Determination of Deoxynivalenol in Feeds by Automatic Immunomagnetic Beads Clean-up Coupled with High-performance Liquid Chromatography

Wang Shi[1], Mo Fan[1], Jin Yinji[2], Tian Jing[1], Yao Ting[3], Zhao Xinxue[1], Zou Yufei[4], Xiao Zhiming[1*]

1. Institute of Quality Standard and Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences, Beijing 100081, China; 2. Beijing General Station of Animal Husbandry, Beijing 100107, China; 3. Beijing Veterinary Drug and Feed Monitoring Center, Beijing 102200, China; 4. Suzhou Beaver Biomedical Engineering Co., Ltd., Suzhou 215123, China

Abstract **[Objective]** The aim of this work was to establish an analytical method for the determination of deoxynivalenol (DON) in feeds using automatic immunomagnetic beads (IMBs) clean-up coupled with high-performance liquid chromatography. **[Method]** Feed samples were extracted using ultra-pure water, purified by automatic IMBs, and subsequently analyzed via high-performance liquid chromatography, employing an external standard method for quantification. **[Result]** A satisfactory linearity was achieved for DON within the concentration range of 0.05 to 2.0 μg/mL, with the corresponding correlation coefficients (R^2) exceeding 0.999 9. The limit of detection (LOD) and limit of quantification (LOQ) for the proposed method were determined to be 0.03 and 0.1 mg/kg, respectively. The average recoveries of the fortified samples (0.1, 0.2 and 1.0 mg/kg) were 88.5%–100.6%, with the relative standard deviations (RSD) ranging from 2.1% to 9.7%. **[Conclusion]** In comparison with the traditional solid-phase extraction and immunoaffinity column purification methods, the IMBs technique consolidates the extraction, separation, and purification into a single process. This approach enables fully automated processing, which significantly enhances work efficiency and mitigates result deviations that may arise from manual operations. Consequently, this technique is particularly well-suited for the determination of DON in a large number of feed samples.

Keywords Immunomagnetic beads (IMBs) clean-up; High-performance liquid chromatography; Feed; Deoxynivalenol (DON)

Deoxynivalenol (DON), frequently referred to as vomitoxin, is one of the most prevalent mycotoxins found in animal feeds. Research has demonstrated that DON exerts considerable negative effects on the immune function of both humans and animals, resulting in either immunosuppression or immunostimulation, contingent upon the dosage and duration of exposure to this mycotoxin[1]. This exposure can lead to acute or chronic poisoning in humans and animals, as well as carcinogenic, teratogenic, and mutagenic effects[2]. The detection rate of DON is notably high in bulk crops such as corn, soybeans, and peanuts in China. Among these, corn is recognized as one of the most significant staple grains globally, with corn and its by-products, soybean meal and peanut meal serving as essential feed ingredients[3]. Consequently, the research and development of a rapid, straightforward, and reliable quantitative analysis method for DON in animal feed is of considerable importance for the prevention, control, and risk assessment of DON in feed.

At present, the prevalent methods for the quantification of DON in grain and feed comprise thin-layer chromatography (TLC)[4], enzyme-linked immunosorbent assay (ELISA)[5], gas chromatography[6], high-performance liquid chromatography (HPLC)[7–8], and liquid chromatography-tandem mass spectrometry (LC-MS/MS)[9–10]. TLC is characterized by low precision and a complex operational procedure. ELISA is susceptible to cross-reactivity and the occurrence of false positives. Gas chromatography necessitates derivatization and intricate pre-treatment processes. In contrast, HPLC is the most widely employed technique for the quantitative analysis of mycotoxins, owing to its stability, reliability, ease of operation, and commendable reproducibility[4]. Furthermore, LC-MS/MS is increasingly employed for the detection of mycotoxins in grains and animal feeds. However, its widespread application in the feed industry is constrained by the complexity of the instrumentation and the associated high costs. Prior to conducting quantitative analysis of DON using HPLC or LC-MS/MS, it is necessary to perform sample purification. The commonly utilized purification methods include solid-phase extraction (SPE) and immunoaffinity chromatography (IAC)[11–12]. The prevailing national standard in China's feed industry, GB/T 30956–2014 *Determination of De-*

Received: 2025–04–01 Accepted: 2025–06–25

Supported by National Key Research and Development Program of China (2023YFD1301001) & Central Public-interest Scientific Institution Basal Research Fund (1610072023005) & Agricultural Science and Technology Innovation Program of CAAS (CAAS-ASTIP-IQSTAP-04).

*Corresponding author. E-mail: xiaozhiming@caas.cn

oxynivalenol in Feed: *Immunoaffinity Column Clean-up-High Performance Liquid Chromatography*, adopts IAC for sample purification. While IAC demonstrates superior selectivity and purification efficacy compared to traditional SPE and is less susceptible to interference from various sample matrices, it presents several drawbacks, including reduced stability due to antibody influence, high costs, and the necessity for manual operation, which contributes to an extended processing time.

Immunomagnetic beads (IMBs) are innovative nanomaterials synthesized through core-shell technology, exhibiting superparamagnetic properties. These beads can be modified with various active groups, including carboxyl, amino, or sulfhydryl groups, on their surfaces. They possess the capability to swiftly capture and separate target compounds when subjected to a magnetic field, thereby facilitating rapid sample pre-treatment processes that integrate extraction, purification, and concentration into a single operation[13–14]. Previous research on IMBs has predominantly concentrated on biochemical applications, including microbial detection[15–16], cell separation[17], and nucleic acid extraction[18]. In recent years, IMBs technology has garnered increasing attention in the domain of food safety testing, attributed to its notable characteristics such as ease of operation, rapid processing, and the capability for batch automation. Consequently, IMBs have been progressively utilized for the detection of small molecules, including veterinary drugs and mycotoxins. Currently, IMBs for aflatoxin B_1, zearalenone, and DON have been developed, and the immunomagnetic separation technique utilizing these beads has been successfully implemented for the detection of associated toxins in cereals[19–22]. In comparison to IAC, the purification process using IMBs is more cost-effective and can be fully automated, thereby alleviating the manual workload for researchers and significantly enhancing operational efficiency[23–24].

This study employs automatic IMBs clean-up coupled with HPLC to establish a rapid, convenient, batch-processing, and automated method for the determination of DON in feed and feed ingredients. Furthermore, the research aims to develop a standardized protocol for the Feed Industry Association of China regarding the IMBs clean-up technology. This initiative seeks to provide technical support to fulfill the requirements for automated, high-throughput, and high-precision determination of DON in various feed ingredients, including corn, soybean meal, peanut meal, and other feeds, as well as in compound feeds for livestock such as pigs, cows, goats, and poultry.

1 Materials and Methods

1.1 Laboratory instruments The instruments utilized in the study comprised the Agilent 1100 High-Performance Liquid Chromatograph, equipped with either a UV or diode-array detector (DAD); the Deoxynivalenol IMBs Kit and the Mycotoxin Automatic Purification Instrument from Suzhou Beaver Biomedical Engineering Co., Ltd.; the N-EVAP 112 Termovap Sample Concentrator manufactured by Organomation, USA; the Nitrogen Generator provided by Anpel Laboratory Technologies (Shanghai) Inc.; the SK5200LHC Ultrasonic Cleaner from Shanghai Kudos Ultrasonic Instrument Co., Ltd.; the Milli-Q plus Ultrapure Water Meter produced by Millipore, USA; the MS2 Minishaker Vortex Oscillator from IKA, Germany; the BS 210S Analytical Balance manufactured by Sartorius, Germany; and the 3K15 High-Speed Refrigerated Centrifuge from Sigma, Germany.

1.2 Materials DON standard solution (100 μg/mL) was procured from Alta Scientific Co., Ltd. Acetonitrile and methanol, both of chromatographic purity, were obtained from Fisher Scientific, USA. The water utilized for testing was first-grade water (18.2 MΩ) purified through the Milli-Q system. Sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and potassium chloride (KCl) were of analytical grade and sourced from Beijing Chemical Reagent Company. Aqueous filter membranes with a pore size of 0.45 μm were supplied by Anpel Laboratory Technologies (Shanghai) Inc. The phosphate buffer solution (PBS, pH 7.0) was prepared by weighing and dissolving 8.0 g of NaCl, 1.2 g of Na_2HPO_4, 0.2 g of KH_2PO_4, and 0.2 g of KCl in water, and then diluting the solution to a final volume of 1 000 mL. Samples of feedstuffs, including corn, soybean meal, and peanut meal, as well as feed samples for pigs, cattle, goats, and poultry, were provided by the China National Feed Quality Control Center (Beijing).

1.3 Experimental methods

1.3.1 Sample extraction. According to the national standard GB/T 30956–2014 *Determination of Deoxynivalenol in Feed: Immunoaffinity Column Clean-up-High Performance Liquid Chromatography*, the following procedure was employed: A precise weight of 10 g of feed samples (accurate to 0.001 g) was placed into a 50 mL centrifuge tube. Subsequently, 20 mL of ultrapure water was added, and the mixture was vortexed thoroughly. The samples were then subjected to ultrasonic extraction in an ultrasonic cleaner for 20 min, during which they were shaken 2–3 times to ensure homogeneity. Following this, the samples were centrifuged at 10 000 r/min for 5 min, and the supernatant was collected for further analysis.

1.3.2 Sample purification. 1 mL of supernatant was transferred into sample well ① of the deoxynivalenol IMBs clean-up kit. Subsequently, the mycotoxin IMBs clean-up instrument was initiated to execute the DON clean-up program, with the components of the kit illustrated in Fig.1. Upon completion of the clean-up program, 0.8 mL of the eluent was extracted from well ⑤ and evaporated to dryness under a nitrogen stream at 40 ℃. Following this, 0.4 mL of a 10% acetonitrile/water solu-

tion was added to re-dissolve the eluent, which was then mixed using a vortex mixer. The resulting solution was filtered through a 0.45 μm aqueous filter membrane prior to analysis by HPLC.

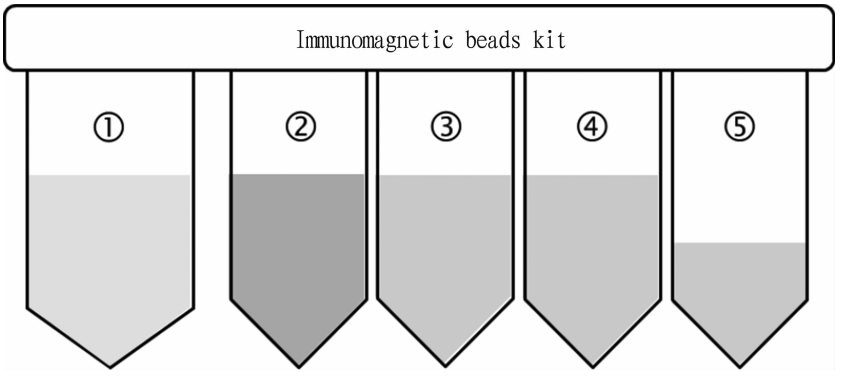
The procedure for the fully automated purification of DON IMBs was conducted as follows: (1) The IMBs were transferred from well ② to sample hole ① to capture the target analyte. The mixture was then automatically stirred for 12 min, followed by a 1 min magnetic separation using a magnetic bar; (2) The IMBs were subsequently moved to washing hole ③, where they underwent a 1 min wash with automatic stirring, followed by a 1 min magnetic separation; (3) The beads were then transferred to washing hole ④, where they were washed for an additional minute with stirring and subjected to another 1 min magnetic separation; (4) Finally, the IMBs were moved to elution hole ⑤, where they were eluted with methanol for 1 min and separated magnetically for an additional minute. The immune beads were then transferred to the bead recovery hole ②, completing the purification process, which lasted approximately 20 min.

1.3.3 HPLC conditions. Chromatographic column: Agilent Zorbax SB-C_{18} column (4.6 mm×250 mm, 5 μm); mobile phase: water/acetonitrile (90/10), isocratic elution; flow rate: 0.8 mL/min; column temperature: 40 ℃; injection volume: 50 μL; ultraviolet or diode array detector (DAD) detection wavelength: 218 nm.

2 Results and Analysis

2.1 Optimization of IMBs reaction time IMBs must achieve complete binding to the target analyte within the sample to ensure optimal separation, and the duration of their interaction with the sample extract is crucial for effective IMBs purification. The IMBs were initially reacted with a standard solution of DON at a concentration of 500 ng/mL. The binding efficiencies were assessed at various reaction times (1, 2, 3, 5, 8, 10, 15, and 20 min), and the results are presented in Fig.2. When the reaction time was 10 min or longer, the adsorption of the IMBs onto the DON was stabilized, with recoveries ranging from 99.8% to 100.2%. This finding suggests that a reaction time of 10 min is sufficient to fulfill the interaction requirements between the IMBs and the target analyte. In light of the impact of various sample matrices in the detection process, the reaction time was extended to 12 min. This adjustment was made to ensure that the IMBs could effectively achieve optimal separation and enrichment across different sample matrices.

2.2 Linearity and sensitivity of the method The DON series standard working solutions were prepared using a 100 μg/mL DON standard solution, which was diluted to create six different concentration gradients: 0.05, 0.10, 0.20, 0.50, 1.0, and 2.0 μg/mL. Linear regression standard curves were generated with concentration plotted on the horizontal axis and peak area on the vertical axis. The results of this analysis are presented in Tab.1. The results indicated that the method exhibited a high degree of linearity, as evidenced by a correlation coefficient of 0.999 9 within the concentration range of 0.05-2.0 μg/mL, thereby fulfilling the criteria for the determination of DON. Utilizing 3 times signal-to-noise ratio (S/N) as the limit of detection (LOD) and 10 times S/N as the limit of quantification (LOQ), the LOD and LOQ of the method were determined to be 0.03 and 0.1 mg/kg, respectively. Furthermore, the LOQ of the method complies with the requirements set forth by the mandatory national standard, *Feed Hygiene*



Note: ① Dilution solution: 9 mL PBS buffer (pH 7.0); ② 0.7 mL IMBs solution; ③ Wash solution 1:1 mL PBS buffer (pH 7.0); ④ Wash solution 2:1 mL ultrapure water; ⑤ Elution solution: 0.9 mL methanol.

Fig.1 Schematic diagram of the deoxynivalenol IMBs clean-up kit

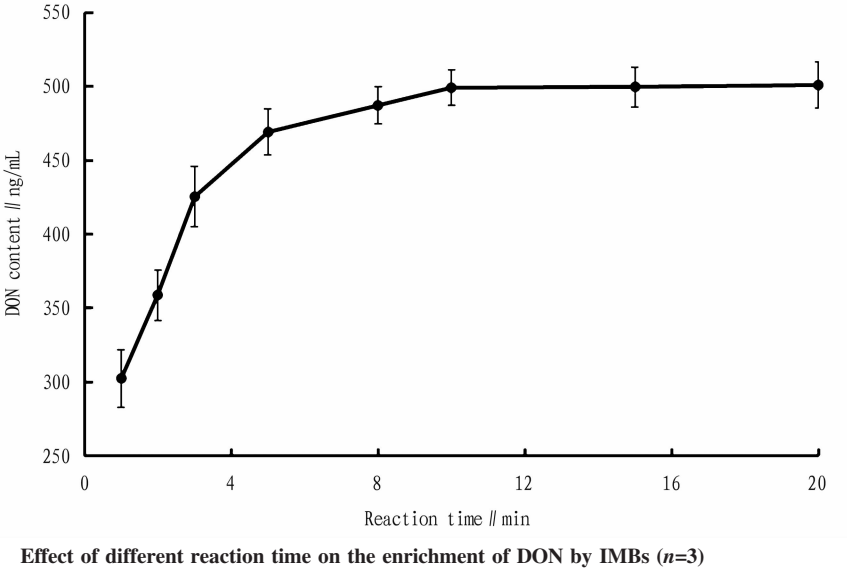


Fig.2 Effect of different reaction time on the enrichment of DON by IMBs (*n*=3)

Standard (GB 13078–2017), regarding the permissible levels of DON in feeds and feedstuffs.

2.3 Accuracy and precision of the method A selection of representative blank samples was made for the fortified recovery test, which included corn flour, soybean meal, peanut meal, distillers dried grains with soluble (DDGS), barley flour, rice bran, secondary flour, and various other feed ingredients, compound feeds and concentrated feeds for pig, chicken, and fish, as well as concentrate supplements for cattle and sheep. In accordance with the guidelines in the FDA's *Bioanalytical Method Validation Guidance for Industry*[25], three concentration levels were established: LOQ, 2×LOQ, and 10×LOQ. For each concentration level, six replicates were prepared, and the samples were processed and analyzed following the prescribed method. The accuracy, precision, and reproducibility of the method are presented in Tab.2, while the representative chromatogram is illustrated in Fig.3. As presented in Tab.2, the average recoveries varied between 88.5% and 100.6% at fortification levels of 0.1, 0.2, and 1.0 mg/kg. The intra-batch coefficients of variation ranged from 2.1% to 8.6%, while the inter-batch coefficients of variation ranged from 3.9% to 9.7% . These results satisfied the established criteria for method accuracy and precision.

2.4 Comparison with IAC clean-up In accordance with the national standard GB/T 30956–2014 *Determination of Deoxynivalenol in Feed: Immunoaffinity Column Clean-up-High Performance Liquid Chromatography*, four standard substances of corn flour, wheat flour, animal feed and pet dog food purchased from FAPAS, and 36 samples of feeds and feed ingredients provided by the China National Feed Quality Control Center (Beijing) were analyzed using both the IAC method and the IMBs method established in this study. The results pertaining to the FAPAS standards and the samples that tested positive for DON are presented in Tab.3. A two-tailed *t*-test was employed to statistically analyze the data from the two groups[26–27]. The results indicated that there was no significant difference between the outcomes of the two groups, suggesting that

Tab.1 Standard curve, LOD and LOQ of DON

Linearity range// μg/mL	Regression equation	Correlation coefficient	LOD// mg/kg	LOQ// mg/kg
0.05–2.0	y=0.130 8x+4.396 8	0.999 9	0.03	0.1

Tab.2 Determination results of accuracy and precision of the method

Feed sample	Fortified concentration mg/kg	Recovery %	Intra-batch coefficients of variation (n=6, %)	Inter-batch coefficients of variation (n=18, %)
Corn flour	0.1	94.7	3.3	4.2
	0.2	97.4	4.9	6.7
	1.0	96.8	4.5	6.5
Soybean meal	0.1	97.7	7.6	8.3
	0.2	96.2	4.4	5.6
	1.0	98.7	7.9	9.7
Peanut meal	0.1	99.0	7.2	7.4
	0.2	97.2	4.6	5.9
	1.0	95.9	3.0	6.1
DDGS	0.1	88.5	5.7	7.0
	0.2	90.6	7.6	9.3
	1.0	95.4	7.1	8.8
Barley flour	0.1	96.3	8.6	9.5
	0.2	100.6	8.3	8.9
	1.0	98.2	3.4	4.9
Compound feed for growing pigs	0.1	98.9	5.3	7.6
	0.2	99.8	7.4	8.7
	1.0	97.4	2.1	4.3
Compound feed for laying hens	0.1	95.2	7.5	7.9
	0.2	88.7	5.2	7.6
	1.0	91.0	2.8	3.9
Compound feed for perches	0.1	100.4	7.0	9.5
	0.2	98.8	4.8	6.0
	1.0	97.5	3.6	4.9
Concentrated feed for sows	0.1	99.3	8.2	8.9
	0.2	98.6	5.6	6.7
	1.0	96.5	4.5	5.2
Concentrated feed for broilers	0.1	97.8	4.7	6.5
	0.2	96.3	7.5	8.1
	1.0	97.7	7.1	8.4
Concentrate supplements for cattle	0.1	96.8	8.0	9.3
	0.2	99.9	4.3	6.2
	1.0	94.5	6.6	7.1
Concentrate supplements for sheep	0.1	89.6	3.6	5.6
	0.2	94.0	5.5	6.8
	1.0	94.9	6.3	7.9

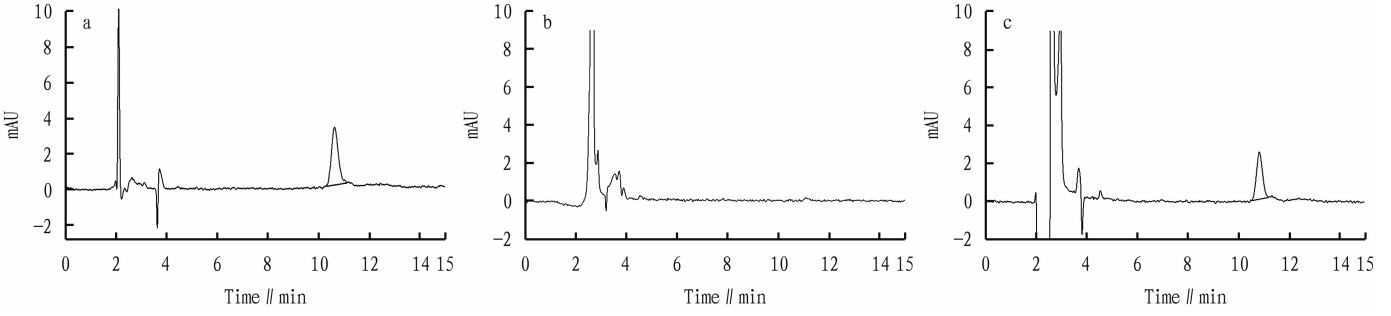


Fig.3 Representative chromatograms of DON standard solution (a), blank (b) and fortified samples (c)

Tab.3 Comparison of IMBs and IAC clean-up results

No.	Sample name	DON determination results by IAC (n=3, μg/kg)	DON determination results by IMBs (n=3, μg/kg)	Labeled DON content // μg/kg
1	Corn flour standard substance (No.TET030RM)	1 279±134	1 287±59	1 208±117
2	Quality control samples of wheat flour (No.T22204QC)	733±41	757±29	665±226
3	Animal feed standard substances (No.TYG087RM)	918±55	903±23	905±55
4	Quality control samples of pet dog food (No.T04447QC)	1 128±91	1 173±52	1 090±344
5	Compound feed for growing pigs	816±51	832±22	–
6	Compound feed for sows	1 213±107	1 227±48	–
7	Compound feed for broilers	653±60	669±25	–
8	Compound feed for laying hens	572±66	569±32	–
9	Compound feed for dairy cattle	403±37	411±29	–
10	Concentrate supplements for high yielding lactating cows	786±72	798±21	–
11	Feed ingredient corn	1 003±119	1 092±58	–
12	Full-price breeding dog food	367±47	377±23	–
13	Full-price adult dog food	448±48	456±26	–
14	Full-price working dog puppy food	349±40	353±19	–
15	Full-price working dog training dog food	418±31	425±15	–

the IMBs clean-up method developed in the present study aligns well with the IAC clean-up method as outlined by the current valid national standards. The mycotoxin automatic purification instrument employed in the IMBs clean-up method utilized in this study was capable of processing 10 samples simultaneously. The automatic purification program for DON required approximately 20 min per batch, resulting in an average processing time of only 2 min per sample. This efficiency facilitates fully automated and high-throughput analysis of DON in feeds and feedstuffs. The traditional IAC purification method requires approximately 2 h for manual execution, and the potential for bias associated with entirely manual processing is considerable. Consequently, the implementation of automatic IMBs clean-up can markedly enhance efficiency and minimize detection bias when handling substantial volumes of feed and feed ingredient samples.

3 Conclusions

In this study, an automatic IMBs clean-up method was developed coupled with HPLC for the quantification of DON in animal feeds. The calibration curves demonstrated a strong linearity within the concentration range of 0.05–2.00 μg/mL, with the LOD established at 0.03 mg/kg and LOQ at 0.1 mg/kg. The average recoveries of DON at three concentration levels (0.1, 0.2, and 1.0 mg/kg) ranged from 88.5% to 100.6%, while the relative standard deviations varied between 2.1% and 9.7%. The method is user-friendly and can be automated for the batch processing of samples, thereby significantly enhancing work efficiency. It is particularly suitable for the quantitative analysis of DON in large volumes of feed samples.

References

[1] Cao JJ, Liu Y, Wang YN, *et al.* Comparative study on the determination of Aflatoxin B_1 in edible oil by automated immunomagnetic beadpurification method and immunoaffinity column purification method [J]. Science and Technology of Cereals, Oils and Foods, 2023, 31(3): 153–158.

[2] Chen JN, Ye J, Guo XG, *et al.* Determination of zearalenone in grains by high-throughput automatic purification with immunomagnetic beads coupled with ultra-high performance liquid chromatography[J]. Food Science, 2021, 42(22): 318–323.

[3] Guo JL, Du J, Li ZS, *et al.* Application of immunomagnetic separation technology in pretreatment of foodborne pathogenic bacteria[J]. Journal of Food Safety and Quality, 2023, 14 (21): 97–106.

[4] Lei YP, Zhou JC, Deng WG, *et al.* A survey report on the mycotoxin contamination of compound feed and its raw materials of China in 2019–2020 [J]. Feed Industry, 2022, 43(20): 59–64.

[5] Li JW, Du MH, Yang Y. Effect of the size of immunomagnetic beads on capture efficiency for foodborne pathogenic bacteria[J]. Journal of Food Science and Biotechnology, 2020, 39(9): 46–52.

[6] Liu Y, Jin YP, Guo SL, *et al.* A method for simultaneous determination of Aflatoxin B_1, Zearalenone and Deoxynivalenol in feed by high performance liquid chromatography [J]. Chinese Journal of Animal Nutrition, 2021, 33 (8): 4684–4693.

[7] Liu XM, Tian W, Xiao ZL. Preparation of immunomagnetic beads and their application in food safety detection[J]. Journal of Food Safety and Quality, 2018, 9(18): 4775–4780.

[8] Wang M, Chen JN, Ye J, *et al.* Immunomagnetic bead high-throughput automatic purification-ultra-high performance liquid chromatography for determination of zearalenone in feed [J]. Journal of the Chinese Cereals and Oils Association, 2023, 38(9): 29–34.

[9] Zhou J, Chen XH, Jin MC, *et al.* Determination of deoxynivalenol and its derivatives in cereals and its products by ultra-high performance liquid chromatography-tandem mass spectrometry with modified pass-through solid phase extraction column[J]. Physical Testing and Chemical Analysis (Part B:Chemical Analysis), 2023, 59(4): 437–443.

[10] Zhu HH, Zhang MX, Hu XF, *et al.* Research progress on the detection method of vomitoxin in food [J]. Food Science and Technology, 2021, 46(11): 314–320.

[11] Clark KD, Varona M, Anderson JL. Ion-tagged oligonucleotides coupled with a magnetic liquid support for the sequence-specific capture of DNA [J]. Angewandte Chemie International Edition, 2017, 56: 7630–7633.

[12] Ganesan AR, Mohan K, Rajan DK, *et al.*Distribution, toxicity, interactive effects, and detection of ochratoxin and deoxynivalenol in food: A review[J]. Food Chemistry, 2022, 378: 131978.

[13] Hu L, Chen X, Chen M, *et al.* Enrichment and detection of circulating tumor cells by immunomagnetic beads and flow cytometry [J]. Biotechnology Letters, 2021, 43: 25–34.

[14] Hu Y, Hassan Y I, Shao S, *et al.* Employing immuno-affinity for the analysis of various microbial metabolites of the mycotoxin deoxynivalenol[J]. Journal of Chromatography A, 2018, 1556: 81–87.

[15] Huang J, Zhao K, Li M, *et al.* Development of an immunomagnetic bead clean-up ELISA method for detection of maduramicin using single-chain antibody in chicken muscle [J]. Food and Agricultural Immunology, 2021, 32 (1): 820–836.

[16] Keskin E, Eyupoglu OE. Determination of mycotoxins by HPLC, LC-MS/MS and health risk assessment of the mycotoxins in bee products of Turkey[J]. Food Chemistry, 2023, 400: 134086.

[17] Kuzdraliński A, Solarska E, Muszyńska M. Deoxynivalenol and zearalenone occurence in beers analysed by an enzyme-linked immunosorbent assay method[J]. Food Control, 2013, 29(1): 22–24.

[18] Li Y, Chen A, Mao X, *et al.* Multiple antibodied based immunoaffinity columns preparation for the simultaneous analysis of deoxynivalenol and T -2 toxin in cereals by liquid chromatography tandem mass spectrometry[J]. Food Chemistry, 2021, 337: 127802.

[19] Liu D, Wang Q, He W, *et al.* Two-way immune effects of deoxynivalenol in weaned piglets and porcine alveolar macrophages: Due mainly to its exposure dosage [J]. Chemosphere, 2020, 249: 126464.

[20] Ni B, Ye J, Xuan Z, *et al.* Automatic pretreatment of dispersive liquid liquid microextraction based on immunomagnetic beads coupled with UPLC-FLD for the determination of zearalenone in corn oils [J]. Toxins, 2023, 15: 337.

[21] Stastny K, Stepanova H, Hlavova K, *et al.* Identification and determination of deoxynivalenol (DON) and deepoxy-deoxynivalenol (DOM-1) in pig colostrum and serum using liquid chromatography in combination with high resolution mass spectrometry (LC-MS/MS) (HR)[J]. Journal of Chromatography B, 2019, 1126–1127: 121735.

[22] Tang K, Liu H, Li XZ, *et al.* An efficient gas chromatography-mass spectrometry approach for the simultaneous analysis of deoxyni-valenol and its bacterial metabolites 3-keto-DON and 3-epi-DON[J]. Journal of Food Protection, 2018, 81: 233–239.

[23] U. S. Food and Drug Administration (US FDA). Bioanalytical Method Validation-Guidance for Industry, 2018.http://www.fda.gov/Drugs/Guidance Compliance RegulatoryInformation/Guidances/default.htm.

[24] Wang W, Zhang Q, Ma F, *et al.* Simultaneous determination of aflatoxins, fumonisin B_1, T-2 and cyclopiazonic acid in agri-products by immunomagnetic solid-phase extraction coupled with UHPLC-MS/MS[J]. Food Chemistry, 2022, 378: 132020.

[25] Xiao Z, Wang R, Suo D, *et al.* Trace analysis of bisphenol A and its analogues in eggs by ultra-performance liquid chromatographytandem mass spectrometry [J]. Food Chemistry, 2020, 327: 126882.

[26] Zeng K, Yang J, Su H, *et al.* Enhanced competitive immunomagnetic beads assay assisted with PAMAM-gold nanoparticles multienzyme probes for detection of deoxynivalenol[J]. Biosensors , 2023, 13(5): 536.