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Generation of a panel of high affinity antibodies and development of a biosensor-based immunoassay for the detection of okadaic acid in shellfish

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Abbreviated title: Biosensor-based assay for okadaic acid

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Abstract

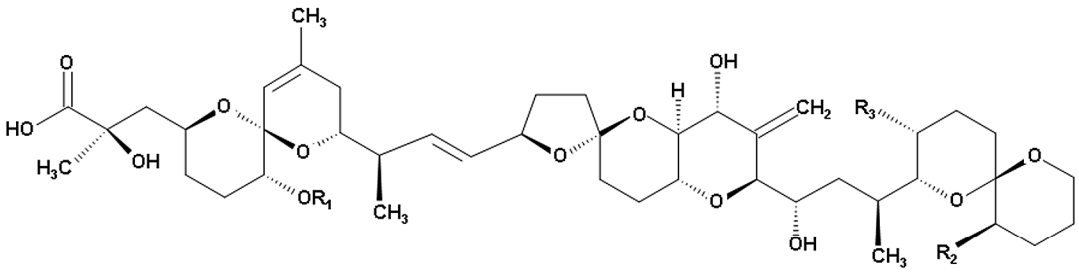
Okadaic acid (OA) and its derivatives, DTX-1 and DTX-2, are marine biotoxins associated with diarrhetic shellfish poisoning. Routine monitoring of these toxins relies on the mouse bioassay. However, due to the technical unreliability and animal usage of this bioassay, there is a need for convenient and reliable alternative assay methods. A panel of monoclonal antibodies against OA was generated and the most suitable was selected for surface plasmon resonance (SPR)-based assay development. SPR-based biosensors have been shown to be highly reproducible immuno-analytical tools, with potential for large scale screening applications. The cross reactivity of the selected antibody with DTX-1 was found to be 73%, confirming its suitability for assay development. The OA and derivative assay was designed as an inhibition assay covering the concentrations 1 to 75 ng/ml, with a sensitivity of 22.4 ng/ml. The regulatory limit for OA and DTXs is 160 ng/g, equivalent to 12.8 ng/ml in crude extract, and this concentration was within the linear range of the assay which was 11.2 to 38.8 ng/ml. The assay was highly reproducible with a coefficient of variability (%CV) of <7.3 %. Preliminary validation showed no matrix interference from mussel extracts and good recovery of added standard in mussel extracts, with %CV of <9.35%. This assay could provide a useful and convenient screening tool for OA and its derivatives with a comprehensive extraction protocol for shellfish monitoring programmes.

Keywords

biosensor, okadaic acid, DSP, monoclonal antibody, shellfish, immunoassay

1. Introduction

Okadaic acid (OA) and its derivatives, the dinophysis toxins DTX-1 and DTX-2, are structurally related, lipophilic, toxic, polyether compounds produced by dinoflagellates of the genera Prorocentrum and Dinophysis (Lee *et al*., 1989) (Figure 1). These biotoxins are associated with diarrhetic shellfish poisoning (DSP). OA was first isolated from the sponge *Halichondria okadaii* (Tachibana *et al*., 1981) and later, OA and its derivatives, including a third derivative DTX-3, were purified from contaminated shellfish (Yasumoto *et al*., 1984). DTX-3, the 7-*O*-acyl derivative, was found to be a metabolic by-product of the parent toxins in the shellfish and not a *de novo* product synthesised by phytoplankton (Suzuki *et al*., 1999).



Toxin	R_1	R_2	R_3
Okadaic acid (OA)	H	H	CH_3
dinophysistoxin-1 (DTX-1)	H	CH_3	CH_3
dinophysistoxin-2 (DTX-2)	H	CH_3	H
dinophysistoxin-3 (DTX-3)	acyl	CH_3	CH_3

Figure 1. Chemical structure of okadaic acid and the dinophysis toxins, DTX-1, -2 and -3.

Filter-feeding marine species which are consumed by humans, such as mussels (*Mytilus edulis*), clams (*Siliqua patula*) and scallops (*Pecten maximus*), accumulate these toxins in their digestive tissues, facilitating their entry into the human food chain and causing DSP. Although no fatalities have been reported, the worldwide occurrence of DSP has made it a serious threat for the

shellfish industry and public health. National shellfish monitoring programmes have been implemented to protect consumers, as well as the shellfish industry, and to promote international harmonisation of biotoxin monitoring. In Europe, the level of OA must not exceed 160 ng/g of shellfish (European Communities decision, 2002/225/EC).

Shellfish monitoring for the presence of DSP toxins relies on the mouse bioassay (MBA) (Yasumoto *et al.*, 1984) and rat bioassay (Kat, 1983), in which three mice or rats are fed with shellfish extract as stipulated by EU regulations (EU Commission regulation No. 15/2011, amending EC regulation No. 2074/2005). A sample is considered positive if two out of three mice die and if a diarrhetic response is observed in any of the three rats. However, the MBA test lacks specificity and is recognised as having poor reproducibility and high variability (Jellett, 1993; Campbell *et al.*, 2011). The assay is also prone to interference from free fatty acid, leading to false positive (Suzuki *et al.*, 1996). Alternative methods of detection have to be used for routine monitoring of shellfish as of the end of 2014 due to technical and ethical problems associated with the MBA to fulfill requirements set by the European Union Reference Laboratory (EU-RL) (EU Commission regulation No. 15/2011, amending EC regulation No. 2074/2005). A number of alternative methods have already been proposed for the detection of OA and derivatives including liquid chromatography-mass spectrometry (LC-MS)-based analysis, which inter-laboratory validation study demonstrated its suitability as alternative detection method (Van den Top *et al*., 2011), and a colorimetric protein phosphatase 2A (PP2A) assay was also demonstrated suitable as alternative method (Smienk *et al.*, 2012, 2013). Immunoassays have also been developed for the detection of OA, such as ELISA-based assay developed by Kreuzer *et al*. (1999), which relied on commercial antibodies. Automated Surface plasmon resonance (SPR) - based biosensors are attractive alternative to these assays which can

be time consuming. The technology initially developed for research, such as screening of biological samples for binding partners and kinetic analysis, is a very useful and reliable quantitative tool for detection of contaminants in biological fluids. When compared to other analytical quantitative methods, such as high performance LC (HPLC), LC-MS and plate-based ELISAs, SPR-based biosensors offer significant advantages in reproducibility, speed, automation, simplicity and the possibility for high throughput analysis with minimal sample preparation.

This work describes the generation of a panel of high-affinity monoclonal anti-OA antibodies, and the development and optimisation of an inhibition biosensor-based immunoassay using SPR.

2. Materials and methods

2.1. Instrumentation

A BIACORE 2000™ biosensor instrument and CM5 sensor chips (research grade) were used (Biacore Life Science, GE Healthcare, UK). The BIACORE 2000™ was controlled by BIACORE control software version 3.2 running under Windows XP. The instrument running temperature was 25 °C.

2.2. Reagents

HEPES buffered saline supplemented with EDTA and surfactant (HBS-EP; 10 mM HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4), and the amine coupling kit (containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'*(3-ethylaminopropyl) carbodiimide (EDC), and ethanolamine hydrochloride) were obtained from Biacore Life Science. OA was purchased from LC Laboratories (U.S.A.) and DTX-1 from Wako Laboratories (Japan). Sodium hydroxide

(pellet, NaOH) was purchased from BDH Chemical Ltd. (UK) and acetonitrile analytical grade from Romil (Lennox, Ireland). Protein G Sepharose™ was a product of Amersham Biosciences (Sweden). Bicinchoninic acid (BCA) protein assay kit was obtained from Thermo Scientific (Ireland). Bovine serum albumin (BSA), ovalbumin (OVA), EDC, NHS, *N*,*N*-dimethylformamide (DMF) and all other reagents were purchased from Sigma and were of the highest grade available.

2.3. Generation of mouse monoclonal antibodies against OA

OA was conjugated to bovine serum albumin (BSA) for immunisation using EDC and NHS coupling, according to the method of Kreuzer *et al*. (1999). In brief, EDC and NHS were added to OA in DMSO, at 20 and 3.3 molar excess over OA, respectively. Following 30 min activation at 37 °C, BSA was added at a 50:1 BSA to OA ratio and the conjugation mixture was incubated for 24 h at 37 °C. The OA-BSA conjugate was purified by dialysis against phosphate buffered saline (PBS), pH 7.2 overnight at 4 °C. OA was coupled to OVA for use in screening assays following the same procedure.

Six to eight weeks old Balb/C mice were injected three times at four week intervals with OA-BSA at a concentration of 50 µg in 150 µl of PBS and emulsified by addition of an equal volume of Freund's complete adjuvant. A week after the last intraperitoneal boost, the tail vein was bled and the serum tested for the presence of anti-OA antibody using antibody capture and indirect competitive ELISA (see section 2.4 below). The mouse with the highest affinity of the antiserum for its antigen, as determined by effective dose 50 (ED-50; the concentration of free OA required to inhibit the binding of the antiserum or antibody to the immobilised antigen by 50%), was selected for the generation of monoclonal antibodies by cellular fusion. Myeloma SP2/mIL-6

cells (Harris *et al*, 1992) were fused with spleen cells of the selected animal in the presence of polyethylene glycol (Köhler and Milstein, 1975). Individual clones were isolated by limiting dilution and antibody-producing clones were selected by their ability to bind and displace free OA on a competitive indirect immunoassay. A panel of eight clones were then cultured for bulk antibody production as previously described (Ker-hwa Ou and Patterson, 1997).

Anti-OA antibodies were purified from tissue culture supernatant by affinity chromatography on a 5 mL Protein G Sepharose 4 Fast Flow column. The tissue culture supernatant was bound to the matrix and washed with 0.02 M sodium phosphate buffer, pH 7.4. IgG was eluted with 0.1 M glycine-HCl, pH 2.5 until absorbance at 280 nm reached 0.05 and fractions were immediately neutralised with 1 M Tris, pH 9. The fractions containing antibody were determined by absorbance reading at 280 nm and dialysed against PBS at 4 °C. The yield per flask (150 ml) was approximately 20 mg, as determined by BCA assay.

2.4. Screening immunoassays

An antibody capture immunoassay was used to screen the anti-sera response and tissue culture supernatant. OA-OVA conjugate, prepared in the same manner as OA-BSA above, was coated at 2 µg/ml in 0.05 M carbonate buffer pH 9.6 onto microtitre plate wells (Nunc Maxiporp) and incubated for 90 min at 37 °C. The plates were blocked with 3% non-fat milk powder for 1 h at 37 °C. Serial dilutions of serum or tissue culture supernatant in PBS with 0.05% BSA (PBS-B) or neat tissue culture supernatant were added (100 µl/well) and the plate was incubated for 1.5 hr at 37 °C. Bound antibody was detected with 100 µl of horse radish peroxidase- (HRP) labelled rabbit anti-mouse IgG diluted at 1:2000 in PBS-B. Between each step the plate was washed four times with 300 µl PBS/ 0.05% tween 20. The diluted serum or tissue culture supernatant or

antibody was added to the wells with free OA (0.1 to 10 ng/ml) standards for the indirect competitive immunoassay to test the specificity of the anti-sera.

2.5 Immobilisation of OA on CM5 sensor chip

Simultaneous immobilisation of OA onto all flow cells of the CM5 sensor chip was performed following modifications of a previously described method (Gillis *et al.*, 2002). For covalent immobilisation, carboxyl groups of the sensor surface were activated by derivatisation with NHS mediated by EDC. EDC and NHS were mixed (1:1) as per kit and 50 µl of the mixture was deposited on the surface for 20 min activation. This step was repeated once. The amine functionalised surface was then prepared by adding 50 µl of 1 M ethylene diamine, pH 8.5, to the activated surface for 1 h. Any remaining activated groups were deactivated with 1 M ethanolamine, pH 8.5, for 20 min. OA (1 mg) was dissolved in 250 µl of DMF and mixed with 225 µl of 10 mM sodium acetate, pH 4.5, containing 5 mg of EDC and 2 mg of NHS. OA was then immobilised on the surface by placing 50 µl of this solution in static contact with the amine-functionalised surface for 2 h. The surface was then conditioned with repeated injection of 25 µl of 100 mM NaOH, to remove any non-covalently bound material.

2.6. Antibody selection

To examine the binding kinetics of the eight antibodies selected, each antibody was injected over the OA immobilised on the sensor chip surface and conditions to remove the bound antibody were investigated. Preliminary binding data were collected for each antibody by injecting a known concentration of antibody over the chip (0.7 µg/ml of antibody for 12 min at 20 µl/min) and then allowing it to dissociate in HBS-EP buffer for 15 min. The interaction curve for each

antibody on the chip was then compared to select the most suitable candidate for concentration assay development.

OA standards of 1 – 75 ng/ml were prepared in HBS-EP buffer. Standard curves were obtained by mixing the antibody in HBS-EP buffer with OA standards to a 200 µl final volume. The mixture was injected for 1 min over the chip at 25 µl/min, and regenerated by 1 min injection of 20 % acetonitrile in 100 mM NaOH. All curves were fitted using a four-parameter equation with BIAevaluation software and ED-50s determined to select the antibody that gave the most sensitive standard curve for assay development. Cross-reactivity of the selected antibody to DTX-1 was then evaluated by assaying the antibody with DTX-1 standards on the biosensor-based assay. DTX-1 standards ranging from 1 to 75 ng/ml in HBS-EP were prepared from a 100 µg/ml stock. The percentage cross-reactivity was defined as the ED-50 of the standard curve divided by the ED-50 of the cross reactant curve and multiplied by 100 (O'Fegan, 2000).

2.7. Concentration assay

Non-contaminated mussels were purchased from a local outlet (Oyster Creek Seafood Ltd., Ireland) and contaminated mussels were obtained from the Marine Institute (Galway, Ireland) as part of their routine screening programme. Hepatopancreas were excised for extraction and crude methanolic extracts were prepared as follows: 2 g of hepatopancreas was homogenised in 12.5 ml 100 % methanol using a vortex. The mixture was centrifuged and methanolic extracts were collected. Methanol extraction was repeated once more on pellets and extracts were pooled with a final volume of 25 ml and filtered through a 0.2 µm membrane (Hess *et al.*, 2004). OA standards were also prepared in different dilutions of methanol (50 %, 80 % and 100 %) to

examine the effect of the solvent on the assay. The curves were then compared against the curve in HBS-EP buffer

All sample analysis optimisation was aimed to achieve the required sensitivity with binding between 200 and 500 resonance units (RU) on the sensor surface. The optimised assay conditions were as follows: the antibody was diluted at 1:750 in HBS-EP buffer and then mixed 9:1 with the OA standard or sample, injected for 2 min over the chip at a flow rate of 25 µl/min and regenerated with 1 min injection of 20% acetonitrile in 100 mM NaOH. Preliminary validation of the assay was carried out following guidelines from Wong *et al*. (1997) and O'Fegan (2000). The desired characteristics of the standard curves were defined as follows: the sensitivity (ED-50), lower limit of detection (LLOD; standard concentration corresponding to B0 minus three times its standard deviation, with B0 being the antibody binding with no antigen), working range (ED-20 to ED-80) and the reproducibility (%CV for each standard). Repeated assay of three quality control standards (11, 20 and 62 ng/ml, n = 5) in one run determined the intra-assay variation. Inter-assay variation was determined over four consecutive assays, using the same set of quality control standards. Recovery of added standards was carried out by spiking crude methanolic extracts with OA concentrations ranging from 10 to 200 ng/ml and extrapolating the concentration of the spiked extract from the standard curve. A linearity study was carried out by diluting OA-positive sample extracts with known OA concentration in HBS-EP buffer to determine the ability of the assay to obtain results directly proportional to the concentration of the analyte in the sample.

3. Results and discussion

3.1. Generation of OA-specific monoclonal antibodies

Five mice were immunised with the OA-BSA conjugate. One week after the last boost, the sera were screened for the presence of anti-OA antibody in an antibody capture immunoassay using plates coated with OA-OVA conjugate. Titres, defined by dilution of sera giving an absorbance reading of 1, ranging from 1:32,000 to 1:64,000 were obtained. To compare responses between mice, dose-response curves were constructed with each of the sera using OA standards, ranging from 10 to 1000 ng/ml (Figure 2). All mice gave a positive response, but serum from an individual mouse, designated 253 with a titre of 1:64,000 gave the most sensitive dose-response curve (Figure 2). Mouse 253 was therefore selected for fusion with myeloma cell line to generate monoclonal antibodies. 176 clones generated from mouse 253 were initially tested positive in the screening assay and 28 showing displacement at 5 ng/ml were selected for further evaluation. The 8 most sensitive clones (labelled Ab 1 to 8) were kept for further displacement studies and bulk antibody production. Displacement at 0.5 ng/ml ranged from 40 to 75%, which was considered adequate sensitivity for analysis of OA. The binding of the antibodies to OA was then evaluated on the biosensor.

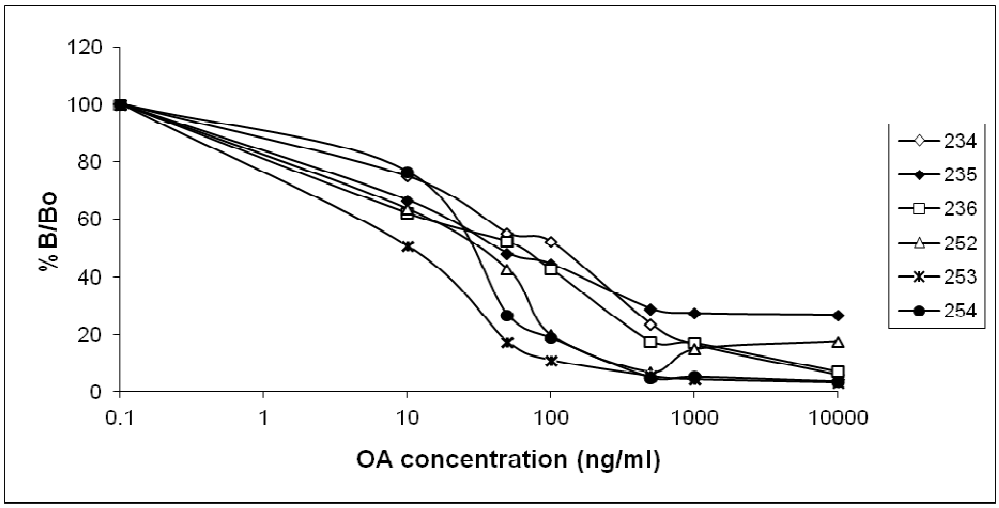


Figure 2. Dose-response curves obtained with serum from OA-BSA immunised mice.

3.2. Preliminary evaluation on the SPR platform

As a preliminary to the development of an SPR-based assay for OA, an evaluation of binding kinetics of the eight monoclonal antibodies was undertaken on sensor surface immobilised OA. The chip was initially conditioned with repeated injection of 100 mM NaOH to remove any remaining non-covalently bound material. A typical analysis cycle is presented in Figure 3. The optimal regeneration condition for each antibody was initially determined where the regeneration step removes any bound material without affecting the ligand activity. Each antibody was injected over the chip and regeneration conditions were optimised. The surface was fully regenerated at 10% acetonitrile in 100 mM NaOH for the antibodies Ab 2, 3, 4, 5, 7, 8 and at 20% acetonitrile in 100 mM NaOH for Ab 1 and Ab 6. The difference between regeneration solutions suggested a slight difference between antibody affinities to the immobilised OA: the stronger the binding of the antibody to the antigen, the higher its affinity to the antigen and therefore, the more concentrated the regeneration solution required to remove the bound antibody. As Ab1 and Ab 6 needed a slightly more concentrated regeneration solution in

acetonitrile for removal from the surface (20% versus 10% for the other six antibodies), suggesting that Ab 1 and 6 represent the best antibody choice to further develop a robust assay (Dillon *et al.*, 2003).

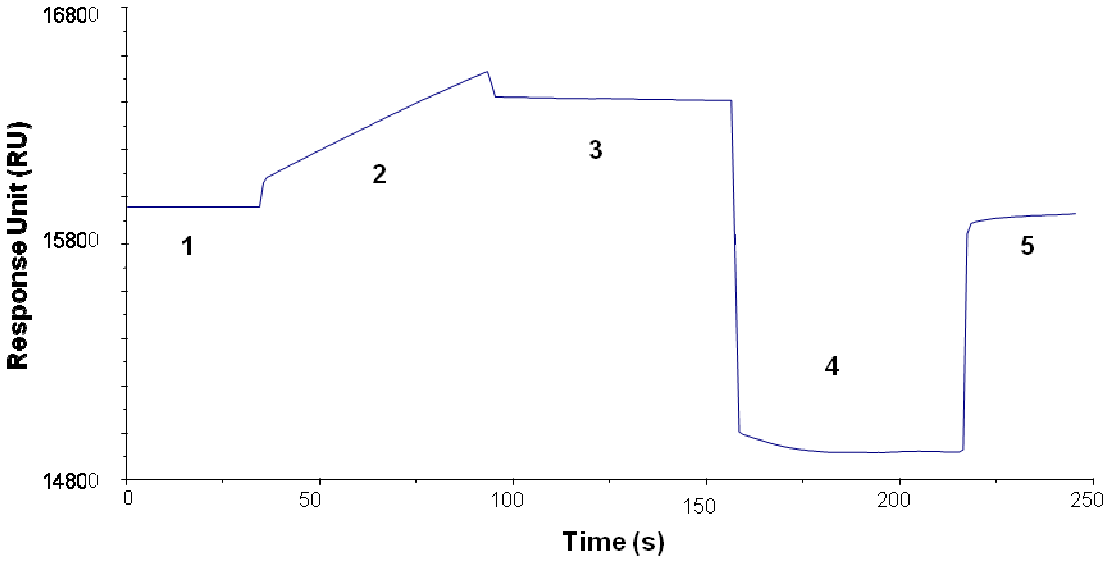


Figure 3. Typical analysis cycle using SPR-based biosensor. 1) Flow of buffer over the immobilised OA on the sensor surface (baseline). 2) Injection of the antibody: sample/standard mixture, the response increases as the antibody binds onto the immobilised OA (association phase). 3) The injection is finished and the buffer flows over the bound antibody (dissociation phase). 4) The surface is regenerated; all the non-covalently bound material is removed. The dip in the response measured is due to the difference between the refractive index of the regeneration buffer and the refractive index of the regular HBS-EP buffer. 5) The baseline returns to its normal level as the buffer flows over the cleaned surface.

Qualitative kinetic data were obtained by comparing the dissociation part of the interaction curves, after injecting one concentration of antibody onto the OA surface. Although this is not the recommended procedure for acquiring binding data, qualitative comparison of interaction curves can be a useful tool in selecting the most suitable antibody for assay development (Karlsson *et a.l*, 1991). Figure 4 shows the binding profiles of the eight antibodies. The dissociation part of the curve is reported to be the most critical value as a lower dissociation rate reflects a more stable binding (Karlsson *et al.*, 1991). A visual comparison of the dissociation part of the curve showed no discernible difference between the eight antibody binding stabilities, which suggested that all eight antibodies had equally strong binding affinities for OA.

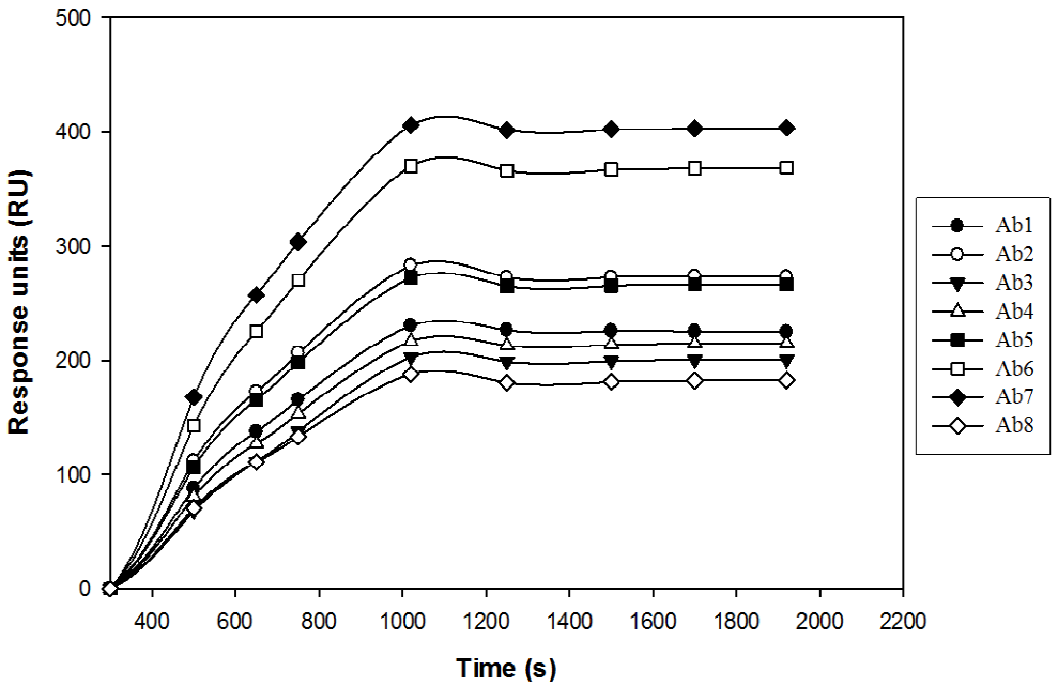


Figure 4. Comparison of the interaction curves of the eight antibodies after injection of 0.7 µg/ml of antibody over immobilised OA for 12 min at 20 µl/min and 15 min dissociation in HBS-EP buffer. The dissociation part of the curve is reflective of the antibody stability.

Separate standard curves were generated for each antibody and ED-50s for each curve were compared (Figure 5). Ab 6 was found to produce the most sensitive curve, as determined by the lowest ED-50 and was thus selected for further assay development. The cross-reactivity of Ab 6 towards the commercially-available DTX-1 was 73%, which indicated that the antibody selected could bind other similar structures. Ab 6 was therefore suitable for assay development for the detection of OA and its co-occuring derivative DTX-1, said to have a relative toxicity of 1 when compared to OA (Aune *et al.*, 2006) and higher toxicity *in vitro* than the other DTXs (Fernández *et al.*, 2014)

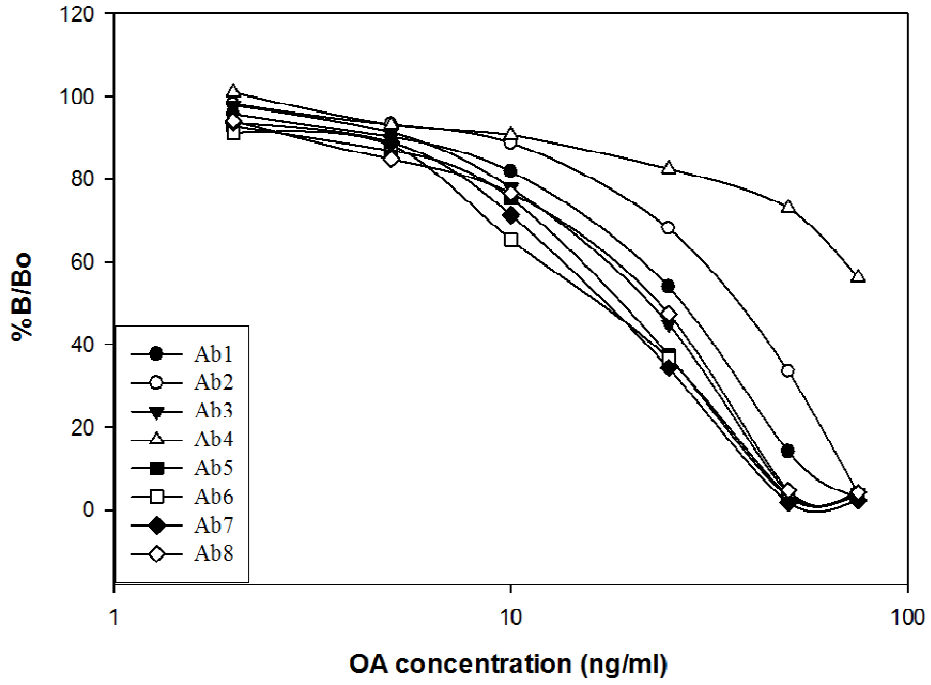
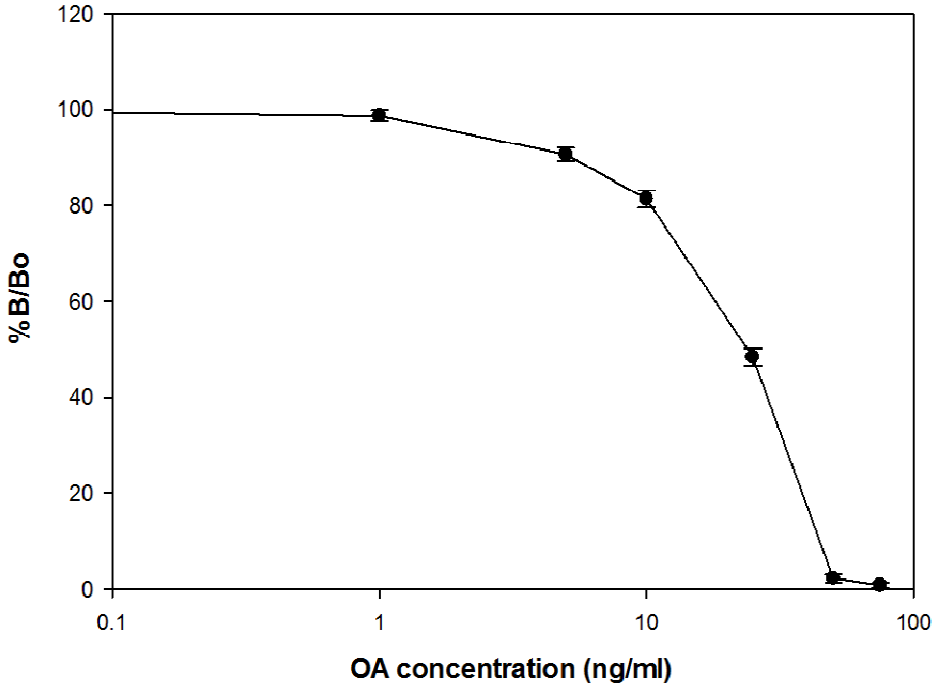


Figure 5. Comparison of standard curves from eight monoclonal anti-OA antibodies on a SPR-based immunoassay.

3.3. Concentration assay

The OA and DTX assay was designed as an inhibition assay with the selected antibody, Ab 6, mixed with the sample or standard at a ratio of 9:1 and to a final volume of 200 µl. Figure 6 represents the mean of 10 curves obtained separately with duplicate concentration of standards. The curve was highly reproducible, with %CV between the 10 curves less than 5%. The assay covered the concentration range 1 to 75 ng/ml, had a linear range between 11.2 and 38.8 ng/ml and ED-50 was 22.4 ng/ml. The regulatory limit of OA and DTXs, set at 160 ng/g, is equivalent to 12.8 ng/ml in crude extract (regulatory cut-off point) which was was within the linear range of the assay. Repeated assays in one run (n=4) of three quality control samples resulted in the following concentration-dependent intra-assay %CVs: at 11 ng/ml, 2.8%; at 20 ng/ml, 1.4% and at 62 ng/ml, 0.9%. Inter-assay variation (n=5) was determined over 5 consecutive assays using the same set of control standards and was 7.3%, 1.9 % and 2.1% at 11, 20 and 62 ng/ml respectively. The assay shows good precision with %CV less than 7.3%, considered acceptable in assay validation (DeSilva *et al.*, 2003)



306

307 **Figure 6.** Composite standard curves for analysis of OA in HBS-EP buffer Ab 6. The standard
308 curve is derived from the mean results for 10 sets of standards analysed in duplicate. The error
309 bars indicate the standard deviations for the 10 sets of standards.

310

311 **3.4. Application of the assay to marine sample analysis**

312 Potential interference from the methanolic extraction buffer and from shellfish extracts was
313 evaluated. The optimised protocol was adjusted to minimise any interference noted. Crude
314 mussel extracts were prepared in 100% methanol and the assessment of the interference of
315 methanol on the assay was carried out by preparing sets of OA standards in different
316 concentrations of methanol (50, 80 and 100%) to compare with the standard curve in HBS-EP
317 buffer (Figure 7). Minimal interference from methanol was noted, especially when standards
318 were prepared in 50% methanol and 50% buffer. Hence, a 1:2 of the sample extracts in HBS-EP
319 buffer following extraction was used for sample analysis in the assay.

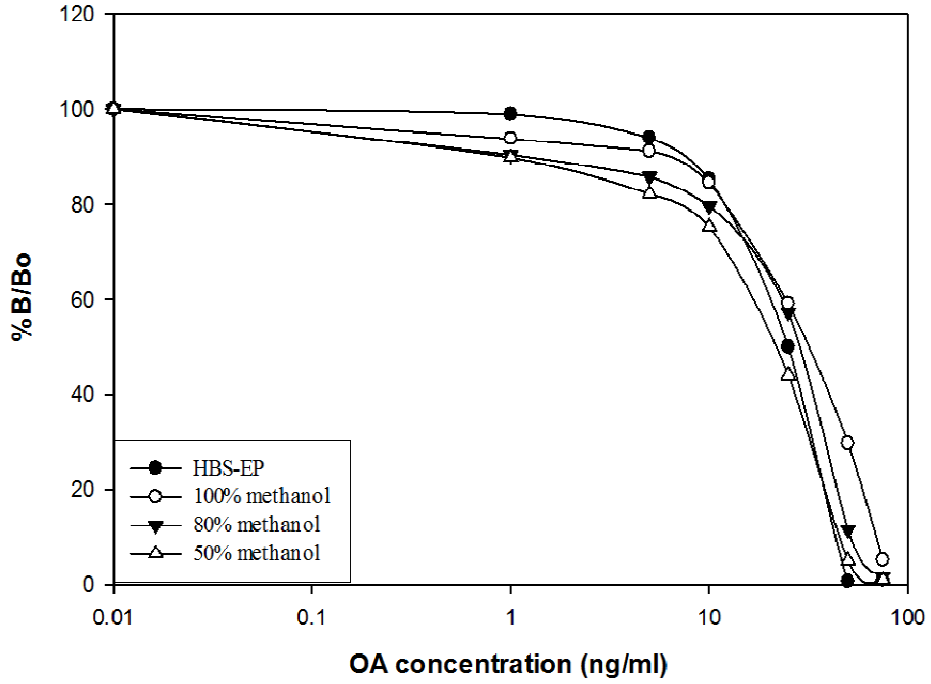


Figure 7. Influence of the extraction buffer on the characteristics of the standard curve: comparison of standard curves obtained with OA standards prepared in different concentration of methanol (50%, 80% and 100%) and OA standards prepared in HBS-EP buffer.

A batch of OA- and DTX-negative mussels was purchased and pooled hepatopancreas extracts were prepared to examine the effect of mussel extract matrices on the assay performance. OA was added to negative mussel extract at concentrations ranging from 10 to 200 ng/ml. Spiked extracts were quantified on SPR-based assay and recoveries of 90.3 – 97.9% were obtained (Table 1). Accurate recovery of added standard indicated minimum matrix interference.

334 **Table 1.** Recovery of added standard in negative mussel extracts.

Spiked OA standards (ng/ml)	Mean conc. determined (n=4, ng/ml)	% CV	% Recovery
10	9.1	9.3	91
20	19.6	3.6	98
40	37.7	1.6	94.2
80	77.6	4.4	97
160	146.5	3.1	91.6
200	180.6	6.8	90.3

335

336

337 Three OA- and DTX-positive mussel homogenates were provided by the Marine Institute as part
338 of their routine monitoring programme. Crude extracts were prepared and a range of different
339 dilutions were made to a final volume of 20 µl in buffer before assay. The concentration of OA
340 measured was directly related to the effective volume assayed over the range examined, with R^2
341 values greater than 0.85 confirming the linearity of the assay (Figure 8). These data also
342 confirmed that there was minimal matrix interference arising from the sample extract in the assay
343 and the suitability of the assay for OA detection in mussel samples. Experiments carried out to
344 evaluate matrix interference during this study, such as effect of methanol on standard curve,
345 linearity and recovery experiments all supported the absence of matrix effects in our assay.

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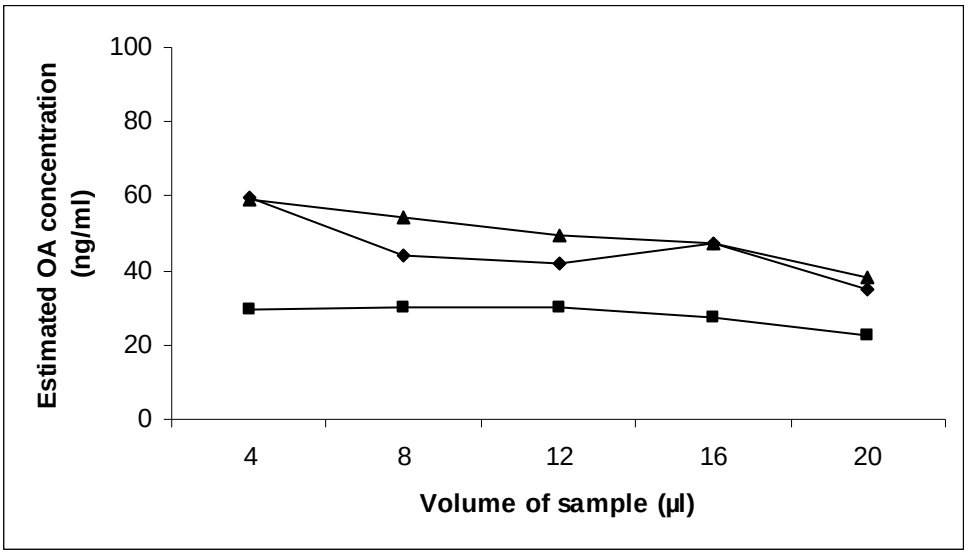

Figure 8. Relationship between the effective volume of sample extract assayed and the concentration of OA measured for three positive mussels extracts. The volume of diluted sample assayed in each case was 20 µl. The final OA concentration in the sample was estimated each time from the measured concentration multiplied by the dilution factor.

Although the developed SPR-based assay described is not as sensitive as ELISA-based assays commercially available and previously described in the literature, such as DSP ELISA Kit (L35000420-096, Biosense Laboratories AS, Norway) and the indirect competitive ELISA developed by Lu *et al.* (2011), it has proven to be a robust (% CV<7.3%) and highly reproducible assay, detecting OA at nanogram concentrations and around the mandated cut-off point. No extensive sample clean-up procedure is required, by comparison to HPLC and MS-based methods, which makes the assay more convenient with a comprehensive extraction protocol. The instrument is fully automated and results are available within minutes after injection, as no incubation time is needed and a high throughput option is feasible.

362 The ability of the same antibody (Ab 6) to detect OA on a different analytical platform was also
363 previously evaluated. The antibody was applied to an electrochemical biosensor and its ability to
364 detect OA produced in this study in shellfish extract was demonstrated. Although the assay had
365 lower sensitivity, the automation of the SPR-based assay in this study allowed for reduced assay
366 time (minutes as opposed to hours) (Campas *et al*., 2008). Various biosensor applications have
367 been previously developed for the detection of OA. Kreuzer *et al.* (2002) presented a screen-
368 printed electrode system for the measurement of a variety of phytotoxins including OA. The
369 assay had a sensitivity of 32 ng/ml, was simple, cost-effective and rapid but was low throughput.
370 Similarly, a quartz crystal microbalance immunosensor was developed but the assay sensitivity
371 was not good enough to fulfill EU requirements (Tang *et al*., 2002). By contrast, the SPR-based
372 assay presented here is suitable for high throughput analysis with a level of sensitivity in
373 accordance with the EU legislation. Antibodies against OA were also produced by Stewart *et al.*
374 (2009a) and the single laboratory validation for routine monitoring of OA using SPR biosensor
375 (Biacore Q) was also presented; however the sample preparation required evaporation to dryness
376 prior to re-suspension in compatible analysis buffer (Stewart *et al.*, 2009b). In the assay
377 presented, minimal interference from the extraction buffer and shellfish matrix with simple
378 sample preparation was demonstrated.. This study presents a convenient, time-effective and
379 confirms the usefulness of SPR biosensing for detection and monitoring of environmental
380 contaminants.
381
382 **4. Conclusion**
383 A panel of monoclonal antibodies against OA was produced and one was selected to develop a
384 fully automated SPR-based immunoassay. The antibody showed the desired ability to recognise

structurally related biotoxins (DTX-1), and good sensitivity, allowing the detection of OA in the nanomolar range. The optimised assay was highly reproducible and was successfully applied to crude mussel extracts and is thus suitable for application to high throughput analysis of OA and DTX-1 in shellfish. As no extensive clean-up is required, the assay is time-effective (5 min per sample). This assay could provide a useful and convenient screening tool with a comprehensive extraction protocol for shellfish monitoring programmes.

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