

# Analysis of shellfish samples for the amnesic shellfish poisoning toxin – domoic acid using high performance liquid chromatography and uv detection

## Introduction

Within the scope of the National Biotoxin Monitoring Programme for England and Wales, and under European Union legislation, Cefas is required to monitor the amnesic shellfish poisoning (ASP) toxin – domoic acid (DA) in harvested shellfish. The analytical method used at the Cefas, Weymouth laboratory and for the detection of DA has held UKAS Accreditation since July 2003 and all aspects of the method are carried out to this standard.

Domoic Acid has an action limit of 20µg[DA]/g (wet weight). This limit was originally set by the EC Shellfish Hygiene Directive 91/492/EEC, and has been amended by Regulation (EC) No 853/2004. The regulation does not permit any shellfish with concentrations that exceed this value onto the market. Where this level is measured in commercial shellfish, harvesting is stopped and further samples are requested and analysed. Once the DA level have declined to below the action limit, management decisions are made and the shellfishery is 'opened'.

Domoic acid is a natural product derived from several species of the diatom genus *Pseudo-nitzschia*. On consumption of mildly contaminated shellfish by humans, it can cause mild gastroenteritis whereas at the higher levels it can lead to neurotoxicological symptoms including dizziness, disorientation, weakness, permanent short term memory loss and in extreme cases, death.<sup>1</sup>

Harmful algal blooms (HABs) can occur in coastal waters where shellfish are commercially grown. Shellfish as well as other higher chain species feeding upon the nutrient rich environment can take up the toxin through filter feeding mechanisms and subsequently accumulate the toxin in their tissues. The concentration of toxicity in the flesh depends on the bioaccumulation of this toxin (Figure 1).

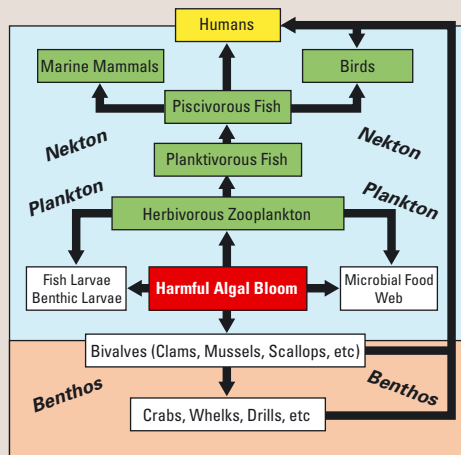


Figure 1: Overview of HABs transport and fate in the marine environment

## HPLC Method

The method used is based on the Quilliam et al method<sup>2</sup> using a 4:1 ratio of shellfish homogenate to 50% aqueous methanol with a strong anion exchange, solid phase extraction clean up. Determination of DA is performed by HPLC using UV detection (wave length 242nm). A typical chromatogram is shown in Figure 2. All DA concentrations are calculated against a set of calibration standards prepared from a certified reference standard of DA. Using a reporting limit of 1 µg[DA]/g, all sample results are reported to the Food Standards Agency on a weekly basis. These results are then used in decision making processes regarding the safety of the seafood product.

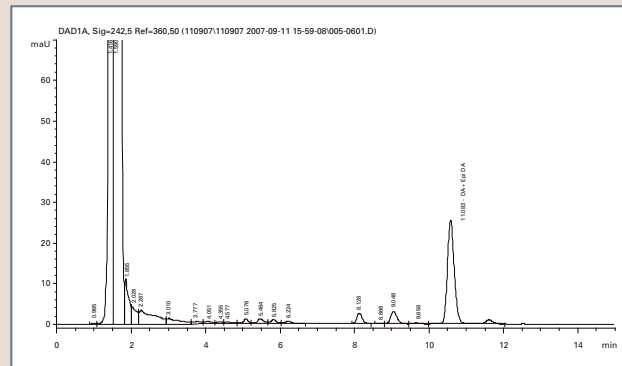


Figure 2: A HPLC UV chromatogram showing the elution of DA and its epimer (epi-DA)

## Quality Tools

A variety of quality assurance tools are used to assess the performance of the ASP method by HPLC. Using reference materials, we enable each sample to be quality checked and toxin amount to be calculated. The method uses both a certified reference material (CRM) and a laboratory reference material (LRM). The CRM is a mussel homogenate purchased externally and analysed on a monthly basis. On a batch-by-batch basis, we analyse a LRM prepared in house from naturally contaminated scallops. The DA concentration determined from the LRM and the monthly CRM are plotted on quality control charts to ensure that the results are within acceptable limits, the analytical process is in control and the results from the batch of samples can be reported.

Cefas has been participating in proficiency testing of the ASP method by interlaboratory comparison for the past three years. The QUASIMEME (Quality Assurance of Information in Marine Environmental Monitoring) performance studies are a quality assurance tool in which Cefas have produced excellent results. In the last round of studies, the matrix/determinands were assigned three satisfactory z-scores for the exercise.

Z-score key: |z| <2- Satisfactory performance

|z| <3- Questionable performance

|z| >3- Unsatisfactory performance

This provides us with assurance that the method used to quantify ASP samples is both accurate and within control.

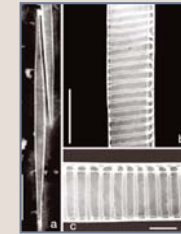
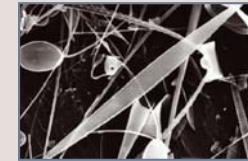


Figure 3: Scanning Electron Micrograph of a *Pseudo-nitzschia* diatom



Figure 4: *Pseudo-nitzschia* species

## Summary

The Biotoxin Monitoring Programme analyses approximately 1000 samples for DA each year, a number that is now on the increase. In 2006/07 there were 954 samples analysed for this toxin, of these, approximately 2% of the samples were reported as above the reporting limit or limit of detection (1 µg[DA]/g) however, none were reported over the action limit of 20µg[DA]/g of shellfish flesh. An overview of harvesting areas demonstrating DA-contamination is shown in Figure 5.



Figure 5: Distribution map of shellfish harvesting areas showing the presence of DA > limit of detection 1µg[DA]/g

## References

<sup>1</sup> Quilliam et al., (1995) *Journal of AOAC International* Vol.78, No.2

<sup>2</sup> Cefas *Marine Algal Toxins in Shellfish Information Leaflet*

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union* L 139, Section VII, Chapter V, pp 60-61.