Determination of Microcystin-LR in Drinking Water Using UPLC Tandem Mass Spectrometry–Matrix Effects and Measurement

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Abstract

A simple detection method using ultra-performance liquid chromatography electrospray ionisation tandem mass spectrometry (UPLC–ESI–MS–MS) coupled with the sample dilution method for determining trace microcystin-LR (MC-LR) in drinking water is presented. The limit of detection (LOD) was 0.04 µg/L and the limit of quantitation (LOQ) was 0.1 µg/L. Water matrix effects of ionic strength, dissolved organic carbon (DOC) and pH were examined. The results indicate that signal detection intensity for MC-LR was significantly suppressed as the ionic strength increased from ultrapure water condition, whereas it increased slightly with solution pH and DOC at low concentrations. However, addition of methanol (MeOH) into the sample was able to counter the signal suppression effects. In this study, dilution of the tap water sample by adding 4% MeOH (v/v) was observed to be adequate to compensate for the signal suppression. The recoveries of the samples fortified with MC-LR (0.2, 1, and 10 µg/L) for three different tap water samples ranged from 84.4% to 112.9%.

Introduction

Blooms of cyanobacteria in surface waters such as rivers, lakes, and reservoirs are a world-wide concern in water resource and quality management (1). Approximately 50–70% of cyanobacterial blooms have been proven to be acutely toxic since some of the cyanobacteria species had the ability to produce toxins (2,3). Microcystins (MCs) are common toxins produced mainly by cyanobacteria belonging to the genera *Microcystis, Anabaena, Planktothrix,* and *Nostoc* (3,4). To date, nearly 80 variants of MCs have been reported in cyanobacteria in natural or laboratory cultivated waters. Microcystin-LR (MC-LR) (Figure 1) has been identified as the most common and most toxic of those in the natural waters (2,5). Acute exposure to high levels of MCs can lead to massive haemorrhage and even death within a few hours; while an exposure to low levels of MCs might lead to acute or chronic liver hepatocyte injury (6,7). A β-amino acid Adda moiety [3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid] is largely responsible for the toxicity of MC-LR (8). The World Health Organization (WHO) put forward a guideline value of 1 µg/L to regulate MC-LR in drinking water, and the guideline has been accepted by many countries around the world (9).

The two commonly used biochemical methods for detection of MC-LR are based on enzyme-linked immunoassays assays (ELISA) and protein phosphatase inhibition assays (PPIA). Although these methods have low limits of detection (LOD) (0.05–0.1 µg/L), they may lack detection specificity and lead to false-positive results due to the presence of similar structures to MCs (8,10). In addition, detection methods using reversed-phase liquid chromatography (LC) coupled with ultra-violet detection (UV), mass spectrometry (MS), tandem mass spectrometry (MS–MS) and time-of-flight mass spectrometry (TOF) have been developed. For HPLC-UV, the LOD is 100 µg/L without preconcentration (8), but can be reduced to 1 µg/L with sample preconcentration (11,12). Without sample preconcentration, LC–MS–MS and LC–TOF-MS can provide a LOD of 0.08 and 0.1 µg/L, respectively (13,14). Ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC–ESI–MS–MS) has also been proposed for analyzing the MCs (8,15). Direct injection methods using UPLC–ESI–MS–MS coupled with isotope-labeled analogs (internal standards) have

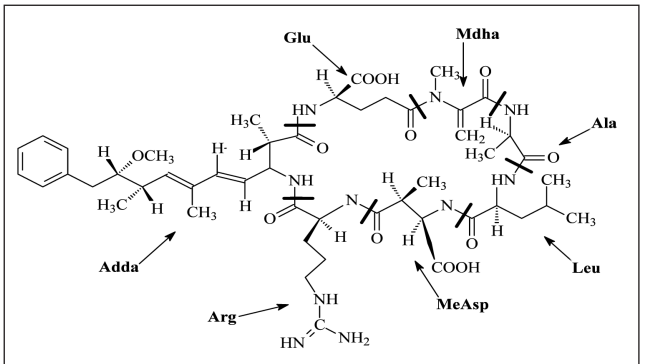

Figure 1. Molecular structure of MC-LR.

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been reported with detection limits of 0.5 µg/L (15), and LOD of 0.29 µg/L and LOQ of 0.11 µg/L (8). This appears to be a time-saving and more sensitive method.

For direct sample injection analysis using LC–MS or LC–MS–MS, the co-eluting matrix components may decrease ionization efficiency of analytes leading to signal suppression and affecting precision and accuracy (16–18). It has been noted previously that the signal suppression by water matrices vary according to the type of instrumentation used for analysis and the optimal dilution factors (17). Several approaches have been used to cope with such negative matrix effects including matrix-matched standards, standard addition, isotope-labeled analogs as internal standards and the sample dilution (17). For water quality analysis, the matrix-matched standard method may be difficult to use due to the variety of matrices in different samples. The standard addition method would be the most accurate, but might increase the number of injections (18). In comparison, addition of isotope-labeled internal standards for the correction of signal deviation has been proved to be an ideal method. However, its high cost and low commercial availability may make it less applicable. Among these methods, the sample dilution method is the simplest approach, which may actually minimize or even eliminate the signal suppression (17).

The objective of this paper is to examine the water matrix effects on signal detection in direct injection analysis of MC-LR in drinking water using UPLC–ESI–MS–MS. Solution effects such as ionic strength, dissolved organic carbon (DOC) and pH on signal detection of MC-LR have been examined. Sample dilution methods were tested to counter the matrix effects. This led to the development of a simple and rapid detection method for MC-LR in drinking water without the need of sample preconcentration, while achieving a low LOD.

Experimental

Chemicals

MC-LR (≥ 95%) was purchased from Alexis Biochemicals (Lausen, Switzerland). Acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific (Hampton, NH) (HPLC grade). Formic acid (FA) (HPLC grade) and ammonia solution (analytical grade) were obtained from Kermel Corporation (Tianjin, China). Humic acid (HA) was purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water (UPW) was produced using an Elga Purelab Ultra Analytic system (Bucks, UK). It had a resistance of 18.2 MΩ cm^{-1} and total organic carbon (TOC) < 2 µg/L.

A 25 mg/L stock solution of MC-LR was prepared in pure MeOH. The stock solution was diluted to 1 mg/L using 10% MeOH solution and this in turn was further diluted in UPW to produce standard solutions ranging in concentrations from 0.2 µg/L to 20 µg/L. The solutions were stored in amber glass bottles at 4°C.

NaF, NaCl, KCl, $MgSO_4$, $CaSO_4$, $CaCO_3$, and $CaCl_2$ (analytical grade) used for synthesizing electrolyte UPW solutions for examination of the ionic effects on signal detection were purchased from Sinopharm Chemical Reagent Co., Ltd, Beijing, China.

Instrumentation

The UPLC–MS–MS system consisted of an Acquity ultra-performance liquid chromatography system and TQD tandem mass spectrometer (Waters Corporation, Milford, MA). The resolution of MS detection was 1.0 Dalton. The analytical column was a Waters ultra-performance LC column (Acquity UPLC HSS T3) (50 mm × 2.1 mm i.d., 1.8 µm).

The concentration of ions was measured by Metrohm ion chromatograph (Herisau, Switzerland). Dissolved organic carbon (DOC) was determined by IL500 TOC analysis (Hach, Loveland, CO). Total dissolved solids (TDS) and alkalinity were measured following the standard methods of the US EPA (19). The residual chlorine in the tap water samples was measured by a chlorine analyzer (Hach, Loveland, CO).

Chromatographic conditions

The column temperature was set at 35°C and the injection volume was 10 µL. The flow rate was 0.2 mL/min. The binary mobile phase consisted of ACN and UPW, both with a content of 0.035% FA. The elution process started with a 3 min isocratic elution at an eluent strength of 2% ACN (98% UPW); and then was followed by a gradient elution, in which the percentage of ACN in mobile phase increased to 100% within 3 min. This condition was maintained for 1 min isocratic elution at the strong eluent strength before finally returning to the original eluent strength of 2% ACN for 2 min column equilibration before next injection.

Mass spectrometry conditions

MC-LR was analyzed using ESI MS in positive ion mode and with multiple reaction monitoring (MRM). The ions monitored were precursor ion ($[M+H]^+$) *m/z* 996.1 and product ions 135.1 (quantitative ion), 107.2 (qualitative ion), and 86.3 (qualitative ion). The product ions were obtained from collision energies of 73 eV, 63 eV, and 70 eV, respectively. The desolvation temperature, source temperature, capillary voltage, and cone voltage were set at 350°C, 110°C, 3.56 kV, and 65 V, respectively. The desolvation and cone gases consisted of nitrogen (99.999%) at flow rates of 400 L/h and 50 L/h, respectively. The collision gas was argon (99. 999%) with a flow rate of 0.2 mL/min.

Evaluation of matrix effects

The concentrations of the main ions ($C_{i,TW}$) of tap water in this laboratory are presented in Table I. The pH and DOC of the tap

Table I. The Tap Water Quality

	Concentration (mg/L)
Na^+	5.0
K^+	2.1
Ca^{2+}	40.0
Mg^{2+}	5.0
SO_4^{2-}	29.4
Cl^-	16.2
F^-	0.9
Alkalinity*	74.1

* Alkalinity: as $CaCO_3$.

water was 7.9 and 1.0 mg/L, respectively. Matrix effects were evaluated following the method of a previous study (20). MS–MS detection intensities (or areas) of known concentrations of standard UPW solutions (A) were compared with those of an analyte-spiked blank tap water sample (B). The ratio of (B/A × 100%) was defined as the absolute matrix effect (ME %). The value of 100% thus indicates no matrix effect.

Ionic strength

Seven ionic species that commonly present in tap water (Table I) were selected and added into UPW to evaluate the ionic strength effect. Three solutions of ionic concentrations 0.5 × $C_{i,TW}$, 1 × $C_{i,TW}$ and 2 × $C_{i,TW}$ were prepared with alkalinity backgrounds (add as 0.38, 0.75, 1.5 mM $CaCO_3$, respectively). Each was dosed with 5 µg/L MC-LR to assess the effect of ionic strength on the signal suppression for MC-LR during mass spectrometry analysis.

Natural organic matter

HA is the main component of natural organic matters in aqueous environment. Five HA UPW solutions with DOC concentrations of 1, 2, 3, 4, 5 mg/L were prepared for examination of their effects on signal intensity for MC-LR. Each of the solutions was spiked with 5 µg/L MC-LR.

pH

At room temperature (24.2°C), the pH of the laboratory tap water was 7.9 while that of the UPW was 5.8. The pH of UPW and tap water were adjusted to 5, 6, 7, 8 and 9 by addition of FA and ammonia solution for examination of its effects on signal detection for MC-LR.

Results and Discussions

Method development and standard calibration curves

Liquid chromatographic separation procedures are important for HPLC–MS and HPLC–MS–MS analyses. According the methodology of pre-cleanup in C_{18} column analyte extraction (21), the initial elution of aqueous eluent (normally 90% or higher of water) after injection of the MC-LR fortified tap water samples may have an influence on the reduction of negative real water sample matrix effects since it may wash out of the column before the analyte ionic species and some organic materials. For this reason, several initial isocratic aqueous mobile phase (ACN–UPW = 2:98) elution times (i.e., 1.5, 3, and 4.5 min) (coupled with the standard gradient elution, see Chromatographic conditions) were selected for evaluation upon the injection of the MC-LR fortified tap water samples. Detection intensities of triplicate mean values of measurements of the tap water samples spiked with 5 µg/L MC-LR are presented in Figure 2. It is clear that the initial isocratic elution time at the high water percentage in the mobile phase possessed an evident effect in the alleviation of the tap water matrix effects on signal intensity. An improvement on signal detection was evident at a longer time 3 min as compared with that of a shorter time 1.5 min. However, it seemed that there was no further improvement when the time was further increased from 3 to 4.5 min. This indicated that 3 min was an optimal value for the initial aqueous mobile phase elution for the purpose of alleviating the water matrix effects. It was thus selected for the following method development procedures.

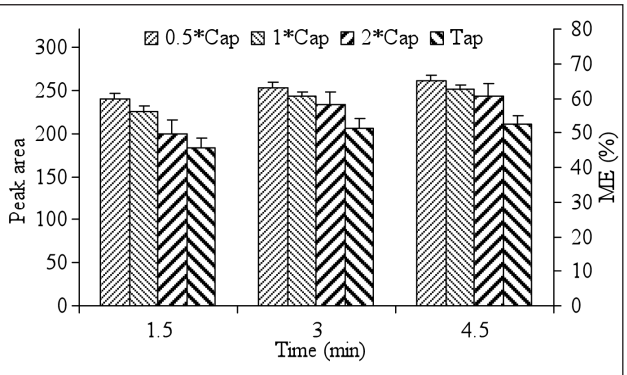

Figure 2. Effects of initial isocratic aqueous mobile phase (ACN–UPW = 2:98) elution time on signal intensities of MC-LR in aqueous solutions of different ionic strengths using UPLC–ESI–MS–MS. MC-LR: 5 µg/L.

Standard MC-LR UPW solution curves were established in the concentrations range from 0.2–20 µg/L. A linear relationship was observed in the calibration concentration range with a correlation coefficient (r) of 0.9997. The LOD was calculated at a signal-to-noise (S/N) = 3 and the LOQ was estimated as S/N=10. The LOD and LOQ of the method were 0.04 and 0.1 µg/L, respectively. The detection limits are the same as that of previous studies (8,13).

The precision of the method was assessed based on intra- and inter-day assays. Three concentrations (0.2, 1, 10 µg/L) were selected for the analyses. For each concentration, measurements of six injections were made for the intraday assays; the average values of five day's measurements were used for the interday assays. The relative standard deviations (RSD) were calculated based on all the measurements, which are listed in Table II. The intraday RSD ranged from 1.34% to 4.56%, and interday RSD ranged from 4.93% to 10.55%.

Matrix effects

Effect of ionic strength

The effect of salt concentration on the detection signal intensity of LC–MS-ESI analysis had been reported by Constantopoulos et al. (22). At high concentration, the presence of electrolytes may decrease ion transmission efficiency and suppress the detection signal of analytes. From our experimental results, signal intensity of the analyte decreased dramatically as a certain

Table II. Analysis Precisions of UPW Standard Solutions of MC-LR Using UPLC–ESI–MS–MS at 0.2, 1.0, and 10.0 µg/L.

MC-LR	Intra-day RSD (%) *n* = 6	Inter-day RSD (%) *n* = 5
0.2 µg/L	1.34	4.93
1.0 µg/L	2.92	8.07
10.0 µg/L	4.56	10.55

ionic strength was added in UPW MC-LR solutions (Figure 2). When the concentration of the ionic species in UPW increased to 0.5 and 2 times the content of the tap water, the detection intensities of the signals of the analyte were suppressed respectively to 65% and 58% of that obtained with the UPW standard solution. The suppression was even higher with the tap water background, which was decreased to 51.5% likely to be due to an even more complicated ionic background. The decrease may also be a result of the presence of co-eluting organic species competing for ionization.

We note that since the concentration of free chlorine in tap water was only 0.08 mg/L, reduction of MC-LR by the residual chlorine oxidation in tap water sample was unlikely (5).

The effect of the DOC

A previous study (23) has shown that organic matter can result in analyte signal suppression during mass spectrometry analysis. As can be seen from Figure 3, when the concentration of DOC was increased from 0 to 1 mg/L in UPW analyte solutions, a 5% increase in detection signal for MC-LR was observed. However, on further increase of DOC concentration from 1 to 5 mg/L, the detection signal intensity for MC-LR remained almost the same. In comparison, in the case of tap water conditions, a slightly larger effect of signal increase was seen when concentration of DOC increased from 1 mg/L to 3 mg/L; and then levelled off. It was clear that HA had a slightly positive effect for MC-LR MS–MS detection at low concentration.

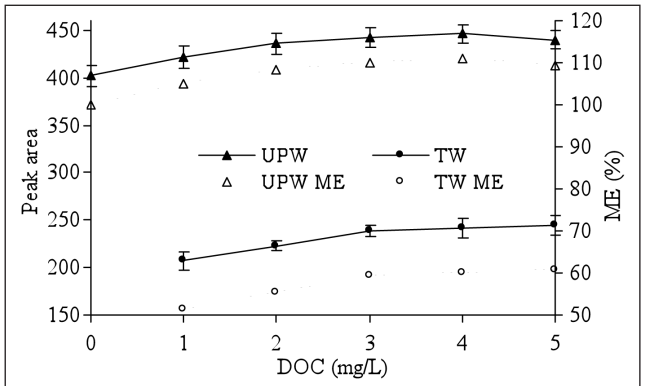

Figure 3. Effect of HA (measured as DOC mg/L) on signal intensities (signal peak areas) and ME of MC-LR (5 µg/L) in UPW and tap water (TW) solutions.

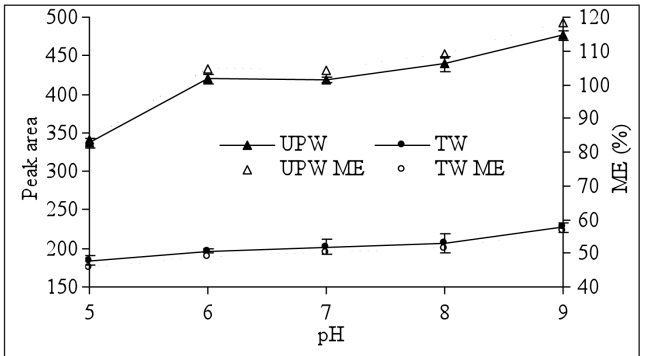

Figure 4. Detection intensities (signal peak areas) and ME of MC-LR (5 µg/L) in UPW and tap water (TW) solutions as a function of pH.

Effects of pH

The influence of pH on the signal intensity of MC-LR prepared in UPW and tap water is presented in Figure 4. In both UPW and tap water solutions, the analyte signal intensity increased with increase in pH with a more significant effect observed with UPW solutions than in tap water solutions. For UPW analyte solutions the detection signal decreased 20.7% when sample pH decreased from its original value of 5.8 to 5; while the signal increased 14.3% when pH increased from 6 to 9. In comparison, when the pH of the tap water sample was adjusted from its original value of 7.9 to 5 the response of MC-LR reduced 11%; whereas when pH was adjusted from 7.9 to 9 the signal response increased 9.6%.

The dilution effects of methanol and water

Dilution of methanol sample extracts using MeOH has been shown to alleviate or even eliminate the negative matrix effect on detection of some organic compounds (17). In this study, we also found that dilution of tap water analyte solutions with MeOH can increase the detection signal for MC-LR, which can compensate for the signal loss caused by the real water matrix (see Figures 5 and 6). From Figure 6, it is clear that the detected signal intensities increased as the dilution factor increased. A 4% addition of MeOH into the analyte-spiked tap water sample

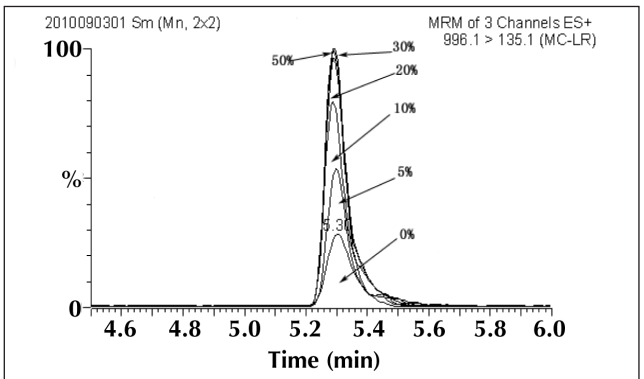

Figure 5. Comparison of chromatograms of MC-LR (5 µg/L) in MeOH-diluted tap water (TW) solutions versus percentage of MeOH (v/v%).

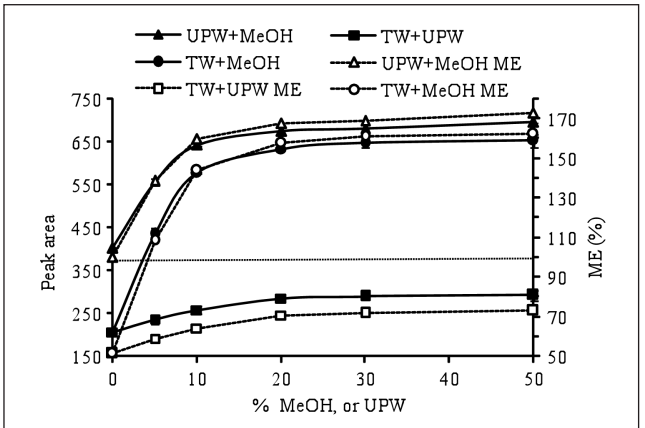

Figure 6. Signal intensities (peak areas) and ME of MC-LR in UPW solutions as function of percentage of MeOH addition (UPW + MeOH); and of MC-LR in tap water (TW) samples as functions of percentage of methanol (TW + MeOH) or UPW addition (TW + UPW). MC-LR: 5 µg/L.

restored the detection intensity of MC-LR up to the level detected with the UPW standard solutions. Surprisingly, when methanol addition was increased to 5%, the peak area was found to exceed the signal obtained from the standard solution at an identical analyte concentration (5 µg/L of MC-LR). When MeOH was added to tap water to give a final concentration of 20% MeOH, the signal intensity for MC-LR was 60% higher than that of the UPW standard solutions. However, on further increase in the amount of MeOH, only a slight increase in signal intensity was observed. Compared with methanol dilution, dilution of the tap water samples using UPW provided much smaller improvement in the detection signal for MC-LR. On addition of a large volume of UPW (50%, v/v) into tap water, the signal intensity for MC-LR only increased by 41%, far below the signal intensity for MC-LR obtained from the standard UPW solution. A previous study (24) reported that when a standard solution of MC-LR (0.5% MeOH) was diluted with 50% (v/v) UPW, there was no improvement in detection intensity of MC-LR by a photodiode array detector, whereas the signal intensity increased about 20 % when the sample was diluted with 50 % (v/v) methanol. This improvement was suggested to be related to the molecular properties of MC-LR other than simply solubility (21,24). However, the improvement by dilution with either water or methanol in this study may also be related to the effects of the solvent and the water matrix on the ionization efficiency of MC-LR in the tandem mass ESI system.

Method validation

To further validate the method, tap water samples of fortified MC-LR at concentrations of 0.2, 1, 10 µg/L were assessed for precision and accuracy by addition of 4% of methanol in the samples. Tap water samples were obtained from three different water treatment plants sourced by river, reservoir and groundwater in the Xi'an region. The total dissolved solids (TDS) of the three waters were 145 ± 3, 118 ± 3, and 362 ± 1 mg/L, respectively. The recoveries and RSDs obtained are listed in Table III. The recoveries were found to be in the range from 84.4 ± 2.5 to 112.9 ± 3.6 %, indicating that the method has high precision and accuracy.

Table III. Recoveries and Precisions of MC-LR Prepared in Various Tap Waters (TW) at 0.2, 1.0, and 10.0 µg/L When Analyzed by UPLC-ESI–MS–MS

TW Samples sources	Spiked MC-LR (µg/L)	Recovery ± RSD (%) (n = 3)
Wei River	0.2	112.9 ± 3.6
	1.0	86.8 ± 4.9
	10.0	94.5 ± 1.5
Black River	0.2	108.8 ± 0.8
Reservoir	1.0	85.2 ± 3.5
	10.0	95.2 ± 1.6
Groundwater	0.2	104.7 ± 1.6
Xian western District	1.0	84.4 ± 2.5
	10.0	94.7 ± 4.9

Conclusion

This study presents a detection method for MC-LR to cope with water matrix effects in UPLC-ESI–MS–MS without sample preconcentration. Tap water matrix effects were examined for the MS–MS signal intensities for MC-LR. Ionic strength showed signal suppression. Application of a longer initial isocratic aqueous mobile phase elution can alleviate the signal suppression, but was not able to restore the detection signal as compared with that under UPW standard solution conditions. At very low concentration, the presence of DOC in water samples may slightly increase the detection signal intensities for MC-LR, but such effects levelled off at a DOC concentration of approximately 1 mg/L. In addition, increasing solution pH led to a small increase of the signal intensity of MC-LR. Importantly, addition of a small percentage of MeOH (4%) to the tap water sample proved to be adequate to restore the detection intensity for MC-LR to levels equal to that with UPW standard solutions. The results obtained for three different tap water samples showed high recoveries ranging from 84.4% to 112.9% with high precisions. The LOD and LOQ for MC-LR in tap water were 0.04 and 0.1 µg/L.

Acknowledgements

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