Rapid and ultrasensitive detection of 3-amino-2-oxazolidinone in catfish muscle with indirect competitive enzyme-linked immunosorbent and immunochromatographic assays

Xi Ding¹,², Liqiang Liu¹,², Shanshan Song¹,², Hua Kuang¹,² and Chuanlai Xu¹,²

¹State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, People’s Republic of China; ²International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, People’s Republic of China

ABSTRACT
In this study, we developed an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and an immunochromatographic assay to detect the furazolidone metabolite, 3-amino-2-oxazolidinone (AOZ). The detection of AOZ was based on the AOZ derivative 3-[[2-nitrophenyl]methyleneamino]-2-oxazolidinone (2-NPAOZ). 3-[[3-Carboxyphenyl)methylene]-amino)-2-oxazolidinone (2-CPAOZ) was used as the immunizing hapten and a conjugate of AOZ and glyoxylic acid (AOZ-A) was used as the coating hapten. The monoclonal anti-2-NPAOZ antibody, 5G12, was generated with a half-inhibitory concentration (IC₅₀) of 0.2 ng/mL and a line of 0.06–0.66 ng/mL. Its cross reactivity with other analogues was less than 8%. Spiked catfish samples (0.1, 0.2, and 0.5 μg/kg) were analyzed with the proposed system. The ic-ELISA showed a recovery range of 86.2–118.5% and the intra-assay and inter-assay coefficients of variation ranged from 4.3% to 9.4%. Under the optimum assay conditions, the immunochromatographic assay had a visual cut-off value of 0.5 μg/kg in catfish samples. Both methods can be used to detect AOZ in catfish samples.

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Introduction
Furazolidone is one of the most frequently used nitrofuran broad-spectrum antibiotics. Because it is cheap and effective, it has been widely used both prophylactically and therapeutically against various bacterial and protozoan infections in the livestock and poultry industries and aquaculture (Barbosa et al., 2011). The metabolism of furazolidone in organisms is particularly rapid, whereas its metabolite, 3-amino-2-oxazolidinone (AOZ), is discharged from the body for several weeks. Therefore, it occurs in the food chain and remains in the natural environment for a long time (Kelly, Heneghan, Connolly, & O’Gorman, 1998; Li, Li, & Xu, 2017). Extensive research suggests that furazolidone and AOZ have carcinogenic and mutagenic properties (Auro, Sumano, Ocampo, & Barragán, 2004). Therefore, these drugs have been prohibited in the treatment and feed of food...
animals in many countries, including the European Union, the United States, and China. Although the Chinese Government and related organizations attach great importance to residues of these kinds of drugs, the illegal use of furazolidone still occurs.

The detection of furazolidone residues is mainly based on the AOZ derivative 3-([2-nitrophenyl]methyleneamino)-2-oxazolidinone (2-NPAOZ) (Cooper, Caddell, Elliott, & Kennedy, 2004; Vass et al., 2005). Various instrumental methods have been used to evaluate furazolidone residues, including high-performance liquid chromatography (HPLC; Connolly, Nugent, & O’Keeffe, 2002), LC–mass spectrometry (LC–MS; McCracken & Kennedy, 1997), and LC–tandem mass spectrometry (LC–MS/MS; Cooper et al., 2005; Hu, Xu, & Yediler, 2007; Zhang, Qiao, Chen, Wang, & Xia, 2016). Although these methods are accurate, they are difficult to use in developing countries and cannot be carried to the field for on-site detection. Most importantly, the sample pretreatment processes required for these methods are very complex, and the methods themselves are expensive, time-consuming, and inconvenient for processing large numbers of samples. Immunological analytical methods are also used to detect furazolidone residues, including enzyme-linked immunosorbent assays (ELISAs) and colloidal gold immunochromatographic assays. The immunoassay method has the advantages of rapidity, high sensitivity, high specificity, and high throughput (Gu, Liu, Song, Kuang, & Xu, 2016; Liu et al., 2010; Peng, Song, Liu, Kuang, & Xu, 2016), so many ELISAs have been developed to detect AOZ in aquatic animals, chicken, pork, beef, and eggs (Chang, Peng, Wu, Wang, & Yuan, 2008; Cheng et al., 2009; Cooper et al., 2004; Diblikova, Cooper, Kennedy, & Franek, 2005; Franek et al., 2006; Jester, Abraham, Wang, El Said, & Plakas, 2014; Le & Yu, 2015; Liu et al., 2010; Vass et al., 2005). Nonetheless, the ELISA is relatively cumbersome and requires expertise. In contrast, the results of the colloidal gold immunochromatographic assay are evident to the naked eye after a few minutes. A single Colloidal gold strip costs about 0.5–1 yuan and a colloidal gold detection card production costs about 1.0–1.5 yuan (R-Biopharm), and this method is simpler and cheaper than the ELISA. At present, the colloidal gold immunochromatographic assay is well accepted and performed worldwide. Therefore, it is essential and exigent to develop an immunochromatographic assay for the rapid detection of AOZ.

In this study, we generated an ultrasensitive and specific monoclonal antibody (mAb), 5G12, directed against 2-NPAOZ. Based on this mAb, an immunochromatographic assay was developed to detect AOZ in catfish samples.

**Materials and methods**

**Chemicals**

1-Aminohydantoin hydrochloride (AHD), 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ), AOZ, semicarbazide, furazolidone, nitrofurantoin, furaltadone, nitrofurazone, 2-nitrobenzaldehyde (2-NBA), 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, 2-carboxybenzaldehyde (2-CBA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N,N’-dicyclohexylcarbodiimide (DCC), and glyoxylic acid monohydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), ovalbumin (OVA), keyhole limpet hemocyanin (KLH; MW 5,000,000 Da), Freund’s complete and incomplete adjuvants, enzyme-immunoassay-grade horseradish peroxidase (HRP)-labeled goat anti-mouse immunoglobulin, 3,3′,5,5′-tetramethylbenzidine (TMB), Tween20, HRP,
and gelatin were also purchased from Sigma-Aldrich. Cell culture reagents (polyethylene glycol solution, HAT supplement, HT supplement, and RPMI 1640 cell culture medium) were obtained from Life Technologies Corporation (Shanghai, China). Catfish samples were obtained from a local supermarket (Wuxi, China).

**Instruments**

A Waters Maldi Synapt Q-Tof mass spectrometer (Waters, Shanghai, China) was used to identify the derivatives of AOZ (3-[(3-carboxyphenyl)methylene]-amino)-2-oxazolidinone [2-CPAOZ], AOZ-A, and 2-NPAOZ). Other instruments were obtained from the following suppliers: UV–VIS scanner (Bokin Instruments, Tsushima, Japan), vortex machine (Shanghai Huxi Analysis Instrument Factory Co., Ltd, Shanghai, China), Multiskan MKS microplate reader (Thermo Labsystems Company, Beijing, China), MilliQ Water Purification System (Millipore, Shanghai, China), and water bath (Shanghai Instrument Group Co., Ltd, Supply & Sales Co., Shanghai, China).

**Buffers and solutions**

Coating buffer (CB; 0.05 M carbonate bicarbonate, pH 9.6), blocking buffer (0.2% gelatin in coating buffer), washing buffer (PBST; 0.05% Tween20 [v/v] in 0.01 M phosphate-buffered saline [PBS]), and stop solution (2 M sulfuric acid) were prepared. Substrate buffer consisted of solutions A (citric acid, H2O2, and Na2HPO4) and B (0.06% [v/v] TMB in glycol) in a 5:1 (v/v) ratio.

**Synthesis and characterization of AOZ derivatives: 2-CPAOZ, AOZ-A, and 2-NPAOZ**

Distilled water (2.6 mL) and 120 μL of 1 M HCl were added to 2-CBA (23.0 mg) in the round bottom flask. Dimethylformamide (DMF) was dropped into the 2-CBA with magnetic stirring until they were dissolved completely. The solution was then mixed with AOZ (30 mg) and the mixture was placed in a constant water bath at 60°C and refluxed overnight to produce a white precipitate. After the mixture was cooled to room temperature, the solid product was centrifuged for 10 min at 5000 revolutions per minute, and the supernatant was discarded. The white precipitate was dried completely in a 60°C oven to produce 2-CPAOZ. Ultra-high-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was used to demonstrate the identity and purity of the target product. Using the same method, we replaced 2-CBA with glyoxylic acid monohydrate to generate AOZ-A or with 2-NBA to generate 2-NPAOZ.

**Preparation of immunogen and coating antigen**

The immunogen was KLH, which was linked to 2-CPAOZ with the DCC method. 2-CPAOZ (2.4 mg) was dissolved in anhydrous DMF (0.6 mL), to which 5.2 mg of DCC and 2.9 mg of NHS were added. The solution was stirred continuously for 4 h at room temperature (solution 1). KLH (10 mg) was mixed with 2 mL of 0.1 M CB (pH 9.6) (solution 2). Solution 1 was poured slowly into solution 2. The reaction was continued for 12 h at room temperature, and then the reaction mixture was dialyzed against PBS (0.01 M pH
7.4) for 3 days at 4°C. UV–VIS spectroscopy was used to confirm the success of the coupling reaction, and the product was stored at −20°C.

The coating antigen was OVA, which was linked to AOZ-A with the EDC method and used as the heterogeneous coating antigen (AOZ-A–EDC–OVA). Briefly, 9.5 mg of EDC and 5.7 mg of NHS were added to 3.2 mg of AOZ-A dissolved in 0.6 mL of anhydrous DMF, and stirred continuously for 4 h at room temperature to form active esters. The solution was then added dropwise to 30 mg of OVA dissolved in 5 mL of CB. The mixture was stirred overnight at room temperature, dialyzed against PBS for 3 days at 4°C, characterized with UV–VIS spectroscopy, and stored at −20°C.

**Production of mAb**

The immunization procedure was performed as described previously (Lin et al., 2011; Suryoprabowo, Liu, Peng, Kuang, & Xu, 2014; Zhang et al., 2013). Female BALB/c mice (6–8 weeks of age) were subcutaneously immunized with the immunogen 2-CPAOZ–EDC–KLH mixed with Freund’s complete adjuvant (100 μg per mouse). Freund’s complete adjuvant was used for the first immunization and Freund’s incomplete adjuvant was used in the subsequent boost injection. Every 3 weeks, 50 μg of the complete antigen was emulsified with an equal volume of Freund’s incomplete adjuvant and administered as a booster immunization. After the third injection, the serum was collected from the tail of each mouse and analyzed with an indirect competitive (ic-ELISA). The mouse with the highest inhibition and titer was intraperitoneally injected with 25 μg immunogen. Three days later, the mouse’s spleen and blood from the eye were collected for cell fusion.

Hybridoma cells were produced by the fusion of the mouse splenocytes with Sp 2/0 myeloma cells. The cells were selected with ic-ELISA and subcloned three times. The optimal cell line was selected for amplification culture and was injected intraperitoneally into mice for the production of ascites. After 7 days, the ascites were collected and purified with caprylic acid and ammonium sulfate precipitation (Yan, Liu, Xu, Kuang, & Xu, 2015). The concentration of the mAb was calculated with the Bradford method.

**M Ab sensitivity and specificity**

To determine the sensitivity of the mAb, an ic-ELISA was developed, using the checkerboard method to optimize the concentration of the coating antigen, AOZ-A–EDC–OVA (1–0.01 μg/ml). AOZ-A–EDC–OVA was added to a 96-well microtiter plate and diluted with coating solution. The assay was performed with the conventional protocol (Peng et al., 2008). Standard mAb curves were generated by plotting B/B₀ against log [2-NPAOZ] (log of the 2-NPAOZ concentration). The sensitivity of the mAb was determined as the IC₅₀. The specificity of the mAb was determined from its CR with other compounds and calculated as: CR (%) = ([IC₅₀ of 2-NPAOZ]/[IC₅₀ of competitor]) × 100%.

**Sample pretreatment**

Three catfish samples were taken for the experiments. About two years old, 2–3 kg of wild type fresh catfishes were purchased from a local supermarket and processed to get
shredded catfish muscle samples; then, they were stored at −20°C before use. The catfish muscle samples were selected for analysis with LC–MS, and no nitrofurans or their metabolites were detected. The samples were prepared with the procedure described by Luo et al. (2012) and Pimpitak, Putong, Komolpis, Petsom, and Palaga (2009). The catfish muscles were finely chopped and hydrolyzed with 1 M HCL, and 2-CBA was added as the derivatization reagent. The sample was then extracted repeatedly to remove any potentially interfering impurities. Finally, the extracts were tested with the ic-ELISA, and each test was repeated five times.

**Preparation of colloidal gold and gold-labeled mAb**

Colloidal gold was prepared by reducing chloroauric acid with sodium citrate, as described previously (Chen, Liu, Kuang, Song, & Xu, 2013; Sun et al., 2012; Yang et al., 2015). First, 1 mL of 1% sodium citrate was added to 100 mL distilled water, and after boiling, 1 mL of 1% chloroauric acid was dissolved immediately in it. When the color became clear claret red, the mixture was heated for a further 10 min, and then cooled to room temperature to produce the colloidal gold solution, which was stored at −4°C.

The pH of 10 mL of colloidal gold solution was adjusted to 7.8, and 0.8 mg of the anti-2-NPAOZ mAb was dropped into the magnetically stirred solution. After 30 min, the reaction was terminated with 20% BSA. The solid product was centrifuged for 30 min at 8000 revolutions per minute, and the precipitated gold-labeled mAb was resuspended in borate solution and stored at 4°C (Guo et al., 2015).

**Strip preparation**

A test line of the coating antigen and a control line of goat anti-mouse IgG antibody were applied to a nitrocellulose (NC) membrane with a membrane dispenser, using 1.0 μL/cm. The distance between the two lines was 0.5 cm. The NC membrane was dried at 37°C for 30 min before use. A sample pad, the NC membrane, and an absorbent pad were sequentially pasted onto a polyvinyl chloride (PVC) plate, and the assembled plate was cut into individual strips (2.9 mm wide).

**Immunochromatographic assay to detect AOZ in catfish samples**

A series of standard AOZ samples were prepared to evaluate the linearity and limit of detection of the assay. The series concentrations were 0, 0.25, 0.5, and 1 μg/kg. The sample solution (100 μL) was mixed with the labeled mAb and reacted for 5 min at room temperature. After incubation for 5 min in a dry heat machine at 37°C, a strip was added to the microwell with the sample pad face down. The effect of capillary action allowed the labeled mAb and the sample solution to move from the sample pad, across the NC membrane, and into the absorbent pad. In AOZ-negative catfish samples, the labeled mAb was captured by the coating antigen, so a red test line became visible. In AOZ-positive catfish samples, the immobilized antigen and the free AOZ antigen competed for the gold-labeled mAb. Consequently, the color intensity of the test line correlated inversely with the concentration of the AOZ present in the
catfish samples. The results were determined with the naked eye after 5 min. The cut-off value was 0.5 μg/kg.

Results and discussion

Preparation of AOZ derivatives and conjugated antigen

2-CPAOZ, AOZ-A, and 2-NPAOZ analytes were synthesized with condensation reactions using aldehyde and ammonia (Figure 1), and their structures were confirmed with UPLC–MS/MS.

The immunogen was KLH, which was linked to 2-CPAOZ with the DCC method. In our research, DCC method, EDC method and mixed aldehyde method were evaluated (Table 1). From the table, we can find that DCC method induced the highest titers and the best inhibitory effect; the DCC method induced a better immune response in mice than the EDC or mixed anhydride method. The immunogen (2-CPAOZ–DCC–KLH) was confirmed with UV–VIS spectroscopy and the results are shown in Figure 2(a). The absorbance peaks for 2-CPAOZ–DCC–KLH, 2-CPAOZ, and KLH all occurred at 280 nm, so the concentrations of the proteins were adjusted to 0.5 mg/mL with UV–VIS spectrophotometry. The ratios for the coupling reaction between 2-CPAOZ and

![Figure 1. The synthesis routines of the haptens 2-CPAOZ, AOZ-A and the analyte 2-NPAOZ.](image-url)
KLH were set at 2k, 4k, 6k, and 8k. Figure 2(a) shows that the absorbance peak for 2-CPZOA–DCC–KLH increased linearly as the ratio of the coupling reaction increased, confirming that the 2-CPZOA hapten was successfully linked to the carrier protein KLH.

The coating antigen was OVA, which was linked to AOZ-A with the EDC method to produce the heterogeneous coating antigen. The UV–VIS results for the hapten AOZ-A, the carrier protein OVA, and the AOZ-A–EDC–OVA conjugate are shown in Figure 2(b). From the spectra in Figure 2(b), the absorbance peak of AOZ-A–EDC–OVA occurred at 260 nm, whereas the peaks for AOZ-A and OVA occurred at 246 and 280 nm, respectively. Thus, the peak for AOZ-A–EDC–OVA was quite different from those of AOZ-A and OVA, so the production of the coating antigen was completely successful.

**Preparation and identification of mAb**

The mAb was prepared with cell fusion technology, and confirmed with ic-ELISA. Cell lines 2A8, 2C9, 4E9, 5E11, 5H11, and 5G12, each of which secrete antibody directed against 2-NPZOA, were generated. The ascites were purified with the caprylic acid–ammonium sulfate precipitation method. The IC50 of these antibodies were all about 0.2 ng/mL, as shown in Figure 3. As the function of linking arm 2-CBA, the chemical structure of AOZ was maximally exposed to the immune system. Therefore, the antibody was highly sensitive and highly specific. Cross-reactivity with analogues was less than 8% as shown in Table 2. Cell line 5G12 best met the selection criteria, and was selected for the immunochromatographic assay.

**Recovery in catfish samples tested with ic-ELISA and LC–MS/MS**

Spiked catfish samples were used in this experiment. The catfish samples were spiked with different standard AOZ concentrations (0.1, 0.2, or 0.5 μg/kg), recovery experiments

| Method         | Titer of mice serum | IC50 (ng/mL) |
|----------------|---------------------|--------------|
| DCC            | 81,000              | 2            |
| EDC            | 27,000              | 10           |
| Mixed aldehyde | 27,000              | 10           |

Table 1. The evaluation of different synthetic immunogen methods.

*Figure 2.* (a) UV–VIS spectra of immunogen (2-CPZOA–DCC–KLH), 2CPZOA and KLH; (b) UV–VIS spectra of coating (AOZ–A–EDC–OVA), AOZ–A and OVA.
measured by ic-ELISA and LC–MS/MS and the results are shown in Table 3. The mAb showed satisfactory repeatability, with recoveries ranging from 86.2% to 118.5%, and the intra-assay and inter-assay coefficients of variation (CV) ranged from 4.3% to 9.4%.

**Optimization of colloidal gold-labeled mAb**

The results of the immunochromatographic assay were determined rapidly (5–10 min) based on the red color of the gold nanoparticles. The size of the nanoparticles may affect the sensitivity of the method, and pH is also an important factor. Based on our previous studies (Wang et al., 2015; Xu et al., 2015), 20 nm gold nanoparticles were used to label the mAb, and the conjugation reaction between the mAb and the colloidal gold nanoparticles was performed at pH 8.2. In this study, the resuspension solution and amount of mAb used were optimized. The basic buffer contained 20 mM Tris (pH 8.2), 0.1% PEG, 0.1% Tween, 5% sucrose, 5% trehalose, and 0.2% BSA. Thirteen kinds of resuspension solutions (1% or 5%) were tested: PVP, PEG, BSA, casein, sucrose, trehalose, sorbitol, mannitol, glucan, Tween20, Brij-35, Triton X-100, and On-870. The results are shown in Figure 3.

![Standard curve obtained by ic-ELISA based on antibody 5g12.](image)

Table 2. CR of antibody with various drugs and marker residues.

| Competitor         | IC50 (ng/ml) | CR (%) |
|--------------------|--------------|--------|
| 2-NPAOZ            | 0.2          | 100    |
| 3-NPAOZ            | 3.0          | 6.67   |
| 4-NPAOZ            | 2.5          | 8.00   |
| AOZ                | 5.0          | 4.00   |
| Furazolidone       | 450          | 0.04   |
| 2-NPAHD            | >1000        | <0.02  |
| 2-NPAMOZ           | >1000        | <0.02  |
| 2-NPSEM            | >1000        | <0.02  |
| AHD                | >1000        | <0.02  |
| AMOZ               | >1000        | <0.02  |
| SEM                | >1000        | <0.02  |
| Nitrofurantoin     | >1000        | <0.02  |
| Furaladone         | >1000        | <0.02  |
| Nitrofurazone      | >1000        | <0.02  |
Table 3. Recovery results in spiked catfish samples measured by ic-ELISA and LC–MS/MS.

| Samples  | Spiked level of AOZ (µg/kg) | Intra-assay ($n = 5$) | Inter-assay ($n = 5$) | LC–MS/MS ($n = 5$) |
|----------|-----------------------------|------------------------|------------------------|---------------------|
|          |                             | Recovery (%) | CV (%) | Recovery (%) | CV (%) | Recovery (%) | CV (%) |
| Catfish  | 0.1                         | 86.2        | 4.3    | 90.4        | 5.6    | 89.5         | 8.0    |
|          | 0.2                         | 94.5        | 7.8    | 100.7       | 9.1    | 92.7         | 6.2    |
|          | 0.5                         | 111.2       | 8.0    | 118.5       | 9.4    | 96.6         | 4.5    |

Figure 4. Based on the results, resuspension solution 4 (5% BSA) was used for subsequent experiments. To assess the optimal amount of mAb to be used in the assay, 8 and 10 µg/mL mAb were tested. The results are shown in Figure 5 and indicate that 10 µg/mL was the better choice. The optimal concentrations were confirmed to be 0.2 mg/mL coating antigen, 1 mL of gold nanoparticles, 4 µL of K$_2$CO$_3$, 5% BSA, and 10 µg/mL mAb.

Figure 5. Optimization of the amount of mAb ($1 = 0$, $2 = 0.25$ ng/ml, $3 = 0.5$ ng/ml, and $4 = 1$ ng/ml).
AOZ detection in catfish samples with the immunochromatographic assay

To validate the method developed, catfish samples were spiked with AOZ to simulate real samples. The catfishes were obtained at a local supermarket and analyzed with LC–MS to confirm that no AOZ was present. In this experiment, we added 0, 0.25, 0.5, or 1 ng/mL 2-NPAOZ in PBS to the strip and the results are shown in Figure 6(a). When the concentration of 2-NPAOZ exceeded 0.5 ng/mL, the test line disappeared completely. We also spiked the catfish samples with 0, 0.25, 0.5, or 1 μg/kg AOZ and applied the test strip to them. The results when the immunochromatographic assay was used to detect AOZ in the catfish samples were the same as those for 2-NPAOZ in PBS. The results are shown in Figure 6(b) and Table 4 (Kong, Liu, Song, Kuang, & Xu, 2016). Thus, this method is practicable and the test strip can be used as a fast on-site screening tool to detect the furazolidone metabolite AOZ in catfish samples.

Conclusions

In this study, we prepared an ultrasensitive mAb for AOZ detection, with an IC$_{50}$ of 0.2 ng/ml. Its CR was very low, indicating that the developed mAb was highly specific. More importantly, we developed an immunochromatographic assay based on the mAb

| Table 4. Accuracy and reproducibility evaluation of the AOZ strip. |
|---------------------------------------------------------------|
| AOZ concentration (μg/kg) | 0  | 0.25 | 0.5 | 1 |
| Control line           | ++ | ++   | ++  | ++ |
| Test line              | ++ | +    |     | ++ |
| Results                | Negative | Weakly positive | Positive | Positive |

Notes: ++: Highly red color; +: weakly red color; --: no test line is observed.

**AOZ detection in catfish samples with the immunochromatographic assay**

To validate the method developed, catfish samples were spiked with AOZ to simulate real samples. The catfishes were obtained at a local supermarket and analyzed with LC–MS to confirm that no AOZ was present. In this experiment, we added 0, 0.25, 0.5, or 1 ng/mL 2-NPAOZ in PBS to the strip and the results are shown in Figure 6(a). When the concentration of 2-NPAOZ exceeded 0.5 ng/mL, the test line disappeared completely. We also spiked the catfish samples with 0, 0.25, 0.5, or 1 μg/kg AOZ and applied the test strip to them. The results when the immunochromatographic assay was used to detect AOZ in the catfish samples were the same as those for 2-NPAOZ in PBS. The results are shown in Figure 6(b) and Table 4 (Kong, Liu, Song, Kuang, & Xu, 2016). Thus, this method is practicable and the test strip can be used as a fast on-site screening tool to detect the furazolidone metabolite AOZ in catfish samples.

**Conclusions**

In this study, we prepared an ultrasensitive mAb for AOZ detection, with an IC$_{50}$ of 0.2 ng/ml. Its CR was very low, indicating that the developed mAb was highly specific. More importantly, we developed an immunochromatographic assay based on the mAb
raised against 2-NPAOZ, and used it to detect AOZ in spiked catfish samples. The cut-off value was 0.5 μg/kg. At present, it has been widely used in the market of rapid detection. This assay provides a very fast and cost-effective alternative tool for screening for AOZ.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Notes on contributors**

**Xi Ding** got his bachelor from Wuhan Polytechnic University, Wuhan, China in 2014 and then he began to study in Jiangnan University (Wuxi, China) for as a graduate student in food science. His research interests are immunoassay applications in food.

**Liqiang Liu** got his Ph.D in Food science in 2014 from Jiangnan University, Wuxi, China and then became a faculty in college of Food science and technology of Jiangnan University. His research interests are immunochromatographic strip design and application.

**Shanshan Song** got her Master degree in Food science in 2012 from Jiangnan University, Wuxi, China and then became a research assistant in college of Food science and technology of Jiangnan University. Her research interests are monoclonal antibody development.

**Hua Kuang** got her Ph.D from China Agricultural University in 2009 and then began to work as a faculty in college of Food science and technology of Jiangnan University. She is currently a full professor in food safety. Her research interests are biosensor development.

**Chuanlai Xu** is a full professor of Food science and technology of Jiangnan University. He got his Ph.D in food science in 2002. His research interests are fast detection technology and food safety evaluation.

**References**

Auro, A., Sumano, H., Ocampo, L., & Barragán, A. (2004). Evaluation of the carcinogenic effects of furazolidone and its metabolites in two fish species. *Pharmacogenomics Journal, 4*, 24–28.

Barbosa, J., Freitas, A., Moura, S., Mourão, J. L., Noronha da Silveira, M. I., & Ramos, F. (2011). Detection, accumulation, distribution, and depletion of furaltadone and nifursol residues in poultry muscle, liver, and gizzard. *Journal of Agricultural and Food Chemistry, 59*, 11927–11934.

Chang, C., Peng, D. P., Wu, J. E., Wang, Y. L., & Yuan, Z. H. (2008). Development of an indirect competitive ELISA for the detection of furazolidone marker residue in animal edible tissues. *Journal of Agricultural and Food Chemistry, 56*, 1525–1531.

Cheng, C. C., Hsieh, K. H., Lei, Y. C., Tai, Y. T., Chang, T. H., Sheu, S. Y., … Kuo, T. F. (2009). Development and residue screening of the furazolidone metabolite, 3-amino-2-oxazolidinone (AOZ), in cultured fish by an enzyme-linked immunosorbent assay. *Journal of Agricultural and Food Chemistry, 57*, 5687–5692.

Chen, X. J., Liu, L. Q., Kuang, H., Song, S. S., & Xu, C. L. (2013). A strip-based immunoassay for rapid determination of fenpropathrin. *Analytical Methods, 5*, 6234–6239.
Conneely, A., Nugent, A., & O’Keeffe, M. (2002). Use of solid phase extraction for the isolation and clean-up of a derivatised furazolidone metabolite from animal tissues. Analyst, 127, 705–709.

Cooper, K. M., Caddell, A., Elliott, C. T., & Kennedy, D. G. (2004). Production and characterisation of polyclonal antibodies to a derivative of 3-amino-2-oxazolidinone, a metabolite of the nitrofuran furazolidone. Analytica Chimica Acta, 520, 79–86.

Cooper, K. M., Mulder, P. P. J., van Rhijn, J. A., Kovacsics, L., McCracken, R. J., Young, P. B., & Kennedy, D. G. (2005). Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV. Food Additives and Contaminants, 22, 406–414.

Diblikova, I., Cooper, K. M., Kennedy, D. G., & Franek, M. (2005). Monoclonal antibody-based ELISA for the quantification of nitrofuran metabolite 3-amino-2-oxazolidinone in tissues using a simplified sample preparation. Analytica Chimica Acta, 540, 285–292.

Franek, M., Diblikova, I., Vass, M., Kotkova, L., Stastny, K., Fragalova, K., & Hruska, K. (2006). Validation of a monoclonal antibody-based ELISA for the quantification of the furazolidone metabolite (AOZ) in eggs using various sample preparation. Veterinarni Medicina, 51, 248–257.

Gu, H. Y., Liu, L. Q., Song, S. S., Kuang, H., & Xu, C. L. (2016). Development of an immunochromatographic strip assay for ractopamine detection using an ultrasensitive monoclonal antibody. Food and Agricultural Immunology, 27, 471–483.

Gu, H. Y., Liu, L. Q., Song, S. S., Kuang, H., & Xu, C. L. (2015). Development of a monoclonal antibody-based immunochromatographic strip for cephalixin. Food and Agricultural Immunology, 26, 282–292.

Hu, X. Z., Xu, Y., & Yediler, A. (2007). Determinations of residual furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in fish feeds by HPLC-UV and LC-MS/MS, respectively. Journal of Agricultural and Food Chemistry, 55, 1144–1149.

Jester, E. L. E., Abraham, A., Wang, Y. S., El Said, K. R., & Plakas, S. M. (2014). Performance evaluation of commercial ELISA kits for screening of furazolidone and furaltadone residues in fish. Food Chemistry, 145, 593–598.

Kelly, B. D., Heneghan, M. A., Connolly, C. E., & O’Gorman, T. A. (1998). Nitrofurantoin-induced hepatotoxicity mediated by CD8+ T cells. American Journal of Gastroenterology, 93, 819–821.

Kong, D., Liu, L. Q., Song, S., Kuang, H., & Xu, C. (2016). Development of an immunochromatographic strip for the semi-quantitative and quantitative detection of biotin in milk and milk products. Analytical Methods, 8, 1595–1601.

Le, T., & Yu, H. (2015). Determination of 3-amino-2-oxazolidinone in animal tissue by an enzyme-linked immunosorbent assay and a time-resolved fluoroimmunaoassay. Analytical Letters, 48, 1275–1284.

Li, Z. H., Li, Z. M., & Xu, D. K. (2017). Simultaneous detection of four nitrofuran metabolites in honey by using a visualized microarray screen assay. Food Chemistry, 221, 1813–1821.

Lin, F., Song, S. S., Liu, L. Q., Kuang, H., Wang, L. B., & Xu, C. L. (2011). Development of the detection of benzophenone in recycled paper packaging materials by ELISA. Food and Agricultural Immunology, 22, 39–46.

Liu, Y., Peng, D. P., Huang, L. L., Wang, Y. L., Chang, C., Ihsan, A., … Yuan, Z. H. (2010). Application of a modified enzyme-linked immunosorbent assay for 3-amino-2-oxazolidinone residue in aquatic animals. Analytica Chimica Acta, 664, 151–157.

Luo, P. J., Jiang, W. X., Beier, R. C., Shen, J. Z., Jiang, H. Y., Miao, H., … Wu, Y. N. (2012). Development of an enzyme-linked immunosorbent assay for determination of the furaltadone etabolite, 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) in animal tissues. Biomedical and Environmental Sciences, 25, 449–457.

McCracken, R. J., & Kennedy, D. G. (1997). Determination of the furazolidone metabolite, 3-amino-2-oxazolidinone, in porcine tissues using liquid chromatography-thermospray mass spectrometry and the occurrence of residues in pigs produced in northern Ireland. Journal of Chromatography B, 691, 87–94.

Peng, C. F., Chen, Y. W., Chen, W., Xu, C. L., Kim, J. M., & Jin, Z. Y. (2008). Development of a sensitive heterologous ELISA method for analysis of acetylgestagen residues in animal fat. Food Chemistry, 109, 647–653.
Peng, S., Song, S. S., Liu, L. Q., Kuang, H., & Xu, C. L. (2016). Rapid enzyme-linked immunosorbent assay and immunochromatographic strip for detecting ribavirin in chicken muscles. *Food and Agricultural Immunology*, 27, 449–459.

Pimpitak, U., Putong, S., Komolpis, K., Petsom, A., & Palaga, T. (2009). Development of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of the furaltadone metabolite, AMOZ, in fortified shrimp samples. *Food Chemistry*, 116, 785–791.

Sun, F. X., Liu, L. Q., Ma, W. W., Xu, C. L., Wang, L. B., & Kuang, H. (2012). Rapid on-site determination of melamine in raw milk by an immunochromatographic strip. *International Journal of Food Science and Technology*, 47, 1505–1510.

Suryoprabowo, S., Liu, L. Q., Peng, J., Kuang, H., & Xu, C. L. (2014). Development of a broad specific monoclonal antibody for fluoroquinolone analysis. *Food Analytical Methods*, 7, 2163–2168.

Vass, M., Kotkova, L., Diblikova, I., Nevorankova, Z., Cooper, K. M., Kennedy, D. G., & Franek, M. (2005). Production and characterisation of monoclonal antibodies for the detection of AOZ, a tissue bound metabolite of furazolidone. *Veterinarini Medicina*, 50, 300–310.

Wang, W. B., Feng, M., Kong, D. Z., Liu, L. Q., Song, S. S., & Xu, C. L. (2015). Development of an immunochromatographic strip for the rapid detection of pseudomonas syringae pv. Maculicola in broccoli and radish seeds. *Food and Agricultural Immunology*, 26, 738–745.

Xu, N. F., Xu, L. G., Ma, W., Liu, L. Q., Kuang, H., & Xu, C. L. (2015). An ultrasensitive immunochromatographic assay for non-pretreatment monitoring of chloramphenicol in raw milk. *Food and Agricultural Immunology*, 26, 635–644.

Yang, X. D., Zhang, G. P., Wang, F. Y., Wang, Y. B., Hu, X. F., Li, Q. M., … Zeng, X. Y. (2015). Development of a colloidal gold-based strip test for the detection of chlorothalonil residues in cucumber. *Food and Agricultural Immunology*, 26, 729–737.

Yan, H. J., Liu, L. Q., Xu, N. F., Kuang, H., & Xu, C. L. (2015). Development of an immunoassay for carbendazim based on a class-selective monoclonal antibody. *Food and Agricultural Immunology*, 26, 659–670.

Zhang, X., Feng, M., Liu, L. Q., Xing, C. R., Kuang, H., Peng, C. F., … Xu, C. L. (2013). Detection of aflatoxins in tea samples based on a class-specific monoclonal antibody. *International Journal of Food Science and Technology*, 48, 1269–1274.

Zhang, Y. B., Qiao, H. O., Chen, C., Wang, Z. L., & Xia, X. D. (2016). Determination of nitrofuran metabolites residues in aquatic products by ultra-performance liquid chromatography-tandem mass spectrometry. *Food Chemistry*, 192, 612–617.