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Determination of pesticide residues in ginseng by dispersive liquid–liquid microextraction and ultra high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A procedure involving acetonitrile-based extraction combined with dispersive liquid–liquid microextraction (DLLME) and detection by ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was used for determination of 39 pesticides in ginseng. The extraction of pesticide residues in ginseng was performed with acetonitrile, applying QuEChERS methodology, and the extract was further disposed by DLLME method before analyzed by UHPLC–MS/MS. The average recoveries ranged from 70 to 120% for 82% of the analytes with RSD lower than 15%. The calibration curves obtained with blank matrices were linear with a correlation coefficient of over 0.99. The limits of detection were between 0.01 and 1.0 μg/kg. Matrix effects were studied by comparing solvent calibration curves and matrix-matched calibration curves. The results indicate the feasibility of this method for the determination of 39 pesticides in ginseng.

1. Introduction

Ginseng as a Chinese medicine or dietary supplement has been used for thousands of years. Ginseng and its constituents have been ascribed antineoplastic, antistress, and antioxidant activity. The growth period of ginseng is quite long. Usually, it will take 4–6 years. During these years, the pesticides, such as organochlorine, organophosphorous and carbamates, have often been used [1,2], and therefore, it will cause environmental pollution and endanger human health.

To improve the detection of pesticides and contaminants in dried and powdered ginseng, effective methods and analytical techniques are needed. The test for various pesticides involving individual organochlorine (OCP) [3] and OCPs multiresidues in ginseng [4] has been reported. The test for multiclass-pesticide residues were also performed by Hayward and Wong using both gas chromatography–single quadrupole mass spectrometry with selected ion monitoring (GC–qMS-SIM) and gas chromatography–high resolution time-of-flight mass spectrometry (GC–HR-TOFMS) [5]. Although polar pesticides, such as organophosphorous (OPs), carbamate, strobilurin and triazole, are generally less persistent than OCPs and extremely toxic to animals and humans, they are still frequently used on agricultural crops as easily degradable. Therefore, more attention was paid to these pesticides with the increasing need for the polar and less volatile pesticides recently. Thus HPLC hyphenated to tandem mass spectrometry (HPLC-MS/MS) in multi-class pesticide residues analysis has become more increasingly appropriate than gas chromatograph hyphenated to tandem mass spectrometry (GC-MS/MS).

The analysis of pesticide residues in ginseng, which contains amino acids, carbohydrate, ginsenoside and volatile oil, is a challenging issue, due to high complexity of the matrix, low concentrations of analytes and wide range of physico-chemical properties of pesticides. Generally, the most efficient analytical approach is the multiclass-pesticide residues analysis method. While an inherent difficulty with multiclass-residue analysis is that more matrix co-extractives and potential interferences should occur with the increase of polarity range of targets [6]. The extraction and clean-up procedures are therefore critical steps to improve the determination speed and the sensitivity.

An ideal pre-treatment technique should able to isolate the analytes from a matrix, clean up the extract, as well as concentrate the analytes in one step. For the treatment of solid sample, homogenization with organic solvent is the first step in most methods. Subsequently, the extract could be cleaned up and targets could be enriched using a suitable solid-phase extraction (SPE) [7,8], liquid–liquid extraction (LLE) [9,10] or other procedures [11–17]. However, SPE and LLE suffer from the disadvantages of expensive,

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laborious, large amount of samples and toxic organic solvents. The further performance of rotary evaporation is time-consuming. For overcoming these problems, other techniques, such as solid-phase microextraction (SPME) [11,12], stir bar sorptive extraction (SBSE) [13] and liquid-phase microextraction techniques (LPME) [14–16] seem very promising. However, the disadvantages, such as sample carry-over, relatively high cost, fibre fragility, and, also, time consuming are often encountered.

Recently, the extraction procedure named as "*quick, easy, cheap, effective, rugged and safe*" (QuEChERS) is the most common technique used in analysis of multi-pesticide residues in samples such as vegetables, olive oils and cereals [18–20]. Their main advantages include high recoveries obtained for a wide range of pesticide polarities, adequate trueness and precision, high sample throughput, simple instrumentation and materials, and low cost per sample. This method is based on liquid–liquid partition with acetonitrile. After that is dispersive SPE clean-up with primary secondary amine (PSA). The major drawback of this technique is the poor enrichment factor to lead to a higher detection limits than other techniques. In our previously work [21], we have improved the detection limits by extra concentration procedure. To overcome this deficiency, some authors proposed that a dispersive liquid–liquid microextraction (DLLME) was carried out after QuEChERS [22,23].

In 2006, as a new microextraction technique, DLLME based on LPME was firstly introduced by Rezaee et al. [24]. The advantages of the DLLME method are short extraction time, low cost, simplicity of operation and high recovery and enrichment factor. To date, most of the works about DLLME are mainly used for samples in aqueous. Therefore, a further exploration of the potential applications of DLLME in more complex samples, such as fruit, vegetables and food, is very significant. Cunha and Fernandes have reported the results for the determination of forty-one pesticide residues in maize samples [23] using QuEChERS combined with DLLME, followed by GC–MS.

In this paper, a QuEChERS-DLLME-UHPLC-MS/MS was introduced to analyze 39 pesticide residues in ginseng. In which, most of the pesticides are more polar, less volatile, and may be unstable. As their degradations are easy, thus, the pesticides were widely used in agriculture in recent years. But, it is a difficult task to analyze the pesticide residues in ginseng by GC–MS. The analysis method in the present work is UHPLC–MS/MS possessing excellent chromatographic performance and high selectivity. Several important parameters for DLLME, such as type and volume of extraction solvent, volume of dispersive solvent, effects of salt and extraction time were investigated. The method in this research firstly used for multi-pesticide residues in ginseng and can be expected to extend to the other Chinese herbal medicine.

2. Experimental

2.1. Regents and solutions

Pesticide standards with purity >98% or higher purity grade were obtained either from Ehrensdorfer (Augsburg, Germany) or from Sigma–Aldrich (USA). Individual stock solutions of the pesticides at a concentration of 1.0 mg/mL were prepared in acetonitrile, methanol or acetone. Working solutions of multiple pesticides (1.0 μg/mL) were prepared by dilution of the stock solution in acetonitrile. Acetonitrile was HPLC grade from Fisher (USA). Ultrapure water was produced by a Millipore (Bedford, MA, USA) MilliQ water purification system. Anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were all ACS grade and obtained from Beijing Chemical Reagents Company (Beijing, China). $MgSO_4$ was activated by heating at 650 °C for 4 h and NaCl at 105 °C for 4 h before use and kept in desiccator. Extraction solvents dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), and chlorobenzene (C_6H_5Cl) were obtained from Beijing Chemical Reagents Company (Beijing, China).

2.2. Instrumentation and UPLC–MS/MS analytical conditions

The analytes were determined using a Waters Acquity UPLC combined with a Xevo™ TQ mass spectrometer (Waters, USA) fitted via an electrospray ionization interface (ESI). The Data acquisition and processing were performed by Masslynx 4.1 software. In this study, a Waters UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size) was used. Gradient elution was performed with acetonitrile (LC grade) as mobile phase A and 0.1% formic acid in water as mobile phase B. The gradient program was started with 10% component A (90% B) at injection time and increased linearly to 30% A (70% B) in 1 min, further to 90% A (10% B) over 9 min, where it was held for 2 min before it returned to the initial stating condition. The temperature of the column was kept at 35 °C and sample manager was at 4 °C. The flow rate of the mobile phase was set at 0.3 mL/min. The sample volume injected was maintained at 7.5 μL.

Mass spectrometric analysis was performed using the multiple reaction monitoring (MRM) modes and operated in both positive and negative ion modes. All MS parameters were optimized in the combine mode except for the poorly ionized pesticides. The ESI ion source parameters were as follows: capillary voltage, 3.20 kV; extractor voltage, 3.00 V; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas (nitrogen) flow, 50 L/h; desolvation gas (nitrogen) flow, 800 L/h; collision gas (argon) flow, 0.16 mL/min. The LC–MS/MS parameters, such as precursor-to-product ion transitions, cone voltage and collision energy, are listed in Table 1.

2.3. Sample preparation

Pesticides were extracted from ginseng using a modified QuEChERS method combined with DLLME procedure. Roughly summarized it consisted of the following steps: (1) 2.00 g of dry powdered ginseng was weighed and transferred into a 50 mL Teflon centrifuge tube; (2) 10 mL of ultra-pure water was added into the tube and vigorously vortexed, followed by 10 mL acetonitrile after 1 h; (3) the Teflon centrifuge tube was vortexed for 1 min with a vortex mixture; (4) 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the tube and shaken immediately for 1 min by vortex mixer; (5) the tube was centrifuged at 3500 rpm for 10 min. Then a DLLME procedure was carried out: (6) 1 mL of the MeCN extract was transferred into a 4 mL vial tube and mixed with 100 μL of chloroform ($CHCl_3$); (7) the mixture was injected rapidly into a 15 mL screw cap plastic tube containing 5 mL of deionized water with salt concentration of 8%; (8) the tube was centrifuged at 3500 rpm for 10 min after vortexed for 30 s; (9) the sedimented phase was completed transferred into another test tube and then evaporated to dryness with a mild nitrogen stream; (10) the residue was re-dissolved in 200 μL of MeOH.

3. Results and discussion

3.1. Selection of the extraction method

For the performance of DLLME method, the first step is to extract analytes from ginseng samples. Four extraction methods have been investigated. (a) 30 g sample of ginseng was added with 1 L of water and then the mixture was ultrasonicated for 1 h. The mixture was kept overnight (at 4 °C) and filtered through filter paper [25]. This method needs large amount of sample and requires a lot of time. (b) Traditional extraction methods with organic solvents. A well-known drawback of them is demanding for too much organic solvent which results in high cost and pollution. (c) 1.0 g of sample

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

Compound	Molecular mass	Molecular formulas	Retention time (min)	Primary trace[a]	Secondary trace	Cone (V)	CE1[b] (eV)	CE2[c] (eV)	Scan mode
Acetamiprid	222.67	$C_{10}H_{11}ClN_4$	2.62	223.1/126.1	223.1/56.1	20	20	16	ESI+
Alachlor	269.77	$C_{14}H_{20}ClNO_2$	6.38	270.0/238.1	270.0/162.0	19	12	21	ESI+
Azoxystrobin	403.39	$C_{22}H_{17}N_3O_5$	5.67	404.1/372.0	404.1/343.8	20	13	23	ESI+
Buprofezin	305.44	$C_{16}H_{23}N_3OS$	9.41	306.2/201.1	306.2/116.1	19	11	15	ESI+
Butachlor	311.85	$C_{17}H_{26}ClNO_2$	8.54	312.4/162.1	312.4/238.2	20	21	11	ESI+
Carbofuran	221.25	$C_{12}H_{15}NO_3$	3.93	222.2/165.2	222.2/123.1	17	12	20	ESI+
Cyprodinil	225.29	$C_{14}H_{15}N3$	6.88	226.2/93.1	226.2/108.2	41	30	23	ESI+
Difenoconazole	406.26	$C_{19}H_{17}Cl_2N_3O_3$	7.01	406.1/251.0	406.1/337.0	31	24	17	ESI+
Diniconazole	326.22	$C_{15}H_{17}Cl_2N_3O$	6.52	326.2/70.1	326.2/158.9	33	25	29	ESI+
Ditalimfos	299.28	$C_{12}H_{14}NO_4PS$	6.34	300.1/148.0	300.1/244.0	19	18	13	ESI+
Ethoprophos	242.34	$C_8H_{19}O_2PS_2$	5.92	243.0/173.1	243.0/130.9	20	15	21	ESI+
Etrimfos	292.28	$C_{20}H_{17}N_2O_4PS$	7.19	293.1/265.0	293.1/125.0	29	17	23	ESI+
Fenarimol	331.20	$C_{17}H_{12}Cl_2N_2O$	5.56	331.0/268.1	331.0/81.1	32	23	27	ESI+
Fenchlorphos-oxon	305.49	$C_8H_8Cl_3O_4P$	5.76	305.0/109.1	305.0/258.0	35	24	28	ESI+
Fenobucarb	202.27	$C_{12}H_{17}NO_2$	5.35	208.2/95.1	208.2/152.1	18	13	7	ESI+
Fenoxaprop-ethyl	333.72	$C_{16}H_{12}ClNO_5$	7.86	361.9/288.1	361.9/244.0	32	18	24	ESI+
Fenthion-sulfoxide	294.33	$C_{10}H_{15}O_4OS_2$	3.83	295.2/280.0	295.2/109.1	29	18	32	ESI+
Fludioxonil	248.19	$C_{12}H_6F_2N_2O_2$	5.44	247.0/126.1	247.0/180.1	37	29	27	ESI−
Fomesafen	438.76	$C_{15}H_{10}ClF_3N_2O_6S$	3.40	437.1/285.9	437.1/221.8	35	24	27	ESI−
Isazofos	313.70	$C_9H_{17}ClN_3O_3PS$	6.69	314.0/161.9	314.0/271.9	23	16	13	ESI+
Malaoxon	314.29	$C_{10}H_{19}O_7PS$	3.79	315.1/127.1	315.1/99.1	17	12	25	ESI+
Metalaxyl	279.33	$C_{15}H_{21}NO_4$	4.38	280.2/220.2	280.2/248.1	20	13	9	ESI+
Metolachlor	283.79	$C_{15}H_{22}ClF_3NO_4$	6.37	284.1/252.1	284.1/176.2	18	15	24	ESI+
Myclobutanil	288.78	$C_{15}H_{17}ClN_4$	5.74	289.1/70.1	289.1/125.1	25	16	34	ESI+
Napropamide	271.35	$C_{17}H_{21}NO_2$	6.01	272.1/129.1	272.1/171.1	21	16	18	ESI+
Phosphamidon	299.69	$C_{10}H_{19}ClNO_5P$	3.11	300.0/127.0	300.0/174.1	21	21	13	ESI+
Pirimicarb	238.29	$C_{17}H_{18}N_4O_2$	4.40	239.1/72.1	239.1/182.2	25	20	17	ESI+
Pirimiphos-ethyl	333.39	$C_{13}H_{24}N_3O_3PS$	8.84	334.2/198.2	334.2/182.1	29	21	24	ESI+
Promecarb	207.27	$C_{12}H_{17}NO_2$	5.61	208.1/151.1	208.1/109.1	17	8	19	ESI+
Propanil	218.08	$C_9H_9Cl_2NO$	5.11	218.1/162.0	218.1/127.1	28	15	27	ESI+
Propoxur	209.24	$C_{11}H_{15}NO_3$	3.85	210.2/168.1	210.2/111.1	13	7	15	ESI+
Pyrazophos	373.36	$C_{14}H_{20}N_3O_5PS$	7.20	374.2/222.1	374.2/346.0	33	21	15	ESI+
Quizalofop-ethyl	372.80	$C_{19}H_{17}Cl_2N_2O_4$	7.88	373.1/299.1	373.1/91.0	30	18	30	ESI+
Simazine	186.64	$C_7H_{11}ClN_4$	5.43	202.1/104.1	202.1/96.1	30	26	23	ESI+
Tebuconazole	307.82	$C_{16}H_{22}ClN_3O$	6.04	308.2/70.1	308.2/125.1	27	19	36	ESI+
Tebufenozide	352.47	$C_{22}H_{28}N_2O_2$	6.64	353.3/297.1	353.3/133.1	10	7	20	ESI+
Tetrachlorvinphos	365.96	$C_{10}H_9Cl_4O_4P$	6.36	366.7/127.0	366.7/240.9	23	14	20	ESI+
Triadimefon	293.75	$C_{14}H_{16}ClN_3O_2$	5.81	294.1/197.1	294.1/69.1	23	16	20	ESI+
Triflumuron	358.70	$C_{15}H_{10}ClF_3N_2O_3$	6.91	356.9/154.0	356.9/176.1	19	13	23	ESI−

[a] The primary trace was use to quantification.
[b] CE1: collision energy of primary trace.
[c] CE2: collision energy of secondary trace.

was added with 2 mL of acetonitrile and n-hexane mixture (250:3), and magnetically stirred for 45 min at 42 °C [26]. This procedure cannot be followed in our experiment because insufficient extract have been obtained. d. Extraction with QuEChERS procedure. The extraction solvent, acetionitrile, could be used as disperser solvent in the DLLME method. Other advantages of QuEChERS are fewer impurities were obtained and small amount of sample and solvent were needed. All these merits proved that QuEChERS is suitable for DLLME method.

3.2. Optimization of the DLLME conditions

Conventional DLLME is based on a ternary system, namely, an aqueous solution containing the analytes, a water-immiscible extraction solvent and a water-miscible disperser solvent. A mixture of the disperser and the extraction solvent is rapidly introduced into the aqueous solution, and then, the equilibrium is quickly reached due to the great surface contact between the droplets of the extraction solvent and the aqueous sample. After centrifugation, the extraction solvent including the targets is normally collected at the bottom of the tube. In this experiment analytes were initially extracted with acetonitrile by the previous QuEChERS procedure and then disposed by DLLME which serves as a procedure for clean-up and enrichment of the extract. In DLLME, most of polar impurities were removed with double distilled water. Major factors affecting the extraction performance of DLLME, such as type and volume of the extraction solvent, amount of the dispersive solvent, salt addition, extraction time and shake mode were evaluated. All the experiments were repeated for three times and the means were used as the final results.

3.2.1. Extraction solvent selection

The selection of extraction solvent is crucial for the DLLME procedure. In this study four halogenated hydrocarbons including carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and chlorobenzene (C_6H_5Cl) were investigated. 1 mL of the extract (dispersive solvent) obtained by the previous QuEChERS extracted from a spiked sample (all the pesticides at 0.1 mg/kg) was mixed with 100 μL of each extractive solvent and rapidly injected into 5 mL of deionized water. For CH_2Cl_2, no cloudy solution was observed and no separated phases were obtained after centrifugation, so CH_2Cl_2 was rejected. The effects of the extraction solvents (CCl_4, $CHCl_3$ and C_6H_5Cl) on the peak area with the use of acetonitrile as dispersive solvent are shown in Fig. 1. The results in this figure showed that $CHCl_3$ gave the highest extraction efficiency for all the pesticides investigated except for few polar pesticides. Thereby, $CHCl_3$ was selected as the extraction solvent.

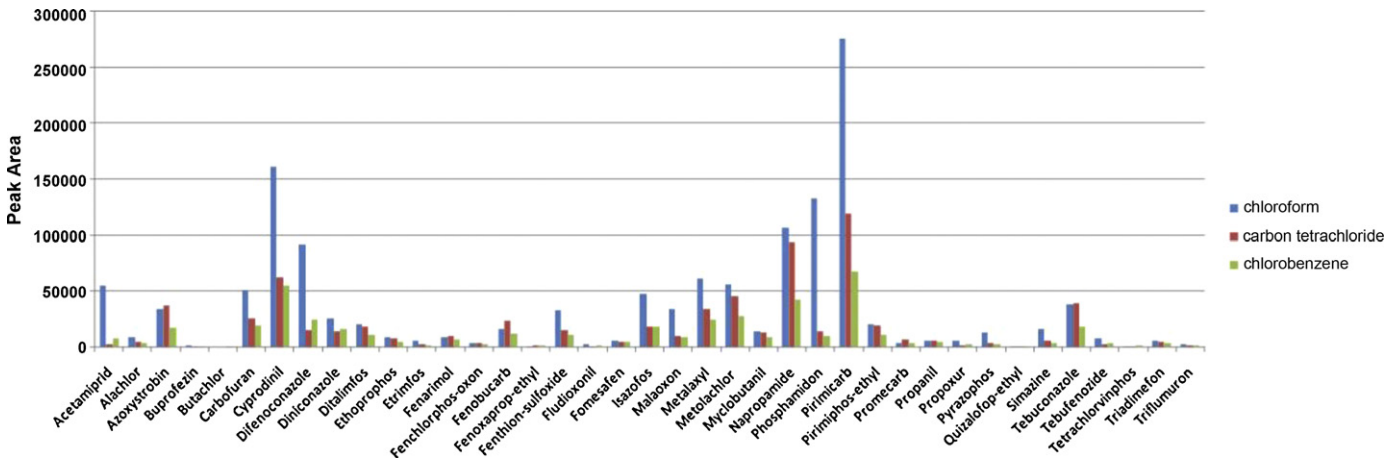

Fig. 1. Comparison of average peak area response obtained with the different extractive solvents ($n = 3$).

3.2.2. The effect of extraction solvent volume

The volume of extraction solvent is another important factor that affects the extraction efficiency. 1 mL of QuEChERS extract (obtained from a spiked ginseng sample) mixed with five different volumes of $CHCl_3$ (50 μL, 100 μL, 150 μL, 200 μL and 250 μL) were observed. The amount of sediment obtained was small when 50 μL extraction solvent was used, and therefore the poor reproducibility was resulted. Extraction efficiency remained almost constant or slightly fluctuated for all the target analytes with the increment of the volume of $CHCl_3$ from 100 μL to 250 μL. Extra vortex was needed with higher volumes of $CHCl_3$ because the cloudy suspension of droplets may not formed well [22]. Therefore, 100 μL of $CHCl_3$ was selected in the following studies.

3.2.3. The effect of dispersive solvent volume

The next step performed in the optimization of the proposed method was the evaluation of the dispersive solvent volume. In order to study the effect of the volume of the dispersive solvent on the performance of the presented DLLME procedure, different volumes of acetonitrile (containing same amount of pesticides) varied in the range from 1 to 3 mL in 0.5 mL intervals, mixed with 100 μL of extractive solvent were added into 5 mL of deionized water. With more than 2 mL of acetonitrile, no two phase system was observed. The results shown in Fig. 2 revealed that increasing the volume of acetonitrile will decrease the extraction efficiency for all the targets. This may be because the solubility of the targets in water was increased with the increase of the volume of dispersive solvent. Based on the above results, 1 mL of acetonitrile was selected as the optimized dispersive solvent volume.

3.2.4. The effect of extraction time and shake mode

Dispersion is the key step for effective extraction. The effects of extraction time from 0 to 20 min and shake modes including no shake, vortex, and ultrasonic shake were observed. The results showed that the time and shake modes did not affect significantly the extraction efficiency. The reason may be that the surface area between the extraction solvent and the aqueous phase is "infinitely" large after the formation of a cloudy solution. Subsequently, equilibrium state could be achieved quickly, and therefore the extraction time was very short. This is a remarkable advantage of DLLME technique.

3.2.5. Effect of salt

The solubilities of the target complex and organic extraction solvent in the aqueous phase usually decrease with the increase of ionic strength [27]. To validate this phenomenon, sodium chloride as a salt regent with the concentrations from 0 to 20% (w/v) was observed. As shown in Fig. 3, by increasing NaCl concentration up to 8%, the peak areas of some polar pesticides, such as acetamiprid, malaoxon and pirimicarb were increased. However, no significant changes were observed for others in this concentration range of sodium chloride. On the other hand, the stability of the ternary component solvent system was reduced with the further increase of salt concentration. When the salt concentration was higher than

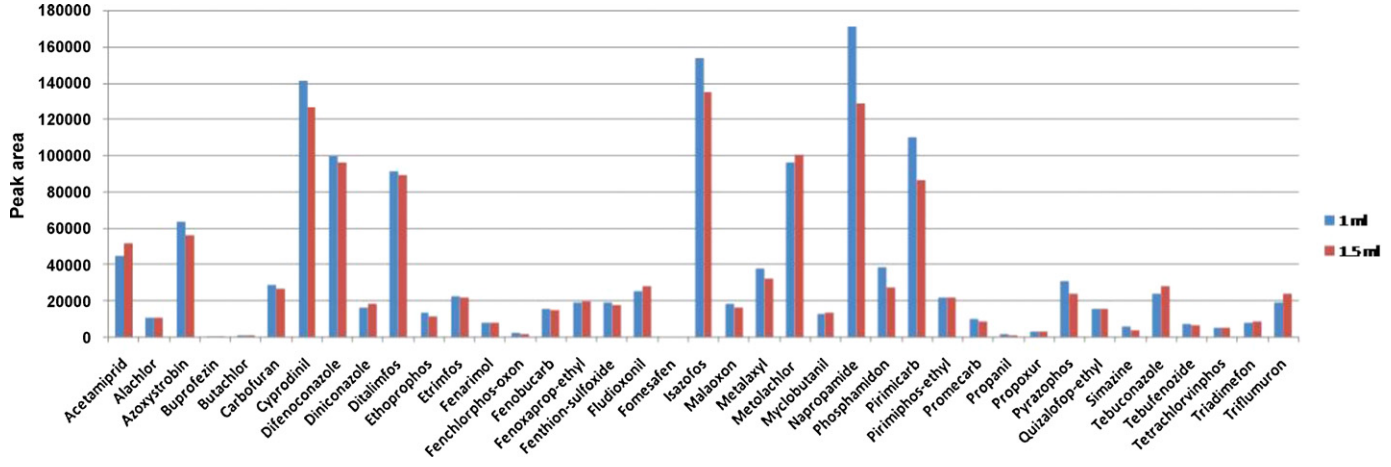

Fig. 2. Comparison of average peak area response obtained with different volumes of the dispersive solvent ($n = 3$).

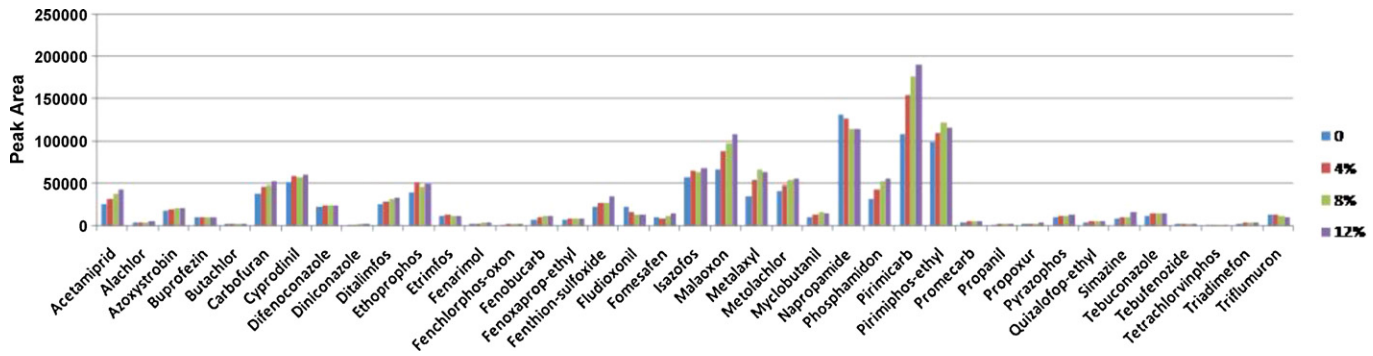

Fig. 3. Comparison of average relative peak area response obtained with different salt addition ($n = 3$).

12%, the sediment could not be formed at the bottom of the centrifuge tube. Therefore, the concentration of 8% of NaCl was used in further experiments.

3.3. Matrix effects

In order to circumvent errors associated with matrix co-eluting components, matrix effects were evaluated by comparing solvent calibration curves and matrix-matched calibration curves. In this study, matrix effects were calculated by the ratio of the slopes of the calibration curves obtained in matrixes (ginseng free of pesticides) and in pure standard solutions, which was then multiplied by 100 to get the enhancement or suppression in percentage [23]. The results are shown in Fig. 4. The majority of the analytes, about 64%, reported signal enhancement (between 100% and 150%) while 26% showed signal suppression. Strong signal increase (higher than 200%) only occurred for propanil. This is consistent with the literature results [23]. Fig. 5 shows the matrix and solvent match calibration curves obtained for three tested pesticides: alachlor, triflumuron and pirimiphos-ethyl. Alachlor and triflumuron were chosen for illustrating the positive (signal enhancement) and negative (signal suppression) matrix effects, respectively (see Fig. 5a and b). The behavior of pirimiphos-ethyl (see Fig. 5c) indicates the absence of matrix effects. In this study, the matrix-matched standards were used in order to obtain more realistic results.

3.4. Method validation

To evaluate the overall analytical method, linear range, analytical limits (including LODs and LOQs), accuracy and precision were investigated. Matrix matched calibration curves were prepared using matrix-matched calibration solutions (standards added to blank samples) in at least 5 points. Calibration curves were constructed by plotting peak area of the primary trace ion of the analyte obtained against the concentration values. The results are shown

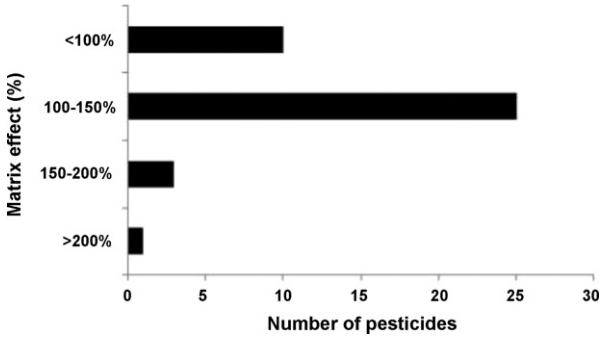

Fig. 4. Distribution of pesticide response difference to matrix-effect.

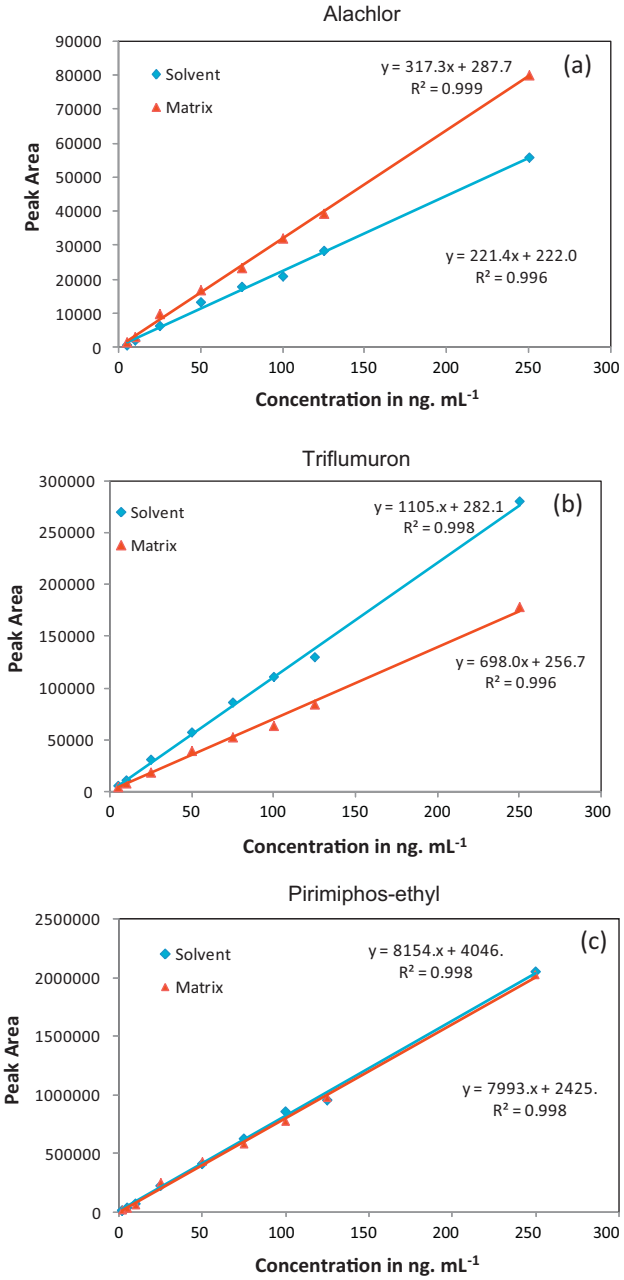

Fig. 5. Comparison of solvent (quadrangle) and matrix-matched (triangle) calibration curves, illustrating the presence of matrix effects: (a) positive or signal enhancement for Alachlor, (b) negative or ion suppression for triflumuron, and (c) no effect for pirimiphos-ethyl.

Table 2
Linearity, limit of detection (LOD, μg/kg), mean recoveries (Rev %) and repeatability (RSD %).

Compound	Linearity range (mg/kg)	R^2	LOD (μg/kg)	Level of concentration						RSD[b] (%)
				0.02 mg/kg		0.05 mg/kg		0.1 mg/kg		
				Rev (%)	RSD[a] (%)	Rev (%)	RSD[a] (%)	Rev (%)	RSD[a] (%)	
Acetamiprid	2–250	0.9995	0.3	93	7	95	5	88	5	4
Alachlor	2–250	0.9992	1	85	5	96	9	82	4	8
Azoxystrobin	5–250	0.9991	0.3	70	10	84	10	73	14	10
Buprofezin	2–250	0.9977	0.3	97	5	91	16	98	13	4
Butachlor	5–125	0.9950	1	76	3	82	3	94	10	11
Carbofuran	2–125	0.9974	0.3	100	5	97	4	83	5	10
Cyprodinil	2–125	0.9987	0.3	82	18	129	44	92	12	24
Difenoconazole	2–250	0.9989	0.1	72	3	72	1	60	3	10
Diniconazole	2–250	0.9983	0.3	84	10	96	8	97	13	8
Ditalimfos	2–125	0.9992	1.0	87	16	90	1	89	3	2
Ethoprophos	2–250	0.9980	1.0	90	7	83	5	107	12	13
Etrimfos	2–125	0.9971	0.3	93	9	92	8	90	6	2
Fenarimol	2–250	0.9990	0.3	94	3	91	6	87	4	3
Fenchlorphos-oxon	2–250	0.9987	1.0	110	5	101	7	90	6	10
Fenobucarb	2–125	0.9976	0.3	89	5	88	1	86	6	1
Fenoxaprop-ethyl	5–100	0.9979	0.01	90	5	71	11	79	7	12
Fenthion-sulfoxide	2–250	0.9991	0.1	87	6	87	6	75	3	8
Fludioxonil	2–250	0.9985	0.3	86	11	90	27	96	10	5
Fomesafen	5–252	0.9978	0.3	94	1	88	12	80	2	8
Isazofos	2–125	0.9968	0.3	93	8	94	4	89	10	3
Malaoxon	2–250	0.9981	0.1	100	7	87	5	96	1	7
Metalaxyl	2–125	0.9996	0.3	95	7	103	6	91	7	6
Metolachlor	2–250	0.9981	0.3	94	2	105	2	86	3	10
Myclobutanil	2–125	0.9982	0.3	98	12	99	4	86	10	7
Napropamide	2–250	0.9980	0.1	84	9	117	12	113	3	17
Phosphamidon	2–250	0.9977	0.1	113	2	100	4	93	3	10
Pirimicarb	2–125	0.9992	0.1	102	9	91	8	83	5	10
Pirimiphos-ethyl	2–125	0.9990	0.1	97	6	93	4	80	5	10
Promecarb	2–250	0.9994	1.0	95	12	84	5	127	3	22
Propanil	2–250	0.9986	0.3	115	7	91	11	86	6	16
Propoxur	2–250	0.9985	0.3	85	8	94	11	82	9	7
Pyrazophos	2–250	0.9960	0.1	83	1	87	8	90	8	4
Quizalofop-ethyl	2–125	0.9972	1.0	90	4	92	10	100	4	6
Simazine	2–250	0.9995	0.3	93	7	97	2	95	10	2
Tebuconazole	2–250	0.9979	0.3	83	6	87	3	54	10	24
Tebufenozide	2–250	0.9987	1.0	93	6	96	11	88	11	5
Tetrachlorvinphos	5–250	0.9985	1.0	90	8	83	2	86	24	4
Triadimefon	2–125	0.9987	0.3	90	12	100	6	89	8	7
Triflumuron	2–250	0.9991	0.3	91	10	104	2	98	11	7

[a] Intra-day repeatability.
[b] Inter-day repeatability.

in Table 2. The linearity of the pesticides in the studied range, with determination coefficient higher than 0.99, is good.

A rough limit of detection (LOD) values were evaluated by injecting blank sample extracts spiking at the 0.01–0.03–0.1–0.3–1.0–2.0 and 5.0 μg/kg concentration levels. The LODs were settled as the lowest concentrations whose signal to noise ratio was greater than 3 (see Table 2). The LODs were in the range from 0.01 to 1.0 μg/kg for all the cases. LOQs were determined as the lowest concentration level of calibration curves in this experiment. Most of the pesticides have LOQs 2.0 μg/kg, except for azoxystrobi, butachlor, fenoxaprop-ethyl, fomesafen and tetrachlorvinphos which were 5.0 μg/kg (see Table 2).

The recovery and repeatability were determined on blank (free of pesticides) samples of ginseng spiked with pesticides at three concentration levels. The mean recoveries and repeatability of the pesticides ($n = 3$) at the spiking levels 0.02, 0.05 and 0.1 mg/kg are shown in Table 2. Approximately 82% of the pesticides presented recoveries between 70% and 120% with RSD lower than 15%. The results provide evidences that the optimized method achieves acceptable recoveries in line with criteria set by the DGSANCO/2007/3131 of the European Quality Control Guidelines: 70–120% [28]. Pesticides with recoveries not satisfying these criteria were cyprodinil, difenoconazole, promecarb and tebuconazole. This may be due to degradation during extraction procedure. Cyprodinil, fludioxonil, promecarb, tebuconazole and tetrachlorvinphos were not exactly determined by this method for higher RSD values than 20%.

3.5. Application to real samples

The proposed method was applied to the analysis of different samples, including aerial parts of ginseng, taproot of ginseng, fibrous root of ginseng, codonopsis pilosula, figwort root, american ginseng and heterophylly falsestarwort root. All these samples are famous traditional Chinese herbal medicine. In the real samples, fludioxonil, azoxystrobin and cyprodinil were detected and they were confirmed by spiking multi-pesticides into the real samples. But all the concentrations of the obtained pesticides were lower than the MRL (0.05 mg/kg). Other pesticides were not observed for all the samples.

4. Conclusions

In this work, a QuEChERS-DLLME-UHPLC-MS/MS method for the determination of 39 pesticides in ginseng was described. Less time was spent here to evaporate 100 μL $CHCl_3$ than original method we

have developed. The results demonstrate that the proposed method has good recoveries and reproducibilities. The proposed method offers many practical advantages, including simplicity, cheapness, rapidity of extraction and high sensitivity. The method presented in this research can be applied to the analysis of pesticides in more complex matrixes such as codonopsis pilosula, figwort root, american ginseng and heterophylly falsestarwort root. This implies that the method has the potential to be popularized as a preparation strategy for the analysis of pesticides in Chinese herbal medicine.

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