Talanta 97 (2012) 131–141

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous determination of organophosphorus, organochlorine, pyrethriod and carbamate pesticides in *Radix astragali* by microwave-assisted extraction/dispersive-solid phase extraction coupled with GC–MS

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ARTICLE INFO

Article history:
Received 9 January 2012
Received in revised form
26 March 2012
Accepted 1 April 2012
Available online 24 April 2012

Keywords:
Chinese herbal medicines
Pesticides
Gas chromatography–mass spectrometry
Microwave-assisted extraction
Dispersive-solid phase extraction
Multi-residue analysis

ABSTRACT

A simple, rapid and reliable method was proposed for the simultaneous determination of 27 pesticides (organophosphorus, organochlorine, pyrethroid and carbamate pesticides) in *Radix astragali*. The pesticides were extracted by acetonitrile and the experimental variables, such as temperature, extraction time and volume of acetonitrile, were optimized through orthogonal array experimental design. Cleanup of extracts was performed with dispersive-solid phase extraction using primary secondary amine (PSA) as the sorbent. The determination of pesticides in the final extracts was carried out by gas chromatography–mass spectrometry in selected ion monitoring mode (GC–MS, SIM). The linearity of the calibration curves is good in matrix-matched standard, and yields the coefficients of determination (R^2) $\geq$ 0.99 for approximately 96% of the target analytes. Under optimized conditions, the average recoveries (six replicates) for most pesticides (spiked at 0.02, 0.1 and 0.2 mg kg^{-1}) range from 70% to 120%, and RSDs are less than 17.2%.

1. Introduction

Chinese herbal medicines (CHMs) have been widely used as a means of medication or dietary supplement for their mild pharmaceutical effects and minimum side effects [1,2]. The commercial cultivation of CHMs receives frequent application of diverse pesticides to prevent, repel or mitigate the effects of pest [3]. These utilized pesticides can be mainly categorized into four classes, namely organophosphorus pesticides (OPPs), organochlorine pesticides (OCPs), pyrethroid pesticides (PYRs) and carbamate pesticides (CBs). These pesticides can be concentrated and stabilized in CHMs, causing a potential risk to human health. Therefore, it is imperative to monitor these pesticides in CHMs [3,4]. Recently, a number of works on the determination of pesticide residues in CHMs have been published [5–12]. However, most of them usually dealt with one or two classes of pesticides. It means that simultaneous detection of the four classes of pesticide residues in CHMs needs two or more methods [13], which require extensive labor, time and cost. Thus, it is necessary to develop a simple and reliable method for simultaneously determining the four classes of pesticide residues in CHMs. The simultaneous determination of multi-residues in CHMs represents an analytical challenge, due to the broad physicochemical properties of these pesticides and the complexity of CHMs matrixes [14].

Gas chromatography (GC) coupled with selective detectors, such as flame photometric detector (FPD) [15,16], nitrogen phosphorous detector (NPD) [17,18], and electron capture detector (ECD) [19,20], has been widely utilized to determine the pesticides in complex samples. But they are not suitable for simultaneous determination of the four classes of pesticides for their selectivity, and often suffer from interference of matrixes. For these reasons, mass spectrometry (MS), as a good multi-residue analysis technique [21,22], was introduced for its universal property and higher sensitivity. The four classes of pesticides can be simultaneously detected in MS, and the results can be identified and quantified via full scan or selected ion monitoring (SIM) spectra. Furthermore, the use of MS detector in the SIM mode can effectively discriminate the signals between analyte and impurity, which improves the selectivity and yields low background noise [23].

In order to eliminate interferences and keep the chromatographic system in good working order, an effective sample preparation

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http://dx.doi.org/10.1016/j.talanta.2012.04.007

process is necessary. There are many pretreatment techniques for the extraction and clean-up of the pesticides in CHMs. Although many of these techniques are suitable and effective, they are relatively time-consuming and require large volumes of organic solvents. This is hazardous to human health and causes serious pollution problems [20]. Simple, rapid and solvent-saving sample preparation is one of modern trends in analytical chemistry [24]. In our previous study [25], a simple and reliable sample pretreatment technique, microwave-assisted extraction (MAE) coupled with dispersive solid-phase extraction (D-SPE), has been developed. The MAE technique provided satisfactory recoveries for the extraction of pesticides in CHMs, and the D-SPE method greatly simplified the sample clean-up process and minimized the consumption of organic solvent.

Radix astragali (Huang-qi) is the root of Astragalus membranaceus Bunge, which belongs to the family of Leguminosae, and is one of the most frequently used crude drugs for oriental medicine in China, Taiwan, Japan, Korea, and other Asian areas [26]. It plays an important role in agricultural production and people's living. Pharmacological studies and clinical practices have proven that *Radix astragali* is used as an immunostimulant, hepatoprotective, antidiabetic, analgesic, expectorant, and sedative drug for the treatment of nephritis, diabetes, albuminuria, hypertension, cirrhosis, cancer, etc [27]. Hence, it is significative to monitor pesticide residues in *Radix astragali*.

Herein, MAE coupled with D-SPE pretreatment method was employed to extract 27 pesticides covering OPPs, OCPs, PYRs and CBs from *Radix astragali* and clean up the extracts. The determination of pesticides in the final extracts was carried out by GC–MS. The extraction conditions were optimized via orthogonal array experimental design. The type and amount of sorbents that affected the efficiency of D-SPE were also investigated. To the best of our knowledge, OPPs, OCPs, PYRs and CBs have never been simultaneously determined in CHMs. Moreover, the combination of MAE/D-SPE coupled with GC–MS has not been applied to the simultaneous analysis of pesticides in CHMs. The established method is effective, simple, rapid and environmentally-friendly, being well suitable for simultaneous determination of the four classes of pesticide residues in *Radix astragali*.

2. Experimental

2.1. Chemicals and reagents

Pesticide standards including isoprocarb, cadusafos, hexachlorobenzene, BHC-alpha, diazinon, quintozene, BHC-gamma, BHC-beta, pirimicarb, heptachlor, pirimiphos-methyl, malathion, parathion, bromophos, butachlor, *p,p′*-DDE, dieldrin, *o,p′*-DDT, *p,p′*-DDD, sulprofos, *p,p′*-DDT, bifenthrin, carbosulfan, fenpropathrin, cyhalothrin, benfuracarb and permethrin were purchased from Agro-Environment Protection Institute (Tianjin, China). Stock solutions of each pesticide were 100 mg L^{-1} and standard working solutions at various concentrations were obtained by dilution of the stock solutions in *n*-hexane. Matrix-matched standard solutions were prepared via serial dilution of standard solutions by blank sample extracts. These solutions were stored at 0–4 °C. Acetone, acetonitrile, ethyl acetate and *n*-hexane were of HPLC grade. Ultrapure water was produced by a Milli-Q system (Millipore, USA). Analytical grade anhydrous magnesium sulfate ($MgSO_4$) was purchased from Sinopharm Chemical Reagent Limited Company (Shanghai, China). Neutral aluminum (Alumina N), Primary secondary amine (PSA) and Graphitized carbon black (GCB) were obtained from Beijing Zhenxiang Industrial Foreign Trade Limited Company (Beijing, China). They were stored in a desiccator before use. The *Radix astragali* samples were purchased from different markets in China. They were ground using a mixer-grinder, ranked by mesh screen (0.42 mm), and then stored in a desiccator at room temperature. The samples were analyzed following the procedure described below, and the sample collected from Jiangxi province in China showing the absence of target analytes was used as blank sample in the preparation of standards and in the recovery study.

2.2. Extraction and clean-up

For extraction, MAE was performed with an ETHOS E Microwave Apparatus (Milestone, Italy) in temperature-controlled mode. 1.0000 g of sample was accurately weighed into a teflon-lined extraction vessel, and 20 mL acetonitrile was added. The extraction temperature was 80 °C and programmed as follows: ramp to 80 °C for 10 min, holding at 80 °C for 5 min and decrease to room temperature for 10 min. After extraction, the extracts were transferred into a 50 mL round-bottomed flask and concentrated to dryness using a RE-52A rotary vacuum evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) in a water bath at 50 °C, and then 1 mL *n*-hexane was added. The mixture was vortex mixed for 2 min at 1800 r min^{-1} with a MS2 mini shaker (Guangzhou Yike Lab Technology LTM Co., Guangzhou, China).

For clean-up, the extracts were transferred to a 5 mL microcentrifuge tube containing 150 mg PSA and 100 mg $MgSO_4$, followed by vortexing for 1 min. The 5 mL microcentrifuge tube was centrifuged at 5000 r min^{-1} for 2 min. Subsequently, the mixture was filtered through a 0.45 μm organic membrane. Finally, the solution was transferred to the 2.0 mL sample vial and then placed in the autosampler for GC–MS analysis.

2.3. GC–MS analysis

GC–MS analysis was carried out using an Agilent 6890 N GC system integrated with an Agilent 7683 series autosampler, and a 5973 massselective detector (MSD). The analytes were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-35MS fused-silica capillary column. The injector was set at 250 °C and the carrier gas was helium at a flow rate of 1.0 mL min^{-1}. The oven temperature was initially at 60 °C for 2 min, increased at a rate of 15 °C min^{-1} up to 220 °C; held for 4 min; increased at a rate of 10 °C min^{-1} up to 260 °C; held for 4 min; increased at a rate of 5 °C min^{-1} up to 280 °C and held for 4 min. The ion source, quadrupole and transfer line temperature were set at 230, 150 and 280 °C, respectively. The mass spectrometer was operated at 70 eV in electron impact (EI) mode. Solvent delay was 8 min and the injection volume was 2 μL.

Analysis was performed in the selected ion monitoring mode (SIM) based on the use of one target and two or three qualifier ions, and the total ion chromatogram of the 27 pesticides in standard solution is shown in Fig. 1. Groupings were defined to increase the sensitivity of the MS analysis, and the SIM programme used to analyze pesticides is indicated in Table 1. Target and qualifier abundances were determined by injection of individual pesticide standards under the same chromatographic conditions in full-scan mode with the mass/charge ratio ranging from m/z 50 to 500. Table 1 also summarizes the pesticides studied with their chemical structures, retention times, the target and qualifier ions, and the qualifier to target abundance ratios.

2.4. Qualitative and quantitative GC–MS

For the sample determination, three injections were required to analyze all the pesticides via GC–MS under the conditions

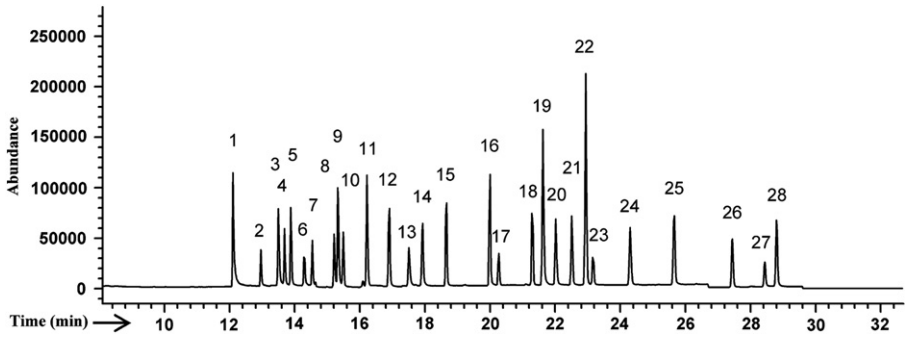


Fig. 1. GC–MS-SIM chromatogram of standard mixture of the 27 pesticides (0.2 μg mL^{-1}). Peak identification: 1 isoprocarb, 2 cadusafos, 3 hexachlorobenzene, 4 BHC-alpha, 5 diazinon, 6 quintozene, 7 BHC-gamma, 8 BHC-beta, 9 pirimicarb, 10 heptachlor, 11 pirimiphos-methyl, 12 malathion, 13 parathion, 14 bromophos, 15 butachlor, 16 *p,p′*-DDE, 17 dieldrin, 18 *o,p′*-DDT, 19 *p,p′*-DDD, 20 sulprofos, 21 *p,p′*-DDT, 22 bifenthrin, 23 carbosulfan, 24 fenpropathrin, 25 cyhalothrin, 26 benfuracarb, 27 Permethrin-I, 28 Permethrin-II.

Table 1
Chemical structures, retention times, segments, scan time windows, target ions, and qualifier ions of the selected pesticides.

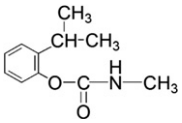
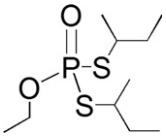
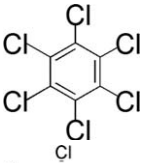
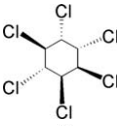
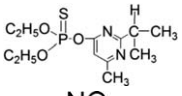
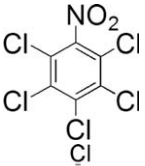
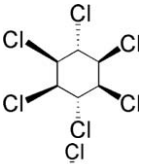
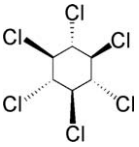
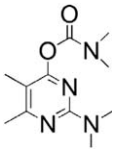
Pesticide	Structure	Retention time	Segment	Time window	Target ion	Qualifier ion 1	Qualifier ion 2	Qualifier ion 3
Isoprocarb		12.10	1	8.00–13.25	121 (1 0 0)	136 (45.3)	91 (10.4)	
Cadusafos		12.97			158 (1 0 0)	97 (48.6)	121 (1.0)	
Hexachlorobenzene		13.51	2	13.25–14.65	284 (1 0 0)	249 (29.4)	142 (30.7)	
BHC-alpha		13.71			183 (1 0 0)	219 (108.6)	109 (14.3)	
Diazinon		13.90			179 (1 0 0)	137 (1 5 2)	304 (6.6)	
Quintozene		14.31			249 (1 0 0)	237 (1 2 5)	265 (63.5)	
BHC-gamma		14.56			183 (1 0 0)	219 (84.6)	109 (57.8)	
BHC-beta		15.23			183 (1 0 0)	219 (98.6)	109 (57.8)	
Pirimicarb		15.36	3	14.65–16.00	166 (1 0 0)	238 (15.5)	72 (54.9)	

Table 1 (*continued*)

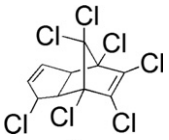
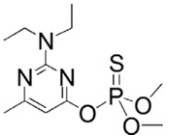
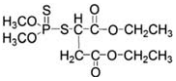
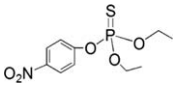
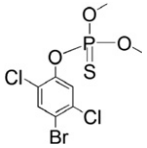
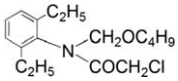
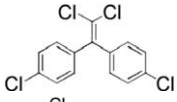
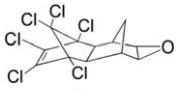
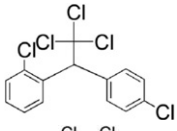
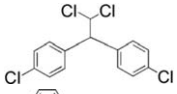
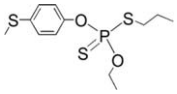
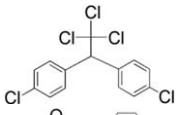
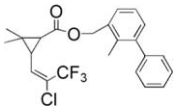
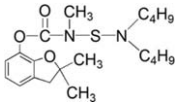
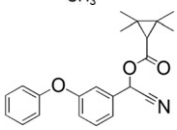
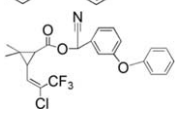
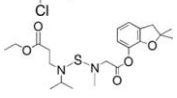
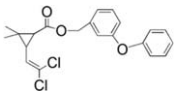
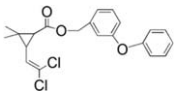
Pesticide	Structure	Retention time	Segment	Time window	Target ion	Qualifier ion 1	Qualifier ion 2	Qualifier ion 3
Heptachlor		15.51			272 (1 0 0)	100 (45.1)	237 (32.2)	337 (25.5)
Pirimiphos-methyl		16.24	4	16.00–17.30	290 (1 0 0)	276 (86.8)	305 (65.3)	
Malathion		16.91			173 (1 0 0)	125 (77.4)	127 (74.8)	93 (47.3)
Parathion		17.53	5	17.30–19.40	291 (1 0 0)	109 (93.3)	97 (80.6)	125 (44.1)
Bromophos		17.94			331 (1 0 0)	333 (27.4)	125 (23.2)	
Butachlor		18.67			176 (1 0 0)	160 (71.7)	188 (45.2)	237 (25.4)
p,p′-DDE		20.01	6	19.40–24.00	246 (1 0 0)	318 (82.1)	176 (23.4)	210 (13.2)
Dieldrin		20.29			263 (1 0 0)	277 (76.5)	235 (34.7)	
o,p′-DDT		21.32			235 (1 0 0)	165 (22.2)	199 (12.4)	
p,p′-DDD		21.65			235 (1 0 0)	165 (59.6)	199 (13.4)	
Sulprofos		22.03			156 (1 0 0)	322 (87.9)	140 (88.0)	
p,p′-DDT		22.54			235 (1 0 0)	165 (19.6)	199 (8.8)	
Bifenthrin		22.96			181 (1 0 0)	165 (30.4)	166 (29.7)	
Carbosulfan		23.19			164 (1 0 0)	118 (263.1)	135 (64.8)	
Fenpropathrin		24.33	7	24.00–26.68	181 (1 0 0)	97 (92.3)	265 (47.3)	141 (26.6)
Cyhalothrin		25.67			181 (1 0 0)	146 (20.5)	97 (2.7)	265 (0.4)
Benfuracarb		27.45	8	26.68–29.60	164 (1 0 0)	190 (199.4)	144 (43.6)	

Table 1 (*continued*)

Pesticide	Structure	Retention time	Segment	Time window	Target ion	Qualifier ion 1	Qualifier ion 2	Qualifier ion 3
Permethrin-I		28.45			183 (1 0 0)	163 (19.0)	165 (16.8)	
Permethrin-II		28.82			183 (1 0 0)	163 (24.2)	121 (20.2)	

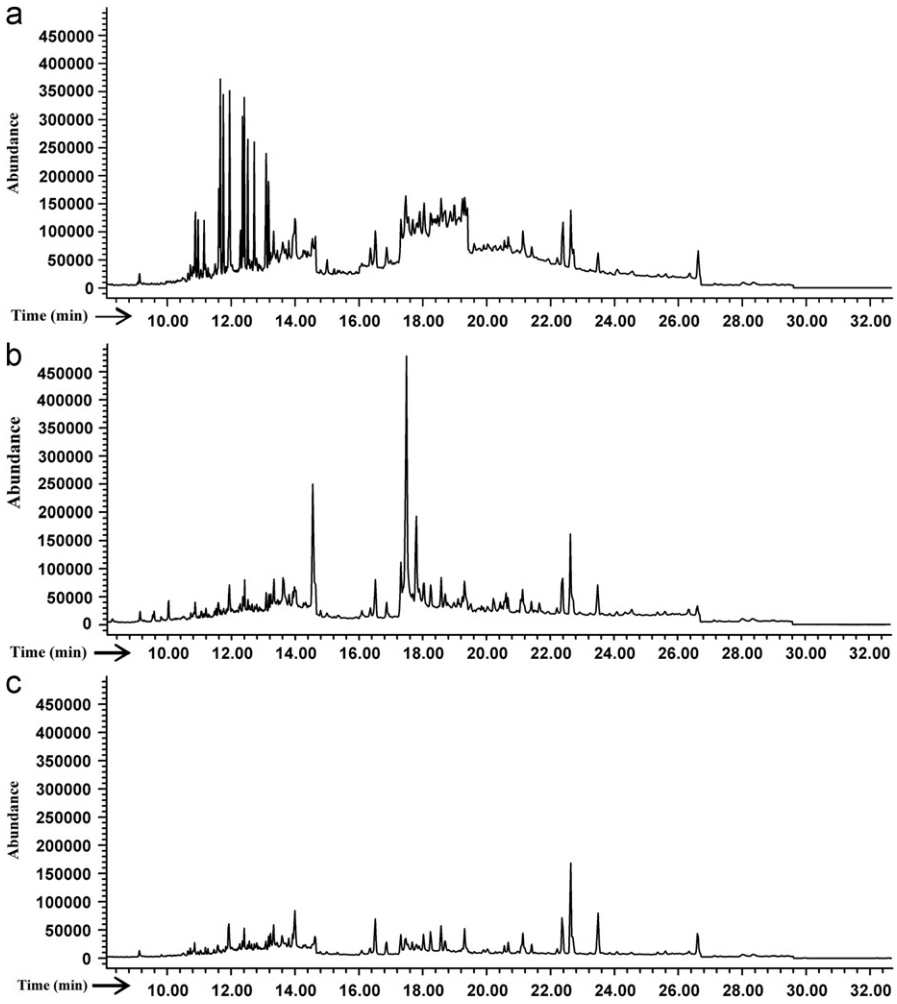


Fig. 2. GC–MS-SIM chromatograms of *Radix astragali* extracts using acetone (a), ethyl acetate (b), and acetonitrile (c) as extraction solvents. Sample preparation conditions: extraction temperature: 80 °C; extraction volume: 20 mL; extraction time: 5 min; dissolving solvent: 1 mL *n*-hexane; sorbents: 100 mg PSA and 100 mg $MgSO_4$.

given above. Pesticides were identified according to the retention times, the target and qualifier ions, and the qualifier to target abundance ratios. Quantification was performed using peak areas of each target ion, respectively (shown in Table 1). Exceptionally, permethrin was quantified by summing the peak areas of permethrin-I and permethrin-II. Considering matrix effects, matrix-matched standards were used for quantification.

2.5. Validation study

The validation of the analytical method was performed by the following parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. The calibration curves were evaluated with matrix-matched standard calibration in blank extracts of *Radix astragali* in the concentrations 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 and 0.5 mg kg^{-1}. Three injections were performed at each of the concentration levels. The peak areas of each analyte were plotted against the concentrations, and linear regression was performed on the resulting curves using the minimum least squares method. The LOD and LOQ were evaluated based on a signal-to-noise ratio of 3 and 10, respectively. For sample matrix testing, 1.0000 g of *Radix astragali* was separately spiked and tested for accuracy and precision at three fortification levels (0.02, 0.1, 0.2 mg kg^{-1}). Each sample was mixed thoroughly and stored overnight in darkness before extraction assay. For each fortification level, six replicate experiments were carried out.

3. Results and discussion

3.1. Optimization of microwave-assisted extraction

3.1.1. Selection of extraction solvent

Acetone, ethyl acetate and acetonitrile have often been used in multi-residue methods as extraction solvents [28–30]. To test their extraction ability for *Radix astragali*, parallel experiments were carried out. Fig. 2 shows the total ion chromatograms of extracts obtained from the three solvents. It can be seen that acetone and ethyl acetate extracts yield much more impurities than that of acetonitrile. Thus, acetonitrile was chosen as the extraction solvent to simplify the cleanup process and keep the chromatographic system from contamination.

Table 2
Average recoveries of 27 pesticides obtained from optimization experiments using an L_9 (3^4) orthogonal array design.

Trial No.	Factor			Average recovery (%)
	A[a]	B[b]	C[c]	
1	1	1	1	72.2
2	1	2	2	75.6
3	1	3	3	85.4
4	2	1	2	61.7
5	2	2	3	69.1
6	2	3	1	59.0
7	3	1	3	73.7
8	3	2	1	56.7
9	3	3	2	57.2
K_1	77.7	69.2	62.6	
K_2	63.3	67.1	64.9	
K_3	62.5	67.2	76.1	
Range	15.2	2.1	13.5	
Optimization level	A_1	B_1	C_3	

K_i, mean effect of each factor at level i (i=1, 2, 3).
[a] Factor A, temperature; level 1, 80 °C; level 2, 100 °C; level 3, 120 °C.
[b] Factor B, extraction time; level 1, 5 min; level 2, 10 min; level 3, 15 min.
[c] Factor C, volume of acetonitrile; level 1, 10 mL; level 2, 15 mL; level 3, 20 mL.

3.1.2. Optimization of extraction conditions

The extraction conditions were optimized using orthogonal array experimental design which was a cost-effective optimization strategy with minimum number of experiments [12,25,31]. In this study, effects of three factors on the recoveries were studied and optimized by a L_9 (3^4) orthogonal array. Table 2 illustrates factor allocation for the orthogonal matrix. In the matrix, the letters A, B and C represent temperature, extraction time and volume of acetonitrile, respectively. The numbers 1, 2 and 3 denote three different experimental levels.

A portion (100 μL) of the 1.0 mg L^{-1} standard mixture solutions was added to 1.0000 g of the *Radix astragali*. For each experimental trial, three replicate experiments were performed, so 27 samples were prepared in this way and extracted according to the orthogonal array design. Table 2 provides data on the average recoveries for 27 pesticides used in each experimental trial, as well as the mean effects (K_1, K_2 and K_3) for each factor at different levels. The range in K observed with the changes in A and C are 15.2 and 13.5, respectively, which are higher than 2.1 that resulted from changes in B. In other words, extraction temperature and volume of acetonitrile are more significant than extraction time. Deducing from the orthogonal array design, the optimum level extraction conditions are $A_1C_3B_1$, namely temperature 80 °C, extraction time 5 min and extraction volume 20 mL.

3.2. Optimization of cleanup conditions

There are many fatty acids in *Radix astragali* extract, such as tetradecanoic acid and pentadecanoic acid, which could be confirmed by their high responses in the total ion chromatogram of GC–MS (Fig. 3(a)). PSA, Alumina N and GCB can selectively adsorb organic acids, sugars, pigments and some other co-extracts.

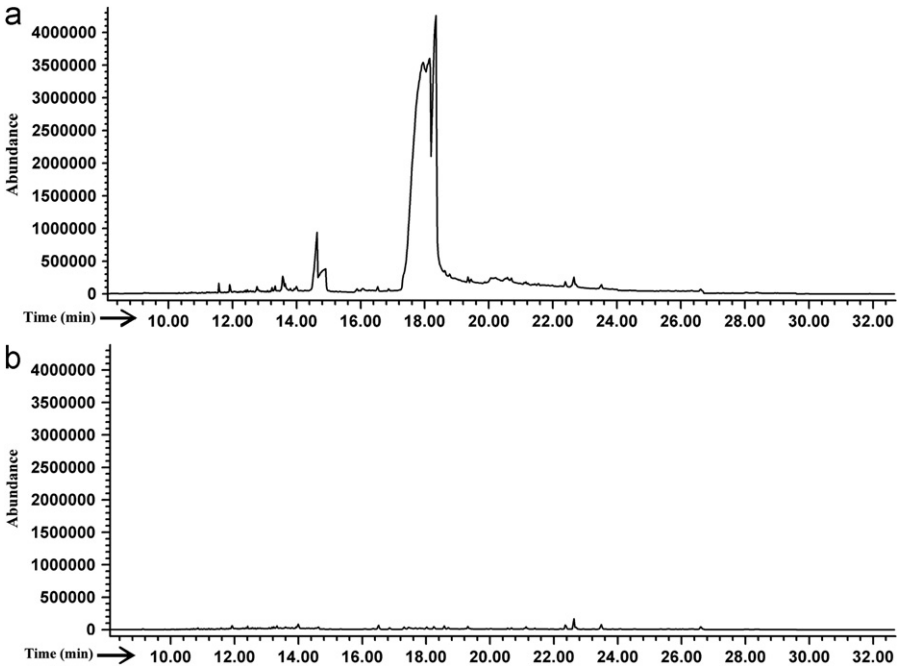


Fig. 3. GC–MS-SIM chromatograms of *Radix astragali* extracts of no cleanup (a), and cleanup with D-SPE using 150 mg PSA and 100 mg $MgSO_4$ as the sorbents (b). Sample preparation conditions: extraction temperature: 80 °C; extraction solvent: acetonitrile; extraction volume: 20 mL; extraction time: 5 min; dissolving solvent: 1 mL *n*-hexane.

Their adsorption abilities were studied in this experiment. As revealed in Fig. 4, PSA and Alumina N do a better job of removing additional matrix components from the extracts than GCB, but Alumina N adsorbs pesticides, such as isoprocarb, cadusafos, diazinon, BHC-gamma, BHC-beta, pirimiphos-methyl, butachlor, carbosulfan, fenpropathrin and cyhalothrin. Therefore, PSA was chosen as the adsorbent in the dispersive-SPE process. Meanwhile, the amount of PSA was optimized and its influence on the recoveries of the 27 pesticides is listed in Table 3. When 50 mg of PSA was added, the recoveries for most pesticides reached above 130%. This is due to the interference of sample matrix co-extractives. Increasing the amount of PSA to 100 and 150 mg, the interference co-extractives can be efficiently removed and the recoveries for most pesticides are in the range of 70–130%. Further increasing the PSA to 200 mg, some pesticides are undesirably adsorbed, for example, the recovery of pirimiphos-methyl falls to 36.7%. Ultimately, 150 mg of PSA was selected as the optimal amount to keep a balance between maximal removal of the matrix compounds and minimal adsorption of the analytes. In addition, 100 mg of anhydrous $MgSO_4$ was added to adsorb the residual water in the extraction process. Results show that there are less impurity residues in the final extract and interferences are efficiently avoided in the GC–MS chromatograms when using 150 mg PSA and 100 mg $MgSO_4$ (Fig. 3(b)). It is noted that the low recoveries of isoprocarb, BHC-beta and pirimicarb are probably due to the strong adsorption of PSA, which was confirmed by spiking PSA with mixture standard solutions.

3.3. Selection of dissolving solvent

Acetonitrile possesses many advantages in extraction; however, it is seldom used in GC analysis for its larger solvent expansion volume during GC vaporization, high toxicity and low volatility [30]. The use of acetone, *n*-hexane and ethyl acetate in a GC system will be a much better choice. Thus, acetone, *n*-hexane and ethyl acetate were considered to replace acetonitrile and tested. As shown in Fig. 5, besides pesticides, acetone and ethyl acetate can dissolve amounts of impurities, which may shorten the capillary GC column life and have a deleterious effect on the detection of pesticides at trace levels. Finally, *n*-hexane was chosen as dissolving solvent.

3.4. Matrix effect

Matrix effect is an important aspect in multi-residue analysis, which may decrease or enhance the analyte signals in matrix extracts as compared to a matrix-free solution [32,33]. It will influence the quantitative results. The ratio of the slopes (Rs) of the corresponding calibration curves can be used to investigate

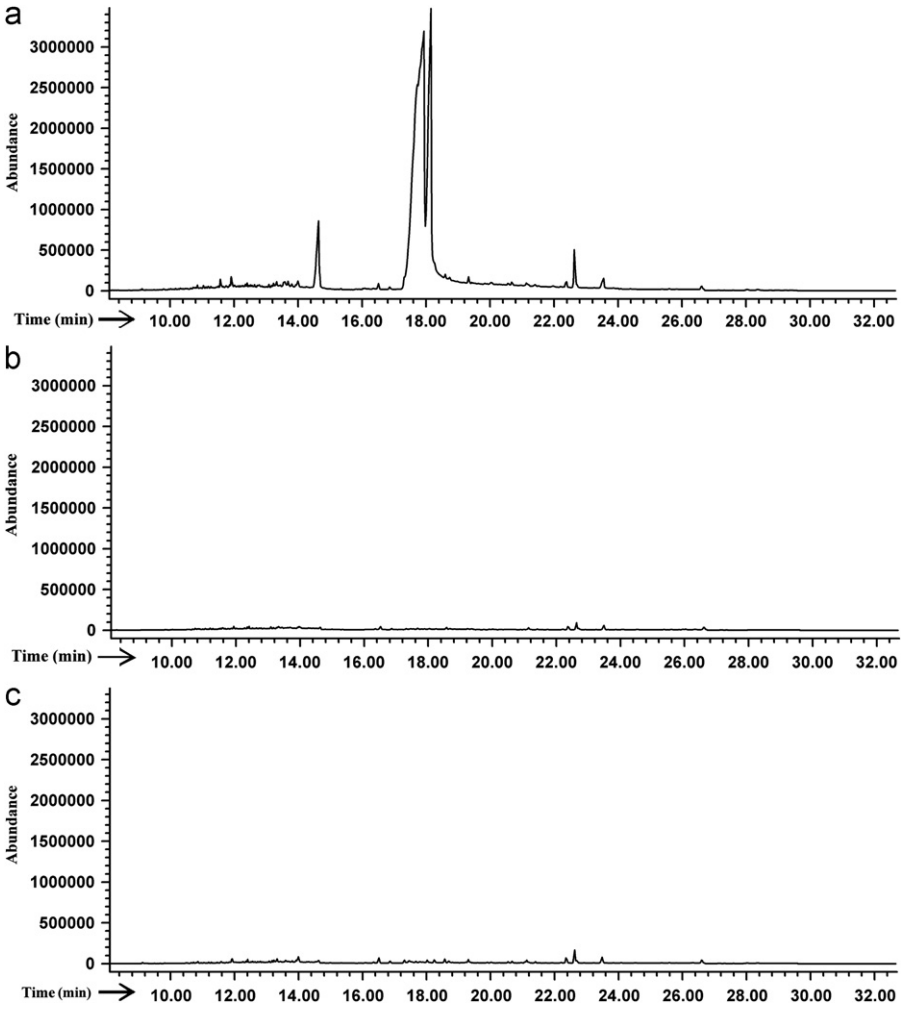


Fig. 4. GC–MS-SIM chromatograms of *Radix astragali* extracts of cleanup with D-SPE using 150 mg GCB (a), 150 mg Alumina N (b), and 150 mg PSA (c) as the sorbents. Sample preparation conditions: extraction temperature: 80 °C; extraction solvent: acetonitrile; extraction volume: 20 mL; extraction time: 5 min; dissolving solvent: 1 mL *n*-hexane.

Table 3
Recoveries of 27 pesticides with different amounts of PSA ($n=3$).

Pesticide	Average recovery (%)			
	50 mg PSA	100 mg PSA	150 mg PSA	200 mg PSA
Isoprocarb	79.5	46.0	27.8	0
Cadusafos	126.6	124.8	98.2	100.5
hexachlorobenzene	118.3	119.2	98.8	114.1
BHC-alpha	109.4	120.8	93.4	96.2
Diazinon	124.0	122.1	100.8	93.7
Quintozene	130.2	128.3	106.2	121.1
BHC-gamma	135.4	102.9	80.8	80.0
BHC-beta	75.7	46.6	37.2	26.2
Pirimicarb	40.5	36.2	46.3	6.1
Heptachlor	132.4	132.2	107.6	120.1
Pirimiphos-methyl	83.8	92.6	88.7	36.7
Malathion	152.8	125.7	95.0	89.5
Parathion	156.6	113.9	115.7	115.3
Bromophos	134.4	126.5	103.6	107.0
Butachlor	130.3	121.3	99.5	101.4
p,p′-DDE	117.1	122.1	105.4	113.4
Dieldrin	120.2	116.4	97.0	104.5
o,p′-DDT	166.0	132.9	123.3	136.5
p,p′-DDD	97.1	90.9	78.4	66.7
Sulprofos	136.2	129.9	106.2	109.3
p,p′-DDT	179.1	118.6	93.7	130.8
Bifenthrin	128.3	123.6	105.4	115.1
Carbosulfan	118.8	111.7	98.4	76.6
Fenpropathrin	135.0	116.5	95.6	95.1
Cyhalothrin	136.3	102.3	79.1	67.0
Benfuracarb	83.8	91.8	77.0	67.3
Permethrin	174.4	147.2	128.7	127.9

the influence of the matrix on the signal response. If Rs is around 1, matrix effect can be neglected. If Rs is smaller or larger than 1, it indicates suppressing or enhancing effects of the matrix. Thus, a comparison between matrix-free and matrix-matched calibration was performed. Calibration curves for standards in solvents were plotted versus that for matrix-matched standards, and the difference in slope was calculated. As listed in Table 4, most of pesticides exhibit signal enhancement effects. Moreover, Matrix-free and matrix-matched calibration is compared using Student's t test [34,35], and the experimental P values are given in Table 4. It can be seen that most of P values are lower than 0.05, indicating significant difference between the matrix-free and matrix-matched calibration. Therefore, matrix-matched standard curves were used in the quantitative analysis to counter the matrix effect.

3.5. Validation of the method

The calibration curves all represent good linearity, with correlation coefficients in the range of 0.9835–0.9986 (Table 4). Pesticides can be detected at the level of 0.0002–0.01 mg kg^{-1} depending on the type of the analytes, and the LOQ values range from 0.0008 to 0.03 mg kg^{-1} (Table 5). The LODs determined for the pesticides are lower than maximum residue limits (MRLs, 0.01–5.0 mg kg^{-1}) set by European Union (EU), United States Environmental Protection Agency (EPA) and Japan [36]. Furthermore, comparative study was carried out between the present technique and some reported methods where the pesticides were sensitively analyzed. The comparative results are listed in Table 6

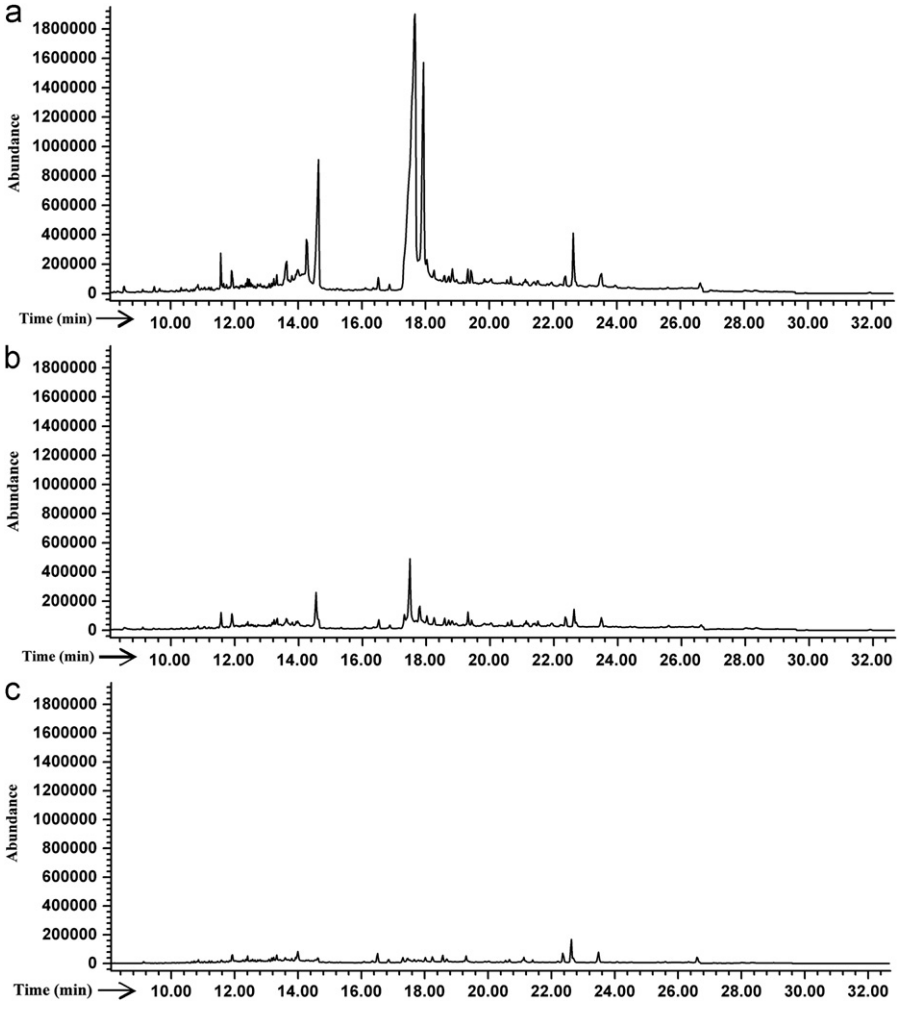


Fig. 5. GC–MS-SIM chromatograms of *Radix astragali* extracts using acetone (a), ethyl acetate (b), and *n*-hexane (c) as dissolving solvents. Sample preparation conditions: extraction temperature: 80 °C; extraction solvent: acetonitrile; extraction volume: 20 mL; extraction time: 5 min; sorbents: 150 mg PSA and 100 mg $MgSO_4$.

Table 4
Calibration data in matrix and matrix-free solvent of 27 pesticides.

Pesticide	Calibration data in matrix	R^2	Calibration data in solvent	R^2	Slope in matrix/slope in standard	P value
Isoprocarb	$y=1.86\times10^7\times-5.26\times10^4$	0.9977	$y=1.44\times10^7\times-1.00\times10^5$	0.9996	1.29	0.002
Cadusafos	$y=4.39\times10^6\times+1.96\times10^4$	0.9956	$y=3.55\times10^6\times-2.22\times10^4$	0.9997	1.24	0.009
hexachlorobenzene	$y=9.45\times10^6\times-5.75\times10^3$	0.9973	$y=8.28\times10^6\times-3.31\times10^4$	0.9998	1.14	0.044
BHC-alpha	$y=3.43\times10^6\times+1.93\times10^4$	0.9969	$y=2.85\times10^6\times-1.01\times10^4$	0.9998	1.20	0.006
Diazinon	$y=4.62\times10^6\times+4.40\times10^4$	0.9955	$y=3.70\times10^6\times-2.04\times10^4$	0.9997	1.25	0.006
Quintozene	$y=1.48\times10^6\times-1.63\times10^3$	0.9969	$y=1.23\times10^6\times-7.84\times10^3$	0.9996	1.20	0.016
BHC-gamma	$y=3.05\times10^6\times-3.22\times10^3$	0.9979	$y=2.51\times10^6\times-9.14\times10^3$	0.9998	1.22	0.008
BHC-beta	$y=2.94\times10^6\times+6.48\times10^3$	0.9968	$y=2.42\times10^6\times-9.99\times10^3$	0.9998	1.21	0.013
Pirimicarb	$y=1.25\times10^7\times-2.62\times10^4$	0.9973	$y=1.07\times10^7\times-5.29\times10^5$	0.9804	1.17	0.017
Heptachlor	$y=3.77\times10^6\times-7.27\times10^3$	0.9973	$y=3.17\times10^6\times-1.84\times10^3$	0.9997	1.19	0.013
Pirimiphos-methyl	$y=6.92\times10^6\times-1.37\times10^4$	0.9977	$y=5.72\times10^6\times-4.92\times10^4$	0.9995	1.21	0.016
Malathion	$y=6.15\times10^6\times-1.45\times10^3$	0.9977	$y=4.68\times10^6\times-4.14\times10^4$	0.9993	1.31	0.003
Parathion	$y=3.93\times10^6\times-3.40\times10^4$	0.9984	$y=2.97\times10^6\times-4.67\times10^4$	0.9971	1.32	0.004
Bromophos	$y=9.22\times10^6\times-3.05\times10^4$	0.9979	$y=7.41\times10^6\times-5.82\times10^4$	0.9995	1.24	0.013
Butachlor	$y=8.07\times10^6\times-1.22\times10^4$	0.9972	$y=6.55\times10^6\times-5.50\times10^4$	0.9994	1.23	0.014
p,p′-DDE	$y=9.36\times10^6\times-6.27\times10^2$	0.9966	$y=7.61\times10^6\times-3.24\times10^4$	0.9999	1.23	0.015
Dieldrin	$y=1.22\times10^6\times+3.03\times10^3$	0.9969	$y=1.01\times10^6\times-2.31\times10^3$	0.9998	1.21	0.004
o,p′-DDT	$y=1.23\times10^7\times-5.74\times10^4$	0.9978	$y=1.00\times10^7\times-1.15\times10^4$	0.9990	1.23	0.014
p,p′-DDD	$y=1.60\times10^7\times-1.14\times10^3$	0.9973	$y=1.25\times10^7\times-5.06\times10^3$	0.9999	1.28	0.005
Sulprofos	$y=4.91\times10^6\times-1.61\times10^4$	0.9978	$y=3.75\times10^6\times-2.87\times10^4$	0.9996	1.31	0.002
p,p′-DDT	$y=1.28\times10^7\times-9.34\times10^4$	0.9984	$y=1.01\times10^7\times-1.49\times10^5$	0.9983	1.27	0.006
Bifenthrin	$y=2.82\times10^7\times-4.32\times10^4$	0.9973	$y=2.12\times10^7\times-8.52\times10^4$	0.9998	1.33	0.001
Carbosulfan	$y=1.35\times10^6\times+3.70\times10^4$	0.9835	$y=1.23\times10^6\times-6.45\times10^3$	0.9998	1.10	0.055
Fenpropathrin	$y=5.08\times10^6\times-1.81\times10^4$	0.9980	$y=3.77\times10^6\times-2.35\times10^4$	0.9996	1.35	0.002
Cyhalothrin	$y=7.45\times10^6\times-3.36\times10^4$	0.9982	$y=5.40\times10^6\times-4.62\times10^4$	0.9994	1.38	0.002
Benfuracarb	$y=2.27\times10^6\times-2.41\times10^3$	0.9977	$y=1.89\times10^6\times-3.51\times10^4$	0.9994	1.20	0.018
Permethrin	$y=1.72\times10^7\times+5.84\times10^4$	0.9986	$y=1.24\times10^7\times-6.24\times10^4$	0.9998	1.39	0.001

Table 5
LODs, LOQs and recoveries of 27 pesticides in *Radix astragali* at three fortified levels ($n=6$).

Pesticide	Class	LODs mg kg^{-1}	LOQs mg kg^{-1}	0.02 mg kg^{-1}		0.1 mg kg^{-1}		0.2 mg kg^{-1}	
				Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Isoprocarb	CBs	0.0008	0.0030	26.4	14.2	14.8	8.2	11.6	5.2
Cadusafos	OPPs	0.0040	0.0100	93.1	4.6	84.3	5.1	77.4	5.4
Hexachlorobenzene	OCPs	0.0010	0.0030	95.4	4.7	98.4	5.8	94.4	5.0
BHC-alpha	OCPs	0.0100	0.0300	92.6	16.0	101.9	11.3	81.9	7.2
Diazinon	OPPs	0.0080	0.0200	115.5	11.2	107.6	3.9	97.2	5.7
Quintozene	OCPs	0.0020	0.0060	88.2	9.0	101.5	4.7	100.0	5.6
BHC-gamma	OCPs	0.0100	0.0200	89.4	2.4	72.7	11.3	67.4	8.6
BHC-beta	OCPs	0.0020	0.0050	40.8	17.2	13.8	8.6	13.0	3.4
Pirimicarb	CBs	0.0030	0.0100	46.6	16.1	59.9	5.8	49.8	11.4
Heptachlor	OCPs	0.0030	0.0060	124.3	7.0	105.1	4.5	93.9	5.0
Pirimiphos-methyl	OPPs	0.0003	0.0010	114.7	3.6	96.3	5.5	90.0	6.1
Malathion	OPPs	0.0030	0.0100	88.5	9.7	91.1	5.3	72.3	4.7
Parathion	OPPs	0.0080	0.0020	115.5	6.1	97.0	7.0	79.1	5.4
Bromophos	OPPs	0.0050	0.0020	115.3	5.1	111.2	4.4	95.6	6.2
Butachlor	CBs	0.0020	0.0040	110.3	5.1	96.1	4.8	88.6	4.7
p,p′-DDE	OCPs	0.0003	0.0010	110.6	4.7	108.2	4.8	106.2	4.7
Dieldrin	OCPs	0.0080	0.0200	86.3	5.5	90.2	3.6	83.8	4.7
o,p′-DDT	OCPs	0.0010	0.0040	70.8	5.0	114.5	4.3	101.2	6.3
p,p′-DDD	OCPs	0.0004	0.0010	71.5	5.0	70.3	3.5	71.5	5.7
Sulprofos	OPPs	0.0030	0.0100	103.2	6.6	92.6	10.7	92.3	7.1
p,p′-DDT	OCPs	0.0006	0.0020	86.3	10.1	116.7	5.3	85.6	8.9
Bifenthrin	PYRs	0.0002	0.0008	107.8	3.5	108.2	3.4	103.1	6.0
Carbosulfan	CBs	0.0050	0.0100	71.5	15.4	82.4	10.2	79.9	8.6
Fenpropathrin	PYRs	0.0020	0.0070	86.8	5.0	85.6	3.5	82.0	4.9
Cyhalothrin	PYRs	0.0030	0.0100	76.3	5.4	66.6	4.3	64.5	4.0
Benfuracarb	CBs	0.0030	0.0090	61.8	5.3	68.4	6.8	60.7	5.4
Permethrin	PYRs	0.0020	0.0060	107.6	6.5	112.0	3.0	102.0	5.7

and demonstrate that LODs of the present method are lower than or comparable to those of the reported methods [4,21,37–41]. These confirm the sensitivity of the present method. Method accuracy and reproducibility were evaluated via recovery experiments. Recovery validation experiments were conducted in matrix at three fortified levels (0.02, 0.1 and 0.2 mg kg^{-1}). The average recoveries and relative standard deviations (RSDs) from these experiments are given in Table 5. The RSDs of all pesticides are lower than 17.2%, which can fulfill the requirements of pesticide residue analysis [42,43]. And the recoveries of all OPPs (72.3–116%) and most of OCPs, PYRs, CBs are in the range of 70–120% set by EU guidelines [42,43]. For the BHC-beta (OCPs), isoprocarb (CBs), pirimicarb (CBs)

Table 6
Comparison of the present technique with reported methods.

Chemical class	LOD	Analytical technique	Reference
OPPs, CBs	0.2–13.5 μg kg^{-1}	HPLC/MS/MS	[4]
OPPs, PYRs	1.0–10.0 μg kg^{-1}	GC–MS SIM	[21]
PYRs	2.2–3.1 μg kg^{-1}	GC-FID	[37]
OCPs, OPPs	1.0–14.3 μg kg^{-1}	GC-ECD, GC-FPD	[38]
OCPs	1.0–6.0 μg kg^{-1}	GC-ECD	[39]
OPPs, CBs	0.5–10.0 μg L^{-1}	LC–MS	[40]
OCPs, OPPs	0.1–50.0 μg kg^{-1}	GC–MS SIM	[41]
OCPs, OPPs, CBs, PYRs	0.2–10.0 μg kg^{-1}	GC–MS SIM	The present method

Table 7
The concentrations of 27 pesticide residues in *Radix astragali*.

Pesticides	Analytical results (mg kg^{-1})					
	Gansu *Radix astragali* 1[a]	Gansu *Radix astragali* 2[a]	Gansu *Radix astragali* 3[a]	Inner Mongolia *Radix astragali* 1[a]	Inner Mongolia *Radix astragali* 2[a]	Inner Mongolia *Radix astragali* 3[a]
Isoprocarb	ND[b]	ND	ND	ND	ND	ND
Cadusafos	ND	ND	ND	ND	ND	ND
hexachlorobenzene	ND	0.01	ND	ND	ND	ND
BHC-alpha	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	ND	ND	ND	ND
Quintozene	ND	ND	ND	ND	ND	ND
BHC-gamma	ND	ND	ND	0.02	ND	0.03
BHC-beta	ND	ND	ND	ND	ND	ND
Pirimicarb	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	0.01	ND	ND	ND
Pirimiphos-methyl	ND	ND	0.01	ND	ND	ND
Malathion	ND	ND	ND	ND	ND	ND
Parathion	ND	ND	ND	ND	ND	ND
Bromophos	ND	ND	ND	ND	ND	ND
Butachlor	ND	ND	ND	ND	ND	ND
p,p′-DDE	ND	ND	ND	ND	ND	ND
Dieldrin	ND	0.02	ND	ND	0.03	0.03
o,p′-DDT	ND	ND	ND	0.01	ND	ND
p,p′-DDD	ND	ND	ND	ND	ND	ND
Sulprofos	ND	ND	ND	ND	ND	ND
p,p′-DDT	ND	ND	ND	ND	0.02	0.02
Bifenthrin	ND	ND	0.01	ND	ND	0.01
Carbosulfan	ND	ND	ND	ND	ND	ND
Fenpropathrin	ND	ND	ND	ND	ND	ND
Cyhalothrin	ND	ND	ND	ND	ND	ND
Benfuracarb	ND	ND	0.01	ND	ND	ND
Permethrin	0.02	ND	0.02	0.01	0.01	0.02

[a] From different markets.
[b] Not detectable or lower than limits of detection.

and benfuracarb (CBs), their recoveries are lower than 70% at the three spiked levels, possibly due to the adsorption of these pesticides by PSA. On the whole, the optimized method achieves the requirements for simultaneously routine screening of pesticide residues, except for several pesticides. Thus, the developed method is reliable for the simultaneous detection of OPPs, OCPs, PYRs and CBs pesticide residues in *Radix astragali*. For seeking a better recovery of isoprocarb, BHC-beta and pirimicarb, other sorbents will be investigated in our future work.

4. Real sample analysis

Six different *Radix astragali* samples were collected from Gansu and Inner Mongolia province in China. These samples were determined via the method established above, and the concentrations of pesticide residues are listed in Table 7. BHC-gamma, dieldrin, *p,p′*-DDT, bifenthrin and permethrin are detected in two or more samples. Hexachlorobenzene, heptachlor, pirimiphos-methyl, *o,p′*-DDT and benfuracarb are detected in one sample. BHC-gamma, dieldrin, *o,p′*-DDT and *p,p′*-DDT may come from the soil for plants. Hexachlorobenzene, heptachlor, pirimiphos-methyl, bifenthrin, benfuracarb and permethrin may originate from either the environmental soil or the necessity of the pesticide administration for the purpose of controlling pests. And the others are not found in these samples.

5. Conclusions

Twenty-seven pesticides covering OPPs, OCPs, PYRs and CBs in *Radix astragali* were first determined by GC–MS. MAE coupled with D-SPE technique was applied in the pretreatment process. The MAE technique provides satisfactory recoveries for the extraction of pesticides in *Radix astragali*. The D-SPE method greatly simplifies the sample clean-up process, and minimizes the consumption of organic solvents. To compensate the matrix-induced response enhancement effect, matrix-matched standards were employed. The linear range reaches 0.01–0.5 mg kg^{-1} for

each pesticide. The LOD and LOQ values range from 0.0002 to 0.01 mg kg^{-1} and 0.0008 to 0.03 mg kg^{-1}, respectively. The recoveries of all OPPs and most of OCPs, PYRs and CBs are between 70% and 120% with the RSDs less than 17.2%, meeting the requirements for routine screening of pesticide residues. Thus, this work demonstrates a simple, solvent-saving and reliable method for the simultaneous determination of OPPs, OCPs, PYRs and CBs in *Radix astragali*. This might be helpful for monitoring the four classes of pesticide residues in other CHMs.

Acknowledgements

The authors gratefully acknowledge the financial support of this study by the Natural Science Foundation of China (20965005), the Science and Technology Planning Project of Jiangxi Province (2008BB22400), and the State Key Laboratory of Food Science and Technology in Nanchang University (SKLF-TS-200918 and SKLF-MB-201002).

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