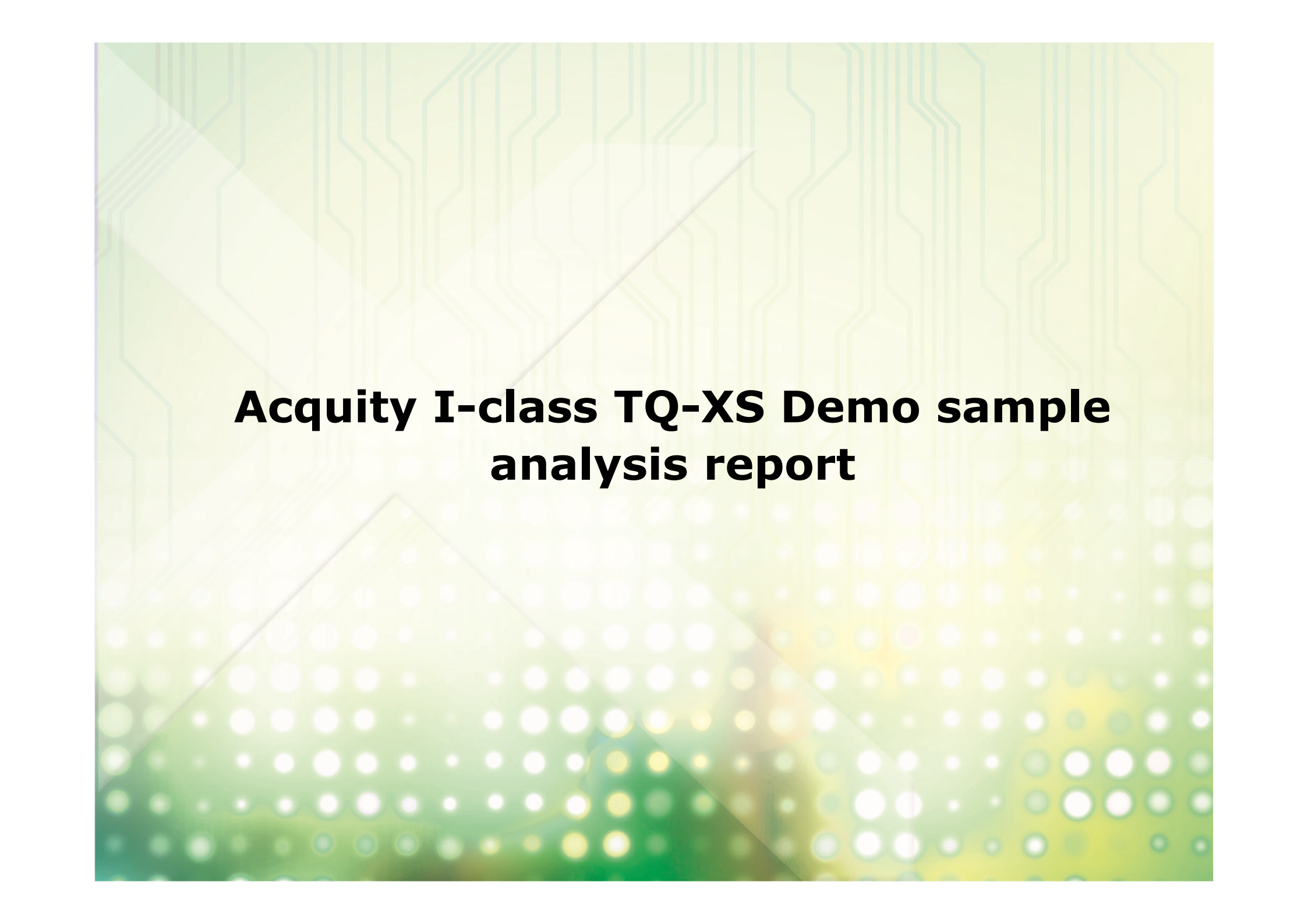


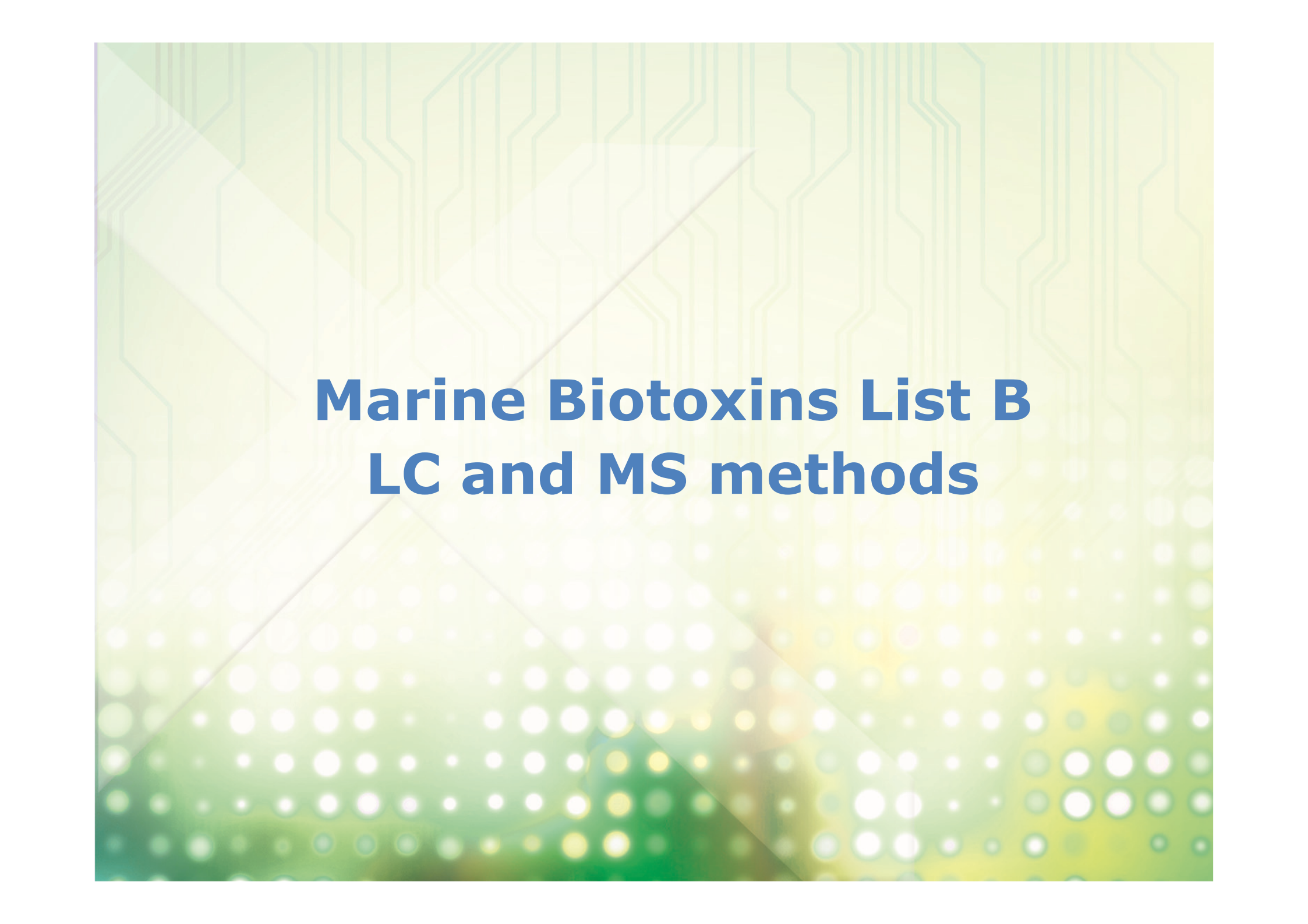
The background of the slide features a light green to yellow gradient. Overlaid on this are faint, light green circuit board traces that run vertically and horizontally. In the lower half of the slide, there is a grid of small, semi-transparent white circles. A large, semi-transparent white diagonal line runs from the top-left towards the bottom-right, passing behind the text.

Acquity I-class TQ-XS Demo sample analysis report

Introduction

AVA has provided us with four groups of samples.

- Group -1 : Sudan Dyes
 - Group -2 : Sweeteners
 - Group -3 :Marine Biotoxins List A
 - Group -4 :Marine Biotoxins List B
-
- This system must be able to run a variety of applications, including a sensitive assay for all the group of compounds mentioned in the above samples.
 - This report demonstrates the potential for the I-Class FTN/XEVO TQ-XS to provide a robust and sensitive results for the above mentioned compounds.



Marine Biotoxins List B

LC and MS methods

Samples Provided

Samples :

- Two samples, one blank and one spiked (BLK-B and SPK-B) were provided.
- One stock concentration sample 100ppb in Methanol was given for the preparation of the calibration curve samples.

Samples were ran on the XEVO TQ-XS in order to assess the systems in the most efficient way, with sample stability not influencing results

Experimental

Sample Analysis :

- The MS parameters were obtained from literature.
- Calibration standards were prepared from 100ppb in neat solvent (60:40 Acetonitrile:Water) at 0.2ppb, 0.5ppb, 1ppb, 5ppb, 10ppb levels.
- Samples BLK-B and SPK-B were diluted 10x in neat solvent before injection.

Quantification

- BLK-B and SPK-B were analyzed on the calibration curve.
- Concentration was back-calculated for the unknown sample from the calibration curve.

MS Method: Marine Biotoxins List B

Analyte	Parent	Daughter	Dwell	Cone Voltage	Collision energy	Mode of Ionization	References
GYM	508.2	162.2	0.005	60	55	ES+	1
GYM	508.2	490.2	0.005	60	40	ES+	1
Domoic acid	312.00	161	0.005	30	30	ES+	2
Domoic acid	312.00	266	0.005	30	25	ES+	2
AZA1	842.5	672.4	0.005	35	40	ES+	1
AZA1	842.5	824.5	0.005	35	30	ES+	1
AZA2	856.5	672.4	0.005	35	40	ES+	1
AZA2	856.5	838.5	0.005	35	30	ES+	1
AZA3	828.5	658.4	0.005	35	40	ES+	1
AZA3	828.5	792.5	0.005	35	30	ES+	1
PTX2	876.5	213.1	0.005	40	30	ES+	1
PTX2	876.5	823.5	0.005	40	30	ES+	1
PTX11	892.5	213.1	0.005	40	30	ES+	1
PTX11	892.5	839.5	0.005	40	30	ES+	1
P-CTX-1	1128.70	1075.7	0.005	50	50	ES+	3
P-CTX-1	1128.70	1093.7	0.005	50	40	ES+	3
P-CTX-3	1112.7	1059.6	0.005	50	50	ES+	3
P-CTX-3	1112.7	1077.6	0.005	50	55	ES+	3

MS Method: Marine Biotoxins List B

Analyte	Parent	Daughter	Dwell	Cone Voltage	Collision energy	Mode of Ionization	References
YTX	570.40	396.4	0.005	75	30	ES-	1
YTX	570.40	467.4	0.005	76	30	ES-	1
Homo-YTX	577.4	403.4	0.005	75	30	ES-	1
Homo-YTX	577.4	474.4	0.005	75	30	ES-	1
45-OH-YTX	578.4	396.4	0.005	75	30	ES-	1
45-OH-YTX	578.4	467.4	0.005	75	30	ES-	1
45-OH-homo-YTX	585.4	403.4	0.005	75	30	ES-	1
45-OH-homo-YTX	585.4	474.4	0.005	75	30	ES-	1
DTX1	817.5	113	0.005	80	60	ES-	1
DTX1	817.5	255.2	0.005	80	45	ES-	1
OA/ DTX2	803.5	113.1	0.005	80	60	ES-	1
OA/ DTX2	803.5	255.2	0.005	80	45	ES-	1
Brevetoxin-3	897.0	725.0	0.005	50	60	ES-	4
Brevetoxin-3	897.0	752.0	0.005	50	50	ES-	4
Microcystin YR	1046.5	135.0	0.005	55	60	ES-	5
Microcystin YR	1046.5	213.0	0.005	55	60	ES-	5

Literature for Marine Biotoxins List B

References

1. UPLC/MS/MS Method for the Routine Quantification of Regulated and non-regulated Lipophilic Marine Biotoxins in Shellfish Arjen Gerssen et al (Waters Corporation, 720004601en)
2. Analysis of Marine Toxins in Shellfish. Andrew Turner, Cefas
www.rsc.org/images/analysis-of-marine-toxins-in-shellfish_tcm18-245151.pdf
3. Update on Methodologies Available for Ciguatoxin Determination: Perspectives to Confront the Onset of Ciguatera Fish Poisoning in Europe. J. Diogene et al, Marine Drugs 2008, 8(6), 1838-1907
4. Comparative Analysis of Three Brevetoxin-Associated Bottlenose Dolphin (Tursiops truncatus) Mortality Events in the Florida Panhandle Region (USA). M.Twiner et al, PLOS one, 2012
5. MassLynx, Quanpedia Library

Inlet Method

LC Conditions	
Column	Acquity UPLC BEH C8 2.1x100 mm, 1.7µm
Solvent A	0.05% NH3 in Water
Solvent B	90:10 Acetonitrile:Water (0.05% NH3)
Injection volume	10 µL

Time	Flow rate	% B
0	0.4	30
1	0.4	30
5	0.4	90
6	0.4	90
6.1	0.4	30
8	0.4	30



Results

Calibration Curve - GYM

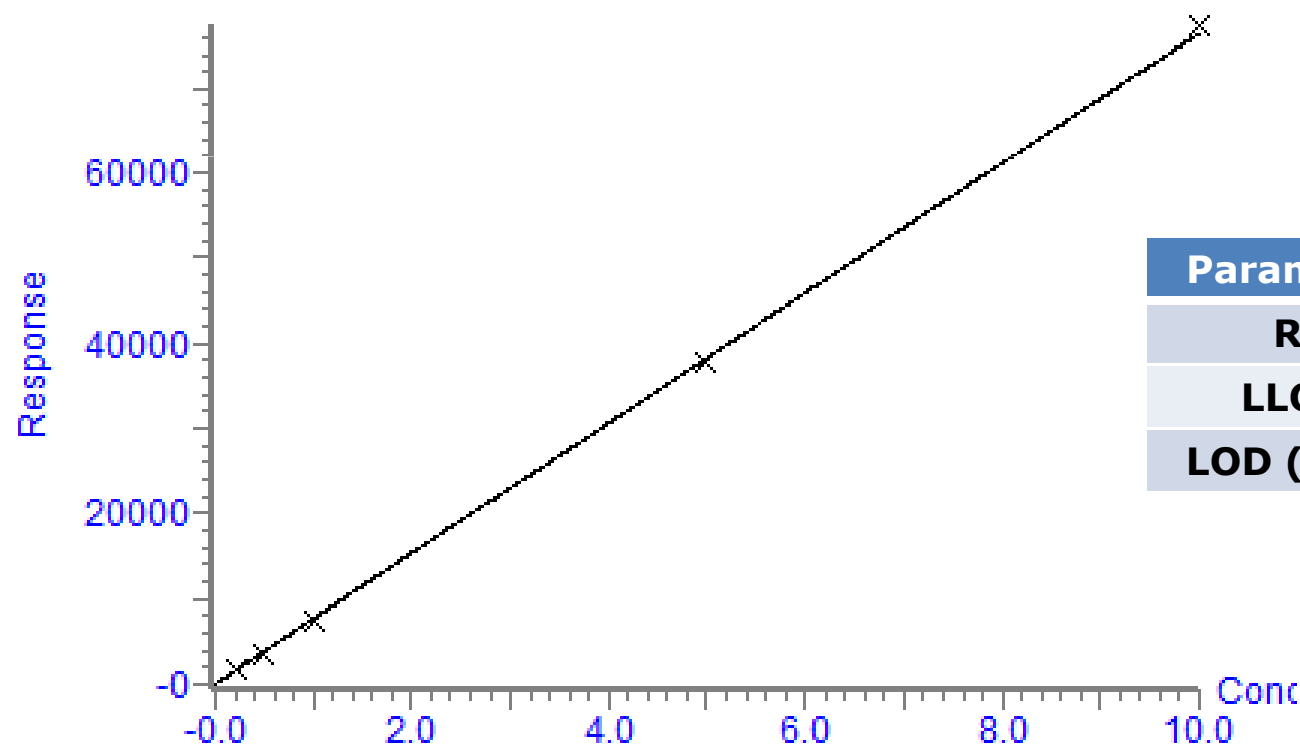
Compound name: GYM

Correlation coefficient: $r = 0.999484$, $r^2 = 0.998969$

Calibration curve: $7640.9 * x + 87.0339$

Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans:



Parameter	Result
R^2	0.998
LLOQ	0.2 ppb
LOD (calc)	0.06ppb

Calibration Curve – PTX2

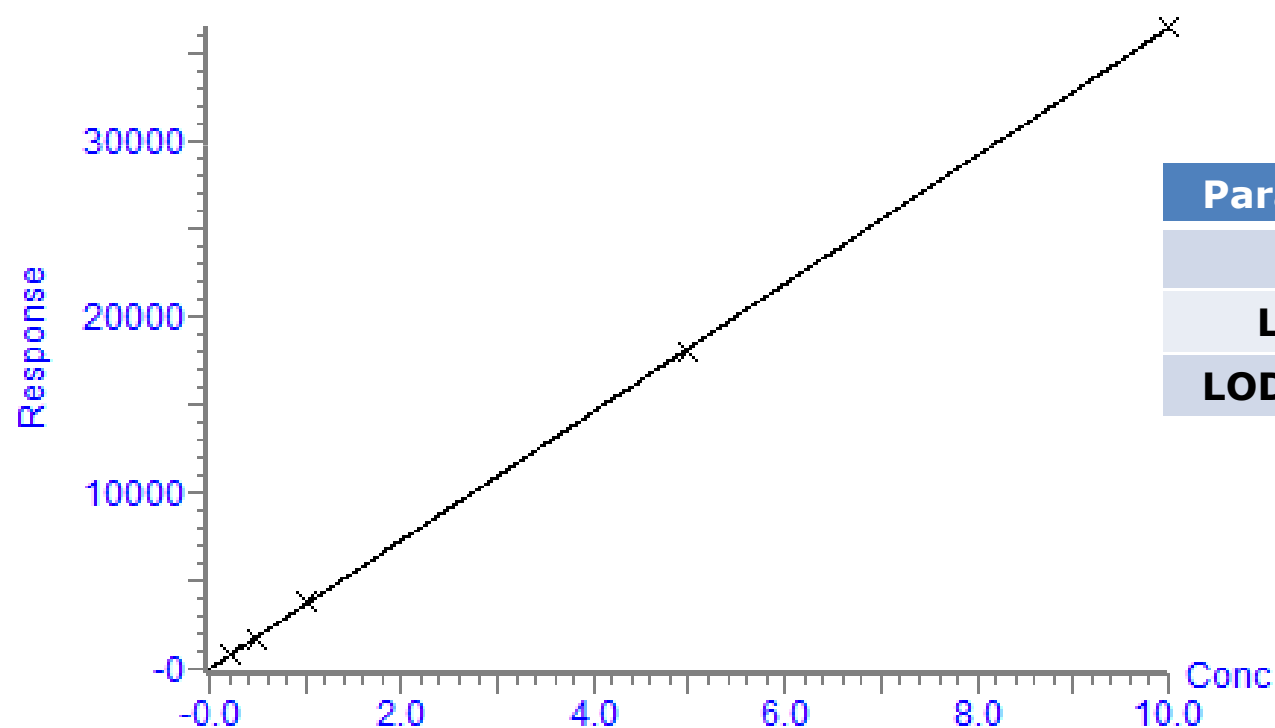
Compound name: PTX-2

Correlation coefficient: $r = 0.999814$, $r^2 = 0.999627$

Calibration curve: $3647.6 * x + 26.0374$

Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: No



Parameter	Result
R^2	0.999
LLOQ	0.2 ppb
LOD (calc)	0.04ppb

Calibration Curve – AZA2

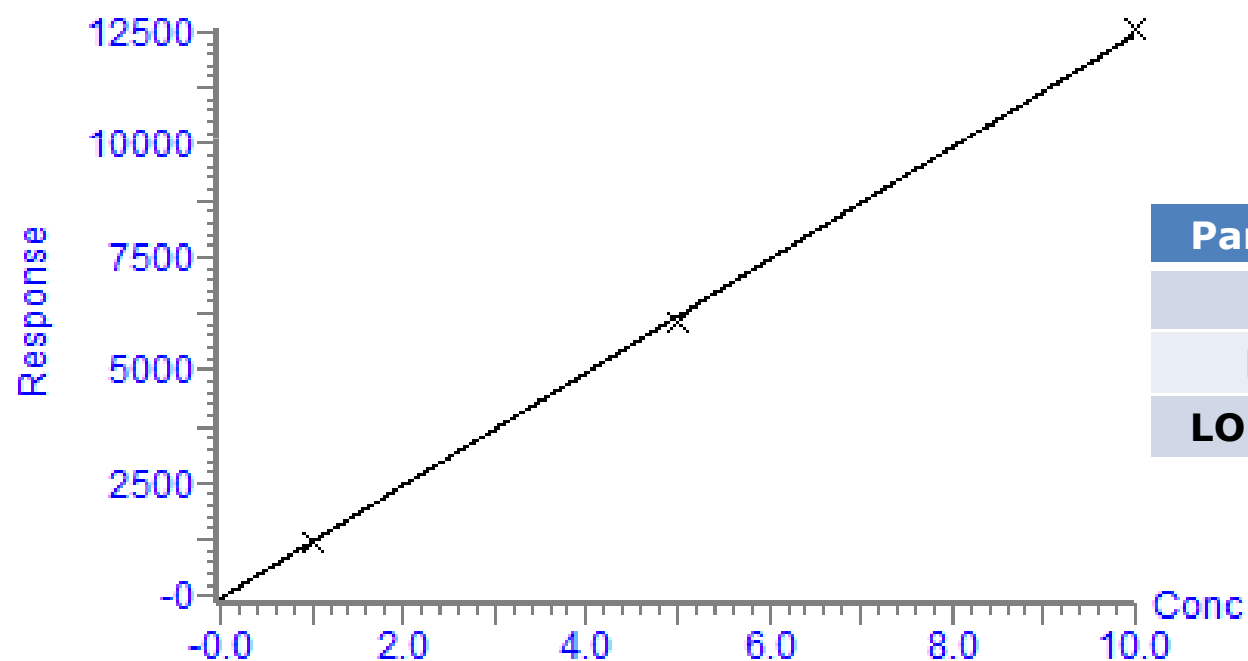
Compound name: AZA2

Correlation coefficient: $r = 0.999773$, $r^2 = 0.999546$

Calibration curve: $1248.03 * x + -49.9633$

Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

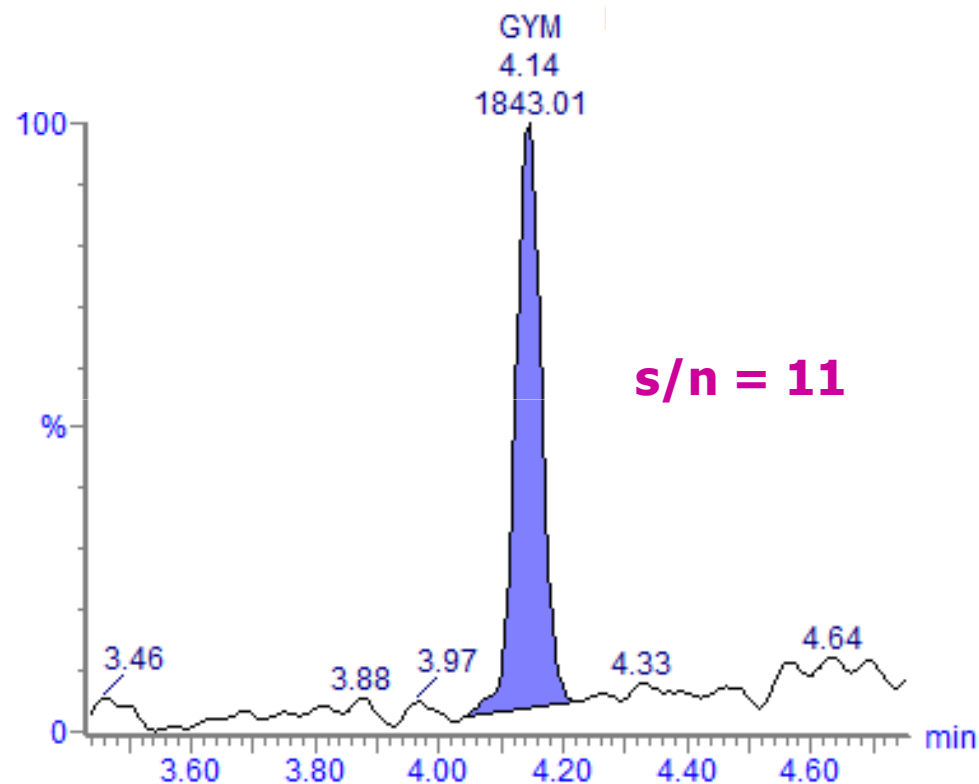


Parameter	Result
R²	0.999
LLOQ	1 ppb
LOD (calc)	0.2ppb

***Higher sensitivity may be achieved with MS method development**

Sensitivity at LLOQ - GYM

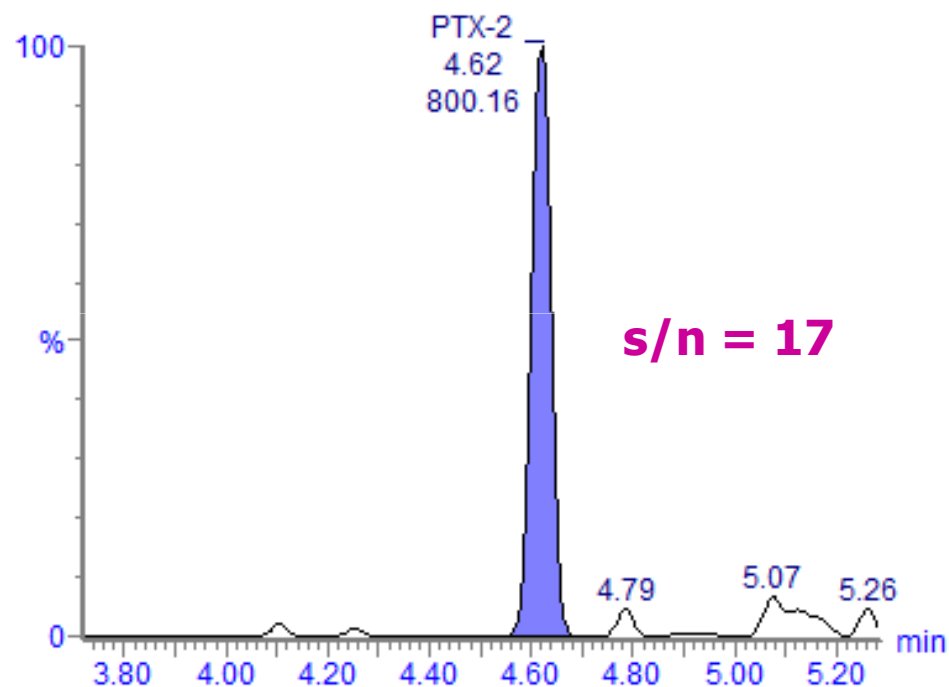
- A signal-to-noise value of 11 was observed for GYM standard of 0.2ppb.



GYM standard 0.2ppb

Sensitivity at LLOQ – PTX2

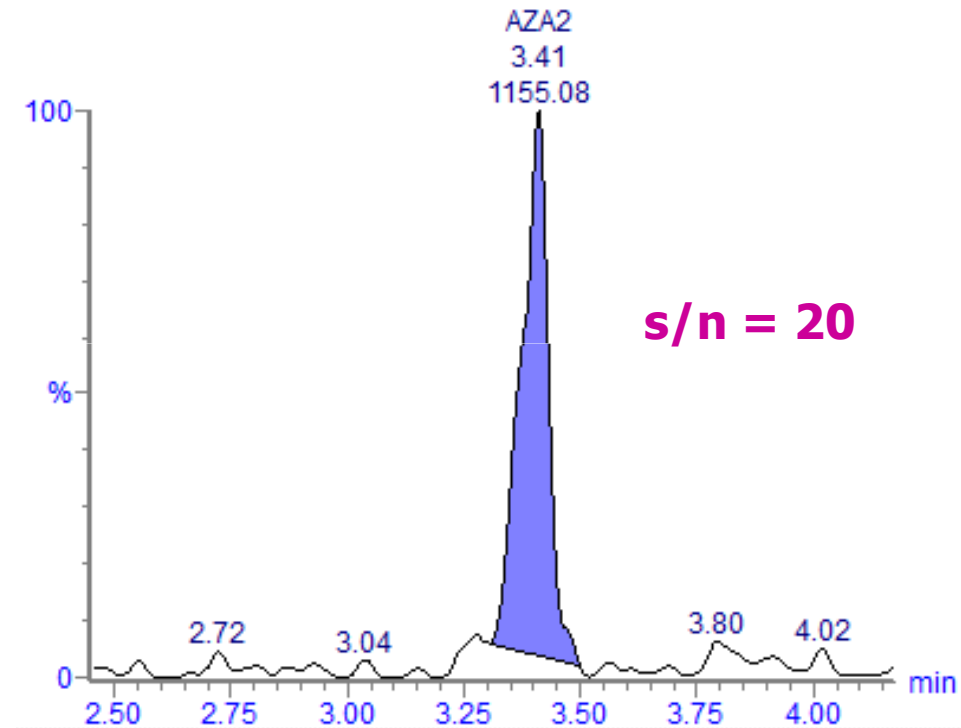
- A signal-to-noise value of 17 was observed for PTX2 standard of 0.2ppb.



PTX-2 standard 0.2ppb

Sensitivity at LLOQ – AZA2

- A signal-to-noise value of 20 was observed for AZA2 standard of 1ppb*.



AZA2 standard 1ppb

*Higher sensitivity may be achieved with MS method development

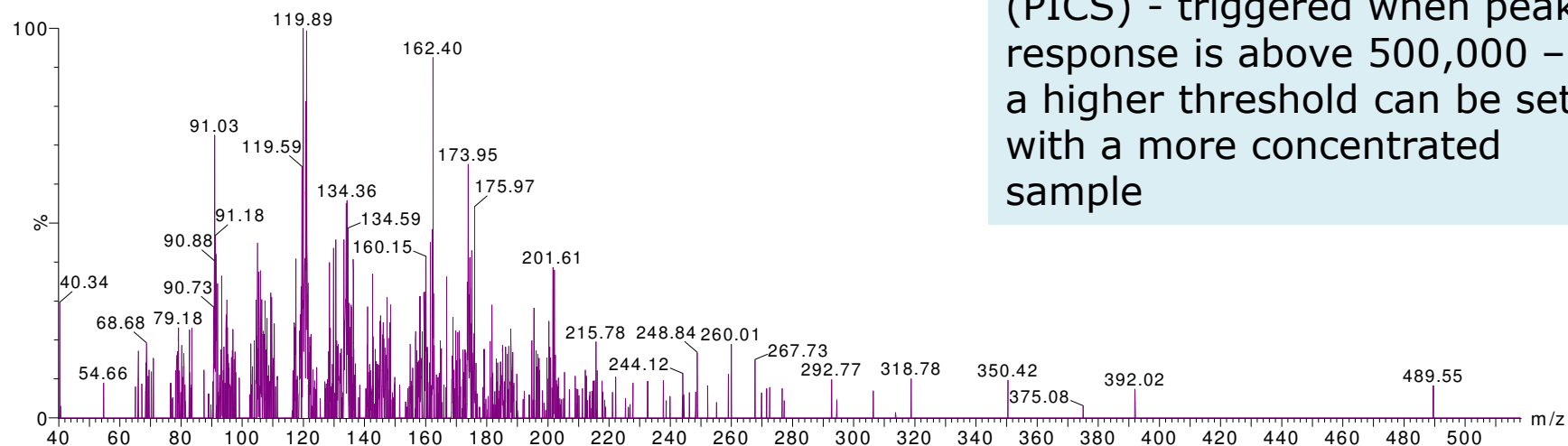
Quantitation Results

Detected Biotoxins	BLK-B (ppb)	SPK-B (ppb)
Gymnodimine (GYM)	<LOQ	20.5
Pentenotoxin-2 (PTX2)	<LOQ	19.6
Azaspiracid-2 (AZA2)	<LOQ	20.0

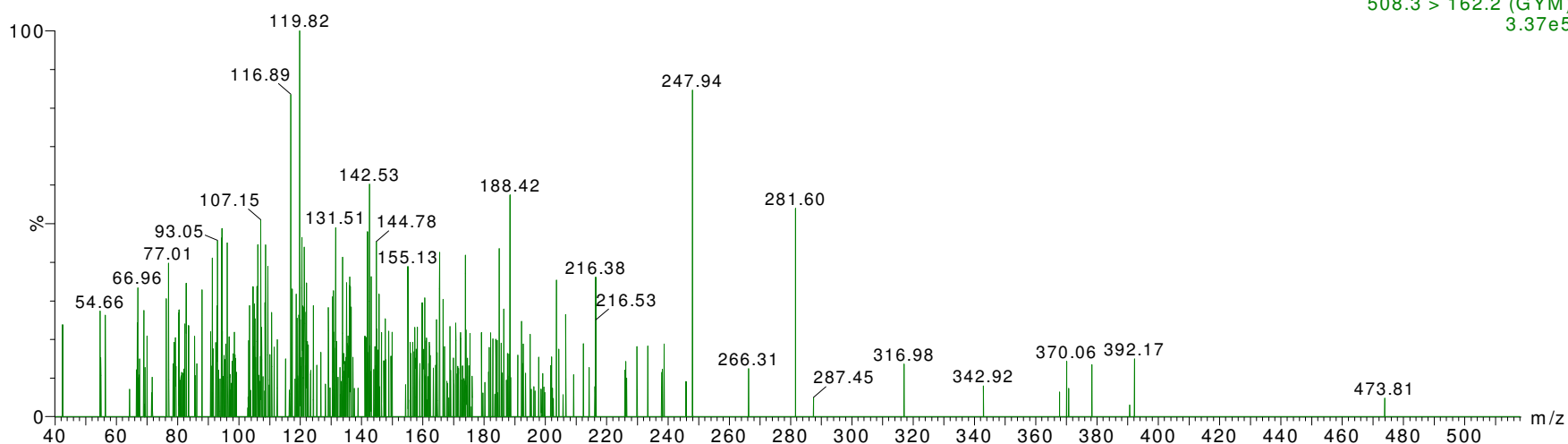
MS2 Full Scan - GYM

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5ppb_full
13OCT201631 1 (4.186)



SPK-B_full
13OCT201632 1 (4.186)

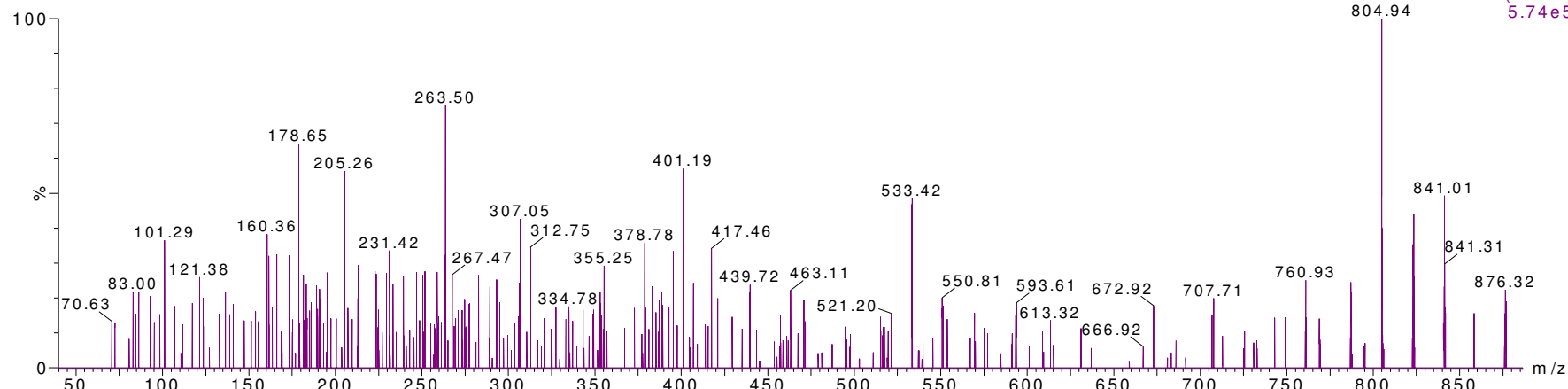


MS2 Full Scan – PTX2

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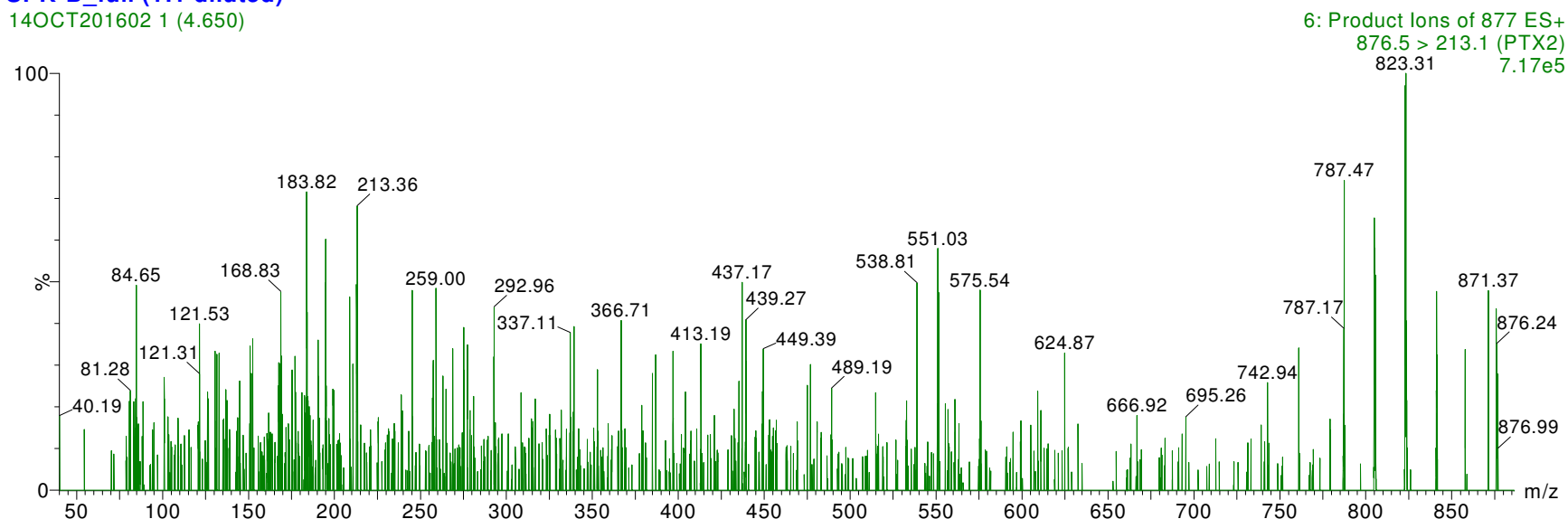
5ppb_full

13OCT201631 1 (4.655)



SPK-B_full (1:1 diluted)

14OCT201602 1 (4.650)



Conclusion

- A LC-MS/MS method under 8 minutes was used to analyse ppb levels of lipophilic marine biotoxins. MRM transitions of 18 biotoxins were obtained from literature. There was not a sufficiently high concentration of standards available for tuning.
- In the semi-blind demo analysis, 3 biotoxins GYM, PTX2 and AZA2 were detected in standard and spiked sample.
- The spiked sample was found to contain 20.5ppb of GYM, 19.6ppb of PTX2 and 20.0ppb of AZA2.
- In summary, the analysis has shown that the Xevo TQ-XS demonstrates good potential to provide high quality data in terms of linearity and sensitivity for detection and quantitation of marine biotoxins.