

## ➔ PARALYTIC SHELLFISH TOXINS

### ANALYSIS OF DINOFLAGELATE DERIVED NEUROTOXINS IN BIVALVE MULLUSKS USING HPLC POSTCOLUMN FLUORESCENCE METHOD

The paralytic shellfish toxins are a group of 18 secondary metabolites deposited in bivalve mollusks by dinoflagelates. Dinoflagelates blooms are seasonal, occurring during warm months. Since it is unpredictable whether an 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.

Mouse bioassay is the official method of AOAC International, but the drawbacks associated with this method have led to exploration of chemical methods. The most common HPLC post-column method is to oxidize the separated toxins under alkaline conditions to a fluorescent compound. Sullivan et al.<sup>1</sup> used this method to determine the gonyautoxins 1-6 (GTX1-6), saxitoxin (STX) and neosaxitoxin (neoSTX) but not the N-sulfocarbamoyl-11-hydroxysulfate toxins (C1-C4). Oshima et al.<sup>2,3</sup> modified this method to determine the 3 toxin groups separately using isocratic elution with 3 different mobile phases. Further improvement by Jeffery van de Riet et al.<sup>4,5</sup> has led to a shorter analysis time to determine the 3 groups of toxins using step gradient and a switching valve. This method abstract describes the use of Pickering Laboratories Pinnacle PCX post-column derivatization system for the HPLC post-column determination of paralytic shell fish toxins.

#### METHOD

##### Equipment:

LC with a Binary Pump

Fluorescence Detector

Pickering Laboratories dual reagent

Pinnacle PCX post-column derivatization unit  
(1153-1061 – 120 V, 1153-1062 – 240 V)

Agilent Technologies Zorbax Bonus RP column,  
3.5 mm, 4.6 x 150 mm (863668-901)

##### Reagents:

Heptane Sulfonate

Phosphoric Acid

Ammonium Hydroxide

Acetonitrile

Periodic Acid

Sodium Hydroxide

Nitric Acid

##### LC Conditions

Sample Injection Volume: 10  $\mu$ L

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.

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

##### Post-Column Conditions

Column Temperature: 40  $^{\circ}$ C

Reactor Volume: 1.0 mL (knitted)

Reactor Temperature: 85  $^{\circ}$ C

Reagent Flow Rate: 0.4 mL/min

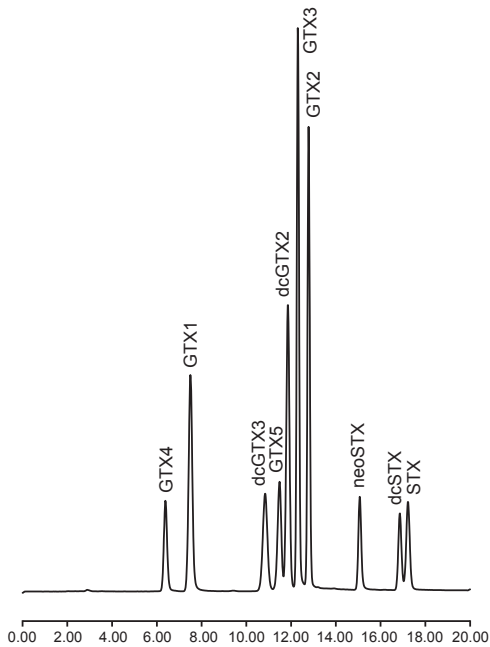
##### Reagents:

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

2. 0.75 M nitric acid

##### Detection: Fluorometer

$\lambda_{\text{ex}}$ : 330 nm,  $\lambda_{\text{em}}$ : 390 nm



*Chromatogram of GTX and STX mixed working standard*

REFERENCES:

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- 4) Wade A. Rourke, et. al  
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