



General

Domoic acid (DA) and DA isomers are water-soluble neurotoxins produced by a number of marine algae, in particular the genus Pseudo-nitzschia. Blooms of Pseudo-nitzschia spp. may lead to the accumulation of DA in shellfish filter feeders and other marine species [1,2]. Ingestion of DA contaminated shellfish may lead to amnesic shellfish poisoning (ASP) by affecting the central nervous system, and has caused the death of both animal and human consumers in severe cases [3]. The European Commission Directive 2002/226/EC has implemented a maximum permitted level (MPL) of 20 mg DA/kg shellfish flesh intended for human consumption, and this level has been adopted by the regulatory authorities in most other countries.

The ASP ELISA

Enzyme Linked Immunosorbent Assay (ELISA) has proved to be a sensitive and rapid method for detection of DA in the marine environment [4]. Originally developed by AgResearch (New Zealand) based on antibodies described by Garthwaite *et al.*, 1998 [5], the direct ASP ELISA is primarily intended for use in routine monitoring of DA levels in cultured bivalve molluscs to comply with the regulatory MPL, but is also applicable for quantification of DA in other matrices like algal samples, seawater and body fluids of marine mammals. The assay has been subject to an international collaborative validation study, and is officially approved by the AOAC[®] International as First Action Official MethodSM number 2006.02[6].

New, stripbased format offers flexibility

The ASP ELISA is offered in a 8x12-strip well format, to offer the end-user flexible analysis. The kit can now be used in 2 separate rounds to analyze 12 samples each time, or the full plate can be used to analyze 36 samples in one round of analysis. To ensure accurate and reliable sample analysis, a free software is provided for the automatic QA of the calibration and sample calculation.

Summary of ASP ELISA Performance parameters	
Assay running time	2.5 hours
Calibration range	10-260 pg/mL
Assay working range- shellfish	0.01 mg/kg up to at least 250 mg/kg
Limit of Detection	0.003 mg/kg shellfish
Limit of Quantification	0.011 mg/kg shellfish
Interlaboratory - Repeatability - RSDr	15±4%
Interlaboratory - Reproducibility - RSDR	23±6%
HORRAT	1.7±0.5
Correlation to expected CRM spike values 0.1-20 mg/kg (DA plus isomers)	R ² 0.992 Slope of regression 1.015
Interlaboratory Recovery	104±10%
Correlation to instrumental LC methods DA concentration range 0.1-20 mg/kg	R ² 0.984 Slope of regression 1.29



References

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- 2) Scholin C.A. *et al.* (2002) *Nature* 403, 80-84.
- 3) Wright J.I.C. *et al.* (1989) *Can. J. Chem* 67, 481-490.
- 4) Garthwaite I., Ross K.M., Miles C.O., Briggs L., Towers N., Borell T. & Busby P. (2001) *J. AOAC* 84, 1643-1648.
- 5) Garthwaite I., Ross K.M., Miles C.O., Hansen R.P., Foster D., Wilkins A.L. & Towers N. (1998) *Nat. Toxins* 6, 93-104.
- 6) Official Methods of Analysis of AOAC INTERNATIONAL (2006) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD USA, Official Method 2006.02.