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Research and Application of In Situ Sample-Processing Methods for Rapid Simultaneous Detection of Pyrethroid Pesticides in Vegetables

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Abstract: A novel rapid and cost-effective pre-processing method for the simultaneous determination of pyrethroid pesticides in vegetables has been developed and validated. The process of pesticide extraction was carried out by the QuEChERS (quick, easy, cheap, effective, rugged and safe) method combined with filtration by filter paper, and cleanup was carried out by the multi-plug-filtration-cleanup (m-PFC) method with no centrifuge program during the whole process. The pre-processing method is optimized for gas chromatography (GC). The process is convenient and time saving, requiring just a few seconds per sample. The recovery rate (70–120%), limit of detection (0.0001–0.007 mg/kg), precision (0.2–9.3%) and accuracy for each analyte were determined in 10 representative vegetables with good results. Finally, the feasibility of the developed method was further confirmed by the successful determination of pyrethroid-pesticide residues in pyrethroid-containing practical samples within the processing method coupled with thin-layer chromatography and a colloidal-gold test strip.

Keywords: pesticides; processing methods; centrifuge; QuEChERS; multi-plug-filtration cleanup

1. Introduction

Pyrethroid pesticides are a class of synthetic organic insecticides derived from naturally occurring pyrethrums, such as permethrin, fenvalerate, and deltamethrin, etc. [1]. Pyrethroid pesticides have been widely used in agriculture to protect fruit and vegetable crops since the 1980s because of their predominant characteristics of selective insecticidal activity, low toxicity to mammals and birds and lower environmental persistence [2–4]. However, the widespread application of pyrethroids has caused contamination of environmental compartments and has led to the ongoing possibility of food-chain contamination, leading to the bioaccumulation of these insecticides in food products of animal origin, which will pose a threat to environmental and food quality [2,5]. Because of this risk to public health, many countries and organizations have set maximum-residue limits (MRLs) for pyrethroid pesticides in vegetables [6].

Because of the weak polarity, moderate boiling point and easy gasification, pyrethroid pesticides are typically detected using gas chromatography (GC). Now, many methods have been developed to analyze pyrethroid pesticides, mainly including gas chromatography–electron-capture detector (GC-ECD) [7], GC coupled to mass spectrometry (GC/MS or GC-MS/MS) [8,9], high-performance liquid chromatography–mass spectrometry (HPLC-MS/MS) [10].

Pesticide-residue analysis is a complex technology in which the pre-processing method plays a vital role in the detection process. Appropriate pre-processing methods can improve the sensitivity, detection range, precision, and accuracy of the detection technology [11,12]. Several pre-processing methods have been developed for the analysis of pyrethroid pesticides in vegetables, including solid-phase extraction (SPE) [13,14], solid-phase microextraction (SPME) [15], dispersive solid-phase extraction (d-SPE) [16], matrix solid-phase dispersion (MSPD) [17], liquid–liquid extraction (LLE) [18], dispersive liquid–liquid microextraction (DLLME) [19,20], accelerated solvent extraction (ASE) [21] and gel-permeation chromatography (GPC) [22]. However, these pre-processing methods demand a large volume of organic solvents and several cleanup procedures, which are mostly time consuming, labor intensive, and require frequent instrument maintenance. It is of great significance to establish a rapid, simple, and high throughput method for pesticide-residue analysis in order to meet the requirements of modern pesticide-residue analysis.

Nowadays, the QuEChERS method developed by Anastassiades et al. in 2003 [23] has received great attention and achieved wide application [24]. The method combines traditional LLE with SPE methods and involves an initial solvent extraction with acetonitrile, partitioning between acetonitrile and the aqueous phase through the addition of sodium chloride (NaCl) and magnesium sulfate ($MgSO_4$), and purification of the extract through the d-SPE cleanup process with loose sorbents such as primary secondary amine (PSA) and octadecylsilane (C18) [25–27]. As we know, PSA has been used as the d-SPE sorbent, which is aimed at removing polar pigments, fatty acids, polar organic acids and some sugars [28], and C18 has also been used as the d-SPE sorbent in the modified QuEChERS method to remove sterols and nonpolar interfering substances [29,30]. The QuEChERS method is an official AOAC (Association of Official Analytical Chemists) method for the extraction of pesticides from plant matrices [31,32]. However, as a general pre-processing method, it needs two centrifugation steps in order to separate and purify the acetonitrile phase, which requires a large centrifuge machine. Therefore, the scope of the application of the QuEChERS method is restricted in routine operations.

Carbon nanotubes (CNTs) are a kind of one-dimensional nanomaterial with special structures, which are made up of graphene sheets. CNTs can be divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) according to the number of layers. CNTs have the characteristics of a large surface area, narrow pore-size distribution, low density and a high-binding functional group; MWCNTs especially have a large number of groups and unique structures on their large specific surface, so they have strong adsorption ability to be used in the QuEChERS pretreatment process to absorb interfering substances (detergents) and remove substances such as high-percentage pigments and hydrophobic substances from samples as an alternative d-SPE adsorbent [33,34]. Recently, MWCNTs [35,36], aminated MWCNTs [37], and magnetic aminated MWCNTs [38,39] have attracted much attention due to their high chemical stability, large surface area, strong adsorption capacity, wide pH application range and low cost [40,41]. Pan et al. [42] utilized MWCNTs as d-SPE adsorbents to improve and optimize the QuEChERS method. Its excellent cleanup effect has been verified for more than 100 pesticides and a variety of representative substrates, especially for vegetable and fruit substrates. Compared with the classic QuEChERS method, it has obvious advantages in interference removal, matrix effect (ME), and processing speed. In-depth studies on more complex substrates such as shallot, ginger, garlic, and leek have shown that the use of MWCNTs in d-SPE is significantly better than traditional solid-phase materials in terms of purification. Furthermore, it has a dehydration function, and the method eliminates the vortex and centrifugation steps without any solvent evaporation during the purification process, thereby greatly reducing the purification time.

The multi-plug-filtration cleanup (m-PFC) based on QuEChERS was initially developed to purify interfering substances in the matrix. This method uses filtration-type-purification syringe tubes that combine solid-phase sorbents, such as MWCNTs, PSA, C18, and $MgSO_4$ into syringe tubes to complete the purification step. Compared with the SPE process, the m-PFC procedure can be done in a few seconds without the leaching and elution steps. In comparison to the conventional QuEChERS method, the m-PFC procedure eliminates the vortex and centrifugation process in the cleanup procedure [43]. This method has been successfully applied to the detection of pesticide residues in vegetables [44,45].

In situ analysis is a technique to analyze the composition, content, and distribution of target analytes in a specified area. The detection method required for rapid in situ analysis must be convenient and fast, with high sensitivity and low cost, in order to be more widely applied to the substratum. At present, the main methods of rapid in situ analysis involve either rapid detectors or test strips (or color cards) [46]. The pre-processing method is generally used to shred the sample and directly detect it after extraction. In this way, not only is the extraction not uniform, but the pigment content of the extract is also high, which will lead to inaccurate detection results. The QuEChERS method and the m-PFC method have the potential to be pre-processing methods for rapid in situ analysis, but they both require centrifugation during pretreatment, which will limit their applications in the field of rapid in situ analysis. Therefore, it is particularly important to develop a more convenient and fast pre-processing method for rapid in situ analysis.

This study aimed to develop a novel, rapid, cost-effective, and time-saving pre-processing method for the simultaneous in situ determination of 10 pyrethroid pesticides in vegetables. Based on the previous work, this study developed a more practical pre-processing method suitable for in situ analysis by further improving and optimizing the m-PFC method. Factors influencing the extraction efficiency of the m-PFC method were investigated and optimized by GC. The verification results of recovery rate, linearity, matrix effect, detection limit, precision, and accuracy show that this pre-processing method is feasible. Ultimately, 10 pyrethroid pesticides in vegetables were successfully and rapidly determined in situ by this method coupled with thin-layer chromatography (TLC) and a colloidal-gold test strip.

2. Materials and Methods

2.1. Chemicals

A total of 10 standard pyrethroid-pesticide (Bifenthrin, Deltamethrin, Cyfluthrin, Permethrin, Cyfluthrin, Cypermethrin, Flucythrinate, Fenvalerate, Flumethrin, Deltamethrin) products (1000 mg/L) were purchased from the Environmental Protection Research Monitoring Institute, Ministry of Agriculture, People's Republic of China (Tianjin, China). The purity of the standard samples was higher than 98% and they were stored in a refrigerator at −20 °C. Acetonitrile, acetone, and petroleum ether of chromatography grade was supplied by Merck KgaA Co. Ltd. (Darmstadt, Germany). NANO agricultural special-cartridge products (NANO) were purchased from Tianjin Bona Ajer Technology Co. Ltd. (Tianjin, China). LUMTECH MPFC-QuEChERS (complex matrix) (MPF1) and LUMTECH MPFC-QuEChERS (simple matrix) ultrafiltration-purification columns (MPF2) were purchased from Lumtech Technology Co. Ltd. (Beijing, China); QUICLEAR syringe filters (QUI) were purchased from Alta Technology Co. Ltd.) (Tianjin, China). The QuEChERS purification package (QUE) made up of anhydrous magnesium sulfate, sodium chloride, sodium citrate, and disodium hydrogen citrate was purchased from Dikma Technology Instrument Co. Ltd. (BeiJing, China).

2.2. GC Analytical Conditions

The determination of 10 pyrethroid pesticides was performed using the Agilent 7890A GC Ultra gas chromatographer with an electron-capture detector (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved using an HP-5MS capillary column (30 m × 0.32 mm, 0.25 μm film thickness) (J&W Scientific, Santa Clara, CA, USA), with the temperature program set as follows: 100 °C hold for 1 min, 100–220 °C at 25 °C min^{-1} (hold for 2 min), 220–240 °C at 10 °C min^{-1} (hold for 6 min), then 240–260 °C at 4 °C min^{-1} (hold for 2 min), and lastly 260–300 °C at 10 °C min^{-1} (hold for 2 min). The total run time was 30.33 min. The electron-capture detector (ECD) temperature was set at 300 °C. Nitrogen (99.999% purity) was used as a carrier gas with a constant flow of 1.0 mL min^{-1}. The splitless mode was used with splitless injection and the inlet temperature was set at 220 °C. GC chromatograms of 10 pyrethroid pesticides can be seen Figure 1.

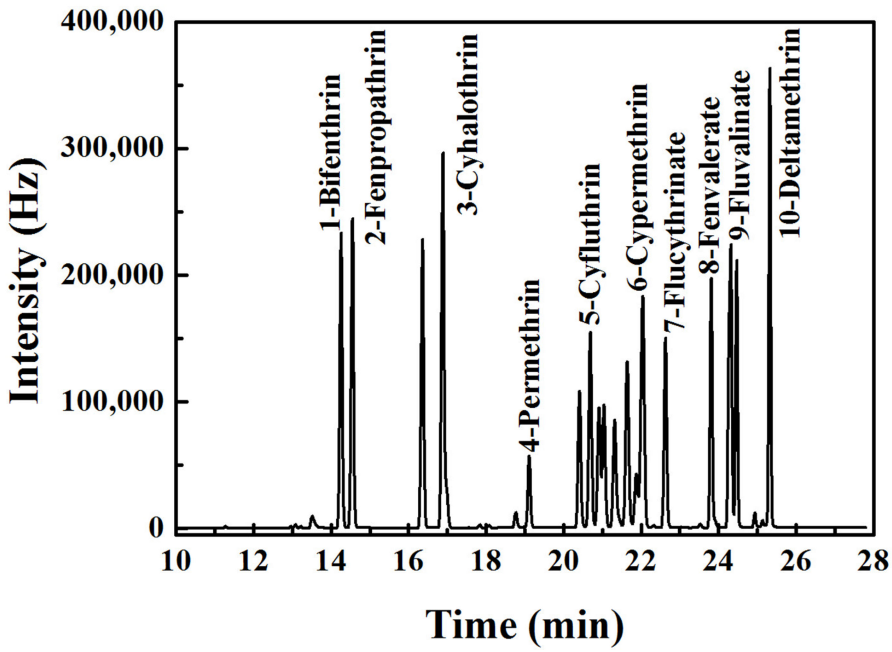


Figure 1. Chromatograms of 10 pyrethroid pesticides.

2.3. TLC Analytical Conditions

The analysis of TLC was finished by a BC-D1 efficient and multi-functional pesticide-residue rapid-measuring instrument (Shanghai Ruixin Technological Instrument Co. Ltd., Shanghai, China) that was made up of a concentrator, color-developing instrument, and imaging analyzer. Next, 2 mL filtrate was dried using the measuring instrument, and then 100 µL of petroleum ether was added to the reconstitution. An entire tube of reconstituted solution using thin capillary glass pipettes was spotted on the TLC plates, then put into the TLC tank to expand. The TLC plates were taken out of the thin-layer plate and dried after the developing agent reached the front line. At last, the TLC plates were put into the imaging analyzer for imaging analysis.

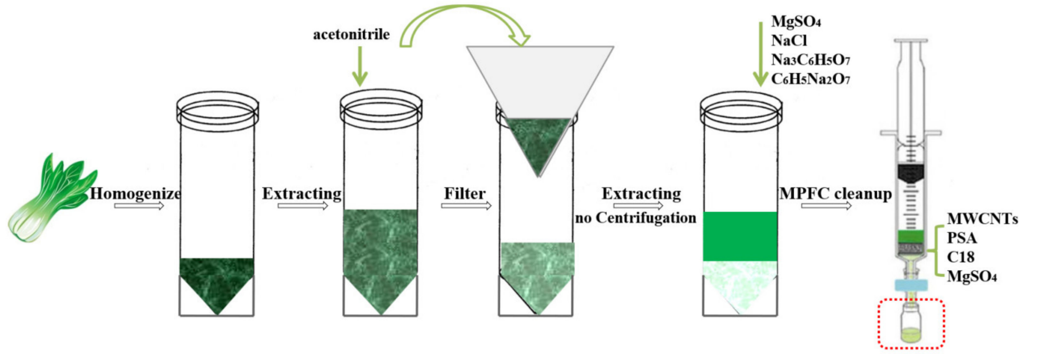
2.4. Analysis of Quick Test Strip

The analysis of the quick test strip was finished by a colloidal-gold test strip (Shenzhen Bioeasy Biotechnology Co. Ltd., Shenzhen, China). Generally speaking, a mixture of 20 µL of filtrate and 180 µl of ultra-pure water was directly detected using a colloidal-gold test strip.

2.5. Sample Preparation

Vegetable samples were collected from farmers' markets in Shanghai. Then, the samples were ground into powder and homogenized by a food processor. The prepared samples were stored at 4 °C and analyzed within 24 h following the procedure described below. Pyrethroid-free samples were used as the blank matrix with which to prepare matrix-matched standards for calibration.

Samples were extracted following the typical QuEChERS procedure with some modifications as shown Scheme 1. In brief, homogenized samples (10 ± 0.01 g) were exactly weighed into a 50 mL polypropylene centrifuge tube, followed by the addition of 20 mL of acetonitrile. Then, the centrifuge tube was shaken manually for 1 min and filtered with filter paper into another 50 mL polypropylene centrifuge tube. Subsequently, the QuEChERS extraction-salt pack (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, and 0.5 g disodium hydrogen citrate) and a homogenizer were added, and the mixture was then shaken vigorously for 1 min. After remaining at room temperature for 1–3 min, 1.5 mL of the acetonitrile supernatant was filtered through the MPFC column (the specific classification of simple and complex samples can be seen in Supplementary Materials, Table S1) and a 0.22 μm Nylon syringe filter (Dikma Technologies Instrument Co. Ltd., Beijing, China). The filtrate was either directly used for analysis or stored for further use.



Scheme 1. Schematic diagram of in situ sample-processing methods for rapid simultaneous detection of pesticides in vegetables.

2.6. Matrix Effects

MEs are defined as the different effects of components other than the target analyte on the response value of the target compound in the sample by The National Committee for Clinical Laboratory Standardization (NCCLS) [47]. ME is caused by the co-elution of matrix constituents that play an important role in the multi-residue analysis of pesticides, which can affect the accuracy of the analysis results by matrix enhancement and attenuation effects caused by the amount and the type of the sample matrix and sample-preparation procedure [48]. For the vegetable matrix, there are numerous components that will influence ME value, such as polar pigments, fatty acids, sugars and so on. Therefore, ME is a very effective indicator by which to identify the purification effect of the pre-processing method for the detection of pesticide residue in vegetables.

ME was calculated by the equation: $ME = K_{matrix}/K_{standard}$ or $ME = A_{matrix}/A_{standard}$, where K_{matrix} is the slope of the matrix standard-calibration curve, $K_{standard}$ is the slope of the pure-solvent standard-calibration curve, A_{matrix} is the peak area of the matrix standard solution and $A_{standard}$ is the peak area of the solvent standard solution. Generally, the closer the ME value is to 1, the smaller the matrix effect. Usually, ME is considered to be ignored if the ME value is between 0.85–1.15, while it was regarded to be matrix suppression or enhancement effect when the value is less than 85% or greater than 115%, respectively [49,50].

3. Results and Discussion

3.1. Optimization of the Extraction Procedure

The QuEChERS procedure reduces the analytical error due to the application of few steps and good recoveries for most of the pesticides, with only slight differences in the multi-class pesticide analysis in vegetables. However, Lehotay and Maótovská reported that low recoveries of some pH-sensitive analytes prompted the need for a buffering medium. The result of the application of sodium acetate in response to this need increased recoveries remarkably and led to the adoption of the official methods AOAC 2007.01 [51]. Lehotay et al. suggested that there is no need to optimize the QuEChERS method for one particular class of analyte or matrix [52]. In 2015, González-Curbelo et al. mentioned that the AOAC 2007.01 method has been adopted as a routine method in many laboratories, although many other modified versions have also been developed [53,54]. Referring to China National Standard «GB 23200.113-2018» [55], this study choose an extraction-salt pack (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, and 0.5 g disodium hydrogen citrate) as the extraction salt, and 20 mL acetonitrile the as extraction solution.

3.2. Optimization and Comparison of the Cleanup Procedure

3.2.1. Optimization of Purification Column

A suitable purification column is crucial to the rapid filtration-purification method. This study firstly compared the purification effects of three commercially available filtration-purification columns and the QuEChERS purification kits in terms of recovery rate and matrix effect. The solid-phase sorbents of NANO and MPF filtration-purification columns are made up of MWCNTs, PSA, C18, and anhydrous $MgSO_4$, and QUI filtration-purification columns contain PSA, C18, and anhydrous $MgSO_4$. The greatest difference between NANO, MPF1, MPF2, QUI and QUE is the amount of MWCNTs.

In this study, cucumber was used as a simple substrate, and greengrocery was used as a complex substrate. The cleanup effects of NANO, MPF1, MPF2, QUI and QUE were investigated under a spiked level of 0.01 mg/kg. The results are shown in Figures 2 and 3. For simple matrices (Figure 2), it can be observed that the MPFC1 ultrafiltration-purification column has a poor recovery rate (70–87%), the other four purification methods have good recovery rates (81–99%), and the MPFC2 ultrafiltration-purification column has a recovery rate of 81–94%, which is the same as the recovery rate of the conventional QuEChERS method, while slightly higher than that of NANO column and QUE column. At the same time, the ME value of MPFC1 is between 0.88–1.12 (the matrix effect is negligible), and the matrix effect of MPFC2 is close to the conventional QuEChERS method. For complex matrices (Figure 3), the recovery rates of the five methods are the same (70–116%). The matrix effect of MPFC1 is the smallest; the ME value is between 1.03–1.22, and the ME values of the remaining four methods are between 1.03–1.75.

The main substance which plays a major role in the five purification methods is PSA. PSA is a weak anion exchanger, which can effectively remove organic acids, sugars, fatty acids, polar pigments, and other substances that easily form hydrogen bonds in the sample, but its adsorption capacity is limited. As shown in Figure S1, the QUICLEAR syringe filter only contains PSA and has the worst pigment-removing ability. The purification effect of the QuEChERS purification package is better than that of the QUICLEAR syringe filter because the QuEChERS purification package contains PSA and graphitized carbon black (GCB). GCB can effectively remove pigments in vegetables, such as chlorophyll and carotenoids. However, the strong adsorption ability will lead to a low target-recovery rate. The samples purified by NANO and MPFC purification columns are almost colorless because the two purification columns contain PSA and an appropriate amount of MWCNTs. MWCNTs is a nano-grade high-strength carbon fiber material with a large specific surface area, better adsorption, and purification capabilities, which can effectively remove impurities such as pigments while presenting little effect on the recovery rate of the target.

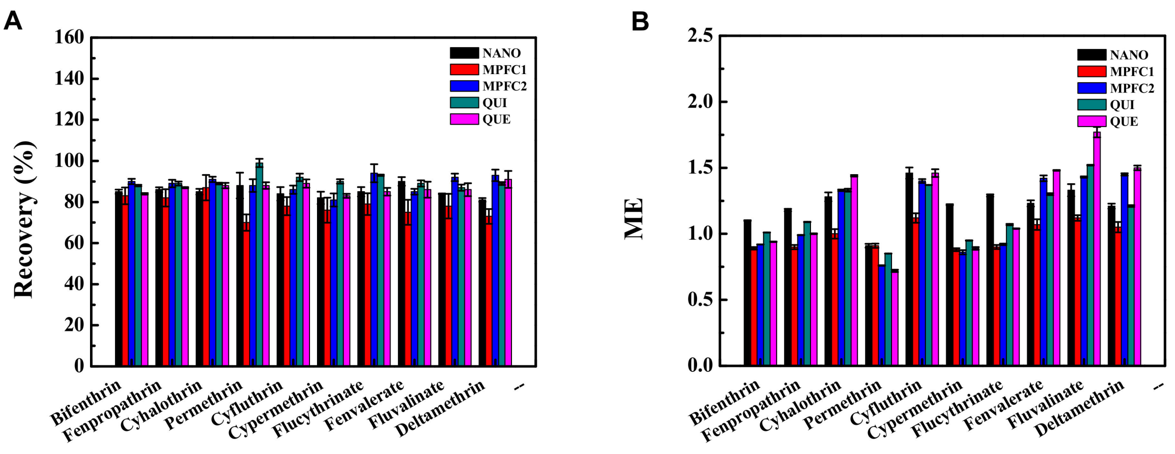


Figure 2. (**A**) The effect of different purification columns on the recovery rate of pyrethroid pesticides in a simple matrix (cucumber) (n = 3). (**B**) Matrix effect of pyrethroid pesticides in the simple matrix (cucumber) under different purification conditions.

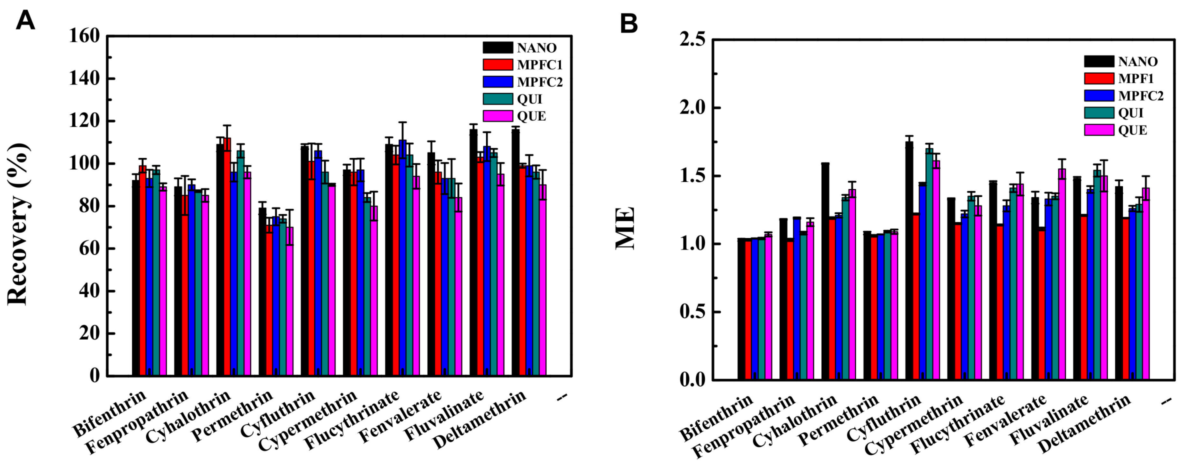


Figure 3. (**A**) The effect of different purification columns on the recovery rate of pyrethroid pesticides in a complex matrix (greengrocery) (n = 3). (**B**) Matrix effect of pyrethroid pesticides in a complex matrix (greengrocery) under different purification conditions.

The above experimental results show that the combination of PSA and MWCNTs with NANO and MPFC purification columns can effectively remove impurities in the sample, reduce the interference with the target and increase the recovery rate. The purification effect of the MPFC1 (complex matrix) purification column is the best; however, it will absorb pyrethroid pesticides to a certain extent and cause the recovery rate to decrease, which can be ignored in the complex matrix. Thus, MPFC columns (MPFC2 ultrafiltration-purification columns for simple matrices and MPFC1 ultrafiltration-purification columns for complex matrices) were selected as the cleanup unit for further experiments.

3.2.2. Effect of Centrifugation

One of the requirements for the detection method of the rapid in situ analysis is that it is simple and suitable for substratum. Therefore, using the fewest instruments as well as ensuring the accuracy of the measured results is very essential. In the QuEChERS method, a high-speed centrifuge is used to accelerate the stratification of the acetonitrile phase and the water phase. The method of China National Standard NY/T 761-2008 [56] is to naturally separate the acetonitrile phase and the water phase by remaining at room temperature after filtration. The centrifuge is an instrument of large dimensions that is not suitable for on-site inspection at the base course. During the experiment, it was found that after the filtered filtrate was added to the extraction-salt packet and left at room temperature, the acetonitrile phase and the water phase could be separated in a short time. This study also used cucumber as a simple substrate and greengrocery as a complex substrate to research the effect of the centrifuge. The effects of the two methods of centrifugation and remaining at room temperature on the recovery rate and matrix effect of 10 pyrethroid pesticides were compared under a spiked level of 0.01 mg/kg. The results were shown in Table 1. It can be observed that under the remaining-at-room-temperature conditions, except for permethrin, the recovery rate of MPF1 is 88–111%, the ME value is 0.93–1.29, and the recovery rate of MPF2 is 84–94%, the ME value is 0.92–1.43. The experimental results show that regardless of whether the matrix is simple or complex, the recovery rates and matrix effects for the 10 pyrethroid pesticides are similar, which proves that the method does not require centrifugation. Hence, remaining at room temperature for natural layering was chosen as the extraction method.

Table 1. Influence of centrifugation on the recovery rate and matrix effect of pyrethroid pesticides.

Pesticides	Recovery Rate/% (RSD/%)				ME			
	Cucumber (MPF2)		Greengrocery (MPF1)		MPF2		MPF1	
	N	Y	N	Y	N	Y	N	Y
Bifenthrin	94 (3.0)	90 (1.3)	95 (0.2)	99 (3.2)	0.92	0.92	0.93	1.03
Fenpropathrin	87 (3.7)	89 (1.8)	88 (2.5)	85 (9.2)	0.99	0.99	1.04	1.03
Cyhalothrin	87 (4.6)	91 (1.3)	111 (1.4)	112 (6.0)	1.29	1.33	1.16	1.19
Permethrin	86 (4.6)	88 (3.1)	71 (0.1)	74 (3.5)	0.76	0.76	0.96	1.06
Cyfluthrin	84 (3.5)	86 (2.0)	105 (2.1)	101 (8.4)	1.38	1.4	1.29	1.22
Cypermethrin	86 (6.0)	81 (3.2)	96 (8.1)	96 (6.2)	0.84	0.86	1.07	1.15
Flucythrinate	85 (3.2)	94 (4.4)	108 (5.6)	104 (4.4)	0.94	0.92	1.15	1.14
Fenvalerate	87 (4.7)	85 (1.4)	99 (0.8)	96 (5.5)	1.37	1.42	1.18	1.11
Fluvalinate	88 (2.9)	92 (1.8)	104 (1.7)	103 (2.4)	1.38	1.43	1.21	1.21
Deltamethrin	90 (1.1)	93 (2.8)	98 (3.6)	99 (1.0)	1.43	1.45	1.21	1.19

N: The sample was processed by pre-processing method without centrifugation. Y: The sample was processed by pre-processing method with centrifugation.

3.2.3. Effect of Filtration

In the previous work, we removed centrifugation. The intention of filtration is to remove the fibrous material in the matrix and promote the separation of the organic and aqueous phases. This study continued to use cucumber as a simple substrate and greengrocery as a complex substrate to compare the effects of filtration on the recovery rates and matrix effects of pyrethroid pesticides under a spiked level of 0.01 mg/kg. The results are shown in Table 2. For simple substrates, the range of the recovery rate without filtration is 73–84%, and the recovery rate of filtration is 76–84%, which is the same. For complex matrices, the recovery rate of filtration ranges from 70 to 89%. Simultaneously, the recovery rates without filtration of permethrin, cyfluthrin, and deltamethrin are 56%, 58%, and 58%, respectively. The recovery rates of the rest range from 62 to 72%, which is much lower than the recovery rate under filtration conditions. The above analysis results show that for complex matrices, filtration has a great influence on the recovery rate, so filtration is essential for complex matrices. For simple substrates, the effect of filtration is very small, therefore filtration is not necessary.

Table 2. Influence of filtration on the recovery rate of pyrethroid pesticides.

Pesticides	Recovery Rate/% (RSD/%)			
	Cucumber (Simple Substrate)		Greengrocery (Complex Substrate)	
	N	Y	N	Y
Bifenthrin	81 (3.5)	82 (3.0)	72 (4.2)	80 (2.0)
Fenpropathrin	79 (4.9)	82 (3.2)	70 (9.6)	80 (8.5)
Cyhalothrin	81 (6.4)	81 (4.3)	71 (6.6)	85 (4.3)
Permethrin	79 (6.5)	84 (2.8)	56 (9.2)	70 (9.3)
Cyfluthrin	79 (3.5)	81 (3.1)	58 (8.6)	75 (3.1)
Cypermethrin	80 (8.5)	81 (5.5)	62 (8.2)	80 (4.0)
Flucythrinate	81 (4.1)	83 (4.3)	62 (12.0)	79 (8.4)
Fenvalerate	80 (4.9)	76 (4.7)	69 (10.2)	89 (5.5)
Fluvalinate	84 (3.0)	84 (1.5)	63 (9.9)	78 (9.2)
Deltamethrin	73 (8.7)	77 (5.0)	58 (8.3)	71 (8.9)

N: The sample was processed by pre-processing method without filtration. Y: The sample was processed by pre-processing method with filtration.

3.2.4. Effect of Standing Time

During the experiment, it was found that the acetonitrile phase and the water phase could achieve preliminary separation after adding sodium chloride and magnesium sulfate by vigorous shaking. To ensure that target samples were completely eluted, this study tested the evolution of the recovery rate in the range of 1–10 min after the acetonitrile phase and the water phase had separated. As shown in Figure 4, it can be observed that the recovery rate of bifenthrin is close to 100% when standing for 1–10 min. For the other four pyrethroid pesticides, the recovery rate is higher when the standing time is in the range of 1–3 min. With the increase in standing time, the recovery rate decreases slightly. After standing for 7 min, there is no significant improvement in the recovery rate, which remains relatively stable (within the acceptable range). It means that the recovery rate is the highest and remains relatively stable when the standing time is 1–3 min. Thus, 1–3 min was used as the standing time.

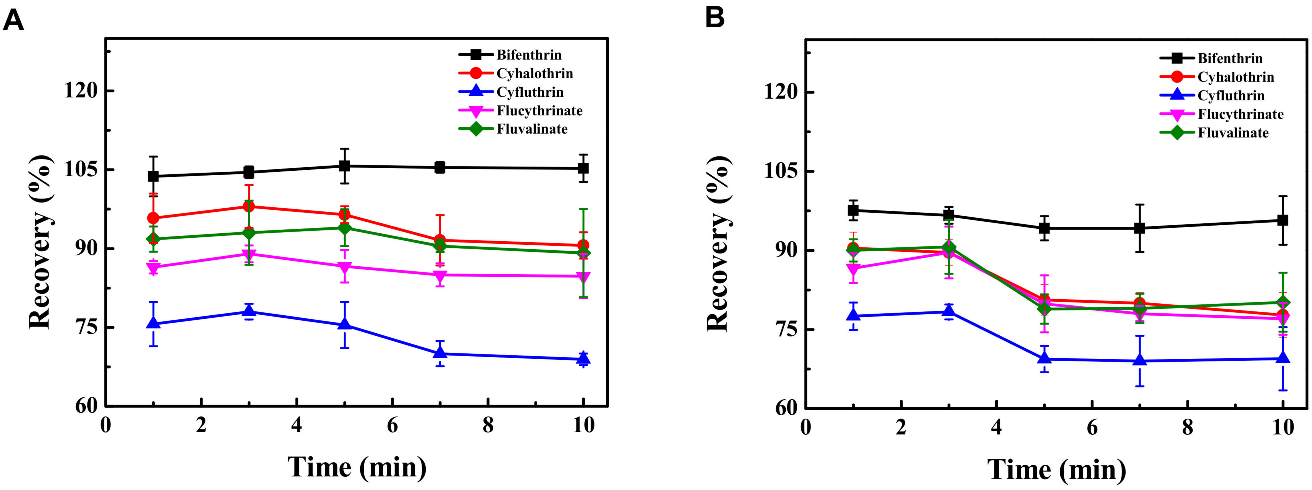


Figure 4. The recovery rate changes with the standing time after the acetonitrile phase was separated from the water phase for a simple substrate (**A**) and a complex substrate (**B**).

3.3. Method Validation

Linearity, the limits of detection (LOD), the limits of quantification (LOQ), accuracy, and the precision of this method for the determination of 10 pesticides were validated under optimized conditions. A total of 10 representative vegetables were selected as substrates in this experiment, including Chinese cabbage (simple substrate), tomato (simple substrate), wax gourd (simple substrate), bitter gourd (simple substrate), asparagus (simple substrate), water spinach (complex substrate), cowpea (complex substrate), eggplant (complex substrate), leek (complex substrate), and cabbage (complex substrate). Linearity was examined through the calibration curves of five matrix-matched standards (0.005, 0.01, 0.05, 0.1, 0.5 mg/kg) for the 10 pesticides, and the accuracy and precision were evaluated through the recovery experiments, which were carried out at three spiked levels (0.05, 0.1 and 0.2 mg/kg) in different substrates. The results of method validation are given in Table 3. The LOD and LOQ of the proposed method were calculated as the concentration giving signal-to-noise ratios of 3 (S/N = 3) and 10 (S/N = 10), respectively.

As shown in Table 3, all 10 pyrethroid pesticides have a good linear relationship in the range of 0.005–0.5 mg/kg, and the determination coefficient (R^2) is higher than 0.99. It was evaluated that the LOD of 10 pyrethroid pesticides are all between 0.0001 and 0.007 mg/kg, and the LOQ are all between 0.0002 to 0.02 mg/kg. This developed method shows that the average recoveries of the ten pesticides in different vegetables range from 70% to 120% with relative standard deviations (RSD) from 0.2% to 9.3%. The results comply with the European Union guide lines SANTE/12682/2019 [57] (spike recovery should be in the range 70–120% with an RSD less than or equal to 20%). For most substrates, the recovery rate of deltamethrin is relatively low, and the recovery rate of other pyrethroid pesticides is better. The method has good applicability and meets the guidelines of pyrethroid pesticides in actual vegetable samples.

Table 3. Correlation coefficients, detection limits, quantification limits, spike-recovery rates and relative standard deviations of pyrethroid pesticides in 10 vegetables (n = 3).

Pesticides	Chinese Cabbage (Simple Substrate)						Tomato (Simple Substrate)					
	R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)			R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)		
				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg
Bifenthrin	0.9968	0.0002	0.0006	101 (3.8)	99 (1.2)	89 (2.5)	0.9959	0.0001	0.0004	99 (3.5)	100 (1.1)	101 (4.3)
Fenpropathrin	0.9946	0.0002	0.0006	90 (5.8)	89 (1.6)	78 (4.0)	0.9969	0.0001	0.0004	101 (3.4)	100 (0.7)	102 (2.0)
Cyhalothrin	0.9937	0.0001	0.0004	70 (9.0)	71 (3.1)	72 (7.2)	0.9949	0.0002	0.0007	93 (5.6)	107 (1.4)	110 (8.9)
Permethrin	0.9971	0.0005	0.002	107 (8.7)	110 (2.2)	96 (0.7)	0.9981	0.0003	0.001	106 (5.5)	96 (1.9)	89 (1.3)
Cyfluthrin	0.9949	0.0001	0.0003	78 (9.3)	72 (4.2)	79 (8.2)	0.9964	0.0001	0.0003	99 (7.9)	97 (0.9)	103 (6.9)
Cypermethrin	0.9918	0.0001	0.0002	93 (1.9)	94 (2.5)	85 (5.5)	0.9963	0.0001	0.0002	102 (4.0)	99 (2.6)	100 (6.8)
Flucythrinate	0.9909	0.0003	0.0009	81 (9.1)	85 (1.4)	76 (5.3)	0.9975	0.0002	0.0008	94 (3.2)	100 (0.4)	106 (1.1)
Fenvalerate	0.9736	0.0006	0.002	73 (9.2)	76 (3.6)	78 (7.3)	0.9962	0.001	0.004	95 (3.9)	100 (0.1)	107 (7.1)
Fluvalinate	0.9647	0.0003	0.0009	86 (9.6)	77 (5.7)	79 (5.3)	0.9928	0.0005	0.002	106 (5.0)	107 (1.0)	108 (5.5)
Deltamethrin	0.9958	0.0007	0.002	76 (2.5)	73 (5.4)	79 (8.3)	0.9932	0.002	0.005	97 (6.2)	108 (2.4)	105 (8.2)
Pesticides	**Wax Gourd (Simple Substrate)**						**Bitter Gourd (Simple Substrate)**					
	R^2	**LOD/mg/kg**	**LOQ/mg/kg**	**Recovery Rate/% (RSD/%)**			**R^2**	**LOD/mg/kg**	**LOQ/mg/kg**	**Recovery Rate/% (RSD/%)**		
				0.05 mg/kg	**0.10 mg/kg**	**0.20 mg/kg**				**0.05 mg/kg**	**0.10 mg/kg**	**0.20 mg/kg**
Bifenthrin	0.9999	0.0002	0.0005	93 (0.6)	92 (3.4)	77 (3.1)	0.9999	0.0003	0.0009	82 (2.1)	77 (1.0)	83 (6.8)
Fenpropathrin	0.9997	0.0002	0.0005	98 (1.2)	91 (4.5)	76 (7.3)	0.9999	0.0003	0.0008	87 (1.5)	75 (1.3)	79 (1.4)
Cyhalothrin	0.9993	0.0001	0.0005	96 (2.7)	89 (3.2)	76 (7.5)	0.9998	0.0001	0.0003	85 (0.7)	72 (1.9)	77 (5.2)
Permethrin	0.9997	0.0004	0.001	78 (4.6)	84 (4.0)	70 (4.3)	0.9998	0.0005	0.002	83 (5.1)	77 (7.8)	76 (7.7)
Cyfluthrin	0.9980	0.0001	0.0002	98 (1.8)	85 (3.6)	76 (7.2)	0.9997	0.0001	0.0003	86 (8.5)	77 (2.9)	79 (2.0)
Cypermethrin	0.9981	0.0001	0.0002	106 (1.4)	89 (3.4)	79 (4.2)	0.9997	0.0001	0.0003	83 (4.7)	78 (4.3)	71 (4.8)
Flucythrinate	0.9977	0.0003	0.0009	107 (0.5)	90 (3.5)	70 (2.2)	0.9996	0.0003	0.001	88 (1.4)	80 (2.5)	72 (2.3)
Fenvalerate	0.9976	0.0004	0.001	104 (1.5)	86 (3.0)	76 (2.8)	0.9996	0.0004	0.001	83 (3.3)	76 (6.4)	78 (8.3)
Fluvalinate	0.9969	0.0002	0.0006	113 (1.2)	90 (3.3)	76 (3.2)	0.9994	0.0002	0.0007	96 (9.5)	79 (2.6)	70 (2.8)
Deltamethrin	0.9974	0.0004	0.001	90 (3.5)	77 (3.6)	70 (7.1)	0.9996	0.0004	0.001	78 (3.5)	71 (6.7)	74 (3.1)

Table 3. *Cont.*

Pesticides	Asparagus (Simple Substrate)						Water Spinach (Complex Substrate)					
	R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)			R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)		
				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg
Bifenthrin	0.9947	0.0001	0.0005	91 (4.7)	90 (4.2)	90 (2.4)	0.9960	0.001	0.005	103 (3.3)	104 (7.4)	97 (6.8)
Fenpropathrin	0.9945	0.0001	0.0004	97 (4.1)	99 (1.5)	101 (6.1)	0.9963	0.002	0.005	93 (5.3)	99 (3.2)	92 (6.9)
Cyhalothrin	0.9951	0.0002	0.0005	93 (1.6)	95 (7.4)	104 (3.5)	0.9956	0.0002	0.0007	87 (3.7)	86 (7.3)	83 (7.4)
Permethrin	0.9918	0.0005	0.002	88 (4.8)	81 (2.4)	72 (1.1)	0.9958	0.003	0.01	103 (9.9)	96 (5.6)	98 (5.4)
Cyfluthrin	0.9944	0.0001	0.0003	97 (2.5)	82 (1.1)	85 (8.4)	0.9959	0.0009	0.003	83 (1.6)	87 (8.8)	78 (6.8)
Cypermethrin	0.9945	0.0001	0.0002	101 (3.8)	109 (1.4)	115 (5.8)	0.9968	0.0007	0.002	93 (4.4)	98 (7.6)	98 (7.4)
Flucythrinate	0.9943	0.0003	0.0009	102 (1.1)	107 (1.1)	105 (1.5)	0.9967	0.002	0.008	89 (3.8)	98 (8.8)	94 (2.5)
Fenvalerate	0.9949	0.0007	0.002	97 (3.3)	104 (1.3)	102 (4.4)	0.9958	0.003	0.01	71 (6.3)	87 (7.2)	81 (6.8)
Fluvalinate	0.9942	0.0003	0.001	105 (1.0)	109 (0.8)	111 (6.3)	0.9967	0.001	0.005	90 (3.4)	99 (3.3)	82 (1.7)
Deltamethrin	0.9944	0.0008	0.003	88 (7.6)	92 (9.3)	102 (6.6)	0.9937	0.004	0.01	72 (5.7)	78 (7.6)	72 (9.1)
Pesticides	**Cowpea (Complex Substrate)**						**Eggplant (Complex Substrate)**					
	R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)			R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)		
				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg
Bifenthrin	0.9961	0.0001	0.0005	104 (1.1)	103 (0.3)	107 (1.4)	0.9951	0.0006	0.002	102 (0.9)	100 (0.7)	102 (4.4)
Fenpropathrin	0.9963	0.0001	0.0005	106 (1.3)	105 (1.0)	112 (1.2)	0.9961	0.0007	0.002	104 (3.9)	102 (6.8)	107 (4.7)
Cyhalothrin	0.9967	0.0002	0.0005	96 (2.1)	102 (0.4)	105 (1.8)	0.9987	0.0007	0.002	89 (4.6)	95 (6.4)	115 (2.3)
Permethrin	0.9917	0.0005	0.002	103 (1.8)	100 (1.7)	106 (5.6)	0.9874	0.007	0.02	103 (6.6)	97 (4.8)	103 (9.5)
Cyfluthrin	0.9964	0.0001	0.0003	102 (8.6)	97 (2.3)	108 (7.3)	0.9982	0.004	0.01	86 (4.4)	86 (1.4)	106 (6.6)
Cypermethrin	0.9963	0.0001	0.0003	96 (1.8)	106 (0.9)	114 (7.4)	0.9957	0.002	0.008	103 (5.5)	95 (7.5)	108 (3.5)
Flucythrinate	0.9964	0.0003	0.001	105 (2.6)	109 (1.0)	103 (8.6)	0.9963	0.007	0.02	95 (1.2)	98 (6.3)	115 (4.8)
Fenvalerate	0.9955	0.001	0.003	105 (2.6)	95 (1.9)	117 (3.9)	0.9986	0.003	0.009	94 (7.8)	83 (5.4)	105 (5.6)
Fluvalinate	0.9963	0.0004	0.001	107 (3.9)	101 (0.2)	106 (1.6)	0.9984	0.002	0.005	104 (4.1)	98 (1.6)	99 (4.4)
Deltamethrin	0.9961	0.001	0.004	75 (2.5)	74 (7.7)	89 (3.5)	0.9980	0.005	0.02	76 (4.5)	80 (8.1)	89 (8.6)

Table 3. *Cont.*

Pesticides	Leeks (Complex Substrate)						Cabbage (Complex Substrate)					
	R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)			R^2	LOD/mg/kg	LOQ/mg/kg	Recovery Rate/% (RSD/%)		
				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg				0.05 mg/kg	0.10 mg/kg	0.20 mg/kg
Bifenthrin	1.0000	0.002	0.007	102 (2.1)	100 (1.1)	98 (1.1)	0.9999	0.0005	0.002	99 (1.9)	97 (2.6)	95 (2.8)
Fenpropathrin	0.9997	0.002	0.007	107 (5.1)	98 (1.2)	79 (2.9)	0.9999	0.0005	0.002	101 (1.6)	96 (1.4)	95 (4.9)
Cyhalothrin	0.9997	0.0007	0.002	92 (2.8)	98 (0.8)	101 (2.5)	0.9997	0.0001	0.0005	90 (2.3)	96 (2.5)	101 (5.4)
Permethrin	0.9995	0.003	0.01	101 (2.8)	94 (2.4)	75 (5.6)	0.9990	0.0005	0.002	95 (8.5)	89 (4.8)	81 (1.4)
Cyfluthrin	0.9994	0.0007	0.002	97 (5.6)	92 (0.8)	92 (2.1)	0.9996	0.0001	0.0004	90 (4.6)	93 (6.0)	91 (3.0)
Cypermethrin	0.9995	0.0006	0.002	98 (1.7)	100 (1.9)	100 (1.1)	0.9996	0.0001	0.0004	90 (3.0)	93 (4.8)	96 (7.6)
Flucythrinate	0.9994	0.003	0.008	97 (1.4)	100 (2.8)	101 (3.0)	0.9996	0.0005	0.002	91 (2.4)	93 (3.4)	94 (8.7)
Fenvalerate	0.9994	0.0007	0.002	120 (4.0)	107 (3.8)	91 (1.7)	0.9996	0.0006	0.002	86 (2.9)	88 (4.4)	89 (5.1)
Fluvalinate	0.9989	0.0004	0.001	105 (4.3)	92 (4.0)	87 (3.5)	0.9993	0.0003	0.001	115 (2.0)	96 (6.2)	90 (5.3)
Deltamethrin	0.9988	0.0009	0.003	82 (7.7)	84 (7.5)	81 (9.1)	0.9989	0.0007	0.002	73 (4.3)	79 (8.8)	77 (9.3)

3.4. Comparison with Standard Methods

Cucumber was used as a simple substrate and greengrocery was used as a complex substrate. When the addition level was 0.01 mg/kg, the quick inspection method and the AOAC2007.1 and GB23200.113 standard methods were used for the recovery of 10 pyrethroid pesticides. For comparison, the results are shown in Table 4. The test results show that for simple matrices, the recovery rate of this quick test method is 84–94%, the recovery rates of the AOAC2007.1 and GB23200.113 standard methods are 87–94% and 88–98%, respectively, and the RSDs are both less than 5%; for complex matrices, the recovery rate of this quick test method is 71–111%, and the recovery rates of the AOAC2007.1 and GB23200.113 standard methods are 82–106% and 74–100%, respectively, with both RSDs being less than 10%, which meets the actual testing requirements. The recovery rates of the three methods are relatively small and the accuracies are the same, but compared with the AOAC2007.1 and GB23200.113 standard methods, this quick inspection method does not require other auxiliary equipment, and the pretreatment time can be controlled within 10 min, which is more convenient and more efficient than the standard method. It can be seen that the rapid inspection method can accurately realize the pretreatment of extraction and purification of pyrethroid pesticides in vegetables in a relatively short time.

Table 4. Comparison with AOAC2007.1 and GB23200.113-2018.

Pesticides	Recovery Rate/% (RSD/%)					
	Cucumber (Simple Substrate)			Greengrocery (Complex Substrate)		
	This Method	AOAC2007.1	GB23200.113	This Method	AOAC2007.1	GB23200.113
Bifenthrin	94 (3.0)	91 (1.4)	88 (1.1)	95 (0.2)	94 (8.8)	93 (4.9)
Fenpropathrin	87 (3.7)	89 (2.3)	89 (0.2)	88 (2.5)	90 (3.9)	88 (3.7)
Cyhalothrin	87 (4.6)	90 (4.0)	94 (0.6)	111 (1.4)	103 (3.5)	100 (4.3)
Permethrin	86(4.6)	89 (1.4)	98 (4.7)	71 (0.1)	82 (7.9)	74 (4.4)
Cyfluthrin	84 (3.5)	90 (3.8)	92 (2.1)	105 (2.1)	99 (0.8)	95 (3.4)
Cypermethrin	86 (6.0)	88 (1.8)	90 (1.2)	96 (8.1)	89 (0.9)	84 (1.8)
Flucythrinate	85 (3.2)	87 (3.5)	90 (2.2)	108 (5.6)	102 (7.6)	96 (7.9)
Fenvalerate	87 (4.7)	92 (2.5)	97 (1.5)	99 (0.8)	92 (3.4)	85 (9.3)
Fluvalinate	88 (2.9)	94 (4.2)	95 (4.4)	104 (1.7)	106 (0.2)	99 (4.5)
Deltamethrin	90 (1.1)	91 (4.9)	97 (2.3)	98 (3.6)	98 (1.4)	93 (9.2)

3.5. Application of Pre-Processing Method in Pyrethroid-Containing Practical Samples

The applicability of the developed method was evaluated by analyzing a total of 20 vegetable samples collected from the 2020 Routine Vegetable Monitoring Project of Shanghai Agricultural Products Quality and Safety Center and each sample was tested three times. All analyzed samples included at least one pyrethroid pesticide, and a total of 10 pyrethroid-pesticide residues were detected in 20 samples. All of the samples were processed with the new pre-processing method and then examined by different methods, such as TLC and gold-colloidal test strips. Table 5 shows the analytes detected in 20 vegetable samples. Residues of Cypermethrin, Cyhalothrin, Fenpropathrin, Bifenthrin, and Flucythrinate were detected in greengrocery, water spinach, bell pepper, potato leaves, Chinese cabbage, and feminine, respectively. For the illustration, the results of TLC and the gold-colloidal test strips were consistent, which also corresponded with GC methods. All methods provided satisfying results and were in accordance with the validation requirements.

Table 5. The detection results of 20 pyrethroid-containing practical vegetable samples.

Number	Sample	Pesticides	Results (mg/kg)			MRLs (mg/kg)
			GC	TLC	Gold Colloidal Test Strip	
1	Greengrocery 1	Fenpropathrin	0.16	−	−	1
		Cypermethrin	0.94	−	−	2
2	Greengrocery 2	Cypermethrin	0.65	−	−	2
3	Greengrocery 3	Cypermethrin	0.80	−	−	2
4	Greengrocery 4	Cypermethrin	0.79	−	−	2
5	Greengrocery 5	Cypermethrin	0.84	−	−	2
6	Greengrocery 6	Cypermethrin	0.27	−	−	2
7	Water spinach 1	Cyhalothrin	0.051	−	−	/
		Cypermethrin	1.27	+	+	0.7
8	Water spinach 2	Cyhalothrin	0.035	−	−	/
		Cypermethrin	1.29	+	+	0.7
9	Water spinach 3	Cyhalothrin	0.034	−	−	/
		Cypermethrin	1.24	+	+	0.7
10	Water spinach 4	Cypermethrin	0.69	+	+	0.7
11	Bell pepper	Bifenthrin	0.22	−	−	/
12	potato leaves 1	Bifenthrin	0.034	−	−	/
13	potato leaves 2	Bifenthrin	0.75	+	+	/
14	Chinese cabbage 1	Cyhalothrin	0.16	−	−	2
		Flucythrinate	0.23	−	−	-
15	Chinese cabbage 2	Cypermethrin	0.50	−	−	2
16	Chinese cabbage 3	Cypermethrin	0.35	−	−	2
17	Chinese cabbage 4	Cypermethrin	0.32	−	−	2
18	Chinese cabbage 5	Cypermethrin	0.12	−	−	2
19	Feminine 1	Cyhalothrin	0.096	−	−	2
		Cypermethrin	0.10	−	−	2
20	Feminine 2	Cypermethrin	0.13	−	−	2

"+": positive; "−": negative; "/": no.

4. Conclusions

A rapid and efficient multi-residue method was developed for the determination of 10 pyrethroid pesticides in vegetables using new technology. This new pre-processing method provided a good extraction and purification effect with the QuEChERS method combined with filtration by filter paper and an m-PFC process. The method validation in terms of analytical range, precision, and recovery showed that the proposed method met the requirements for pesticide analysis. This pre-processing method was proven to be very rapid, which took just a few seconds to perform without the vortex and centrifugation steps. Although the current study was focused on 10 pyrethroid pesticides in vegetables, the underlying principle and strategy of the proposed methods have the potential to become a common pre-processing method that takes into account both the purification effect and the recovery rate, which should be suitable for the routine analysis and monitoring of a large number of pesticides in different matrices. This method is expected to be widely applied to market monitoring at trace levels in our future study.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/separations9030059/s1, Table S1: Comparison table of LUMTECH MPFC-QuEChERS purification column. Figure S1: Color comparison diagrams of purification solutions (green grocery) corresponding to different methods.

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