Validation of HPLC method for scallops, clams and razors, and Pacific and Native oysters

Area of research interest: Chemical hazards in food and feed
Study duration: 2005-04-01
Planned completion: 1 November 2009
Project code: ZB1807
Conducted by: CEFAS

Background

The aim of the study was to test the validity of the HPLC method for detection of shellfish biotoxins in scallops, clams and razors, and pacific and Native oysters, and essentially this was a comparative study of the HPLC and MBA methods.

Research Approach

In the investigation of the AOAC HPLC method for scallops, HPLC results showed acceptable method selectivity and linearity in both scallop extracts. Method performance characteristics were acceptable for the non-N-hydroxylated toxins oxidised by peroxide prior to HPLC quantitation. This included good evidence for acceptable toxin recovery, precision, ruggedness and sensitivity and with no evidence of matrix-related signal suppression. However, with poor method performance for N-hydroxylated toxins, the HPLC method would not be safe to implement for scallops in its current form and further work to improve the method performance is recommended.

With regard to clams and razors, validation results showed that the HPLC method was selective and sensitive enough to detect and quantify the presence of each toxin peak in both hard clams and razors. The linearity of the method was shown to be good over a wide range of toxin concentrations and toxin recoveries were similar to those described previously for other species. The precision of the method for both razors and hard clams was shown to be statistically acceptable over the short, medium and long – term and comparable to values reported previously for other species. Ruggedness experiments showed that the method was robust for all parameters investigated. Method performance results obtained throughout the study were used to calculate levels of Measurement Uncertainty (MU) for the analysis of PSTs in hard clams and razors, with results being generally lower and more consistent than the range of uncertainties reported previously for mussels.

With regard to oysters and cockles, validation results showed that the analysis of PSTs in oysters and cockles was selective and sensitive enough to detect and quantify the presence of each toxin peak. The relationship between HPLC instrumental response and toxin concentration was shown to be linear over an appropriate working range and toxin recoveries were similar to those described previously for mussels. The precision of the method for oysters and cockles was shown to be acceptable over the short, medium and long - term. Variability in method performance during these assessments was similar to or improved from values reported previously for mussels and all found to be statistically acceptable. Ruggedness experiments showed that the method was robust for all parameters investigated. Method performance results obtained throughout the

study were used to calculate levels of Measurement Uncertainty (MU) for the analysis of PSTs in cockles and oysters, with results being generally lower and more consistent than the range of uncertainties reported previously for mussels.

Results

An important part of all the validation studies carried out included parallel comparative analysis of the HPLC & MBA methods. Comparative results generally showed good correlation between the two methods for razors, clams and cockles, as determined previously in mussels. However, a high positive HPLC bias as compared with the MBA was observed in the quantitative analysis for pacific and native oysters, which was found not to be solely related to the use of highest toxicity equivalent factors (TEFs) or artificial enhancement of the HPLC response. Further work is therefore recommended to identify the potential cause of these differences. Comparative results for scallops were variable, but insufficient sample numbers were available to calculate any statistical significance. Relative performance of the HPLC method was particularly poor in queen scallops, where there were known recovery and sensitivity problems.

Overall, the results presented show the refined AOAC 2005.06 HPLC method in cockles, oysters, razors and clams behaves similarly to the method applied to mussels, where it has already been implemented for use. However, with poor method performance for N-hydroxylated toxins in scallops, the HPLC method would not be safe to implement in its current form for this species and further work to improve the method performance is recommended.

Additional Info

FS235002A (ZB1807): Refinement and validation of the AOAC method (2005.06) to improve the determination of toxins in scallops

This refines and validates AOAC HPLC method 2005.06, making it suitable for use in the UK Official Biotoxins Monitoring Programme for the analysis of PSP toxins in whole scallops.

FS235002B (ZB1807): Method performance verification for the analysis of minor clam species for paralytic shellfish poisoning toxins

This extends the validation of AOAC HPLC method 2005.06 for use in the UK Official Biotoxins Monitoring Programme for the analysis of PSP toxins in minor clam species.

FS235002S (ZB1807): Summary of investigations conducted at Cefas into the effects of oyster matrix on HPLC and MBA PSP results

This confirms the validity and suitability of AOAC HPLC method 2005.06 for the analysis of PSP toxins in oysters, recommending it as a far more accurate replacement for the oyster MBA test in the UK Official Biotoxins Monitoring Programme.

ZB1803: Refinement and in-house validation of the AOAC HPLC method (2005.06): the determination of paralytic shellfish poisoning toxins by liquid chromatography and fluorescence detection, is a further related report

Published Papers

1. 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. Journal of

Analytical and Bioanalytical Chemistry 399(3),1257-1270
2. Turner, A.D. Hatfield R.G., Rapkova-Dhanji M., Norton D.M., Algoet M., Lees D.N. (2010) Single laboratory validation of a refined AOAC HPLC method (2005.06) for oysters, cockles and clams in UK shellfish. Journal of AOAC International, 93(5),1482-1493
3. Algoet, M., Luque-Perez, E., Rowland, S., Hatfield, R., Norton, D., Philo, M., Turner, A. & Lees, D. (2009) Application of the AOAC HPLC official method for the qualitative screening of PSP toxins in shellfish. Proceedings of the 6th International Conference on Molluscan Shellfish Safety (ICMSS 07), 141-46, 18–23 March 2007, Blenheim, New Zealand.
4. Higman, W.A., Algoet, M., Stubbs, B. & Lees, D. (2009) Overview of developments of the algal biotoxin monitoring programme in England, Scotland and Wales. Proceedings of the 6th International Conference on Molluscan Shellfish Safety (ICMSS 07), 41-45, 18–23 March 2007, Blenheim, New Zealand.
5. Turner, A.D., Norton, D.M., Hatfield, R.G., Morris, S., Reese, A.R., Algoet, M. & Lees, D.N. (2009) Single laboratory validation of the AOAC HPLC method (2005.06) for mussels: refinement and extension of the method to additional toxins. Journal of AOAC International, 92(1), 190-207.

Research reports

England, Northern Ireland and Wales

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