Analytical Methods

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

A sensitive method for the total microcystins determination in water and sediment samples by liquid chromatography with fluorescence detection

C. Wang,[ab] C. Tian,[ab] Y. Tian,[ab] B. Feng,[ab] S. We,[c] Y. Li,[c] X. Wu,*[a] B. Xiao,*[a]

[a]Key Laboratory of Algal Biology of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.

[b]University of the Chinese Academy of Sciences, Beijing 10085, China.

[c]Key Laboratory for Applied Toxicology, Hubei Provincial Center for Disease Control and Prevention, Wuhan 430079, China

† Electronic supplementary information (ESI) available.

Abstract:

Microcystins (MCs) are potent hepatotoxins that comprise a family of more than 90 different variants. MCs can bind to matrices, after which they cannot be completely extracted by solvent. Currently, the MMPB (2-methyl-3-methoxy-4-phenylbutyric acid) method is the only effective way of determining total MCs without solvent extraction. In this study, a sensitive method to determine the total MCs in water and sediment was developed based on the MMPB method. Specifically, MCs were oxidized to MMPB with improved oxidation reagent (20 mg mL^{-1} $NaIO_4$, 4000 mg L^{-1} $KMnO_4$, pH ~9) and a stable MMPB yield of about 35% was obtained for both water and sediment samples. The minimum volume of oxidation reagent could be determined by the organic content in the matrix. After concentrated, the MMPB was derivatized with 1,2-benzo-3,4-dihydrocarbazole-9-ethyl-p-toluenesulfonate (BDETS) and detected by liquid chromatography using a fluorescence detector. Quantification limits of MCs in water and sediment samples were 125 ng L^{-1} and 100 ng g^{-1} respectively. The method was successfully applied to Lake Dianchi and the soluble MCs concentrations determined by this method were well correlated with those obtained by enzyme-linked immunosorbent assay (ELISA).

Introduction

Microcystis spp., which frequently occurs as the dominant bloom-forming organism in eutrophic freshwater systems, is widely known for their production of the potent hepatotoxins known as microcystins (MCs).[1] MCs irreversibly inhibit eukaryotic serine/threonine protein phosphatases 1 and 2A, resulting in liver disease as well as nephro- and neurotoxicity.[2]

Analytical Methods Accepted Manuscript

Analytical Methods Accepted Manuscript

Different techniques have been applied to quantify MCs in the water column, mainly liquid chromatography with a diode array detector or mass spectrometry (HPLC-PDA, LC-MS), protein phosphatase inhibition assay (PPIA) or enzyme-linked immunosorbent assay (ELISA).[3] There are more than 90 different MC variants in natural samples;[4] therefore, measuring the total MC content is the most economic and feasible method. HPLC-PDA is the most widely-used approach for quantification of MCs, while LC-MS can provide more sensitive detection and accurate identification.[5] However, both chromatography and mass-spectrometric methods neglect unknown MCs variants that are not available as standards. PPIA and ELISA can detect MCs present at levels below the WHO guideline without the need for sample pre-concentration. However, false positives may be obtained in PPIA from other phosphatase inhibitors that may occur in environmental samples including okadaic acid, tautomycin and calyculin-A.[6] Moreover, it is doubtful that PPIA and ELISA are equally sensitive to different MC variants.[7]

Although these methods are generally effective for measuring the dissolved toxins in water, their application to analysis of MCs in other matrices (algae, sediment and tissue) requires prior solvent extraction, which may significantly underestimate total toxin content.[8] Meissner et al. found that a considerable portion of MCs binds to protein in algae and is neglected by current analysis techniques based on solvent extraction.[9] Bieczynski et al. found that protein-bound MC-LR (L = leucine, R = arginine) represented 66–100% of total MC-LR in the intestine and liver.[10] Our earlier studies also revealed that MCs can be strongly adsorbed by sediment, but only be partly recovered by solvent extraction.[11]

The MMPB (2-methyl-3-methoxy-4-phenylbutyric acid) method has been successfully employed for determination of total MCs content in various matrices.[11,12] This method is especially well-suited to analysis of animal tissues[3, 14, 15] and sediments[12, 16, 17], where toxins are bound to matrix components.[18] This technique involves cleavage of the chemically unique C20 amino acid, (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl–10-phenyldeca-4,6-dienoic acid (Adda), to form MMPB, which is then quantified.[11] A stable oxidation yield of MMPB is essential for the precise quantification. However, the MMPB yields were only mentioned in four studies and the reported values were inconsistent.[3, 11, 17 19] On the other hand, the direct quantification of MMPB using HPLC with a UV detector was not sensitive enough.[16, 17] GC-MS[12, 20] and LC-MS[14] have also been used to quantify MMPB, but the instrument and operation cost of mass spectrometry is much higher than HPLC and GC.

Derivatization followed by HPLC with a fluorescence detector (FLD) has been commonly used to measure organic acids.[21, 22] As a small molecular organic acid, derivatization may also be feasible for sensitive quantification of MMPB. 1,2-benzo-3,4-dihydrocarbazole-9-ethyl-p-toluenesulfonate (BDETS) was synthesized by You et al. and used in the determination of bile acids and fatty acids.[21, 22] In the present study, we investigated the feasibility of using this derivatization reagent to improve the sensitivity of MMPB.

This study was conducted to develop a comprehensive method that sensitively and accurately analyzes the total MCs in water and sediments. The specific goals were to (i) optimize the oxidation procedures for a stable MMPB yield, (ii) improve the detection sensitivity of MMPB by fluorescence derivatization, and (iii) apply the optimized

Analytical Methods Accepted Manuscript

method for the detection of total MCs in spiked and natural samples.

Experimental

Reagents and chemicals

The MC-LR used in the experiment were isolated and purified in the laboratory as previously described.[16] The concentrations were determined according to the MCs standards purchased from Sigma-Aldrich (St. Louis, MO, USA). MMPB sodium salt standards were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). BDETS was kindly provided by the Northwest Plateau Institute of Biology (Chinese Academy of Sciences, Xining, China). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Reagents other than HPLC grade methanol and acetonitrile were of analytical grade.

Sample Collection and Preparation

Sediment and water samples for toxin analysis were obtained from 18 sites in Lake Dianchi on April 25, 2014 (**Fig. S1** of the supplementary material). Sediment and water samples for spike experiments were obtained from Lake Donghu in Wuhan. Sediments from Lake Caohai and Lake Taihu were also used.

All sediment samples were transported to the laboratory as soon as possible. After homogenizing, the samples were stored at −20 °C and then freeze-dried at 0.05 mbar in an Alpha 1–2/LD Freeze Dryer (Martin Christ, Germany). Next, the dried samples were crushed, passed through an 80 mesh sieve and stored at −20°C until analysis.

For water samples, 400 mL of water were filtered through Whatman 0.45 μm nitrocellulose membranes, after which the MCs were concentrated using a Sep-pak C18 cartridge (500 mg, 6cc, Waters, Milford, MA, USA). The cartridge was pre-conditioned with 10 mL methanol followed by 10 mL water. After loading the filtered water, the cartridge was washed with 10 mL water and 10 mL 20% methanol, respectively, and then eluted with 10 mL methanol. The eluate was evaporated to dryness by rotary vacuum evaporation at 40 °C and reconstituted with 0.5 mL 50% v/v methanol. Aliquots of the concentrated MCs solutions were used for ELISA or the following oxidation procedure.

Oxidation of MCs in aqueous solutions and sediments

Sediment and water samples containing natural MCs or spiked with MCs were oxidized by Lemieux reagent according to Wu et al.[11] Each test was conducted in triplicate.

For sediment samples, 1 g of freeze-dried sediment was oxidized with 10 mL oxidation reagent containing 20 mg mL^{-1} sodium periodate ($NaIO_4$) and 4000 mg L^{-1} potassium permanganate ($KMnO_4$) for 1 h under weakly alkaline conditions (pH ~9) unless otherwise specified. After centrifugation, the supernatants were quenched with 40% sodium bisulphite ($NaHSO_3$) solution (50 μl $NaHSO_3$ for 1 mL oxidation reagent). The oxidation yields of MCs in sediments from Lake Tai, Lake Caohai and Lake Dianchi were then compared. The organic contents of the three

sediments were 12.78, 76.75 and 438.21 g kg^{-1}, respectively. For water samples, aliquots of the concentrated MCs solutions (0.1 mL) were added to 1 mL oxidation reagent with the same composition. One hour later, 50 μl of 40% $NaHSO_3$ were added to terminate the reaction.

The pretreatment of MMPB in reaction solution

The MMPB in the reaction solutions were concentrated and purified with three commercial SPE disposable cartridges, Sep-Pak cartridge (500 mg, 6cc), Oasis MAX cartridge (150mg, 6cc), and Oasis HLB cartridge (200mg, 6cc) (Waters, MA, USA), and with three extraction solutions, n-hexane, diethyl ether and dichloromethane, respectively. Each test was conducted in triplicate.

The cartridge was pre-conditioned with 10 mL methanol followed by 10 mL water. After loading aliquots of reaction solution, the Sep-Pak and Oasis HLB cartridge were washed with 10 mL water and 10 mL 20% methanol, respectively, and then eluted with 10 mL methanol. The Oasis MAX cartridge was subsequently washed with 10 mL 5% ammonia water and 10 mL methanol and eluted with 10 mL 5% formic acid in methanol. In all cases, the eluent was evaporated to dryness and resolved in 0.5 mL acetonitrile.

Derivatization of MMPB

The derivatization of MMPB was conducted according to Wang et al.[20] All reactions were conducted in a 2 mL vial containing 5 mg K_2CO_3, 200 μL dimethyl formamide (DMF), 100 μL BDTES (5×10^{-3} mol L^{-1}) and 100 μL of MMPB acetonitrile solution unless otherwise specified. The vial was then sealed and allowed to react in a microliter thermostat at 100 °C for 60 min unless otherwise specified. Each test was conducted in triplicate. The derivatization procedure is showed in **Fig. 1**.

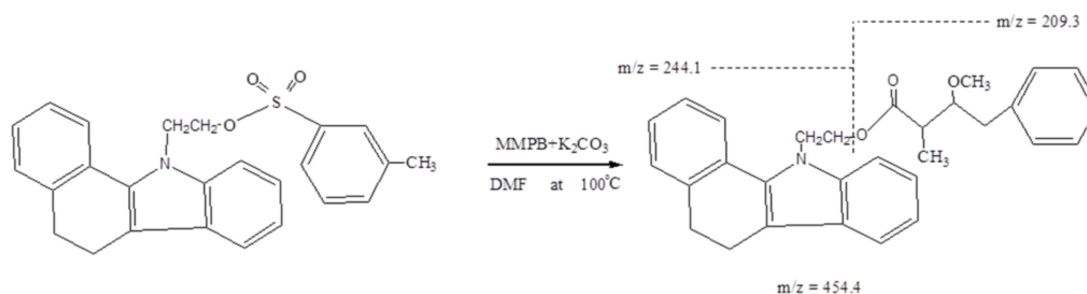


Fig. 1. Derivatization procedure using 1,2-benzo-3,4-dihydrocarbazole-9-ethyl-p-toluenesulfonate (BDETS) as a labeling reagent

Analysis methods

MCs and MMPB were analyzed by HPLC-PDA as described.[16] MCs in water samples were also detected by ELISA based on anti-MCLR monoclonal antibodies.[22, 23]

The MMPB derivative (BDETS-MMPB) was analyzed on a Synergi Hydro-RP C18 column (4 μm, 250 mm × 4.6 mm) using HPLC with FLD at excitation and emission wavelengths of 273 and 365 nm, respectively. The mobile phase consisted of acetonitrile and 0.05% aqueous trifluoroacetic acid with the following gradient applied at a constant flow of 1.0 mL min^{-1}: initial concentration of acetonitrile at 70%, which was increased gradually to

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

100% over 20 min, then decreased back to 70% over the next minute, where it was held until the end of the run at 25 min. The retention time of the MMPB derivative was determined by comparing the chromatograms of standard MMPB solutions with that of a blank control.

The structure of the MMPB derivative was confirmed by Agilent 6460 Triple Quadrupole LC-MS System (Agilent Technologies, Waldbronn, Germany) in positive-ion ESI mode. The ESI source parameters were as follows: gas temperature 300 °C, gas flow 6 L min^{-1}, nebulizer gas pressure 40 psig, sheath gas temperature 300 °C, sheath gas flow 11 L min^{-1}, capillary voltage was 3500 V. Fragmentor voltage and collision energy was 166 V and 45 eV, respectively.

Statistical analysis

All statistical analyses were performed by the software SPSS 13 for Windows (SPSS, Chicago, USA). Graphs were generated with Origin 8.0 software (OriginLab, Northampton, USA).

Results and Discussion

Identification of MMPB Derivative

The BDETS-MMPB derivative produced an intense molecular ion peak at *m/z* $[M+H]^+$ 454.2 (**Fig. 2**). With MS/MS analysis of the derivative, the collision induced dissociation spectra of *m/z* 454.2 produced specific fragment ions of *m/z* 436.4, 244.0 and 209.3. The fragment ion at *m/z* 436.4 corresponded to dehydration product of the derivative. The fragment ions of *m/z* 209.3 and 244.0 came from the cleavage of BDETS-MMPB as shown in **Fig. 1**.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

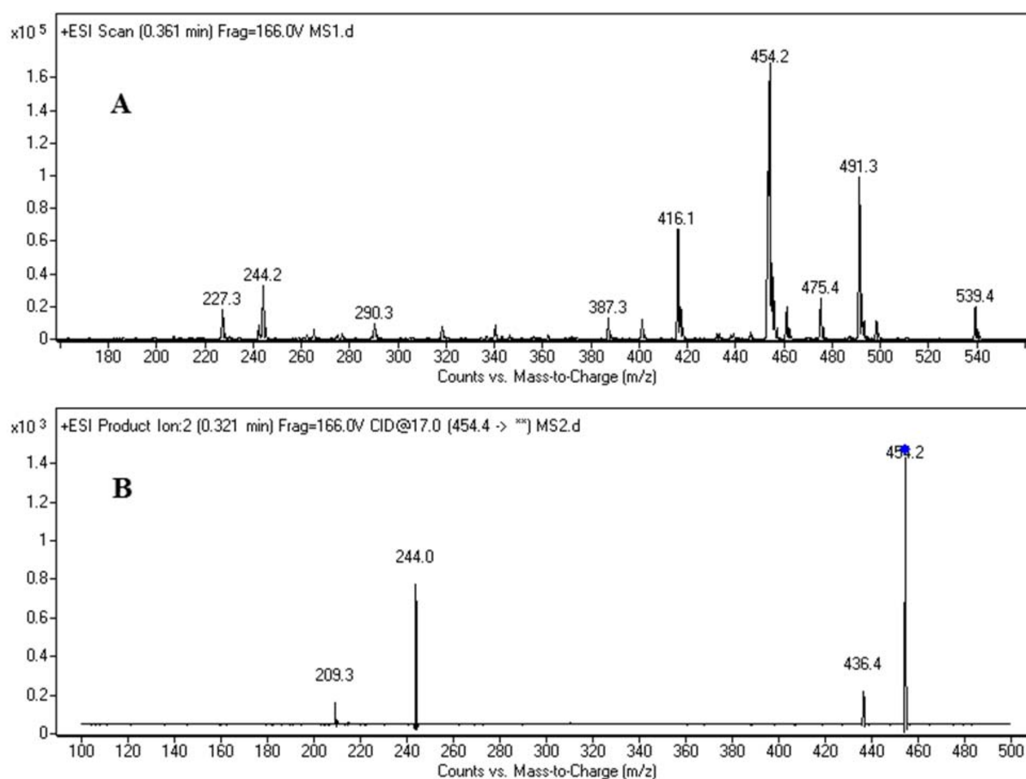


Fig. 2. Mass spectra of the BDETS-MMPB derivative.

Optimization of Derivatization Procedure of MMPB with BDETS

The effects of reaction temperature and time on the derivatization yield were investigated as shown in **Fig. 3a**. The rate of derivatization was positively correlated with temperature. Maximum fluorescence responses were observed at 90°C for 60 min or 100°C for 40min. The latter was adopted for further study and deemed optimum. You et al. derived the carboxyl groups of bile acids with BDETS in 30 min at 95°C.[22] Wang et al. derived the carboxyl groups of free fatty acids with BDETS in 30 min at 90°C.[21] We adopted a slightly longer time and higher temperature to derive MMPB with BDETS.

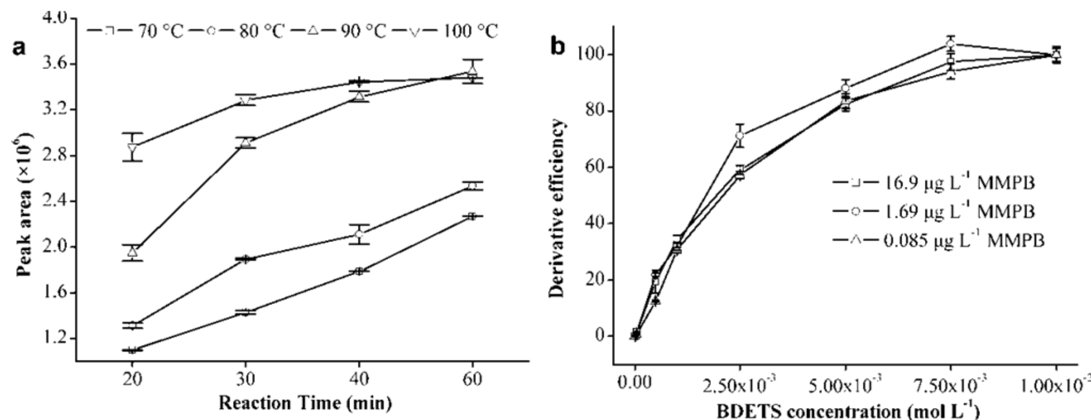


Fig. 3. Effects of reaction temperature (a), time (a) and BDETS concentrations (b) on the derivatization of MMPB.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Measurement of the derivatization yields of the three different concentrations of MMPB with varying concentrations of BDETS revealed that the yields increased with the concentration of BDETS and became stable at 7.5×10^{-3} mol L^{-1} (**Fig. 3b**); therefore, the concentration of BDETS was set at 7.5×10^{-3} mol L^{-1}. You et al. found that the derivative yield reached about 100% when a 10- to 15-fold molar excess of BDETS was used.[21] Our results showed that the derivative yields relied on the concentration of BDETS rather than the molar ratio between BDETS and MMPB.

The effects of different amounts of potassium carbonate (K_2CO_3) and water content on the derivation yield were also compared (**Fig. S2** of the supplementary material). Changes in the water content had a remarkable effect on the derivation reaction, while no significant differences were observed in response to variations in the K_2CO_3 concentration (P=0.865, LSD test). Indeed, as little as 2.5 μL of water could significantly reduce the fluorescence intensity, and increasing the water content resulted in a decreased peak area. Thus, anhydrous condition is essential for a stable derivation yield.

Analysis of serial dilutions of standard MMPB solutions ranging from 1 μg L^{-1} to 1 mg L^{-1} showed that response was a linear function of the amount of MMPB over a 1000-fold concentration range that could be described by the linear function Y=107364X–8796, where Y is the peak area and X (μg mL^{-1}) is the amount of MMPB (correlation coefficient > 0.9999). The corresponding derivatives were stable and enabled further HPLC analysis for at least 2 weeks with normalized peak areas <0.32%.

Oxidation of MCs

During Lemieux oxidation, $KMnO_4$ is renewable as the product, manganite (K_2MnO_4), can be oxidized to $KMnO_4$ by $NaIO_4$. Thus, sufficient $NaIO_4$ and small amounts of $KMnO_4$ are needed in the Lemieux oxidation. As shown in **Table 1**, saturated $NaIO_4$ solution (~20 mg mL^{-1}) has been used for oxidation in most previous studies. In all but one study, the MCs oxidation was conducted in weak base (pH ~9). In contrast, the $KMnO_4$ concentrations varied significantly among studies. Thus, we investigated the effects of different $KMnO_4$ concentrations on the MMPB yield with consistent $NaIO_4$ (20 mg mL^{-1}) concentrations and pH (9).

Table 1 Summarized oxidation conditions of MCs to produce MMPB.

Matrix	NaIO4 (mg mL^{-1})	KMnO4 (mg L^{-1})	pH	Yield	Origin
Water	20	50	1.6		16
Water	12.5	1896	9		20
Water	20	4000	9	96.5%	19
Algae	20	8000	9	75%	17
Sediment	20	8000	9	33-45%	11

Analytical Methods Accepted Manuscript

Tissue	18.5	365	9		25
Tissue	10	8000	9		7
Tissue	20	16,000	9	22.4%-40%	3, 26
Tissue	4.3	3160	9		14
Tissue	20.6	562	9		8

$KMnO_4$ concentrations of 50 and 8000 mg L^{-1} were too low or high for the oxidation of MCs (**Fig. 4**). The oxidation yields in $KMnO_4$ solutions with concentrations ranging from 500 to 4000 mg L^{-1} showed no significant differences (P = 0.124, SNK test). In this range of $KMnO_4$ concentrations, the effects of oxidation time were compared and no significant differences were observed (P = 0.124, SNK test).

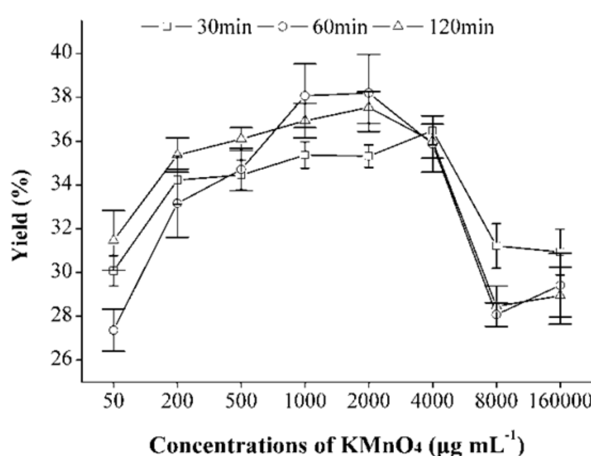


Fig. 4. Effects of $KMnO_4$ concentration on the yield of MMPB.

Since $KMnO_4$ is not a selective oxidant, other organics in matrixes can affect the formation of MMPB. This was verified by Wu et al., who found that the yield of MMPB decreased as the organic material in sediments increased.[11] Thus, we tested the effects of sediments on the yields of MMPB.

There were no significant differences in the oxidation yields of MCs in water solution and 1 g sediments from Lake Taihu and Lake Dianchi (**Fig. 5a**). However, significant reductions were found in 0.2 and 0.4 g sediments from Lake Caohai (**Fig. 5a**). By increasing the volume of oxidation reagent or repeating the oxidation procedure with more oxidation reagent, the loss of oxidation yield in sediment from Lake Caohai could be recovered (**Fig. 5b**).

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Analytical Methods Accepted Manuscript

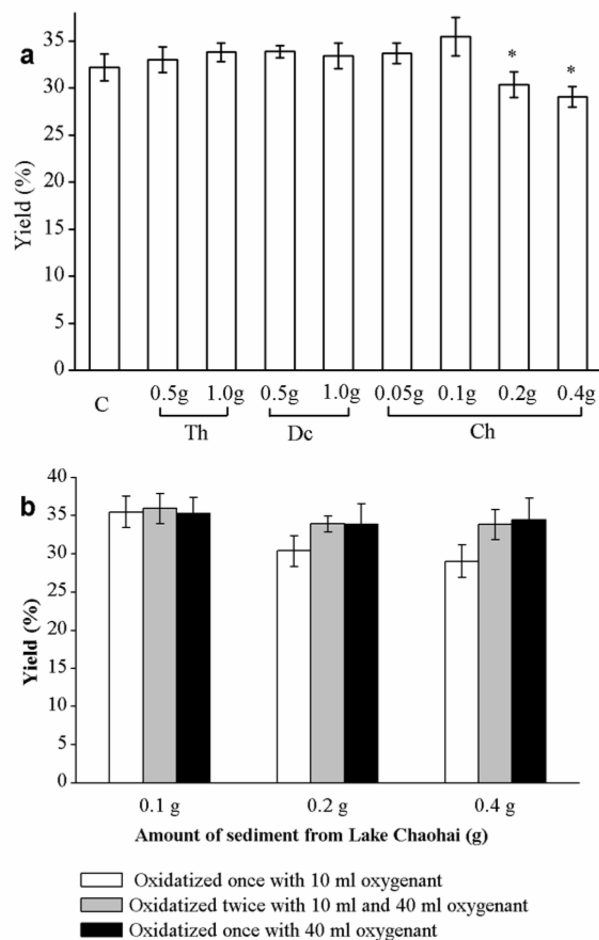


Fig. 5. The MMPB yield of the oxidation of MCs in different sediments (C, Control; Th, Lake Taihu; Dc, Lake Dianchi; Ch, Lake Caohai). a, the MCs were oxidized once in 10 mL oxidation reagent; b, the MCs were oxidized different times using various volumes of oxidation reagent.

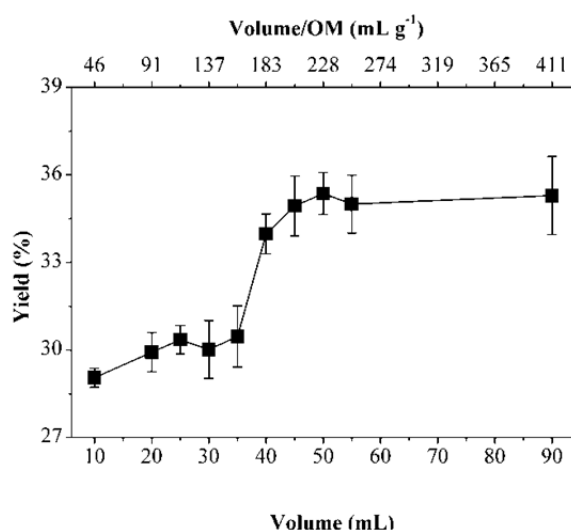


Fig. 6. The yields of MMPB from oxidation of 0.5 g of sediments from Lake Caohai (organic content, 438.21 g kg^{-1}) in different volumes of oxidation reagent. OM indicates organic matters in sediment.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

The relationship between the volume of oxidation reagent and organic content of sediment was tested by oxidizing 0.5 g spiked sediments from Lake Caohai with different volumes of oxidation reagent (**Fig. 6**). Stable yields were obtained when more than 45 mL of oxidation reagent was used. Since the organic content was 438.21 g kg^{-1}, 205 mL of oxidation reagent would be needed for sediments with 1 g organic matter.

A stable MMPB yield of 35% could be obtained when the MCs were oxidized by an appropriate volume of oxidation regent with fixed concentrations. The MMPB yield was reported to be 29 ~ 40% for animal tissues spiked with MCs,[3] and 33% ~ 45% for sediment samples.[11] Our results were in accord with these studies. Maximum MMPB yield from the oxidation of algae powder was found to be higher than 85% by Wu et al.[17] However, the MMPB yield was overestimated for the MCs concentrations in the algae powder was underestimated in the study.[11] Xu et al. oxidized MCs solutions under the similar oxidation conditions with this study[19] and the MMPB yield, 96.5%, was inconsistent with this study and other researches.

Extraction, concentration and clean-up of MMPB in environmental samples

To decrease the adverse impact of impurities of environmental samples on the determination of MMPB derivatives, the extraction capabilities of liquid-liquid extraction or solid-phase extraction were compared. As shown in **Table 2**, the extraction efficiencies of the SPE cartridges were significantly higher than those of the solvents, and there were no significant differences in the extraction efficiency of the three SPE cartridges for MMPB. The extraction efficiencies of diethyl ether and dichloromethane did not differ significantly, but the extraction efficiency obtained by n-hexane was significantly lower. Consequently, solid-phase extraction with SPE cartridges is more efficient for MMPB, and Sep-Pak cartridges were chosen for the subsequent procedures owing to their high cost-effectiveness.

Table 2. Recoveries of MMPB by liquid-liquid extraction or solid-phase extraction.

Material	Recovery (%)	Material	Recovery (%)
n-hexane	9±0	Sep-Pak cartridge	96±2
Diethyl ether	77±2	HLB cartridge	96±2
Dichloromethane	70±2	MAX cartridge	96±2

Analysis of spiked samples

A standard curve between the MCs amount (10 ng to 2.5 μg) and peak area determined by HPLC-FLD was generated (**Fig. 7.**) and the formula describing the curve was found to be $Y=(15.298X+0.265)\times10^6$, where X and Y are the amount of MCs (μg) and peak area, respectively. The R-Square was 0.9995.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

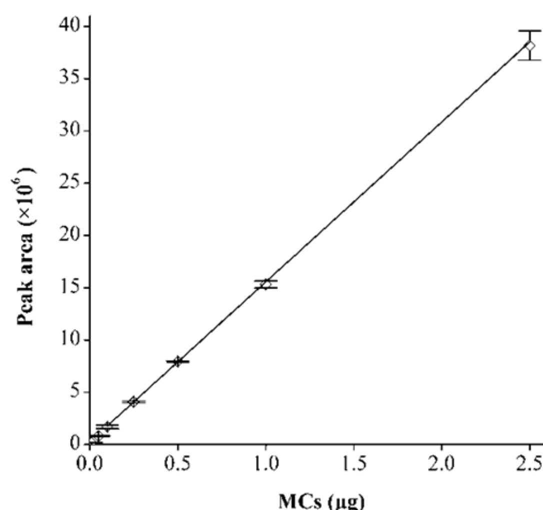


Fig. 7. Plot of peak area versus MCs content in deionized water.

Water and sediment samples spiked with different amounts of MCs were also detected by optimized procedures and the recoveries were determined by the standard curve. The detection limits for water and sediment samples were respectively 10 ng (80 mL water sample) and 100 ng (1 g sediment), which were equivalent to 125 ng L^{-1} and 100 ng g^{-1} (**Table 3**). The recoveries were 98 ~ 109% and most of the relative deviations were below 10%. Derivatization followed by GC-MS detection was adopted by Xu et al. and the detection limit was 0.56μg L^{-1} when 100 mL water sample was used for detection.[19] Roy-Lachapelle et al. detected MMPB with LDTD-APCI-MS/MS and the quantification limit was 0.2 μg L^{-1}.[7] Thus, the sensitivity of the proposed method was comparable with published methods based on mass spectrometry. As the low test cost and high sensitivity, this method may a good option in the routine detection of MCs.

Table 3. Recoveries of MCs from lake water and sediments.

Spiked MCs (μg)	Recovery from lake water (%)	Recovery from sediment (%)
2.5	106±4	107±1
1	99±2	102±5
0.5	109±3	100±5
0.25	101±2	98±12
0.1	107±5	104±15
0.05	109±9	Not detected
0.01	103±3	Not detected

Application to field samples

The total MCs concentrations in water samples of Lake Dianchi determined by HPLC-FLD ranged from 0.21

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

µg L^{-1} at point 6 to 1.01 µg L^{-1} at point 18 (**Fig. 8**). The MCs concentration was only higher than 1 µg L^{-1} at point 18. The results of the two methods were significantly correlated, with a relationship defined by Y=0.666X+0.052, where X and Y are the MCs concentrations determined by HPLC-FLD and ELISA, respectively. The correlation coefficient was 0.743. The average concentrations of MCs determined by HPLC-FLD and ELISA were 0.541 and 0.455 µg L^{-1}, respectively. The results of HPLC-FLD were higher than those obtained by ELISA (paired sample T test, p=0.044). Wu et al. found that the average soluble MCs content measured by ELISA was 0.5 µg L^{-1} in 2009,[27] which was consistent with our results.

Since anti-MCLR monoclonal antibody was used in the ELISA, other variants may not have the same cross-reactivity. It was reported that the antibody had good cross-reactivities with MC-LR, MC-RR, and MC-YR but lower reactivities with MC-LY and MC-LA (Y = tyrosine, A = alanine).[28] Thus, the total MCs content may be underestimated by ELISA.

The World Health Organization has proposed a provisional upper limit for microcystins LR of 1 mg L^{-1} in drinking water.[29] Our result revealed the low potential risk of MCs for human health in Lake Dianchi.

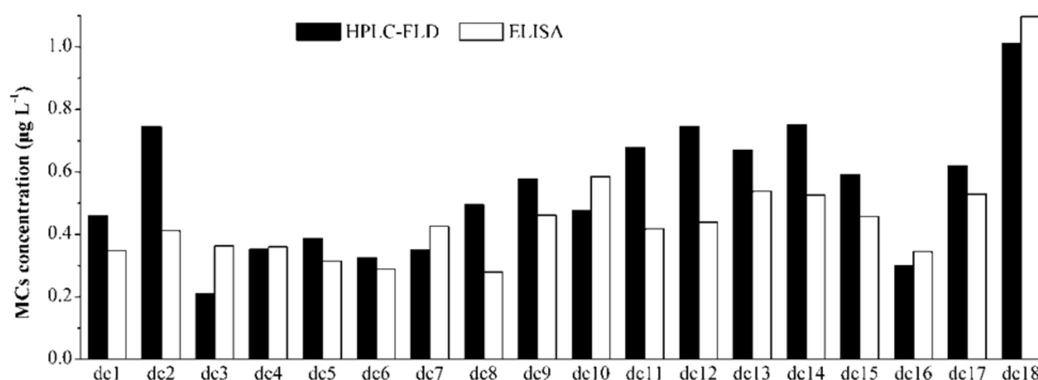


Fig. 8. The MCs contents at the 18 sampling points in Lake Dianchi determined by HPLC-FLD and ELISA.

No MCs were detected in sediment samples from any sampling points. After adsorption by sediment, MCs may be rapidly degraded by microorganisms in sediment as reported by Chen et al., who found that MC-LR could be degraded anoxically from 5 mg L^{-1} to below the detection limit within 2 days.[30] It seems that MCs are quickly degraded in sediments.

Conclusion

A sensitive method for the total microcystins determination in water and sediments of a eutrophic lake was developed. MCs were oxidized to MMPB with consistent yield (35%). After concentrated with a Sep-pak SPE cartridge, the MMPB was derivatized with BDETS and detected by HPLC with FLD. The detection limits for water and sediment samples were 125 ng L^{-1} and 100 ng g^{-1} respectively. The results of this method were well correlated with those obtained by ELISA. The developed methods were then successfully applied to field samples in Lake Dianchi.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31270509 & 31370504), and the Major Science and Technology Program for Water Pollution Control and Treatment (2013ZX07102005). We are indebted to Prof. Lirong Song, Prof. Nanqin Gan and Jin Liu of the Institute of Hydrobiology, CAS for their help of MCs detection by ELISA.

References

1. N. Gan, Y. Xiao, L. Zhu, Z. Wu, J. Liu, C. Hu and L. Song, Environmental Microbiology, 2012, 14, 730-742.
2. S. Wood, A. Rueckert, D. Hamilton, S. Cary and D. Dietrich, Environmental Microbiology Reports, 2010.
3. M.-R. Neffling, E. Lance and J. Meriluoto, Environmental Pollution, 2010, 158, 948-952.
4. M. Welker and H. von Döhren, Fems Microbiol Rev, 2006, 30, 530-563.
5. N. Khreich, P. Lamourette, P. Y. Renard, G. Clave, F. Fenaille, C. Creminon and H. Volland, Toxicon, 2009, 53, 551-559.
6. M. Masango, J. Myburgh, C. Botha, L. Labuschagne and D. Naicker, Water Res, 2008, 42, 3241-3248.
7. A. Roy-Lachapelle, P. B. Fayad, M. Sinotte, C. Deblois and S. Sauvé, Analytica Chimica Acta, 2014, 820, 76-83.
8. P. Suchy and J. Berry, International Journal of Environmental Analytical Chemistry, 2012, 92, 1443-1456.
9. S. Meissner, J. Fastner and E. Dittmann, Environmental Microbiology, 2013, 15, 1810-1820.
10. F. Bieczynski, V. A. Bianchi and C. M. Luquet, Fish Physiol Biochem, 2013, 39, 1309-1321.
11. X. Wu, C. Wang, B. Xiao, Y. Wang, N. Zheng and J. Liu, Analytica Chimica Acta, 2012, 709, 66-72.
12. K. Tsuji, H. Masui, H. Uemura, Y. Mori and K. Harada, Toxicon, 2001, 39, 687-692.
13. W. Chen, L. Li, N. Gan and L. Song, Environmental Pollution, 2006, 143, 241-246.
14. J. L. Ott and W. W. Carmichael, Toxicon, 2006, 47, 734-741.
15. E. Lance, M. R. Neffling, C. Gerard, J. Meriluoto and M. Bormans, Environmental Pollution, 2010, 158, 674-680.
16. X. Wu, B. Xiao, Y. Gong, Z. Wang, X. Chen and R. Li, Environmental Toxicology and Chemistry, 2008, 27, 2019-2026.
17. X. Wu, B. Xiao, R. Li, Z. Wang, X. Chen and X. Chen, Analytica Chimica Acta, 2009, 651, 241-247.
18. F. A. Dörr, E. Pinto, R. M. Soares and S. M. Feliciano de Oliveira e Azevedo, Toxicon, 2010, 56, 1247-1256.
19. X. Xu, R. Yu, L.-p. Wang, S. Wu and Q. Song, Analytical Methods, 2013, 5, 1799-1805.
20. K. Kaya and T. Sano, Analytica Chimica Acta, 1999, 386, 107-112.
21. H. Wang, Y. Suo, X. Wang, Y. Li, J. You and X. Zhao, Separation and Purification Technology, 2007, 56, 371-377.

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

22. J. You, Y. Shi, Y. Ming, Z. Yu, Y. Yi and J. Liu, Chromatographia, 2004, 60, 527-535.

23. L. M. Lei, Y. S. Wu, N. Q. Gan and L. R. Song, Clin Chim Acta, 2004, 348, 177-180.

24. D. Y. Ou, L. R. Song, N. Q. Gan and W. Chen, Environmental Toxicology, 2005, 20, 373-380.

25. D. E. Williams, M. Craig, S. C. Dawe, M. L. Kent, C. F. B. Holmes and R. J. Andersen, Chemical Research in Toxicology, 1997, 10, 463-469.

26. S. Cadel-Six, D. Moyenga, S. Magny, S. Trotereau, M. Edery and S. Krys, Environ Pollut, 2014, 185, 333-339.

27. Y. Wu, L. Li, N. Gan, L. Zheng, H. Ma, K. Shan, J. Liu, B. Xiao and L. Song, Journal of Environmental Sciences, 2014.

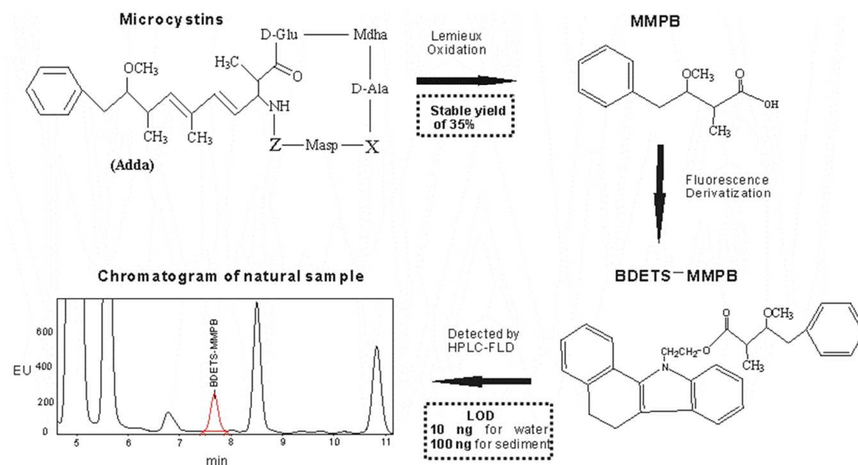
28. F. S. Chu, X. Huang, R. D. Wei and W. W. Carmichael, Applied and Environmental Microbiology, 1989, 55, 1928-1933.

29. World Health Organization., Guidelines for safe recreational water environments, World Health Organization, Geneva, 2003.

30. X. Chen, X. Yang, L. Yang, B. Xiao, X. Wu, J. Wang and H. Wan, Water Research, 2010, 44, 1884-1892.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



The procedures for the determination of total microcystins in water and sediment samples
254x190mm (96 x 96 DPI)

Analytical Methods Accepted Manuscript