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Determination of Microcystin-LR in Water from Lake Tai, China

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Abstract Microcystin-LR (MC-LR) is a heptapeptide hepatotoxin produced by cyanobacteria. Immunoaffinity chromatography (IAC) column was prepared with CNBr-activated Sepharose 4B and monoclonal antibody of MC-LR. Water sample was cleaned up by IAC column and the content of MC-LR in water was determined by liquid chromatography-mass spectrometry (LC-MS). The results suggested that the IAC column exhibited highly selective specificity for MC-LR and selective removed interference from complex water sample. Water sample was concentrated for 2,000-fold through once purification. Cyanobacterial blooms had broken out in 2007 in Lake Tai, the third largest freshwater lake in China. Water samples from two parts of Lake Tai had been analyzed. The highest concentration of MC-LR in water from Lake Tai was 0.522 μg/L.

Keywords Cyanobacterial blooms · Immunoaffinity chromatography · Lake Tai · Liquid chromatography-mass spectrometry · Microcystin-LR

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Lake Tai is the third largest freshwater lake in China. However, due to the increasing population and rapid industrial and agricultural pollution in the lake's drainage basin, Lake Tai has undergone rapid eutrophication (Jin and Hu 2003). In recent years, heavy cyanobacterial blooms frequently occur in the eutrophic lake (Xie and Liu 2001), and the dominant species is *Microcystis aeruginosa*, which could produce microcystins (MCs), a family of hepatotoxic heptapeptides. There are more than 70 MCs isoforms that have been identified (Barco et al. 2004). The most frequent variant is microcystin-LR (MC-LR). MCs are potential specific protein phosphatase 1 and 2A inhibitors, and have been considered as potential tumor promoters (Eriksson et al. 1990; Runnegar et al. 1993). The resulting imbalance in protein phosphorylation disrupt liver cytoskeleton, which lead to massive hepatic haemorrhages that cause death in rodents and fish (de Figueiredo et al. 2004; Pichardo et al. 2005). Contamination of MCs in drinking water has been suggested as a risk factor for liver cancer (Ueno et al. 1996). Furthermore, the deaths of more than 50 haemodialysis patients in Caruaru, Brazil, have been linked to the presence of MCs in water used for dialysis (Pouria et al. 1998). To protect human health, the World Health Organization (WHO) has proposed a provisional guideline level of 1.0 μg/L for MC-LR in drinking water (Falconer et al. 1999). This guideline level has been adopted by many countries.

Immunoaffinity chromatography (IAC) was found to be an excellent sample cleanup technique for a large variety of toxic chemicals in biological samples at trace concentrations using little or free organic solvents. IAC column selective removed interference from complex matrices and exhibited highly selective specificity for object. Most interferents were eliminated after purification using IAC column (Stroka et al. 2000). IAC column was prepared

with CNBr-activated Sepharose 4B and monoclonal antibody (MAb) against MC-LR in this paper, and it was used to determine MC-LR in water from Lake Tai.

Materials and Methods

MC-LR was purchased from Alexis Corporation (Lausen, Switzerland); CNBr-activated Sepharose 4B was purchased from Amersham Biosciences (Uppsala, Sweden); water was deionized water; methanol and acetonitrile were of HPLC grade. Phosphate buffered saline (PBS): 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 were dissolved in 800 mL of distilled H_2O, and adjusted the pH to 7.4 with HCl, and added H_2O to 1 L, then sterilized by autoclave.

MAb against MC-LR was produced as described previously (Zhao et al. 2006). IAC columns were prepared with CNBr-activated Sepharose 4B and MAb against MC-LR. The preparation methods were according to the guide of CNBr-activated Sepharose 4B.

Meiliang Bay and Wuli Lake are the parts of Lake Tai. There are two sampling points in Wuli Lake and Meiliang Bay (Fig. 1). Water samples were collected since March to December, 2007. And water samples were tested in one day or these samples were kept under freezing temperature prior for analysis.

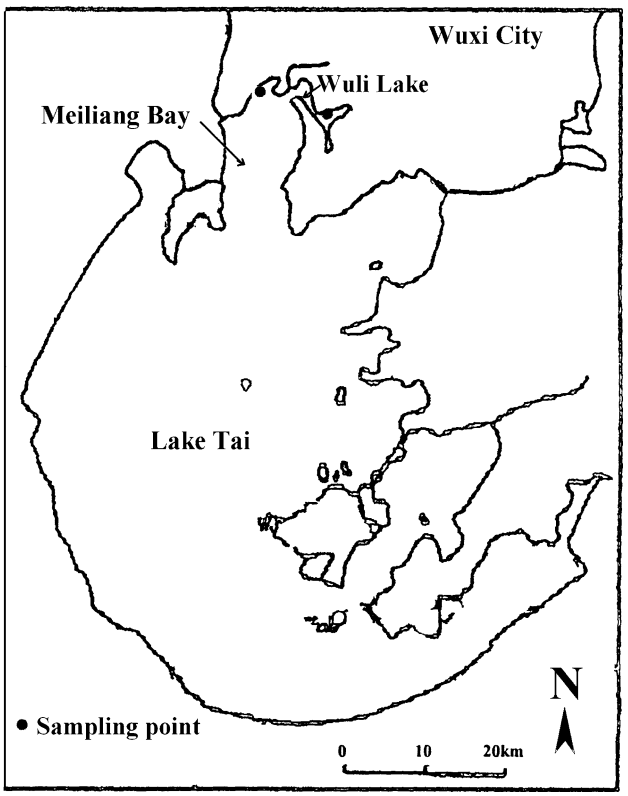

Fig. 1 The map of sampling point

Water sample (200 mL) was first filtered through a 0.45-μm disc filter. After purification, the contaminated water was applied directly to IAC column, which had been preconditioned with 5 mL of PBS and 3 mL of deionized water. Then, the column was washed with 5 mL of deionized water, 3 mL of 10% methanol in water to eliminate interferents. MC-LR was eluted from the cartridge with 5 mL of 100% methanol. The elution fraction was evaporated to dryness under reduced pressure in 40°C water bath, and then re-dissolved in 100 μL (water sample was concentrated for 2,000-fold) of 30% methanol–water prior to LC-MS analysis. If the concentration of MC-LR exceeded the limit of detection for LC-MS, it would be adjusted and analyzed again. The IAC column was then rinsed with 5 mL of deionized water and 5 mL of PBS and stored at 4°C for later use (Mhadhbi et al. 2006).

The LC-MS system used consisting of a model 2690 solvent and sample manager, a model 996 photodiode-array detector and a ZMD4000 mass spectrometer (all from Waters). The separation was performed on an Symmetry C18 column (2.1 mm × 150 mm, 5 μm), maintained at 35°C, using a mobile phase of acetonitrile and water containing 0.1% formic acid (Table 1), at the flow rate of 0.3 mL/min. The volume of sample injected was 20 μL. A post column flow splitter was used to produce the flow rate of 0.1 mL/min into the instrument.

MS detection was performed in electrospray ionization (ESI) mode. The cone voltage was set at 45 V. The desolvation gas (nitrogen) temperature and flow rate were set at 300°C and 350 L/h, respectively. The ion source temperature was set at 120°C. The system was optimized for transmission of $[M + H]^+$ ions. Acquisitions were made in full scan (310–1,200 amu, one spectrum per second) and selected ion recording (SIR). The cone voltages and masses monitored for analysis were optimized in determination. SIR was performed for the specific masses of interest.

Results and Discussion

The molecular weight (MW) of MC-LR is 995 D, *m/z* of $[M+H]^+$ is 996, and *m/z* of $[M-H]^-$ is 994. The

Table 1 Gradient elution conditions

Time (min)	*B*% (*B*: 20% acetonitrile, 0.1% formic acid)	*A*% (*A*: 80% acetonitrile, 0.1% formic acid)
0.00	20.0	80.0
15.00	50.0	50.0
20.00	100.0	0.0
30.00	20.0	80.0

quantitative analysis of the following sample is in model of SIR of ES + (996).

The standards of MC-LR were prepared by diluting the concentrated standards with water. Five levels from 0.005 to 0.5 μg/mL were prepared. Duplicate injections were made, and an external, linear calibration was performed to obtain a correlation coefficient (R^2) of 0.9994, the regression equation of MC-LR is as follows:

$$y = 116307x - 598.57$$

where x is the concentration of MC-LR (μg/mL); y is the peak area of MC-LR (μAU·s); linear range of MC-LR is 0.005–0.5 μg/mL; the limit of detection ($S/N = 3$) for MC-LR is 0.001 μg/mL. Water sample is concentrated for 2,000-fold, linear range of MC-LR is 2.5–250 ng/L; the limit of detection ($S/N = 3$) for MC-LR is 0.5 ng/L.

The retention behavior of MC-LR on an IAC column was examined, using the following method. A mixture containing 10 ng MC-LR dissolved in 50 mL of deionized water was applied to an IAC column, after washing and elution, the eluate was evaporated to dryness and re-dissolved in 100 μL (concentrated for 500-fold) of 30% methanol–water and then was tested by LC-MS five times. The recovery of MC-LR from IAC column was from 90.8% to 98.1%, the relative standard deviation (RSD) was less than 3.2%. 10 ng MC-LR was added to lake water (50 mL), other operations were same with the above. The recovery of MC-LR added to lake water from IAC column was from 87.7% to 102.3%, the RSD was less than 7.9%.

Water samples were collected from Meiliang Bay and Wuli Lake, two sites of Lake Tai, and these were tested in one day.

The general chromatogram of lake water after cleaned up by IAC columns is shown in Fig. 2. The results suggested that MC-LR could be recognized especially by IAC column, most interferents could be removed through once purification.

The data of MC-LR in water samples from Meiliang Bay and Wuli Lake are shown in Table 2. MC-LR occurred in water from Meiliang Bay since 4-May to 20-July in 2007. The highest concentration of MC-LR in water from Meiliang Bay was 522 ng/L, and it occurred on 5-June, 2007.

MC-LR was not detected in water sample from Wuli Lake up to the present. However, the concentration of MC-LR in water sample from Meiliang Bay was very high after May, 2007.

Meiliang Bay is located in the northern part of Lake Tai with a surface area of 100 km^2 and a depth of 1.8–2.3 m. It is not only as principal water resource for Wuxi City, but also as an important tourist attraction. This region has been listed as one of the hypertrophic parts in Lake Tai. During the past few decades, it has been contaminated by domestic and industrial polluted water from Wuxi City. Meiliang Bay has undergone rapid eutrophication. In recent years, heavy cyanobacterial blooms have occurred regularly and may last 8 months each year.

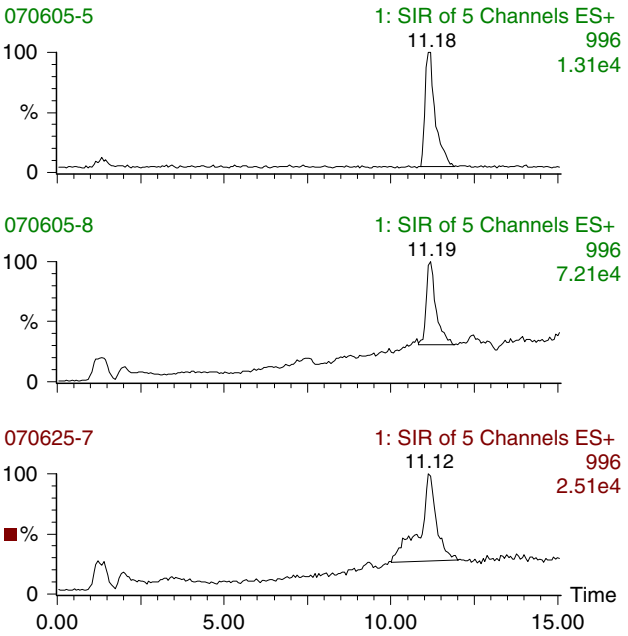

Fig. 2 The SIR Chromatograms of microcystin-LR standard and lake water after cleaned up by immunoaffinity chromatography column From First to Last: microcystin-LR standard and two samples of lake water

Table 2 Concentration of microcystin-LR in water samples from Lake Tai in 2007

Date	Concentration of MC-LR in water samples(ng/L)	
	Meiliang Bay	Wuli Lake
21-Mar	ND	ND
5-Apr	ND	ND
20-Apr	ND	ND
4-May	14.1 ± 0.5	ND
15-May	219.5 ± 0.6	ND
25-May	271.7 ± 0.5	ND
5-Jun	522.1 ± 0.2	ND
15-Jun	316.4 ± 0.9	ND
25-Jun	177.6 ± 1.1	ND
5-Jul	92.9 ± 0.6	ND
20-Jul	9.2 ± 0.3	ND
20-Aug	ND	ND
20-Sep	ND	ND
21-Oct	ND	ND
20-Nov	ND	ND
20-Dec	ND	ND

ND not detected

Cyanobacterial blooms broke out at the end of April, 2007. In May heavy cyanobacterial blooms made a water inlet of waterwork shut down in Meiliang Bay. During this study, MC-LR concentration was relatively high in the water of Meiliang Bay in June, 2007, which could be risky to both the ecosystem and human health of this region.

Correspondingly, cyanobacterial blooms had not occurred in Wuli Lake in 2007 (there was a little cyanobacterial blooms occurred in 2006). MC-LR was not detected in the water samples from Wuli Lake (Table 2).

Wuli Lake is a part of Lake Tai with a surface area of 10 km^2, it is near to Wuxi city. Five years ago, the lake eutrophication was severe, heavy cyanobacterial blooms occurred regularly each year, which was worse than Meiliang Bay. Wuli Lake was restored in the last four years. Some measures were put in practice, including repair of lakeshore, sludge dredging, waste water cut-off, ecological rehabilitation, water transfer, stop fish-farming in lake (Gu and Lu 2004; Zhu and Qu 2005). The environment and water quality of Wuli Lake was improved obviously at present. There was a dam built between Wuli Lake and Meiliang Bay, and a strobe to control water flowing. It was allowed that the water of Meiliang Bay flowed into Wuli Lake when the water quality of Meiliang Bay was better than Wuli Lake. It was not allowed if it was opposite. So the water quality of Wuli Lake could keep up better than Meiliang Bay. And it was proved from the results of determination of MC-LR in Wuli Lake and Meiliang Bay.

The determination method of MC-LR in water using the IAC column in conjunction with LC-MS was presented in the paper, the limit of detection was low and the sensitivity was improved. Lake Tai is the principal water resource for the surrounding cities. It is difficult to eliminate MC-LR in tap water by general treatment technology, so the determination of MC-LR in source water and tap water is very important. For ensuring the health of people around Lake Tai, it is necessary to monitoring MC-LR in source water and tap water, and monitoring MC-LR in Lake Tai. The determination method presented in the paper make this question easy.

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