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Analytical Methods

Development of direct competitive biomimetic immunosorbent assay based on quantum dot label for determination of trichlorfon residues in vegetables

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ABSTRACT

A direct competitive biomimetic immunosorbent assay method based on molecularly imprinted polymer was developed for the determination of trichlorfon. A CdSe/ZnS quantum dot label was used as the marker. The hydrophilic imprinted film was synthesized directly on the surface of a 96-well plate, and characterized by Fourier-transform infrared spectroscopy and thermo-gravimetric analyses. The method exhibited high stability, selectivity, and sensitivity. Under optimal conditions, the limits of detection and sensitivity of the biomimetic immunosorbent assay method were 9.0 μg L^{-1} and 5.0 mg L^{-1} (0.1 mg kg^{-1} and 62.5 mg kg^{-1} for vegetable sample), respectively. Low cross-reactivity values of 19.2% and 15.6% were obtained for the structural analogues. Spinach and rape samples spiked with trichlorfon were extracted and determined by this method with recoveries ranging from 83.6% to 91.1%. The method was applied for the detection of trichlorfon residues in leek and cucumber samples, and results correlated well with those obtained using GC.

1. Introduction

Organophosphorus pesticides (OPs) are widely utilized in agriculture for improving crop productivity and quality (Bala et al., 2017; Xu, Li, Hu, & Su, 2017). However, OP residues can contaminate agricultural products, and result in toxic effects on humans such as poisoning (Dehghani et al., 2017; Kim, Kim, & Park, 2015). A sensitive and simple method for the detection of OPs is therefore necessary.

Many methods for detecting OP residues have been reported in the past few decades. These include gas chromatography (GC) (Mahpishanian & Sereshti, 2016; Ramasubramanian & Paramasivam, 2016; Xiao, He, Chen, & Hu, 2016), high performance liquid chromatography (Pirsaheb, Fattahi, & Shamsipur, 2013; Seebunrueng, Santaladchaiyakit, & Srijaranai, 2014), GC or liquid chromatography coupled to mass spectrometry (Chen et al., 2016; Hamelin et al., 2014; Nedaei, Salehpour, Mozaffari, Yousefi, & Yousefi, 2014; Ueyama et al., 2014) and biosensors (Yu, Wu, Zhao, Wei, & Lu, 2015; Zhang, Asiri, Liu, Du, & Lin, 2014). However, these methods have disadvantages, including large material consumptions, high personnel skill requirements, expensive equipment, and time-consuming procedures. They are therefore unsuitable for rapid, on-site testing (Gupta, Yola, & Atar, 2014; Liu et al., 2014; Suri et al., 2009). The immunosorbent assay is an alternative method which has been widely used for the detection of OPs, with high sensitivity and efficiency (Kim, Park, Lee, Lee, & Lee, 2007; Wang, Li, & Liu, 2010; Xu et al., 2010). However, the monoclonal and polyclonal antibodies have low stability and short lifetime. The high commercial cost and difficulties associated with antibody production have restricted application of the immunosorbent assay in the determination of OP residues (Liang, Xie, Wang, Gui, & Zhu, 2013; Shi, Liu, Song, Qiao, & Xu, 2015).

The artificial antibodies have been designed and synthesized as replacements for natural antibodies, in attempt to overcome these issues. Molecular imprinting technology is an attractive method for obtaining artificial antibodies. The molecularly imprinted polymers (MIPs) as antibodies possess good rigidity and stability, and high specific recognition ability (Tang et al., 2016). Many biomimetic enzyme-linked immunosorbent assay (BELISA) methods have been reported in recent years, which have improved the recognition ability and reduced the cost (Chianella et al., 2013; Fang et al., 2011; Meng, Qiao, Xu, Xin, & Wang, 2012; Sun, Xu, Ma, Qiao, & Xu, 2014; Tang, Fang, Wang, Sun, & Qian, 2013; Tang et al., 2017; Wang, Tang, Fang, Pan, & Wang, 2011; Wang et al., 2009; Whitcombe, Kirsch, & Nicholls, 2014). BELISA methods were used to detect dieldrin in dairy products, olaquindox in chick feed and so on (Morsy, Ibrahim, & Hewedi, 1996; Sun et al., 2014; Zhao, Qiao, Xu, Xu, & Yan, 2013). In these BELISA methods enzyme conjugate (enzyme-labeled antigen) is used as marker. However, the

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enzyme is a macromolecule, and the structure of enzyme-labeled antigen differs significantly from that of the antigen. Thus, the recognition ability of the biomimetic antibody toward the enzyme labeled antigen is limited, which results in low detection sensitivity. A better marker is needed to replace the enzyme in the biomimetic immunosorbent assay (BIA).

Quantum dots (QDs) are nanocrystals of semiconductor materials which have received much more attention in recent years. QDs have numerous useful optical and chemical properties, including narrow and symmetric emission profiles, tunable emission color, high quantum yield, brightness, photostability, and broad absorption profiles (Hooshyar & Bardajee, 2017; Tian et al., 2017). They have been widely used in various detection applications (Deng, Lu, Cao, & Tian, 2016; Yan, Li, Han, & Su, 2015; Yu et al., 2016; Zaid et al., 2017). More importantly, QDs are much smaller than enzymes, so can potentially be used as a marker in the BIA.

In this study, a sensitive direct competitive BIA method was developed, using the hydrophilic imprinted film as artificial antibody and CdSe/ZnS QD label as marker. The conditions were optimized. The applicability and accuracy of the presented method were evaluated.

2. Materials and methods

2.1. Materials

Spinach and rape samples were obtained from Taishan Yaxiya Food Co., Ltd. (Tai'an, China) in January 2017. Leek and cucumber samples were purchased randomly from a market in Tai'an (Shandong, China) in February 2017.

2.2. Reagents and chemicals

CdSe/ZnS QDs were obtained from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). Trichlorfon (PubChem CID: 5853), acephate (PubChem CID: 1982) and monocrotophos (PubChem CID: 5371562) (> 99%) were obtained from the Institute for the Control of Agrochemicals of the Ministry of Agriculture (Beijing, China). Ethylene glycol dimethacrylate (PubChem CID: 7355) was supplied by Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride was obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Methacrylic acid (MAA) (PubChem CID: 4093), 2,2-azobisisobutyronitrile (AIBN) (PubChem CID: 6547), methanol (PubChem CID: 887), acetic acid (PubChem CID: 176), acetonitrile (PubChem CID: 6342) and anhydrous pyridine (PubChem CID: 7355) were purchased from Tianjin Chemical Reagent Factory (Tianjin, China). MAA was vacuum distilled and AIBN was recrystallized before use. Double distilled water (DDW, 18.2 $M\Omega cm^{-1}$) used throughout the experiments was prepared using an Aike ultrapure water instrument (Tangshi Kangning Technology, Chengdu, China).

Solutions of phosphate-buffered saline (PBS, 50 mmol L^{-1} sodium phosphate, 154 mmol L^{-1} NaCl, pH 7.0), PBS with 0.05% Tween-20 (PBS/T) and borate buffer saline (BBS, 180 mmol L^{-1} boric acid, 5 mmol L^{-1} borax, pH 7.4) were used in this study.

The stock solution of trichlorfon (1000 mg L^{-1} in DDW) was diluted to 1/10, and the solution of 100,000 μg L^{-1} (in BBS) was sequentially diluted to give trichlorfon concentrations of 20,000, 4000, 800, 160, 32 and 6.4 μg L^{-1}. These solutions were used to obtain a calibration curve for the detection.

2.3. Apparatus

Microlon 96-well plates (Costar®, Corning) were purchased from Beijing Biolead BiologySci & Tech Co., Ltd (Beijing, China). The 96-well plates were washed in a ST-36wt microplate washer (Kehua Bio-engineering Co., Ltd., Shanghai, China). Fluorescence measurements were performed using Multimode Plate Reader (Molecular Devices, Sunnyvale, CA, USA). The excitation and emission wavelengths were set at 354 and 625 nm, respectively.

Trichlorfon was analyzed using a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame photometric detector and a PC-based data acquisition system. Separation was conducted on a Rtx-1 capillary column (30 m length × 250 μm i.d. × 0.1 μm film thickness, Shimadzu, Kyoto, Japan). Nitrogen was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1} with an injection volume of 1.0 μL. The injection port temperature was held at 180 °C, and injection was performed in split mode with a split ratio of 2:1. The detector temperature was 250 °C. The makeup flow was 30.0 mL min^{-1}.

2.4. Synthesis of trichlorfon hapten

Trichlorfon hapten was synthesized following our previously reported method (Meng et al., 2012). First, 5.66 g (22 mmol) of trichlorfon and 2.00 g (20 mmol) of succine anhydride were dissolved in 5 mL of anhydrous pyridine. The mixture was allowed to react in the dark for 18 h. After that, the yellowish/brown mixture was condensed to dryness under a gentle flow of nitrogen at room temperature. Then, the mixture was re-dissolved in 50 mL of DDW, and the pH was adjusted from 8 to 9 with saturated $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (3 × 30 mL), and the organic fractions were collected and evaporated to dryness under reduced pressure. The resulting extract was re-dissolved in 30 mL of BBS, and the solution was then diluted with BBS in a 50 mL flask.

2.5. Preparation of the CdSe/ZnS QD conjugate

Conjugation of trichlorfon hapten and the CdSe/ZnS QDs was performed as follows: 40 μL of trichlorfon hapten and 80 μL of BBS (10 mM, pH = 7.4) were added to a reaction container, and the mixture was stirred for 5 min at room temperature. Then, 40 μL of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10 mM, pH = 7.4, dissolved in BBS) were added, and the mixture was stirred for 10 min. When 10 μL of CdSe/ZnS QDs were added, the mixture was placed in the dark for 2 h at room temperature, and then was centrifuged to remove the caking. Finally, the product was filtrated and concentrated five times. The end product was preserved at 2–8 °C.

2.6. Preparation of hydrophilic molecularly imprinted film on the surface of 96-well plate

The hydrophilic molecularly imprinted film was synthesized directly on the 96-well plate. First, 1.03 g (4 mmol) of trichlorfon and 0.67 g of MAA were dissolved in a mixed solution of 8 mL of DDW and 12 mL of acetonitrile. The mixture was stirred at room temperature for 30 min. Next, 1.51 mL of ethylene glycol dimethacrylate and 0.08 g of AIBN were added. The mixture was continuously stirred for 60 min. Then, 200 μL of the mixture was placed in each well of a 96-well plate, and the plate was sealed in nitrogen gas at 38 °C for 18 h in the dark. After that, the plate was washed with DDW and followed by methanol to remove the unreacted solution. Finally, the plate was firstly extracted with 320 mL of methanol/acetic acid (7:1, v/v) for 8 h, and then with 300 mL of methanol for 4 h, after which it was dried at 37 °C for 4 h.

For comparison, a non-imprinted film was synthesized by the same procedure as above, except that no trichlorfon was added.

2.7. Direct competitive BIA procedure

The direct competitive BIA was performed according to the following procedure. First, the plate was washed with PBS/T solution three times. Next, 10% methanol BBS was added to the blank (200 μL) and control (100 μL) wells. Gradient standard solutions or sample extracts were added to the allocated wells (100 μL $well^{-1}$). Then, 100 μL of CdSe/ZnS QD conjugate was applied immediately to all wells,