Total microcystins analysis in water using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry

Audrey Roy-Lachapelle, Paul B. Fayad, Marc Sinotte, Christian Deblois, Sébastien Sauvé

**Highlights**
- Total microcystins are analyzed in water using the MMPB oxidation product.
- Samples are subjected to a simple oxidation and analyzed in 15 s.
- Limits of detection for total microcystins are 0.2 µg L⁻¹ in field-collected water samples.
- The calibration curve showed good linearity (R² > 999).
- Interday and intraday variation coefficients were below 15%.

**Graphical Abstract**

**Abstract**

A new approach for the analysis of the cyanobacterial microcystins (MCs) in environmental water matrices has been developed. It offers a cost efficient alternative method for the fast quantification of total MCs using mass spectrometry. This approach permits the quantification of total MCs concentrations without requiring any derivatization or the use of a suite of MCs standards. The oxidation product 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) was formed through a Lemieux oxidation and represented the total concentration of free and bound MCs in water samples. MMPB was analyzed using laser diode thermal desorption-atmospheric pressure chemical ionization coupled to tandem mass spectrometry (LDTD-APCI-MS/MS). LDTD is a robust and reliable sample introduction method with ultra-fast analysis time (<15 s per sample). Several oxidation and LDTD parameters were optimized to improve recoveries and signal intensity. MCs oxidation recovery yield was 103%, showing a complete reaction. Internal calibration with standard addition was achieved with the use of 4-phenylbutyric acid (4-PB) as internal standard and showed good linearity (R² > 0.999). Limits of detection and quantification were 0.2 and 0.9 µg L⁻¹, respectively. These values are comparable with the WHO (World Health Organization) guideline of 1 µg L⁻¹ for total microcystin-LR congeners in drinking water. Accuracy and interday/intray variation coefficients were below 15%. Matrix effect was determined with a recovery of 91%, showing no significant signal suppression. This work demonstrates the use of the LDTD-APCI-MS/MS interface for the screening, detection and quantification of total MCs in complex environmental matrices.

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1. Introduction

Microcystins (MCs) are cyclic heptapeptide hepatotoxins produced by cyanobacteria (blue-green algae), and are the most frequently observed cyanobacterial toxins [1]. A cyanobacterial bloom can occur in surface waters rich in nutrients and the toxins can be released from the cells to the natural water or even in drinking water reservoirs [2,3]. The cyclic structure of MCs consists of uncommon amino acids, of which two vary from one species to another (X and Z in Fig. 1) thus potentially generating over 80 known MCs structures [2,4]. The β-amino acid Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E), 6(E)-dienoic acid [5]) is the only part common to all MCs hepatotoxin congeners, and is responsible for their toxicity. This toxicity is caused by the toxin’s ability to inhibit specific protein phosphatases in animal tissues, and it is aggravated by its tendency to bioaccumulate in the liver, thus potentially leading to liver failure [6,7]. MCs LD₅₀ values range from 45 to 100 μg kg⁻¹ for mice, depending on the specific congener tested [8]. The World Health Organization (WHO) recommends an upper limit of 1 μg L⁻¹ for microcystin-LR in drinking water, the most studied and thought to be the most frequently occurring MC [9]. In a recent study, it was mentioned that it was possible to observe significant cyanobacterial bloom within a water treatment facilities, suggesting significant risks of toxin release, thus potentially contributing to high MC concentrations in drinking water [10].

There are many techniques available for the screening of MCs in natural water. Solid phase extraction for preconcentration and cleanup and quantification using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) is used for the specific analysis of several MCs species [7,11–13]. However, this analysis is limited to the MCs for which MCs standards are available. Currently, HPLC-MS analysis can detect roughly 10 different congeners over more than 80 possible forms. Gas chromatography coupled with mass spectrometry is used for the analysis of total MCs with the oxidation product 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) [14–16]. Since the toxin itself is not volatile enough to be separated by gas chromatography, an oxidation reaction prior to analysis is necessary to obtain the volatile compound MMPB from the Adda structure common to all the different MC congeners. However, the technique requires an extra derivatization step to make the MMPB more volatile using an ester addition. Traditional chromatography techniques are also time consuming, requiring sample pre-treatment, solid phase extraction and a chromatographic separation which takes several minutes for each analysis, as well as relying on a restricted number of available standards and not taking into account other MCs congeners. In mass spectrometry analysis, MALDI-TOF-MS has also been used recently for the identification of microcystins. However, the technique can be only used for qualitative purposes and the possibility for automated routine HPLC-MALDI interface is still limited for the quantification of microcystins [17]. Finally, the most commonly used technique for the nearly instant detection of MCs is enzyme-linked immunosorbent assays (ELISA) [12,18–20]. This technique is fast and sensitive, but encounters cross-selectivity which tends to overestimate MCs concentration. The ELISA method mostly uses the microcystin-LR standard, so the quantification assumes that every congener is reacting like MC-LR and results are expressed in MC-LReq.

The laser diode thermal desorption-atmospheric pressure chemical ionization interface coupled to tandem mass spectrometry (LDTD-APCI-MS/MS) is an alternative technique proposed for the quantitative analysis of total MCs. The LDTD is a sample introduction method using thermal desorption, thus eliminating the use of chromatographic separation prior to mass spectrometry detection. This approach results in ultra-fast sample analysis (<15 s sample⁻¹) with simple sample preparation, reducing time and material costs otherwise required for chromatography while also reducing solvent consumption. The LDTD method was previously developed for the analysis of a cyanobacterial neurotoxin, anatoxin-a, in water matrices [21]. It was also developed for several pharmaceutical and pesticide compounds in different environmental matrices including wastewater, sludge, sediments and soil samples [22–26]. The schematic and assembly of the LDTD-APCI source apparatus have been detailed previously [27].

The aim of this study is to develop and validate a new method using the LDTD-APCI-MS apparatus allowing for a simple, rapid and high-throughput detection and quantification of total MCs in complex environmental water matrices. MCs oxidation and MMPB extraction were optimized as a function of the MCs reaction yield and MMPB recovery yield. Several LDTD parameters were studied in order to optimize the thermal desorption and enhance the compound signal: the solvent position, the deposition volume, the laser power, the laser pattern and the carrier gas flow. The method was validated using 4-phenylbutyric acid (4-PB) as the internal standard. The method validation was done by evaluating the detection and quantification limits (MDL and MQL), linear dynamic range, accuracy, precision and matrix effect. This ultrafast technique could be used for the quicker screening and quantification of MCs applied for environmental and public health purposes.

2. Materials and methods

2.1. Chemicals, reagents and stock solutions

MMPB (2-methyl-3-methoxy-4-phenylbutyric acid) sodium salt standards, 94%, were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-PB (4-phenylbutyric acid) standard, 99%, was obtained from Sigma-Aldrich (Oakville, ON, Canada). Sodium (meta) periodate (purity ≥ 99.0%), sodium bisulfate (A.C.S. reagent), potassium carbonate (purity ≥ 99.0) and sulfuryl acid standard solution (1.000 mol L⁻¹) were obtained from Sigma–Aldrich (Oakville, ON, Canada). Potassium permanganate (A.C.S. reagent) was obtained from Biopharm (Montreal, QC, Canada). Methanol (MeOH) and ethyl acetate (EtAc) were of analytical grade purity from Fisher Scientific (Whitby, ON, Canada). Deionized/distilled water (dd-H₂O) was used for dilution. Individual stock standard solutions were prepared in MeOH at a concentration of 100 mg L⁻¹ and kept at −20 °C for 12 months for the.

![Fig. 1. Reaction scheme of Lemieux oxidation reaction to produce the MMPB used for total microcystin analysis.](image-url)
MMPB standards with no significative degradation ($P>0.5$), and a maximum of 6 months for the 4-PB standards (see supplementary, Fig. A-1).

2.2. Cyanobacterial bloom samples

Environmental samples were provided from the monitoring program realized by the Ministère du Développement Durable, de l’Environnement, de la Faune et des Parcs (MDDEFP – The province of Quebec Ministry of the Environment) (Québec, Canada). The samples were collected from lakes encountering cyanobacterial blooms and were sampled in glass bottles that were kept in the dark at 4°C until analysis. The lakes were targeted in different regions from the province of Québec, Canada, and were chosen for their high occurrence of cyanobacterial blooms. Microcystins (HiIR, HtYR, LA, LF, LR, LR (D-Asp3), LW, LY, RR, RR (D-Asp3), WR, YR) were previously quantified by LC-MS/MS by the Centre d’Expertise en Analyse Environnementale du Québec (CEAEQ), the analytical services of the MDDEFP.

2.3. Optimization of Lemieux oxidation and MMPB extraction

Microcystins (MCs) were obtained from a cyanobacterial bloom culture where the supernatant was sampled. The cells were sub-mitted to freeze–thaw lysis and then filtered with nylon filters of 0.2 μm obtained from Whatman (Florham Park, NJ, USA). Solutions were kept at −20°C for 6 months. The solutions were analyzed by LC-MS/MS and the MCs (MC-RR, MC-YR, MC-LR, MC-LY, MC-LW and MC-LF) were quantified with a total concentration of 22 mg L$^{-1}$. These MCs were used to optimize the Lemieux oxidation parameters. The reaction scheme of the Lemieux oxidation is presented in Fig. 1. The MCs solutions were spiked into a river water blank matrix to obtain a total MCs concentration of 150 μg L$^{-1}$. All reactions were conducted with KMnO$_4$ (50 mM) and NaIO$_4$ (50 mM) in alkaline conditions (pH 9) by adding K$_2$CO$_3$, at room temperature for 1 h, in the absence of light. The reaction was quenched with a saturated sodium bisulfite solution until all the purple color of the solution disappeared. A solution of 10% sulfuric acid was added until the pH became acidic (−2) [15]. Each reaction test consisted of three replicates and all solutions were conducted with a total volume of 1 mL. A volume of 100 μL of a saturated NaCl solution was added in the aqueous phase to enhance the liquid–liquid extraction. The liquid–liquid extraction was done with 1 mL of EtAc, and then the solutions were submitted to a vortex at 2500 rpm for 2 min. The supernatants were then used directly for analysis by LDTD-APCI-MS/MS.

2.4. Oxidation recovery yield and matrix effect

The sample oxidation recovery yield was measured as the proportion of MCs converted to MMPB and extracted by liquid–liquid extraction. Recovery was determined by spiking blank environmental water matrix with a known amount of MCs (120 μg L$^{-1}$ and 483 μg L$^{-1}$) and of internal standard (4-PB – 150 μg L$^{-1}$) before oxidation and compared to the same blank samples spiked with the corresponding amount of MMPB (25 μg L$^{-1}$ and 100 μg L$^{-1}$) and 4-PB (150 μg L$^{-1}$) after the oxidation and the liquid–liquid extraction with ethyl acetate. The concentration ratios were expressed as recovery percentage. The matrix effect was determined by comparing blank environmental water matrix samples with pure solvent samples both spiked with MMPB (25 μg L$^{-1}$ and 100 μg L$^{-1}$) and 4-PB (150 μg L$^{-1}$). The concentration ratios gave the signal recovery and were expressed as percentage.

2.5. LDTD-APCI-MS/MS

Desorption and ionization of MMPB and 4-PB were achieved with the T-960 LDTD-APCI interface model, controlled by the LazSoft 4 Software (Phytronix Technologies, Quebec, QC, Canada) integrated with the Excalibur 2.0 software (Thermo Fisher Scientific, Waltham, MA, USA). Negative mode ionization was achieved with the APCI source. Compound detection was performed using a Quantum Ultra AM triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Sample solution aliquots for the extraction supernatants (2 μL) were spotted into a LazWell 96-well plate containing inserts made of a thin proprietary stainless steel alloy. The samples were heated at 40°C for 5 min until complete evaporation of solvent. Details on the operation of the LDTD apparatus [27] (Fig. A-2) were described in previous studies [21,23,27]. Briefly, an infrared laser (980 nm, 20 W, continuous) heats the back of the hexagonal shaped concave well, and a thermal desorption of the compounds occurs. The uncharged sample analytes, in gas phase, are transported through a transfer tube by a carrier gas (medical grade purified air) and are then ionized in the APCI corona discharge. The ionized analytes are then transported to the MS detector.

The optimization of the LDTD-APCI parameters was conducted by adding 2 μL of a MMPB (250 μg L$^{-1}$) and 4-PB (150 μg L$^{-1}$) solutions made from blank environmental water matrix subjected to oxidation and extraction to take into account matrix effects. The LDTD-APCI desorption parameters were optimized to the following settings: the maximum laser power used was 35% for the laser pattern (2 s at 0%, 2 s ramping from 0% to 35%, 0.1 s from 35% to 0%, and 2 s at 0% for a total of 6 s), the carrier gas flow rate was 2 L min$^{-1}$ with a temperature of 50°C. The ion source parameters were set with the following settings: ion sweep gas 0.3 (arbitrary unit) and sheath gas, auxiliary gas, skimmer offset and vaporizer temperature were set to 0. Capillary temperature was set to 350°C with a discharge current of 4 μA in NI mode.

Selected reaction monitoring (SRM) mode was used, and two product ions for MMPB and 4-PB were selected as quantification and confirmation ions. The quantification ion, with the highest signal intensity, was used to determine the limits of detection and quantification. The relative intensity ratios of product ions, with constant values, were used for confirmation to the targeted compound and to avoid false positives. Optimization of MS/MS parameters was done with the following settings: collision gas (Ar) pressure at 1.5 mTorr, resolution for Q1/Q3 was set at 0.7 amu and scan time was set at 0.005 s. The different SRM transitions and their optimized parameters for the quantification and confirmation of MMPB are shown in Table 1. MMPB and 4-PB structures are presented with their respective fragments in Fig. A-3.

2.6. Method validation

The method validation was done according to the recommendation of validation protocol for environmental chemistry analysis from the Quebec’s MDDEFP guidelines [28]. Data analysis was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Selected reaction monitoring (SRM) optimal parameters for the MMPB analysis by APCI-MS/MS in negative ionization mode ($[M^-]$)</th>
<th>Tube lens (V)</th>
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</thead>
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<tr>
<td>Compound</td>
<td>Precursor ion $→$ product ion (m/z)</td>
<td>Collision energy (V)</td>
</tr>
<tr>
<td>MMPB</td>
<td>207.1 $→$ 131.1$^a$</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>207.1 $→$ 175.1$^a$</td>
<td>13</td>
</tr>
<tr>
<td>4-PB</td>
<td>163.1 $→$ 119.1$^b$</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>163.1 $→$ 119.1$^b$</td>
<td>12</td>
</tr>
</tbody>
</table>

$^a$ Quantification ion.

$^b$ Confirmation ion.
performed with LCQuan 2.5 software (Thermo Fisher Scientific, Waltham, MA, USA). Internal calibration with standard addition method was used with a seven-point calibration curve, ranging from 1 to 500 μg L⁻¹ in triplicates with a bloom matrix submitted to Lemieux oxidation, liquid–liquid extraction and spiked with MMPB and 4-PB. Method detection limit (MDL) and method quantification limit (MQL) were evaluated as 3 and 10 times the standard deviation of the mean value of 10 matrix samples spiked with about 10 times the estimated detection limit (we targeted 10 μg L⁻¹). Accuracy was determined by the relative error (RE – %) using the MC spiked bloom sample previously quantified by LC-MS/MS and then quantified by internal calibration with standard addition method. Precision (interday/intraday) and accuracy were expressed as the percentage relative standard deviation (RSD), and were then evaluated using concentrations of 25 μg L⁻¹, 100 μg L⁻¹ and 250 μg L⁻¹ of MMPB as quality control (QC). The solutions were spiked in a blank river water matrix submitted to oxidation and extraction and analyzed 6 times with internal standard (IS) with a concentration of 150 μg L⁻¹.

For a statistical comparison, the Statistical Package for Social Science (SPSS 17.0, Chicago, IL) for Windows was used, when needed, for the ANOVA test as well as the Tukey’s post-hoc test with statistical significance defined as \( P < 0.05 \).

3. Results and discussion

3.1. Microcystins oxidation and MMPB extraction optimization

All the Lemieux oxidation parameters were optimized in order to have the best reaction efficiency. It was shown that weakly alkaline conditions (pH ~9) were the most efficient for MC oxidation. The different pH conditions during the oxidation reaction were tested between 2 and 10.5; pH 9 was confirmed as the best value (Fig. 2a). Potassium permanganate and sodium periodate concentrations, as well as reaction time, were optimized in order to have the best reaction yield. Each oxidant was tested with a concentration between 10 mM and 100 mM, and for each concentration the reaction lasted between 0.5 h and 5 h. The optimal concentration was set at 50 mM for a reaction time of 1 h (Fig. 2b). The use of a higher oxidant concentration could induce compound degradation and provoke higher signal variability. Between 1 and 3 h for each concentration, no significant reaction yield enhancement was observed (\( P > 0.05 \)) and it was decided to choose the fastest reaction time in order to minimize sample preparation time. It was also important to choose a reaction time that would not induce compound degradation, which was observed after 3 h with higher oxidant concentrations (Fig. 2b). Finally, in several studies, it was shown that the temperature was not a significant parameter when maintained higher than 25 °C for the Lemieux oxidation reaction yield [29]. These results were similar to those of several previous studies [15,16,30,31]. Thus, the optimal reaction conditions used for the determination of total MCs in natural water matrices were as follows: an alkaline condition of pH 9, 50 mM of KMnO₄ and NaIO₄ each, and a total reaction time of 1 h at room temperature.

A liquid–liquid extraction was used after MCs oxidation to extract the formed MMPB. Several organic solvents were tested according to their non-miscibility with water; EtAc, hexane, cyclohexane, heptane, diethyl ether, methylene chloride and chloroform. However, only EtAc and hexane showed good recovery values for MMPB extraction. Finally, EtAc was chosen as a compromise with the optimal deposition solvent for the LDTD (Fig. 3). To enhance extraction recovery of MMPB and 4-PB, the addition of a solution of...
Fig. 3. Effect of using different solvents for the deposition of the analytes in the LDTD Lazwell plate wells with MMPB at 250 μg L⁻¹. Vertical error bars represent standard deviations from the mean (n = 6).

saturated NaCl in the aqueous phase was tested (Fig. A-4). By adding saturated salt in the aqueous phase of the reaction solution, neutral compounds are less soluble and the organic extraction is enhanced [32]. In addition, since the oxidative solution was adjusted to pH 2 following the quenching reaction, the compounds were now in their neutral form which improved the migration from the aqueous phase to the organic phase.

3.2. Optimization of LDTD/APCI parameters

In order to obtain optimal LDTD desorption and APCI ionization of the target compounds, several LDTD parameters were optimized to achieve signal enhancement while minimizing variability. The laser power, laser pattern, deposition solvent, deposition volume and carrier gas flow were all evaluated. All LDTD parameters were optimized in negative ionization (NI) mode by spiking 250 μg L⁻¹ of MMPB in river water matrix that was subjected to the Lemieux oxidation and liquid–liquid extraction and sample aliquots were deposited in Lazwell plates. Each sample was analyzed 6 times (n = 6), in SRM mode using the optimized MS/MS parameters corresponding to parent and fragment m/z with indicated optimized tube lens and collision energies (Table 1).

The laser power along with the laser pattern are compound dependent parameters that will have an impact on how the compounds are desorbed when subjected to heat, thus affecting signal intensity. The laser efficiently heats the metal back of the well and some of that energy is transferred to the compounds which are thermally desorbed and transferred by the gas flow to the APCI ionization. The amount of energy transferred is dependent on the laser power setting of the instrument (%), a fairly critical parameter since it must give enough energy to desorb the compound but not so much so as to cause its degradation or fragmentation. Usually, a stable working laser power is obtained with the range of 5–65% and this is estimated to correspond to generated well temperature between approximately 50 and 220 °C [33]. Depending of the nature of the matrix, increasing laser power can cause a decrease in signal to noise response because of a higher amount of interfering compounds being desorbed and transferred to the corona discharge along with the compounds of interest [23]. By adjusting the laser ramp pattern and the hold time at maximum laser power, it is possible to increase the amount of desorbed compounds and control the amount of simultaneously desorbed interfering analytes. For MMPB, these parameters were evaluated by spiking 250 μg L⁻¹ of MMPB in blank river water matrix submitted to oxidation and extraction in order to properly consider any matrix interference. The tested parameters were optimized using 6 replicates. Laser

Fig. 4. Effect of LDTD laser power on average peak area of the target compound, MMPB at 250 μg L⁻¹. Vertical error bars represent standard deviations from the mean (n = 6).

Fig. 5. Illustration of the LDTD laser pattern with its corresponding SRM signal and peak shape obtained using APCI-MS/MS analysis.
power was evaluated between 10 and 60% (Fig. 4). The optimal laser power for maximum compound desorption was set at 35%. Lower laser power did not allow for maximum compound desorption and a higher laser power caused higher variability and decreased signal intensity which we presume can be attributed to heat-promoted compound degradation. Optimized laser pattern was 2 s at 0%, with a ramp of 2 s from 0 to 35%, with no hold time required at maximum power, and a direct decrease of 0.1 s from 35 to 0% with finally 2 s at 0%. Fig. 5 shows graphically the laser pattern with its corresponding signal. Overall, with a 6 s LDTD desorption, a chromatogram-like signal is observed covering a time span of less than 15 s. This signal is subsequently quantified the same way a chromatogram would be, illustrating the fast analysis time the LDTD can offer.

Using an adequate deposition solvent is an important parameter affecting compound desorption [23]. Depending on the solvent used for deposition, the nanocrystals formed after drying in the well can have a different effect upon crystallization, repartition during the APCI discharge and also a chance of trapping the compounds into nonvolatile matrix products, all of which would affect signal intensity [23]. The deposition volume was tested between 1 and 8 μL in steps of 1 μL with the spiked matrix. Fig. 6a illustrates the MMPB signal intensity as a function of the deposition volume. The signal response progressively decreases by increasing the volume above 2 μL. These results suggest a signal suppression by matrix effect, and this is in concordance with previous work [22,23]. As a result, 2 μL was set as the optimal deposition volume with significantly higher signal intensity (P < 0.05).

Carrier gas flow rates have an impact on the transfer of the thermally desorbed molecules to the corona discharge region for ionization. It also allows thermalization of the desorbed and vaporized analytes, thus reducing thermal degradation [34]. Carrier gas flow was optimized between 1 and 8 L min⁻¹ (Fig. 6b). This parameter is also optimized for sensitivity enhancement. Carrier gas flow was optimal at 2 L min⁻¹ with a significantly higher signal response (P < 0.05), and a significant signal drop occurs above 3 L min⁻¹. These results are in concordance with a previous study of BTEX analysis in air where carrier gas flow had an influence on the APCI ionization rate [35]. In fact, it was shown that compound signal intensity was dependent of the residence time in the corona discharge region where ionization occurs. It was also discussed that optimal flow was between 2 and 3 L min⁻¹, as high as possible to maximize the mass of analytes but not so fast as to prevent ionization from occurring, as discussed earlier [21–24,34]. This parameter is not expected to be compound-dependent but rather a physical factor necessary to optimize ionization in the APCI source. Since LDTD-APCI ionization occurs in dry conditions, the amount of available reactive water molecules becomes an important factor in terms of signal enhancement. In previous work, the possibility of increasing water concentration in the corona discharge area was tested, but had no significant impact above a minimal level [36]. It is therefore important to control the time of residence in the ionization area to optimize ionization rate, thus, allowing sensitivity enhancement.

### 3.3. Method validation

We used 4-phenylbutyric acid as IS because of the unavailability of isotopically-labeled MMPB, however, 4-PB has been successfully used in previous work [37]. We used a seven-point internal calibration curve based on a standard addition method of MCs.

![Graph showing average peak area of the target compound, MMPB at 250 μg L⁻¹, as influenced by: (a) the deposition volume in the LDTD (1–8 μL), and (b) LDTD gas flow (1–8 L min⁻¹).](image)

**Fig. 6.** Average peak area of the target compound, MMPB at 250 μg L⁻¹, as influenced by: (a) the deposition volume in the LDTD (1–8 μL), and (b) LDTD gas flow (1–8 L min⁻¹). Vertical error bars represent standard deviations from the mean (n = 6).

### Table 2

<table>
<thead>
<tr>
<th>QC concentrations (μg/L)</th>
<th>Accuracy RE (%)</th>
<th>Intraday RSD (%)</th>
<th>Interday RSD (%)</th>
<th>Oxidation recovery yield (%)</th>
<th>Signal recovery from matrix effect (%)</th>
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<td>25</td>
<td>10</td>
<td>5</td>
<td>13</td>
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<td>6</td>
<td>4</td>
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showed a good linearity with 500 extraction so as to account for matrix effects and validate the spiked in river water submitted to the optimized oxidation and ND – Not detected.

RR, RR (D-Asp3), WR, YR) using LC–MS/MS. tentatively identified using LC–MS/MS. characteristics are slightly different from the available standard, there is a very high probability that these are isomers of LC-RR and LC-YR. /H9262 tion levels (25 /H9262 Validation parameters were determined with three concentra-

lution (1 μg L\(^{-1}\)). The MDL is comparable to other similar analytical techniques ranging from 0.02 to 15 μg L\(^{-1}\) [30,38]. Validation parameters were determined with three concentration levels (25 μg L\(^{-1}\), 100 μg L\(^{-1}\) and 250 μg L\(^{-1}\) with calibration curves and results are shown in Table 2. Accuracy, expressed in terms of relative error (RE – %), was below 10%. Intraday precision was below 6% and interday precision was below 11%. The Lemieux oxidation reaction yield is 103% with a completed reaction and signal recovery of MMPB at 91%, thus showing no significant matrix effect in the analysis. The precision of the reaction yield and the signal recovery were 7% and 6% respectively. The method was tested on MCs present in a real cyanobacterial bloom where the supernatant was sampled and submitted to a cell lysis, filtered to remove excess cells debris and quantified in parallel by LC-MS/MS. This method was applied to determine the total microcystins present in nine natural cyanobacterial bloom samples during the proliferation season in order to assess their levels in several water sources. Table 3 shows the different results obtained from the quantification of total microcystins using LDTD-APCI-MS/MS. These results are compared with previous analysis of the same samples using standard LC-MS/MS analysis. We observe that for 5 out of 6 samples for which results from both methods were above detection limits, the LC-MS/MS method which is relying on only the 12 MC standards available, significantly underestimated total MCs when compared to LDTD-APCI-MS/MS. For these five samples, the percentage of MC certified and quantified with standards using LC-MS/MS by CEAEQ represents between 15 and 38% of the total MC via MMPB (Table 3). Although CEAEQ had tentatively identified what seems to be isomers of MC-RR and MC-YR, they could not certify them in the absence of the specific standards. When concentrations of MC and MC isomers are added to give total MC via LC-MS/MS, the results of both methods are quite similar exception made of sample no. 8 for which total MC still only represents 22% of MC via MMPB since no congener isomers were detected by the CEAEQ laboratory. Our results show that the presence of isomers of MC or other congeners for which standards are not available could be missed altogether, potentially leading to substantial underestimations of MCs in water samples. Overall, the LDTD-APCI-MS/MS is a robust method that allows high throughput analysis with less than 15 s sample\(^{-1}\), and could be a powerful tool for a fast screening of cyanobacterial toxins including some MC congeners that would otherwise go undetected.

4. Conclusion

A new method for the screening of total microcystins in natural water has been demonstrated and validated using LDTD-APCI-MS/MS. This method uses an oxidation step but this is a simple sample pre-treatment that has been kept relatively easy to implement. The MS analysis is simple, fast and allows high-throughput analysis (<15 s sample\(^{-1}\)). Oxidation and instrumental parameters were optimized in order to enhance sample treatment efficiency and signal response. An internal calibration with standard addition of MMPB and using 4-PB as IS gave an excellent linearity with a correlation coefficient (R\(^2\)) above 0.999. MDL and MQL were measured to be 0.2 and 0.9 μg L\(^{-1}\) respectively. Accuracy and precision were below 10%. Intraday and interday precision were evaluated below 15%. The limit of quantification is below the WHO microcystin-LR guideline for drinking water making the proposed method a useful approach for a rapid total microcystins screening in drinking water, surface waters and water treatment facilities. It could give a fast quantification response prior to applying any LC–MS analysis for characterization of the MCs congeners, eliminating costly and lengthy analysis while providing more selectivity and accuracy than ELISA detection which does not utilize MS/MS detection. The method is based on total MCs and could therefore also identify contaminated samples related to MC congeners which are not usually included in the LC–MS analytical protocol, thus preventing some false negative. The ultrafast analysis combined with a robust quantification and relatively simple sample pre-treatment makes this method a usable alternative for environmental safety and health concerns caused by the presence of microcystins in cyanobacterial blooms occurring in surface waters.

Acknowledgments

The Fond de Recherche Québec Nature et Technologies and the Natural Sciences and Engineering Research Council of Canada (NSERC) are acknowledged for financial support. We thank Thermo

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Location</th>
<th>Date</th>
<th>Total MC via MMPB (μg L(^{-1})) (RSD – %)</th>
<th>Total MC with standards (μg L(^{-1}))</th>
<th>MC isomer without standards (μg/L)</th>
<th>Total MC with standards (μg/L)</th>
<th>Percentage of MC with standards (%)</th>
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<td>425 (9)</td>
<td>70</td>
<td>340 (RR(^{+}))</td>
<td>0.5 (RR(^{+}))</td>
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<td>1.0 (5)</td>
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.12.001.

References


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