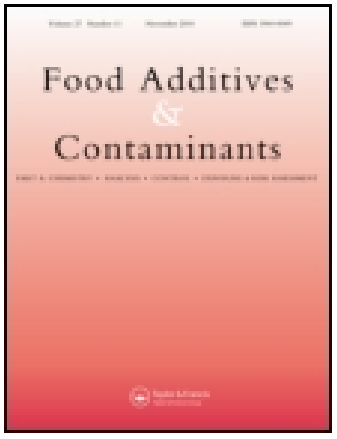

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Detection of the hepatotoxic microcystins in 36 kinds of cyanobacteria Spirulina food products in China

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Detection of the hepatotoxic microcystins in 36 kinds of cyanobacteria *Spirulina* food products in China

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Gel filtration chromatography, ultra-filtration, and solid-phase extraction silica gel clean-up were evaluated for their ability to remove microcystins selectively from extracts of cyanobacteria *Spirulina* samples after using the reversed-phase octadecylsilyl ODS cartridge for subsequent analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The reversed-phase ODS cartridge/silica gel combination were effective and the optimal wash and elution conditions were: H_2O (wash), 20% methanol in water (wash), and 90% methanol in water (elution) for the reversed-phase ODS cartridge, followed by 80% methanol in water elution in the silica gel cartridge. The presence of microcystins in 36 kinds of cyanobacteria *Spirulina* health food samples obtained from various retail outlets in China were detected by LC-MS/MS, and 34 samples (94%) contained microcystins ranging from 2 to 163 ng g^{-1} (mean = 14 ± 27 ng g^{-1}), which were significantly lower than microcystins present in blue green alga products previously reported. MC-RR – which contains two molecules of arginine (R) – (in 94.4% samples) was the predominant microcystin, followed by MC-LR – where L is leucine – (30.6%) and MC-YR – where Y is tyrose – (27.8%). The possible potential health risks from chronic exposure to microcystins from contaminated cyanobacteria *Spirulina* health food should not be ignored, even if the toxin concentrations were low. The method presented herein is proposed to detect microcystins present in commercial cyanobacteria *Spirulina* samples.

Keywords: cyanobacteria *Spirulina* health food; microcystins; liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS); clean-up

Introduction

Spirulina, a blue–green microalga, has been widely used for algal health food since the 1990s due to its fast growth, non-toxicity, assimilability (85–95%), high protein content (60–70%), well-balanced amino acid composition, richness in vitamins, and a great variety of biologically active agents which are present in appreciable amounts (Belay et al. 1993; Mosulishvili et al. 2002). World Health Organization (WHO) experts have indicated that *Spirulina* as a health-improving agent surpasses all so-far-known food components and medication (Mosulishvili et al. 2002). However, the cultivation environments of cyanobacteria *Spirulina* are also suitable for the growth of some toxic cyanobacteria species, such as *Anabaena*, *Microcystis*, *Oscillatoria*, and *Nostoc* (Bittencourt-Oliveira et al. 2005; Moore 2005; Babicca et al. 2006). These cyanobacteria can produce microcystins (MCs), a group of monocyclic heptapeptides that have more than 60 structural variables generally differing in the nature of the two L-amino acids and in the degree of methyl substitution (Soares et al. 2004). Microcystins are named according to their variable L-amino acids. For example, MC-RR contains two molecules of arginine (R), MC-YR contains tyrose (Y) and arginine (R), and MC-LR contains leucine (L) and arginine (R).

MCs mainly cause morphological and functional changes in hepatocytes (Gulledge et al. 2002), inhibiting the activity of protein phosphatases, especially types 1 and 2A, both *in vivo* and *in vitro* (Runnegar et al. 1995; Barford 1996; Codd et al. 2005), resulting in cell proliferation and cancer or an apoptotic process and cell death (Chen et al. 2005). More attention should be paid to the risk of consuming cyanobacteria *Spirulina* supplements which were cultivated in these systems. Based on the fact that increasingly more people around the world are beginning to prefer cyanobacteria *Spirulina* products, there is an urgent need to investigate the concentration of MCs in cyanobacteria *Spirulina* health foods.

Until now, the most important health concerns about MCs have been in relation to their contamination of drinking water supplies, and there is only limited information on the potential risks from

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exposure through the ingestion of contaminated dietary supplements made from cyanobacteria *Spirulina*, which are commonly consumed for their putative beneficial effects in the USA, Canada, and Europe (Gilroy et al. 2000). So far there have been only occasional reports on the presence of microcystin contamination in *Spirulina* health food and on the pretreatment of *Spirulina* samples for the detection of MCs. Enzyme-linked immunosorbent assay (ELISA), protein phosphatase inhibition assay (PPIA), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) were used to detect MCs in *Spirulina*, *Aphanizomenon flos-aquae*, or other unidentified blue–green algae (BGA) samples (Gilroy et al. 2000; Lawrence et al. 2001; Lawrence and Menard 2001; Xu et al. 2003; Saker et al. 2005). ELISA and PPIA measure total microcystins; they do not provide any information on the actual microcystin composition in the sample (Lawrence and Menard 2001). Barco et al. (2002) discussed the lack of specificity of ultraviolet light detection of HPLC that resulted in some problems in identifying MCs in the presence of matrix interferences, especially at low toxin concentrations. LC-MS/MS is sensitive and has a high specificity; the technique of MS/MS detection where the fragmentation pattern can be used greatly to assist in the identification of the toxins is useful (Edwards et al. 1993; Robillot et al. 2000). However, the ion suppression from co-eluting background components greatly affect accurate detection of MCs in *Spirulina* samples using LC-MS, which are difficult to eliminate (Lawrence et al. 2001). For an accurate detection of MCs in cyanobacteria *Spirulina* samples, it is necessary to develop an effective clean-up method to eliminate the matrix effects.

The purposes of the present study were (1) to develop an effective clean-up method to detect accurately small amounts of three common microcystins, MC-RR, -YR, and -LR, in cyanobacteria *Spirulina* health food products; and (2) to examine the MC contents in 36 kinds of cyanobacteria *Spirulina* health food products distributed in China using LC-MS/MS with comments on the potential risk to human consumption.

Materials and methods

Materials and reagents

Commercial quantitative standards (MC-RR, MC-YR and MC-LR) were obtained from Wako Pure Chemical Industries, Ltd (Chuo-Ku, Osaka, Japan). LC-MS-grade methanol and acetonitrile, and trifluoroacetic acid (TFA) for ultraviolet light spectroscopy were purchased from TEDIA Co. (Fairfield, OH, USA). Deionized water was obtained from a Labconco water purification system (Kansas City, MO, USA). Reversed-phased ODS silica gel and silica gel cartridges were manufactured by Waters Corporation (Milford MA, USA). Toyopearl resin (HW-40F) for a gel filtration chromatography column was obtained from TOSOH Corporation (Minato-Ku, Tokyo, Japan). A Vivaspin ultra-filtration centrifugal tube (2 ml, 3000D MWCO) was purchased from Beijing Genosys Tech-Trading Co., Ltd (Beijing, China). Thirty-six kinds of cyanobacteria *Spirulina* health food mainly in forms of tablets and capsules were obtained from various retail outlets in China, which were numbered from 1 to 36 (Table 1). All the cyanobacteria *Spirulina* samples were from different commercial growers. The cyanobacteria *Spirulina* for numbers 2, 6, 8, and 32 were cultivated in Lake Chenghai (Yunan Province, China) based upon the product descriptions. The species of *Spirulina* present in numbers 8, 13, 16, 21, 25, 26, and 29 were *Spirulina platensis* (Nords.) Geitler 1925.

Extraction of MCs from Spirulina samples

Samples (2.0 g) were homogenized, dried (30°C) for 24 h and then extracted with 20 ml 75% (v/v) methanol in water (Fastner et al. 1998) for 3 h while stirring three times. The supernatant was filtered through a GF/C microfibre filter (Whatman, Maidstone, UK) and then diluted with about 170 ml distilled water to ensure a methanol concentration less than 20%.

Optimization of the reversed-phase ODS cartridge clean-up procedure

The diluted extract from the *Spirulina* samples mixed with MCs standard solution was directly applied to 5 g of a reversed-phase ODS cartridge, which had been preconditioned with 30 ml 100% methanol and 30 ml deionized water. The cartridge was washed with 30 ml deionized water, then a wash/elution experiment on the reversed-phase ODS cartridge was performed. A total of 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% methanol in water were evaluated in the first elution step, and then 100% methanol was used in the second elution step. Elution from the column yielded the toxin-containing fraction. The toxin-containing fraction was evaporated to dryness. This residue was dissolved with 100% methanol, and the methanol solution was subjected again to a final clean-up procedure for precise quantification.

Optimization of the clean-up procedure after the reversed-phase ODS cartridge

An elution experiment on the silica gel cartridge was performed using *Spirulina* samples (spiked with MC standard solution). A total of 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% methanol in water and

Table 1. Concentrations of microcystins in 36 *Spirulina* samples collected in China.

Sample number	MC-RR ($ng\,g^{-1}$) ($n=3$)	MC-YR ($ng\,g^{-1}$) ($n=3$)	MC-LR ($ng\,g^{-1}$) ($n=3$)	Total ($ng\,g^{-1}$)	A ($g\,day^{-1}$)	B ($ng\,day^{-1}$)
1	26 ± 5	n.d.	n.d.	26	7.5	195.0
2	4 ± 3	n.d.	n.d.	4	4.2	16.8
3	5 ± 3	n.d.	n.d.	5	4.5	22.5
4	8 ± 2	n.d.	n.d.	8	5.0	40.0
5	8 ± 3	n.d.	n.d.	8	1.5	12.0
6	4 ± 2	n.d.	5 ± 4	9	3.5	31.5
7	8 ± 3	n.d.	n.d.	8	4.0	32.0
8	4 ± 2	3 ± 2	n.d.	7	6.0	32.0
9	7 ± 3	n.d.	4 ± 5	11	4.2	46.2
10	159 ± 11	n.d.	4 ± 2	163	7.5	1222.5
11	6 ± 4	n.d.	34 ± 5	40	6.0	240.0
12	37 ± 4	4 ± 3	4 ± 4	45	4.5	202.5
13	4 ± 2	n.d.	n.d.	4	4.5	18.0
14	4 ± 2	n.d.	n.d.	4	1.2	4.8
15	2 ± 2	n.d.	n.d.	2	3.8	9.6
16	2 ± 2	n.d.	n.d.	2	8.0	16.0
17	6 ± 4	n.d.	n.d.	6	3.0	18.0
18	4 ± 3	n.d.	n.d.	4	1.5	6.0
19	4 ± 3	n.d.	5 ± 4	9	3.0	27.0
20	6 ± 2	3 ± 3	n.d.	9	6.0	54.0
21	5 ± 3	4 ± 3	5 ± 4	14	3.8	53.2
22	7 ± 4	n.d.	5 ± 4	12	2.6	31.2
23	4 ± 3	7 ± 5	n.d.	11	2.6	28.6
24	2 ± 1	5 ± 4	3 ± 3	12	6.0	72.0
25	5 ± 3	5 ± 4	3 ± 3	13	2.5	32.5
26	5 ± 4	n.d.	3 ± 3	8	3.0	24.0
27	5 ± 2	5 ± 4	n.d.	10	9.0	90.0
28	2 ± 1	n.d.	n.d.	2	3.0	6.0
29	8 ± 4	7 ± 5	n.d.	15	3.9	58.5
31	5 ± 4	n.d.	n.d.	5	4.0	20.0
32	9 ± 3	n.d.	n.d.	9	4.5	40.5
33	5 ± 3	n.d.	n.d.	5	4.5	22.5
34	3 ± 3	n.d.	n.d.	3	1.0	3.0
35	4 ± 3	2 ± 1	n.d.	6	2.0	12.0

Note: A, maximum amounts of each sample it is suggested that people consume per day.
B, maximum total amounts of microcystins that people might consume per day according to the experiment results.
n.d., Toxins not detected.

100% methanol were evaluated in an elution step. After the toxin-containing fraction was eluted from the reversed-phase ODS cartridge and evaporated to dryness, the residue was dissolved with 5 ml 100% methanol and then eluted with 20 ml 80% methanol in water. The toxin-containing fraction was also evaporated to dryness.

Microcystin analysis

Qualitative and quantitative analyses of MCs were also performed using a Finnigan LC-MS system comprising a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion-trap mass spectrometer equipped with an electrospray ionization source (ESI). The instrument control, data processing, and analysis were conducted using Xcalibur software. Separation was carried out under the reversed-phase on a Hypersil GOLD 5 μm column (2.1 mm i.d. × 150 mm). The isocratic mobile phase consisted of solvent A water + 0.05% (v/v) formic acid/solvent B acetonitrile + 0.05% formic acid. The linear gradient programme was as follows: 0 min 30% B, 2 min 30% B, 7 min 50% B, 11 min 100% B, 14 min 100% B, 15 min 30% B, and 25 min 30% B. Sample injection volumes were typically 10 μl. MS tuning and optimization were achieved by infusing microcystin-RR and monitoring the $M+2H^{2+}$ ion at m/z 520. MS conditions were as follows: ESI spray voltage 4.54 kV, sheath gas flow rate 20 units, auxiliary gas flow rate zero units, capillary voltage 3.36 V, capillary temperature 250°C, and multiplier voltage −853.19 V. Tube lens offset, 55 V. Data acquisition was in the positive ionization centroid mode. MS detection was operated in four segments: (1) full-scan mode

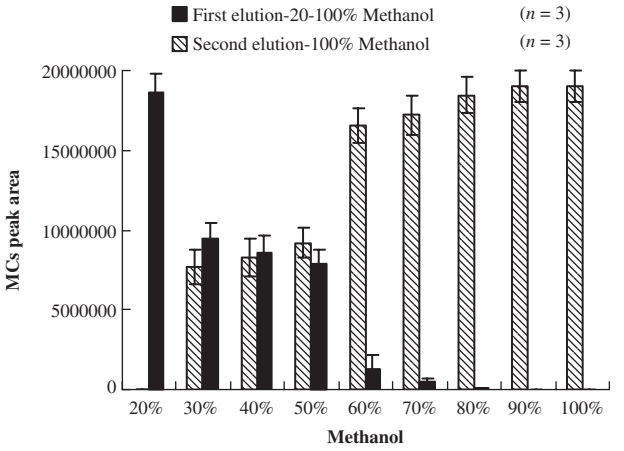

Figure 1. Determination of the wash and elution steps for MCs in *Spirulina* samples using a reversed-phase ODS cartridge. Proposed SPE procedure: H_2O (wash), 20% methanol (wash) and 90% methanol (elution).

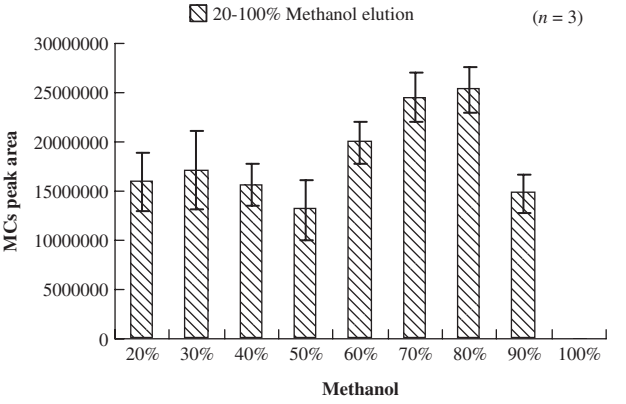

Figure 2. Determination of the elution step for MCs in *Spirulina* samples using a silica gel cartridge after the reversed-phase ODS cartridge. Proposed SPE procedure: 100% methanol (wash) and 80% methanol (elution).

with a mass range between m/z 400 and 1400, 4.2 min; (2) two scan events: full-scan mode as same as segment 1 and full MS/MS mode for MC-RR with parent ion: 520; isolation width: 1; normalized collision energy: 37%; 4.8 min; mass range of MS/MS was between 140 and 1100; (3) three scan events: full-scan mode the same as segment 1 and full MS/MS mode with parent ion: 995.5 for MC-LR and 1045.5 for MC-YR, respectively; mass range of MS/MS was between 270 and 1100 for MC-LR and between 285 and 1100 for MC-YR, respectively; isolation width: equal for both, 1; normalized collision energy: equal for both, 35%; 4.8 min; (4) full-scan mode as same as segment 1 in the rest time. All the values present in the text were measured by ESI-LC/MS/MS. The limit of detection (LOD) was considered as three times the signal-to-noise ratio (S/N) and the LOQ was ten times the S/N ratio. LOD and LOQ were calculated by spiking standards into SPE extracts of blank samples with $0.1\,\mu g\,ml^{-1}$ of MCs. The LODs for MC-RR, MC-YR, MC-LR were similar and slightly below $0.5\,\mu g\,kg^{-1}$ and the similarly for LOQs which were below $1.5\,\mu g\,kg^{-1}$. The LOD and LOQ for the three MCs were, therefore, considered wholly as 0.5 and $1.5\,\mu g\,kg^{-1}$, respectively, for LC-MS/MS.

Results

Optimization of the wash and elution conditions for the reversed-phase ODS cartridge

MCs were detected in 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% methanol elution. The MC concentrations increased with the increase of the elution concentrations of methanol. Low concentrations of methanol had a negative effect on the elution of MC-RR (data not shown). The MC content in 90% methanol solution became very close to the content in 100% methanol. The chromatograms of 100% methanol elution obtained by HPLC showed much more baseline noise than that of 90% methanol elution. An optimal wash/elution pattern is proposed (Figure 1): H_2O (wash), 20% methanol in water (wash) and 90% methanol in water (elution).

Optimization of the clean-up procedure after the reversed-phase ODS cartridge

MCs were detected in 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% methanol elution. An 80% methanol elution step was subsequently used (Figure 2). Figure 3 shows the peaks of three MCs in chromatograms of a *Spirulina* sample containing analytical standard of MCs (A) before and (B) after clean-up with the reversed-phase ODS cartridge/silica combination. The peak areas (AA) of the three MCs were greatly increased after the clean-up with the reversed-phase ODS cartridge/silica, indicating that the matrix effect had decreased which facilitated accurate quantification of MCs.

Microcystin analysis

An optimal extraction procedure is schematically proposed in Figure 4. The proposed procedure was used to determine the MC content of 36 *Spirulina* samples from various retail outlets in China. Of the total of 36 samples, some 34 (94%) contained concentrations ranging from 2 to $163\,ng\,g^{-1}$ (mean = $14 \pm 27\,ng\,g^{-1}$) (Table 1). MC-RR was present in 94.4% of the samples, MC-YR in 27.8%, and MC-LR in 30.6%. The percentage of MC-RR in the total MC concentration ranged from 15.0 to 100% (mean = 76.2%), MC-YR ranged from zero to 63.6%

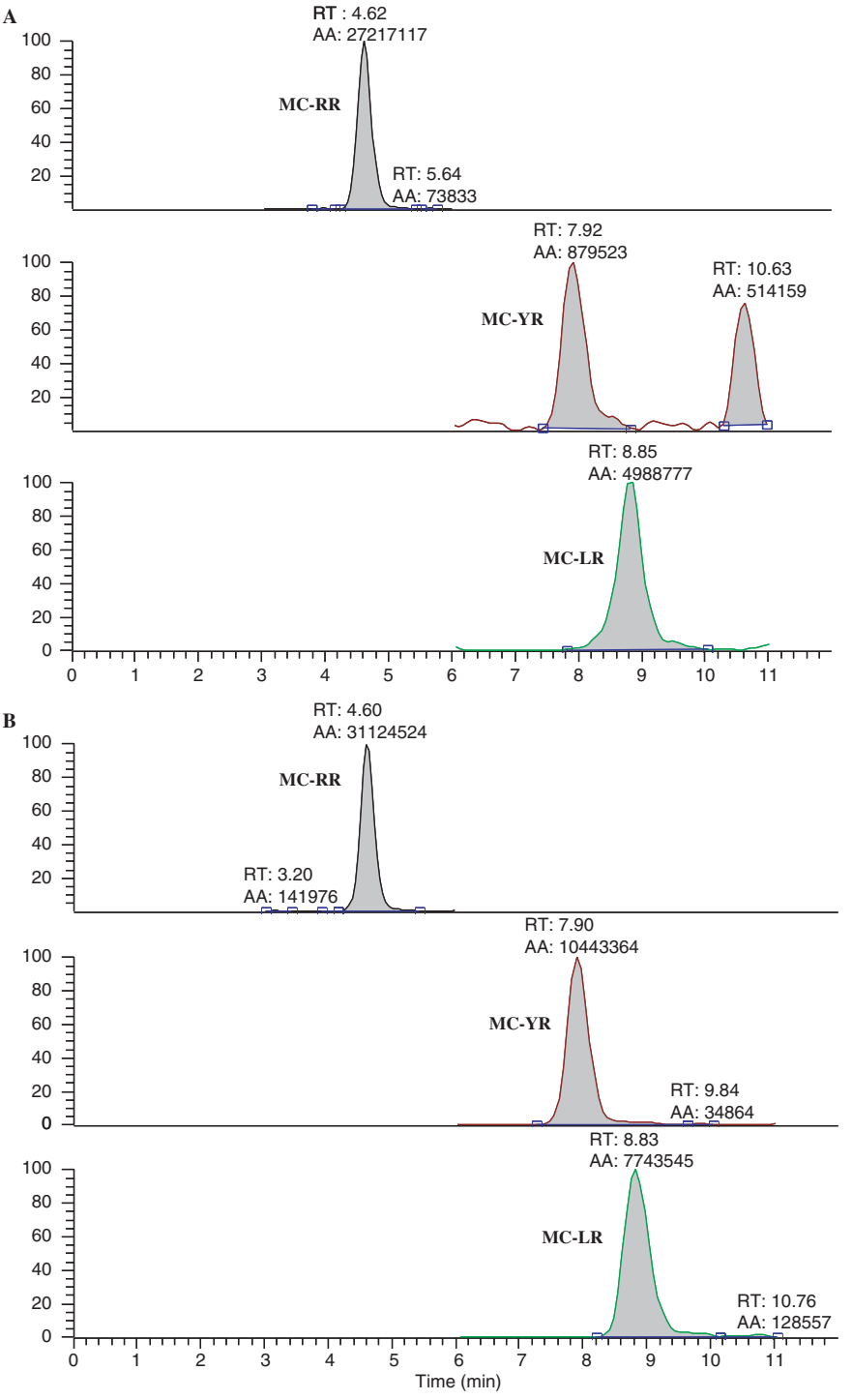

Figure 3. Comparison of high-performance liquid chromatograms and mass chromatograms of a *Spirulina* sample spiked to contain 0.5 μg g^{-1} analytical standard of MCs (A) before and (B) after clean-up with the reversed-phase ODS cartridge/silica combination.

(mean = 11.4%), and MC-LR from zero to 85.0% (mean = 12.5%). Only four samples contained all three MC variants, 19 samples only contained MC-RR, seven samples comprised both MC-RR and MC-LR, and six samples only comprised MC-RR and MC-YR. No MC variants were detected in two samples that were not given in Table 1.

Recovery (%) for Spirulina samples extracted by the optimal proposed procedure

A mixture of MC-RR, -YR, and -LR (152.5, 76.3, and 152.8 ng, respectively) was added to the extract and subjected to the analytical procedure shown in Figure 4. Recoveries ($n = 3$) were 82.3% ± 2.5% for MC-RR, 40.3% ± 3.0% for MC-YR, and

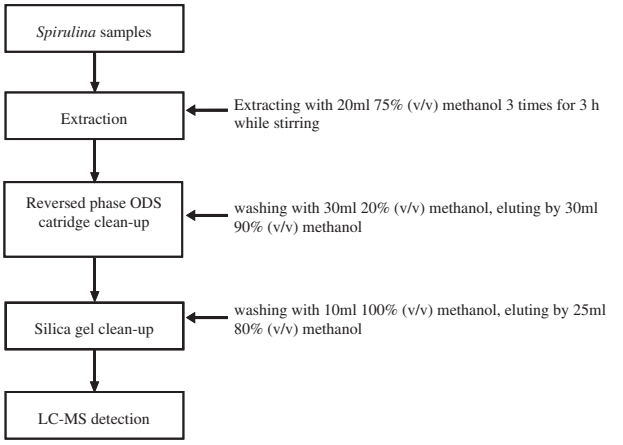

Figure 4. Schematic diagram of the analytical procedure for microcystins in *Spirulina* samples.

43.3% ± 2.7% for MC-LR, respectively. Losses were observed when the reversed-phase ODS cartridge was used before LC-MS analysis by Lawrence et al. (2001) when the MC standard solution was spiked at the $1\,\mu g\,g^{-1}$ level each. The low recoveries in the present study obtained for MC-YR and -LR could be caused by many factors, mainly the very low concentrations in *Spirulina* samples, the complexity of the matrix, possible retention in the cartridges, or losses in the evaporation step.

Discussion

This study has established optimal wash and elution conditions for extraction for MCs present in cyanobacteria *Spirulina* samples using reversed-phase ODS cartridge: H_2O (wash), 20% methanol in water (wash) and 90% methanol in water (elution). A combination of H_2O (wash), 20% methanol in water (wash) and 100% methanol (elution) was suggested to concentrate MCs in water samples and biological samples using a C18 reverse-phase silica cartridge, independently (Krishnamurthy et al. 1986; Zhang et al. 2003). Lawrence et al. (2001) described the C18 SPE clean-up procedure for *Spirulina* samples as: H_2O (wash), 20% methanol in water (wash) and 50% methanol in water (elution). However, the present results indicated that 50% methanol in water could not elute all the MCs present in *Spirulina* samples; 90% and 100% methanol almost had the same ability to elute MCs from the reversed-phase ODS cartridge, whereas the matrix effects were more obvious with 100% than in 90% methanol elution.

In the present study the chromatograms of cyanobacteria *Spirulina* samples obtained by HPLC showed excessive variable baseline noise, suggesting that *Spirulina* samples had significant co-extracted material that could interfere with the measurement of MCs when we used a clean-up step of the traditional reversed-phase ODS cartridge. Lawrence et al. (2001) pointed out that it was impossible to determine MCs accurately at the low concentrations present in BGA products by LC/MS without a clean-up step and the SPE C18 clean-up alone did not provide a clean enough extract to enable the detection of the toxins at low $\mu g\,g^{-1}$ concentrations. The extracts of the BGA products contained too much co-extracted material that interfered in LC-UV detection (Lawrence and Menard 2001). The present study was able to detect accurately very small amounts of MCs present in *Spirulina* samples by LC-MS/MS after eliminating co-extracted substances.

The present study, for the first time, attempted gel filtration chromatography and ultra-filtration centrifugation in a clean-up procedure after using the reversed-phase ODS cartridge for the determination of MCs present in cyanobacteria *Spirulina* samples. However, the results indicated that it was difficult to apply these two methods to clean-up *Spirulina* samples. It is well known that cyanobacteria *Spirulina* is rich in protein, and it is probable that the toxin-containing fraction eluted from the reversed-phase ODS cartridge mainly consisted of unknown proteins or peptides. These co-extracted materials resulted in distorted peak shapes or multiple peaks in the LC-UV detection. The stationary phase for gel filtration has a fractionation range, meaning that molecules within that molecular weight range can be separated. Gel filtration chromatography is relatively amenable to protein molecules and has frequently been used in protein separation (Al-Mashikhi and Nakai 1987; Yoshida 1990; Fee 2003; Roufik et al. 2005). Meanwhile, ultra-filtration is a pressure-driven membrane separation process that is increasingly being used in water and wastewater treatment processes, and in the pharmaceutical and liquid food industries. Ultra-filtration can remove dissolved macromolecules with MWCO between 1000 and 100,000 Da (Vigneswaran and Kiat 1988). Hegbrant et al. (1995) separated neuropeptide Y from plasma consisting of multiple peptide fragments using ultra-filtration. In the present study, the agreement between gel filtration chromatography and ultra-filtration centrifugation suggests that the molecular sizes of impurities in *Spirulina* were close to MCs and that it is impossible to use these two methods to clean up the *Spirulina* samples according to molecular size.

In the present study, the interfering substances in *Spirulina* samples were effectively eliminated by the reversed-phase ODS cartridge/silica gel cartridge combination. The optimal wash/elution conditions of extraction for MCs in *Spirulina* samples using the silica gel cartridge were as follows: the silica gel cartridge was washed with 100% methanol, followed by an 80% methanol elution. A total of 100%

Table 2. Microcystins in blue–green algae dietary supplements detected from 2000 to 2006.

Reference	Sample	Sample source	Extraction	Method	Concentration range ($\mu g\,g^{-1}$)	Contaminated samples (%)
Gilroy et al. (2000)	*Aphanizomenon flos-aquae*, *Spirulina*	USA	0.1 M ammonium bicarbonate, C-18 cartridge	ELISA	undetected, 18.4	
Lawrence et al. (2001)	*Aphanizomenon flos-aquae*, *Spirulina*, unidentified BGA samples	Canada	75% methanol/water, C-18 cartridge	ELISA, PPIA, LC-MS	0.5–35	100
Lawrence and Menard (2001)	*Spirulina*, unidentified BGA samples		75% methanol/water, immunoaffinity cartridge	HPLC	1.3–5.8	100
Xu et al. (2003)	*Spirulina*	China	5% acetic acid/water, Oasis cartridge	ELISA	undetected, 0.72	95
Saker et al. (2005)	*Aphanizomenon flos-aquae*	USA, Canada, Europe	Milli-Q ultrapure water	ELISA	0.1–4.72	100
Present work	*Spirulina*	China	75% methanol/water, C-18 cartridge/silica	LC-MS^2	undetected, 0.16	94

methanol (wash) and 70% methanol (elution) was suggested for the silica gel cartridge to analyse MCs in organs of aquatic animals (Xie et al. 2004). The reversed-phase ODS cartridge/silica gel cartridge combination can provide excellent sample clean-up for MCs in *Spirulina* samples at trace concentrations before correct LC-MS/MS detection in the present study.

Lawrence and Menard (2001) applied anti-microcystin LR immunoaffinity cartridges to remove MCs from extracts of blue–green algae, fish and water samples for analysis by HPLC. Compared with the immunoaffinity cartridges, not only could the commercially available reversed-phase ODS cartridge/silica combination in the present study selectively remove MCs from extracts of *Spirulina* samples, but also they greatly decreased the cost of cartridges, which could make the method adopted in present work more popular.

In the present study, MC-RR was present in 94.4% of the samples; the percentage of MC-RR in the total MC concentration ranged from 15.0% to 100% (mean = 76.2%). Kemp and John (2005) showed that lakes with a higher percentage of MC-LR were dominated by *Microcystis aeruginosa* and those with a higher percentage of MC-RR were dominated by *M. flos-aquae*. The presence of MCs in cyanobacteria *Spirulina* samples during the present study suggests that the cultivation of some *Spirulina* was polluted by some MC-producing cyanobacteria such as *M. flos-aquae*.

In the present study, the MC concentrations of the 36 cyanobacteria *Spirulina* health food samples from China ranged from undetected to 163 ng g^{-1}, which were significantly lower than MCs in those BGA (some including *Spirulina*) products previously reported (Table 2). The reasons for this might because that: (1) some BGA products detected previously were harvested from an open lake environment where other cyanobacteria can grow freely during the period of an extensive cyanobacteria bloom, whilst *Spirulina* is a commonly consumed BGA that is often grown under controlled cultured conditions (Gilroy et al. 2000); (2) there might be differences between *Spirulina* and other BGA products that had different sources or origins, and MC content might be influenced by spatial variability; and (3) differences existed among different MCs detected and the methods adopted to quantify MCs. For example, the reaction between antibodies and non-toxic microcystin-LR methyl ester will produce a false-positive from the toxicological point of view in ELISA (An and Carmichael 1994; Nagata et al. 1995) and the cyanobacterial sample itself might contain phosphatase activity that masks the presence of toxins in PPIA (Sim and Mudge 1993, 1994). Therefore, both ELISA and PPIA might overestimate the testing results.

There is a wide range of MC contents in BGA (including *Spirulina*) products from various distributors and analysed by different methods, and MC contents of most BGA samples exceeded the safe level of 1 μg g^{-1}, which was established for MCs in BGA products for adults by the Oregon Health Division (OHD) investigators (Gilroy et al. 2000). Although the concentrations of MCs in the present study did not exceed this safe level, attention should be paid to the possible health risks from chronic exposure to low doses of MCs. MCs chronically administered promote liver cancer in mammals by inducing oxidative DNA damage (Ito et al. 1997; Zegura et al. 2003). Long-term exposure to even very low levels of MCs is related to chronic human intoxication such as primary liver cancer (Yu 1989, 1995). In addition, there are groups more sensitive to MC poisoning that require special attention such as B-hepatitis patients, but also children and old people who are mostly likely to be the cyanobacteria *Spirulina* health food consumers.

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