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Comparison of Liquid Chromatography/Mass Spectrometry, ELISA, and Phosphatase Assay for the Determination of Microcystins in Blue-Green Algae Products

JAMES F. LAWRENCE, BARBARA NIEDZWIADEK, CATHIE MENARD, BENJAMIN P.Y. LAU, DAVID LEWIS, and TINE KUPER-GOODMAN
Health Canada, Health Protection Branch, Food Research Division, Banting Research Centre, 2203D, Ottawa, Ontario, K1A 0L2, Canada
SUSAN CARBONE
EnviroLogix, Inc. 55 Industrial Way, Portland, ME 04103
CHARLES HOLMES
University of Alberta, Department of Biochemistry, Edmonton, AB, T6G 2H7, Canada

More than 100 samples of blue-green algae products (consisting of *Aphanizomenon*, *Spirulina*, and unidentified blue-green algae) in the form of pills, capsules, and powders were collected from retail outlets from across Canada. The samples were extracted with 75% methanol in water and centrifuged to remove solids. Aliquots of the extracts along with spiked blank sample extracts were sent to each participating laboratory and independently analyzed for microcystins by enzyme-linked immunosorbent assay (ELISA), protein phosphatase inhibition assay, and by liquid chromatography–tandem mass spectrometry (LC–MS/MS) after sample cleanup using C_{18} solid–phase extraction. The results obtained by ELISA and LC–MS/MS agreed very well over a concentration range of about 0.5–35 μg/g. The colorimetric phosphatase results generally agreed with the other 2 methods. While the 2 biochemical assays measured total microcystin content compared with a standard of microcystin LR, the LC–MS/MS method measured specific microcystins (LA, LR, RR, YR) using external standards of these for identification and quantitation. Microcystin LR was found in all positive samples by LC–MS/MS. Microcystin LA was the only other microcystin found in the samples analyzed. These 2 microcystins represent essentially all the microcystins that were present in the extracts. Otherwise, the LC–MS/MS results would have been significantly lower than the results of the biochemical assays had other unknown microcystins been present.

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Microcystins are a group of monocyclic heptapeptides produced by several species of freshwater cyanobacteria (popularly referred to as blue-green algae) such as *Microcystis*, *Oscillatoria*, and *Anabaena*. They are potent hepatotoxins (1) and have been shown to be strong inhibitors of protein phosphatases 1 and 2A (2). In addition, they have been shown to be liver tumor promotors (3, 4). The most important health concerns related to these compounds have been in relation to their contamination of drinking water supplies and this has been comprehensively reviewed (5). However, in recent years blue-green algal health food products have become available to consumers through health food stores or direct mailing sources, for example via the Internet. Many of these products are consumed in the form of tablets, capsules, or powders. Because some of these blue-green algae are harvested from natural freshwater lakes, there exists the possibility of contamination with microcystins or other toxins. In 1999, Health Canada performed a national survey of blue-green algae health food products to determine if microcystin contamination presented a health concern in these commodities. A risk assessment based on these findings has been published elsewhere (6).

In order to obtain the most accurate information possible, it was necessary to use reliable analytical methods capable of detecting sub μg/g concentrations of microcystins in the algal products. Most of the analytical techniques developed for microcystins have focussed on their determination in algal cultures of *Microcystis* where the concentrations of microcystins were relatively high (100 μg/g or higher concentrations) or in water. Three types of methods have found the most application for these purposes: liquid chromatography (LC) with UV (7–10) or mass spectrometric (MS; 11–15) detection, enzyme linked immunosorbent assay (ELISA; 16–20), and phosphatase bioassay (21–23). None of these methods have been validated by interlaboratory studies and their application to quantitating sub μg/g concentrations in blue-green algae has been very limited. We report here the development and application of an LC/MS method for determin-

Table 1. Conditions for the selected ion monitoring for screening of microcystins by LC/MS

Ions monitored	Identity	Compounds (microcystins)	Cone voltage, v
519.8	$[M+2H]^{+2}$	RR	40
530.8	$[M+2Na]^{+2}$	RR	40
825.4	$[M+H]^{+}$	Nodularin (I.S.)	60
1045.6	$[M+H]^{+}$	YR	70
995.5	$[M+H]^{+}$	LR	70
1017.5	$[M+Na]^{+}$	LR	70
1009.5	$[M+H]^{+}$	MeLR	70
910.5	$[M+H]^{+}$	LA	70
932.5	$[M+Na]^{+}$	LA	70
986.5	$[M+H]^{+}$	LF	70
1002.5	$[M+H]^{+}$	LY	70
1025.5	$[M+H]^{+}$	LW	70

ing microcystins in blue-green algal health food products at concentrations down to 0.1 μg/g. In addition, the resulting data were compared with results obtained independently in 3 additional laboratories that used an ELISA method or a colorimetric phosphatase assay.

Experimental

Apparatus

(**a**) *Coffee grinder*.—Braun.
(**b**) *Homogenizer*.—Polytron.
(**c**) *Centrifuge*.—Mistral 2000.
(**d**) *Centrifuge tubes*.—Teflon, 50 mL.
(**e**) *Syringe filters*.—0.45 μm; Acrodisc.
(**f**) *Syringes*.—5cc, single use.
(**g**) *Test tubes*.—Glass, graduated, 5 and 50 mL.
(**h**) *Solid-phase extraction (SPE) C_{18} cartridges*.—3 mL, Supelco.
(**i**) *SPE vacuum box with manifold*.
(**j**) *Rotary evaporator*.—Buchi.

Reagents

(**a**) *Solvents*.—LC grade methanol; Milli-Q water.
(**b**) *Microcystin stock solutions*.—All microcystin standards were used as received to prepare the standard solutions. Microcystin LA and YR standards (Calbiochem); microcystin RR and LR (Sigma). Primary stock solutions of each of the microcystins LA, YR, RR, and LR were prepared by dissolving solid standards in methanol to obtain a concentration of 500 μg/mL for YR, LR, and RR and 200 μg/mL for LA. Nodularin was received from Health Canada as a working standard solution of 10 μg/mL in methanol.
(**c**) *Spiking standard solutions (each microcystin separately)*.—100 μL stock solution of YR, LR, or RR were added to 900 μL water and mixed; 250 μL stock solution of LA were added to 750 μL water and mixed.

Table 2. Conditions for multiple reaction monitoring for the quantitative analysis of 4 microcystins by LC–MS/MS

Microcystins	Precursor ions	Precursor/product ion pairs	Cone voltage, v	Collision energy, eV
RR	$[M+2H]^{+2}$	519.8→135	40	25
RR	$[M+2H]^{+2}$	519.8→213	40	25
Nodularin (I.S.)	$[M+H]^{+}$	825.5→135	60	40
Nodularin (I.S.)	$[M+H]^{+}$	825.5→227	60	40
YR	$[M+H]^{+}$	1045.5→135	70	60
YR	$[M+H]^{+}$	1045.5→213	70	55
LR	$[M+H]^{+}$	995.5→135	65	60
LR	$[M+H]^{+}$	995.5→213	65	55
LA	$[M+H]^{+}$	910.5→135	35	50
LA	$[M+H]^{+}$	910.5→213	35	45

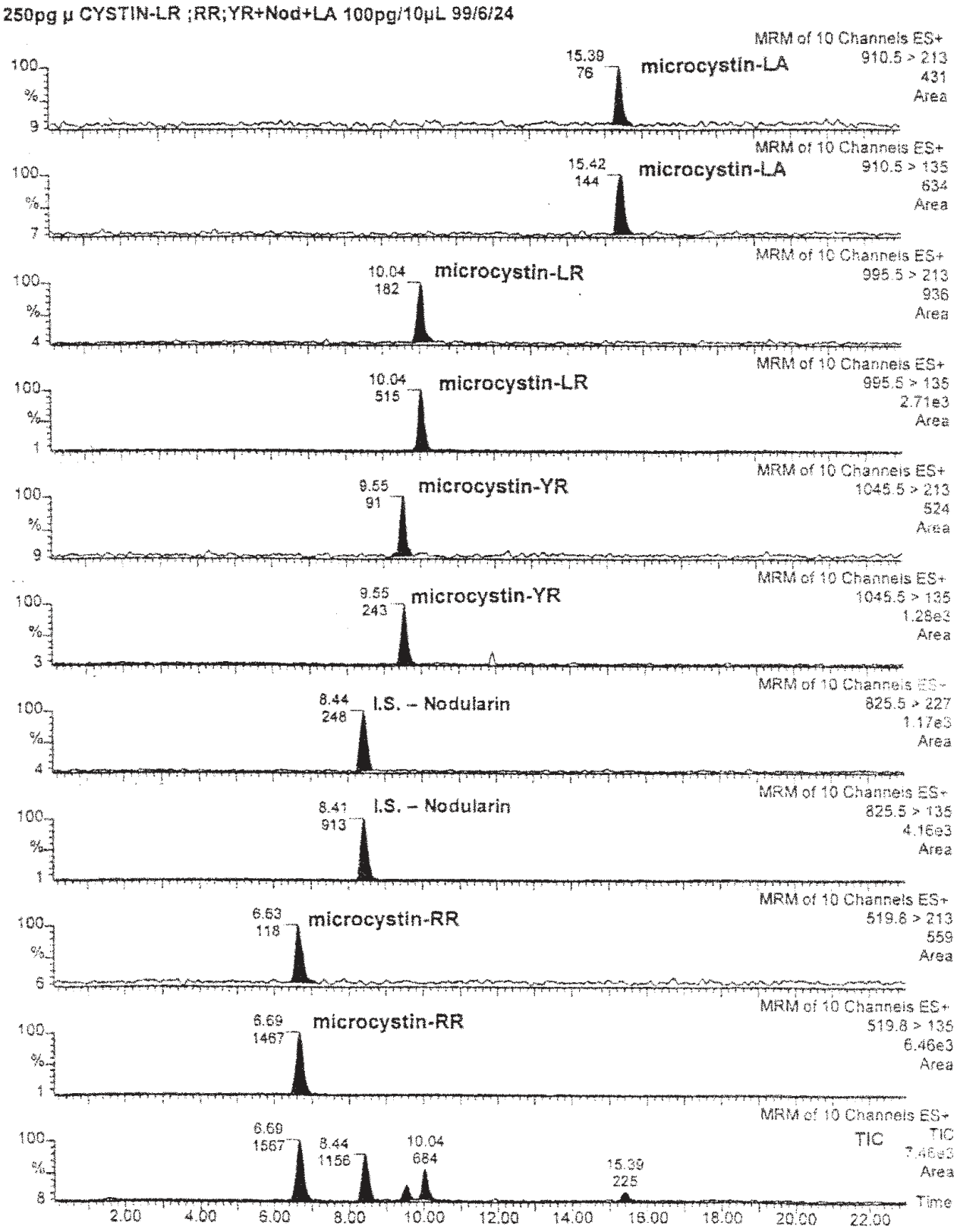

Figure 1. MRM mass chromatograms from an injection of 250 pg of a mixture of microcystins RR, YR, LR, nodularin, and 100 pg LA.

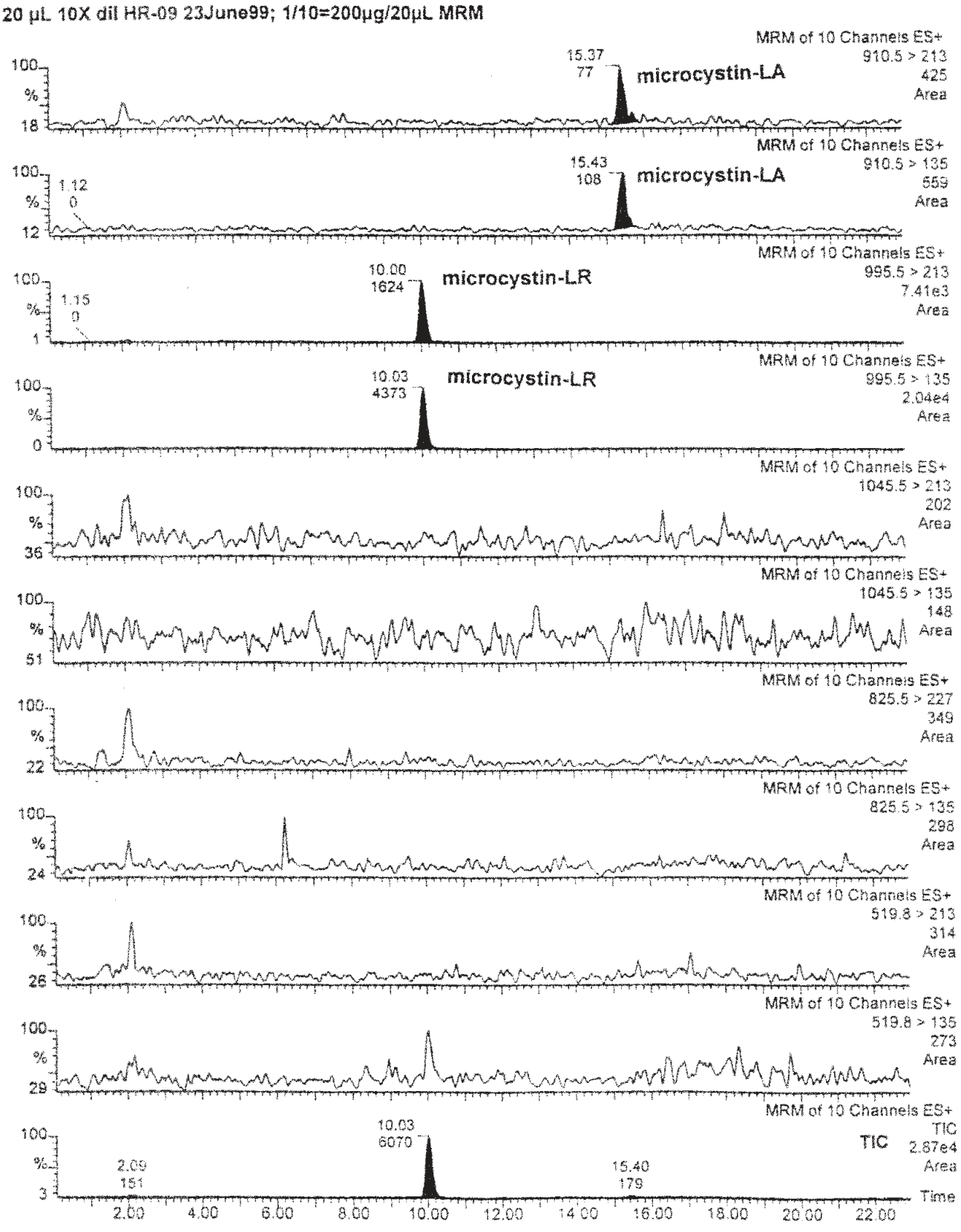

Figure 2. MRM mass chromatograms of a blue-green algae tablet containing 11.9 µg/g LR and 0.5 µg/g LA.

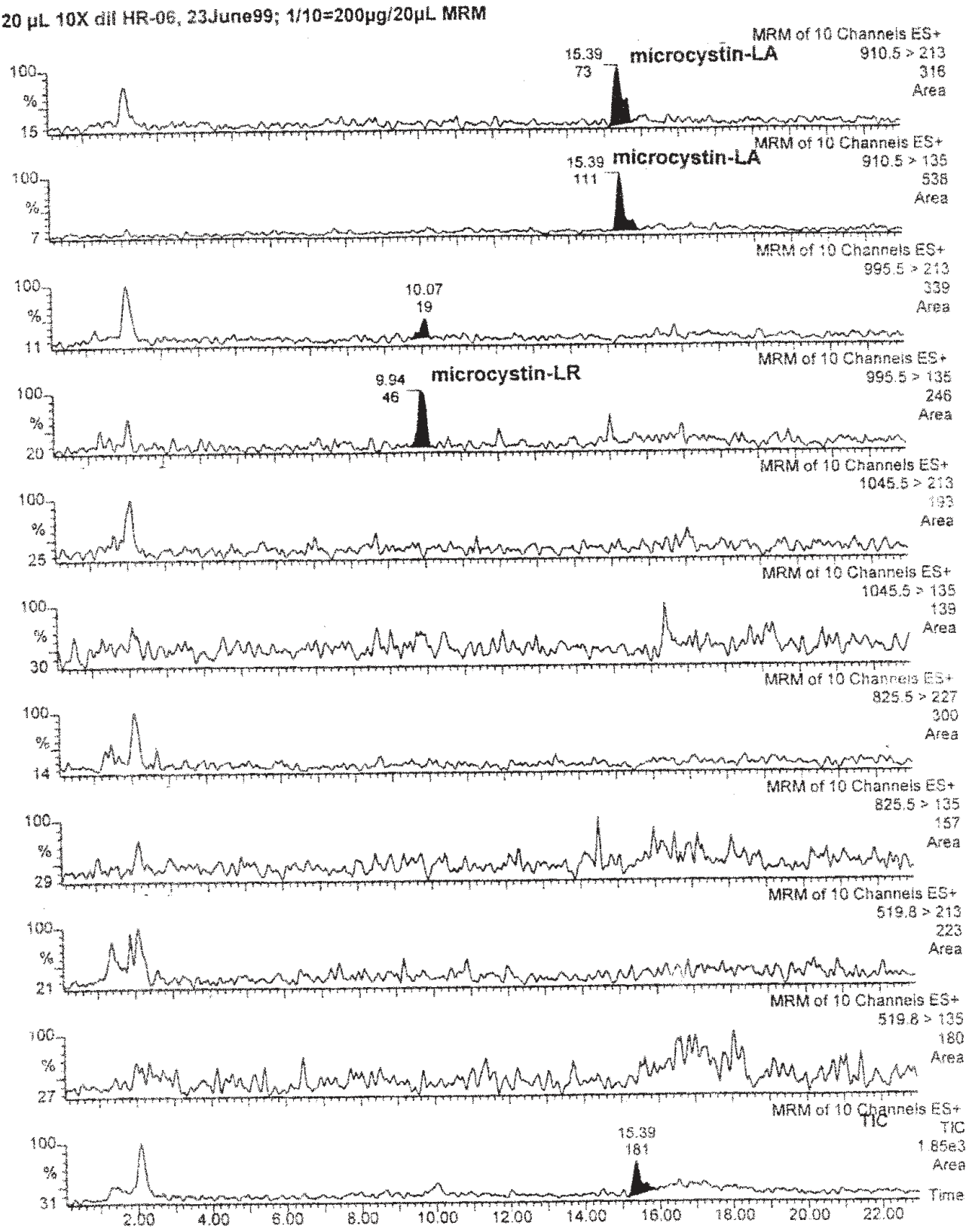

Figure 3. Blue-green algae sample containing 0.01 µg/g LR and 0.5 µg/g LA.

Table 3. Comparison of quantitative results for microcystins by LC/MS, LC–MS/MS ELISA, and phosphatase[a]

Samples	LC/MS (LR + LA in μg/g)	LC/MS/MS (LR + LA in μg/g)	ELISA (μg/g)	Phosphatase (μg/g)
AT-05	<0.2	<0.2	<0.3	0.1
AT-12	Interference	<0.2	<0.3	<0.3
AT-14	0.5	1.3	2.2	1.7
AT-18	1.4(-YR)+5.9	<0.2, <0.2, <0.2, <0.2	<0.3	<0.3
CR-30	0.7	1.3	2.1	1.5
CR-41	0.3		0.7	
OR-01	1.4		2.2, 2.1	1.9
OR-02	1.5, 1.4	1.9, 1.6, 1.3	2.6, 2.3, 2.1	1.1
OR-03	0.8		1.1	
OR-04	1.9	3.2	2.9	2.3
OR-05	3.7	0.3	0.3,0.3	
OR-06	5.6	0.3, 0.2, 0.3	0.4	
OR-07	0.2		<0.3	
OR-09	0.5	0.5, 0.5	<0.3, <0.3	
OR-10	0.4		0.7	
OR-11	0.3		0.7	0.6
OR-14	1.4	1.5	2.2	0.9
OR-20	0.9	0.7	1.9	2.1
OR-22	5.7	5.5	5.8, 6.2	6.6
OR-23	Interference	0.3, 0.2	0.4	
OR-24	6.1	5.7	9.9	6.3
OR-25	1	1.1	1.6	2.1
OR-26	Trace	0.4	0.4	0.9
OR-27	Interference	0.4	2.3	
OR-28	1.8	1.9	2.3	
OR-29	Trace		<0.3	
OR-30	Trace		<0.3	
OR-31	Trace		0.4	
OS-02	0.8		1.9	
OS-04	0.7, 0.5, 0.5	0.4, 0.8	0.5, 0.6	0.5, 0.5
OS-05	27.3, 26.3, 25.0	32.8, 35.7	33.5, 35.7	49
QR-08	<0.2	<0.2	<0.3	<0.3
QR-27	<0.2	<0.1	<0.3	<0.3
WR-02	3	4.1, 3.6	4.2	5.4
WR-06	<0.2	<0.2	<0.3	
WR-11	0.1	0.2	<0.3	0.4
WR-20	1.4		2.7	
WR-21	2.8, 4.2	4.9, 6.6	6.1	6.3
WR-23	0.1	0.5	0.6	1
WR-24	0.3		0.6	
WR-25	0.1	0.4	0.4, 0.6, 1.7	1.4
WR-26	2.4	2.8	3.3, 2.1	2.9
WR-28		6.2, 7.2	8.9	
WR-29	1.7,1.8	3.1	2.85	2.7
WR-35		1.4, 1.6	0.5	
WR-36		1.1	0.9	

Table 3. (*continued*)

Samples	LC/MS (LR + LA in μg/g)	LC/MS/MS (LR + LA in μg/g)	ELISA (μg/g)	Phosphatase (μg/g)
WR-37		0.5	0.5	
WR-39		2.2	1.8	
WR-42		<0.1	<0.3	

[a] LC/MS and LC–MS/MS data are not corrected for recoveries. "Trace" represents amount between LOD (S/N = 3) and LOQ (S/N = 9). Total microcystins were measured by ELISA and phosphatase using LR as a standard.

Sample Collection and Preparation

Samples of blue-green algae products were collected from retail outlets across Canada. They included tablets, powder, and capsules. Tablets were ground using a coffee grinder and mixed. Capsules were emptied and mixed. Powdered samples were likewise mixed. For each sample container, the complete contents were processed and a representative subsample was taken for extraction.

Sample Extraction

A 3 g portion of sample was placed into a 50 mL beaker and 20 mL 75% methanol (v/v) in water were added. The mixture was homogenized for 3 min with a Polytron homogenizer. The contents were transferred to a 50 mL teflon centrifuge tube and centrifuged at 4500 rpm ($3632 \times g$) for 10 min. The supernatant was transferred to a clean 50 mL graduated cylinder. A 10 mL volume of extracting solution was added to the 50 mL beaker, and the contents homogenized for 30 s to clean the homogenizer blades. The solution was transferred to the residue in the centrifuge tube, mixed, and the tube centrifuged again. The supernatant was combined with the first supernatant and the volume made up to exactly 30 mL. For analysis by ELISA and phosphatase, 1 mL aliquots of this solution were sent to the participating laboratories for independent analysis. For LC/MS, an aliquot of this solution was cleaned up using SPE C_{18}.

C_{18} SPE Cleanup for Non-Spirulina Samples

If an extract was not clear, it was filtered through a 0.45 μm Acrodisc filter before proceeding with the C_{18} cleanup. A 0.5 mL aliquot of this solution (equivalent to 50 mg sample) was diluted to 2 mL with water (to reduce the methanol concentration to less than 20%) and this solution was passed through a 3 g C_{18} SPE cartridge (previously conditioned with 10 mL methanol followed by 10 mL water) and the effluent discarded. For convenience, a reservoir was attached to the top of the cartridge and the flow rate regulated using a plastic syringe or an SPE vacuum manifold. The cartridge was then washed with 5 mL 30% methanol in water and this wash also discarded. The microcystins were eluted from the cartridge with 4 mL 80% methanol in water and collected in a 50 mL round bottom flask. The eluate was evaporated to dryness on a rotary evaporator at 45°C. The dry residue was reconstituted with 400 μL methanol. Then 600 μL water was subsequently added, resulting in a final volume of 1 mL consisting of a 40% methanol–water mixture. For analysis by LC/MS, a 0.1 mL volume of the solution was diluted to 1 mL with water.

C_{18} SPE Cleanup for Spirulina Samples

The same procedure as described above was used for *Spirulina* blue-green algal samples, except for modification of the wash and elution conditions. After addition of the sample extract, the cartridge was rinsed with 3 mL water followed by 3 mL 20% methanol in water, which was discarded. The microcystins were eluted with 6 mL 50% methanol in water and the fraction collected in a 50 mL round bottom flask. The eluate was evaporated to dryness and reconstituted as described above.

LC/MS

(**a**) *LC conditions*.—Separations were performed on a Hewlett-Packard HP-1100 LC system (Mississauga, Canada) consisting of a binary pump, autosampler, degasser, and a variable wavelength UV detector set to 238 nm. The LC column was a C_8 Lightning (2 × 100 mm, 3 μm particle size; Jones Chromatography, Mid Glamorgan, UK). The mobile phase consisted of a gradient at a constant flow rate of 0.2 mL/min using the following conditions: Mobile phase A, 0.08% HCOOH in water. Mobile phase B, acetonitrile. Gradient program: 30–40% B from 0.0–5.0 min; 40% B (isocratic) from 5.0–11.0 min; 40–75% B from 11.0–13.0 min; 75% B (isocratic) from 13.0–19.0 min: 75–30% B from 19.0–21 min; 30% B (isocratic) from 21–30 min. Sample injection volume was 10–20 μL.

(**b**) *Mass spectrometer conditions*.—A Micromass (Manchester, UK) Quattro II triple quadrupole tandem mass spectrometer was used for the analyses under the following conditions: Ionization mode, electrospray; detection mode, positive ion; capillary voltage, +3.0 KV; source temperature, 140°C; MS1 resolution, ~85% valley at base; MS1 ion energy, 1.0 V; MS2 resolution, ~80% valley at base; MS2 ion energy, 1.0 V; collision gas, argon; collision gas pressure, 1.8×10^{-3} torr; drying gas, 450 L/h; nebulizer gas, 15 L/h.

Data acquisition and processing were performed on a Windows NT 4.0 based MassLynx software (Micromass) version 3.0). Data smoothing of 2 adjacent data points (2 × 1 smoothing) was performed prior to peak integration in order to improve the baseline determination and peak detection. Quantitation was based on peak areas using the external calibration curve method. The instrumental limit of detection (LOD) was defined as 3 times the signal-to-noise

(peak-to-peak) ratio and the limit of quantitation (LOQ) was 3 times LOD. Signals between the LOD and LOQ were reported as "trace" to reflect positive identification but with uncertainty regarding the accuracy of the value.

Eight microcystins (RR, YR, LR, LA, methylated LR, LF, LY, and LW) were initially screened by LC/MS using electrospray (ESI) and selected ion monitoring (SIM) of 12 ions (Table 1) corresponding to their singly or doubly charged [M+H] and [M+Na] ions. Because standards for the latter 4 compounds were not available, optimal conditions for SIM of these compounds were extrapolated from the first 4 microcystins.

Quantitation and confirmation of 4 microcystins (RR, YR, LR, and LA) along with the internal standard, nodularin (added occasionally to sample extracts to note any matrix effects on signal response), were performed by LC–MS/MS in the multiple reaction monitoring (MRM) mode. Detailed MRM parameters are listed in Table 2. The cone voltage and collision energy of each precursor/product ion pair was optimized individually and incorporated into the MRM acquisition cycle.

Immunoassay

Immunoassays were performed with a commercially available ELISA test kit (EnviroLogix, Portland, ME; Cat. No. EP 022) exactly as described in the kit instructions. Aliquots of the aqueous methanol sample extracts were appropriately diluted with water and quantitatively analyzed by comparison with a microcystin LR standard.

Phosphatase Assay

The colorimetric protein phosphatase inhibition assay was performed according to An and Carmichael (23). Aliquots of the aqueous methanol extracts were appropriately diluted with water before analysis. Quantitation was based on a comparison with a known standard solution of microcystin LR.

Results and Discussion

Sample Extraction and Cleanup

A variety of extraction procedures for microcystins have been reported in the literature and these have been recently reviewed (5). The extraction procedure chosen for the present work was based on earlier work performed by Fastner et al. (24) who found that 75% methanol in water was the most effective for extraction of microcystins from lyophilized field samples. We found this solvent mixture functioned well for extraction of the 4 microcystins studied in this work. However, with this extractant, it was impossible to accurately determine microcystins at low μg/g concentrations in blue-green algae by LC/MS without a cleanup step. For this purpose we evaluated SPE C_{18} (7, 9) and a combination of SPE C_{18}/silica (8, 25, 26).

It was hoped that some of the preliminary method development work on algal extracts could have been performed using LC with UV detection. However, the SPE C_{18} cleanup alone did not provide a clean enough extract to enable detection of the toxins at low μg/g concentrations. To further purify the extracts, we evaluated the SPE C_{18}/silica combination reported earlier (8). Unfortunately, we found that the SPE silica cleanup was very sensitive to the conditioning and elution solutions. We obtained reproducible elution patterns for pure standards, but with sample extracts, the elution patterns changed and were not reproducible for the types of products that were included in this study. As a result, the attempt to develop an LC–UV method was abandoned and all further work used LC/MS using only the SPE C_{18} for extract cleanup using the conditions described in *Experimental*.

Even using the SPE C_{18} cleanup we observed matrix effects on the recoveries of the microcystins from spiked sample extracts. The elution patterns of the toxins were affected by the type and quantity of sample matrix passed through the cartridges. This was particularly evident with spiked samples of *Spirulina*, where the microcystin elution patterns were affected by even the smallest amount of sample matrix. For example, many *Spirulina* samples caused the microcystins to elute in the wash fraction (30% methanol) and not in the 80% methanol, as was determined using pure standards. Blue-green algal products originating from the Klamath Lake area of Oregon in the United States did not show this strong matrix effect. However, slight changes in elution patterns were observed if quantities of spiked sample greater than 100 mg were passed through the SPE C_{18} cartridge. To ensure minimal influence of the sample on the microcystin elution patterns, we limited the amount of matrix passed through the cartridges to 50 mg. Even with this small amount some *Spirulina* samples still exhibited a significant matrix effect. The reason for this is not known, although we noted that the pH of aqueous slurries of the *Spirulina* samples were for the most part above pH 8, while the non-*Spirulina* blue-green algal products exhibited a pH of less than 6. The *Spirulina* matrix effects were not eliminated by adjusting the pH before passage through the SPE cartridges. It is possible that other components in these samples caused these effects, although this was not investigated further. However, for *Spirulina* samples we modified the SPE elution procedure as described in *Experimental*. As a result of these studies, we found that verification of the SPE C_{18} elution patterns in different algal products using spiked samples was important to ensure accuracy of the method. This is particularly important from a regulatory point of view because significant changes in elution pattern can result in a substantial underestimation of the concentrations of microcystins present in the algal products.

The effectiveness of the extraction solution was tested on spiked samples of *Spirulina* and non-*Spirulina* products. The crude extracts before C_{18} cleanup were analyzed directly by ELISA independently in 2 different laboratories using the same commercial ELISA test. Each laboratory obtained virtually 100% recovery (range 90–113%, all values) from both types of algal products for RR, LR, and LA spiked at the 1 μg/g level each. However, losses were observed when the C_{18} cleanup was used before LC–MS/MS (MRM) analysis. Determinations in 3 different non-*Spirulina* algal samples at a

spiking level of 3 μg/g yielded average recoveries of 48% (range 43–50%) for RR, 89% (range 75–113%) for YR, 80% (range 69–96%) for LR, and 60% (range 50–75%) for LA. At 1 μg/g spiking level, recoveries of LR and LA through the complete procedure were 66 and 78%, respectively, from one non-*Spirulina* sample. Microcystin-RR consistently showed about a 50% recovery through the procedure. This was observed even when blank extracts were spiked immediately before C_{18} cleanup. The RR was not found in other fractions. When blank extracts were similarly spiked at 1 μg/g with the other microcystins just before the C_{18} cleanup, the recoveries were the same as those reported above for the complete procedure. Although not studied further, it appears that the C_{18} cleanup still allows enough sample matrix to be present in the 80% methanol fractions to create minor matrix effects (which can vary from sample to sample) on the LC/MS responses to the toxins. The extracts were usually colored pale yellow to pale green after the C_{18} cleanup.

LC/MS

The conditions described in the *Experimental* section were considered optimum after varying many parameters. Initially we performed the analyses using LC/MS in the SIM mode. Among the 8 microcystins monitored by LC/MS in the SIM mode, only LR, LA, and low levels of RR were found. While this proved to be generally selective enough to detect the toxins in the algal samples to below 1 μg/g concentrations, interferences even at such high masses occasionally occurred as evident by high background and/or broad and badly tailed peaks. Few false-positive peaks were observed. However, the additional specificity provided by the MRM (MS/MS) improved the quantitative aspects significantly. As a result, LC–MS/MS with MRM was considered to be the preferred approach for quantitation for regulatory purposes.

Two characteristic product ions, *m/z* 135 and 213, produced by the collision induced dissociation (CID) processes from the protonated molecules of the 4 microcystins (RR, YR, LR, and LA) were used in the MRM. The electrospray positive ion MS/MS spectra of RR and LR agreed very well with that reported by Edwards et al. (14). The ion at *m/z* 135 corresponds to the $PhCH_2CH(OCH_3)$ fragment, resulting from the α-cleavage of the methoxy group of the "Adda" residue. The *m/z* 213 corresponds to [Glu.Mdha + H]. Assuming the *m/z* 135 ion is the common fragment for most microcystins and using high collision energy, a parent MS/MS scan of *m/z* 135 was also performed with the anticipation of detecting all microcystins in the most highly contaminated samples. The results indicated the presence of LR and LA. No other microcystins were detected.

Over the course of 3 months during which time hundreds of samples were analyzed by LC–MS/MS, the absolute detection limits of the instrument for the 4 microcystins were maintained in the low picogram range. Considering the variability of recoveries and background noise observed in different matrixes, the overall limits of detection ranged from 0.02–0.17 μg/g for RR, 0.01–0.14 μg/g for YR, 0.04–0.36 μg/g for LR, 0.05–0.75 μg/g for LA, and 0.02–0.17 μg/g for the internal standard, nodularin.

A set of typical MRM mass chromatograms from an injection of 250 pg of a mixture of RR, YR, LR, nodularin, and 100 pg LA is shown in Figure 1. The MRM mass chromatograms of a blue-green algae tablet containing 11.9 μg/g LR and 0.5 μg/g LA are shown in Figure 2. Figure 3 shows another blue-green algae sample which contained 0.01 μg/g of LR and 0.5 μg/g of LA.

Comparison of Methods

Table 3 lists results obtained by LC/MS, LC–MS/MS, ELISA, and colorimetric protein phosphatase inhibition assay for microcystins in a number of naturally contaminated algal products. Overall there is a good agreement among the methods for most of the results. The LC–MS/MS results generally agreed better with the other 2 methods than the LC/MS results. This was due to the improved selectivity of the MS/MS as mentioned above. Two clear false-positives were obtained (for samples OR-5 and OR-6) using only LC/MS. However, it was observed with these samples that the peaks obtained for LR were broad and somewhat tailing, which was uncharacteristic of that compound leading to suspicion that they were not due to LR. This was confirmed by LC–MS/MS.

The agreement among the methods is important and provides strong evidence that the results accurately represent the true concentrations of microcystins in the samples. The 3 methods base their detection on entirely different principles. The LC–MS/MS approach is based on chromatographic retention and molecular fragmentation patterns while the ELISA is based on molecular recognition by antibodies and the protein phosphatase inhibition assay on inhibition of enzymatic activity by the microcystins. One limitation of the LC–MS/MS method is that it is best suited to detecting microcystins for which analytical standards are available. There was some concern that other microcystins might be present that would not be detected by LC–MS/MS. Similarly, the ELISA and phosphatase assays may produce incorrect results due to the differences in cross reactivity of the different members of the microcystin family and to other compounds that may be present in the extracts. It is very possible that the 3 methods could produce different results on unknown samples not because of poor performance characteristics of the methods, but due to the fact that they measure different things. The good agreement in the present comparison study indicates with very good confidence that the microcystins found are the only ones present in the samples.

From the results presented in Table 3 and from the results of an additional 67 samples analyzed as part of a national survey of blue-green health food products that showed a good correlation between the ELISA and LC–MS/MS method, these 2 techniques have been incorporated into a routine screening approach as part of a regulatory program for monitoring these substances in blue-green algal products. Because LC–MS/MS is relatively expensive for routine screening, initial analyses of the samples are performed by analyzing crude methanolic extracts of the products by ELISA. Positive sam-

ples and selected negative samples are subsequently confirmed by LC–MS/MS after aliquots of the extracts are cleaned up by SPE C_{18}.

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