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Direct competitive fluoroimmunoassays for detection of imidaclothiz in environmental and agricultural samples using quantum dots and europium as labels

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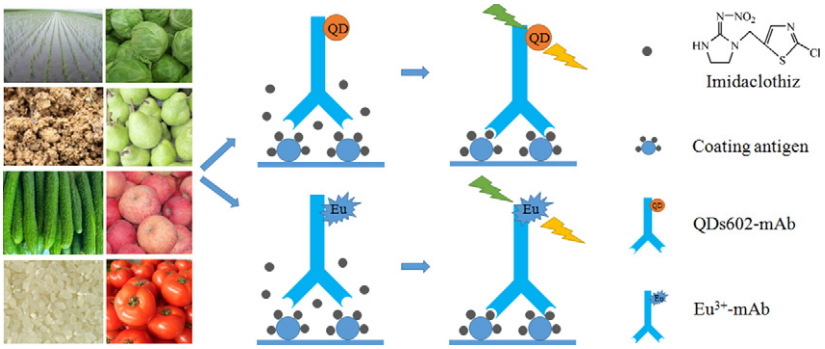
HIGHLIGHTS

- QDFIA and TRFIA were developed and applied to the monitoring of imidaclothiz.
- QDFIA and TRFIA showed higher sensitivity than reported ELISA and FPIA.
- Eight kinds of matrix were studied by spiked recovery experiment.
- The amounts of imidaclothiz in real samples detected by QDFIA and TRFIA were correlated with that detected by HPLC.

GRAPHICAL ABSTRACT



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ABSTRACT

A direct quantum dots-based fluoroimmunoassay (QDFIA) and a time-resolved fluoroimmunoassay (TRFIA) for imidaclothiz (IMI) were developed by using the quantum dots (QDs)-labeled antibody and the europium (Eu^{3+})-labeled antibody, respectively. After optimization, the half-maximal inhibition concentration (IC_{50}) and the limit of detection (LOD, IC_{10}) are 20.41 and 0.52 μg L^{-1} for the QDFIA, while 6.91 and 0.018 μg L^{-1} for the TRFIA, respectively. The cross-reactivities (CRs) with the analogues are negligible except for imidacloprid with CRs of 29.0% for the QDFIA and 26.6% for the TRFIA. The spiked recoveries of IMI in paddy water, soil, pear, tomato, rice, apple, cabbage and cucumber are 72.7–117.6% with a standard deviation (RSD) of 2.4– 13.5% for the QDFIA, and 81.3–117.7% with a RSD of 1.6– 7.5% for TRFIA. The detection results of the analyses for the real paddy water and pear samples are markedly correlated with these of high-performance liquid chromatography (HPLC).

1. Introduction

Imidaclothiz (IMI) is a new neonicotinoid insecticide with greater systemic activity, which acts on nicotinic acetylcholine receptor (Feng et al., 2008). It has great effect on controlling thrips, whiteflies, plant

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hoppers, leafhoppers and other insects (Zhang, 2005; Lan, 2006; Xu et al., 2007). However, neonicotinoid insecticides are high risk contaminants to environmental ecosystem because of the high toxicity to earthworm, bees and other pollination insects (Dussaubat et al., 2016; Wu-Smart and Spivak, 2016; Beketov et al., 2013). Therefore, it is necessary to monitor the residual IMI in environmental samples and agroproducts. Instrument-based methods, such as high-performance liquid chromatography (HPLC) (Wu et al., 2010) and HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) (Jiao et al., 2016; Zhang et al., 2012; Zheng et al., 2015) have been successfully used to detect IMI. But these methods depend on expensive instrumentations and may be not suitable for rapid detection of a large number of samples.

Immunoassays, with the advantages of high specificity and sensitivity, simplicity, rapidity, low cost, high-throughput and suitability for on-site analysis, have been successfully developed for detection of pesticides (Morozova et al., 2005; Knopp, 2006). Among them, fluoroimmunoassays (FIA) have been developed rapidly because they provide the advantages of high sensitivity, low matrix interference and wide detection range (Li et al., 2009). Fluorescence labels may be organic or inorganic materials, such as fluorescent proteins, quantum dots (QDs), lanthanide chelates, and so on. QDs have been considered as a promising fluorescent labels due to their resistance to photodegredation, high quantum yields and narrow emission with a broad excitation wavelength range (Probst et al., 2013; Stanisavljevic et al., 2015). Currently, many studies on the development of quantum dots-based fluoroimmunoassays (QDFIA) for pesticides and other contaminations such as clothianidin, thiacloprid (Li et al., 2015), fenvalerate (Liu et al., 2014), chloramphenicol (Berlina et al., 2013) and tetracyclines (Garcia-Fernandez et al., 2014) have been reported. Lanthanide chelates have the unique properties of large Stokes shifts, narrow emission, long decay lifetimes and high quantum yields (Gui et al., 2009; Hagan and Zuchner, 2011). Lanthanide chelates with advantage of long fluorescence lifetime can effectively eliminate background fluorescence from sample matrix by using a time-resolved measurement mode. Besides, lanthanide chelates can be dissociated by pH, and converted into a new chelate with high fluorescence to enhance the fluorescence intensity. So, time-resolved fluoroimmunoassays (TRFIA) based on lanthanide chelates usually have higher sensitivity and lower interference. To date, TRFIAs have been developed to detect pesticides and other contaminations such as organophosphorus pesticides (Xu et al., 2012), chlorpromazine (Huang et al., 2012), paclobutrazol (Liu et al., 2016), fluoroquinolones (Zhang et al., 2013), and so on.

In our previous study, enzyme-linked immunosorbent assays (ELISAs) (Fang et al., 2011; Li et al., 2014) and a fluorescence polarization immunoassay (FPIA) (Ma et al., 2016) have been developed for detection of imidaclothiz. But their half maximal inhibition concentrations (IC_{50}) were in the range of 58.2 μg L^{-1} to 87.94 μg L^{-1}, which might be not suitable for the monitoring imidaclothiz at trace levels. In this study, the QDs and Eu^{3+} were employed to develop fluoroimmunoassays (QDFIA and TRFIA) for imidaclothiz in environmental and agricultural samples. The influence of assay conditions including pH, ionic strength, and organic solvent were studied. The optimized immunoassays were compared and applied to detect environmental and agricultural samples, and the results were validated with HPLC.

2. Materials and methods

2.1. Chemicals and instruments

IMI (97%) was provided by Nantong Jiangshan Agrochemical and Chemicals Co., Ltd. (Jiangsu, China). Ovalbumin (OVA), bovine serum albumin (BSA), tris (hydroxymethyl) aminoethane (Tris), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and polyoxyethylene sorbitan monolaurate (Tween-20) were provided by Aladdin (Shanghai, China). The carboxylic groups-modified CdSe/ZnS core-shell QDs (emission at 602 nm) were provided by Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). *N*′-[*p*-isothiocyanatobenzyl]-diethylene-triamine-N^1, N^2, N^3, N^4-tetraacetate-Eu^{3+} (DTTA-Eu^{3+}) was provided by Tianjin Radio-Medical Institute (Tianjin, China). The 6% cross-linked agarose (sepharose CL-6B) was provided by GE healthcare Life Sciences (Pittsburgh, USA). The coating antigen and monoclonal antibody (mAb) were prepared previously (Fang et al., 2011).

Ultraviolet absorbance was detected by using a NanoDrop-1000 spectrophotometer (Thermo, USA). Black microplates (96-well) for QDFIA were provided by Corning Costar Corporation (New York, USA). Microplates (96-well) for TRFIA were purchased from Jiangsu Institute of Nuclear Medicine (Jiangsu, China). The centrifugation was performed by an Allegra TM 64R Centrifuge (Beckman, USA). The microplates were wished using an Immuno Wash 12 (Thermo, USA). The fluorescence was determined by a SpectraMax M5 (Molecular Devices, Sunnyvale, USA). The QDFIA and TRFIA were validated with an Agilent 1260 HPLC equipped with ultraviolet detector (Agilent, Wilmington, USA).

2.2. Buffers and solutions

Tris-HCl buffer (TBS, pH 8.0, 0.05 mol L^{-1}, containing 0.9% NaCl), TBS containing 0.05% Tween-20 (TBST), sodium borate buffer (BB, pH 9.0, 0.05 mol L^{-1}), BB containing 0.05% Tween-20 (BBT) and carbonate-buffered saline buffer (CBS, pH 9.6, 0.05 mol L^{-1}) were used. The enhanced solution (0.1 mol L^{-1} potassium biphthalate-acetic acid buffer containing 0.1% Triton X-100 and 15 μmol L^{-1} β-naphthoyltrifluoroacetone, pH 3.2) was purchased from Jiangsu Institute of Nuclear Medicine (Jiangsu, China).

2.3. Preparation of the labeled antibodies

The freeze-dried powder of antibody was dialyzed and adjusted by BB with the concentration of 5 mg mL^{-1}. Two hundred and seventy microliters of monoclonal antibody was mixed with 30 μL of QDs-602 in a small centrifuge tube, and then 15 μL EDC (10 mg mL^{-1}) was added. The mixture was stirred for 2 h at room temperature (RT) and kept in the dark. After centrifugation at 18,000 rpm for 30 min at 4 °C, the supernatant was removed carefully. The loose sediment was resuspended with 300 μL of BB, and centrifuged again. Finally, the sediment of QDs602-antibody was resuspended with 500 μL of BB (containing 0.05% sodium azide and 1% BSA) and stored at 4 °C.

The europium-labeled antibody was prepared as described previously with some modifications (Xu et al., 2012). Briefly, the freeze-dried powder of antibody was dialyzed by carbonate buffer (0.05 mol L^{-1}, pH 8.5), and then adjusted to 1 mg mL^{-1}. One milliliter of antibody and 0.5 mg of DTTA-Eu^{3+} were added into a centrifuge tube. The mixture was reacted for 24 h at 4 °C with stirring. The Eu^{3+}-labeled antibody was purified by sepharose CL-6B, and the eluate was collected 200 μL per tube. The tubes which possessed maximum ultraviolet absorbance and fluorescence intensity as purified Eu^{3+}-labeled antibody were pooled and stored at −20 °C containing 50% glycerol and 0.1% BSA.

2.4. Procedures of QDFIA and TRFIA

The microplates were coated with the coating antigen (hapten-OVA) diluted with CBS (100 μL per well) and incubated 2 h at 37 °C or overnight at 4 °C. The microplates were washed five times with BBT and blocked 0.5 h at 37 °C with 1% OVA in BB (200 μL per well). After washing again, 50 μL of the sample or standard solution and 50 μL of QDs602-mAb were added and incubated 1 h at 37 °C. The fluorescence intensity (*F*) of QDs was determined by SpectraMax M5 at excitation/emission wavelengths of 260/606 nm after washing and padding dry.

The procedure of TRFIA was the same with QDFIA expect the labeled mAb, diluted solution and washing buffer. The labeled mAb, diluted solution and washing buffer for TRFIA were Eu^{3+}-antibody, TBS and TBST, respectively. In addition, an enhancement solution was added to the plates (200 μL per well) after the final step of washing for TRFIA and