

Abstract

Capillary Electrophoresis–Tandem Mass Spectrometry as an Analytical Technique for the Simultaneous Determination of Multiclass Cyanotoxins †

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Abstract: Cyanotoxins are toxic metabolites produced by most cyanobacteria. In recent years, the occurrence of cyanobacterial blooms in aquatic ecosystems has temporally and spatially increased because of nutrient oversupply caused by human and also by climatic changes. This increase has a negative impact on water quality, ecosystem integrity, and human health. Cyanotoxins constitute a group of compounds with diverse physicochemical properties and their presence in drinkable, fishable, and recreational water is the main health-damaging cause. They are also able to bioaccumulate in plants and vegetables irrigated with contaminated water. Research on the development of suitable analytical methods is needed to establish early-warning strategies for the improved protection of humans and ecosystems health. Liquid chromatography coupled with mass spectrometry (LC-MS) has been the preferred option for the control of these compounds, mainly using reverse-phase mode or hydrophilic interaction liquid chromatography (HILIC) in order to separate multiclass cyanotoxins of varying polarity, which cannot be handled by the commonly used reverse phase columns. In this work, we propose the use of capillary electrophoresis (CE) coupled with tandem mass spectrometry using triple quadrupole and positive electrospray ionization (CE-(ESI)-MS/MS) to determine a mixture of cyanotoxins with different polarity. CE is an advantageous alternative to LC given its short analysis times, high resolution, low sample and reagent volumes, and the use of silica capillaries and buffers as separation media, resulting in lower cost and low environmental impact. Moreover, CE allows the analysis of molecules hardly affordable by LC, such as polar and very similar compounds (e.g., isomers). The method is designed for the simultaneous determination of eight cyanotoxins belonging to three different classes: cyclic peptides (microcystin-LR, microcystin-RR, and nodularin), alkaloids (cylindrospermopsin, anatoxin-a), and three non-protein amino acids isomers (β -methylamino-L-alanine, 2,4-diaminobutyric acid, and N-(2-aminoethyl) glycine). Separation was achieved using an acidic background electrolyte (BGE) consisting in 2 M of formic acid (FA) and 20% acetonitrile in water. The proper separation and resolution of the three non-protein amino acid isomers was one of the main challenges of the method. This was overcome by applying a voltage of 30 kV in a 90 cm length capillary at 20 °C. Parameters affecting MS detection and the sheath–liquid interface were also studied. Finally, the fixed values were: a sheath gas flow rate of 5 L/min at 195 °C; sheath–liquid consists of MeOH/H₂O/FA (50:49.95:0.05 v/v/v), a flow rate of 15 μ L/min; and a nozzle voltage of 2000 V; N₂ dry gas rate of 11 L/min at 150 °C; a nebulizer pressure of 10 psi; and a capillary voltage of 2000 V. Online pre-concentration approaches were tested in order to achieve higher sensitivity, obtaining an enrichment factor of 4 with a mixed technique of pH-junction and Field Amplified Sample Stacking (FASS).

Keywords: capillary electrophoresis; multiclass cyanotoxins; online pre-concentration; tandem mass spectrometry

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