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Fast, rugged and sensitive ultra high pressure liquid chromatography tandem mass spectrometry method for analysis of cyanotoxins in raw water and drinking water—First findings of anatoxins, cylindrospermopsins and microcystin variants in Swedish source waters and infiltration ponds

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ABSTRACT

Freshwater blooms of cyanobacteria (blue–green algae) in source waters are generally composed of several different strains with the capability to produce a variety of toxins. The major exposure routes for humans are direct contact with recreational waters and ingestion of drinking water not efficiently treated. The ultra high pressure liquid chromatography tandem mass spectrometry based analytical method presented here allows simultaneous analysis of 22 cyanotoxins from different toxin groups, including anatoxins, cylindrospermopsins, nodularin and microcystins in raw water and drinking water. The use of reference standards enables correct identification of toxins as well as precision of the quantification and due to matrix effects, recovery correction is required. The multi-toxin group method presented here, does not compromise sensitivity, despite the large number of analytes. The limit of quantification was set to 0.1 μg/L for 75% of the cyanotoxins in drinking water and 0.5 μg/L for all cyanotoxins in raw water, which is compliant with the WHO guidance value for microcystin-LR. The matrix effects experienced during analysis were reasonable for most analytes, considering the large volume injected into the mass spectrometer. The time of analysis, including lysing of cell bound toxins, is less than three hours. Furthermore, the method was tested in Swedish source waters and infiltration ponds resulting in evidence of presence of anatoxin, homo-anatoxin, cylindrospermopsin and several variants of microcystins for the first time in Sweden, proving its usefulness.

1. Introduction

Toxin producing cyanobacteria can be a hazard for drinking water treatment plants (WTPs) using surface waters as source water [1,2]. The World Health Organization (WHO) has established a guideline value of 1 μg/L microcystin-LR in drinking water, including both the free and cell bound forms [1]. In addition, other national authorities have implemented guidelines for anatoxin at 3 μg/L and cylindrospermopsin at 1–15 μg/L [1,3]. Potentially toxin producing cyanobacteria have been identified in 45% of the Swedish source waters [4,5] and microcystins and nodularin have been frequently detected in fresh water and brackish waters, respectively [5,6].

The major human exposure routes are considered to be direct contact with recreational waters and ingestion of drinking water, not sufficiently treated [1]. Acute poisoning of cyanotoxins may lead to gastroenteritis, liver damage, jaundice and neurotoxic effects [1]. At worst, exposure to contaminated water can cause

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severe intoxication such as in Caruaru, Brazil, where 52 patients died after receiving renal dialysis with contaminated water [7].

During drinking water treatment, the removal efficiency of cyanotoxins depends on the degree of toxins present in intracellular and extracellular forms [1,2]. Intracellular toxins are commonly removed by separation processes such as coagulation, sedimentation/flocculation and filtration [2]. Extracellular toxins are removed by adsorption on active carbon, membrane filtration, or destruction using free chlorine, ozone or potassium permanganate [2,8]. Rupture of cell membranes may cause release of cyanotoxins from cells during the drinking water treatment processing steps [9,10]. Since drinking water treatment steps are different for removing intra- and extra cellular cyanotoxins, fast analytical methods are needed that are capable of distinguishing and quantifying toxins in the both states. Furthermore, fast analysis is crucial for WTPs experiencing cyanobacterial blooms at their water intake, to prevent toxic water from reaching the consumers.

Studies of microcystins in surface waters suggest that at high concentrations, the toxin is present almost solely in intracellular form [1]. Despite this, many recently developed analytical methods fail to address the analysis of intracellular cyanotoxins in source water to WTPs, since the validated methods lack a cell lysing step [11,12]. Cells from cyanobacteria in water samples can be lysed using ultrasonication, lyophilization, freeze–thaw cycles, bead beating or by chemicals treatments such as using surfactants or commercial lysing agents [13,14].

For the first time, an in-house validated fast, rugged and sensitive ultra high pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for analysis of 22 intra- and extracellular cyanotoxins in raw water and drinking water is presented. The toxins include anatoxins, cylindrospermospins, nodularin, and microcystins. The quantification is based on pure reference standards, using external calibration and delivers results on intra- and extra-cellular basis for all 22 toxins within three hours. Direct analysis of a large volume of water after cell lysing using UPLC-MS/MS, as proposed in this method, is fast and provides the advantage that several toxin groups can be analyzed simultaneously. Compared to UPLC-MS/MS methods developed solely for microcystins using advanced sample clean-up, the proposed multi-toxin group method presented here does not compromise sensitivity [11,15]. Earlier methods that have been developed for multi-toxin group analyses did not evaluate the cell lysing step, were less sensitive and covered significantly fewer cyanotoxins [16,17]. Direct analysis of a large volume of water has also been performed previously for anatoxin-a and cylindrospermopsin but not in the context of multi-toxin analysis [18,19]. The validation study, presented here covers all steps of the analysis. The functionality of the method is proven by the identification of anatoxins, cylindrospermopsins, nodularin and variants of microcystin for the first time in Swedish source waters and infiltration ponds.

2. Material and methods

2.1. Chemicals and consumables

Methanol (LiChrosolve) and formic acid (98%) was purchased from Merck (Darmstadt, Germany), and LC–MS grade acetonitrile from Fisher Scientific (Loughborough, United Kingdom). LC–MS grade water was produced by a Milli-q purification system from Millipore (Billerica, MA, USA). Discardit II 10 mL polypropylene syringes were ordered from Beckton, Dickinson and Company (Franklin Lakes, NJ, USA) and Ø 13 mm PVDF 0.20 μm filters from Whatman (Maidstone, United Kingdom).

2.2. Reference standards

The cyanotoxins included in this method are listed in Table 1. Standards of anatoxins, cylindrospermopsins, nodularin, and microcystins were ordered from several sources as described in supplementary material (Table S1). When possible, standards were purchased as solution (concentrations ranging from 2.0 to 12 μg/mL). However, some of the substances were only available as solid standards. Stock solutions of 5000 μg/L in methanol were therefore prepared in-house. All stock solutions were stored in darkness at −20 °C. Three separate mixed standard solutions were prepared, and the cyanotoxins were divided between the solutions as described in supplementary material (Table S1). The three mixed standard solutions had a concentration of 625 μg/L, and were prepared in methanol (MeOH). Next, the three mixed standard solutions were pooled and diluted to 62,5 μg/L using Milli-q water/MeOH 97/3 (v/v). The calibration standards were prepared from this mix and the calibration points were 0.1, 0.5, 1.0, 5.0 and 10.0 μg/L. All calibration standards were diluted in 97/3 (v/v) Milli-q/MeOH.

2.3. UPLC-MS/MS method

Chromatography was performed on an ACQUITY UPLC system (Waters, Manchester, United Kingdom). Separation was achieved with an ACQUITY BEH C_{18} UPLC column, 2.1 × 100 mm fitted with a VanGuard ACQUITY BEH C_{18} UPLC pre-column, 2.1 × 5 mm, both having a particle size of 1.7 μm (Waters, Manchester, United Kingdom). The columns were kept at 35 °C during analysis, and injection volume was 100 μL. Mobile phase A was 0.1% formic acid (FA) in Milli-q and mobile phase B 0.1% FA in acetonitrile (ACN). The gradient elution was performed as follows: 0–0.7 min, 2% B, flow 0.3 mL/min; 0.80 min, 2% B, starting from here, flow increased to 0.45 mL/min; 9.0 min, 70% B; 9.1 min, 90% B; 10.0 min, 90% B; 10.1 min, 2% B; 12.0 min, 2% B.

Quantification of cyanotoxins was performed in dynamic Multiple Reaction Monitoring (MRM) mode using a triple quadrupole mass spectrometer (MS/MS), Xevo TQ-S from Waters (Manchester, United Kingdom). The mass spectrometer was used in positive electrospray mode (ES +), with a capillary voltage of 3.0 kV. The source offset was 50 V and the source temperature 150 °C. Nitrogen was generated from pressurized air, and used as desolvation and cone gas at flows of 650 and 150 L/Hr, respectively. The desolvation gas temperature was 350 °C. The nebulizing gas was also N_2 at a pressure of 7.0 bars. Argon (Alphagas™, Malmö, Sweden) was used as collision gas at a flow of 0.15 mL/min. The compound specific mass spectrometric parameters such as cone voltage (CV), collision energy (CE) and mass transitions are presented in Table 1. The mass spectrometric parameters of individual cyanotoxins were optimized using 1 μg/mL solutions in methanol with a constant flow of mobile phase. The individual solutions of microcystin-[D-Asp3]-LR, microcystin-[D-Asp3]-RR, microcystin-[D-Asp3, (*E*)-Dhb7]-77 and microcystin-[Dha7]-RR at the concentration 1 μg/mL was also utilized to perform product ion scans at 10 eV, 30 eV, 60 eV and 90 eV in an attempt to find unique fragments for quantification of the co-eluting isomers. The scan range was *m*/*z* 70–*m*/*z* 1250.

2.4. Identification and quantification

Criteria for positive identification and quantification were set in accordance with guidance for analytical methods used in food control [20]. Compounds were considered positively identified when the relative intensities of the quantification product ion in relation to the qualifier product ion, expressed as a ratio, corresponded to those of the calibration standard. For positive identification, the ion ratios differed no more than ±30% between specific