Original Article

Determination of Saxitoxin by Receptor Binding Assay: A New Radiolabeled Kit in Thailand

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ABSTRACT

Objective: A receptor binding assay for detection of saxitoxin, a paralytic shellfish poisoning toxin, was formatted for use in a high throughput detection system using microplate scintillation counting. The RBA technology was transferred from the NOAA National Ocean Service, SC USA, to the Thailand Institute of Nuclear Technology, which uses a Perkin Elmer Microbeta TriLux 1450 microplate scintillation counter. **Methods:** Validation of method was performed by participating in the regional inter-laboratory program. The result yielded all parameters within the critical control point (i.e. RSD less than 30%). **Results:** The slope of the calibration graph = 1.2 + 0.26 (RSD = 21.7%) (criteria = 0.8 - 1.2), half maximal inhibition (IC50) = 3.1 + 0.47 nM (RSD = 15.2%) (criteria = 3.0 nM), dynamic range = 1.2 - 10 nM and the limit of detection (IC80) from graph = 1.2 nM, equivalent to the limit of quantification of 2.57 mg/100 g shellfish. The result of analysis of unknown samples yielded the RSD between assays ranging from 6 - 23% and the recovery compared to the expected value ranging from 79 -133%. The application of RBA for determination of PSP in shellfish samples yielded RSD in assays less than 30% and between assays ranging from 1.4-15.2%. **Conclusion:** RBA for STX has showed a valuable for rapid, reliable, cost-effective alternative to live animal testing and high throughput screen prior to testing by the conventional mouse bioassay (MBA) and its suitability for providing an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

Keywords: saxitoxin, receptor binding assay, paralytic shellfish poisoning, tritium

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Introduction

The paralytic shellfish poisoning (PSP) syndrome is caused by the consumption of shellfish containing high levels of saxitoxin (STX) (Figure 1) and its numerous analogs. Toxicity of saxitoxin has been reported in many countries such as USA, Canada, Japan, Australia, New Zealand, Malaysia, the Philippines and Thailand[1,2,3] and Southeast Alaska[4,5]. In human, recognizable PSP symptoms include tingling and numbness of peripheral area and extremities, following ingestion of about 0.72 mg STX equivalents; while severe intoxication include respiratory paralysis and possible death, resulting from the exposure to 0.9 - 3.6 mg STX equivalents. Renal clearance of the toxin from the blood is completed within 24 hours[6].

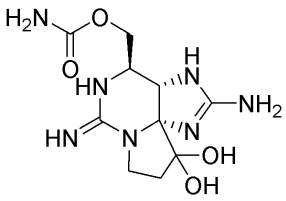

Figure 1 Structure of saxitoxin (STX).

These PSP toxins exploit the highly specific interaction with their biological receptor, i.e. voltage-dependent sodium channel, specifically site 1 of α-subunit[7]. The assay on STX is thus based on functional activity rather than antigenic determinant[8]. This receptor binding assay provides a radioactive endpoint, and is performed in a microtiter filter plate format with results determined by liquid scintillation counting. The K_i for the assay is 3.66 $\pm$ 0.86 nM saxitoxin,

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with a limit of detection of 5 ng saxitoxin/ml in a sample extract[8].

The mouse bioassay (MBA) has historically been the most universally applied technique for examining shellfish, especially for PSP. However, MBAs have some limitations including low sensitivity, somewhat large variability of test results (±20%)[8], the use of large numbers of live animals, and the resulting toxicity measure of all toxins combined instead of individual toxins. Furthermore there are some critics of the mouse toxicity method from animal protection group[6,8,9].

In recent years, considerable effort has been applied to development of chemical assays to replace these bioassays. As a result, a good high performance liquid chromatography (HPLC) coupled with post-column electrochemical oxidation procedure has been developed to identify individual PSP toxins[10]. Nevertheless HPLC technique was not sufficiently rapid or robust to handle a large number of samples generated during bloom events and the system requires a considerable amount of skill and dedicated time to make it operate routinely[11]. Numerous other techniques have been examined as possible replacements including receptor binding assay (RBA)[12,13], enzyme immunoassays[14,15], biosensor technology[16] and neuronal network[17]. Among these methods, RBA is considered a very promising alternative to the mouse bioassay.

The RBA technique employs the microplate scintillation technology[8,12]. It expresses PSP toxin concentrations in nM STX equivalents; while in the MBA method, concentrations are expressed in µg STX equiv./100 g shellfish toxin[6]. Theoretically, RBA is a functional assay based on the toxins with their pharmacological receptors and gives a measure of toxic potency. RBAs are appropriate for those toxin classes that interact with membrane receptors, including the voltage dependent sodium channel and are the method of choice when a rapid, high throughput measure of total toxic potency is desired. RBA is carried out by incubating the known receptor for the toxin (R), in the presence of the radiolabeled toxin analog (T*) which together form a radiolabeled receptor toxin complex (T*R).

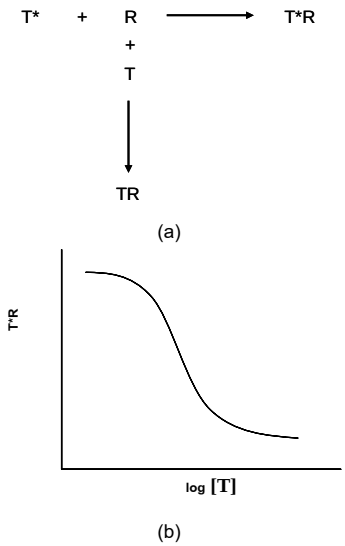

Figure 2 Receptor-toxin complex (a) and associating competition curve between labeled and unlabeled toxin (b). **Note:** T = unlabeled toxin, T* = radiolabeled toxin, T*R = radiolabeled–receptor complex, R = receptor and TR = unlabeled–receptor complex.

With the addition of unlabeled toxin (T) to this incubation mixture, in the form of toxin standard or unknown sample, the unlabeled toxin competes with the radiolabeled toxin for the receptor, forming unlabeled complex (TR). The amount of radiolabeled complex formed in this mixture is quantified by liquid scintillation counting. With increasing amount of unlabeled toxin, the amount of radiolabeled complex decreases relatively to the amount of radiolabeled complex formed in the absence of unlabeled toxin. This competition between labeled and unlabeled toxin for the receptor generates a competition curve which can then be used to quantify the amount of toxin present in an unknown sample.

The objectives of this research were to establish a laboratory with its ability to perform RBA for determination of PSP toxins especially saxitoxin, and to produce a ready-to-use radiolabeled kit for STX toxicity in seafood with a high throughput screening for STX.

Materials and Methods

Materials

Chemicals and equipments with their sources were as follows: Motorized tissue homogenizer, high speed centrifuge with rotor, centrifuge tubes, 1 and 8 channel micropipettes,

MOPS (UltraPure, BioBasic); choline chloride (Sigma); phenyl methylsulfonyl fluoride (PMSF) (Sigma); bicinchoninic acid protein assay (BCA) kit (Novagen); 96-well microtiter filter plate; Multiscreen vacuum manifold (Millipore); microplate reader with 562 nm wavelength (Tecan); microplate scintillation counter (Perkin Elmer Microbeta TriLux 1450); saxitoxin reference standard (STX dihydrochloride) (NRC, Canada), ^{3}H-STX (I.I.C.H., USA); Optiphase cocktail (Perkin Elmer); shellfish samples; Wistar rat brains (6 weeks old male rats, National Animal Center, Mahidol University); absorbent toweling, disposable bench paper; curve-fitting software package (Graph Pad Prism).

Methods

A) Sample Preparations

- **Samples collection**

Shellfish samples were collected in March 2008, in Gulf of Thailand by Department of Marine Coastal Resources. Each sample was weighed and measured for its length and homogenized with blender. Samples were frozen immediately after transferred to TINT laboratory on ice, and stored at –20 $^{\circ}$C until analyzed for the presence of toxin by receptor binding assay.

- **Samples extraction**

Samples were homogenized with blender then accurately weighed for 5.0 g and put into a conical tube. The sample was added with 3.0 ml of 1% acetic acid, mixed on vortex, heated in water bath for 5 minutes, then capped loosely to avoid pressure buildup. The sample was removed from water bath, mixed on vortex mixer, let cool to room temperature, and centrifuged for 10 minutes at 4,500 rpm. Finally supernatant in conical tube was collected. The solid residue in the original tube was added with 3.0 ml of 1% acetic acid, mixed well on vortex mixer and centrifuged for 10 minutes at 4,500 rpm. This supernatant was collected and combined with the first portion. The combined supernatant was mixed and diluted to 6 ml with ddH_20, and centrifuged to clarify supernatant and transferred to a new tube. The sample extract was stored at -80 $^{\circ}$C until assay.

- **Preparation of rat brain membrane**

Medulla and cerebellum from rat brain was removed. The brain (cerebral cortex) was placed in a small amount of 100 mM MOPS/100 mM choline chloride/0.1 mM PMSF buffer, pH 7.4 in a petri dish which was placed on ice. In a glass tube, 25 ml of MOPS/choline chloride/PMSF buffer and 2 cerebral cortices were put in, then homogenized at 3,000 rpm with 20 up and down strokes or more, if necessary. There should be no chunks left in the homogenate. The tube was kept in ice at all times. Homogenized tissue was poured into a beaker on ice and the procedure was repeated with remaining cortices. This pooled homogenized tissue was transferred to centrifuge tubes balanced with ice cold buffer, then centrifuged at 20,000 xg for 15 min at 4 $^{\circ}$C. Supernatant was aspirated and discarded. Re-suspend pellet was pooled in ice cold MOPS/choline chloride/PMSF buffer. The centrifuge was thoroughly rinsed with a small amount of buffer and the rinsed sample was pooled in pool in beaker. For each brain sample, a final volume of 10 ml was made using buffer solution while kept on ice. This pooled homogenized tissue was transferred to a glass tube, homogenized at 3,000 rpm with 10 up and down strokes or more, if necessary. This homogenized tissue was then poured into a beaker and stirred with magnetic bar on ice with a low speed. The preparation was then aliquoted for 1 ml per tube into microtubes or cryotubes on ice and stored at -80 $^{\circ}$C. This preparation was stable for at least 6 months.

- **Protein determination in rat membrane by BCA protein assay kit**

Rat brain membrane was removed for determination of protein concentration by BCA protein assay kit. The method was performed according to manufacturer's instructions (BCA protein assay kit 71285-3, Novagen). The protein concentration (in stock 1 ml/tube) was approximately 7.315 mg/ml. The stock rat membrane preparation was diluted 1:8 in ice cold assay buffer (100 mM MOPS/100 mM choline chloride buffer, pH 7.4) to yield a final protein concentration of approximately 0.128 mg in the assay, before use.

B) The Assay Procedure

• Preparation of Stock Solution and Standards for Assay

♦ ***Assay buffer solution (100 mM MOPS/100 mM choline chloride buffer, pH 7.4)***

MOPS of 20.9 g and choline chloride of 13.96 g were added to 900 ml ultra pure H_20 or ddH_20 for a buffer with pH of 7.4 while stirring with 0.1 N HCl or 0.1 NaOH. The final volume of 1 L was made with ultra pure H_20 or ddH_20.

♦ ***Radioligand solution***

^{3}H-STX stock solution was provided in 50 µCi/ampoule, 21 Ci/mmol, or 0.1 mCi/ml. This was equivalent to 4.17 µM.

a) ^{3}H-STX working solution: The solution of 15 nM ^{3}H-STX was freshly prepared from ^{3}H-STX stock solution for each assay daily. Fifteen µl of stock ^{3}H-STX solution was transferred and 4.155 ml assay buffer was added and mixed with vortex mixer. Since specific activity of ^{3}H-STX usually varied between lots, the calculation on concentration was required and the volume used for dilution was adjusted accordingly.

b) Total radioactivity measurements on each working solution prior to the assay: Fifty µl of Optiphase® cocktail was added to 35 µl of working solution. Using liquid scintillation counter, the radioactivity count should be at least 1,000 counts. The count measurement was performed to determine if the isotope was degrading over time relative to the initial value and/or to detect an incorrect dilution.

♦ ***Unlabelled STX reference standard preparation***

STX standard was provided at a concentration of 268.8 µM (100 µg/ml). Serial dilutions for the standard curve were prepared as shown in Table 1. These STX standards could be prepared in advance and stored at 4 °C for 1 month.

• Determination of Tissue Linearity

Dilutions of membrane preparation were made at 1/4, 1/8, 1/16, 1/32, 1/64 in assay buffer. 140 µl of each dilution was added to triplicate wells of 96-well microtiter filter plate containing 35 µl assay buffer and 35 µl ^{3}H-STX working solution. The plate was covered and incubated at 4 °C for 1 h to achieve equilibrium binding. The mixture was then filtered using a Multiscreen vacuum manifold system. The count was carried out using microplate scintillation counter. Counts per minute (CPM) against dilutions was plotted to determine the maximum dilution that gave adequate binding (≥ 1000 CPM), such that a minimum number of membrane preparation was used in each assay. This dilution had to be on the linear part of the curve.

Table 1 Serial dilutions for standard curve of saxitoxin (STX).

	STX standard (M)	0.003 M HCl (ml)	Final concentration (M)	In assay* concentration (M)
A	100 µl 268.8 µM STX	4.38	$6x10^{-6}$	$1x10^{-6}$
B	500 µl from tube A	4.5	$6x10^{-7}$	$1x10^{-7}$
C	1.5 ml from tube B	3.5	$1.8x10^{-7}$	$3x10^{-8}$
D	500 µl from tube B	4.5	$6x10^{-8}$	$1x10^{-8}$
E§	500 µl from tube C	4.5	$1.8x10^{-8}$	$3x10^{-9}$
F	500 µl from tube D	4.5	$6x10^{-9}$	$1x10^{-9}$
G	500 µl from tube F	4.5	$6x10^{-10}$	$1x10^{-10}$
H	500 µl from tube G	4.5	$6x10^{-11}$	$1x10^{-11}$
Ref	0	5.0	0	Reference

* All standards were diluted 1:6 in the assay (35 µl standard in 210 µl of total reaction mixture).

§ Inter-assay calibration standard: A reference standard containing $1.8x10^{-8}$ M STX standard. It was dispensed with 120 µl in a 0.5 ml microtube for 20 microtubes and stored at -80 °C. It was critical that aliquot was thawed and kept on ice before use. This served as a quality control for the day-to-day performance of the assay.

C) Performing the Assay

Standards and samples were run in triplicate. Samples were undiluted and diluted 1:2 with assay buffer (1 part sample + 1 part assay buffer) (see Table 2 for plate layout). Rat brain membrane preparation was thawed and kept on ice. Membrane preparation was diluted at 1:8 with ice cold

assay buffer (one part of membrane + 7 parts of buffer). Diluted membranes were kept on ice until used. Each of the following was added to each of the 96-wells microfilter plate; 35 µl STX standard or QC or sample, 35 µl ^{3}H-STX, 140 µl rat brain membrane preparation (equiv. 0.128 mg protein). All were then covered and incubated at 4 ^{o}C for 1 hour. After that, 96-well plate was placed on the multiscreen vacuum manifold and empty wells of the plate were filled with 210 µl of assay buffer.

The vacuum was run until liquid was removed. Each well was rinsed twice with 210 µl ice cold assay buffer using 8 multichannel pipettor, then the plastic bottom was removed from the plate. The plate was blotted on absorbent toweling and bottom of counting cassette was sealed with sealing tape and microplate was placed in a counting cassette. Fifty µl Optiphase cocktail was added to the wells using 8 multichannel pipettor. The plate was sealed with sealing tape and allowed to sit for 30 minutes at room temperature and then counted in microplate scintillation counter (1 minute per well).

D) Data Analysis

Curve fitting was carried out using Graph Pad Prism® software in the one-site receptor competition assay mode. The concentration of PSP toxins(in nM STX equivalent) in the sample was determined from a competitive binding curve generated by transforming the percent total binding data (B/B_0) using a logit transformation and graphing the result values on a linear y-axis scale against a logarithmic x-axis scale of the unlabelled STX concentration. The best-fitted line was generated using a non linear regression, and the STX concentrations in the unknown sample were obtained by solving the regression equation for (x) using the logit transformation of B/Bo for the sample (y). Sample concentration was then calculated in µg STX equivalents/100 g shellfish, using the following formula:

Table 2 Plate layouts.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10^{-6} M	10^{-6} M	10^{-6} M	Ref	Ref	Ref	Sam 4	Sam 4	Sam 4	Sam 8	Sam 8	Sam 8
B	10^{-7} M	10^{-7} M	10^{-7} M	QC	QC	QC	Sam 4 1:2	Sam 4 1:2	Sam 4 1:2	Sam 8 1:2	Sam 8 1:2	Sam 8 1:2
C	$3x10^{-8}$ M	$3x10^{-8}$ M	$3x10^{-8}$ M	Sam 1	Sam 1	Sam 1	Sam 5	Sam 5	Sam 5	Sam 9	Sam 9	Sam 9
D	10^{-8} M	10^{-8} M	10^{-8} M	Sam 1 1:2	Sam 1 1:2	Sam 1 1:2	Sam 5 1:2	Sam 5 1:2	Sam 5 1:2	Sam 9 1:2	Sam 9 1:2	Sam 9 1:2
E	$3x10^{-9}$ M	$3x10^{-9}$ M	$3x10^{-9}$ M	Sam 2	Sam 2	Sam 2	Sam 6	Sam 6	Sam 6	Sam 10	Sam 10	Sam 10
F	10^{-9} M	10^{-9} M	10^{-9} M	Sam 2 1:2	Sam 2 1:2	Sam 2 1:2	Sam 6 1:2	Sam 6 1:2	Sam 6 1:2	Sam 10 1:2	Sam 10 1:2	Sam 10 1:2
G	10^{-10} M	10^{-10} M	10^{-10} M	Sam 3	Sam 3	Sam 3	Sam 7	Sam 7	Sam 7	Sam 11	Sam 11	Sam 11
H	10^{-11} M	10^{-11} M	10^{-11} M	Sam 3 1:2	Sam 3 1:2	Sam 3 1:2	Sam 7 1:2	Sam 7 1:2	Sam 7 1:2	Sam 11 1:2	Sam 11 1:2	Sam 11 1:2

Note: Sam = sample; The standard curve may be used for multiple plates.

$$\text{(nM equiv STX)} \times \text{(sample dilution)} \times \frac{\text{(210 µl total volume)}}{\text{35 µl sample}} = \text{nM equiv STX in extract} \quad (1)$$

$$\text{(nM equiv STX in extract)} \times \frac{\text{1 L}}{\text{1000 ml}} \times \frac{\text{372 ng}}{\text{nmol}} \times \frac{\text{1 µg}}{\text{1000 ng}} = \text{µg STX equiv/ml} \quad (2)$$

$$\text{µg STX equiv/ml} \times \frac{\text{ml extract}}{\text{g shellfish extract}} \times 100 = \frac{\text{µg STX equiv}}{\text{100 g shellfish}} \quad (3)$$

E) Assay Quality Control

This process consisted of 3 critical control points. **First**, for a ligand which interacted specifically at one receptor site, the slope of the resulting competition curve should theoretically be 1.0. If the slope of the curve for a given assay was outside of the acceptability range of 0.8 - 1.2, linearity of the assay would be compromised and quantitation of the unknowns would be incorrect. Therefore, the assay should be re-run. **Second**, the QC check standard should fall within ±30% of the stated value of 3.0 nM (2.1 - 3.9 range). If the QC check standard did not fall within an acceptable limit of 2.1-3.9, the assay should be re-run. **Third**, the following criteria must be met for acceptability of a sample measurement. 1) Sample quantification should be done only on dilutions that on the linear part of the curve (B/Bo = 0.2 - 0.7). 2) In the event that no sample dilutions fell within the linear range (i.e., concentration was too high, or B/Bo < 0.2), further dilution must be made and the sample reanalyzed. In the event that the sample concentration is too low to be quantified (i.e. B/Bo > 0.7), the sample must be reported as below limit of detection. 3) The % relative standard deviation (RSD) of calculated value must be less than 30%.

Results

Tissue linearity

Based on CPM vs. dilutions plot, the dilution at 1/8 was the maximum dilution that gave adequate binding (≥ 1,000 CPM) and easy to filter from multi-screen vacuum manifold (data not shown).

Validity of the method

The test of validity was done by running the assay on 7 unknown samples provided by National Oceanic and Atmospheric Administration (NOAA), USA. The assays were run in triplicate, at 3 dilutions on 3 different days. The calibration curve is shown in Figure 3. All parameters fell within the acceptable critical points for quality control on the method: i.e., IC 50 = 3.1 + 0.47 nM (RSD = 15.2%); slope = -1.2 + 0.26 (RSD = 21.7%); and dynamic range = 1.2 - 10 nM (b/b_0: 0.2 - 0.8). Limit of quantification was found on the linear part of the curve (b/b_0: 0.2 - 0.8) as follows:

LOD = IC 80 = 1.2 + 0.04 nM

LOQ = 1.2 * 6 (assay dilution) * 5 (sample dilution) = 36 nM

36 nM = 13.17 ng/ml = 2.57 µg/100g

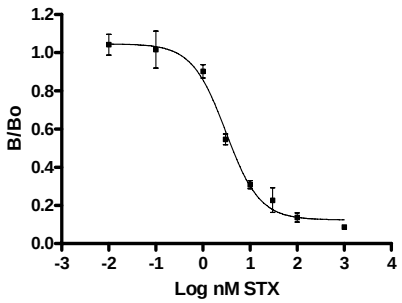

Figure 3 Calibration curve from 3 assays run on 3 different days. Conditions: [^{3}H- STX] 1.5 nM, FDA di HCl reference standard, crude rat brain membrane, microplate assay format, Perkin Elmer Microbeta TriLux 1450.

Linearity of the method was found from the plot of the measured values vs. the expected values of STX concentrations (Figure 4). The curve shows a high correlation with r^2 of 0.9964.

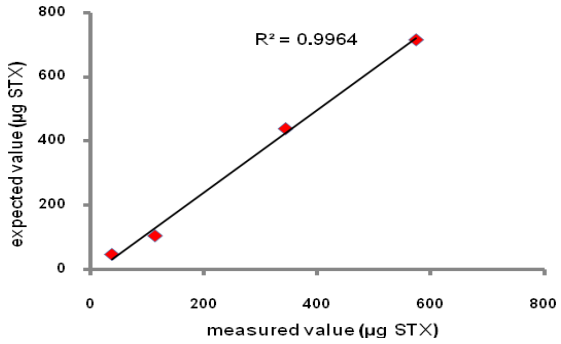

Figure 4 Plot of expected vs. measured STX concentrations showing a linearity of the RBA method.

The results of inter-comparison exercises compared to the expected values were shown in Table 3. All results exhibited good precision with the RSD between assays of less than 30%, and acceptable accuracy with the recovery varied from 79% to 133%.

Application of RBA technique on the determination of PSP in samples

Shellfish samples from Gulf of Thailand were determined for PSP using a typical run of the receptor binding assay protocol. The concentration of PSP toxins (in µg STX equiv. /100 g sample) was determined using the Prism software (GraphPad Software Inc.) The analytical results are shown in Table 4. All results were acceptable with RSDs in assay of less than 30% and RSDs between assays ranging from 1.4% to 15.2%.

Discussion and Conclusion

The RBA technique is a competitive binding assay based on pharmacological properties of PSP toxins. Because the affinity of binding to their biological receptor is directly proportional to the toxic potency of the PSP toxins, measurements based on sodium channel binding yield a very

Table 3 Results of inter-comparison exercises of RBA techniques compared to the expected values.

	Assay 1	Assay 2	Assay 3	Mean	SD	% RSD	Expected	% Recovery
QC	3	3	4	4	1	23	3	133
RR1	39	41	35	38	3	7	46	83
RR2	346	547	479	457	102	21	480	95
RR3	515	540	667	574	81	14	715	80
RR4	569	519	654	581	69	13	518	112
RR5	<DL	<DL	<DL	<DL	0	0	0	100
RR6	317	347	369	344	26	6	438	79
RR7	108	110	123	114	8	8	104	110

Note: RR = shellfish homogenates; QC = Quality control; DL = Detection limit.

Table 4 PSP toxicity (as STX equivalent) in shellfish samples.

Sample no.	Sample code	Dilution*	nM STX from curve	nM STX equiv.	Extract volume (ml)	Sample weight (g)	µg STX equiv./sample	µg STX equiv./100g shellfish
1	B-BTB-08-03-13-1	1	33.121	198.726	10	5.04	0.074	14.668
		1/2	13.028	156.336	10	5.04	0.058	11.539
2	R-MK-08-03-04-1	1	1.305	7.830	10	5.08	0.0029	0.573
		1/2	0.584	7.008	10	5.08	0.0026	0.513
3	H-MK-08-03-04-1	1	0.388	2.328	10	4.98	0.0009	0.174
		1/2	0.207	2.484	10	4.98	0.0009	0.186
4	G-BT-08-03-25-1	1	4.231	25.386	10	5.02	0.0094	1.881
		1/2	2.347	28.164	10	5.02	0.0105	2.087
5	O-BT-08-03-25-1	1	1.771	10.626	10	5.05	0.0040	0.783
		1/2	0.957	11.484	10	5.05	0.0043	0.846
6	G-BT-08-03-24-1	1	9.059	54.354	10	5.03	0.0202	4.020
		1/2	5.096	61.152	10	5.03	0.0227	4.523
7	O-AS-08-03-24-1	1	0.643	3.858	10	5.05	0.0014	0.284
		1/2	0.273	3.276	10	5.05	0.0012	0.241
8	G-BS-08-03-24-1	1	1.579	9.474	10	5.05	0.0035	0.6979
		1/2	0.581	6.972	10	5.05	0.0026	0.5136
9	G-TK-08-03-19-1	1	19.34	116.04	10	5.19	0.0432	8.317
		1/2	7.123	85.476	10	5.19	0.0318	6.127
10	O-PD-08-03-18-1	1	3.345	20.07	10	4.99	0.0075	1.496
		1/2	1.626	19.512	10	4.99	0.0073	1.455

* The sample was run with no dilution and with a 1:2 dilution. All RSDs of calculated values were less than 30%. The quality control check standard on 3 assays was 2.482, 2.537 an d 2.695 nM, respectively, with the value of 3.0 nM.

accurate estimate of sample's overall toxicity. In the RBA method, tritiated STX (^{3}H-STX) and unlabeled toxin (STX) in the form of standard or sample compete for available receptors contained in a rat brain membrane preparation. The extent of radiolabeled receptor-toxin complex formation, which is inversely proportional to the amount of unlabeled toxin present, is determined by the detection of tritium, a low energy beta emitter (18.6 keV), using the highly sensitive and reliable liquid scintillation counting method.

Compared with the MBA, where external factors such as the health of the mice can affect the measures and the response is from a gross measurement (death time), the RBA is dependent on the receptor-based toxic activity and radioisotopic counting statistics. Thus, the RBA method can be considered a more refined technique with enhanced specificity, precision, and high sensitivity. Although the MBA has been reliable as an indicator of human oral potency, it cannot predict a bloom in advance because of its low sensitivity. The limit of detection for The MBA is 40 µg STX equiv/100g shellfish tissue, a level only two-fold less than the federal action level of 80 µg STX equiv/100g shellfish tissue, the point that harvesting of shellfish is prohibited. In contrast the limit of detection for The RBA is 0.2 µg STX equiv/100g shellfish tissue; this method is therefore capable of detecting the early stages of toxic algal blooms.[6] Thus, RBA for PSP has showed a valuable for rapid, reliable, cost-effective alternative to live animal testing and high throughput screening - prior to testing by the conventional mouse bioassay (MBA) and its suitability for providing an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.[8]

This study showed the validity of method on determination of PSP as STX equivalent in shellfish samples based on linearity, precision, accuracy and limit of quantification. The results from analyzing 7 unknown samples in triplicate in three different days revealed a high linearity with R^2 of 0.9964, RSD in assay of < 30%, a recovery ranging from 79 % to 133% and a limit of quantification of 2.57 µg STX equiv. /100 g shellfish. Based on 10 shellfish samples from the Gulf of Thailand, the result showed a good precision with RSDs between assays of 1.4 - 15.2%. In this study all shellfish samples collected from Gulf of Thailand showed low levels of STX, which were less than the maximum tolerance levels established by the European Union and the Food and Drug Administration (40 - 80 µg per 100 g edible portion of fresh, frozen, or tinned shellfish).[15] Based on the purpose of our institute, to promote the usage of radioisotope technique in marine biotoxins assay in Thailand, we thus have chosen the radioisotope technique to determine STX in shellfish extracted samples. In conclusion, we successfully set up a Marine Biotoxins Laboratory in TINT and produce a new radiolabeled kit to detect STX in Thailand with a high throughput screening for STX according to France van Dolah experience and her previous report.[8,18]

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