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Abstract

Determination of Multiclass Cyanotoxins in Spirulina-Based Dietary Supplements Using a SLE-Tandem-SPE Procedure Followed by HILIC-MS/MS †

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Abstract: Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes, which are believed to be one of the oldest life forms on Earth. They live in a wide range of ecosystems and withstand extreme environmental conditions. An important proportion of cyanobacteria is known to be producers of harmful cyanotoxins, which are toxic secondary metabolites that can impact the ecosystem and human health. The oral route is one of the main ways whereby humans can be exposed to cyanotoxins. Therefore, the consumption of contaminated algae-based food supplements is becoming more relevant due to its upsurge, which underlines the importance of controlling these toxins in this kind of products. This work describes the simultaneous determination of seven cyanotoxins belonging to three different classes: the cyclic peptides microcystin-LR (MC-LR), microcystin-RR (MC-RR) and nodularin (NOD); the alkaloid anatoxin-a (ANA) and three non-protein amino acids isomers β-methylamine-L-alanine (BMAA), 2,4-diaminobutyric acid (DAB) and N-(2-aminoethyl)glycine (AEG). These have been determined in spirulina-derived food supplements using a novel solid–liquid extraction coupled with a solid phase extraction procedure for clean up and preconcentration (SLE-tandem-SPE) and analysis by hydrophilic interaction liquid chromatography with tandem mass spectrometry detection (HILIC-MS/MS). A SeQuant®Zwitterionic Hydrophilic Interaction Liquid Chromatography (ZIC-HILIC) column (EMD Millipore, Billerica, MA, USA) was employed to achieve the chromatographic separation in less than 12 min using water and acetonitrile, both acidified with 0.3% of formic acid, as mobile phase. Previously, an SLE was developed, using 4 mL of aqueous 5% formic acid to extract the most polar compounds, followed by 4 mL of 80% MeOH. Both extracts were combined and submitted to a tandem-SPE using mixed-mode cation exchange (MCX) and Strata-X cartridges. Elution from both cartridges was performed using 10% $NH_3 \cdot H_2O$ in MeOH. Method validation was carried out in terms of linearity, limit of detection (LOD) and quantification (LOQ), recoveries, matrix effect and repeatability and intermediate precision. LOQs in the range of 50–300 $\mu g \cdot kg^{-1}$ and recoveries ranging between 64.2% and 102.9% with an associated relative standard deviation < 19.2% were achieved. Satisfactory precision was obtained with RSD values lower than 19.6% in all cases, with the exception of BMAA, which reported the highest RSD values, reaching 25.1%. The method was satisfactorily applied to determine the occurrence of cyanotoxins in blue green algae (BGA) dietary supplements. DAB was the most frequently detected cyanotoxin, at concentrations up to 2408 $\mu g \cdot kg^{-1}$, and AEG was found in few samples at concentrations up to 194 $\mu g \cdot kg^{-1}$. However, MC-LR and MC-RR were found in one sample at concentration levels higher than 5 $mg \cdot kg^{-1}$, which underlines the need to control these substances in these matrices.

Keywords: cyanotoxins; HILIC-MS/MS; spirulina-based dietary supplements; tandem-SPE

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