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

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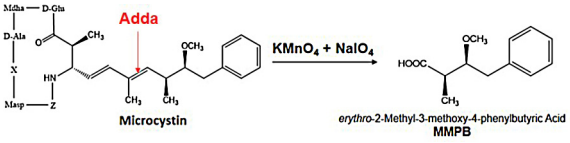
HIGHLIGHTS

- 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 $\mu g L^{-1}$ in field-collected water samples.
- The calibration curve showed good linearity ($R^2 > 999$).
- Interday and intraday variation coefficients were below 15%.

GRAPHICAL ABSTRACT



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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 $sample^{-1}$). 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 ($R^2 > 0.999$). Limits of detection and quantification were 0.2 and 0.9 $\mu g L^{-1}$, respectively. These values are comparable with the WHO (World Health Organization) guideline of 1 $\mu g L^{-1}$ for total microcystin-LR congener in drinking water. Accuracy and interday/intraday 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 LD_{50} values range from 45 to 1000 $\mu g\,kg^{-1}$ for mice, depending of the specific congener tested [8]. The World Health Organization (WHO) recommends an upper limit of 1 $\mu g\,L^{-1}$ 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/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-phenylburytic 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 sulfuric 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-H_2O) was used for dilution. Individual stock standard solutions were prepared in MeOH at a concentration of 100 mg^{-1} and kept at −20 °C for 12 months for the

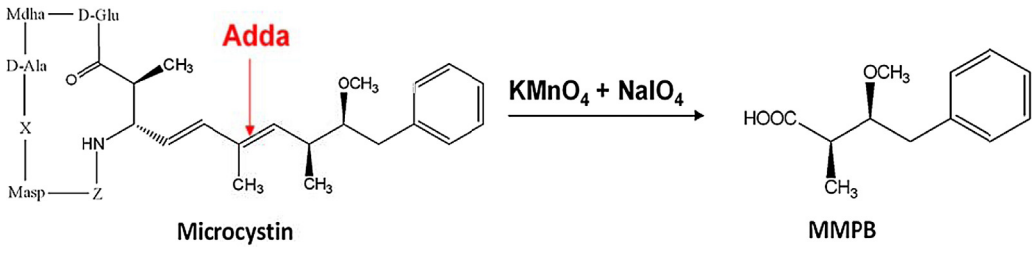

Fig. 1. Reaction scheme of Lemieux oxidation reaction to produce the MMPB used for total microcystin analysis.