Development of an ic-ELISA and Immunochromatographic Strip Assay for the Detection of Diacetoxyscirpenol in Rice

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ABSTRACT: Diacetoxyscirpenol (DAS) is a highly toxic type A trichothecene, a natural contaminant in food and animal feed, which is a serious hazard to human and animal health. An anti-DAS mAb, 3H10, with high sensitivity and specificity, was prepared, and an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and a lateral-flow immunochromatographic strip (ICA strip) were developed for rapid testing of DAS in rice samples. The 50% inhibitory concentration and limit of detection of ic-ELISA were 5.97 and 0.78 ng/mL, respectively. The recovery of rice samples ranged from 99.4 to 110.7%, demonstrating that the analytical method can be used to detect rice samples. The cutoff value of the lateral-flow ICA strip to DAS was 500 ng/g. The developed immunoassay method can provide an effective method of initially detecting and screening DAS in food and feed samples.

1. INTRODUCTION

Trichothecenes are highly toxic secondary metabolites produced by Fusarium fungi, which are widely distributed in nature and seriously endanger human and animal health. Diacetoxyscirpenol (DAS, anguidine), T-2 toxin, and HT-2 toxin are among the most toxic mycotoxins of the trichothecene group. Like other trichothecenes, DAS exerts an amalgam of acute and chronic effects on humans and animals, such as hematoxicity, immunotoxicity, growth retardation, pulmonary disorders, and cardiovascular effects. The potential for induction of anorexia by type A trichothecenes through targeting the appetite center is of particular concern from the perspective of human and animal health. For intraperitoneal administration, comparing anorexia efficacy, DAS is stronger than other type A trichothecenes. DAS is a dangerous natural contaminant of feed and agricultural products worldwide, being commonly found in cereals, particularly in wheat, barley, rice, and maize. Although there are no regulatory limits for DAS, clinical studies have shown its high toxic potency in different animal species. In 2016, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted the first toxicological assessment of DAS. Finally, it was decided that DAS should be involved in the group with a temporary allowable daily intake, which includes T-2 and HT-2 toxins (0.06 µg/kg bw). Consequently, a highly sensitive and specific DAS assay is critical to assess the safety of food and animal feed.

There are many analytical methods for the detection of DAS, including gas chromatography-tandem mass spectrometry, thin-layer chromatography, chromatography-mass spectrometry, ultraefficient UPLC–MS/MS, and high-resolution mass spectrometry. Roma et al. used UPLC–MS/MS to analyze feed samples from a Spanish market and tested the DAS for an LOD of 25 µg/kg. The sample was ground and sieved, and then 2 g of the sample was extracted with 8 mL of an extract (acetonitrile:water:formic acid = 80:19:1 v/v/v) under an orbital shaker (4-time dilution). After centrifugation at 5500 rpm for 5 min, 2 mL of the supernatant was filtered through a 0.22 µm filter and injected into a UPLC–MS/MS system. Although the traditional instrument detection method is sensitive and reliable, it also has the disadvantages of being time-consuming and requiring expensive equipment, complicated sample preparation, and professional operation. This makes it impossible to achieve rapid, low-cost, simple detection of a large number of samples on the market. Enzyme-linked immunoassays are widely used in food testing due to their high sensitivity, high selectivity, high throughput, and low cost. However, only a small number of immunoassays have been established for the test of DAS, for instance, radioimmunoassays and enzyme-linked immunosorbent assays. Schubring and Chu developed an ic-ELISA to detect DAS in wheat and corn samples. However, the antibody was highly susceptible to sample matrix interference, and the sensitivity was not high enough to be commercialized.

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In this study, we developed a monoclonal antibody (mAb) against DAS. Based on this monoclonal antibody, a highly sensitive ic-ELISA and lateral-flow ICA strips were prepared to determine DAS concentrations in rice.

2. RESULTS AND DISCUSSION

2.1. Identification of Complete Antigen. For the immunizing antigen, first of all, HS-DAS was formed by the reaction of DAS with succinic anhydride, which was then conjugated to the protein. The resulting material was identified by LC−MS/MS (Figure 2). The molecular weight of the main product was 466 as determined by negative-ion mass spectrometry, which was consistent with the molecular weight (MW 466) of the target substance HS-DAS. It can be seen that HS-DAS synthesis was successful. DAS was combined with KLH and BSA by the carbodiimide and the CDI methods, respectively, and the antigens DAS-HS-EDC-BSA and DAS-CDI-BSA were confirmed by electrophoresis (Figure 3). The synthesized antigen and the standard concentration of BSA strips showed significant band shifts, demonstrating successful synthesis of the complete antigen.

2.2. Development and Optimization of ic-ELISA. DAS-HS-EDC-KLH was used as the immunizing antigen to immunize mice, and DAS-CDI-BSA was used as the coating antigen. After the cells were fused, hybridoma cells were obtained, the ascites was prepared, and the antibody was purified. The mAbs 4F4, 4H8, 3H10, and 3H10 were identified by ic-ELISA, and the monoclonal antibody 3H10 was found to be the most sensitive.

The titer and 50% inhibitory concentration (IC50) are two important indicators indicating the activity and sensitivity of the antibody, respectively, and the best optimization conditions are obtained by measuring them. The pH value is an important factor affecting the results of ic-ELISA. This is because a pH that is too high or too low will affect the physical and chemical properties of antigens and antibodies. The results of optimization are revealed in Figure 4A. From the test results, the pH value was in the range of 4.0−8.6, the antibody titer was basically unchanged, and the highest IC50 value was at pH 7.4, so pH 7.4 was selected. Since ethanol has low toxicity properties relative to other organic solvents, the ethanol content was selected for optimization in view of the safety of sample extraction. Once the ethanol content was higher than 20%, the antibody titer was remarkably reduced. With increasing ethanol content, the antibody inhibition effect first increased and then decreased, and the IC50 value was the lowest at 5%. Therefore, 5% ethanol was selected, at which concentration the antibody titer was high and the inhibition...
was good (Figure 4B). Therefore, the best test factors were pH 7.4 and 5% ethanol-PBS. Under optimal optimization conditions, the standard curve established by ic-ELISA was represented by $Y = 0.207 + 1.631/[1 + (x/5.967)^{1.039}]$, and the linear regression correlation coefficient ($R^2$) was 0.99946. The $IC_{50}$ was 5.97 ng/mL, and the LOD ($IC_{10}$ value) was 0.78 ng/mL. The linear range was 1.57–22.6 ng/mL (Figure 4C).

Comparing the properties of the antibodies with those in the literature,

Table 1. Published Literature on Immunoassay Methods for Detecting Diacetoxyscirpenol

| antibody type | $IC_{50}$ (ng/mL) | LOD (ng/mL) | matrix resistance | immunoassays | ref |
|---------------|------------------|-------------|-------------------|--------------|-----|
| mAb           | 1                | 1           | ic-ELISA          | 17b          |     |
| pAb           | 15               | 1–3         | RIA               | 17a          |     |
| mAb           | 10               | 0.1         | cannot            | ic-ELISA     | 15  |
| mAb           | 5.97             | 0.78        | can               | ic-ELISA     | this study |

this study not only has high sensitivity but also resists sample matrix interferences. Different trichothecene toxins were tested by ic-ELISA, such as DAS, T-2, HT-2, DON, and NIV, and the results can be seen from Table 2. The results indicate that the cross-reactivity of DAS mAb with other *Fusarium* toxins was less than 1%, so this antibody was highly specific.

Table 2. Cross-Reactivity of DAS mAb 3H10 with Trichothecene Toxins

| analyte | $IC_{50}$ (ng/mL) | cross-reactivity (%) |
|---------|------------------|----------------------|
| DAS     | 5.97             | 100                  |
| T-2     | >10000           | <0.1                 |
| HT-2    | >10000           | <0.1                 |
| DON     | >10000           | <0.1                 |
| NIV     | >10000           | <0.1                 |

2.3. Spiked and Recovery Tests by ic-ELISA. The rice sample was detected by extracting and diluting with the sample extract. The DAS spike concentrations were 100, 250, and 500 ng/g. As shown in Table 3, the recovery of DAS in rice samples was 99.4 to 110.7%, and the coefficient of variation was less than 6.9%. Therefore, the antibody is not easily interfered by the sample matrix, and this method can reliably detect DAS residues in rice for commercial application.

Table 3. Recovery of DAS in Rice Determined by ic-ELISA

| sample | spiked level (ng/g) | detection level (ng/g), mean ± SD | recovery rate (%) | CV (%) |
|--------|---------------------|----------------------------------|------------------|--------|
| rice   | 100                 | 1.987 ± 0.025                    | 99.35 ± 1.25     | 6.89   |
| (DAS)  | 250                 | 5.226 ± 0.156                    | 104.52 ± 3.12    | 3.42   |
| 500    | 11.069 ± 0.301      | 110.69 ± 3.01                    | 110.69 ± 3.01    | 4.57   |

*Rice samples were diluted 50 times.*

Figure 4. Standard curves of DAS by ic-ELISA: (A) standard curves for DAS with ic-ELISA based on different pH values; (B) standard curves for DAS with ic-ELISA based on different ethanol contents; (C) standard inhibition curve of DAS.
2.4. Optimization of the Immunochromatographic Strip. According to the results of ic-ELISA, DAS-CDI-BSA as the coating antigen had higher titer than DAS-HS-EDC-BSA and could save antigen synthesis time and cost, so the subsequent trials selected the coating antigen DAS-CDI-BSA.

Parameters such as the quantity of gold-nanoparticle-labeled monoclonal antibody and the coating antigen, as well as the suspension buffer, have a certain influence on the analytical performance and sensitivity of a lateral-flow ICA strip. Each condition was analyzed with a negative sample (0 ng/mL DAS) and a positive sample (25 ng/mL DAS).

The contents of the suspension buffer determine the intensity, clarity, flow rate, and background color of the T line. The basic suspension buffer was prepared by adding 5% sucrose, 2% sorbitol, 1% mannitol, 0.1% Tween, 0.1% PEG, and 0.04% sodium azide in 0.02 M PBS. Four different surfactants were then incorporated, including 5% polyvinylpyrrolidone (PVP), BSA, PEG, and Tween-20. The results in Figure 5 show that the basic suspension buffer containing PEG produced a deep red T-line color for DAS-negative samples, while others were light red or colorless. So, PEG was selected as the optimal resuspension buffer.

Concentrations (0.3 and 0.7 mg/mL) of coating antigens onto the T line were used to detect different amounts of gold-nanoparticle-labeled monoclonal antibody (8 and 10 μg/mL, respectively). As can be seen from Figure 6A, when the negative sample was tested, as the amounts of antigen and antibody (8, 10 μg/mL) increased, the color intensity on the test line increased and the inhibitory effect was the same. Therefore, the amounts of coating antigen and gold-nanoparticle-labeled monoclonal antibody on the lateral-flow ICA strip were 0.7 μg/mL and 10 μg/mL, respectively.

PBS samples spiked with different concentrations of DAS (0, 0.5, 1, 2.5, 5, and 10 ng/mL) were detected by the lateral-flow ICA strip. As can be seen from Figure 6B, for DAS, the cutoff value of the lateral-flow ICA strip was 10 ng/mL

2.5. Sample Analysis. To analyze the rice samples, 2 g samples were weighed from seven different points. DAS at concentrations of 0, 12.5, 25, 50, 125, 250, and 500 ng/g was added, and the solutions were extracted with 20 mL of 25% ethanol-PBS (10-time dilution). After vigorous shaking for 1 h, the supernatant was centrifuged, diluted five times, and then detected by ic-ELISA and the lateral-flow ICA strip. DAS at concentrations of 8, 20, and 40 ng/g was added to the rice samples (4-time dilution), which were prepared according to Romera et al.’s method and detected by the UPLC−MS/MS system. It can be seen from the results of Figure 7 that, as the concentration of DAS increased, the color of the T line began to weaken at a concentration of 1 ng/g and the color disappeared completely at a concentration of 10 ng/g. Since the dilution factor of the sample was 50, for rice samples, the LOD and cutoff value of the lateral-flow ICA strip were 50 and 500 ng/g, respectively. The results in Table 4 demonstrated a good quantitative consistency between ic-ELISA and UPLC−MS/MS, further confirming the reliability of the analytical method.

Table 4. DAS Concentration in the Rice Samples Quantified by ic-ELISA and UPLC−MS/MS

| sample         | ic-ELISA   | UPLC−MS/MS  |
|----------------|------------|-------------|
| spiked DAS (ng/g) | result (ng/g), mean ± SD | spiked DAS (ng/g), result (ng/g), mean ± SD |
| rice 100 | 1.987 ± 0.025 | 8 | 1.992 ± 0.009 |
| 250      | 5.226 ± 0.156 | 20 | 5.032 ± 0.021 |
| 500      | 11.069 ± 0.301 | 40 | 10.027 ± 0.015 |

“Rice samples were diluted 50 times for ic-ELISA. Rice samples were diluted four times for UPLC−MS/MS.

3. CONCLUSIONS

In this study, a highly sensitive and specific mAb 3H10 against DAS was obtained. By optimizing the sample extraction solution, we established a simple and safe ic-ELISA and lateral-flow ICA strip for the detection of diacetoxyscirpenol. The IC_{50} of ic-ELISA was 5.97 ng/mL, the LOD (IC_{10} value) was 0.78 ng/mL, and the linear range was 1.57−22.6 ng/mL. An ic-ELISA method was established to determine DAS in rice samples, and the recovery of rice samples ranged from 99.4 to 110.7%. The lateral-flow ICA strip has a cutoff value of 500 ng/g for rice samples. Our developed immunoassay method
provides an effective method for initially detecting and screening DAS in food and feed samples.

4. MATERIALS AND METHODS

4.1. Chemicals and Instruments. Standards, including DAS, nivalenol (NIV), deoxynivalenol (DON), T-2 toxin, and HT-2 toxin, were purchased from J&K Scientific Co., Ltd. (Shanghai, China). 4-Dimethylaminopyridine, succinic anhydride, N-hydroxysuccinimide (NHS), 1-ethyl-carbodiimide hydrochloride (EDC), carbodiimide (CDI), bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), goat anti-mouse immunoglobulin (IgG) antibody, Freund’s incomplete adjuvant, and Freund’s complete adjuvant were obtained from Sigma-Aldrich (Shanghai, China). Other reagents were analytical-grade pure reagents produced by National Pharmaceutical Group Chemical Reagents Co., Ltd. (Shanghai, China). The nitrocellulose (NC) high-flow plus membrane (PuraBind RP) was purchased from Whatman-Xinhu Filter Paper Co. (Hangzhou, China). Sample pad (CB-SB08), absorbance pad (SX18), and polyvinylchloride (PVC) backing materials were provided by Goldbio Tech Co. (Shanghai, China).

4.2. Solutions and Buffers. The buffer solutions were prepared as follows: coating buffer solution (0.05 M sodium carbonate buffer solution), blocking buffer (0.2% gelatin, v/v in coating buffer containing), PBS buffer (0.01 M phosphate buffer solution), stop solution (2 M sulfuric acid), and washing buffer (PBS buffer solution containing 0.05% Tween 20). The substrate buffer was prepared by mixing solutions A (glycol containing 0.01% TMB) and B (0.1 M citrate phosphate buffer containing 0.3% H2O2) at a ratio of 1:5.

4.3. Synthesis of Hapten and Antigens. 4.3.1. Preparation of DAS Hapten. One milligram of DAS was weighed and dissolved in pyridine, and then 7.2 mg of succinic anhydride and 3.4 mg of catalytic DMAP were added. The reaction was magnetically stirred at 50 °C for 5 h in the dark. The reaction was then quenched by the addition of 50 μL of the stop solution, the absorbance was measured at 450 nm. A standard curve of the DAS LOD values were calculated. A standard curve of the DAS antibody against the DAS standard was plotted, and IC50 and LOD values were calculated.

4.4. ic-ELISA Process. In this experiment, DAS-BSA was serially diluted three times with coating buffer, and then 100 μL per well was added to 96-well microtiter ELISA plates and incubated for 2 h at 37 °C. After washing the plate three times with washing buffer, 200 μL of blocking solution was added to each well and blocked at 37 °C for 2 h. The sealed plates were washed three times, dried, and stored at 4 °C. DAS standards and antibodies were diluted with potassium salt containing 0.01 M PBS and antibody dilution buffer, respectively. Then DAS standard (50 μL) and DAS mAb (50 μL) were sequentially added to each well and incubated in a 37 °C oven for 0.5 h. After washing three times, the goat anti-mouse IgG was diluted 3000-fold with the antibody dilution, 100 μL was added to each well, and the solution was incubated at 37 °C for 0.5 h. After washing, 100 μL of the substrate buffer was added to each well and incubated at 37 °C for 15 min in the dark. After adding 50 μL of the stop solution, the absorbance was measured at 450 nm. A standard curve of the DAS antibody against the DAS standard was plotted, and IC50 and LOD values were calculated.

4.5. Cross-Reactivity. A cross-reactivity (CR) study was performed to determine the specificity of this mAb. DAS, T-2, HT-2, DON, and NIV were subjected to ic-ELISA. The CR values were calculated by the following formula:

\[
CR = \frac{IC_{50} \text{ of DAS}}{IC_{50} \text{ of tested compound}} \times 100
\]

4.6. Preparation of GNP-Labeled Monoclonal Antibodies. This experiment synthesized gold nanoparticles (GNPs) and GNP-labeled mAb. As described above, colloidal gold nanoparticles having an average radius of 7.5 nm were completed. To prepare GNP-labeled monoclonal antibodies, the pH value of the GNP solution (10 mL) was first adjusted to 8.0 with 0.1 M K2CO3, and then anti-DAS mAb (0.1 mg) was added dropwise. After reacting for 60 min at RT, BSA solution (1 mL) was further added dropwise. After incubation for 2 h followed by centrifugation at 7000 × g for 30 min, the resulting pellet was washed three times with 0.02 M PBS (containing 5% sucrose, 1% BSA, 0.5% PEG 6000, pH 7.4). The GNP-labeled mAb was then stored in 4 mL of gold-labeling resuspension buffer (0.02% NaN3, v/v in 0.02 M PBS) at 4 °C.

4.7. Preparation of the Lateral-Flow ICA Strip. In order to prepare the lateral-flow ICA strip, a PVC backing card, an NC film, a sample pad, and an absorbent pad were layered and assembled. The coating antigen was sprayed onto the NC membrane near the sample pad at a rate of 1 μL/cm with a membrane dispenser to form the test line, and the goat anti-mouse IgG antibody was sprayed onto the NC membrane near the sample pad at a rate of 1 μL/cm with a membrane dispenser to form the test line, and the goat anti-mouse IgG antibody was sprayed onto the NC membrane near the sample pad at a rate of 1 μL/cm with a membrane dispenser to form the test line, and the goat anti-mouse IgG antibody was sprayed onto the NC membrane near the sample pad at a rate of 1 μL/cm with a membrane dispenser to form the test line, and the goat anti-mouse IgG antibody was sprayed onto the NC membrane near the sample pad at a rate of 1 μL/cm with a membrane dispenser to form the test line.
adjacent to the absorbent pad to form the control line. After drying at 37 °C for 0.5 h, the strip was stored in a desiccator.

4.8. Principle of the Lateral-Flow ICA Strip. A 50 μL aliquot of the GNP-labeled mAb was mixed with 150 μL of a sample solution containing the DAS standard in microwells. The mixed solution was added to the sample pad after incubation for 5 min at room temperature, and the solution slowly infiltrated into the absorbent pad due to capillary action of the microporous membrane. Since the negative sample did not contain the DAS standard, the GNP-labeled mAb bound only to the coated antigen, making the T line appear red. In the positive sample, the GNP-labeled mAb bound to DAS was not captured by the coating antigen, and as a result, the T line was light red or colorless. Both free and bound GNP-labeled mAbs were captured by the goat anti-mouse IgG antibody on the C line, forming a deep red color. Negative and positive samples were judged by comparison between the T line and the C line, and the results could be visually observed within 5 min. Testing of a range of different concentrations of DAS standards confirmed the sensitivity of the immunochromatographic strip.

4.9. Sample Preparation and UPLC−MS/MS Analysis. Fusarium toxin samples were typically extracted by using organic reagents. The harmful reagents methanol and acetonitrile are commonly used. Consequently, ethanol has the advantages of low toxicity and safety, which ensures not only safe and clear sample pretreatment but also human health. Therefore, sample extraction was performed using 25% ethanol-PBS solution. Rice was bought at a local supermarket. After the rice without DAS (determined by UPLC−MS/MS) was ground to 20 mesh, 2 g samples were weighed from seven different points, and DAS of different concentrations was added while shaking. Each sample was then extracted with 20 mL of 25% ethanol-PBS (10-fold dilution). The mixture was oscillated on an electric shaker for 1 h and then centrifuged at 5000 × g for 20 min. The supernatant was diluted five times and further subjected to ic-ELISA and immunochromatographic analysis. The rice samples were prepared by the sample preparation method by Romero et al. and subjected to UPLC−MS/MS analysis to compare the quantitative consistency of ic-ELISA and UPLC−MS/MS.

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Notes
No potential conflict of interest was reported by the authors. The authors declare no competing financial interest.

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■ REFERENCES

(1) Nesci, K.; Ivanovic, S.; Nesci, V. Fusarial toxins: secondary metabolites of Fusarium fungi. Rev. Environ. Contam. Toxicol. 2014, 228, 101−120.
(2) Zhang, J.; Sheng, K.; Wu, W.; Zhang, H. Anorectic responses to T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol correspond to plasma elevations of neurotransmitters S-hydroxytryptamine and substance P. Ecotoxicol. Environ. Saf. 2018, 161, 451−458.
(3) Antonissen, G.; Martel, A.; Pasmans, F.; Ducatelle, R.; Verbrugghe, E.; Vandenbroucke, V.; Li, S.; Haesebrouck, F.; Van Immerseel, F.; Crousels, S. The impact of Fusarium mycotoxins on human and animal host susceptibility to infectious diseases. Toxins 2014, 6, 430−452.
(4) Vidal, A.; Mengelers, M.; Yang, S.; De Saeger, S.; De Boeye, M. Mycotoxin Biomarkers of Exposure: A Comprehensive Review. Compr. Rev. Food Sci. Food Saf. 2018, 17, 1127−1155.
(5) Lebrun, B.; Tardivel, C.; Félix, A.; Abysique, A.; Troadej, J.-D.; Gaïg, S.; Dallaporta, M. Dysregulation of energy balance by trichothecene mycotoxins: Mechanisms and prospects. Neurotoxicology 2015, 49, 15−27.
(6) Zhang, J.; Zhang, H.; Liu, S.; Wu, W.; Zhang, H. Comparison of Anorectic Potencies of Type A Trichothecenes T-2 Toxin, HT-2 Toxin, Diacetoxyscirpenol, and Neosolaniol. Toxins 2018, 10, 179.
(7) Gonzalves, R. A.; Hofstetter, U.; Schatzmayr, D.; Jenkins, T. Mycotoxins in Southeast Asian aquaculture: plant-based meals and finished feed products. World Mycotox. J. 2018, 11, 265−275.
(8) Yang, S.; De Boeye, M.; Zhang, H.; De Ruyck, K.; Sun, F.; Wang, Z.; Cao, X.; Shen, J.; De Saeger, S.; Zhang, S. Unraveling the in vitro and in vivo metabolism of diacetoxyscirpenol in various animal species and human using ultra-high-performance liquid chromatography-quadrupole/time-of-flight hybrid mass spectrometry. Anal. Bioanal. Chem. 2015, 407, 8571−8583.
(9) Chilaka, C. A.; De Boeye, M.; Atanda, O. O.; De Saeger, S. Prevalence of Fusarium mycotoxins in cassava and yam products from some selected Nigerian markets. Food Control 2018, 84, 226−231.
(10) Yoshinari, T.; Takeda, N.; Watanabe, M.; Sugita-Konishi, Y. Development of an Analytical Method for Simultaneous Determination of the Modified Forms of 4,15-Diacetoxyscirpenol and their Occurrence in Japanese Retail Food. Toxins 2018, 10, 178.
(11) Escrivá, L.; Manyes, L.; Font, G.; Berrada, H. Analysis of trichothecenes in laboratory rat feed by gas chromatography-tandem mass spectrometry. Food Addit. Contam., Part A 2016, 33, 329−338.
(12) Carballo, D.; Font, G.; Ferrer, E.; Berrada, H. Evaluation of Mycotoxin Residues on Ready-to-Eat Food by Chromatographic Methods Coupled to Mass Spectrometry in Tandem. Toxins 2018, 10, 243.
(13) Romero, D.; Mateo, E. M.; Mateo-Castro, R.; Gómez, J. V.; Gimeno-Adelantado, J. V.; Jiménez, M. Determination of multiple mycotoxins in feedstuffs by combined use of UPLC-MS/MS and UPLC-QTOF-MS. Food Chem. 2018, 267, 140−148.
(14) Han, X.; Sheng, F.; Kong, D.; Wang, Y.; Pan, Y.; Chen, M.; Tao, Y.; Liu, Z.; Ahmed, S.; Yuan, Z.; Peng, D. Broad-spectrum monoclonal antibody and a sensitive multi-residue indirect competitive enzyme-linked immunosorbent assay for the antibacterial synergists in samples of animal origin. Food Chem. 2019, 280, 20−26.
(15) Schubring, S. L.; Chu, F. S. An indirect enzyme-linked immunosorbent assay for the detection of diacetoxyscirpenol in wheat and corn. Mycotoxin Res. 1987, 3, 97−106.
(16) Kong, D.; Xie, Z.; Liu, L.; Song, S.; Zheng, Q.; Kuang, H. Development of an immunochromatographic assay for the detection of alternariol in cereal and fruit juice samples. Food Agric. Immunol. 2017, 28, 1082−1093.
(17) (a) Chu, F. S.; Liang, M. Y.; Zhang, G. S. Production and characterization of antibody against diacetoxyscirpenol. Appl. Environ. Microbiol. 1984, 48, 777−780. (b) Pauly, J. U.; Bitter-Suermann, D.; Dose, K. Production and characterization of a monoclonal antibody to the trichothecene mycotoxin diacetoxyscirpenol. Biol. Chem. Hoppe-Seyler 1988, 369, 487−492.
(18) Zeng, L.; Jiang, W.; Liu, L.; Song, S.; Kuang, H. Development of ic-ELISA and lateral-flow immunochromatographic strip for detection of vitamin B2 in an energy drink and vitamin tablets. Food Agric. Immunol. 2018, 29, 121−132.
(19) Ceballos-Alcantarilla, E.; Agulló, C.; Abad-Somovilla, A.; Abad-Fuentes, A.; Mercader, J. V. Highly sensitive monoclonal antibody-based immunoassays for the analysis of fluopyram in food samples. *Food Chem.* 2019, 288, 117–126.

(20) Li, H.; Ma, S.; Zhang, X.; Li, C.; Dong, B.; Mujtaba, M. G.; Wei, Y.; Liang, X.; Yu, X.; Wen, K.; Yu, W.; Shen, J.; Wang, Z. Generic Hapten Synthesis, Broad-Specificity Monoclonal Antibodies Preparation, and Ultrasensitive ELISA for Five Antibacterial Synergists in Chicken and Milk. *J. Agric. Food Chem.* 2018, 66, 11170–11179.

(21) Lei, X.; Xu, L.; Song, S.; Liu, L.; Kuang, H. Development of an ultrasensitive ic-ELISA and immunochromatographic strip assay for the simultaneous detection of florfenicol and thiamphenicol in eggs. *Food Agric. Immunol.* 2018, 29, 254–266.

(22) Ding, X.; Liu, L.; Song, S.; Kuang, H.; Xu, C. Rapid and ultrasensitive detection of 3-amino-2-oxazolidinone in catfish muscle with indirect competitive enzyme-linked immunosorbent and immunochromatographic assays. *Food Agric. Immunol.* 2017, 28, 463–475.

(23) Ni, T.; Peng, D.; Wang, Y.; Pan, Y.; Xie, S.; Chen, D.; Wang, Y.; Tao, Y.; Yuan, Z. Development of a broad-spectrum monoclonal antibody-based indirect competitive enzyme-linked immunosorbent assay for the multi-residue detection of avermectins in edible animal tissues and milk. *Food Chem.* 2019, 286, 234–240.

(24) Feng, M.; Kong, D.; Wang, W.; Liu, L.; Song, S.; Xu, C. Development of an Immunochromatographic Strip for Rapid Detection of Pantoea stewartii subsp. stewartii. *Sensors* 2015, 15, 4291–4301.

(25) Guo, M.; Sun, L.; Liu, L.; Song, S.; Kuang, H.; Cui, G. Ultrasensitive immunochromatographic strip for detection of cyproheptadine. *Food Agric. Immunol.* 2018, 29, 941–952.

(26) Lin, L.; Jiang, W.; Xu, L.; Liu, L.; Song, S.; Kuang, H. Development of IC-ELISA and immunochromatographic strip assay for the detection of flunixin meglumine in milk. *Food Agric. Immunol.* 2018, 29, 193–203.

(27) Kong, D.; Wu, X.; Li, Y.; Liu, L.; Song, S.; Zheng, Q.; Kuang, H.; Xu, C. Ultrasensitive and eco-friendly immunoassays based monoclonal antibody for detection of deoxynivalenol in cereal and feed samples. *Food Chem.* 2019, 270, 130–137.

(28) Hendrickson, O. D.; Zvereva, E. A.; Shanin, I. A.; Zherdev, A. V.; Dzantiev, B. B. Development of a multicomponent immunochromatographic test system for the detection of fluoroquinolone and amphenicol antibiotics in dairy products. *J. Sci. Food Agric.* 2019, 99, 3834–3842.

(29) Peng, J.; Liu, L.; Kuang, H.; Cui, G.; Xu, C. Development of an icELISA and immunochromatographic strip for detection of norfloxacin and its analogs in milk. *Food Agric. Immunol.* 2017, 28, 288–298.

(30) Guo, J.; Liu, L.; Xue, F.; Xing, C.; Song, S.; Kuang, H.; Xu, C. Development of a monoclonal antibody-based immunochromatographic strip for cephalexin. *Food Agric. Immunol.* 2014, 26, 282–292.

(31) Wang, J.; Peng, T.; Zhang, X.; Yao, K.; Ke, Y.; Shao, B.; Wang, Z.; Shen, J.; Jiang, H. A novel hapten and monoclonal antibody-based indirect competitive ELISA for simultaneous analysis of alternariol and alternariol monomethyl ether in wheat. *Food Control* 2018, 94, 65–70.