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Rapid and Sensitive Detection of 3-Amino-2-oxazolidinone Using a Quantum Dot-Based Immunochromatographic Fluorescent Biosensor

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ABSTRACT: To monitor the levels of furazolidone in edible animal tissues, a fluorescent sensor was developed for the determination of 3-amino-2-oxazolidinone (AOZ), the metabolite of furazolidone, featuring an immunochromatographic test strip assay (ITSA) integrated with a quantum dot (QD) label. The optimal QD-based ITSA sensor exhibits good dynamic linear detection for AOZ over the range of 0.1−100 μg/L, with a 50% inhibitory concentration (IC_{50}) of 1.06 μg/L. The decision limit and the detection capability were 0.14−0.15 and 0.27−0.33 μg/kg, respectively, for this analyte using the QD-based ITSA sensor. These values represent an improvement over a previously reported gold nanoparticle-based immunochromatographic assay. The recoveries of AOZ in kinds of animal tissues were between 76.3 and 98.4% at the levels of 1.0, 5.0, and 10.0 μg/kg. The performance and practicality of our QD-based fluorescent immunosensor were confirmed by commercial ELISA kit and LC-MS/MS. In conclusion, the proposed sensor was a feasible detection method for AOZ analysis on site.

KEYWORDS: *furazolidone, 3-amino-2-oxazolidinone, immunochromatographic test strip, quantum dot, biosensor, animal tissue*

■ INTRODUCTION

Furazolidone, nitrofurantoin, furaltadone, and nitrofurazone (Figure 1) are antibiotics of the nitrofuran family, a group of broad-spectrum antibiotics that are widely employed to treat gastrointestinal and dermatological infections in animal husbandry.[1,2] Residual nitrofurans and their metabolites are serious threats to human and animal health, such as potential carcinogenic and mutagenic effects and bacterial resistances.[3,4] As a consequence, nitrofurans have been prohibited for use in food-producing animals in the European Union (EU), China, the United States, and other countries.[3,5−7] For instance, the minimum required performance limits (MRPLs) for each nitrofuran metabolite in food of animal origin has been set at 1 μg/kg in the EU.[8,9] Nitrofuran antibiotics are quite unstable in vivo and are rapidly metabolized and do not persist as a residue of the parent drug in animal tissues.[10] The nitrofuran metabolites can bind covalently to cellular proteins and may persist in animal tissues for weeks. These metabolites are 3-amino-2-oxazolidinone (AOZ), 1-amino-hydantoin, 3-amino-5-morpolinomethyl-2-oxazolidone, and semicarbazide derived from furazolidone, nitrofurantoin, furaltadone, and nitrofurazone, respectively.[4,11] AOZ can be used as a marker for monitoring the levels of furazolidone in edible animal tissues. The AOZ metabolite is bound covalently to proteins and must be released from the cellular proteins under mildly acidic conditions and derivatized with *o*-nitrobenzaldehyde in vitro to form the nitrophenylAOZ.[12]

Various instrumental reference and screening methods, including liquid chromatography−mass spectrometry and liquid chromatography−tandem mass spectrometry (LC-MS/MS), for the determination of furazolidone and AOZ have since been developed.[13−17] These analytical methods are sensitive and accurate. However, costly equipment, skilled personnel, and complex sample pretreatment procedures are involved in the methods mentioned above. In the past decade, a few enzyme-linked immunosorbent assays (ELISAs) have been reported for the determination of furazolidone and their metabolites.[10,12,18−22] ELISA offers advantages of simplicity and high-throughput screen ability. However, ELISA devices are expensive, and the procedure involves multiple steps of complicated solution addition and plate washing, requiring at least 2 h to obtain results.

Recently, a gold nanoparticle-based immunochromatographic test strip assay (ITSA) has been published for the detection of metabolites of nitrofurans. Compared to other methods, ITSA offers great potential with no instrument requirements, a simple procedure, immediate results, and low cost.[2,23−25] However, fluorophore-gold nanoparticles have intrinsic limitations such as unstable fluorescence intensity and photobleaching, which compromise their sensitivity and limit their potential applications. Compared with fluorophore-gold nanoparticles, quantum dots (QDs) feature bright narrow fluorescent emission spectra, can be excited across a broad range of UV wavelengths with large optical absorption coefficients, and provide high fluorescence quantum yields, excellent stability, and biocompatibility.[22,26] Moreover, a QD-based ITSA integrated with a fluorescence reader could provide a simple, rapid, accurate, sensitive, and inexpensive field-monitoring tool. However, the use of a QD-based fluorescent

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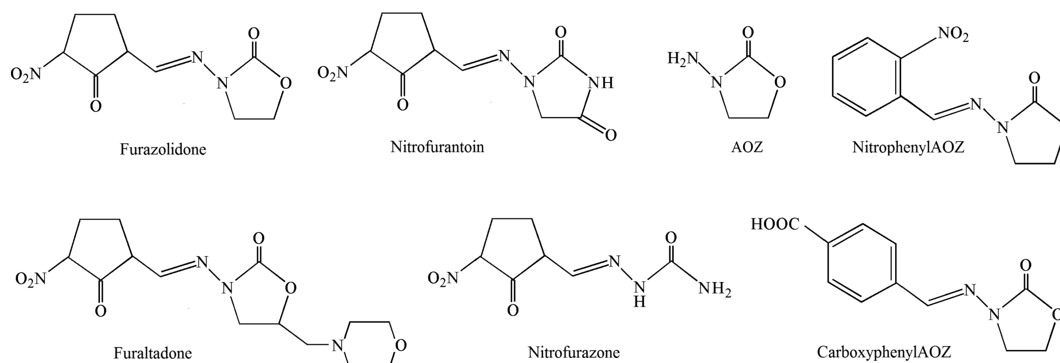

Figure 1. Chemical structures of nitrofuran parent drug, furazolidone metabolite residue AOZ, the former derivative carboxyphenylAOZ, and the target analyte nitrophenylAOZ.

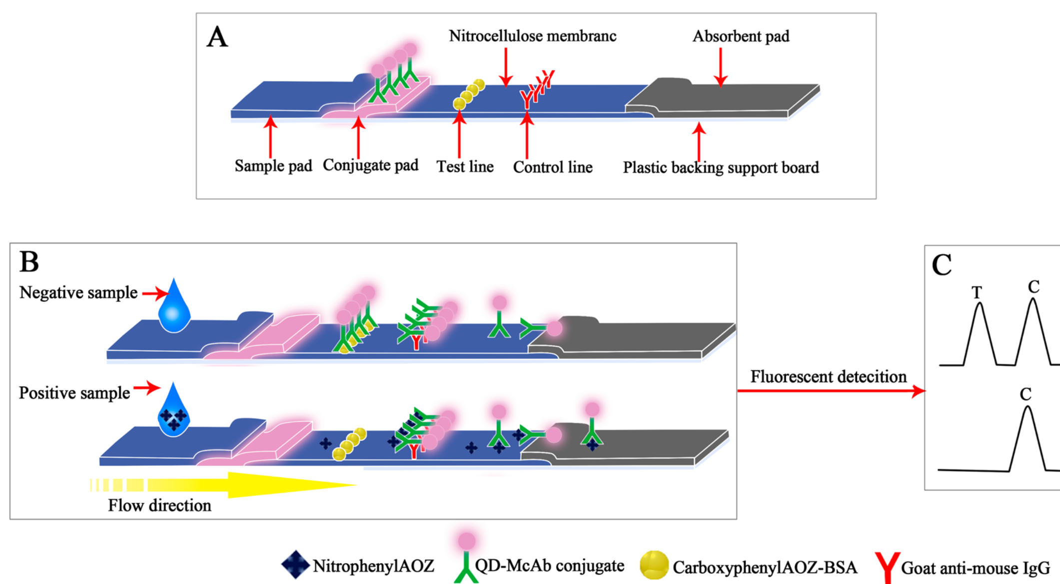

Figure 2. Schematic illustration of fluorescent ITSA detection of AOZ: (A) typical assembly of the fluorescent ITSA; (B) negative tests show two lines, and positive tests show only one line (the control line); (C) negative and positive tests displayed by the strip reader.

ITSA for the detection of AOZ has not been previously reported.

In this study, we developed a QD-based fluorescent ITSA for the simple, sensitive, specific, and quantitative detection of AOZ in tissue samples based on a monoclonal antibody (McAb) against nitrophenylAOZ.[22] Our developed sensor displayed high sensitivity, simplicity, low cost, accuracy, precision, and stability for the determination of AOZ in edible animal tissues.

MATERIALS AND METHODS

Materials and Reagents. Furazolidone, AOZ, nitrophenylAOZ, and *o*-nitrobenzaldehyde were purchased from WITEGA Laboratorien Berlin-Adlershof GmbH (Berlin, Germany). Anti-nitrophenylAOZ McAb, carboxyphenylAOZ, and carboxyphenylAOZ–bovine serum albumin (carboxyphenylAOZ-BSA) conjugates were produced in our laboratory.[27] Goat anti-mouse IgG antibodies were obtained from Sino-American Biotechnology (Shanghai, Branch, China). QD was purchased from Invitrogen Corp. (Carlsbad, CA, USA). A Ridascreen nitrophenylAOZ kit was purchased from R-Biopharm AG (R-Biopharm, Darmstadt, Germany). Sample pads, glass fibers, absorbent pads, and nitrocellulose membranes were purchased from Millipore Corp. (Billerica, MA, USA). Amicon Ultra (100 kDa) ultra-centrifugation tubes were from Millipore (Bedford, MA, USA). All other chemicals were of analytical grade or better.

Apparatus. The BioDot TSR3000 membrane strip reader, CM4000 programmable strip cutter, and BioDot XYZ Platform combining motion control with an AirJet Quanti3000 dispenser were all purchased from Bio-Dot (Richmond, CA, USA). An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a Quattro Premier XE tandem quadrupole mass spectrometer was obtained from Waters (Manchester, UK).

Synthesis of QD-McAb Conjugates. QD-McAb conjugates were prepared using a previously reported method.[28] Briefly, anti-nitrophenylAOZ McAb was dialyzed and dissolved in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Fifty microliters of QDs (8 μM) and 100 μL of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (0.8 mM) were stirred for 10 min at room temperature, and then 100 μL of *N*-hydroxysulfosuccinimide (0.8 mM) was added. Then 600 μL of McAb (0.2 mg/mL) was added, and the mixture was reacted for 1.5 h at room temperature. The mixture was centrifuged at 10,000 rpm for 15 min at room temperature, and then the precipitates were resuspended with 0.1 M PBS (pH 7.4). The resuspended conjugate compound was then stored in a refrigerator at 4 °C for further experiments.

ITSA Sensor Preparation. The fabrication and function of the ITSA sensor are shown in Figure 2. The AOZ competitive assay test strip consisted of a blotted nitrocellulose membrane, sample pad, conjugate pad, and absorbent pad. The sample pads (300 × 15 mm) were treated with a buffer (pH 8.0) containing 20 mM sodium borate, 2.0% (w/v) sucrose, 2.0% (w/v) BSA, and 0.1% (w/v) NaN_3. Then it

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was dried in a nitrogen box for 1 h and stored at 4 °C. The conjugate pad was made by dispensing the QD-labeled McAbs (20 nM) onto a 300 × 7 mm glass fiber (Millipore) at a speed of 2 μL/cm using a BioDot XYZ dispensing platform and then dried for 1 h at 56 °C. As shown in Figure 2, on the nitrocellulose membrane, 0.4 mg/mL of carboxyphenylAOZ-BSA and 1 mg/mL of goat anti-mouse IgG dissolved in 50 mM phosphate buffer (pH 7.6) were dispensed as test and control lines using the dispensing platform at 4 μL/cm. Later, the nitrocellulose membrane was dried at room temperature. Subsequently, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were laminated onto a backing card, in 1−2 mm overlapping positions. Then, the assembled boards was cut into 3 mm wide pieces using a CM4000 programmable strip cutter. The strip products were mounted in plastic cassettes with desiccant and stored at room temperature until used.

Sample Preparation. Extraction procedures for sample were characterized according to the procedure described in the literature.[10] Briefly, muscle tissues from chicken, pork, carp, and shrimp were homogenized, and the homogenate was distributed (1.0 g each) into 10 mL microcentrifuge tubes. Then the samples were spiked with AOZ standard solution, and the mixtures were vortexed for 10 s at room temperature and processed for 10 min in a boiling water bath. After cooling to room temperature, 150 μL of HCl (5 M) and 25 μL of *o*-nitrobenzaldehyde (50 mM) in dimethyl sulfoxide were added to the samples, and each tube was incubated at 37 °C overnight. Then samples were extracted by mixing with 5 mL of K_2HPO_4 (0.1 M), 0.35 mL of NaOH (1 M), and 5 mL of ethyl acetate and vortexed thoroughly for 20 s. Samples were centrifuged at 5000 rpm at 4 °C for 20 min. The ethyl acetate extracts were collected and dried under a stream of nitrogen. The evaporates were dissolved in 2 mL of hexane and 1 mL of PBS (0.1 M, pH 7.4). The mixture was centrifuged at 4000 rpm for 5 min. The remaining evaporates were redissolved in 1 mL of PBS and analyzed with a fluorescent immunosensor and ELISA kit.

Quantitative Procedure and Sensor Calibration Standard. Stock nitrophenylAOZ solution (50 μL; 1 μg/L in dimethyl sulfoxide) was dissolved in 21.56 mL of PBS to obtain a nitrophenylAOZ concentration of 2314 μg/L. A 10 μL nitrophenylAOZ standard solution was added to 990 μL of matrix supernatant to obtain an immunosensor calibration standard having a concentration of 23.14 μg/L. The matrix supernatant was spiked with nitrophenylAOZ standard solution to obtain immunosensor standards in matrix-matched concentration standards of 0.02, 0.07, 0.23, 0.69, 2.31, 6.94, 23.14, 69.42, and 231.4 μg/L. After correcting for the increases in mass arising from derivatization with *o*-nitrobenzaldehyde using the coefficient 2.314, the concentrations (as AOZ equivalents) were 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μg/L.

Properties of ITSA. The fluorescence signals of the test line were measured with a strip reader. The standard curve was established by plotting $F/F_0 \times 100\%$ against the logarithm of the AOZ concentration, where F and F_0 represent fluorescence intensity and were referred to as the positive and negetive samples, respectively. The 50% inhibitory concentration (IC_{50}) was determined as a measure of the sensitivity of the ITSA sensor.

To evaluate the performance of the fluorescent ITSA, important parameters such as the decision limit (CCα) and the detection capability (CCβ) at different false-positive rates, coefficients of variation, and percent recovery were determined. Animal tissues were assayed using fluorescent ITSA to determine the CCα and CCβ. The CCα was established by analyzing 20 blank tissue samples, accepting no false-positive rates, with an average plus 3 times the standard deviation (SD). The CCβ was determined by analyzing the above-mentioned 20 blank samples, with an average plus 10 times the SD. The recoveries were measured using the following equation: (concentration measured/concentration spiked) × 100. By analysis of the above-mentioned samples, intra-assay and interassay coefficients of variation (CVs) for ITSA procedures were determined. Samples were spiked with AOZ at levels of 1.0, 5.0, and 10.0 μg/kg, with five replicates at each level. The samples were analyzed on five different days with the same instrument and operator. The visual detection limits of the ITSA were determined as the antibiotic concentration when the test line disappeared.

Comparison of ITSA with ELISA Kit and LC-MS/MS for Spiked Sample Analysis. To further demonstrate the capability and accuracy of the fluorescent ITSA, a side-by-side comparison between fluorescent ITSA, a commercial nitrophenylAOZ ELISA kit (Ridascreen Nitrofuran (AOZ) kit),and LC-MS/MS was conducted using the same spiked tissue samples. Five spiked tissue samples with AOZ, including carp (spiked at 150 and 300 μg/kg), beef (spiked at 10 and 60 μg/kg), shrimp (spiked at 100 and 250 μg/kg), chicken (spiked at 80 and 200 μg/kg), and pork (spiked at 20 and 180 μg/kg) were performed simultaneously by ITSA, ELISA kit, and LC-MS/MS analyses, and the result were compared. Sample pretreatment for the nitrophenylAOZ ELISA kit was performed according to the manufacturer's instructions. The LC-MS/MS procedure is described in the literature.[16]

Animal Experiments. To prove the advantage of the fluorescent ITSA, the incurred tissue samples from an animal feeding experiment were analyzed for AOZ residues using fluorescent ITSA, ELISA kit, and LC-MS/MS, and the measured results were compared. Twenty-four carp weighing 250 ± 10 g were randomly collected from a fish farm in Chongqing, China, and were maintained in the laboratory for a period of 2 weeks for them to acclimatize before the experiment started. The carp were divided into control (n = 6) and test groups (n = 18). For 7 consecutive days, the test group was treated with furazolidone, contained in pelleted dry feed, at a dose of 100 mg/kg body weight per day. The control group was maintained without furazolidone treatment. Three carp from the test group and one from the control group were sacrificed at each withdrawal period (0, 4, 7, 14, 21, and 28 days). The carp muscle and liver samples were collected separately for analyses by ITSA, ELISA kit, and LC-MS/MS.

RESULTS AND DISCUSSION

Principle and Evaluation of Fluorescent ITSA. The principle of fluorescent ITSA in this study is illustrated in Figure 2. It is based on the integration competitive reaction of antibody−antigen and the signal intensity of QD. When the standard solution or sample extract is added to the sample pad, the QD-labeled antibodies dissolve and move to the membrane by capillary action. When the concentrations of AOZ target analyte in the sample were below the threshold, the free QD-McAbs would be trapped by immobilized carboxyphenylAOZ-BSA conjugates in the test line, and the QDs emit a clear fluorescent signal on the test line.The free QD-McAbs are subsequently captured by the specific secondary goat anti-mouse IgG at the control line. Consequently, the development of two colored fluorescent lines indicates a negative result. When the concentrations of AOZ in the sample exceeded the threshold, the QD-McAbs immobilized on the conjugate pad will be all captured, preventing their binding in the test line and leading to a lower fluorescent signal at the test line, indicating that the sample is positive for AOZ residues. Thus, the degree of intensity of the QD-McAb of the test line was the reverse of the concentration of AOZ. If there is no fluorescent signal at the control line, the test strip is invalidated. Using the strip reader, the fluorescence signal intensity was measured for AOZ quantitative analysis.

Determination of Working Concentrations of Captured Reagents for Immobilization. CarboxyphenylAOZ-BSA and QD-McAb were diluted with 50 mM PBS at pH 7.6. The various working concentrations were then dispensed onto a nitrocellulose membrane for assembly of the fluorescent ITSA. The fluorescent ITSA test system operates on the principal of competitive binding to the QD-labeled antibodies between AOZ in the sample and the carboxyphenylAOZ-BSA conjugates immobilized on the surface of nitrocellulose

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Figure 3. Series of diluted derivative solutions tested by fluorescent ITSA for nitrophenylAOZ in buffer and visual results examined after 10 min. Concentrations are expressed as equivalent underivatized AOZ. Sample was spiked with AOZ at levels of 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μg/L.

membranethe. Hence, the fluorescence signal intensities of the corresponding test lines gradually decreased to nothing as the analyte concentration in the sample increased. An ideal test system should have a well-defined minimum limit of detection and allow precision. We found that the optimal application of carboxyphenylAOZ-BSA to nitrocellulose membrane was 4 mL/cm at a concentration of 0.4 mg/mL diluted with 50 mM phosphate buffer (pH 7.6) solution. The optimum concentration of the QD-McAb conjugate was 20 nM. These optimum concentrations can obtain a wide linear portion in the response curve and be fixed for the rest of the experiment.

Sensor Performance. In this study, a fluorescent ITSA method was developed for the determination of AOZ residue in tissue samples. The AOZ residue was derivatized into nitrophenylAOZ for the detection. For detection of AOZ by ITSA, diluted derivatized solutions of AOZ, at final concentrations of 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μg/L, were tested (Figure 3). At 10 min after the additionn of a sample solution to the sample pad, the fluorescent color could be visualized by the naked eye. As shown in Figure 3, upon the addition of higher concentrations of AOZ, the fluorescence of the test lines gradually decreased. The fluorescent color of the test line became similar to or weaker than that of the control line for a negative sample, becoming obviously weaker at 3 μg/L. The fluorescent color of the test line disappeared in the presence of >10 μg/L AOZ (Figure 3). Therefore, under the optimized detection conditions, the visual detection limit of the strip to AOZ was 10 μg/L. At concentrations >10 μg/L, the weaker fluorescent signal of the test line could be measured using the strip reader.

Detection of the fluorescence intensity of the test line was performed using a strip reader. The sensitivity of the fluorescent ITSA for AOZ was expressed by an IC_{50} value. A low IC_{50} value indicated higher assay sensitivity. The standard curves plotted by fluorescent ITSA analysis (Figure 4) showed that the working concentration was in the range of 0.01−100 μg/L. The IC_{50} value of the method for AOZ was 1.06 μg/L in the linear range of 0.01−100 μg/L with acceptable correlation coefficients (R^2 = 0.9621, y = −25.897x + 50.626). The respective intra- and interassay CVs were 3.1−6.3%. The sensitivity of the fluorescent ITSA was compared with that of the conventional ELISA method, which was reliable.[10,18,22]

The animal tissue samples (including pork, chicken, carp, and shrimp), collected from local retail stores, were analyzed by fluorescent ITSA. On the basis of the results from 20 blank samples, the CCα of the ITSA for muscles and livers of pork, chicken, carp, and shrimp samples was 0.14−0.15 μg/kg, and the CCβ was 0.27−0.33 μg/kg. Table 1 compares the accuracy and precision of the fluorescent ITSA obtained in blank samples spiked with AOZ, at levels of 1.0, 5.0, and 10.0 μg/kg. The mean recovery values range from 76.3 to 98.4%, with CV values of <15% for spiked samples, which were within an acceptable levels.

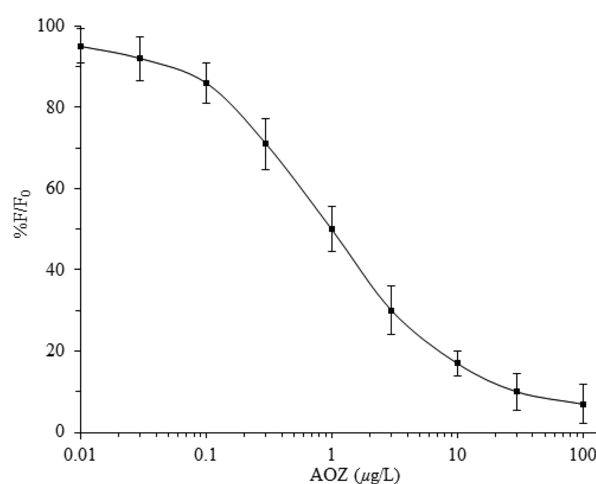

Figure 4. Standard curve of the immunosensor for nitrophenylAOZ in buffer. Concentrations are expressed as equivalent underivatized AOZ. Normalized signals expressed as $100(F/F_0)$ (where F and F_0 are the peak fluorescence signal intensity obtained with the AOZ analyte and the blank sample, respectively) were plotted versus the logarithm of AOZ concentration. The bars show the standard deviation for nine replicate assays performed over 3 months.

Measurements were carried out using standard assay protocols for the ITSA methods. The CCα (0.14−0.15 μg/kg) measured for our ITSA method is lower than the MRPL set by the EU.[8]

Spiked Sample Analysis and Comparison of the ITSA with Commercial ELISA Kit and LC-MS/MS. Five food samples were analyzed by fluorescent ITSA, commercial ELISA kit, and LC-MS/MS. As shown by a side-by-side comparison, there was excellent correlation between ITSA and ELISA kit (y = 0.8919x + 2.4916, R^2 = 0.9932) and ITSA and LC-MS/MS (y = 0.9628x − 0.5479, R^2 = 0.9976). Thus, there were no major differences among the three methods. These results showed that our fluorescent immunosensor was convenient, sensitive,

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Table 1. Recovery and Coefficients of Variation for AOZ Determination from Spiked Animal Muscle Tissue Samples by the QD-Based Fluorescent ITSA (n = 5)

sample	spiked (μg/kg)	mean ± SD (μg/kg)	recovery (%)	CV (%)
chicken	1.0	0.76 ± 0.08	76.3	10.5
	5.0	4.38 ± 0.56	87.6	12.8
	10.0	8.17 ± 0.89	81.7	10.9
pork	1.0	0.91 ± 0.08	90.8	8.8
	5.0	4.66 ± 0.58	93.2	12.4
	10.0	9.42 ± 0.87	94.2	9.2
carp	1.0	0.92 ± 0.09	90.1	9.8
	5.0	4.53 ± 0.48	90.6	10.6
	10.0	8.86 ± 0.89	88.6	10.0
shrimp	1.0	0.77 ± 0.08	77.2	10.4
	5.0	4.92 ± 0.65	98.4	13.2
	10.0	8.42 ± 0.94	84.2	11.2

and accurate and suitable for the qualitative and quantitative determination of AOZ residue in animal tissues.

Analysis of Animal Experiment Sample by ITSA, Commercial ELISA Kit, and LC-MS/MS. The results of fluorescent ITSA, commercial ELISA kit, and LC-MS/MS analyses are compared in Table 2, which demonstrates the performance of our fluorescent ITSA technique compared with the ELISA kit and LC-MS/MS methods for AOZ in muscle and liver tissues of carp. According to these data, 0−28 days of withdrawal were required for the drug to reach a concentration below the MRPLs for the tissue-bound residues of AOZ at 1 μg/kg in the EU. Table 2 shows excellent correlation between ITSA and ELISA kit (R^2 = 0.9965) and ITSA and LC-MS/MS (R^2 = 0.9979) results in a side-by-side comparison. The values measured by fluorescent ITSA were higher than those of the ELISA kit and were much closer to the values measured by LC-MS/MS. These data also demonstrate that the detection ability of our immunosensor was as good as that of the ELISA kit. Our results also demonstrate that fluorescent ITSA is reliable compared with the ELISA kit and LC-MS/MS methods for the detection of AOZ residue.

Table 2. Result Analyses of Fluorescent ICTA, ELISA Kit, and LC-MS/MS for the Detection of AOZ in Carp Muscle and Liver Samples from Animal Experiments (n = 3)[a]

		AOZ concentration (μg/kg)		
matrix	withdrawal (days)	ITSA	ELISA kit	LC-MS/MS
carp muscle	0	241.2	286.2	265.7
	4	118.9	163.4	133.8
	7	60.1	85.4	76.5
	14	31.2	47.2	41.2
	21	14.9	22.3	17.6
	28	4.7	7.2	5.4
carp liver	0	420.5	476.8	458.6
	4	293.5	326.5	316.5
	7	178.6	207.8	198.3
	14	64.7	89.6	77.5
	21	25.7	37.3	31.2
	28	11.5	15.6	12.3

[a]Results are presented as means (n = 3). Equation curves and correlation coeficients were as follows: ITSA/ELISA kit (carp muscle, $y = 0.8419x - 7.3299$, $R^2 = 0.9937$); ITSA/LC-MS/MS (carp muscle, $y = 0.9188x - 3.634$, $R^2 = 0.9955$); ITSA/ELISA kit (carp liver, $y = 0.9041x - 8.0759$, $R^2 = 0.9989$); ITSA/LC-MS/MS (carp liver, $y = 0.9764x - 5.3243$, $R^2 = 0.9997$).

In summary, QD-based ITSA provides a simple, effective, and quantitative tool for AOZ residue detection. Under optimal conditions, this sensor is capable of detecting a minimum of 0.04−0.15 μg/kg AOZ standard analyte in 10 min. The results of CCα, CCβ, accuracy, and precision are consistent with European Commission MRPLs (2002). The proposed immunosensor showed a good agreement with a confirmatory ELISA kit and LC-MS/MS method. Overall, this study demonstrated that our fluorescent immunosensor provides a simple, sensitive, rapid, cost-effective, and convenient way to detect AOZ in food samples.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AOZ, 3-amino-2-oxazolidinone; ITSA, immunochromatographic test strip assay; QD, quantum dot; IC_{50}, 50% inhibitory concentration; CCα, decision limit; CCβ, detection capability; MRPLs, minimum required performance limits; LC-MS/MS, liquid chromatography−tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; McAb, monoclonal antibody; carboxyphenylAOZ-BSA, carboxyphenylAOZ−bovine serum albumin; PBS, phosphate-buffered saline

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