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New SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins

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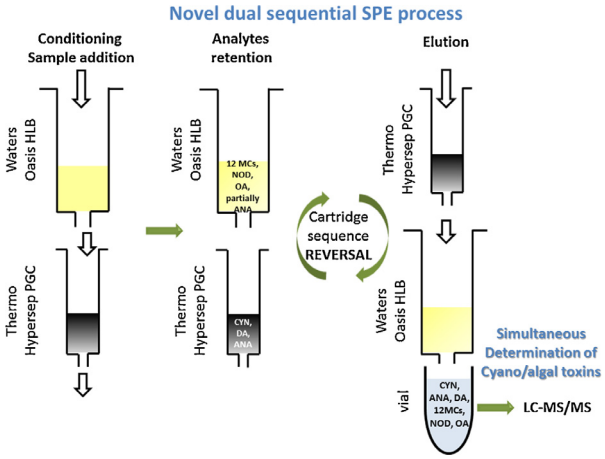
HIGHLIGHTS

- Simultaneous determination of various classes of cyanobacterial/algal toxins in water.
- Dual SPE cartridge assembly for the determination of multi-class toxins.
- Determination of 12 MCs, NOD, CYN, ANA, DA and OA in one run by SPE-LC–MS/MS.
- Validation data of the method to demonstrate its suitability.
- Identification of a wide range of MCs in Greek lakes for the first time demonstrated the method's applicability.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacterial and algal toxins comprise a large group of harmful metabolites, belonging to different chemical classes, with a variety of chemical structures, physicochemical properties and toxic activities. In this study, a fast, simple and sensitive analytical method was developed for the simultaneous determination of multi-class cyanobacterial and algal toxins in water. The target compounds were: Cylindrospermopsin, Anatoxin-a, Nodularin, 12 Microcystins ([D-Asp3]MC-RR, MC-RR, MC-YR, MC-HtyR, [D-Asp3]MC-LR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW and MC-LF), Okadaic acid and Domoic acid. Analytes were determined using liquid chromatography–tandem mass spectrometry (LC–MS/MS). A dual Solid Phase Extraction (SPE) cartridge assembly was applied for the extraction of target compounds from water. Optimized SPE parameters included cartridge material, initial sample pH, sequence of the cartridges in the SPE assembly as well as composition and volume of the elution solvent. The method was validated, providing acceptable mean recoveries and reproducibility for most analytes. Limits of

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detection were at the ng L^{-1} level. The method was successfully applied in real lake water samples from Greece, where a wide range of Microcystins were detected for the first time, at concentrations ranging from 0.034 to 63 μg L^{-1}.

1. Introduction

Cyanobacteria under favorable conditions are able to produce harmful secondary metabolites, called cyanotoxins, which pose a significant threat to human health and the environment [1–4]. Cyanotoxins comprise a large variety of compounds with various structural and physicochemical properties (Fig. 1). Microcystins (MCs) [5] and Nodularins (NODs) [6] are cyclic peptides, both containing the unusual β-amino acid Adda ((2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl deca-4,6-dienoic acid) which is responsible for their hepatotoxic activity [7–9]. Cylindrospermopsin (CYN), is an alkaloid cyanotoxin with cytotoxic, dermatotoxic, hepatotoxic and possibly carcinogenic potency [10,11]. The alkaloid Anatoxin-a (ANA), also known as "Very Fast Death Factor", is a bicyclic secondary amine (2-acetyl-9-azabicyclo[4,2,1]non-2-ene) with acute neurotoxicity [9]. Domoic acid (DA) and Okadaic acid (OA) are algal toxins (Fig. 1), that have been reported to accumulate in shellfish, consequently consumed by humans [12,13].

The release of cyanotoxins into water during cyanobacterial blooms has been reported worldwide [14], with serious incidences regarding animal and human poising [15–18]. World Health Organization (WHO) has proposed the provisional limit of 1 μg L^{-1} in drinking water for MC-LR, the most common MC variant [19]. Recently, the US EPA has included cyanotoxins (with special consideration for ANA, CYN and MC-LR) to the Contaminant Candidate List 3 (CCL3) for drinking water [20] and has issued drinking water health advisories for MCs and CYN [21]. The European Union has also established regulatory limits for the presence of DA and OA in live bivalve mollusks, since these algal toxins mainly accumulate in shellfish [22]. The necessity to determine OA and DA, along with freshwater toxins, has been demonstrated [23–28] and the presence of these compounds in brackish and drinking water has been a scientific concern.

There are numerous analytical methods for the determination of discrete classes of cyanobacterial/algal toxins [4,29,30]. However, the separate isolation and identification of toxins belonging to each discrete class is laborious, expensive and time consuming. Therefore a multi-class toxin analytical approach is necessary for the simultaneous screening, detection, identification and quantification at low concentration levels. Nevertheless, the simultaneous analysis of various classes of cyanobacterial/algal toxins is challenging, due to the largely different structures and physicochemical properties of the analytes.

Existing literature data indicate that analytical methods for simultaneous determination of multi-class cyanobacterial/algal toxins in water are limited, generally not involving sample extraction/preconcentration steps [31–34] to achieve limits of detection (LODs) in the ng L^{-1} range, and also sometimes lacking definitive identification (e.g. Tandem mass spectrometry, MS/MS) and validation data. The use of a single solid-phase extraction (SPE) sorbent [23,35,36] in most cases is not sufficient to provide satisfactory recoveries for the various classes of cyanotoxins.

Multiple SPE materials in a single pretreatment stage are known to be useful, in order to efficiently recover a wide range of pollutants from water samples. The multi-cartridge SPE approach has been employed in the past for several classes of micropollutants, including pesticides [37], PAHs [38], endocrine disruptors [39] etc. However, studies employing multiple SPE materials for the extraction of various classes of cyanotoxins, lack detailed description and validation of the applied procedures, which are essential for standardization and reproducible performance between laboratories. Specifically, the combination of SPE cartridge materials, such as C18 and graphitized carbon, has initially been proven effective for the analysis of CYN [40,41]. Yen et al. [42] have developed a method utilizing a dual cartridge SPE assembly for the determination of various cyanotoxins. Nevertheless, large volumes of extraction eluents (40 mL) and a complicated evaporation procedure were applied while the prolonged chromatography program was used lacked definitive identification and high sensitivity, since only single stage mass spectrometry was used. Additionally, initial sample pH was not adjusted to a value >10, which is known to promote ANA sorption on SPE sorbent [36,43].

The aim of the present study was to develop a simple and sensitive method for the simultaneous determination of several classes of cyanotoxins and algal toxins, including CYN, ANA, NOD, several MC variants ([D-Asp3]MC-RR, MC-RR, [D-Asp3]MC-LR, MC-LR, MC-HilR, MC-YR, MC-HtyR, MC-WR, MC-LA, MC-LW, MC-LY and MC-LF), DA and OA in water. The method was based on the dual SPE cartridge approach, providing a clear description of the extraction steps and mechanisms involved, in combination with definitive identification (LC–MS/MS). Throughout the study, the role of each individual sorptive material was clarified and the effect of separate SPE parameters were assessed, in order to determine the optimized conditions for the extraction of the multi-class set of toxins. A large number of various toxins were included in the study, possibly originating from different water types, so as to provide a basis of a standardized analytical method, able to be reproduced by relevant laboratories according to their individual needs. Even if all the analytes are not possibly found in the same water sample, the selection of toxins with different physicochemical properties and origin can provide a useful analytical template, for various classes of toxins. Analysis of real surface water samples using the developed method was carried out in order to prove its suitability for the simultaneous determination of different classes of cyanotoxins and algal toxins in water.

2. Material and methods

2.1. Chemicals and reagents

[D-Asp3]MC-LR, [D-Asp3]MC-RR, MC-WR, MC-HtyR, MC-HilR, MC-LY, MC-LW, MC-LF and OA standards were supplied by ENZO Life Science (Lausen, Switzerland). MC-RR, MC-LR, MC-YR, MC-LA and NOD standards were supplied by Sigma-Aldrich (Steinheim, Germany). CYN was purchased from Abraxis (Warminster, USA), ANA fumarate from TOCRIS Bioscience (Bristol, UK) and DA from CALBIOCHEM (Darmstadt, Germany). All substances had purity >95%. Methanol (MeOH) of HPLC grade (99.99%) and dichloromethane (DCM) of analytical reagent grade (99.9%) were obtained from Fischer Scientific (Leics, UK), acetonitrile (ACN) of gradient grade for HPLC (≥99.9%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). High purity water (18.2 MΩ) was produced on-site using a Temak TSDW10 system (Temak S.A.).

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