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A double-label time-resolved fluorescent strip for rapidly quantitative detection of carbofuran residues in agro-products

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ABSTRACT

A rapid and quantitative time-resolved fluorescent immunochromatographic assay (TRFICA) for detecting carbofuran residues in agro-products was reported in this paper. This assay was developed based on double-label immunoprobes, one of which was a carbofuran-specific antibody coupled with europium microbeads for the test (T) line signal while the other was mouse IgG coupled with europium microbeads for the control (C) line signal. Quantitative relationships between carbofuran concentrations and T/C ratios were established to determine the analyte concentration. To increase assay accuracy, four standard curves were established for the agro-products (green bean, cabbage, apple, and pear). The limits of detection (LODs) ranged from 0.04 to 0.76 mg L^{-1}. The spiked recoveries of carbofuran in the agro-products were in the range of 81–103%, which was in good agreement with a standard HPLC method. Therefore, we provided a new and reliable method for determination of *N*-methylcarbamate pesticide carbofuran residues in agro-products including vegetables and fruits.

1. Introduction

Carbofuran (or furadan), one of the most toxic *N*-methylcarbamate pesticides, has been used worldwide for agricultural and domestic pest control for many years. Considering its high toxicity to humans, birds, livestock, poultries and fish, and its long half-life in environment (Evert, 2000; Gupta, 1994; Yang et al., 2007), carbofuran residues in agro-products are a threat to consumer safety and human health. To eliminate this threat, many countries set rigid regulations on carbofuran residues in agro-products. For example, China set its maximum residue limit (MRL) at 0.1 or 0.2 mg kg^{-1} in grains and oilseeds on August 1, 2014 (GB 2763 – 2014, 2014), and the United States Environmental Protection Agency (EPA) set its MRL at 0.1 mg kg^{-1} in selected agro-products including green bean, banana, coffee and rice on April 17, 2015 (Environmental Protection Agency, 2015). Therefore, effective analytical methods become the last defense for safety of agro-product consumers.

An immunoassay has become an important on-site monitoring tool because of its high sensitivity, simplicity, cost-effectiveness and efficiency (Li et al., 2012). In particular, immunochromatographic methods for detecting agricultural contaminants have been developed quickly in both developed and developing countries, and many strips and even readers have been reported (Dzantiev, Byzova, Urusov, & Zherdev, 2014). The dip strip test is quite a nice screening tool for the target analyte, and is usually considered as a qualitative or semi-quantitative assay.

As for carbofuran, a nanocolloidal gold based immunochromatographic assay has also been developed for qualitative detection, whose sensitivity reached 0.25 mg L^{-1} (Zhou et al., 2004), but it is only used for detecting carbofuran residues in water samples.

To find new methods to improve quantitative detection of an immunochromatographic assay, we used europium particles to decrease the background signals because of their time-resolved fluorescence feature (Cummins et al., 2006). Besides, we used carbofuran as a target analyte to develop a rapid and quantitative double-label time-resolved fluorescent immunochromatographic assay (TRFICA) for detecting carbofuran residues in agro-products, which may provide a new immune research approach for other chemical pesticides.

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2. Experimental

2.1. Main reagents and instruments

Europium microbeads were provided by Shanghai Uni Biotechnology Company. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), boric acid, rabbit anti-mouse IgG, bovine serum albumin (BSA) and carbofuran standards were all purchased from Sigma-Aldrich. Nonspecific mouse IgG were prepared in our lab. Nitrocellulose membranes, sample pads, and absorbent pads were purchased from Millipore Corp. (Bedford, MA, USA). An XYZ3050 Dispensing Platform, CM4000 Guillotine Cutter and LM4000 Batch Laminator (Bio Dot, Irvine, CA, USA) were used to prepare test strips. Sonicator 3000 (Misonix, USA) was used to synthesize immunoprobes. The vacuum freeze drier (Thermo Electron Corporation, Rockford, IL, USA) was used to dry immunoprobes that were divided into small bottles. A portable reader previously described by Zhang et al. (2015) was used to read fluorescence signals of both the T and C lines on a strip.

2.2. Preparation of carbofuran-specific antibody and nonspecific mouse IgG

As double labels, two antibodies were prepared, which were carbofuran-specific antibody and nonspecific mouse IgG.

The carbofuran-specific antibody was prepared according to the reported reference (Zhang et al., 2008). Briefly, two female New Zealand white rabbits were immunized with the conjugates of BSA and carbofuran-*N*-caproic acid which were previously synthesized in our laboratory (Qu, Chen, & Liu, 2013). A total of six injections were carried out, and then the antisera were obtained and purified. An indirect competitive ELISA was used to characterize the specificities of the antibodies, indicating less cross-reactivity to other *N*-methylcarbamate pesticides.

The antibody for the control line which does not bind to carbofuran or carbofuran conjugates was prepared using our hybridoma cell line 10C9 (previously developed for aflatoxin) (Li et al., 2009).

2.3. Preparation of time-resolved fluoresce probes

Double immunoprobes were respectively prepared using the above two antibodies conjugated to europium microbeads. The microbeads with an average size of 190 nm have a carboxyl acid group which can bind to a carrier protein through a dehydration reaction.

For conjugation, the beads (200 μL; 1.9%, w/v) were mixed with 800 μL borate buffer (pH 8.18, a mixture of 65 mL 0.2 mol L^{-1} boric acid solution and 35 mL 0.05 mol L^{-1} sodium borate solution) using a vortex mixer, and then treated by a sonicator for 12 s at room temperature. EDC, *N*-1-((ethylimino)methylene)-*N*-3,*N*-3-dimethylpropane-1,3-diamine and 40 μL of 15 mg/mL solution were added, mixed fully, and then shaken for 15 min. The activated beads were then centrifuged at 13,000*g* for 10 min at 10 °C. After the supernatant was removed, 1 mL borate buffer (pH 8.18) was added and the precipitate was resuspended. Then, the carbofuran-specific antibody or nonspecific mouse IgG (60, 80 or 120μg from 1 mg mL^{-1} solution in 0.01 mol L^{-1} phosphate buffer at pH 7.4) was added. The mixture was shaken overnight at 250 rpm at room temperature, and then centrifuged at 19,000*g* for 10 min at 10 °C. After the supernatant was removed, 1 mL borate buffer (pH 8.18) with 0.5% BSA was added to resuspend the precipitate. The resuspended beads were shaken at 250 rpm at room temperature for 2 h, and the final solution was stored at 4 °C for use.

2.4. Preparation of immunochromatographic strips

Along with the probes, an immunochromatographic strip containing only a test (T) line and a control (C) line was prepared. On a nitrocellulose membrane (Millipore HF095, Millipore HF135 or Millipore HF180), the T line was previously coated with the carbofuran-ovalbumin prepared (Qu et al., 2013) and the C line was coated with the rabbit anti-mouse IgG prepared in our laboratory. During the coating step, an XYZ3050 Dispensing Platform (Bio Dot, Irvine, CA, USA) was used with the spray speed set to 0.6 μL cm^{-1}. Besides the type of membrane, the following previously prepared blocking formulas were also investigated according to the previous description (Zhang, Li, Zhang, & Zhang, 2011):

1) 0.01 mol·L^{-1} pH 7.4 PBS + 2% BSA
2) 0.01 mol·L^{-1} pH 7.4 PBS + 2% BSA + 0.5% polyvinyl pyrrolidone (PVP-K 30)
3) 0.01 mol·L^{-1} pH 7.4 PBS + 2% BSA + 0.1% Trion X-100
4) 0.01 mol·L^{-1} pH 7.4 PBS + 2% BSA + 0.5% PVP-K 30 + 2.5% sucrose
5) 0.01 mol·L^{-1} pH 7.4 PBS + 2% BSA + 2.5% sucrose + 0.02% NaN_3

Finally, the prepared strips were dried at 37 °C before use.

2.5. Establishment of standard curves

To establish standard curves for determining carbofuran residues, we also optimized the compositions of the sample extraction solution and dilution solution as well as the most suitable dilution times for analysis.

To select a suitable sample extraction solution, we compared effects of acetone-water, acetonitrile-water, and methanol-water on carbofuran recoveries.

To select a suitable dilution solution for the extraction, we investigated the phosphate buffer (pH 7.4, 0.01 mol L^{-1}), 0.4% Tween-phosphate buffer, 0.5% TEGOTENS® FG 40-phosphate buffer, borate buffer (pH 8.18), 0.4% Tween-borate buffer and borate buffer (pH 9.2), and compared their effects on the assay sensitivity.

To establish standard curves, we used a standard high performance liquid chromatographic (HPLC) method to make sure that the samples of green bean, cabbage, apple and pear were free from detectable residues with the limit of quantification (LOQ) being ~0.02 mg/kg (NY/T 761-2008, 2008). Then, we used these samples to prepare extraction solutions, with which serial concentrations of carbofuran standards were prepared. The above optimized conditions were used for sample extraction, dilution and competitive reaction in the application with the dipstick. After obtaining the results with a TRFICA reader previously described by our group (Zhang et al., 2015), quantitative relationships between analyte concentrations and T/C ratios were established.

2.6. Validation

Four samples free from detectable residues, including green bean, cabbage, apple and pear, were used for validation experiments. These samples were cut into small pieces and carbofuran standards in methanol were spiked into each sample (25.0 g). The optimized extraction solution (40.0 mL) was added into each sample and homogenized for 2 min. The supernatants were then diluted for competitive immunoreactions on TRFICA strips. On the same day, the spiked samples were also detected using the standard HPLC method (NY/T 761-2008, 2008).