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A rapid optical immunoassay for the screening of T-2 and HT-2 toxin in cereals and maize-based baby food

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ABSTRACT

A rapid surface plasmon resonance (SPR) screening assay has been developed for the combined detection of T-2 and HT-2 toxins in naturally contaminated cereals using a sensor chip coated with an HT-2 toxin derivative and a monoclonal antibody. The antibody raised against HT-2 displayed high cross-reactivity with T-2 toxin while there was no cross-reaction observed with other commonly occurring trichothecenes. A simple extraction procedure using 40% methanol was applied to baby food, breakfast cereal, and wheat samples prior to biosensor analysis. Limits of detection (LOD) for each matrix were determined as 25 $\mu g\,kg^{-1}$ for baby food and breakfast cereal and 26 $\mu g\,kg^{-1}$ for wheat. Intra-assay precision ($n=6$) was calculated for each matrix. The results were expressed as the relative standard deviation and determined as 2.8% (100 $\mu g\,kg^{-1}$) and 1.8% (200 $\mu g\,kg^{-1}$) in breakfast cereal, 4.6% (50 $\mu g\,kg^{-1}$) and 3.6% (100 $\mu g\,kg^{-1}$) in wheat and 0.97% (25 $\mu g\,kg^{-1}$) and 6.3% (50 $\mu g\,kg^{-1}$) in baby food. Between run precision ($n=3$) performed at the same levels yielded relative standard deviations of 6.7% and 3.9% for breakfast cereals, 3.3% and 1.6% for wheat and 6.8% and 0.08% for baby food, respectively.

1. Introduction

The trichothecenes are a major class of mycotoxins found in agricultural crops such as cereals, oilseeds, fruit and vegetables and in the products derived from them. Many of these toxins have been associated with disease in animals and humans raising food safety concerns and having a huge impact on the global trade of food and feed [1]. They are produced by various fungal genera including *Fusarium*, *Cephalosporium*, *Myrothecium*, *Trichoderma*, *Stachybotrys* and *Verticimonosporium* [1]. The most food relevant species is *Fusarium* which produces the greatest range of trichothecenes and is commonly found in tropical and temperate regions but has also been observed in harsher environments such as deserts, alpine and arctic regions [1,2]. Typical trichothecenes identified in agricultural products are deoxynivalenol (DON), nivalenol (NIV), HT-2, T-2, T-2 triol, T-2 tetraol, diacetoxyscirpenol (DAS), monoacetoxyscirpenol, neosolaniol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and fusarenon X [3]. Deoxynivalenol (vomitoxin) is the most prevalent trichothecene while the macrocyclic trichothecenes e.g. satratoxins, T-2 and diacetoxyscirpenol although not as frequently detected are amongst the most toxic [4].

The pathological effects of trichothecenes in animals are well documented. Swine, cattle, poultry, horses, rats, dogs, mice and cats are all affected and symptoms include digestive disorders, haemorrhage, oedema, oral lesions, dermatitis, blood disorders and depression of the immune response [1].

To date links between human disease and trichothecenes are less well elucidated. However one of the most notable human mycotoxicoses attributed to T-2 toxin is Alimentary toxic aleukia. The disease is caused by consumption of over wintered grains such as millet, wheat, oats, rye and buckwheat infected with *Fusarium sporotrichoides*. During the period from 1932 to 1947 thousands of the population in the former Soviet Union's grain belt became ill following ingestion of contaminated grains and the fatalities reached 60% [1,5,6]. In more recent times there have been outbreaks of mycotoxicoses in Japan, China and India. These have been linked to ingestion of wheat corn and rice contaminated with several trichothecenes including T-2 toxin. Fortunately no fatalities occurred but the symptoms consisted of abdominal pain, nausea, vomiting, diarrhoea, headache and dizziness [7].

There have also been implications that trichothecenes have been used in biological/chemical warfare in Afghanistan, Kampuchea and Laos [8] and that other countries were researching the use of trichothecenes for development of biological weapons [9].

Currently there are no regulatory limits for T-2 and HT-2 toxins in Europe, however it is anticipated that these will be agreed in the near future and the legislation will refer to the sum of T-2 and

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HT-2 [10]. This is due to the fact that T-2 is rapidly deacetylated to HT-2 in vivo and the acute toxicities are within the same range [11]. It is also believed that the levels set, particularly for infant food will be low reflecting the toxicity of the metabolites, therefore requiring sensitive analytical techniques capable of supporting the legislative demands. Within the European Union there have been suggestions that regulatory limits of 100, 500, 200 and 50 $\mu g\,kg^{-1}$ will be agreed for unprocessed cereals, unprocessed oats, oat products and cereal based infant and baby foods, respectively [12]. The number of countries that have established regulations for T-2 and HT-2 toxins is relatively low. No regulatory limits exist in North America, Latin America or in the Asia/Oceania regions, in fact only 13 countries have legislated for these trichothecenes. Five countries within the European Union currently regulate the toxins including Bulgaria, Hungary, Latvia, Slovakia and Slovenia while legislation has also been set in Armenia, Belarus, Canada, Iran, Moldova, Russia, Serbia and Montenegro and Ukraine. This logic is based on risk assessment of these trichothecenes coupled with perhaps inadequate methodology.

A wide variety of analytical methods exist for the detection and quantitative measurement of trichothecenes in foods. Rapid screening tests often employ immunochemical techniques which require little or no sample clean-up or concentration steps and are finding wide application in this field to complement confirmatory methods. The majority of rapid methods used are enzyme linked immunosorbent assays (ELISA) [13,14] due to their speed, ease of use and high sample throughput, however problems associated with many of these tests are lengthy incubation periods, lack of sensitivity and over-estimation [15]. One of the most important aspects to be considered when using immunoassays is specificity of the antibody. Since the legislation expected will cover the sum of T-2 and HT-2, it is important for the accuracy of the method that the antibody displays as close as 100% cross-reactivity with the two toxins; this is not the case with several of the commercial kits available and therefore under-estimation of the contaminants may result. Such test kits include RIDASCREEN® T-2 Toxin, Veratox® T-2 Toxin and Eurodiagnostica T-2 Toxin EIA each having 100% cross-reactivity with T-2 toxin and cross-reactivities of 7%, 17% and 3.4% with HT-2, respectively. Other screening methods applied to the analysis of type A trichothecenes include thin layer chromatography [16] and dipstick enzyme immunoassay [17]. The use of surface plasmon resonance (SPR) immunoassay has been evaluated for the detection of type B trichothecenes, i.e. deoxynivalenol [18], however to date this technology has not been applied to type A trichothecenes. SPR has been used for many years in drug discovery and development, biotherapeutics and life science research and over the past few years has emerged as an important analytical tool in food analysis with successful applications in vitamins, veterinary drug testing and shellfish toxins [19–23].

Quantitative methods of analysis for trichothecenes use high performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with a variety of detectors such as fluorescence detection, ultraviolet detection, diode array detection, electron capture detection and mass spectrometry [24–28]. While these analytical tools allow for sensitive determination of trichothecenes they are extremely expensive and require long and tedious sample preparation limiting the sample throughput. LC–MS/MS [29–32] has become more popular than GC–MS for the analysis of mycotoxins due to its sensitivity and the fact that samples do not require derivatisation, but probably of greater significance is the applicability of the technology to simultaneous determination of mycotoxins having great chemical diversity [15].

Due to the inevitable presence of such naturally occurring toxins in our food and feed the aim of this work was to develop a rapid, simple screening assay for the simultaneous determination of T-2 and HT-2 toxins in cereals.

2. Safety

T-2 and HT-2 toxins are known to be hazardous to humans; therefore when handling toxin standard solutions, reference samples or naturally contaminated materials care should be taken. Gloves should be worn at all times and appropriate disposal methods should be used.

3. Materials and methods

3.1. Equipment

An optical surface plasmon resonance (SPR) biosensor system (Biacore®Q) was obtained from GE Healthcare, Uppsala, Sweden. Biacore Q Control Software, version 3.1 was used for instrument operation and data handling.

3.2. Chemicals and reagents

T-2, HT-2, T-2 triol, T-2 tetraol, deoxynivalenol, nivalenol, neosolaniol and diacetoxyscirpenol toxin standard solutions and HT-2 (solid material) were purchased from Biopure Referenzsubstanzen GmbH, Tulln, Austria. GPR grade methanol, acetonitrile, hydrochloric acid, sodium dodecyl sulphate, sodium acetate, carbonyldiimidazole, anhydrous dimethylsulphoxide, jeffamine (2,2′-(ethylenedioxy)bis(ethylamine)), boric acid, sodium hydroxide were purchased from Sigma–Aldrich, Gillingham, England.

A Monoclonal antibody raised against HT-2 toxin was supplied by The University of Natural Resources and Applied Life Sciences, Department for Agrobiotechnology (IFA), Vienna, Austria. The procedure has been described in detail by Molinelli et al. [33]. Sensor chips CM5 Research grade, HBS-EP buffer and an Amine Coupling kit were purchased from GE Healthcare, Uppsala, Sweden. Reference blank and naturally contaminated wheat, baby food and breakfast cereals were supplied by several partners in the BioCop Project as few reference materials particularly for the trichothecenes T-2 and HT-2 are available commercially. Wheat test materials were produced in a Standards Measurements and Testing (SMT) project at the Swedish University of Agricultural Sciences, Uppsala, Sweden [34] and excess material made available for this study. Baby food based on 97% maize was produced by the collaboration of the Nestlé Research Centre, Lausanne, Switzerland, the Institute of Chemical Technology, Prague, Czech Republic and the EU Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Geel, Belgium. All partners were involved in the production of the breakfast cereal test material which was based on 60.7% graham wheat, 15% rice flour, 15% crystal sugar and flavourings of cocoa powder, malt extract and salt. These materials were characterized using established mass spectrometry methods. Naturally contaminated samples were provided by Neogen Europe Ltd., Ayr, Scotland and the Department IFA-Tulln, University of Natural Resources and Applied Life Sciences, Vienna, Austria.

3.3. Coupling of HT-2 to the CM5 sensor chip

The immobilization procedure was performed under static conditions on the bench, the volumes of solutions for each step were 40 μl unless stated otherwise and between each stage the solutions were removed by tissue, taking care not to touch the centre of the sensing surface. Briefly the CM5 sensor chip was allowed to equilibrate to room temperature and primed using HBS-EP buffer. The surface was activated by placing a 1:1 mixture of 0.4 M EDC/0.1 M NHS in water onto the surface for 30 min. An amine i.e. jeffamine (2,2′-(ethylenedioxy)bis(ethylamine)) (20% v/v in 50 mM borate buffer, pH 8.5) was applied for 1 h followed by deactivation of un-reacted sites using 1 M ethanolamine, pH 8.5 for 30 min. The

reactive HT-2 carbamate was prepared by reacting HT-2 toxin (1 mg) with CDI (1.5 mg) in anhydrous DMSO (100 μl) for 4 h, then diluted 1:1 in 10 mM sodium acetate solution, pH 4.6. This solution was allowed to react with the amine (on the surface) overnight at room temperature after which the sensor chip was washed with deionized water and dried under a gentle stream of nitrogen. When not in use the sensor chip was placed in a storage package containing desiccant and stored at +4 °C.

3.4. Immobilization reproducibility

Reproducibility of the immobilization protocol was investigated by performing the procedure on four CM5 Research grade chips of varying batches on different days. Assessment of the results included measuring the maximum binding capacity (R_{max}) of each surface by passing a concentrated solution of antibody (dilution 1:5 in HBS-EP buffer) over the surface (contact time 15 min, flow rate 5 μl min^{-1}) until the plateau was reached. In addition calibration curves were constructed and run over the surface of the four chips.

3.5. Antibody cross-reactivity

The cross-reactivity profile of the antibody was determined in buffer and in the presence of a range of matrices. HBS-EP buffer, reference blank wheat, baby food and breakfast cereal extracts were spiked with structurally related and commonly occurring trichothecenes (T-2, T-2 triol, T-2 tetraol, deoxynivalenol, nivalenol, neosolaniol and diacetoxyscirpenol) and run in the biosensor against an HT-2 buffer curve. The HT-2 calibration solutions used were 0, 2, 4, 8, 20 and 80 ng ml^{-1} (cereal equivalents of 0, 25, 50, 100, 250 and 1000 μg kg^{-1}), while the other trichothecene calibration solutions were 0, 2, 4, 8, 20, 80, 160 and 800 ng ml^{-1} (cereal equivalents of 0, 25, 50, 100, 250, 1000, 2000 and 10,000 μg kg^{-1}). The midpoint relative response value of the HT-2 calibration curve was used to calculate the midpoint concentration (IC_{50}) for HT-2 and the other compounds. Cross-reactivity was calculated as a percentage relative to HT-2.

3.6. Preparation of assay reagents and samples

3.6.1. Antibody

The monoclonal antibody was diluted 1:200 (6.15 μg ml^{-1}) in HBS-EP buffer.

3.6.2. Regeneration solution

250 mM hydrochloric acid/0.5% sodium dodecyl sulphate.

3.6.3. Calibrants

Serial dilutions of the Biopure HT-2 toxin standard solution in HBS-EP buffer were prepared and the calibration curve consisted of 6 calibrants ranging from 0 to 80 ng ml^{-1} (cereal equivalent of 0–1000 μg kg^{-1}).

3.6.4. Sample extraction

Wheat, breakfast cereal and baby food: 5.0 ± 0.02 g of sample was weighed into a plastic centrifuge tube (TPP, Techno Plastic Products AG, Switzerland) and mixed with 25 ml of 40% methanol. The sample was mixed by vortex for 10 s followed by centrifugation at 4369 × *g* for 10 min. 1 ml of the supernatant (wheat and breakfast cereal) or 2 ml of the supernatant (baby food) was evaporated to dryness under a stream of nitrogen at 60 °C, the residue reconstituted in 5 ml of HBS-EP buffer and mixed by vortex.

3.6.5. Assay conditions

The samples were transferred to the wells of a microtitre plate and mixed with antibody solution, (1:1 antibody/sample) by the autosampler via the control software and injected immediately over the surface of the sensor chip The flow rate was maintained at 20 μl min^{-1} for injection of the calibrants/samples and regeneration. Contact times for the calibrants and samples were 4 min and the surface was regenerated by 2 × 30 s pulses of the solution detailed above.

3.7. Assay validation

The limit of detection (LOD) of the assay was determined from the mean of the measured response units (RU) of negative samples (n = 20) minus 3 times the standard deviation (SD) of the mean (mean − 3SD). The precision of the assay was investigated by calculating the intra-assay (within run) and inter-assay (between run) variation expressed as relative standard deviation (RSD%). To ascertain these baby food was spiked at 25 and 50 μg kg^{-1}, wheat was spiked at 50 and 100 μg kg^{-1} and breakfast cereal was spiked at 100 and 200 μg kg^{-1} (n = 6 at each concentration). The analyses were repeated on three separate occasions using three 3 different biosensor instruments (a total of n = 18 samples for each). The levels were chosen with respect to the forthcoming legislation. The accuracy of the method was evaluated by analyzing naturally contaminated samples and reference samples and comparing these with confirmatory test results.

3.8. Confirmatory analysis

LC–MS/MS-based reference analysis was performed using a method that had been described in detail by Sulyok et al. [35]. In brief, 10 g sample were extracted with 40 ml (for bran samples and some of the oat samples, 80 ml were added) of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) for 90 min using a GFL 3017 rotary shaker (GFL, Burgwedel, Germany). After filtration, the extracts were transferred into glass vials using Pasteur pipettes and 350-μl aliquots were diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v) and directly injected into the LC–MS/MS instrument.

Detection and quantification in the Selected Reaction Monitoring (SRM) mode was performed with a QTrap 4000 LC–MS/MS System (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray electrospray ionization (ESI) source and an 1100 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C_{18} column, 150 mm × 4.6 mm i.d., 5-μm particle size, equipped with a C_{18} 4 mm × 3 mm i.d. security guard cartridge (all from Phenomenex, Torrance, CA, USA). All related instrumental parameters can be found in Sulyok et al. [35]. For quantification, external calibration was performed using multi-analyte standards prepared and diluted in a 1:1 mixture of extraction and dilution solvent. Results were not corrected for incomplete extraction or for matrix effects as the apparent recoveries of T-2 and HT-2 were found not to deviate significantly from 100% in the grain-based matrices that have been validated so far with the exception of HT-2 in maize.

4. Results and discussion

This screening assay was designed as a competitive inhibition assay and detects the toxin-specific antibody as it binds to the HT-2 immobilized onto the surface of the sensor chip. Prior to injection a known amount of antibody is mixed with the sample. Any HT-2 or T-2 toxin present in the sample binds to the antibody thus preventing it from binding to the immobilized toxin on the sensor chip surface. The more contaminated the sample, the greater the level

Table 1
Reproducibility of the immobilization procedure.

Chip number	Maximum binding capacity (R_{max}) (response units, RU)
1	1094.5
2	1033.9
3	948.6
4	945.6
Mean	1005.65
SD	72.00
RSD (%)	7.2

of inhibition, resulting in a lower response being recorded. Binding responses resulting from the analyses of unknown sample extracts were measured against a six-point calibration curve to obtain the concentration in $\mu g\,kg^{-1}$.

Amine coupling is the simplest means of attaching a ligand to the surface of a CM5 surface and exploits primary amine groups. For many immobilization protocols, the carboxyl groups on the surface of the sensor chip are activated with mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.05 M N-hydroxysuccinimide (NHS) in water to produce reactive succinimide esters. These succinimide esters react spontaneously with amines or other nucleophilic groups allowing direct immobilization. In the case of HT-2 toxin there is no group which can be directly immobilized onto the surface following activation, therefore an amine was attached to the surface and the toxin modified to enable reaction with the amine linked to the surface. The immunogen preparation approach utilized the hydroxyl group at position 4 coupled directly to amino groups of proteins using the carbonyldiimidazole (CDI) reaction [36,37]. Evidence has shown that the hydroxyl group at position 4 is more reactive than that at position 3 [38] therefore this procedure was followed for attachment of HT-2 to the CM5 surface. The immobilization procedure proved to be reproducible, displaying an RSD of 7.2%, when four chips were immobilized and tested (Table 1). In addition calibration curves were run on these chips demonstrating reproducible curves with almost identical sensitivities. The results of which are highlighted in Fig. 1 and Table 2.

To determine if we could employ a buffer curve in the screening assay thus negating the need for matrix matched curves and therefore separate analyses depending on the sample matrix, calibration curves were prepared in HBS-EP buffer, wheat, breakfast cereal and baby food extracts and assayed together in the SPR biosensor instrument. Using the Biacore Q Evaluation Software, the curves were overlaid and the results indicated that there was good agreement between the curves particularly in the linear part of the curve where the levels of concern are measured; therefore a buffer calibration curve could reliably be used for the assay (Fig. 2). Table 3 displays the midpoint concentration (IC_{50}) of the buffer and matrix curves in $ng\,ml^{-1}$ units and the equivalents in sample terms in $\mu g\,kg^{-1}$.

Robustness is a key element for both screening and confirmatory assays therefore a number of parameters were assessed during the study. To ascertain this for the SPR assay we initially

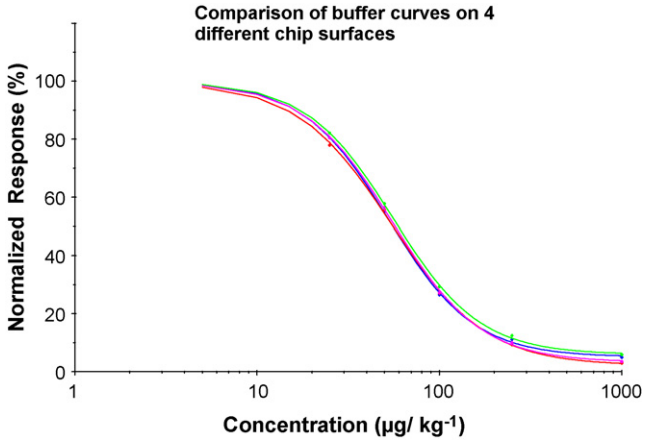

Fig. 1. Comparison of buffer curves over 4 different HT-2 sensor surfaces.

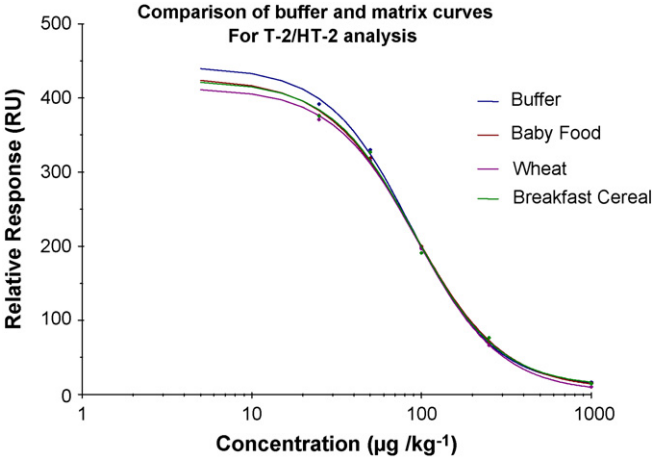

Fig. 2. Comparison of buffer and matrix curves for T-2/HT-2 analysis.

Table 3
Midpoint concentrations measured in $ng\,ml^{-1}$ and the relating $\mu g\,kg^{-1}$ equivalent, highlighting the similarity in the buffer and matrix calibration curves.

Matrix	IC_{50} ($ng\,ml^{-1}$)	IC_{50} ($\mu g\,kg^{-1}$)
Buffer	5.6	70.3
Baby food	6.3	78.8
Breakfast cereal	6.6	83.1
Wheat	6.2	77.5

evaluated not only the longevity of the sensor surface whereby 40 sequential analyses of each blank matrix were performed but also the immobilization reproducibility as described. Minimal drift was observed for both the baseline and sample responses (i.e. 80 and 66 resonance units, respectively) and is detailed for wheat in Fig. 3. In addition (for validation purposes) the assay was evaluated on different days with fresh calibration solutions, extraction

Table 2
Reproducibility of the calibration curves.

Concentration ($\mu g\,kg^{-1}$)	Chip 1 (normalised response %)	Chip 2 (normalised response %)	Chip 3 (normalised response %)	Chip 4 (normalised response %)
0	100	100	100	100
25	78	81	82	81
50	56	57	58	55
100	27	27	29	27
250	9	10	12	11
1000	3	4	6	5
IC_{50} ($\mu g\,kg^{-1}$)	51.5	51.8	53.0	52.5

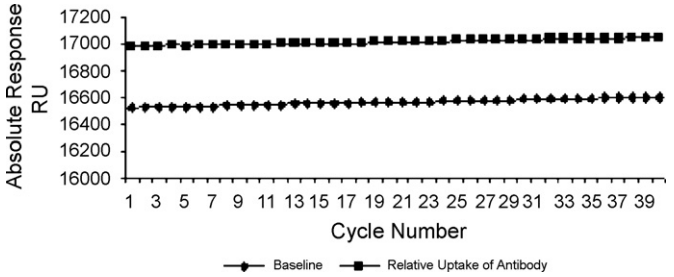

Fig. 3. Baseline and relative uptake of antibody for 40 sequential cycles of wheat free from T-2 and HT-2 toxins.

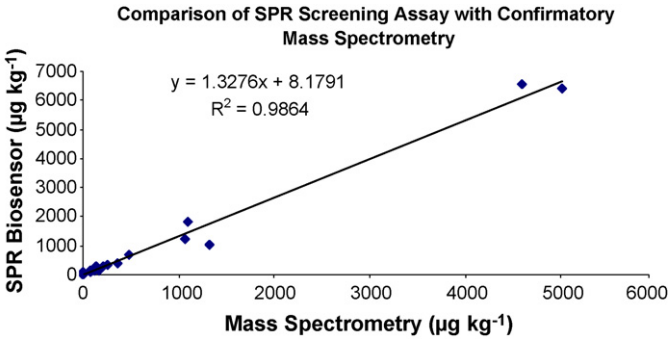

Fig. 4. Correlation between the screening and confirmatory assays.

solutions and sample extracts and the use of different instruments. Calibration curves (in duplicate) run over 5 consecutive days demonstrated excellent repeatability, the results of which are displayed in Table 4. Of equal importance from an end-user's point of view, it was possible to perform over 300 analyses cycles on a single flow cell of the sensor chip without any deterioration of the signal.

The antibody demonstrated high specificity for HT-2 and T-2 toxins. Cross-reactivities of 100% were exhibited for HT-2 in buffer, baby food, breakfast cereal and wheat while for T-2 the results were 92%, 121%, 117% and 110%, respectively. No measurable cross-reaction was demonstrated with T-2 triol, T-2 tetraol, deoxynivalenol, nivalenol, neosolaniol or diacetoxyscirpenol.

Table 5 outlines the validation data generated for the three matrices tested. The Limits of detection (LOD) for each matrix were determined from the mean response of representative blank samples minus 3 times the standard deviation of the mean. Assay precision was investigated by determination of the intra-assay (within run) and inter-assay (between run) variation, expressed as the relative standard deviation (RSD). Inter-assay variation was evaluated over 3 days. All intra-assay and inter-assay RSDs fell below 7% while the Limits of detection for all matrices were between 25 and 26 $\mu g\,kg^{-1}$, indicating that low level matrix effects were being observed or that the reference blank materials were not completely free from T-2 and HT-2 toxins, or a combination of both. Toxin recovery from the sample preparation procedure employed was determined by spiking 6 blank samples of each food matrix at two concentrations; the levels of which varied depending upon the foodstuff tested as described. The recoveries achieved ranged from 88% to 128% for all matrices.

The accuracy of this assay was evaluated by comparing results of naturally contaminated samples analyzed by both the SPR method and the LC–MS/MS method detailed previously. Excellent correlation was observed between the two methods, exhibiting an R^2 value of 0.99 (Fig. 4). Information regarding the samples and the concentrations determined are detailed in Tables 6 and 7. In addition to providing the quantitative data, the qualitative data has also been included in the table to display the accuracy of this method. There was extremely good agreement between the methods and in particular no false negatives were observed within the sample population.

A simple, rapid, specific and sensitive assay has been developed for the determination of T-2 and HT-2 toxins in cereals. The assay was validated in wheat, breakfast cereals derived from wheat and baby food derived from maize. The SPR–LC–MS/MS comparison also indicates that the method may be applied to other sample matrices such as oats and barley. The vast majority of published immunoassay based screening tests are limited by the fact that the antibody shows little or no specificity towards the HT-2 toxin. It is widely anticipated that within the European Union legislation will be based on the sum of T-2 and HT-2. It is also predicted that other global regions will follow suit. Once legislation has been established within the European Union these assays will become obsolete since it has been demonstrated that T-2 and HT-2 toxins occur simultaneously in cereals [39]. The same problem applies to the numerous

Table 4
Repeatability data for the calibration curves (in duplicate) ran over 5 consecutive days.

Concentration ($\mu g\,kg^{-1}$)	Mean relative response (RU) ($n=2$)					Mean	SD	RSD (%)
	Day 1	Day 2	Day 3	Day 4	Day 5			
0	497	494	475	466	455	478	18	4
25	461	455	433	399	405	430	28	7
50	390	384	358	315	333	356	32	9
100	242	233	215	177	190	211	28	13
250	90	87	78	71	75	80	8	10
1000	31	26	21	27	28	26	4	14
IC_{50} ($\mu g\,kg^{-1}$)	92	90	87	70	79	84	9	11

Table 5
Validation data for the 3 food matrices using SPR technology.

	Wheat		Breakfast cereal		Baby food	
	50[a]	100[a]	100[a]	200[a]	25[a]	50[a]
Intra-assay RSD (%) ($n=6$)	4.6	3.6	2.8	1.8	1.0	6.3
Inter-assay RSD (%) ($n=18$)	3.3	1.6	6.7	3.9	6.8	0.1
Mean recovery (%)	125	113	95	98	122	103
Limit of detection (LOD) ($\mu g\,kg^{-1}$)	26		25		25	

[a] Spiked concentration ($\mu g\,kg^{-1}$).

Table 6
Comparison of results obtained using SPR technology and LC–MS/MS (*LOD LC–MS/MS ($\mu g\,kg^{-1}$) = 20 and 2 for HT-2 toxin and T-2 toxin, respectively based on spiked samples).

Sample number	Matrix	LC–MS/MS ($\mu g\,kg^{-1}$)	Neg/Pos	SPR ($\mu g\,kg^{-1}$)	Neg/Pos	SPR RSD (%)
1	Wheat	<LOD*	Negative	46	Negative	3.4
2	Wheat	<LOD	Negative	49	Negative	10.5
3	Wheat	93	Negative	136	Positive	7.0
4	Wheat	5012	Positive	6390	Positive	2.5
5	Wheat	<LOD	Negative	59	Negative	1.6
6	Wheat	120	Positive	147	Positive	2.1
7	Wheat	1316	Positive	1040	Positive	4.4
8	Wheat	<LOD	Negative	46	Negative	0.9
9	Wheat	<LOD	Negative	<25	Negative	4.8
10	Maize	<LOD	Negative	54	Negative	3.9
11	Maize	<LOD	Negative	58	Negative	4.0
12	Maize	26	Negative	83	Negative	4.9
13	Maize	207	Positive	302	Positive	4.5
14	Maize	1092	Positive	1813	Positive	3.8
15	Maize	170	Positive	159	Positive	1.4
16	Oats	135	Negative	303	Negative	1.7
17	Oats	4592	Positive	6575	Positive	1.8
18	Oats	1067	Positive	1225	Positive	6.6
19	Oats	<LOD	Negative	68	Negative	1.7
20	Oats	<LOD	Negative	87	Negative	1.0
21	Fine Bran	<LOD	Negative	69	Negative	1.1
22	Light Bran	<LOD	Negative	66	Negative	4.2
23	Barley	252	Positive	348	Positive	7.0
24	Horse Feed	363	Positive	400	Positive	0.9

Table 7
Comparison of the BioCop reference sample results obtained using SPR technology and mass spectrometry.

Sample	Matrix	Mass spectrometry ($\mu g\,kg^{-1}$)	Neg/Pos	SPR ($\mu g\,kg^{-1}$)	Neg/Pos
1	Baby food (maize)	<LOD	Negative	<LOD	Negative
2	Baby food (maize)	69	Positive	128	Positive
3	Breakfast cereal (wheat)	<LOD	Negative	<LOD	Negative
4	Breakfast cereal (wheat)	81	Negative	81	Negative
5	Wheat	<LOD	Negative	<LOD	Negative
6	Wheat	162	Positive	161	Positive
7	Wheat	484	Positive	672	Positive

commercial ELISA kits which are used for monitoring purposes. In contrast the monoclonal antibody employed in the SPR assay displayed virtually equal specificity for both T-2 and HT-2 toxin in both buffer and matrices making it the only viable immunochemical screening option for both government and commercial testing laboratories. Solvent extraction of the toxins from cereals is required for all the screening assays and in the majority of cases high concentrations of acetonitrile or methanol are employed, thus significantly increasing the cost per test and adding to the solvent burden in a laboratory. These important issues have been addressed during assay development. Extensive extraction and incubation times, common to various published and commercial methods have also been avoided to permit rapid analysis, again favorably impacting on cost. The simple extraction procedures developed within this study coupled with the high performance of the SPR assay in terms of accuracy, precision and low limits of detection allow for reliable, rapid and cost effective screening of the sum of T-2 and HT-2 in cereals and maize-based baby food.

All fungal species and their ability to produce mycotoxins is heavily influenced by environmental conditions, the most important of these are temperature and moisture, however insect and bird damage, pathogenic organisms and other adverse conditions to the plant such as drought, lack of nutrients, etc. encourage fungal growth and therefore increase the probability of mycotoxin production. Climate change is expected to have a huge impact on all of these factors and therefore on fungal growth and the production of mycotoxins. With this in mind it will be necessary to have accurate and rapid monitoring systems in place to ensure a safer global food supply for both animals and humans alike.

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