

Study of Analytical Methods for Determining 16 Organophosphorus Pesticide Residues in *Panax Notoginseng* and *Aucklandia Lappa Decne* by Automated Solid-Phase Extraction System and GC/MS

Na Wu

Honghe University

Gaozhang Gou

Honghe University

Dachao Liang

Honghe University

Jinru Ruan

Hong He Institute for Food and Drug Control

Wei Liu

Honghe University

Shaoping Feng (✉ shaopingfeng@126.com)

Honghe University

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Abstract

A fast and sensitive multi-residue analytical method was developed for the determination of organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa* decne by comparing an ultrasonic extraction and homogenate extraction approach coupled with automated solid-phase extraction system and gas chromatography-mass spectrometry (GC/MS). Various factors affecting the homogenate extraction and purification efficiency were investigated in detail. The optimum conditions for homogenate extraction of organophosphorus pesticide residues from *Panax notoginseng* and *Aucklandia lappa* decne were 60 mL dichloromethane, 10000 r/min of extracting revolution speed and 2 min of extracting time. The extracts were cleaned up by automated solid-phase extraction system and determined by GC/MS. Under the optimum conditions, the results of the homogenate extraction approach were compared with those of the ultrasonic extraction, and the homogenate extraction approach was used to extract the samples. The average recoveries of the method by a homogenate extraction approach coupled with automated solid-phase extraction system and GC/MS ranged from 72.56% to 96.70%, and RSDs were 0.05~0.14%, which allowed the determination of 16 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa* decne. The results indicated that the proposed method can apply for the determination of organophosphate pesticides residues in *Panax notoginseng* and *Aucklandia lappa* decne with high accuracy and precision.

1. Introduction

Pesticide residues refer to pesticides, their toxic metabolites and degradation products and any impurities that may live on or in an biosome, farm products, or the environment after pesticides are used¹⁻³.

Sometimes pesticide residues are inevitable; however, once the residue reaches its limitation, it can make a series of danger to humans and animals or other organism in the ecological system by the food cycle⁴. The organophosphate pesticides (OPPs) were widely used and led to the repeated incidents of poisoning, and therefore, self-poisoning with organophosphate pesticides is a significant global health problem⁵⁻⁷. Hence, a ban was imposed on the use of toxic pesticides, including OPPs, such as methamidophos, parathion, monocrotophos, methyl parathion, etc⁸⁻¹⁰.

The rules linking pesticides on plants and in agricultural products have been set up so far by a lot of international organizations and countries. With the remarkable enhancements in testing methods and analytical techniques of pesticide residues and the extensive attention of consumer safety in recent years, the developed countries have become strictly monitoring both the terms of pesticide class or amount and maximum residue levels (MRLs) in foodpesticide residues¹¹⁻¹⁴. Multiresidue analytical systems, which have been developed worldwide, can simultaneously determine over three hundred pesticides¹⁵. The MRLs of 9 organochlorines (OCPs) were prescriptive in the Chinese pharmacopoeia, which involved benzene hexachloride (BHC), dichlorodiphenyltrichloroethane (DDT) and pentachloronitrobenzene (PCNB). *Radix et Rhizoma Glycyrrhizae* and *Radix Astragali* in the Pharmacopoeia of the People's Republic of China (Ch.P) were involved¹⁶. The first draft of the quality standards from China in July 2001, which had something to do with green professional standards for import and export of officinal plants and products, was issued and enforced in the ministry of foreign trade and economic cooperation. The 9 OCPs and aldrin were included according to the standards. The analytical methods of 3 pyrethroids and 12 OPPs were supplied separately in the Ch.P, but MRLs were not prescriptive. In order to improve the international regulations and standards of pesticide residues from Chinese traditional medicine, further research has been needed¹⁷. The main food safety issues focused on pesticide residues. Especially the MRLs of pesticide residues from many countries and health organizations had published. People have paid a more attention to analytical methods of remnant pesticides, so the analytical methods possessing sureness, simpleness, speediness, relatively steady, highly sensitive and repeatability is required. The sample pre-treatment and detected techniques have a crucial effect on the accuracy and precision of the analytical methods.

The sample pre-treatment techniques include liquid-liquid extraction, liquid-solid extraction, shaking extraction, Soxhlet extraction, supercritical fluid extraction, pressurized solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, gel permeation chromatography, solid phase extraction, solid phase microextraction, molecularly imprinted polymers, matrix solid-phase dispersion, QuEChERS, cloud-point extraction, liquid phase microextraction, etc¹⁸⁻²². Automated solid-phase extraction system achieves artificial intelligence in the sample pre-treatment techniques.

With the significantly improving requirements in environmental quality and food safety, quantity of submitted test samples is increasing. Hence, this increase requires that the analytical method is fast, ingenious, accurate, and convenient. In order to accelerate the process of internationalizing and modernizing traditional Chinese herbal medicine, analysis methods, including GC/LC, GC/MS, LC/MS, SFC, CE, CE/MS and ELISAs for the detection of pesticide residues have been used to determine pesticide residues²³⁻²⁶. Great progress has been made on new analytical methods and techniques at present, especially in pesticides multiresidue analyses method. The analyses of multipesticide residues from the same class of pesticide and a trial sample of new single pesticides in *Panax notoginseng* were also reported^{27,28}.

Panax notoginseng belonging to araliaceae species which mainly produced in the any counties of WenShan prefecture from Yunnan provinces of China. The origin of artificially cultivated *Panax notoginseng* are expanding from WenShan prefecture to other areas in Yunnan provinces. The dry roots and rhizomes of *Panax notoginseng* were position and has the functions of removing stasis, stopping blood and pain, activating blood^{29,30}.

Aucklandia lappa decne belonging to asteraceae perennial herb which mainly produced in Yunnan places like Lijiang Naxi Autonomous County and Ludian County, Zhaotong City. The origin of artificially cultivated *Aucklandia lappa decne* are distributed mainly over Sichuan, Yunnan, Guangxi and Guizhou provinces. *Aucklandia lappa decne* has the functions of stomach, tocolysis, relieving pain, regulating Qi and soothing the nerves. The application of OPPs in processes of artificially cultivated *Panax notoginseng* and *Aucklandia lappa decne* were unavoidable, but there are few standard of detective pesticide residue in *Panax notoginseng* and *Aucklandia lappa decne*. For these reasons above, a rapid and simple analytical method with high accuracy and precision for determining 17 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne* by automated solid-phase extraction system and GC/MS was developed in this paper. The rapid and simple method for the simultaneous determination of 16 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne* was developed by comparing an ultrasonic extraction and homogenate extraction and optimizing purification conditions of automated solid-phase extraction system, which can meet the requirement of determining pesticide residues from *Panax notoginseng* and *Aucklandia lappa decne*.

2. Experimental

2.1 Apparatus, Chemicals and Materials

A Shimadzu GC/MS-QP2010 Ultra (Shimadzu, Japan), a J2 automated solid-phase extraction system (J2 Scientific, USA), a rotary evaporator (Switzerland, Germany), an electronic balance (Switzerland, Germany), a vortex mixer (Beijing, China), a universal pulverizer (Tianjin, China), an ultrasound cleaner (Shanghai, China) and the homogenate approach (Jiangsu, China) were used.

Acetone, dichloromethane, and n-hexane, all of analytical reagent grade, were from Tianjin University and were purchased from Tianjin Chemical Experiment Plant (Tianjin, China). Methylbenzene, acetonitrile, and methyl alcohol were all of HPLC grade and were purchased from Tedia (Fairfield, OH, USA). Anhydrous sodium sulfate and sodium chloride (Shanghai, China) were baked at 450 °C for 4 h and stored in a desiccator before use. The standards of 17 OPPs (methamidophos, dichlorvos, acephate, omethoate, monocrotophos, phorate, phosphamidon, methyl parathion, fenitrothion, malathion, fenthion, chlorpyrifos, parathion, profenofos, triazophos, imidan, phosalone) with a solution concentration of 1000 mg/L were purchased from Quality Testing and Inspection Centre of the Ministry of Agriculture for Agricultural Products (Beijing, China).

Panax notoginseng and *Aucklandia lappa decne* samples were purchased from local markets (Yunnan, China). Solid-phase extraction cartridges (SPE cartridges) containing C₁₈, NH₄ and Florisil were purchased from Agela Technologies (Tianjin, China).

2.2 GC/MS conditions

The separation was performed on an Rtx-5MS quartz capillary column (30 m × 0.25 mm, I.D. 0.25 μm) with a helium flow rate of 1.20 mL·min⁻¹ in a constant flow mode using the following oven temperature program: 70 °C (1 min) to 250 °C (2 min) at a rate of 8 °C·min⁻¹ and 250 °C (2 min) to 300 °C (10 min) at a rate of 5 °C·min⁻¹. The injection inlet temperature was set at 250 °C, and a 1.0 μL volume was injected. Helium was the carrier gas. Using the splitless injection, the total analysis time was 45.5 min. The mass spectrometer was operated in electron ionization mode with an ionizing energy of 70 eV, an ion source temperature of 230 °C, and an MS quad temperature of 280 °C. The scan ranged from m/z 45 to 500, and the solvent delay was 3.5 min. Under the above GC-MS conditions, the total ion chromatogram (TIC) of 17 OPPs was obtained (Fig. 1).

2.3 Standard solution preparation

The 0.90 mg (accurate to 0.01 mg) individual pesticide standards of 17 OPPs were weighed and redissolved in methylbenzene to make 10 mL, which was to obtain the mixed standard reserve fluid of 17 OPPs. The standard stock mixtures were then diluted to 4.5 μg·mL⁻¹ with acetone. The series of mixed standard working solutions for 2.00, 1.00, 0.50, 0.25, 0.10, and 0.05 μg·mL⁻¹ were made up by the dilution method step by step with acetone. The standard solutions were stored in the dark at -4 °C before use.

2.4 Sample preparation

The samples were pulverized into fine powders, sieved through 80 mesh and dried at 60 °C for 4 h. One gram of powder was then weighed accurately, and 60 mL of dichloromethane was added. The mixture was extracted for 2 min via homogenate extraction at 10000 r/min, and then 1.0 g sodium sulfate anhydrous and 1.0 g sodium chloride were added. After filtration, the extracts were transferred into a flat-bottomed flask and condensed on a rotary evaporator at 40 °C under vacuum until a volume of 5 mL was reached. The concentrated solutions were purified with Florisil solid-phase extraction cartridges by an automated solid-phase extraction system. The solid-phase extraction cartridges with the adsorbed extracts were eluted with 5 mL of methylbenzene-acetonitrile (1:3, v/v) three times. The eluate solution was collected and evaporated until near dryness by a rotary evaporator with a water bath at 38 °C. Finally, the residues were resuspended in 1.00 mL of acetone and filtered through a 0.45 μm PTFE filter for GC-MS analysis.

2.5 Investigation of extraction and purification profiles

2.5.1 Investigation of ultrasonic extraction profiles

The extraction technology implemented for the analysis of 17 OPPs from *Panax notoginseng* was under study, and the effects of factors such as extraction repetition and duration, extraction *solvent and solvent* volume on the extraction rate of 17 OPPs were examined through orthogonal experiments. The extraction repetition and duration, extraction *solvent and solvent* volume were screened, and an L9(3⁴) (Table 1) orthogonal test was applied.

Table 1 The factors and levels of the ultrasonic extraction orthogonal experiment

Factors Levels	1	2	3
A (Solvent)	Methanol	Acetonitrile	Dichloromethane
B (Volume)/mL	20	40	60
C (Repetitions)	1	2	3
D (Duration)/min	15	30	45

2.5.2 Investigation of homogenate extraction profiles

The homogenate extraction approach of 17 OPPs from *Panax notoginseng* was under study, and the effects of factors such as time, revolution speed of homogenate extraction, extraction *solvent and solvent* volume on the extraction rate of 17 OPPs were examined through orthogonal experiments. The factors were screened, and an L9(3⁴) (Table 2) orthogonal test was applied.

Table 2 The factor and level of the homogenate extraction orthogonal experiment

Factors Levels	1	2	3
A (Solvent)	Methanol	Acetonitrile	Dichloromethane
B (Volume)/ mL	30	40	60
C (Revolution speed)/ r/min	10000	15000	20000
D (Time)/min	2	3	5

2.5.3 Investigation of purification profiles

The most effective purifying condition for 17 OPPs from *Panax notoginseng* was obtained experimentally by the orthogonal test design method. The influence of factors such as eluate, elution volume and times, and different solid-phase cartridges on the purification profiles were investigated. The factors were screened, and an L9(3⁴) (Table 3) orthogonal test was applied.

Table 3 The factors and levels of the purification orthogonal experiment

Factors Levels	1	2	3
A (Eluate) (volume batching)	Acetone and N-hexane (1:1,V/V)	Methylbenzene and Acetonitrile (1:3,V/V)	Cyclohexane and Ethyl acetate (1:1,V/V)
B (Volume)/mL	5	10	20
C (Solid-phase cartridges)	C ₁₈	NH ₄	Florisil
D(times)	1	2	3

3. Results And Discussion

3.1 Selection of Quantization and qualifying ion

Single standard solutions of 17 OPPs was monitored with the full-scan mode (SCAN) by GC/MS. One quantitative ion and two or three qualitative ions of each compound were confirmed. The detection method for 17 OPPs from 4.5 µg/m was established with the selected ion monitoring mode (SIM). The results are shown in Table 4.

Table 4 Parameters for the determination of OPPs in *Panax notoginseng* by GC/MS

NO.	Pesticides	Retention time	The qualitative and quantitative ions
		(min)	(* the quantitative ions) (m/z)
1	Methamidophos	6.983	94*, 141, 47, 64
2	Dichlorvos	7.241	109*, 79, 185, 145
3	Acephate	9.795	136*, 94, 47, 125
4	Omethoate	11.817	156*, 110, 79, 126
5	Monocrotophos	13.164	127*, 97, 67, 192
6	Phorate	13.330	75*, 121, 260, 231
7	Phosphamidon	15.417	127*, 264, 15, 205
8	Methyl parathion	16.965	109*, 125, 263, 79
9	Fenitrothion	18.228	125*, 109, 260, 277
10	Malathion	18.712	173*, 127, 93, 185
11	Fenthion	19.111	278*, 125, 169, 245
12	Chlorpyrifos	19.146	314*, 197, 28, 268
13	Parathion	19.259	291*, 109, 137, 97
14	Profenofos	23.330	139*, 208, 337, 269
15	Triazophos	26.695	161*, 257, 285, 172
16	Imidan	30.185	160*, 317, 133, 77
17	Phosalone	32.368	182*, 121, 154, 367

3.2 Selection of ultrasonic extraction and homogenate extraction conditions

Based on the orthogonal test and multiple standard addition method, the data in Table 5 were obtained using the ultrasonic and homogenate extraction methods. Both of intuitionistic analysis and square-difference analysis is applied to analyze the data from Table 5, and the results are shown in Table 6, from which indicated that the dominant influence on the investigation factors for 17 OPPs from *Panax notoginseng* were the type of solvent used for the ultrasonic and homogenate extraction methods. For other factors with the ultrasonic and homogenate extraction, the effects on extraction efficiency were ranked as follows: C>B>D. The results showed the optimal conditions (A₃B₁C₁D₂) for the extraction of 17 OPPs from *Panax notoginseng* by ultrasonic extraction were as follows: *Panax notoginseng* was extracted with approximately 20 mL of dichloromethane, and the extraction replication and duration times were one and 30 min, respectively. The results showed that the optimal conditions (A₃B₃C₁D₁) for the extraction of 17 OPPs from *Panax notoginseng* by the homogenate extraction method were as follows: *Panax notoginseng* was extracted with approximately 60 mL of dichloromethane, and the extraction time was 2 min at 10000 r/min.

Table 5 The recovery results of the extraction rates in ultrasonic and homogenate extraction by L9(3⁴) (%)

Extraction method	NO.	Methamidophos	Dichlorvos	Acephate	Omethoate	Monocrotophos	Phorate	Phosphamidon	Methyl parathion
Ultrasonic extraction	1	67.95	77.32	75.59	68.27	64.52	73.27	69.66	93.43
	2	67.89	76.7	75.45	67.88	63.98	72.9	69.39	91.36
	3	59.72	74.43	75.14	67.42	61.66	70.51	67.99	58.99
	4	62.95	62.9	65.65	64.26	78.27	79.55	71.33	75.83
	5	67.96	63.71	66.84	65.50	81.51	76.62	72.3	76.83
	6	61.37	61.31	70.96	66.18	70.89	65.36	63.99	86.66
	7	67.68	76.72	75.56	67.63	63.32	72.32	69.23	95.43
	8	64.31	62.85	72.56	63.98	81.52	65.29	69.54	85.13
	9	65.13	61.58	73.82	65.86	80.44	62.48	69.56	86.84
Homogenate extraction	1	69.06	75.21	75.97	74.75	66.23	62.58	71.23	68.91
	2	45.94	76.28	75.99	75.28	67.09	63.49	71.91	69.76
	3	55.78	75.61	75.97	75.25	67.34	62.57	71.73	69.70
	4	59.45	63.01	73.03	74.42	66.03	85.53	78.11	71.91
	5	67.96	65.08	71.84	73.85	62.35	91.91	81.76	69.72
	6	62.96	73.71	71.62	73.34	60.63	96.29	92.89	69.68
	7	64.78	79.96	67.62	73.12	61.98	95.95	97.03	80.35
	8	65.76	72.06	71.95	7346	63.33	97.32	80.52	72.88
	9	66.99	62.22	74.38	73.93	65.03	80.88	67.52	65.04

Table 5 The recovery results of the extraction rates in ultrasonic and homogenate extraction by L9(3⁴) (%) (Continued)

Extraction method	NO.	Fenitrothion	Malathion	Fenthion	Chlorpyrifos	Parathion	Profenofos	Triazophos	Imidan	Phosalone
Ultrasonic extraction	1	68.96	65.78	63.49	61.01	67.43	67.40	76.02	61.84	66.2
	2	68.55	65.20	63.25	61.37	67.50	66.9	75.91	61.95	65.88
	3	66.35	62.46	60.24	63.69	64.36	65.02	74.42	62.24	63.99
	4	84.54	83.55	94.05	101.1	59.87	60.41	62.89	63.11	64.94
	5	83.18	85.15	92.11	101.22	51.04	60.40	63.71	62.79	64.47
	6	71.28	71.68	79.14	85.30	60.00	67.39	61.31	62.85	61.15
	7	67.94	64.76	62.54	62.08	68.00	52.43	67.72	50.25	49.33
	8	75.60	80.81	86.96	98.35	62.17	67.23	62.85	61.82	66.39
	9	74.72	79.16	84.76	95.43	68.41	66.39	61.58	61.82	66.39
Homogenate extraction	1	27.00	67.2	64.34	60.04	63.66	68.33	48.78	61.87	66.38
	2	43.98	67.94	65.06	61.63	62.54	67.59	60.87	61.91	66.41
	3	45.66	67.44	64.12	61.08	63.14	67.19	67.38	61.85	66.42
	4	74.25	91.53	90.27	100.74	117.98	46.72	67.37	34.77	60.38
	5	66.94	94.66	101.5	107.36	131.25	68.35	56.89	59.19	45.67
	6	64.85	100.25	103.16	110.81	135.37	68.38	67.37	57.94	65.76
	7	70.70	88.31	85.18	101.53	128.93	68.27	67.37	49.57	48.77
	8	70.23	93.24	97.05	103.67	125.92	68.37	67.35	49.76	54.32
	9	81.83	79.52	97.98	89.73	105.79	68.41	67.36	61.82	61.54

Table 6 The analysis results of intuitionistic analysis and square-difference analysis through orthogonal experiment with ultrasonic and homogenate extraction

Extraction method	NO.	A	B	C	D	Result
Ultrasonic extraction	1	1	1	1	1	30.5099
	2	1	2	2	2	8.5829
	3	1	3	3	3	6.4947
	4	2	1	2	3	58.7942
	5	2	2	3	1	24.9327
	6	2	3	1	2	72.5786
	7	3	1	3	2	53.5518
	8	3	2	1	3	67.4066
	9	3	3	2	1	57.8401
	Averaged 1	15.196	47.619	56.832	37.761	–
	Averaged 2	52.102	33.641	41.739	44.904	–
	Averaged 3	59.600	45.638	28.326	44.232	–
	Range	44.404	13.978	28.506	7.143	–
Homogenate extraction		A	B	C	D	Result
	1	1	1	1	1	32.7974
	2	1	2	2	2	13.8500
	3	1	3	3	3	18.8223
	4	2	1	2	3	4.0959
	5	2	2	3	1	76.1613
	6	2	3	1	2	102.4241
	7	3	1	3	2	69.8418
	8	3	2	1	3	78.4418
	9	3	3	2	1	80.2859
	Averaged 1	21.823	35.578	71.221	63.082	–
	Averaged 2	60.894	56.151	32.744	62.039	–
	Averaged 3	76.190	67.177	54.942	33.787	–
	Range	54.367	31.599	38.477	29.295	–

3.3 Selection of purification conditions

Chinese medicinal materials contain a variety of matrices; thus, a clean-up procedure is necessary. ^[19] Based on the orthogonal test and multiple standard addition method, the data in Table 7 are the purification profiles of the orthogonal L9(3⁴) method implemented. The recovery results of the purification profiles are shown in Table 7. The results of the intuitionistic analysis and square-difference analysis are shown in Table 8. The results of the experiments and the intuitionistic analysis and square-difference analysis of experimental (Table 7 and Table 8) results showed that the dominant influence on the purification recoveries of 17 OPPs was the type of eluting solvent. For other factors, the effects of the purification profiles were ranked as follows: B>D>C. The optimal purification conditions were a combination of A₃B₃C₁D₁ that 3 extractions with approximately 5 mL of methylbenzene-acetonitrile (1:3, V/V) each time from Florisil solid-phase cartridges met the requirement for the purification of the 17 OPPs.

Table 7 The recovery results of the purification profiles by L9(3⁴) (%)

NO.	Methamidophos	Dichlorvos	Acephate	Omethoate	Monocrotophos	Phorate	Phosphamidon
1	55.99	65.76	62.46	64.34	54.51	53.54	57.99
2	51.77	63.56	73.44	61.17	51.79	50.77	54.93
3	62.49	65.95	63.49	73.91	52.06	54.38	54.24
4	56.39	66.31	58.79	60.14	150.58	53.71	88.13
5	50.91	66.48	63.25	53.36	88.13	54.52	96.50
6	55.11	51.99	55.65	63.04	65.29	53.79	69.44
7	51.18	63.02	50.80	54.65	63.48	54.36	74.60
8	56.66	67.46	65.34	64.26	55.13	54.78	73.59
9	57.62	67.07	65.06	62.75	54.55	54.67	52.88

Table 7 The recovery results of the purification profiles by L9(3⁴) (%) (Continued)

NO.	Methyl parathion	Fenitrothion	Malathion	Fenthion	Chlorpyrifos	Parathion	Profenofos	Triazophos	Imidan	Phosalone
1	58.47	59.34	57.59	54.43	51.41	52.05	53.84	55.65	52.78	54.15
2	56.54	51.29	53.49	50.25	51.02	54.46	50.52	50.62	54.57	50.31
3	59.41	75.46	58.56	55.44	52.83	51.31	56.61	55.09	52.17	55.47
4	54.17	79.81	54.86	55.74	108.09	51.69	50.89	58.53	52.34	53.79
5	55.42	71.84	52.56	55.80	51.77	51.61	54.71	55.72	52.33	54.82
6	51.95	70.51	53.82	55.91	136.44	52.31	56.58	53.31	52.25	54.01
7	76.11	97.41	56.22	54.64	60.13	50.87	64.04	60.66	55.63	52.50
8	57.47	62.31	57.61	55.97	53.58	51.20	55.74	56.39	52.32	54.78
9	57.78	81.35	58.05	55.94	53.67	50.96	57.56	56.57	51.97	55.66

Table 8 The analysis results of intuitionistic analysis and square-difference analysis through orthogonal experiment with automated solid-phase extraction system

NO.	A	B	C	D	Result
1	1	1	1	1	104.7890
2	1	2	2	2	104.7031
3	1	3	3	3	106.3391
4	2	1	2	3	114.3657
5	2	2	3	1	108.2106
6	2	3	1	2	108.4004
7	3	1	3	2	111.6991
8	3	2	1	3	106.3132
9	3	3	2	1	105.2920
Averaged 1	105.277	110.285	106.501	106.097	–
Averaged 2	110.326	106.409	108.120	108.268	–
Averaged 3	107.767	106.676	108.750	109.006	–
Range	5.049	3.876	2.249	2.909	–

3.4 Methodology validation

3.4.1 Calibration curves, determination coefficients and LODs

To confirm that the optimized method was suitable for application, precision, recovery, linear range and detection limits (LODs) were evaluated for the analytical approach developed using samples of *Panax notoginseng*. Quantitative analysis was achieved using an external standard. The calibration curve was obtained by analyzing blank *Panax notoginseng* samples spiked with pesticides at the same levels (Table 9). For detection of the 17 OPPs by GC/MS, the LODs were $0.12\sim0.52\times10^{-3}\mu\text{g}$. The acceptable linearity was indicated by the determination coefficients, which ranged from 0.9928 to 0.9999.

Table 9 Calibration curves, determination coefficients and LODs of the pesticides analyzed by GC/MS

NO.	Pesticide	Calibration curve	Determination coefficient/r	Linear range (g/mL)	LODs ($\times10^{-3}\mu\text{g}$)
1	Methamidophos	$Y = 1578831X + 125793.7$	0.9941	0.005~2.0	0.12
2	Dichlorvos	$Y = 2852786X + 455151.9$	0.9945	0.005~2.0	0.17
3	Acephate	$Y = 662784.3X + 104183.2$	0.9986	0.005~2.0	0.2
4	Omethoate	$Y = 585230.1X + 82190.18$	0.9928	0.005~2.0	0.31
5	Monocrotophos	$Y = 1810437X + 138459.1$	0.9947	0.005~2.0	0.26
6	Phorate	$Y = 3379836X + 149327.7$	0.9980	0.005~2.0	0.38
7	Phosphamidon	$Y = 296661.7X + 36655.06$	0.9909	0.005~2.0	0.21
8	Methyl parathion	$Y = 1037505X + 100506.8$	0.9973	0.005~2.0	0.46
9	Fenitrothion	$Y = 906542.2X + 315367.7$	0.9954	0.005~2.0	0.52
10	Malathion	$Y = 1514504X + 128704.2$	0.9967	0.005~2.0	0.32
11	Fenthion	$Y = 2456014X + 139159.0$	0.9973	0.005~2.0	0.45
12	Chlorpyrifos	$Y = 661122.3X + 28795.83$	0.9971	0.005~2.0	0.13
13	Parathion	$Y = 661122.3X + 28795.83$	0.9971	0.005~2.0	0.37
14	Profenofos	$Y = 635449.9X - 4061.267$	0.9999	0.005~2.0	0.42
15	Triazophos	$Y = 1102994X + 73458.66$	0.9974	0.005~2.0	0.18
16	Imidan	$Y = 6699876X - 109404.20$	0.9987	0.005~2.0	0.41
17	Phosalone	$Y = 1403894X + 81005.27$	0.9969	0.005~2.0	0.22

3.4.2 Results of methodology validation

Under the optimized extraction, purification and detection conditions for 17 OPPs in *Panax notoginseng* determined by orthogonal design, two analytical methods were developed. In the first method, *Panax notoginseng* was extracted by ultrasonic extraction. The extracts were cleaned up by an automated solid-phase extraction system and determined by GC/MS. The second method used homogenate extraction. Experiments in which standards were added to the samples to determine the recovery rate were performed. The standard recovery test results for the two methods and calculation of the recovery rate are shown in Table 10. The average recoveries of the first method ranged from 43.37% to 85.63%, with the relative standard deviations (RSDs) of 0.07~0.17% (n=3). The average recoveries of the second method ranged from 67.88% to 96.70%, with RSDs of 0.05~0.14% (n=3). The results showed that the average recoveries of the first method were often relatively lower than the second method. Therefore, the analytical method was developed by homogenate extraction and automated solid-phase extraction system (the specific condition shown in 3.2 and 3.3). The average recovery (67.88%) of methyl parathion was just below 70%. The average recoveries of other OPPs ranged from 70% to 120%, with RSDs of less than 10%. So the established analytical method can be applied to determining 16 organophosphorus pesticide residues in *Panax notoginseng*. Similarly, to further verify the viability of the method for *Aucklandia lappa decne*, recovery experiments were carried out, the results were satisfactory. The rapid and simple method for the simultaneous determination of 16 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne* was developed, which can meet the requirement of determining pesticide residues from *Panax notoginseng* and *Aucklandia lappa decne*.

Table 10 The average recoveries of two analytical methods

Ultrasonic extraction					Homogenate extraction		
Pesticides	Spiked	Detection value	The average recoveries	RSD	Detection value	The average recoveries	RSD
	(µg)	(µg)	(%)	(%)	(µg)	(%)	(%)
Methamidophos	1.00	0.87	75.23	0.14	0.80	82.75	0.12
		0.66			0.74		
		0.72			0.94		
Dichlorvos	0.90	0.49	43.37	0.25	0.76	83.00	0.07
		0.38			0.69		
		0.30			0.79		
Acephate	0.98	0.85	84.44	0.12	0.89	86.77	0.12
		0.72			0.92		
		0.91			0.73		
Omethoate	0.90	0.54	53.36	0.12	0.77	83.34	0.10
		0.43			0.67		
		0.47			0.81		
Monocrotophos	0.90	0.83	85.27	0.09	0.76	85.95	0.05
		0.78			0.82		
		0.69			0.74		
Phorate	0.90	0.75	85.63	0.13	0.87	96.69	0.07
		0.68			0.93		
		0.88			0.81		
Phosphamidon	0.90	0.76	80.08	0.07	0.85	95.58	0.05
		0.74			0.91		
		0.66			0.82		
Methyl parathion	0.99	0.54	53.73	0.10	0.68	67.88	0.14
		0.58			0.57		
		0.47			0.76		
Fenitrothion	0.90	0.55	62.69	0.09	0.69	82.65	0.08
		0.62			0.73		
		0.52			0.81		
Malathion	0.90	0.74	72.28	0.15	0.90	94.45	0.05
		0.66			0.81		
		0.55			0.84		
Fenthion	0.90	0.75	63.97	0.09	0.74	72.56	0.09
		0.63			0.80		
		0.69			0.71		
Chlorpyrifos	1.08	0.51	60.46	0.17	0.77	80.43	0.06
		0.47			0.68		

		0.65			0.72		
Parathion	0.90	0.84	84.44	0.09	0.76	78.16	0.08
		0.73			0.70		
		0.71			0.65		
Profenofos	0.90	0.58	59.67	0.23	0.85	86.35	0.09
		0.40			0.77		
		0.63			0.71		
Triazophos	0.90	0.71	67.08	0.15	0.93	96.70	0.06
		0.56			0.82		
		0.54			0.86		
Imidan	0.90	0.62	74.95	0.07	0.76	90.38	0.06
		0.71			0.83		
		0.69			0.85		
Phosalone	0.90	0.73	82.65	0.10	0.79	88.20	0.07
		0.68			0.74		
		0.82			0.85		

3.5 The sample test

The established analytical method can be applied to determining 16 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne* from yunnan. The test indicated that Methamidophos, Dichlorvos, Acephate, Omethoate, Monocrotophos, Phorate, Phosphamidon, Fenitrothion, Malathion, Fenthion, Chlorpyrifos, Parathion, Profenofos, Triazophos, Imidan and Phosalone in *Panax notoginseng* and *Aucklandia lappa decne* were not detected.

4. Conclusion

Two sample pretreatment methods for the extraction of 16 OPPs from the traditional Chinese medicinal herb *Panax notoginseng* and *Aucklandia lappa decne* were developed. A comparison of the two methods of analyzing 16 OPPs in *Panax notoginseng* was performed. We can see that the homogenate extraction method is effective, with a high extraction rate and shortened extraction time. Therefore, a fast and sensitive multi-residue analysis method was developed for the determination of organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne* by a homogenate extraction approach coupled with automated solid-phase extraction system and GC/MS. Under the optimum conditions, the results of the homogenate approach were compared with the ultrasonic extraction method, and the homogenate approach was used to extract the samples. The average recoveries of the method ranged from 72.56% to 96.70%, and RSDs were 0.05~0.14%, which meets the requirements for the determination of 16 organophosphorus pesticide residues in *Panax notoginseng* and *Aucklandia lappa decne*.

Declarations

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Conflicts of interest

There are no conflicts of interest to declare.

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Figures

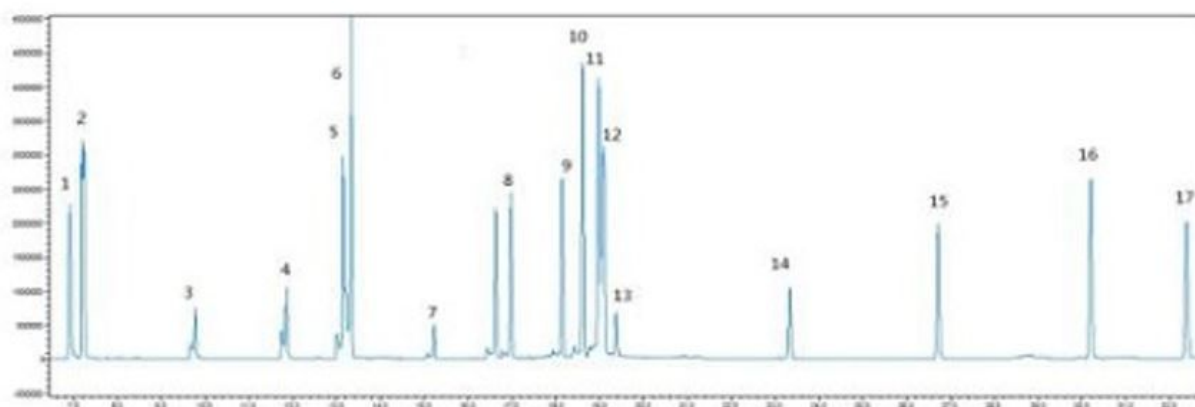


Figure 1

The TIC of 17 OPPs