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Comparison of biosensor platforms for surface plasmon resonance based detection of paralytic shellfish toxins

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ABSTRACT

Paralytic shellfish poisoning (PSP) toxins are produced by certain marine dinoflagellates and may accumulate in bivalve molluscs through filter feeding. The Mouse Bioassay (MBA) is the internationally recognised reference method of analysis, but it is prone to technical difficulties and regarded with increasing disapproval due to ethical reasons. As such, alternative methods are required. A rapid surface plasmon resonance (SPR) biosensor inhibition assay was developed to detect PSP toxins in shellfish by employing a saxitoxin polyclonal antibody (R895). Using an assay developed for and validated on the Biacore Q biosensor system, this project focused on transferring the assay to a high-throughput, Biacore T100 biosensor in another laboratory. This was achieved using a prototype PSP toxin kit and recommended assay parameters based on the Biacore Q method. A monoclonal antibody (GT13A) was also assessed. Even though these two instruments are based on SPR principles, they vary widely in their mode of operation including differences in the integrated μ-fluidic cartridges, autosampler system, and sensor chip compatibilities. Shellfish samples ($n = 60$), extracted using a simple, rapid procedure, were analysed using each platform, and results were compared to AOAC high performance liquid chromatography (HPLC) and MBA methods. The overall agreement, based on statistical 2×2 comparison tables, between each method ranged from 85% to 94.4% using R895 and 77.8% to 100% using GT13A. The results demonstrated that the antibody based assays with high sensitivity and broad specificity to PSP toxins can be applied to different biosensor platforms.

1. Introduction

The development of alternative methods and the applicability of a variety of technology platforms for paralytic shellfish poisoning (PSP) toxin analysis in seafood is currently a highly topical debate, particularly within the European Union in relation to both established and new directives on the protection of animals used for scientific purposes [1]. Shellfish are eaten as a stable, nutritional diet in many parts of the world, though they are exposed to potential hazards in their natural environment. For example, molluscan bivalves and other filter feeders may accumulate and concentrate high levels of PSP toxins, which are produced by specific dinoflagellates and some cyanobacteria [2]. More than 20 analogues of saxitoxin (Fig. 1) are known that contribute to varying degrees of PSP [3]. All are neurotoxins which bind to voltage-dependent sodium channels and result in the blockage of channels and prevention of sodium ion movement across the membrane, which may lead to paralysis followed by death. However, each analogue displays a different toxic potency [4], and in the past the method of testing deemed to be most applicable for measuring combined toxicity was an animal bioassay. The Mouse Bioassay (MBA) is the AOAC Official Method [5] and internationally accepted technique for testing of PSP toxins in shellfish, but this method faces increasing technical and ethical concerns. Difficulties arise with the application of toxicity equivalent factors in developing alternative methods to animal bioassays for marine-toxin detection [6], particularly if analytical standards are unavailable. However, there is some progress in moving away from animal bioassays [7].

Recently, high performance liquid chromatography (HPLC) methods [4,8–11] have been developed and improved. The Lawrence HPLC method [12] has achieved AOAC approval, though it is not validated for all PSP toxins due to the unavailability of certain

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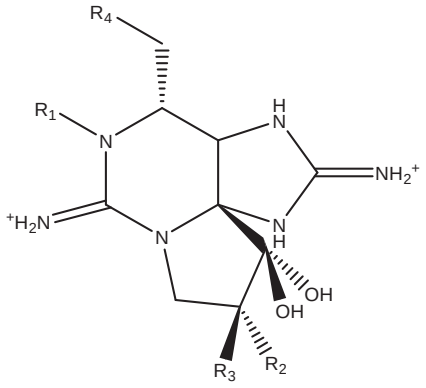


			Carbamate Toxins	*N*-Sulfocarbamoyl toxins	Decarbamoyl toxins	Deoxydecarbamoyl toxins
R_1	**R_2**	**R_3**	**R_4-** $OCONH_2$	**R_4-** $OCONHSO_3^-$	**R_4-** OH	**R_4-** H
H	H	H	STX[a]	B1 (GTX 5)	dc-STX	do-STX
H	H	OSO_3^-	GTX[b] 2	C1	dc-GTX 2	do-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3	do-GTX 3
OH	H	H	NEO[c]	B2 (GTX 6)	dc-NEO	
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1	
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4	

[a]STX: Saxitoxin [b]GTX: Gonyautoxin [c]NEO: Neosaxitoxin

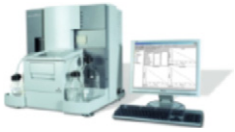
Fig. 1. Chemical structure of paralytic shellfish poisoning toxins; those with commercially available reference standards (National Research Council, Halifax, Canada) are highlighted.

certified reference materials (Fig. 1); however, it has been recently extended to include decarbamoyl neosaxitoxin and decarbamoyl gonyautoxin 2,3 [13]. Despite the method's AOAC validation, it has not been widely implemented as a regulatory tool given its time-consuming pre-column oxidation and sample preparation procedures as well as complex data analysis [14].

Within the European Union (EU) a regulatory limit of 800 μg of STX equivalents per kilogram of shellfish meat [15] has been implemented by the European Food Safety Authority (EFSA) [16]. However, EFSA has recommended that this limit should be lowered so that a 60 kg adult avoids exceeding the acute reference dose of 0.5 μg STX equiv./kg body weight. This in turn means that a 400 g portion of shellfish should not contain more than 30 μg STX equiv. corresponding to 75 μg STX equiv./kg shellfish meat. If the EFSA opinion is adopted, a method capable of detecting such a lower regulatory action level would be required.

The advent of the surface plasmon resonance (SPR) biosensor has allowed the development of rapid assays that can monitor interactions in real time. SPR biosensor based assays (i) offer rapid screening of analytes, (ii) require less sample preparation than most other methods, (iii) achieve low limits of detection and (iv) have undergone some level of validation. These advantages make SPR assays amenable to improvement, transferability and further validation that would allow for wider implementation as a monitoring tool. Assays have been developed using SPR instrumentation to test for a wide range of analytes including veterinary drug residues [17], mycotoxins [18], paralytic and diarrhetic shellfish poisoning toxins [19,20] and vitamins [21]. These particular assays were exclusively designed for and developed on one instrument, the Biacore Q SPR biosensor. However, the number of SPR biosensors available is increasing each year with variations in fluidics, automation, high-throughput sampling and multiplexing capabilities. One such instrument is the Biacore T100. Although both the Q and T100 instruments are SPR based, there are differences between the devices including the integrated μ-fluidic cartridges, autosampler system, pump system, sensor chip compatibilities, recommended buffer systems, and certain operating parameters (Table 1). These parameters essentially create two platforms: one that can be used for a wide range of research-grade analyses with the other more suited towards rapid quantification. The T100 instrument performs basic research and benefits from more elaborate software that allows for kinetic and thermodynamic analysis. In addition, the T100 has a design that enables higher throughput (4 flow cells at one time), continuous buffer degassing, 4-buffer selection capability, analysis temperature control, and in-line reference subtraction. The Q instrument is dedicated to concentration analysis, and, as such, requires less rigorous software and design elements which allow for a lower cost, easier-to-use instrument for food analysis. In addition, the Q system is designed to work with the Q-flex kits for vitamin and veterinary drug residue analysis as well as prototype kits such as the one for PSP analysis used herein. The transferability of SPR assay kits to different SPR platforms would allow this PSP toxin assay to be more amenable in regulatory and monitoring laboratories, as this would offer a choice of equipment that could be employed. To investigate this transferability and applicability of methods between two different SPR instruments in two organisations, the recently developed and validated screening assay [19,22] for PSP toxins on the Biacore Q, as part of the BioCop project [23], was chosen as a model assay. The previous single laboratory validation of this method on the Biacore Q deemed it to be a valid screening tool for the detection of PSP toxins in bivalve molluscan shellfish. The aim of the current study was to investigate the technology transfer of this SPR assay between laboratories using two different SPR platforms with two different antibodies in an inter-laboratory study.

Table 1
Comparison of the SPR Biosensors used in the current study. (Information courtesy of www.biacore.com).

SPR systems	 Biacore Q	 Biacore T100
Uses	System dedicated for screening or quantification, assay development and concentration analysis	Used for concentration analysis (with/without calibration), kinetic and thermodynamic analysis, specificity and affinity analysis, epitope mapping
Sensor surface	One needle enables injection over one flow cell at a time, 4 flow cells in parallel	One needle with single, pair wise and serial injection options over 4 flow cells which are in series
Sensor chips	Reusable sensor chips: classic format 	Reusable sensor chips: series S format 
Sample capacity	96-well microplate format	96-well/384-well microplate format
Dynamic range	0–40,000 RU	0–70,000 RU
Temperature control	25 °C	4–45 °C
Injection volume	5–325 μL	2–350 μL
Pump system	Electronic syringe driven pumps	Electronic syringe and peristaltic driven pumps
Analysis time per cycle	Typically 2–10 min	Typically 2–15 min
Wizard based methods	Yes	Yes
In-line degassing	No	Yes
Interfacing	LIMS export capability	LIMS export capability
Automation	Up to 24 h unattended	Up to 48 h unattended with potential for laboratory robot interface

2. Materials and methods

2.1. Instrumentation

A Biacore Q SPR biosensor with control software version 3.0.4, Biacore Q Evaluation software version 1.0, and BIAevaluation software version 4.1 were purchased from Biacore (GE Healthcare, Uppsala, Sweden). Separately, a Biacore T100 with control software version 2.0 and Biacore T100 Evaluation software version 1.1.1 were obtained from Biacore (GE Healthcare, Piscataway, NJ). Classic CM5 and Series S CM5 sensor chips were purchased from Biacore for each instrument, respectively.

2.2. Chemicals and reagents

Saxitoxin dihydrochloride (STXdiHCl, 65 μM), as certified reference standard material, was obtained from the Institute for Marine Biosciences, National Research Council (NRC), Halifax, Canada. Saxitoxin dihydrochloride was also supplied by the FDA (College Park, MD, USA) and used for immobilisation of the toxin onto sensor chips. An amine coupling kit, HBS-EP buffer (pH 7.4, 0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20, ready-for-use) and HBS-EP+ (0.1 M HEPES, 1.5 M NaCl, 30 mM EDTA and 0.5% v/v Surfactant P20, diluted 10× in water before use to yield pH 7.4) were obtained from GE Healthcare. For each system, buffer was either HBS-EP (Biacore Q) or HBS-EP+ (Biacore T100), referred to as running buffer henceforth. Formaldehyde (37%), 2,2-(ethylenedioxy)bis-(ethylamine) (Jeffamine), glacial acetic acid, sodium acetate (minimum 99%), boric acid, and hydrochloric acid solution 1 M were purchased from Sigma–Aldrich (UK and USA). A prototype PSP toxin kit containing antibodies (polyclonal (R895) and monoclonal (GT13A)), HBS-EP buffer, instructions for use and accessories was produced at the Institute of Agri-Food and Land Use (IAFLU) at the Queen's University of Belfast (Northern Ireland).

2.3. Shellfish samples

PSP toxin-free shellfish homogenates, pre-determined by both the AOAC HPLC and MBA methods, were obtained from the Agri-Food and Biosciences Institute, Belfast, Northern Ireland. The species obtained were mussels (*Mytilus edulis*), cockles (*Cerastoderma edule*), clams (*Veneridae* spp.), oysters (*Crassostrea gigas*) and scallops (*Pecten maximus*). Naturally contaminated shellfish samples were collected from a number of regulatory laboratories including: (i) UK National Reference Laboratories: the Fisheries Research Centre (FRS), Scotland; (ii) the Agri-Food and Biosciences Institute, Belfast, Northern Ireland; and (iii) the Autonomous Government Laboratory for shellfish monitoring in Andalucía, Spain to ensure that tissues containing variable PSP toxin profiles were included in the study.

2.4. Immobilisation of STX on the sensor chip surface

STX was covalently immobilised to the surface of CM5 Classic (Biacore Q) and Series S (Biacore T100) chips by a modification of the amino-amino coupling methods described by Campbell et al. [22] and Yakes et al. [24]. For the Biacore Q, EDC and NHS, as prepared according to the amine coupling kit, were mixed (1:1, v/v) and applied to the chip surface for 30 min. Excess solution was removed, and Jeffamine solution was added to the chip surface and allowed to react for 1 h. The chip surface was deactivated by addition of ethanolamine (1 M, pH 8.5) for 30 min leaving an amine modified surface. For the Biacore T100, jeffamine immobilisation and ethanolamine blocking was performed in the instrument [22]. For both sensor chips, the amine surface preparation was followed by immobilisation of STXdiHCl onto the amine surface via amino–amino coupling using 37% formaldehyde (overnight, 37 °C in a humidity chamber). The sensor chip surface was then washed with deionized water, dried using a stream of nitrogen gas, and stored desiccated at 4 °C when not in use.

2.5. Polyclonal antibody (R895)

Rabbit polyclonal antibody (R895) produced from a STX-Jeffamine-BSA protein conjugate was prepared as previously detailed by Campbell et al. [22]. The polyclonal antibody (R895) was diluted to 1/50 (1 mg/mL) for the prototype kit and to a final working dilution of 1/250 (0.2 mg/mL) in running buffer for use in the assay. Full characterisation of this antibody in terms of specificity/cross-reactivity was previously reported by Campbell et al. [19].

2.6. Monoclonal antibody (GT13A)

Monoclonal antibody GT13A, raised to gonyautoxin 2/3 (GTX2/3)-keyhole limpet hemocyanin (KLH) protein conjugate, was produced as previously described [25] and assessed on the SPR biosensor by Campbell et al. [22]. This antibody was used at a final working dilution of 1/500 (0.004 mg/mL) for the SPR assays.

2.7. Preparation of assay calibration curve

STXdiHCl calibrants (0, 1.0, 2.5, 5.0, 7.5, 10.0 and 100.0 ng/mL) were prepared in running buffer from the NRC stock solution. These are equivalent to 0, 120, 300, 600, 900, 1200 and 12000 μg of STXdiHCl per kilogram shellfish based on the assay parameters. For the SPR assays with the polyclonal antibody (R895), calibrants 0–10 ng/mL were used, whereas for assays using the monoclonal antibody (GT13A) calibrants 0–100 ng/mL were used to construct the respective calibration curves.

2.8. Extraction method for shellfish samples

The extraction procedure was carried out as recommended by Campbell et al. [19]. Shellfish samples were removed from their shells and drained. Shellfish meat (100 g) was homogenised using a Waring blender, and the homogenised tissue was stored frozen at −20 °C until required. Samples (1 g) of homogenised shellfish tissue were weighed into centrifuge tubes and 0.1 M sodium acetate buffer pH 5 (5 mL) was added. Each tube was vortexed for 10 s and roller mixed for 30 min. Following mixing, samples were centrifuged at 3600 *g* for 10 min at room temperature, and the supernatant was collected and diluted 1 in 20 in HBS-EP buffer.

2.9. SPR analysis–instrumental parameters

Analyses were performed using the Biacore Q SPR biosensor at the Queen's University of Belfast (Belfast, Northern Ireland) and on the Biacore T100 SPR biosensor at the US Food and Drug Administration (College Park, MD, USA). The optical biosensor uses the phenomenon of SPR to exploit the behaviour of light at boundaries of different refractive indices to monitor biomolecular reactions, in this case between gold coated glass and sample solution. The technology detects the change in reflected light angle from the side of the chip opposite of the flow channel according to the mass bound to the surface of the chip. The SPR biosensor technique does not require any labelling of the interacting components, and the interactions are measured as they occur (real time) with an analysis time of minutes. The speed of analysis and low running costs of the biosensor make it well suited for high-throughput toxin screening.

The SPR biosensor method used herein is an inhibition assay. A known dilution of saxitoxin binding protein (R895 or GT13A, SBP) is mixed with the sample and injected over the surface of a sensor chip to which saxitoxin has been immobilised. If no PSP toxins are present in the sample then the SBP is free to bind to the immobilised saxitoxin thus producing a response. If PSP toxins are present in the sample then these will bind to the SBP, thereby inhibiting it from binding to the immobilised saxitoxin on the chip surface and thus producing a lower response. The level of inhibition is proportional to the concentration of PSP toxins present in the sample. The binding events are monitored in the form of a sensorgram (plot of binding response versus time).

The following assay parameters were developed and validated on the Biacore Q biosensor and then replicated on the Biacore T100 instrument without further validation. The assay parameters on the instrument were set to mix the relevant antibody with an equal volume of STXdiHCl calibrant (or sample) prior to injection over the STX sensor chip surface. The flow rate across this chip surface was 12 μL/min and the contact time of the antibody-calibrant (sample) mixture with the surface was 120 s. Report points were recorded before (10 s) and after each injection (30 s), and the relative response units (RU) were determined. The chip surface was regenerated with 8 μL injections of hydrochloric acid (50 mM) at a flow rate of 12 μL/min.

2.10. Analysis of samples

Samples ($n = 60$), including mussels, cockles and scallops, both toxin-free and naturally contaminated with PSP toxins, were analysed with both SPR instruments (using 2 different antibodies) for the detection of PSP toxins and concentrations were determined against STXdiHCl calibration curves. Where possible, samples were also analysed using both the AOAC HPLC and mouse bioassay methods performed at the UK National Reference Laboratory AFBI, Northern Ireland.

3. Results and discussion

A SPR biosensor assay for PSP toxin screening had been previously developed and validated on the Biacore Q instrument [19,22]. The Biacore T100 instrument was chosen as an alternative platform to examine the transferability and applicability of the assay between laboratories with different instrumentation. Although both instruments are based on SPR detection they vary widely in the operation parameters and specifications (Table 1). The important differences between the instruments are that the Biacore T100 system has the potential for additional automated high-throughput screening and multiplexing capabilities due to the potential interface with a robot, the use of 384-microwell plates and the capability to use serial injections over the four flow cells. This potential of increased throughput on the Biacore T100, as well as in-line buffer degassing along with temperature control, means that three analytical measurements can be run in the same time it takes to run one on the Biacore Q. However, if capital equipment costs and ease of operation and maintenance are the main considerations for routine monitoring laboratories, then the less expensive Biacore Q would be the instrument of choice. The PSP toxin assay is based on the inhibition of antibody binding to immobilised STX in the presence of PSP toxins in the sample. Fig. 2 illustrates an overlay of the typical sensorgrams obtained for the STXdiHCl calibration curve on the Biacore Q and Biacore T100 SPR biosensors. A baseline reading followed by a bulk effect and binding of the antibody, a sharp drop at the end of injection and a consistent deterioration in response (due to initial dissociation of the antibody) was observed. The sensorgram is completed by the regeneration of the chip surface in preparation for the next sample injection (data not shown). The biosensors are real-time instruments and each stage can be monitored as it occurs. Report points can be recorded at any point and set to record the amount of binding at a fixed time window for each analysis cycle. The major difference between the sensorgrams for each instrument was where the absolute response started, i.e. ~15,500 RU (Biacore Q) and ~36,000 RU (Biacore T100), due to differences in the set up for the optical detection units. It is also of note that the Biacore T100 shows a higher matrix effect due to the mismatch of the HBS-EP+ running buffer with the background from antibody stock being in HBS-EP.

Fig. 3 illustrates %-normalised response versus concentration calibration curves obtained on the Biacore Q and Biacore T100 biosensors using STXdiHCl as the calibrant with both the polyclonal (R895) and monoclonal (GT13A) antibodies (average error value <1%, data not shown). From these calibration curves, the IC_{20}–IC_{80} dynamic ranges and IC_{50} values were calculated. Using the R895, the range was found to be 2.02–6.40 ng/mL

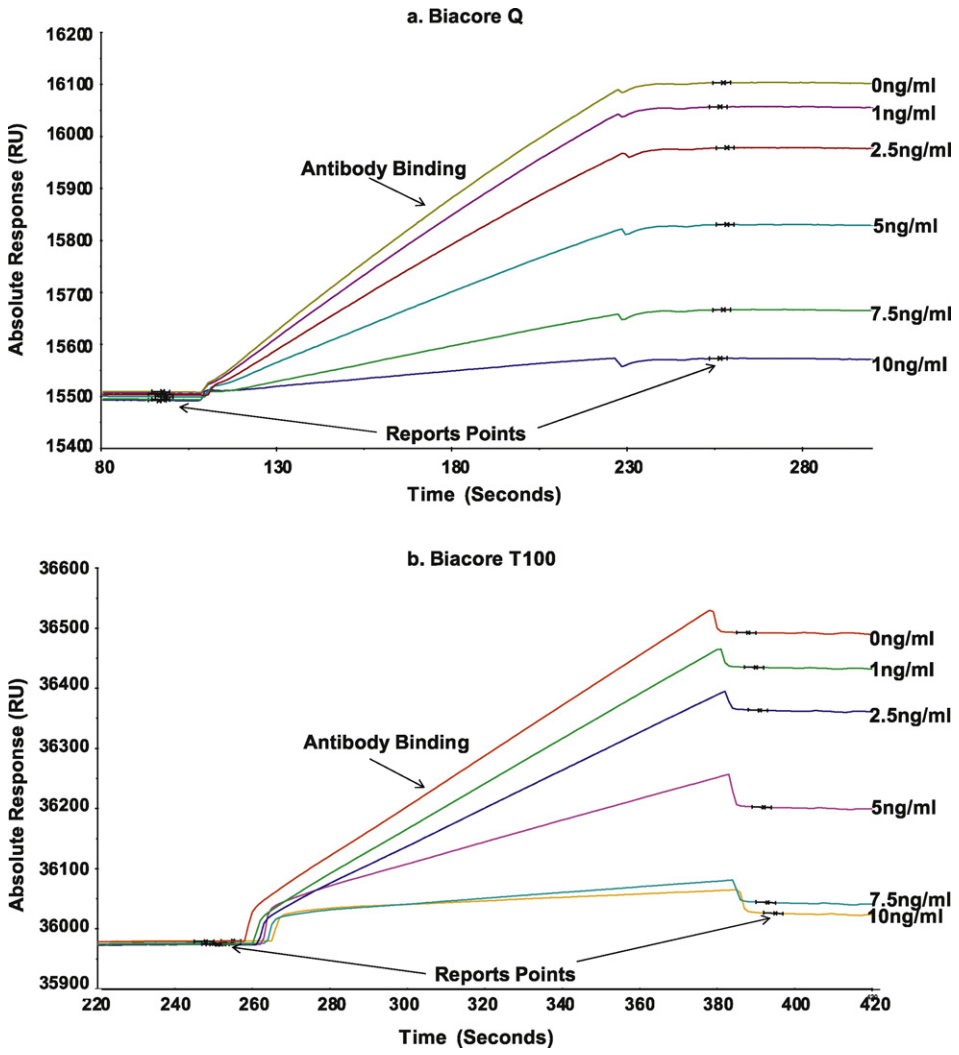


Fig. 2. Typical sensorgrams obtained on: (a) Biacore Q and (b) Biacore T100 using the R895 polyclonal antibody.

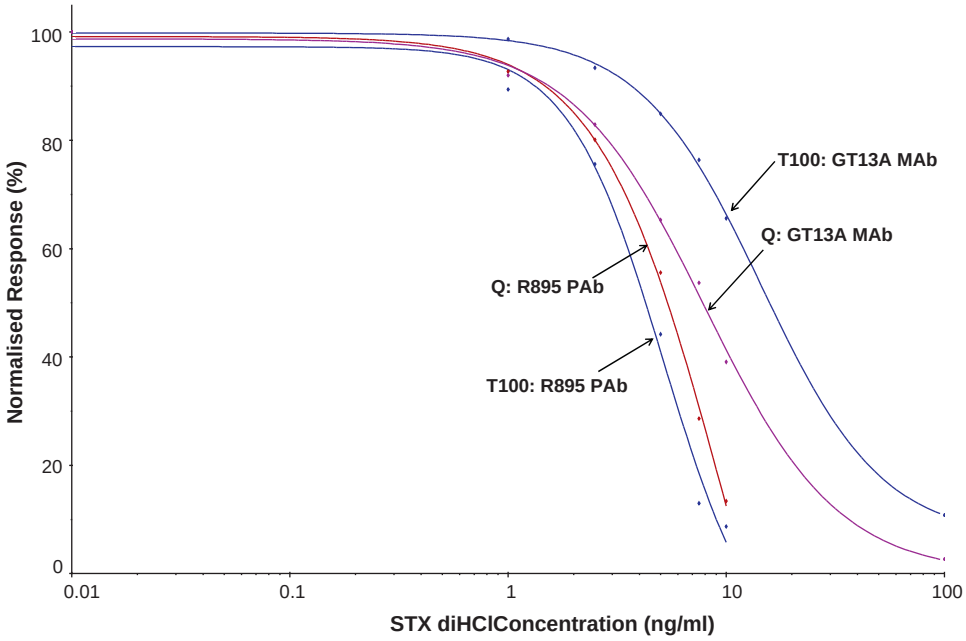


Fig. 3. Comparison of calibration curves obtained on each SPR biosensor with both of the antibodies.

Table 2
Qualitative results of the shellfish samples analysed by the Biacore Q and T100 assay (using R895 and GT13A antibodies), HPLC, and MBA. Positive (+) and negative (−) results are indicated based on the 800 μg STX equiv./kg regulatory action limit.

Sample No	Shellfish species	Biacore Q (μg STXdiHCl/kg) (R895)	T100 (μg STXdiHCl/kg) (R895)	Biacore Q (μg STXdiHCl/kg) (GT13A)	T100 (μg STXdiHCl/kg) (GT13A)	HPLC (μg STX equiv./kg)[a]	MBA (μg STX equiv./kg)
1	Cockles	−	−	−	−	−	NR
2	Mussels	−[b]	−[b]	+	+	+	−[b]
3	Mussels	+	+	+	+	+	+
4	Scallops	−[b]	−[b]	+	+	−	−
5	Mussels	−[c]	+	−[b]	−[b]	+	NR
6	Mussels	+	+	+	+	−[b]	+
7	Cockles	+	+	+	+	−	−[b]
8	Mussels	+	+	−[c]	−[b]	+	NR
9	Mussels	−	−	−	−	−	−
10	Scallops	−[b]	−[b]	+	+	−	−
11	Mussels	+	+	+	+	+	+
12	Mussels	+	+	+	+	+	+
13	Cockles	−	−	−	−	−	NR
14	Mussels	+	+	+	+	+	+
15	Clams	+	+	+	+	+	NR
16	Cockles	+	+	+	+	−	−[b]
17	Mussels	−	−	−	−	−	−
18	Mussels	+	+	+	+	+	+
19	Clams	+	+	+	+	+	NR
20	Mussels	−[b]	−[b]	+	+	+	−[b]
21	Mussels	−[b]	−[b]	+	+	−[b]	−[b]
22	Mussels	−	−	−	−	−	−
23	Mussels	+	−[b]	+	+	−[b]	+
24	Scallops	−[b]	−[b]	+	+	−	−[b]
25	Scallops	−[b]	−[b]	+	+	+	−[b]
26	Clams	−	−	−	−	−	−
27	Clams	−	−	−	−	−	−
28	Scallops	+	−[c]	+	+	+	−[b]
29	Mussels	+	−[c]	+	+	+	+
30	Scallops	−	−	−	−	−	−
31	Clams	−	−	−	−	−	−
32	Clams	−	−	−	−	−	−
33	Mussels	+	−[b]	+	+	−[b]	+
34	Scallops	−	−	−	−	−	−
35	Cockles	−	−	−	−	−	−
36	Scallops	−	−	−	−	−	−
37	Scallops	−	−	−	−	−	−
38	Scallops	−[b]	−[b]	+	+	−	−
39	Scallops	−	−	−	−	−	−
40	Scallops	−	−	−	−	−	−
41	Scallops	−	−	−	−	−	−
42	Mussels	+	+	+	+	+	+
43	Mussels	−[b]	−[b]	+	+	−[b]	−[b]
44	Mussels	+	+	+	+	+	+
45	Mussels	+	+	+	+	+	+
46	Mussels	−	−	−	−	−	−
47	Cockles	+	+	+	+	+	+
48	Cockles	+	+	+	+	+	+
49	Cockles	+	+	+	+	+	+
50	Cockles	+	+	+	+	+	+
51	Oysters	−	−	−	−	−	−
52	Oysters	−	−	−	−	−	−
53	Oysters	−	−	−	−	−	−
54	Scallops	−	−	−	−	−	−
55	Scallops	−	−	−	−	−	−
56	Cockles	+	+	+	+	+	+
57	Cockles	+	+	+	+	+	+
58	Oysters	−	−	−	−	−	−
59	Oysters	−	−	−	−	−	−
60	Cockles	+	+	+	+	+	+

NR Not Run by MBA method due to lack of sample.
[a] HPLC results based on NRC toxic equivalency factor (TEF) [4].
[b] Value >400 μg/kg (half the action limit).
[c] Value >750 μg/kg but <800 μg/kg indicating the presence of a PSP toxin, although negative due to being just below the action limit.

(equivalent to 242–768 μg STXdiHCl/kg) with IC_{50} of 3.95 ng/mL (473 μg STXdiHCl/kg) on the Biacore T100, whereas the range was found to be 2.24–7.51 ng/mL (269–901 μg STXdiHCl/kg) with IC_{50} of 4.96 ng/mL (563 STXdiHCl/kg) on the Biacore Q. Using the GT13A, the range was found to be 5.72–30.5 ng/mL (686–3663 μg STXdiHCl/kg) with IC_{50} of 13.63 ng/mL (1635 μg STXdiHCl/kg) on the Biacore T100, whereas the range was found to be 2.80–18.93 ng/mL (335–2271 μg STXdiHCl/kg) with IC_{50} of 7.51 ng/mL (901 STXdiHCl/kg) on the Biacore Q. These results highlight the differences in sensitivity between the two

Table 3
Statistical 2 × 2 comparative analysis of the qualitative results for each analytical method showing the overall agreements between techniques for (a) the polyclonal antibody (R895) and the monoclonal antibody (GT13A) and (b) MBA/HPLC correlation.

(a) SPR assays with polyclonal antibody (R895) and monoclonal antibody (GT13A)

Polyclonal Ab R895					Monoclonal Ab GT13A				
		Biacore Q					Biacore Q		
		+	–	Total			+	–	Total
T100	+	21	1	22	T100	+	33	0	33
	–	4	34	38		–	0	27	27
	Total	25	35	60		Total	23	27	60
		Overall agreement		91.7%			Overall agreement		100%
		Biacore Q					Biacore Q		
		+	–	Total			+	–	Total
HPLC	+	20	4	24	HPLC	+	22	2	24
	–	5	31	36		–	11	25	36
	Total	25	35	60		Total	33	27	60
		Overall agreement		85.0%			Overall agreement		78.3%
		Biacore Q					Biacore Q		
		+	–	Total			+	–	Total
MBA	+	19	0	19	MBA	+	19	0	19
	–	3	32	35		–	12	23	35
	Total	22	32	54		Total	31	23	54
		Overall agreement		94.4%			Overall agreement		77.8%
		T100					T100		
		+	–	Total			+	–	Total
HPLC	+	19	5	24	HPLC	+	22	2	24
	–	3	33	36		–	11	25	36
	Total	22	38	60		Total	33	27	60
		Overall agreement		86.7%			Overall agreement		78.3%
		T100					T100		
		+	–	Total			+	–	Total
MBA	+	16	3	19	MBA	+	19	0	19
	–	2	33	35		–	12	23	35
	Total	18	36	54		Total	31	23	54
		Overall agreement		90.7%			Overall agreement		77.8%

(b) MBA/HPLC qualitative correlation

		MBA		
		+	–	Total
HPLC	+	16	4	20
	–	3	31	34
	Total	19	35	54
			Overall agreement	87.0%

antibodies on each instrument. This could be due mainly to differences in the preparation of the sensor chip surfaces and μ-fluidics systems but highlights the need for calibration curves.

3.1. Analysis of naturally contaminated samples

Sixty shellfish samples were extracted and analysed at the Queen's University of Belfast and at the FDA on the Biacore Q and Biacore T100 instruments, respectively. The extraction procedure is very simple compared to the alternative HPLC and MBA sample preparation [5,12]. For example, the extraction procedure used in this study involves the mixing of homogenised shellfish with acetate buffer followed by centrifugation and dilution (1/20) of the supernatant with HBS-EP buffer compared to having pre-column oxidation, solid phase extraction and boiling steps. The final dilution (1/20) is required to correlate the concentration of the PSP toxin in a sample to the buffer based STXdiHCl calibration curve. This also reduces matrix effects from the shellfish extract. However, the assay does not distinguish between PSP toxins or assess toxicity of the samples but simply indicates the presence or absence of these toxins with respect to their antibody reactivity. The SPR assay on both instruments was run with the polyclonal antibody (R895) and the monoclonal antibody (GT13A) as a further comparison of the transferability and applicability of the assays between instruments. The cross-reactivity profile of both these antibodies for commercially available PSP toxins has been published previously [22,26].

Table 2 shows the qualitative data for the PSP toxin-contaminated samples including mussels, cockles, clams, oysters and scallops analysed using both SPR instruments, HPLC and MBA. Some samples were not analysed by the MBA due to the limited amounts of sample material available. All results found close to the action limit and at greater than half the action limit are also indicated in the table. It should be noted that the cross reactivity profile of the two antibodies in relation to the saxitoxin analogues as previously published [22,26] accounts for the variability in some samples particularly those at close to the threshold level. GT13A has substantial cross reactivity to the non-hydroxylated toxins compared to the hydroxlated toxins. In relation to toxicity, this antibody cross reacts highly with the less toxic toxins such as C1/C2 and GTX5 which may cause elevated levels in the results data compared to other methods. If high levels of hydroxylated toxins are present in the sample this antibody could underestimate the toxin level due to the low cross reactivity to these toxins. However the cross reactivity profile for R895 shows a better correlation with toxicity of each analogue as in the MBA compared to GT13A.

Statistical 2 × 2 comparative analyses were performed for the sample data between each of the detection methods based on

whether the sample was determined as being above (+) or below (−) the regulatory action limit of 800 μg STX equiv./kg (Table 3). For the sample set analysed using the polyclonal antibody (R895) for the SPR assays, the highest overall agreement was found between the Biacore Q and MBA data (94.4%), followed by the overall agreement between the Biacore T100 and the Biacore Q data (91.7%). It should be noted that four of the Biacore Q/T100 results were found to be >780 μg STX equiv./kg but <800 μg STX equiv./kg and therefore considered negative for this dataset. However, regulatory laboratories finding results so close to the regulatory limit, using a screening test, would consider these as suspect and worthy of further confirmatory analysis. The lowest overall agreements were found to be between the Biacore Q and HPLC data (85.0%) and between Biacore T100 and HPLC data (86.7%). However, it should be noted that some of the results again occurred close to the regulatory action level.

For the sample set analysed using the monoclonal antibody (GT13A) for the SPR assays, the highest overall agreement was found between the Biacore Q and T100 data (100%), followed by the overall agreement between both the Biacore T100 and Biacore Q data with the HPLC dataset (78.3%). The lowest overall agreements were found to be between both the Biacore Q and T100 data with MBA data (77.8%). The lower overall agreement between both the MBA and HPLC data compared with the SPR data is probably due to the high cross-reactivity of GT13A with GTX2,3 (163%), C1/C2 (175%), dcGTX2,3 (216%) and GTX5 (137%) which does not correlate with the toxicity factors for the MBA [22].

The high values obtained for the overall agreement (77.8% to 100%) between all techniques is significant since these include biological, biochemical and physiochemical analytical methods with different extraction procedures. The wide range of matrices analysed (cockles, mussels, clams, oysters and scallops) demonstrated that the simple analysis protocol herein is applicable to a variety of shellfish species.

Considering that the prototype kit assay was not developed for the Biacore T100 and that no validation of the protocols on this instrument was undertaken before analysis, the results and correlation between the two SPR instruments were found to be excellent. The results indicate that, with use of well characterised and specific antibodies and good replication of surface immobilisation chemistry between different sensor chips, the transferability and applicability of methods between laboratories and different SPR instruments is very much achievable. Future work could include: (i) multiplexing, high-throughput screening, and validation of the PSP toxin assay on the Biacore T100; (ii) investigating the transferability of the prototype kit to SPR instruments manufactured by other companies; (iii) exploring the technology transfer of the prototype kit to other non-SPR biosensors.

4. Conclusions

A validated assay for the screening of PSP toxins in shellfish using the Biacore Q SPR instrument in one laboratory was transferred to the high-throughput Biacore T100 biosensor in another laboratory. The technology transfer was conducted through use of a prototype PSP toxin kit, containing polyclonal and monoclonal antibodies with assay parameters based on the Biacore Q method. Shellfish samples were successfully analysed on both instruments and results compared to HPLC and MBA results with statistical overall agreements ranging from 77.8% to 100% indicating very good to excellent correlation between the qualitative datasets. This data shows that this technology is highly suitable as a rapid screening test for PSP toxin analysis particularly if used in tandem with the AOAC HPLC method for confirmation of toxin analogue and quantification for PSP contaminated samples. This combination could potentially reduce the number of samples for confirmatory analysis and the need for the MBA for this particular test.

In addition the successful transfer of this model screening assay illustrates the possibility for the technology transfer of other Biacore Q assays, such as those for mycotoxins, veterinary drugs and vitamins, to the high throughput Biacore T100 biosensor and potentially other SPR platforms.

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