UHPLC-HR-Orbitrap mass spectrometry for quantitative analysis of lipophilic marine toxins in shellfish

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Up to now, analytical methods used for the detection of marine toxins have generally been focussing on the analysis of 13 lipophilic toxins. However, to date more than 200 lipophilic marine toxins have been described (Gerssen *et al.*, 2011). Edible shellfish can bioaccumulate marine toxins when grazing on toxic marine microorganisms, particularly marine microalgae such as certain species of dinoflagellates and diatoms, creating a potential food safety risk to humans. During the last years, many analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have been consolidated by inter-laboratory validations. However, the main drawback of LC-MS/MS methods remains the limited number of compounds that can be analysed in a single run. Moreover, due to the targeted nature of LC-MS/MS only known toxins for which methods have been previously optimized will be detected. Therefore in this study, a method based on ultra high-performance liquid chromatography (UHPLC) coupled to high resolution (HR) Orbitrap mass spectrometry was developed and its quantitative performance evaluated for confirmatory analysis of regulated lipophilic marine toxins in shellfish flesh (*Mytilus edulis, Crassostrea gigas, Cerastoderma edule,* and *Ensis ensis*) according to Commission Decision 2002/657/EC (CD) and EU Reference Laboratory for Marine Biotoxins SOP 2011. Okadaic acid (OA), dinophysistoxin-1 (DTX-1), pectenotoxin-2 (PTX-2), azaspiracid-1 (AZA-1), yessotoxin (YTX) and 13-desmethyl spirolide C (SPX-1) were quantified using matrix-matched calibration curves. The obtained results for recovery, repeatability, within-laboratory reproducibility (RSD_R), decision limit , linearity and ruggedness were compliant with CD recommendations. The linearity was evaluated by preparing 8-point calibration curves in matrix and correlation coefficients (R^2) obtained for each compound were $\geq$ 0.99. For all compounds the RSD_R ranged from 2.9% to 4.9%, repeatability from 2.9% to 4.8% and recovery from 90% to 112% for three spiked levels. In addition, a first confirmatory identification of the compounds was performed by detecting the $[M+H]^+$ or $[M-H]^-$ ion with their specific retention times and accuracies. Secondly, the $^{13}C/^{12}C$ diagnostic isotopic ratio was selected for confirmation of a compound's identity. In conclusion, UHPLC-HR-Orbitrap MS allowed a more accurate and faster (less than 4min.) detection of the target toxins than previously described LC-MS/MS methods. Furthermore, HRMS allows to retrospectively screen for many toxin analogues and metabolites using its full scan capabilities but also untargeted screening through the use of metabolomics software.

References

Gerssen A., P.P.J. Mulder And J. De Boer. 2011. Screening of lipophilic marine toxins in shellfish and algae: Development of a library using liquid chromatography coupled to Orbitrap mass spectrometry. Analytica Chimica Acta 685:176-185.