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Separation of paralytic shellfish poisoning toxins on Chromarods-SIII by thin-layer chromatography with the Iatroscan (mark 5) and flame thermionic detection

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Abstract

Thin-layer chromatography (TLC) on Chromarods-SIII with the Iatroscan (Mark-5) and a flame thermionic detector (FTID) was used to develop a rapid method for the detection of paralytic shellfish poisoning (PSP) toxins. The effect of variation in hydrogen (H_2) flow, air flow, scan time and detector current on the FTID peak response for both phosphatidylcholine (PC) and PSP were studied in order to define optimum detection conditions. A combination of hydrogen and air flow-rates of 50 ml/min and 1.5-2.0 l/min respectively, along with a scan time of 40 s/rod and detector current of 3.0 A (ampere) or above were found to yield the best results for the detection of PSP compounds. Increasing the detector current level to as high as 3.3 A gave about 130 times more FTID response than did flame ionization detection (FID), for PSP components. Quantities of standards as small as 1 ng neosaxitoxin (NEO), 5 ng saxitoxin (STX), 5 ng B1- toxins (B1), 2 ng gonyautoxin (GTX) 2/3, 6 ng GTX 1/4 and 6 ng C-toxins (C1/C2) could be detected with the FTID. The method detection limits for toxic shellfish tissues using the FTID were 0.4, 2.1, 0.8 and 2.5 μg per g tissue for GTX 2/3, STX, NEO and C toxins, respectively. The FTID response increased with increasing detector current and with increasing the scan time. Increasing hydrogen and air flow-rates resulted in decreasing sensitivity within defined limits. Numerous solvent systems were tested, and, solvent consisting of chloroform: methanol–water–acetic acid (30:50:8:2) could separate C toxins from GTX, which eluted ahead of NEO and STX. Accordingly, TLC/FTID with the Iatroscan (Mark-5) seems to be a promising, relatively inexpensive and rapid method of screening plant and animal tissues for PSP toxins.

Keywords: Chromarod; Flame thermionic detection; Detection, TLC; Toxins; Paralytic shellfish poisoning toxins; Neosaxitoxin; Saxitoxin; Gonyautoxin

1. Introduction

Paralytic shellfish poisoning (PSP) is a neurological illness resulting from the consumption of shellfish contaminated with saxitoxin and/or its derivatives (Fig. 1) which are formed intracellularly from marine dinoflagellate algal cells such as *Alexandrium* (formerly *Gonyaulax* or *Protogonyaulax*) *tamarensis, A. catanella* and *Gymnodinium catenatum* or from fresh water cyanobacterium *Aphanizomenone*

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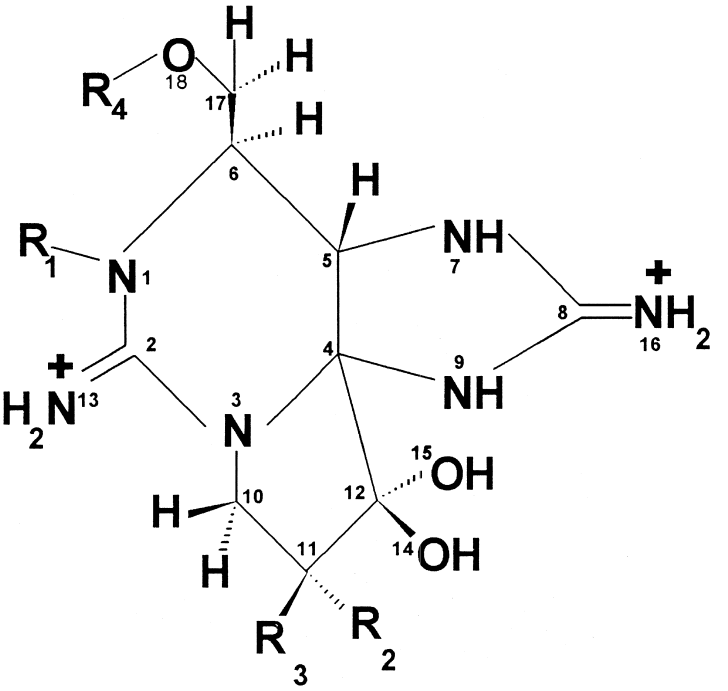

	Molecular weight	R_1	R_2	R_3	R_4
STX	301.31	H	H	H	$CONH_2$
B1 (GTX5)	380.36	H	H	H	$CONHSO_3^-$
GTX 2	396.36	H	OSO_3^-	H	$CONH_2$
C1	475.41	H	OSO_3^-	H	$CONHSO_3^-$
GTX 3	396.36	H	H	OSO_3^-	$CONH_2$
C2	475.41	H	H	OSO_3^-	$CONHSO_3^-$
NEO	317.31	OH	H	H	$CONH_2$
B2 (GTX6)	396.36	OH	H	H	$CONHSO_3^-$
GTX 1	412.36	OH	OSO_3^-	H	$CONH_2$
C3	491.41	OH	OSO_3^-	H	$CONHSO_3^-$
GTX 4	412.36	OH	H	OSO_3^-	$CONH_2$
C4	491.41	O	H	OSO_3^-	$CONHSO_3^-$
dcSTX	258.28	H	H	H	H
dcGTX 2	353.33	H	OSO_3^-	H	H
dcGTX 3	353.33	H	H	OSO_3^-	H
dcNEO	274.28	OH	H	H	H
dcGTX 1	369.33	OH	OSO_3^-	H	H
dcGTX 4	369.33	OH	H	OSO_3^-	H

Fig. 1. Structure of Paralytic Shellfish Poisoning (PSP) toxins.

flos-aquae [1]. Toxic dinoflagellates and their toxins can accumulate in filter feeding shellfish such as oysters, mussels, clams, cockles and scallops resulting in illness or death to birds, fish, marine mammals and humans. Records of human illness from consumption of shellfish dates back to the 1600s [2] and PSP has been reported to be a public health problem since 1793 [3]. There are no known antidotes to the toxins and chemical detection is difficult.

Several detection methods including chemical, biochemical and bioassays have been developed since the toxins were first diagnosed as a primary cause of shellfish poisoning. The mouse bioassay [4] that established the basis for the standardized assay has been used world-wide for the detection of PSP in shellfish. Although this method provides adequate sensitivity for the detection of total PSP toxin in shellfish extracts [5], its narrow dynamic range, inherent variability and the social pressure to ban animal bioassays are presently increasing the demand for alternative methods for analysis.

Several spectrophotometric methods involving various colour reactions of the PSP toxins have been reported [6,7] and fluorometric oxidation methods [8–15] have also been developed for the detection of PSP in shellfish tissues. These tests are nonspecific in nature and suffer from inadequacies in terms of sensitivity and specificity.

HPLC was used to separate saxitoxin from its other reaction products [16], and this technique was further improved by post-column derivatization [17] and by pre-chromatographic oxidation [18–21] for the separation of wide array of PSP toxins. Even though the HPLC techniques separate most of the PSP derivatives and are relatively sensitive, they are time consuming, require rather expensive instrumentation and rely on relatively difficult pre- or post-column derivatization procedures.

Various expensive electrophoretic [22–24] and time consuming TLC methods in combination with peroxide oxidation [12,25] have been used to detect PSP toxins although C toxins which are common in Atlantic shellfish, were not recorded using any of these TLC methods.

TLC with Iatroscan flame ionization detection (FID) has been commonly used for the analysis of lipid classes [26], vitamins, amino acids and a variety of other lipophilic compounds [27]. The TLC–FID technique has also been used for the detection of tetrodotoxins in biological fluids [28], although this technique has never been used for the detection of PSP toxins. Iatroscan analyses have several advantages over classical planar thin-layer techniques including simple, rapid and more accurate quantitative analysis, thus making it a natural choice for the analysis of PSP's even though they are usually present in very low concentrations. It should be noted that the core element of PSP toxins includes nitrogen atoms, and some may be found in the various groups that create the different toxins. The recent development of a flame thermionic detector (FTID) for the Iatroscan enables the detection of nitrogenous compounds with much higher sensitivity than previously possible with the FID [26]. The presence of up to seven nitrogen atoms in the various PSP toxins suggests that FTID detection may provide improved sensitivity as compared to existing methods of PSP detection.

The objectives of this study were to examine the feasibility of using the Iatroscan with FTID to detect PSP toxins, and to attempt to identify at least the major toxin compounds by TLC–FTID.

2. Materials and methods

2.1. Detection by FTID

The Iatroscan (TH-10) Mark-5 (Iatron Laboratories, Tokyo, Japan) with a FTID (Detector Engineering & Technology, Walnut Creek, CA, USA) was used to detect PSP toxins and the peak response was determined by integrating the peak area. Operational conditions for the FTID were determined in preliminary studies using phosphatidylcholine (PC) which is non-volatile and contains one nitrogen atom per molecule. Minimum peak width of detection (MWD), tangent triggering percentage (TTP) and maximum noise level (MNL) were varied to improve the baseline, and the effect of variation in H_2 and air flow-rates, detector current and scan speed were studied for PC prior to testing with STX, neosaxitoxin (NEO), gonyautoxin 2 and 3 (GTX 2/3), and C toxins (C1/C2).

Typically, about 60–300 ng/μl of individual PSP

compounds (STX, NEO, GTX 2/3 and C1/C2) and PC were spotted on silica gel coated rods (Chromarods-SIII) with a Hamilton syringe and after oven drying for about 10 min, the rods were scanned initially with constant air flow-rate of 1.5 l/min, varying the H_2 flow-rate from 50 to 100 ml/min and the detector current from 2.6 to 3.3 A. FTID and FID responses were obtained in the same manner keeping the air flow-rate constant at 1.5 and 2.0 l/min while varying the H_2 flow-rate and detector current from 50 to 100 ml/min and 2.6 to 3.3 A, respectively. Once the optimum H_2 flow, air flow and detector current levels were obtained, the effect of scan time on the FTID response was studied while varying the detector current from 2.6 to 3.3 A at constant H_2 and air flow-rates of 50 ml/min and 1.5 l/min respectively.

2.2. Standards and toxin samples

Commercial standards of PSP toxins (STX, NEO, GTX 2/3 and GTX 1/4) and purified B1 and C1/C2 toxins were obtained from the National Research Council (NRC), Halifax, Nova Scotia. Toxins were also extracted from cells of *Alexandrium excavatum* and scallop digestive glands. Concentrated *A. excavatum* cells were ultrasonicated with an equal amount of water for 30 min, and 16 g of scallop homogenates were homogenized for 10 min at high speed settings using a Polytron homogenizer. The extracts were centrifuged at 6000 *g* for 30 min, the supernatants were defatted and the separated aqueous layers were filtered through 1000 Da MW cutoff membranes prior to examination by TCL–FTID. Partially purified toxins rich in NEO and STX extracted from a strain of *Alexandrium tamarensis*, were also obtained from NRC and used for TLC–FTID. Toxin profiles of each extract were obtained by HPLC [29].

2.3. Separation of toxins on chromarods-SIII

A series of 35 different solvent systems, including the pyridine-ethyl acetate-based systems conventionally used in planar TLC for the separation of PSP compounds, were tested for individual toxins. A chloroform–methanol–water based system was finally chosen to elute individual components of PSP. Initially about 0.2 μl of each compound (commercial standards) were individually spotted on the pre-scanned Chromarods-SIII and developed in chloroform–methanol–water–acetic acid (30:50:8:2) for 55 min. The rods were then oven dried at 105°C for about 10 min, and scanned at 40 s/rod using a H_2 flow-rate of 50 ml/min, an air flow-rate of 1.5 l/min and a detector current of 3.0 A. Two microlitres of a mixture of toxin standards consisting of 4 ng STX, 12 ng NEO, 22 ng GTX 2/3, 13 ng C1/C2 were applied to each rod for scanning. In addition, 1.6 μl of an *A. excavatum* cell extract consisting of 22 ng C toxins, 3 ng GTX 2/3, 3 ng STX was also spotted and scanned. Two microlitres of NEO and STX-rich *A. tamarensis* extract (16 ng NEO and 14 ng STX) were also applied, developed and scanned in the same manner. Two microlitres of scallop extract consisting of 26 ng GTX 2/3, 5 ng STX, 5 ng NEO and 12 ng C toxins, were initially developed in acetone (100%) for 30 min, dried at 105°C for about 10 min and partially scanned under similar conditions. Chromarods were then developed in chloroform–methanol–water–acetic acid (30:50:8:2) for 55 min, dried and fully scanned as above.

2.4. Calibration

Different solution volumes (0.2-1.2 μl) of STX (850 μg/ml), NEO (100 μg/ml), GTX 2/3 (6.34 μg/ml, GTX 1/4 (113 μg/ml), B1 (0.15 mg/ml) and C toxins (C1/C2, 0.1 mg/ml) were individually spotted on pre-scanned rods, developed in chloroform–methanol–water–acetic acid (30:50:8:2) for 55 min, oven-dried for about 10 min, and scanned at 40 s/rod, a detector current of 3.3 A and H_2 and air flow-rates of 50 ml/min and 1.5 l/min respectively. Calibration curves were compared by analysis of covariance (ANCOVA) via multiple regression using indicator variables in MINITAB version 11.21.

3. Results and discussion

The FTID/FID detection system for the Iatroscan (Mark-5), consists of a combination of FID and FTID detectors in series. The FID provides a universal response to virtually all organic compounds, whereas the FTID provides specific responses pri-

marily only to compounds containing nitrogen and/or halogen atoms [30]. In the present study, PC and all PSP individual standards had significantly higher FTID responses ($p<0.01$) than the FID responses, especially at any given level of detector current and scan time, and low levels of hydrogen and air flow. Under optimum conditions, the sensitivity of the FTID for pure toxin standards was 1, 5, 5, 2, 6 and 6 ng for NEO, STX, B1, GTX 2/3, GTX 1/4 and C1/C2 respectively. However, the method detection limits for toxic shellfish tissues using the current extraction protocol, gave significantly poorer sensitivity. In comparison to the quarantine limit of 0.8 μg STX equivalent per g edible tissue, the method detection limits for NEO, STX, GTX 2/3 and C1/C2 were 0.8, 2.1, 0.4 and 2.5 μg/g tissue, respectively. Although the sensitivity is perhaps not as high as that reported using other techniques, the findings reported here should be considered as preliminary and no doubt may be improved by manipulation of a number of parameters.

3.1. Baseline

The factors such as flow-rates of H_2 and air, detector current, amplitude, age of the rods, cleanliness of the rod, and the sample load affected the baseline. High H_2 (120 ml/min) and air flow (2.8 l/min) levels recommended by the operational instruction manual resulted in high noise levels. It became necessary to alter levels recommended by the manufacturer for MWD, TTP and MNL to 1%, 1.2% and 2 mm in order to achieve a baseline with minimum noise when scanning PC or PSP. These settings were often different from those recommended by the manufacturer.

3.2. Detector current and H_2/air flow

The major parameters which control the magnitude of the FID/FTID signal are the H_2 and air flow, and the scan rate of the Chromarod [27]. The variations in H_2 flow change the thermochemistry of the flame – Chromarod interaction, and this affects both the vaporization and ionization efficiencies of the sample on the rod.

The FTID responses to individual toxins were also influenced by the variation in H_2 and air flow-rates as well as detector current levels. The FTID responses to all toxins tested were similar with regard to the effects of detector current and H_2 flow for any fixed air flow-rate (see Fig. 2 for example). When the detector current and H_2 flow were changed from 2.8 to 3.3 A and 100 to 50 ml/min respectively, while the air flow-rate was maintained at 1.5 l/min, the FTID response to STX increased exponentially from

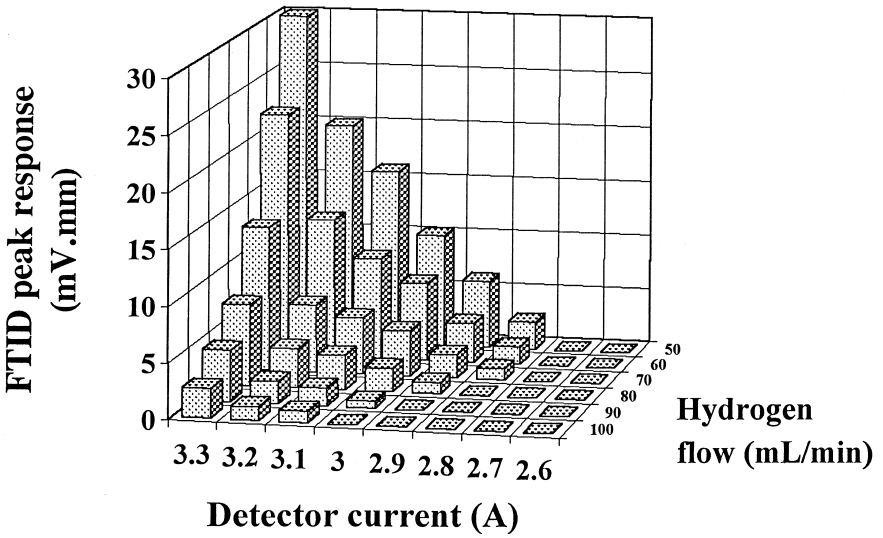

Fig. 2. A. Effect of H_2 flow and detector current on the FTID peak response to NEO (air flow=1.5 l/min).

2.1 to 182 mV mm. STX was not detectable at detector currents below 2.8 A due to a noisy baseline. However, when the air flow-rates were increased from 1.5 to 3.0 l/min, the exponential relationship became more ''flattened''. The maximum FTID response recorded for STX using the air flows of 2.0 and 3.0 l/min (detector current of 3.3 A and H_2 flow-rate of 50 ml/min) were 125 and 86 mV mm, respectively. It is interesting to note that the peak response increased rapidly, at any air flow-rate (from 1.5 to 3.0 l/min), when the detector current was increased from 2.6 to 3.3 A with the simultaneous decrease of H_2 flow from 100 to 50 ml/min.

The FTID response to NEO, GTX 2/3 and C1/C2 followed the same general pattern with regard to detector current, H_2 and air flow-rates except that the response was 4-6 times higher for NEO than for STX. NEO, GTX 2/3 and C1/C2 also could be detected only at detector current settings ≥2.8 A due to excessive noise and, H_2 flow-rates above 70 ml/min had to be maintained. Air flow-rates ≥3.0 l/min further limited the sensitivity of the detector.

3.3. Scan time

Varying the scan times for the rods mainly affects the efficiency of vaporization of the sample. Once the optimum operational conditions of the Iatroscan Mark 5 FTID were established, the effect of scan time on sensitivity was studied using H_2 and air flow-rates of 50 ml/min and 1.5 l/min, respectively, while varying the current from 2.6 to 3.3 A.

STX was detectable with a detector current as low as 2.6 A when the scan time was increased from 25 to 60 s/rod. Increasing scan times improved detector response up to a point. For STX, increasing scan times dramatically improved FTID sensitivity and this effect was most apparent at high detector current levels (2.9–3.2 A, Fig. 3). However, if the scan time was extended too long (>50 s/rod) at high detector current settings, there was a reduction of the FTID response which was probably due to rapid disappearance of combustion products from the flame. High temperature resulting from high current (3.3 A) and short scan times (50–60 s/rod) reduced the efficiency of rods in subsequent scannings. Therefore, moderate scan times such as 40 s/rod should be selected for higher responses in routine Iatroscan use.

3.4. Separation of PSP toxins/solvent systems

By examining the development of individual PSP toxin standards in different solvent systems, the chloroform–methanol–water–acetic acid (30:50:8:2)

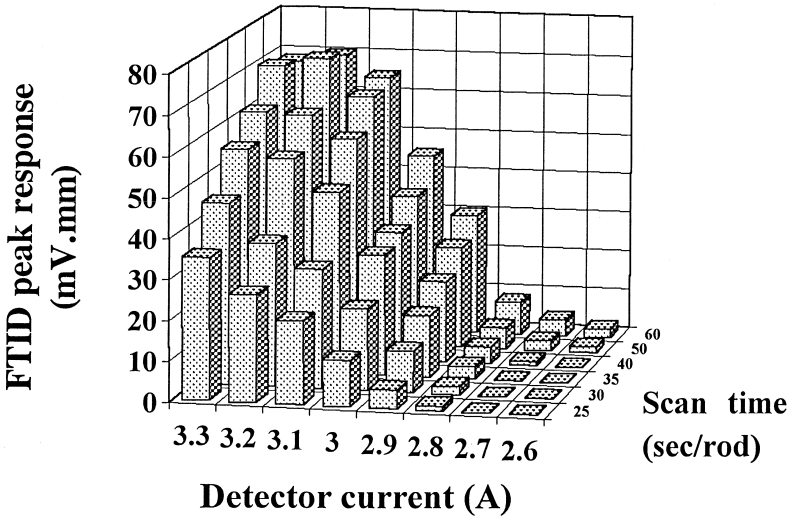

Fig. 3. Effect of scan time and detector current on STX response (air flow = 1.5 l/min, H_2 flow = 50 ml/min).

system was found to be the best to separate at least the major PSP components (Table 1). Although pyridine, ethyl acetate, acetic acid and water in varied proportions were used to separate STX from other toxins by TLC [25], a complete separation of all six toxins on Chromarods could not be achieved when the development was attempted in pyridine–water–ethyl acetate–acetic acid (70:30:25:15) for 1.5 h.

When standard toxin mixtures were spotted and developed in a chloroform–methanol–water–acetic acid (30:50:8:2) solvent system for 1 h, C1/C2 eluted to the solvent front (Fig. 4) followed by GTX 2/3 but it was difficult to achieve a complete separation of NEO from STX. Although GTX 2/3 were eluted as one sharp peak followed by a small broad peak, these peaks were not able to be distinguished individually. However, GTX 2/3 were well separated from NEO and STX, and NEO moved very close to STX which remained at the origin. It is also interesting to note that when these co-spotted samples were developed only in H_2O for 50 min, C1/C2 moved ahead of GTX 2/3 which co-eluted with NEO and STX.

C toxins extracted from *A. excavatum* eluted to the top of the solvent front (Fig. 5) when developed in chloroform–methanol–water–acetic acid (30:50:8:2) for 55 min as in the case of C1/C2 standards. NEO derived from *A. tamarensis,* partially separated from STX which did not move in this solvent system (Fig. 6). Toxic scallop homogenates were first developed in acetone and some unknown compounds, possibly hydrophilic phytopigment derivatives, were eluted (Fig. 7A). After the partial scanning of these compounds, rods were developed in chloroform–methanol–water–acetic acid (30:50:8:2) to separate C toxins from GTX 2/3 (Fig. 7B). NEO and STX were present in relatively small amounts in the scallop extracts. It was observed that the cleanliness of the rods and the sample is critical since the baseline noise can be increased with any contaminated nitrogenous or halogenated compounds.

Table 1
Relative mobilities of PSP individual components on Chromarods-SIII, developed in different solvent systems

Solvent system	Relative mobilities
Pyridine–ethyl acetate–water (45:15:18:12)[a], Pyridine–ethyl acetate–water–acetic acid (45:15:10:5)[a]	All moved together with same retention
Methanol–water–chloroform–acetic acid (45:45:10:5)	All moved together with same retention
Acetic acid–water (40:40)	All moved together with same retention
Ethyl acetate–water–formic acid (40:40:5)[a]	All moved together with same retention
Methanol–ethyl acetate–water (35:35:10)	All moved partially from NEO and STX. NEO and STX did not move
Methanol–ethyl acetate–water (40:20:20), Methanol–ethyl acetate–water–formic acid (35:35:10:2), Methanol–ethyl acetate–water (35:40:5)	All moved with same retention
Methanol–ethyl acetate–water (35:45:10)	None moved
Butanol–methanol–water–ammonium hydroxide (40:20:20:2) or (35:45:10:10)	Very noisy base line and none moved. Double development didn't improve.
Chloroform–methanol–water–isopropanol (35:45:8:2)[a]	GTXs and Cs together partially moved from NEO and STX
Chloroform–methanol–water (70:30:5)	None moved
Chloroform–methanol–water (40:40:20)	All moved at same retention time
Chloroform–methanol–water (35:45:8)	GTXs separated from NEO and STX. NEO and STX didn't move
Chloroform–methanol–water–acetic acid (30:50:8:2)[a]	Cs separated from GTXs and GTXs completely separated from NEO and STX. NEO partially separated from STX. STX didn't move
Pyridine–water–ethyl acetate–acetic acid (70:30:25:15)[a]	All moved with same retention time
Water[a]	Only Cs separated and GTXs,NEO,STX moved together
Methanol–water (50:50)[a]	All moved with same retention
Chloroform–methanol–water–acetic acid (40:40:8:2)[a]	Cs partially separated from GTXs and GTXs completely separated from NEO and STX. NEO partially separated from STX. STX didn't move

[a] Same solvent system was used on Chromarods-A, but the resolution was not improved, in most cases even worse than Chromarods-SIII.

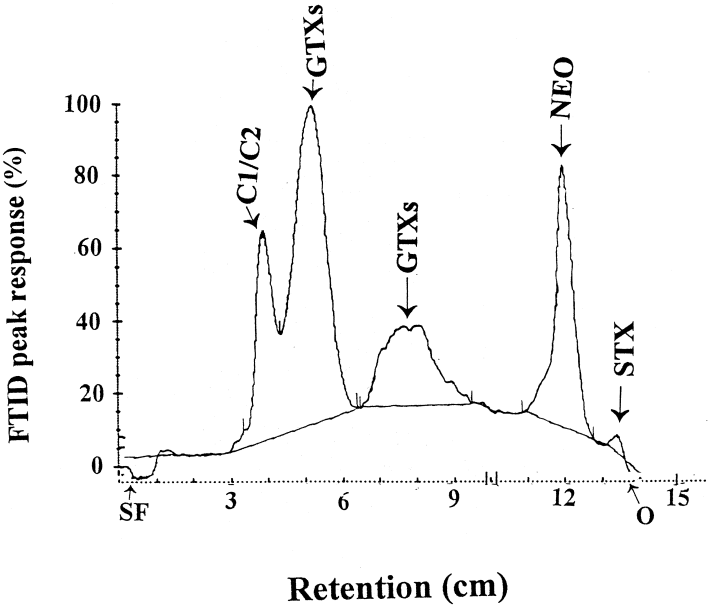

Fig. 4. Chromatogram of a mixture of standard PSP toxins developed in chloroform–methanol–water–acetic acid (30:50:8:2) (air flow = 1.5 l/min, H_2 flow = 50 ml/min, detector current = 3.3 A, SF = solvent front, O = origin).

4. Calibration

Curvilinear calibration curves relating peak areas to amounts of spotted authentic standards were obtained for PSP toxins, and the calibration equations were ''best fitted'' using quadratic models. Some typical calibration curves are shown in Fig. 8, and calibration equations for PSP compounds are given in Table 2. However, quantification of very low concentrations of any PSP component in a mixture of toxins may not be very accurate due to the nature of broad peaks and noise. The separation technique should be further improved to reduce background noise.

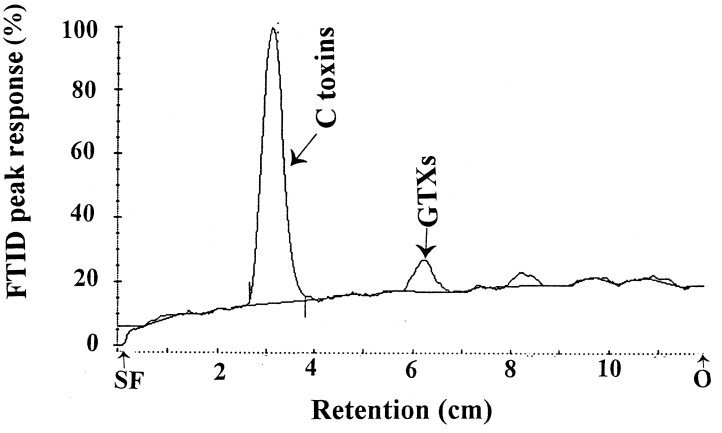

Fig. 5. Chromatogram of PSP toxins in *Alexandrium excavatum* cell extracts developed in chloroform–methanol–water–acetic acid (30:50:8:2) (same conditions as in Fig. 4).

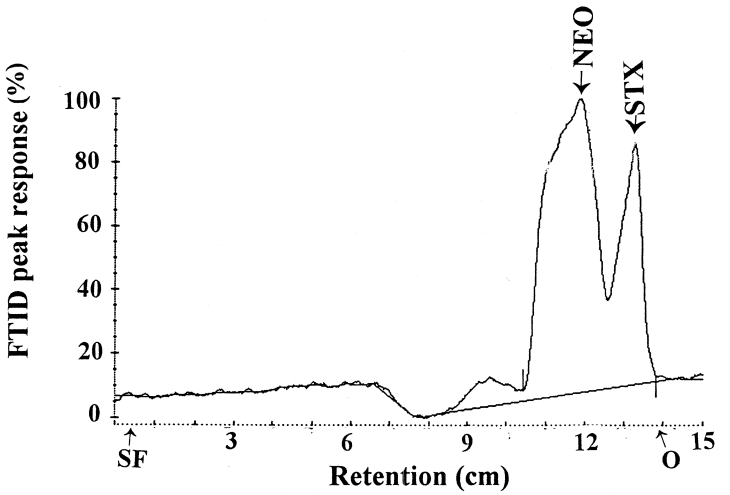

Fig. 6. Chromatogram of PSP toxins in partially purified mixture of STX and NEO extracted from *Alexandrium tamarensis* after developed in chloroform–methanol–water–acetic acid (30:50:8:2) (same conditions as in Fig. 4).

The slopes of linearized FTID responses of STX, NEO, GTX 2/3 and C1/C2 clearly indicate that the FTID response for NEO was higher than that of STX at any given current, hydrogen and air levels. To date, other chemical detection methods have suffered due to poor sensitivity for the *N*-1-hydroxy compounds such as NEO, C 3/4, B2 and GTX 1/4. However, with the present technique, the *N*-1-hydroxy PSP compounds gave responses similar to or greater than those of STX, GTX 2/3 and C1/2.

4.1. Inter and intrarod variability

The FTID response for the same amounts of

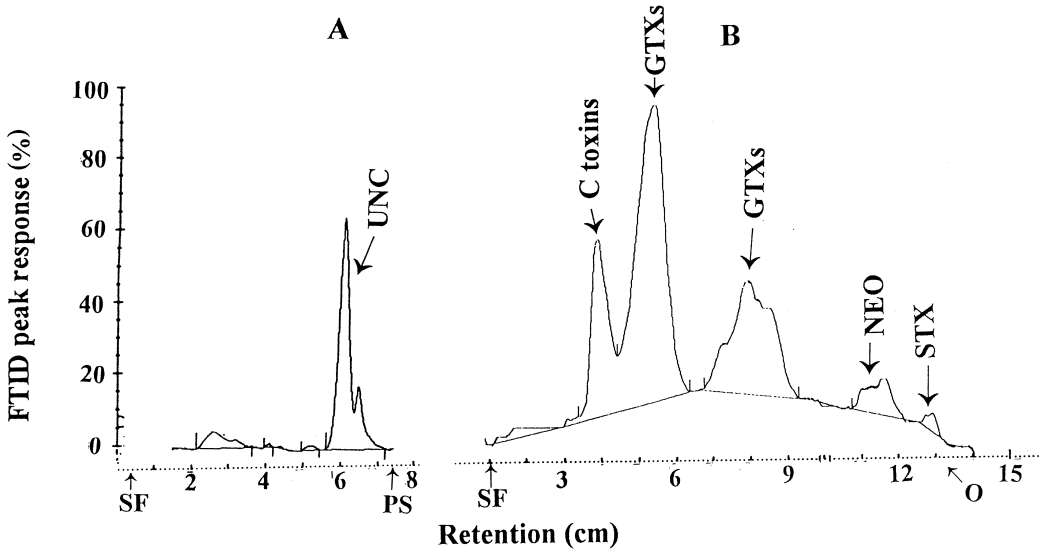

Fig. 7. Chromatogram of PSP toxins in scallop digestive glands. (A) Partial scan after developed in acetone, (B) full scan after developed in chloroform–methanol–water–acetic acid (30:50:8:2) (same conditions as in Fig. 4).

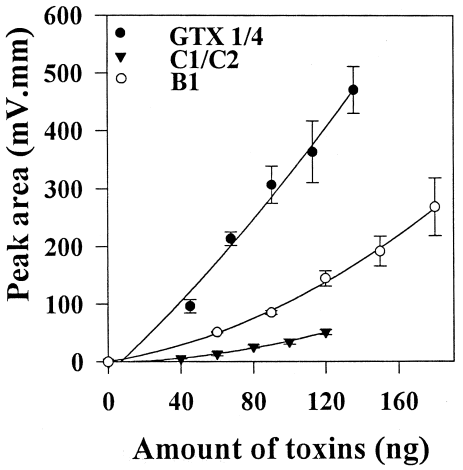

Fig. 8. Calibration curves for GTX1/4, C1/C2 and B1 based on the FTID peak response for different amounts of toxins (Vertical lines show upper and lower 95% confidence limits, $n=150$).

individual PSP compounds may vary not only from one Chromarod to another (interrod) but also for the same rod (intrarod) at different times. The coefficient of variation of the FTID response, which is the standard deviation expressed as percentage of the mean for each rod was calculated at low and high loading levels for each PSP compound to check inter- and intrarod precision (Table 3). It was found that the coefficient of variation of FTID response for different rods as well as for the same rod for each toxin was higher at low amounts of spotting than high amounts, indicating better precision at higher loading levels.

Table 3
Mean coefficient variation of FTID response for inter- and intrarod precision at two different loading levels of PSP toxins

Type of toxin	Interrod coefficient of variation (%)		Intrarod coefficient of variation (%)	
	Low load[a]	High load[b]	Low load[a]	High load[b]
STX	4.3	1.4	0.5	0.1
NEO	11.1	4.2	2.2	2.0
GTX 1/4	16.7	8.4	3.2	0.8
GTX 2/3	13.8	10.8	1.2	1.0
B1	9.9	2.6	1.0	0.8
C1/2	7.4	5.5	1.8	0.9

[a] Low load: STX – 17 ng, NEO – 4 ng, GTX 1/4 – 45.2 ng, GTX 2/3 – 2.3 ng, B1 – 60 ng, C1/2 – 40 ng.
[b] High load: STX – 51 ng, NEO – 12 ng, GTX 1/4 – 135.6 ng, GTX 2/3 – 7.0 ng, B1 – 180 ng, C1/2 – 120 ng.

4.2. Other factors affecting sensitivity

The sensitivity of FTID decreased as the number of operational hours increased. Maximum sensitivity was always achieved with a new detector. Detector response data presented here was always obtained with a ''new'' detector. Sensitivity was also adversely affected by the continuous use of high current levels. Although FTID responses were highest at high detector current settings, settings above 3.0 A are not recommended because it decreases the ionization efficiency of the detector and results in poor sensitivity. Also high current settings lead to more frequent detector replacement and continuous use of high current deteriorates the physico-chemical nature of the Chromarods resulting in poor resolution. It is

Table 2
Calibration equations for PSP toxins

Type of toxin	Amount of toxin spotted (range, ng)	Regression equation x=amount of toxin (ng) y=log FTID response (mV mm)	R^2
B1	0–180	$y=0.7+0.5+0.01x^2$	0.94
C1/2	0–120	$y=0.8+0.6x+0.003x^2$	0.97
GTX 1/4	0–136	$y=-20+2.9x+0.01x^2$	0.95
GTX 2/3	0–7	$y=-0.2+0.6x+0.1x^2$	0.94
NEO	0–12	$y=-0.01+0.4x-0.2x^2$	0.95
STX	0–51	$y=-2.6+1.5x-0.01x^2$	0.98

also important to note that no two FTID detectors gave identical response sensitivities even if both were of the same age.

5. Summary and conclusions

The Iatroscan Mark 5 equipped with FTID is capable of detecting of nitrogenous lipophilic compounds such as PC and hydrophilic organic compounds such as PSP toxins. H_2 and air flows, scan speed and detector current affect the FTID response. Hydrogen and air flow rates of 50 ml/min and 1.5 l/min coupled with a detector current of 3.3 A and scan time of 40 s/rod gave highest FTID responses and were 25 and 130 times higher for PC and PSP toxins than signals achieved by FID. This chemical detection technique involves relatively inexpensive equipment (Iatroscan system), is fairly rapid (detects a minimum of 60 samples in 1 h, and more if multiple development chambers are used), using sample sizes as small as 0.2 μl, and the solvent system used may also be used to purify PSP toxins by TLC. All PSP components tested can be detected with more or less similar sensitivity. Standard toxin amounts as small as 1 ng NEO, 5 ng STX and B1, 2 ng GTX 2/3, 6 ng GTX 1/4 and C1/C2 can be detected by TLC/FTID, however the method sensitivity for extracts from tissue requires improvement. These detection limits can perhaps be further improved by increasing the detector current level. Two major advantages of the Iatroscan-Chromarod analytical system are the handling of ten Chromarods as a unit, facilitating rapid screening of multiple samples, and the flexibility offered by a partial scan and redevelopment in the same or a different solvent system.

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