THAILAND

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I. Introduction

The Department of Fisheries (DOF) has legal authority for classifying approved and harvesting areas of fishery include bivalves. DOF has imposed the Notification on Classification of bivalve harvesting areas under the authority of Fisheries Act.1947. The main objective is to ensure that fishery products exported from Thailand have been harvested from approved areas and further processed in a safe, clean and wholesome manner by approved establishments.

DOF laboratories perform the analyses required in Council Directives 91/493/EEC, 91/492/EEC and 79/923/EEC. Samples are taken to the laboratory for the DOF control of sanitary quality of fishery products and for the monitoring of marine biotoxins, trace elements and bacteriological contamination of bivalve mollusks. Since 1997, DOF has been submitting reports on bivalve production and sanitation program to the EU which covers the monitoring results of bivalve mollusks flesh from the approved harvesting areas. The reports on biotoxin contents including PSP (Paralytic Shellfish Poisoning), DSP (Diarrhetic Shellfish Poisoning), ASP (Amnesic Shellfish Poisoning) and Lipophilic toxins such as Yessotoxin (YTX), Pectenotoxin (PTX) and Azaspiracids (AZA).

These monitoring programs are for routine surveillance testing of bivalve mollusks, improving our knowledge and understanding on the levels of biotoxins occurrences in the ASEAN region, and enhancing our capabilities to prevent of marine biotoxins occurrence to ensure that fish & fishery products are safe for human consumption.

II. Objectives and Goals

1) To strengthen the laboratory capacity to detect and control outbreak of Biotoxins in Thailand and ASEAN region.
2) To strengthen the laboratory capacity for analysis of Marine Biotoxins in Bivalve mollusks.

III. Survey Methodologies

a. Sampling Method, Sampling Site, Target Species *(include scientific name)*, Number of Samples and Sampling Size

The samples are taken from the approved harvesting area in Chonburi province. Green mussel samples are taken from the top, middle and bottom regions. The total weight of flesh samples should be not less than 300 g at each sampling point.

b. Method of Analysis *(e.g. sample preparation method, analytical method used, quality control measures)*

Sample Preparation

Sample was collected from the approved harvesting area in Chonburi province. Clean the outside of the shellfish with fresh, running water, if necessary. Open by cutting the adductor muscles. Rinse inside, only if necessary (e.g. the excessive presence of sand), with fresh, running water. Remove the tissue from the shell with a suitable knife. Collect required weight of tissue (approx. 150g) and place on a sieve to drain. Transfer to suitable container for weighing (beaker). Transfer to blender and blend the tissue until homogeneous**.**

Determination method for ASP by HPLC

Reference method: In house method based on M.A. Quilliam. 2003. Chemical methods for domoic acid, the amnesic shellfish poisoning (ASP) toxic. In: G.M. Hallegraeff, D.M. Anderson & A.D. Cembella (Eds), Manual on Harmful Marine Microalgae, Monographs on Oceanographic Methodology, Vol. II. Chapter 9. Intergovernmental Oceanographic Commission (UNESCO), Paris, 247 – 266.

Sample extraction

Homogenized samples (approximately 4.0 g) were extracted with MeOH/H2O (1:1, 16 mL) at 10,000 rpm for 5 min. After 40 min of centrifugation at 4,000 rpm, supernatant (5 mL) was filtered through a 0.45 µm membrane filter. Then sample solutions were analyzed immediately by HPLC with PDA at WL 242 nm.

HPLC Condition:

- Column: Symmetry C18, 3.9x150 um
- Mobile phase: 10% Acetonitrile: 0.1% TFA
- Flow rate: 10 ml/min
- Column Temperature: 40 degrees Celsius
- 5 Injection volume: 20 µl

Quality Control:

1) Reagent Blank
2) Spiked sample
3) Duplicate sample

Determination method for AZA by LC/MS/MS

Reference method: D. Faulkner, S.A. Hewitt and J. Cooper. 2012. The determination of Lipophilic (DSP) shellfish toxins in shellfish species by LC-MS-MS. Veterinary Sciences Division. Agri-Food and Biosciences Institute (AFBI). Standard Operating Procedure CSD 379 Version 4.

Sample extraction:

Weigh individual aliquots (2.00 ± 0.05 g) of each sample into 50 ml screw capped plastic centrifuge tubes. Add 9 ml of methanol to each tube and vortex mix for 3 minutes**.** Centrifuge at 2800 rpm for 10 minutes and transfer the supernatants to individually labelled 20 ml volumetric flasks. Add a further 9 ml of methanol to the sample tube and homogenise for 1min**.** Combine with the previous supernatants. Make up to the 20 ml graduation with methanol. Filter and transfer to vials and now ready for LCMSMS analysis as un-hydrolysed.

For hydrolysed, pipette 1.0 ml of each extract to individually labelled vials. Then pipette 125µl of 2.5M NaOH into each vial. Vortex mix. Heat the mixture at 76°C ±3°C for 40 minutes in a water bath. Pipette 125µl of 2.5M HCl into each vial, re-cap and vortex mix for 10 seconds. Centrifuge the vials for 5 minutes at 5000 rpm. The hydrolysed extract is now ready for LC-MS-MS analysis.

HPLC Condition:

- Column: Waters X-Bridge C18 (3.5µm), 100 x 2.1mm
- Mobile phase: 10% Acetonitrile: 0.1% TFA
- Flow rate: 0.3 ml/min
- Column Temperature: 20 degree Celcius
- Injection volume: 10 µl

Quality Control:

1) Reagent Blank
2) Spiked sample
3) Duplicate sample

c. Limit of Detection and Limit of Quantification

ASP: LOD = 0.03 ug/g and LOQ = 0.25 ug/g.

AZA: LOD = 0.003 ug/g and LOQ = 0.01 ug/g.

d. National Regulatory Limits

ASP = 20 ug/g

AZA = 0.16 ug/g

IV. Results and Discussions

a. Participation in Inter-Laboratory Proficiency Testing and Results *(if any)*

1. Proficiency test for ASP by HPLC with QUASEMEME; The Netherland
   Results: Z score = - 0.40 to 0.60

2. Proficiency test for AZA by LC/MS/MS with QUASEMEME; The Netherland
   Results: Z score = 1.05 to 2.24

b. Survey Results and Discussion

Table for Survey Results

Sampling Location	Month & Year of Sampling (MM/YYYY)	Analyte Tested	No. of Samples Analysed	Min Concentration (ug/100g of meat)	Max Concentration (ug/100g of meat)	Average Concentration (ug/100g of meat)
At Chonburi province	October 2014 – December 2017	ASP (HPLC)	28 samples	0.00 ug/100g (Not detected)	0.00 ug/100g (Not detected)	0.00 ug/100g (Not detected)
		AZA (LC/MS/MS)	28 samples	0.00 ug/100g (Not detected)	0.00 ug/100g (Not detected)	0.00 ug/100g (Not detected)

Discussion Results

The results of this survey during October 2014 – December 2017 indicated that ASP and AZA are not detected in all bivalve mollusk samples. However, the region still needs to continue monitoring the level of Biotoxins to ensure that the bivalve mollusk products are free from contamination of Biotoxins.

c. Corrective Actions

No corrective action is deemed necessary for this survey.

V. Problems and Challenges Encountered

No problems occurred during survey.

VI. Recommendations and Suggestions for Future Follow-Up Action(s)

Shellfish Monitoring Programme which also includes the Biotoxins Survey, is a very important programme for ensuring the safety and quality of bivalve or shellfish for both local consumption and export purposes. The future follow up action for this project should be focused on updating and training on new analytical methods, especially quantitative method for all biotoxins, in order to improve the testing capabilities on quality and safety of fishery products in Southeast Asia.

In addition, it is important to set up a networking system of Harmful Algae Blooms (HAB) at this regional level to support the sharing of knowledge and exchanging information related to shellfish toxins.