

Introduction

Liquid chromatography – mass spectrometry (LC-MS) has emerged as the preferred analytical tool for accurate and reliable monitoring of shellfish biotoxins. This is especially true for toxins such as the dinophysistoxins, pectenotoxins, and azaspiracids, which are structurally diverse and thus do not contain a common UV chromophore or reactive functional group for fluorescence derivatization. In addition, these toxins tend to yield unreliable results by the mouse bioassay.

In general, LC-MS analysis of biotoxins is performed in selected reaction monitoring (SRM) mode on a triple-quadrupole MS, whereby the first quadrupole is programmed to transmit a selected mass for fragmentation and the final quadrupole transmits a single fragment ion to the detector. For monitoring multiple analytes simultaneously, the quadruples can rapidly toggle between pre-defined masses in multiple reaction monitoring (MRM) mode. This approach is well suited for quantification due to its inherent selectivity and high sensitivity. However, the relatively low resolution of quadrupoles (typically unit resolution) renders the technique prone to interference from ions of similar mass in complex samples. In addition, due to the targeted nature of MRM, only known toxins specified in the method will be detected. Therefore, new or modified biotoxins could remain undetected indefinitely, even at high abundance.

This presentation describes the high-resolution analysis of marine biotoxins using a bench-top mass spectrometer based on Orbitrap™ technology.

Experimental & Instrumentation

Materials

Toxin standards were acquired from the National Research Council of Canada's Certified Reference Materials Program, Halifax, Nova Scotia, Canada

LC Column

Thermo Fisher Gold, 2.1 x 50 mm
1.8 µm particle size
Injection volume: 3 µL
5 replicate injections

LC-MS system

Thermo Exactive mass spectrometer coupled with Accela liquid chromatography system

MS Conditions

Full scan m/z 150-1000
Positive mode
Electrospray ionization
External calibration
Ultra-high resolution setting

Sheath gas : 50
Auxiliary Gas : 10
Capillary temperature : 360°C
heater temperature : 250°C



Mobile phase

A stock solution of 1% formic acid was pH adjusted to 3.0 with concentrated ammonium hydroxide and diluted accordingly for the following mobile phases:

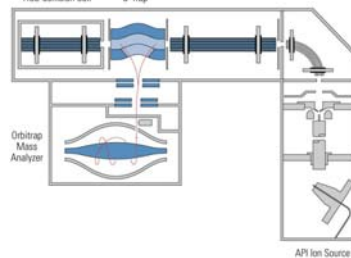
Solvent A : water 0.1% formic acid
Solvent B: acetonitrile 0.1% formic acid

LC Conditions

Time (min)	%A	%B
0.0	90	10
2.0	10	90
3.0	10	90
3.5	90	10
6.00	90	10

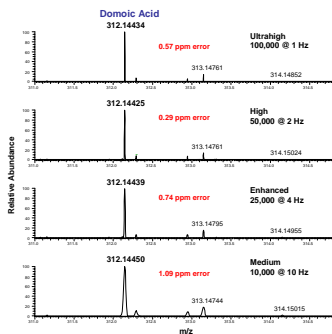
Instrumentation

The Thermo Exactive offers high resolution and mass accuracy over a broad mass range (m/z 50 – 4000). The optional HCD collision cell provides "all ion fragmentation", which can be used to confirm peak identity in screening applications.



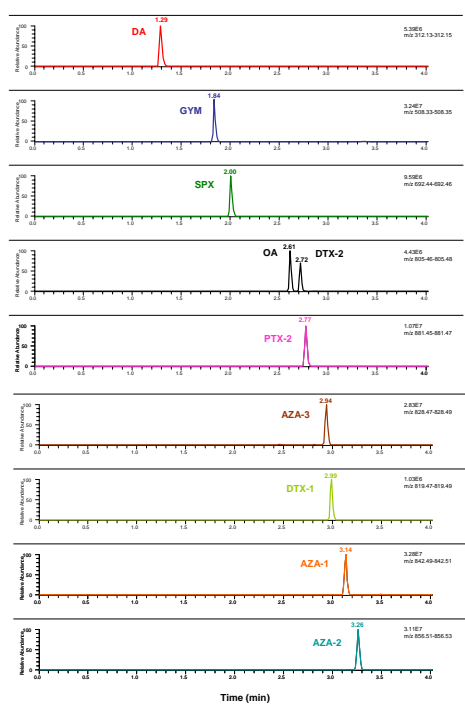
Comparison of different resolution settings

Resolution up to 100,000 can be achieved with a 1 second cycle time, while faster scanning can be performed at lower resolution settings.



Results

Exactive LC-MS analysis of a marine biotoxin standard mixture

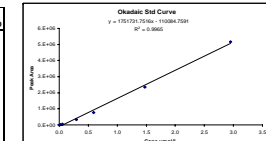


Results

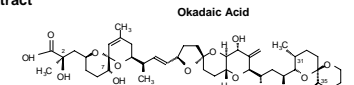
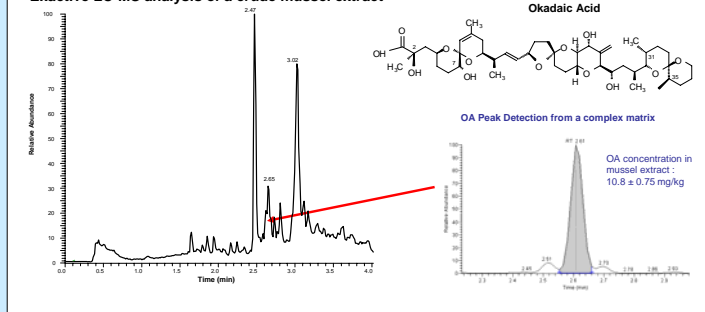
Accurate mass and quantification performance

TOXIN	DA	GYM	SPX	OA	DTX-2	PCTX-2	AZA-3	DTX-1	AZA-1	AZA-2
Expected Mass	[M+H] ⁺ 312.14416	[M+H] ⁺ 508.34214	[M+H] ⁺ 692.45208	[M+H] ⁺ 895.47327	[M+H] ⁺ 895.47327	[M+Na] ⁺ 891.46633	[M+H] ⁺ 898.48925	[M+H] ⁺ 819.48892	[M+H] ⁺ 842.50490	[M+H] ⁺ 856.52555
Observed Mass	312.14392	508.34194	692.45193	895.47297	895.47274	891.46491	898.48839	819.48846	842.50403	856.51968
ppm error	1.73	0.39	0.22	0.24	0.66	1.61	1.15	0.993	1.03	1.02
Std Curve R ²	1.0000	0.9993	0.9992	0.9965	0.9751	0.9998	0.9996	0.9983	0.9995	0.9996
Std Curve Midrange RSD	5.9	7.1	3.6	5.1	7.6	6.5	5.1	8.4	9.6	2.6

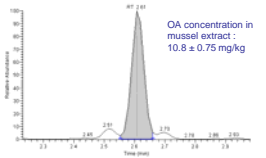
Typical calibration curve



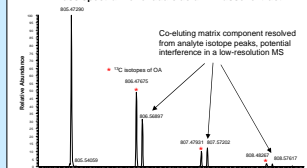
Exactive LC-MS analysis of a crude mussel extract



OA Peak Detection from a complex matrix



Mass spectrum of okadaic acid in mussel extract



Conclusions

- The Exactive represents a viable alternative to conventional mass spectrometers for screening and quantification
- Little method development as settings are not tuned for each analyte
- Non-targeted approach so unknown compounds are also detected
- Comparable quantitative performance to triple quadrupole systems

Acknowledgements

Thermo-Fisher Scientific for the loan of the Exactive mass spectrometer.