



Broad screening of phycotoxins in tissue, water and food supplements with LC-hrMS

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Background

Phycotoxins such as azaspiracids, microcystins, saxitoxins and ciguatoxins are produced by algae which are naturally occurring in marine and fresh waters. Different types of phycotoxins can end up in matrices like tissue (shellfish or fish), water or food supplements. When these toxins are consumed or there is contact with some of the toxins i.e. in swimming water intoxication such as skin irritation, paralysis, diarrhoea or even death may occur.

Analytical methods are available to analyse regulated phycotoxins, however these methods are only suitable for a small specific group of toxins and/or a specific matrix (mainly shellfish). Therefore, a screening method is developed for all kinds of phycotoxins in different matrices such as tissue (shellfish), fresh and sea water and food supplements. This method can be applied in case of an incident or when symptoms can not be directly related to the regulated phycotoxins.

Methods

Extraction methods

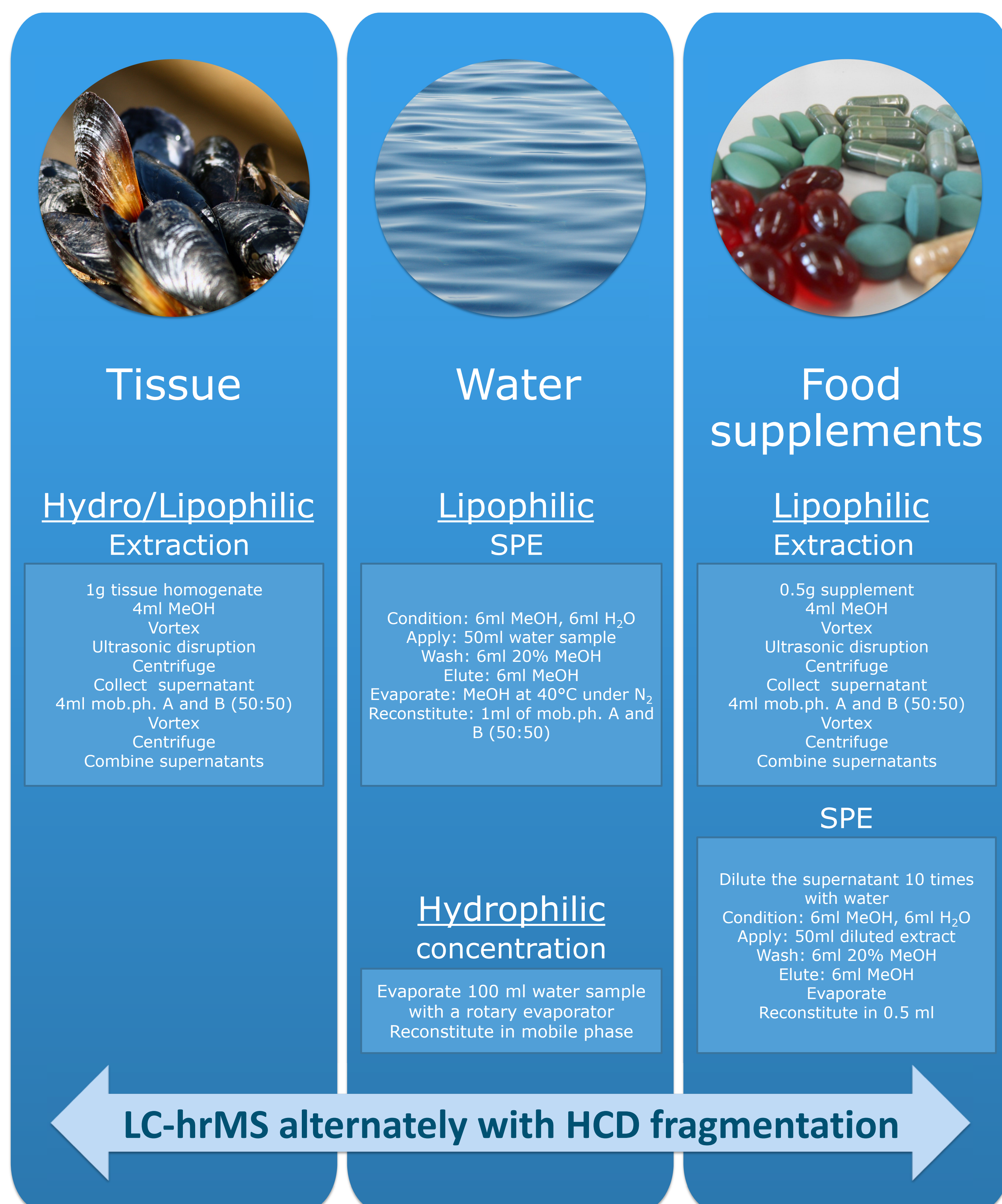


Figure 1. Extraction methods for phycotoxins in tissue (shellfish/fish), water and food supplements.

LC-hrMS methods

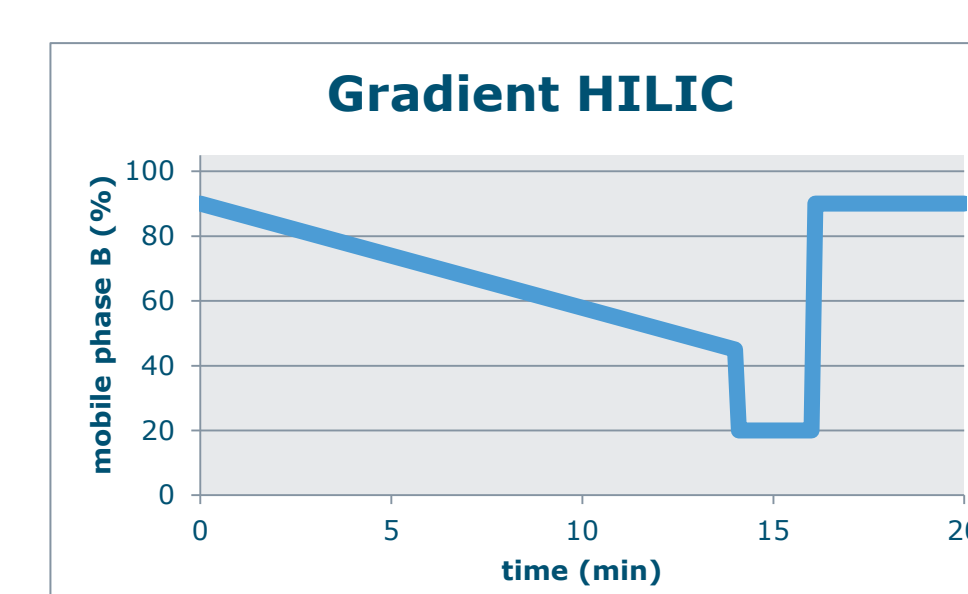
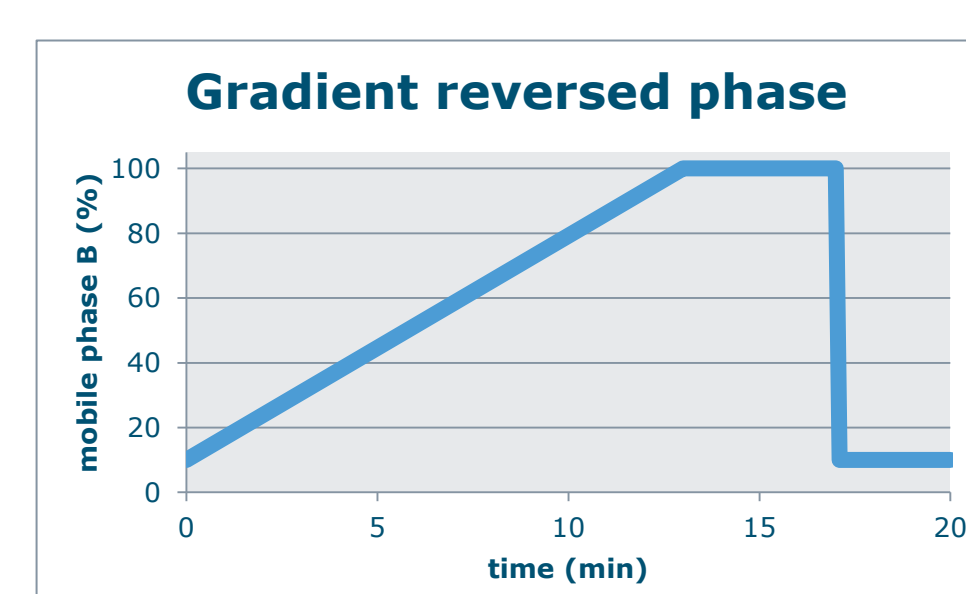
- Mobile phase A H₂O and B ACN/H₂O (90/10) both containing 2mM ammonium formate and 0.5mM formic acid
- Column temperature: 35°C; Injection volume: 10µl

Lipophilic phycotoxins (Reversed Phase):

- Column: BEH C₁₈ 100mm x 2.1mm, 1.7µm; Flow: 0.3ml/min

Hydrophilic phycotoxins (HILIC):

- Column: TSKgel Amide-80 150mm x 2mm, 3µm; Flow: 0.5ml/min

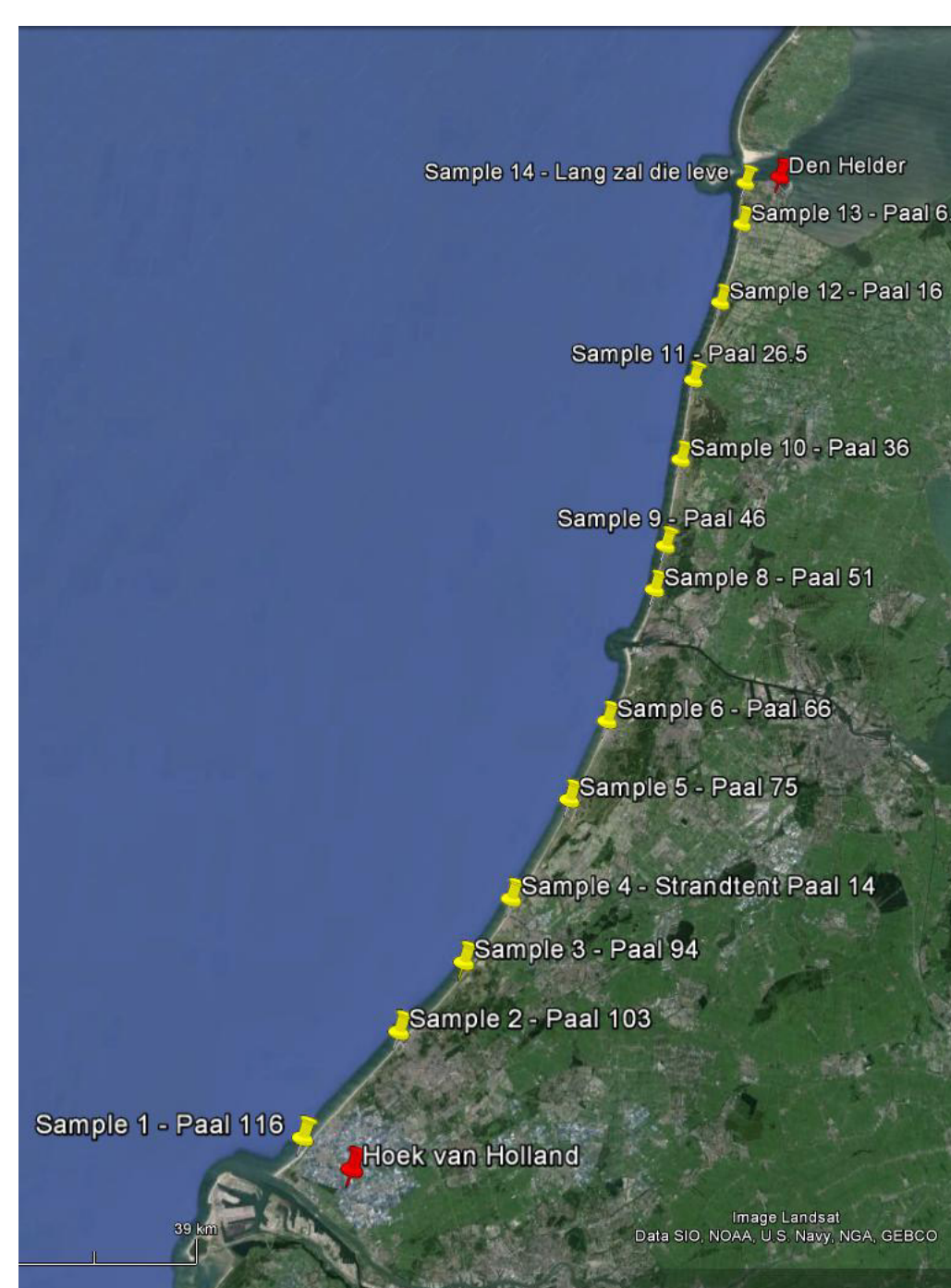


- Detection with full scan high-resolution single stage MS Orbitrap
- Mass range: m/z 100 – 1,500; Resolution: 50,000 FWHM
- Collision energy HCD: 70 eV (lipophilic toxins, >500 Da)
30 eV (hydrophilic toxins <500 Da)
- Data is searched for over 300 different phycotoxins using Metalign software

Results

Method development

- ~50 standards available for method development (LC and extraction)
- Lipophilic toxins, good recoveries (70-120%), RSDs < 30%
- Hydrophilic toxins, good recoveries (70-120%), RSDs < 30%
- Exception for hydrophilic toxins in seawater and food supplements (due to salt content and relative low concentrations)



Application of developed method

- Dutch waters (coast line sampled)
 - First time Azaspiracids found
 - Spirolides and saxitoxins in brakish water lake
- Food supplements
 - Pinnatoxins, spirolides and gymnodimines in NZ green lipped mussels
 - Gymnodimines in kelp
 - Okadaic acid in krill oil
 - Microcystins in Klamath lake supplements

Conclusions

- A generic screening method is developed for different kinds of phycotoxins in various matrices
- Library constructed containing over 300 phycotoxins, based on available standards and literature
- Application revealed toxins not shown before in Dutch waters
- Method development on-going for the concentration of hydrophilic toxins in water and food supplements

