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Analytical Methods

Development of a bi-enzyme tracer competitive enzyme-linked immunosorbent assay for detection of thiacloprid and imidaclothiz in agricultural samples

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ABSTRACT

A bi-enzyme tracer direct competitive enzyme-linked immunosorbent assay (dc-ELISA) was developed based on anti-imidaclothiz and anti-thiacloprid antibodies. Several affected physicochemical factors were optimised, including methanol concentration, ionic strength and pH value. Under the optimised conditions, 50% inhibiting concentration (IC_{50}) values for thiacloprid and imidaclothiz were 182.62 and 58.17 μg/L, with a limit of detection (LOD) of 4.25 and 2.12 μg/L, respectively. There was no obvious cross reactivity (CR) between the two pesticides with most neonicotinoids pesticides except imidacloprid. The method analyzing the spiked samples of tomato, pear and cabbage showed satisfying recoveries (85.27–113.07%). Comparable dissipation of thiacloprid and imidaclothiz in authentic tomato samples determined with the dc-ELISA and high performance liquid chromatography (HPLC) indicated that dc-ELISA is suitable for monitoring thiacloprid and imidaclothiz residues simultaneously in agricultural samples.

1. Introduction

Thiacloprid and imidaclothiz, belong to neonicotinoid insecticides, are now largely recruited in agricultural products due to their high effectiveness and broad spectrum (Sharma & Parihar, 2013; Wu, Xiao, et al., 2010). As synergistic insecticidal compositions, they are used against sucking insects in fruits, vegetables, and lawn for increasing food productivity (Chen et al., 2012). These substances are promising insecticides. Therefore, it is vital to establish a highly sensitive, accurate and rapid method for imidaclothiz and thiacloprid residues detection. Several sufficiently sensitive analytical methods have been developed to screen the residues of pesticides, including liquid chromatography/mass spectrometry (Zhang, Li, Yu, & Pan, 2012) and high-performance liquid chromatography (HPLC) (Mohan, Kumar, Madan, Saxena, & Assess, 2010; Wu, Cai, Yao, Dai, & Lu, 2010). Although, instrumental analyses are sensitive and selective, they are time-consuming and expensive. Enzyme-linked immunosorbent assay (ELISA), as an alternative to chromatography analytical method (Hennion & Barcelo, 1998), has wide application to development of analytical methods for pesticide residues because it can very sensitively and accurately determine them in samples. Currently, many researches have been developed for detection of neonicotinoid insecticides in food or environmental samples (Watanabe, Miyake, & Yogo, 2013). However, those general ELISA assays developed only to recognise a single analyte with high specificity (Watanabe, Baba, & Miyake, 2011). As the number of different types of residues increases, detection of each individual residue is limited to a certain degree. To enhance detecting speed and reduce testing costs, multi-residue analysis has gradually become the mainstream of residue analysis. Thus it would be more significant to develop an easy and efficient ELISA method to detect two or more analytes simultaneously (Zhang, Wang, & Fang, 2011).

At present, many efforts have been concentrated on developing multi-residue ELISA, with preparing an antibody recognising a group of compounds (Yan, Li, Yan, & Su, 2014). Broad-selective immunoassay has been developed by using more than one antibody (Sun, Dong, Zhang, & Wang, 2010; Sun, Zhang, & Wang, 2010; Yan et al., 2013) or class-selective antibody derived from general-structure hapten (Schneider, Goodrow, Gee, & Hammock, 1994), and it has also been established by using a multi-hapten antigen antibody (Yan, Shi, & Wang, 2012) or bispecific monoclonal antibody (Jin, Guo, Wang, Wu, & Zhu, 2009). However, as multi-analyte analysis, all the mentioned methods possess some

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disadvantages. The general-structure haptens are difficult to synthesis as the molecular structures of analytes are quite distinct from each other. ELISA based on a generic antibody cannot identify individual target or distinguish the components of the samples. Bispecific monoclonal antibody by using hybrid hybridomas is difficult to obtain and had disadvantages in sensitivity and stability. Using more than one antibodies and multi-hapten antigen antibody rarely have good performance in sensitivity and specificity. For example, Liu and Yan developed a novel strategy for the direct quantitative detection of imidaclothiz and thiacloprid (Liu, Yan, Xu, & Wang, 2013). This method using one standard curve quantitatively analysed the total quantity of imidaclothiz and thiacloprid for the first time. However, this method cannot well distinguish the components in samples when imidaclothiz and thiacloprid coexist, and it has narrow working range for analyzing pesticides. Furthermore, a quite similar affinity constant of antibodies and hapten density of hapten-HRP conjugates were prerequisites for the development of such an assay. So, these drawbacks have restricted its application. Compared to those multi-residue screening methods, the bi-enzyme tracer dc-ELISA could simultaneously detect imidaclothiz and thiacloprid with broad liner range in authentic samples. Moreover, the only requisite for such a system to work is to avoid shared-reactivity, that is, each antibody should only recognise its own enzyme tracer.

In this study, we aim to develop a multi-residue bi-enzyme tracer ELISA for quantitative detection of thiacloprid and imidaclothiz in agricultural samples and to evaluate the analytical reliability using tomato as the model sample. As shown in Fig. 1, the ELISA plate were directly coated with anti-imidaclothiz and anti-thiacloprid antibodies, then bi-enzyme tracers were added to analyse pesticides. Thus, an antibody-coated direct competitive ELISA was optimised to render it more general application. The bi-enzyme tracer dc-ELISA for multi-residues showed more superiority in saving detection time and workload using two mixed enzyme tracers in the same condition. Moreover, the assay for monitoring of thiacloprid and imidaclothiz residues provided a potential application in the quick and simple determination of agricultural products.

2. Materials and methods

2.1. Reagents

Imidaclothiz and thiacloprid were obtained from Nanjing Agricultural University (Jiangsu, China). Freund's complete and incomplete adjuvants, horseradish peroxidase (HRP), 3,3′,5,5′-tetramethylbenzidine (TMB), bovine serum albumin (BSA), skim milk and polyoxyethylene sorbitan monolaurate (Tween-20) were purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Buffers and solutions

Phosphate-buffered saline (PBS, 0.01 mol/L, pH 7.4), phosphate-buffered saline containing 0.05% Tween-20 (PBST), carbonate-buffered saline (CBS, 0.05 mol/L, pH 9.6), the substrate solution containing 0.025 mol/L of citrate and 0.062 mol/L of sodium phosphate (pH 5.4), the TMB solution containing 10 g/L TMB and 0.75% H_2O_2 in substrate solution were used. The water used in all experiments had a resistivity higher than 18 MΩ/cm. All chemicals were used of analytical reagent grade without further purification.

2.3. Instruments

Ninety-six well polystyrene microplates (Maxisorp) were purchased from Nunc (Roskilde, Denmark). ELISA plates were washed with a Well wash Plus (Thermo, USA). Immunoassay absorbance was read using an Infinite M200 microtiter plate reader (Tecan, Switzerland). Thiacloprid and imidaclothiz were detected using Agilent 1260 HPLC chromatography (Agilent, USA).

2.4. Hapten synthesis

Thiacloprid hapten (Th) was synthesized according to Liu et al. (2013), and imidaclothiz hapten (Im) was synthesized according to Fang et al. (2011). The structures of haptens are showed in Fig. S1.

2.5. Preparation of protein conjugates

Protein conjugates were synthesized by the activated ester method (Wang, Allan, Skerritt, & Kennedy, 1998). Haptens (Th and Im) were conjugated to BSA for use as immunogen and conjugated to the carrier HRP as enzyme tracers, respectively. The conjugates were dialyzed in PBS at 4 °C in darkness over 72 h and stored at −20 °C. Conjugated formation was confirmed by UV–Vis spectroscopy.

2.6. Immunization and antibody preparation

The polyclonal antibodies were produced in rabbits according to Sheth and Sporns (1991). The rabbits had free access to drinking water and commercial standard laboratory diet. It was housed

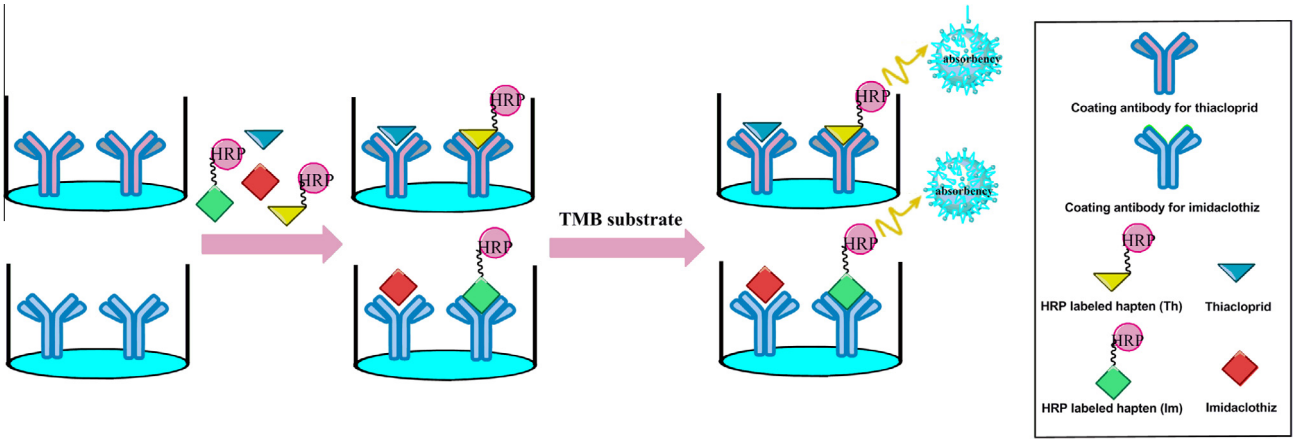

Fig. 1. Schematic illustration of the bi-enzyme tracer dc-ELISA.