Dispersive Solid-Phase Extraction Cleanup Combined with Accelerated Solvent Extraction for the Determination of Carbamate Pesticide Residues in *Radix Glycyrrhizae* Samples by UPLC–MS–MS

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Abstract

Dispersive solid-phase extraction (DSPE) cleanup combined with accelerated solvent extraction (ASE) is described here as a new approach for the extraction of carbamate pesticides in *Radix Glycyrrhizae* samples prior to UPLC–MS–MS. In the DSPE–ASE method, 15 carbamate pesticides were extracted from *Radix Glycyrrhizae* samples with acetonitrile by the ASE method at 60°C with a 5 min heating time and two static cycles. Cleanup of a 1 mL aliquot of the extract by the DSPE method used 20 mg PSA (primary secondary amine), 50 mg Al_2O_3-N, and 20 mg GCB (graphitized carbon black) (as cleanup sorbents) under the determined optimum conditions. The linearity of the method was in the range of 10 to 200 ng/mL with correlation coefficients (r^2) of more than 0.996. The limits of detection were approximately 0.2 to 5.0 µg/kg. The method was successfully used for the analysis of target pesticides in *Radix Glycyrrhizae* samples. The recoveries of the carbamate pesticides at the spiking levels of 50, 100, and 200 µg/kg ranged from 79.7% to 99.3% with relative standard deviations lower than 10%. This multi-residue analytical method allows for a rapid, efficient, sensitive and reliable determination of target pesticides in *Radix Glycyrrhizae* and other medicinal herbs.

Introduction

Radix glycyrrhizae (Glycyrrhiza glabra L.) has been used as medicinal herb for over 2,000 years. In China and Japan, it is used as a raw material in tobacco, food, confectionery, and the worldwide pharmaceutical industry (1–4). It is cultivated on a large scale in China. Pesticides are usually applied in the field to eliminate damage and infestation by insects and pathogens. To ensure safe consumption, pesticide residues in the herbs are routinely monitored before putting them on the market.

Carbamate pesticides are efficient broad-spectrum insecticides, similar to organochlorine and organophosphorus (5). The most widely used method for the analysis of carbamate pesticides is high-performance liquid chromatography (HPLC) with post-column hydrolysis and derivatization by fluorescence detection (6–8). However, this method is time-consuming and requires skillful additional confirmatory techniques. In recent years, an HPLC-based method combined with sensitive mass spectrometric detection (LC–MS) and versatile tandem mass spectrometry (LC–MS–MS) using multiple reaction monitoring (MRM) mode have both become reliable and acceptable analytical tools for the simultaneous sensitive quantification and the unequivocal confirmation of a wide range of target pesticides in complex matrices. Sample pre-treatment is a crucial procedure in the analytic process, especially when a large number of samples is involved and where rapid extraction becomes even more essential (9).

The commonly used extraction techniques for pesticides are liquid–liquid extraction (LLE) (10), solid-phase-extraction (SPE) (11), solid-phase micro-extraction (SPME) (12), supercritical fluid extraction (SFE) (13), microwave-assisted extraction (MAE) (14), and ASE (15–17). Of these methods, ASE is the most suitable for solid and semi-solid samples because it provides advantages such as lower solvent volumes, an automatic procedure for the simultaneous extraction of multiple samples, short sample preparation time, and higher extraction recoveries. It has been accepted as the standard method for solid sample extraction by the U.S. EPA [Method 3545A (18)]. In the ASE system, conventional solvents at elevated temperatures and pressures are used, resulting in improved extraction kinetics to achieve quantitative extraction from solid and semi-solid samples in a short time with a small amount of solvent (19–22).

The extraction step is usually followed by a cleanup procedure. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method based on DSPE is commonly used. This method has many advantages, including a high recovery of pesticides with a wide range of polarities and volatilities, high sample throughput, smaller volumes of organic solvent and no chlorinated solvents. Very little labor is required, and the safety of laboratory

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workers is improved (23–26). However, little research on ASE in combination with a DSPE cleanup technique for the analysis of carbamate pesticide residues in herbs such as *Radix glycyrrhizae* has been reported.

This study was designed to develop a rapid, sensitive, and practical analytical method to simultaneously identify and quantify multiple classes of carbamate pesticides in *Radix glycyrrhizae*. The ASE experimental parameters (i.e., extraction solvent, temperature, extraction time and cycles) were optimized to achieve the maximum extraction efficiencies for carbamate pesticides. DSPE was applied to pre-concentrate and clean up the ASE extracts. The amounts of carbamate pesticide residues were quantified by an ultra performance liquid chromatography–electrospray ionization tandem mass spectrometry (UPLC–MS–MS) equipped with an electrospray ionization (ESI) source.

Experimental

Reagents and materials

Acetonitrile and ethyl acetate (HPLC grade) were obtained from J.T. Baker (Phillipsburg, PA); analytical grade acetone was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China); analytical grade formic acid was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO); HPLC-grade ammonium acetate was obtained from Fisher (New York). A milli-Q-Plus ultra-pure water system (Millipore, Milford, MA) provided HPLC-grade water. Sodium chloride (NaCl) was obtained from the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Beijing, China); and the analytical grade sorbents used for QuEChERS (primary secondary amine (PSA, 40–60 µm), octadecylsilane (ODS, 40–60 µm), alumina-N (Al_2O_3-N, 150 Mesh), florisil (60–100 Mesh), and graphitized carbon black (GCB)) were purchased from Agela Technologies (Beijing, China).

Standards and samples

15 pesticide standards with purity greater than 95.1% were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The standards are listed in Table I. Individual stock standard solutions of pesticides (approximately 1000 µg/mL) were prepared by dissolving 10 mg of a compound in 10 mL of acetonitrile. Stocks were stored at –20°C. Mixed compound stock standard solutions at a concentration of 10 µg/mL were prepared in acetonitrile and stored in brown bottles at –20°C.

The samples of *Radix Glycyrrhizae* were purchased from Tong Ren Tang Chinese Traditional Medicine Imports and Exports Co., Ltd. (Beijing, China). The samples were crushed and sieved through a 200-mesh filter and stored at temperatures below 20°C. These samples were used as controls in the spiked experiments because of the absence of phytosanitary treatment in growing and processing.

Instruments and chromatographic conditions

An ASE 300 system (Dionex, Sunnyvale, CA) was used for sample extraction. The ASE 300 was equipped with a solvent controller and AutoASE software. The solvent controller is an easy-to-use module that allows automated solvent mixture and delivery from up to four solvents. The AutoASE software is both a controller and a reporting application software package. The ASE 300 was also equipped with an auto-sampler carousel and a collection tray that allowed 12 separate samples to be extracted sequentially. Stainless steel extraction cells and glass collection vials of 66 and 200 mL volumes were used.

The separation of the analytes from the extracts was performed on a UPLC system consisting of a vacuum degasser, an auto-sampler, a column heater and a binary pump (Acquity Ultra Performance LC, Waters, Milford, MA). The UPLC was equipped with a reversed-phase rapid resolution C18 analytical column of 50 mm × 2.1 mm i.d. with a 1.7 µm particle size (Waters). For each analysis, 5 µL of extract was injected. Mobile phase A consisted of 0.1% (v/v) formic acid and ammonium acetate in water (5 mmol/L); mobile phase B was acetonitrile. All mobile phases were pumped at a flow rate of 0.25 mL/min. The gradient elution program began at 70% A, with a linear decrease to 45% A over 3 min and to 10% A over 6 min. The mobile phase was then kept at 10% A for 0.2 min, followed by a return to 70% A over 7 min, where it was held for 2 min to re-equilibrate the column prior to the next injection. The auto-sampler tray was kept at 10°C.

The UPLC system was connected to a triple quadrupole mass spectrometer (Waters Quattro Premier XE, Manchester, UK) equipped with an electrospray ionization (ESI) interface (Z-spray). The system was operated in the positive ion mode, using the following operation parameters: capillary voltage, 3500 V; desolvation gas (nitrogen, 99.999% purity), 50 L/h; cone gas (nitrogen, 99.999% purity), 798 L/h; source temperature, 110°C; desolvation temperature, 350°C; and collision cell pressure, 3.2 × 10^{-3} mbar. Optimization of the cone voltage and the collision

Table I. UPLC–MS–MS Parameters for 15 Carbamate Pesticides

Peak No. in Fig. 1	Analyte	CAS No.	Retention time (min)	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (ms)	Cone voltage (V)	Collision energy (eV)
1	Methomyl	16752-77-5	0.86	162.9	87.6*, 105.7	50	15	10/10
2	Aldicarb	116-06-3	1.55	191.0	115.8*, 88.8	50	13	10/15
3	Metolcarb	1129-41-5	1.76	284.2	176.0*, 252.14	50	15	25/15
4	Pirimicarb	23103-98-2	1.80	239.1	181.9*, 71.7	50	20	15/15
5	Propoxur	114-26-1	2.05	210.0	110.7*, 167.8	50	18	15/8
6	Carbofuran	1563-66-2	2.11	222.1	122.8*, 164.9	50	25	20/12
7	Bendiocarb	22781-23-3	2.09	224.0	108.7*, 166.8	50	18	18/10
8	Carbaryl	63-25-2	2.29	202.0	126.9*, 144.8	50	20	25/13
9	Isoprocarb	2631-40-5	2.69	194.0	94.6*, 151.7	50	20	15/10
10	Methiocarb	2032-65-7	3.28	226.0	120.8*, 168.8	50	20	20/10
11	Fenobucarb	3766-81-2	3.29	208.1	94.8*, 151.8	50	20	15/10
12	Thiobencarb	28249-77-6	5.00	258.0	99.8*, 124.79	50	20	15/15
13	Indoxacarb	173584-44-6	5.29	528.0	150.0*, 218.2	50	31	20/20
14	Furathiocarb	65907-30-4	5.63	383.2	166.9*, 195.0	50	10	25/20
15	Triallat	2303-17-5	6.16	304.0	85.8*, 127.9	50	23	15/15

* Quantitative ion.

energy (CE) for each individual pesticide was done by infusion of the pesticide directly into the LC effluent using a syringe pump (Harvard, Kent, UK) at a flow rate of 10 µL/min in the respective mobile phase composition. For instrument control, data acquisition and processing, MassLynx software v4.1 was used. The analytes were measured in the multiple reaction monitoring (MRM) mode using scheduled time windows. The compound-specific mass spectrometry parameters (i.e., fragmentor voltage and collision energy) are summarized in Table I. The total ion current (TIC) chromatograms of 15 carbamate pesticides are shown in Figure 1.

Sample preparation

Accelerated solvent extractions of *Radix glycyrrhizae* samples were performed using the ASE 300 system. An aliquot (5 g) of spiked sample or blank sample was put into the 66 mL extraction cell with a cellulose disk at the bottom; the cell was then capped and placed on the extractor. The extraction process was sequentially performed with acetonitrile under the following conditions: extraction temperature, 60°C; extraction pressure, 10.3 MPa; heating time, 5 min; and static extraction period, 5 min. The sample was then rinsed with a cell volume of 60% acetonitrile and purged with nitrogen for 120 s with two static cycles. The extraction efficiencies of three solvent systems were compared under the fixed ASE conditions: acetonitrile, ethyl acetate, and acetone. The extract was transferred to a cone-bottomed flask and condensed almost to dryness on a rotavap at 40°C. The residue was dissolved in 10 mL of acetonitrile. A 1-mL aliquot of the supernatant (acetonitrile phase) was transferred to a 2-mL micro-centrifuge tube containing 20 mg PSA, 50 mg Al_2O_3-N and 20 mg GCB and shaken energetically for 1 min. The extract was centrifuged for 5 min at 10,000 rpm. Finally, an extract in 100% acetonitrile was obtained. Prior to analysis, the extract was filtered through a 0.20 µm PTFE filter and transferred to a vial. Five µL of the filtrate was injected into the UPLC–MS–MS. Matrix extracts were used for validation of the method by appropriate spiking with the pesticide mix in the subsequent analysis.

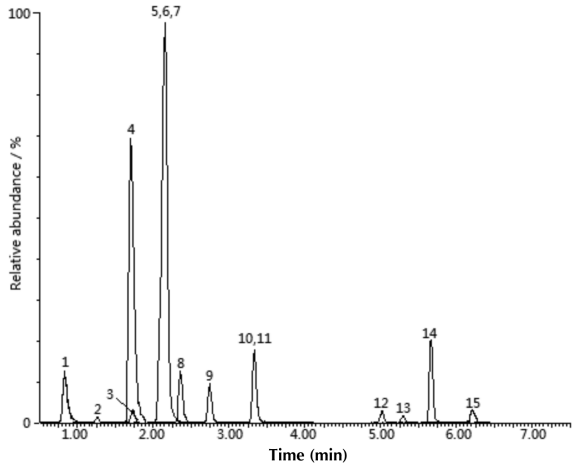

Figure 1. UPLC–MS–MS total ion current chromatograms of the mixed standard solution of 15 carbamate pesticides (with a concentration of 0.1 mg/L for each compound). Note: For peak identifications, see Table I.

Calibration and quantitation

In order to reduce matrix effects, matrix-matched standards were used in this study. The *Radix glycyrrhizae* sample was used as the blank for preparation of the standards. To obtain the respective calibration curves, a series of standard solutions containing different concentrations (10, 20, 50, 100, 150, and 200 ng/mL) of target analytes were determined. Eight replications were conducted for each concentration. Peak areas of spiked samples were measured and plotted against the spiked concentration to generate the calibration curves. The extraction, cleanup and analysis procedures were as described above.

Table II. Calibration Curves, Correlation Coefficients (r^2), Spiked Recoveries, LODs, and LOQs for 15 Carbamate Pesticides in *Radix Glycyrrhizae* samples

Analyte	Calibration curve*	r^2	LOD (mg/kg)	LOQ (mg/kg)	Average recoveries ± RSDs (n = 8) (mg/kg)		
					0.05	0.1	0.2
Methomyl	Y = 103.0389X – 403.805	0.998	0.0012	0.0040	89.6 ± 4.6	95.8 ± 6.8	92.2 ± 2.9
Aldicarb	Y = 5.32897X – 22.0778	0.997	0.0050	0.0166	89.0 ± 6.0	90.8 ± 4.4	99.1 ± 5.1
Metolcarb	Y = 115.872X + 183.585	0.999	0.0006	0.0021	79.7 ± 4.3	91.4 ± 4.8	97.2 ± 4.6
Propoxur	Y = 94.8865X – 280.885	0.996	0.0010	0.0035	94.6 ± 3.7	95.3 ± 3.3	98.2 ± 2.0
Pirimicarb	Y = 597.004X – 1163.06	0.998	0.0004	0.0014	90.0 ± 4.1	94.1 ± 3.0	97.1 ± 4.8
Carbofuran	Y = 165.89X – 297.223	0.997	0.0008	0.0028	95.5 ± 3.6	96.7 ± 4.9	97.2 ± 2.6
Bendiocarb	Y = 246.914X – 571.827	0.998	0.0002	0.0007	89.9 ± 3.6	94.6 ± 3.3	95.8 ± 2.9
Carbaryl	Y = 131.854X – 746.302	0.997	0.0043	0.0144	93.3 ± 2.9	96.0 ± 3.5	96.8 ± 5.0
Isoprocarb	Y = 196.68X – 363.369	0.999	0.0004	0.0012	87.9 ± 5.1	96.9 ± 3.4	98.5 ± 2.7
Methiocarb	Y = 74.902X – 147.022	0.999	0.0011	0.0037	88.4 ± 4.5	94.4 ± 5.2	97.9 ± 2.8
Fenobucarb	Y = 88.0739X – 100.394	0.999	0.0012	0.0040	90.1 ± 4.8	94.8 ± 4.6	99.3 ± 3.9
Thiobencarb	Y = 50.9404X – 262.941	0.999	0.0004	0.0015	87.9 ± 5.3	93.0 ± 4.2	97.3 ± 2.9
Furathiocarb	Y = 365.895X – 77.0772	0.999	0.0002	0.0008	90.7 ± 4.9	93.0 ± 3.7	99.2 ± 4.0
Triallat	Y = 22.7162X + 46.6744	0.999	0.0009	0.0029	87.8 ± 6.6	97.0 ± 3.6	98.8 ± 4.2
Indoxacarb	Y = 32.0203X – 62.4099	0.999	0.0005	0.0016	87.2 ± 5.3	95.2 ± 4.2	97.6 ± 3.5

*linear range: 10–200 ng/mL. Y = peak area and X = mass concentration (ng/mL).

Results and Discussion

Selection of extraction solvent

The choice of the solvent is critical for developing a new multi-class, multi-residue method. The extraction solvents most commonly used for multi-residue analysis of pesticides are acetonitrile (27–29), acetone (29,30), and ethyl acetate (31); each has been shown to give high recoveries of a wide range of pesticides. Therefore, acetonitrile, acetone and ethyl acetate mixture were compared in this study. Of these, the highest extraction efficiencies were achieved with acetonitrile. The results showed that the recoveries of 15 carbamate pesticides spiked in *Radix glycyrrhizae* samples were significantly different.

Under the same extraction and cleanup conditions, the color sequential brightness of the extract was as follows: ethyl acetate > acetonitrile > acetone. As shown in Figure 2, the

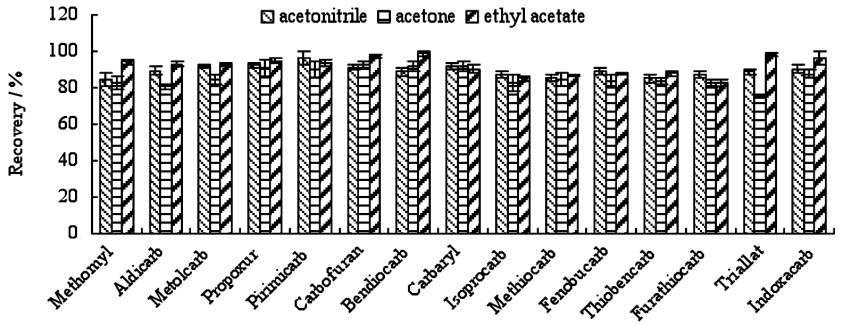

Figure 2. Effect of three different extraction solvents on the recoveries of 15 carbamate pesticides spiked in *Radix glycyrrhizae* samples at 0.1 mg/kg.

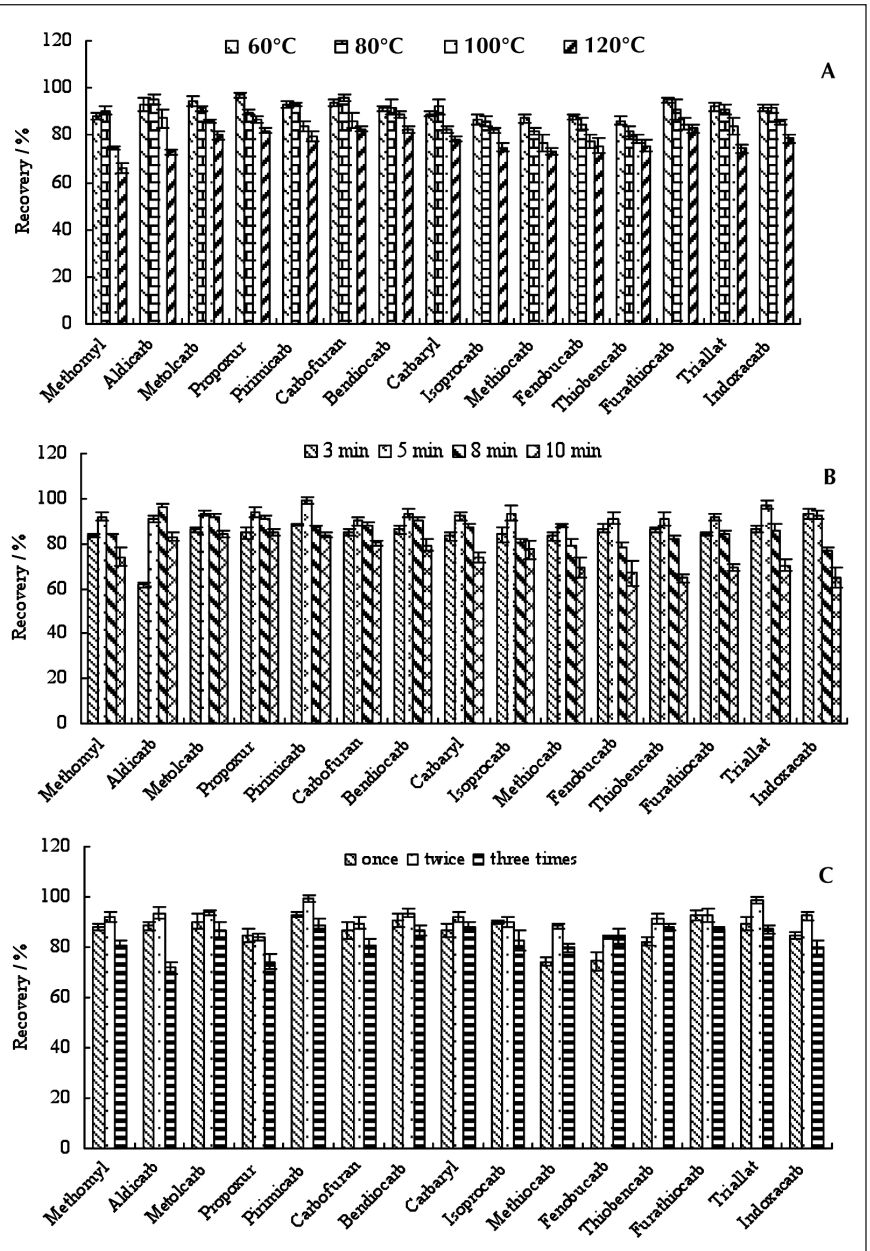

Figure 3. Effect of different factors [(A) extraction temperature, (B) static extraction period, and (C) static cycle] on ASE extraction efficiency (expressed as relative recovery of 15 carbamate pesticides spiked in *Radix glycyrrhizae* samples at 0.1 mg/kg.).

efficiency of cleanup was: ethyl acetate > acetonitrile > acetone. However, compared with ethyl acetate, acetonitrile does not extract as much lipophilic material, e.g., waxes, fat, and lipophilic pigments (23). Using acetonitrile, the average recoveries of the 15 carbamate pesticides ranged from 84.4% to 96.0%, which met the requirements of 70% to 120% established in SANCO/10684/2009 (32). Therefore, acetonitrile was selected as the extraction solvent.

Optimization of ASE operating parameters

With the aim of identifying the most efficient conditions, the key parameters of ASE that affect extraction efficiency [extraction temperature, static extraction period and static cycles (19–22)], were evaluated. These parameters were optimized in three sets of experiments. Extraction temperature was first optimized while keeping the other parameters constant (extraction pressure, 10.3 MPa; heating time, 5 min; static extraction period, 5 min; flush volume, 60%; purge time, 120 s; static cycles, twice). After determining the optimal extraction temperature of 60°C (Figure 3A), the optimal static extraction period was determined to be 5 min (Figure 3B). Optimization of the static cycle was then evaluated (Figure 3C). Finally, the combination of extraction parameters was selected: two static extractions in acetonitrile of 5 min each at 60°C and 10.3 MPa, followed by purging with nitrogen for 120 s.

Optimization of cleanup procedure

The method of DSPE is based on the SPE method. The sorbent in DSPE is added directly to the extract, and the cleanup is easily carried out by shaking and centrifugation. In the SPE method, the column should be conditioned. DSPE, in which the sorbent acts as a "chemical filter" to remove matrix components, is therefore much more convenient than SPE. Nevertheless, it requires the identification of the optimal sorbent combination and determination of the capacity of different sorbents.

Selection of sorbent combination

The cleanup sorbents PSA, GCB, NH_2, alumina-N, florisol, and ODS were compared. The sorbents PSA and -NH_2 are weak anion exchangers with the ability to remove fatty acids, sugars and other matrix co-extractives. PSA removes more matrix co-extractives than -NH_2 per given quantity, because PSA has both a primary and a secondary amine.

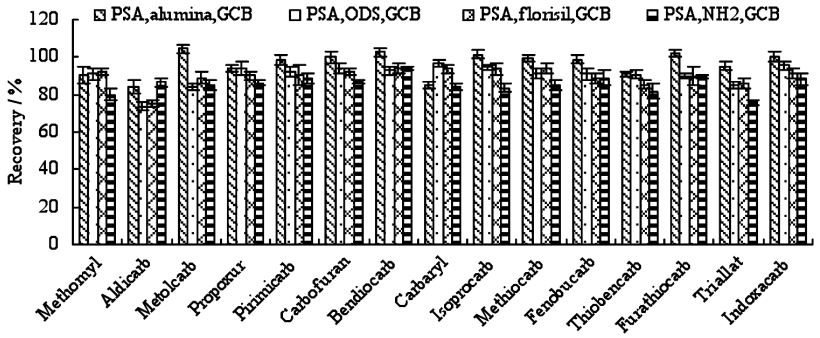

Figure 4. Effect of different sorbent combinations on the recoveries of 15 carbamate pesticides spiked in *Radix glycyrrhizae* at 0.1 mg/kg in the cleanup procedure.

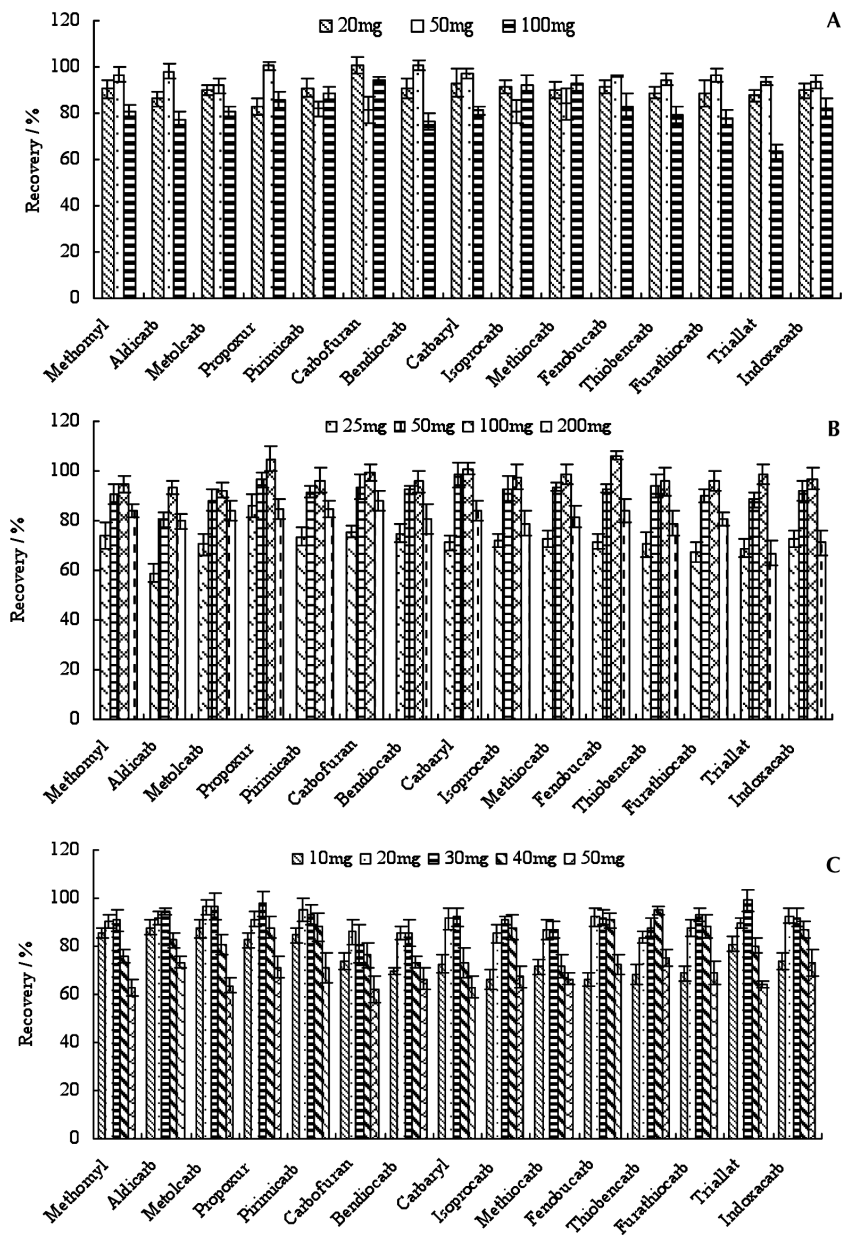

Figure 5. Effect of different amounts of sorbent [(A) PSA, (B) Al2O3-N and (C) GCB] on the recoveries of 15 carbamate pesticides spiked in *Radix glycyrrhizae* at 0.1 mg/kg in the cleanup procedure.

ODS is a reversed-phase sorbent that retains lipophilic plant lipids and sterols in fat-containing samples. Alumina-N and florisol are normal-phase sorbents, and their role is similar to ODS. GCB removes planar molecules such as natural pigments (e.g., chlorophyll and carotenoids) from sample matrices (23,33). In order to identify the most suitable sorbent combination for maximizing the efficiency of cleanup without affecting recovery, the effects of sorbent type and composition on cleanup efficiency and extraction recovery were evaluated. For improved evaluation, the content of fat in *Radix glycyrrhizae* was tested according to GB/T 5009.6-2003 (34). The fat content of *Radix glycyrrhizae* was 2.9%, which is higher than the criteria of high and low fat content [2.5% (35)].

According to the results shown in Figure 4, the DSPE protocol using a ternary mixture of PSA/Al_2O_3-N/GCB was the most appropriate sorbent combination for efficient cleanup. The sequential color brightness of the extract was as follows: PSA/Al_2O_3-N/GCB > PSA/-NH_2/GCB > PSA/Florisil/GCB > PSA/ODS/GCB. The efficiency of cleanup was as follows: PSA/ Al_2O_3-N /GCB > PSA/ODS/GCB > PSA/Florisil/GCB > PSA/-NH_2/GCB. Using the sorbent combination of PSA/Al_2O_3-N/GCB, all targets had satisfactory recoveries (ranging from 70–120%). This combination was therefore selected for the subsequent analysis.

Capacity of different sorbents in DSPE

To determine the best sorbent amount for maximizing the effectiveness of cleanup, different amounts of sorbent were investigated. For optimizing the dosage of PSA, 3 recovery experiments were performed, in which dosages of 25, 50, and 100 mg were compared and the dosages of other sorbents (50 mg Al_2O_3-N and 20 mg GCB to cleanup 1 mL of the sample extracts) were kept constant.

Figure 5A shows the effect of different amounts of PSA on the purification of *Radix Glycyrrhizae* extracts. All targets achieved satisfactory recoveries (ranging from 70% to 120%) when 20/50 mg PSA in combination with 50 mg Al_2O_3-N and 20 mg GCB was used. The recovery of Triallat was less than 70% among the carbamate pesticides when 100 mg PSA was used. Therefore, 20 mg PSA was chosen as the most cost-effective technique. After comparing the cleanup effectiveness of 25, 50, 100, and 200 mg of Al_2O_3-N, 50 mg was chosen as the optimal dosage (Figure 5B). The optimum GCB dosage was deter-

Table III. Quantitative Analysis of Carbamate Pesticides in Real *Radix Glycyrrhiza*e Samples by UPLC–MS–MS

Sample No.	Isoprocarb (mg/kg)	Methiocarb (mg/kg)	Fenobucarb (mg/kg)	Thiobencarb (mg/kg)	Furathiocarb (mg/kg)	Triallat (mg/kg)	Indoxacarb (mg/kg)
1	0.007	0.007	N.D.*	0.013	0.011	0.003	0.010
2	N.D.*	N.D.*	0.005	N.D.*	N.D.*	N.D.*	0.015
3	0.009	0.011	0.004	0.010	0.015	0.007	0.004
4	0.005	N.D.*	0.009	0.016	N.D.*	0.005	0.004

* ND means not detected.

Table IV. Comparison of ASE-DSPE-UPLC–MS–MS with Other Extraction Methods for the Determination of Carbamate Pesticides in *Radix Glycyrrhiza*e Samples

Methods	LODs (mg/kg)	Volume of organic solvent required (mL)	Analytical period by instrument (min)	Ref.
SPE–HPLC	0.0003–0.068	>70	50	(36)
LLE–LC–MS	0.01–0.5	>500	20	(37)
GPC–HPLC	0.0001–0.001	>80	45	(38)
DSPE-ASE–UPLC–MS–MS	0.0002–0.005	60	9	This method

mined to be 20 mg (Figure 5C). Based on these results, the DSPE protocol uses the sorbent combination of PSA/Al_2O_3-N/GCB (20 mg/50 mg/20 mg per mL of extract) for the efficient removal of various interfering substances in *Radix glycyrrhizae* extracts.

Analytical method performance

Using matrix-matched standards, the linearity of the method was detected in the range of 10 to 200 ng/mL for all targets with correlation coefficients higher than 0.996 (see Table II). The limits of detection (LOD) and quantitation (LOQ) for target analytes were calculated when the signal-to-noise ratio was 3 and 10, respectively. The LOD and LOQ obtained for different carbamate pesticides are shown in Table II. The LODs ranged from 0.2–5.0 µg/kg and the LOQs were in the range of 0.7–16.6 µg/kg.

The accuracy and reproducibility of the quantitative method were investigated by means of recovery tests performed on samples of *Radix glycyrrhizae* spiked with target analytes at three concentration levels: 0.05, 0.1 and 0.2 mg/kg, with eight replicates for each level. Mean recoveries and the corresponding relative standard deviations (RSD) are indicated in Table II. Recoveries ranging from 79.7% to 99.3% with RSD values < 10% were obtained for all the carbamate pesticides studied. The good recoveries and the repeatability of the proposed method make it suitable for the quantification of a wide polarity range of carbamate pesticides present in *Radix glycyrrhizae* at trace levels.

Application to real samples

In order to investigate the performance of the proposed method, 4 samples collected from different planting areas in China were extracted by ASE using acetonitrile, purified by the DSPE technique and analyzed by UPLC–MS–MS. The results showed that all the *Radix glycyrrhizae* samples contained detectable levels of one of the target analytes, ranging from 0.004 to 0.016 mg/kg (see Table III).

The results demonstrate that the recommended method can be used for the trace analysis of carbamate pesticides in *Radix glycyrrhizae* samples.

Comparison of ASE-DSPE-UPLC–MS–MS with other Published Methods

Table IV summarizes the limit of detection (LOD), analytical period by instrument and volume of organic solvent required in the liquid–liquid extraction (LLE), solid-phase-extraction (SPE), gel permeation chromatographic (GPC) cleanup and DSPE-ASE (represented method) for the extraction and determination of carbamate pesticides in *Radix glycyrrhizae* samples. The DSPE-ASE method has a low LOD comparable to the LLE, GPC and SPE methods. It has the advantage of using less organic solvent (about 60 mL) than the other kinds of methods. In addition, the analysis time using UPLC–MS–MS was approximately one-half to one-fifth of the other methods. Thus, DSPE-ASE-UPLC–MS–MS is the most efficient method, in addition to being more environmentally benign, for the analysis of carbamate pesticide residues in *Radix glycyrrhizae* samples.

Conclusions

A simple, sensitive and relatively fast analytical method for carbamate pesticides was developed. DSPE cleanup combined with ASE is a new approach for the extraction of carbamate pesticides in *Radix glycyrrhizae* samples prior to UPLC–MS–MS. In the present study, four different extraction solvents, three important ASE operating parameters (extraction temperature, static extraction period and static cycles) and six different sorbent materials were compared for their ability to remove interfering materials from the extracts. The proposed method makes simultaneous analyses of 15 pesticides possible, with satisfactory recoveries, low detection limits, high throughput and short analytical time (9 min). This simple, rapid, efficient, and environmentally friendly method facilitates the analysis of carbamate pesticides in herbs such as *Radix glycyrrhizae* and could be applied to the routine analysis of *Radix glycyrrhizae* samples.

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References

1. X.J. Pan, H.Z. Liu, G.H. Jia, and Y.Y. Shu. Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochem. Eng. J.* **3:** 173–177 (2000).
2. J. Cong, L. Gao, and B. Lin. Progress in studies of flavonoid compounds of licorice. *Fine chemicals*. **S1:** 121–124 (2004).
3. G. Xing, N. Li, T. Wang, and M. Yao. Adcances in stuides on flavonoids of licorice. *China Journal of Chinese Materia Medica*. 7: 593–597 (2003).
4. F. Rauchensteiner, Y. Matsumura, Y. Yamamoto, S. Yamaji, and T. Tani. Analysis and comparison of *Radix Glycyrrhizae* (licorice) from Europe and China by capillary-zone electrophoresis (CZE). *J. Pharm. Biomed. Anal.* **4:** 594–600 (2005).
5. C. Przybylski, and V. Bonnet. Use of spermine and thiabendazole as analyte protectants to improve direct analysis of 16 carbamates by gas chromatography-mass spectrometry in green vegetable matrices. *Analytical and Bioanalytical Chemistry*. **4:** 1147–1159 (2009).
6. H. Mori, M. Kobayashi, K. Yagi, M. Takahashi, T. Gondo, and N. Umetsu. A High-Performance Liquid-Chromatographic Method for Determination of Benfuracarb and Carbofuran Residues in Soil and Water. *Journal of Pesticide Science*. **3:** 491–497 (1987).
7. R.T. Krause. High-Performance Liquid-Chromatographic Determination of Aryl N-Methylcarbamate Residues Using Post-Column Hydrolysis Electrochemical Detection. *J. Chromatogr.* **442:** 333–343 (1988).
8. B.D. Mcgarvey. High-Performance Liquid-Chromatographic Methods for the Determination of N-Methylcarbamate Pesticides in Water, Soil, Plants and Air. *J. Chromatogr.* **1-2:** 89–105 (1993).
9. Y.J. Ding, W.H. Zhang, C. Gu, I. Xagoraraki, and H. Li. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. *J. Chromatogr. A.* **1:** 10–16 (2011).
10. L.Y. Fu, X.J. Liu, J. Hu, X.N. Zhao, H.L. Wang, and X.D. Wang. Application of dispersive liquid-liquid microextraction for the analysis of triazophos and carbaryl pesticides in water and fruit juice samples. *Anal. Chim. Acta.* **2:** 289–295 (2009).
11. R.M. Gonzalez-Rodriguez, R. Rial-Otero, B. Cancho-Grande, and J. Simal-Gandara. Determination of 23 pesticide residues in leafy vegetables using gas chromatography-ion trap mass spectrometry and analyte protectants. *J. Chromatogr. A.* **1196-1197:** 100–109 (2008).
12. J. Schurek, T. Portoles, J. Hajslova, K. Riddellova, and F. Hernandez. Application of head-space solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the determination of multiple pesticide residues in tea samples. *Anal. Chim. Acta*. **2:** 163–172 (2008).
13. Y.C. Ling, H.C. Teng, and C. Cartwright. Supercritical fluid extraction and clean-up of organochlorine pesticides in Chinese herbal medicine. *J. Chromatogr. A.* **1-2:** 145–157 (1999).
14. Z. Hu, M. Cai, and H.H. Liang. Desirability function approach for the optimization of microwave-assisted extraction of saikosaponins from *Radix Bupleuri*. *Sep. Purif. Technol*. **3:** 266–275 (2008).
15. S. Sporring, S. Bowadt, B. Svensmark, and E. Bjorklund. Comprehensive comparison of classic Soxhlet extraction with Soxtec extraction, ultrasonication extraction, supercritical fluid extraction, microwave assisted extraction and accelerated solvent extraction for the determination of polychlorinated biphenyls in soil. *J. Chromatogr. A.* **1-2:** 1–9 (2005).
16. J. He, R. Balasubramanian, S. Karthikeyan, and U.M. Joshi. Determination of semi-volatile organochlorine compounds in the atmosphere of Singapore using accelerated solvent extraction. *Chemosphere*. **5:** 640–648 (2009).
17. H.A. Yu, Y.F. Tao, T. Le, D.M. Chen, A. Ishsan, Y. Liu, Y.L. Wang, and Z.H. Yuan. Simultaneous determination of amitraz and its metabolite residue in food animal tissues by gas chromatography-electron capture detector and gas chromatography-mass spectrometry with accelerated solvent extraction. J. Chromatogr. *B-Anal. Technol. Biomed. Life Sci.* **21:** 1746–1752 (2010).
18. EPA METHOD 3545A: Pressurized Fluid Extraction (PFE). Available from: <http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3545a.pdf>.
19. H. Yu, Y. Tao, D. Chen, Y. Wang, and Z. Yuan. Development of an HPLC-UV method for the simultaneous determination of tetracyclines in muscle and liver of porcine, chicken and bovine with accelerated solvent extraction. *Food Chem*. **3:** 1131–1138 (2011).
20. Y. Li, T. Pang, Z.M. Guo, Y.L. Li, X.L. Wang, J.H. Deng, K.J. Zhong, X. Lu, and G.W. Xu. Accelerated solvent extraction for GC-based tobacco fingerprinting and its comparison with simultaneous distillation and extraction. *Talanta*. **1-2:** 650–656 (2010).
21. E. Conte, R. Milani, G. Morali, and F. Abballe. Comparison between accelerated solvent extraction and traditional extraction methods for the analysis of the herbicide diflufenican in soil. *J. Chromatogr. A.* **1:** 121–125 (1997).
22. M. Herrero, P.J. Martin-Alvarez, F.J. Senorans, A. Cifuentes, and E. Ibanez. Optimization of accelerated solvent extraction of antioxidants from Spirulina platensis microalga. *Food Chem.* **3:** 417–423 (2005).
23. M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J. Schenck. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2:** 412–431 (2003).
24. S.J. Lehotay, K. Mastovska, and A.R. Lightfield. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables (vol 88, pg 615, 2005). *J. AOAC Int.* **3:** 60a–60a (2005).
25. I. Ferrer, and E.M. Thurman. Multi-residue method for the analysis of 101 pesticides and their degradates in food and water samples by liquid chromatography/time-of-flight mass spectrometry. *J. Chromatogr. A.* **1:** 24–37 (2007).
26. A. Valverde, A. Aguilera, C. Ferrar, F. Camacho, and A. Cammarano. Analysis of Forchlorfenuron in Vegetables by LC/TOF-MS after Extraction with the Buffered QuEChERS Method. *J. Agric. Food. Chem.* **5:** 2818–2823 (2010).
27. R. Yang, J. Wang, R. Zhang, M. Wang, and M. Huang. Determination of 11 carbamate pesticide residues in Glycyrrhizae radix et rhizoma and its extracts using ultra performance liquid chromatography-tandem mass spectrometry with gel permeation chromatographic clean-up. *Chinese J. Chromatogr.* **8:** 769–775 (2010).
28. L.B. Liu, Y. Hashi, Y.P. Qin, H.X. Zhou, and J.M. Lin. Development of automated online gel permeation chromatography-gas chromatograph mass spectrometry for measuring multiresidual pesticides in agricultural products. *J. Chromatogr. B-Anal. Technol. Biomed. Life Sci.* **1:** 61–68 (2007).
29. I.R. Pizzutti, A. de Kok, M. Hiemstra, C. Wickert, and O.D. Prestes. Method validation and comparison of acetonitrile and acetone extraction for the analysis of 169 pesticides in soya grain by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* **21:** 4539–4552 (2009).
30. Q.B. Lin, H.J. Shi, and P. Xue. MSPD-GC-MS-MS Determination of Residues of 15 Organic Nitrogen-Containing Pesticides in Vegetables. *Chromatographia*. **11-12:** 1143–1148 (2010).
31. C. Müller, F. Bracher, and F. Plössl. Determination of Nicotine in Dried Mushrooms by Using a Modified QuEChERS Approach and GC–MS–MS. *Chromatographia*. **73:** 807–811 (2011).
32. European Commision. Method validation and quality control procedures for pesticide residues analysis in food and feed, Document No. SANCO/10684/2009 (2009). Available from: www.ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf.
33. C. Diez, W.A. Traag, P. Zommer, P. Marinero, and J. Atienza. Comparison of an acetonitrile extraction/partitioning and "dispersive solid-phase extraction" method with classical multi-residue methods for the extraction of herbicide residues in barley samples. *J. Chromatogr. A.* **1-2:** 11–23 (2006).
34. The People's Republic of China national standards. Determination of fat in foods. Document No. G.T. 5009.6-2003 (2003).
35. Modular multiple analytical method for the determination of pesticide residues in foodstuffs (extended and revised version of the DFG Method S 19), Collection of Official Methods under Article 35 of the German Ferderal Food Act (1999).
36. X.H. Mao, Z.W. Jia, S. Miao, J.W. Lu, K. Wang, and S. Ji. HPLC for the simultaneous determination of 13 pesticides in traditional Chinese herbal medicines. *Chinese Traditional Patent Medicine*. **3:** 454–459 (2010).
37. M. Fernández, Y. Picó, and J. Mañes. Simultaneous Determination of Carbamate and Organophosphorus Pesticides in Honeybees by Liquid Chromatography-Mass Spectrometry. *Chromatographia* **58:** 151–158 151–158 (2003).
38. L. Sun, H.Y. Jin, J.G. Tian, and R.C. Lin. HPLC multi-residue analysis of 13 N-methylcarbamates in traditional Chinese medicines with gel permeation chromatography and postcolumn derivatization. *Chinese J. Pharmaceut. Anal.* **4:** 668–672 (2010).

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