Q3 (*m*/*z*)	Declustering potential (V)	Collision energy (V)	Entrance potential (V)	Collision entrance potential (V)	Collision exit potential (V)
MC-RR	520.3/135.2	45	39	8	18	2.8
MC-YR	1046.0/135.5	140	88	8	35	2
MC-LR	996.0/135.3	127	85	11	33	2.1
MC-LA	910.9/135.0	70	83	8	35	2.2
MC-LY	1002.8/135.3	53	84	10	32	2.1
MC-LF	986.9/134.9	67	85	8	36	2.1
MC-LW	1026.0/135.4	67	86	8	35	2
Nod-R	825.8/135.3	90	83	10	27	2

3.2. On-line microSPE-capillary LC optimization

The setup of on-line microSPE-capillary LC is identical to that of on-line SPE-LC in conventional chromatography, except the geometry of the column, microSPE column and connection tubing. In theory, downscaling from conventional columns of 4.6 mm i.d. to capillary columns of 0.3 mm i.d. can result in a gain in sensitivity of two orders of magnitude if the other column parameters

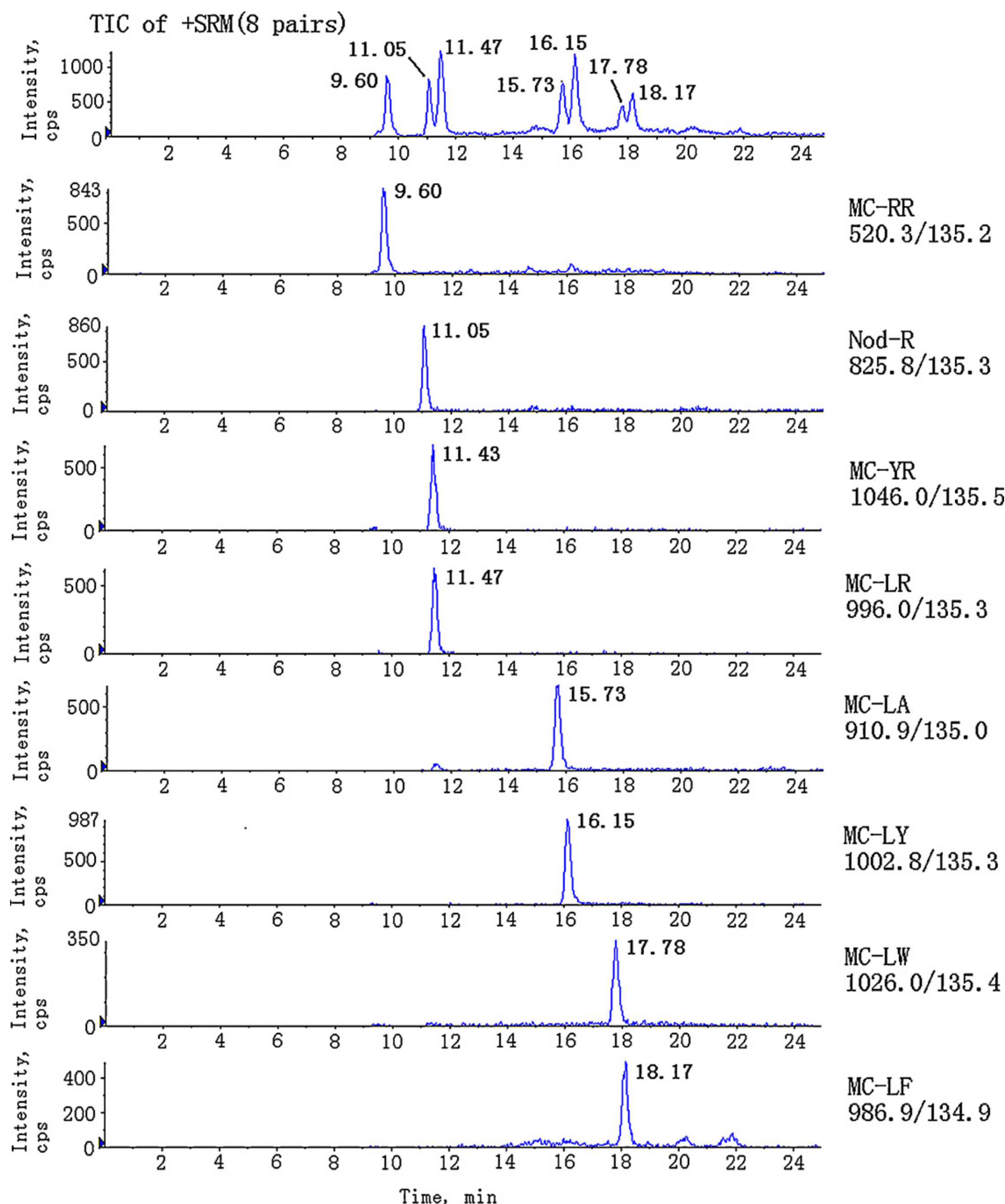

Fig. 1. Total ion current chromatogram and SRM constructed ion current chromatograms of lake water spiked with 500 ng/L microcystins and 250 ng/L nodularin-R.

Table 2
Repeatability, matrix effect and recovery data for toxins.

Toxins	Conc (ng/L)	Lake water				Tap water			
		Intraday (RSD%)	Interday (RSD%)	Matrixes (%)	Recovery (%)	Intraday (RSD%)	Interday (RSD%)	Matrixes (%)	Recovery (%)
MC-RR	100	4.6	6.2	78.6	71.3	5.3	10.0	92.8	64.6
	500	3.1	9.8	60.9	93.2	4.1	6.3	80.7	98.1
	2000	3.3	15.7	63.4	87.1	2.3	3.7	80.7	94.4
MC-YR	100	8.2	13.8	106.3	110.3	8.7	8.1	95.8	103.5
	500	2.6	4.9	99.8	161.2	9.7	8.5	105.3	135.7
	2000	4.2	4.1	115.1	162.8	3.9	3.8	98.3	134.2
MC-LA	100	6.2	13.7	101.8	78.7	7.6	6.6	119.4	76.2
	500	7.0	10.3	90.0	95.6	2.9	3.6	96.5	92.8
	2000	5.5	4.9	86.8	96.9	4.0	4.0	102.3	93.3
MC-LW	100	9.4	12.0	87.8	70.9	9.8	5.2	81.7	88.5
	500	6.0	11.8	81.5	96.4	5.6	2.6	85.8	118.2
	2000	4.9	1.2	92.7	114.4	1.4	4.2	86.4	93.9
MC-LF	100	6.4	11.5	97.0	73.0	8.1	6.1	94.2	85.8
	500	5.8	11.2	88.6	90.5	5.7	2.9	84.8	101.8
	2000	3.2	1.7	89.6	101.4	2.0	3.2	87.4	90.8
MC-LY	100	6.0	8.7	103.3	72.5	8.9	8.3	98.5	76.0
	500	7.6	9.9	90.0	92.8	4.8	6.5	91.7	97.5
	2000	4.6	4.4	91.4	99.1	1.2	2.8	88.0	91.0
MC-LR	100	6.8	8.9	120.5	116.8	10.0	4.4	111.9	88.2
	500	5.5	5.4	104.1	157.1	7.2	7.2	104.4	130.9
	2000	4.0	5.6	107.7	159.7	7.1	7.1	94.7	134.4
Nod-R	50	6.4	7.6	118.2	102.0	9.4	9.3	112.4	85.9
	250	4.0	2.2	97.9	122.2	3.9	5.5	103.3	106.8
	1000	2.9	6.5	113.5	134.7	3.6	2.8	101.4	111.2

remain constant and the equal absolute amounts of analytes are injected. That makes the determination and quantification of trace MCs and nodularins more feasible and accurate. Moreover, the system of on-line microSPE-capillary LC is also more automated and time-saving. The same solvent was used for condition of microSPE column and preconcentration procedure within 6 min and after the valve-switching, analytes were eluted with mobile phase directly to the analytical column for separation. After the reequilibration, the next run can be done automatically with the same process. The high level of automation minimizes possible variations in analytical results caused by different operators. By contrast, more manual steps are needed in the case of off-line SPE. It takes usually several hours to deal with the condition of the SPE column, percolation, cleanup, desorption of the samples and sometimes the lyophilization is also needed. Furthermore, several milliliters or even 1 L of samples are often needed to obtain high sensitivity. Zhang et al. utilized a total 1 L water sample in off-line SPE followed by LC–MS/MS to get the method detection limit of MC-LR 2.6 ng/L [5]. But in the system of on-line microSPE, 100 μL water sample is enough to get the high sensitivity. The detailed parameters optimized with this on-line microSPE is given below.

In capillary LC special care must be taken to avoid dead volumes that may exist in the connections between the valves, the columns, and the detector. All the peek connections used in our experiments had a diameter of 25 μm, a nano six-port valve was used to reduce the dead volumes. As a result, no peak dispersion was observed in this on-line microSPE-capillary LC system, which indicates a good quality coupling.

Two kinds of solution, methanol/pure water (5/95, v/v) and acetonitrile/pure water (5/95, v/v), were tested in order to obtain full preconcentration of toxins in the microSPE column. The results showed that seven MCs and Nod-R were all fully preconcentrated by the two solvents except Nod-R which was not retained by acetonitrile/pure water (5/95, v/v). For the methanol/pure water (5/95, v/v), most of toxins can be clearly eluted except the most hydrophobic MC-LW and MC-LF. It was found adding 15% methanol in the sample solvent can improve the elution of the MC-LF and MC-LW. Besides, owing to the very small sorbent amount contained in the microSPE column, it is necessary to check the injection volume which does not exceed the breakthrough volume. Breakthrough volume (V_b) of these toxins was experimentally determined by preconcentration of increasing volumes of the sample that contained a constant amount of toxins [19]. 20, 50 and 100 μL sample volume of tap water spiked with 10 μg/L, 4 μg/L and 2 μg/L of microcystins and half concentration of Nod-R were applied to find the optimal injection volume, respectively. And whatever the preconcentrated volume, the peak areas of the toxins were constant, which mean that the V_b volume is larger than 100 μL. Therefore, 100 μL sample volume, the largest injection volume of SIL-20A autosampler, was selected for all the applications.

For on-line microSPE-capillary LC, the elution solvent, which is also used as the mobile phase for separation, was optimized to obtain the best elution efficiency and separation results. Acetonitrile was investigated as the best solvent owing to lower viscosity and stronger elution than methanol. Formic acid was selected as the mobile phase modifier because of having a good separation of toxins and supplying H^+ to obtain high MS responses. So 0.2% formic acid was added in both the water and acetonitrile. Fig. 1 gives a typical chromatogram of the spiked lake water under the optimal conditions. Total ion current chromatogram (TIC) and extracted ion chromatograms (XIC) of the MCs (500 ng/L) and Nod-R (250 ng/L) are given, respectively. Since the retention times of MC-LR and MC-YR are almost the same, only one peak with the retention time of 11.47 min can be seen in the TIC chromatogram. But in SRM mode, the transitions of these two analytes are rather different (MC-YR: 1046.0/135.5, MC-LR: 996.0/135.3), so the determination and quantification of these MCs can be performed without any interference by each other.

3.3. *Method validation*

After optimized, the analytical method developed was validated in terms of precision, matrix effect, linearity, recovery and sensitivity.

3.3.1. *Precision*

The precision of this method was evaluated through intraday repeatability and interday repeatability test. Three different con-

Table 3
Linear range and calibration curve data of toxins.

Toxins	Lake water					Tap water				
	Equation of line	Linear range (ng/L)	R^2	LOD (ng/L)	LOQ (ng/L)	Equation of line	Linear range (ng/L)	R^2	LOD (ng/L)	LOQ (ng/L)
MC-RR	$y = 39.645x + 3056.5$	50–2000	0.9984	18.8	62.5	$y = 32.954x + 28.98$	50–2000	0.9996	18.2	60.6
MC-YR	$y = 34.385x - 293.99$	50–2000	0.9971	16.7	55.6	$y = 16.298 + 384.89$	50–2000	0.9986	22.5	75.0
MC-LA	$y = 33.897x + 1765.9$	50–2000	0.9989	33.0	110.0	$y = 22.748x - 240.31$	50–2000	0.9988	28.8	96.2
MC-LW	$y = 26.25x + 703.51$	50–2000	0.9989	29.4	98.0	$y = 10.832x - 260.57$	50–2000	0.9852	56.6	186.8
MC-LF	$y = 34.884x + 1507.9$	50–2000	0.9981	38.9	129.5	$y = 25.408x + 87.401$	50–2000	0.9984	26.5	88.5
MC-LY	$y = 56.004x + 1725.9$	50–2000	0.9991	13.6	45.5	$y = 33.757x - 25.465$	50–2000	0.9976	18.8	62.5
MC-LR	$y = 32.863x - 12.451$	50–2000	0.9979	11.3	37.5	$y = 16.043x - 85.734$	50–2000	0.9961	19.9	66.4
Nod-R	$y = 42.792x + 29.928$	25–1000	0.9993	10.7	35.7	$y = 33.899x + 322.92$	25–1000	0.9978	14.1	46.9

centration of eight toxins solution with two different kinds of matrix were used for this study. Table 2 demonstrates the RSD of intraday and interday repeatability of eight toxins in low, medium and high concentrations. The RSD of all analytes is no more than 10.0% for intraday repeatability. In the interday repeatability test, one-way analysis of variance was used in the data handling and the RSD is also no more than 15.7%. The excellent precision in different concentrations makes the quantification results more trustworthy.

3.3.2. Matrix effect

Matrix effect can lead to either a reduced response (ion suppression) or an increased response (ion enhancement) of the mass spectrometry system, which can severely influence the quantitative analysis of trace samples. In this study, matrix effect was evaluated by a comparison between the peak responses of eight toxins spiked to a blank sample of tap water or lake water at three different concentrations with those obtained after being spiked in the pure water at the same concentration levels. The results are illustrated in Table 2. No significant matrix effect was observed with this on-line SPE procedure except that the response of MC-RR was slightly suppressed in the lake water matrix.

It was found that matrix effect could be reduced remarkably by adding 15% methanol in the tap water or lake water. This may due to less of the polar interferents retained on microSPE column. In fact, the aim of cleanup has been achieved at the same time of preconcentration. But for MC-RR, it is the most polar analyte among eight toxins owing to the special structure with two arginine residues and its retention time is near to the dead time. So it's hard to avoid some matrix constituents co-eluted with MC-RR from the microSPE column and may have a slight influence on the ionization efficiency of MC-RR.

3.3.3. Recovery

The recovery test of the on-line microSPE-capillary LC–MS method was carried out by comparing the peak areas obtained using on-line preconcentration of a spiked sample and direct loop injection of the same amount of toxins. So 5 μL spiked samples with 2 μg/L, 10 μg/L and 40 μg/L MCs and half-concentration Nod-R were used in the direct loop injection method and 100 μL spiked samples with 100 ng/L, 500 ng/L and 2000 ng/L MCs and half-concentration Nod-R were used in the method with microSPE column. The ratios of the peak areas of all the toxins in the two tests (a latter-to-former ratio) are used as recovery data and summarized in Table 2. Acceptable recoveries were obtained in the three concentrations with both tap water matrix and lake water matrix. It was found that the recoveries with tap water matrix are better than those with lake water matrix. The possible reason is that the lake water matrix is more complicated and may have an influence

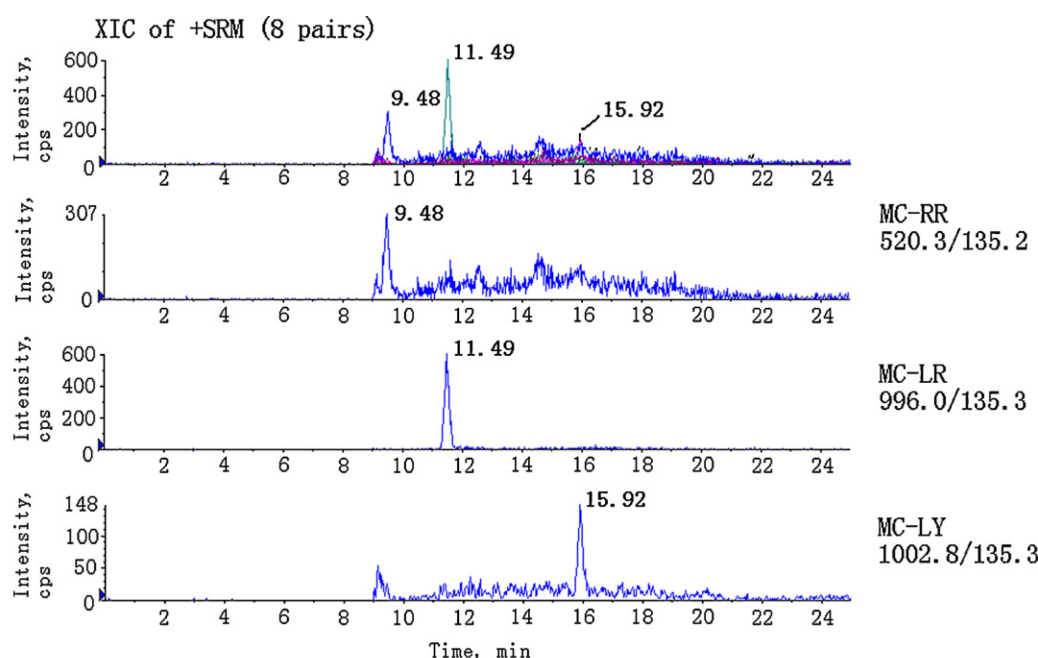

Fig. 2. SRM constructed ion current chromatograms of natural water sample from Tai lake (Dapukou, Jiangsu, China).

on the recovery results. In addition, the recoveries of eight toxins are varied partially because the different polarities of these toxins induce discrepant extraction efficiency using the same on-line microSPE method.

3.3.4. Linearity

The linear range of this method was investigated using eight toxins in the matrixes of tap water and lake water. In order to guarantee the accuracy of the determination and quantification in the nanogram per liter level of toxins, the linear range for the MCs was chosen to test from 50 to 2000 ng/L and from 25 to 1000 ng/L for Nod-R. Table 3 illustrates the calibration curve parameters for each of the toxins. The coefficient of determination (R^2) values for the calibration curves were all above 0.99 except for MC-LW of 0.9852 with tap water matrix. Good linearity was observed over the concentration ranges of 50–2000 ng/L for MCs and 25–1000 ng/L for Nod-R.

3.3.5. Limits of detection and quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for eight toxins in tap water were calculated as 3 times and 10 times of ratio of signal to noise, respectively. Under the tap water matrix, the LOD of eight toxins is in the range of 14.1–56.6 ng/L, and LOQ is in the range of 46.9–186.8 ng/L, which are all far below 1 μg/L given in the WHO provisional guideline for MC-LR in drinking water. The similar results were obtained under the blank lake water matrix with LOD lower than 39.0 ng/L and LOQ lower than 130.0 ng/L.

In spite of the similar structure of these toxins, the LOD and LOQ are different. The toxins with one or two arginine residues, such as MC-RR, MC-LR, Nod-R and MC-YR, always have lower LOD and LOQ than the others. The reasonable explanation is that the guanidino group of the arginine increases the ionization efficiency in the ESI^+ mode and results in high MS response.

3.4. Application

The usefulness of the method was assessed through the analysis of the microcystins and nodularin-R in tap water and surface water matrices from Tai lake, Jiangsu, China. None of eight toxins was detected from tap water. Three different surface water samples were collected from three places of the Tai lake, named Puzhuang, Yuyangshan and Dapukou, respectively. All the lake samples were filtered to get rid of cyanobacteria and other interferents before frozen. When the samples were prepared, all the water samples were filtered through 0.22 μm filters again and 150 μL methanol was added to 850 μL water sample as the solution for injection. Under the optimized conditions, each sample was detected twice. Only trace MC-RR, MC-LR and MC-LY were detected from the sample of Dapukou. Using the calibration curves, the concentration of MC-LR in the sample was calculated as 196.0 ng/L. The signals of MC-RR and MC-LY in the MS detector were under the LOQ, which means that the concentration of MC-RR is lower than 62.5 ng/L and that of MC-LY is lower than 45.5 ng/L. All of them were under 1 μg/L, the WHO provisional guideline for MC-LR in drinking water. The SRM chromatogram of toxins from the sample of Dapukou is shown in Fig. 2. It is evident that MC-RR is also a common hepatotoxin found in lake waters just like MC-LR from our results and from the previously published results [7,14]. But for MC-LY, it was rarely found in real lake water and little focus was on it in previous work. However, the results of this research demonstrate that more attention should be paid on MC-LY and its concentration in natural water is worth monitoring in the future.

4. Conclusions

A novel analytical method based on on-line microSPE-capillary LC–MS was developed to realize the fast and sensitive detection of eight toxins in natural water. Compared to the existing methods, this method is more automated than off-line SPE analysis and more sensitive than conventional on-line SPE-LC analysis. Furthermore, just 100-μL sample is sufficient for the detection of natural toxins in water at concentrations below 60 ng/L level. In the validation test, this method gave excellent results in precision, recovery and linearity. This method was successfully applied to the analysis of lake water samples and proved to be useful for water quality monitoring.

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