

## PARALYTIC SHELLFISH TOXINS



The group of paralytic shellfish toxins (PST) consists of 18 substances, which are secondary metabolites stemming from algae and are mainly produced during the algal bloom ("Red Tide"). During this time, PST accumulate in shellfish.

Since it is unpredictable whether an infestation will occur, the shellfish population needs to be regularly tested for these toxins. The consumption of contaminated shellfish can cause paralytic shellfish poisoning, which is a life-threatening illness.

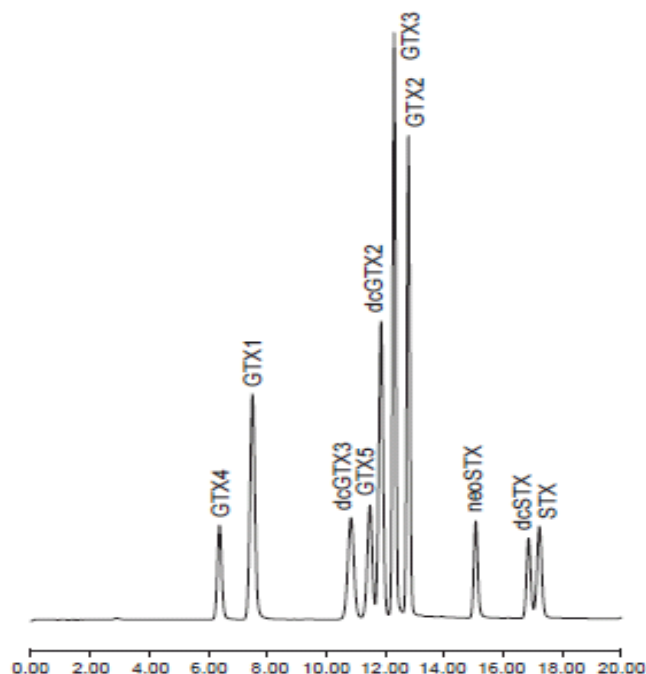
The Mouse Bioassay is the official method of AOAC International, but the drawbacks associated with this method have led to exploration of chemical methods. The most common HPLC post-column method is to oxidize the separated toxins under alkaline

conditions to a fluorescent compound. Sullivan et al. [1] 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. [2, 3] 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. [4, 5] 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.

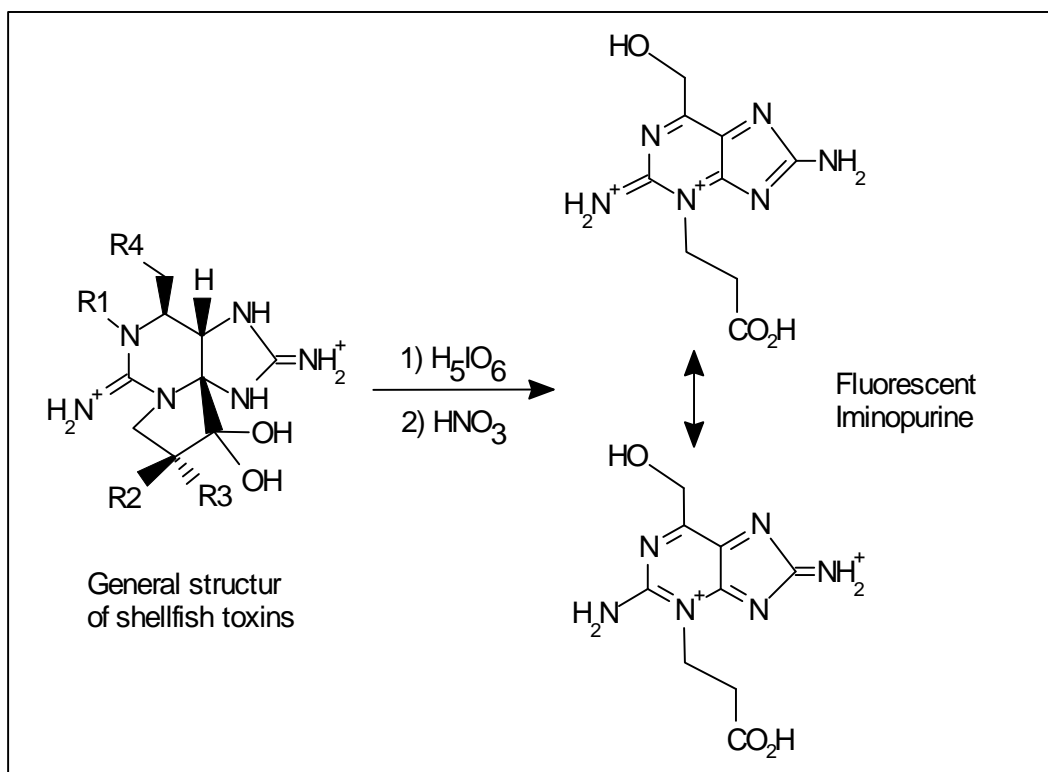
### Description of the Method

After separation on a reversed-phase column, the toxins are converted in a two-step derivatization to fluorescing imino purine derivatives. The first step consists of the oxidation with periodic acid in an alkaline environment and in the second step; the pH-value is adjusted with acid. Afterwards, the imino purine derivatives are measured with a fluorescence detector.

## Chromatogram



Chromatogram of GTX and STX mixed working standard



## APPLICATION NOTE

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Toxin	N-Sulfocarbamoyl	Decarbamoyl	R1	R2	R3
Saxitoxin	B1	dc-Saxitoxin	H	H	H
Neosaxitoxin	B2	dc-Neosaxitoxin	OH	H	H
Gonyautoxin I	C3	dc-Gonyautoxin I	OH	H	OSO <sub>3</sub> <sup>-</sup>
Gonyautoxin II	C1	dc-Gonyautoxin II	H	H	OSO <sub>3</sub> <sup>-</sup>
Gonyautoxin III	C2	dc-Gonyautoxin III	H	OSO <sub>3</sub> <sup>-</sup>	H
Gonyautoxin IV	C4	dc-Gonyautoxin IV	OH	OSO <sub>3</sub> <sup>-</sup>	H

PICKERING offers the complete post-column derivatization system PINNACLE PCX for the analyses of PST. The user, however, has to supply columns, eluents, reagents, etc..

## HPLC Conditions and Derivatization Parameters

<b>HPLC</b>	
Operating mode	Binary Gradient
Eluent	<p>A. 11 mM heptane sulfonate, 5.5 mM phosphoric acid, adjusted to pH 7.1 with ammonium hydroxide</p> <p>B. 11 mM heptane sulfonate, 16.5 mM phosphoric acid, 11.5 % acetonitrile, adjusted to pH 7.1 with ammonium hydroxide</p>
Degassing	Helium- or vacuum-degassing
HPLC column	Zorbax Bonus RP column, 3.5 $\mu$ m, 4.6 x 150 mm
Flow rate	0.8 mL/min
Sample injection volume	10 $\mu$ L
<b>Post-Column Derivatization</b>	
Pinnacle PCX	Dual-pump
Column oven	40 °C
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	0.4 mL/min each

# APPLICATION NOTE

Detection	
Detection mode	Fluorescence detection
Excitation wavelength	330 nm
Emissions wavelength	390 nm
Cell	Analytical; pressure stable up to 7 bar

## HPLC Gradient Program

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

## Literature

- 1) J.J.Sullivan et. al J.Food Sci. 50(1) (1985) 26-29.
- 2) Yasukatsu Oshima "Post-Column Derivatization Liquid Chromatographic Method for Paralytic Shellfish Toxins." Journal of AOAC International Vol.78, No.2, (1995) 528-532.
- 3) Yasukatsu Oshima, et. al in *Mycotoxins and Phytotoxins* 88, Elsevier Science Publishers, Amsterdam, The Netherlands, 319-326.
- 4) Wade A. Rourke, et. al "Rapid Post-Column Methodology for Determination of Paralytic Shellfish Toxins in Shellfish Tissue." Journal of AOAC International Vol.91, No.3, (2008) 589-597.
- 5) Jeffrey M. van de Riet, et. al "Liquid Chromatographic Post-Column Oxidation Method for Analysis of Paralytic Shellfish Toxins in Mussels, Clams, Scallops and Oysters: Single-Laboratory Validation." Journal of AOAC International Vol.92, No. 6, (2009) 1690-1704.

## Order Information

Order number	Description
1153-1062	PINNACLE PCX – Dual-pump; 1.0 mL reactor