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SIMULTANEOUS MULTICLASS PRE-CONCENTRATION AND HPLC-MS/MS QUANTIFICATION OF ALGAL TOXINS IN ENVIRONMENTAL WATERS

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Global warming and anthropogenically-induced eutrophication of aquatic ecosystems are linked with increased frequency and magnitude of algal reproduction and thereby potentially harmful algal blooms. Marine biotoxins possess notable structural and physico-chemical properties different from each other. Domoic acid (DA) is a neurotoxic tricarboxylic amino acid, belonging to the class of kainoids, able to interfere with neurotransmission mechanisms by irreversible binding to glutamate receptors.

Okadaic acid (OA), a member of a group of molecules called polyketides, is an algal toxin responsible for diarrhetic shellfish poisoning.

Microcystins (MCs) are hepatic cyanotoxins which contain five fixed amino acids and two variable amino acids (X and Y) in positions 2 and 4, which characterize each MC.

Increasing concern regarding the presence of algal toxins in environmental waters has resulted in the need for reliable analytical methods for their monitoring at nanograms per litre levels. [1]

Aim of the present study is to develop a simple and sensitive method for the simultaneous screening, detection and quantification of several classes of algal toxins at environmental concentration levels, using SPE followed by HPLC-HESI-MS/MS.

Pre-concentration tests were undertaken on tap water samples (250 mL) enriched with 0.8 $\mu\text{g L}^{-1}$ of each analyte (DA, OA, MC-LW, MC-LR, MC-RR, MC-YR) using a carbonaceous sorbent material, recently proposed by our research group, HA-C@silica [2], evaluating different parameters, such as sorbent amount, extraction pH, composition and volume of the eluent. Adsorption is pH independent for all the analytes except for DA, which needs acid conditions. Therefore, the pre-concentration was carried out at pH close to 3, using 400 mg of HA-C@silica packed in 3 mL SPE tubes. MeOH with or without different percentages of Formic Acid (FA, 0-10% v/v) or Ammonia (NH₃, 0-5% v/v) was tested as eluent. The results highlighted that a single elution (2.5 mL) with MeOH 5% v/v FA provided recovery higher than 75% for all the analytes except for MC-RR (<20%). This toxin is strongly retained by the sorbent material probably due to the presence of an aromatic structure and polar groups, as highlighted by XPS analysis. So, further tests are now on going to assess the performance of different eluents and sorbent materials.

After SPE, algal toxins were separated and quantified by HPLC-HESI-MS/MS. Three reversed phase chromatographic columns were tested (Zorbax Eclipse Plus C18, 4.6 mm \times 100 mm, 3.5 μm ; Zorbax Eclipse Plus C18, 2.1 mm \times 50 mm, 1.8 μm ; Adamas C18-B 2.1 mm \times 150 mm,

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3.0 μm) and better chromatographic separations were obtained by the last one, using a gradient elution program with water and ACN both containing 0.5% v/v FA.

The quantitative analysis of the target compounds was performed in multiple-reaction monitoring (MRM) mode, using the two most intense and characteristic precursor/product ion transitions of each compound obtained from the MS/MS. The ionisation is performed in positive mode and the single protonated molecular ion $[\text{M}+\text{H}]^+$ is observed as precursor ion for all the analytes, except for MC-RR. This MC presented doubly protonated $[\text{M}+2\text{H}]^{2+}$ as precursor ion, because it contains two arginine residues in its molecular structure.

The main figures of merit - selectivity, sensitivity, linearity, recovery and precision – are evaluated and pre-concentration tests on lake and standing water are in progress to investigate the applicability to different environmental matrices.

References

[1] Zervoua S.K., Christophoridisa C., Kaloudisb T., Triantisa T.M., Hiskiaa A., Journal of Hazardous Materials, 2017, 323, 56.

[2] A. Speltini, F. Merlo, F. Maraschi, M. Sturini, M. Contini, N. Calisi, A. Profumo, J. Chromatogr. A, 2018, 1540, 38.