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Analysis of individual and total microcystins in surface water by on-line preconcentration and desalting coupled to liquid chromatography tandem mass spectrometry

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ABSTRACT

A fast and high-throughput method is proposed for the determination of total microcystins (ΣMC) in environmental surface waters. After a 1-h Lemieux-von Rudloff oxidation step to yield the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) moiety, samples were quenched, filtered, and directly analyzed. This was achieved via solid phase extraction (SPE) coupled on-line to ultra-high performance liquid chromatography electrospray ionization triple stage quadrupole mass spectrometry. The choice of on-line SPE settings was conducted using experimental designs. Given the matrix complexity of oxidation extracts, the on-line desalting step was found to be a critical parameter to ensure suitable method robustness. The on-line sample loading volume was 5 mL, and the wash volume applied for on-line desalting was 3 mL. Instrumental analysis was performed in just 8 min. The method limit of quantification was 0.5 ng L^{-1} ΣMC (i.e. 2000 times lower than the current World Health Organization – WHO drinking water guideline). Excellent determination coefficients were observed for matrix-free and matrix-based calibration curves alike, and the linearity range tested spanned ~4 orders of magnitude. Accuracy and intermediate precision did not depend on the spike level and proved satisfactory (in the range of 93–110% and 3–6%, respectively). A thorough assessment of instrumental matrix effects was conducted by comparing standard additions curves in several lake and river oxidation extracts with the matrix-free reference. Regardless of the internal standard used (4-PB or D3-MMPB), instrumental matrix effects were efficiently compensated. The matrix effect that may occur at the earlier sample preparation stage was evaluated separately. While the oxidation step was generally not complete (yield ~65%), the conversion rates of MCs into MMPB remained within a consistent range of values regardless of matrix type. No significant back-pressure was observed upon consecutive injections of oxidation-based samples, while the instrumental sensitivity remained unaffected. The herein described method could therefore be eligible for future large-scale monitoring surveys. The method was applied to a selection of surface water samples (n = 30) collected across the province of Québec, Canada, and the results were compared to those achieved by an individual variant analysis of 8 MC congeners and a commercial ELISA kit.

1. Introduction

Anthropogenically-induced eutrophication of aquatic ecosystems, in conjunction with rising water temperatures worldwide, has been linked with increased frequency and magnitude of harmful algal bloom events [1–3]. The proliferation of waterborne cyanobacteria may result in blue-green or red layers forming intermittently on freshwater or marine habitats, a harbinger of further risks to come when the bacteria release endotoxins upon senescence [4] or rupturing. Early reports in the scientific literature on the poisonous effects of blue-green algae date back to the late XIXth century [1,5]. Since then, harmful algal blooms (HABs) have been linked with mild to lethal poisonings of wild and domestic animals worldwide [6].

Among the wide array of naturally-occurring cyanotoxins, microcystins (MCs) are the most frequently monitored. They are commonly produced by genera such as *Microcystis*, *Dolichos-*

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permum and Planktothrix. Microcystins are hepatotoxic cyclic heptapeptides of molecular mass generally comprised between 900 and 1100 Da. In vertebrates, they are readily accumulated in the liver where they cause potent inhibition of two protein phosphatases (PP1 and PP2A), disrupting cellular homeostasis [6]. MC poisoning may entail hepatic swelling, hemorrhaging, and death in the most acute cases [6]. The LD_{50} of microcystin-LR (MC-LR), the most commonly monitored MC variant, has been reported as low as 50–250 ng g^{-1} body weight for mice, reflecting a highly toxic potential [6]. Microcystin bioaccumulation has been reported in aquatic food webs and exposure routes are multifold, possibly including trophic transfer [7–10]. Microcystins have also been implicated in human poisonings in several countries, including one incident in Brazil when patients died of acute liver failure after exposure to contaminated hemodialysis water [11,12].

In surface waters used for recreational activities, drinking water production or irrigation purposes, MC-LR concentrations are highly variable both spatially and temporally [3]. Although very high concentrations (> mg L^{-1}) may be punctually observed, MC-LR has been reported to occur in the range 0.68–9.1 μg L^{-1} in some lakes from Sweden [13], between <0.02–36 μg L^{-1} in water bloom samples across lakes from Québec, Canada [14], 17–344 ng L^{-1} in water reservoirs from Southern Portugal [15], and up to 28 μg L^{-1} in an Italian surface water basin [16]. In recent years, exceedances of the World Health Organization (WHO) drinking water guideline for microcystins (1 μg L^{-1}) have led to temporary "do not use" notices, including in Northern America. For instance, in August 2014, the Toledo water utility (Ohio, USA) withheld distribution for three days as a result of a particularly intense HAB event in Lake Erie [17].

A standard method for microcystin surveillance in water is the enzyme-linked immunosorbent assay (ELISA) that may achieve detection limits as low as 40 ng L^{-1} for its most sensitive versions [18]. Despite these acknowledged benefits, the ELISA approach has been reported to produce false positives, a potentially contentious issue for water managers who need to correctly and readily identify risks before taking the appropriate measures [6,19]. In addition to ELISA and other bioassays (such as the Protein Phosphatase Inhibition Assay), the analysis of microcystins and nodularins may be achieved by a variety of instrumental methods, chief among which are liquid chromatography based methods. The latter are often coupled with a diode array detector (HPLC-DAD) or mass spectrometry (LC–MS or LC–MS/MS) [6]. Analytical methods may be designed to target individual microcystins or total microcystins (ΣMCs). In the latter case, samples may be oxidized to yield the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) moiety common to most microcystins and nodularins [20]. The rationale for the use of the ΣMC approach via MMPB is the considerable lack of certified standards, since only one tenth of the ~250 or so reported individual MCs are currently available [13,21–23]. Recently, Wang et al. [24] explored the possibilities of coupling liquid chromatography with fluorescence detection for the determination of ΣMCs in environmental samples, with suitable limits of detection (LOD = 125 ng L^{-1} in water samples). Roy-Lachapelle et al. [25] proposed an innovative approach for the analysis of ΣMCs in water using an ultrafast laser diode thermal desorption interface (LDTD/APCI-MS/MS). Following the selection of optimal settings, a LOD of 200 ng L^{-1} could be reached. Foss and Aubel [26] reported detection limits of ΣMCs down to 50 ng L^{-1} via Lemieux oxidation of 5 mL raw water samples, subsequent clean-up through off-line solid phase extraction (off-line SPE), and concentration to 0.5 mL prior to HPLC–MS/MS analysis.

In order to lower method LODs, analysts may resort to higher sample intake (e.g. 100–1000 mL) and sensitive instrumental techniques (e.g. HPLC–MS/MS). In the particular case of the total microcystin procedure based on the Lemieux-von Rudloff reaction, a high sample volume would imply the use of considerable amounts of oxidation reagents, scarcely compatible with the principles of green analytical chemistry. Instead, samples could be first concentrated through off-line SPE prior to performing the oxidation step. This approach is not, however, without its pitfalls. Conducting SPE prior to oxidation may indeed be complicated when many different microcystins with varying properties are present, resulting in possible losses that may not be compensated. Following the generation of MMPB, oxidation extracts may be subjected to further clean-up, either by liquid–liquid extraction or a second off-line SPE step. In order to reach a suitable sample concentration factor, an evaporation step would follow prior analysis of a small sample aliquot. As can be inferred from the aforementioned analytical scheme, sample preparation may be relatively cumbersome to implement and take several hours. An alternative solution would be to keep a minimal sample amount but to operate at higher injection volumes. This could be achieved via a fully-automated method bypassing the numerous off-line sample preparation steps.

In the present study, a fast and ultrasensitive method was proposed for the determination of ΣMCs in surface water. The method required a small sample intake and minimal sample preparation handling. After completion of the initial permanganate and periodate oxidation step, samples were quenched, filtered, and the MMPB moiety was analyzed. To this end, we used on-line SPE and desalting coupled to ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). To the knowledge of the authors, this is the first attempt at analyzing ΣMCs via Lemieux oxidation and using an on-line SPE – UHPLC–MS/MS workflow. Given the challenging matrix complexity, experimental designs proved useful in identifying the critical robustness factors. The method was validated following the selection of the most suitable operating conditions. Matrix-based LODs, linearity, accuracy, precision, and oxidation yields were therefore characterized. Matrix effects were assessed on a subset (~20%) of the surface water samples surveyed, covering 6 different locations, and the performance of internal standards was evaluated. The newly-developed on-line SPE – UHPLC–MS/MS method was applied to a selection of 30 surface water samples collected across lakes and rivers from the province of Québec, Canada. The results generated were compared with those achieved by an on-line SPE – UHPLC–MS/MS specific analysis of 8 individual microcystin variants [14] and a commercial ELISA kit. Potential limitations of the method were also examined and discussed.

2. Experimental

2.1. Chemicals and materials

MMPB was obtained from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Native standards for individual cyanotoxins were obtained from the National Research Council of Canada (Ottawa, ON, Canada) for microcystins MC-RR, MC-LR and [D-Asp^3] MC-LR. Microcystins MC-YR, MC-LY, MC-LW, MC-LA and MC-LF were from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). ELISA kits (Microcystins-ADDA SAES ELISA) were obtained from Abraxis, Inc. (Warminster, PA, USA). Nodularin-R (NOD-R, henceforth referred to as "NOD") was purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). 4-phenylbutyric acid (4-PB) was purchased from Sigma-Aldrich (Oakville, ON, Canada). Isotopically labelled D3-MMPB was kindly provided by the National Research Council of Canada (Halifax, Nova Scotia, Canada) (D3-MMPB standard originally obtained from Wako Pure Chemicals Industries, Ltd). Stock