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Received February 14, 2012
Revised March 24, 2012
Accepted April 17, 2012

Research Article

Rapid determination of pesticide residues in herbs using selective pressurized liquid extraction and fast gas chromatography coupled with mass spectrometry

A selective pressurized liquid extraction and gas chromatography coupled with triple quadrupole mass spectrometer method was developed for simultaneous determination of 52 pesticide residues in medicine and food dual-purpose herbs. The developed extraction method integrated extraction and cleanup processes for sample preparation. The sorbents, 5 g Florisil and 100 mg graphitized carbon black, were placed inside the extraction cell to remove matrix interferences. Optimized conditions of selective pressurized liquid extraction were ethyl acetate as extraction solvent, 120°C of extraction temperature, 6 min of static extraction time, 50% of flush volume extracted for two cycles. An ultra inert capillary GC-MS HP-5 UI column (20 m × 0.18 mm id, 0.18 μm) and column backflush system were used for the analysis. Multiple-reaction monitoring was employed for the quantitative analysis with electron ionization mode. All calibration curves showed good linearity ($r^2 > 0.995$) within the test ranges. The average recoveries of most pesticides were from 81 to 118%. The validated method was successfully applied for the determination of pesticide residues in four herbs. The results indicate that selective pressurized liquid extraction and GC-MS/MS is a sensitive and reliable analytical method for the simultaneous determination of multiple pesticide residues in herbs.

Keywords: GC-MS/MS / Medicine and food dual-purpose herb / Pesticide residue / Selective pressurized liquid extraction
DOI 10.1002/jssc.201200169

1 Introduction

In China, lots of traditional food and herbal products are consumed for both medicinal and food purposes. Likewise, "Let food be thy medicine and medicine be thy food" was also espoused by Hippocrates, the father of modern medicine, nearly 2500 years ago [1]. With the increasing demand for healthy benefits, a large quantity of medicine and food dual-purpose herbs (MFDPHs) are used throughout the world today [2]. However, during the herbs growing process, pesticides including organochlorine (OC), organicphosphorus (OP), pyrethroid (PYR), carbamate (CAR) and other types (OT) are frequently used as chemotherapeutants in agriculture for destroying or controlling any pests. Therefore, MFDPHs are liable to contain pesticide residues, which are accumulated from agricultural practices and storage period. In fact, pesticides have already been found in Chinese herbal medicines and over-the-counter herbal dietary supplements sold in the market [3]. Therefore, it is necessary to develop methods for the rapid and sensitive determination of multiple pesticide residues in MFDPHs.

In general, low content and complicated matrix are the two obstacles of the determination of pesticide residues in MFDPHs. Furthermore, the contamination of injection inlet and column by high boiling matrix compounds during GC analysis should be also considered, which could affect the chromatographic separation and reduce column life [4]. So far, a series of sample preparation methods, including liquid–liquid extraction (LLE) [5], pressurized liquid extraction (PLE) [6, 7], solid phase extraction (SPE) [8, 9], gel permeation chromatography (GPC) [10, 11], QuEChERS (quick, easy, cheap, effective, rugged, and safe) [12, 13], and solid phase micro extraction (SPME) [14, 15] have been employed for the extraction and enrichment of pesticides in Chinese herbal medicine. However, LLE-SPE and PLE-GPC are tedious and complicated since the extraction and cleanup are respective procedures. Meanwhile, LLE and GPC need a large amount of organic solvents. Although QuEChERS is a quick and easy method, the requirement of sample with more than

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Abbreviations: GCB, graphitized carbon black; **MFDPH**, medicine and food dual-purpose herb; **SPLE**, selective pressurized liquid extraction

75% moisture is a limit [12, 16]. The SPME process is also very simple, but the recovery of this method depends on the property of SPME fiber for pesticides [14, 17].

PLE, an extraction technique under elevated temperature and pressure, has been recently used in the extraction of residual organic pollutants from different matrices such as tea, vegetables, and compost for its rapid extraction process and high extraction efficiency [18–20]. Meanwhile, PLE with different sorbents could be used as selective pressurized extraction approach. Integration of the PLE and cleanup process has also been achieved by loading sorbent at the bottom of the PLE stainless cell [21–23]. Comparing with traditional extraction and cleanup approaches (LLE-SPE, SPME, and PLE-GPC), the selective pressurized liquid extraction (SPLE) can simplify extraction step and significantly reduce extraction time and solvent consumption.

Gas chromatography coupled with triple quadrupole mass spectrometer (GC-MS/MS) has been intensively used for the determination of organic pollutants [24, 25]. The detector can focus on characteristic precursor and product ions. Typically, the multiple reaction monitoring of GC-MS/MS gives the possibility of simultaneous confirmation and quantification with excellent selectivity and sensitivity. In addition, a column backflush offered potential advantages to reduce run time and prevent high boiling contaminants to GC-MS system [26, 27].

In this paper, it is the first time to report an SPLE and GC-MS/MS method with column backflush for the determination of multiple pesticides residues in four MFDPHs including the root of *Pueraria thomsonii* Benth., *Pogostemon cablin* Benth., *Houttuynia cordata* Thunb., and *Disoscorea opposita* Thunb.

2 Materials and methods

2.1 Chemicals, materials, and standards

Ethyl acetate, *n*-hexane, cyclohexane, acetonitrile, toluene, and acetone (HPLC grade) were purchased from Merck (Darmstadt, Germany). Petroleum ether (bp 40–60°C) was purchased from Fluka Analytical (Sigma–Aldrich Corp., St. Louis, USA). Deionized water was purified through a Milli-Q synthesis system (Millipore, USA).

Primary and secondary amine (PSA) (particle size 50 μm), Florisil (particle size 200 μm, pestanal), octadecylsilyl packing (C_{18}) (particle size 12 μm), and graphitized carbon black (GCB) (particle size 45 μm) were obtained from Supelco (Sigma–Aldrich Corp.). Diatomaceous earth was purchased from Dionex (Sunnyvale, CA, USA)

Twenty MFDPHs were collected from 11 different places (Table 3). The four pesticide-free samples including *P. thomsonii* Benth., *P. cablin* Benth., *H. cordata* Thunb., and *D. opposita* Thunb. came from Meishan, Sichuan; Nantong, Jiangsu; Yibin, Sichuan; and Dujiangyan, Sichuan, respectively. They were dried in a universal oven with forced convection (FD115, Tuttlingen, Germany) at 40°C for 2 days. The dried sample was ground using Sample Mill (model YF102, Ruian Yongli Pharmacy Machinery Company, China). The botanical origins of the material were identified by Professor Yuecheng Li. The voucher specimens were deposited at Sichuan Provincial Institute for Food and Drug Control, Chengdu, Sichuan, China.

Pesticide standards (Table 1) were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Fluka and Riedel-de-Haën (Sigma–Aldrich Corp.). The purities of the pesticide standards were from 96 to 99%. Individual pesticide stock solutions (1 mg/mL) were prepared in acetonitrile and kept at 0°C, protected from light. A mixed standard solution (0.02 mg/mL) was prepared by diluting an appropriate volume of each individual stock standard solution with acetonitrile. Internal standard triphenylphosphate (TPP) was purchased from Aldrich (Sigma–Aldrich Corp.). Individual stock internal standard solutions (0.01 mg/mL) were prepared in acetonitrile.

2.2 Sample preparation

2.2.1 Selective pressurized liquid extraction

SPLE was carried on an ASE 350 system (Dionex Company, Sunnyvale, CA, USA), fitted with 34 mL stainless steel cells. 5 g powder of sample with 500 μL internal standard (TPP, 500 ng/mL) was mixed with diatomaceous earth in a proportion of 2:1. One cellulose filter was placed at the bottom of cell, 5 g of Florisil and 0.1 g of GCB, as cleanup adsorbents, were placed to the cell, and followed by the introduction of dispersed sample. Finally the empty space above the mixture was filled with diatomaceous earth (Fig. 1). The extraction cell was extracted under the optimum conditions: solvent, ethyl acetate; temperature, 120°C; static extraction time, 6 min; pressure, 1500 psi; flush volume, 50%; static cycle, 2. The extract was evaporated to near-dryness at 40°C using a gentle stream of nitrogen in a TurboVap LV concentration workstation (Hopkinton, MA, USA). The residue was transferred into a 5 mL volumetric flask, which was brought up to its volume with petroleum ether, and filtered through a 0.22 μm nylon membrane filter (Tianjin Jinteng Co., Ltd., China) before GC-MS/MS analysis.

2.2.2 PLE and gel permeation chromatography cleanup

Sample preparation was performed on an ASE 350 system and a fully automated GPC ULTRA System (LCTech, Bahnweg, Germany) as described by Wu et al. [10]. In brief, 5 g powder with 500 μL internal standard was mixed with diatomaceous earth in a proportion of 2:1 and transferred into cells. The extraction procedure was the same as in Section 2.2.1 (without sorbents). The residue was dissolved in 10 mL cyclohexane–ethyl acetate (1:1, v/v) for injection into GPC system.

The cleanup condition of GPC system: mobile phase was cyclohexane–ethyl acetate (1:1, v/v) in isocratic mode.

Table 1. List of pesticides, chemical classes, retention time, and the parameters of MRM for the tested pesticides

Peak No.	Pesticide	Chemical class	Retention time (min)	Time window	Dwell time (ms)	Precursor ions (1) [a)]	Product ions	Collide energy (eV)	Product ions	Collide energy (eV)	Precursor ions (2)	Product ions	Collide energy (eV)
1	Methomyl	CAR	3.977	1	150	**105.0**	88.1	5	58.1	25			
2	Methamidophos	OP	5.814	2	70	**141.0**	95.0	5	80.0	25			
3	Dichlorvos	OP	6.002	2	70	**185.0**	93.0	15	109.0	15			
4	Mevinphos	OP	8.002	3	70	**127.0**	109.0	10			192.0	127.0	10
5	Metolcarb	CAR	8.378	3	70	**108.0**	77.1	25	89.1	25			
6	Isoprocarb	CAR	9.140	4	30	**136.0**	121.0	6			121.0	103.0	10
7	Omethoate	OP	9.670	4	30	**156.0**	110.0	10	79.0	25			
8	Propoxur	CAR	9.784	4	30	**110.0**	92.0	10	82.0	8	152.0	110.0	6
9	Sulfotep	OP	10.502	5	40	**322.1**	146.0	30	265.8	5			
10	Monocrotophos	OP	10.566	5	40	**127.0**	109.1	10	95.0	20			
11	Phorate	OP	10.724	5	40	**260.1**	74.8	10					
12	Dimethoate	OP	11.086	6	15	**125.0**	47.0	25	79.0	5			
13	Carbofuran	CAR	11.219	6	15	**164.0**	149.0	10	103.9	15			
14	β-BHC	OC	11.348	6	15	**181.0**	145.0	15	109.0	30	219.0	183.0	10
15	Quintozene	OC	11.422	6	15	**236.9**	142.9	30	118.9	25			
16	Lindane	OC	11.551	6	15	**180.9**	145.0	12	109.0	30	218.8	183.0	5
17	Fonofos	OP	11.779	7	40	**246.0**	109.1	16	137.0	5	137.0	109.0	5
18	Diazinon	OP	11.863	7	40	**304.0**	179.0	10					
19	Chlorothalonil	OC	11.942	7	40	**265.9**	133.0	40	168.0	25			
20	δ-BHC	OC	12.284	8	70	**181.0**	145.0	15	109.0	30	219.0	183.0	10
21	Iprobenfos	OP	12.566	9	35	**204.0**	91.1	10	122.0	10	204.0	171.0	2
22	Pentachloroaniline	OC	12.709	9	35	**265.0**	194.0	24	158.0	40	194.0	165.0	40
23	Propanil	OC	13.021	10	20	**161.0**	98.8	40	89.9	40			
24	Methylparathion	OP	13.343	10	20	**263.0**	109.1	15	127.0	6	263.0	246.0	2
25	Heptachlor	OC	13.576	10	20	**271.9**	236.8	25	116.9	40	274.0	239.0	20
26	Metalaxyl	OT	13.630	10	20	**206.0**	105.2	25	132.0	10			
27	Pirimiphos-methyl	OP	14.091	11	70	**305.2**	180.2	5	289.7	10			
28	Methyl-pentachlorophenyl sulfide	OC	14.204	11	70	**296.0**	262.7	15	280.6	15			
29	Malathion	OP	14.487	12	15	**173.1**	99.0	15	117.1	5	173.0	127.0	4
30	Chlorpyrifos	OP	14.690	12	15	**314.0**	257.8	10	285.9	5			
31	Aldrin	OC	14.690	12	15	**263.0**	193.0	30	191.0	30	298.0	263.0	8
32	Fenthion	OP	14.803	12	15	**278.0**	109.1	20	245.0	10			
33	Isocarbophos	OP	15.071	12	15	**136.0**	108.0	14			289.0	136.0	6
34	Bromophos-methyl	OP	15.363	12	15	**330.9**	315.9	16	285.9	34	331.0	93.0	34
35	Phenthoate	OP	16.368	13	30	**274.0**	121.1	10	93.0	15			
36	Procymidone	OT	16.462	13	30	**283.0**	96.0	10	255.0	10	283.0	67.1	40
37	trans-Chlordane	OC	16.853	13	30	**373.0**	265.6	25	301.0	10			
38	α-Endosulfan	OC	17.274	14	40	**241.0**	206.0	15	171.0	15			
39	*p,p′*-DDE	OC	17.838	14	40	**246.0**	176.0	30	211.0	20	248.0	176.0	30
40	Dieldrin	OC	17.882	14	40	**262.8**	193.2	30	227.7	25			

Table 1. Continued

Peak No.	Pesticide	Chemical class	Retention time (min)	Time window	Dwell time (ms)	Precursor ions (1) [a]	Product ions	Collide energy (eV)	Product ions	Collide energy (eV)	Precursor ions (2)	Product ions	Collide energy (eV)
41	Endrin	OC	18.229	15	15	**263.0**	227.9	20	193.0	10			
42	β-Endosulfan	OC	18.392	15	15	**236.9**	142.8	25	118.9	30			
43	*p,p′*-DDD	OC	18.486	15	15	**235.0**	200.0	8	199.1	15	237.0	165.0	20
44	Oxadixyl	OT	18.496	15	15	**233.1**	146.1	5	118.0	25			
45	*o,p′*-DDT	OC	18.516	15	15	**235.0**	165.0	20	199.1	20	237.0	165.0	20
46	Carbophenothion	OP	18.823	15	15	**157.0**	45.0	15	122.1	10			
47	*p,p′*-DDT	OC	18.937	15	15	**235.0**	165.0	20	199.1	20	237.0	165.0	20
	TPP	IS	19.150	16	25	**326.1**	215.1	30	170.0	30			
48	Fenpropathrin	PYR	19.665	16	25	**265.0**	210.0	15	89.0	35			
49	Tetradifon	OC	19.828	16	25	**353.7**	159.0	10	159.0	10			
50	λ-Cyhalothrin	PYR	20.145	16	25	**197.0**	161.0	10	171.0	15	181.1	152.1	30
51	Coumaphos	OP	20.897	17	25	**362.1**	109.0	15	225.9	15			
52	Esfenvalerate	PYR	23.031	17	25	**125.0**	89.1	25	99.2	25	419.0	125.0	30

a)The quantitative ions are in bold.

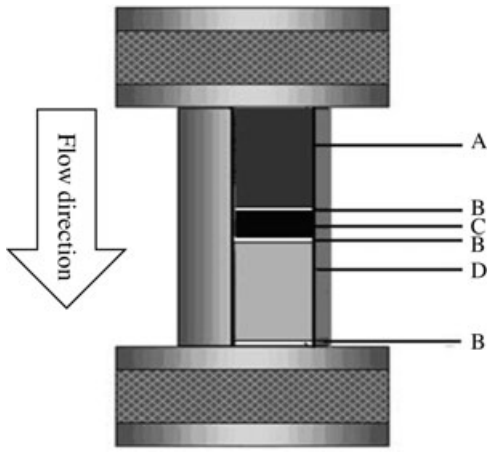

Figure 1. Packing of stainless steel cell in the developed SPLE method. The contents of developed SPLE including the mixture of sample and diatomaceous (A), cellulose filter (B), graphitized carbon black (C), and Florisil (D).

Bio-Beads S-X3 (40 g) was packed in the column (300 mm × 10 mm id). The flow rate was 5 mL/min. The fraction from 8 to 20 min was collected. The collected GPC fraction was on-line evaporated to 5 mL and subjected to analysis.

2.2.3 Solid phase extraction

SPE was performed on GX-274 ASPEC (Gilson, Middleton, USA) as described by Yang et al. [8]. In brief, the mixture of 5 g sample with 500 μL internal standard and 20 mL acetonitrile was vortexed for 2 min, then added 5 g sodium chloride and vortexed for 2 min again. The mixture was centrifuged for 5 min at 4000 rpm, and then 10 mL supernatant was evaporated at 40°C until nearly dryness for cleanup.

The residue was dissolved with 2 mL acetonitrile–toluene (3:1, v/v) and loaded onto a PestiCarb/NH_2 mixed phase SPE column (500 mg GCB and 500 mg NH_2, 6 mL, Agela, China). The extract solution was passed through the columns at the flow rate of 1 mL/min. The retained analytes were eluted with 25 mL of acetonitrile–toluene (3:1, v/v) at 1 mL/min. The eluent was collected and evaporated until nearly dryness. Finally, the residues were redissolved with 2.5 mL acetone.

2.2.4 Solid phase microextraction

SPME was performed using manual sampling device from Supelco (Bellefonte, PA, USA) and it consisted of a holder assembly and a replaceable 100 μm thickness polydimethylsiloxane fibers (PDMS). The extraction condition was described by Campillo et al. [15]. In brief, 5 g powder with 500 μL internal standard was immersed in 50 mL water for 3 min. Thirty-five milliliters of the infusion and 15 mL of phosphate buffer solution were placed in 50 mL vial, which was sealed by cap after adding the magnetic stir bars. With stirring set at 1400 rpm, the PDMS fiber was totally immersed in sample solution for 40 min at 90°C. In the desorption process, the

fiber was inserted into GC system of splitless injection mode at 280°C for 7 min. The fiber was then kept in injector for 10 min after opening the split valve, to ensure total desorption and no memory effects.

2.3 GC-MS/MS analysis

GC-MS/MS was carried on an Agilent 7890A gas chromatograph coupled with 7000B triple quadrupole mass spectrometer (Waldbronn, Germany). Injection was carried out using a CTC PAL sample injector and injection volume was 1 μL. All analytes were separated on a HP-5 MS UI column (20 m × 0.18 mm id, 0.18 μm) and a restrictor column (1.3 m × 0.180 mm id., 0.18 μm) (Agilent Technologies). Three-way splitter with analytical column in and restrictor out to mass spectrometer was used, and helium pressure was provided by auxiliary electronic pressure control (Aux EPC) at 4 psi (Supporting Information Fig. S1). The oven temperature program was: initial temperature was set at 80°C (hold 1 min), to 186°C at 12°C/min, to 190°C at 3.5°C/min, to 210°C at 4°C /min, to 280°C at 38°C/min, and hold for 6 min at 280°C. High-purity helium gas (>99.999%) was used as carrier gas with the flow rate of 1 mL/min. Backflush parameters: hold time, 5 min; inlet pressure, 1 psi; three-way splitter pressure, 60 psi; oven temperature, 280°C.

The mass spectrometer operated in electron ionization mode at 70 eV. The analyses were performed in multiple reactions monitoring (MRM) mode (Table 1). The GC–MS transfer line and mass source temperature were 300 and 230°C, respectively. The scanned mass range was set at 45–500 *m/z*.

2.4 Linearity, LOD, and LOQ

Stock solution containing 52 pesticides standards and TPP were diluted to appropriate concentrations using matrices extracted from blank MFDPHs for the construction of calibration curves. The mixed standard solutions of seven concentrations were injected in duplicates, and then the calibration curves were constructed by plotting the ratios of the peak areas of each standard to IS versus the concentration of each analyte. The LOD and LOQ under the GC-MS/MS conditions were determined at an S/N of about 3 and 10, respectively.

2.5 Precision and repeatability

Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day precision, the mixed standards solution (approx. 0.1 μg/mL) was analyzed for six replicates within 1 day, while for inter-day precision test, the solution was examined in duplicates for consecutive 6 days. Variations were expressed by RSD.

To confirm repeatability, 5 g of MFDPHs was extracted and purified by SPLE method into five replicates and determined by GC-MS/MS system as mentioned above. The RSD value was calculated as a measurement of method repeatability.

2.6 Accuracy

Pesticide-free samples including *P. thomsonii*, *P. cablin*, *H. cordata,* and *D. opposite* monitored by our laboratory were used as the blank matrix for spiking to determine recoveries. The recovery was used to evaluate the accuracy of the method and determine it at three different concentration levels (10, 50, and 200 μg/kg). Three replicates were performed at each level. The percentage recoveries were calculated according to the following equation:

(total detected amount − original amount) × 100/added amount.

3 Results and discussion

3.1 Optimization of SPLE

3.1.1 Selection of extraction solvent

Polarity is one of the major factors that may influence extraction efficiency. In our work, four solvents including acetonitrile, ethyl acetate, *n*-hexane/acetone (1:1, v/v), and *n*-hexane/ethyl acetate (4:1, v/v), were compared. PLE parameters were performed as described by Blasco et al [28]. When *n*-hexane/acetone or *n*-hexane/ethyl acetate were used as extraction solvent, the extraction efficiencies of dichlorvos, iprobenfos, and heptachlor are very low and recoveries of other pesticides are in the range of about 30–60% (Supporting Information Table S1). When acetonitrile or ethyl acetate was used as extraction solvent, the recoveries of most pesticide residues were increased significantly. However, the acetonitrile can extract more complex matrices from MFDPHs under the conditions of high temperature and pressure (Supporting Information Fig. S2). Therefore, ethyl acetate was chosen as extraction solvent in the SPLE.

3.1.2 Selection of sorbent

In order to achieve effective one step sample preparation, it is crucial to choose suitable sorbent for pesticide residue analysis. Four sorbents including Florisil, PSA, C_{18}, and GCB were investigated. According to previous reports, PSA and Florisil were usually used as the major sorbents in dispersive SPE for the cleanup step of pesticides in vegetables, fruits, and medicinal plants [29–31]. PSA can effectively remove saccharide, polar organic acids and lipids from food samples, while Florisil can preferentially absorb polar and low-fat components [32]. The C_{18} and GCB were usually used as auxiliary sorbents for pesticide analysis.

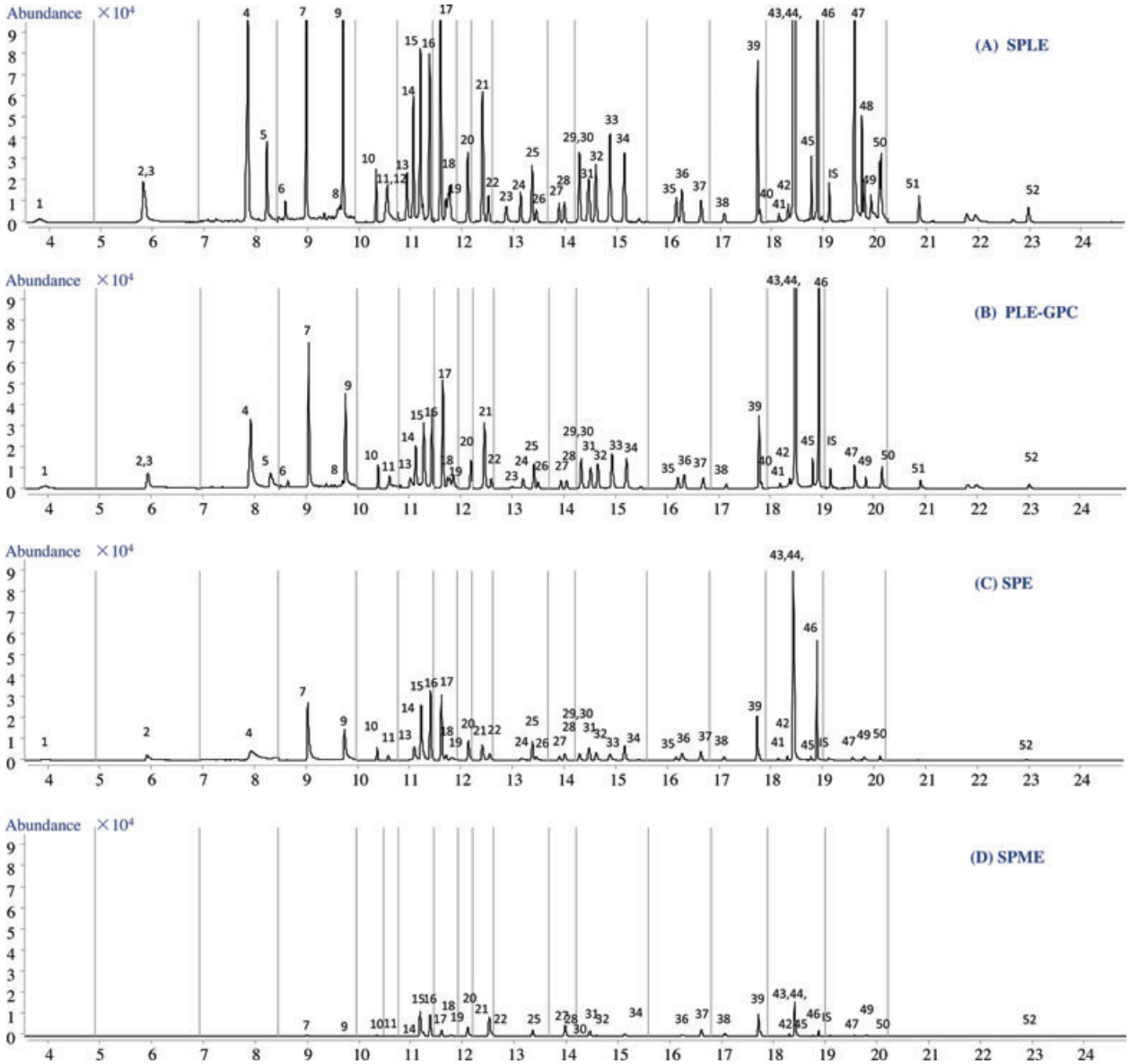

Figure 2. The typical total ion chromatograms of 52 pesticide residues were acquired by multiple reactions monitoring mode. Spiked Samples were performed by different methods including SPLE (A), PLE-GPC (B), SPE (C), and SPME (D).

Result showed that the extract was turbid when using PSA (0.1–5 g, six levels) alone. However, the extract became clear when the loading amount of Florisil (3–9 g, six levels) alone was >3 g. The loading amount of Florisil was >5 g; the matrix interference was not to be further reduced significantly. Therefore, 5 g Florisil was used as the major absorbent. Octadecylsilyl (C_{18}) and GCB were used as additional sorbents to be studied. The C_{18} material can remove fat compound, but it fails to show good performance for removing the chromatographic interference and pigments. The ability of GCB is to remove planar molecules such as chlorophylls, carotiniods, and sterols. The mixture sorbents of 5 g Florisil and GCB (0.05–0.4 g) were studied at five levels. Results showed that the mixed sorbents of 5 g Florisil and 0.1 g GCB can produce clear extracts, clean chromatographic profiles, and better recoveries for the investigated pesticide residues (Supporting Information Fig. S3, Fig. 2A, Supporting Information Table S2). Therefore, 5 g Florisil and 0.1 g GCB were used as sorbents for SPLE procedure.

3.1.3 Optimization of SPLE parameters

The parameters including temperature (80, 90, 100, 110, 120, and 130°C), static extraction time (2, 4, 6, and 8 min), total flush volume (40, 50, and 60%), and number of cycles (1, 2, and 3) were studied by using univariate approach while other conditions were kept constant (temperature, 100°C; static extraction time, 4 min; flush volume, 40%, and one extraction

cycle). Fluid delivery pressure 1500 psi is a fixed parameter on ASE 350 instrument. Therefore, pressure was set at 1500 psi. The recovery of *P. thomsonii* spiked mixed standard at 50 μg/kg level was used for evaluation of extraction efficiency. The optimization results are shown in Supporting Information Fig. S4. Considering the results, the conditions of the SPLE method proposed were temperature, 120°C; static extraction time, 6 min; flush volume, 50%; cycle, 2.

3.2 Comparison of SPLE, SPE, SPME, PLE-GPC, and QuEChERS

The extraction and cleanup are two important steps of sample preparation for the determination of trace pesticide residues. According to previous reports, SPE, SPME, and PLE-GPC methods were utilized to extract and clean up pesticides from different complicated matrices [8–11, 15]. In this study, the developed SPLE method was compared with SPE, SPME, and PLE-GPC. The performances of different approaches were evaluated by MRM chromatograms, S/N of investigated pesticides and recoveries. Figure 2 shows the MRM chromatograms of 52 pesticide residues extracted by different methods. Among these chromatograms, the response of SPLE is the strongest. The S/Ns of multiclass pesticides residues indicated that the sensitivities of the developed SPLE are higher than those of PLE-GPC, SPE, and SPME (Supporting Information Table S3). Table 2 shows that the recovery range of the developed SPLE is 65–121%, which is better than others. SPLE has the advantage of PLE, which can accelerate the extraction kinetics and force the solvent into matrix pores under high temperature and pressure condition. Therefore, it can provide good extraction performance. Meanwhile, the extracts of SPLE are clearer than PLE (Supporting Information Fig. S3). Comparing SPLE with PLE-GPC, SPE, and SPME, the major difference is that SPLE integrates the extraction and cleanup in one step. The sample preparation process is simplified, which leads to reduce the loss of preparation. Meanwhile, sample preparation time and organic solvent consumption are reduced, too (Supporting Information Table S4). In addition, QuEChERS method has attracted great attention for pesticide analysis studies, but the method is suitable for samples with more than 75% moisture [12, 16]. It has to be used for the dried root herbs, the sample amount may have to be reduced and water has to be added to make sample pores more accessible to the extraction solvent. In other words, the dry sample amount is reduced, which leads to decrease method sensitivity. However, SPLE method can directly extract pesticides from dried sample without moisture limit. The QuEChERS method was used to determine pesticide residues in the same *P. thomsonii* from Meishan, Sichuan and Yifeng, Jiangxi [33]. Results show that the contents of λ-Cyhalothrin extracted by QuEChERS method are lower than those of SPLE method. Therefore, the extraction performance of QuEChERS is lower than that of SPLE. In a word, SPLE is a quick, simple, and efficient sample preparation method and it could be a better choice for the pesticides analysis.

3.3 Optimization of GC-MS/MS

The relevant parameters including precursor ions, product ions, and collision energies were optimized to obtain optimal specificity and sensitivity. The mixed standard solution was infused by GC system into the mass spectrometer. After analyzing the full scan spectra, the precursor ion for every analyte was selected, and then subjected to collision energy voltages to generate MS/MS product ions. The choice of the precursor ion was rather based on selectivity than signal intensity. For most pesticides, ions could potentially serve as the precursor ion, but preferably ions at the higher mass range (m/z >200) were chosen because this usually afforded the highest S/Ns for the selected product ions. Based on the confirmation of precursor ions, more than two product ions should be selected when using MS/MS analysis in accordance with relevant legislation [34]. In this work, two product ions resulting from fragmentation of one precursor ion or two product ions each resulting from two different precursor ions were monitored. But there were a few exceptions of pesticides, this was the case with phorate and diazinon, for which only one MRM transition could be recorded due to either poor intensity or insufficient specificity of the second transition.

Based on pesticides' MRM chromatogram, a time-scheduled acquisition method was constructed. Finally, the developed method included 17 retention time windows, each comprising between one and seven MRM transitions. Start and end times were defined and scan time parameter was set for each segment, resulting in dwell times in the range of about between 15 and 150 ms for particular MRM transitions throughout the chromatographic run. The GC–MS/MS parameters are given in Table 1 and some typical chromatograms are shown in Fig. 2. Figure 2A shows that interfering substances (11–12 and 19–20 min) affected chromatographic separation, but the extracted ion chromatograms (EIC) could evade the problem of poor separation (Supporting Information Fig. S5).

3.3.2 Optimization of backflush column

Due to complex matrix of MFDPHs, the high boiling point compounds and dark brown residues will be accumulated in the liner of inlet and column head after hundreds of injections. Therefore, a backflush program was developed in the present study. Operation parameters are described in Section 2.3. Supporting Information Fig. S1 shows the diagram of column backflush system. Compared with traditional GC system, the column backflush system was added a three-way splitter coupled with electronic pressure control (EPC), and 1.3 m capillary column was used as restrictor column. When GC system was in the forward elution, the pressures of injection inlet, three-way splitter, and detector were 50 psi, 4 psi, and vacuum, respectively. The investigated compounds can pass through the inlet to MS/MS detector. The restrictor column material is the same as analytical column. Therefore, separation efficiency would not be affected by restrictor

Table 2. Comparison of the recoveries of investigated pesticide residues using different preparation method ($n = 5$)

Pesticide	SPLE		PLE-GPC		SPE		SPME	
	Recovery[a)] (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Methomyl	101.29	8.48	86.96	27.53	79.78	21.20	-[b)]	–
Methamidophos	83.45	13.26	79.27	11.08	–	–	–	–
Dichlorvos	65.08	4.48	46.60	23.56	40.22	23.12	–	–
Mevinphos	78.36	7.58	68.24	4.30	–	–	–	–
Metolcarb	103.30	5.81	81.49	0.56	–	–	–	–
Isoprocarb	107.25	4.45	76.88	5.55	68.33	15.93	–	–
Omethoate	64.58	5.81	42.98	11.60	–	–	–	–
Propoxur	104.85	6.34	92.28	15.78	76.75	30.96	–	–
Sulfotep	95.64	7.84	100.30	16.35	71.06	17.42	122.73	6.82
Monocrotophos	65.19	19.71	45.35	27.49	–	–	–	–
Phorate	96.16	7.89	75.47	14.07	81.31	16.66	130.74	27.13
Dimethoate	107.63	11.54	61.84	14.13	74.40	34.08	–	–
Carbofuran	109.45	7.18	100.17	12.85	81.42	17.27	–	–
β-BHC	115.52	3.13	97.92	19.03	84.89	7.06	112.61	11.19
Quintozene	105.73	8.18	92.54	12.68	79.16	13.28	68.68	15.67
Lindane	111.33	4.96	86.17	21.92	74.25	9.30	155.57	21.95
Fonofos	105.06	6.02	75.39	19.93	84.34	15.50	87.46	6.25
Diazinon	107.08	7.52	103.06	14.82	68.85	15.68	122.74	19.43
Chlorothalonil	79.96	3.28	67.21	25.67	60.04	14.48	–	–
δ-BHC	111.75	3.99	86.69	5.28	63.72	12.26	108.91	15.20
Iprobenfo	86.18	14.73	116.95	7.84	76.65	17.47	–	–
Pentachloroaniline	113.52	3.32	52.48	0.31	78.50	12.04	46.76	28.46
Propanil	111.23	6.25	100.72	13.11	–	–	–	–
Methylparathion	82.89	11.52	65.05	14.91	53.77	18.30	–	–
Heptachlor	103.62	5.05	84.08	18.06	76.97	5.90	37.42	30.52
Metalaxyl	101.59	6.41	68.69	11.05	42.81	14.83	–	–
Pirimiphos-methyl	92.81	14.84	103.66	13.44	60.12	10.21	293.72	32.78
Methyl-pentachlorophenyl sulfide	111.61	3.16	80.19	19.43	74.79	8.32	29.74	9.42
Malathion	92.31	12.34	103.89	5.80	61.56	14.09	–	–
Chlorpyrifos	103.27	6.84	90.66	17.15	88.23	9.12	132.38	6.94
Aldrin	113.24	5.08	71.91	18.41	66.08	7.57	40.49	16.14
Fenthion	101.27	5.94	81.39	10.71	78.86	11.99	208.34	22.31
Isocarbophos	80.77	12.69	133.32	2.67	62.87	15.32	–	–
Bromophos-methyl	104.08	4.29	83.95	13.26	58.83	6.80	59.50	24.83
Phenthoate	89.41	8.58	77.05	13.11	71.23	8.61	107.98	20.86
Procymidone	111.25	4.90	113.69	12.21	83.14	9.15	109.57	27.33
trans-Chlordane	111.69	4.42	101.48	15.24	71.31	3.96	40.06	11.11
α-Endosulfan	114.14	8.22	91.33	15.19	76.45	8.42	45.89	29.23
p,p′-DDE	111.32	7.18	73.53	17.15	70.92	1.63	67.97	8.88
Dieldrin	121.14	3.94	86.74	19.85	62.77	1.83	89.99	3.04
Endrin	101.15	7.90	87.90	19.98	63.92	3.94	63.09	28.57
β-Endosulfan	88.36	16.87	75.93	19.93	76.76	17.99	76.31	17.14
p,p′-DDD	104.43	5.94	100.37	5.86	56.54	5.90	29.65	34.14
Oxadixyl	101.64	5.86	82.59	19.15	64.58	22.59	–	–
o,p′-DDT	111.77	3.54	95.34	13.58	79.70	18.48	68.33	16.36
Carbophenothion	97.13	6.95	87.88	6.68	85.32	23.19	69.21	13.15
p,p′-DDT	115.78	4.25	54.98	17.84	56.08	5.61	63.59	25.12
Fenpropathrin	105.22	5.73	98.26	20.07	69.37	10.88	–	–
Tetradifon	112.97	3.79	10.34	11.53	60.54	12.10	81.31	13.29
λ-Cyhalothrin	97.70	10.49	87.94	12.11	62.15	2.61	79.45	22.83
Coumaphos	88.33	10.36	78.65	18.53	102.71	3.18	–	–
Esfenvalerate	107.20	9.03	105.64	21.51	101.74	2.27	–	–

a) The spiking level of the pesticide residues was 50 μg/kg.
b) No pesticide was detected.

Table 3. The contents (mg/kg) of the found pesticide residues in four MFDPHs

Sample	Source	Contents of pesticides detected (mg/kg) (RSD%, $n = 4$)									
		Monocrotophos	Carbofuran	Quintozene	Lindane	Chlorothalonil	Endrin	Metalaxyl	Fonofos	*p,p′*-DDT	λ-Cyhalothrin
P. thomsonii	Yuexi, Anhui	0.026 (7)	-[a)]	–	–	–	–	–	–	<LOQ	0.200(4)
P. thomsonii	Yichang, Hubei	0.029 (9)	–	–	0.035(6)	–	<LOQ	–	–	<LOQ	0.258(4)
P. thomsonii	Meishan, Sichuan	–	–	–	–	0.031(6)	–	–	–	<LOQ	0.035(6)
P. thomsonii	Meishan, Sichuan	–	–	–	–	–	–	–	–	–	0.047(8)
P. thomsonii	Yifeng, Jiangxi	–	–	–	–	0.041(7)	–	–	–	–	<LOQ
P. cablin	Zhanjiang, Guangdong	0.127(6)	0.024(9)	–	–	–	–	0.037(5)	0.012(6)	<LOQ	–
P. cablin	Zhanjiang, Guangdong	0.056(8)	<LOQ	–	0.062(7)	0.018(8)	–	–	–	<LOQ	0.043(6)
P. cablin	Zhanjiang, Guangdong	0.107(5)	–	–	–	0.208(5)	–	0.116(4)	–	<LOQ	–
P. cablin	Nantong, Jiangsu	<LOQ	–	<LOQ	–	0.042(7)	–	–	<LOQ	0.011(7)	–
P. cablin	Nantong, Jiangsu	0.077(8)	0.028(9)	–	–	<LOQ	–	0.204(3)	<LOQ	–	–
H. cordatia	Yaan, Sichuan	–	0.023(8)	–	<LOQ	–	<LOQ	–	–	0.004(13)	<LOQ
H. cordatia	Yaan, Sichuan	<LOQ	–	–	–	–	–	0.104(3)	–	–	–
H. cordatia	Yaan, Sichuan	0.033(8)	0.017(7)	–	–	–	0.061(4)	–	<LOQ	<LOQ	<LOQ
H. cordatia	Yibin, Sichuan	–	–	–	–	–	–	–	–	–	–
H. cordatia	Yibin, Sichuan	–	–	–	–	–	–	–	–	–	–
D. opposita	Jiaozuo, Henan	–	0.086(6)	0.064(4)	–	0.023(6)	0.047(6)	0.156(3)	0.015(5)	0.086(7)	0.061(6)
D. opposita	Jiaozuo, Henan	0.017(6)	–	–	–	–	–	–	–	<LOQ	–
D. opposita	Qinyang, Henan	<LOQ	–	–	0.007(9)	0.042(7)	–	0.267(4)	–	<LOQ	0.037(7)
D. opposita	Dujiangyan, Sichuan	0.025(5)	–	–	–	–	<LOQ	<LOQ	–	–	0.049(6)
D. opposita	Dujiangyan, Sichuan	0.031(5)	–	–	–	0.042(7)	0.109(4)	–	–	–	<LOQ

a) No pesticide was detected.

column. When forward elution program is over, backflush program can be activated. The pressure of inlet immediately fell to 1 psi, the pressure of three-way splitter rose up to 60 psi, and the column temperature was changed to 280°C. The flow rate of analytical column was about 6 mL/min. On the high temperature and high flow rate condition, high boiling compounds can be removed from the split of injection inlet. Supporting Information Fig. S6 showed that a 5 min backflush cleaned column as well as a 20 min bake-out and the residues on liner were significantly reduced too.

3.5 Validation of method

All calibration curves for the 52 target compounds were linear in a relatively wide concentration in the ranges of 2–400 μg/kg (Supporting Information Table S5). The correlation coefficient values ($r^2 > 0.996$) indicated good correlations between the investigated compounds concentrations and their ratios of the peak areas of each standard to IS within the tested ranges. The LOD and LOQ were in the range of 0.2–5 μg/kg and 1–10 g/kg, respectively (Supporting Information Table S5). Method precision was also evaluated by determining reproducibility, and the intra- and inter-day precisions (RSD) of the 52 analytes were less than 2.61% and 3.11%, respectively. The repeatability presented as RSD ($n = 5$) was between 3.26 and 11.54%. Recovery experiments with spiked blank samples were performed at three concentrations. Supporting Information Table S6 shows the recoveries of the 52 investigated pesticides were 62–127% and RSD < 19%.

3.6 Real sample analysis

The developed SLPE and fast GC-MS/MS method in this work was applied to simultaneously determine 52 pesticides in 20 samples including *P. thomsonii*, *P. cablin*, *H. cordata,* and *D. opposite*. Ten pesticides were identified in the MFDPHs. Retention time and monitor ions of the ten pesticide residues detected in MFDPHs were consistent with matrix-matched standards. Table 3 showed the contents of detectable pesticides including monocrotophos, carbofuran, quintozene, lindane, chlorothalonil, endrin, metalaxyl, fonofos, *p,p′*-DDT, and λ-cyhalothrin.

Unfortunately, four forbidden pesticides including monocrotophos, endrin, lindane, and *p,p′*-DDT were found in the tested MFDPHs. Monocrotophos is extremely dangerous organophosphate insecticide, which has acute toxicity to human body. Endrin, lindane, and *p,p′*-DDT are persistent organic pollutants. Therefore, these pesticides have been banned in many countries. In European Pharmacopoeia (EP), the maximum residue limits (MRLs) of monocrotophos, endrin, lindane, and *p,p′*-DDT are 0.1, 0.05, 0.6, and 1 mg/kg, respectively [35]. Table 3 shows that the contents of monocrotophos in one batch of sample and endrin in two batches of samples exceeded MRL. Although the contents of lindane and *p,p′*-DDT are lower than the MRL of EP, the detection rates of them are very high.

Furthermore, carbofuran and fonofos are classified as restricted use pesticides by United States Environmental Protection Agency (EPA) [www.epa.gov]. They are found in multibatches MFDPHs and the contents are in the range of 0.012–0.086 mg/kg. Quintozene, chlorothalonil, and metalaxyl are classified as a general use pesticide by EPA and they are used as fungicide to control a wide range of fungi species in vegetables, crops, and soil. λ-Cyhalothrin is a pyrethroid insecticide. Table 3 shows that the contents of λ-cyhalothrin are lower than MRL (1 mg/kg) of EP.

4 Concluding remarks

In this study, an optimized SPLE and fast GC-MS/MS method was developed. The method was applied for the determination of 52 pesticide residues in MFDPHs. Results showed that the optimized SPLE can simplify sample preparation and obtain high recoveries for most pesticide residues. The column backflush was used to decrease analysis time and extend the life of GC-MS/MS system. Therefore, SPLE and fast GC-MS/MS method is a good method for the determination of multiple pesticide residues in MFDPHs.

The research was supported by grants from Macao Science and Technology Development Fund (049/2005/A-R1; UL016/09-Y1/CMS/WYT01/ICMS; 013-2008-A1).

The authors have declared no conflict of interest.

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