

Summary of investigations conducted at Cefas into the effects of oyster matrix on HPLC and MBA PSP results

Maes o ddiddordeb ymchwil: [Chemical hazards in food and feed](#)

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Background

Previous validation work on the AOAC 2005.06 HPLC method highlighted significant differences in method performance between the High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) and Mouse Bioassay (MBA) official control methods for the quantitation of PSP toxins (PSTs) in oysters. Results showed that the total PSP toxicities determined using HPLC are on average 200% and 280% higher than the MBA toxicity results for Pacific oysters and native oysters respectively, as compared with a better correlation between the two methods for mussels and cockles (mean HPLC/MBA = 101% and 133% respectively).

Research Approach

This discrepancy in results could not be explained through any method performance issues associated with the HPLC analysis and so (further to some previously documented research findings) it was assumed that matrix components within the oysters may be influencing toxicity estimations. Given the importance of demonstrating the equivalence of the two methodologies prior to any potential implementation of HPLC methods into official control programmes, the potential causes of these findings were further investigated.

Results

Initial results showed no evidence for any artificial signal enhancement in the HPLC analysis of oysters, which may occur through the effects of co-extracted matrix components. Similarly, no effects were attributable to the variable toxin concentrations extracted using either acetic acid or hydrochloric acid extraction solvents. Additional MBA conducted at a separate international laboratory showed there were no issues relating to the reproducibility of the bioassay which may have affected the observed differences.

A period of comparative testing on a range of oyster and non-oyster samples involved the use of four different methodologies in comparison to the MBA. The AOAC 2005.06 pre-column oxidation HPLC method, a post-column oxidation HPLC method (now AOAC 2011.02), an LC-MS/MS method and an electrophysiological assay were all utilised. On average results between the four non-bioassays agreed well and all showed significantly higher total toxicity results than the MBA. As such, there was good evidence for the potential under-estimation of toxicities by MBA as

opposed to over-estimation of toxicity by HPLC.

Further work examined the potential presence of matrix components which may affect either of the assays. Whilst the overall salinity and nutritional content of the sample extracts showed no correlation with the observed effects, concentrations of some metals (notably zinc and manganese) were shown to be present at much higher levels in the oyster samples which exhibited the largest differences in toxicity results between the two assays. Further experimental work involving the removal of metals from oyster samples and the addition of zinc to mussel and cockle samples provided strong evidence for the suppressive effect of zinc and potentially other metals on the performance of the bioassay, whilst not affecting the performance of the HPLC.

Overall, the work has demonstrated the accuracy of the official reference MBA method to be compromised in samples containing high concentrations of metals, typically observed naturally in both UK species of oysters. As such the recommendation is to replace the oyster MBA test with the HPLC method in the UK official control monitoring programme.

Published Papers

1. Turner, A.D., Dhanji-Rapkova, M., Algoet, M., Suarez-Isla, B.A., Cordova, M., Caceres, C., Murphy, C.J., Casey, M. & Lees, D.N. (2012) Investigations in matrix components affecting the performance of the official bioassay reference method for quantitation of paralytic shellfish poisoning toxins in oysters. *Toxicon*, 59(2), 215-230 doi: 10.1016/j.toxicon.2011.11.013
2. Turner, A.D., Hatfield, R.G., Rapkova, M., Higman, W., Algoet, M., Suarez-Isla, B.A., Cordova, M., Caceres, C., van de Riet, J., Gibbs, R., Thomas, K., Quilliam, M. & Lees, D.N. (2011) Comparison of AOAC 2005.06 LC official method with other methodologies for the quantitation of paralytic shellfish poisoning toxins in UK shellfish species. *Anal. Bioanal. Chem.* 399, 1257-1270

Research report

England, Northern Ireland and Wales

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