

Xinxin MENG^{1*} and Dan ZHAO^{1*}

A HIGHLY SENSITIVE DETECTION METHOD FOR FIVE ORGANOPHOSPHORUS PESTICIDE RESIDUES IN TEA BASED ON THE COMBINED TECHNOLOGY OF SPME-LC-MS/MS

Abstract: Excessive pesticide residues seriously threaten the safety of agricultural products and the health of consumers. As a widely consumed beverage, the monitoring of pesticide residues in tea is of vital importance. To improve the sensitivity and accuracy of organophosphorus pesticide residue detection in tea, an integrated method combining solid-phase microextraction pretreatment and high-performance liquid chromatography-tandem mass spectrometry analysis was researched and developed. The research optimised the sample pretreatment process. Tea powder was extracted by vortex using a mixed solvent of ethyl acetate and n-hexane with a volume ratio of 9 : 1. The isotope internal standard method was used for quantification. By optimising the chromatographic conditions and mass spectrometry parameters, the detection methods for five target pesticides were established, and the linear range, quantification limit, accuracy and precision of the methods were systematically evaluated. The results show that the linear relationship of the five organophosphorus pesticides is good within the range of (5.0 - 500.0) ng/mL, and the quantitative limit of the method is extremely low, ranging from 0.0011 mg/kg to 0.0026 mg/kg. The correlation coefficients of the standard curves were all greater than 0.99, and the average recovery rates ranged from 72 % to 109 %. The results show that the pretreatment of the research method is simple, rapid and has low solvent consumption, effectively improving the detection efficiency and accuracy. The research provides efficient and reliable analytical means for ensuring the quality and safety of tea, safeguarding consumers' health and promoting the sustainable development of the tea industry.

Keywords: solid - phase microextraction, LC-MS/MS, pesticide residues, accuracy

Introduction

Pesticide residue (PR) refers to the amount of PR in crops during the cultivation of agricultural products that exceeds the limits set by national or regional regulations. PRs pose potential risks to human health, so monitoring and controlling PRs in agricultural products is crucial [1, 2]. Currently, commonly used PR detection methods include Gas Chromatography - Mass Spectrometry (GC-MS), *Liquid Chromatography-Mass Spectrometry Tandem (LC-MS)*, and *High Performance Liquid Chromatography (HPLC)*. (LC-MS), *Liquid Chromatography - Mass Spectrometry Tandem (LC-MS)*, and *High Performance Liquid Chromatography (HPLC)* [3-5]. However, these methods have some disadvantages in the sample pretreatment process, such as cumbersome operation, long time, and high solvent consumption. In recent years, Solid-Phase Microextraction (SPME) has been introduced into the field of PR detection. SPME is a sample pretreatment technique that uses an adsorbent immobilised on a fiber to extract and enrich analytes from

¹ Instrument and Equipment Management Services Testing Center, Jilin Agricultural University, Changchun 130118, China, ORCID: XM 0009-0005-0589-2727, DZ 0009-0003-9245-2978

* Corresponding author: MengXinxin_mxx@outlook.com and 17704317096@163.com

samples, followed by thermal or solvent desorption into a chromatographic system for analysis. Compared with traditional pretreatment methods, SPME offers advantages such as simple operation, high extraction efficiency, minimal sample loss, and low solvent consumption [6-9]. However, the single use of SPME in PR detection still faces limitations, including restricted choices of adsorbent coatings and insufficient sensitivity. To improve detection sensitivity and accuracy, researchers have begun combining SPME with LC-MS/MS, a highly sensitive, selective, and high-throughput analytical method capable of simultaneously detecting and quantifying multiple pesticide residues [10-13]. The enrichment capability of SPME, coupled with the powerful separation and detection capabilities of LC-MS/MS, not only enhances sample pretreatment efficiency but also significantly improves the detection sensitivity and accuracy for trace pesticide residues in complex matrices.

Previous studies have confirmed the potential of this combined approach in agricultural product analysis. Xin et al. [14] improved the SPME fiber coating to enhance instrumental sensitivity, achieving recoveries of 80 % - 101.6 % in samples including green tea. Jouyban et al. [15] developed an efficient SPME extraction method for hydroxylated polycyclic aromatic hydrocarbons (PAHs), achieving a wide linear range (26 - 500,000) ng/L, low limits of detection (3.6 - 7.2) ng/L, and quantification (11 - 26) ng/L, well below relevant standards. Wang et al. [16] fabricated a magnetic covalent organic framework ($\text{Fe}_3\text{O}_4@\text{TpPa-NO}_2$) as an SPME adsorbent for pyrethroid insecticides, achieving recoveries of (85.7 - 109.8) %. Zhang et al. [17] developed an in-tube SPME device based on poly (4-vinylphenylboronic acid-co-ethyleneglycol dimethacrylate) coupled with ultra-high-performance LC-MS/MS for trace paraben analysis in sewage and soil samples, achieving limits of detection of (0.64 - 1.4) ng/L and recoveries of (86.4 - 113) %, with relative standard deviations between 2.1 % and 6.0 %. These results demonstrated excellent applicability for environmental monitoring of trace contaminants. In LC-MS/MS applications, Dwaity et al. [18] assessed PR levels in organic and conventional lemons, both below regulatory limits. Dong et al. [19] investigated the transfer rates of water-soluble pesticides from dry tea to infusion, showing rates above 70 % after three infusions. Naik et al. [20] standardised a method for removing pesticide residues in curry leaves, achieving up to 98.47 % removal after soaking in 2 % salt solution followed by boiling. Despite these advancements in SPME adsorbent development and LC-MS/MS sensitivity enhancement, studies specifically targeting a rapid, low-solvent, high-sensitivity combined detection method for organophosphorus pesticide residues in tea remain limited. In particular, there is a lack of research that systematically optimises extraction conditions, cleanup procedures, and mass spectrometry parameters to accommodate the complex tea matrix.

Therefore, this study proposes a method that combines SPME with LC-MS/MS for the sensitive determination of five organophosphorus pesticides in tea. The approach involves optimising extraction solvent composition, selecting an appropriate cleanup column, fine-tuning mass spectrometry parameters, and employing an isotope internal standard method for accurate quantification. The method is validated in terms of linearity, limits of quantification, recovery, and precision. The objective is to provide an efficient, accurate, and feasible analytical tool for pesticide residue monitoring in tea, thereby supporting quality assurance, consumer health protection, and the sustainable development of the tea industry.

Materials and methods

Rationale for selecting SPME technology

SPME is a rapid, efficient, and low-solvent-consumption sample pretreatment technique that integrates sampling, extraction, concentration, and injection into a single step [21, 22]. The SPME pretreatment workflow used in this study is shown in Figure 1.

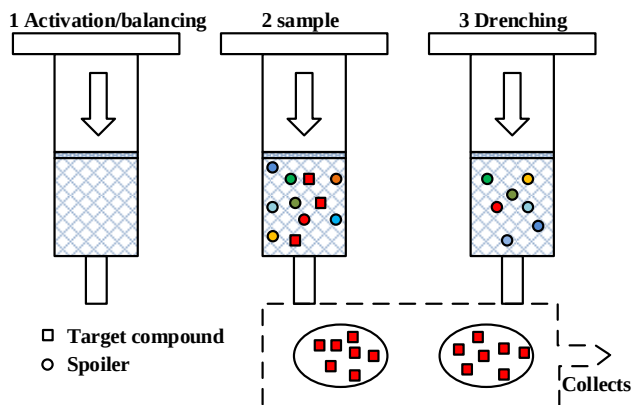


Fig. 1. Pretreatment workflow of solid-phase microextraction

As shown in Figure 1, SPME mainly consists of four steps: column activation, sample extraction, impurity removal, and target analyte elution. First, methanol and deionised water were used to activate the sorbent material, thereby enhancing its binding capacity with the target analytes. The tea extract was then slowly passed through the extraction column to ensure sufficient binding of the organophosphorus pesticides. Next, an appropriate solvent was selected to remove pigments, sugars, and other matrix interferences present in the tea. Finally, the target pesticides were eluted using ethyl acetate-n-hexane (9 : 1, v/v) and directly introduced into the analytical system. After comparing the purification performance of different sorbents, Carb-NH₂ was selected for this study, as it effectively removed pigments and sugars from the tea matrix while maintaining high pesticide recovery rates, thus providing reliable assurance for subsequent high-sensitivity detection.

Application of LC-MS/MS in PR detection in tea

LC-MS/MS offers high sensitivity and selectivity, enabling simultaneous quantification of multiple pesticide residues in complex tea matrices [23]. In this study, electrospray ionisation (ESI) was employed as the ionisation mode, which is suitable for highly polar and thermally labile organophosphorus pesticides. The principle of ESI is illustrated in Figure 2.

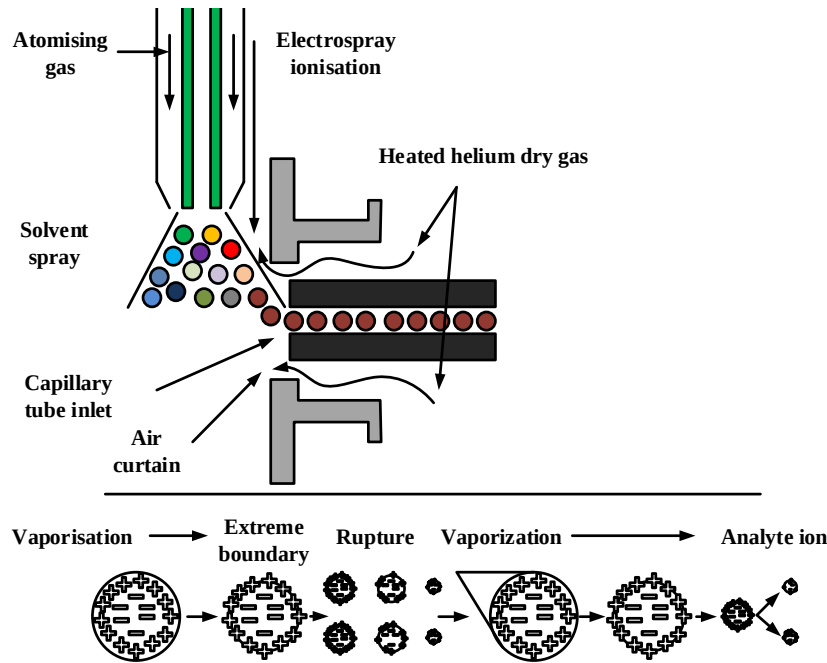


Fig. 2. Schematic diagram of electrospray ionisation

For detection, a triple quadrupole mass spectrometer was operated in multiple reaction monitoring (MRM) mode. This process involves the selection of precursor ions by Q1, fragmentation through collision-induced dissociation in Q2, and screening of product ions by Q3. This multi-stage filtering approach achieves highly selective detection and accurate quantification while minimising signal interference from the complex tea matrix. The working principle is shown in Figure 3.

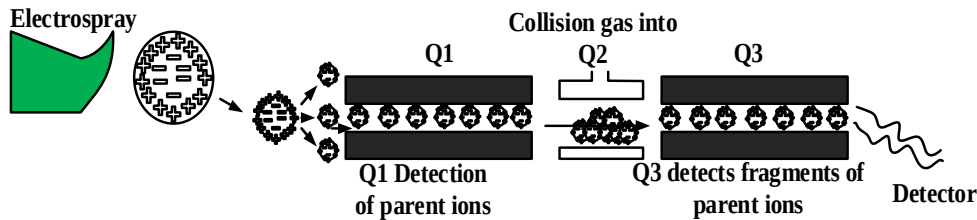


Fig. 3. Schematic diagram of triple quadrupole mass spectrometry

By combining the efficient purification of SPME with the high sensitivity of LC-MS/MS, the method established in this study enables accurate determination of trace levels of organophosphorus pesticide residues in tea, achieving high recovery rates and good stability.

Determination of five organophosphorus pesticide residues in tea by fusion of solid-phase microextraction and LC-MS/MS with isotope internal standard method

In order to accurately detect the five organophosphorus pesticide residues of fenthion, phorate, malathion, dichlorvos and loxapro in tea, it is necessary to select suitable reagents, materials, instruments, analytical conditions and sample pretreatment methods.

Reagents and materials

The purity of the organophosphorus standards used in the experiments was greater than 98 % (purchased from Dr. Ehrenstorfer-Germany); the deuterated diazinon standards (purchased from AccuStandard-USA); and the organic reagents used were high-performance liquid chromatography (HPLC) pure (purchased from TEDIA-USA). All pesticide standards were prepared in a standard stock solution of 10 µg/mL using methanol and were formulated into a series of mixed standard working solutions at the time of use. Each mL of this mixed standard working solution contained 5 ng of the isotopic internal standard diazinon. The solid phase extraction columns used included a neutral alumina (Alumina-N) column (1 g, 3 mL), a graphitised carbon black (Carb) column, and a composite amino-graphitised carbon black (Carb-NH) column (500 mg, 6 mL). Tea was purchased from the market and analysed by LC-MS/MS treatment to confirm negative samples.

Instrumentation and analytical conditions

Agilent LC-MSMS (1260-G6460) (Agilent, USA), AB Triple Quadrupole LC-MS Liquid Mass Spectrometer API4000 (Applied Biosystems, USA), and Agilent MassHunter software for data acquisition and processing.

(1) Liquid chromatography conditions

The chromatographic column was an Agilent XDB-C18 (2.1 mm i.d. · 100 mm, 3.5 µm) column. The mobile phase A was methanol; B was 0.05 % formic acid aqueous solution (v/v). The gradient conditions were (0.0 - 2.0) min, (0 - 10) % A; (0.0 - 4.0) min, (10 - 80) % A; (4.0 - 5.0) min, (80 - 95) % A; (5.0 - 12.0) min, (95) % A; (12.0 - 12.1) min, (95-10) % A; (12.1-20.0, 10) % A. The flow rate was 0.25 mL/min; the injection volume was 10 µL; and the column temperature was 30 °C.

(2) Mass spectrometry conditions

The electrospray voltage was 5500 V; the atomising gas pressure was $4.14 \cdot 10^5$ Pa; the air curtain gas pressure was $1.38 \cdot 10^5$ Pa; the auxiliary gas flow rate was $4.14 \cdot 10^5$ Pa; the ion source temperature was 450 °C; the collision gas I Medium; the dwell time was 200 ms; and the electrospray was positive mode MRM monitoring.

Sample pre-treatment

(1) Sample extraction

The tea samples were finely ground into powder, and 1 g of the powder was precisely weighed into a 50 mL centrifuge tube. Next, 10 mL of an extraction solution consisting of ethyl acetate and hexane in a ratio of 9 : 1 (v/v) was added to the tube. The mixture was vigorously vortexed for 1 minute and then centrifuged at 8000 rpm for 5 minutes.

The resulting supernatant was carefully transferred to a flask. Then 10 mL of the extraction solution was used for another repetition of the extraction, and the supernatants of the two times were combined in a chicken-centered flask. It was then concentrated under reduced pressure in a water bath environment at 35 °C until less than 2 mL of liquid remained in the bottle.

(2) Calculation of pesticide residues

Because of the internal standard method used in the experiment, the corresponding calculation formula also needs to use the calculation formula of the internal standard method, which is shown in the equation:

$$\omega = \frac{\rho A \rho_i A_{si} V}{A_s \rho_{si} A_i m} \quad (1)$$

where ω indicates the residue amount of the measured material in the sample [mg/kg]; ρ - the mass concentration of the measured material in the matrix standard working solution [$\mu\text{g/mL}$]; A - the chromatographic peak area of the measured material in the sample solution; ρ_i - the mass concentration of the internal standard in the sample solution [$\mu\text{g/mL}$]; A_{si} indicates the chromatographic peak area of the internal standard in the matrix standard working solution; V - the final volume of the sample solution [mL]; A_s - the chromatographic area of the measured material in the matrix standard working solution; ρ_{si} - mass concentration of the internal standard in the working solution of the matrix standard [$\mu\text{g/mL}$]; A_i - chromatographic area of the internal standard in the sample solution; m - mass of the sample represented by the sample solution [g].

Sample quantity and preparation

A total of 30 batches of tea samples were collected for this study, comprising 10 batches each of green tea, black tea, and oolong tea. The samples were sourced from large supermarkets (60 %) and specialised tea wholesale markets (40 %) in the city where the experiments were conducted, ensuring representativeness of the sample sources. All samples were immediately sealed in polyethylene zip-lock bags upon purchase, stored at 4 °C, and processed within 48 h. Prior to processing, the tea samples were ground using a stainless-steel grinder and passed through a 40-mesh sieve. The quartering method was applied to ensure sample homogeneity. Each portion of (1.00 ± 0.01) g was weighed into a 50 mL centrifuge tube, and an isotopically labeled diazinon-d10 internal standard solution (final concentration: 5 ng/mL) was added prior to extraction to correct for potential signal drift and matrix effects during analysis.

Replication and experimental design

To ensure the reliability and reproducibility of the results, three independent biological replicates were conducted for each batch of tea samples (i.e., re-weighing, independent extraction, and purification from the same batch), and each extract was analysed in triplicate by LC-MS/MS (technical replicates). All experiments were performed under consistent environmental conditions (room temperature: (22 ± 1) °C; relative humidity: 45 % - 55 %) using the same batch of reagents and standards to minimise batch-to-batch variability. Quality control (QC) checks were performed daily before analysis, including chromatographic system suitability tests (retention time, peak shape, signal-to-noise ratio) and mass spectrometric signal stability assessments ($\text{RSD} < 5 \%$).

Data processing and statistical analysis

Pesticide residue levels were calculated using the internal standard method, with the calculation formula provided in equation (1). All data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). Differences in pesticide residues among different tea types were assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons, with statistical significance set at $p < 0.05$. Recovery and precision validation were conducted in accordance with the SANTE/12682/2019 guidelines, using three spiking levels (0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg) with six replicates per level. The recovery rate and relative standard deviation (RSD) were calculated and compared with the international acceptance criteria (recovery: 70 % - 120 %; RSD $\leq$ 20 %) to confirm the applicability and stability of the method.

Result and discussion

To verify the fusion SPME-LC-MS/MS effectiveness in the study for pesticide residue detecting, the study utilised the method to do detecting of five organophosphorus PRs in tea and corroborated the effectiveness of the application of the method by the precision and accuracy of the detection of the five organophosphorus pesticide residues.

Optimisation of extraction conditions

In our experiments, we compared the efficiency of three extraction methods: vortex mixing, oscillatory extraction and ultrasonic extracting. The extracting efficiency of vortex mixing was the highest, with an extraction time of only 1 min and a recovery of 85 %. In contrast, the time of oscillation extraction was longer than 30 min, and the recovery rate should reach 80 %. And the efficiency of ultrasonic extraction was relatively low, which could only reach 70 %. Therefore, based on the experimental results, we chose the vortex mixing method to extract the organophosphorus pesticide residues in tea. The experiments compared ethyl acetate and solvent systems in which ethyl acetate and n-hexane were mixed in a certain proportion. Figure 4 shows the extraction effect of these four solvent systems on five organophosphorus compounds.

As can be seen from Figure 4, the recoveries of the extraction solvents were different under different reagents and different mixing ratios. By comparison, it can be seen that ethyl acetate and n-hexane with 9 : 1 volume ratio as the extraction solvent results were more satisfactory than other solvent systems, and the recovery can reach more than 85 %.

Selection of extraction solvents

In order to select and obtain the suitable extraction solvents, the study utilised vortex mixing extraction and homogenised extraction for comparison in order to obtain the most suitable extracting method for five organophosphorus pesticide residues in tea. Vortex mixing extraction and homogenisation extraction are two common extraction methods, and the results of the recovery comparison of the two extraction methods are shown in Figure 5.

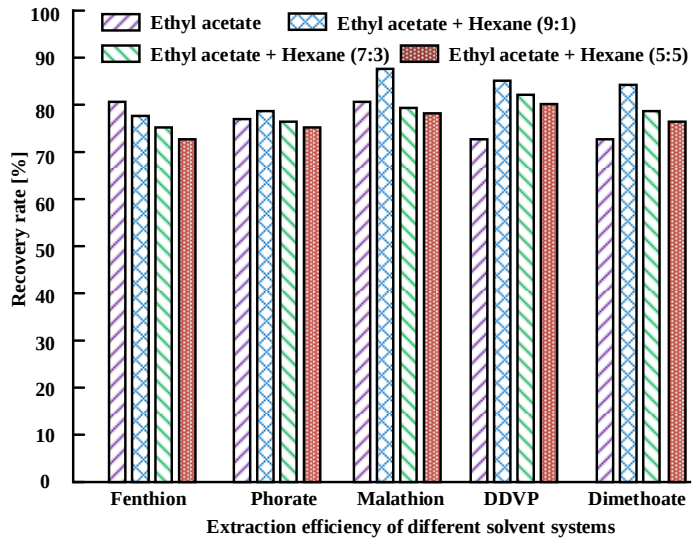


Fig. 4. Extraction effect of four solvent systems

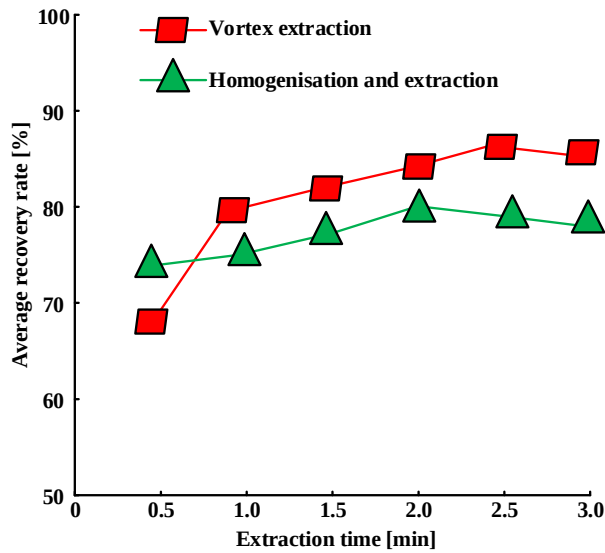


Fig. 5. Comparative results of the effect of extraction time on the recovery of organophosphorus pesticides by two methods

Combined with the analysis in Figure 5, it can be seen that when the extraction time is less than 1 min, the recovery of homogeneous extraction is higher than that of vortex mixing extracting, which indicates that the efficiency of homogeneous extraction is also higher in the case of shorter extraction time. However, when the extraction time was greater than 1 min, the recoveries of the two extraction methods did not differ much. For the five

organophosphorus pesticides, the average recovery could reach more than 80 %, and the increase of the average recovery was not obvious with the extension of the extraction time. Meanwhile, considering that the vortex mixing extraction was simpler and more convenient in operation, the vortex mixing method was chosen to extract the organophosphorus PRs in tea.

Selection of solid phase extraction columns

Since tea samples have a complex matrix and contain a large number of pigments, sugars, organic acids and other substances, these substances will not only interfere with the analysis of the target, but also cause serious damage to the column and mass spectrometry. Therefore, the study compared the cleanup effects of three solid phase extraction columns, Alumina-N, Carb and Carb-NH₂, and the results are shown in Figure 6.

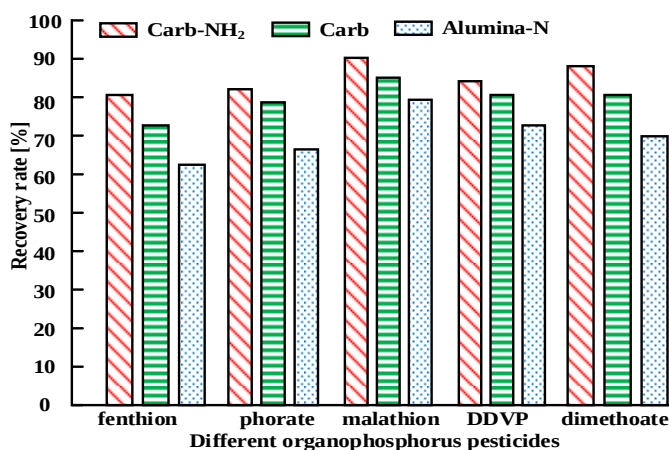


Fig. 6. Comparison of the purification effect of three kinds of solid phase extraction columns

From the analysis of Figure 6, it can be seen that the Carb-NH₂ column has the best purification effect, followed by the Carb column, while the Alumina-N has the worst effect. Specifically, Alumina-N could only remove part of the sugars, while Carb could remove most of the pigments; however, the Carb-NH₂ column could not only remove the pigments in the tea, but also remove the substrates such as sugars at the same time. Therefore, the Carb-NH₂ solid phase extraction column was chosen as the clean-up column for tea samples.

Optimisation of analytical conditions

A mobile phase with a small amount of formic acid added to the aqueous phase was used to promote the ionisation efficiency of organophosphorus pesticides during electrospraying to enhance the mass spectrometry response values. The mass spectrometry conditions were optimised by injecting the standards into the mass spectrometer via a micro injection plug pump, selecting the two more responsive daughter ions as the monitoring fragmentation ions and the higher response value of one of them as the quantification ion. Figure 7 shows the extracted MRM chromatogram of 0.05 mg/kg pesticide added to the blank matrix under optimised conditions.

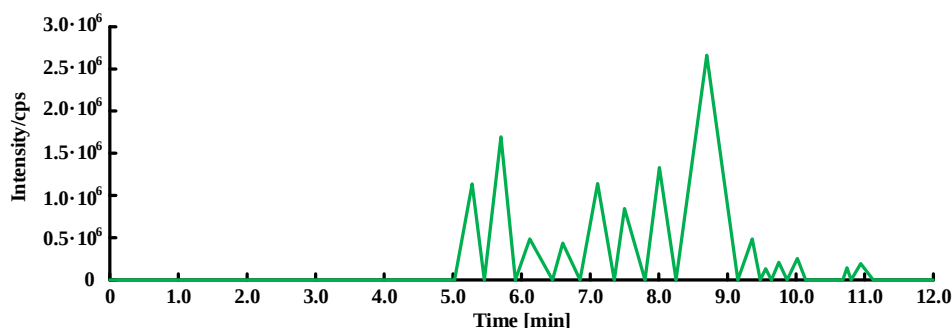


Fig. 7. Extracted MRM chromatogram of 0.05 mg/kg pesticide added to blank substrate

Combined with the experimental conditions and the analysis of Figure 7, it can be seen that although the experiments analysed the same class of substances and their properties are similar, the DP voltage and CE voltage of different compounds also have great differences. The molecular weight has a large effect on the DP voltage, and in addition, the CE voltages of the daughter ions are very different even when they are ionised from the same molecule. Therefore, in order to obtain the strongest signals of the daughter ions, it is necessary to optimise the DP voltage and CE voltage of different organophosphorus.

Standardised working curves and limits of quantification

The response peak areas of the five pesticides at mass concentrations of 5.0, 10.0, 50.0, 100.0 and 500.0 ng/mL were determined in a blank matrix. The standard curves were plotted with the response peak areas as vertical coordinates and mass concentrations as horizontal coordinates. The limit of quantification (LOQ) results were calculated based on the signal-to-noise ratio $S/N = 10$ as Table 1.

Limit of quantification results of the method

Table 1

Serial number	Name	Retention time [min]	Correlation coefficient R^2 [-]	Method limit of quantification [mg/kg]	GB2763 limit [mg/kg]
1	fenthion	13.26 $\pm$ 0.04	0.9997	0.0014 $\pm$ 0.0001	0.05
2	phorate	10.08 $\pm$ 0.03	0.9997	0.0026 $\pm$ 0.0001	0.01
3	malathion	11.83 $\pm$ 0.05	0.9995	0.0011 $\pm$ 0.0001	0.5
4	DDVP	15.92 $\pm$ 0.04	0.9993	0.0013 $\pm$ 0.0001	0.2
5	dimethoate	14.88 $\pm$ 0.05	0.9996	0.0016 $\pm$ 0.0001	0.01

As shown in Table 1, the obtained coefficients were all larger than 0.99, which indicated that the method is qualified to do quantitative analysis of the compounds in this concentration range. Meanwhile, the limits of quantification (LOQs) of five organophosphorus pesticides, namely, fenthion, phorate, malathion, dichlorvos and phosmet, were lower than the relevant national standards, which also verified the feasibility of the method.

Accuracy and precision of methods

Spiking recovery experiments evaluate the method. Three concentrations of mixed standard solutions of 0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg were added prior to sample pretreatment. The extraction was then carried out according to the prescribed procedure and five average determinations were made. Table 2 gives the calculation outcome about relative standard deviation (RSD) and average recovery.

Table 2

Mean recovery and standard deviation of the method

Num	Name	0.05 mg/kg (mean $\pm$ SD) [%]	0.1 mg/kg (mean $\pm$ SD) [%]	0.5 mg/kg (mean $\pm$ SD) [%]
1	fenthion	86.0 $\pm$ 5.1	83.6 $\pm$ 6.8	87.1 $\pm$ 3.7
2	phorate	93.2 $\pm$ 3.9	91.0 $\pm$ 3.5	89.5 $\pm$ 1.0
3	malathion	98.6 $\pm$ 4.0	108.4 $\pm$ 5.7	69.8 $\pm$ 6.1
4	DDVP	96.4 $\pm$ 1.2	93.4 $\pm$ 7.7	73.0 $\pm$ 4.6
5	dimethoate	105.9 $\pm$ 4.3	86.9 $\pm$ 6.2	98.9 $\pm$ 3.2

As shown in Table 2, the average recoveries of the five organophosphorus pesticides at three spiking levels ranged from 69.8 % to 108.4 %, with standard deviations ranging from 1.0 % to 7.7 %. These results indicate that the proposed method had good accuracy and precision for the determination of organophosphorus pesticide residues in tea.

Determination of actual samples

Three samples of commercially available black tea, green tea and white tea were randomly picked, and the samples were processed and tested using the testing method established in the study. The outcomes obtained are in Table 3.

Table 3

Measurement results of actual samples

Serial number	Name	Black tea [mg/kg]	Green tea [mg/kg]	White tea [mg/kg]
1	fenthion	< LOQ	< LOQ	< LOQ
2	phorate	< LOQ	< LOQ	< LOQ
3	malathion	< LOQ	0.0015	< LOQ
4	DDVP	< LOQ	< LOQ	< LOQ
5	dimethoate	< LOQ	< LOQ	0.0036

As shown in Table 3, the residue contents of the five organophosphorus pesticides in the three tea samples were all below the method limit of quantification (LOQ), among which malathion in green tea and loxapro in white tea were detected at 0.0015 mg/kg and 0.0036 mg/kg, respectively. Although detected, the values of the detected pesticides were below the MRLs of the national standards. This indicates that the practical application of the method is excellent.

Discussion

The study established a SPME combined with LC-MS/MS method for the simultaneous determination of five organophosphorus pesticides in tea samples. The method achieved LOQ of (0.0011 - 0.0026) mg/kg, which are significantly lower than

the maximum residue limits (GB 2763). Average recoveries ranged from 72.99 % to 108.41 %, indicating good sensitivity, accuracy, and reproducibility. Compared with existing studies, this method shows advantages in detection limits and matrix interference control. For instance, Dong et al. [24] used multi-plug filtration cleanup (m-PFC) combined with LC-MS/MS to detect 14 pesticides in tea, with LOQs ranging from 3 µg/kg to 50 µg/kg, some of which were higher than those obtained in this study. Xu et al. [25] proposed a modified QuEChERS-GC-MS/MS method for detecting 10 pyrethroid pesticides, which has a wider application range but requires more solvent and cleanup steps, whereas the present method achieves higher sensitivity while reducing organic solvent use.

The complex tea matrix poses a major challenge for pesticide residue analysis, as abundant pigments and polyphenols can cause matrix effects. Chen et al. [26] emphasised that establishing tea matrix reference materials is essential to ensure comparability of results among different laboratories. The use of SPME combined with isotope-labelled internal standards in this study effectively reduced matrix interference and improved quantification accuracy across different tea types. In terms of application, Gao et al. [27] developed colloidal gold-based lateral flow immunochromatographic strips (LFICS), which can complete sample pretreatment and detection within 30 min, making them suitable for on-site rapid screening, though their confirmatory quantitative capability is limited, thus complementing the present method. Pesticide residue levels in tea vary across regions. Fan et al. [28] detected 18 pesticide residues in 105 green tea samples from Hangzhou, with only one sample exceeding the limit for carbofuran, indicating low overall risk. Heshmati et al. [29] analysed 33 pesticides in black tea samples from Iran, finding that 40 % exceeded the European Commission limits, and some pesticides migrated into tea infusions during brewing, highlighting the need for risk assessment at the consumption stage. Miao et al. [30] summarised migration patterns of water-soluble and fat-soluble pesticides during tea cultivation, processing, and brewing, emphasising the higher dietary exposure risk of water-soluble pesticides, which is consistent with the experimental findings of Heshmati et al. [29] Reducing pesticide residues relies not only on detection methods but also on preventive measures during production.

It should be noted that the detection scope of this study covered only five organophosphorus pesticides, whereas a wider range of pesticide types are used in actual production, including neonicotinoids, pyrethroids, and fungicides. Future work may adopt multi-residue detection strategies to expand the range of analytes and investigate pesticide migration into tea infusions, providing more comprehensive data for dietary exposure risk assessment. In conclusion, the proposed SPME-LC-MS/MS method demonstrates excellent sensitivity, accuracy, and matrix interference control, and can serve as a reliable technical tool for monitoring pesticide residues, quality control, and risk assessment in tea.

Conclusion

By integrating SPME and LC-MS/MS techniques, the study successfully developed a rapid, sensitive and accurate method to determine organophosphorus PRs in tea. The study firstly selected suitable SPME materials for enrichment. Then, the quantitative analysis was carried out using LC-MS/MS technique. The outcomes tell that the detection of tea samples' organophosphorus pesticides revealed the prevalence of organophosphorus PRs in tea. However, the contents of five organophosphorus pesticide residues in the three tea samples detected were under the LOQ of the method, but malathion in green tea and

dimethoate in white tea were detected at 0.0015 mg/kg and 0.0036 mg/kg, respectively, which demonstrated that the method has high sensitivity and selectivity as well as rapid analytical speed. However, there are still shortcomings in the study, the method only determined the residues of organophosphorus pesticides, and did not determine the residues of other types of pesticides, the next step can be to determine other pesticide residues to verify the method's adaptability.

References

- [1] Odewale GO, Sosan MB, Oyekunle JAO, Adeleye AO. Assessment of systemic and carcinogenic health risks of persistent organochlorine pesticide residues in four fruit vegetables in south-western Nigeria. *British Food J.* 2022;124(5):1755-74. DOI: 10.1108/BFJ-05-2021-0548.
- [2] Xia Q, Du Z, Lin D, Huo L, An Y. Review on contaminants in edible oil and analytical technologies. *Oil Crop Science.* 2021;6(1):23-7. DOI: 10.1016/j.ocsci.2021.03.003.
- [3] El-Saeid MH, Hassanin AS, Bazeyad AY. Rapid analytical method for the determination of 220 pesticide with their isomers by GCMS-TIC. *Saudi J Biol Sci.* 2021;28(8):4173-82. DOI: 10.1016/j.sjbs.2021.04.007.
- [4] Sharma S, Kaur A, Warangtiwar RK. Dissipation and residual bioefficacy of chlorantraniliprole in tomato and brinjal fruits. *Pesticide Res J.* 2019;31(2):249-58. DOI: 10.5958/2249-524X.2019.00032.5.
- [5] Wang Y, Gao W, Li Y, Xiao Y, Hou R. Establishment of a HPLC-MS/MS detection method for glyphosate, glufosinate-ammonium, and aminomethyl phosphoric acid in tea and its use for risk exposure assessment. *J Agricultural Food Chem.* 2021;69(28):7969-78. DOI: 10.1021/acs.jafc.1c01757.
- [6] Osinova ES, Melekhin AO, Bulkatov DP, Tishchenko VV, Khalyavin IA. Determination of pesticide residues in wheat grains by GC-MS/MS after their extraction by a modified QuEChERS method using hyper-cross-linked polystyrene at the purification stage. *J Anal Chem.* 2025;80(6):1068-76. DOI: 0.1134/S1061934825700418.
- [7] Wang Y, Zhou W, Pawliszyn J. Sequential solid-phase microextraction with biocompatible coating materials to address displacement effects in the quantitative analysis. *Anal Chem.* 2023;95(16):6718-24. DOI: 10.1021/acs.analchem.3c00508.
- [8] Sereshti H, Karami F, Nouri N, Farahani A. Electrochemically controlled solid phase microextraction based on a conductive polyaniline-graphene oxide nanocomposite for extraction of tetracyclines in milk and water. *J Sci Food Agricult.* 2021;101(6):2304-11. DOI: 10.1002/jsfa.10851.
- [9] Lin X, Lu K, Hardison AK, Liu Z, Xu X, Gao D, et al. Membrane inlet mass spectrometry method (REOX/MIMS) to measure 15N-nitrate in isotope-enrichment experiments. *Ecol Indicators.* 2021;126:107639. DOI: 10.1016/j.ecolind.2021.107639.
- [10] Feeney W, Menking-Hoggatt K, Arroyo L, Curran J, Bell S, Trejos T. Evaluation of organic and inorganic gunshot residues in various populations using LC-MS/MS. *Forensic Chem.* 2022;27:100389. DOI: 10.1016/j.forc.2021.100389.
- [11] Song JG, Cao C, Li J, Yuan F. Development and validation of a QuEChERS-LC-MS/MS method for the analysis of phenolic compounds in rapeseed oil. *J Agricult Food Chem.* 2019;67(14):4105-12. DOI: 10.1021/acs.jafc.8b0725x.
- [12] Kuchipudi A, Angappan S, Kaithamalai B. Method validation, dissipation and decontamination of flubendiamide residues in lettuce using LC/MS/MS. *Pesticide Res J.* 2019;31(1):34-41. DOI: 10.5958/2249-524X.2019.00009.8.
- [13] Tang T, Zhou M, Lv J, Cheng H, Wang H, Qin D, et al. Sensitive and selective electrochemical determination of uric acid in urine based on ultrasmall iron oxide nanoparticles decorated urchin-like nitrogen-doped carbon. *Colloids Surfaces B: Biointerfaces.* 2022;216:112538. DOI: 10.1016/j.colsurfb.2022.112538.
- [14] Xin J, Xu G, Zhou Y, Wang X, Zhao RS. Ketoenamine covalent organic framework coating for efficient solid-phase microextraction of trace organochlorine pesticides. *J Agricult Food Chem.* 2021;69(28):8008-16. DOI: 10.1021/acs.jafc.1c02895.
- [15] Jouyban A, Nemati M, Farazajdeh MA, Nabil AAA, Mogaddam MRA. A polymer-based dispersive solid phase extraction combined with deep eutectic solvent based-dispersive liquid-liquid microextraction for the determination of four hydroxylated polycyclic aromatic hydrocarbons from urine samples. *J Separation Sci.* 2021;44(21):4025-36. DOI: 10.1002/jssc.202100269.
- [16] Wang Q, Chen L, Li Y, Yang J, Yang R, Yang X. Magnetic nanocomposite-based TpPa-NO₂ covalent organic framework for the extraction of pyrethroid insecticides in water, vegetable, and fruit samples. *Food Anal Methods.* 2023;16(1):71-82. DOI: 10.1007/s12161-022-02394-0.

- [17] Zhang Q, Yang B, Wen M, Wang X. Analysis of trace parabens in environmental samples by highly efficient solid-phase microextraction coupled with ultra-high-performance liquid chromatography-tandem mass spectrometry. *Separation Sci Plus*. 2022;5(6):228-36. DOI: 10.1002/sscp.202200011.
- [18] Dwaiby J, Cheaib D, Jammoul A, Darra NE. Assessment of phenolic content, pesticide residue and biological activities of organic lemon and cucumber in Lebanese market. *Nutrition Food Sci*. 2021;51(8):1207-23. DOI: 10.1108/NFS-01-2021-0013.
- [19] Dong X, Zhu B, Zhao X, Wang H, Liu S. Transfer rates on nine pesticides from dry tea to infusion by QuEChERS purification followed by LC-MS/MS analysis. *Int J Environ Anal Chem*. 2023;103(13):2931-47. DOI: 10.1080/03067319.2021.1900149.
- [20] Naik RH, Pallavi MS, Bheemanna M. Dissipation and decontamination of dimethoate in curry leaf using LC-MS/MS (ESI) technique. *Pesticide Res J*. 2021;33(1):30-8. DOI: 10.5958/2249-524X.2021.00016.9.
- [21] El-Maghrabi HH, Hassanein TF, Younes AA. Chelating solid-phase polymeric adsorbent for the removal of Hg²⁺ ions from aqueous solutions: preparation, characterisation and adsorption optimisation studies. *J Dispersion Sci Technol*. 2023;44(7):1278-87. DOI: 10.1080/01932691.2021.2013866.
- [22] Zhang G, Zhao Z, Yin X, Zhu Y. Impacts of biochars on bacterial community shifts and biodegradation of antibiotics in an agricultural soil during short-term incubation. *Sci Total Environ*. 2021;771:144751. DOI: 10.1016/j.scitotenv.2020.144751.
- [23] Chen J, Huang X, Wang L, Ma C, Wu S, Wang H. The synthesis of a dual-template surface molecularly imprinted polymer based on silica gel and its application in the removal of pesticides from tea polyphenols. *Anal Methods*. 2020;12(7):996-1004. DOI: 10.1039/C9AY02708D.
- [24] Dong X, Lan T, Tian X, Li Y, Zhao Y, Zong Q, et al. Simultaneous determination of 14 pesticide residues in tea by multi-plug filtration cleanup combined with LC-MS/MS. *J Environ Sci Health, Part B*. 2021;56(8):771-81. DOI: 10.1080/03601234.2021.1944962.
- [25] Xu R, Xie Q, Li X, Zhao H, Liu X, Wei Y, et al. Modified QuEChERS method based on multi-walled carbon nanotubes coupled with gas chromatography-tandem mass spectrometry for the detection of 10 pyrethroid pesticide residues in tea. *Chinese J Chromatography*. 2022;40(5):469-76. DOI: 10.3724/SP.J.1123.2021.11015.
- [26] Chen Y, Qiu X, Feng C, Xu Q, Lin Y, Le S, et al. Preparation of a reference material for tea containing five pesticide residues and its evaluation in an interlaboratory comparison study in China. *Accreditation Quality Assurance*. 2022;27(2):93-101. DOI: 10.1007/s00769-022-01494-y.
- [27] Gao W, Guo J, Xie L, Peng C, He L, Wan X, et al. Washing fresh tea leaves before picking decreases pesticide residues in tea. *J Sci Food Agric*. 2020;100(13):4921-9. DOI: 10.1002/jsfa.10553.
- [28] Fan JC, An J, Ren R, Liu SY, He HL, Zhao G. Occurrence and exposure risk assessment of pesticide residues in green tea samples cultivated in Hangzhou area, China. *Food Additives Contaminants: Part B*. 2023;16(1):8-13. DOI: 10.1080/19393210.2022.2103746.
- [29] Heshmati A, Mehri F, Mousavi Khaneghah A. Simultaneous multi-determination of pesticide residues in black tea leaves and infusion: a risk assessment study. *Environ Sci Pollut Res*. 2021;28(11):13725-35. DOI: 10.1007/s11356-020-11658-3.
- [30] Miao S, Wei Y, Pan Y, Wang Y, Wei X. Detection methods, migration patterns, and health effects of pesticide residues in tea. *Comprehensive Rev Food Sci Food Safety*. 2023;22(4):2945-76. DOI: 10.1111/1541-4337.13167.