Determination of 3-Methyl-quinoxaline-2-carboxylic Acid and Quinoxaline-2-carboxylic Acid in Pork Based on a Background Fluorescence Quenching Immunochromatographic Assay

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A novel rapid method based on a background fluorescence quenching immunochromatographic assay (bFQICA) was established to achieve simultaneously the quantitative detection of 3-methyl-quinoxaline-2-carboxylic acid (MQCA) and quinoxaline-2-carboxylic acid (QCA), which were efficiently extracted and enriched 4 times using immunomagnetic beads from pork. The analysis of field pork samples by bFQICA was in accordance with that of LC-MS/MS; especially, the proposed bFQICA exhibited great advantages in convenience and efficiency, which only takes 30 min for the detection of MQCA and QCA.

Keywords Background fluorescence quenching immunochromatographic assay (bFQICA), 3-methyl-quinoxaline-2-carboxylic acid (MQCA), quinoxaline-2-carboxylic acid (QCA), immunomagnetic beads, pork

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Olaquindox (OLA) and carbadox (CBX) (Fig. S1, Supporting Information) are the well-known members of the quinoxaline antibacterial, which have been widely used as medicinal feed additives and growth promoters for the prevention dysentery and bacterial enteritis in swine. However, OLA and CBX can be rapidly metabolized and converted into a marker of drug residue in animal tissues, 3-methyl-quinoxaline-2-carboxylic acid (MQCA) and quinoxaline-2-carboxylic acid (QCA), respectively. Moreover, MQCA and QCA have carcinogenic, mutagenic and photo allergenic effects on people, threatening the food safety.

Indeed, many strategies have been devoted to establish sensitive, fast and reliable analytical methods applicable to determine OLA, CBX and their metabolites (MQCA and QCA), such as high-performance liquid chromatography and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). However, it has limitations in concerning food testing because of expensive instruments, a long sample preparation time and professional knowledge of operators. In addition, immunoassay is considered as to be a rapid technique for veterinary drug residue analysis with high throughput tests. Some strategies were proposed, such as time-resolved fluoroimmunoassay (TR-FIA), dual-label direct competitive fluorescence-linked immunosorbent assay (dc-FLISA) and enzyme-linked immunosorbent assay (ELISA), but these methods required tedious washing steps and long times for consumption. The traditional colloidal gold immunochromatographic assay (GICA) is fast, simple and low cost, but always shows disadvantages of a narrow quantitative range or just a qualified determination. In this study, we firstly proposed a quantitative analysis method for a background fluorescence quenching immunochromatography assay (bFQICA) with immunomagnetic beads separation and enrichment based on a streptavidin-biotin system for the simultaneous determination of MQCA and QCA residues in pork (Fig. 1). This method utilizes the ability of gold nanoparticles to efficiently quench fluorescence, and to measure the background fluorescence value by the instrument to achieve the rapid quantitative detection of analytes. In addition, immunomagnetic beads capturing could decrease the sample pretreatment time, increase the assay sensitivity and address the any interferences from complex samples.

A schematic illustration of the bFQICA strip is shown in Fig. 2. AuNPs-anti-MQCA mAb immunoprobes were bound to MQCA and/or QCA in the standard, or samples extraction solution; the mixture was dripped onto the sample pad and could move towards the absorbent pad through a capillarity. Based on the competitive binding format, when there were no MQCA and QCA (negative) in the solution, AuNPs-anti-MQCA mAb immunoprobes bound to the MQCA-NH2-OVA immobilized on the NC membrane and the gathered AuNPs could obviously quench the fluorescence of T line (F2). The remaining AuNPs-anti-MQCA mAb immunoprobes continued to move towards to the C line and combined with the goat anti-mouse IgG, resulting in less fluorescence quenching at the C line (F1). At this point, the ratio of F1/F2 was minimum (min). Conversely, when MQCA and/or QCA (positive) were present, the AuNPs-anti-MQCA mAb immunoprobes bound with the analytes, resulting in a decreased fluorescence quenching degree of the T line. Moreover, these probes (the unbound AuNPs-anti-MQCA mAb immunoprobes and AuNPs-anti-MQCA mAb-analyses complex) could combine with goat anti-mouse IgG at C line and obviously quenched the fluorescence of C line. At this point, the ratio of F1/F2 was maximum (max). The value of the fluorescence signal ratio (F1/F2) decreased with the increased concentration of analytes present in the standards or sample solution. Interestingly, F1 waxed and F2 waned with increased concentration of the analytes.

Under the optimized conditions (Supporting Information), the standard solutions of MQCA were diluted in 0.02 M PBS to produce a concentration range from 0 to 6.4 μg L⁻¹ (0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 μg L⁻¹), and were measured by the developed bFQICA strip. Two arrows indicate the position of the T line

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and C line on strips, and could serve as a quick, qualitative determination by eyes according to the color intensity (Fig. 3a). The color intensity gradient from low to high represented increasing aggregation of AuNPs-anti-MQCA mAb probes, and a decreasing value of the fluorescence signal. The regression equation was \( y = 1.518 + (6.706 - 1.518)/(1 + (x/0.356)^{2.273}) \), with a correlation coefficient of 0.9636 (Fig. 3b). The sensitivity of bFQICA was evaluated by using the IC\(_{50}\) value of the obtained calibration curve, which was 0.34 \(\mu g\) L\(^{-1}\), and the dynamic linear range determined as the concentrations causing 20 – 80% inhibition of F1/F2 was 0.1 – 1.6 \(\mu g\) L\(^{-1}\). This performance was superior to that of reported studies in terms of the sensitivity.\(^{17,18}\)

Matrix elimination was considered in the proposed bFQICA method for MQCA/QCA detection. MQCA and QCA in pork samples were extracted and enriched by immunomagnetic beads (Fig. 1),\(^{19}\) and separated from the sample interference matrix by a magnetic field, and then the analytes (MQCA/QCA) were eluted into 0.02 M PB by heating at 85°C. The superimposition of the calibration curves indicated that there was no significant matrix effect (Fig. S2). Therefore, the pretreatment of immunomagnetic beads can basically eliminate matrix interference, and the pork sample can be analyzed using the standard inhibition curve instead of the sample matrix curve. The LOD was calculated as the mean value of 20 blank pork samples plus three-times the standard deviation (mean + 3SD). The LODs of the developed bFQICA were 0.03 \(\mu g\) kg\(^{-1}\) for MQCA and
This newly developed bFQICA strategy is a promising approach for the rapid field detection of MQCA and QCA in animal-derived foods.

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Supporting Information

It contains detailed experimental procedures in this study and supplementary figures and tables. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Fig. 3 (a) Typical responses of the bFQICA strips to MQCA with different concentrations; (b) standard curves of the bFQICA for MQCA.