PICKERING LABORATORIES

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 when 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 the exploration of chromatographic methods of analysis. An HPLC method that utilizes post-column oxidation of the toxins under acidic conditions has been approved as a new official AOAC method – OMA 2011.02. Two different columns and gradients are utilized to separate GTX/STX toxins and C-toxins. The derivatized analytes 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, reagents, and mobile phase for analysis of paralytic shellfish toxins according to AOAC Method 2011.02 in the below abstract.

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 for GTXs and STXs

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 (Cat No PSP-0001)

Mobile Phase B: 11 mM heptane sulfonate, 16.5 mM phosphoric acid, 11.5% acetonitrile, adjusted to pH 7.1 with ammonium hydroxide (Cat No PSP-0002)

Table 1. HPLC Gradient Program for GTXs and STXs

Time (Min)	%A	%B
0	100	0
7.9	100	0
8	0	100
18.5	0	100
18.6	100	0
24	100	0

Sample Injection Volume: 10 uL

Analytical Conditions for C-toxins

Column: Thermo BetaBasic 8, 5 um, 4.6 x 250 mm (Thermo Fisher Scientific)

Flow Rate: 0.8 mL/min

Mobile Phase A: 2 mM tetrabutylammonium phosphate, pH 5.8 (Cat. No PSP-C003)

Mobile Phase B: 2 mM tetrabutylammonium phosphate, pH 5.8 with 4% MeCN (Cat No PSP-C004)

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 (Cat No PSP-R1)

Reagent 2: 0.75 M nitric acid (Cat No PSP-R2)

Reagent Flow Rates: 0.4 mL/min

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

Table 2. HPLC Gradient Program for C-toxins

Time (Min)	%A	%B
0	100	0
8	100	0
15	0	100
16	0	100
19	100	0
24	100	0

Sample Injection Volume: 5 uL

Analytical conditions for GTX-6 toxin

GTX-6 toxin is less toxic than other STX and GTX toxins and is rarely detected in contaminated shellfish. However, some regulatory agencies require its identification and quantification. Under the standard AOAC 2011.02 conditions, GTX-6 coelutes with GTX-4. By using a modified first eluent (2.75 mM heptane sulfonate, 1.4 mM phosphoric acid, adjusted to pH 5.7 with ammonium hydroxide) under STX/GTX analytical conditions, GTX-6 can be effectively separated and accurately quantified when reporting is required.

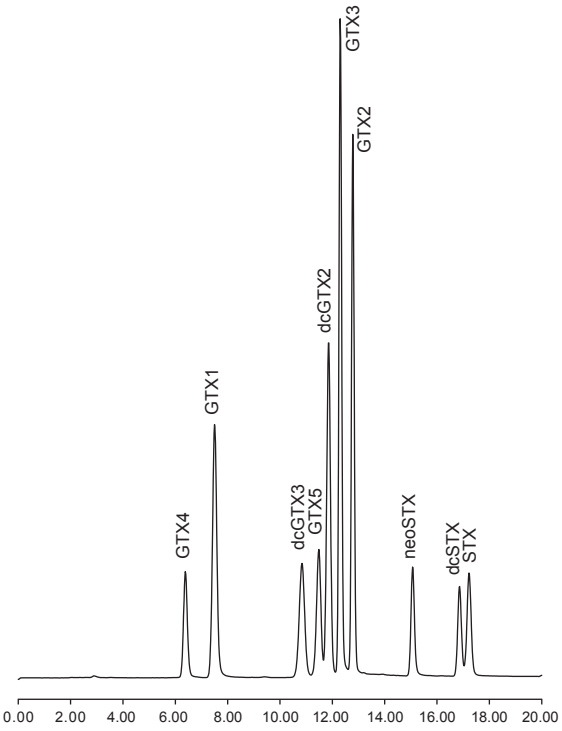

Fig 1. *Chromatogram of GTX and STX mixed toxins standard*

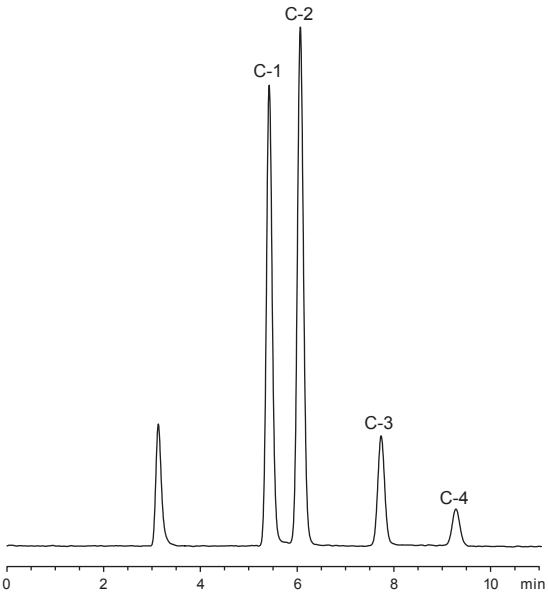

Fig 2. *Chromatogram of C toxins standard*

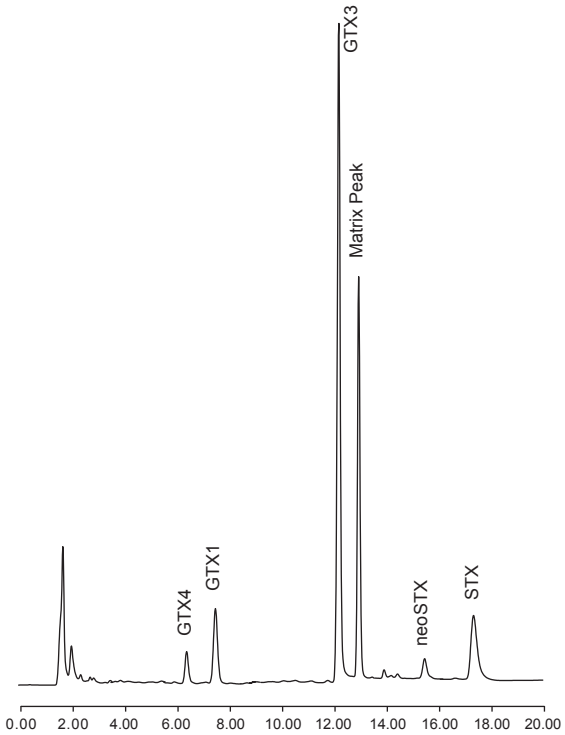

Fig 3. *Chromatogram of mussels sample naturally contaminated with paralytic shellfish toxins*

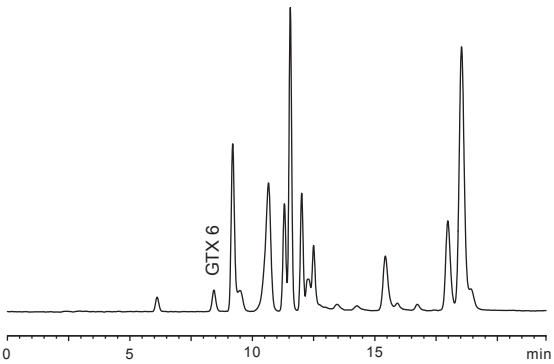

Fig 4. *Chromatogram of GTX and STX mixed toxins standard containing GTX-6 using modified Mobile Phase A*