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Original article

On-site rapid detection of multiple pesticide residues in tea leaves by lateral flow immunoassay

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ABSTRACT

The application of pesticides (mostly insecticides and fungicides) during the tea-planting process will undoubtedly increase the dietary risk associated with drinking tea. Thus, it is necessary to ascertain whether pesticide residues in tea products exceed the maximum residue limits. However, the complex matrices present in tea samples comprise a major challenge in the analytical detection of pesticide residues. In this study, nine types of lateral flow immunochromatographic strips (LFICSs) were developed to detect the pesticides of interest (fenpropathrin, chlorpyrifos, imidacloprid, thiamethoxam, acetamiprid, carbendazim, chlorothalonil, pyraclostrobin, and iprodione). To reduce the interference of tea substrates on the assay sensitivity, the pretreatment conditions for tea samples, including the extraction solvent, extraction time, and purification agent, were optimized for the simultaneous detection of these pesticides. The entire testing procedure (including pretreatment and detection) could be completed within 30 min. The detected results of authentic tea samples were confirmed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), which suggest that the LFICS coupled with sample rapid pretreatment can be used for on-site rapid screening of the target pesticide in tea products prior to their market release.

1. Introduction

Tea is a globally popular, nutritious beverage. Based on its degree of fermentation, tea can be divided primarily into green tea, oolong tea, and black tea. China is the largest tea producer, exporter, and consumer worldwide. In 2020, China produced 2,740 metric tons of tea, accounting for about 45% of the global tea production (https://www.statista.com). However, tea plants are susceptible to pests like tea cicada, tea inchworm, tea gall, and anthracnose. The primary method for resolving these issues in tea cultivation is the use of pesticides. Till the end of 2021, a total of 68 pesticides had been registered and allowed for use in the cultivation of tea in China [1]. However, multiple pesticide residues have been detected frequently in various tea products in China [2]. For instance, when testing 45 green tea samples in Jiangxi Province, China, 30 samples were found to contain pesticide residues, representing 21 kinds of pesticides. Furthermore, 40% of the tea samples contained pesticide residues that exceeded the maximum residue limits (MRLs) permitted by European Community Regulation No. 396/2005 [3]. Since some pesticide residues in tea leaves can be transferred to the tea infusion after brewing, the consumption of pesticide-laden tea brews present a potential dietary risk to human health due to the cumulative effect of various pesticides [4]. Therefore, multiple pesticide residues must be monitored in tea products to ensure the safety of tea consumption.

There are a variety of techniques for analyzing pesticide residues; the primary approaches include instrumental methods and rapid detection techniques. Commonly-used instrumental methods include chromatography and chromatography-mass spectrometry (MS) [5–8]. However, they are expensive, time-consuming, and

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unsuitable for rapid on-site detection. Methods for rapid detection of pesticide residues include the enzyme inhibition test and antibody-based immunoassays [9]. Among them, the enzyme inhibition method is simple, convenient, rapid, and inexpensive, but it is limited in its applicability to pesticide types. Conversely, immunoassays (such as enzyme-linked immunoassay and colloidal gold immunochromatography) have a high level of sensitivity and specificity, making them suitable for identifying a variety of pesticides. On the other hand, enzyme-linked immunoassay (ELISA) requires more time and operating steps, while colloidal gold immunochromatography is better suited for the on-site rapid detection of pesticides due to its simple operation, fast testing, and low cost [10,11]. Whereas, there are very few reports on the rapid detection of multiple pesticide residues in tea samples using immunoassays.

Tea leaves contain complex matrices such as pigments, alkaloids, and polyphenols, which can easily interfere with the precise detection and accurate estimation of pesticide residues in tea samples. Therefore, it is essential to take reasonable pretreatment to eliminate the influence of these matrices before detection. Pretreatment methods typically comprise solid phase extraction (SPE) [12], solid-phase micro-extraction [13], and dispersive solid-phase extraction [5], which are used alone or in conjunction with purification, concentration, and other processes to achieve the desired effect. However, most of the aforementioned techniques have the disadvantages of a limited application range, limited coupling techniques, and a high cost. In this regard, although the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is deemed suitable for detecting numerous pesticides, it still requires multiple operation steps and appears to be complicated for on-site rapid detection [7,14,15]. In contrast, the extract-dilute method is straightforward, saves time, and is typically utilized for sample preparation prior to immunoassays [16,17]. For samples with complex substrates, such as tea leaves, the purification step is also necessary to ensure the pretreatment effect. Additionally, the method of extract-purify-dilute (EPD) should be optimized to reduce the total test time.

In this study, nine types of colloidal gold-labeled lateral flow immunochromatographic strips (LFICSs) were developed to detect multiple pesticides (fenpropathrin, chlorpyrifos, imidacloprid, carbendazim, thiamethoxam, acetamiprid, chlorothalonil, pyraclostrobin, and iprodione) in tea samples, using the EPD method for sample rapid pretreatment. Following step-by-step optimization, the entire test was completed in 30 min (Scheme 1). In addition, the detected pesticide residues in authentic tea samples were further verified using ultra-performance liquid chromatography-tandem MS (UPLC-MS/MS).

2. Experimental

2.1. Chemicals and materials

Standards for chlorpyrifos (98.5%), fenpropathrin (98.0%), chlorothalonil (97.5%), carbendazim (98.0%), iprodione (97.5%), acetamiprid (99.2%), thiamethoxam (99.0%), as well as their analogs were purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Imidacloprid (98.8%) was obtained from Bayer (Leverkusen, Germany), while pyraclostrobin (99.9%) was obtained from BASF (Ludwigshafen, Germany). The corresponding mouse monoclonal antibodies (mAbs) and their coating antigens for LFICSs were produced in our laboratory previously.

Dimethylsulphoxide (DMSO), chloroaurate, trisodium citrate, bovine serum albumin (BSA), ovalbumin (OVA), polyvinylpolypyrrolidone (PVPP), polyethylene glycol (PEG, 20000), florisil, activated carbon (AC), and *N*-propylethylenediamine (PSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitrocellulose (NC) membrane CN-140 was purchased from Sartorius (Gottingen, Germany). Additionally, sodium chloride, sucrose, acetone, ethanol, methanol, chloroauric acid, acetonitrile, Tween-20, boric acid, hydrogen chloride, sodium tetraborate, concentrated sulfuric acid, hydrogen peroxide, potassium carbonate, sodium hydroxide, sodium bicarbonate, anhydrous disodium hydrogen phosphate, and potassium dihydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemical reagents were of the analytical reagent grade or higher.

Goat-anti-mouse IgG was purchased from Jiening Biology (Shanghai, China). In addition, accurate pH test papers were purchased from Sanaisi Reagent Co., Ltd. (Shanghai, China). Ultra-pure water was prepared using a Millipore instrument (Boston, MA, USA). Cleanert^{TM} triple phase SPE for tea (TPT)-SPE was purchased from Agela Technologies (Tianjin, China).

For the preparation of the 1 mg/mL stock solution, the pesticide standards were dissolved in methanol (except carbendazim, which was dissolved in DMSO). Phosphate buffer saline (PBS; 0.01 mol/L, pH 7.4), Tris-HCl (0.02 M, pH 7.4), solution 1 (PEG:PBS (1:99,*V*/*V*)), solution 2 (BSA:PBS (1:9,*V*/*V*)), and solution 3 (5% (*V*/*V*) sucrose,1% (*V*/*V*) BSA, 0.1% (*V*/*V*) PEG, and 0.02 M Tris-HCl, pH 7.4) were prepared in our laboratory.

2.2. Preparation of LFICS

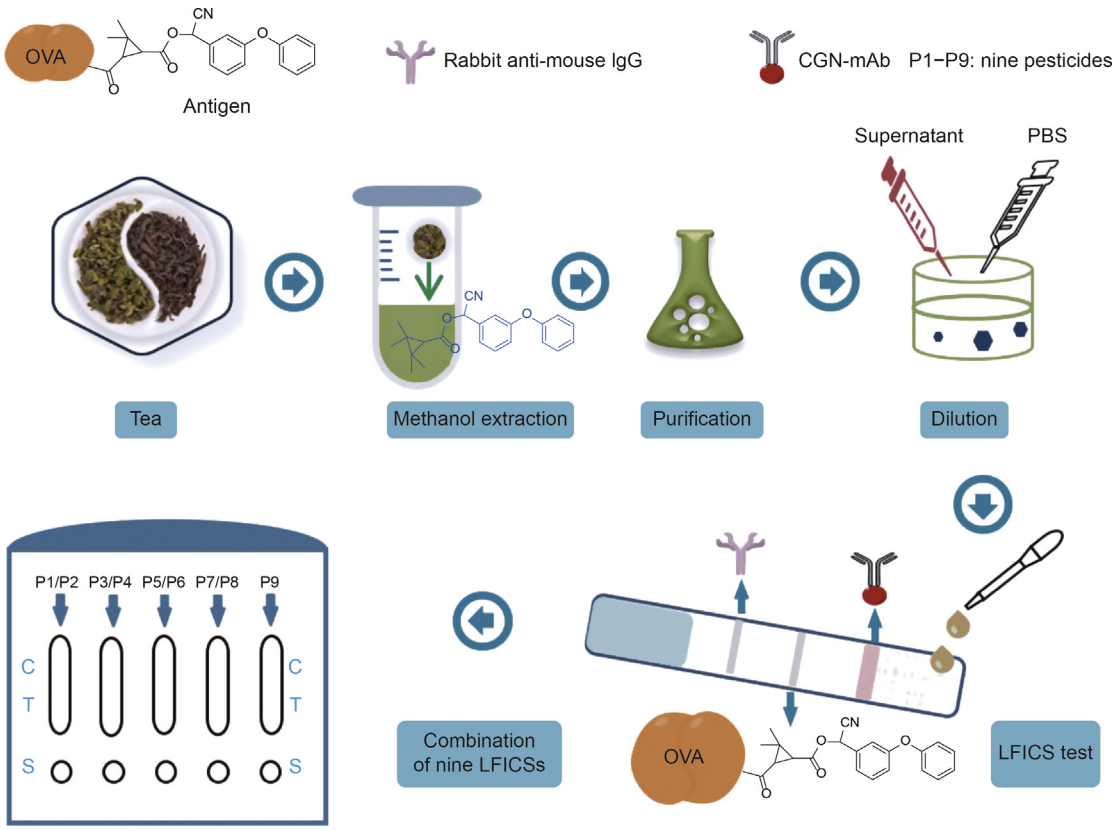
2.2.1. Preparation of gold-labeled antibody

A colloidal-gold nanoparticle (CGN) solution with an average diameter around 28 nm was prepared by trisodium citrate reduction in accordance with our previous study [18]. Each 1 mL of CGN was added to a 2-mL centrifuge tube, its pH was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 using a 10 mM K_2CO_3 solution, and then it was mixed with 0.1 mL of each anti-pesticide mAb diluted to 50 mg/L with ultrapure water. After 1 h of incubation, solution 1 (0.1 mL) and solution 2 (0.1 mL) were added to the mixture, which was then incubated for an additional 30 min. After centrifuging the mixture at 8,000 *g* for 20 min with an Allegra 64R centrifuge (Beckman, Brea, MO, USA), the supernatant was discarded and the precipitate was redissolved with solution 3 (0.1 mL), followed by applying to each LFICS test. When observing the color rendering index (CRI) of LFICS tested with 10% methanol-PBS (1:9, *V*/*V*), the pH corresponding to the darkest color on the test line (T-line) would be used to determine the optimal pH for gold-labeled antibody.

Each 1 mL of CGN was added to a 2-mL centrifuge tube, the pH of the CGN was adjusted to the optimal pH with 10 mM K_2CO_3 solution, and it was then mixed with mAb solutions of varying concentrations. The subsequent steps were identical to that described above. Comparing the LFICS results of the PBS and the pesticide standard solution helped determine the optimal concentration of the mAb for labeling.

2.2.2. Optimization of LFICS parameters

The prepared CGN-mAb conjugate was evenly sprayed on the conjugate pad and then dried at 37 °C. Goat anti-mouse IgG and pesticide coating antigens were assigned to the NC membrane as the control line (C-line) and the T-line using the T2DDA platform dispenser (Hangzhou Hangan Technology Co., Ltd., Hangzhou, China). A polyvinyl chloride (PVC) sheet, an NC membrane, an absorbing pad, a conjugate pad, and a sample pad were assembled as usual [11]. The LFICSs were then cut to a width of 3.3 mm using a C6 cutter (Hangzhou Hangan Technology Co., Ltd.) after being dried for 9 h at 37 °C. Subsequently, we determined the optimal concentrations of reagents for C-line and T-line by analyzing the material consumption and the sensitivity of the assay.



Scheme 1. Rapid and simultaneous detection for nine pesticide residues in tea leaves by lateral flow immunochromatographic strips (LFICSs). OVA: ovalbumin; CGN: colloidal-gold nanoparticle; mAb: monoclonal antibodies; PBS: phosphate-buffered saline; C: control; T: test; S: sample.

Individual LFICSs for detecting nine types of pesticides (fenpropathrin, chlorpyrifos, imidacloprid, carbendazim, thiamethoxam, acetamiprid, chlorothalonil, pyraclostrobin, and iprodione) were fabricated and stored in a drying cabinet (Zhuhai Aipo Electric Appliance Co., Ltd., Zhuhai, China) for use when needed.

2.2.3. Judgment criteria

For the LFICS test, 0.1 mL of analyte solution was placed on the sample pad. During the assay, the coating antigen on T-line captured the CGN-mAb conjugate that did not react with the target analyte, and the LFIC displayed corresponding color changes. When the C-line was colorless, the LFICS was deemed invalid. Additionally, when the C-line appeared steady red and the T-line color disappeared or lightened, the presence of the target analyte in the sample solution (positive) was confirmed; the more analyte in the sample, the weaker the red of the T-line. Moreover, when the C-line and T-line were both red, the target-pesticides were deemed absent in the sample (negative).

A portable strip-reader (Suzhou Hemai Precision Instrument Co., Ltd., Suzhou, China) was used to determine the CRI values of LFICS for precise measurement. Accordingly, when the T-line/C-line ratio (T/C) $\geq$ 1.0, the test solution was deemed negative; when T/C $<$ 0.5, the smaller the value, the stronger the positive test result. Additionally, both C-line and T-line readings less than 800 indicated an unsuitable test system.

2.3. Pretreatment of tea samples

2.3.1. Sample collection and preparation

Green tea, oolong tea, and black tea leaf samples were provided by the Tea Research Institute of the Chinese Academy of Agricultural Sciences (Hangzhou, China). All samples were ground with a pulverizer and sieved through a 50-mesh screen and kept at 4 °C. As previously described [19], UPLC-MS/MS was aforehand utilized to detect the target pesticides. The tea samples devoid of the aforementioned analytes were regarded as blank samples and were spiked with mixed solutions of nine pesticide standards at gradient concentrations.

2.3.2. Selection of extraction solvent

Using fenpropathrin as an example, four blank and four spiked (with 5 mg/kg of fenpropathrin) black tea samples (1.0 g) were weighed and placed in eight centrifuges (50-mL), labeled Nos. 1–4 and 5–8, respectively. After overnight standing at room temperature, 3 mL of methanol, ethanol, acetone, and acetonitrile were added to centrifuges Nos. 1 and 5, Nos. 2 and 6, Nos. 3 and 7, and Nos. 4 and 8, respectively. The mixtures were vigorously shaken for 1 min before being allowed to rest for 5 min. The 300 μL of supernatant was absorbed and placed in a 2-mL centrifuge tube before being diluted 5, 10, or 50 times with 0.01 M PBS. Each 0.1 mL of the liquid was added dropwise to the LFICS. After 10 min, the CRI values of LFICS were used to evaluate the extraction effect of pesticides in tea samples.

2.3.3. Evaluation of purifying agent

Each sample of spiked green tea (1.0 g, 15 replicates) was weighed and placed in a 50-mL centrifuge tube. Subsequently, 3 mL of methanol was then added to the sample (15 replicates), which was shaken for 1 min and allowed to stand for 5 min. Then, 1 mL of sample extract was taken and loaded into 2-mL centrifuge tubes (15 replicates). The extract solution was subsequently treated with florisil (1%, 2%, and 4%), AC (1%, 2%, and 4%), PSA (1%, 2%, and 4%), and PVPP (1%, 2%, 4%, 6%, 8%, and 10%). After 1 min of vortexing, the mixtures were centrifuged at 2,000 *g* for 1 min, after which the absorbed supernatants were diluted 10-fold with

0.01 M PBS and tested with LFICS. The CRI values of C-line and T-line were read by the strip reader in order to select the most effective purifying agent.

2.4. UPLC-MS/MS analysis and validation

In order to validate the accuracy of LFICS analysis, UPLC-MS/MS was utilized to detect imidacloprid, acetamiprid, thiamethoxam, carbendazim, fenpropathrin, and chlorpyrifos in authentic tea samples. In this study, the sample pretreatment method was based on the Chinese National Standard GB 23200.13-2016 [20], with some modifications.

Each 1.0 ± 0.01 g homogenized sample was placed in a 50 mL polypropylene tube. After alternatingly adding 5 mL of deionized water and 5 mL of acetonitrile to the tube, the sample was extracted using ultrasonic extraction for 30 min. The mixture was then vigorously shaken for 2 min with a vortex mixer and centrifuged at 4,000 r/min for 5 min. The supernatant was transferred carefully into a pear-shaped evaporation flask. Subsequently, the above extraction procedure was repeated one time from the addition of the acetonitrile step. The resulting supernatant was combined and evaporated using a water bath at 40 °C. The residue was dissolved in 2 mL of acetonitrile in preparation for TPT-SPE purification. Prior to sample loading, TPT cartridges were preconditioned with 5 mL of acetonitrile:toluene (3:1, *V/V*). The targeted compounds were collected with 25 mL of acetonitrile:toluene (3:1, *V/V*) as the elution. Before UPLC-MS/MS analysis, residues were reconstituted in 1 mL of acetonitrile and filtered through a 0.2 mm one-off polytetrafluoroethylene (PTFE) syringe filter after drying under a stream of nitrogen at 40 °C.

The analyses were conducted utilizing a Waters Acquity UPLC (Milford, MA, USA) and a Quattro Premier XE (Waters, Manchester, UK) triple quadrupole mass spectrometer. Chromatographic separation was done using a Waters Acquity HSS T_3 (100 mm × 2.1 mm, 1.8 μm) at 40 °C. The mobile phase consisted of 0.1% (*V/V*) formic acid aqueous solution (A) and methanol (B) with a flow rate of 0.3 mL/min, and the gradient was set as follows: initial, 20% B; 4 min, 90% B; and 7 min, 20% B. The injection volume was 5 μL and the sample temperature was maintained at 8 °C.

The MS/MS system featured an electrospray positive-ion multiple reaction mode: capillary voltage of 3.5 kV; cone voltage of 40 V; source temperature of 150 °C; desolvation temperature of 350 °C; cone gas flow of 50 L/h; and desolvation gas flow of 650 L/h. The analysis parameters are listed in Table S1. The data was processed using the MassLynx 4.1 Software (Waters). The method validation, including linearity, detection limit, accuracy, precision, matrix effects, and recovery, was conducted in accordance with Chinese National Standard GB 23200.13-2016 method validation recommendations [16,21].

3. Results and discussion

3.1. Conjugation of gold-labeled antibody

As evaluated by the CRI of the T-line, the CGN-mAb conjugate probe was confirmed to be a key component for LFICS, as allowing for the determination of the optimal pH and antibody dosage for labeling. As listed in Table S2, for fenpropathrin and pyraclostrobin, the optimal pH for the CGN-mAb conjugate was 6.5, whereas the optimal pH for the remaining seven pesticides was 7.0. The optimal antibody concentrations for the nine types of pesticides are as follows: fenpromethrin at 40 mg/L; chlorpyrifos at 120 mg/L; imidacloprid at 80 mg/L; carbendazim at 40 mg/L; thiamethoxam at 30 mg/L; acetamiprid at 50 mg/L; chlorothalonil at 40 mg/L; pyraclostrobin at 20 mg/L; and iprodione at 10 mg/L. The observed disparity can be attributed to the different property, activity, or affinity of these antibodies to their corresponding antigens for various pesticides.

3.2. Optimal parameters of LFICS

When the CGN-mAb conjugate interacted with various concentrations of goat-anti-mouse IgG and hapten-OVA conjugate, the CRI of C-line and T-line varied. Taking into account the combination of C-line and T-line with similar color (CRI values above 800) and the LFICS sensitivity, Table S2 displays the optimal parameters of the nine types of LFICSs. For fenpropathrin, chlorpyrifos, imidacloprid, carbendazim, thiamethoxam, acetamiprid, chlorothalonil, pyraclostrobin, and iprodione, the most appropriate T-line concentrations are 0.8, 4.5, 0.3, 0.8, 0.1, 0.7, 5, 1, and 0.4 mg/mL respectively, and the most appropriate C-line concentrations are 0.1, 0.09, 0.1, 0.04, 0.02, 0.07, 0.04, 0.1, and 0.3 mg/mL, respectively. These parameters were strongly correlated with the coating antigen quality and the CGN-mAb conjugated probe.

3.3. Assay sensitivity and selectivity

A series of pesticide standard solutions were used to investigate the sensitivity of the assay. As shown in Table 1, the visual limits of detection (LODs) of fenpropathrin, chlorpyrifos, imidacloprid, carbendazim, thiamethoxam, acetamiprid, chlorothalonil, pyraclostrobin, and iprodione in standard solutions by CGN-LFICSs were 0.1, 0.05, 0.005, 0.05, 0.001, 0.01, 0.025, 0.05 and 0.1 mg/L, respectively.

In order to determine the selectivity of the assay, cross-reactions between the nine pesticides and their structural analogs were examined. The visual LODs for detecting clothianidin and imidaclithiz using imidacloprid LFICS are both 0.005 mg/L. The LFICS method for detecting acetamiprid could also be used to detect thiacloprid at a LOD of 0.02 mg/L. In addition, fenpropathrin LFICS could be used to detect cypermethrin, with a LOD of 1 mg/L, which could be attributed to the broad-specific antibodies developed in our previous research [17,22,23].

Above all, this study developed nine kinds of LFICSs based on the optimal combinations of antigen and antibody, which can be used for rapid screening of 13 pesticide residues, including fenpropathrin (cypermethrin), chlorpyrifos, imidacloprid (clothianidin, imidaclithiz), carbendazim, thiamethoxam, acetamiprid (thiacloprid), chlorothalonil, pyraclostrobin, and iprodione.

3.4. Extraction solvent for tea samples

Since the matrix component is greater in green tea than in black tea or oolong tea, black tea samples were initially used to determine the extraction solvent in the present study. LFICS tests of fenpropathrin by ethanol, acetone, and acetonitrile extraction (Fig. 1) yielded false negative results (×10, Nos. 6–8), only positive by methanol extraction (×10, No. 5). Thus, methanol was employed as the extraction solvent. In the pretreatment, the dilution ratio of the matrix must be considered alongside the sensitivity of LFICS; therefore, a dilution factor of 10 times is more appropriate.

The effects of extracting the nine pesticides at four different times (30 s, 1 min, 2 min, and 5 min) were also examined in the current study. The LFICS results demonstrated that high-polarity pesticides such as acetamiprid could be completely extracted after 30 s of oscillation. In contrast, pesticides with low-polarity, such as chlorothalonil, can be completely extracted after 2 min. Therefore, the optimal extraction duration for the nine pesticides was determined to be 2 min.

3.5. Purifying agent for tea samples

Even though the extract effect of pesticides in black tea samples was guaranteed under the optimal extraction solvent and dilution factor (Fig. 1, Nos. 1 and 5), there are still issues. For example, the

Table 1
The visual limit of detection (LOD) of lateral flow immunochromatographic strip (LFICS) to nine standard solutions of target pesticides.

Pesticide	Concentration of standard solution (mg/L)						Visual LOD (mg/L)	Picture
Fenpropathrin	0.4 ++	0.2 ++	0.1 +	0.05 −	0.025 −	0 −	0.1	
Chlorpyrifos	0.1 ++	0.05 +	0.025 −	0.012 −	0.006 −	0 −	0.05	
Imidacloprid	0.04 ++	0.02 ++	0.01 +	0.005 +	0.002 −	0 −	0.005	
Carbendazim	0.2 ++	0.1 ++	0.05 +	0.025 −	0.012 −	0 −	0.05	
Thiamethoxam	0.002 ++	0.001 +	0.0005 −	0.0002 −	0.0001 −	0 −	0.001	
Acetamiprid	0.08 ++	0.04 ++	0.02 ++	0.01 +	0.005 −	0 −	0.01	
Chlorothalonil	0.4 ++	0.2 ++	0.1 +	0.05 +	0.025 +	0 −	0.025	
Pyraclostrobin	0.2 ++	0.1 ++	0.05 +	0.025 −	0.012 −	0 −	0.05	
Iprodione	0.1 +	0.05 −	0.025 −	0.012 −	0.006 −	0 −	0.1	

− means negative, +means weak positive, and ++ means strong positive.

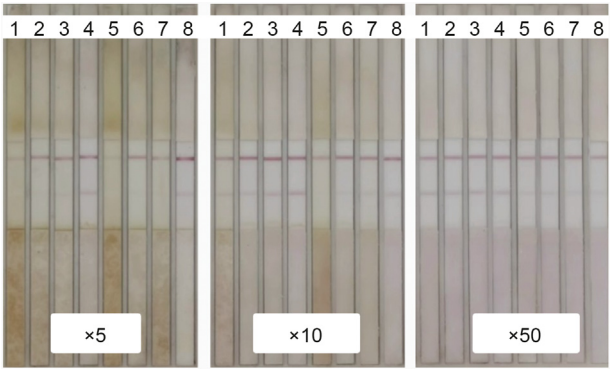


Fig. 1. Test results of fenpropathrin lateral flow immunochromatographic strips (LFICSs) using samples from different extract conditions. Samples Nos. 1–4 and 5–8 were the blank and spiked (with 5 mg/kg of fenpropathrin) black tea samples, extracted with 3 mL of methanol, ethanol, acetone, and acetonitrile, respectively. ×5, ×10, and ×50 mean the extract of black tea samples was diluted 5, 10, and 50 times with 10% methanol-phosphate buffer saline (PBS) (1:9, *V/V*), respectively.

strips tested with extracts of black tea (Fig. 1, ×10, No. 1) and green tea (Fig. 2, B1) exhibited weaker color development than those tested without a tea matrix (using PBS as a negative control in Fig. 2, A1). After extraction, tea samples must be purified to resolve this issue of matrix interference.

After green tea sample extracts were treated with various purifying agents, the color saturation of LFICS was variable (Fig. 2). The strip reader was utilized to record the CRI values of the T-lines and C-lines and to calculate the T/C ratio, as detailed in Table S3. For strips C1–F3 (Fig. 2), C-line and T-line readings less than 800 indicate an unsatisfactory test system (1%, 2%, and 4% florisil, AC, PSA, and PVPP). Only the spiked green tea samples that were purified with 4% PVPP were positive. The matrix interference of the test solution after 6%, 8%, and 10% PVPP purification was drastically reduced, and the LFICS was able to develop color normally, indicating that the test result was highly positive. Among them, the C-line CRI values of green tea samples treated with 6% and 8% PVPP (Fig. 2, F4 and F5, C-line value = 1590 and 1686) were very close to those of the blank positive treatment (Fig. 2, A2, C-line value = 1672). As a result, 6% PVPP was chosen as the ideal

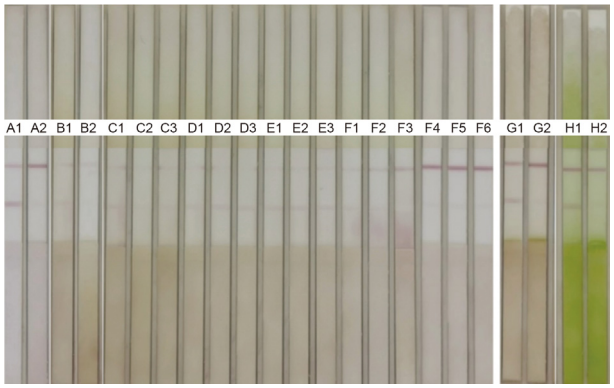


Fig. 2. Test results of fenpropathrin lateral flow immunochromatographic strips (LFICSs) using green tea sample extracts treated with different purifying agents. C1–C3: 1%, 2%, and 4% *N*-propylethylenediamine (PSA); D1–D3: 1%, 2%, and 4% florisil; E1–E3: 1%, 2%, and 4% activated carbon (AC); F1–F6:1%, 2%, 4%, 6%, 8%, and 10% polyvinylpolypyrrolidone (PVPP). A1: 10% methanol-phosphate buffer saline (PBS) (1:9, *V/V*) as negative control; A2: 10% methanol-PBS (1:9, *V/V*) spiked with 0.2 mg/L fenpropathrin as positive control; B1, G1, and H1: non-spiked tea sample; B2, G2, and H2: spiked tea sample with 5 mg/kg of fenpropathrin; B1 and B2: green tea sample (not purified); G1 and G2: black tea sample (purified with 6% PVPP); H1 and H2: fresh green tea sample (purified with 6% PVPP).

purifying agent. The selection of PVPP as the purifying agent of tea matrix is consistent with that in the literature [15].

In particular, the optimal steps for tea sample pretreatment are as follows: the tea sample (1.0 g) and methanol solvent (3 mL) were combined in a 15-mL tube and vortexed or shaken for 2 min. After standing for 5 min, the supernatant (1.0 mL) was absorbed and transferred to a new 2-mL centrifuge tube. Subsequently, PVPP (60 mg) was added, oscillated vigorously for 1 min, and then centrifuged at 2,000 *g* for 1 min. The supernatant was then diluted 10-fold using PBS and was used for the LFICS test.

3.6. Method validation by spiked sample detection

Black tea, oolong tea, and green tea were respectively subjected to pesticide-spiked testing. Each pesticide was fortified at five concentrations. According to the coloration of the LFICS test, for fenpropathrin, chlorpyrifos, imidacloprid, carbendazim, thiamethoxam, pyraclostrobin, and iprodione, the visual limit of quantitation (LOQ) of pesticide LFICSs corresponding to three kinds of tea is the same, i.e., 5, 2, 0.5, 2, 0.05, 2, and 5 mg/kg (Table 2). For certain pesticides, the sensitivities of pesticide LFICSs corresponding to three types of tea samples were distinct (the visual LOQ of acetamiprid LFICSs for black tea, oolong tea, and green tea is 0.5, 0.5, and 0.2 mg/kg; the visual LOQ of chlorothalonil LFICSs for black tea,

Table 2
Different pesticides spiked in three types of tea samples tested by lateral flow immunochromatographic strips (LFICSs).

Pesticides	Sample	Spiked concentration of pesticides (mg/kg)						LOQ (mg/kg)
Fenpropathrin	/	0	0.5	1	2	5	10	MRL[a]: 5
	Black tea	−	−	−	−	+	++	5
	Oolong tea	−	−	−	−	+	+	5
	Green tea	−	−	−	−	+	++	5
Chlorpyrifos	/	0	0.5	1	2	5	10	MRL[a]: 2
	Black tea	−	−	−	+	+	++	2
	Oolong tea	−	−	−	+	++	+	2
	Green tea	−	−	−	+	++	++	2
Imidacloprid	/	0	0.2	0.5	1	2	5	MRL[a]: 0.5
	Black tea	−	−	+	++	++	++	0.5
	Oolong tea	−	−	+	++	++	++	0.5
	Green tea	−	−	+	++	++	++	0.5
Carbendazim	/	0	0.25	0.5	1	2	5	MRL[a]: 5
	Black tea	−	−	−	−	+	++	2
	Oolong tea	−	−	−	−	+	++	2
	Green tea	−	−	−	−	+	++	2
Thiamethoxam	/	0	0.0125	0.025	0.05	0.1	0.2	MRL[a]: 10
	Black tea	−	−	−	++	++	++	0.05
	Oolong tea	−	−	−	++	++	++	0.05
	Green tea	−	−	−	++	++	++	0.05
Acetamiprid	/	0	0.02	0.05	0.1	0.2	0.5	MRL[a]: 10
	Black tea	−	−	−	−	−	++	0.5
	Oolong tea	−	−	−	−	−	++	0.5
	Green tea	−	−	−	−	+	++	0.2
Chlorothalonil	/	0	0.25	0.5	1	2	5	MRL[a]: 10
	Black tea	−	−	−	+	+	+	1
	Oolong tea	−	−	+	+	+	++	0.5
	Green tea	−	−	+	+	++	++	0.5
Pyraclostrobin	/	0	0.25	0.5	1	2	5	MRL[a]: 10
	Black tea	−	−	−	−	+	+	2
	Oolong tea	−	−	−	−	+	++	2
	Green tea	−	−	−	−	++	++	2
Iprodione	/	0	0.25	0.5	1	2	5	MRL: none
	Black tea	−	−	−	−	−	++	5
	Oolong tea	−	−	−	−	−	++	5
	Green tea	−	−	−	−	−	+	5

− Means negative, + means weak positive, and ++ means strong positive. [a] Refer to maximum residue limits (MRLs) of tea in GB 2763-2021. LOQ: the visual limit of quantitation.

Table 3
Analysis of target pesticide residues in tea samples with ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and lateral flow immunochromatographic strips (LFICSs).

Sample	Test method	Fenpropathrin (mg/kg)	Chlorpyrifos (mg/kg)	Imidacloprid (mg/kg)	Carbendazim (mg/kg)	Thiamethoxam (mg/kg)	Acetamiprid (mg/kg)
1	UPLC-MS/MS	0.063	ND	0.076	ND	0.047	0.072
	LFICS	−	−	−	−	++	−
2	UPLC-MS/MS	0.28	ND	0.016	0.069	ND	0.17
	LFICS	−	−	−	−	−	+
3	UPLC-MS/MS	ND	ND	ND	ND	ND	ND
	LFICS	−	−	−	−	−	−
4	UPLC-MS/MS	0.03	ND	0.12	ND	0.031	0.34
	LFICS	−	−	−	−	++	++
5	UPLC-MS/MS	0.054	ND	0.021	0.47	0.3	0.21
	LFICS	−	−	−	−	++	+
6	UPLC-MS/MS	ND	ND	ND	ND	ND	ND
	LFICS	−	−	−	−	−	−
7	UPLC-MS/MS	ND	ND	ND	ND	ND	ND
	LFICS	−	−	−	−	−	−
8	UPLC-MS/MS	0.219	ND	0.062	0.14	0.034	0.27
	LFICS	−	−	−	−	++	++

− Means negative, + means weak positive, and ++ means strongly positive. ND means no pesticide residue was detected, and those with data were the detected pesticide contents (mg/kg).

Table 4
Comparison of the proposed lateral flow immunochromatographic strip (LFICS) assay and other rapid detection methods for pesticide.

Method	Analyte	Limit of detection	The entire test time	Application sample	Refs.
QD-LFICS	Imidacloprid Imidaclothiz clothianidin	0.5–1 ng/mL	1 h	Green tea, black tea, and oolong tea	[19]
SERS-based sensor	Carbendazim	100 ng/mL	>2 h	Oolong tea	[23]
SERS-based sensor	Imidacloprid	4.55×10^{-5} μg/mL	>2 h	Green tea	[24]
UCNPs-LFICS	Imidacloprid	0.45 ng/mL	1 h	Cucumber, honey, and tea	[25]
CGN-LFICS	Acetamiprid	10 ng/mL	45 min	Tea	[26]
CGN-LFICS	Thiamethoxam, fenpropathrin, chlorpyrifos, imidacloprid, acetamiprid, carbendazim, chlorothalonil, pyraclostrobin, and iprodione	0.001–0.1 mg/L	30 min	Green tea, black tea, and oolong tea	This study

QD: Quantum-dot; SERS: surface-enhanced Raman spectroscopy; UCNPs: up-converting nanoparticles; CGN: colloidal-gold nanoparticle.

oolong tea, and green tea is 1, 0.5, and 0.5 mg/kg). This must be due to the fact that the specific co-extracts in different species of tea products have different matrix effects, similar to a previous study of other agro-products containing complex matrices [22].

3.7. Analysis of authentic tea samples

Table 3 displays the analysis results of target pesticide residues in the eight authentic tea samples. Seen from the results of UPLC-MS/MS, six samples (75%) contained pesticide residues, five samples contained five types of pesticide residues, and none of them exceeded the Chinese MRLs. Among the six pesticides analyzed, fenpropathrin, imidacloprid, and acetamiprid were found in 62.5% of the tea samples, with residues ranging from 30 to 280 μg/kg, from 16 to 120 μg/kg, and from 72 to 340 μg/kg, respectively. However, due to the insufficient sensitivity of the LFICS, some pesticides (fenpropathrin, imidacloprid, carbendazim, and acetamiprid) can be detected by the instrument but not by the LFICS. The main reason for the high detection rate of pesticides in tea samples may be the abuse of multiple pesticides by tea farmers. As a result, although the pesticide residue level is below the MRL, it is necessary to regularly monitor the multi-residue pesticides in tea samples to ensure food safety.

In contrast, the majority of previously reported methods for rapidly detecting pesticide residues in tea samples require at least 1 h and their application was typically restricted to a small number of water-soluble pesticides (Table 4) [19,23–26]. In this study, the EPD method for tea pretreatment was simple and suitable for on-site fast screening by LFICS, with the entire test taking less than 30 min and involving nine insecticides or fungicides commonly used in tea planting.

4. Conclusion

After optimizing a number of parameters, including pH and antibody dose, nine distinct CGN-LFICSs based on antigen-antibody reactions were developed in the current study. In consideration of the complexity of the tea matrix, a simple pretreatment method was developed by optimizing the extraction solvent, the type and dosage of the purifier, etc., and combined with the appropriate LFICS to simultaneously detect nine pesticides in tea. Satisfyingly, the rapid-test of these nine pesticides could meet the Chinese pesticide MRL standards outlined in Chinese National Standard GB 2763-2021. In addition, the detection results of actual samples were generally consistent with those obtained by UPLC-MS/MS. This method is user-friendly, cost-effective, and quick (the entire process within 30 min), indicating that it is suitable for on-site field testing of tea leaves.

CRediT author statement

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

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