

## 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

### 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

### MS Conditions

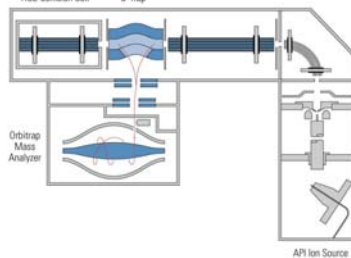
Full scan m/z 150-1000  
Positive mode  
Electrospray ionization  
External calibration  
Ultra-high resolution setting

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



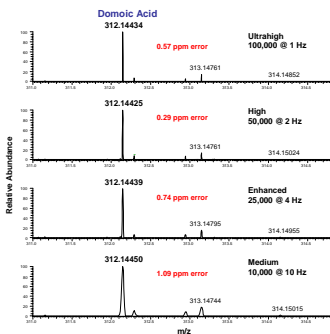
## Instrumentation

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.



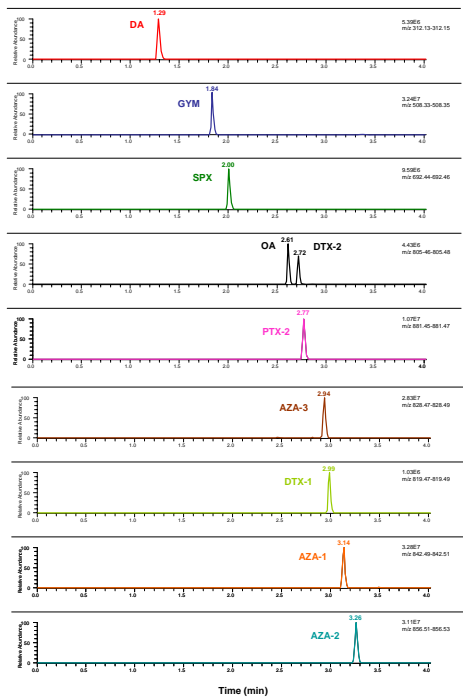
### Comparison of different resolution settings

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

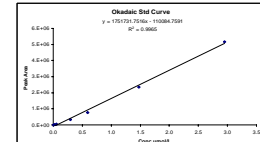


## Results

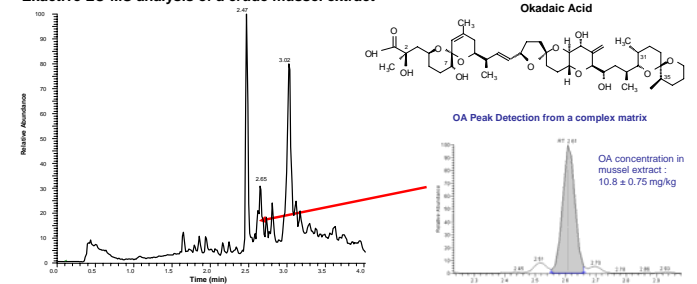
### Accurate mass and quantification performance

TOXIN			Expected Mass	Observed Mass	ppm error	Std Curve R <sup>2</sup>	Std Curve Midrange RSD
Domoic acid	DA	[M+H] <sup>+</sup>	312.14416	312.14362	1.73	1.0000	5.9
Gymnodimine	GYM	[M+H] <sup>+</sup>	508.34214	508.34194	0.39	0.9993	7.1
Desmethyl Spiroside C	SPX	[M+H] <sup>+</sup>	692.45208	692.45193	0.22	0.9992	3.6
Okeadonic acid	OA	[M+H] <sup>+</sup>	895.47327	895.47267	0.74	0.9965	5.1
Dinophysistoxin-2	DTX-2	[M+H] <sup>+</sup>	895.47327	895.47274	0.66	0.9751	7.6
Pectenotoxin-2	PTX-2	[M+Na] <sup>+</sup>	891.46633	891.46491	1.61	0.9998	6.5
Azaspiracins 1	AZA-1	[M+H] <sup>+</sup>	828.48925	828.48839	1.15	0.9996	5.1
Dinophysistoxin-1	DTX-1	[M+H] <sup>+</sup>	819.48892	819.48846	0.56	0.9983	8.4
Azaspiracins 2	AZA-2	[M+H] <sup>+</sup>	842.50490	842.50403	1.03	0.9995	9.6
Azaspiracins 2	AZA-2	[M+H] <sup>+</sup>	856.52555	856.51968	1.02	0.9996	2.6

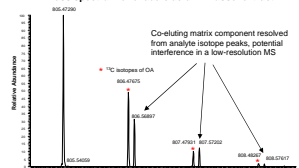
### Typical calibration curve



### Exactive LC-MS analysis of a crude mussel extract



### Mass spectrum of okeadonic 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.