



Strip-based immunoassay for the simultaneous detection of the neonicotinoid insecticides imidacloprid and thiamethoxam in agricultural products

Ting Xu^a, Qi Gong Xu^b, Hao Li^a, Jia Wang^a, Qing X. Li^c, Weilin L. Shelver^d, Ji Li^{a,*}

^a College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

^b Chinese Research Academy of Environmental Sciences, Beijing 100012, China

^c Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 96822, USA

^d USDA-ARS Biosciences Research Laboratory, 1605 Albrecht Boulevard, Fargo, ND 58102, USA

ARTICLE INFO

Article history:

Received 13 June 2012

Received in revised form

26 August 2012

Accepted 28 August 2012

Available online 4 September 2012

Keywords:

Imidacloprid

Thiamethoxam

Insecticide analysis

Strip immunoassay

Food safety

ABSTRACT

A semiquantitative strip immunoassay was developed for the rapid detection of imidacloprid and thiamethoxam in agricultural products using specific nanocolloidal gold-labeled monoclonal antibodies. The conjugates of imidacloprid–BSA, thiamethoxam–BSA and goat anti-mouse IgG were coated on the nitro-cellulose membrane of the strip, serving as test lines and control line, respectively. The flow of the complexes of gold labeled antibodies and insecticides along the strip resulted in intensive color formed on the test lines inversely proportional to the concentrations of imidacloprid and thiamethoxam. The visual detection limits of imidacloprid and thiamethoxam in assay buffer were 0.5 and 2 ng mL⁻¹, respectively. Matrix interference of cucumber, tomato, lettuce, apple, and orange on the strip assay could be eliminated by diluting sample extracts with assay buffer. The strip analysis of imidacloprid and thiamethoxam in these samples was compared to liquid chromatography–mass spectrometry and the results were in good agreement. The strip was stable for storage more than 5 months at 4 °C. The strip assay is a rapid and simple method for the simultaneous screening of imidacloprid and thiamethoxam in agricultural products.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Neonicotinoids are among the most effective insecticides for the control of sucking insects such as aphids, whiteflies, leaf- and plant-hoppers, thrips, some micro-lepidoptera and a number of coleopteran insects. Their physico-chemical properties make them useful for a wide range of applications, including foliar, seed treatment, soil drench and stem injection. Neonicotinoids act as agonists on the insect nicotinic acetylcholine receptor [1]. The broad spectrum of efficacy, together with systemic and translaminar action, pronounced residual activity and a unique mode of action, makes the neonicotinoids the most rapidly expanding insecticidal class in the world. Imidacloprid and thiamethoxam belong to the first and the second generation neonicotinoids, respectively, and have been used to protect a wide range of crops such as vegetables, rice, cotton, fruit, tobacco and cereals [2–5]. Imidacloprid is the most widely used insecticide of this class [2].

One of the most serious problems of the neonicotinoids is their toxicity to honey bees [6,7]. As a consequence of the wide application of neonicotinoids, their residues may occur in agricultural products such as fruits and vegetables and, potentially, pose a hazard for consumers. Various analytical procedures have been utilized for the determination of neonicotinoid insecticides in foods, agricultural and environmental samples. Modern instrumental methods such as liquid chromatography–mass spectrometry (LC–MS) have shown excellent sensitivity and selectivity that enable analysis of neonicotinoid insecticides in diverse samples at trace levels [8–11]. They also provide solid evidence to confirm both the identity and quantity of the residues detected. Although being sensitive and specific, these methods can be time-consuming and expensive. Therefore, it is necessary to develop a portable and rapid method for the detection of neonicotinoids. Enzyme-linked immunosorbent assays (ELISAs) have been developed for the rapid monitoring of the neonicotinoids in different matrices [12–18]. However, it is a challenge to make a one step multianalysis using these assays. Additionally, these assays are not portable. Specific nanocolloidal gold-labeled antibodies have been widely used in immunochromatographic assays for low molecular weight analytes [19–23]. Compared with ELISA, this

* Corresponding author. Tel./fax: +86 10 6273 2017.
E-mail address: lijl@cau.edu.cn (J. Li).

strip format possesses several advantages, including portability, simplicity, and the ability to be used without specialized laboratory equipment in an on-site location.

Two specific monoclonal antibodies (MAbs) against imidacloprid and thiamethoxam have been produced in our previous studies [12,14]. The MAbs based ELISAs have been applied to the determination of imidacloprid and thiamethoxam in various matrices [24–26]. The aim of this work was to develop a multi-analysis of imidacloprid and thiamethoxam in one step. Immunochromatographic strips were prepared using the nanocolloidal gold-labeled specific MAbs. This new method was compared with the LC–MS method for the multianalysis of imidacloprid and thiamethoxam in agricultural products.

2. Materials and methods

2.1. Materials and reagents

All reagents were of analytical grade unless specified otherwise. Reference standards of imidacloprid (99.5%) and thiamethoxam (99.7%) were purchased from the Institute for the Control of Agrochemicals, Ministry of Agriculture, Beijing, China. Chemicals purchased from Sigma-Aldrich (St. Louis, MO, USA) were bovine serum albumin (BSA), goat anti-mouse IgG, and 3,3',5,5'-tetramethylbenzidine (TMB). Haptens of imidacloprid and thiamethoxam (Fig. 1) and two MAbs specific to either imidacloprid or thiamethoxam were prepared in our previous studies [12,14].

A protein A IgG purification kit was obtained from Pierce (Rockford, IL, USA). Chloroauric acid (HAuCl₄) and trisodium citrate were obtained from Shanghai Chemical Reagents (Shanghai, China). Nitro-cellulose (NC) membranes were purchased from Millipore Corp. (Billerica, MA, USA). Other reagents were purchased from Beijing Reagent Corp. (Beijing, China). Phosphate buffered saline (PBS, 0.01 M phosphate, 0.137 M NaCl, and 0.003 M KCl, pH 7.4) was used as the assay buffer and washing buffer. ELISAs were carried out in 96-well polystyrene microplates (Nalge Nunc International, Copenhagen, Denmark).

2.2. Instrumentation

Absorbances of microplate wells were determined with a microplate reader (Wellscan MK3, Labsystems Dragon, Finland). Nanocolloidal gold particles were scanned with a H-7650 transmission electron microscope (TEM; Hitachi High-Technologies America, Inc., Pleasanton, CA, USA).

LC–MS analysis of imidacloprid and thiamethoxam was performed on an Agilent 1100 series LC–MS system. The LC instrument was equipped with a LichroCart 125–4 LiChrosphere 100 RP-18 (5 μm) column. The MS system was a quadrupole MS equipped with an electrospray ionization (ESI) source and operated in positive ionization mode. The LC–MS system was operated according to the published conditions [27] with slight modifications as follows: the mobile phase was a linear gradient elution of methanol/water with the following methanol content: 0–3 min, 5%; 3–10 min, 5–40%; 10–15 min, 40%; 15–20 min, 40–60%; 20–25 min, 60%; 25–30 min,

60–5%; and 30–35 min, 5% at a flow rate of 1.0 mL min⁻¹. The column temperature was maintained at 40 °C. An aliquot of 20 μL of samples was injected. The operating conditions for ESI were drying gas (nitrogen) flow 10 L min⁻¹; capillary voltage 3500 V; and gas temperature 300 °C. The fragmentor voltage was kept at 20 V.

2.3. Nanocolloidal gold suspension preparation

All glassware used in this section was thoroughly cleaned in aqua regia (HCl/HNO₃ (3/1, v/v)), rinsed in Milli-Q purified water, and oven-dried prior to use. Nanocolloidal gold particles were prepared by slightly modifying the procedures described by Zhou et al. [28]. Briefly, 100 mL of 0.01% HAuCl₄ solution (in Milli-Q purified water) in a 250-mL flask was boiled thoroughly, and then 1.5 mL of 1% trisodium citrate solution was added while stirring. After the color of the solution changed to dark red in about 1 min, it was boiled for additional 5 min, and then the nanocolloidal gold solution was cooled to ambient temperature under stirring. Particles obtained were scanned by a TEM, showing that the particles were relatively uniform in size and the average diameter was around 20 nm. The solution was stored in a brown bottle at 4 °C and used to prepare nanocolloidal gold-MAb conjugate as soon as possible. With the scan between 400 and 600 nm, the above solution has only one maximum absorbent wavelength at around 520 nm.

2.4. Labeling MAb with nanocolloidal gold

MAb was conjugated to nanocolloidal gold according to the methods described by Kranthi et al. [20]. The pH of colloidal gold solution for MAb conjugation was adjusted to 9.0 with 0.1 M K₂CO₃. The optimal concentrations of MAb for conjugation with nanocolloidal gold were determined by titrating aliquots of diluted IgG with colloidal gold. Ten different concentrations (0.01–0.5 mg mL⁻¹) of the diluted MAbs were prepared in 0.2 mL borate buffer (2 mM, pH 9.0), and added separately to 1 mL of the nanocolloidal gold solution. After incubation of the mixture for 10 min, 0.1 mL of 10% NaCl was added to the tubes and the absorbance was measured at 520 nm. The least amount of MAb required to stabilize the colloidal gold was determined from the abscissa in the curve drawn from the concentration and the absorbance.

With gentle stirring, 1 mL of MAb against imidacloprid (0.2 mg mL⁻¹) or MAb against thiamethoxam (0.15 mg mL⁻¹) was added dropwise to 50 mL of nanocolloidal gold solution. The mixture was gently mixed for 10 min, blocked with 5 mL of 1% BSA solution for 30 min and centrifuged at 10,000 rpm, 4 °C for 30 min. The pellet was resuspended in 10 mL of conjugate storage buffer (2 mM sodium borate containing 0.1% BSA and 0.1% sodium azide, pH=7.2) and diluted for use.

2.5. Immobilization of reagents

The hapten–BSA conjugates (imidacloprid–BSA and thiamethoxam–BSA) and goat anti-mouse IgG prepared in PBS were separately immobilized on the NC membrane with a CAMAG Automatic TLC Sampler 4 (CAMAG Muttenz, Switzerland) and served as test lines (T1 and T2) and control line (C), respectively (Fig. 2). The membranes were dried at 37 °C for 1 h. The remaining protein binding sites of the membranes were blocked by immersing them in PBS containing 1% BSA and 2% sucrose for 30 min at ambient temperature and then dried again at 37 °C for 1 h. The optimal amount of each of the two MAb–gold complexes was mixed and then blotted on the glass-fiber conjugate pad, which had been pretreated by soaking in a medium of 0.01 M PBS (containing 2% sucrose and 0.05% Tween 20) and airing at

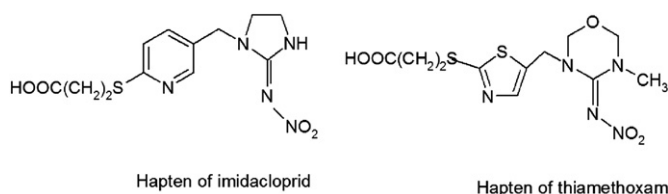


Fig. 1. Chemical structures of the haptens of imidacloprid and thiamethoxam.

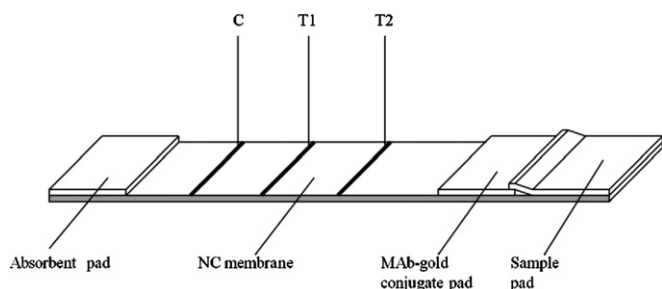


Fig. 2. Schematic diagram of one-step test strip. The control line (C) was coated with goat anti-mouse IgG, and two test lines (T1 and T2) were separately coated with imidacloprid-BSA and thiamethoxam-BSA conjugates. Gold-labeled anti-imidacloprid MAb and gold-labeled anti-thiamethoxam MAb were dispensed on the conjugate pad.

ambient temperature. The MAb–gold conjugate pads were finally lyophilized.

2.6. Assemblage of the one-step strips

An one-sided adhesive polyvinyl chloride (PVC) sheet was used as a support of the strip compositions. The absorbent pad and the MAb–gold conjugates pad were pasted on the sheet by overcrossing 2 mm with the two ends of NC membrane mentioned above. The sample pad was also stuck on the sheet by overcrossing 2 mm with the MAb–gold conjugate pad (Fig. 2). The well-assembled sheet was then cut length-wise into strips ($45 \times 3 \text{ mm}^2$). The strips were stored in a desiccator at 4°C before use.

2.7. Strip assay of imidacloprid and thiamethoxam

Each test solution ($100 \mu\text{L}$) was pipetted onto the sample pad and allowed to migrate by capillary action along the strip. The MAb–gold complexes redissolved by the sample solution were allowed to react with imidacloprid and thiamethoxam (if they existed in the test solution) while the whole mixture was passing along the membrane. After the solution migrated toward the test lines (T1 and T2), different intensities of color on the test lines could be visually observed. The colors of the test lines were compared with those of the negative control (zero imidacloprid and thiamethoxam) and the results could be interpreted to be positive or negative. As the developed assay is a competitive assay, the color intensity of test lines is reversely corrected with the concentration of imidacloprid and thiamethoxam. If the control line is colorless, the test is invalid.

2.8. Sample preparation

Cucumber, tomato, lettuce, apple and orange samples were purchased from local markets. Samples free from residual imidacloprid and thiamethoxam confirmed by LC–MS analysis were used for the matrix effect and recovery studies. For strip determinations, 10 g of the homogenized samples was weighed and placed in 100-mL flasks. The mixtures of imidacloprid and thiamethoxam were fortified into the samples at different levels. The samples were extracted with 20 mL of 70% aqueous methanol for 10 min while shaking at ambient temperature. An aliquot of the extract was filtrated through a Whatman syringe filter ($0.45 \mu\text{m}$). The filtrate was diluted with PBS (20-fold for the cucumber extract and 50-fold for the other extracts) prior to strip test.

For LC–MS determination, an aliquot of 10 g of homogenized sample was weighed in a 100-mL beaker and diluted with 20 mL of deionized water. The sample was transferred to a separation funnel, and then extracted with methylene chloride ($100 \text{ mL} \times 2$).

The organic phases were combined and evaporated to dryness with a rotary evaporator under vacuum at 40°C . The residue was dissolved with approximately 5 mL of ethyl acetate/n-hexane (1:1, v/v) and then loaded on a silica gel column, which was packed with 5 g of silica gel containing 10% water and 2 g of anhydrous sodium sulfate on the top. After washing with 40 mL of ethyl acetate/n-hexane (1:1, v/v), the column was eluted with 100 mL of ethyl acetate. The eluate was dried under vacuum followed by reconstitution of the residue in 1 mL of methanol, which was filtrated prior to the LC–MS.

3. Results and discussion

3.1. Feasibility of multianalysis of imidacloprid and thiamethoxam

As each MAb is selective to the target insecticide, either imidacloprid or thiamethoxam, with negligible cross reaction (less than 0.1%) to others [12,14], it should be feasible to detect multiple insecticides by combining different detections on one NC membrane. The different corresponding coating antigens for each insecticide could be fixed at different sites as respective test lines on the strip. As shown in Fig. 3, in the absence of insecticides, when the mixture of two gold antibody conjugates moved from the glass-fiber conjugate pad to the reaction zone, both test lines (T1 and T2) and control line (C) had color development. For the single insecticide (imidacloprid or thiamethoxam) strip assay, the presence of imidacloprid (or thiamethoxam) at a level of 1000 ng mL^{-1} did not interfere in the binding of gold-labeled MAbs against thiamethoxam (or imidacloprid) to T2 line (or T1 line) (Fig. 3). The results demonstrated no cross-reaction between imidacloprid and thiamethoxam even at these relatively high concentration.

3.2. Optimization of strip assay

The concentrations of immunoreagents were optimized to achieve a good sensitivity and reliability of the test. For this purpose, experiments similar to “checkerboard titration” of microplate format were performed [29]. The amount of antibody and conjugates should be kept low enough to achieve good sensitivity, but must be sufficient to provide an acceptable signal. For further investigation of the assay the following optimal values were chosen: on each strip, the optimal amounts of gold-labeled MAbs against imidacloprid and thiamethoxam blotted on the glass-fiber conjugate pad were 20 ng and 30 ng, respectively. The T1, T2, and C lines were coated with 20 ng of imidacloprid-BSA, 20 ng of thiamethoxam-BSA, and 10 ng of goat anti-mouse IgG, respectively. A $100\text{-}\mu\text{L}$ aliquot of sample solution was dropped on

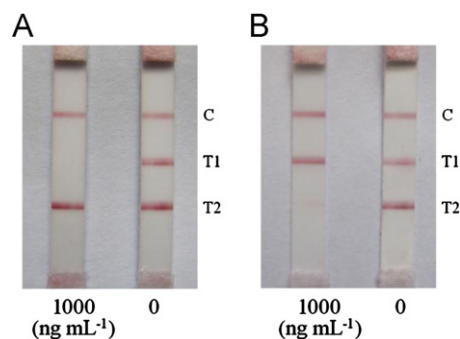


Fig. 3. Strip assays for single insecticide imidacloprid (A) or thiamethoxam (B) at the levels of 0 and 1000 ng mL^{-1} .

the sample pad and the color intensity was read visually after 10 min.

3.3. Visual assessment of assay sensitivity

Serial mixtures of imidacloprid and thiamethoxam standard prepared in PBS (each insecticide having same final concentrations of 0, 0.25, 0.5, 1, 2, 4, and 8 ng mL⁻¹) were assayed in triplicate using the optimized strip test. The visual detection limits of the assay were defined here as the minimum imidacloprid and thiamethoxam concentrations causing the color density of the test lines distinguishably weaker than those in the assay of the negative control sample (it means 100% agreement between result assessments of three repetitions of the same test by three observers).

As shown in Fig. 4, 0.5 ng mL⁻¹ of imidacloprid and 2.0 ng mL⁻¹ of thiamethoxam caused a slight but distinguishable difference compared to the negative control. It can be concluded that the strip assay had visual detection limits for imidacloprid and thiamethoxam at 0.5 and 2.0 ng mL⁻¹, respectively. For imidacloprid concentration > 2 ng mL⁻¹ and thiamethoxam concentration > 8 ng mL⁻¹, the T1 line and T2 line were invisible. It indicated that the strip allows distinguishing each insecticide semiquantitatively in three concentration intervals: < 0.5 ng mL⁻¹, 0.5–2 ng mL⁻¹, and > 2 ng mL⁻¹ for imidacloprid; < 2 ng mL⁻¹, 2–8 ng mL⁻¹, and > 8 ng mL⁻¹ for thiamethoxam. The microplate-based ELISAs for imidacloprid and thiamethoxam were performed similar to the previous studies [12,14] and the limits of detection (LODs) of imidacloprid and thiamethoxam were 0.2 and 0.08 ng mL⁻¹, respectively. It is noteworthy that the sensitivity of imidacloprid is lower than that of thiamethoxam in the microplate-based ELISA format, but the reverse results were observed in the strip assay. The difference possibly resulted from the differential loss of MAb activity caused by the colloidal gold labeling as well as differences in the assay formats—in ELISA the MAb was in homogenous solution while in the strip assay the MAb was particle bound. Although the strip assay sensitivity was lower than that of the microplate-based ELISA format, the strip assay is fast, simple, and portable, providing a much needed tool for on-site monitoring of food sources.

3.4. Cross reactivity of strip assay with neonicotinoid insecticides

The neonicotinoid insecticides including acetamiprid, nitenpyram, thiacloprid, clothianidin, dinotefuran and 6-chloronicotinic acid (the major metabolite of imidacloprid) were used to evaluate the cross reactivity of the test strip. Because the strip assay is semiquantitative, cross reactivity value was defined as the percentage of the LOD of the imidacloprid or thiamethoxam relative to that of the cross-reactant

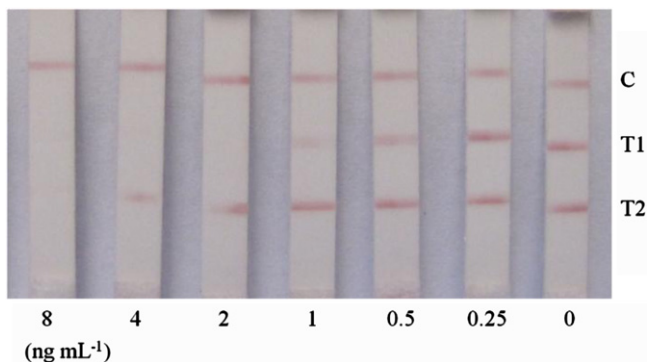


Fig. 4. Strip assays for the multianalysis of standard solutions of imidacloprid and thiamethoxam. The concentrations of two insecticides in each standard solution were the same (0, 0.25, 0.5, 1, 2, 4, and 8 ng mL⁻¹).

by the strip assay. The standard solution containing 1000 ng mL⁻¹ of each compound listed above did not cause the color intensity of either of the test lines visually weaker than the negative control (data not shown). Thus, the cross reactivity of this strip assay was < 0.05% or < 0.2%, based on the LOD values of imidacloprid and thiamethoxam, respectively. The cross reactivity of the strip assay format was similar to those of other immunoassay formats reported previously [12,14].

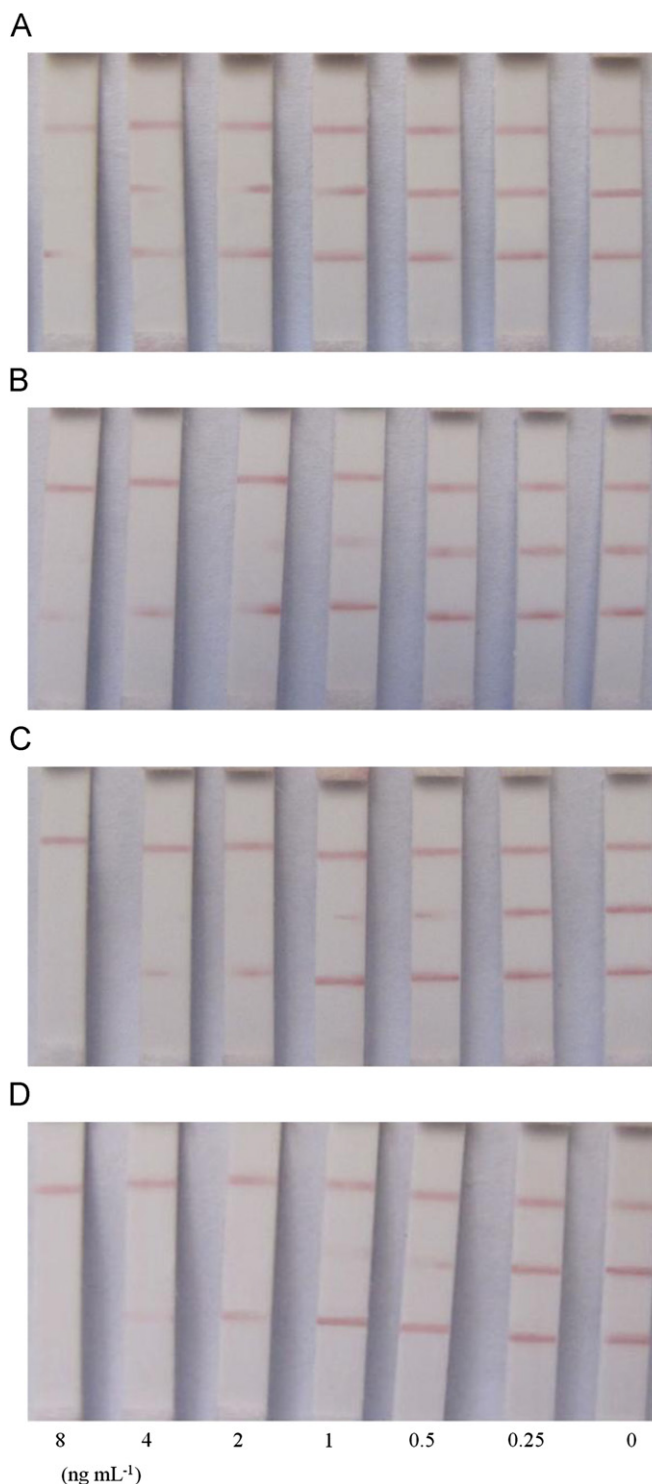


Fig. 5. Strip assays for imidacloprid and thiamethoxam prepared in PBS containing different percentages of orange extract. (A) 10% extract; (B) 5% extract; (C) 2% extract; and (D) no extract. The concentrations of two insecticides in each assay solution were the same (0, 0.25, 0.5, 1, 2, 4, and 8 ng mL⁻¹).

3.5. Evaluation of matrix effects

The possibility of analyzing liquid samples or solid sample extracts with minimum clean-up is one of the most outstanding advantages of immunoassays over commonly used instrumental methods [17,26]. However, immunochemical interactions are not completely free from interferences caused by unidentified compounds of environmental matrices. Moreover, sample matrix can be the cause of an intensive background color generated on membrane during a strip test procedure [30]. Consequently, it could interfere in result interpretation as false positive or negative. Therefore, the influence of the selected matrices on any proposed assay should be determined prior to the application of the method to samples with imidacloprid and thiamethoxam.

The influence of the matrix on the strip test performance was tested by comparing the inhibition by insecticides in PBS and in extracts diluted with PBS [30]. Similar patterns of matrix effects on the strip assay were observed for all sample extracts except cucumber extract. As demonstrated in orange sample, 10% extract (10-fold dilution) provided a more visually intensive background coloring compared with that in assay buffer (Fig. 5A). Five percent extract (20-fold dilution) provided a similar inhibition pattern, but had a slight interference of background coloring as compared with those in assay buffer (Fig. 5B). To eliminate the matrix effect of sample extract, it is necessary to improve the dilution factor to 50-fold, i.e. 2% extract in PBS (Fig. 5C). However, the matrix effect of cucumber extract can be eliminated by 20-fold dilution. It seems cucumber has less matrix effect on the strip assay than the others. These results suggested that imidacloprid and thiamethoxam could

be directly analyzed in properly diluted extracts without a need of clean-up. Based on the dilution factors of extracts, the theoretical LODs of imidacloprid and thiamethoxam in cucumber were calculated as 20 ng g⁻¹ and 80 ng g⁻¹, respectively, and in the other samples were 50 ng g⁻¹ and 200 ng g⁻¹, respectively.

The maximum residue limits (MRLs) of imidacloprid and thiamethoxam in very few agricultural products have been regulated in China. The MRLs of imidacloprid in apples, oranges, and tomatoes set up by the Ministry of Agriculture of China were 0.5, 1.0 and 1.0 mg kg⁻¹, respectively (NY 1500.1.1~1500.30.4-2007). The MRL of thiamethoxam in cucumber was set up at 0.5 mg kg⁻¹ (GB 26130-2010). The LODs of strip assay for two insecticides in corresponding samples are well below the MRLs.

3.6. Detection of imidacloprid and thiamethoxam in agricultural products

This strip was applied to the multianalysis of imidacloprid and thiamethoxam fortified in several agricultural products (Table 1). The concentration intervals distinguished by the strip in samples were adjusted according to the dilution factors in sample extracts. The extract of cucumber was diluted 20-fold prior to the assay, and then negative results (–) were obtained for samples with levels of imidacloprid and thiamethoxam less than 20 ng g⁻¹ and 80 ng g⁻¹, respectively; the weakly positive results (±) were obtained for samples with imidacloprid concentrations of 20–80 ng g⁻¹ and those with thiamethoxam concentrations of 80–320 ng g⁻¹; while samples with imidacloprid concentrations over 80 ng g⁻¹ and those with thiamethoxam over 320 ng g⁻¹

Table 1
Comparison of results determined by the strip assay and LC-MS method.

Samples	Spiked concentration (ng g ⁻¹), imidacloprid/thiamethoxam	Strip assay, ^a n=4 T1/T2	LC-MS (ng g ⁻¹), ^b n=3 imidacloprid/thiamethoxam
Cucumber	0/0	----/----	< LOD
	10/10	----/----	9.5/9.3
	50/50	± ± ± ± / ----	52/50
	200/200	++++/± ± ± ±	195/207
	1000/1000	++++/++++	996/939
Orange	0/0	----/----	< LOD
	20/20	----/----	19/19
	100/100	± ± ± ± / ----	88/90
	500/500	++++/± ± ± ±	505/496
	2000/2000	++++/++++	1800/1970
Apple	0/0	----/----	< LOD
	20/20	----/----	20/18
	100/100	± ± ± ± / ----	115/111
	500/500	++++/± ± ± ±	491/488
	2000/2000	++++/++++	2160/2070
Tomato	0/0	----/----	< LOD
	20/20	----/----	17/23
	100/100	± ± ± ± / ----	103/109
	500/500	++++/± ± ± ±	521/476
	2000/2000	++++/++++	2110/1980
Lettuce	0/0	----/----	< LOD
	20/20	----/----	22/24
	100/100	± ± ± ± / ----	97/117
	500/500	++++/± ± ± ±	562/505
	2000/2000	++++/++++	1790/2120

Visual assessment of test lines was based on three insecticide concentration intervals in cucumber samples: <20 ng g⁻¹ (–), 20–80 ng g⁻¹ (±), and >80 ng g⁻¹ (+) for imidacloprid; <80 ng g⁻¹ (–), 80–320 ng g⁻¹ (±), and >320 ng g⁻¹ (+) for thiamethoxam.

The three insecticide concentration intervals in orange, apple, tomato and lettuce samples: <50 ng g⁻¹ (–), 50–200 ng g⁻¹ (±), and >200 ng g⁻¹ (+) for imidacloprid; <200 ng g⁻¹ (–), 200–800 ng g⁻¹ (±), and >800 ng g⁻¹ (+) for thiamethoxam.

^a Prior to an assay, a cucumber extract was diluted 20-fold with assay buffer and the other sample extracts were diluted 50-fold.

^b Data were the means of triplicate measurements of LC-MS.

were evaluated as positive (+). As the other sample extracts were diluted 50-fold, the three concentration intervals in these samples were $< 50 \text{ ng g}^{-1}$ (-), $50\text{--}200 \text{ ng g}^{-1}$ (\pm), and $> 200 \text{ ng g}^{-1}$ (+) for imidacloprid; $< 200 \text{ ng g}^{-1}$ (-), $200\text{--}800 \text{ ng g}^{-1}$ (\pm), and $> 800 \text{ ng g}^{-1}$ (+) for thiamethoxam. These samples were analyzed by the LC–MS method and the results of the LC–MS method were close to the spiked levels (Table 1). These experiments demonstrated the good correlation between the methods of visually assessed strips and LC–MS.

This strip assay was finally applied to survey the presence of imidacloprid and thiamethoxam in 31 samples purchased from local markets including 7 cucumbers, 7 tomatoes, 5 lettuces, 6 apples, and 6 oranges. Except one tomato recognized as imidacloprid weakly positive (\pm), all samples were imidacloprid and thiamethoxam negative (-). These samples were further confirmed by the LC–MS method and the concentrations of both insecticides in most of the samples were below the LOD. However, imidacloprid was detectable in two tomato samples with the concentrations of 68 ng g^{-1} and 10 ng g^{-1} , respectively. The sample with higher level of imidacloprid corresponded to the weakly positive sample by the strip assay, while the sample with a lower level of imidacloprid was below the LOD of the strip assay. These results confirmed the validity of the strip as well as its value as a screening procedure.

3.7. Stability of the strip assay

The stability of the strip assay was evaluated by comparing the analysis of the imidacloprid and thiamethoxam standard solutions before and after the strip storage. The strips prepared from the same batch were stored at 4°C under desiccated conditions. After 5 months of the storage of the strips, the LOD and color intensity did not show significant differences from those using the fresh strips. It indicated that the strip assay was stable for at least 5 months at 4°C .

4. Conclusion

A semiquantitative one-step strip immunoassay has been developed and applied to the rapid detection of imidacloprid and thiamethoxam residues in agricultural products such as cucumber, tomato, lettuce, apple, and orange. The insecticides could be directly determined without troublesome sample pre-treatment procedures but only diluting sample extracts with assay buffer. The visual results of the assay for all samples are in a good agreement with those of the LC–MS method. Although the sensitivities of the strip assay described here are lower than those of the microplate-based ELISA and LC–MS methods, it is clearly easy, rapid and convenient to perform and requires no equipment. With respect to its overall speed and simplicity, more economical to perform for large numbers of samples, this method

can be used for screening and complemented with the LC–MS method.

Acknowledgments

This project was supported in part by the Ministry of Environmental Protection of the People's Republic of China (201109009), Chinese Universities Scientific Fund (2010JS115), the US National Institute on Minority Health and the National Center for Research Resources (5 G12 RR003061-26) and the US National Institute on Minority Health and Health Disparities (8 G12 MD007601-26).

References

- [1] M. Tomizawa, J.E. Casida, *Annu. Rev. Pharmacol.* 45 (2005) 247–268.
- [2] A. Elbert, M. Haas, B. Springer, W. Thielert, R. Nauen, *Pest Manag. Sci.* 64 (2008) 1099–1105.
- [3] C. Luo, C.M. Jones, G. Devine, F. Zhang, I. Denholm, K. Gorman, *Crop Prot.* 29 (2010) 429–434.
- [4] N. Kaur, B.S. Sohal, K. Singh, *Pestic. Biochem. Phys.* 99 (2011) 280–284.
- [5] D.G. Bottrell, K.G. Schoenly, *J. Asia–Pac. Entomol.* 15 (2012) 122–140.
- [6] M. Henry, M. Béguin, F. Requier, O. Rollin, J.F. Odoux, P. Aupinel, J. Aptel, S. Tchamitchian, A. Decourtye, *Science* 336 (2012) 348–350.
- [7] P.R. Whitehorn, S. O'Connor, F.L. Wackers, D. Goulson, *Science* 336 (2012) 351–352.
- [8] A.D. Muccio, P. Fidente, D.A. Barbini, R. Dommarco, S. Seccia, P. Morrica, *J. Chromatogr. A* 1108 (2006) 1–6.
- [9] W. Xie, C. Han, Y. Qian, H. Ding, X. Chen, J. Xi, *J. Chromatogr. A* 1218 (2011) 4426–4433.
- [10] Z. Xiao, X. Li, X. Wang, J. Shen, S. Ding, *J. Chromatogr. B* 879 (2011) 117–122.
- [11] F. Zhang, Y. Li, C. Yu, C. Pan, *Bull. Environ. Contam. Toxicol.* 88 (2012) 885–890.
- [12] H.J. Kim, W.L. Shelver, Q.X. Li, *Anal. Chim. Acta* 509 (2004) 111–118.
- [13] E. Watanabe, H. Eun, K. Baba, T. Arao, Y. Ishii, S. Endo, M. Ueji, *Anal. Chim. Acta* 521 (2004) 45–51.
- [14] H.J. Kim, W.L. Shelver, E.C. Hwang, T. Xu, Q.X. Li, *Anal. Chim. Acta* 571 (2006) 66–73.
- [15] E. Watanabe, K. Baba, H. Eun, S. Miyake, *Food Chem.* 102 (2007) 745–750.
- [16] S. Fang, B. Zhang, K.W. Ren, M.M. Cao, H.Y. Shi, M.H. Wang, *J. Agric. Food Chem.* 59 (2011) 1594–1597.
- [17] E. Watanabe, K. Baba, S. Miyake, *Talanta* 84 (2011) 1107–1111.
- [18] R. Wang, Z. Wang, H. Yang, Y. Wang, A. Deng, *J. Sci. Food Agric.* 92 (2012) 1253–1260.
- [19] C. Zhang, Y. Zhang, S. Wang, *J. Agric. Food Chem.* 54 (2006) 2502–2507.
- [20] K.R. Kranthi, M. Davis, C.D. Mayee, D.A. Russell, R.M. Shukla, U. Satija, M. Kshirsagar, D. Shiware, S. Kranthi, *Crop Prot.* 28 (2009) 428–434.
- [21] Y.R. Guo, S.Y. Liu, W.J. Gui, G.N. Zhu, *Anal. Biochem.* 389 (2009) 32–39.
- [22] N.A. Byzova, E.A. Zvereva, A.V. Zherdev, S.A. Eremin, B.B. Dzantiev, *Talanta* 81 (2010) 843–848.
- [23] D. Zhang, P. Li, Y. Yang, Q. Zhang, W. Zhang, Z. Xiao, X. Ding, *Talanta* 85 (2011) 736–742.
- [24] T. Xu, C.M. Jacobsen, A.H. Hara, J. Li, Q.X. Li, *Pest Manag. Sci.* 65 (2009) 163–169.
- [25] H.X. Ma, Y.J. Xu, Q.X. Li, T. Xu, X.T. Wang, J. Li, *Food Addit. Contam.* 26 (2009) 713–718.
- [26] T. Xu, K.Y. Wei, J. Wang, H.X. Ma, J. Li, Y.J. Xu, Q.X. Li, *J. AOAC Int.* 93 (2010) 12–18.
- [27] P. Fidente, S. Seccia, F. Vanni, P. Morrica, *J. Chromatogr. A* 1094 (2005) 175–178.
- [28] Y. Zhou, F.G. Pan, Y.S. Li, Y.Y. Zhang, J.H. Zhang, S.Y. Lu, H.L. Ren, Z.S. Liu, *Biosens. Bioelectron.* 24 (2009) 2744–2747.
- [29] S. Wang, C. Zhang, J. Wang, Y. Zhang, *Anal. Chim. Acta* 546 (2005) 161–166.
- [30] M. Blažková, P. Rauch, L. Fukal, *Biosens. Bioelectron.* 25 (2010) 2122–2128.