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Screening of Lipophilic Marine Toxins using LC-Orbitrap-MS

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Introduction

Most liquid chromatography (LC) mass spectrometric (MS) methods used for routine monitoring of lipophilic marine toxins focus on the analysis of the 13 toxins that are stated in European Union legislation. However, to date over 200 lipophilic marine toxins have been described in the literature. To fill this gap, a screening method using LC coupled to high resolution (HR) orbitrap MS (resolution 100 000) for lipophilic marine toxins has been developed.

Method

LC Column Waters XBridge C18 (150 × 3mm, 5µm)
Mobile phase; A) water, B) acetonitrile both containing 6.7 mM ammonium hydroxide (pH = 11).
Flow; 0.4 ml/min with a 30 min gradient
Injection volume; 10 µl

MS Thermo Exactive Orbitrap operating at ultra high resolution (FWHM 100 000) in both electrospray positive (ESI^+) and negative (ESI^-). Sample extracts were analyzed with and without fragmentation using the HCD cell.

Data processing Data reduction was done with *metAlign* and library searching was done with *Search_LCMS* (both programs are available as freeware at http://www.metalign.nl/UK/). The search library for *Search_LCMS* consisted of a CSV file with information about the compound name, *m/z*, mass window, retention time and retention time window (Table 1). When only the *m/z* and mass window are defined and the compound name is "all", all compounds with this mass will be shown.

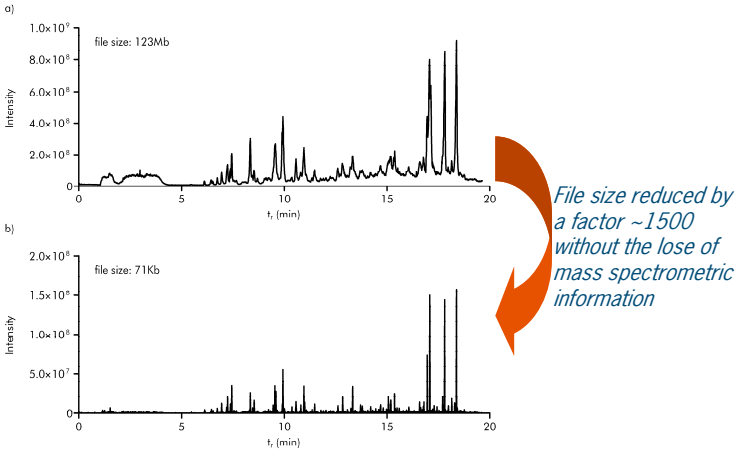

Figure 1. Full scan chromatogram of a) raw data file and b) metAlign reduced data file of a shellfish extract.

Results

A library was constructed by analyzing various samples (shellfish and algae) with different toxin profiles. Data files were processed, reduced (Fig. 1) and searched for 1) a set of 200 known toxins found in Scifinder and 2) unknown toxins with comparable fragmentation patterns. In total 85 different toxins were tentatively identified in the contaminated samples. Based on the specific fragments some "unknowns" were found (Fig 2).

Table 1. Part of constructed CSV search library.

Compound	*m/z*	Mass window (Da)	t_r (min)	t_r window (min)
Okadaic acid	803.4587	0.0040	8.4	0.2
all	255.1238	0.0013		
all	785.4482	0.0039		

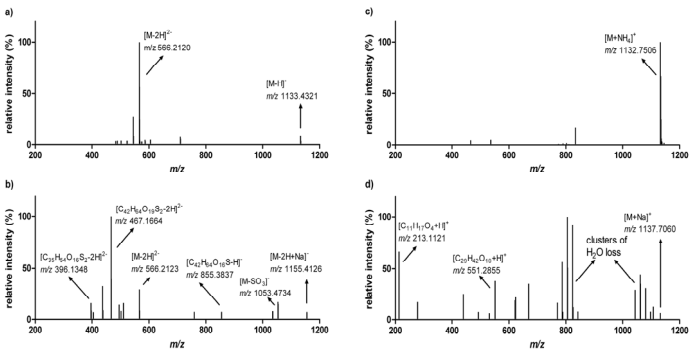

Figure 2. New toxins found with searching for common fragments. A YTX derivative $C_{52}H_{78}O_{23}S_2$ a) without and b) with fragmentation and a 16:0 PTX2sa ester c) without and d) with fragmentation.

Conclusion

- Search library constructed for 85 identified toxins
- Results for samples analyzed with this approach were in line with the tandem LC-MS and rat bioassay used for routine monitoring
- Library searching is very fast
- Library is easily exchangeable between labs even with other LC-HRMS instruments (Fig. 3)

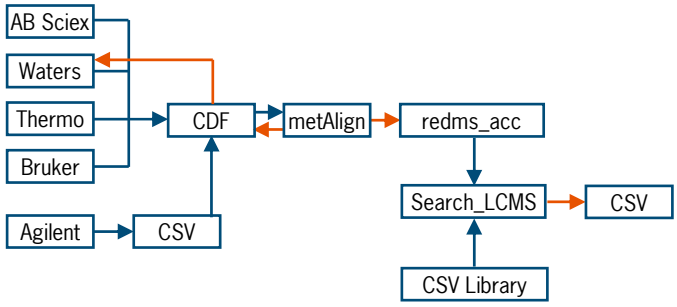

Figure 3. Scheme of data processing procedure.

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