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Multi-residue method for fast determination of pesticide residues in plants used in traditional chinese medicine by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry

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ABSTRACT

An ultra-high-performance liquid chromatographic–tandem mass spectrometry (UHPLC–MS/MS) method for the simultaneous quantification and identification of 116 pesticide residues which were most widely used in plants used in Traditional Chinese Medicine (TCM) in 15 min has been developed and validated. Samples were extracted and cleaned up with modified QuEChERS method and detected by UHPLC–MS/MS under multiple reactions monitoring mode, and quantified by matrix-match calibration. The validation study was carried out on five different matrixes following DG SANCO/2007/3131 of the European Quality Control Guidelines. The linearity of the calibration was good between 5 and 100 ng ml^{-1} concentration ranges, and the limits of quantification (LOQs) less than 0.01 mg/kg for most pesticides. The mean recoveries of almost all pesticides were in the range from 70% to 120% at three concentration levels ranging from 0.01 mg/kg to 0.1 mg/kg with relative standard deviations (RSD) better than 15%. The method was applied on 138 real samples from 102 different kinds of Chinese herbal medicine. 95 positive samples were detected. This method is fast, robust, accurate, selective, sensitive and easy to operate.

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1. Introduction

Plants used in Traditional Chinese Medicine (TCM), which has been used in China for thousands of years, shows very good therapeutic effects in China as well as other countries. In the Western World, botanical products are widely used as food ingredients, supplements, over the counter drug products, and phytomedicines. Today more plants used in TCM crops than ever are being cultivated in large-scale farming operations. So like other agricultural products, the materials must be subjected to safety control because of the pesticides used in their cultivation in order to control pest and improve the plant quality. Pesticide residues are not only harmful to human health, but also a major concern to the stakeholders of the plants used in TCM industry [1].

In contrast to the past when more pesticides used were non-polar, recently introduced pesticides are often more polar and less volatile. This complicated nature of pesticides gives rise to the development of special methods which are intended for analysis of a certain pesticide or group of pesticides. In this sense, the application of HPLC hyphenated to tandem mass spectrometry (LC–MS/MS) in multi-class pesticide residue analysis has become more increasingly appropriate than gas chromatograph hyphenated to tandem mass spectrometry (GC–MS/MS) [2,3]. UPLC uses a new generation of columns filled with particles of size 1.7 μm which can operate at higher back pressures. This technique has generated higher chromatographic performance, such as improved resolution, speed, sensitivity, and peak capacity. Thus, UPLC coupled with triple quadrupole (MS/MS) in MRM mode can be used as the most promising technique for the analysis of pesticide residues in food and other matrixes, since it allows their quantification and confirmation at trace levels. Up to now, UPLC- MS/MS has already been applied in analysis of multiple pesticides in foods [4–9] and water [10,11]. Nevertheless, only a limited number of these studies describe a comprehensive (>50 pesticides) multi-residue method for the screening of pesticides belonging to various classes. No papers have been found for the determination of multi-residue pesticides in plants used in TCM samples using this method.

In addition, for the analysis of pesticides in plants used in TCM, sample preparation is the bottleneck because of the complex matrixes. The extraction and clean-up procedures are often critical steps that improve the speed of the whole analytical determination and the sensitivity. Several methods have been developed, such as solid–liquid extraction (SLE) [12,13], solid phase extraction (SPE) [14,15], matrix solid-phase dispersion (MSPD) [16,17], solid-phase microextraction (SPME) [18,19], pressurized liquid extraction (PLE) [20,21], and stir bar sorptive extraction (SBSE) [22].

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However, many of them are complicated, time-consuming, expensive, require large amounts of solvents, and fail in performance in multi-residue applications. In recent years, trends in sample treatment are towards the miniaturization and simplification of the methodologies [23]. Therefore, several approaches, such as single-drop microextraction (SDME) [24,25], supported-liquid membrane (SLM) [26], membrane-assisted solvent extraction (MASE) [27] and direct injection of the sample previous dilution with pure water [28], have been proposed. Recently, a sample preparation method named QuEChERS has been introduced [29]. This method has been shown to be a powerful technique in analysis of pesticide residues in foodstuffs [23] and other compounds, like drugs in blood [30], and has become very popular in the last few years. While to our knowledge, there are few reports on pretreatment of pesticides in plants used in TCM using this method.

This paper describes a rapid multi-residue method for determination of 116 pesticides and pesticide metabolites from several classes in 15 min in different types of plants used in TCM using modified QuEChERS extraction and UPLC–MS/MS analysis, which is not yet documented in literature. In order to illuminate the applicability of the proposed procedure, sample matrixes representative of a variety of plants used in TCM have been selected. *Radix Ginseng* was chosen as a representative for root, *Flos Loncerae* for flower, *Semenpersicae* for seed, *Herba Lophatheri* for leaf, and *dogwood* for fruit. It must be remembered that the high resolving capacity of the UPLC method combined with the fast sample preparation approach will offer significant benefits for pesticide residues analysis, which can be used in routine analysis requiring high sample throughput.

2. Experimental

2.1. Materials and reagents

Certified reference standards of all the test pesticides were of >98% purity purchased from the Ehrensdorfer (Augsburg, Germany) or Sigma–Aldrich (USA). Acetonitrile and methanol of HPLC quality were from Fisher (USA). Water was deionized in the laboratory using a Millipore (Bedford, MA, USA) MilliQ water purification system. Formic acid and acetic acid were HPLC grade which from TEDIA company (ING, USA). Bulk sorbents (50 μm particle size) for dispersive-SPE including primary secondary amine (PSA), octadecylsilane (C18) and graphitized carbon black (GCB) were obtained from Sigma–Aldrich (USA). SPE cartridges were Supelclean Envi-Carb II/PSA (500 mg/500 mg, 6 ml size) and Supelclean C18 (500 mg, 6 ml size) from Sigma–Aldrich (USA). Anhydrous magnesium sulfate and sodium chloride were all ACS grade and obtained from Beijing Chemical Works (Beijing, China). Anhydrous magnesium sulfate ($MgSO_4$) was activated by heating at 650 °C for 4 h and sodium chloride at 105 °C for 4 h before use and kept in desiccator.

2.2. Preparation of standard solutions

Individual stock solutions of the pesticides at a concentration of 1.0 mg/ml were prepared in acetonitrile, methanol or acetone, according to their solubility. An intermediate stock standard of 1.0 μg/ml were prepared by diluting the appropriate volume of the individual stock solutions. An ultimately mixed working standard solution containing different concentration of each pesticide whose portions owing to their signal to noise was prepared by dilution of the stock solutions in methanol (see Table 1).

2.3. Instrumentation and UPLC–MS/MS conditions

Chromatographic analysis was performed with an Acquity UPLC system (Waters, Xevo™ TQ, USA) equipped with a binary solvent delivery system, vacuum degasser, a solvent delivery compartment with high pressure mixing, an autosampler and column heater. Chromatographic separations were achieved using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size) from Waters with a binary mobile phase composed of 0.1% (v/v) formic acid in water (eluent B) and acetonitrile (eluent A) pumped at a flow rate of 0.3 ml/min. In front of the separation column was a Van Guard™ pre-column 31PK, BEH C18 (1.7 μm, 2.1 mm × 5 mm, Waters, USA) to reduce the amount of matrix components entering the separation column. The sample manager temperature was at 4 °C to prevent thermal labile pesticides from degradation. All the analyses were kept in the same temperature condition, and the temperature of the column heater was maintained at 35 °C.

The gradient program was started with 5% component A (95%B) at injection time and increased linearly to 10% A (90%B) in 1 min, further to 30%A (70%B) over 4 min. It was then changed to 60%A (40%B) after 8 min and gradually changing to 90% until 13.5 min after injection. This eluent composition was shifted within 1.5 min back to the starting conditions and kept there for 2 min. The injection volume was 5 μl considering the sensitivity of the instrument and to avoid carry-over. The auto-sampler was flushed with weak wash and strong wash, which were composed of acetonitrile and water in ratio 1:9 in the former and 9:1 in the later, after sample injected. Then the auto-sampler was kept in weak flush when there were no samples injected.

API-MS detection was achieved using a Xevo™ TQ mass spectrometer (Waters, USA) equipped with an electrospray ionization interface (Z-spray) operating in the positive and negative modes. The capillary voltage and extractor voltage were set at 3.2 kV and 3.00 V, respectively. The source temperature and desolvation temperature were held at 150 and 400 °C, respectively. Nitrogen was used for the cone and desolvation gas flows set at 50 and 800 l h^{-1}, respectively, and argon was used as collision gas at a pressure of 0.1 MPa and flows 0.16 ml/min. MS/MS experiments were carried out in multiple reaction monitoring modes (MRM) for simultaneous detection of all the target pesticides, with two precursor-to-product ion transitions monitored for each analyte. The cone voltage and the collision energy were optimized for the different pesticides in direct flow-injection mode. Precursor and corresponding product ions for the MRM detection are listed in Table 1. The date acquisition was made using Masslynx 4.1 software with Instrument and date processing was performed using MassLynx 4.1 with TargetLynx.

2.4. Extraction procedure

Considering different chemical components in different plants used in TCM, QuEChERS-based approach with different cleanup steps was evaluated in the analysis of various sorts of plants used in TCM.

2.4.1. Extraction method A

Plants used in TCM raw materials were purchased from a medicine store, including *Herba Lophatheri*, *dogwood*, *Radix Ginseng*, *Semenpersicae* and *Flos Loncerae*. All materials were cut and ground to fine pieces. For routine analysis, 2 g of each sample (1 g for *Herba Lophatheri*) was weighed into a 50 ml Teflon centrifuge tube and soaked with 10 ml of Ultra-pure water at ambient temperature for 1 h. Ten milliliters of 0.1% HAC/acetonitrile were then added to the samples, and the mixture were shaken for 1 min. Afterwards, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, and the mixtures were shaken immediately for 1 min by vortex mixer to prevent the formation of coagulated Magnesium sulfate and centrifuged for 10 min at 3500 rpm at 4 °C. 7 ml of the upper layer of the extracts were transferred into a 10 ml centrifuge tube which contained 210 mg PSA, 70 mg GCB and 1.05 g anhydrous $MgSO_4$, except for *dogwood* and *Semenpersicae* which we added extra 200 mg C18.

Table 1
UPLC–ESI–MS–MS conditions and molar weight for the different pesticides: primary trace, secondary trace, retention time, cone voltage (V) and collision energy (eV).

Pesticide	Molecular weights	Molecular formulas	Retention time	Primary trace[e]	Secondary trace	Cone (V)	CE1[b] (eV)	CE2[c] (eV)	Concentration of pesticide[a] (μg/ml)	Scan mode
Acephate	183.17	$C_4H_{10}NO_3PS$	1.61	184.1/143.0	184.1/125.0	14	9	18	0.1	ESI+
Acetamiprid	222.67	$C_{10}H_{11}ClN_4$	4.86	223.1/126.1	223.1/56.1	20	20	16	2	ESI+
Acetochlor	269.77	$C_{14}H_{20}ClNO_2$	9.40	270.0/224.0	270.0/148.0	10	9	32	0.1	ESI+
Acifluorfen	361.66	$C_{14}H_{12}ClF_3NO_5$	13.48	362.4/300.3	362.4/256.1	39	22	23	1	ESI+
Alachlor	269.77	$C_{14}H_{20}ClNO_2$	9.37	270.0/238.1	270.0/162.0	19	12	21	2	ESI+
Aldicarb-sulfone	222.26	$C_7H_{14}N_2O_4S$	2.95	240.2/223.1	240.2/76.1	11	8	13	0.1	ESI+
Atrazine	215.68	$C_8H14ClN_5$	6.93	216.0/174.0	216.0/96.1	29	18	24	0.1	ESI+
Azinphos-ethyl	345.38	$C_{12}H_{16}N_3O_3PS_2$	9.47	346.0/160.0	346.0/289.0	10	8	7	0.1	ESI+
Azinphos-methyl	317.32	$C_{10}H_{12}N_3O_3PS$	8.28	318.4/132.0	318.4/124.9	11	16	16	0.1	ESI+
Azoxystrobin	403.39	$C_{22}H_{17}N_3O_5$	8.59	404.1/372.0	404.1/343.8	20	13	23	0.1	ESI+
Buprofezin	305.44	$C_{16}H_{23}N_3OS$	13.55	306.2/201.1	306.2/116.1	19	11	15	0.1	ESI+
Butachlor	311.85	$C_{17}H_{26}ClNO_2$	12.41	312.4/162.1	312.4/238.2	20	21	11	1	ESI+
Butamifos	332.36	$C_{13}H_{21}N_2O_4PS$	11.24	333.2/180.1	333.2/152.0	12	10	18	0.1	ESI+
Carbaryl	201.22	$C_{12}H_{11}NO_2$	6.87	202.2/145.1	202.2/127.1	16	10	27	0.1	ESI+
Carbendazim	191.19	$C_9H_9N_3O_2$	3.76	192.1/160.1	192.1/132.1	21	17	27	0.1	ESI+
Carbofuran	221.25	$C_{12}H_{15}NO_3$	6.55	222.2/165.2	222.2/123.1	17	12	20	0.1	ESI+
Carbofuran-3-hydroxy	237.25	$C_{12}H_{15}NO_4$	4.39	238.1/163.1	238.1/181.1	19	14	10	0.1	ESI+
Chlorfenvinphos	359.57	$C_{12}H_{14}Cl_3O_4P$	9.78	359.0/155.0	359.0/127.1	17	13	17	0.1	ESI+
Chlorpyrifos	350.59	$C_9H_{11}Cl_3NO_3PS$	12.68	350.3/197.9	350.3/96.9	22	26	27	1	ESI+
Chlorpyrifos-methyl	322.53	$C_7H_7Cl_3NO_3PS$	10.93	321.9/125.0	321.9/289.8	24	19	16	2	ESI+
Coumaphos	362.77	$C_{14}H_{16}ClO_5PS$	10.50	362.9/227.0	362.9/307.0	35	28	18	0.1	ESI+
Cymoxanil	198.18	$C_7H_{10}N_4O_3$	5.21	199.1/128.1	199.1/158.0	12	10	7	2	ESI+
Cyprodinil	225.29	$C_{14}H_{15}N_3$	9.02	226.2/93.1	226.2/108.2	41	30	23	0.1	ESI+
Cyromazine	166.18	$C_6H_{10}N_6$	2.29	167.1/85.1	167.2/125.2	26	18	17	0.1	ESI+
Demeton methyl	230.28	$C_6H_{15}O_3PS_2$	6.17	231.1/89.1	231.1/61.0	7	10	26	0.1	ESI+
Diazinon	304.35	$C_{12}H_{21}N_2O_3PS$	10.47	305.2/169.1	305.2/153.1	26	21	22	2	ESI+
Dichlofenthion	315.15	$C_{10}H_{13}Cl_2O_3PS$	12.61	315.2/258.9	315.2/186.8	18	17	11	2	ESI+
Dichlorvos	220.98	$C_4H_7Cl_2O_4P$	6.16	221.1/109.1	221.1/145.0	25	18	12	2	ESI+
Dicrotophos	237.19	$C_8H_{16}NO_5P$	3.64	238.0/112.1	238.0/192.9	19	11	11	0.1	ESI+
Diethofencarb	267.32	$C_{14}H_{21}NO_4$	8.33	268.2/226.1	268.1/180.1	14	10	19	0.1	ESI+
Difenoconazole	406.26	$C_{19}H_{17}C_{12}N_3O_3$	10.08	406.1/251.0	406.1/337.0	31	24	17	0.1	ESI+
Dimethoate	229.26	$C_5H_{12}NO_3PS_2$	4.84	230.1/145.1	230.1/189.1	27	22	11	0.1	ESI+
Diniconazole	326.22	$C_{15}H_{17}Cl_2N_3O$	9.50	326.2/70.1	326.2/158.9	33	25	29	1	ESI+
Disulfoton	274.40	$C_8H_{19}O_2PS_3$	4.40	275.1/141.0	275.1/109.0	20	20	22	1	ESI+
Ditalimfos	299.28	$C_{12}H_{14}NO_4PS$	9.30	300.1/148.0	300.1/244.0	19	18	13	0.1	ESI+
EPN[d]	323.20	$C_{14}H_{14}NO_4PS$	10.27	324.0/296.0	324.0/156.9	22	14	24	1	ESI+
Ethoprophos	242.34	$C_8H_{19}O_2PS_2$	8.82	243.0/173.1	243.0/130.9	20	15	21	0.1	ESI+
Etrimfos	292.28	$C_{20}H_{17}N_2O_4PS$	10.34	293.1/265.0	293.1/125.0	29	17	23	1	ESI+
Fenamiphos	303.36	$C_{13}H_{22}NO_3PS$	8.61	304.2/217.1	304.2/234.0	26	21	16	0.1	ESI+
Fenarimol	331.20	$C_{17}H_{12}Cl_2N_2O$	8.44	331.0/268.1	331.0/81.1	32	23	27	1	ESI+
Fenchlorphos-oxon	305.49	$C_8H_8Cl_3O_4P$	10.49	305.0/109.1	305.0/258.0	35	24	28	0.1	ESI+
Fenitrothion	277.23	$C_{10}H_{12}NO_5PS$	9.33	278.1/109.0	278.1/125.1	22	20	21	1	ESI+
Fenobucarb	207.27	$C_{12}H_{17}NO_2$	8.23	208.2/95.1	208.2/152.1	18	13	7	0.1	ESI+
Fenoxaprop-ethyl	361.78	$C_{18}H_{16}ClNO_5$	11.47	361.9/288.1	361.9/244.0	32	18	24	2	ESI+
Fensulfothion	308.35	$C_{11}H_{17}O_4PS_2$	7.40	308.9/252.8	308.9/280.9	25	18	15	0.1	ESI+
Fenthion	278.33	$C_{10}H_{15}O_3PS_2$	4.30	279.1/264.0	279.1/104.1	29	19	32	0.1	ESI+
Fenthion-sulfone	310.33	$C_{10}H_{15}O_5PS_2$	7.65	311.1/125.1	311.1/279.0	29	20	17	1	ESI+
Fenthion-sulfoxide	294.33	$C_{10}H_{15}O_4OS_2$	6.54	295.2/280.0	295.2/109.1	29	18	32	0.1	ESI+
Fipronil	437.15	$C_{12}H_4Cl_2F_6N_4OS$	5.98	454.0/368.0	454.0/436.6	20	22	6	0.1	ESI+
Fludioxonil	248.19	$C_{12}H_6F_2N_2O_2$	8.40	247.0/126.1	247.0/180.1	37	29	27	0.1	ESI−
Flufenoxuron	488.77	$C_{21}H_{11}ClF_6N_2O_3$	12.73	487.2/304.0	487.2/410.3	24	19	15	0.1	ESI−
Fluroxypyr	255.00	$C_7H_5O_3N_2FCl_2$	13.34	255.0/208.9	255.0/181.0	27	15	20	1	ESI+
Fomesafen	438.76	$C_{15}H_{10}ClF_3N_2O_6S$	8.87	437.1/285.9	437.1/221.8	35	24	27	0.1	ESI−
Hymexazol	99.09	$C_4H_5NO_2$	1.77	100.1/54.2		4	20		2	ESI+
Imidacloprid	255.66	$C_9H_{10}ClN_5O_2$	4.55	256.4/175.1	256.4/209.1	19	17	16	1	ESI+
Indoxacarb	527.83	$C_{22}H_{17}ClF_3N_3O_7$	11.64	528.0/150.0	528.0/249	28	25	18	0.1	ESI+
Iprobenfos	288.34	$C_{13}H_{21}O_3PS$	9.20	306.2/289.1	306.2/264.8	9	6	6	0.1	ESI+
Isazofos	313.70	$C_9H_{17}ClN_3O_3PS$	9.31	314.0/161.9	314.0/271.9	23	16	13	0.1	ESI+
Isofenphos-methyl	331.37	$C_{14}H_{22}NO_4PS$	10.52	332.0/273.2	332.0/231.1	7	5	20	0.1	ESI+
Isoprocarb	193.24	C11H15NO2	7.42	211.2/194.1	211.2/95.1	9	6	19	0.1	ESI+
Isoprothiolane	290.40	$C_{12}H_{18}O_4S_2$	9.20	291.1/207.1	291.1/91.1	12	10	21	0.1	ESI+
Malaoxon	314.29	$C_{10}H_{19}O_7PS$	6.45	315.1/127.1	315.1/99.1	17	12	25	0.1	ESI+
Malathion	330.36	$C_{10}H_{19}O_6PS_2$	9.23	331.1/127.0	331.1/284.9	16	12	7	0.1	ESI+
Metalaxyl	279.33	$C_{15}H_{21}NO_4$	7.14	280.2/220.2	280.2/248.1	20	13	9	0.1	ESI+
Methacrifos	240.21	$C_7H_{13}O_5PS$	5.85	241.1/209.0	241.1/125.1	14	8	19	1	ESI+
Methamidophos	141.13	$C_2H_8NO_2PS$	1.30	142.1/94.0	142.1/125.1	20	13	14	0.1	ESI+
Methidathion	302.33	$C_6H_{11}N_2O_4PS_3$	8.23	303.1/145.0	303.1/85.0	16	8	21	0.1	ESI+
Methiocarb	225.31	$C_{11}H_{15}NO_2S$	8.21	243.1/226.1	243.1/169.2	8	7	14	0.1	ESI+
Methomyl	162.21	$C_5H_{10}N_2O_2S$	3.27	162.9/88.1	162.9/106.2	18	10	10	0.1	ESI+
Metolachlor	283.79	$C_{15}H_{22}ClF_3NO_4$	9.36	284.1/252.1	284.1/176.2	18	15	24	0.1	ESI+
Metolcarb	165.19	$C_9H_{11}NO_2$	6.20	166.0/109.1		14	10		0.1	ESI+
Mevinphos	224.15	$C_7H_{13}O_6P$	5.05	225.1/127.0	225.1/193.2	12	18	9	0.1	ESI+
Monocrotophos	223.16	$C_7H_{14}NO_5P$	3.30	224.1/193.0	224.1/127.1	16	9	15	0.1	ESI+
Myclobutanil	288.78	$C_{15}H_{17}ClN_4$	8.64	289.1/70.1	289.1/125.1	25	16	34	0.1	ESI+
Napropamide	271.35	$C_{17}H_{21}NO_2$	8.94	272.1/129.1	272.1/171.1	21	16	18	0.1	ESI+

Table 1 (*Continued*)

Pesticide	Molecular weights	Molecular formulas	Retention time	Primary trace[e]	Secondary trace	Cone (V)	CE1[b] (eV)	CE2[c] (eV)	Concentration of pesticide[a] (μg/ml)	Scan mode
Omethoate	213.19	$C_5H_{12}NO_4PS$	2.34	214.0/183.0	214.0/155.0	20	10	12	0.1	ESI+
Paraquat dichloride	257.25	$C_{12}H_{14}N_2$ + 2.2HCl	6.11	256.3/88.1	256.3/102.0	53	23	21	2	ESI+
Parathion-ethyl	291.26	$C_{10}H_{14}NO_5PS$	10.16	292.0/235.9	292.0/264.0	20	13		2	ESI+
Parathion-methyl	263.21	$C_8H_{10}NO_5PS$	13.30	362.1/300.1	362.1/256.1	34	22	23	2	ESI+
Phenthoate	320.36	$C_{12}H_{17}O_4PS_2$	10.31	321.0/274.9	321.0/163.0	14	10	12	2	ESI+
Phorate	260.38	$C_7H_{17}O_2PS_3$	10.89	261.1/75.0	261.1/198.8	9	12	7	1	ESI+
Phosalone	367.81	$C_{12}H_{15}ClNO_4PS_2$	10.91	368.0/181.9	368.0/321.9	18	18	9	0.1	ESI+
Phosphamidon	299.69	$C_{10}H_{19}ClNO_5P$	5.71	300.0/127.0	300.0/174.1	21	21	13	0.1	ESI+
Phoxim	298.30	$C_{12}H_{15}N_2O_3PS$	10.87	299.1/129.1	299.1/125.0	15	11	10	0.1	ESI+
Piperonyl butoxide	338.44	$C1_9H_{30}O_5$	11.86	356.4/177.1	356.4/147.1	13	16	32	0.1	ESI+
Pirimicarb	238.29	$C_{17}H_{18}N_4O_2$	6.80	239.1/72.1	239.1/182.2	25	20	17	0.1	ESI+
Pirimiphos-ethyl	333.39	$C_{13}H_{24}N_3O_3PS$	12.63	334.2/198.2	334.2/182.1	29	21	24	0.1	ESI+
Pirimiphos-methyl	305.33	$C_{11}H_2ON_3O_3PS$	10.84	306.1/164.1	306.1/108.1	38	23	30	0.1	ESI+
Profenofos	373.63	$C_{11}H_{15}BrClO_3PS$	11.75	372.9/302.8	372.9/344.8	23	18	13	1	ESI+
Promecarb	207.27	$C_{12}H_{17}NO_2$	8.51	208.1/151.1	208.1/109.1	17	8	19	0.1	ESI+
Propamocarb	188.27	$C_9H_2ON_2O_2$	3.52	190.1/145.1	190.1/182.2	22	18	17	0.1	ESI+
Propanil	218.08	$C_9H_9Cl_2NO$	7.94	218.1/162.0	218.1/127.1	28	15	27	0.1	ESI+
Propetamphos	281.31	$C_{10}H_{20}NO_4PS$	9.47	282.1/156.0	282.1/138.1	13	10	18	1	ESI+
Propiconazole	342.22	$C_{15}H_{17}Cl_2N_3O_2$	9.52	342.1/158.9	342.1/69.1	31	25	23	0.1	ESI+
Propoxur	209.24	$C_{11}H_{15}NO_3$	7.47	210.2/168.1	210.2/111.1	13	7	15	0.1	ESI+
Pymetrozine	217.23	$C_{10}H_{11}ClF_3NO_4$	2.50	218.3/105.1	218.3/88.1	24	18	20	0.1	ESI+
Pyrazophos	373.36	$C_{14}H_{20}N_3O_5PS$	10.36	374.2/222.1	374.2/346.0	33	21	15	0.1	ESI+
Quinalphos	298.30	$C_{12}H_{15}N_2O_3PS$	9.93	299.0/147.0	299.0/163.0	21	23	20	0.1	ESI+
Quizalofop-ethyl	372.80	$C_{19}H_{17}Cl_2N_2O_4$	11.86	373.1/299.1	373.1/91.0	30	18	30	0.1	ESI+
Simazine	201.66	$C_7H_{12}ClN_5$	5.93	202.1/104.1	202.1/96.1	30	26	23	0.1	ESI+
Sulfotep	322.32	$C_8H_{20}O_5P_2S_2$	10.76	323.0/171.0	323.0/294.8	17	13	9	0.1	ESI+
Tebuconazole	307.82	$C_{16}H_{22}ClN_3O$	8.96	308.2/70.1	308.2/125.1	27	19	36	0.1	ESI+
Tebufenozide	352.47	$C_{22}H_{28}N_2O_2$	9.67	353.3/297.1	353.3/133.1	10	7	20	0.1	ESI+
Tetrachlorvinphos	365.96	$C_{10}H_9Cl_4O_4P$	6.50	366.7/127.0	366.7/240.9	23	14	20	0.1	ESI+
Thiamethoxam	291.71	$C_8H_{10}ClN_5O_3S$	3.84	292.1/211.0	292.1/131.9	17	13	20	0.1	ESI+
Thiodicarb	354.47	$C_{10}H_{18}N_4O_4S_3$	6.41	355.1/88.0	355.1/108.0	16	16	15	0.1	ESI+
Thiophanate-methyl	342.39	$C_{12}H_{14}N_4O_4S_2$	6.28	343.1/151.1	343.1/311.0	18	23	11	1	ESI+
Tolclofos-methyl	301.13	$C_9H_{11}Cl_2O_3PS$	10.85	301.1/269.0	301.1/125.0	17	18	20	1	ESI+
Triadimefon	293.75	$C_{14}H_{16}ClN_3O_2$	8.74	294.1/197.1	294.1/69.1	23	16	20	0.1	ESI+
Triadimenol	295.76	$C_{14}H_{18}ClN_3O_2$	8.02	296.1/70.1	296.1/227.1	13	10	10	1	ESI+
Triazophos	313.31	$C_{12}H_{16}N_3O_3PS$	9.72	314.0/162.0	314.0/286.0	22	18	13	0.1	ESI+
Trichlorfon	257.44	$C_4H_8Cl_3O_4P$	4.12	256.9/109.0	256.9/221.0	23	16	10	0.1	ESI+
Triflumizole	345.75	$C_{17}H_{15}CLF_3N_3O$	10.47	346.4/284.1	346.4/88.1	15	23	28	2	ESI+
Triflumuron	358.70	$C_{15}H_{10}ClF_3N_2O_3$	10.01	356.9/154.0	356.9/176.1	19	13	23	0.1	ESI-
Triphenyl phosphate	326.28	$C_{18}H_{15}O_4P$	10.31	327.1/152.1	327.1/77.0	40	37	39	0.1	ESI+
Vamidothion	287.34	$C_8H_{18}NO_4PS_2$	9.14	288.3/106.2	288.3/88.1	17	22	27	0.1	ESI+

[a] Concentrations of pesticide standard in mixed working standard solution.
[b] CE1: collision energy of primary trace.
[c] CE2: collision energy of secondary trace.
[d] EPN: English name is ethylphenylphosphonothioic acid O-(4-nitro-phenyl)ester;O-Ethyl-O-p-nitrophenyl-phenylphosphono thionate.
[e] The primary trace was use to quantification.

The tubes were shaken vigorously for 1 min and then centrifuged for 10 min at 3500 rpm at 4 °C. After centrifugation, 5 ml cleaned extracts were transferred into test tubes and evaporated to dryness under a stream of nitrogen. The residues were immediately reconstituted in 1.0 ml 0.1% formic acid in methanol/water (3:2) to give a final matrix concentration of 1 g/ml.

2.4.2. Extraction method B

In case of serious matrix effect for some plants used in TCM with complex matrix obtained with extraction method A, re-analysis using an alternative purification method was applied. In this method, SPE instead of DSPE was used for sample clean-up. Multi-layer Supelclean Envi Carb-II/PSA SPE cartridge was used for most plants used in TCM. Supelclean C18 SPE cartridge was added to the top of the columns for some plants used in TCM that have more fat or waxes. An aliquot of 5 ml extracts were transferred into a 200 ml pearshaped flask and evaporated to 1 ml (30 °C) on the rotary evaporator followed by dissolution in acetonitrile–toluene (3:1, v/v). Envi Carb-II/PSA SPE cartridge was conditioned with 10 ml acetonitrile–toluene (3:1, v/v). When the conditioning solution reached the top of filler the cartridge was connected to a pearshaped flask and the concentrated sample obtained as described above was added to the cartridge. The pearshaped flask was rinsed with 3× 2 ml acetonitrile–toluene (3:1, v/v), and the washings were also applied to the cartridge. A reservoir was attached to the cartridge and the pesticides were eluted with 20 ml acetonitrile–toluene (3:1, v/v). The eluate was evaporated to 1 ml by rotary evaporation at 30 °C, exchanged with acetone (2× 5 ml) and concentrated to 1 ml. Finally, the ultimately cleaned extracts were transferred into test tubes and evaporated to dryness under a stream of nitrogen. The residues were immediately reconstituted in 1.0 ml 0.1% formic acid in methanol/water (3:2) to give a final matrix concentration of 1 g/ml.

2.5. Method validation

The suitability of the method was properly verified through the analysis of negative plants used in TCM samples obtained from different classes prior to its application in real samples in order to ensure that the results obtained were reliable. Method validation was implemented by evaluated performance characteristics in terms of linear range, matrix effects, analytical limits including method limits of quantification (LOQs) and instrument limits of detection (LODs), accuracy and precision using extraction method

A. The limits of detection (LODs) were considered to be the concentration that produced a signal to noise (S/N) ratio of 3, and the limits of quantification (LOQs) a S/N ratio of 10.

To test the linearity of the method, a series of fortified samples for matrix-matched calibration curves were prepared at five concentration levels which covered a wide range including MRLs for the analytes. Accuracy and precision of the method were evaluated by three-fold determinations of each spiking blank samples fortified at three different levels in the individual matrixes. The latent interfering effect from co-eluting matrix constituents on ESI response was investigated by comparing the slopes of linear calibration curves from matrix-matched experiments with that obtained from pure solvent standards. The slope ratios (slope matrix/slope solvent) of 1 indicates that matrix does not significantly suppress or enhance the response of the MS, otherwise denoting ionization suppression (<1) or enhancement (>1) [12].

3. Results and discussion

3.1. UPLC-ESI–MS/MS analysis

Special attention must be paid to the optimization of the LC–MS system in order to optimize the multi-residue pesticide analysis method. A suitable compromise between resolution and analysis time should be obtained in the UPLC conditions and the MS instrumentation parameters should collect sufficient data across the peak to enable reliable integration. Thus, the mobile phase composition, additives and gradient procedures were carefully optimized in the selection of UPLC conditions. In the preliminary experiments, different mobile phases consisting of methanol, acetonitrile and water with formic, acetic acid, ammonium acetate or ammonium formate at different concentrations were checked.

For most pesticides, wider peak shape was observed with methanol as organic solvent in the mobile phase instead of acetonitrile. Furthermore, acetonitrile is preferred to the multi-residues analysis when both ESI+ and ESI− are used in the same analytical run. On the other hand, the addition of formic acid provided better results than acetic acid and was used to improve the ionization efficiency. Ammonium acetate and ammonium formate did not significantly improve the ionization efficiency than formic acid. The optimal separation of 116 compounds was achieved using a gradient elution with acetonitrile and an aqueous solution of formic acid at 0.1% (v/v) within 15 min. Due to the high selectivity of MRM detection, it is not necessary to achieve the complete resolution between the pesticides. Other parameters such as flow rate, injection volume and column temperature were ascertained based on the principle of the instrument in order to stabilize the retention times. Chromatogram of ginseng fortified at 0.02 mg/kg is shown in Fig. 1.

The instrument used in the study can immediately (required 20 ms) switch between positive and negative modes and was found to be suitable to allow sensitive determination of the 112 ESI+ and 4 ESI− analytes for each sample in a single injection. In order to perform the detection at sufficient instrument sensitivity, the MRM detection was separated into 22 overlapping functions each acquiring 4–7 substances based on analytes' retention times. In these functions, the chromatographic peaks of the analytes were in the centre of the time window, consequently, minimize the risk of peak loss due to unexpected slight changes in retention time. Because peak widths for UPLC were approximately 6–12 s, the number of spectral data points across the peaks were much smaller than for conventional HPLC, it could lead to deleterious effect on the spectral quality. So we keep the dwell times in the range 5–20 ms by lessening the compounds number in the same function in order to obtain reproducible results for determination and confirmation. Table S1 shows the time-scheduled data acquisition sequence of the LC–MS/MS method.

The MS parameters were optimized with the objective of obtaining two ion pairs for identification and quantification the target. This was done by injecting the mixed pesticide standard solutions with no more than 10 compounds. It was noticed that the pesticide standards with same or similar molecular weight could not be mixed together. Also, the conditions for some pesticides with poor ionization were optimized by injecting their individual standard solutions. Only two most intense transitions were chosen for creating MS method. The most intense transition was used as a quantifier while the other was used as a qualifier peak for the confirmatory analysis. The MS/MS transitions for quantification and confirmation, as well as the optimized parameters for all the studied pesticides are indicated in Table 1. In some cases, there were not enough transitions obtained, like hymexazol and metolcar, which had only one transition, thus make it impossible to obtain an adequate identification. MS parameters, such as source temperature, desolvation gas flows were optimized in the combine mode which were more consistent with the real samples, except for the poorly ionized pesticides.

3.2. Extraction

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method is well known for its applicability in simultaneous analysis of a large number of pesticides in a variety of food matrixes with water content more than 80% [29]. It is a method meant for isolating pesticide residues from fortified hydrated samples using acetonitrile (10 ml) as an extraction solvent and anhydrous $MgSO_4$ (4 g) and NaCl (1 g) as phase separation agents. Anhydrous $MgSO_4$ is also used to remove the water from the extract and subsequent cleanup of the acetonitrile extract is performed by vortexing an aliquot of the extract with a small quantity of solid phase extraction sorbent.

Plants used in TCM is a special product with a water content <10% therefore it cannot be used in the QuEChERS method directly. Previous report had shown that some processing methods must be adopted for particular matrixes [31]. In this experiment the QuEChERS method was modified by reducing the amount of the sample and adding some water. Considering the complexity of chemical composition in plants used in TCM, more interference will transfer into the extract with the addition of water. So in order to decrease this interference effect, different extraction modes were evaluated. (a) Ultrasonic extraction with 10 ml acetonitrile, (b) Ultrasonic extraction with 10 ml acetonitrile (with some salt to increase recovery), (c) Vortexed extraction with 10 ml acetonitrile after being soaked in water (added some salts mixture to achieve phase separation and remove water). The result showed that the first method gave the least amount of co-extractives and the third one gave the most in the final extracts without cleanup. Also, the recovery of the third method was the best as shown in Fig. 2(A). This proved that extraction with acetonitrile combined with water is the best (see Fig. 4(B)). The ratio of sample to water was also examined via several tests with many special pesticides in this work. Because the PH of solvent will be rise after clean-up by PSA, acetonitrile with 0.1% acetic acid instead of acetonitrile neat was employed as extraction solvent in order to stabilize problematic pesticides and to expand the applicability.

Other experiment factors, like amounts of salt, PSA, C18 and GCB were also examined in order to gain the good purification and recoveries.

The ratios $MgSO_4$ to NaCl have been tested using (1) 4.5 g $MgSO_4$ to 1.5 g NaCl; (2) 3.5 g $MgSO_4$ to 1.5 g NaCl; (3) 4.5 g $MgSO_4$ to 1 g NaCl; (4) 4 g $MgSO_4$ to 1 g NaCl. The result can be seen in Fig. 2(B). Recovery yields of 8 mentioned pesticides were best with method 4. We also discussed the amounts of PSA. For most of matrixes

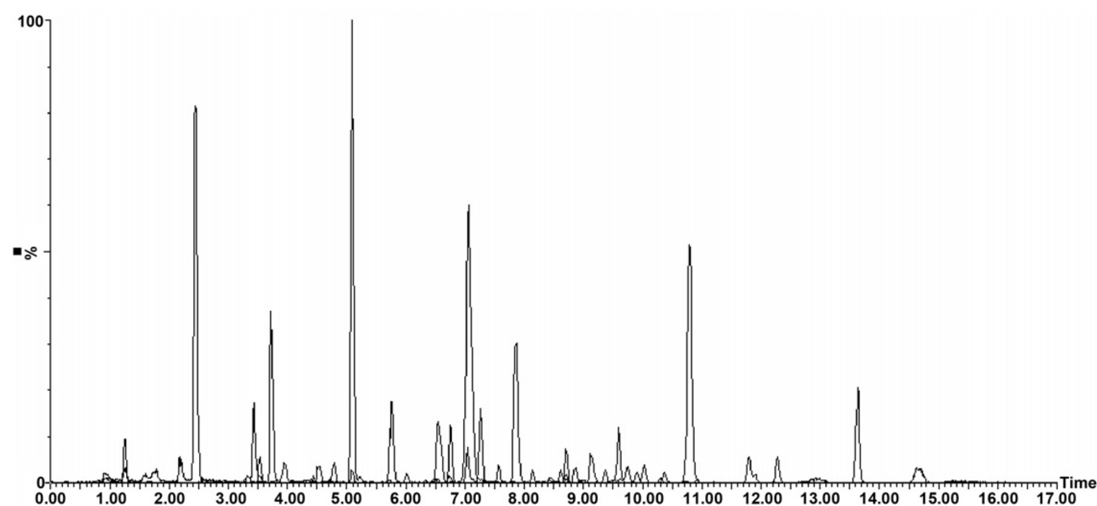

Fig. 1. Total ion chromatogram of ginseng fortified at 0.02 mg/kg.

in this experiment purification was accepted when 30 mg/ml PSA were used. The amounts of C18 have been compared to discuss the effects on the purification and recoveries (see Fig. 2(C)). When 200 mg C18 for 7 ml extract were applied, the residues were least and the recoveries were accepted. The effects of the mass of GCB on the recoveries were also tested in order to prevent the significant losses of low polarity and planar pesticides (see Fig. 2(D)). Recoveries were accepted for *Ginseng* which contains sterols when the amounts of GCB were 10 mg/ml. For other matrixes containing more pigments than *Ginseng*, 10 mg/ml will not affect recoveries. Appending concentrating procedure was the last one which modified in this experiment. In primal QuEChERS method the final extract was injected into the LC–MS/MS system directly. While in this method the amount of the sample had to be reduced to get the right water to sample ratio. Consequently, the final extract was concentrated before injection to increase the sensitivity.

3.3. Comparation of two clean-up procedures

In Traditional Chinese Medicine formulation, different edible parts of TCM plants can be chosen to make each dose. Moreover, the plants used in TCM matrixes are versatile in chemical constituents,

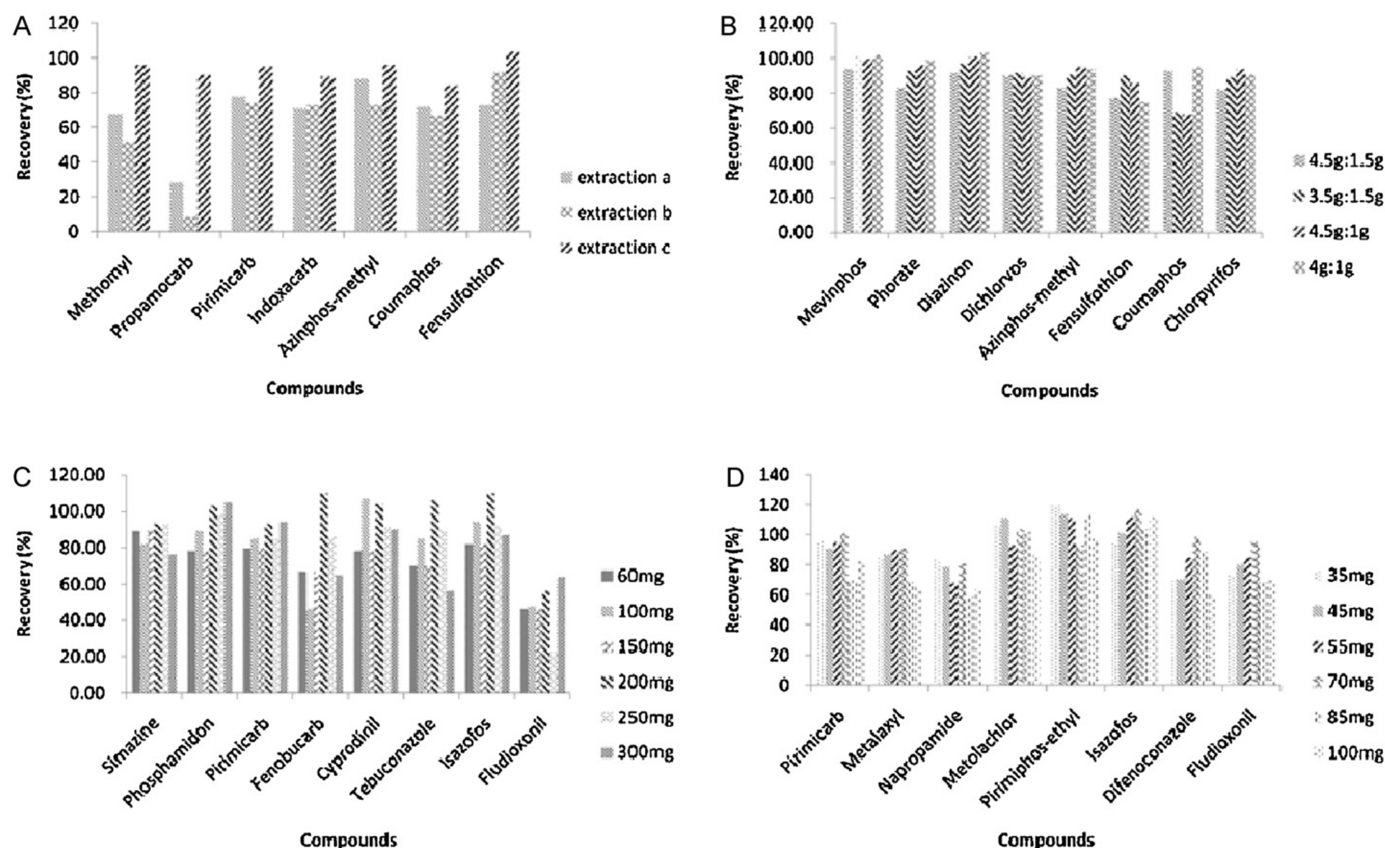

Fig. 2. (A) Comparation of the three methods; (B) comparation of the ratios $MgSO_4$ to NaCl; (C) the effect of the amounts of C18; (D) the effect of the amounts of GCB.

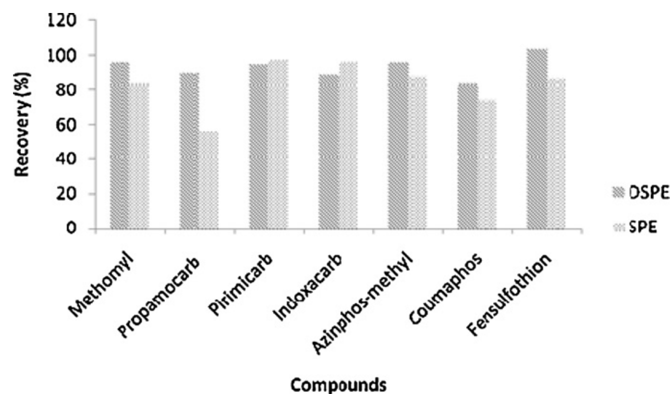

Fig. 3. Comparation of DSPE to SPE method.

such as flavonoids, polysaccharide, saponin, alkaloid, essential oils, etc. Therefore, it is difficult to determine the pesticide residues in such kinds of complex matrixes as plants used in TCM.

In our study, both DSPE and SPE were investigated in their recoveries and matrix effects. *Radix Ginseng* was investigated at first, then other different kinds of plants used in TCM were discussed which were representative of different matrix chemical constituents and plants used in TCM classes. The comparation of two methods in recoveries for *Ginseng* was shown in Fig. 3. *Flos Loncerae* for flower, *Semenpersicae* for seed, *Herba Lophatheri* for leaf, *Radix Ginseng* for root, and *dogwood* for fruit are the five selected plants used in TCM. It could be concluded that both DSPE and SPE were found to give less co-extractive in the plants used in TCM analysis in this study, however, for some plants used in TCM with most complex matrixes, SPE is better than DSPE because of the better purification.

The procedure A is simple and easy to operate in routine pesticide analysis and has been proven to give good recoveries for a wide scope of pesticides and matrixes. The major advantage of this extraction method, apart from its speed, is that the small quantities of reagents can be used in analysis, and also allows the simultaneous determination and confirmation of very large number of pesticides. The only disadvantage of extraction method A is relatively stronger matrix effect when the plants used in TCM have most complex matrixes. As an alternative clean-up procedure, we have developed on the basis of SPE, which was frequently available nowadays in routine pesticide analysis laboratories. In the method B solid phase extraction (SPE) cartridge layered with GCB/PSA was applied as complementary to QuEChERS method in order to reduce the matrix effects from plants used in TCM. For seed and fruit herbal which contain much fat and wax, C18 SPE cartridge was essential. Nevertheless, method B is also insufficient, for example, more eluent was needed to avoid the loss of target compounds because the pesticides in research covered a wide range of polarity and volatility, and must be concentrated before injected into the LC–MS/MS in order to improve the sensitivity of the approach.

3.4. Method validation

3.4.1. Linearity and matrix effects

The linearity and matrix effects of the analytical procedure were studied in calibration standards prepared in neat solvent (0.1% formic acid in methanol/water: 3/2) and in blank TCM matrix extract, which were prepared with modified QuEChERS. The seven-point-calibration curves in solvent and five-point-calibration curves in the five matrixes (*Radix Ginseng*, *Flos Loncerae*, *Semenpersicae*, *Herba Lophatheri*, *dogwood*) were constructed and compared, respectively. The linear calibration curves were not forced though the origin and the regression line calculated by a weighting of $1/x$ when deviation is large in small calibration point, and x in large calibration point.

The linear regressions showed that 90% of the pesticides presented good linearity with coefficients of determination (r^2) better than 0.99 except for some particular pesticides, such as butamifos, chlorpyrifos, fomesafen, disulfoton and sulfotep exhibiting a narrower linear range or bad linearity in the range studied ($r^2 < 0.99$). This phenomena may be caused by low sensitivity [32] in electrospray ionization, especially in the *Herba Lophatheri*, which depends on the properties of the analytes and the presence of other ionizable compounds [33]. So these 15 pesticides with unreliable information for further research were deleted from the list of pesticides quantified. These results are included in Table S2.

To evaluate matrix effects, slope ratios (slope matrix/slope solvent) of the five plants used in TCM in different class (root, seed, flower, leaf and fruit) are compared for selected pesticides in Table S3 and it can be observed that the relative response for most of the investigated pesticides were smaller than 1. These results showed that signal suppression was prevalent. It is noticeable that in some cases, strong suppression appeared in all five matrixes, like methamidophos, tolclofos-methyl and monocrotophos, while for pirimiphos-ethyl, cyromazine, mevinphos, propamocarb and cymoxanil, strong suppression only appeared in some special matrixes. This might not be due to matrix effects in the MS, but rather the interaction of pesticides with the matrix. Strong signal enhancement was not very important in any of the matrixes or compounds. Only pesticides, such as imidacloprid, acetamiprid, fomesafen and propamocarb, strong signal enhancement occurred in some given matrixes. Pyrazophos and pirimiphos-methyl were the one which have strong enhanced effect in all five matrixes, pyrazophos was significantly higher in *Radix Ginseng*, while pirimiphos-methyl in *Flos Loncerae*. To sum up, soft, medium and strong signal suppression or enhancement were observed in Fig. 4(A).

Dogwood is a special case with strong enhancement in signal in Table S3 and it may be due to the following reason: One standard

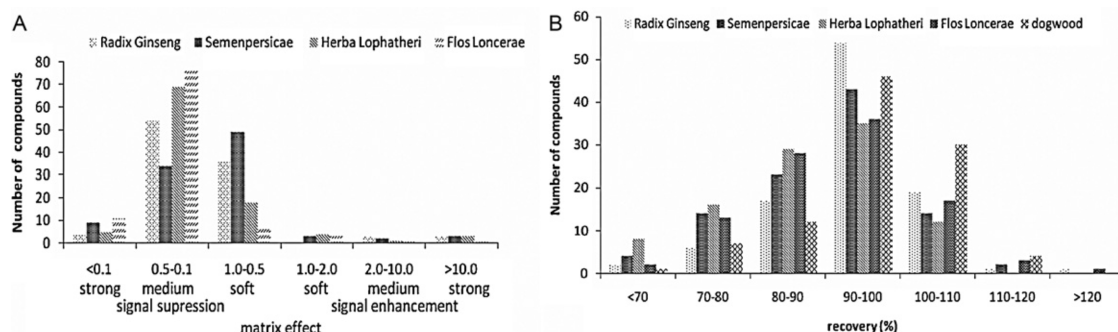

Fig. 4. (A) Distribution of matrix effects; (B) distribution of the recoveries.

curve cannot be used in long term due to the fluctuations in instrument performance, while in this text only one solvent calibration was selected in order to discuss the matrix effect in five plants used in TCM simultaneously. This indicated that the same calibration has not been used in long term because of the fluctuation of the instrument. So within each batch of samples analyzed, a matrix-matched calibration standard was prepared freshly to quantify pesticides in corresponding plants used in TCM. This is not only compensating the matrix effect but also preventing the instrument errors.

3.4.2. Accuracy and precision

Accuracy and precision were evaluated by recovery and repeatability experiment established by extraction method A. In order to get more accurate results, the recoveries were calculated using matrix-matched single-level calibration standards at concentration levels same as the spiking level of the pesticides. Detailed recovery and repeatability data for all pesticides ($n = 3$) analyzed in the five matrixes at the three spiking levels 0.01, 0.05 and 0.1 mg/kg are appears from Table S4. The distribution of the mean recoveries is shown in Fig. 4(B). The recoveries of most pesticides were in the accepted range of the DG SANCO/2007/1313 of the European Quality Control Guide lines: 70–120%, with associated relative standard deviations (RSDs) less than 15%, except for cyprodinil, demeton methyl, phosalone, carbendazim, etc., which showed recoveries lower than 70% or RSDs higher than 15% (see Table S4). In the case of *dogwood*, a precise quantification is impossible due to very high RSD for cyprodinil, carbendazim, phosalone, chlorpyrifos-methyl, etc. These lower recoveries or large RSD values could be linked to pesticides that may be unstable or decomposed during sample extraction. Generally, the good recoveries and RSD obtained in this study support the adequacy of the method with the exception of only a few pesticides.

3.4.3. Analytical limits

The analyte quantification limits (LOQs) evaluated as the lowest concentrations were tested by analyzing matrix-matched standards and checking for peak signals with a signal to noise greater than 10. The results obtained are shown in Table S3, and it can be observed that more than 90% of the cases were below or equal to 10 μg/kg and the most frequent LOQ was 5 μg/kg. Furthermore, it can be observed that LOQs were higher in *Herba Lophatheri* than in the other matrixes, because of the reduction of the sample weight. Some particular pesticides, such as EPN, methacrifos, dichlorvos, chlorpyrifos, chlorpyrifos-methyl, and tolclofos-methyl, have higher LOQ (50–100 μg/kg) in each matrix, this may be relative to the ionization efficiency. For most of the pesticides, the detection limits were not affected, or were only slightly affected, by the studied matrixes, except for thiophanate-methyl (0.1 mg/kg LOQ in *Herba Lophatheri*), thiophanate-methyl (0.1 mg/kg in *Herba Lophatheri*), *dichlorvos* (0.005 mg/kg in *Semenpersicae*), etc. It could be emphasized that for all of the pesticides in the plants used in TCM matrixes investigated the quantification limits were equal to or lower than the maximum residue limits established by European Union [34].

3.4.4. Application to real samples

The proposed method was applied to the analysis of 138 samples containing 102 varieties of matrixes. 55 compounds in 95 positive samples were found. While most of them showed a concentration lower than the MRL (0.05 mg/kg). Carbendazim, carbofuran, propoxur, triazophos, acetamiprid were most frequently detected. No pesticides were observed in the other 43 samples.

4. Conclusions

In this study, a new multi-residue method was developed and validated for rapid and simultaneous determination of more than 100 pesticides in plants used in TCM by UPLC–MS/MS, using modified QuEChERS methodology as the proposed extraction method. For modified QuEChERS, two different sample cleaning steps, namely DSPE and SPE, were optimized for the purification of plants used in TCM extracts. It was found that DSPE was more convenient and inexpensive than SPE which is advantage of cleaning efficient. While in this work, both cleanup steps were able to provide suitably clean extracts for the five select plants used in TCM. The linearity, matrix effect, recovery, limit of analysis and repeatability were studied in *Radix Ginseng*, *Flos Loncerae*, *Semenpersicae*, *Herba Lophatheri* and *dogwood*. For most pesticides, the linearity of the calibration was good between 5 and 100 μg/kg concentration ranges, and the limits of quantification (LOQs) less than 0.01 mg/kg. The mean recoveries of almost all pesticides were in the range from 70% to 120% at three concentration levels ranging from 0.01 mg/kg to 0.1 mg/kg with the relative standard deviations (RSD) better than 15%, except for some problematic pesticides. Furthermore, there were no significant differences between the relative responses of different matrixes except for *dogwood*. Lastly, the determination time was only 15 min, which is shorter compared to traditional methods and suitable to allow determination of all 116 analytes in a single injection at both ESI+ and ESI− modes. The developed method combines the selectivity, high resolution capacity and fast analysis of UPLC–MS/MS with the advantages of QuEChERS (fast, easy, cheap, robust and efficient), providing a simple, rapid and reliable method to analysis the pesticides in plants used in TCM with high quality of results (good linearity, sensitivity, selectivity, recovery, repeatability, and wide analytical scope) and practical benefits (low cost, high sample throughput, little labour, hardly any waste and few labwares and space demands).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.071.

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