

## PARALYTIC SHELLFISH TOXINS – OSHIMA METHOD



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 flower period ("Red Tide"). During this time, PST accumulate in shellfish.

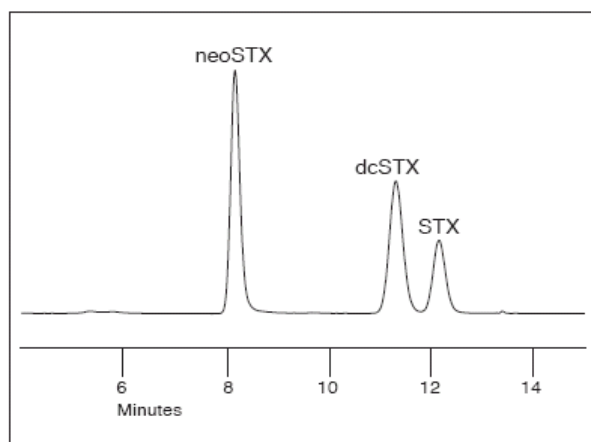
Since it is difficult to predict when infestation occurs, the shellfish population needs to be tested regularly for these toxins. The consumption of contaminated shellfish can cause paralytic shellfish poisoning, which is a life-threatening illness.

### Description of the Method

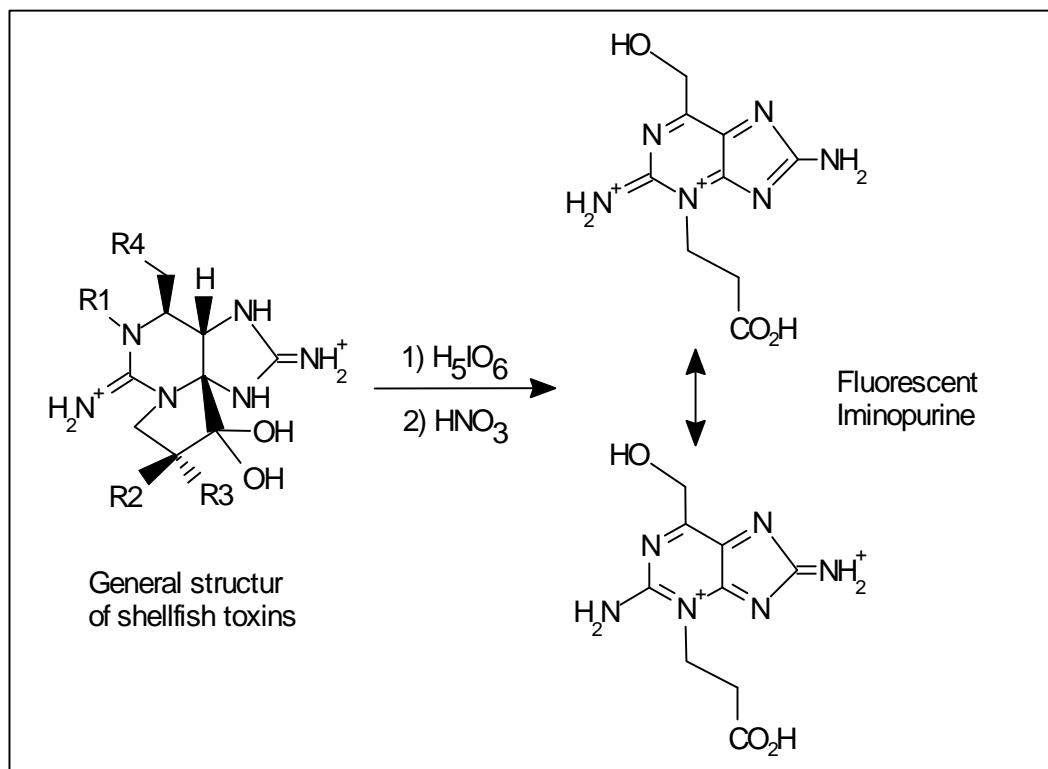
After separation on a reversed-phase column, the toxins are converted in a two-step derivatization to fluorescing imino purine derivatives. The first step consists of the oxidation with periodic acid in an alkaline environment and in the second step, the pH-value is adjusted with acid. Afterwards, the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

The PST react differently to the pH-value of the oxidation solution (pH 7 – 11). Optimal wavelengths for fluorescence measurements of the individual compounds vary also. Ideally, one should orientate oneself by comparison to the values stated in the literature.

### Chromatogram



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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	$OSO_3^-$
Gonyautoxin II	C1	dc-Gonyautoxin II	H	H	$OSO_3^-$
Gonyautoxin III	C2	dc-Gonyautoxin III	H	$OSO_3^-$	H
Gonyautoxin IV	C4	dc-Gonyautoxin IV	OH	$OSO_3^-$	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	Gradient
Eluent	A. For C1-C4 toxins: Tetrabutylammonium phosphate (1 mM) adjusted to pH 5.8 with acetic acid B. For GTX-1 to GTX-6, dcGTX2, and dcGTX3. Sodium 1-heptanesulfonate (2 mM) in 10 mM ammonium phosphate, pH 7.1 C. For STX, neoSTX, and dcSTX: Sodium 1-heptanesulfonate (2 mM) in 10 mM ammonium phosphate, pH 7.1: Acetonitrile (2:1)
Degassing	Helium- or vacuum-degassing
HPLC column	RP C8, 4 x 150 mm (Order No 0846150)
Guard column	18ECG001
Flow rate	0.8 mL/min
<b>Post-Column Derivatization</b>	
Pinnacle PCX	Dual-pump
Column oven	40 °C
Reactor volume	2.0 mL
Reactor temperature	75 °C
Reagent 1	Periodic acid (7 mM) in 50 nM potassium phosphate buffer; pH 9.0
Reagent 2	0.5 M acetic acid
Reagent flow	0.4 mL/min each

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

## Literature

- 1) PICKERING LABORATORIES, *Application Note 105*.
- 2) B. Luckas, *Chemie in unserer Zeit* **1995**, 2, 68 – 75.
- 3) G. Thielert, Dissertation, Universität Hohenheim, **1993**.
- 4) G. W. Langlois, R. E. Danielson, S. K. Perera, M. V. Pickering, *LC GC* **1991**, 9, 838-844.
- 5) J. J. Sullivan, M. M. Wekell, L. L. Kentala, *J. Food. Sci.* **1985**, 50, 26 – 29.
- 6) H. A. Bates, H. Rappoport, *J. Agric. Food. Chem.* **1978**, 26, 252 – 254.
- 7) Y. Oshima, K. Sugino, T. Yasumoto, "Latest Advanced in HPLC Analysis of Paralytic Shellfish Toxins in Bioactive Molecules" 10, (**1989**) 319-326.
- 8) Y. Oshima, *JOAC, INTL.* 78 (2) **1995**.

## Order Information

Order number	Description
1153-1062	PINNACLE PCX – Dual-pump; 2.0 mL reactor