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A comparison of the determination of multiple pesticide residues in fruits, vegetables, and edible fungi using gas chromatography combined with filtration purification and solid-phase extraction

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The multiplug filtration clean-up (m-PFC) and solid-phase extraction (SPE) pretreatment methods were employed to process 8 representative matrices in fruits, vegetables, and edible fungi, respectively. 37 pesticide residues were determined using gas chromatography equipped with ECD and FPD detectors. The measurement data were compared and analyzed following m-PFC purification and gas chromatography analysis, and both accuracy and precision met the (EU) 2021/808 requirements, achieving recovery rates for the 8 matrices ranging from 67.0% to 112.8% (averaging over 83.8% recovery), and RSDs between 0.2% and 15.2%. The 37 pesticides exhibited good linearity between 0.05 and 1.6 μg mL^{-1}, and the matrix effect was found to be weaker compared to that of the Florisil solid-phase extraction method. The detection limits ranged from 0.0001 to 0.03 μg kg^{-1}, with 31 pesticides showing lower detection limits compared to the SPE method. The application of this method to 150 real samples resulted in the detection of 17 pesticides across all samples. Fewer pigments were detected in m-PFC purified solutions compared to Florisil PR SPE when analyzed by liquid chromatography. m-PFC achieved more thorough adsorption of endogenous substances like pigments, reducing instrument contamination, utilizing less organic solvent, and simplifying the operation. This purification step offers clear advantages, allowing for the processing of larger sample batches in a short time. It can serve as a replacement for SPE methods like Florisi PR in batch detection of fruit and vegetable samples.

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Introduction

Food is considered the cornerstone of life, with agricultural products like vegetables, fruits, and edible fungi being essential components of daily sustenance. However, in agricultural production, the growing dependence on chemical inputs to boost yields and improve harvests has resulted in escalating concerns regarding pesticide residue levels.[1,2] Excessive pesticide residues pose potential risks to human health. Therefore, formulating maximum residue limits (MRLs) standards for pesticides has become crucial for many countries and organizations to strengthen risk management and control of pesticide residues in fruits and vegetables.[3–5] Common methods for testing pesticide residues include gas chromatography (GC),[6] liquid chromatography (LC),[7] gas chromatography-tandem mass spectrometry (GC-MS/MS),[8,9] liquid chromatography-tandem mass spectrometry (LC-MS/MS).[10,11] Mass spectrometry, known for its high selectivity and sensitivity, is particularly suited for detecting trace substances. However, its high equipment cost poses significant operational financial pressures on small and medium-sized laboratories. In contrast, gas chromatography, with its excellent qualitative and quantitative capabilities and ease of operation, also offers the advantage of relatively low equipment costs. Therefore, it has become the preferred method for pesticide residue testing in small to medium-sized laboratories.

By employing appropriate instruments, equipment, and pretreatment methods, the requirements for determining specific compounds can be met by researchers. Many organic phosphorus compounds, characterized by their thermal stability and volatility, are found particularly suitable for analysis using gas chromatography.[12,13] IIn the determination of pesticide residues in fruits, vegetables, and edible fungi by gas chromatography (GC), sample pretreatment is recognized as a key step to ensure accurate qualitative and quantitative analysis. Purification, a crucial core step in the pretreatment process, is utilized to eliminate interference from various sample matrices. Fruits, vegetables, and edible fungi, which contain different amounts

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of water, pigments, sugars, lipids, organic acids, vitamins, and other components, are subjected to traditional methods such as solid-phase extraction (SPE),[14] liquid–liquid extraction (LLE),[15,16] solid-phase microextraction (SPME),[17] gel permeation chromatography (GPC) cleanup,[18] matrix solid-phase dispersion (MSPD),[19] and disperse-SPE (d-SPE).[20,21] These methods often suffer from low efficiency and incomplete removal of impurities, leading to inaccurate quantification and false-positive results. Conversely, excessive purification is often found to result in significant pesticide adsorption, leading to false-negative results. Moreover, these processes, which are cumbersome and time-consuming, fail to quickly qualify and quantify contaminants. Consequently, the detection report may be issued long after the products have been sold, falling short of the goal of risk prevention and control.

In 2013, Zhao and colleagues[22] first utilized multi-walled carbon nanotubes (Multi-Walled Carbon Nanotubes, MWCNTs) as a purification material to establish a method for analyzing 186 kinds of pesticide residues in tomatoes and tomato products. MWCNTs are a kind of novel carbonaceous material composed of multiple layers of graphene curled and closely stacked to form concentric cylindrical layers of carbon atoms. Their highly ordered nanometer–diameter structure provides a large specific surface area, extremely high mechanical strength, and elasticity, as well as excellent thermal and electrical conductivity. These characteristics endow MWCNTs with superior adsorption capabilities compared to other adsorbent materials, enabling them to effectively remove a variety of interfering substances in pesticide residue analysis, including pigments, organic acids, sugars, and sterols. Furthermore, MWCNTs address the issue of adsorption of planar pesticides, improving upon the limitations observed with graphitized carbon black (GCB).[23–28] Han *et al.*[29] established a pesticide residue analysis method for 124 pesticides in liquor, sorghum, and rice hulls by combining m-PFC with GC-MS/MS. Qin *et al.*[30] established an m-PFC clean-up method for 25 pesticides in six typical matrices, including wheat, spinach, and carrot, and compared it with the QuEChERS method, which stands for quick, easy, cheap, effective, rugged, and safe. Song *et al.*[31] established a pesticide residue analysis method for 48 pesticides in green tea by combining m-PFC with LC-MS/MS.

Currently, existing reports show that the rapid filtration purification technology, known as multiplug filtration clean-up (m-PFC), has been applied as a pretreatment in detecting various pesticide residues in fruits, vegetables, and edible fungi using mass spectrometry analyses such as GC-MS/MS and LC-MS/MS.[32–34] There have been no reports of applying this method as a pretreatment for gas chromatography (GC) alone. To address the needs of small and medium-sized laboratories and enterprises lacking mass spectrometers and requiring rapid monitoring results, this experiment introduces m-PFC, primarily utilizing MWCNTs, to establish a rapid detection method for 37 pesticide residues, including organophosphates (OPPs), organochlorines (OCs), and pyrethroids (PYs), in eight representative matrices of fruits, vegetables, and edible fungi, combined with gas chromatography detectors (GC-FPD, GC-ECD). Compared to solid-phase extraction (SPE), m-PFC eliminates the activation and elution steps of SPE,[22] significantly shortening the purification time and effectively addressing the problems associated with SPE, such as numerous purification steps, large reagent consumption, and lengthy processes.

To the best of our knowledge, this study is first to establish a rapid method for the determination of organophosphorus and organochlorine (pyrethroids) in fruits, vegetables and edible mushrooms using m-PFC clean-up combined with GC-FPD/ECD. This is intended to provide a method reference for small and medium-sized micro-laboratories and enterprises to detect multi-pesticide residues in plant source samples in large quantities. These conditions are designed to ensure optimal recovery, minimize potential matrix effects, and adhere as closely as possible to the principles of green analytical chemistry, which emphasize reducing time, cost, the number of steps, and the amount of reagents used. Our study also seeks to validate the proposed method for various fruits, vegetables, and edible mushrooms in accordance with European legislation.

Experiments

Instruments and reagents

Instruments. GC-7890A with FPD and ECD (Agilent, USA); T-25 High-speed Shaker (IKA, Germany); EFCG-11155 Nitrogen Evaporator (Organomation, USA); TP-602 Centesimal Balance (Beijing Sartorius Instrument and System Co., Ltd, BSISL, China); TGL-16 High-speed Refrigerated Centrifuge (Sichuan Shuke Instrument Co., Ltd, China); KN-026S Multi-Tube Vortex Mixer (Beijing Knorth Technology Co., Ltd, China); and food grinder (Braun, Germany).

Drug reagents. Florisi PR-SPE column (1000 mg/6 mL), carbon-SPE column (500 mg/6 mL) from Agilent Technologies Inc.; m-PFC B-5 column (750 mg/6 mL), m-PFC C-5 column (800 mg/6 mL), GCB-SPE column (500 mg/6 mL), and buffer extraction from Beijing Knorth Technology Co., Ltd; acetonitrile, acetone, and *n*-hexane (chromatographically pure) from Fisher Chemical; additional reagents included analytical reagent (AR) and pure water, both produced by the ultrapure water machines in the laboratory. All the standard substances, purchased from NCRMN, are liquid solutions of 1000 μg mL^{-1} and include 19 organophosphorus types (OPPs), 9 organochlorine types (OCs), and 9 pyrethroid types (PYs), totaling 37 pesticides. First, a single-standard stock solution of 100 μg mL^{-1} was prepared using acetone for organophosphorus and *n*-hexane for organochlorine and pyrethroid standard solutions. Subsequently, four groups of mixed standard solutions were prepared at a concentration of 4 μg mL^{-1} in the order of appearance. These solutions were then diluted with acetone, *n*-hexane, or various matrices to create working solutions and calibration curves with different concentrations. Table 1 lists the names and CAS numbers of the pesticides.

Samples. The plant-derived samples: *Pleurotus ostreatus* (edible fungus), tomato (solanaceous vegetable), radish (root vegetable), Chinese cabbage (leaf vegetable), sweet cherry (fruit), cucumber (melon), string beans (bean), and spinach (leaf vegetable)—totaling 8 samples of 5 vegetable species, 1

Table 1 Pesticide compound CAS information and retention time for gas chromatography analysis

Name	CAS	*R*/*T* (min)	Name	CAS	*R*/*T* (min)
Dichlorvos	62-73-7	4.84	α-BHC	319-84-6	7.31
Methamidophos	10265-92-6	5.83	β-BHC	319-85-7	7.76
Acephate	30560-19-1	8.39	γ-BHC	58-89-9	7.94
Diazinon	333-41-5	10.58	δ-BHC	319-86-8	8.46
Dimethoate	60-51-5	13.06	Vinclozolin	50471-44-8	9.07
Parathion-methyl	298-00-0	15.13	Triadimefon	43121-43-3	10.11
Fenitrothion	122-14-5	15.75	Procymidone	32809-16-8	10.90
Isofenphos-methyl	99675-03-3	16.37	Fenpropathrin	39515-41-8	13.99
Triazophos	24017-47-8	19.37	Cyfluthrin	68359-37-5	14.33
Phosmet	732-11-6	21.28	Flucythrinate	70124-77-5	16.44–16.54
Phorate	298-02-2	9.45	Tau-fluvalinate	102851-06-9	16.95–17.17
Omethoate	1113-02-6	10.53	Deltamethrin	52918-63-5	17.95–18.03
Monocrotophos	6923-22-4	12.64	Dicofol	115-32-2	18.85
Chlorpyrifos	2921-88-2	14.967	Iprodione	36734-19-7	10.218–14.411
Malaoxon	121-75-5	15.62	Bifenthrin	82657-04-3	14.14
Parathion	56-38-2	16.22	Cyhalothrin	91465-08-6	15.11
Isocarbophos	24353-61-5	16.63	Cypermethrin	52315-07-8	16.78–16.97
Profenofos	41198-08-7	17.42	Fenvalerate	51630-58-1	17.82–18.08
Phosalone	2310-17-0	22.22			

fruit type, and 1 edible fungus—were sampled randomly from plantation bases and the market. Subsequently, the samples were promptly processed into a homogenized mixture using a blender and packed in plastic sample boxes until analysis. The prepared backup samples were labeled and stored at −20 °C until the test (for less than 1 month), then removed for natural thawing and thorough mixing before analysis.

Sample pretreatment

Extraction process. The extracted fresh samples were ground in the food grinder, and 10.00 g (±0.05) of the ground fresh samples were weighed precisely and put into a 50 mL centrifuge tube. Since the moisture content of fresh fruits, vegetables, and edible fungi samples exceeds 75%, there is no need to add moisture. Add 20.00 mL of acetonitrile reagent and homogenize at high speed for 2 minutes. Then, add an extraction reagent pack for m-PFC, mix uniformly for 1 minute by oscillation, and centrifuge at 6000 rpm for 5 minutes. The supernatant was used for further clean-up.

Rapid filtration purification method (m-PFC). After centrifugation, take the upper layer solution in the 50 mL centrifuge tube and transfer 3.2 mL each to the m-PFC B-5 and m-PFC C-5 column tubes. Control the flow rate at 1 drop per second and wait for all the purified solution to pass through, collecting it in a 10 mL polypropylene centrifuge tube. Maintain the temperature below 55 °C and blow with nitrogen until nearly dry. Afterwards, extract 1.6 mL of a 1 : 1 (v/v) mixture of *n*-hexane and acetone, pack it respectively into 2 bottles for testing OPPs and OCs (including PYs), after filtering through a 0.22 μm filter head. Fig. 1 illustrates the specific clean-up procedures.

Florisi PR-SPE and C18-SPE purification. Aspirate the supernatant 2.4, 2.8, 3.2, 3.6, and 4 mL of the supernatant after centrifugation into a tube. Evaporate to near dryness at 55 °C using nitrogen, then dissolve in 2 mL of *n*-hexane for sample loading. Sequentially take 5 mL of a solvent mixture (acetone : *n*-hexane, V : V = 1 : 9) and 5 mL of *n*-hexane, activate the Florisi PR-SPE column, collect and dispose of the waste according to the safety regulations for chemical reagents. Inject the sample into the Florisi PR-SPE column. Take 10 mL of a solvent mixture (acetone : *n*-hexane, V : V = 1 : 9) into the sample tube, vortex for 1 minute to dissolve the residue. Then, inject all the dissolved solution from the sample tube into the Florisi PR-SPE column, elute, and repeat process once. Collect all eluates in a 15 mL centrifuge tube, and evaporate to near dryness with nitrogen at 55 °C. Dissolve 1.6 mL of acetone : *n*-hexane (V : V = 1 : 1) mixture, vortex mix, and filter through a 0.22 μm × 13 mm PTFE membrane into a 2 mL sample vial for detection of OPPs by GC-FPD, and detection of OCs and PYs by GC-ECD.

The use of the C18-SPE column for solid phase extraction complies with the established protocol.

GCB-SPE and carbon-SPE purification. Aspirate 2.4, 2.8, 3.2, 3.6, and 4 mL of the supernatant after centrifugation into a tube. Evaporate to about 2 mL at 55 °C. Prepare 5 mL of a solvent mixture (acetonitrile : toluene, V : V = 3 : 1) for activation of the GCB-SPE column, collect and dispose of the waste according to the safety regulations for chemical reagents. Inject the sample solution from tube B into the GCB-SPE column. Next, dissolve the sample in tube B with 25 mL of a solvent mixture (acetonitrile : toluene, V : V = 3 : 1) to dissolve the sample tube B, and then inject it into the GCB-SPE column. Elute, and evaporate the eluate to near dryness with nitrogen at 55 °C. Dissolve the residue in 1.6 mL of an acetone : *n*-hexane mixture (V : V = 1 : 1), mix well, then filter through a 0.22 μm × 13 mm PTFE membrane into a sample vial. Use this for the detection of OPPs by GC-FPD and OCs and PYs by GC-ECD.

The use of the carbon-SPE column for solid phase extraction aligns with the GCB-SPE step in the protocol.

Due to the strong toxicity of toluene, it is combined with acetonitrile to serve as the eluent for GCB-SPE and carbon-SPE, enabling the exploration of their purification efficiency and recovery rates.

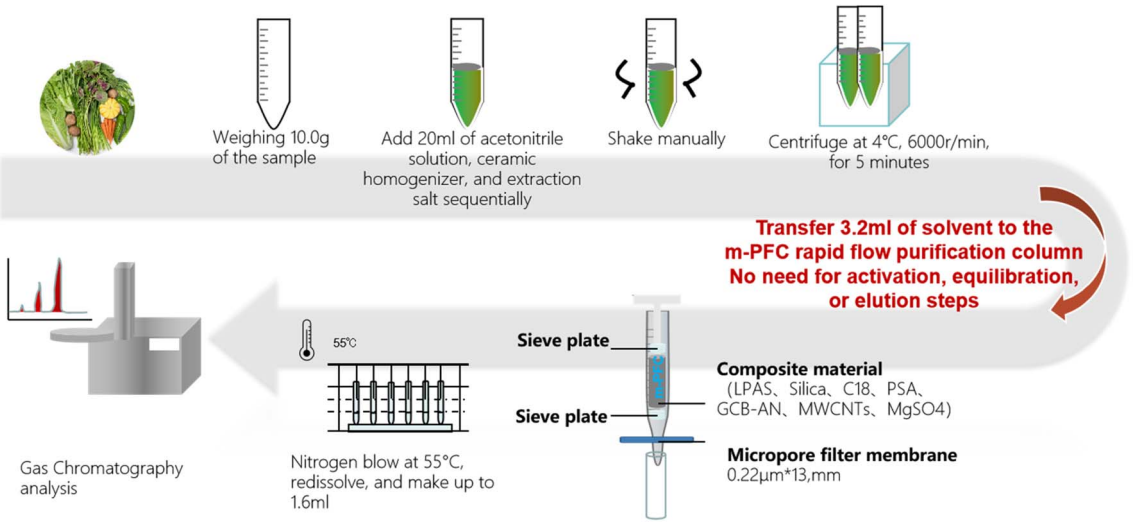

Fig. 1 Pre-treatment process of rapid filtration purification of m-PFC samples.

The above purification methods each utilize 2.4, 2.8, 3.2, 3.6, and 4 mL of the supernatant, passed through various solid-phase extraction columns, to explore the impact of different purification volumes on purification efficiency and recovery rates.

The operator maintains controls over the flow rate throughout the process, ensuring that the solid-phase extraction column remains wet. All waste chemical reagents are disposed of properly in accordance with chemical safety regulations and are stored for recycling by a professional organization.

Ref. 35 discusses taking 3.2 mL of the upper clear liquid, evaporating it to dryness using a nitrogen evaporator, then dissolving it in 1.6 mL of an acetone : *n*-hexane mixture, mixing well, and filtering through an organic membrane before bottling. This solution can be directly analyzed using GC-FPD for OPPs and GC-ECD for OCs and PYs.

Instrument conditions

Gas chromatography conditions. In sample testing, a gas chromatography-flame photometric detector (GC-FPD) was used for analyzing organophosphorus compounds, while a gas chromatography-electron capture detector (GC-ECD) was employed for organochlorine and pyrethroids analysis. The specific conditions included:

GC-FPD: DB-1701 MS chromatography column, 30 m × 0.32 mm × 0.25 μm; gasification temperature: 230 °C; detector temperature: 250 °C, hydrogen 75 mL min^{-1}, air 100 mL min^{-1}; column heating program: begin at 120 °C, hold for 0.5 minutes, then ramp up at a rate of 10 °C min^{-1} of 10 °C min^{-1} to 200 °C, hold for 2 minutes, followed by an increase at 15 °C min^{-1} to 275 °C, and hold for 5 minutes; carrier gas: 99.999% nitrogen; flow rate: 1.5 mL min^{-1}.

GC-ECD: DB-5 MS chromatography column: 30 m × 0.32 mm × 0.25 μm; gasification temperature: 250 °C; detector temperature: 310 °C; column heating program: begin at 80 °C, hold for 1 minute, then increase at a rate of 15 °C min^{-1} to 300 °C, and hold for 6 min; carrier gas: 99.999% nitrogen; flow rate: 1.5 mL min^{-1}.

Detection of natural pigments in the matrix. Natural pigments, such as chlorophyll and carotenoids, were detected in the matrix solution using high-performance liquid chromatography (HPLC) after various purification methods to assess their effects on purification. The instrument conditions were set according to the referenced methods.[36,37]

Method validation and comparison

Following the requirements of (EU) 2021/808,[38] it is necessary to validate the accuracy, precision, limit of detection (LOD), linear range, and matrix effects (MEs) of the established m-PFC method. Using spinach and Chinese cabbage as representatives of dark and light matrices, the accuracy and precision of the m-PFC method were validated at three addition levels: 0.05, 0.1, and 0.5 mg kg^{-1}. MRL standard solutions at 0.25 times were added to blank matrix samples (0.05 mg kg^{-1} added into MRL-free pesticides). The formula LOD = 3.3 × (SDMRL × 0.25/S)[39] was used to calculate the quantitative limit of each matrix. The ME[40] was determined by slope ratio between the matrix-matched and the comparing the slope ratio between the matrix-matched and the solvent calibration curves. Additionally, the method established was also used to analyze 150 real samples.

Comparisons between the two methods were made regarding pretreatment, clean-up effects, blank sample chromatograms, device contamination, and method performance. Additionally, the matrix effects (MEs) of the eight samples and the accuracy and precision of the eight matrices treated by the two methods at the 0.1 mg kg^{-1} level were also compared.

Results and discussion

Comparison of pretreatment procedures

Numerous reports have identified the optimal extraction solvent for pesticide residues in fruits and vegetables.[41–43] Based

on this literature, we chose acetonitrile as the extraction solvent for this experiment.

Comparisons were made on the reagent consumption, time consumption, and waste liquid generated amount (Table 2). Among them, the m-PFC filtration purification method demonstrated higher efficiency in reagent consumption, vessel usage, and experiment duration compared to the other four pretreatment methods. Additionally, this method produced the least amount of waste, at 15 mL, compared to the other four methods, indicating a significant advantage in reducing pollution emissions. The operation is simpler; after homogenization and centrifugation extraction, only the sample application step is required to complete the purification. Compared to the complex process of SPE single-sample consumption, which can take 200 minutes or more, m-PFC purification does not require steps such as activation, equilibration, or multiple rinses. Fig. 1 illustrates the process: the supernatant was removed and introduced into an m-PFC injector; the solution was transported to a centrifuge tube after one-by-one filtration and clean-up (1 drop per s). The one-stop clean-up procedure was completed in just 2 minutes. The subsequent within-laboratory testing was conducted by concentrating and replacing solutions; the time required for performing a single-sample test was less than 30 min. Thus, the m-PFC method outperforms the SPE method in clean-up efficiency, processing a larger volume of samples in less time, which potentially benefits inspectors' health and promotes environmental sustainability.

Comparison of different purification methods

The experiment compared the purification effects and recovery rates of commonly used solid phase extraction (SPE) methods including Florisi PR-SPE, carbon-SPE, GCB-SPE, and C18-SPE in pesticide residue detection. As shown in Fig. 2 and 4. It was found that spinach, after purification with m-PFC C-5, appeared clearer than when purified with Florisi PR-SPE and C18-SPE, exhibiting a significant decrease in visible pigment content. In contrast, after purification with carbon-SPE and GCB-SPE, it was almost colorless. Similarly, Chinese cabbage, representing light-colored vegetables, appeared colorless after purification with all five methods. The purified solutions were further analyzed using high-performance liquid chromatography (HPLC) to determine the residual plant pigments at a wavelength of 425 nm (Fig. 3). Six distinct natural pigments were detected in the unpurified spinach solution, while none were detected in the GCB-SPE and m-PFC (C-5) purification solutions. A small amount of lutein was detected in the carbon-SPE purification solution, whereas phacophytin b, phacophytin a, and β-carotene were detected in the Florisi PR-SPE purification solution, albeit with significantly reduced content. Lutein, these pigments were also detected in the C18-SPE purification solution. No pigments were detected in the Chinese cabbage after purification using any of the five methods. In summary, GCB-SPE, carbon-SPE, and m-PFC (C-5) showed significant advantages in pigment adsorption.

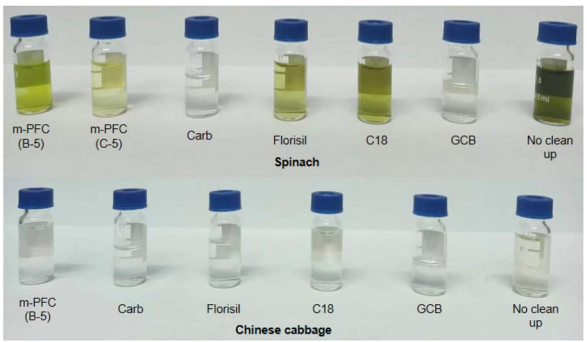

Fig. 2 Comparison of different purification materials after purification (3.2 mL).

Interestingly, we from that although Carbon-SPE and GCB-SPE effectively recovery most pigments after purification, the recovery rates for most pesticides remained relatively high, especially for organochlorine and pyrethroid pesticides. Conversely, the overall recovery rate after C18-SPE purification was relatively low, suggesting poorer performance. This might be related to the presence of four pigments not completely removed during pigment detection in spinach. After purification with Florisi PR-SPE, six organophosphorus pesticides showed no recovery, whereas other pesticides demonstrated good recovery effects. This finding was further verified by subsequent experiments, as depicted in Fig. 4 and 5. Florisi PR-SPE and m-PFC (C-5) exhibited clear advantages in pesticide recovery rates in these experiments.

Table 2 Comparison of consumption of two pretreatment methods

Project	m-PFC	FLorisi PR SPE	C18 SPE	GCB SPE	Carbon SPE
Sample dosage	10.0 g	10.0 g	10.0 g	10.0 g	10.0 g
Acetonitrile dosage	20 mL	20 mL	20 mL	42.5 mL	42.5 mL
Hexane dosage	1.0 mL	22.5 mL	22.5 mL	1.0 mL	1.0 mL
Acetone dosage	1.0 mL	2.5 mL	2.5 mL	1.0 mL	1.0 mL
Toluene	—	—	—	7.5 mL	7.5 mL
Liquid waste	15 mL	22.0 mL	22.0 mL	25 mL	25 mL
Utensil consumption	Less 50 mL PP tube × 1-pk, 10 mL PP tube × 1-pk	Multiple (such as funnels, plug measuring cylinder, *etc.*)	Multiple (such as funnels, plug measuring cylinder, *etc.*)	Multiple (such as funnels, plug measuring cylinder, *etc.*)	Multiple (such as funnels, plug measuring cylinder, *etc.*)
Average processing time/sample	Approx.30 min	Approx. 100 min	Approx. 100 min	Approx. 200 min	Approx. 150 min

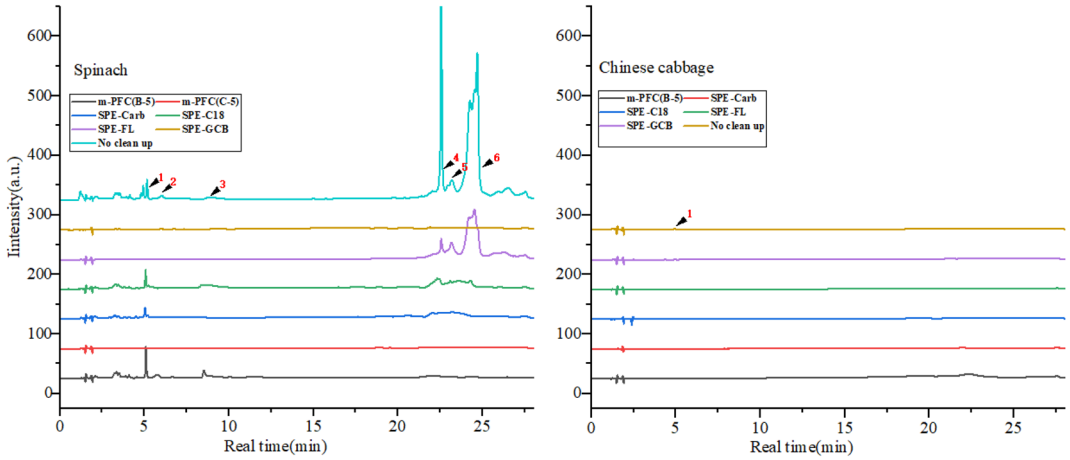

Fig. 3 Natural colours after cleanup using different cleanup columns. Note: (1) lutein; (2) chlorophyll b; (3) chlorophyll a; (4) phacophytin b; (5) phacophytin a; (6) β-carotene.

Optimization of m-PFC purification volume

Commercial clean-up columns were selected because filler contents could be fixed. Therefore, the clean-up effect depended on the volume of clean-up liquid added. Clean-up volumes were optimized for spinach and Chinese cabbage, representatives dark and light matrices respectively. The spiking amount of pesticides was 0.1 mg kg^{-1}, (n = 3). Filtration clean-up was conducted at the five-volume gradients, which were 2.4, 2.8, 3.2, 3.6, and 4 mL; Fig. 4 and 6 illustrate the clean-up effects, while Table 3 summarizes the recovery rates. Fig. 4 reveals that the larger the clean-up volume was, the darker the color was after clean-up; Table 3 shows that the recovery rate of multi-pesticides was also increased. In the range of 3.2 mL to 3.6 mL of purification volume, both Chinese cabbage and spinach showed good pesticide recovery rates (70–130%), and the results were relatively stable. Especially when the purification volume reached 3.2 mL, the recovery rates of 59.5% (22 pesticides) in spinach and 48.6% (18 pesticides) in Chinese cabbage reached their highest levels. However, when the clean-up volume exceeded 3.6 mL, the recovery rates of 4 pesticides (iprodione, dicofol, acephate, and fenitrothion) in spinach and 2 pesticides (procymidone and cyfluthrin) in Chinese cabbage continued to increase. Conversely, the recovery rates of the remaining pesticides began to decline. Therefore, when the clean-up volume was smaller (2.4 and 2.8 mL), the clean-up volumes of most pesticides were not enough and a small amount of pesticide residues was on the m-PFC clean-up column. Conversely, when the volume was expanded to 3.6 or 4 mL, the m-PFC clean-up column became overloaded, leading to incomplete clean-up and compromised recovery rates (Fig. 6). Additionally, the clean-up liquid darkened with larger volumes, further indicating inadequate clean-up. Therefore, the 3.6 mL extraction liquid was selected for clean-up in the experiment.

Solid phase extraction purification volume optimization

When purified with Florisi PR-SPE, the pesticides acephate, dichlorvos, acetamiprid, oxydemeton-methyl, and diazinon showed no recovery across all tested gradients in both spinach and Chinese cabbage. The recovery rates for spinach remained within the method validation requirements (70–130%), with little variation as the purification volume increased. The highest recovery count (30) with rates within 70–130% was observed at

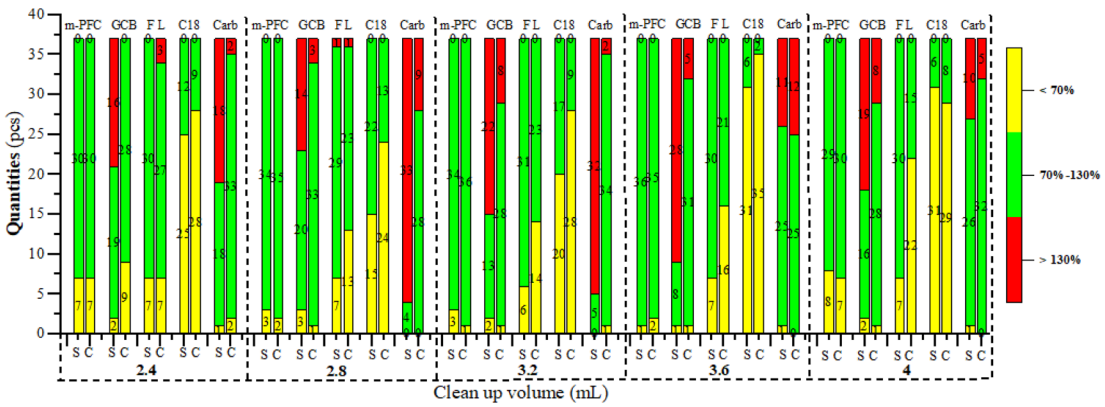

Fig. 4 The effect of different purification column on the recovery rates at different purification volumes.

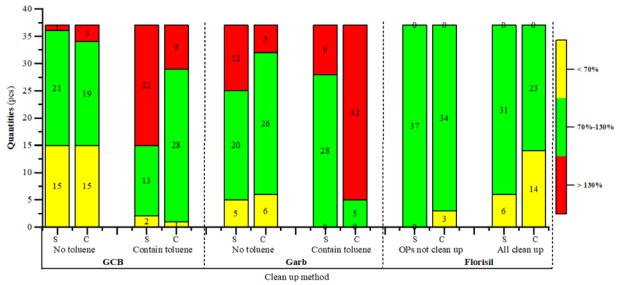

Fig. 5 Recovery rates by different cleanup volumes.

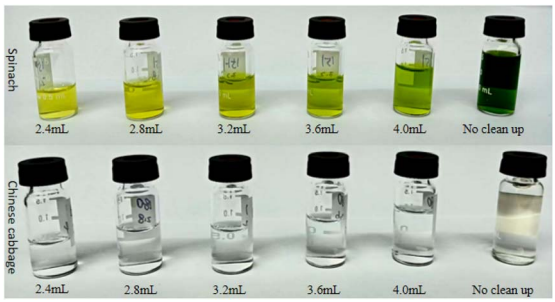

Fig. 6 Cleanup effects of different cleanup volumes (m-PFC).

3.2 mL, for Chinese cabbage, the overall trend of recovery rates decreased as the purification volume increased, with the highest number of good recovery rates (27) at 2.4 mL. This resulted from using the same Florisi PR-SPE column amount of 1000 mg/6 mL for both dark and light matrices.

When purified with C18-SPE both spinach and Chinese cabbage exhibited a pattern where recovery rates initially increased and then decreased, peaking at 2.8 mL. For purification with carbon-SPE, spinach reached its maximum recovery rates at 4 mL (34 recoveries), while Chinese cabbage showed its highest recovery at 3.2 mL (34 recoveries) without significant fluctuations. Conversely, with GCB purification, both spinach and Chinese cabbage displayed a general decline in recovery effectiveness, with spinach peaking at 2.8 mL (20 recoveries) within a range of 70–120%, and Chinese cabbage achieving its best at 3.6 mL (31 recoveries).

When purified with m-PFC from 2.4 mL to 4 mL, the method demonstrated higher recovery rates and greater effectiveness compared to the other four solid phase extraction methods. Notably, at 3.2 mL and 3.6 mL, both Chinese cabbage and spinach exhibited high recovery rates, with minimal variation between the two. Specifically, Chinese cabbage showed 34 and 36 recoveries, respectively, while spinach showed 36 and 35. Given the superior purification performance of Florisi PR-SPE at 3.2 mL, this volume was selected for further experiments.

Comparison of purification methods

Due to the adsorption of planar pesticides by GCB-SPE and carbon-SPE, 25% toluene (3 : 1) was initially added to the acetonitrile eluent in preliminary experiments for protection. However, due to toluene is highly toxicity and environmental impact, the experiment was further conducted using only acetonitrile without toluene. The results indicated that omitting toluene generally increased the recovery rates of organophosphorus and organochlorine pesticides with GCB-SPE and carbon-SPE. Nonetheless, the results. Whether with or without toluene, were not ideal. These results are illustrated in Fig. 5.

In preliminary experiments, it was found that six organophosphorus pesticides had no recovery when purified with Florisi PR-SPE. Literature review revealed that Florisi PR-SPE is not suitable for purifying organophosphorus pesticides consequently, the experiment was limited to using Florisi PR-SPE for purification organochlorine and pyrethroid pesticides. It was observed that when organophosphorus pesticides were not subjected to purification, their recovery rates increased significantly. Recovery rates for spinach and cabbage ranged between 70% and 120%, with a total of 34 pesticides recovered. Research using GC-FPD to detect organophosphorus pesticides (OPPs)

Table 3 Comparison of the limit of detection of 37 pesticides in two matrices

	m-PFC		
Pesticide name	Spinach	Chinese cabbage	SPE
Dichlorvos	0.01	0.01	0.01
Methamidophos	0.005	0.005	0.01
Acephate	0.01	0.01	0.03
Diazinon	0.01	0.01	0.02
Dimethoate	0.01	0.01	0.02
Parathion-methyl	0.01	0.01	0.02
Fenitrothion	0.01	0.01	0.02
Isofenphos-methyl	0.01	0.01	—
Triazophos	0.01	0.01	0.01
Phosmet	0.03	0.03	0.06
Phorate	0.01	0.01	0.02
Omethoate	0.01	0.01	0.02
Monocrotophos	0.02	0.01	0.03
Chlorpyrifos	0.02	0.01	0.02
Malaoxon	0.02	0.01	0.03
Parathion	0.01	0.01	0.02
Isocarbophos	0.01	0.01	0.03
Profenofos	0.01	0.01	0.04
Phosalone	0.03	0.03	0.05
α-BHC	0.0001	0.0001	0.0001
β-BHC	0.0001	0.0001	0.0004
γ-BHC	0.0001	0.0001	0.0002
δ-BHC	0.0001	0.0001	0.0001
Vinclozolin	0.0002	0.0002	0.0004
Triadimefon	0.0002	0.0002	0.001
Procymidone	0.0006	0.0005	0.002
Fenpropathrin	0.0004	0.0004	0.002
Cyfluthrin	0.0006	0.0005	0.002
Flucythrinate	0.0007	0.0006	0.001
Tau-fluvalinate	0.0005	0.0005	0.002
Deltamethrin	0.0004	0.0004	0.001
Dicofol	0.0005	0.0004	0.0008
Iprodione	0.0015	0.0023	0.001
Bifenthrin	0.0005	0.0004	0.0006
Cyhalothrin	0.0002	0.0002	0.0005
Cypermethrin	0.001	0.0009	0.003
Fenvalerate	0.0005	0.0004	0.002

has indicated that matrix purification is unnecessary, aligning with findings from 'Determination of Multiple Residues of Organophosphorus, Organochlorine, Pyrethroid, and Carbamate Pesticides in Vegetables and Fruits: NY/T 761-2008'.[35] However, there are significant differences in matrices among fruits, vegetables, and edible fungi. Leafy vegetables have higher contents of N, P, and K;[44] Sweet cherries have higher contents of anthocyanins and sugars;[45] Mushrooms have polysaccharides as their main active components.[46] The presence of these substances enhances the matrix effect, making the recovery rates of pesticides such as malathion, dichlorvos, acephate, oxydemeton-methyl, and prothiofos normal after purification. Therefore, in subsequent experiments, the Florisi PR-SPE solid-phase extraction method was used, and the organophosphorus pesticides were not purified, following the method described.

Comparison of clean-up effects

Comparison of visual clean-up effects. Eight samples were processed respectively in the two methods; Fig. 3 presents the clean-up effects. The clean-up column used in SPE was 1000 mg/6 mL Florisi PR, the same amount for dark and light pigments resulted in incomplete clean-up for dark pigments and excessive clean-up for light ones. The m-PFC column combined anhydrous magnesium sulfate, C18, MWCNTs, and Florisi, leveraging the advantages of d-SPE. The MWCNTs amount added into injectors differed for samples containing different pigments. Additional 50 mg of MWCNTs was added to samples with darker pigments than to those with lighter pigments to more effectively remove pigments. For example, sweet cherry, cucumber, string beans, and spinach were effectively cleaned using m-PFC (C-5) injectors for darker pigments, while *Pleurotus ostreatus*, tomato, radish, and Chinese cabbage achieved optimal results with m-PFC (B-5) injectors for lighter pigments (Fig. 7).

Comparison of blank chromatograms. Eight within-laboratory samples were analyzed to determine the pretreatment effects using two different methods: all pesticides in the added samples were well-separated, and none of the 37 pesticides were detected in blank matrices, indicating that the instrument conditions were properly optimized. We randomly selected blank chromatograms of the two vegetables to compared the cleanup effects of the two pretreatment methods (Fig. 8a and b).

As shown in Fig. 4a, displays the detection of organophosphorus in the radish sample, after purification by the m-PFC method, the chromatogram revealed only three impurity peaks (No. 1, 3, and 4 in Fig. 8a). However, after purification using the SPE method, the chromatogram showed four impurity peaks, with both peak areas and heights greater than those observed after purification using the m-PFC method. The same effect also occurred in chromatograms of organochlorine (Fig. 8b): the matrix impurity peak height of the blank samples of *Pleurotus ostreatus* was reduced by half after m-PFC treatment and the area was reduced by five times compared to the samples treated with SPE. Accordingly, m-PFC clean up more thoroughly, has a stronger capacity to remove impurities, and is more conducive to reducing false positives.

Comparison of instrument contamination. During organophosphorus analysis using SPE, the supernatant was dried with nitrogen, followed by redissolving in acetone. Subsequently, within-laboratory testing was conducted after thorough mixing in the filter head; the matrices were not subjected to any clean-up, instead, their concentration was enhanced as the solvent volume was reduced. In the m-PFC method, similar concentration steps are conducted, but the impurities in the matrices are significantly reduced after m-PFC clean-up. Comparisons analyses of 100 μL aliquots (random samples) were performed after treat by both methods, along with evaluations of the cleanliness of the liner tubes used in GC-FPD (Fig. 9).

Fig. 9 (the enlarged figure on the right) reveals that the liner tube was clean after m-PFC treatment but dirty with many contaminants after SPE treatment. The accumulation of contaminants directly impacted the accuracy and sensitivity of test results, leading to contamination at the gasification end of the chromatographic column. Frequent replacement of liner

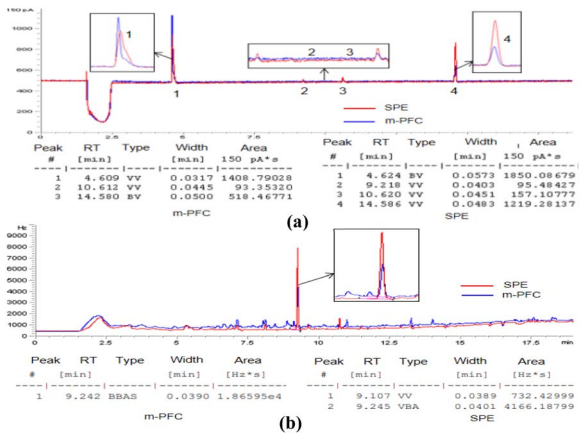

Fig. 8 Comparison of blank sample chromatograms after clean-up in two methods.

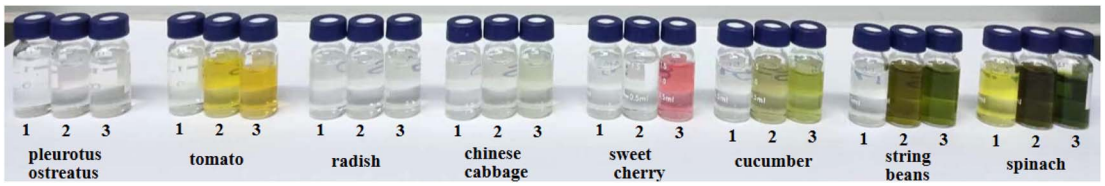

Fig. 7 Visual comparison of cleanup effects using two methods. Note: (1) cleaned up by m-PFC (B-5 or C-5); (2) cleaned up by SPE; (3) uncleaned.

tubes increased testing costs and further compromised the accuracy and sensitivity of test results due to column contamination. This suggests that m-PFC treatment results in less contamination to instruments compared to SPE treatment, reduces maintenance frequency and costs, and is more instrument-friendly.

Comparison of method performance

Accuracy and precision. Spinach (darker) and Chinese cabbage (lighter) were selected as representatives for cleanup, each in different types of m-PFC columns (C-5 and B-5), to verify the recovery rates and accuracy (Fig. 10). Fig. 10 demonstrates that the average recovery rate of 37 pesticide residues from the two samples, at three spiking levels – 0.05, 0.1, and 0.5 mg kg^{-1} – was in the range of 66.4% to 113.6%, and the relative standard deviation (RSD) ranged from 0.1% to 14.9% for m-PFC. These results conformed to the accuracy and precision requirements of EU regulation 2021/808.

Linear range and LOD. Spinach (darker) and Chinese cabbage (lighter) were selected as representatives for cleanup using two types of m-PFC injectors (C-5 and B-5). Working curves were prepared with blank matrices for concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 μg mL^{-1} in groups (Table 1). Calibration curves were plotted with the response value of target compounds on the vertical axis and coordinate on the horizontal axis. The R^2 values for all pesticides was ≥0.995, indicating strong linearity.

Table 3 shows the LOD as quoted from a document by FLorisi PR-SPE;[35] For each pesticide tested in spinach and Chinese cabbage using m-PFC, the LODs indicated that iprodione was 0.0015 mg kg^{-1} in spinach and 0.0023 mg kg^{-1} in Chinese cabbage, slightly higher than the SPE value of 0.001 mg kg^{-1}. The LODs of five pesticides – dichlorvos, isofenphos-methyl (unavailable in SPE), triazophos, α-BHC, and δ-BHC – were identical to those form SPE; and the LODs of the other 31 pesticides were lower than their corresponding SPE values. Thus, the method meets the requirements for multi-pesticide residue testing in various plant products.

Matrix effects (MEs). MEs refer to phenomena in which interfering substances in matrices cause varying degrees of signal enhancement or attenuation for analytes.[33] These effects are widespread in instrumental analysis, such as GC and GC-MS/MS, and impact the accuracy and precision of determination results.[47] When using GC to analyze organic phosphorus pesticides in complex samples like spinach and mushroom, the ME is significant and directly impacts quantification accuracy.

Herein, 0.1 mg L^{-1} standard solutions were prepared using the blank matrices of eight samples after they had been treated with m-PFC and SPE. Additionally, identical standard solutions were prepared with acetone and *n*-hexane. The slope ratio of the calibration curve generated using blank matrix solutions (k_1) was compared with that of the reagent calibration (k_2) to calculate the matrix effect (ME = k_1/k_2). It is commonly accepted that no ME occurs when ME = 1, matrix enhancement occurs when ME > 1, matrix attenuation occurs when ME < 1. ME values are typically found within the range of 0.8–1.2, which is considered an acceptable range.[44] In the experiment, the MEs of 37 pesticides in 8 types of plants were compared (Fig. 11).

Fig. 11 shows that the ME values for the eight matrices determined using FLorisi PR-SPE ranged from 0.8 to 1.2. However, the ME values obtained using the m-PFC method were more varied. Matrix enhancement of organophosphorus pesticides was observed in 89.5% of cases (except for phorate, dichlorvos, and chlorpyrifos) across eight matrices in both m-PFC and FLorisi PR-SPE treatments. Matrix effects, whether enhancement or attenuation, occurred simultaneously for the same pesticide, such as methamidophos, acephate, and, omethoate, when pretreated in the same matrix under both Florisi PR-SPE and m-PFC. Additionally, the MEs of 6 pesticides (phorate, dichlorvos, triazophos, chlorpyrifos, phosalone, and phosmet) among the 19 tested organophosphorus pesticides were lower in m-PFC than in FLorisi PR-SPE. In contrast, the MEs of 15 organochlorine pesticides after m-PFC treatment were smaller than those in FLorisi PR-SPE. This shows that after using m-PFC, organophosphorus demonstrated strong matrix enhancement effects, but organochlorine demonstrated weak matrix attenuation effects and had weaker MEs after the treatment in m-PFC than SPE. Therefore, the blank matrix-matched standard curve was used to test samples to remove MEs.

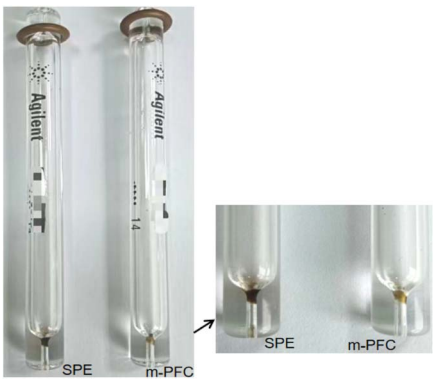

Fig. 9 Comparison of liner tubes for 100 qrganophosphorus injections after processing by two methods.

Comparison of multi-sample accuracy and precision

Given that different MEs are exhibited by different samples and various interfering substances are contained, the testing results of the eight samples (the additive amount was 0.1 mg kg^{-1}, n = 3) after treatment in m-PFC and SPE to verify the clean-up effects of m-PFC in different matrices, and calculated their average recoveries and RSDs.

Table 4 shows good recovery effects were obtained in the eight samples in m-PFC; the average recoveries were 67.0–112.8%, and the RSDs were 0.2–15.2%. The average recoveries and mean values show that the average recoveries of the eight samples were above 83.8%, and this declared that the determination result deviations of the method were small, the precision was good, and the recovery rate was high. The recoveries of SPE testing were 60.2–111.2%, and RSDs were 0.5–

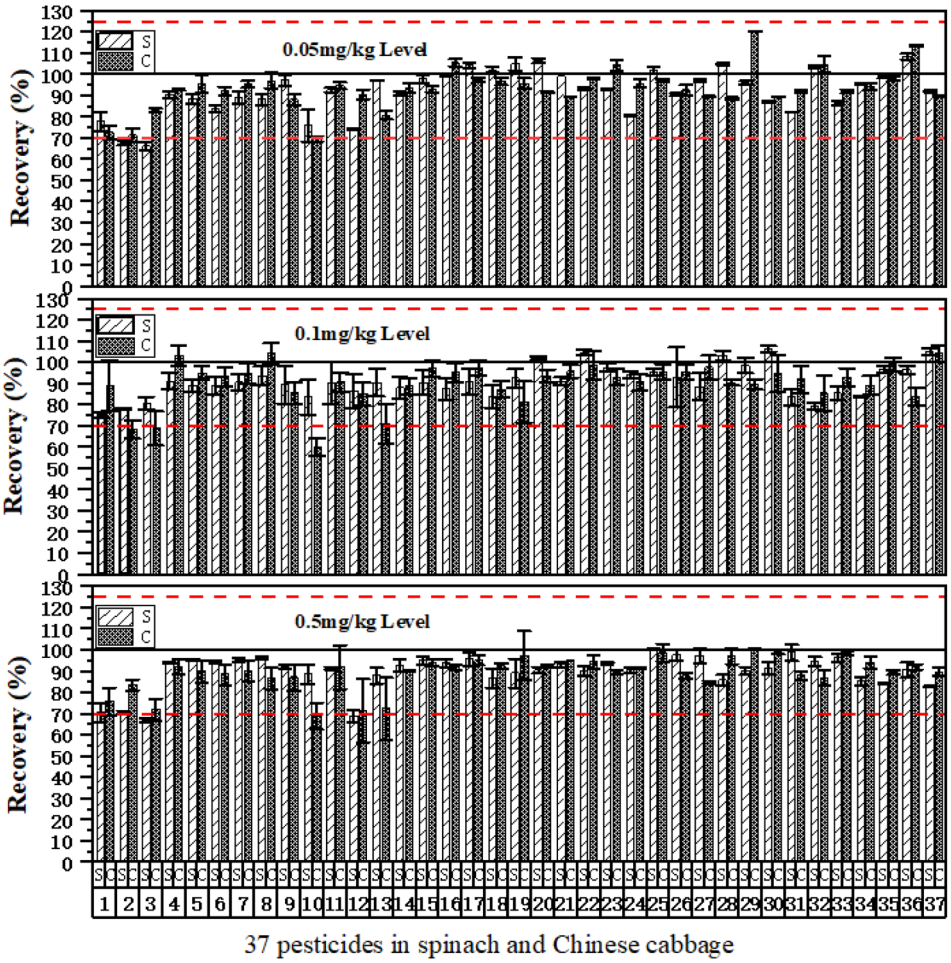

Fig. 10 The relative standard deviations for 37 pesticides in spinach and Chinese cabbage were determined using the m-PFC method at three supplemental levels ($n = 3$). Note. S is spinach; C is Chinese cabbage. Table 1 lists the 37 pesticide names.

14.2%; there were no significant differences in the average recovery rate and RSD after comparing the two methods. The multi-type fruit and vegetable sample results revealed that m-PFC could replace SPE to determine multi-pesticide residues of organophosphorus, organochlorine, and pyrethroids in multiple agricultural products.

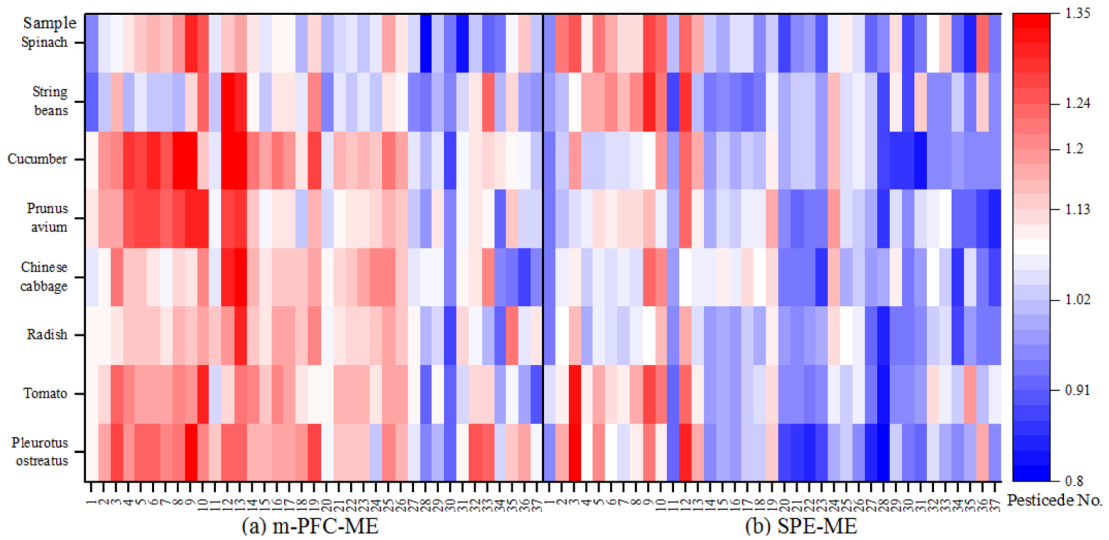

Fig. 11 Matrix effects of 37 pesticides in 8 matrices detected in two methods.

Table 4 Comparison of 37 pesticides in eight samples with two pretreatment methods (added 0.1 mg kg^{-1}, $n = 3$)

No.	Sample name	Method	Average recovery rate (%)	Median recovery rate (%)	Range of recovery rates (%)	RSD (%)
1	*Pleurotus ostreatus*	SPE	85.4	85.8	67.0–103.6	1.4–13.5
		m-PFC	87.6	87.3	72.1–103.0	0.9–9.6
2	Tomato	SPE	88.0	88.6	72.5–105.2	1.1–10.1
		m-PFC	90.0	90.9	69.4–104.0	0.5–9.5
3	Radish	SPE	90.1	91.5	71.9–105.4	1.3–15.2
		m-PFC	90.3	90.3	73.5–110.1	1.1–11.9
4	Chinese cabbage	SPE	88.1	90.4	69.4–102.8	0.5–8.5
		m-PFC	90.3	93.2	60.2–104.3	1.4–11.9
5	Sweet cherry	SPE	84.7	83.8	68.3–102.8	0.5–9.5
		m-PFC	83.3	83.1	67.4–102.7	1.1–6.9
6	Cucumber	SPE	87.5	86.6	77.3–108.5	0.2–9.1
		m-PFC	88.2	87.0	73.4–107.8	0.6–8.3
7	String beans	SPE	92.7	93.7	77.3–108.5	1.0–15.5
		m-PFC	88.9	89.2	66.7–111.1	0.7–12.2
8	Spinach	SPE	95.8	97.7	73.7–112.8	0.6–13.2
		m-PFC	91.1	90.6	75.4–105.2	0.4–14.2

Real sample testing

Using m-PFC, tests was conducted on 150 agricultural product derived from plants, randomly selected from cultivation bases and market. The samples comprised 75 vegetable (including 10 bulbs, 10 brassicas, 45 leafy, 6 solanaceous, and 4 root vegetables), 15 edible fungi (6 *Lentinula edodes* and 9 *Pleurotus ostreatus*), and 60 fruit (10 citruses, 15 cherries, 15 strawberries, and 25 loquats). The results indicated that among 37 pesticides, 17 pesticides were detected from 258 sample times; 3 restricted drugs were detected from 12 sample times; 13 common pesticides were detected from 242 sample times; 2 prohibited pesticides (dicofol and fenvalerate) were detected from 4 sample times, and no other pesticides were detected. The predominant pesticides are the low-toxicity pyrethroid but with low toxicity levels, which met the National Food Safety Standard-Maximum Residue Limits for Pesticides in Food (China Limit Standard GB2763-2021).[48] Therefore, the overall pass percent of the 150 samples was 100.0%; consequently, the fruit and vegetable samples in the batch were of quality safety.

Conclusion

In total, 37 pesticides, comprising 19 organophosphorus, 9 organochlorine, and 9 pyrethroids, were analyzed in 8 agricultural products utilizing m-PFC combined with GC-ECD. The results were compared with those obtained using the traditional SPE method. Additionally, the method was applied to testing 150 real samples.

It has been shown by the results that the accuracy and precision of the samples treated with m-PFC at three addition levels have been confirmed by GC-ECD to meet the requirements of (EU) 2021/808. The recoveries of the eight samples were 67.0–112.8%, the RSDs were 0.2–15.2%, and the average recoveries were above 83.8%. Furthermore, strong linearity was observed for 37 pesticides in the range of 0.05–1.6 μg mL^{-1}; it was found that the matrix effects (MEs) of most pesticides treated with m-PFC were weaker than those treated with SPE, and the MEs for the eight matrices ranged from 0.8 to 1.2. LODs were recorded from 0.0001 to 0.03 μg kg^{-1}, and for 31 pesticides, they were found to be lower than those obtained *via* SPE. The m-PFC method was successfully employed to test 150 real samples; resulting in the detection of 17 pesticides with low detected contents, and a pass rate of 100% was achieved. Therefore, the pretreatment method of m-PFC can replace SPE for testing multi-pesticide residues in multiple agricultural products in GC-ECD.

Impurities such as proteins and fats can be removed by hydroxyl (–OH), which can also react with both acids and bases to form salts and water, effectively improving hydrophilicity and salinization. Acidified substances such as saturated fatty acids, unsaturated fatty acids, and amino acids can be removed by alkyl (C_nH_{2n+2}), a long-chain alkyl carboxylic acid consisting of carbon and hydrogen atoms. Sterols and pigments are highly absorbed by hollow MWCNTs, which are hydrophobic. The conductivity and stability are further enhanced by magnetic MWCNTs, which are prevented from agglomeration or oxidation in organic solvents and aqueous environments. The m-PFC purification tube was introduced with the above bifunctional groups (–OH and C_nH_{2n+2}) as purification materials, around which the magnetic MWCNTs containing Fe^{+} were wrapped. The capture and adsorption of specific molecular impurities in the matrix are facilitated by the introduction of the above three substances.

Compared with the SPE method, liquid pigments were cleaned up more thoroughly by m-PFC, achieve fewer impurity peaks in blank matrices and higher multi-pesticide separation, and misjudgments of false positives were effectively reduced. Contamination to instruments was lighter, instrument maintenance times and costs were lowered, and it was made more instrument friendly by m-PFC. Meanwhile, fewer solvent reagents, utensils, and significantly fewer organic solvents were consumed by m-PFC than by SPE, and higher safety was achieved in eradicating result errors caused by component volatilization. The amount of waste liquid generated by m-PFC was

only 1/10 of that generated by SPE; therefore, pollution emissions were significantly reduced. With much easier operation, compared to 200 min for a single-sample test in SPE, a one-stop clean-up was completed by m-PFC in only 2 minutes; the single-sample test time for m-PFC was less than 30 minutes, indicates a significant advantage in clean-up procedures. A larger batch of samples could be processed by m-PFC in a shorter period, which was beneficial for inspectors' physical health and was environmentally friendly. The m-PFC has been recognized as an effective testing method for primary testing laboratories that are only equipped with GC-ECD and lack funds, and do not have GC-MS/MS for conducting risk monitoring of pesticide residues in agricultural products, substantially improving the testing efficiency.

Nearly 100 articles on m-PFC technology have been published by researchers. It a new way of thinking for analysing trace substances by mass spectrometry and Raman spectroscopy is provided by it. Through extensive experiments conducted over one year, it has been determined that m-PFC can achieve a significant breakthrough in the field of chromatographic analysis, which is considered to be an unprecedented innovation.

Author contributions

Data curation, Yu Zhang; formal analysis, Xiaxue Li; funding acquisition, Xiaxue Li and Yan Zeng; validation, Bin Qu, Qiao Hui Yang, and Yan Zeng; investigation, Bin Qu; methodology, CanPing Pan and Tao Lan; resources, Ya Chen; supervision, Ya Chen; writing – original draft, Yan Zeng; writing – review & editing, CanPing Pan and Tao Lan. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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