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A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay for chlorpyrifos residue determination in Chinese agricultural samples

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A monoclonal antibody-based competitive antibody-coated enzyme-linked immunosorbent assay (ELISA) was developed and optimized for determining chlorpyrifos residue in agricultural products. The IC₅₀ and IC₁₀ of this ELISA were 3.3 ng/mL and 0.1 ng/mL respectively. The average recoveries in six agricultural products were between 79.5% and 118.0%, with the intra-assay coefficient of variation being less than 8%. The limit of detection for all tested products was 30 ng/g. To the best of our knowledge, this assay has the best specificity among all the published research on ELISAs for chlorpyrifos.

Keywords: Chlorpyrifos; monoclonal antibody; enzyme-linked immunosorbent assay (ELISA); agricultural products.

Introduction

Due to its broad spectrum, chlorpyrifos is one of the most intensively used organophosphate insecticides in agriculture and urban pest management. Unfortunately, although chlorpyrifos is only moderately toxic to mammalian species, it is extremely toxic to a wide range of non-target aquatic organisms. Furthermore, it is a suspected endocrine disruptor.[1] Due to its widespread use, high persistence and toxicity, chlorpyrifos has been listed as a candidate for priority review under the National Registration Authority's Existing Chemical Review Program by the U.S. National Drugs and Poisons Schedule Committee in 2000. However, in China, chlorpyrifos is still recommended by the government to replace some organophosphorus pesticides which have even higher toxicity than chlorpyrifos, which has made chlorpyrifos one of the most used pesticides in China. As a result, the high chlorpyrifos residue in food[2,3] and in the environment[4] has frequently been reported. It is necessary to develop a simple, rapid, and selective method for determining chlorpyrifos residue.

Immunoassays, especially the enzyme-linked immunosorbent assay (ELISA), have been emerging as an attractive alternative to the traditional chromatographic methods.[5] Due to their simplicity and cost-effectiveness, immunoassays can be used for the high sample throughput and on-site screening of pesticide residue.[6] Several polyclonal or monoclonal antibodies-based ELISAs have been developed for chlorpyrifos;[1,7−11] however, they were mainly used to detect chlorpyrifos in water, soil and oil samples. Manclus[7,10] used a monoclonal antibody (Mab) coated to microtiter plates to analyze chorpyrifos in water and achieved a limit of detection (LOD) of 6.0 ng/mL. Using polyclonal antibody, Brun[9] established an indirect competitive immunoassay, with a LOD of 10 ng/g in olive oil.

Immunoassays for pesticides are usually carried out under physiological conditions, and many factors will influence their performance,[12−14] such as pH, ionic strength and presence of detergent. These factors can directly affect the assay sensitivity by changing the interaction of the antibody with the conjugated hapten. Continuous effort has to be made in optimizing the assays. Information obtained in the optimization process using well-controlled samples may be greatly useful in solving problems arising from validation studies with real samples. In our previous study, we produced a chlorpyrifos-specific Mab with IC₅₀ of 80.8 ng/mL (obtained from the indirect competitive ELISA).[15] In this work, we developed and optimized a direct competitive ELISA, which was successfully utilized in determining chlorpyrifos residues in six agricultural products.
Materials and methods

Chemicals and equipment

All reagents were of analytical grade unless specified otherwise. Chlorpyrifos was obtained from the National Standards Company (Beijing, China). Ovalbumin (OVA, MW 45 000), Bovine serum albumin (BSA, MW 67 000), N-hydroxysuccinimide (NHS), and N,N′-dicyclohexylcarbodiimide (DCC) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Complete and incomplete Freund’s adjuvants were obtained from Institute of Bio-products (Beijing, China). Tetramethylbenzidine (TMB), tri-n-butyl amine (TBA), triethyl amine (TEA), iso-butyl chloroformate and other chemical reagents were purchased from Shanghai Chemical Reagents Company (Shanghai, China). The ELISA was carried out in 96-well polystyrene microplates (COSTAR, New York, USA). Ultraviolet-visible (UV-vis) spectra were recorded on a spectrophotometer (Xinmao, Shanghai, China). ELISA plates were washed with a dispenser electric microplate washer (Beijing Tuopu Analytical Instruments, Beijing, China). The absorbances were measured with a 550 plate reader (Bio-Rad, Hercules, California, USA.).

Preparation of hapten-protein conjugate

The structures of chlorpyrifos and haptens are shown in Figure 1. The synthesis of hapten and the preparation of Mab were reported in our previous publication.[15] The synthesis strategy and Mab production procedures were described in our studies on parathion ELISA determination.[16, 17] The hapten synthesis method is simply described as follows: A solution of 3,5,6-trichloropyridin-2-ol (21.6 mmol) in 15 mL of acetonitrile was added dropwise to a stirred mixture of ethyl dichlorothiophosphate (27.8 mmol), 20 g of finely ground K2CO3 and 20 mL of acetonitrile. After stirring for 1 h at room temperature, the mixture was filtered through celite, and the solvent was removed under reduced pressure. The residue (1.78 mmol) was dissolved in 3 mL of MeOH and a 2 mL solution of KOH (4.45 mmol) and 6-aminohexanoic acid (1.96 mmol) in MeOH was then added dropwise. The reaction was carried out in an ice-water bath. After stirring for 5 min, the reaction mixture was filtered and extracted with 1 M HCl-chloroform. The extract was dried over Na2SO4 and the solvent was evaporated. The residue was subjected to column chromatography [silica gel, petroleum ether: ethyl acetate: formic acid (90:10:1)] to obtain the final product.

Hapten-OVA and HRP-hapten conjugates were prepared by mixed anhydride method and isobutyl chloroformate method, respectively. Conjugate formations were confirmed spectrophotometrically.

Antibody-coated ELISA

Unless otherwise mentioned, PBS is 10 mM phosphate buffer, pH 7.4. PBST is PBS with 0.05% Tween 20. Citrate buffer is a 40 mM solution of sodium citrate, pH 5.5. The substrate solution contains 0.01% TMB and 0.004% H2O2 in citrate buffer.

All incubations were carried out at 37°C. All assay buffers and solutions were kept at room temperature to avoid the effects of temperature changes on assay precision. After each step, plates were washed four times with PBST.

ELISA plates were coated with 100 µL per well of the appropriate concentration of antibody in coating buffer (PBS). Then, 50 µL per well of the analyte solution was added, followed by 50 µL per well of the appropriate HRP-hapten concentration in assay buffer. The mixture was incubated for 1 h and then 100 µL per well of substrate solution was added. The reaction was stopped after 15 min by adding 50 µL of 2 M H2SO4 and the absorbance was measured at 450 nm. Standard curve data were fitted to a four parameter logistic equation.

Assay optimization

Solvent effect

Competitive curves were performed from chlorpyrifos standards and HRP-hapten dissolved in PBS buffers [containing different proportions of methanol, acetone and acetonitrile (from 0.6 to 20 %)].

pH studies

PBS solutions were adjusted to different pH values (ranging from 4.5 to 9.0) by adding 0.01 M HCl or 0.01 M NaOH. These buffers were used to prepare chlorpyrifos standards.

Fig. 1. Structure of chlorpyrifos and hapten used for immunogen and coating antigen.
Sensitive monoclonal antibody-based ELISA

and HRP-hapten solutions, which were used for the competitive immunoassay to obtain the standard curves.

Ionic strength studies
To build competitive curves, chlorpyrifos standards and HRP-hapten were dissolved in PBS buffers (containing the selected solvent and pH value) of different ionic strength, which were prepared by adding NaCl (from 0 to 0.2 M) to PBS buffer solution.

Detergent studies
To build competitive curves, chlorpyrifos standards and HRP-hapten were dissolved in PBS buffers containing different detergents, which were prepared by adding detergent (0–0.2% for Tween 20, 0–0.5% for BSA, 0–2.0% for milk) to PBS buffer solution. Optimized values were used for other parameters, such as solvent, ionic strength and pH.

Cross-reactivity determination
The specificity of the optimized assay was evaluated against several organophosphorus insecticides, and calculated as follows: CR(%) = [IC$_{50}$ (chlorpyrifos) / IC$_{50}$ (interferent)] × 100.

Data analysis
Standards and samples were run in triplicate wells, and the mean absorbance values were calculated. The competition curves were obtained by plotting absorbance against the logarithm of analyte concentration, and were fitted to a four-parameter logistic equation: $y = (A-B)/(1-(x/C)^D) + B$, where A is the maximal absorbance, B is the minimum absorbance, C is the concentration producing 50% of the maximal absorbance, D is the slope at the inflection point of the sigmoid curve, x is the concentration of pesticide, and y is the OD value. From the equation, IC$_{50}$ values (concentrations at which the binding of the antibody to the coating antigen are inhibited by 50%) were determined.

Fig. 2. Effect of three kinds of solvents on ELISA performance.

Spiked samples analysis
Standards and samples were run in triplicate wells. Determination of spiked samples was performed by interpolating their mean absorbance values in the standard curve run in the same plate. Using selected ELISA, the chlorpyrifos level was determined in six agricultural samples, including carrot, Chinese cabbage, tomato, potato, green pepper and savoy cabbage, all purchased from the local market. The samples were first chopped or ground into fine pieces and each 5.0 g sample was then spiked with 0.5 mL chlorpyrifos standard solution in acetonitrile. Next, the spiked samples were placed in darkness for 24 h. To each fortified sample, acetonitrile (9.5 mL) was added, followed by 1 min vortex. After being centrifuged for 5 min (4000 rpm), 1.0 mL of the superstratum was transferred and evaporated under a gently nitrogen flow. The residue was dissolved with in 5.0 mL of 5% methanol-PBS. After being vigorously shaken for 5 min by a rotation shaker, the residue was determined by the developed ELISA. After the procedures
described above, the matrix of the samples had been diluted 10 times to decrease the matrix interference. No other clean-up procedures were used. The validation experiment was also carried out by gas chromatography (GC) according to NY/761-2004 as required by Criterion of Ministry of Agriculture, China.

Results and discussion

The sensitivity of the ELISA is represented by the IC₅₀ value. The lower IC₅₀ indicates higher sensitivity. An ideal ELISA method should be fast and easy to perform. Unfortunately, the properties of each sample may influence the reliability of the analysis. Consequently, a successful ELISA method very often has to be optimized for each specific sample. Among other factors, some buffer-related factors such as solvents, ionic strength, pH and detergent were the most common ones which can be manipulated to enhance the analytical sensitivity.

**Organic solvent tolerance of the ELISA**

Chlorpyrifos is a non-polar pesticide, its stock solution is commonly prepared using organic solvents. The standards are then prepared in PBS or water by serial dilutions from the stock solution. Moreover, solvents are often used to extract analytes from samples in immunoassays. Therefore, it is necessary to examine the effect of the solvents on the immunoassays. In this study, methanol, acetone and acetonitrile were examined (Fig. 2). A similar trend was observed for acetone and acetonitrile, Maximum signal (A_max) decreased while more organic solvents were added to the assay buffer. The sensitivity decreased with the increase of the solvent concentration; however, it increased gradually after the solvent concentration reached beyond 5 % for acetonitrile and 10 % for acetone. For methanol, the A_max was also decreased with the increasing methanol concentration; however, A_max changed only slightly (from 1.05 to 0.93) when the solvent concentration increased from 0.6 % to 5.0 %. The IC₅₀ ranged from 14.9 ng/mL to 22.3 ng/mL, indicating the assay sensitivity using methanol as a solvent was higher than those using the other two solvents. In general, adding organic solvents at the competition step has a negative impact on assay sensitivity, probably due to their influence on the antibody-analyte interactions, and/or on the conformational changes of the macromolecules participating in the interaction.

Taking into account of A_max, IC₅₀ and solvent concentration necessary for sample analysis, 5 % methanol was selected as the optimal organic solvent parameter.

**Effect of pH on the ELISA performance**

Like many other ELISAs, the immunoassay for chlorpyrifos was also pH-sensitive (Fig. 3); however, A_max and IC₅₀ were relatively constant at pH from 5.8 to 7.2. When the system was adjusted to be more acidic (from 5.8 to 4.5), the A_max was decreased from 1.0 to 0.7 and the corresponding IC₅₀ increased from 12.4 to 35.3 ng/mL. On the other hand, when the pH changed from 7.2 to 9.0, A_max was reduced from 1.1 to 0.7, and IC₅₀ increased from 9.4 to 51.2 ng/mL. The most preferable pH is 7.2, at which IC₅₀ is the lowest and A_max close to the maximum. Considering the nonionizable nature of chlorpyrifos, the effect observed may be related to the conformational changes of the macromolecules participating in the interaction. Finally, pH 7.2 was also preferred for the buffer in the competition step of the immunoassays. The IC₅₀ and IC₁₀ of the optimized
ELISA were 9.4 and 0.5 ng/mL, respectively. In previous studies on chlorpyrifos ELISA, a slight acidic pH (6.0 or 6.5) was selected as the optimal value.\[^9,21\] It was found that further increasing pH decreased or had no effect on sensitivity. The difference may be due to the various tolerance of different antibodies to acidic or basic condition.

**Effect of ionic strength on the ELISA performance**

Imunoassay performance was investigated in media with ionic strength from 0 to 0.2 M (Fig. 4). $A_{\text{max}}$ was 1.1 at zero ionic strength and it reduced slightly when the ionic strength increased. $A_{\text{max}}$ was 0.9 at 0.2 M, indicating the ionic strength had a strong impact on the sensitivity. The IC$_{50}$ was the lowest at 0.01 M, which was selected for the buffer in the competition step of the chlorpyrifos immunoassays. The IC$_{50}$ and IC$_{10}$ of the optimized ELISA were 3.3 ng/mL and 0.1 ng/mL, respectively.

**Effect of detergents on the elisa performance**

Tween 20, BSA and skimmed milk, were commonly used detergents in ELISA development. The influence of these detergents on both the signal and sensitivity of chlorpyrifos ELISA was studied, using the optimized buffer concentration and pH conditions. It turned out that detergents had a significantly negative influence on the sensitivity (Fig. 5). IC$_{50}$ increased with the increase of the detergent concentrations. It reached up to 147.2 ng/mL with 0.05% Tween 20 in buffer. In addition, absorbance did not fit well to a sigmoidal curve while the Tween 20 concentration was increased to 0.1% or 0.2%. Although the $A_{\text{max}}$ was improved by adding BSA or skimmed milk in the competition buffer, the sensitivity was not. Thus it could be concluded that the buffer without any detergent was the best for this particular assay.

Vanderlaan had reported that the nonionic detergent Tween 20 in buffers reduced nonspecific binding and improved sensitivity.\[^{22}\] Brun\[^9\] selected 0.5% Tween as the optimal detergent for chlorpyrifos ELISA determination. However, the opposite effect of Tween 20 has also been reported.\[^{23,24}\] It was believed that nonspecific hydrophobic interactions between the detergent and the non-polar analytes disturbed the specific analyte-antibody interaction. Sometimes adding BSA instead of Tween 20 in the buffer would obtain satisfactory result.\[^{24}\] Skimmed milk powder has been used to enhance the sensitivity, by reducing well to well variability or lowering the background. This study showed that the detergent had a negative effect on chlorpyrifos ELISA, which could also be explained by the nonspecific hydrophobic interactions between the detergent and chlorpyrifos.

In conclusion, the optimal condition for the chlorpyrifos immunoassay was 5% methanol in the 0.01 M PBS buffer at pH of 7.2. The IC$_{50}$ of the non-optimized assay was 14.9 ng/mL. The IC$_{50}$ of the optimized assay was 3.3 ng/mL, which was almost a 4.5-fold improvement on the assay sensitivity in comparison with that of the non-optimized one.
Cross-reactivity studies

Assay specificity was evaluated by using organophosphorus insecticides and their metabolites which are structurally similar to chlorpyrifos. Several chemically related pesticides were also selected, considering their widespread agricultural and domestic uses. IC\textsubscript{50} and CR data for each compound are given in Table 1. Although the non-optimized and optimized assay had a negligible CR with many tested compounds, the latter was less interfered by chlorpyrifos-methyl and chlorpyrifos-oxon. Brun et al.[9] developed two optimized ELISAs for chlorpyrifos with IC\textsubscript{50} 271 ng/mL and 7 ng/mL, but both showed a higher interference from chlorpyrifos-oxon (140 % and 50 %). Such interference had been reported by many other researchers.[8,21] The lowest CR for chlorpyrifos-methyl that was ever been reported was 26 % in a magnetic particle–based enzyme immunoassay.[25] The CR for the optimized assay in our study was 12.5 %. Previous immunoassays for chlorpyrifos showed variable CR for fenchlorphos, which was up to 75 %.[26] In this study, both optimized and non-optimized assays have negligible CRs for fenchlorphos. Chlorpyrifos-oxon, the biologically active phosphate ester analogue of chlorpyrifos, had poor reactivity in the optimized assay (0.6 %) with a CR which is smaller than the values in other studies.[9,11] The immunoassay developed in this study may be highly specific for chlorpyrifos (except for the metabolite chlorpyrifos-methyl). In addition, the direct ELISA developed in this study is faster and simpler than indirect ELISA developed by other researchers. The assay time for an indirect ELISA format is at least 3.5 h. In contrast, it is only 1.5 h for a direct ELISA. It is crucial to have a short assay time for an ELISA kit.

### Table 1. Cross reactivity of chlorpyrifs-related compounds, their metabolites and other extensively used pesticides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Optimized ELISA</th>
<th>Non-optimized ELISA</th>
<th>Gabaldon’s ELISA [8]</th>
<th>Brun’s ELISA 1 [11]</th>
<th>Brun’s ELISA 2 [11]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC\textsubscript{50} (ng/mL)</td>
<td>CR (%)</td>
<td>IC\textsubscript{50} (ng/mL)</td>
<td>CR (%)</td>
<td>IC\textsubscript{50} (ng/mL)</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>3.3</td>
<td>100</td>
<td>14.9</td>
<td>100</td>
<td>2.13</td>
</tr>
<tr>
<td>chlorpyrifos-methyl</td>
<td>26.5</td>
<td>12.5</td>
<td>27.6</td>
<td>54</td>
<td>2.08</td>
</tr>
<tr>
<td>chlorpyrifos-oxon</td>
<td>565.4</td>
<td>0.6</td>
<td>153.7</td>
<td>9.7</td>
<td>164</td>
</tr>
<tr>
<td>3,5,6-trichloro-2-pyridinol</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>&gt;10\textsuperscript{3}</td>
</tr>
<tr>
<td>fenchlorphos</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>14.9</td>
</tr>
<tr>
<td>atrazine</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>/</td>
</tr>
<tr>
<td>parathion</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>/</td>
</tr>
<tr>
<td>parathion-methyl</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>/</td>
</tr>
<tr>
<td>diazinon</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>&gt;10\textsuperscript{3}</td>
</tr>
<tr>
<td>fenthion</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>/</td>
</tr>
<tr>
<td>fenitrothion</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.03</td>
<td>&gt;10\textsuperscript{3}</td>
<td>&lt;0.1</td>
<td>/</td>
</tr>
</tbody>
</table>

\*IC\textsubscript{50} value could not be determined accurately due to the limited solubility at high concentrations, however, it was clear that inhibition was less than 50 % at 1000 ng/mL.

Agricultural samples analysis

Six agricultural samples were spiked with chlorpyrifos and analyzed by the optimized assay. Carrot, Chinese cabbage, tomato, potato, green pepper and savory cabbage were fortified with chlorpyrifos at 30.0, 50.0, and 100.0 ng/g, respectively. The fortifications of agricultural samples were carried out by incubating the chopped vegetable leaves or grounded slurry in fortification solutions for 24 h. Thus, pesticides might have penetrated deeply into the interior of the leaves or grains during the incubation period. Results in Table 2 show that pesticides could be well recovered by the extraction procedure used in this study. The mean recoveries ranged from 79.5 % to 117.9 % for the six samples, with the intra-assay CV (coefficient of variation) of less than 8 %. As a result, the LOD of this ELISA was 30 ng/g. For all the samples, the GC data was well correlated to the ELISA results (r\textsuperscript{2} = 0.9741, P < 0.05) and the slope of the linear regression curve was 1.06 with the intercept of 0.04. The validation data from GC suggested that the assay was suitable for the detection of chlorpyrifos in all six agricultural samples.

Gabaldon et al.[11] developed an acetone direct extraction procedure for chlorpyrifos residue determination in pepper and tomato. The procedure was described as follows. A 5 g sample and 10 mL of acetone were blended for 10 min, and the supernatant vacuum filtered through 0.45 μm nylon filters using a Millipore extraction device. The extracts were then conditioned by diluting 1/25 (v/v) in PBS and checked for chlorpyrifos with ELISA. The mean recovery was 94 %, and the LOD was 400 ng/g. It was evident that the acetonitrile direct extraction procedure developed in this study was simpler and more efficient.
Table 2. Recoveries of chlorpyrifos from spiked samples by the optimized ELISA and GC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked (ng/g)</th>
<th>Measured(^b) (ng/g)</th>
<th>Mean Recovery(^b) (%)</th>
<th>Measured(^c) (ng/g)</th>
<th>Mean Recovery(^c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>30.0</td>
<td>35.2 ± 1.8</td>
<td>117.3</td>
<td>30.7 ± 1.8</td>
<td>102.4</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>56.8 ± 3.4</td>
<td>113.5</td>
<td>49.3 ± 2.5</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>94.0 ± 1.3</td>
<td>94.0</td>
<td>92.8 ± 2.7</td>
<td>92.8</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>34.3 ± 1.2</td>
<td>114.2</td>
<td>31.4 ± 0.8</td>
<td>104.8</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>50.0</td>
<td>51.2 ± 2.2</td>
<td>102.4</td>
<td>49.3 ± 1.0</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>92.1 ± 1.4</td>
<td>92.1</td>
<td>95.3 ± 2.1</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>29.0 ± 1.8</td>
<td>96.7</td>
<td>27.2 ± 2.3</td>
<td>90.8</td>
</tr>
<tr>
<td>Tomato</td>
<td>50.0</td>
<td>45.6 ± 2.5</td>
<td>91.3</td>
<td>47.8 ± 3.0</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>79.5 ± 5.6</td>
<td>79.5</td>
<td>90.0 ± 2.4</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>27.7 ± 1.1</td>
<td>92.2</td>
<td>28.1 ± 2.9</td>
<td>93.6</td>
</tr>
<tr>
<td>Potato</td>
<td>50.0</td>
<td>55.2 ± 0.8</td>
<td>110.3</td>
<td>49.3 ± 2.8</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>93.6 ± 3.1</td>
<td>93.6</td>
<td>97.4 ± 4.2</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>34.7 ± 2.4</td>
<td>115.7</td>
<td>30.2 ± 2.5</td>
<td>100.7</td>
</tr>
<tr>
<td>Green pepper</td>
<td>50.0</td>
<td>55.3 ± 3.5</td>
<td>110.7</td>
<td>52.4 ± 2.9</td>
<td>104.8</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
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<td>105.3</td>
<td>98.9 ± 6.0</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>33.2 ± 2.3</td>
<td>110.6</td>
<td>29.5 ± 2.5</td>
<td>98.3</td>
</tr>
<tr>
<td>Savoy cabbage</td>
<td>50.0</td>
<td>58.9 ± 3.3</td>
<td>117.9</td>
<td>51.5 ± 1.5</td>
<td>103.1</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>93.9 ± 4.0</td>
<td>93.9</td>
<td>97.3 ± 5.6</td>
<td>97.3</td>
</tr>
</tbody>
</table>

\(^b\): Results were determined by optimized ELISA; \(^c\): Results were determined by GC. The GC was performed according to NY/761-2004 (criterion of Ministry of Agriculture, China).

Conclusions

A fast, sensitive and specific competitive ELISA for determination of chlorpyrifos residue in Chinese agricultural samples was developed. The optimal condition for the chlorpyrifos immunoassay was 5% methanol in the 0.01 M PBS buffer (pH 7.2). The IC\(_{50}\) and IC\(_{10}\) of the optimized ELISA were 3.3 and 0.1 ng/mL, respectively. The LOD of the ELISA for six tested agricultural samples was 30 ng/g, which was less than 100 ng/g, the Chinese official maximum residue limit of chlorpyrifos in agricultural products.

Acknowledgments

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