Analysis of Paralytic Shellfish Toxins in Bivalve Mollusks using HPLC Method with Post-column Derivatization and Fluorescence Detection

The paralytic shellfish toxins are a group of 18 secondary metabolites deposited in bivalve mollusks by dinoflagelates. Dinoflagelate blooms are seasonal, occurring during warm months. Since it is unpredictable whether the infestation will occur, the shellfish population should be regularly monitored for toxins. Ingestion of contaminated shellfish can lead to paralytic shellfish poisoning: a life-threatening illness.

The Mouse Bioassay method used to detect dinoflagelate-derived neurotoxins has major drawbacks, which led to exploration of chromatographic methods of analysis. Recently, HPLC method that utilizes post-column oxidation of the toxins under alkaline conditions has been approved as a new official AOAC method – OMA 2011.02. Three groups of toxins are separated on a C_{18} column using a single step-gradient method. The products of post-column derivatization of the toxins can be detected with high sensitivity using a fluorescence detector, leading to the determination of toxin type and concentration. We describe the use of Pickering Laboratories' post-column derivatization system for analysis of paralytic shellfish toxins according to AOAC Method 2011.02.

Method

Sample Preparation

- Transfer 5 g of homogenized shellfish tissue into a 50 mL centrifuge tube and add 5 mL of 0.1 N HCl
- Vortex the mixture, adjust the pH to be in range of pH 2-4 as necessary
- Heat the mixture in the boiling water bath for 5 min, cool to room temperature, and recheck the pH, adjusting if necessary
- Centrifuge the mixture and transfer 500 uL of supernatant into a microcentrifuge tube
- Add 25 uL of 30% trichloroacetic acid (TCA) to deproteinate the extract, mix well and centrifuge
- Adjust pH with 1 M NaOH to optimum range of pH 2-4
- Filter through 0.2 um filter and inject

Analytical Conditions

Column: Zorbax Bonus RP column, 3.5 um, 4.6 x 150 mm (Agilent Technologies)

Flow Rate: 0.8 mL/min

Mobile Phase A: 11 mM heptane sulfonate, 5.5 mM phosphoric acid, adjusted to pH 7.1 with ammonium hydroxide

Mobile Phase B: 11 mM heptane sulfonate, 16.5 mM phosphoric acid, 11.5% acetonitrile, adjusted to pH 7.1 with ammonium hydroxide

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.0 mL

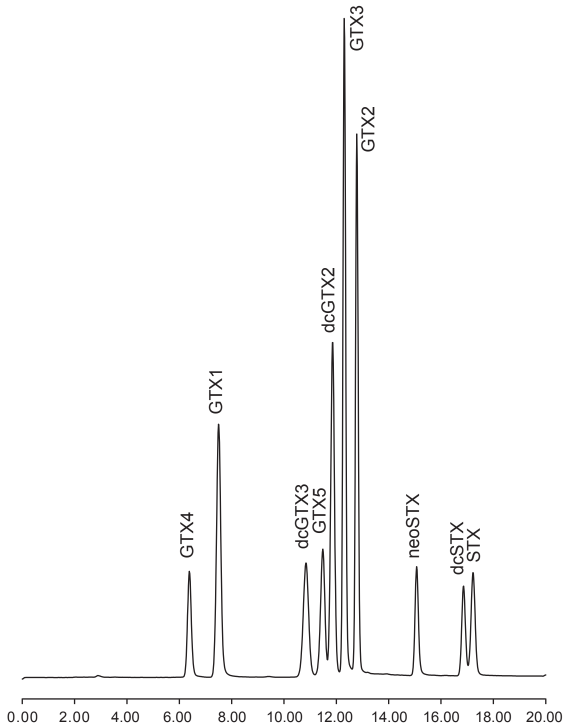
Reactor Temperature: 85 °C

Reagent 1: 100 mM phosphoric acid, 5 mM periodic acid, adjusted to pH 7.8 with 5 M sodium hydroxide

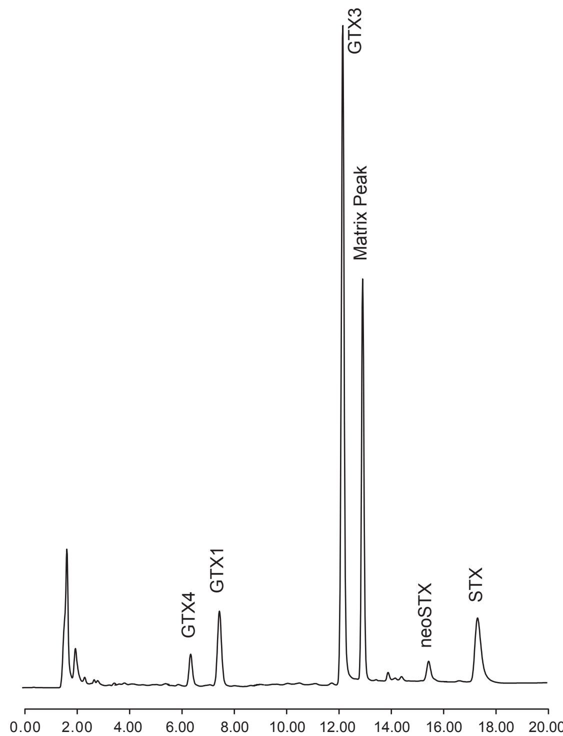
Reagent 2: 0.75 M nitric acid

Reagent Flow Rates: 0.4 mL/min

Detection: Fluorescence detector, λ_{ex} : 330 nm, λ_{em} : 390 nm



Chromatogram of GTX and STX mixed toxins standard



Chromatogram of mussels sample naturally contaminated with paralytic shellfish toxins

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References:

(1) van de Riet, J.M., Gibbs, R.S., Chou, F.W., Muggah, P.M., Rourke, W.A., Burns, G., Thomas, K, Quilliam, M.A. (2009) J. AOAC Int. 92, 1690-1704.

(2) AOAC Official Method 2011.02. Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops. Post-Column Oxidation (PCOX) Method.