Food Chemistry 119 (2010) 834–839

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Development of an immunochromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize

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ARTICLE INFO

Article history:
Received 19 March 2009
Received in revised form 28 August 2009
Accepted 31 August 2009

Keywords:
Deoxynivalenol
Monoclonal antibody
Immunochromatography
Cationic bovine serum albumin
Colloidal gold
Wheat
Maize

ABSTRACT

An anti-DON monoclonal antibody (Mab) was produced from a stable hybridoma cell line, 12D1, generated by the fusion of SP2/O myeloma cells with spleen cells isolated from a Balb/c mouse immunized with DON–cationic bovine serum albumin (CBSA) conjugate. The 12D1 Mab belongs to the immunoglobulin G1 (κ-chain) isotype.

A colloidal gold immunochromatographic strip (ICS) test for rapid detection of deoxynivalenol (DON) in wheat and maize samples was also developed using this Mab. The ICS test, which has a detection limit of 50 ng mL^{-1} for DON and can be completed in 10 min. Analysis of DON in wheat and maize samples revealed that data obtained from ICS test were in a good agreement with those obtained from ELISA and GC/MS. The results demonstrate that the ICS test can be used as qualitative tool for screening technique of DON on-site.

1. Introduction

Mycotoxins are toxic metabolites produced by fungi either in the field or during production, transportation, processing, or storage of food, and are of considerable concern for human and animal diseases as well as economic effects due to crop contamination (Ngundi et al., 2006). Each year ~25% of all food crops are affected by mycotoxin contamination, causing considerable financial damage worldwide (Brenn-Struckhofova, Cichna-Markl, Böhm, & Razzazi-Fazeli, 2007). Owing to their considerable economical and health risk, the occurrence of mycotoxins in food and feed products has been studied extensively and for decades (Bucheli et al., 2008). One of the world significant mycotoxins seems to be a deoxynivalenol (DON).

DON is produced largely by *Fusarium* fungi, which are widespread in nature and commonly contaminate many cereal grains such as wheat, corn, barley, oats, and rye intended for human and animal consumption (Cetin & Bullerman, 2006). The current maximum levels set by the European Commission, the US Food and Drug Administration, are 1750 μg kg^{-1}, 1000 μg kg^{-1}, respectively, for unprocessed durum wheat and maize (Kolosova et al., 2008).

In order to determine the DON levels in foods and feeds, many research efforts have been conducted to develop sensitive and specific methods such as chromatographic methods, near infrared spectroscopy (Pettersson & Aberg, 2003), mid-infrared spectroscopy (Kos, Lohninger, & Krska, 2003) and etc. for DON detection, and detected in several types of grain and cereals throughout the world (Cirillo, Ritieni, Visone, & Cocchieri, 2003). At present, the chromatographic methods of determination of DON are normally carried out using gas chromatography (GC) with either electron capture or mass spectrometric (MS) detection (Mirocha, Kolaczkowski, Xie, Yu, & Jelen, 1998; Schothorst & Jekel, 2003), or high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, fluorescence detection after postcolumn derivatization or MS detection (Brenn-Struckhofova et al., 2007; Valenta & Dänick, 2005). The merit of chromatographic methods is that they allow highly sensitive measurements of individual toxins simultaneously. But they are thought to be unsuitable for measuring many samples within a short time. In addition, some researchers have established enzyme-linked immunosorbent assay (ELISA) for DON (Casale, Pestka, & Hart, 1988; Sinha, Savard, & Lau, 1995), and it is also unsuitable for on-site detection.

In the past few years, ICS test has increasingly gained interest in the field of food safety due to the potential for fast and simple on-site application. For instance, it has been used for the detection of small molecular toxins such as ochratoxin A (Cho et al., 2005; Liu, Tsao, Wang, & Yu, 2008), fumonisin B_1 (Wang, Quan, Lee, & Kennedy, 2006), aflatoxin B_1 (Delmulle, De Saeger, Sibanda, Barna-Vetro, & Van Peteghem, 2005), and T-2 toxin (Molinelli et al., 2008).

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doi:10.1016/j.foodchem.2009.08.049

Recently, an ICS test for the rapid detection of DON (Kolosova, De Saeger, Sibanda, Verheijen, & Van Peteghem, 2007; Kolosova et al., 2008) has been reported. Some parameters, such as the amount of immunoreagents, type of the materials, composition of the blocking solution and of the detector reagent mixture, were investigated. In order to remove the non-specific adsorption, the membranes were blocked with 2% (w/w) case/phosphate buffer for 30 min, and then dried at 37 °C for 60 min in the preparation of the test strip. However, the related preparation schemes of the anti-DON Mab and the colloidal gold-Mab probes were seldom involved in detail in the literature (Kolosova et al., 2008).

In the current study, the monoclonal anti-DON antibody was generated with DON–CBSA conjugated for immunogen, and then a monoclonal antibody-based gold nanoparticle ICS test was established to analyze DON in wheat and maize samples. We improved the preparation of an ICS test for rapid detection of DON in wheat and maize samples. A simple approach was applied to remove the non-specific absorption and ensure the reliable result by using anionic surfactant, which has significant effect on the test performance. Moreover, the optimization of pH values in colloidal gold solution was also carried out so that improvement the conjugation between the colloidal gold and anti-DON antibody. Based on the proposed method, we obtained a series of satisfactory results. In summary, the aim of the presented work was to develop ICS for the rapid screening of DON in wheat and maize. The ICS should allow fast and selective sample screening within a few minutes, requiring no skilled personnel.

2. Materials and methods

2.1. Materials and chemicals

DON, bovine serum albumin (BSA), ethylenediamine, tetrahydrofuran (THF), ovalbumin (OVA), dimethyl sulfoxide (DMSO), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), 1,1′-carbonyl-diimidazole (CDI), 4-dimethylaminopyridine (DMAP), *N*-hydroxysuccinimide, 1-butaneboronic acid (BBA), Tween-20, sucrose, mycose, polyvinylpyrrolidone (PVP), sodium dodecyl sulphate (SDS), sodium dodecylbenzene sulfonate (SDBS), polyethylene glycol (PEG MW = 1500 and 20,000), Goat anti-mouse IgG was obtained from Sino-American Biotechnology Co. (Shanghai, China). The SP2/O myeloma cell line was obtained from Institute of Hematology, Chinese Academy of Medical Sciences (Tianjing, China). A mouse Mab isotyping kit was obtained from Hycult biotechnology (Uden, Netherlands). The anti-DON Mab was purified using Protein-G Sepharose Fast Flow Columns (Amersham, NJ, USA). All other chemicals and solvents were of analytical grade or better and were obtained from Beijing Chemical Reagent Co. (Beijing, China). The CBSA was prepared according to the method described by Chu, Lau, Fan, and Zhang (1982). Stock solution was prepared by dissolving 1.0 mg of DON in 1.0 mL of deionized water and then kept at −20 °C for further dilution.

The NC membranes such as AE99, Prima 40 (P40), Immunopore RP (RP), Immunopore FP (FP), Sartorius CN 140 (CN140), and Millipore 135 (M135) were obtained from Whatman (Middlesex, UK), Sartorius (Göttingen, Germany) or Millipore Co. (Bedford, MA, USA). The colloidal gold (40 nm in diameter), the sample pad, the conjugation pad and the absorbent pad were obtained from Jiening Bio. Co. (Shanghai, China).

2.2. Equipments

The equipment used for spraying and cutting strip tests was purchased from Bio-Dot (Irvine, CA). The guillotine cutter (model CM 4000) was supplied by Bio-Dot (Irvine, CA). A Maxisorp polystyrene 96-well plates were purchased from Nunc (Roskilde, Denmark), and the plates were washed with a 3AWA Wellwash Plus from Thermo (Labsystems, Vantaa, Finland). Immunoassay absorbance was read with a Multiskan MK3 Spectrum purchased from Thermo. UV–Visible spectra were obtained by using an Ultrospec 4300 Pro UV–Visible Spectrophotometer (Amersham, NJ, USA). The GC/MS analysis was performed with Shimadzu QP2010 GC/MS (Kyoto, Japan).

2.3. Mab production and characterisation

2.3.1. Conjugation of DON to CBSA

DON was conjugated with CBSA in the presence of CDI under the following conditions: The CDI solution (8.0 mg of CDI in 0.02 mL of anhydrous tetrahydrofuran (THF)) was freshly prepared and then added to a DON solution (1.0 mg of DON in 0.02 mL of anhydrous tetrahydrofuran (THF)) and stirred at room temperature for 2 h. After reaction, the THF was removed by vacuum pumping, and then the residue was dissolved in 0.1 mL DMSO. The residue was added slowly to 2.0 mg of CBSA, which was dissolved in 0.6 mL of PBS and stirred at 15 °C for overnight. After reaction, the mixture was dialyzed against 2 L of PBS for 72 h, and changed PBS two times 24 h and then lyophilized for storage at −20 °C until further use.

2.3.2. Preparation of anti-DON Mab

Anti-DON Mab was produced according to the slightly modified method described previously by Kohler and Milstein (1975). DON antibodies in ascites were purified by ammonium sulfate precipitation followed by Protein-G Sepharose Fast Flow Column.

2.4. Measurement of cross-reactivity

To evaluate the specificity of the Mab, DON and several related compounds were tested for cross-reactivity ciELISA as described by Dixon, Warner, Hart, Pestka, and Ram (1987). The molar DON concentration that caused 50% inhibition (IC_{50}) were used to calculate cross-reactivities according to the following equation: % cross-reactivity = $[IC_{50}(DON)/IC_{50}(\text{cross-reacting compound})] \times 100$.

2.5. Preparation of colloidal gold probes

Anti-DON Mab purified by affinity chromatography was dialyzed against phosphate buffer (0.01 M, pH 7.4, PB) for 24 h at 4 °C and then centrifuged at 10,000 rpm (8497*g*) for 10 min to get a clear supernatant for conjugation. The pH of the colloidal gold solution for anti-DON monoclonal antibody conjugation was adjusted to pH 7.0 with 0.1 M K_2CO_3 or 0.1 M HCl. With gentle stirring, the anti-DON Mab (100 μg mL^{-1}, 0.7 mL) was added dropwise to the colloidal gold solution (10 mL). After reacting for 30 min, PB containing 10% BSA (1 mL) was added and further reacted for 15 min and then, to remove the excess of antibody, the reaction mixture was centrifuged at 10,000 rpm (8497 g) at 4 °C for 20 min. The clear supernatant was carefully removed, and gold pellets were resuspended in 1 mL of conjugate storage buffer (5% sucrose, 0.2% BSA, 0.3% PVP, 1% mycose, and 0.05% sodium azide in PBS, w/v) and stored at 4 °C until use.

2.6. Preparation of immunochromatographic strip

The sample and the conjugate pads were treated with PBS and the above conjugate storage buffer, respectively, and then vacuum-dried at 37 °C for 4 h. A total of 5 μL cm^{-1} of colloidal gold-labelled anti-DON Mab (OD 10) diluted five times with PBS containing 5% sucrose, 0.2% BSA, 0.3% PVP, 1% mycose and 0.05% sodium azide, was jetted on the treated conjugate pad by using

the Bio-Dot XYZ Platform, and then lyophilized to dryness. The pad was stored in a desiccator at room temperature. A total of 0.74 μL cm^{-1} of DON–CBSA (1 mg mL^{-1}) conjugate and goat anti-mouse IgG antibody (1.5 mg mL^{-1}) were sprayed onto the bottom and the top of NC membrane (Prima 40, 25 × 300 mm) as the test (T line) and control lines (C line), respectively, by Bio-Dot XYZ Platform, and then vacuum-dried at 37 °C for 2 h. The distance between lines was 4–5 mm. The sample pad, conjugate release pad, NC membrane, absorbent pad and backing card were assembled as as described by Zhu et al. (2008).

2.7. Detection of standard solution

Several concentrations of DON (from 0 to 400 ng mL^{-1}) were dissolved with PBS containing 0.5% Tween-20 and 0.05% (w/v) anion surfactant as detergent, and then the detection limit was determined. The application of the strips was put in a 100 μL the solution on the sample pad and allowed to migrate up the membrane.

In order to evaluate the effect of the anion surfactant on the ICS, the concentration of anion surfactant in the solution of groups a (including a_0 and a_1) and b (including b_0 and b_1) was 0, and 0.05%, respectively.

2.8. Sample pretreatment for strip test

Samples of wheat and maize were ground in the mechanical mortar for 5 min to pass a 20-mesh screen, then mixed, and 5 g of the ground samples were weighed into a 50 mL polypropylene centrifuge tube, and then mixed with 25 mL of distilled water. After severe mixing the suspension for 3 min, extracts were filtered through a Whatman 2 V filter. One milliliter of the filtrate was aspirated, diluted with 3 mL distilled water, and then directly subjected to ELISA kits (Ridascreen® Fast DON ELISA). For ICS test, 5 g of the ground samples were weighed into a 250 mL glass beaker, and then mixed with 100 mL of PBS containing 0.5% Tween-20 and 0.05% (w/v) anion surfactant, and then 100 μL of the resulting filtrate was pipetted onto the sample pad of the strip for analysis. A total of 32 natural samples (16 wheat and 16 maize samples) were extracted and tested by the ICS method. Then the results were confirmed by ELISA and gas chromatographic/tandem mass spectrometric (GC/MS).

2.9. Analysis by GC/MS

The GC/MS method was carried out as described by Mirocha, Kolaczkowski, Xie, Yu, and Jelen (1998). Twenty-five grams the finely ground wheat or maize sample was placed into a 250 mL Erlenmeyer flask, and then 100 mL acetonitrile/water (84:16, v/v) was added. The sample was extracted on a shaker for 1 h and centrifuged for 10 min at 4000 rpm (1359g). Four milliliters of the supernatant was passed through a specially prepared column, which mix thoroughly one part C-18 packing (40 μm particle size) material to three parts aluminum oxide neutral (Tacke & Casper, 1996). Next, the filtrate passed through the column was derivatized through HFBI, and then, one microliter of this was injected into the Shimadzu QP2010 GC/MS. Each sample was evaluated three times in duplicate.

3. Results and discussion

3.1. Monoclonal antibody preparation and characterization

DON is too a low molecular to be immunogenic. In order to overcome the limit of small molecule recognition in an immune response, the DON–protein conjugate was constructed and used as the immunogen instead of DON itself. Some researchers found that proper orientation of DON on the carrier protein was critical to antibody production. Since DON cannot be directly conjugated to carrier proteins, chemical groups capable of covalent linkage to protein must be introduced onto the molecule (Casale et al., 1988). In this paper, DON–CBSA conjugate was used as the immunogen. The DON–CBSA was prepared by the CDI method. The protein was easy to denature in this method, but the application of CBSA for carrier protein was able to improving the stability.

The subclass of the anti-DON Mab was identified as IgG1, and the light chains were identified as κ-chains by Mouse Immunoglobulin Isotyping ELISA Kit. The specificity of anti-DON Mab was determined in the indirect ELISA using DON–CBSA as the solid-phase antigen. With concentrations of DON and related compounds required to inhibit 50% of the binding of the anti-DON Mab in the ciELISA as a basis for comparison, the relative cross-reactivities for 15-AC-DON, 3-AC-DON, nivalenol, T-2, HT-2, ZEN, and citrinin were determined with the same ciELISA (Table 1). The results shown that the anti-DON Mab had a higher affinity for 15-*O*-acylated DON (15-acetyl-DON, and 15-HG-DON) than for DON, while 3-AC-DON, nivalenol, T-2, HT-2, ZEN, and citrinin cross-reacted poorly (Table 1). The IC_{50} of binding of Mab to DON–CBSA by free DON was found to be 96 ng mL^{-1} in the ciELISA. The range of detection was 20–460 ng mL^{-1}, lower than previously reported immunoassays for DON (Casale et al., 1988; Sinha et al., 1995).

3.2. Development and optimization of the strip test

The main objective of the ICS test was qualitative detection of DON contamination at threshold levels, and used in on-site screening of wheat and maize samples. For the development of a sensitive ICS test, the optimal various parameters such as detector reagent, capture reagent and NC membrane were as follows: To make a high-quality and reproducible colloidal gold conjugate, many factors, such as purity of antibody, size and quality of gold colloid, pH value of solution and antibody amount, etc. should be considered.

3.2.1. Anti-DON Mab purification

All antibodies, whether monoclonal or polyclonal, should be purified before conjugation. In addition to IgG, the purified preparation will contain a mass of proteins. All of these proteins will compete for binding sites on surface of the labelled gold colloid. This will result in a low degree of sensitivity of the detector reagent in the assay (Christopher, Robinson, & Shaw, 2005, chap. 5). In this study, the Anti-DON Mab from the cleared ascites fluid was purified by ammonium sulfate precipitation (50% saturation for the final solution) twice and then was purified again by affinity chromatography with a Protein-G Sepharose Fast Flow Column.

Table 1
Cross-reactivity and IC_{50} of the Mab to DON and related compounds.

DON analogues	DON	15-AC-DON	3-AC-DON	Nivalenol	T-2	HT-2	ZEN	Citrinin
IC_{50} (ng/mL)	96	24	6194	2300	>10,000	>10,000	>10,000	>40,000
Cross reactivity	100%	400%	1.6%	4.3%	<0.5%	<0.5%	<1%	<0.3%

3.2.2. Gold colloidal particles selection

In a rapid test, the colloidal gold particle must be large enough to be seen. The most common size used is 40 nm. In some previous papers, they represented that 40 nm colloidal gold particles offered maximum visibility due to the least steric hindrance in the case of IgG conjugation (Christopher et al., 2005). Therefore, spherical gold particles with a diameter of 40 nm colloidal gold were also selected in our study.

3.2.3. pH value and antibody amount of colloidal gold conjugate

In this study, several pH values (5.5, 6.0, 6.5 7.0, 7.4, 8.2, and 9.0) of colloidal gold solution were tested for conjugation with anti-DON Mab. After centrifugation, the titer of supernatant and original antibody was compared by ciELISA. It was found that the OD of supernatant decreased with pH value from 5.5 to 7.0, and increased with pH from 7.0 to 9.0 (Fig. 1). On the other hand, through the ICS test for negative solution, the color intensity of T and C line gradually increased with an increasing pH value from 5.5 to 7.0, and decreased from 7.0 to 9.0. As above result, the optimal pH value was found to be 7.0. Any excess antibody present will compete with labelled antibody for binding sites with the capture reagents, and this will lead to reduced sensitivity and potentially false-positives. The antibody amounts (from 1 to 10 μg per milliliter of colloidal gold) were screened for optimum combination in pH 7.0 to obtain the best sensitivity of the ICS test. It is found that the optimum amount of antibody was 7 μg for 1 mL colloidal gold.

3.2.4. Capture reagent selection

In this study, various types DON–carrier protein conjugates such as DON–OVA, DON–BSA and DON–CBSA on NC membrane were compared. Among three carrier protein conjugate, DON–CBSA gave the best sensitivity and higher signal. The application of DON–CBSA for capture reagents has several advantages. Firstly, the CBSA gives higher stability in coupling with DON. Secondly, the CBSA show higher coupling ration than other protein in the coupling reaction with DON. Finally, the DON–CBSA for capture reagents improves the assay sensitivity. However, it was also found the non-specific bindings were high between DON–CBSA and colloidal gold due to electrostatic action (Fig. 2). To remove the non-specific adsorption, a range of blocking reagents such as polymers (PVP, PVA, and PEG), surfactant (Tween-20, Triton-X), and protein (BSA, decreamed milk, casein, etc.) were tested to demonstrate the minimum non-specific binding in ICS test applications (Kaur et al., 2007; Kolosova et al., 2008). In this study, the effect of the three blocking buffers (including 0.1–3% decreamed milk, 0.005–3% BSA, and 0.05–1.0% casein) was evaluated. The results showed that the non-specific adsorption did not remove by the lower concentration of above three blocking buffers. Protein concentration (1–3%), in general, help to reduce the levels of non-specific background signal in ICS test. However, compared with the NC membrane unblocked, blocked membrane with these protein allows both sample solution and gold-labelled antibody to flow more slowly, and hence are not recommended as suitable blocking materials for ICS test applications (Christopher et al., 2005; Kaur et al., 2007). But the non-specific reaction was removed by adding anion surfactants such as SDS and SDBS to the sample solution. As shown in Fig. 2, the T line color intensity of 50 ng mL^{-1} of DON was clearly distinguishable from that of the negative control in group *b*. Apparently, the non-specific reaction was removed by adding 0.05% anion surfactant to the solution of group *b*. It appears that anion surfactant may neutralize the positive charge of the CBSA.

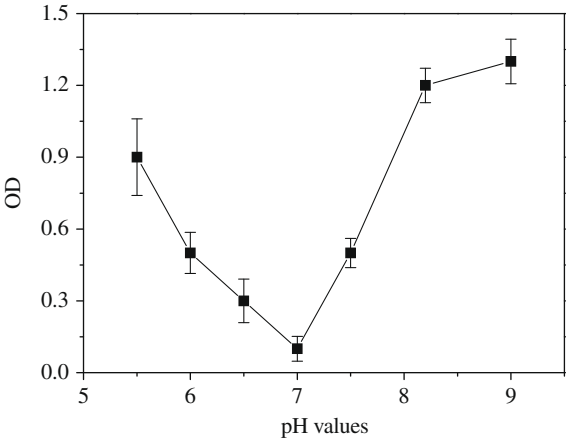

Fig. 1. Effect of the change pH values from 5.5 to 9.0 on the OD of supernatant of colloidal gold-labelled anti-DON Mab.

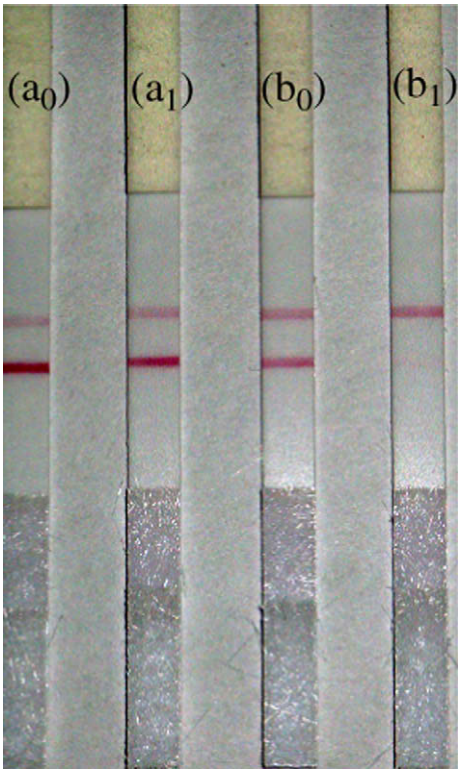

Fig. 2. Effect of the anion surfactant on the ICS: upper line, C line; lower line, T line. The concentration of anion surfactant in the solution of groups a (including a_0 and a_1) and *b* (including b_0 and b_1) was 0, and 0.05%, respectively. The concentration of DON in the solution (a_0) and (b_0), and (a_1) and (b_1) was 0, and 50 ng/mL, respectively.

3.2.5. NC membrane selection

For NC membrane selection, this was established by color intensity of the T line on the various NC membrane such as AE99, P40, RF, FP, CN140, and M135 following the application of a negative sample. It was found that RF, FP, CN140, and M135 produced lower color intensity of T line as compared to AE99 and P40. This finding may indicate that for this application, these membranes (AE99 and P40) may be more efficient in the binding of the protein conjugate increasing the amount of antibody that could attach. It might also be suggested that the slower nominal flow rates of these membranes may determine the interaction of the colloidal gold-labelled anti-DON Mab with the DON–CBSA on NC membrane occurring. Although best results were obtained with the unsupported NC membranes (AE99), giving a strong test signal when incubated

with colloidal gold-labelled anti-DON Mab as detector reagent. But it was more different to assemble the unsupported NC membrane to the blacking card. Therefore, the supported NC membrane (P40) was selected for experiment in this study.

Subsequently, the optimal concentration of capture reagent and the amount of detector reagent were selected as a clear appearing in the negative control with the shortest time and comparison of the intensity of color between positive samples and negative samples, and the positive sample could be easily distinguished from the negative sample by eye (Shim et al., 2006). The optimal conditions for ICS test were as follows: A total of 0.74 μL cm^{-1} of DON–CBSA (1 mg mL^{-1}) conjugate and goat anti-mouse IgG antibody (1.5 mg mL^{-1}) were sprayed onto the bottom and the top of NC membrane (Prima 40, 25 × 300 mm) as the T and C line, and a total of 5 μL cm^{-1} of colloidal gold-labelled anti-DON Mab (OD 10) diluted five times with PBS containing 0.2% BSA, 5% sucrose, 0.3% PVP, 1% mycose and 0.05% sodium azide, was jetted on the treated conjugate pad.

3.2.6. Extraction solvents selection

For extraction solvents selection, although acetonitrile in combination water is often used for the extraction of DON from wheat and maize (Mirocha et al., 1998), acetonitrile was not used as the extraction solvent in this approach because of its toxicity (Cho et al., 2005) and incompatibility with the anti-DON Mab. Furthermore, because this extraction should also be performed on-site in a non-laboratory environment by unskilled personnel, it was avoided as extraction solvent. Sometimes water or PBS was used for the extraction of DON from cereals (Maragos & Plattner, 2002). Therefore, in this study, deionized water or PBS was selected for extraction of DON from wheat and maize samples. After 3–5 min extraction procedure with water for wheat and maize samples, the DON recovery was determined by ELISA. Results shown that the 80–91.5% recoveries were obtained from these spiked samples by water extraction.

3.3. Detection limit of DON ICS test

The detection limit is defined as the concentration of DON in the solution that causes a complete invisibility of the T line. Each sample containing various concentrations of DON (0–400 ng mL^{-1}) and negative sample were assayed ten times by using the ICS test as described previously. The results were judged by at least three person visualization with 10 min after the reaction started. Two red lines on the membrane indicated that the DON concentration was below the detection limit. If only one red line was shown in the C line, the DON concentration was above the detection limit, which could occupy all of the colloidal gold-labelled anti-DON Mab and prevent the colloidal gold-labelled anti-DON Mab from binding with the DON–CBSA on the test zone. As shown in Fig. 3, the detection limit of strip test for DON was about 50 ng mL^{-1}.

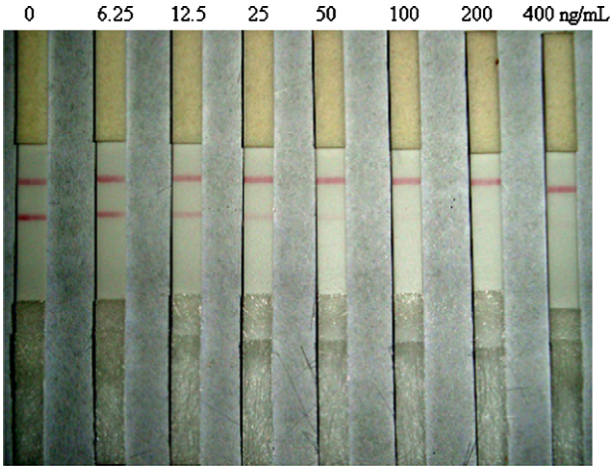

Fig. 3. Detection limit of DON with ICS test: upper line, C line; lower line, T line. A series of dilutions (0–400 ng/mL) of DON was made in 0.01 M PBS containing 0.5% Tween-20 and 0.05% anion surfactant. A concentration higher than 50 ng/mL DON was found to cause a disappearance of red line at the T line. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

3.4. Samples analysis

The DON negative wheat and maize samples determined by GC/MS were spiked with 0, 50, 500, and 1500 μg kg^{-1} of DON, and then analyzed by ICS test, ELISA and GC/MS. The recoveries by GC and ELISA ranged from 82.2% to 91.5% and from 80% to 90%, respec-

Table 2
Results of DON analysis by GC/MS, ELISA, and ICS test in the wheat and maize samples.

Wheat samples	GC/MS (mg/kg)[b]	DON kit[a] (mg/kg)	Visual results of ICS test (n = 4)	Maize samples	GC/MS (mg/kg)	DON kit[a] (mg/kg)	Visual results of ICS test (n = 4)
1	1.11	0.95	+[c], +, +, +	1	0.42	0.54	–, –, –, –
2	1.52	1.43	+, +, +, +	2	0.35	0.44	–, –, –, –
3	4.3	4.6	+, +, +, +	3	0.34	0.39	–, –, –, –
4	0.14	<LOD[e]	–[d], –, –, –	4	ND	<LOD	–, –, –, –
5	0.21	0.38	–, –, –, –	5	0.18	ND	–, –, –, –
6	0.11	<LOD	–, –, –, –	6	ND	<LOD	–, –, –, –
7	0.17	<LOD	–, –, –, –	7	0.10	<LOD	–, –, –, –
8	ND[f]	ND	–, –, –, –	8	ND	<LOD	–, –, –, –
9	0.15	ND	–, –, –, –	9	0.39	0.62	–, –, –, –
10	ND	<LOD	–, –, –, –	10	0.33	0.74	–, –, –, –
11	0.25	<LOD	–, –, –, –	11	0.15	0.33	–, –, –, –
12	0.22	<LOD	–, –, –, –	12	1.51	1.82	+, +, +, +
13	ND	<LOD	–, –, –, –	13	0.35	0.53	–, –, –, –
14	0.17	<LOD	–, –, –, –	14	0.51	0.55	–, –, –, –
15	0.26	<LOD	–, –, –, –	15	0.26	<LOD	–, –, –, –
16	ND	<LOD	–, –, –, –	16	0.32	0.38	–, –, –, –

[a] Screening by Ridascreen® Fast DON ELISA kits.
[b] Mean value (n = 3).
[c] Positive result, T line vanished.
[d] Negative result, both C line and T line appeared clearly.
[e] Limit of determination, 0.2 mg/kg.
[f] Not detected.

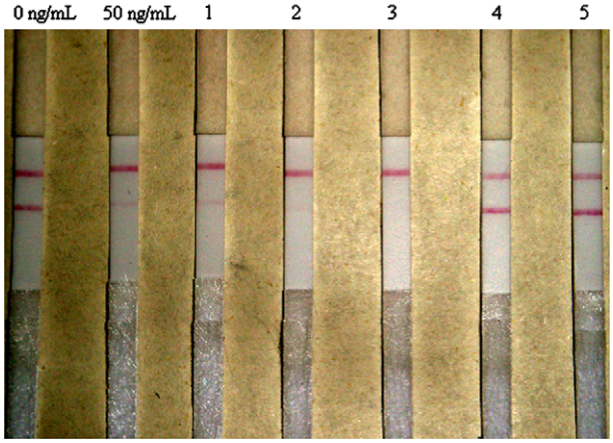

Fig. 4. Detection of DON with ICS test in control, 50 ng/mL, and five wheat samples. Control strip containing no DON shows two red lines on the membrane. Samples 1–3 containing DON at more than 50 ng/mL was found to cause a disappearance of T line. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

tively. Thirty-two natural samples (16 wheat and 16 maize samples) purchased in local markets were analyzed using the ICS test and then confirmed with ELISA and GC/MS. Results were shown in Table 2. Samples 1–3 of wheat containing 1.1, 1.6, and 4.3 mg kg^{-1}, respectively, in ELISA and GC/MS gave a positive result with only one red line on the ICS test (Fig. 4). Sample 12 of maize gave a positive result with only one red line on the ICS test (figure not shown). All the contained wheat and maize samples with DON levels lower than 1 mg kg^{-1}, indicating that they were negative in the ICS test. Results of samples analysis obtained from the ICS were in a good agreement with those obtained form ELISA and GC. This results shows that the three methods corresponded well, and the ICS test gave neither false-positive nor false-negative results.

4. Conclusions

A rapid ICS test was developed to detect DON in wheat and maize samples. The ICS test can be used as qualitative tools for the rapid screening of DON contamination in 10 min on-site. The ICS test had a visual detection limit of 50 ng mL^{-1} for DON. The sensitivities of the current assay methods were sufficient to detect DON at the maximum residue limit of 1 mg kg^{-1} proposed for legislation in China and are suitable for use as rapid screening tests for DON.

Acknowledgements

This work was financially supported by grants from the National High Technology Research and Development Program of China (863 Program, No. 2007AA10Z427) and Program for Changjiang Scholars and Innovative Research Team in University (IRT0540-3).

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