Cefas

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REDACTED
Fish Health Inspectorate
Cefas

Our ref: Testing of crab tissue samples for marine neurotoxins

Dear REDACTED,

This report provides a brief summary of the results obtained following the testing of 14 crab samples received from yourself last week. As discussed between us, we conducted analysis for a range of marine neurotoxins produced naturally at certain times of the year by certain species of marine phytoplankton, using chemical detection methods. There follows, an overall summary of the findings, along with a description of the methods utilised, with additional Annexes to the report providing further details, including chromatographic outputs, which you may find useful for your records.

For further information, the method used for detection of PSP has been fully validated for application to crabs, but no such validation has been performed for ASP. As such, these results are not reported to ISO17025 standard.

Table 1. Summary of samples received (all samples crab hepatopancreas, PM41921)

Sample number	Tissue weight for PST (g)	Tissue weight for ASP (g)
1	1.51	1.00
2	2.47	2.01
3	4.40	1.99
4	0.76	2.00
5	4.83	2.01
6	1.02	0.99
8	4.93	2.01
9	3.27	1.99
10	0.28	0.66
11	0.39	1.01
12	0.32	0.83
13	0.39	0.85
14	0.98	1.99
15	0.31	0.69

Table 2. Summary of methods utilised for testing:

Analytes	Method	Status
Paralytic shellfish toxins (PSTs)	Acetic acid extraction, reverse phase SPE clean-up, pre-column oxidation and LC-FLD analysis	Validated in shellfish and crab, but not accredited
	Acetic acid extraction, carbon de-salting SPE clean-up, dilution and LC-MS/MS analysis	Validated in shellfish and crab, but not accredited
Domoic acid (DA)	50% aqueous methanol (MeOH) extraction with LC-UV analysis	Developmental method, performance characteristics determined in crab, not accredited
	50% aqueous methanol (MeOH) extraction followed by LC-MS/MS analysis	Developmental method, performance characteristics determined in crab, not accredited

Results

Table 3 summarises the results obtained following each of the methods undertaken at the Cefas laboratory.

Table 3. Overall summary of results for each class of toxins

Analytes	Findings	Comments
PSTs[a]	No saxitoxin peaks detected in any of the samples analysed using either method	LODs range from 0.4 to 13 µg STX eq/kg per analogue for shellfish
DA – by LC-FLD	Without SPE, peaks observed, but following SPE clean-up no toxin peak in any of the samples	LOD estimated around 0.4 mg/kg
DA – by LC-MS/MS	Trace levels of domoic acid detected in some of the crab samples (maximum concentration ~ 150 µg/kg)	LOD estimated around 20 µg/kg

[a]STX, dcSTX, NEO, dcNEO, C1-4, GTX1-6, dcGTX1-4

Table 4. Overall summary of results obtained for each crab sample

Sample number	DA by LC-MS/MS (µg/kg)	DA by LC-UV (mg/kg)	PST by LC-FLD	PST by LC-MS/MS
1	155	-	-	-
2	58	-	-	-
3	-	-	-	-
4	121	-	-	-
5	-	-	-	-
6	109	-	-	-
8	-	-	-	-
9		-	-	-
10	64	-	-	-
11	63	-	-	-
12	-	-	-	-

13	-	-	-	-
14	79	-	-	-
15	-	-	-	-

- = not detected

Implications and interpretation

These results showed no evidence for the presence of detectable marine toxins in these environmental samples using official control methods. When using more sensitive mass spectrometric methods, low concentrations of domoic acid were quantified, but no saxitoxins were detected. It is, however, important to stress certain points:

1. These methods are targeted detection methods, i.e. they can only detect specific compounds which are incorporated into the method(s) and are available as certified reference standards. We are not able to conduct non-targeted screening assays – other organisations should be consulted if this approach is required.
2. As discussed previously, these methods are validated only in the matrix of bivalve mollusc shellfish (various species), and crab (for PSP toxins only). As such, we have no evidence for toxin recovery and method performance for the analysis of domoic acid in these samples. Consequently, there is the potential for under or over-estimating toxin concentrations, without any such validation of method performance characteristics.

Conclusions

Overall there were no clear indications of marine neurotoxins being present in the samples received at levels which would cause concern.

Work conducted

- All sample processing and analysis conducted by REDACTED (Principal Chemist, Food Safety Group, Cefas), REDACTED (Analytical Chemist) and REDACTED (Student analyst).
- Sample results reported by REDACTED

Report signed:

Name: REDACTED

Signature:

Cefas

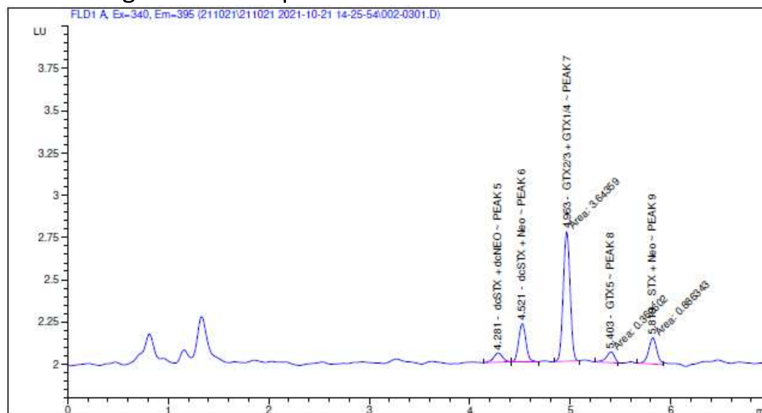
Annexes – specific details and chromatographic outputs from the analyses conducted

1. ***PSTs by LC-FLD***

Extraction was performed using 1% acetic acid as the extraction solvent. Variable weights of crab tissues were weighed and extracted with acid leading to an approximate solvent:sample ratio of 5:1. Sample/solvent mix was subjected to Ultra Turrax mixing at 2,500 rpm and vortex mixing followed by centrifugation (4,500 rpm, 10 mins). Supernatants were subjected to a reverse-phase clean-up using C18 solid phase extraction (SPE) cartridges, prior to pre-column oxidation LC-FLD.

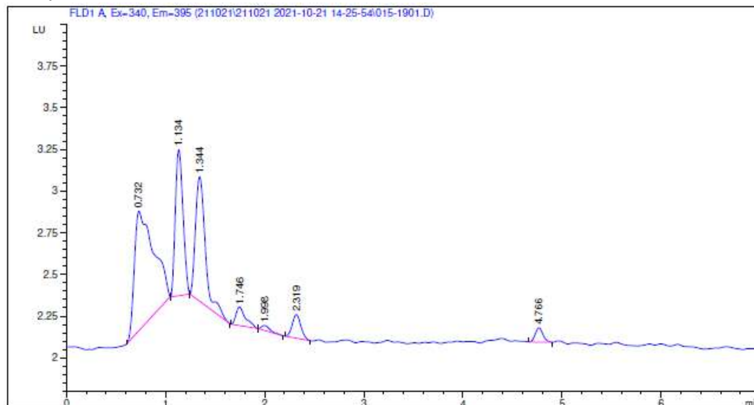
Figures here show the toxin analytes detected in calibration standards, followed by an example chromatogram showing the results obtained for toxins contained in calibrants.

Chromatogram of example PSTs in toxin standard

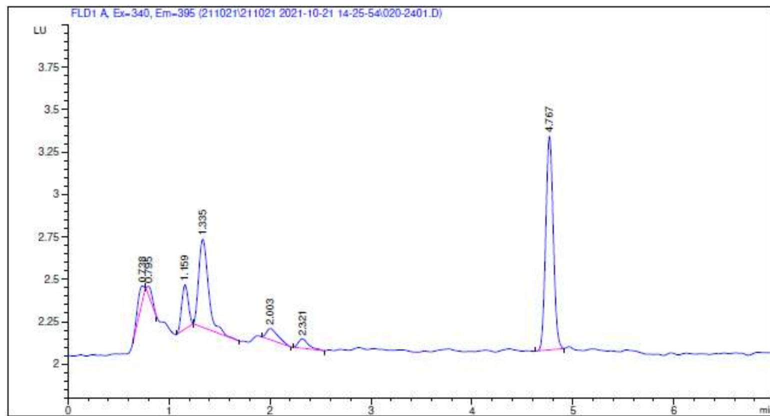


LC-FLD from example crab samples (some matrix peaks observed just before 5 mins, but these did not coincide with toxin peak elution times)

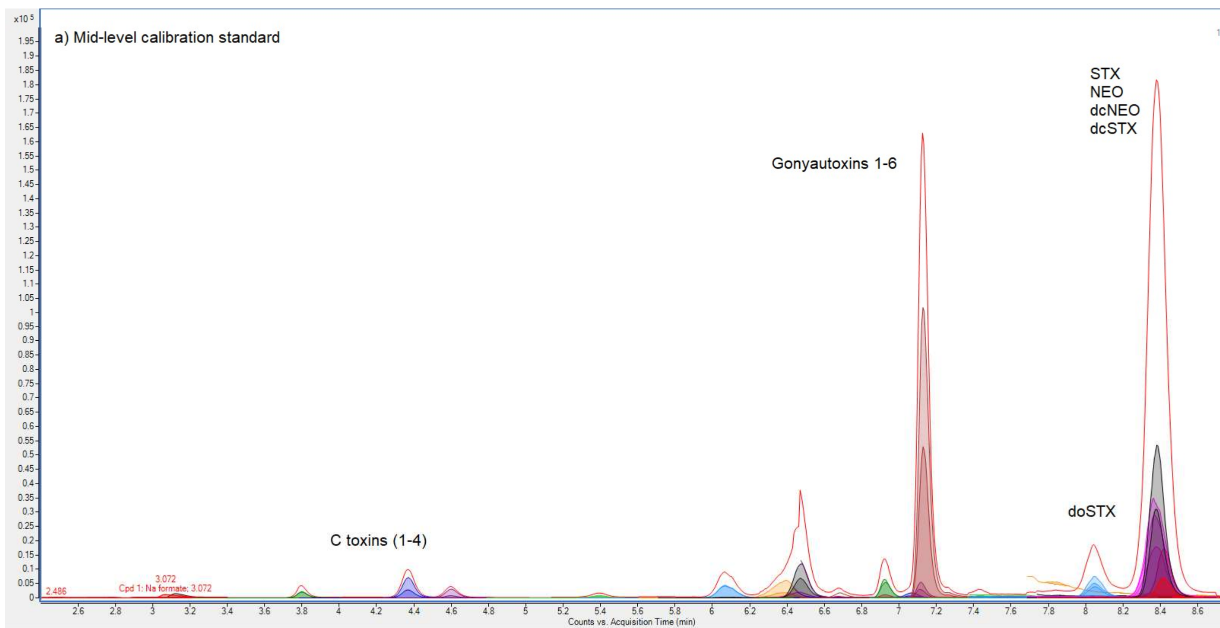
Sample 1

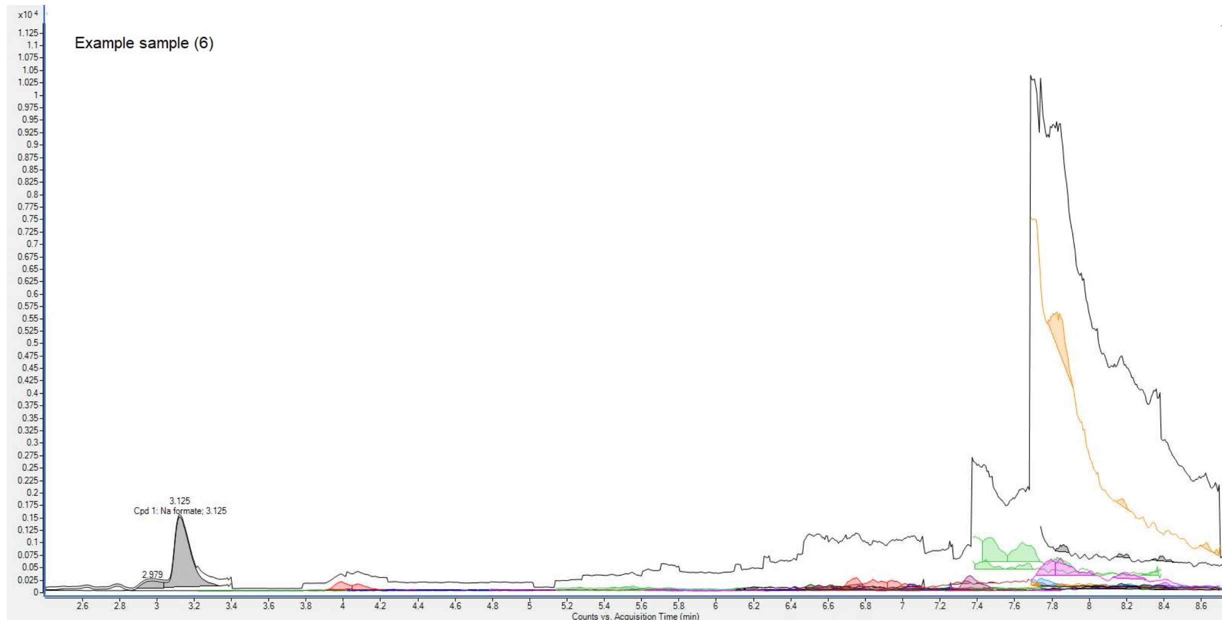


Sample 6



2. *PSTs (saxitoxins) by LC-MS/MS*

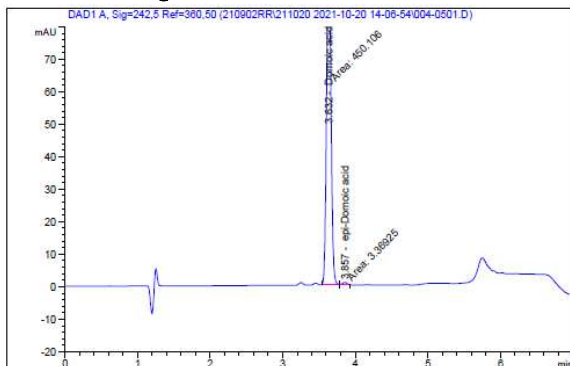




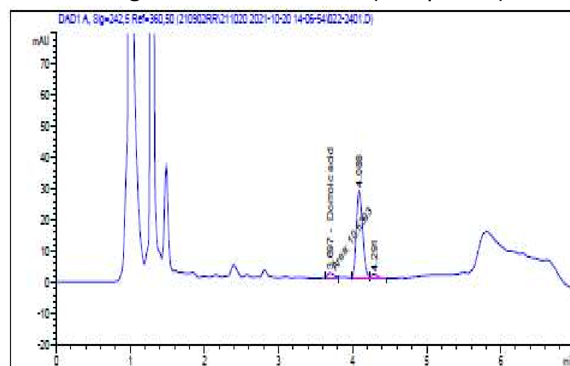
3. *DA by LC-UV*

Samples were extracted using 50% MeOH / 50% Water, using the approach described above. After extraction, centrifuged supernatants were filtered (0.2 µm) and subjected to both LC with UV detection (LC-UV) without SPE as conducted for routine monitoring of bivalves and with an additional SPE step, recently developed for the analysis of fish tissues. Example chromatograms below show the matrix peaks and "toxin peak" eluting at the same retention time as DA, but with the non-detection of DA confirmed in the SPE-cleaned extract.

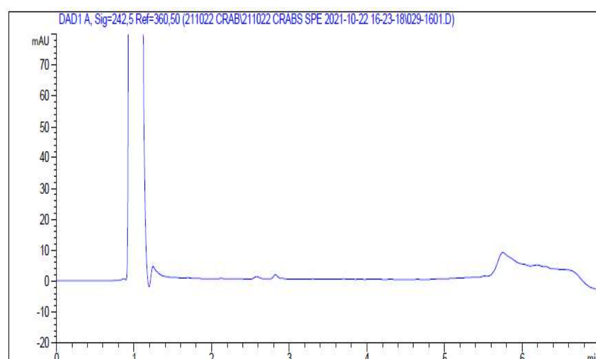
UV chromatogram of standard



Chromatogram of crab extract (sample 10)



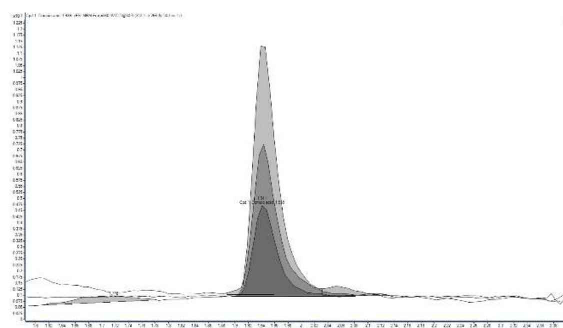
Chromatogram with additional SPE step



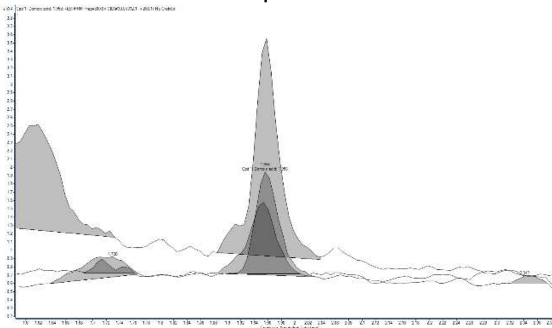
4. DA by LC-MS/MS

LC-MS/MS following an in-house research method. The figures here show firstly the three MRM transitions following LC-MS/MS analysis associated with DA acquisition, for a low-level calibration standard. The second set of traces shows the MRMs obtained in one example sample (sample 6), which shows the presence of small MRM peaks at the same retention time as the DA standard. The ratios between the relative peak areas for each of the 3 MRMs varied between the three transitions. As such, overall there is limited evidence for trace levels of domoic acid in some samples.

3 MRMs for DA in low level standard



3 MRMs for DA in sample 6



References

[1] Turner, A.D., Dhanji-Rapkova, M., Fong, S.Y.T., Hungerford, J., McNabb, P.S., Boundy, M.J. and Harwood, D.T. (2020). Ultrahigh-performance hydrophilic interaction liquid chromatography with tandem mass spectrometry method for the determination of paralytic shellfish toxins and tetrodotoxin in mussels, oysters, clams, cockles and scallops: collaborative study. *J. AOAC International.* 103, 1-35