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DEVELOPMENT OF AN ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR THE SCREENING OF CIANOTOXINS CONTENT IN DRINKING WATER SAMPLES

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More and more frequently waters intended for human consumption, for recreational, drinking, fishing or agricultural purposes, were affected by unusual cyanobacteria proliferation.
About 40 of the 150 known *genera* of cyanobacteria are able to produce, in particular conditions, secondary metabolites (called cyanotoxin) that are different for chemical structure and for toxic effects; unfortunately the studies about their toxicity are scarce and a linear correlation between the presence of a cyanobacteria bloom and the production of cyanotoxins does not exist, for the best of our knowledge.
In this context, it becomes important to develop analytical methods that allow the identification and, if possible, the quantification of as many cyanotoxins as possible.
This work presents the development of an analytical method to detect simultaneously 21 cyanotoxins of different classes (including 12 Microcystins, 5 Microginins, 2 Cyanopeptolins, and 2 Anabaenopeptins) using an ultra performance liquid chromatograph coupled with a high resolution mass spectrometer.
Microcistins are the most diffused cyanotoxin in Europe and World; they are monocyclic heptapeptides with an uncommon aminoacid ADDA, that are able to inhibit the protein phosphatase 1 (PP-1) and 2A (PP-2A) generating hepatotoxic effects [1].
Anabaenopeptide and Cyanopeptolins are cyclic non ribosomal oligopeptides produced by a broad range of cyanobacterial species that inhibit the serine proteases and the protein phosphatases, responsible for the regulation of several vital physiological and metabolic processes, but the studies about their ecological toxicity are scarce [2].
Microginins are linear peptides, characterized by N-terminal β-amino-α-hydroxy-decanoic or octanoic acid that inhibit zinc-containing metalloproteases; thirty-one different microginins had been isolated and fully characterized [3].
A chromatographic gradient was employed using acetonitrile and water as mobile phases, both containing 10 mM formic acid, and an Acquity UPLC system (Waters) equipped with an Acquity UPLC HSS T3 column (2,1 mm ID x 100 mm, 1,7 µm, Waters) at 40°C.
UPLC system was coupled with a XEVO G2S Q-TOF mass spectrometer (Waters) and the experiments have been carried out with a full scan 50-1200 in MS^E mode, positive ionization and resolution mode, with a scan time of 0.1 s.

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This particular acquisition mode allows to alternate low collision energy (MH^+ or MH^{n+} signal) and elevated collision energy (fragment signal) during the same experiment.
In this work, a collision energy ramp was optimized to obtain an optimal fragmentation for all the analytes.
Appling the previously described experimental conditions to a drinking water sample, we are able to identify 4 of 21 selected cyanotoxins and simultaneously, analyzing all the detected signals, to reveal the presence of non target compounds.
The method resulted robust, in terms of repeatability, reproducibility, linearity and detection limits that are at least 20-fold lower than the guideline value proposed by WHO for drinking water.
Moreover, this method allows the simultaneous identification of target and non-target compounds, in short time of analysis (16 minutes) and low injection volume (10 µl) allowing to detect the presence of other compounds potentially harmful to human health.
The analytical method proposed in this study can be applied to assure the prevention and management of emergencies caused by the proliferation of cyanobacteria and the cyanotoxins production in water for human consumption.

References

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