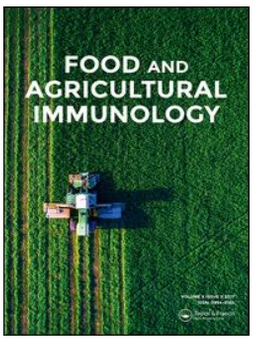

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Development of immunocolloidal strip for rapid detection of pyrimethanil

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ABSTRACT

In this study, we prepared a monoclonal antibody against pyrimethanil, for which we first derived its hapten according to the structural formula of pyrimethanil; we used indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) to screen positive mice for cell fusion; finally, we developed five cell lines (1A5, 1E7, 3A6, 3H12 and 5D4) that produced monoclonal antibodies to pyrimethanil. The monoclonal antibody produced by each cell was detected by ic-ELISA, and the monoclonal antibody produced by the 1E7 cell strain was found to be the most sensitive to pyrimethanil. The IC50 value of this antibody was 4 ng/ml. After using this monoclonal antibody, i.e. 1E7, to perform immunocolloidal gold strip treatment on cucumber samples, the PBS and the cucumber samples cutoff value of Immunochromatographic strips (ICS) was found to be 50 ng/ml; We conclude that ICS can rapidly detect pyrimethanil in cucumber samples.

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KEYWORDS
Pyrimethanil; ic-ELISA; monoclonal antibody; immunochromatographic strips (ICS)

Introduction

In the growing season, harvesting period, and post-harvest storage period of vegetables and fruits, it is necessary to spray fungicides to inhibit the growth of pathogenic fungi, prevent fungal diseases of plants, and prevent fruit from rot. Pyrimethanil (PYR) belongs to the class of aniline pyrimidine bactericides (Esteve-Turrillas, Mercader, Agulló, Abad-Somovilla, & Abad-Fuentes, 2015), which can inhibit fungal secretion of hydrolase, thereby inhibiting the degradation and digestion of infected tissues by fungi (Mercader, Esteve-Turrillas, Consuelo, Antonio, & Antonio, 2012). Pyrimethanil is a broad-spectrum fungicide and can effectively control grey mold on fruits, vegetables, and ornamental plants (Esteve-Turrillas, Mercader, Agulló, Abad-Somovilla, & Abad-Fuentes 2017; Esteve-Turrillas et al., 2015). For example, pyrimethanil is often applied

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in vineyards to inhibit mold and grey mold (Esteve-Turrillas, Agulló, Abad-Somovilla, Mercader, & Abad-Fuentes, 2016). At the same time, the problem of residual bactericide pyrimethanil on the fruit also needs to be resolved.

Studies have shown that fungicides in the natural environment can cause abnormal growth and teratogenicity in amphibians (Bernabò et al., 2016). Pyrimethanil is less toxic in mammals, but studies have shown that pyrimethanil is potentially carcinogenic in mice, rats, dogs, and aquatic organisms (Mandrile, Giovannozzi, Durbiano, Martra, & Rossi, 2018). Therefore, the development of safe, rapid, and efficient detection methods for pyrimethanil in food and the environment is of great significance for food safety.

At present, the most commonly used method for the determination of pyrimethanil residues is gas chromatography, liquid chromatography (Peris-Vicente, Marzo-Mas, Roca-Genoves, Carda-Broch, & Esteve-Romero, 2016), high performance liquid chromatography (HPLC), high performance liquid and gas chromatography-tandem mass spectrometry (Song et al., 2019; Zhang et al., 2019), and tandem mass spectrometry (Samsidar, Siddiquee, & Shaarani, 2017). In recent years, new detection methods have been developed, such as electrochemical sensors (Garrido, Rahemi, Borges, Brett, & Garrido, 2016) and surface enhanced Raman scattering supported by gold nanoparticles (Mandrile, Giovannozzi, Durbiano, Martra, & Rossi, 2018). However, these methods require expensive instruments, professional personnel, and are time consuming. Therefore, it is necessary to develop a cheap, simple, fast, and safe method for detection of pyrimethanil.

In recent years, more and more chemical residues and pollutants have been detected by bioanalytical techniques. Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) is one of the most commonly used immunochemical methods. It is simple to perform, highly sensitive, portable, cost-effective, and uses high sample throughput. However, ic-ELISA also require time and expertise. Therefore, it is not suitable for screening large numbers of samples in the shortest time (Wang et al., 2017). And in practical applications, immunochromatographic methods are simpler and faster than ic-ELISA procedures. It also has the advantage that ic-ELISA does not have: all reagents are included in the strip, and the results are available in 5–10 min. The ICS can be stably stored in various climates for a long time, and the cost is low, the method of use is so simple that even non-professionals can operate (Wang, Gao, Kang, Jia, & Wang, 2011).

In recent years, there have been reports on the development of pyrimethanil ELISA. Among them, Mercader et al. developed an ELISA based on a pyridoxamine polyclonal antibody with a detection limit of 0.024 ng/mL (Mercader, Esteve-Turrillas, Agulló, Abad-Somovilla, & Abad-Fuentes, 2012). Esteve-Turrillas has developed an ELISA based on a pyridoxamine monoclonal antibody with an IC50 value of less than 0.3 ng/mL (Esteve-Turrillas et al., 2015). Cao et al. developed a more sensitive ELISA based on a pyridoxamine monoclonal antibody with an IC50 value of 0.25 ng/mL (Cao, Shi, Le, Tang, & Xie, 2019). However, there has been no research on the development of ICS of pyrimethanil. In this test, we not only prepared a highly specific monoclonal antibody against pyrimethanil, but also developed ICS for pyrimethanil based on this monoclonal antibody. Provide different method for rapid detection of pyrimethanil in foodstuff.

Materials and methods

Chemicals and materials

Pyrimethanil, 2-[(4,6-dimethylpyrimidin-2-yl)amino]benzoic acid, 4-[(4,6-dimethylpyrimidin-2-yl)amino]benzoic acid, 4-[(4,6-dimethylpyrimidin-2-yl)(methyl)amino]benzoic acid, 3-[(4,6-dimethylpyrimidin-2-yl)(methyl)amino]benzoic acid, N1-(4,6-dimethylpyrimidin-2-yl)-N1-ethylbenzene-1,4-diamine, 2-[N-(4-aminophenyl)-N-methylamino]-4,6-dimethylpyrimidine were purchased from J&K Scientific Ltd. (Beijing, China). Bovine Serum Albumin (BSA), Ovalbumin (OVA), 3,3′,5,5′-tetramethylbenzidine, N-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Freund's complete adjuvant, Freund's incomplete adjuvant, and gold chloride trihydrate were purchased from Sigma-Aldrich (St Louis, MO, USA). RPMI-1640 cell culture medium, hypoxanthine-thymidine supplement, polyethylene glycol 1500, hypoxanthine-aminopterin-thymidine supplement, and fetal calf serum were obtained from Gibco BRL (Paisley, UK). Goat anti-mouse IgG (Fc specific) conjugated to horseradish peroxidase was obtained from Jackson Immuno Research (West Grove, PA, USA). All reagents and solvents were analytical grade.

Nitrocellulose (NC) high-flow plus membranes (Pura-bind RP) were purchased from Whatman-Xinhua Filter Paper Co., Ltd. (Hangzhou, China). Polyvinylchloride (PVC) backing material, glass fibre membrane (CB-SB08) used for the sample pad, and absorbance pad (SX18) were obtained from Goldbio Tech Co., Ltd. (Shanghai, China). The strip cutting instrument was CM 4000 (Gene, Shanghai, China).

The hapten and antigen were characterized with a UV/vis scanner (Bokin instruments, Tsushima, Japan). All water used was purified to 18.2 MΩ cm by a water purification system from Millipore (Billerica, MA, USA). The results of ic-ELISA were measured by a Multiskan MKS microplate reader (Thermo Lab systems Company, Beijing, China).

Other instruments used were a vortex (Shanghai Huxi Analysis Instrument Factory Co., Ltd, Shanghai, China), a membrane dispenser (Xinqidian Gene-Technology Co. Ltd, Beijing, China), and water bath (Shanghai Instrument Group Co., Ltd, Supply & Sales Co., Shanghai, China). A BioDot TSR 3000 Membrane Strip Reader (Gene Company Limited, Shanghai Branch, Shanghai (China)).

Hapten synthesis

The structure of the hapten, which was synthesized, is shown in Figure 1. A solution of 2-chloro-4,6-dimethylpyrimidine (713 mg, 5 mmol), 4-aminobenzoic acid (685.7 mg, 5 mmol) in dioxane (20 mL) was stirred at 75°C for 30 h. After cooling to room temperature, it was dried under reduced pressure, and the residue was dissolved with formic acid

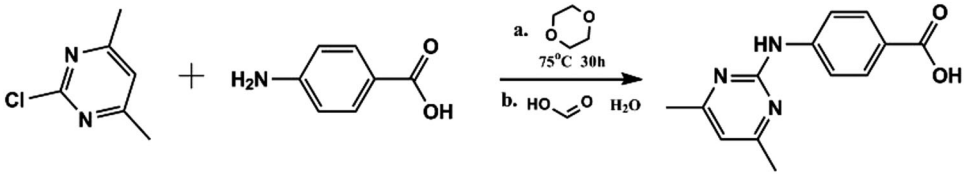


Figure 1. Hapten synthesis of pyrimethanil.

(0.5 ml), and water was slowly added to the precipitate, and the mixture was washed repeatedly with cold water and dried in vacuo. Finally, HPLC was carried out to obtain compound 2 (78 mg) as a pink powder (6% yield).

Antigen preparation

PYR was covalently linked to BSA with the help of EDC to synthesize the immunogen PYR-EDC-BSA (Wang et al., 2017). Briefly, PYR (6 mg), EDC (14 mg), and N-hydroxysuccinimide (8.7 mg) were dissolved in 400μL anhydrous N, N-dimethylformamide (DMF solution A) and stirred at room temperature for 7 h. BSA (10 mg) was dissolved in 3 ml borate buffer solution (solution B). Finally, solutions A and B were mixed and the mixture reacted overnight at room temperature. The product of this reaction was the immunogen PYR-EDC-BSA (Kong et al., 2017). The coating antigen PYR-EDC-OVA was prepared in a similar manner.

Preparation of monoclonal antibodies (mAbs) against PYR

The antigen PYR-EDC-BSA was injected into the subcutaneous tissue of ten 6-week-old female BALB/c mice (Wang et al., 2016). Before immunization, the antigen must be emulsified with Freund's adjuvant. At the first immunization, 100μg antigen was emulsified with Freund's complete adjuvant. In the sequential boosters, 50μg of immunogen was emulsified in Freund's incomplete adjuvant. There was an interval of three weeks between each immunization. After each booster, the serum was collected from the tail vein of each mouse to assess the antibody specificity by ic-ELISA. The mouse with the highest titre and the best specificity to PYR was selected, and intraperitoneal immunization with 20μg immunogen, which was dissolved in 200μL normal saline was done (Yin, Liu, Song, Hua, & Xu, 2015). All animal studies in this work were performed according to institutional ethical guidelines and were approved by the Committee on Animal Welfare of Jiangnan University.

Twenty-one days after intraperitoneal immunization, target mouse splenocytes were fused with SP2/0 myeloma cells by using polyethylene glycol 1500; the fused cells were then distributed into 96-well culture plates (Liu, Yan, Xun, Hua, & Xu, 2015). After one week, we analyzed the supernatants from the plates by ic-ELISA to select the cells with highest titer and the best specificity to PYR. Selected cells were subcloned three times using the limiting dilution method (Cheng, Liu, Song, Hua, & Xu, 2016). Ten 3-week-old BALB/c mice were prepared for producing ascites, and the mAbs were purified by caprylic acid-ammonium sulfate precipitation method, dialyzed for 3 days, and stored at −20°C (Xu et al., 2016).

Development of an ICS assay for pyrimethanil detection

Preparation of gold nanoparticles

As previously reported, sodium citrate was used to reduce gold chloride to produce gold nanoparticles; this was the composition of the immunochromatographic strip (Wang et al., 2017). First, under constant stirring, 30 mL of $AuCl_3{\cdot}HCl{\cdot}4H_2O$ solution (0.01%, w/v) was boiled. Second, 2 mL (1%, w/v) of freshly prepared trisodium citrate was

added under constant stirring. One minute later, the solution turned dark red (Xu et al., 2015); this was then boiled for 15 min, and the volume adjusted to 30 mL with ultrapure water. Finally, the solution was cooled to room temperature and stored at 4°C. We used UV-vis spectrophotometry and transmission electron microscopy to characterize the gold nanoparticles (Yao, Liu, Song, Hua, & Xu, 2017). The UV maximum absorption peak was 520 nm, and the gold nanoparticles' average diameter was 18 ± 2 nm.

Preparation of gold-labelled anti-PYR mAb

We added 1 mL of colloidal gold solution to an optimal amount of 0.1M K_2CO_3 solution. We then added an optimal amount of anti-PYR mAb to the colloidal gold solution and mixed this for 2 h at room temperature. BSA solution (3%, 30 mL) was then added to block unbound sites on the closed colloidal gold to ensure specific adsorption of the gold nanoparticles and stability of the colloidal gold. Centrifugation at 8,000 rpm for 25 min was done 2 h later, and the supernatant discarded. We then dissolved the precipitate in 1 mL ultrapure water and centrifuged it. Finally, were suspended the gold-labelled anti-PYR mAb in borate buffer (BB) (0.002M BB, 1% sucrose, and 0.01% Tween-20, pH 7.2, w/v) and stored at 4°C (Wang et al., 2016).

ICS preparation

The test strip consisted of four parts: a conjugate pad, a nitrocellulose (NC) membrane, a sample pad, and an absorbent pad using a dispensing platform (Bio Dot Inc., Irvine, CA, USA) spray. Goat anti-mouse IgG antibody and the appropriate concentration of coating antigen were applied onto the NC membrane; in this way, a test band (T band) and control band (C band) were obtained. The sample pad immersed in the treatment liquid and the absorbent pad were attached to the dried NC membrane near the end of the T line and C line, respectively, and dried at 37°C for 4 h. The absorbent pad was attached to the upper section of the plate. Finally, the pad was cut into 3-mm wide strips (Isanga et al., 2016; Xu et al., 2016).

ICS assay

The samples and gold-labeled mAbs were mixed; the mixture was allowed to react for 2–3 min, and the test strip was then inserted into the mixture. The liquid was allowed to move from the sample pad to the absorbent pad through capillary action. After 2 min, we checked the control and test lines; if both of the lines turned dark red, the sample was negative (Figure 2(A)). If the control line was dark while the test line was light or devoid of colour, the sample was positive (Figure 2(B)). if both of the lines do not appear (Figure 2(C)), the test procedure is incorrect or the strip is invalid, declaring that the test should be repeated with a new strip. (Mukunzi et al., 2016; Song et al., 2015).

Sample pre-treatment and spiked sample analyses

Placed chopped cucumber samples (3 g) into 50 mL centrifuge tubes, spiked with different amounts of pyrimethanil (0, 60, 120, 240, 480 and 660 ng), and mixed with 9 mL

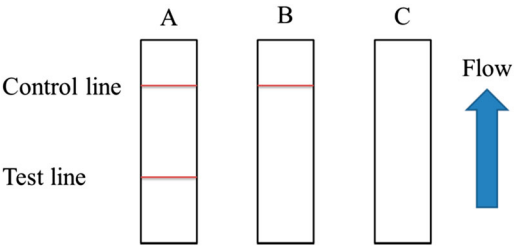


Figure 2. Illustration of typical strip test results. If the sample is negative (A), a positive result could be indicated only if the control line appears (B), and if the control and test line does not appears (invalid) (C).

acetonitrile. The spiked samples were extracted by ultrasonic extraction for 15 min and centrifuged at 2,400×g for 3 min. A volume of 1 mL of the resulting supernatant was dried with nitrogen, and then reconstituted with 5 ml phosphate-buffered saline (PBS), and analyzed by ic-ELISA.

Results and discussion

Synthesis of hapten and artificial antigen

Pyrimethanil has low reactivity with proteins; hence, it is necessary to synthesize haptens to modify small molecules to introduce special active groups such as carboxyl or amino groups. In this study, we used 2-chloro-4,6-dimethylpyrimidine and 4-aminobenzoic acid. After a series of reactions, we obtained an active hapten PYR which contained a carboxyl group with one carbon chain. To synthesize the immunogen and coating antigen, we used EDC to couple PYR with BSA and OVA. The result is shown in Figure 3. The immunogen PYR-EDC-BSA and the coating antigen PYR-EDC-OVA were identified by UV-vis spectroscopy. PYR and BSA had absorption peaks at 271 and 280 nm, respectively; PYR-EDC-BSA had an absorption peak at 285–305 nm. PYR-EDC-OVA also yielded the same results. Therefore, the immunogen and coating antigen were successfully synthesized.

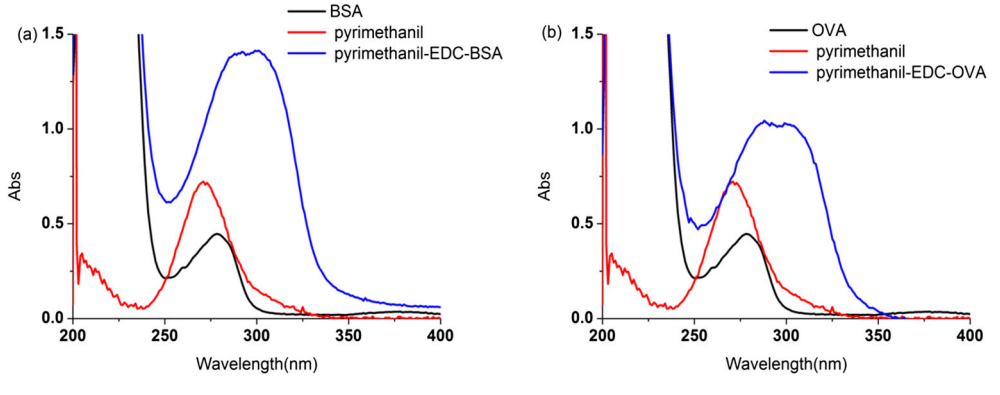


Figure 3. The ultraviolet-visible absorption spectra of artificial antigen. (a) Immunogen PYR-EDC-BSA; (b) Coating antigen PYR-EDC-OVA.

Determination of antibody sensitivity in ic-ELISA

We obtained 1A5, 1E7, 3A6, 3H12, and 5D4 cell lines. Of these five cell lines, mAb 1E7 had the lowest IC_{50} value (4 ng/mL); thus, it was selected for subsequent optimization experiments. The optimal concentrations of the coating antigen and antibody were 0.02 and 0.03 μg/mL, respectively. The antibody had the lowest IC_{50} value and the highest absorbance value (Absmax) at pH 7.4 (Figure 4a) and 0.8% NaCl concentration (Figure 4b). In a series of methanol concentration gradients, the lowest IC_{50} value was obtained with 10% methanol (Figure 4c). Therefore, the optimal conditions for ic-ELISA consisted of pH 7.4, 0.8% NaCl, and 10% methanol in PBS buffer. Under these conditions, IC_{50} had a minimum value of 4 ng/mL. The indirect competitive inhibition non-linear standard curve for pyrimethanil of 1E7 was shown in Figure 4d. The equation of 1E7 was $y = 0.11263 + 178453/ [1+(x/4.00835)^{1.08219}]$ and the correlation coefficient, R^2 was 0.99867. The IC20, IC50, and IC80 for PIC were 1.1939, 4.00835, and 15.47518 ng/mL, respectively. So, we can conduct that the mAb 1E7 was sensitivity to pyrimethanil.

Cross-reactivity (CR)

To assess CR, we analyzed pyrimethanil, 2-[(4,6-dimethylpyrimidin-2-yl)amino]benzoic acid, 4-[(4,6-dimethylpyrimidin-2-yl)amino]benzoic acid, 4-[(4,6-dimethylpyrimidin-2-yl)(methyl)amino]benzoic

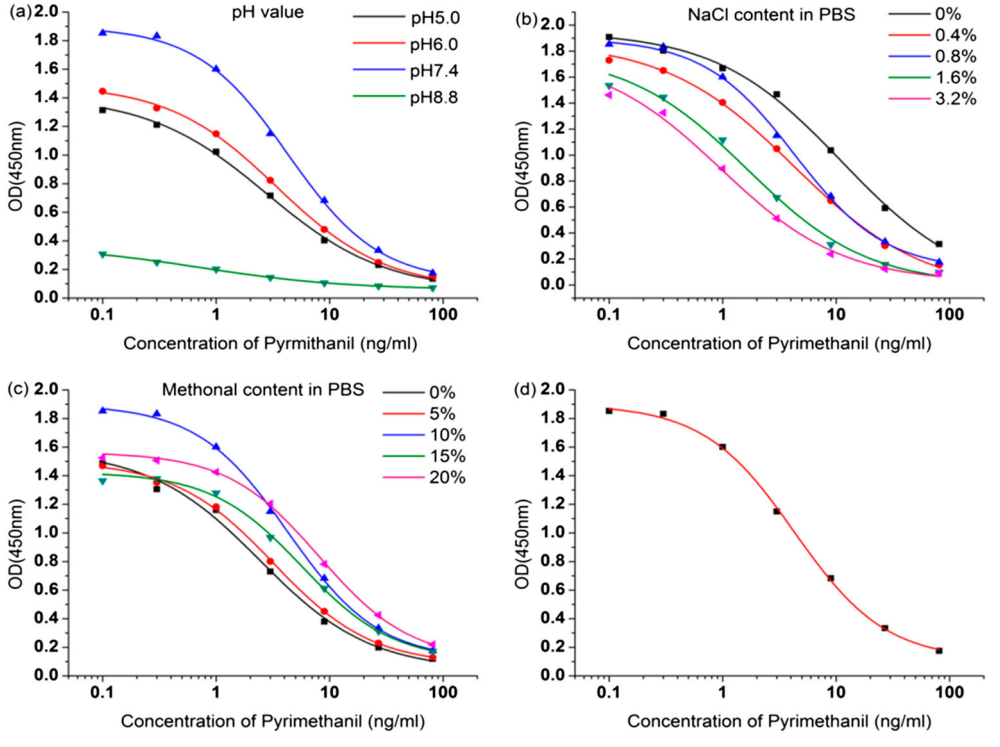
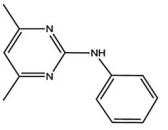
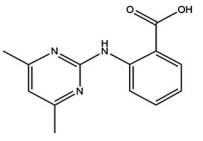
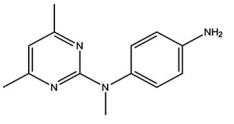
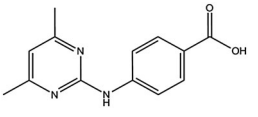
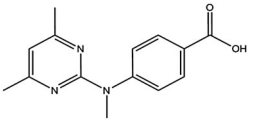
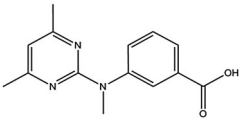
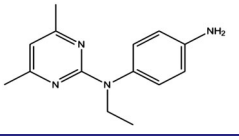


Figure 4. The indirect competitive inhibition non-linear standard curve for pyrimethanil of 1E7. (a) The variable of pH in PBS, (b) the variable of ionic strength in PBS, (c) the variable of methanol content in PBS and (d) standard inhibition curve for the ic-ELISA.

Table 1. Cross-reactivity of monoclonal antibody with pyrimethanil and analogs.

Chemicals	Structure	IC_{50} (ng/mL)	CR (%)
Pyrimethanil		4	100
2-[(4,6-Dimethylpyrimidin-2-yl)amino]benzoic acid		>1000	<0.092
pyrimidine		>1000	<0.092
4-[(4,6-Dimethylpyrimidin-2-yl)amino]benzoic acid		>1000	<0.092
4-[(4,6-Dimethylpyrimidin-2-yl)(methyl)amino]benzoic acid		>1000	<0.092
3-[(4,6-Dimethylpyrimidin-2-yl)(methyl)amino]benzoic acid		>1000	<0.092
N1-(4,6-Dimethylpyrimidin-2-yl)-N1-ethylbenzene-1,4-diamine		>1000	<0.092

acid, N1-(4,6-dimethylpyrimidin-2-yl)-N1-ethylbenzene-1,4-diamine, pyrimidine using ic-ELISA. CR was calculated according to the following equation: CR (%)=(half maximal inhibitory concentration [IC_{50}] of pyrimethanil/IC_{50} of analogs)× 30% (Peng, Liu, Hua, Gang, & Xu, 2016). The results are shown in Table 1. Based on these data we can conclude that the mAb of 1E7 was highly specificity for pyrimethanil, and can be used for further experiments.

Pyrimethanil recovery tests

In order to avoid the impact of acetonitrile volatilization on the experimental results, 1 mL of the extraction solution was dried using nitrogen, then reconstituted with 5 ml PBS. In this way, we transformed the acetonitrile extraction solution to a PBS system and the

Table 2. Accuracy evaluation of immunoassay methods for the samples.

Sample	Spiked level (ng/g)	ic-ELISA Mean ± SD (ng/mL)	Strip Recovery (%)	CV (%)	Control-line	Test-line
Cucumbers	20	1.326 ± 0.038	99 ± 2.8	9.25	+++	++
	40	2.573 ± 0.029	96 ± 1.1	6.32	+++	++
	80	5.103 ± 0.104	96 ± 1.9	4.58	+++	+
	160	9.652 ± 0.094	90 ± 0.9	2.72	+++	+
	220	14.324 ± 0.472	97 ± 3.2	4.40	+++	—

Note: The working range of ic-ELISA was 1.2–15.5 ng/mL. The cucumber samples were diluted fifteen times in the pretreatment. –, negative: the concentration of PYR was <1.2 ng/mL; +, weakly positive: the concentration of PYR was 1.2–15.5 ng/mL; ++, positive: the concentration of PYR was ≥15.5 ng/ml.

extraction solution was five-fold diluted; thus, we obtained a series of concentrations of pyrimethanil. The matrix interference was diluted to a minimum. The recovery of pyrimethanil from positive cucumber samples was calculated (Table 2) and the values ranged from 90% to 99%, with a standard deviation of 0.029–0.472 and a coefficient of variation of 2.72–9.25%. Hence, the sample pretreatment steps were appropriate.

Negative cucumber samples determined by UPLC-MS / MS method

Negative cucumber samples were purchased from supermarkets in Wuxi. The samples were pretreated according to the method in GB 23200.46-2016, and then tested by UPLC-MS / MS method. An Acquity UPLC-TQD instrument (Waters, Milford, MA) was used in this method. Electrospray ionization and MRM techniques are used in the qualitative and quantitative analysis of PYR. The result is shown in Figures 5 and 6. The relative molecular weight of pyrimethanil was 199.2, in MS^e positive ion mode detection, $[M + H]^+$ was 200.1, retention time (RT) was 6.95 min; MS/MS results showed no 200.1 at RT = 6.95 min, indicating that there is no pyrimethanil in the sample.

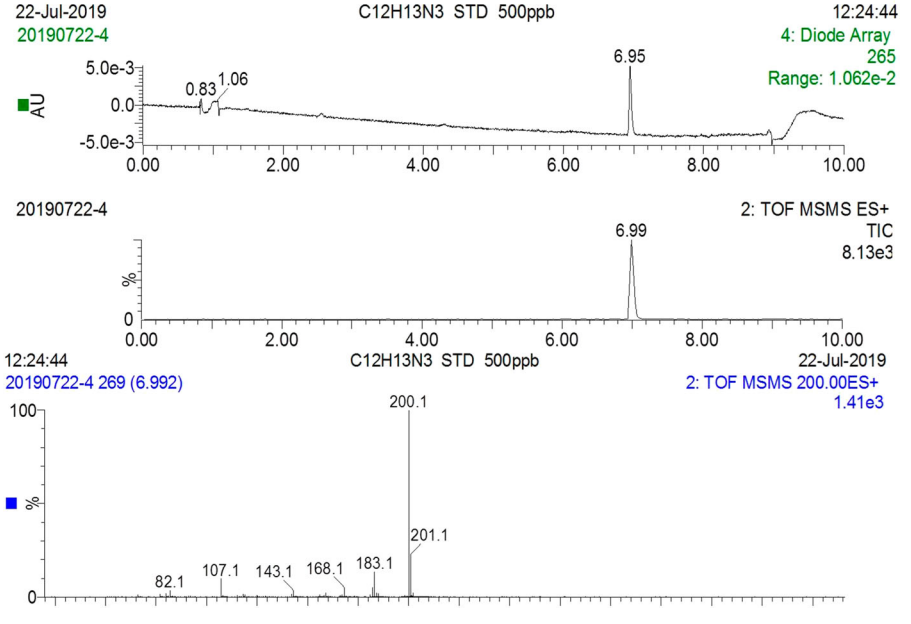


Figure 5. LC-MS diagram of pyrimethanil standard.

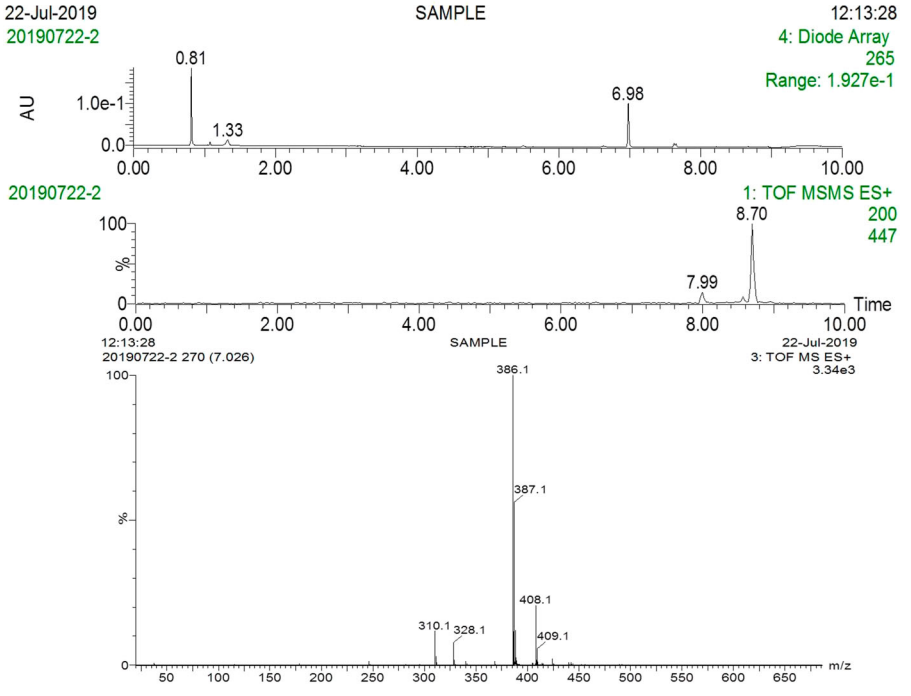


Figure 6. LC-MS diagram of cucumber samples.

Pyrimethanil detection by ICS

Optimization of coating antigen and antibody concentration:
Antigens of PYR-EDC-BSA-30 (coating 1), PYR-EDC-BSA-60 (coating 2) and PYR-EDC-BSA-90 (coating 3) were selected as coating antigens. Different coated antigens were sprayed onto the T line at 0.8 mg / mL. The antibody concentration was chosen to be 8 and 10 μg/mL. Bands were evaluated in 0.01 M PBS pH 7.4 containing 0 or 10 ng / ml PYR. Based on the results shown in Figure 5, we found that when coating 1 was used as the coating antigen and the antibody concentration was 10 μg/mL, the colour intensity of the T line of the negative sample was strong, while the T line of the positive sample had no colour. Therefore, the coating antigen and antibody concentration were selected for further immunochromatographic strip determination.

Evaluation of the strip test

The PYR standard was diluted by PBS to a range of concentrations to study assay sensitivity. The minimum detection limit (LDL) of the naked eye test was set to the amount of PYR, and the intensity of the test strip was clearly visible compared to when no PYR was added to the sample as a negative control. As shown in Figure 6 (a), the signal colour on the test line disappears completely from strong to weak to 50 ng/mL. It is concluded that the cutoff value of the pyrimethanil test strip is 50 ng/ml in the PBS solution system.

Detection of PYR in cucumber samples

A liquid from 40 g of crushed cucumber was added to the PYR standard (1 mg/mL, prepared with 0.01 M PBS pH 7.4), PYR in cucumbers with a final concentration of 2.5, 5, 10, 25, 50. Using optimized test strips prepare and analyze the spiked series. The results are summarized in Figure 6 (b). As the PYR concentration increases, the colour intensity decreases. And the colour disappeared completely at a PYR concentration of 50 ng/mL. It is concluded that the cutoff value of the pyrimethanil test strip is 50 ng/ml in the cucumber sample solution system Figures 7 and 8.

When using ICS to detect PYR in cucumber samples, the test results can be obtained in only 10 min, so the whole process was fast and efficient. And we can also determine the PYR content in the cucumber sample from the test results. When the PYR concentration was <1.2 ng/mL, the result was negative; when the PYR concentration was 1.2–15.5 ng/mL, the result was weakly positive; when the PYR concentration was >15.5 ng/mL, the result was positive. Therefore, the ICS can detect PYR in cucumber samples sensitively and efficiently.

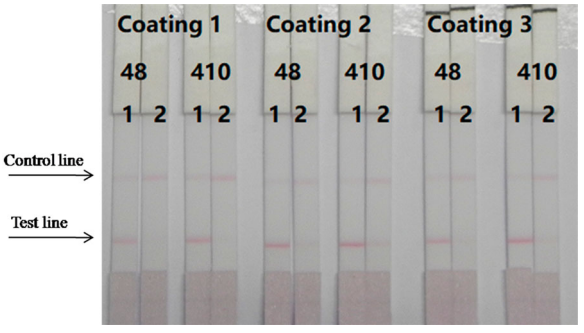


Figure 7. Optimization of different coating antigens by optimized 1 mL gold nanoparticle with 4 μL K_2CO_3, and 8 or 10 μg/mL antibody. Coating 1 was better. 1 = 0 ng/mL, 2 = 50 ng/mL.

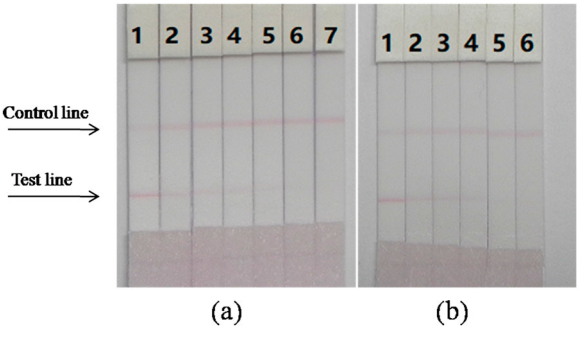


Figure 8. (a) The sensitivity of the immunochromatographic assay in PBS for pyrimethanil. 1 = 0 ng/mL, 2 = 1 ng/mL, 3 = 2.5 ng/mL, 4 = 5 ng/mL, 5 = 10 ng/mL, 6 = 25 ng/mL, and 7 = 50 ng/mL. (b) The sensitivity of the immunochromatographic assay of cucumber sample for pyrimethanil. 1 = 0 ng/mL, 2 = 2.5 ng/mL, 3 = 5 ng/mL, 4 = 10 ng/mL, 5 = 25 ng/mL, and 6 = 50 ng/mL.

Conclusion

We have successfully developed an immunocolloidal gold test strip as rapid methods for the determination of pyrimethanil in cucumber samples. The results showed that the cutoff value of PYR in 50 M PBS pH 7.4 and cucumber samples was 50 ng/mL. In summary, the immunochromatographic test strip test represents a sensitive, simple and rapid method for detecting PYR in 0.01 M. PBS and in cucumber samples.

Disclosure statement

No potential conflict of interest was reported by the authors.

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