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Convenient Analytical Method for Quantitative Determination of 23 Pesticide Residues in Herbs by Gas Chromatography-Mass Spectrometry

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Abstract A convenient analytical method for quantitative characterization of 23 pesticides in three herbs has been developed. Pesticides tested included organochlorine, organophosphorus and pyrethroids. Primary secondary amine and graphitized carbon black as dispersive-SPE sorbent were applied to clean up the sample. Analytical method was established by using the technique of gas chromatography coupled with electron impact mass spectrometry in the selected ion monitoring mode (GC–MS–SIM). The recoveries of all pesticides were in the range of 78.4%–119.2% at three spiked levels of 5, 20 and 50 μg/kg, and the relative standard deviations were below 9.5%. The limits of detections of all pesticides were less than 3.0 μg/kg. This analytical method could be applied to the analysis of commonly used pesticides in commercial herbs.

Keywords Pesticide residues · QuEChERS method · Gas chromatography-mass spectrometry · *Radix Gentianae* · *Cortex Phellodendri* · *Fritillaria ussuriensis Maxim*

Herbal medicines are widely used in China and around world by general public. The development of an analytical method for determination of commonly used pesticide residues in herbs would greatly avoid the possibility of adverse health effects. Pesticide residues analysis in herbs has encountered certain difficulties. In general, herbs have very complicated matrixs with a wide range of biochemical composition, water, and fat content, therefore exhibit quite different polarities, solubilities, and pKa values. Traditionally, the extraction and cleanup of samples were carried out separately and created problems in reproducibility (Tahboub et al. 2005). Thus, the development of a rapid analytical method for the determination of a wide range of pesticides in different samples has been the objective of our study.

Our analytical method adopted the principal of the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) (Lee et al. 2008; Anastassiades et al. 2003), which can provide high-quality results with a minimum number of steps and low solvent and glassware consumptions. This multi-pesticide residues method used an ACN/partitioning extraction and "dispersive SPE" to replace many complicated cleanup steps which were usually employed in traditional methods. Anhydrous magnesium sulfate, primary secondary amine (PSA) and graphitic carbon black (GCB) (Li et al. 2008) were directly added to the extract as sorbents to remove water, fatty acids and sugars.

Only a few analytical methods for the determination of pesticide residues in medicinal plants have been described in the recent literature (Qian et al. 2010; De Carvalho et al. 2009; Zhang et al. 2010). The common medicinal herbs, *Radix Gentianae* (Indications: Jaundice, eczema), *Cortex Phellodendri* (Indications: Dysentery, morbid leucorrhea and anti-inflammation) and *Bulbus Fritillariae Ussuriensis* (Indications: relieve cough and resolve phlegm), are mainly naturally distributed and cultivated in the northeast regions of China. Improper use of pesticides not only pollutes the cultivating soil and ground water but also leads to the accumulation of pesticides in the herbs, therefore, it is essential to establish a convenient quality control method

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in order to assure the safety of the herbal products. To the best of our knowledge, no analytical method has been developed to be able to simultaneously determine multi-pesticide residues in theses herbs. In this paper, we described a convenient analytical method for simultaneous determination of multi-pesticide residues of organochlorine, organophosphorus and pyrethroids in these three medicinal herbs by employing QuEChERS method: gas chromatography coupled with electron impact mass spectrometry (GC–MS–SIM).

Materials and Methods

Reference materials of pesticides were purchased from Agriculture Environment Quality Supervision, Inspection, and Testing Center in Tianjin, China and used as standards: p, p′-DDT(dichloro-diphenyl-trichloroethane), o, p′-DDT, p, p′-DDE(dichloro-diphenyl-dichloroethene), p,p′-DDD(dichloro-diphenyl-dichlorothane), α-BHC(benzenehexachloride), β-BHC, γ-BHC, δ-BHC, aldrin, dieldrin, endrin, heptachlor, hexachlorobenzene, heptachlor epoxide, bifenthrin, fenpropathrin, cyfluthrin, cypermethrin, deltamethrin, dichlorovos, thimet, malathion, parathion. Stock solutions (100 mg/L) were prepared by dissolving reference standards in n-hexane and stored at −20°C. Acetonitrile, n-hexane were obtained from Shandong yuwang group, anhydrous magnesium sulfate, sodium chloride were purchased from Tianjing Damao Chemical Reagent Factory, primary secondary amine(PSA) and graphitic carbon black (GCB) were from Beijing Agela Technologies Inc. Medicinal plants: *Radix Gentianae*, *Cortex Phellodendri, Bulbus Fritillariae Ussuriensis* were purchased from the Tongrentang Pharmacy in Shenyang, China.

A Gas chromatographic system equipped with an autosampler (Agilent 7683) were coupled to a mass spectrometer (GC/MS 6890N/5975i, USA), operating in EI and chemical ionization modes. Data were acquired and processed by GC Chemstation software. The GC separation was performed using a Hp-5MS capillary column with a length of 30 m × 0.25 mm id (0.25 μm film thickness). The oven temperature program was as follows: initial temperature, 90°C (0.5 min), 20°C min^{-1} to 150°C, 3°C min^{-1} to 200°C (2 min), and finally 15°C min^{-1} to 300°C (5 min). The temperature of the injection port was 290°C and a 1 μL volume was injected in pulsed splitless mode. Ion source temperature of the instrument was 230°C, MS Quad temperature of 150°C, and solvent delay was 3 min. The mass spectrometer was in the electron impact ionisation mode, scan from m/z 50 to 500.

Analysis was performed in the selected ion monitoring mode (SIM) based on the use of one quantitative ions. Pesticides were identified according to the retention times, qualitative ions by MS software combines artificially retrieval and references under the same chromatographic conditions in full-scan mode. Chromatogram were presented in Fig. 1.

Approximately 2.0 g of herbal powder (sieved through a No. 40 mesh sieve) was weighed into a 100 mL centrifuge tube for analysis. 8 mL water and 10 mL acetonitrile were added and vortexed for 1 min. After this, 1.0 g NaCl and 4.0 g $MgSO_4$ were added, and the vortexing process was

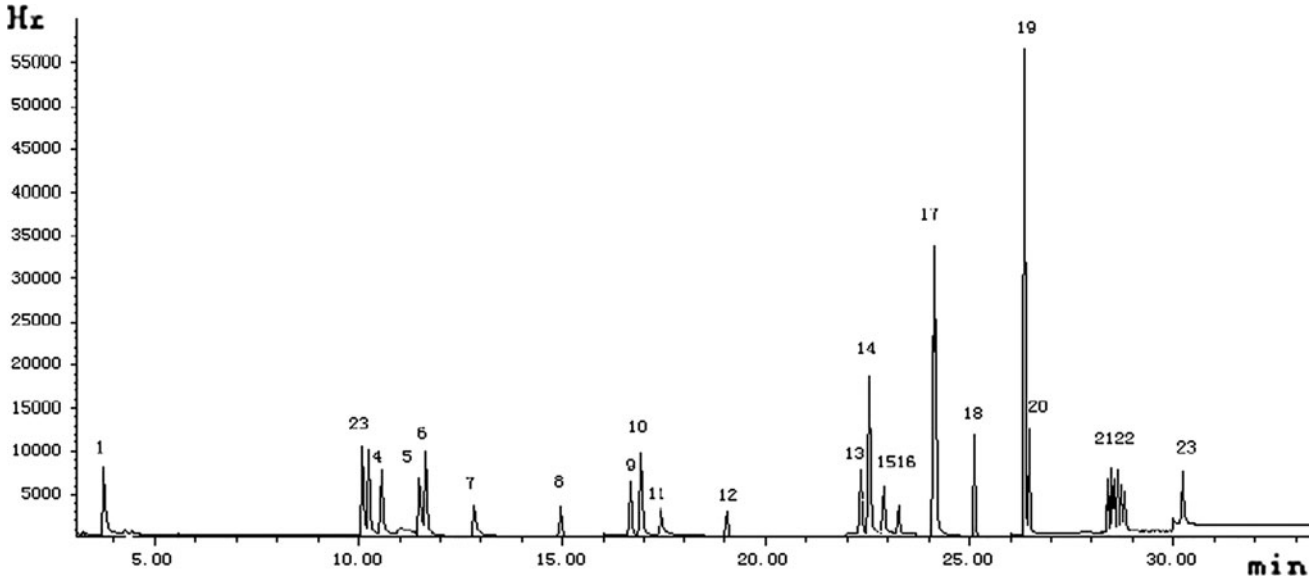

Fig. 1 GC–MS chromatogram of pesticides standard in full-scan mode. *1*. dichlorovos; *2*. thimet; *3*. α-BHC; *4*. hexachlorobenzene; *5*. β-BHC; *6*. γ-BHC; *7*. δ-BHC; *8*. heptachlor; *9*. aldrin; *10*. malathion; *11*. parathion; *12*. heptachlor epoxide; *13*. dieldrin; *14*. p,p′-DDE; *15*. p,p′-DDD; *16*. endrin; *17*. o,p′-DDT; *18*. p,p′-DDT; *19*. bifenthrin; *20*. fenpropathrin; *21*. cyfluthrin; *22*. cypermethrin; *23*. deltamethrin

repeated for 1 min. The extract was then centrifuged (4,000 r/min) for 5 min. A 1.0 mL amount of the supernatant (acetonitrile phase) was then transferred to a PP single use centrifuge tube which contains following sorbents: 300 mg PSA, 50 mg GCB, 50 mg anhydrous magnesium sulfate, after vortex-mixing for 1 min, the mixtures were centrifuged at 12,000 r/min for 5 min and the cleaned extract was transferred into a vial for subsequent analysis.

Results and Discussion

In reference to our earlier report (Zhang et al. 2010), many factors including solvent of extraction, time of extraction, the volume of extraction solvent, etc. were considered in order to achieve the highest recoveries of these pesticides in herbs. In this study, we found that sample cleaned-up was the most important step. For instance, if PSA was used alone in the extraction of *Cortex Phellodendri*, we could only obtain 50–60% recovery with significant matrix interference. Under the condition of co-sorbents (PSA and GCB), the recovery was greatly enhanced with much less matrix interference. In order to optimize the cleanup, the extraction time of 0.5, 1, 2, 3, 5 min have been studied. The result showed that with increasing times, there were no obviously improvement of recovery. In fact, we found that vortex-mixing for 1 min was the best cleanup condition. In our study, acetonitrile was chosen as the extracting solvent and 300 mg of PSA and 50 mg of GCB were added to effectively remove the interferences in the extract.

The calibration solutions were prepared by diluting the stock solutions using n-hexane in concentration range of 5–500 μg/L for organochlorine pesticide, 20–1,000 μg/L for organophosphorus pesticide and 10–1,000 μg/L for pyrethroids. Correlation coefficients were >0.99 in all case.

The limits of detection (LODs) were established by considering a value of three times the background noise of the blank sample at the retention time of each pesticide. The limits of quantification (LOQs) were calculated by considering a value of 10 times that of background noise. The LODs of all pesticides were less than 3 μg/kg and the LOQs were less than 10 μg/kg. Table 1 summarize the calibration data and the LODs and LOQs were obtained for each pesticide.

The reproducibility of the chromatographic method was established by performing the analysis of a herbal sample fortified at 50 μg/L. The sample was injected 5 times. A good reproducibility was expressed as relative standard

Table 1 Regressive equation and correlation coefficient of 23 pesticides

Pesticides	Standard curve equation	Correlation coefficient	LOD (μg/kg)	LOQ (μg/kg)
Dichlorovos	$Y = 193.7X - 137.7$	0.999 5	0.4	1.2
Thimet	$Y = 211.7X - 182.1$	0.999 6	0.1	0.4
α-BHC	$Y = 247.3X + 125.8$	0.999 9	0.3	0.9
Hexachlorobenzene	$Y = 255.4X + 34.86$	0.999 4	0.1	0.3
β-BHC	$Y = 110.5X - 80.59$	0.999 6	0.2	0.8
γ-BHC	$Y = 255.8X - 198.1$	0.999 6	0.2	0.7
δ-BHC	$Y = 172.2X - 162.3$	0.999 4	0.1	0.4
Heptachlor	$Y = 111.8X - 91.23$	0.999 6	0.3	1.0
Aldrin	$Y = 208X - 174.7$	0.999 5	0.2	0.8
Malathion	$Y = 198.8X + 490.9$	0.999 8	0.6	1.9
Parathion	$Y = 152.4X + 1{,}453$	0.998 1	0.8	2.7
Heptachlor epoxide	$Y = 112.7X - 67.97$	0.999 9	1.3	4.4
Dieldrin	$Y = 251.2X - 186.2$	0.999 9	0.1	0.4
p, *p*′-DDE	$Y = 661.2X - 434.3$	0.999 9	0.1	0.3
p, *p*′-DDD	$Y = 250.1X + 198.7$	0.999 8	0.3	0.9
Endrin	$Y = 117.3X - 21.84$	0.999 7	3.0	10.1
o, *p*′-DDT	$Y = 1231X + 1{,}022$	0.999 7	0.08	0.3
p, *p*′-DDT	$Y = 200.6X + 462.5$	0.999 4	0.4	1.4
Bifenthrin	$Y = 1718X - 447.2$	0.999 1	0.05	0.2
Fenpropathrin	$Y = 208.2X - 100.4$	0.999 6	1.7	5.7
Cyfluthrin	$Y = 230.9X - 186.1$	0.999 8	0.1	0.3
Cypermethrin	$Y = 222.9X - 117.4$	0.999 8	0.1	0.4
Deltamethrin	$Y = 178.9X - 136.0$	0.999 9	0.1	0.4

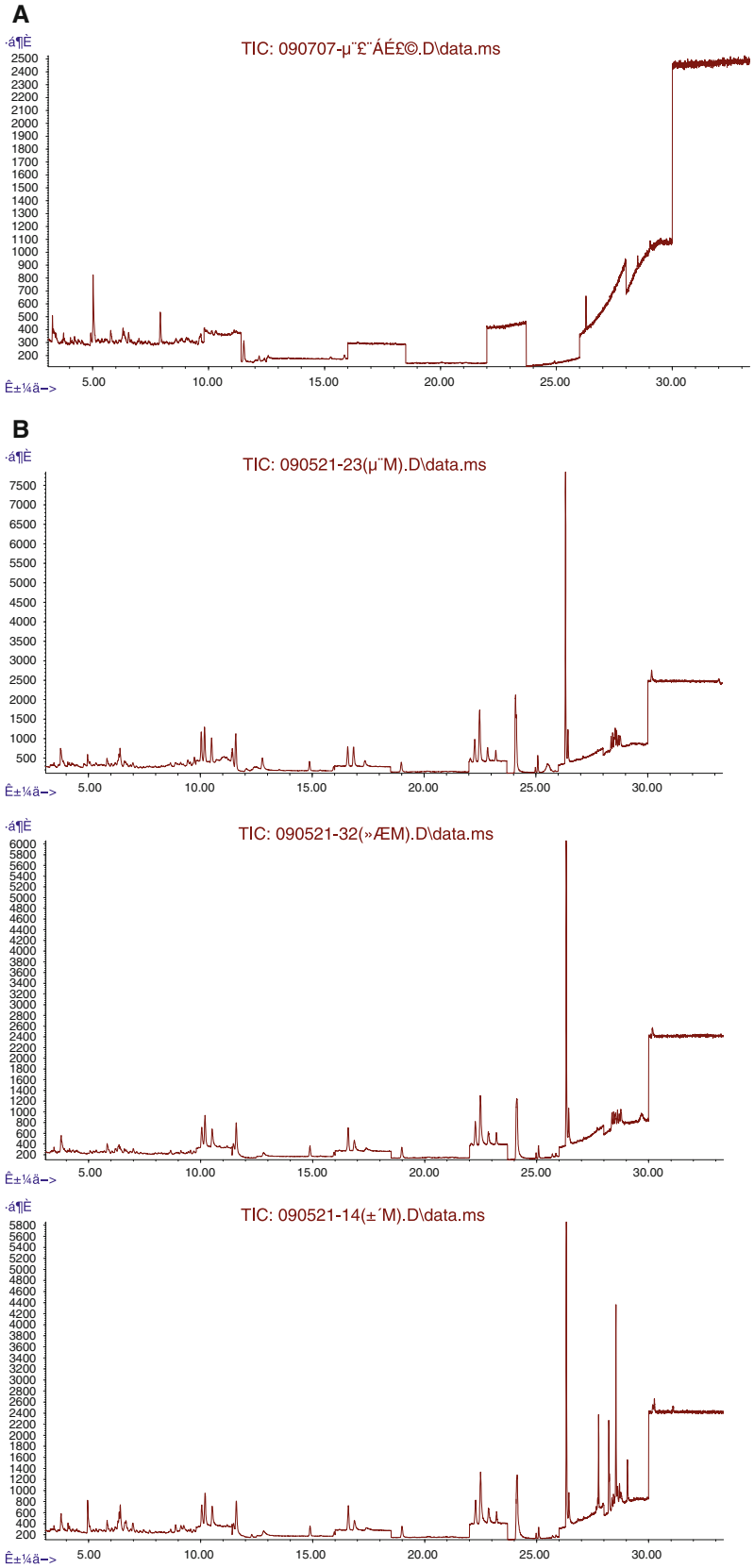

Fig. 2 **a** Blank herb sample and **b** blank herb sample spiked at 50 µg/L, (In order: *Radix Gentianae*, *Cortex Phellodendri*, *Bulbus Fritillariae Ussuriensis*)

deviations (RSD, in%), which was obtained for the peak areas within 5.8%. Moreover, the reproducibility of the complete analytical method, expressed as RSD, was in the range from 3.1% to 8.1% for all the studied compounds and replicate analysis of a fortified herbal sample for 5 times (Fig. 2).

Herbal samples, previously analyzed to verify the lack of the pesticides study, were fortified at 5, 20 and 50 μg/kg

Table 2 Recoveries of pesticides from herbal samples

Pesticides	*Radix Gentianae*			*Cortex Phellodendri*			*Bulbus Fritillariae Ussuriensis*		
	(Mean ± RSD %, n = 3)			(Mean ± RSD %, n = 3)			(Mean ± RSD %, n = 3)		
	5 μg/kg	20 μg/kg	50 μg/kg	5 μg/kg	20 μg/kg	50 μg/kg	5 μg/kg	20 μg/kg	50 μg/kg
Dichlorovos	97.4 ± 4.5	92.3 ± 4.1	100.9 ± 2.5	96.6 ± 3.3	91.8 ± 3.2	94.0 ± 4.6	101.2 ± 3.0	96.1 ± 3.2	95.4 ± 2.6
Thimet	91.3 ± 2.8	86.7 ± 4.9	98.4 ± 3.2	84.9 ± 3.6	90.8 ± 6.7	94.9 ± 6.2	83.4 ± 4.0	97.6 ± 1.6	90.9 ± 1.9
α-BHC	108.6 ± 3.1	96.1 ± 7.5	92.7 ± 2.2	110.9 ± 7.4	95.1 ± 3.7	85.8 ± 3.0	109.7 ± 1.1	97.4 ± 3.9	98.9 ± 3.5
Hexachlorobenzene	110.4 ± 3.0	97.3 ± 5.3	105.6 ± 2.0	107.7 ± 3.8	83.2 ± 3.4	85.0 ± 3.3	104.9 ± 2.8	111.1 ± 3.0	106.1 ± 1.9
β-BHC	91.6 ± 4.7	94.3 ± 3.2	90.1 ± 3.9	90.6 ± 7.2	103.5 ± 3.0	119.1 ± 4.8	90.7 ± 4.2	90.0 ± 3.8	89.1 ± 2.8
γ-BHC	93.7 ± 4.4	96.9 ± 2.8	101.1 ± 5.9	94.5 ± 7.0	96.2 ± 7.4	82.6 ± 3.9	95.5 ± 3.4	101.2 ± 7.0	92.3 ± 3.9
δ-BHC	103.3 ± 4.8	94.5 ± 2.9	92.1 ± 6.0	106.5 ± 9.5	82.6 ± 9.1	93.5 ± 3.0	109.4 ± 4.2	104.2 ± 3.0	102.9 ± 2.7
Heptachlor	102.7 ± 5.2	103.0 ± 1.6	89.4 ± 5.5	85.5 ± 4.2	110.2 ± 6.1	104.1 ± 5.5	95.5 ± 2.6	92.6 ± 2.9	107.5 ± 5.5
Aldrin	110.1 ± 1.2	93.5 ± 5.7	86.6 ± 2.8	101.8 ± 5.1	86.5 ± 4.0	117.9 ± 3.0	100.8 ± 2.4	103.2 ± 3.3	100.7 ± 2.2
Malathion	88.5 ± 5.9	84.9 ± 3.1	87.7 ± 3.9	80.3 ± 8.3	106.6 ± 6.9	88.7 ± 5.1	98.6 ± 5.5	105.6 ± 6.9	88.9 ± 5.6
Parathion	107.5 ± 7.7	85.2 ± 1.9	83.4 ± 4.8	86.8 ± 8.2	83.6 ± 2.9	87.3 ± 9.5	85.9 ± 4.4	113.2 ± 2.2	109.0 ± 3.7
Heptachlor epoxide	84.1 ± 4.3	93.1 ± 1.7	98.0 ± 2.5	78.38 ± 4.5	85.1 ± 3.7	90.3 ± 3.6	86.5 ± 4.5	97.6 ± 3.2	101.8 ± 3.8
Dieldrin	119.5 ± 4.9	115.0 ± 2.1	93.9 ± 1.4	117.1 ± 6.1	107.8 ± 3.2	92.1 ± 4.4	119.6 ± 6.5	84.1 ± 4.9	92.6 ± 4.4
p, *p*′-DDE	105.9 ± 3.9	101.9 ± 4.0	88.9 ± 6.2	94.8 ± 5.1	98.4 ± 4.7	114.0 ± 9.2	83.8 ± 3.4	105.5 ± 3.7	103.9 ± 4.2
p, *p*′-DDD	118.6 ± 3.5	117.3 ± 1.8	101.4 ± 6.7	114.6 ± 6.3	80.2 ± 6.2	103.1 ± 8.2	119.2 ± 2.3	97.5 ± 3.7	97.3 ± 2.6
Endrin	107.2 ± 6.2	103.0 ± 6.1	84.9 ± 4.1	105.3 ± 9.6	101.9 ± 2.5	106.9 ± 5.4	108.6 ± 4.2	110.1 ± 3.7	114.2 ± 3.2
o, *p*′-DDT	112.7 ± 2.4	90.4 ± 1.7	86.8 ± 2.6	79.52 ± 4.8	86.9 ± 8.2	84.9 ± 4.1	85.6 ± 5.3	90.1 ± 2.8	102.4 ± 3.7
p, *p*′-DDT	89.2 ± 6.1	98.3 ± 2.8	87.5 ± 4.9	80.2 ± 4.4	85.6 ± 1.7	96.2 ± 2.3	86.4 ± 2.1	85.6 ± 1.5	91.3 ± 3.1
Bifenthrin	108.4 ± 3.5	101.9 ± 6.3	92.0 ± 2.5	117.9 ± 5.5	90.5 ± 8.7	101.1 ± 6.4	113.6 ± 2.4	97.6 ± 1.9	90.6 ± 4.3
Fenpropathrin	94.4 ± 6.7	105.3 ± 4.8	100.9 ± 3.4	105.3 ± 9.6	101.9 ± 2.5	106.9 ± 5.4	104.4 ± 2.4	107.8 ± 3.6	101.7 ± 3.6
Cyfluthrin	106.1 ± 5.4	84.6 ± 3.1	99.1 ± 1.9	116.9 ± 6.5	104.7 ± 4.0	86.7 ± 2.7	111.9 ± 1.8	85.4 ± 3.7	82.4 ± 4.0
Cypermethrin	113.6 ± 5.0	110.1 ± 4.9	90.7 ± 2.3	96.0 ± 9.3	95.9 ± 3.4	114.0 ± 3.3	95.2 ± 5.1	84.6 ± 3.6	99.0 ± 3.1
Deltamethrin	92.7 ± 2.0	98.5 ± 4.0	87.1 ± 1.3	102.0 ± 8.5	101.7 ± 2.9	96.9 ± 2.3	93.3 ± 4.5	92.0 ± 3.1	106.9 ± 6.4

Table 3 Pesticide levels (μg kg^{-1}) found in herbs samples

Sample	Thimet	α-BHC	Hexachlorobenzene	Aldrin	Heptachlor epoxide	Bifenthrin
Radix Gentianae[a]	ND	ND	ND	ND	ND	15.06
Radix Gentianae[b]	ND	13.78	14.36	ND	ND	7.267
Radix Gentianae[c]	ND	8.524	ND	8.598	ND	13.34
Cortex Phellodendri[a]	18.57	ND	ND	ND	ND	9.336
Cortex Phellodendri[b]	ND	13.38	10.94	ND	8.546	ND
Cortex Phellodendri[c]	ND	10.26	ND	14.33	12.86	ND
Bulbus Fritillariae Ussuriensis[a]	20.47	ND	25.32	ND	ND	7.258
Bulbus Fritillariae Ussuriensis[b]	ND	ND	17.33	ND	9.763	12.64
Bulbus Fritillariae Ussuriensis[c]	ND	ND	ND	22.65	ND	8.034

ND not detected

Three different growing area of each kind of herbs: [a] Liaoning province, [b] Jilin province, [c] Helongjiang province

Pesticides not detected in every herb have been removed from the table

before extraction and prior to the chromatographic analysis. Three replications were developed for each level. The recoveries achieved following the proposed method are shown in Table 2. The recoveries obtained for all pesticides were >80.1% with RSDs <9.5%.

The developed QuEChERS-GC/MS method was applied to the determination of pesticides in three commercial herbs. *Radix Gentianae, Cortex Phellodendri* and *Bulbus Fritillariae Ussuriensis*. Table 3 summarized the pesticide levels found in three herbs on the market in China (three different growing areas of each herb were collected). In general, each sample contains at least one of the pesticides studied. The pesticides most often found were hexachlorobenzene and bifenthrin. However, the detecting levels were much lower than the MRLs established for these pesticides in foods.

In general, a convenient analytical method was established to allow simultaneous determination of 23 pesticides residue in three commercial herbs: *Radix Gentianae, Cortex Phellodendri,* and *Bulbus Fritillariae Ussuriensis*. The mean recoveries were 78.4%–119.2%, and RSDs <9.5%. As evidenced this optimized analytical method could be used to monitor 23 pesticides residue in various commercial herbs. The QuEChERS-GC/MS analysis represents a rapid, simple, and highly sensitive method to serve the quality control purposes.

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