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Immunochromatographic Assay for Simultaneous and Quantitative Detection of 3-Methyl-Quinoxaline-2-Carboxylic Acid and Quinoxaline-2-Carboxylic Acid Residues in Animal Tissues Based on Highly Luminescent Quantum Dot Beads

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Abstract Due to their potential adverse effects on human health, the use of carbadox and olaquindox in feedingstuffs was prohibited by the European Union since 1998. In this work, highly luminescent quantum dot beads (QBs) were synthesized by encapsulating CdSe/ZnS and used as novel fluorescent probes in the immunochromatographic assay (ICA) for simultaneous and quantitative determination of metabolites of olaquindox (3-methylquinoxaline-2-carboxylic acid, MQCA) and carbadox (quinoxaline-2-carboxylic, QCA). The fluorescence intensities of the test lines were recorded using a fluorescence strip reader. The 50% of inhibition for MQCA and QCA was shown to be 8.1 and 10.6 μg L^{-1}, respectively. The whole assay process can be accomplished within 10 min. The immunosensor was used to analyze spiked samples, and analyte intra- and inter-assay recovery rates ranged from 78.7 to 92.2% for MQCA and 80.6 to 95.8% for QCA, and coefficients of variation were all below 12%. The incurred tissues samples were assayed using both QB-based ICA and commercial ELISA kit and were further confirmed with LC-MS/MS. The QB-based ICA results exhibited good agreement with both commercial ELISA and LC-MS/MS methods.

Keywords Immunochromatographic assay · Fluorescent sensor · Quinoxaline-2-carboxylic acid · 3-methyl-quinoxaline-2-carboxylic acid · Quantitative detection

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Introduction

Olaquindox (*N*-(2-hydroxyethyl)-3-methyl-2-quinoxaline-carboxamide-1,4-dioxide) and carbadox (methyl-3-(2-quinoxalinylmethylene)-carbazate-N^1,N^4-dioxide) are synthetic antibacterial drugs, and they were widely used as growth promoters in animal diets and assisted in the prevention of dysentery and/or bacterial enteritis (Jiang et al. 2013; Joint Expert Committee on Food Additives 1991; Wu et al. 2007). Olaquindox and carbadox can be rapidly metabolized into different kinds of metabolites in vivo, such as olaquindox to 3-methylquinoxaline-2-carboxylic acid (MQCA) and carbadox to desoxycarbadox, as well as quinoxaline-2-carboxylic (QCA); the structure of these drugs are depicted in Fig. 1 (Li et al. 2016; Liu et al. 2010; Sin et al. 2004). However, in 1998, due to potential adverse effects on human health, the European Union banned the use of carbadox in food-producing animals (Dibai et al. 2015; Joint Expert Committee on Food Additives 2003). In China, the maximum residue limits (MRLs) for olaquindox in the porcine liver are set at 50 μg kg^{-1} and in the porcine muscle at 4 μg kg^{-1} (Kim et al. 2015). In 2003, the Joint FAO/WHO Expert Committee on Food Additives recommended MRLs and acceptable daily intake for those drugs (Dibai et al. 2015; Sniegocki et al. 2014).

By now, there have been many methods reported to determine residues of MQCA and QCA residues in various animal-

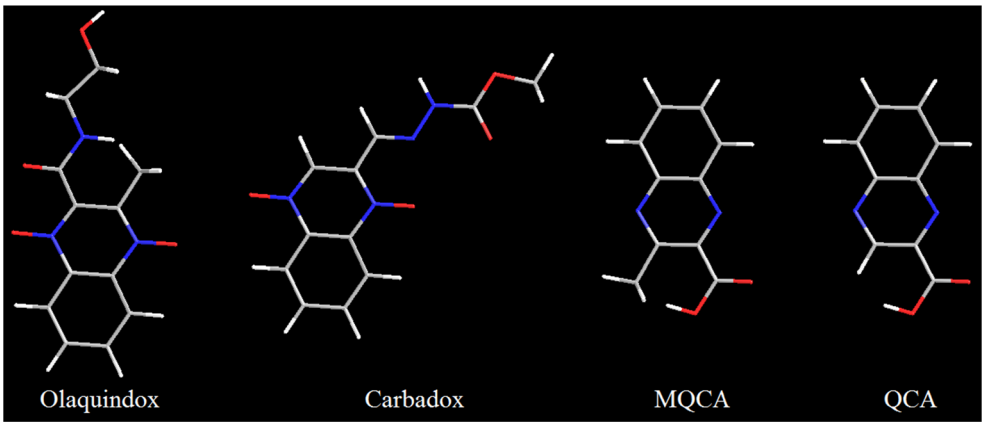

Fig. 1 Chemical structures of olaquindox, carbadox, MQCA, and QCA

derived foods, including liquid chromatography-mass spectrometry (Boison et al. 2009; Hutchinson et al. 2005; Sniegocki et al. 2014; Peng et al. 2017), high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Dibai et al. 2015), high-performance liquid chromatography (Duan et al. 2013; Huang et al. 2008; Wu et al. 2007; Zhang et al. 2005), and gas chromatography-mass spectrometry (Lynch et al. 1991). However, these instrument-based analytical methods rely heavily on complex complicated sample preparation processes, skilled personnel, and expensive equipment.

Recently, many immunochemical methods, including time-resolved fluoroimmunoassay and enzyme-linked immunosorbent assay (ELISA), have been developed for the determination of carbadox and olaquindox metabolites because of their specificity and reliability (Jiang et al. 2013; Kim et al. 2015; Le et al. 2012, 2014; Li et al. 2016; Peng et al. 2011; Song et al. 2011; Zhang et al. 2015). These methods also require a series of complex handling procedures including incubation, washing, and reaction; thus, they are not suitable for on-site detection. Meanwhile, in our previous study, we also reported a gold nanoparticle-based immunochromatographic assay (ICA) to detect QCA (Le et al. 2012). However, use of the gold nanoparticle based ICA for QCA detection is always limited by its relatively low sensitivity (Le et al. 2016a, b).

Compared with other analytical techniques, fluorescence CdSe/ZnS quantum dot (QD) immunoassays have shown many advantages such as high sensitivity, high reproducibility, and unique optical properties for trace analysis. In our previous study, we developed a fluorescence-linked immunosorbent assay based on QDs for the detection of MQCA and QCA residues, as well as achieved high sensitivity (Le et al. 2016a, b). Quantum dot beads (QBs), in which numerous QDs are embedded in a polymer matrix, exhibited approximately 2863 times brighter luminescence than their corresponding QDs. Ren et al. used QBs as luminescent amplification probes and developed immunochromatographic assay (ICA) method for detection of aflatoxin B1, and the limit of detection (LOD) was achieved at 0.42 pg mL^{-1} (Ren et al. 2014). Duan et al. also reported a QB-based ICA (QB-ICA) for detection of zearalenone with a LOD at 3.6 μg kg^{-1} (Duan et al. 2015). However, there are not reported on the use of QB-ICA sensor for the quantitative determination of MQCA or QCA.

Nevertheless, no ICA strip based on QB-label mode and chemiluminescent detection has been developed to detect multiple analytes with two test lines up to now. In this study, highly luminescent QBs were synthesized and applied as ICA signal-amplification probes for ultrasensitive detection of QCA and MQCA in chicken, fish, or other animal tissues. The fluorescent sensor showed higher sensitivity than colloidal gold-based ICA and QD-based immunoassay (Le et al. 2012). Moreover, the QB-ICA sensor was used for the analysis of MQCA and QCA in incurred tissue samples and validated by commercial ELISA kit and LC-MS/MS method.

Materials and Methods

Materials and Reagents

Olaquindox, carbadox, quinocetone, cyadox, mequindox, MQCA, QCA, poly (methyl methacrylate) (PMMA), poly (maleic anhydride-alt-1-octadecene) (PMAO), *N*-(3-(dimethylamino)propyl)-*N*-ethylcarbodiimide hydrochloride (EDC·HCl), bovine serum albumin (BSA), and ovalbumin (OVA) were purchased from Sigma (St. Louis, MO, USA). Goat anti-rabbit IgG antibody was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Polyclonal antibody against MQCA (anti-MQCA PcAb) and polyclonal antibody against QCA (anti-QCA PcAb), MQCA-BSA, and QCA-BSA conjugates were produced in house based on a published protocol (Kim et al. 2015; Le et al. 2012, 2014; Le et al. 2016a, b). CdSe/ZnS QDs with a maximum emission wavelength at 625 nm were purchased from Invitrogen Corp (Carlsbad, CA, USA). Nitrocellulose membranes, glass fibers, sample pads, and absorbent pads were purchased from Millipore Corporation

(Billerica, MA, USA). The commercial ELISA kit was provided by Chongqing Ruofeng Biotech Co., Ltd. (Chongqing, China).

Preparation of QBs

As illustrated in Fig. 2a, carboxyl-modified QBs were synthesized according to previously described procedures with minor modifications (Le et al. 2016a, b; Ren et al. 2014). Briefly, 20 mg of QDs with a maximum emission wavelength at 625 nm was added sequentially to solutions 2 mL of $CHCl_3$ containing 60 mg mL^{-1} of poly(methyl methacrylate) (PMMA), and 40 mg mL^{-1} of poly(maleic anhydride-alt-1-octadecene) (PMAO). The mixtures were reacted for 10 min, and 5 mL and 3 mg mL^{-1} of sodium dodecyl sulfonate aqueous solution were added, reacted for 2 min, after which $CHCl_3$ was evaporated using a rotary evaporator. Subsequently, the mixture was centrifuged for 10 min at 10,000 rpm and washed extensively with water.

Preparation of the QB-Labeled PcAb Probe

Anti-MQCA PcAb and anti-QCA PcAb prepared in our laboratory were purified from rabbit serum by the use of caprylic acid-ammonium sulfate method (Kuang et al. 2013). The QB-labeled antibody was prepared according to a previously published procedure (Duan et al. 2015). Briefly, 5 μg of EDC, 0.5 mg of QBs, and 300 μL 0.5 mg mL^{-1} anti-MQCA PcAb were added sequentially to 1.7 mL of 0.01 M PBS (pH 6.0). The mixture was allowed to react at room temperature for 1 h and was centrifuged for 10 min at 12,000 rpm. The collected anti-MQCA PcAb-labeled QBs were resuspended in 2 mL of PBS (pH 7.4) containing 2% fructose, 5% sucrose, 1% BSA, 1% PEG-20000, and 0.4% Tween-20. The resuspension solution was stored at 4 °C for future use. QB-labeled anti-QCA PcAb was prepared similarly, except that anti-MQCA PcAb was replaced with anti-QCA PcAb (0.4 mg mL^{-1}).

Preparation of QB-ICA Sensor

The formation and principle of the QB-ICA sensor are shown in Fig. 2. The sample pad was made of glass fiber pretreated with 0.01 M PBS (pH 8.0) containing 1.0% BSA, 0.25% Tween-20, and 0.05% NaN_3. The capture reagents consisted of two test lines (T_1 line and T_2 line) positioned at a 4 mm interval. MQCA-BSA (1.3 mg mL^{-1}) and QCA-BSA (1.5 mg mL^{-1}) were spotted by AirJet Quanti3000™ dispenser (Richmond, CA, USA) onto the nitrocellulose membrane at 0.5 μL cm^{-1} to form test lines (T_1 and T_2). Goat anti-rabbit IgG (0.1 mg mL^{-1}) dissolved in 0.02 M coating buffer (pH 9.4) was jet positioned at 1 μL cm^{-1} to act as control line, positioned at 0.5 cm above the T line. After drying for 0.5 h at room temperature, the nitrocellulose membrane was blocked with an appropriate buffer (0.01 M PBS containing 1.0% BSA, and 0.25% PVP) and then dried under nitrogen for 1 h. Subsequently, the sample pad, coated nitrocellulose membrane, and absorbent pad were laminated into a sheet of plastic scaleboard. Finally, the whole assembled plate was cut into 3 mm × 60 mm wide strips using a strip cutter CM4000 (Richmond, CA, USA) and packaged at room temperature for subsequent use.

Procedure of QB-ICA Sensor

As illustrated in Fig. 2b, 2.5 μL of QB labeled with anti-MQCA PcAb (18 μg mL^{-1}), 2.5 μL of QB labeled with anti-QCA PcAb (26 μg mL^{-1}), and 75 μL of standard samples or extracts were premixed and incubated at 37 °C for 2 min and then added to the sample pad of the QB-ICA. The complexes migrated across the nitrocellulose membrane and were captured by MQCA-BSA and QCA-BSA that were immobilized on the test line. After several minutes, two intense fluorescent band colors were developed on T_1 line and T_2 line (Fig. 2b). The excess PcAb-QBs were subsequently captured by the specific secondary goat anti-rabbit IgG, which resulted in the accumulation of QBs on the control line. Based on the detection mechanism, it is clear that the more MQCA or QCA in the extracts, the lower the fluorescence intensity that would appear on the test line (T_1 or T_2). If there were no QBs accumulating on the control line, the sensor result was invalidated. The fluorescence intensity of the test line was proportional to the MQCA or QCA content in the specimens. The fluorescence intensities of QB-ICA were observed by the naked eye with ultraviolet irradiation or read with a BioDot TSR3000 Membrane strip reader (Richmond, CA, USA) to record. The quantitative analysis was calculated according to the calibration curve. According to the principle described above, the competitive inhibitory curves were developed by plotting the F/F_0 values against the concentrations (Log C), where F and F_0 represent the fluorescence values obtained for the positive samples and the negative samples, respectively. The linearity was evaluated based on matrix-matched calibration curves, which were prepared by spiking the standard solutions of MQCA or QCA in 50 mM PBS (pH 7.4, containing 10% methanol) at concentrations of 0, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg L^{-1}. The sensitivity was evaluated based on the 50% of inhibition (IC_{50}) (Le et al. 2016a, b). Intra-variations were measured by eight replicates, and the inter-variations were based on the results of eight independent experiments.

Sample Pretreatment

Samples including the muscle of swine, chicken, cattle, and fish were purchased from a local market and found to be free of olaquindox, carbadox, and their metabolites based on LC-

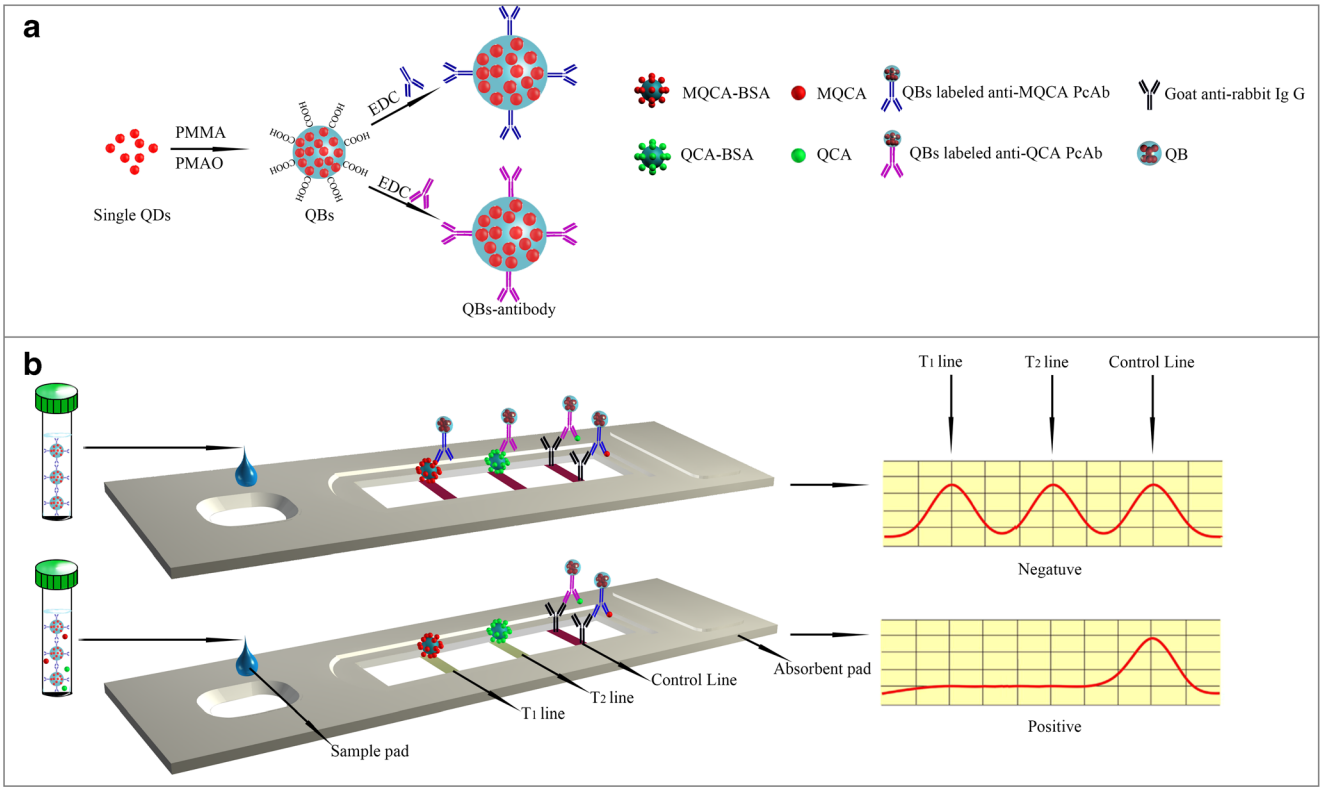

Fig. 2 **a** Schematic illustration of the quantum-dot submicrobead formation. **b** Procedure for the detection of MQCA and QCA using QB-ICA

MS/MS (Manchester, UK) analysis (AQSIQ 2006). After homogenization, the samples (1.00 ± 0.01 g) were spiked with 0.1 mL of MQCA or QCA at different concentrations. To the homogenized samples, 4 mL of deionized water and 2 mL of 2 M H_2SO_4 were added. The samples were thoroughly mixed and ultrasound for 0.5 h. Subsequently, 8 mL of ethyl acetate was added to the sample, and the mixture was vortexed for 5 min and centrifuged at 6000 rpm for 10 min. Subsequently, the 4 mL of supernatants was collected and dried under N_2 at 50 °C. This was followed by re-dissolving the extract in 2 mL of a 1:1 (*v*/*v*) mixture of *n*-hexane and 0.1 M of PBS (pH 7.4) and vortexed thoroughly for 1 min. Finally, the extract solution was centrifuged at 6000 rpm for 10 min at 4 °C, and the supernatants were removed. The extract samples were resuspended in 1 mL of PBS prior to the analysis by QB-ICA sensor and ELISA.

Validation of QB-ICA Sensor Performance

The MQCA and QCA standard solutions were diluted with 0.01 mM PBS (pH 7.4) to obtain a standard curve. The QB-ICA validation was performed using different samples, the samples purchased in retail outlets in Chongqing. All the samples had previously been demonstrated to be MQCA and QCA free by LC-MS/MS. Each sample was assayed using QB-ICA to determine the limit of detection (LOD). The LOD for the sensor was estimated as the concentration of analytes giving a 10% inhibition of the maximum fluorescence intensity (Le et al. 2016a, b).

The accuracy and precision of the QB-ICA were represented by the recovery and coefficient of variation (CV), respectively. To evaluate the recovery and CV of the QB-ICA method, four sets of animal tissue samples were spiked with known amounts of MQCA (2, 4, and 8 μg kg^{-1}) or QCA (2.5, 5, and 10 μg kg^{-1}) and treated as described for the sample preparation. Half of these samples were subjected to the QB-ICA analyses, and the other half were subjected to LC-MS/MS analyses. The mean recovery was calculated as follows: (concentration measured/concentration spiked) × 100%. The CVs were determined via analyses of the above samples spiked with MQCA and QCA at three different levels. Each concentration level was repeated five times over a time span of 3 months. The correlations between the QB-ICA and LC-MS/MS analyses in terms of the detection of MQCA or QCA in the spiked samples were calculated.

Comparison of QB-ICA with ELISA Kit and LC-MS/MS for Incurred Sample Analysis

To evaluate the detection capability and accuracy of the proposed QB-ICA method, tissue samples from animal-feeding experiment were further compared with a commercial ELISA kit and confirmed by reference LC-MS/MS method/GBT 20746−2006 (AQSIQ 2006). The animal-feeding protocol

was described previously (Kim et al. 2015; Le et al. 2014). All animal experiments in this study adhered to the Chongqing Normal University animal experiment center guidelines and were approved by the Animal Ethics Committee (number of the using of Laboratory Animal: SYXK(Yu)2012-0006). These incurred real tissues were minced and homogenized, and frozen at −20 °C until analysis. All samples were subjected to LC-MS/MS and commercial ELISA, as well as LC-MS/MS analyses according to the modified procedure (AQSIQ 2006).

Results and Discussion

Optimization of the Reagents for the QB-ICA

The QB-labeled PcAb probes were achieved by coupling the amino groups of anti-MQCA PcAbs or anti-QCA PcAbs with the carboxyl group of the QBs. To achieve high fluorescence signals and the best sensitivity of the sensor, a checkerboard titration was established to determine the optimal dilution of the coating antigens and QB-labeled antibodies. MQCA-BSA/MQCA-OVA and QCA-BSA/QCA-OVA were evaluated to increase the detection sensitivity for MQCA and QCA, respectively. The results of these studies showed that MQCA-BSA and QCA-BSA had lower cutoff levels than MQCA-OVA and QCA-OVA. Based on indirect competitive fluorescent immunoassay, the lower concentrations of coating antigens (MQCA-BSA or QCA-BSA) resulted in better cutoff levels of analytes (MQCA or QCA). Moreover, the difference between negative and positive samples depended on the appearance of a clear red fluorescence on the T line which could be easily monitored by the naked eye observation under ultraviolet irradiation. In the present study, the best working concentration of coating antigen was 1.3 mg mL^{-1} for MQCA-BSA and 1.5 mg mL^{-1} for QCA-BSA. The optimum concentration of QBs-PcAb probe was 18 μg mL^{-1} for QB labeled anti-MQCA PcAb and 26 μg mL^{-1} for QB-labeled anti-MQCA PcAb. A sample solution (75 μL) was premixed with QB labeled PcAb probe (5 μL) at 37 °C for 2.0 min and then added onto the sensor. After 10 min, the strip was scanned by BioDot TSR3000 Membrane strip reader for MQCA and QCA quantitative analysis.

Table 1 Results of intra- and inter-assays for the QB-ICA (n = 8)

Concentration (μg L^{-1})	Intra-assay CV		Inter-assay CV	
	MQCA	QCA	MQCA	QCA
2.5	5.8	7.2	7.8	8.4
5	7.9	8.5	8.6	7.7
10	8.4	9.5	6.9	8.5
25	9.5	8.6	5.3	9.1
50	6.2	10.2	7.5	9.9

Standard Curve and Specificity for the QB-ICA Sensor

Competitive curves with final MQCA (or QCA) concentrations of 0.5, 1.0, 2.5, 5.0, 25, 10, 50, and 100 μg L^{-1} were run in PBS (Fig. 3a). The response time of the sensor is 10 min; the fluorescence intensity of the test lines (T_1 and T_2) was measured by the strip reader. Good linearity of the standard curve (Fig. 3b) was prepared by using MQCA and QCA concentrations of 2.5, 5.0, 25, 10, and 50 μg L^{-1} in PBS. The curve obtained shows good linearity with R^2 equal to 0.9727 for MQCA and 0.9838 for QCA. The IC_{50} for MQCA and QCA were 8.1 and 10.6 μg L^{-1}, respectively.

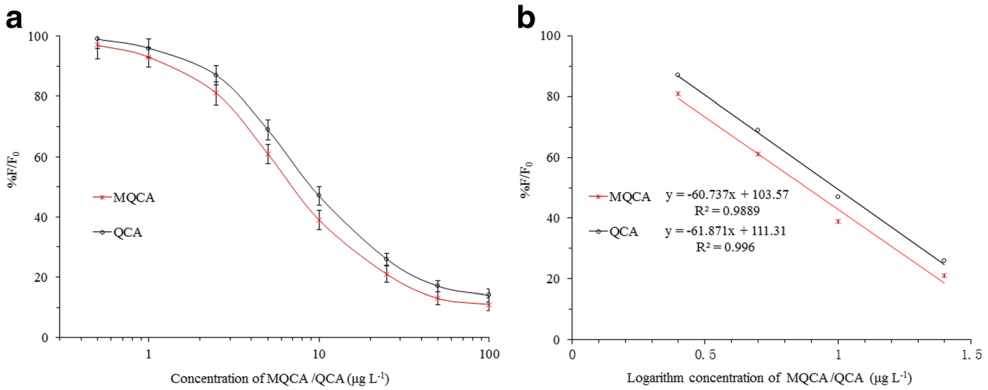

Fig. 3 Competitive curves from the developed QB-ICA for MQCA and QCA in 0.01 mM PBS (pH 7.4). **a** Typical calibration curves of the immunosensor with increasing MQCA and QCA concentrations, from *top* to *bottom*: 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg L^{-1}, respectively. **b** Good linearity of the calibration curves was achieved for MQCA and QCA in the range of 2.5–50 μg L^{-1}. Immunoreaction time was 10 min. Normalized signals expressed as 100 (F/F_0) (where F and F_0 are the peak fluorescence intensities obtained with the MQCA and QCA analyte and the blank sample, respectively) were plotted versus the logarithm of MQCA and QCA concentration

Table 2 Cross-reactivity with MQCA and QCA and analog compounds tested by QB-ICA

Compound	Visual line	Compound concentration (μg L^{-1})								
		1	2.5	5	10	25	50	100	200	300
MQCA	T_1	+	+	±	−	−	−	−	−	−
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
QCA	T_1	+	+	+	+	+	+	+	+	+
	T_2	+	+	+	±	−	−	−	−	−
	C	+	+	+	+	+	+	+	+	+
Olaquindox	T_1	+	+	+	+	+	+	+	±	−
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
Carbadox	T_1	+	+	+	+	+	+	+	+	+
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
Quinocetone	T_1	+	+	+	+	+	+	+	+	+
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
Cyadox	T_1	+	+	+	+	+	+	+	+	+
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
Mequindox	T_1	+	+	+	+	+	+	+	+	+
	T_2	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+

+ red line, ± pale red line, − no red line

Using this standard curve, the reproducibility and stability of the sensor were investigated for MQCA and QCA using MQCA and QCA standard solutions at concentrations of 2.5–50 μg L^{-1}, and the results are summarized in Table 1. The standard solutions described above were analyzed eight times for the intra-assay (n = 8) and daily for eight independent experiments for the inter-assay. The intra-assay CVs were 5.8–10.2%, and the inter-assay CVs were 5.3–9.9%.

In order to evaluate the specificity of this QB-ICA sensor, the interferences of several structurally related compounds (olaquindox, carbadox, quinocetone, cyadox, mequindox, QCA and MQCA) were examined using the sensor. The cross-reactivities with MQCA and QCA and analog compounds in 0.01 mM PBS (pH 7.4) are shown in Table 2 with the range of the concentration from 1 to 300 μg L^{-1}.The results indicated that the QB-ICA sensor was highly selective for MQCA and QCA, and with the exception of olaquindox, there was no cross-reactivity with other analogs.

Visual Detection Limit

As shown in Fig. 4, the red fluorescence of the T_1 line and T_2 line individually decreased as the concentrations of MQCA and QCA in samples increased. As for the simultaneous detection of multiple analytes in the sample by QB-ICA (Fig. 4), mixed MQCA and QCA standard solutions at each final concentration of 0, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg L^{-1} were tested. Under the optimized detection conditions, the cutoff levels with naked eyes could be measured at 5 and 10 μg L^{-1} for MQCA and QCA, respectively.

Validation of ICA Sensor

As shown in Table 3, the developed QB-ICA could detect MQCA and QCA in various biological matrices, including swine muscle, chicken, cattle muscle, and fish. Using above sample preparation procedure, the LODs in various biological matrices ranged from 1.4 to 2.3 μg kg^{-1}.

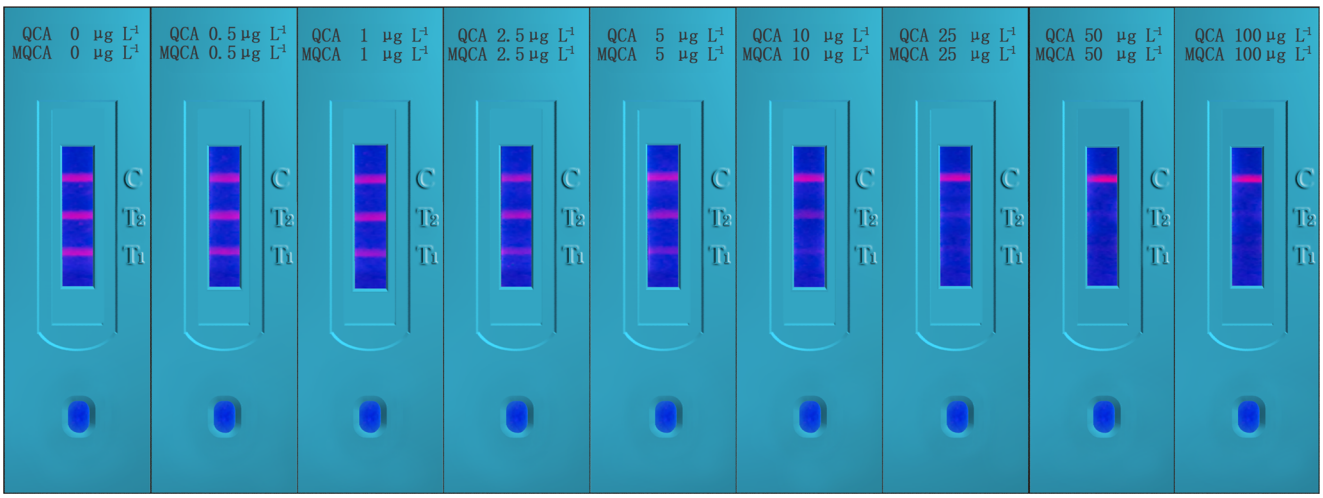

Fig. 4 Dual-antibiotic immunoassays using QB-ICA. Mixed standard solutions of MQCA and QCA at each final concentration of 0, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg L^{-1} were tested

Table 3 The LOD, recoveries, and CVs of MQCA and QCA in edible animal tissues and the correlations (R^2) between the results of QB-ICA and LC-MS/MS

Analyte	Sample	LOD of QB-ICA (μg kg^{-1})	Spiked (μg kg^{-1})	QB-ICA		LC-MS/MS		The correlations (R^2)
				Mean recoveries ± SD (%)	CVs (%)	Mean recoveries ± SD (%)	CVs (%)	
MQCA	Swine muscle	1.4	2.0	90.6 ± 6.8	7.5	88.9 ± 7.3	8.2	0.9944
			4.0	79.6 ± 8.4	10.5	85.6 ± 7.9	9.3	
			8.0	91.9 ± 3.3	3.5	89.5 ± 5.3	5.9	
	Chicken	1.5	2.0	87.8 ± 8.2	9.3	90.2 ± 7.9	8.8	0.9638
			4.0	87.4 ± 9.3	10.6	89.7 ± 8.6	9.5	
			8.0	85.9 ± 8.2	9.5	87.9 ± 7.5	9.0	
	Cattle muscle	1.6	2.0	87.5 ± 8.6	9.9	90.7 ± 7.8	8.6	0.9821
			4.0	80.6 ± 7.0	8.6	84.1 ± 7.1	8.4	
			8.0	81.7 ± 8.8	10.7	85.9 ± 6.9	8.1	
	Fish	1.6	2.0	92.2 ± 9.4	10.2	93.4 ± 7.1	7.6	0.9753
			4.0	84.4 ± 6.1	7.2	87.4 ± 6.9	7.8	
			8.0	78.7 ± 5.4	6.8	81.0 ± 5.9	7.2	
QCA	Swine muscle	2.0	2.5	88.6 ± 7.1	8.0	92.1 ± 7.5	8.1	0.9902
			5.0	95.8 ± 5.7	6.0	97.4 ± 5.3	5.4	
			10.0	81.9 ± 8.5	10.4	84.8 ± 8.6	10.1	
	Chicken	2.2	2.5	87.7 ± 6.1	6.9	90.8 ± 6.1	6.7	0.9865
			5.0	89.9 ± 8.5	9.4	92.8 ± 8.8	9.5	
			10.0	82.0 ± 5.5	6.7	83.6 ± 4.8	5.7	
	Cattle muscle	2.1	2.5	88.5 ± 10.2	11.5	91.2 ± 8.0	8.8	0.9848
			5.0	82.9 ± 5.6	6.6	88.2 ± 4.1	4.7	
			10.0	80.6 ± 8.3	10.3	85.0 ± 6.9	8.1	
	Fish	2.3	2.5	86.4 ± 9.3	10.8	88.6 ± 9.1	10.3	0.9804
			5.0	88.8 ± 8.3	9.3	92.2 ± 8.5	9.2	
			10.0	91.6 ± 10.2	11.1	93.8 ± 8.9	9.5	

SD standard deviation, *CVs* coefficients of variation

The mean recovery and CV for four kinds of animal tissues are shown in Table 3. The blank tissue samples were spiked with known concentrations of MQCA (2, 4, and 8 μg kg^{-1}) or QCA (2.5, 5, and 10 μg kg^{-1}), and the mean recovery ranged from 78.7 to 95.8%. The CVs were less than 12%, which were within an acceptable range (Commission of the European

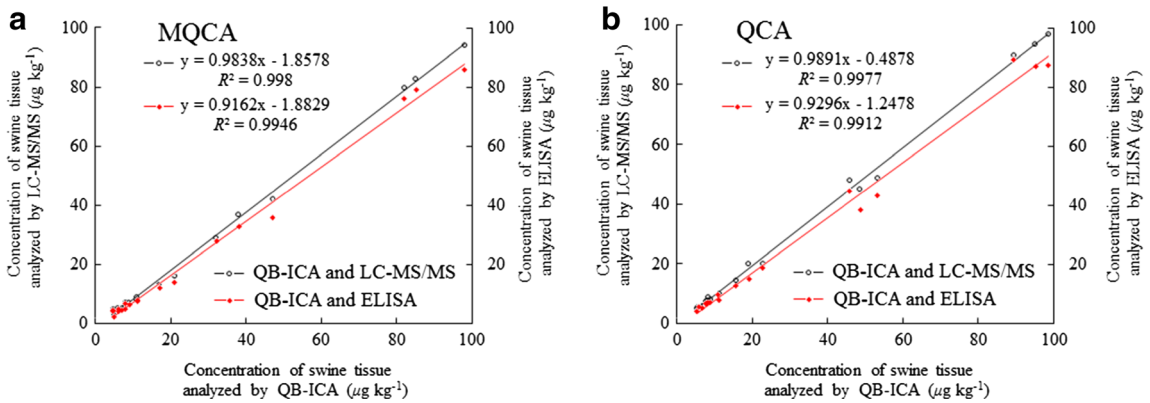

Fig. 5 Correlations between the results of QB-ICA, ELISA kit and LC-MS/MS for **a** MQCA and **b** QCA in animal trial swine muscle and liver. The data are average values of triplicate samples (average ± SD)

Communities 2002). As shown in Table 3, a positive correlation ($r > 0.9638$) between the results of QB-ICA and LC-MS/MS was also observed.

Analysis of Animal Experiment Sample by QB-ICA, Commercial ELISA Kit, and LC-MS/MS

The reliability and accuracy of the QB-ICA method were further studied through the assay of MQCA and QCA in incurred samples from the animal feeding experiment. The concentrations of MQCA and QCA in the swine muscle and liver samples were measured by the QB-ICA method and values were compared with commercial ELISA kit and the reference LC-MS/MS method. MQCA and QCA in animal tissues were determined at varying time points (0, 4, and 10 days) after administration. As can be observed in Fig. 5, the good correlation (R^2) between the QB-ICA and LC-MS/MS results was 0.998 for MQCA and 0.9977 for QCA in the incurred samples, indicating that the QB-ICA is reliable. The good correlation between the QB-ICA and the commercial ELISA kit results were 0.9946 for MQCA and 0.9912 for QCA, demonstrating that the QB-ICA was also consistent with the ELISA kit.

Conclusion

In summary, the proposed method is the first application of the QB-ICA sensor to the determination of MQCA and QCA in edible animal tissue. The sensitivity of sensor was 8.1 and 10.6 μg L^{-1} for MQCA and QCA standard analyte with strip reader in 10 min, respectively. This sensor is capable of detecting MQCA and QCA spiked in edible animal tissues with good recoveries for MQCA (78.7–92.2%) and QCA (80.6–95.8%). The excellent recoveries of MQCA and QCA from spiked in edible animal tissues and satisfactory correlation between data obtained by QB-ICA and LC-MS/MS proved the good accuracy of established sensor for the detection of MQCA and QCA. The consistent results between the established QB-ICA and commercial ELISA kit and LC-MS/MS for survey of MQCA and QCA in incurred tissues demonstrated the excellent capability of detecting MQCA and QCA of proposed QB-ICA. Therefore, the results demonstrate that the developed QB-ICA method is a reliable tool and can be applied for the analysis of MQCA and QCA in various tissues.

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Compliance with Ethical Standards All animal experiments in this study adhered to the Chongqing Normal University animal experiment center guidelines and were approved by the Animal Ethics Committee (number of the using of Laboratory Animal: SYXK(Yu)2012-0006).

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Conflict of Interest Yong Xie declares that he has no conflict of interest. Lei Zhang declares that he has no conflict of interest. Qi Sun declares that he has no conflict of interest. Zhihao Zhang declares that he has no conflict of interest. Tao Le declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human subjects. All animal experiments that were described in the present study were performed in adherence to Chongqing Normal University animal experiment center guidelines and approved by the Animal Ethics Committee.

Informed Consent Not applicable.

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