

# Development of a novel immunobiosensor method for the rapid detection of okadaic acid contamination in shellfish extracts

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**Abstract** The mouse bioassay is the methodology that is most widely used to detect okadaic acid (OA) in shellfish samples. This is one of the best-known toxins, and it belongs to the family of marine biotoxins referred to as the diarrhetic shellfish poisons (DSP). Due to animal welfare concerns, alternative methods of toxin detection are being sought. A rapid and specific biosensor immunoassay method was developed and validated for the detection of OA. An optical sensor instrument based on the surface plasmon resonance (SPR) phenomenon was utilised. A polyclonal antibody to OA was raised against OA-bovine thyroglobulin conjugate and OA-*N*-hydroxy succinimide ester was immobilised onto an amine sensor chip surface. The assay parameters selected for the analysis of the samples were: antibody dilution, 1/750; ratio of antibody to standard, 1:1; volume of sample injected, 25  $\mu\text{l min}^{-1}$ ; flow rate, 25  $\mu\text{l min}^{-1}$ . An assay action limit of 126  $\text{ng g}^{-1}$  was established by analysing of 20 shellfish samples spiked

with OA at the critical concentration of 160  $\text{ng g}^{-1}$ , which is the action limit established by the European Union (EU). At this concentration of OA, the assay delivered coefficient of variations (CVs) of <10%. The chip surface developed was shown to be highly stable, allowing more than 50 analyses per channel. When the concentrations of OA determined with the biosensor method were compared with the values obtained by LC-MS in contaminated shellfish samples, the correlation between the two analytical methods was found to be highly satisfactory ( $r^2=0.991$ ).

**Keywords** Biosensors · Okadaic acid · Diarrhetic shellfish poisons · Mouse bioassay

## Introduction

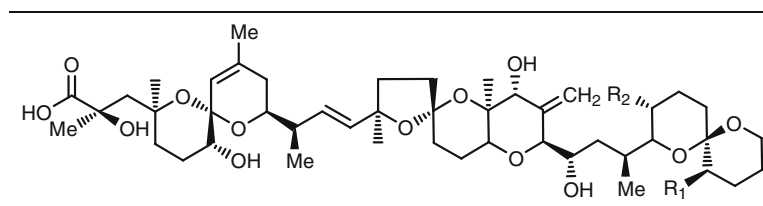
Okadaic acid (OA) has become widely known as a DSP toxin. DSP compounds are produced by a number of dinoflagellates and are responsible for a nonfatal form of food poisoning when consumed following their accumulation in a range of shellfish species. The symptoms of this intoxication include gastrointestinal disorders, vomiting and nausea [1]. Due to this toxicity, there are a wide range of legal requirements regarding the undertaking of monitoring for the presence of this and many other toxins in samples of algal and shellfish origin. The detection of OA (Table 1) is complicated by the fact that OA may be found alone or in combination with several structurally related toxins, such as dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2) (Table 1). Moreover, variations in the toxin profile (both regional and temporal) between incidents of toxin outbreaks are observed. Thus, analytical methods which can detect not only OA but also DTX-1 and DTX-2 are required.

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**Table 1** Chemical structure of diarrhetic shellfish toxins OA, DTX-1 and DTX-2



Substance	R <sub>1</sub>	R <sub>2</sub>
OA	H	H
DTX-1	Me	Me
DTX-2	Me	H

The mouse bioassay, based on the Yasumoto protocol [2], is the most widely used monitoring tool for the detection of DSP toxins in shellfish. The diethyl ether partition stage eliminates some potential interferences, including from saxitoxin and domoic acid, and the extended observation period enables the detection of slow-acting ester forms [3]. Whilst it is a biological test system that can be used to directly demonstrate the presence of unknown toxins, it causes severe suffering to the test animal, with death as an endpoint. Furthermore, the mouse bioassay provides little information on the toxin composition, which is generally varied, and—because the sensitivity is marginal—false negative results can occur [4]. In addition, interference from unsaturated free fatty acids [5, 6] can lead to false positive results. Despite the deficiencies in the procedure, the mouse bioassay remains the reference method within the EU [7].

The European Animal Protection legislation [8] states that, within the EU, animal experiments should not take place if scientifically satisfactory non-animal-based methods are available. Physicochemical methods are permitted under the legislation but must be validated to a recognised international standard, a process impeded by the lack of standard material. The interpretation of these apparently conflicting pieces of legislation has led to a fractured approach to the monitoring of the lipophilic toxins within the EU. Although Germany uses exclusively chromatographic procedures, Belgium, France, Spain and the UK rely on the mouse bioassay. In Ireland and Norway, parallel use of the mouse bioassay and chromatographic procedures is realised, whilst the Netherlands employs a combination of rat bioassay and chromatographic techniques.

A number of physicochemical and colorimetric methods have been described in the scientific literature. Ramstad et al. [9] compared several HPLC-based procedures with the mouse bioassay. The HPLC method appeared to perform as well, if not better than, the mouse bioassay. Nogueiras et al. [10] compared different fluorimetric HPLC methods, where DSP derivatisation was required for the detection of the toxins. In a recent review article, Rossini [11] illustrated that algal toxins structurally related to okadaic acid, such as the DTXs, are potent inhibitors of phosphoprotein phosphatases. This characteristic has allowed the development

of several colorimetric-enzyme-based assays capable of detecting the toxins [12, 13]. The initial problem encountered with these assays was that the analogue DTX-3 was not detected. Detection of DTX-3 was achieved by including a sample hydrolysis step in the test after sample preparation, which made the DTX-3 moiety available [14].

A number of assays based on morphological changes in cultured cells caused by the presence of OA have been described [15, 16]. While these assay systems seem capable of detecting the toxin, there is an ongoing debate about the robustness and reproducibility of such cell-based systems.

Several groups have investigated the potential of biosensor technology to deliver a rapid means of detecting shellfish toxins in samples of shellfish extracts. Kreuzer et al. [17] developed a range of prototype electrochemical biosensors capable of detecting phycotoxins, and Tang et al. [18] utilised piezoelectric technology in a procedure that was capable of detecting OA. Whilst these methods showed some promise, a number of important issues relating to reproducibility and detection capabilities have still to be resolved.

In the field of biosensor analysis, optical technologies have become valuable analytical tools in the areas of food quality and safety analysis. Many studies have described the rapid and reliable detection of veterinary drug residues in recent years [19–25], and a sensitive, robust and reproducible method of analysis for the marine biotoxin domoic acid has recently been reported [26]. In the present study, the development and validation of a rapid and sensitive optical biosensor assay for the detection of OA in shellfish is reported.

## Materials and methods

### Reagents and apparatus

An optical SPR biosensor system (Biacore Q) and Biacore Q control and evaluation software were obtained from Biacore AB (Uppsala, Sweden). A CM5 sensor (research grade), HBS-EP buffer [0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate 20 (v/v), pH 7.4] and 1 M ethanolamine hydrochloride were supplied

by Biacore AB. Bovine thyroglobulin (BTG), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxy succinimide (NHS) were obtained from Sigma-Aldrich (Dorset, UK). OA was acquired from LC Laboratories (Woburn, MA, USA), and DTX-1 from BlueBioTech (Elmshorn, Germany). CRM-DSP-MUS-B certified reference shellfish material was purchased from the National Research Council (NRC, Halifax, NS, Canada) and organic solvents (AnalaR grade) were obtained from Sigma-Aldrich.

#### Source and treatment of samples

Mussel samples that were found to be free from DSP toxins by the mouse bioassay were obtained from the routine monitoring programme carried out on behalf of the Food Standards Agency in Northern Ireland in conjunction with the Agri-Food and Biosciences Institute. Whole mussel tissues were removed from the shell, drained in a sieve (5 min) and homogenised (1 min) using a blender. The homogenates, when not used immediately, were stored at  $-20\text{ }^{\circ}\text{C}$ .

Mussel samples naturally contaminated with DSP toxins were supplied by the Community Reference Laboratory of Marine Biotoxins (CRLMB, Vigo, Spain) and collected in Galicia (northwestern Spain). Mussels arrived fresh with shells and were cleaned and opened by cutting the adductor muscles. The tissue was removed from the shells, transferred to strainers and drained before homogenisation in an Ultra-Turrax. The homogenate was distributed into aliquots and then plastic containers and frozen ( $-20\text{ }^{\circ}\text{C}$ ) until analysis; freeze-dried and wet materials containing okadaic acid and DTX-2 are stable for over eight months at temperatures from  $-20\text{ }^{\circ}\text{C}$  to  $+40\text{ }^{\circ}\text{C}$  [27].

#### Preparation of okadaic acid–BTG (OA–BTG) immunogen

EDC (1.5 mg) and NHS (0.75 mg) were dissolved in MES buffer (0.05 M, 0.5 M NaCl, pH 5.0, 50  $\mu\text{l}$ ) and added to a solution containing OA (1.0 mg) in dimethylsulfoxide (DMSO) (100  $\mu\text{l}$ ). The mixture was incubated for 15 min at  $25\text{ }^{\circ}\text{C}$  to activate the OA. BTG (4.0 mg) was dissolved in phosphate-buffered saline (500  $\mu\text{l}$ , pH 7.2), and the activated OA mixture added along with pyridine (10  $\mu\text{l}$ ) to minimise precipitation. The reaction mixture was incubated for 12 h at  $25\text{ }^{\circ}\text{C}$  and purified by dialysis against saline solution. OA–BTG conjugate (0.1 mg in 1.0 ml saline) was used for each injection. The immunogen was added slowly to Freund's adjuvant with continuous vortexing to produce an emulsion. For the first injection, complete adjuvant containing heat-killed *Mycobacterium tuberculosis* was used, and for subsequent injections incomplete adjuvant was employed. The emulsions were thickened by passing through a narrow-bore 2.0-cm-long cylindrical pyrex block,

drilled at either end to accommodate two syringes. The holes were connected by a channel 1.0 mm in diameter, allowing the flow of emulsion from one syringe to the other.

The emulsions were injected subcutaneously into four sites of the animal (left and right front quarters and left and right hindquarters). The rabbit received a total of six injections on a biweekly basis. Test bleeds were collected two weeks after each booster injection and monitored for the presence of antibodies by immunoassay. After six booster injections, the antiserum was harvested and stored frozen ( $-20\text{ }^{\circ}\text{C}$ ) until required.

#### OA immobilisation onto a sensor chip surface

The carboxymethylated surface of a CM5 sensor chip was equilibrated to room temperature. Fifty microliters of an EDC/NHS mixture (1:1; v/v) was incubated on the chip surface for 30 min. The excess solution was removed and freshly prepared 1 M ethylene diamine (50  $\mu\text{l}$ , pH 8.5) was added to the NHS–ester chip surface and incubated for 1 h. The surface of the chip was washed with HBS-EP buffer and the free carboxyl groups were blocked with 1 M ethanolamine hydrochloride (50  $\mu\text{l}$ , pH 8.5, 20 min).

An EDC (9.0 mg) and NHS (4.0 mg) mixture was dissolved in sodium acetate buffer solution (10 mM, 1.0 ml, pH 4.5). An aliquot of this mixture (10  $\mu\text{l}$ ) was added to OA (50  $\mu\text{g}$ ), which was previously dissolved in DMSO (10  $\mu\text{l}$ ) and sodium acetate buffer solution (10 mM, 30  $\mu\text{l}$  pH 4.5). OA-NHS derivative (50  $\mu\text{l}$ ) was then added to the amine chip surface and incubated for 4 h. The excess solution was removed and the chip surface was washed with deionised water, dried under a gentle stream of nitrogen gas and stored desiccated ( $4\text{ }^{\circ}\text{C}$ ) when not in use.

#### Sample preparation and toxin extraction

One gram of homogenised mussel sample was weighed into screw-cap glass bottles and spiked with 50  $\mu\text{l}$  of the corresponding standard concentration of OA. Methanol (10 ml) was added to each sample, which was then vortexed (5 s), roller mixed (30 min) and centrifuged (3500 $\times$ g, 10 min,  $10\text{ }^{\circ}\text{C}$ ). Supernatant (1.0 ml) was placed in a dry-block sample concentrator (Techne Ltd., Cambridge, UK) and evaporated to dryness ( $45\text{ }^{\circ}\text{C}$ ) under a stream of nitrogen gas. The resulting residue was resuspended in HBS-EP buffer (1.0 ml), vortexed (10 s) and passed through a 0.45- $\mu\text{m}$  filter.

#### Procedure for hydrolysing the esters of DSP toxins

**Procedure A** Methanolic mussel extract (1.0 ml) obtained as described previously (see “[Sample preparation and toxin extraction](#)”) was placed in a screw-cap glass vial and 2.5 N NaOH solution

(125  $\mu\text{l}$ ) was added. The mixture was then heated in a water bath (70  $^{\circ}\text{C}$ , 40 min), cooled (25  $^{\circ}\text{C}$ ) and 2.5 N HCl solution (125  $\mu\text{l}$ ) was added. The content of the vial was dried, redissolved, vortexed and filtered as before (see “Sample preparation and toxin extraction”).

**Procedure B** Methanolic mussel extract (1.0 ml) was prepared as described previously except that, on this occasion, the sample was heated in an oven (70  $^{\circ}\text{C}$ , 40 min). Following treatment under acidic conditions (2.5 N HCl solution, 125  $\mu\text{l}$ , 25  $^{\circ}\text{C}$ ), the reaction mixture was evaporated (45  $^{\circ}\text{C}$ ) under nitrogen gas and the resulting residue dissolved in methanol (1.0 ml) and washed with hexane (1.0 ml). The methanol layer was then dried, redissolved, vortexed and filtered as before (see “Sample preparation and toxin extraction”).

#### Analysis of OA by the biosensor immunoassay (BIA)

Mussel extract was mixed (1:1; v/v) with OA polyclonal antibody diluted 1:750 in HBS-EP buffer. Twenty-five microliters of each sample were injected over the sensor surface (flow rate: 25  $\mu\text{l min}^{-1}$ ) and report points were recorded before and after each injection. Bound antibody was removed from the chip surface by injecting 25  $\mu\text{l}$  of 180 mM NaOH solution containing 15% acetonitrile (flow rate: 25  $\mu\text{l min}^{-1}$ ). A typical analytical cycle for each sample took approximately six minutes to complete, and each sample was analysed in duplicate.

#### Biosensor method validation

A stock solution of OA in methanol (10  $\mu\text{g ml}^{-1}$ ) was used to obtain the calibration curves. Standard concentrations of OA, ranging from 0 to 100  $\text{ng ml}^{-1}$  in HBS-EP buffer and 0–500  $\text{ng g}^{-1}$  in shellfish extracts, were prepared from the stock standard solution of OA diluted with HBS-EP buffer. To establish the action limit for this procedure, negative mussel samples ( $n=20$ ) were spiked with 160  $\text{ng g}^{-1}$  OA and then assayed with the biosensor. Interassay variation ( $n=3$ ) was assessed for mussel replicates spiked with OA at concentrations of 0, 100, 160, 200  $\text{ng g}^{-1}$ . To determine the accuracy of the data, certified reference material (CRM-DSP-MUS-B) and naturally contaminated mussel samples, acquired from the CRLMB, were used as part of the validation study. Preliminary cross-reactivity studies of the polyclonal OA antibody with DTX-1 and DTX-2 were also examined. Throughout assay development, the same biosensor chip was

used in over fifty analytical cycles without any apparent loss of activity being observed.

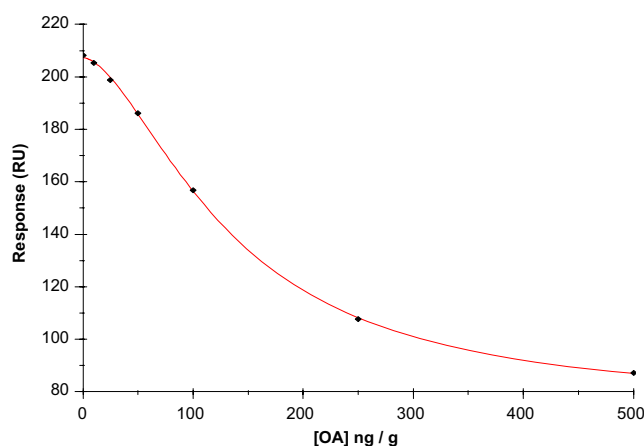
## Results and discussion

#### Generation of OA calibration curve

To determine the specificity of the polyclonal antibody against OA, a calibration curve for OA was produced using mussel extracts (i.e. a matrix curve). Figure 1 shows the calibration curve produced using uncontaminated mussel samples spiked with OA standard concentrations from 0 to 500  $\text{ng g}^{-1}$ . Based on this standard curve, the assay produced a mid-point ( $\text{IC}_{50}$ ) of 148  $\text{ng g}^{-1}$ . The working range of the assay was calculated from the  $\text{IC}_{10}$  and the  $\text{IC}_{90}$  of the curve and was found to be 20–320  $\text{ng g}^{-1}$ .

#### Assay validation

The action limit of the assay (126  $\text{ng g}^{-1}$ ) was calculated as the concentration corresponding to the average instrumental response for twenty negative mussel samples (172  $\text{ng g}^{-1}$ ) fortified with 160  $\text{ng g}^{-1}$  minus three times the standard deviation (SD) (15  $\text{ng g}^{-1}$ ). The subtraction of three times the SD was performed to take into account the uncertainties associated with the assay measurements, such as from extraction efficiency and instrumental performance, to ensure that no false negative results would be reported using the assay as described. Under these conditions a CV % of 8.9 was achieved. Interassay ( $n=3$ ) studies of negative mussel samples spiked with OA concentrations of 0, 100,



**Fig. 1** OA calibration curve obtained with uncontaminated shellfish extracts spiked with OA standards ranging in concentration from 0 to 500  $\text{ng g}^{-1}$ . Calibration curve assay parameters: OA antibody dilution, 1/750; ratio of antibody to standard solution, 1:1; flow rate, 25  $\mu\text{l min}^{-1}$ ; injection volume, 25  $\mu\text{l}$ ; regeneration solution, 15% acetonitrile in 180 mM NaOH. Under these conditions, the mid-point of the calibration curve ( $\text{IC}_{50}$ ) obtained was 148  $\text{ng g}^{-1}$

160 and 200 ng g<sup>-1</sup> produced CV and SD values of 14.4, 9.2 and 13.4, and 1.59, 14.16, 16.47 and 30.12, respectively (Table 2).

The within-assay CV obtained for the analysis of mussel extract spiked at 160 ng g<sup>-1</sup> was found to be an improvement on those obtained with other biosensor methods [17, 18]. The reproducibility observed with the SPR procedure was also superior to those reported for a number of conventional ELISA tests [28–30]. In addition, increased levels of sensitivity could have been achieved with the biosensor method by using alternative assay parameters, such as higher antibody dilution and flow rate (data not included). However, the mid-point of the calibration curve was selected (i.e. the portion of the calibration curve that gives the lowest CV%), as this was the same concentration as the official maximum concentration permitted under EU legislation (160 ng g<sup>-1</sup>).

#### Method comparison (BIA versus LC–MS) and cross-reactivity evaluation of the antibody

Further validation of the biosensor method was performed by analysing naturally contaminated shellfish samples obtained from the CRLMB. Concentrations of OA, DTX-1 and DTX-2 in these samples were determined at CRLMB using liquid chromatography coupled to mass spectrometry (LC–MS). An initial set of four mussel samples, naturally contaminated with OA and esters of OA, were assayed by both procedures. Table 3 outlines the concentrations of OA determined in mussel samples by both the LC–MS and the biosensor methods, prior to and after alkaline hydrolysis of the samples. Sample hydrolysis was performed to convert OA esters to the free form and allow coextraction of these potential sample components. Analysis of these data shows that an extremely good correlation ( $r^2=0.991$ ) was achieved between the two analytical methods for the unhydrolysed mussel extracts. However, after hydrolysis the total concentration of OA present in the samples could not be quantified, as the concentration present in all samples appeared to be greater than the highest calibrant used in the procedure (500 ng g<sup>-1</sup>) (Table 3). These results strongly suggested that,

**Table 2** Interassay parameters calculated for OA with the BIA method ( $n=3$ )

Sample: mussels				
Spiked OA concentration (ng g <sup>-1</sup> )	0	100	160	200
Mean	0.94	98.11	178.13	225.53
SD	1.59	14.16	16.47	30.12
CV (%)		14.4	9.2	13.4

**Table 3** Comparison of the OA concentrations obtained from the analysis of four naturally contaminated mussel samples by the LC–MS and the BIA methods

Sample	OA (ng g <sup>-1</sup> ) by LC–MS	OA (ng g <sup>-1</sup> ) by BIA	OA (ng g <sup>-1</sup> ) by LC–MS	OA (ng g <sup>-1</sup> ) by BIA
	Pre-hydrolysis		Post-hydrolysis*	
1	268	381	458	> 500
2	92	100	198	> 500
3	277	383	491	> 500
4	83	87	175	> 500

\*Hydrolysis of samples performed using Procedure A (see “[Procedure for hydrolysing the esters of DSP toxins](#)”).

during alkaline hydrolysis, additional matrix components were released that caused interference with the OA antibody used in the biosensor assay. In cases where there was a severe matrix effect, an extra sample clean-up procedure could be incorporated to remove the interfering compounds generated. Further work to determine whether this was the problem with the four test samples could not be undertaken as there were insufficient amounts of material available. Nevertheless, during the analysis of further samples (data presented in Table 5), a clean-up procedure was incorporated into the sample preparation procedure (see “[Procedure for hydrolysing the esters of DSP toxins](#)”, Procedure B). This involved washing the methanolic sample extracts with hexane, which proved to be a successful route to overcoming the problem identified during the analysis of the first batch of test materials.

The cross-reactivity of the OA antibody was tested against DTX-1, an analogue of OA which belongs to the same family of toxins. The specificity of the OA antibody was assessed by comparing the binding efficiency of the polyclonal antibody with DTX-1 in buffer conditions. From a DTX-1 stock solution (5 µg ml<sup>-1</sup>), toxin dilutions were made to provide a range of concentrations from 0 to 100 ng ml<sup>-1</sup>. The OA antibody was found to bind DTX-1 with 40% cross-reactivity compared to that of OA. Attempts to reproduce this cross-reactivity in a matrix curve, generated using uncontaminated mussel samples fortified with DTX-1 standard, were unsuccessful. The conformation of the OA molecule is well documented [31] and previous findings have revealed that modifications of the three-dimensional structure of OA affect the affinity of OA for protein phosphatase 2A (PP2A) [32]. The low cross-reactivity value obtained for DTX-1 in the buffer system (40%) may be associated with the presence of a methyl group in the tetrahydropyran ring of DTX-1 (R<sub>3</sub>=Me, Table 1). It is possible that the methyl group modifies the hydrophobicity of DTX-1 in relation to OA, which affects the affinity of OA antibody for DTX-1. The total absence of cross-

reactivity with DTX-1 in the presence of the sample matrix is not unique, as changes in cross-reactivity profiles of antibodies caused by the addition of sample matrix have been reported previously [33]. This phenomenon may be caused by weak molecular interactions between the toxin and a component of the matrix having a greater affinity than the interaction between the toxin and the antibody. Certified reference material was not available to perform cross-reactivity studies on DTX-2.

The cross-reactivity data suggested that the polyclonal OA antibody was not capable of detecting DTX-1 present in sample extracts. This was further investigated by the analysis of certified reference material (CRM-DSP-MUS-B) containing both OA ( $10.1 \mu\text{g g}^{-1}$ ) and DTX-1 ( $1.3 \mu\text{g g}^{-1}$ ), as quantified by LC–MS (both toxins are present only in their acid forms). This reference material (1.0 g) was mixed and vortexed with mussel sample (1.0 g) free from DSP toxins. A subsample (1.0 g) was extracted, following the procedure described earlier (see “Sample preparation and toxin extraction”). The methanol extract (1.0 ml) containing an overall DSP toxin concentration of  $570 \text{ ng g}^{-1}$  was then further diluted to give a range of concentrations from 488 to  $189 \text{ ng g}^{-1}$  of total concentration of OA and DTX-1. Table 4 summarises the biosensor assay and LC–MS results for the determination of the OA and DTX-1 concentrations from these dilutions.

The biosensor method consistently measured lower total concentrations of OA and DTX-1 compared to the reference value for the sample, but the agreement between the two analytical methods was found to be high ( $r^2=0.965$ ). These data suggest that the OA antibody does not detect DTX-1, based on the underestimate of the toxin found with the BIA method compared to the LC–MS procedure.

In a further study, nine mussel samples naturally contaminated with OA and DTX-2 were obtained from CRLMB and analysed both prior to and after alkaline hydrolysis using the biosensor method. The results produced were compared with concentration values determined by the LC–MS method, and are outlined in Table 5. Whilst the results obtained with the biosensor assay for the CRM

**Table 5** Comparison of OA and DTX-2 concentration values obtained by LC–MS and BIA from nine naturally contaminated mussel samples prior to and after hydrolysis

Sample	OA ( $\text{ng g}^{-1}$ ) by LC–MS	OA ( $\text{ng g}^{-1}$ ) by BIA	OA ( $\text{ng g}^{-1}$ ) by LC–MS	OA ( $\text{ng g}^{-1}$ ) by BIA
	Pre-hydrolysis		Post-hydrolysis*	
1	55.1	32.7	162	348
2	66.7	43.0	113	114
3	60.3	60.0	147	160
4	62.6	45.0	390	323
5 <sup>a</sup>	68.0	50.7	no esters	63.4
6 <sup>b</sup>	136.4	137	no esters	154.8
7	24	67	63	114
8	33	61	60	123
9	160	207	226	358

\*Hydrolysis of samples performed using Procedure A (see “Procedure for hydrolysing the esters of DSP toxins”).

<sup>a</sup> Sample 5 contained DTX-2 only; <sup>b</sup> Sample 6 also contained DTX-2 ( $111.6 \text{ ng g}^{-1}$ )

show an underestimation of OA/DTX content, for the naturally contaminated samples there was an overestimate in concentrations when compared with the LC–MS data. The explanation for this may be the presence of OA and DTX isomers/conjugates which are not detected by the LC–MS procedure but are detected by the biosensor procedure due to the cross-reactivity with the antibody employed.

The results obtained upon analysis of the unhydrolysed samples illustrated that the seven samples found to be negative by the LC–MS method (samples 1–5, 7 and 8) were also found to be negative using the biosensor assay (i.e. the OA concentrations were lower than  $126 \text{ ng g}^{-1}$ , which represents the action limit of the assay). Although the biosensor analysis provided an accurate determination of the concentration of OA present in sample 6, based on the concentrations determined by LC–MS ( $136 \text{ ng g}^{-1}$ ), it was not able to detect the presence of DTX-2 ( $112 \text{ ng g}^{-1}$ ). This result suggests that OA antibody does not bind DTX-2. Nevertheless, the biosensor analysis of sample 6 indicated an OA concentration of  $137 \text{ ng g}^{-1}$ , which is above the action limit of the assay ( $126 \text{ ng g}^{-1}$ ) and would therefore have been considered to be a positive sample. Sample 9 was positive by the LC–MS method, which is also in agreement with the result obtained with the biosensor procedure.

The biosensor assay results obtained post-alkaline hydrolysis of the samples consistently reproduced the data provided by the LC–MS method, i.e. samples 1, 4 and 9 were above the action limit ( $126 \text{ ng g}^{-1}$ ) and samples 2, 5, 7 and 8 were below the action limit. However, according to the action limit established with the biosensor method ( $126 \text{ ng g}^{-1}$ ), samples 3 and 6 would be considered to be positives. These data indicate that the biosensor method

**Table 4** Analysis of certified reference material containing OA ( $10.1 \mu\text{g g}^{-1}$ ) and DTX-1 ( $1.3 \mu\text{g g}^{-1}$ ) by LC–MS and BIA methods

Sample	Total OA and DTX-1 by LC–MS ( $\text{ng}^{-1}$ )	Total OA and DTX-1 by BIA ( $\text{ng}^{-1}$ )	Underestimate of biosensor concentration compared to LC–MS (%)
1	189	113	40
2	225	168	25
3	311	226	27
4	443	282	36
5	488	338	31

gives a slight overestimation of the OA present in samples in comparison to the LC–MS procedure. As the biosensor method should be thought of as being a potential screening test, small overestimates would usually be considered to be advantageous, as the possibility of the method generating false negative results is greatly diminished.

## Conclusions

The present study has demonstrated the potential application of an SPR biosensor assay for the rapid and specific detection of OA in shellfish residues. The production of a stable chip surface and a specific binding protein, combined with a quick and simple extraction and hydrolysis procedure, makes the biosensor method an excellent assay for use in the detection of OA in shellfish residues. The precision and accuracy of the method, as judged against the LC–MS data, also points to a method with a high potential value for routine monitoring purposes. The biosensor assay produced a slight overestimation of the OA content in samples; nevertheless, this was not regarded as a limitation to the procedure. A future investigation to improve the developed biosensor assay with respect to the detection of the DTX-1 and DTX-2 isomers by incorporating alternative antibodies is underway.

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