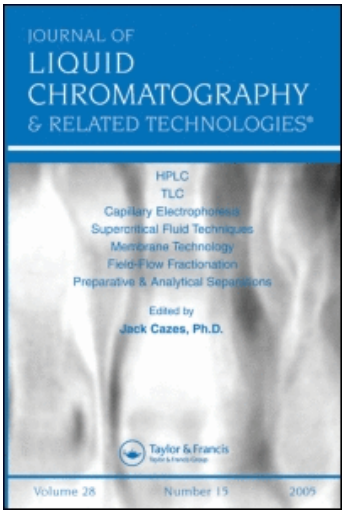

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Analysis of Paralytic Shellfish Toxin (Saxitoxin) in Mollusks by Capillary Zone Electrophoresis

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ANALYSIS OF PARALYTIC SHELLFISH TOXIN (SAXITOXIN) IN MOLLUSKS BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

Contamination of seafood by paralytic shellfish toxins is considered a public health hazard by the U.S. Food and Drug Administration. Human consumption of bivalves including clams, oysters, and mussels which have accumulated significant levels of Saxitoxins (STXs) is known as paralytic shellfish poisoning (PSP). The STXs are a group of structurally related purines which inhibit neurological transmission to nerves and muscles by reversibly binding to sodium channels. The major sources of saxitoxins are the dinoflagellates Alexandrium spp.,Pyrodium bahamense var. compressa, and Gymnodinium catenatum. The official method for the detection of the saxitoxins is a non-specific mouse bioassay. Recently, HPLC procedures have been reported to separate and quantify the saxitoxins. These methods, however involve extensive sample clean-up and derivatizations. A simple and selective analysis of a saxitoxin (STX) in mollusks by capillary zone electrophoresis (CZE) utilizing UV detection, was developed. Detection of saxitoxin was linear over a wide range of STX concentrations, ranging from 0.75ppm to 50ppm (correlation coeffiecient r=0.999), and the coefficient of variation (n=5) for migration and peak area response were less than 1% and 3%, respectively.

INTRODUCTION

Paralytic shellfish poisoning (PSP) is caused by the consumption of mollusks including clams, oysters, and mussels, which are contaminated with neurotoxins known as the saxitoxins.

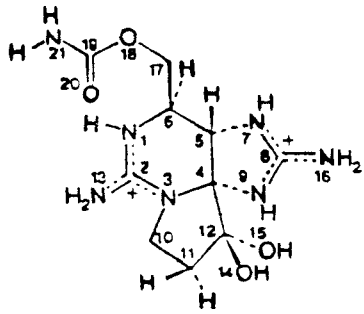

Figure 1: Saxitoxin

PSP generally develops within hours after consuming a contaminated product, resulting in a tingling or burning sensation in the lips, and in severe cases results in paralysis of extremities, loss of motor coordination, and possibly death due to respiratory paralysis. Saxitoxin,the parent molecule, and its derivatives, are a group of water-soluble purines which inhibit neurological transmission to nerves and muscles by reversibly binding to sodium channels (3). The derivatives are formed by 21-sulfo, 11-hydroxysulfate, and N-1-hydroxyl substitutions (see figure 1). Three morphologically different genera of dinoflagellates are regarded as the major sources of the saxitoxins. These include the Alexandrium spp (2), Pyrodinium bahamense var. compressa, and Gymnodinium catenatum (10). Other sources of the saxitoxins include a species of blue-green algae and possibly endosymbiont bacteria (4). Molluscan filter-feeders such as clams, accumulate the saxitoxins from the toxic dinoflagellates, and are relatively insensitive to toxin accumulation.

The mouse bioassay is currently the official method for the detection of the saxitoxins. However, this method cannot separate and quantify related saxitoxins and is subject to variability (7). Recently, high performance liquid chromatographic procedures have reported the isolation and quantification of the saxitoxins,however,

these methods involve extensive sample clean-up and derivatizations (5, 6, 8, 9). Capillary electrophoresis (CE) of marine toxins was previously reported as being useful. Wright et al (11) demonstrated CE with laser induced fluorescence detection, reporting attomole detection limits. Thibault et al (12) used CE with UV detection interfaced with Mass Spectrometry, identifying paralytic shellfish toxins in dinoflagellate and scallop matrices. In this paper we report thedevelopment of a simple and selective method for saxitoxin in fortified mollusk extracts including clam, oyster, and scallop. The utilization of a different buffer system, UV wavelength, and biological matrices resulting in high resolution, sensivity, and general applicability is presented.

EXPERIMENTAL

EQUIPMENT

(A) High performance capillary electrophoresis unit.- Model 270A-HT , UV-vis variable detector (Applied Biosystems, Inc., Foster City, CA 94404).

(B) Column.- Fused silica capillary tube, 50 micrometers id. x 72 cm in length (Applied Biosystems, Inc.).

(C) Data Acquistion - Macintosh IIci (Apple Computer Inc., Cupertino, CA. 95014)
Model 600, ABI Software, Laser Jet III (Hewlett Packard Boise, Idaho 83707)

Reagents and materials

(A) Solvents.- Methanol, distilled in glass

(B) Deionized water.- Milli-Q grade (Millipore Corporation, Marlborough, MA 01752)

(C) Sodium Borate ACS buffer.- 0.06M, pH 9.2

(D) Saxitoxin standard (100 micrograms/ml).- from Dr. Sherwood Hall, Division of Research, Office of Seafood, 200 C St., Washington, DC 20204

Capillary electrophoresis conditions

Voltage: 15,000V

UV detector: 208nm

Temperature: 30 degrees celsius

Vacuum injection time: 10 sec

Rise: 0.1 sec

Range: 0.05

Buffer: 0.06M sodium borate

Capillary distance to detector: 50 cm

Sample Extraction

Blend 10.0 grams of clam, oyster, or scallop composite in a micro-cup blender with approximately 30 ml of 80% methanol at low speeds for one minute, then at higher speeds for 2 minutes. Transfer the contents of the blender into a 100 ml volumetric flask, using additional 80% methanol to retrieve residual sample remaining in the micro-cup blender. Incubate flask in a 60 degree celsius water bath for 20 minutes, with intermittent shaking. Cool flask to ambient temperature and dilute to volume with 80% methanol. Filter solution through Whatman filter paper, then pass filtrate through a Gelman 0.2 micron acrodisc.

Assay

Oyster, clam, and scallop extracts were spiked with saxitoxin standard (100ug/ml) at 3 concentrations of 10, 100, and 500ug STX/g. Spiked extracts were then analyzed by capillary electrophoresis. Calibration curve prepared by diluting 100 ppm STX stock quantitatively, ranging from 0.75 - 50ppm with 80% methanol.

RESULTS AND DISCUSSION

Separation of spiked STX in extracts of raw clam, oyster, and scallops, exhibited no significant interference as demonstrated by

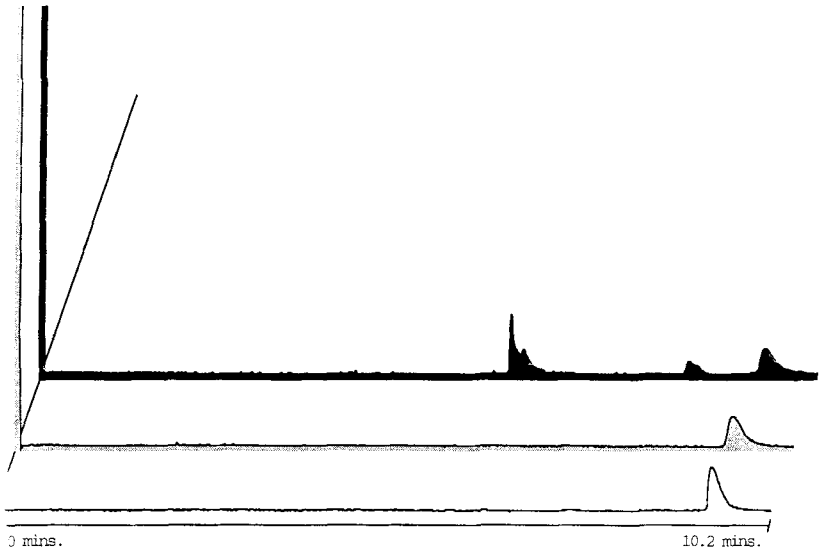

Figure 2: Electropherogram of non-spiked mollusk extracts A. Clam B. Scallop C. Oyster, Conditions: 60mM sodium borate, 15kV, 208nM, 10 sec inj., 30 C

electropherograms in figures 2 and 3 respectively. At an alkaline pH, the electroendosmotic flow (EOF) in the uncoated fused silica capillary is toward the cathode and the order of migration of analytes are as follows: cations, neutrals, and anions. Saxitoxin, at an alkaline pH, migrates faster than most amino acids and other components in these crude extracts. Electropherograms of spiked crude clam extracts demonstrated a comigration of STX and matrix components when using an initial running buffer of 20mM sodium borate. By increasing the concentration of sodium borate, which effectively decreases the EOF, we were able to obtain satisfactory separation in the spiked clam extract. It was determined that a 60mM sodium borate concentration resulted in optimal separation. Decreasing the voltage from 30KV to 15KV further enhanced the resolution of STX. Detection of saxitoxin by UV was at a wavelength

Figure 3: Electropherogram 0f STX spiked (25ppm) extracts A. Clam B. Scallop C. Oyster, Conditions: See figure 1.

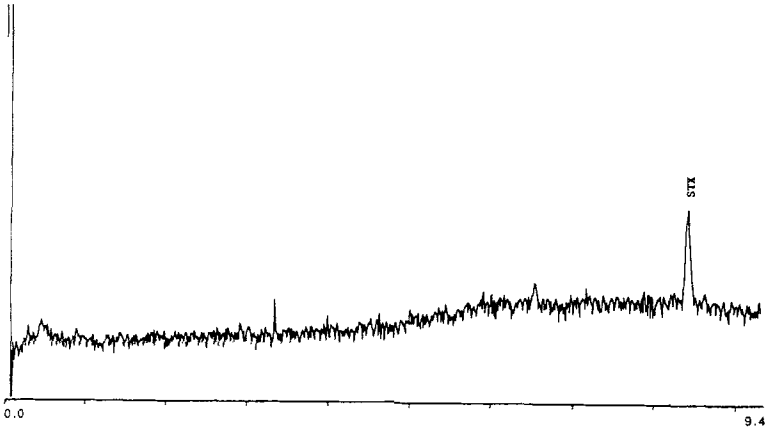

Figure 4: Typical electropherogram of a fortified scallop extract (10ug/g STX) Conditions: See figure 1.

Table 1. Recovery of Saxitoxin from Fortified Molluscan Extracts.

Type	Spiking Level micrograms STX/gram	% Recovery
Clam	10	108
	100	81
	500	97
Scallop	10	105
	100	102
	500	96
Oyster	10	88
	100	106
	500	101

of 208 nm. This wavelength provided the highest response signal and lowest baseline noise. Peak area response was linearly related to a concentration range of 0.75 ppm to 50ppm STX (correlation coefficient, r=0.999). The coefficients of variation of migration and peak area response (n=5) were less than 1% and 3% respectively. Migration time of STX in the clam oyster, and scallop extracts were similar (figure 3). The % recoveries of fortified mollusk extracts were satisfactory (table 1). The average % recoveries for fortified mollusks extracts at 10, 100, and 500 STX ug/g were 100.3, 96.3, and 98, respectively. At the minimal sample clean-up, shorter analytical times, greater reolving power, automation, and small sample size are advantageous characteristics afforded by capillary electrophoresis (CE). CE may not only be useful as a rapid alternative method for multi-screening of PSP extracts, but current investigations also indicate possible applications to other types of marine toxins including the brevetoxins and okadaic acid.

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LITERATURE CITED

1. Anderson, D.M., Sullivan, J.J., and Reguera, B. (1989). Paralytic Shellfish Poisoning in Northwest Spain. The Toxicity of the Dinoflagellate Gymnodinium catenatum. Toxicon 27: 665-674.

2. Bates, H.A., Kostriken, R., and Rapoport,A. (1978). The Occurrence of Saxitoxin and other Toxins in Various Dinoflagellates. Toxicon 16:595-601.

3. Catterall, W.A. (1985). The Voltage Sensitive Sodium Channel: A Receptor for Multiple Neurotoxins. In Toxic Dinoflagellates, D.M. Anderson, A.W. White, and D.G. Baden (Eds.). Elsevier, New York, pp 329-342.

4. Kodama, N., (1990). Possible Links Between Bacteria and Toxin Production in Algal Blooms. In Toxic Marine Phytoplankton, E. Granieli, B. Sundstom, L. Edler, and D.M. Anderson, (Eds), pp.52-61.

5. Lawrence J.F., and Menard, C., (1991). Liquid Chromatographic Determination of Paralytic Shellfish Poisons in Shellfish after Prechromatographic Oxidation. J. Assoc. Off. Anal. Chem. 74:

6. Oshima, Y., Sugino, K., and Yasumoto, T. (1989). Latest Advances in HPLC Analysis of Paralytic Shellfish Toxins. In Mycotoxins and Phycotoxins 88, S. Natori, K. hashimoto, and Y. Yeno (Eds). Elsevier, Amsterdam, pp. 319-336.

7. Park, P.L., Adams, W.N., Graham,S.C., and Jackson, R.C. (1986). Variability of Mouse Bioassay for Determination of Paralytic Shellfish Poisoning Toxins. J. Assoc. Offic. Anal. Chem. 69:547-550.

8. Sullivan, J.J., Wekell,M.M., Kentala,L.L. (1985). Application of HPLC for the Determination of PSP Toxins in Shellfish. J. Food Sci. 50:26-29

9. Sullivan, J.J., and Wekell,M.M. (1987). The Application of High Performance Liquid Chromatography in a Paralytic Shellfish Poisoning Monitoring Program. In Seafood Quality Determination. D.E. Kramer and J. Liston (Eds). pp.357-371.

10. Taylor, F.J.R., (1985). The Taxonomy and Relationships of Red Tide Flagellates. In Toxic Dinoflagellates. D.M. Anderson, A.W. White, and E.G. Baden (Eds). Elsevier Science Publishing, New York,N.Y. pp.11-26.

11. Thibault, P., Pleasance, S., and Laycock, M.V. (1991) Analysis of Paralytic Shellfish Poisons by Capillary Electrophoresis J. Chromatogr., 542:483 - 501.

12. Wright, B.W., Ross, G.A., and Smith, R.D. (1989). Capillary Zone Electrophoresis with Lazer Fluorescences Detection of Marine Toxins. J. Microcolumn Sep. 1(2): 85 - 89.