



Development of a Novel Immunoaffinity Column for the Determination of Deoxynivalenol and Its Acetylated Derivatives in Cereals

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Abstract

Deoxynivalenol (DON) hapten 3-O-hemisuccinyl-deoxynivalenol (3-HS-DON) was synthesized and conjugated to bovine serum albumin (BSA) to prepare immunogen by active ester method. A novel anti-DON monoclonal antibody (mAb) showing good cross-reactivity against both 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) was produced by fusing mouse myeloma cells lines SP 2/0 with spleen cells from Bal b/c mice which were immunized by the immunogen. The mAb was coupled with sepharose-4B, then packed into a solid phase extraction (SPE) cartridge to prepare an immunoaffinity columns (IAC). A method for simultaneous determination of DON, 3-ADON, and 15-ADON in cereals was developed. The cereal samples were extracted by water, cleaned up with IAC, and determined by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). The results showed that the limits of detection (LODs) for DON, 3-ADON, and 15-ADON were 1, 6, and 3 $\mu\text{g kg}^{-1}$, respectively. Recoveries of DON, 3-ADON, and 15-ADON from wheat, oatmeal, and maize were 67.5–93.8, 63.8–113.2, and 75.5–106.6% at spiking levels of 20–2000 $\mu\text{g kg}^{-1}$, and the relative standard deviations (RSDs) were 2.4–13.7, 2.4–12.6, and 7.6–12.2%, respectively. This method was suitable for determination of DON, 3-ADON, and 15-ADON in cereals, with the advantages of simplicity, rapidness, good selectivity and sensitivity, and environmental friendliness.

Keywords Monoclonal antibody · Immunoaffinity column · Deoxynivalenol · 3-acetyl-deoxynivalenol · 15-acetyl-deoxynivalenol · HPLC-MS/MS

Introduction

Deoxynivalenol (DON), also known as vomitoxin, is an epoxy-sesquiterpenoid mycotoxin produced mainly by *Fusarium* species (mainly *F. graminearum* and *F. culmorum*) under appropriate conditions of temperature and humidity. DON is produced and commonly found in cereals, especially wheat, barley, maize, sorghum, and cereal-based products (Li et al. 2012; Wu and Wang 2015; Ennouari et al. 2013). The acetylated derivatives of DON, i.e., 3-acetyl-deoxynivalenol

(3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), are intermediaries in its biosynthesis; hence, they are often described as fungal precursors or as free mycotoxins (Broekaert et al. 2014). In addition, there may exist conversion between the acetylated derivatives (3-ADON and 15-ADON) and DON during the food making process (Wu and Wang 2015). Therefore, 3-ADON and 15-ADON can occur widely in cereals and cereal products; Boevre et al. reported that the presence of 3-ADON and 15-ADON in different cereals and cereal-derived products were 87 and 73%, respectively (De Boevre et al. 2012).

The presence of DON, 3-ADON, and 15-ADON in cereal deals with the quality of foods and feeds, which is becoming a potential threat to humans and livestock. DON displays acute and chronic toxicity, such as anorexia, vomiting, diarrhea, fever, growth retardation, cardiotoxicity, and teratogenicity (Li et al. 2015; Freire and Sant'Ana 2018). In addition, 3-ADON and 15-ADON possess equivalent or much stronger toxicity to animals than DON (Wu and Wang 2015). Although DON and its derivatives are not classified as to carcinogenicity to humans, they can induce cytotoxicity and immunotoxicity. Therefore,

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the provisional maximum tolerable daily intake (PMTDI) of $1 \mu\text{g kg}^{-1}$ body weight (b.w.) for the sum of DON and its acetyl derivatives (3-ADON and 15-ADON) was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2010).

There exist numerous methods for determination of DON in foods. The clean-up procedures for DON analysis, such as QuEChERS (Pereira et al. 2015), liquid-liquid extraction (LLE) (Li et al. 2013), solid phase extraction (SPE) (He et al. 2009), immunoaffinity column (IAC) (Pascale et al. 2014; Trombete et al. 2016), immune-ultrafiltration (IUF) (Böhm et al. 2008), and molecularly imprinted polymers (MIPs) (Choi et al. 2011), have been reported. However, the clean-up procedures for the simultaneous determination of DON, 3-ADON, and 15-ADON were mainly reported with traditional SPE column, which retained analytes by non-specific hydrophobic interactions (Pereira et al. 2015; Devreese et al. 2012). IAC was based on antigen-antibody reactions which provided high selectivity and affinity for isolation, purification, and concentration of target analytes from a complex matrix, and only smaller volumes of sample and organic solvent were required for IAC cleanup procedure. Uhli et al. compared five different commercial IACs for their ability to bind DON and its derivatives and concluded that none of them can retain DON, 3-ADON, and 15-ADON simultaneously (Uhli et al. 2017). Therefore, it is urgent to development of a novel IAC to retain DON, 3-ADON, and 15-ADON efficiently.

Various analytical instruments have been applied to analyze DON. These methods included thin-layer chromatography (Vujanovic and Mansour 2011), gas chromatography (GC) (Simsek et al. 2012), gas chromatography-mass spectrometry (GC-MS) (Pereira et al. 2015), high-performance liquid chromatography (HPLC) (Broekaert et al. 2014), and high-performance liquid chromatographic with tandem mass spectrometry (HPLC-MS/MS) (Infantino et al. 2015). In addition, some fast screening methods have been reported, such as enzyme-linked immunosorbent assay (ELISA) (Li et al. 2015), lateral flow immunoassays (Anfossi et al. 2013), Quenchbody (Yoshinari et al. 2015), and surface plasmon resonance immunoassay (Kadota et al. 2010). Current methods for the simultaneous determination of DON, 3-ADON, and 15-ADON are mainly GC (Weingaertner et al. 1997), HPLC (Buttinger and Krska 2003), GC-MS (Cunha and Fernandes 2012), and HPLC-MS/MS (Flores-Flores and González-Péñas 2015). Currently, the determination method for DON and its derivatives are GC-MS and HPLC-MS, but GC-MS determination needs complicated procedure of derivatization before analysis. Therefore, HPLC-MS/MS has become the major analytical instrument for the simultaneous determination of DON, 3-ADON, and 15-ADON.

The aim of our paper was to screen a monoclonal antibody (mAb) against DON, which had the potential to recognize DON, 3-ADON, and 15-ADON. The sepharose-4B coupled with mAb was used as sorbent and packed into a SPE

cartridge. Then, a simple, rapid, efficient method was developed based on IAC clean-up and HPLC-MS/MS analysis for determination of DON, 3-ADON, and 15-ADON in cereals.

Experimental

Chemicals and Materials

DON (analytical grade, 98.0%) was purchased from Fermentek Ltd. (Jerusalem, Israel). Standards of DON, 3-ADON, and 15-ADON (with purities $\geq 98\%$) were purchased from Sigma-Aldrich Co., Ltd. (Augsburg, Germany). Tris(hydroxymethyl) aminomethane (Tris), 4-dimethylaminopyridine (DMAP), anhydrous tetrahydrofuran (THF), chloroform, ammonium sulfate, succinic anhydride (HS), N,N-dimethyl formamide (DMF, 99.8%), and butylboronic acid (BBA, 96%) were chemical grade and purchased from Sinopharm chemical reagent Co., Ltd. (Shanghai, China). Ultrapure water was generated by the milli-Q ultrapure water system purchased from Millipore Co. (Billerica, USA). N-hydroxy-succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), incomplete Freund's adjuvant, and complete Freund's adjuvant were bought from Sigma company (St. Louis, USA). CNBr-activated sepharose 4B was purchased from GE Healthcare Bio-Sciences Corp. (Piscataway, USA). Female Bal b/c mice were purchased from SPF (Beijing) biotechnology Co., Ltd. (Beijing, China). GF254 silica thin layer plate was purchased from Branch of Qingdao Haiyang chemical Co., Ltd. (Qingdao, China). Protein G affinity column was purchased from Beijing Cankown Bio-tech. Co., Ltd. (Beijing, China). Waterman™ microfiber filter paper was purchased from GE healthcare UK limited, UK.

Apparatus and Equipments

APPS EV 100D protein purification apparatus (Lisure Science (Suzhou) Co., Ltd. China); DYCZ-24DN Electrophoresis (Beijing Liuyi Instrument Factory, China); UV3802 ultraviolet-visible spectrophotometer (UNICO (shanghai) Instrument Co., Ltd. China); HJ-3 magnetic stirrer (Jiangsu Changzhou Ronghua instrument manufacture Co., Ltd. China); Visiprep 24™ DL Solid Phase Extraction Vacuum Manifold (Supelco, USA); API 4000 HPLC-MS/MS (AB Sciex Co., Ltd. USA); Mastersizer-micro particle size analyzer (Malvern Instruments Co., Ltd. UK).

Preparation of Hapten

DON hapten was synthesized according to Zhang Yan (Zhang et al. 2010) with some modifications as shown in

Fig. 1. DON of 5 mg was dissolved in 200 μL of THF, added 0.5 mg BBA, and stirred for 24 h at room temperature under the protection of nitrogen to obtain DON-BBA. DMAP of 22 mg (dissolved in 120 μL THF) and 43 mg HS (dissolved in 380 μL THF) were added and stirred for 6 h at room temperature under the protection of nitrogen, then 1 mL of ultrapure water was added. The solution was evaporated by rotary evaporator to remove THF and extracted with chloroform for three times. The collected chloroform layer was dried by rotary evaporator and dissolved in methanol to obtain hapten 3-O-hemisuccinyl-deoxynivalenol (3-HS-DON).

3-HS-DON was further purified with silica gel GF₂₅₄ thin-layer chromatography (TLC) using chloroform-methanol (9:1, v/v) as a developing solvent, 254 nm as detection wavelength. The retention factor (Rf) of 3-HS-DON was 0.53. The purified hapten was collected by scraping 3-HS-DON band in TLC.

Preparation of Hapten-BSA Conjugate

Hapten-BSA conjugate was prepared as an immunogen for the production of mAb against DON. The hapten was covalently coupled to BSA using the modified active ester method. Three milligrams of purified 3-HS-DON was primarily dissolved in 200 μL of DMF; then, 1.7 mg of NHS and 2.9 mg of EDC were added and stirred overnight at 4 °C to obtain the activated 3-HS-DON solution. In addition, 10 mg BSA was dissolved in phosphate-buffered solution (PBS) (0.01 mol L⁻¹, pH 7.4) to obtain the BSA solution. Subsequently, the activated 3-HS-DON solution was added dropwise to the BSA solution and stirred overnight at 4 °C, then dialyzed with PBS (0.01 mol L⁻¹,

pH 7.4) for 3 days, and the PBS was changed with the 12-h interval. The resulting hapten-BSA conjugate (3-HS-DON-BSA) was stored in PBS containing 0.02% sodium azide (NaN₃) at -20 °C before usage.

Production of a mAb against DON

Twenty female Bal b/c mice (6 to 8 weeks old) were immunized by subcutaneous multiple site injection with 50 μg of 3-HS-DON-BSA conjugate and an equal volume of Freund's complete adjuvants. After 4 weeks, booster doses of 50 μg of 3-HS-DON-BSA conjugate and an equal volume of Freund's incomplete adjuvants were used at 2-week intervals. Ten days after the second booster injection, the blood taken from tail vein was checked for antibody titers by indirect ELISA. All procedures that involved animals were conducted in accordance with the guidelines of the Ministry of Public Health of the People's Republic of China.

The final boost was performed when the titer of tail vein blood was above 10⁻⁴. Three days after the final boost, the generation of hybridomas was carried out using conventional methods with mice splenocytes and mice myeloma cell lines SP 2/0 by polyethylene glycol (PEG) method. The culture supernatant was screened for antibody against DON by an indirect ELISA. Positive cultures undergo single-cell cloning for multiple rounds by limiting dilution until anti-DON-mAb was achieved.

Purification of anti-DON-mAb was performed by saturated ammonium sulfate method and protein G affinity column cleanup method. The purity of the anti-DON-mAb was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The specificity of anti-DON-

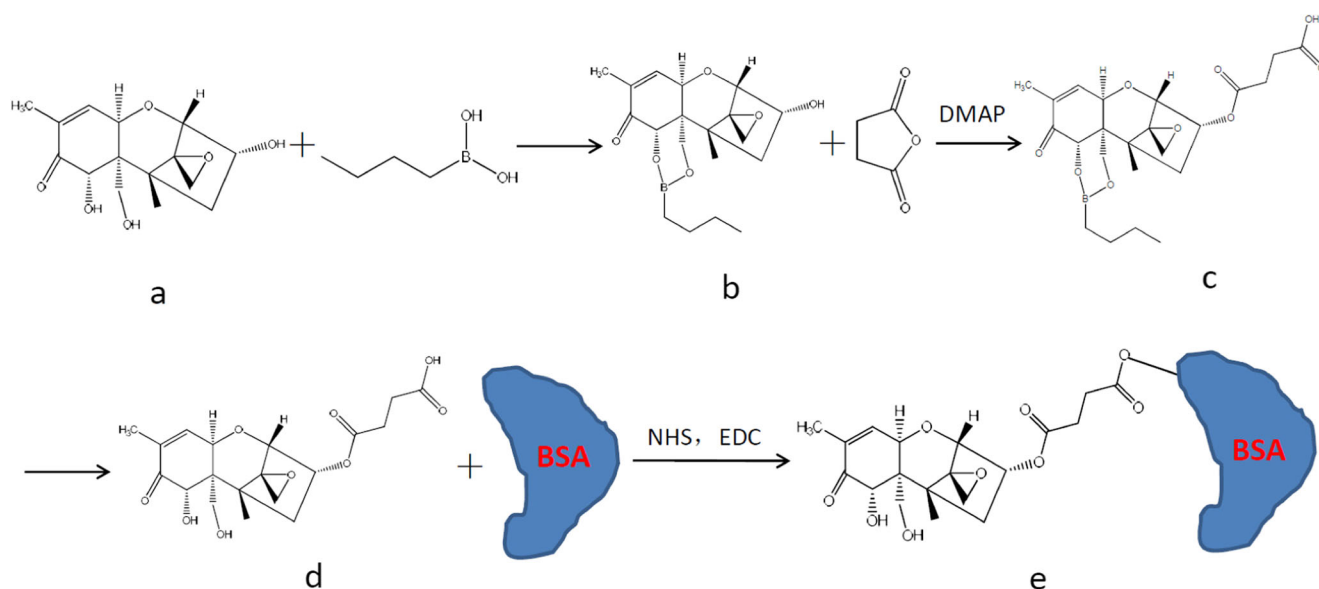


Fig. 1 Schematic diagram of synthesis of DON immunogen. a DON. b DON-BBA. c BBA-DON-HS. d 3-HS-DON. e 3-HS-DON-BSA

mAb was analyzed by indirect ELISA method, and the cross-reactivities (CRs) of anti-DON-mAb against 3-ADON, 15-ADON, and NIV were evaluated by indirect competitive ELISA. According to report, CR values were calculated using the following equation reported by Sakamoto S et al. (Sakamoto et al. 2017):

$$\text{CR}(\%) = \frac{\text{IC}_{50} \text{ of hapten}}{\text{IC}_{50} \text{ of analogues}} \times 100$$

where IC₅₀ was the half maximal inhibitory concentration of target compound.

IAC Particle Size Distribution

Particle size distribution of IAC adsorbent was measured from 0.3 to 300 μm with macro particle size analyzer. Particle size analyses were performed by adding particles into 500 mL ultrapure water until the obscuration value indicates between 10 and 20%, then dispersing the microspheres with the aid of ultra sonication in a water bath for 2 min, and stirring during the measurement. The instrument conditions: helium-neon laser source with the wavelength 633 nm, pump speed 3200 rpm, ultrasonic displacement 20 μm.

Preparation of IAC

One gram CNBr-activated Sepharose 4B was suspended in 10 mL of HCl (1 mmol L⁻¹) to swell completely. The swollen CNBr-activated Sepharose 4B was poured on a sintered glass filter and washed for 15 min with HCl (1 mmol L⁻¹). The anti-DON-mAb was dissolved in coupling buffer solution (pH 8.3, containing 0.1 mol L⁻¹ NaHCO₃, 0.5 mol L⁻¹ NaCl), mixed with the CNBr-activated Sepharose 4B, and stirred at 4 °C overnight for coupling. After that, the mixture was washed sufficiently with the coupling buffer solution in order to remove the unbound mAb. Tris-HCl buffer solution (0.1 mol L⁻¹, pH 8.0) was added and stirred for 2 h to block excess binding sites of the coupled Sepharose 4B. The coupled Sepharose 4B was washed with 0.1-mol L⁻¹ acetic acid buffer solution (pH 4.0, containing 0.5 mol L⁻¹ NaCl) and 0.1 mol L⁻¹ Tris-HCl (pH 8.0, containing 0.5 mol L⁻¹ NaCl) alternately for 4 to 6 cycles.

Finally, the coupled Sepharose 4B was suspended in PBS (0.01 mol L⁻¹, pH 7.4); 0.5 mL of the coupled Sepharose 4B was transferred into an empty SPE cartridge and stored in PBS. The cartridge was capped with polyethylene frits at the top and bottom. The prepared SPE cartridges were stored at 4 °C until use. Coupling efficiency was determined by analyzing the amount of DON in wash solution via UV-Vis spectrometry.

Sample Preparation

Cereal samples were ground thoroughly with a grinder and passed through a 50 mesh sieve. After being mixed thoroughly, samples were sealed in a clean container and stored in desiccator at room temperature. An aliquot of 5 g of sample was mixed with 25 mL of ultrapure water and shaken with a bath oscillator for 30 min at room temperature. The extract was centrifuged at 4200 rpm (3118 g) at room temperature for 5 min, and the supernatant was filtered through a microfiber filter paper.

An aliquot of 2.5-mL extract was loaded on the IAC and dropped through the column about 5 mL min⁻¹. The column was washed with 10 mL water twice and eluted with 1 mL methanol about 3 mL min⁻¹. The elute was collected and evaporated to dryness. The residue was re-dissolved with 1 mL of methanol-water (2:8, v/v), filtered through a 0.22-μm micropore filter for HPLC-MS/MS determination.

Instrument Determination

Purified extracts were analyzed by HPLC-MS/MS. Chromatographic separation was performed on a Luna C18, 150 × 2.0 mm (i.d.), 3 μm analytical column with a mobile phase consisting of mobile phase A (methanol) and mobile phase B (water) at a flow rate of 250 μL min⁻¹. The gradient of mobile phase A was started at 20% and increased to 80% within 6 min, then decreased to 20% from 6 to 6.1 min, and maintained up to 12 min. Column temperature was set to 35 °C. Injected volume was fixed at 10 μL.

Mass spectrometer was operated in the multiple reaction monitoring (MRM) mode with two ion transitions for every target analyte. All compounds were detected in negative electrospray ionization mode (ESI⁻). The dwell time was 100 ms, and the mass analyzers Q1 and Q3 were operated at unit mass resolution. Instrumental and compound specific parameters were optimized by the direct infusion of standard solutions in methanol. The instrumental mass spectrometry parameters were set as follows: collision gas 8 psi, the spray gas (GAS1) 30 psi (nitrogen gas), the auxiliary flow (GAS2) 40 psi (nitrogen gas), the curtain gas (CUR) 20 psi (nitrogen gas), ion spray voltage – 4500 V, and ion source temperature 550 °C. Other parameters are listed in Table 1.

Results and Discussion

DON Immunogen Production and Characterization

DON is a low molecular weight mycotoxin, and it must be coupled with a carrier protein before animal immunization. Therefore, the first and most important step was the synthesis of DON immunogen. As shown in Fig. 1, the hapten 3-HS-

Table 1 Instrument parameters of HPLC-MS/MS

Compound	Parent ion m/z	Daughter ion m/z	DP (V)	EP (V)	CE (eV)	EXP (V)
DON	295.1	264.8	-78	-10	-16	-7
		247.1*	-78	-10	-16	-7
3-ADON	337.4	307.1*	-60	-10	-15	-7
		172.8	-60	-10	-15	-7
15-ADON	337.4	219.0*	-60	-10	-15	-7
		277.0	-60	-10	-12	-7

Note: the daughter ion with “*” mark is the quantification ion

DON was prepared by protection of the C7- and C15-hydroxyls of DON with a cyclic boronate ester, esterified with HS at the C3-hydroxyl of DON, then the boronate ester was removed. 3-HS-DON was activated by active ester method and conjugated to BSA as DON antigen (DON-HS-BSA). The coupling was performed with HS as the space arm to expose the type-feature group better by extending the distance between the position of hydroxyl groups of DON and BSA.

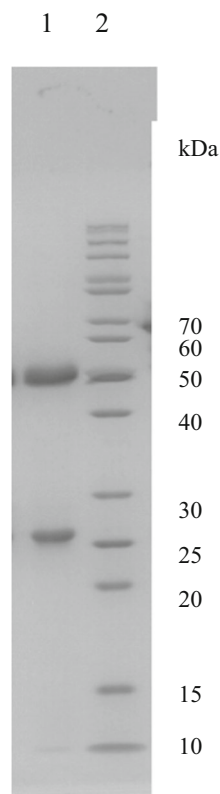
The curve diagram of the conjugate was examined by UV spectroscopy to confirm the coupling reaction. The curve diagram of the conjugated was the combination of hapten and BSA. In order to characterize the DON immunogen, 1 mg mL⁻¹ DON immunogen and 1 mg mL⁻¹ BSA were

determined by UV-Vis spectrum. The full scan range was 200–600 nm (Supporting Information Fig. S1). The results showed that the difference between immunogen and BSA was the adsorption peak at the 280 nm, and the adsorption peak intensity of immunogen was higher than that of BSA, which suggested that 3-HS-DON had successfully coupled with BSA. The content of protein in DON immunogen was determined using bicinchoninic acid (BCA) method (Reichelt et al. 2016), and the results showed that the content of protein in immunogen was 7.0 mg mL⁻¹.

Preparation and Characterization of mAb

There were many methods for antibody purification. In this study, the mAb purification was employed by saturated ammonium sulfate precipitate method due to its simplicity and avoiding loss of antibody activity. The mAb was purified and determined with SDS-PAGE; the SDS-PAGE pattern of purified anti-DON mAb is shown in Fig. 2. The results showed that there were only two strips of 50 and 25 kDa in the SDS-PAGE, which was correspondence to heavy chain and light chain of mAb. Results suggested that mAb was formed with high purity.

The specificity of the mAb was evaluated by the cross-reactivity (CR) values, and it was tested in the ELISA method using 3-ADON, 15-ADON, and Nivalenol (NIV). Some information of these compounds are listed in Supporting Information Table S1. The specificity of the mAb to DON and its analogs is shown in Table 2. Table 2 shows that the CR values of 3-ADON and 15-ADON were 60 and 52%, respectively, while the CR value of NIV was less than 1%. Therefore, the developed mAb could recognize DON, 3-ADON, and 15-ADON.

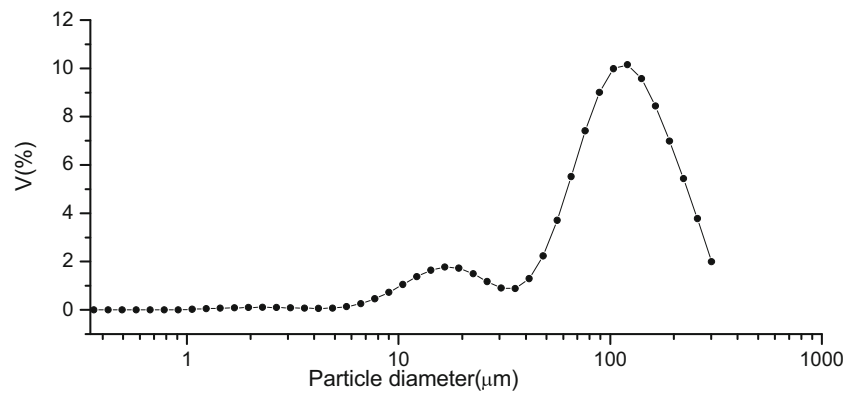


1-mAb; 2-Marker

Fig. 2 SDS-PAGE pattern of purified anti-DON mAb. 1-mAb; 2-Maker

Table 2 IC₅₀ and cross-reactivity of anti-DON mAb

Compound	IC ₅₀ (μg kg ⁻¹)	Cross-reactivity (%)
DON	18.99	100
3-ADON	31.80	60
15-ADON	36.73	52
NIV	> 1800	< 1

Fig. 3 Particle size distribution of IAC adsorbent

Characterization of IAC

Particle Size Distribution

The particle size distribution of IAC adsorbent is shown in Fig. 3. The media for determination of IAC adsorbent size was water, which was in accordance with the wash solvent in IAC cleanup procedure. The results showed that the particle diameter was 50–120 nm and the volume moment mean $D[4,3]$ was 106.18 μm , which was suitable for packing into the SPE cartridge.

Capacity of IAC

The capacity of the IAC was determined for DON, 3-ADON, and 15-ADON by comparing the amount of target compounds added to the IAC with the respective bound amount. Different amounts of DON, 3-ADON, and 15-ADON, from 500 to 4000 ng, were added by loading onto the IAC 5 mL of extract of uncontaminated wheat spiked with the corresponding amount of DON, 3-ADON, and 15-ADON. The IAC was

washed with 10 mL water, eluted with 1 mL methanol, and the maximum binding amounts were obtained. The results showed that the IAC capacities for DON, 3-ADON, and 15-ADON were 4262, 2026, and 2212 ng, respectively.

Stability of IAC

The stability of the IAC was assessed by studying its adsorption performance on DON, 3-ADON, and 15-ADON. IACs were stored at 37 °C in 3 months and assessed its retain performance every month. Appropriate amount of DON, 3-ADON, and 15-ADON was weighted and dissolved in water to obtain 1000 $\mu\text{g L}^{-1}$ of standard solution, 80 μL of above standard solution was loaded on IAC, washed with 10 mL water twice, and eluted with 1 mL methanol. The elute was collected and analyzed. The results showed that the adsorption amount of IAC was more than 80% even placed in 37 °C for 3 months, and one-way ANOVA result indicated that no significance differences were observed. Therefore, the IAC showed high stability performance at 37 °C.

Table 3 Recoveries and RSD of target compounds

Compound	Spike levels ($\mu\text{g kg}^{-1}$)	Recovery (%)			RSD (%)		
		Wheat	Maize	Oatmeal	Wheat	Maize	Oatmeal
DON	20	81.5	81.5	103.3	8.7	9.5	5.9
	40	89.6	92.3	69.3	7.8	10.7	9.2
	80	78.6	89.2	80.1	13.7	9.0	12.5
	2000	80.2	87.8	92.3	9.2	7.6	5.7
3-ADON	20	79.3	90.1	92.2	11.4	12.3	7.6
	40	87.2	99.2	91.6	4.3	9.0	6.6
	80	67.5	106.6	74.8	9.1	8.1	2.4
	2000	79.6	93.5	83.2	10.2	9.4	6.9
15-ADON	20	78.5	83.2	113.2	8.5	12.2	3.6
	40	88.8	78.0	69.5	8.6	10.6	12.6
	80	93.8	75.5	63.8	2.4	10.5	2.8
	2000	92.1	80.4	75.4	7.9	9.8	8.2

Note: data are relevant to 6 replicates

Optimization of IAC Conditions

CNBr-activated Sepharose 4B was employed in this research because it was chemically and biologically inert and could be easily derivatized. The coupling efficiency was used to assess the optimal amount of mAb for coupling. Experiment results showed that when 1 mL gel contained 5 mg mAb and 15 mg mAb, the coupling efficiency was 99.83 and 90.16%, respectively. Therefore, the 1 mL gel containing 5 mg mAb was used for coupling in this study.

DON, 3-ADON, and 15-ADON were soluble in water and could be retained by IAC. In addition, water was selected as solvent for extraction DON, 3-ADON, and 15-ADON in cereal sample determination. Therefore, water was chosen as the loading solvent. With these loading conditions, satisfactory results were obtained. Then, 10 mL of water was used to remove the interfering components which might cause matrix effect and affect the efficiency of ionization of analytes leading to the decrease in the MS/MS responses.

After the washing step, the target compounds were eluted from the antigen-antibody complex by methanol. In this study, different volumes of elution were evaluated to find the best one with high recoveries. The results showed that when the elution volume reached 1 mL, the elute recovery was more than 95%. Therefore, 1 mL of methanol was adopted as the elution solvent.

Real Sample Analysis

Under optimized conditions, the calibration curves of DON, 3-ADON, and 15-ADON were linear within 20–200 $\mu\text{g L}^{-1}$, with correlation coefficients being greater than 0.9986. The limits of detection (LODs) were obtained based on a signal-to-noise ratio of 3. The LODs of the method for DON, 3-ADON, and 15-ADON were 1, 6⁻, and 3 $\mu\text{g kg}^{-1}$, respectively. The limits of quantification (LOQs) for DON, 3-ADON, and 15-ADON were 3, 20⁻, and 10 $\mu\text{g kg}^{-1}$, which was greater than 10 based on signal-to-noise ratio. At the spiked concentrations of 20, 40, 80, and 2000 $\mu\text{g kg}^{-1}$, the average recoveries and RSD ($n = 6$) of oatmeal were 63.8–113.2 and 2.4–12.6%. The average recoveries and RSD ($n = 6$) of wheat were 67.5–93.8 and 2.4–13.7%. The average recoveries and RSD ($n = 6$) of maize were 75.5–106.6 and 7.6–12.2%, respectively (Table 3). The MRM chromatograms of maize are shown in Fig. 4. The MRM chromatograms of maize showed that this method can effectively avoid the interferences of matrix.

Seven cereal samples, including wheat, barley, oatmeal, maize, and rice, were purchased from supermarket in Nanchang city of China. The concentration of DON, 3-ADON, and 15-ADON in these cereal samples was analyzed with this method. Results showed that DON were found in six

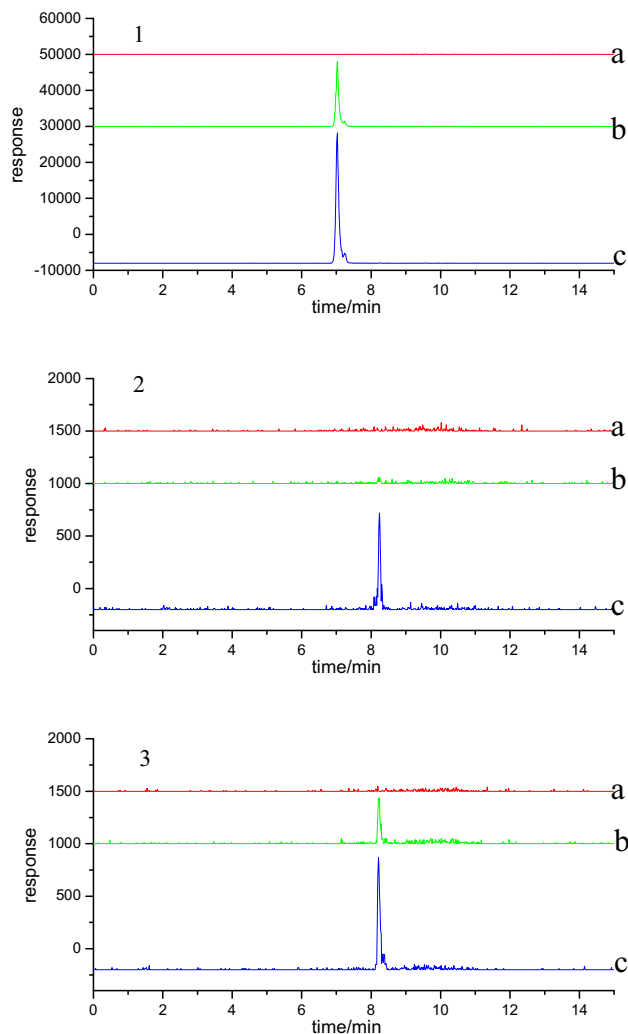


Fig. 4 MRM chromatograms of DON (1), 3-ADON (2), and 15-ADON (3) of maize. **a** Blank maize. **b** Natural occurrence of the DON in a commercial maize (3-ADON under the LOQ; DON corresponded to 10 $\mu\text{g kg}^{-1}$, 15-ADON corresponded to 10 $\mu\text{g kg}^{-1}$). **c** Spiked maize (20 $\mu\text{g kg}^{-1}$). The retention time of DON (1), 3-ADON (2), and 15-ADON (3) were 7.03, 8.24, and 8.24 min

samples with the concentration of 4.1–64.0 $\mu\text{g kg}^{-1}$, and 15-ADON in two samples with 10.0–31.5 $\mu\text{g kg}^{-1}$.

Conclusion

In this paper, a novel IAC was prepared based on DON monoclonal antibody. The novel anti-DON mAb showed good cross-reactivity against both 3-ADON and 15-ADON. The immunosorbent was prepared by coupling anti-DON mAb with CNBr activated sepharose 4B. DON, 3-ADON, and 15-ADON in cereals were extracted with water, cleaned up with IAC, and analyzed by HPLC-MS/MS. The limits of detection (LODs) for DON, 3-ADON, and 15-ADON were 1, 6⁻, and 3 $\mu\text{g kg}^{-1}$, respectively. Thus, method developed was

successfully applied to the determination of DON, 3-ADON, and 15-ADON in cereals with the advantages of simplicity, rapidness, good sensitivity, and environmental-friendly.

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Compliance with Ethical Standards

All animal experiments that were described in the present study were performed in adherence to the guidelines of the Ministry of Public Health of the People's Republic of China and were approved by the Animal Ethics Committee.

Conflict of Interest Hai Gen Zuo declares that he has no conflict of interest. Jian Xin Zhu declares that he has no conflict of interest. Lei Shi declares that she has no conflict of interest. Chun Rui Zhan declares that she has no conflict of interest. Ping Guo declares that she has no conflict of interest. Ying Wang declares that she has no conflict of interest. Yanming Zhang declares that he has no conflict of interest. Jiapeng Liu declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human subjects. All animal experiments that were described in the present study were performed in adherence to the guidelines of the Ministry of Public Health of the People's Republic of China and were approved by the Animal Ethics Committee.

Informed Consent Not applicable.

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