DOI:10.1021/jf101517s

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

# Rapid Surface Plasmon Resonance Immunoassay for the Determination of Deoxynivalenol in Wheat, Wheat Products, and Maize-Based Baby Food

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A rapid screening assay (9 min/sample) has been developed and validated for the detection of deoxynivalenol in durum wheat, wheat products, and maize-based baby foods using an SPR biosensor. Through a single laboratory validation, the limits of detection (LOD) for wheat, wheat-based breakfast cereal, and maize-based baby food were 57, 9, and 6 μg/kg, respectively. Intra-assay and interassay precisions were calculated for each matrix at the maximum and half-maximum European Union regulatory limits and expressed as the coefficient of variation (CV). All CVs fell below 10% with the exception of the between-run CV for breakfast cereal. Recoveries at the concentrations tested ranged from 92 to 115% for all matrices. Action limits of 161, 348, and 1378 μg/kg were calculated for baby food, wheat-based breakfast cereal, and wheat, respectively, and the linear range of the assay was determined as 250−2000 μg/kg.

**KEYWORDS: Deoxynivalenol; trichothecenes; cereals; screening assay; surface plasmon resonance**

## INTRODUCTION

Deoxynivalenol (DON) or vomitoxin belongs to the group of mycotoxins known as the trichothecenes. It is commonly found in agricultural crops such as maize and wheat and is therefore considered a food safety threat (*1*).

Desjardins (*2*) discussed the unequivocal evidence of the link between consumption of DON-contaminated grain and swine feed refusal but highlighted that although *Fusarium graminearum* (responsible for the production of DON) was strongly implicated in various outbreaks of human toxicosis in Japan during the 20th century, there is a lack of scientific evidence to substantiate the claims.

Regulatory limits have been established for DON in many countries, and currently in the European Union, a provisional tolerable daily intake of 1 μg/kg body weight has been established by the Scientific Committee on Food (SCF) (*3*). On the basis of this value, maximum limits for DON have been set at 1750 μg/kg for unprocessed durum wheat, oats, and maize, 1250 μg/kg for unprocessed cereals other than durum wheat, oats, and maize, 750 μg/kg for cereals intended for direct human consumption, cereal flour, bran, and pasta, 500 μg/kg for bread, pastries, biscuits, cereal snacks, and breakfast cereals, and 200 μg/kg for processed cereal-based foods and baby foods for infants and young children (*4*). In the United States a maximum limit of 1000 μg/kg has been established for finished wheat products for human consumption, whereas in Canada the maximum limits set are 2000 μg/kg for wheat, 1200 μg/kg for wheat flour, and 600 μg/kg for wheat flour used for infant food (*5*).

Many analytical methods have been reported for the detection of DON in cereal grains, and these may be divided into rapid/screening assays and confirmatory/reference methods. Today, the majority of screening assays employed are immunochemical methods including enzyme-linked immunosorbent assays (ELISA), lateral flow devices (LFDs), fluorescence polarization immunoassay (FPIA), and biosensor assays. Of these methods the most commonly used is ELISA (*6*−*10*), and there are a wide variety of commercial test kits available for the detection of DON in cereal-based foods and feeds. Similarly, lateral flow devices (*11*) have found widespread application due to their ease of use and the fact that they can be employed in situ, giving a rapid qualitative result. Rapid fluorescence polarization immunoassays (FPIA) for the determination of DON in wheat and wheat-based products have also been published (*12*, *13*). The application of biosensors to food analysis has become extremely popular over the past decade, in particular, optical sensing devices based on the principle of surface plasmon resonance (SPR). This technology has been successfully used for the determination of mycotoxins and phycotoxins in food (*14*−*16*). Several papers relating to the use of SPR for DON analysis have been published (*17*−*19*). Another novel optical biosensor assay described the use of an array biosensor developed by the U.S. Naval Research Laboratory for the detection of DON in foods (*20*). Over recent years alternative binding strategies have been sought for use in screening assays first to negate the need for animal use and second to provide more stable reagents. Maragos (*21*) reviewed the use of

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 Published on Web 07/22/2010 

various novel materials such as antibody fragments, phage display libraries, and synthetic polymers for the detection and determination of DON. He concluded that while recombinant antibody fragments are advantageous and can be incorporated into immunoassay format, animal immunizations are still required at the beginning of the process. The disadvantage of using polymers or synthetic peptides is their lack of selectivity when compared with antibodies. Further novel approaches have included the use of Raman spectroscopy (*22*) and a technique based on acoustic waves (*23*). Confirmatory techniques generally include the use of high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to various detectors, for example, ultraviolet, diode array, fluorescence, electron capture, or mass spectrometry (*24*, *25*). In more recent years there has been a shift from these traditional methods to the use of LC-MS/MS, where there is no requirement for derivatization, but of more importance is the ability to determine many mycotoxins simultaneously, increasing the speed of analysis and therefore reducing cost (*26*−*28*).

The aim of this work was to develop a simple, rapid screening assay for the determination of DON in wheat, wheat-based products, and maize-based baby food using a mouse monoclonal antibody raised against DON, a simple DON sensor chip surface, and the phenomenon of SPR.

## MATERIALS AND METHODS

**Equipment.** A Biacore Q was purchased from GE Healthcare, Uppsala, Sweden. Instrument operation and data handing were performed by the Biacore Q Control Software, version 3.1. This instrument is specifically intended for concentration assays for the measurement of analytes in food. It consists of a processing unit housing the liquid handling and optical units and a PC running the control and evaluation software. The sensor chip is inserted into a port on the detector unit and docks into the instrument, forming one side of the four detector flow cells. The other side is known as the integrated microfluidic cartridge (IFC). Sample and buffer deliveries to the flow cells are accurately controlled by means of the syringe pump system and the valves in the IFC.

**Chemicals and Reagents.** Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), deoxynivalenol glucoside, nivalenol (NIV), T-2 toxin, and HT-2 toxin standards were purchased from Biopure Referenzsubstanzen GmbH, Tulln, Austria. General purpose grade methanol, hydrochloric acid (HCl), sodium dodecyl sulfate (SDS), sodium acetate, carbonyldiimidazole (CDI), anhydrous dimethyl sulfoxide (DMSO), boric acid, 2,2′-(ethylenedioxy)bis-(ethylamine), sodium hydroxide, *N*,*N*′-disuccinimidyl carbonate (DSC), 4-dimethylaminopyridine (DMAP), human serum albumin (HSA), acetone, PBS tablets, sodium chloride, polyethylene glycol, and pyridine were purchased from Sigma-Aldrich, Gillingham, U.K. Research grade CM5 sensor chips, HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4) and an Amine Coupling Kit were purchased from GE Healthcare. Vivaspin centrifugal concentrator tubes were from Sagitorius Stedim Biotech GmbH (Germany) and the MAbTrap kit from Amersham Biosciences AB, Uppsala, Sweden. Quil A and *N*-palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-(*R*)-cysteinyl-seryl-(lysyl)$_3$-lysine (PCSL) were supplied by Superfos Biosector A/S, Frederikssund, Denmark, and EMC Echaz Microcollections, Tuebingen, Germany, respectively.

Blank and naturally contaminated samples were produced and provided by several BioCop Project Partners. The Swedish University of Agricultural Sciences, Uppsala, Sweden, produced wheat materials for the project by infecting durum wheat in the field with *F. graminearum*, and wheat samples high, low, and without DON were selected for use. Maize-based baby food materials (97.7% maize) were produced by 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. The wheat-based breakfast cereal test materials were produced by all of the above partners and contained graham wheat (60.7%), rice flour (15%), and crystal sugar (15%) and were flavored with cocoa powder, malt extract, and salt. All materials were ground or milled, blended, and packaged and subsequently underwent homogeneity and stability studies. Five laboratories experienced in mycotoxin analysis performed the characterization of the test materials using their in-house methods and the mean concentration values used as the assigned values for the project (**Table 2**). Additional naturally contaminated samples were made available by the Department IFA-Tulln, University of Natural Resources and Applied Life Sciences, Vienna, Austria, and Neogen Europe Ltd., Ayr, Scotland.

**Preparation of Deoxynivalenol-DSC-HSA (DON-DSC-HSA) Immunogen.** DON (7.5 mg), DSC (35.5 mg), and DMAP (18.3 mg) were dissolved in dry acetone (10 mL) and allowed to react for 4 h at room temperature, with stirring in the dark. The solvent was removed under nitrogen at 45 °C and the product resuspended in phosphate-buffered saline (pH 7.2) (500 μL) and pyridine (400 μL). HSA (20 mg) was dissolved in PBS (pH 7.2) (1 mL) and DON-DSC (600 μL) added slowly with stirring. The reaction mixture was incubated overnight at 25 °C and purified by dialysis against NaCl (9 g/L).

**Antibody Preparation.** BALB/c mice were immunized every 3 weeks with DON-DSC-HSA immunogen (20 μg). Primary and secondary booster immunizations were administered using Quil A adjuvant by subcutaneous injection, and third and fourth boosters were administered using PCSL adjuvant by interperitoneal injection. A fifth booster injection (25 μg) in PBS was administered by interperitoneal injection. The mice were monitored by biosensor for specific antibody titer using tail bleeds collected 10 days after each booster injection. The mouse showing the highest antibody titer was selected and, 4 days prior to the fusion being performed, received a final booster by interperitoneal injection of the immunogen (50 μg) in PBS (pH 7.2). A single cell suspension was prepared using the spleen of the chosen mouse and fused with SP2 cells using polyethylene glycol according to a modified version of the method published by Kohler and Milstein (*29*). The crude supernatant was concentrated using Vivaspin sample concentrator tubes, purified using a MAbTrap kit using the manufacturer's instructions, and stored in aliquots at −20 °C until required. The antibody was diluted 1:100 in HBS-EP for use in the assay.

**Immobilization of DON onto a CM5 Sensor Chip Surface.** The CM5 sensor chip was equilibrated to room temperature prior to priming with HBS-EP buffer. Activation of the carboxymethylated surface was performed by applying a 1:1 mixture of 0.4 M EDC and 0.1 M NHS in water (40 μL) onto the surface for 30 min. 2,2′-(Ethylenedioxy)bis-(ethylamine) was diluted to 20% in 50 mM borate buffer, pH 8.5 (40 μL), and reacted on the surface for 60 min followed by deactivation of the remaining unreacted sites by 1 M ethanolamine, pH 8.5 (40 μL), for a further 30 min. DON (1 mg) was reacted with CDI (1.5 mg) in anhydrous DMSO (100 μL) for 4 h and diluted 1:1 in 10 mM sodium acetate buffer, pH 4.6. This solution (40 μL) was applied to the sensor surface and the reaction left overnight at room temperature. On completion, the sensor chip was washed with deionized water, dried under a gentle stream of nitrogen, and stored under desiccant at 4 °C.

**Standard Calibration Curve.** The calibration curve consisting of six concentrations was constructed by diluting the Biopure DON stock standard (100 μg/mL) in HBS-EP buffer, ranging from 0 to 80 ng/mL (cereal equivalents of 0−4000 μg/kg).

**Sample Preparation.** All samples were prepared as follows: 5.0 ± 0.02 g was weighed into a plastic centrifuge tube (TPP, Techno Plastic Products AG, Switzerland) and mixed with 40% methanol (25 mL). The sample was vortexed for 10 s followed by centrifugation at 4369*g* for 10 min, and 0.5 mL of the supernatant was transferred to a test tube and evaporated to dryness under a stream of nitrogen at 60 °C; the residue was reconstituted in 5 mL of HBS-EP buffer and vortexed for 30 s. The sample was ready for analysis without the need for further treatment.

**Fortified Samples.** Known blank material was weighed as above and spiked at various levels (100, 200, 250, 500, 875, and 1750 μg/kg) depending on the sample being analyzed using 50 μL of the appropriate standard solution. The described protocol was then adhered to.

**Biosensor Assay Conditions.** The standards and samples were transferred to the wells of a microtiter plate. Under the control software these were mixed 1:1 with the DON monoclonal antibody (1/100 dilution) by the autosampler prior to injection (80 μL) over the surface of the sensor

chip. The flow rate was kept at a constant 20 μL/min throughout the analysis and the surface regenerated to remove the bound material after each cycle by 2 × 30 s pulses of 0.5% SDS/250 mM HCl (i.e., 2 × 10 μL injections at a flow rate of 20 μL/min). Report points were recorded before and after each injection to determine the relative response for each, and these were measured against those of the calibration curve, resulting in a concentration value for each. All analyses were performed at a constant temperature of 25 °C. The time required for each assay cycle was 9 min in total including wash cycles and injection preparation; therefore, the analysis of 20 samples, including sample preparation, could be completed within 6.5 h.

**Antibody Specificity.** The cross-reactivity profile of the antibody was determined in buffer spiked with structurally related and other commonly occurring trichothecenes (3-AcDON, 15AcDON, DON glucoside, NIV, and T-2 and HT-2 toxins). Again, the range used for each was 0−80 ng/mL (cereal equivalents of 0−4000 μg/kg). The midpoint concentration ($IC_{50}$) of each calibration curve was calculated and the cross-reactivity determined as a percentage relative to DON.

**Assay Validation.** Assay precision was evaluated by determining the intra-assay (within run) and interassay (between run) variations expressed as the coefficient of variation (CV) (SD/mean × 100). This was performed by spiking each matrix at the maximum and half-maximum permitted limits. Therefore, baby food was fortified at 100 and 200 μg/kg, breakfast cereal at 250 and 500 μg/kg, and unprocessed wheat at 875 and 1750 μg/kg ($n$ = 6 at each concentration). The analyses were repeated over three different days using three different biosensor instruments totaling $n$ = 18 for each concentration. The limit of detection (LOD) for each was established by the analysis of 20 blank samples and calculated using the mean of the measured response units (RU) minus 3 times the standard deviation (SD) of the mean (mean − 3SD). To assess the accuracy of the method, results of concentrations of naturally contaminated and reference samples determined by SPR were compared with those obtained by confirmatory LC-MS/MS.

**Confirmatory Analysis.** The LC-MS/MS method used has been described in great detail by Sulyok et al. (*26*) and more briefly by Meneely et al. (*14*). In short, mycotoxins were extracted from wheat and maize samples using a solvent mixture of acetonitrile/water/acetic acid (79:20:1 v/v/v) and analyzed by liquid chromatography with electrospray ionization triple-quadrupole mass spectrometry without sample cleanup. This multianalyte method was chosen to enable the collection of information on the DON derivatives.

## RESULTS AND DISCUSSION

The assay described is intended for use as a screening assay providing semiquantitative results. It has been constructed as a competition assay in which interactions of the antibody and toxin are monitored in real time. If a sample is heavily contaminated with DON, it will bind to the antibody in solution, thus preventing the antibody from attaching to the toxin on the surface of the sensor chip, resulting in a low response. Conversely, if the sample tested is negative, the antibody in solution will be able to bind to the sensor chip surface, eliciting a high response. These binding events provided a relative response (relative to the baseline) and were measured against a six-point calibration curve, allowing determination of the sample concentration in micrograms per kilogram.

Amine coupling is one of the most straightforward means of attaching ligands to the surface of CM5 sensor chips. Essentially, the carboxyl groups on the surface of the chip are activated with a mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.05 M *N*-hydroxysuccinimide (NHS) in water, producing reactive succinimide esters that react spontaneously with nucleophiles, resulting in a strong covalent bond. In the case of DON there are no nucleophilic groups to exploit, so it is rather more complex to couple to the sensor chip surface. Previous DON biosensor publications have reported the coupling of DON to proteins such as casein and bovine serum albumin (BSA) (*18*, *19*); although this provides a solution to the problem, often it is more appropriate to derivatize the small molecular weight compound for attachment to avoid possible complications associated with nonspecific binding to the sensor surface. This was the rationale applied in the development of the described assay, and the method of choice, that is, the carbonyldiimidazole (CDI) reaction was exploited. This coupling method has been successfully reported by others (*8*, *30*) for the coupling of T-2 toxin and DON to proteins and more recently used to covalently attach HT-2 toxin to an amine on the surface of a CM5 sensor surface (*14*).

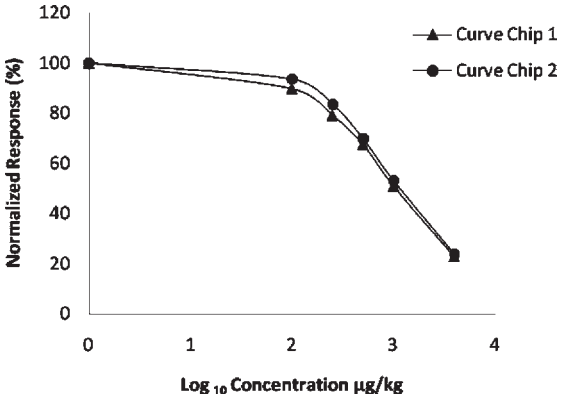


**Figure 1.** Comparison of calibration curves generated on two different DON sensor surfaces.

To ensure this assay/methodology was cost-effective and applicable to the screening of high numbers of samples, it was imperative to ascertain the longevity of the sensor chip surface and robustness of assay parameters. The within-day stability of one flow cell of the sensor chip surface was evaluated by performing 40 sequential analyses of wheat. The results highlight minimal drift throughout for both the antibody uptake of each sample and the corresponding baseline for each, therefore indicating that the surface was viable for the analysis of at least 160 samples (4 flow cells/chip). Two sensor chip surfaces were prepared on separate occasions and calibration curves performed on each. The response values for each calibration solution ($n$ = 2) were normalized by calculating the percentage of bound ligand (antibody) for each calibration point relative to the zero calibration relative response and the mean values plotted, and the results indicate that the surface immobilization procedure was both repeatable and reproducible (**Figure 1**). In addition, during the validation study, fresh calibration curves (in duplicate), extraction solutions, sample extracts, and the use of several instruments were evaluated over different days. Results of the repeatability of the calibrations curves indicated that not only were the curves consistent but they also helped to demonstrate the robustness of the developed assay. Mean relative response (RU) values of 358, 329, 293, 244, 187, and 85 were obtained for standard solutions at concentrations of 0, 100, 250, 500, 1000, and 4000 μg/kg, respectively. The standard deviations associated with these were all $<25$ with CV values of $\leq$11% for all concentrations. The linear range of the immunoassay was determined as 250−2000 μg/kg.

Because DON is a frequent contaminant of cereals and samples totally free of this toxin are difficult to obtain, it was important to develop an assay that did not require the use of matrix-matched curves. This has been achieved, and **Figure 2** highlights that virtually a complete overlay of calibration curves generated in all matrices against a buffer curve could be achieved. Calibration curves were prepared in negative wheat, breakfast cereal, and baby food extracts and compared with a buffer calibration curve. By using the Biacore Q Evaluation software, the curve midpoints were calculated as 13.7, 13.6, 13.4, and 13.1 ng/mL (cereal equivalents of 684, 681, 672, and 653 μg/kg) in buffer, baby food, breakfast cereal, and wheat, respectively. These values indicated that the sensitivity of the assay for all matrices remained virtually

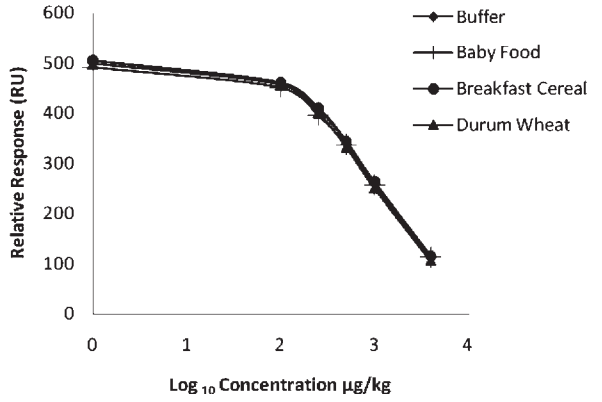


**Figure 2.** Overlay of buffer and matrix curves for DON.

unchanged. The antibody demonstrated a high specificity for DON (100%) and 3-AcDON (221%) in buffer, whereas there was no measurable cross-reactivity with 15-AcDON, NIV, T-2 toxin, HT-2 toxin, or DON-3-glucoside. This is consistent with other reports of antibody production against DON. For the most part, high cross-reactivity has been demonstrated with either 3-AcDON or 15-AcDON (*6*−*9*); however, Schneider et al. (*10*) have reported the production of antibodies against DON in chicken eggs, demonstrating low cross-reactivity with either 3-AcDON or 15-AcDON. Although the regulatory limits refer to the concentration of DON alone, the use of such antibodies for immunological screening tests will lead to some method uncertainty, that is, overestimation; however, it should also be recognized that the metabolites mentioned co-occur with DON at very low levels (*4*).

Limits of detection (LOD) for each matrix were calculated from the mean relative response of the 20 blank samples minus 3SD of the mean. The results were 57, 9, and 6 $\mu$g/kg for wheat, wheat-based breakfast cereal, and maize-based baby food, respectively; all fell well below the maximum permitted limits. Assay precision was evaluated by investigating the within-run and between-run variations, expressed as the CV of blank samples fortified at the regulatory limit and half the regulatory limit ($n = 6$ for within-run repeatability and $n = 18$ for reproducibility). Within-assay CV values for durum wheat were 3.1 and 4.7% spiked at 875 and 1750 $\mu$g/kg, 3% for wheat-based breakfast cereal spiked at 250 and 500 $\mu$g/kg, and 6.9 and 4% for maize-based baby food spiked at 100 and 200 $\mu$g/kg. Between-run variation was examined over three days with blank samples being fortified at the same levels used for the within-run precision study. All CV values fell below 8% with the exception of the between-run precision value for breakfast cereal, which was calculated as 17%. Recovery using the applied sample preparation was ascertained by comparing the concentrations displayed by the fortified samples against the expected concentrations. The results achieved ranged from 92% to 115% for all matrices investigated indicating the efficiency of extraction; however, these results also highlight that there are still some matrix interferences at the levels tested, which is unsurprising considering the matrix curves did not totally match the buffer curve against which the concentrations were calculated. Another factor to consider is that the LOD of the confirmatory method used to determine concentrations in the BioCop materials was 30 $\mu$g/kg, so low levels of DON may be contributing to the higher recovery values. To establish the threshold or cutoff limit for the procedure, negative durum wheat, wheat-based breakfast cereal, and maize-based baby food were spiked at 1750, 500, and 200 $\mu$g/kg (the EU regulatory limits) and analyzed, and the cutoff limits were established by subtracting 3SD from the mean concentration value. The results were 1378 $\mu$g/kg for durum wheat, 348 $\mu$g/kg for wheat-based breakfast cereal, and 161 $\mu$g/kg for maize-based baby food.

**Table 1.** Comparison of the SPR Biosensor Assay with LC-MS/MS

| sample | matrix | LC-MS/MS[a] ($\mu$g/kg) | neg/pos[b] | SPR[c] ($\mu$g/kg) | neg/pos[b] |
|---|---|---|---|---|---|
| 1 | wheat | 201 | negative | 187 | negative |
| 2 | wheat | 1341 | negative | 1190 | negative |
| 3 | wheat | 337 | negative | 355 | negative |
| 4 | wheat | 261 | negative | 356 | negative |
| 5 | wheat | 1357 | negative | 1110 | negative |
| 6 | wheat | 29 | negative | 0.5 | negative |
| 7 | wheat | 90 | negative | 61 | negative |
| 8 | wheat | 299 | negative | 214 | negative |
| 9 | wheat | 224 | negative | 180 | negative |
| 10 | wheat | 33 | negative | 26 | negative |
| 11 | wheat | 864 | negative | 569 | negative |
| 12 | wheat | 198 | negative | 139 | negative |
| 13 | maize | 4048 | positive | 4900 | positive |
| 14 | maize | 114 | negative | 109 | negative |
| 15 | maize | 7384 | positive | 7380 | positive |
| 16 | maize | 2960 | positive | 3050 | positive |
| 17 | maize | 1640 | negative | 1190 | negative |
| 18 | maize | 445 | negative | 370 | negative |
| 19 | oats | 1530 | negative | 1080 | negative |
| 20 | oats | 578 | negative | 321 | negative |
| 21 | oats | 74 | negative | 98 | negative |
| 22 | oats | 1 | negative | 5 | negative |
| 23 | oats | 1 | negative | 0 | negative |
| 24 | barley | 106 | negative | 109 | negative |

[a] LOD wheat =8 $\mu$g/kg; LOD maize =16 $\mu$g/kg. [b] EU regulations, unprocessed durum wheat, maize, and oats (1750 $\mu$g/kg) and unprocessed cereals (1250 $\mu$g/kg). [c] LOD wheat =57 $\mu$g/kg.

**Table 2.** Comparison of the BioCop Reference Materials Obtained Using SPR Technology and Mass Spectrometry

| sample | matrix | mass spectrometry[a] ($\mu$g/kg) | neg/pos[b] | SPR[c] ($\mu$g/kg) | neg/pos[b] |
|---|---|---|---|---|---|
| 1 | baby food (maize) | <LOD | negative | <LOD | negative |
| 2 | baby food (maize) | $211 \pm 42$ | positive | 189 | negative |
| 3 | breakfast cereal (wheat) | <LOD | negative | <LOD | negative |
| 4 | breakfast cereal (wheat) | $485 \pm 61$ | negative | 526 | positive |
| 5 | wheat | <LOD | negative | <LOD | negative |
| 6 | wheat | $750 \pm 177$ | negative | 773 | negative |
| 7 | wheat | $2654 \pm 1029$ | positive | 2750 | positive |

[a] LOD = 30 $\mu$g/kg (all matrices). [b] EU regulations, unprocessed durum wheat, maize, and oats 1750 $\mu$g/kg; unprocessed cereals, 1250 $\mu$g/kg; breakfast cereal, 500 $\mu$g/kg; baby food, 200 $\mu$g/kg. [c] LOD wheat = 57 $\mu$g/kg LOD maize-based baby food = 6 $\mu$g/kg; LOD wheat-based breakfast cereal = 9 $\mu$g/kg.

The accuracy of the assay was evaluated by comparing results of naturally contaminated samples generated by SPR with those determined using the LC-MS/MS method mentioned previously. These samples consisted of durum wheat, wheat-based breakfast cereals, maize, maize-based baby food, one barley sample, and several oat samples. A correlation of $R^2 = 0.98$ was observed (and the results are detailed in **Tables 1** and **2**. Moreover, a few oat samples and one barley sample were also tested by both methods, displaying comparable results. Therefore, it may be possible that the SPR assay can be applied to a wider range of matrices; however, these matrices would need rigorous evaluation, validation, and comparison with a confirmatory method.

Several groups (*17*−*19*) have described the application of optical biosensors for the determination of DON in cereals; however, the present study has many advantages over the other methods described and has presented substantially more validation data to support the performance of the assay. For screening assays to be cost-effective, sample preparation must be rapid and involve little or no cleanup. Whereas Tüdös et al. (*18*) have

employed a simple sample preparation, that is, extraction in 80% acetonitrile followed by dilution and filtration of the sample, the other biosensor publications describe more complicated methods involving extractions using 90% acetonitrile or 10% methanol/6% (w/v) polyvinylpyrrolidone followed by cleanup using solid phase extraction columns (*17*, *19*). Sample extraction times varied between 2 and 30 min (*17*, *19*); however, both methods require the use of MycoSep columns, increasing the cost per analysis significantly. The present method employs rapid sample extraction in 40% methanol and requires no cleanup or filtration prior to analysis. Another advantage of this method is that the antibody and sample are mixed together and immediately injected over the surface of the sensor chip. Others have described the need for incubation of the sample with the antibody prior to injection (*17*, *18*), thereby increasing the time of analysis. Three different matrices were evaluated and validated during this study, highlighting the repeatability and reproducibility of the assay. The accuracy of the method was also evaluated using naturally contaminated samples, a larger population than that used for the other studies, and the correlation observed was much greater (i.e., $R^2 = 0.98$ compared with $R^2 = 0.95$ or $R^2 = 0.91$) (*17*). A major advance described in the present study resides in the preparation of the sensor chip surface, which is simple to perform and highly robust. The methods previously employed to attach DON to the surface of the sensor chip involved coupling to a protein (*18*, *19*) or biotinylating a DON derivative and immobilizing that to a streptavidin-coated surface (*17*). These methods, although they overcome the difficulties of immobilizing DON to the sensor chip surface, are likely to result in increased nonspecific binding to the surface or reduced activity/stability of the surface in the case of the biotinylated DON−streptavidin surface, which could only withstand approximately 100 cycles (*17*).

Therefore, through single-laboratory validation and analysis of naturally contaminated samples, this SPR assay has been proven to offer sensitive, accurate, and reliable results for the determination of DON in durum wheat, wheat-based products, maize, and maize-based baby food. The method comparison also highlights that this screening assay may (with further evaluation and validation) be applied to other food matrices such as oats and barley, meeting the regulatory limits with ease. The sample extraction procedure requires no cleanup, thus significantly reducing the cost per analysis. The method could be transferred into routine testing laboratories offering a cost-effective, user-friendly, rapid screening method ensuring safer food supplies.

## SAFETY

As deoxynivalenol is known to be hazardous to humans and animals, care must be taken when handling this material. Protective clothing and gloves must be worn at all times, and it is important that proper disposal methods are adhered to.

## ACKNOWLEDGMENT

We thank the BioCop Project partners, the Department IFA-Tulln, University of Natural Resources and Applied Life Sciences, Vienna, Austria, and Neogen Europe Ltd., Ayr, Scotland, for the supply of blank and contaminated samples allowing validation of this screening assay.

## LITERATURE CITED

(1) Council for Agricultural Science and Technology. *Mycotoxins: Risks in Plant, Animal, And Human Systems*; Task Force Report 139; **2003**.

(2) Desjardins, A. E. *Fusarium Mycotoxins Chemistry, Genetics, and Biology*, 1st ed.; American Phytopathological Society: St. Paul, MN, 2006.

(3) Commission Regulation (EC) No 856/2005. Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* **2006**.

(4) Commission Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* **2006**.

(5) Food and Agriculture Organization of the United Nations. Worldwide regulations for mycotoxins in food and feed. *FAO Food and Nutrition Paper 81*; FAO: Rome, Italy, **2003**.

(6) Casale, W. L.; Pestka, J.; Hart, L. P. Enzyme-Linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. *J. Agric. Food Chem.* **1998**, *36*, 663–668.

(7) Sinha, R. C.; Savard, M. E.; Lau, R. Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. *J. Agric. Food Chem.* **1995**, *43*, 1740–1744.

(8) Maragos, C. M.; McCormick, S. P. Monoclonal antibodies for the mycotoxins deoxynivalenol and 3-acetyl-deoxynivalenol. *Food Agric. Immunol.* **2000**, *12*, 181–192.

(9) Yoshizawa, T.; Kohno, H.; Ikeda, K.; Shinoda, T.; Yokohama, H.; Morita, K.; Kusada, O.; Kobayashi, Y. A practical method for measuring deoxynivalenol, nivalenol, and T-2 + HT-2 toxin in foods by an enzyme-linked immunosorbent assay using monoclonal antibodies. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2076–2085.

(10) Schneider, L.; Pichler, H.; Krska, R. An enzyme linked immunoassay for the determination of deoxynivalenol in wheat based on chicken egg yolk antibodies. *J. Anal. Chem.* **2000**, *367*, 98–100.

(11) Kolosova, A. Y.; Sibanda, L.; Dumoulin, F.; Lewis, J.; Duveiller, E.; Van Peteghem, C.; De Saeger, D. Lateral-flow colloidal gold-based immunoassay for the rapid detection of deoxynivalenol with two indicator ranges. *Anal. Chim. Acta* **2008**, *616*, 235–244.

(12) Maragos, C. M.; Plattner, R. D. Rapid fluorescence polarization immunoassay for the mycotoxin deoxynivalenol in wheat. *J. Agric. Food Chem.* **2002**, *50*, 1827–1832.

(13) Lippolis, V.; Pascale, M.; Visconti, A. Optimization of a fluorescence polarization immunoassay for rapid quantification of deoxynivalenol in durum wheat-based products. *J. Food Prot.* **2006**, *11*, 2712–2719.

(14) Meneely, J. P.; Sulyok, M.; Baumgartner, S.; Krska, R.; Elliott, C. T. A rapid optical immunoassay for the screening of T-2 and HT-2 toxin in cereals and maize-based baby food. *Talanta* **2010**, *81*, 630–636.

(15) Stewart, L. D.; Hess, P.; Connolly, L.; Elliott, C. T. Development and single-laboratory validation of a pseudofunctional biosensor immunoassay for the detection of the okadeic acid group of toxins. *Anal. Chem.* **2009**, *81*, 10208–10214.

(16) Campbell, K.; Stewart, L. D.; Doucette, G. J.; Fodey, T. L; Haughey, S. A.; Vilarino, N.; Kawatsu, K.; Elliott, C. T. Assessment of specific binding proteins suitable for the detection of paralytic shellfish poisons using optical biosensor technology. *Anal. Chem.* **2007**, *79*, 5906–5914.

(17) Schnerr, H.; Vogel, R. F.; Niessen, L. A biosensor-based immunoassay for rapid screening of deoxynivalenol contamination in wheat. *Food Agric. Immunol.* **2002**, *14*, 313–321.

(18) Tüdös, A. J.; Lucas-van den Bos, E. R.; Stigter, E. C. A. Rapid surface plasmon resonance-based inhibition assay of deoxynivalenol. *J. Agric. Food Chem.* **2003**, *51*, 5843–5848.

(19) Van der Gaag, B.; Spath, S.; Dietrich, H.; Stigter, E.; Boonzaaijer, G.; Van Osenbruggen, T.; Koopal, K. Biosensors and multiple mycotoxins analysis. *Food Control* **2003**, *14*, 251–254.

(20) Ngundi, M. M.; Qadri, S. A.; Wallace, E. V.; Moore, M. H.; Lassman, M. E.; Shriver-Lake, L. C.; Ligler, F. S.; Taitt, C. R. Detection of deoxynivalenol in foods and indoor air using an array biosensor. *Environ. Sci. Technol.* **2006**, *40*, 2352–2356.

(21) Maragos, C. M. Recent advances in the development of novel materials for mycotoxins analysis. *Anal. Bioanal. Chem.* **2009**, *395*, 1205–1213.

(22) Liu, Y.; Delwiche, S. R.; Dong, Y. Feasibility of FT-Raman spectroscopy for rapid screening for DON toxin in ground wheat and barley. *Food Addit. Contam.* **2009**, *26*, 1396–1401.

(23) Juodeikiene, G.; Basinskiene, L.; Vidmantiene, D.; Bartkiene, E.; Kunigelis, V.; De Koe, W. J. Rapid acoustic screening of deoxynivalenol (DON) in grain. *World Mycotoxin J.* **2008**, *1*, 267–274.

(24) Schothorst, R. C.; Jekel, A. A. Determination of trichothecenes in wheat by capillary gas chromatography with flame ionization detection. *Food Chem*. **2001**, *73*, 111–117.
(25) Pussemier, L.; Piérard, J. Y.; Anselme, M.; Tangni, E. K.; Motte, J. C.; Larondelle, Y. Development and application of analytical methods for the determination of mycotoxins in organic and conventional wheat. *Food Addit. Contam*. **2006**, *23*, 1208–1218.
(26) Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom*. **2006**, *20*, 2649–2659.
(27) Vishwanath, V.; Sulyok, M.; Labuda, R.; Bicker, W.; Krska, R. Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. *Anal. Bioanal. Chem*. **2009**, *395*, 1355–1372.
(28) Vendl, O.; Berthiller, F.; Crews, C.; Krska, R. *Anal. Bioanal. Chem*. **2009**, *395*, 1347-1354
(29) Kohler, G.; Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **1975**, *256*, 495–497.
(30) Xiao, H.; Clarke, J. R.; Marquardt, R. R.; Frohlich, A. A. Improved methods for conjugating selected mycotoxins to carrier proteins and dextran for immunoassays. *J. Agric. Food Chem*. **1995**, *43*, 2092–2097.

**Received for review April 21, 2010. Revised manuscript received July 10, 2010. Accepted July 13, 2010. This work was supported by a grant from the European Commission project, "New technologies to screen multiple chemical contaminants in foods" (contract FOOD-CT-2005-006988 BIOCOP).**