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Liquid–solid extraction coupled with magnetic solid-phase extraction for determination of pyrethroid residues in vegetable samples by ultra fast liquid chromatography

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ABSTRACT

In this study, liquid–solid extraction coupled with magnetic solid-phase extraction was successfully developed for the extraction of pyrethroid residues in vegetable samples. The analytes were determined by ultra fast liquid chromatography. The pyrethroids were extracted by liquid–solid extraction and then adsorbed onto magnetic adsorbent. Magnetic adsorbent, C_{18}-functionalized ultrafine magnetic silica nanoparticles, was synthesized by chemical coprecipitation, silanization and alkylation. The analytes adsorbed onto the magnetic adsorbent can be simply and rapidly isolated from sample solution with a strong magnet on the bottom of the extraction vessel. The extraction parameters, such as liquid–solid extraction solvent, liquid–solid extraction time, the amount of magnetic adsorbent, magnetic solid-phase extraction time and magnetic solid-phase extraction desorption solvent, were optimized to improve the extraction efficiency. The analytical performances of this method, including linear range, detection limit, precision, and recovery were evaluated. The limits of detection for pyrethroid were between 0.63 and 1.2 ng g^{-1}. Recoveries obtained by analyzing the four spiked vegetable samples were between 76.0% and 99.5%. The results showed that the present method was a simple, accurate and high efficient approach for the determination of pyrethroids in the vegetable samples.

1. Introduction

Synthetic pyrethroids are broad-spectrum, high-efficiency insecticides [1]. In the past few decades, synthetic pyrethroid insecticides have increasingly replaced organochlorine pesticides due to their relative low mammalian toxicity and selective activity [2]. The pyrethroids are used in urban areas to control pest in house and in rural areas to protect fruit and vegetables such as strawberries, tomato, lettuce and Chinese cabbage [3]. Therefore, the risk of pyrethroid residues in the food consumed is present, which is due to the overuse and accumulation in food chain [4–6]. Although the effects on humans are still unclear, the USA Environmental Protection Agency has classified some of these pyrethroids as possible human carcinogens [7,8]. Agricultural Ministry of Spain has established maximal residue limits (MRLs) for the pesticides in a variety of foods, such as tomato. The MRLs are 0.5 mg kg^{-1} for lambda-cyhalothrin and permethrin, 0.2 mg kg^{-1} for bifenthrin [9]. Because the MRLs are very low the pesticides cannot be directly detected with common analytical instruments. In order to make the low limit of quantification for developed method lower than MRLs, it is necessary to develop sample preparation methods to concentrate the pesticides [10].

Sample preparation before instrumental analysis is one of the most important and crucial steps. Some procedures for extraction of pesticide residues in samples have been described [11]. Soxhlet extraction [12], liquid–solid extraction (LSE) [13] and ultrasonic extraction [14] are the most common techniques for the extraction of pesticides from plant matrices. Matrix solid-phase dispersion is a technique in which the matrix is mixed with a suitable solid adsorbent [15]. Cheng et al. used matrix solid-phase dispersion for extracting the pyrethroid residues in porcine tissues [16]. However, this technique is tedious and time-consuming. In recent years, solid phase extraction (SPE) and microextraction (SPME) are widely applied for the extraction of pesticides in vegetable samples [17–19]. Beltran et al. used SPME for extracting the pyrethroid residues in vegetable samples [9]. In contrast to conventional techniques, SPME is a solvent-free extraction in which the extraction and concentration can be focused into a single step. However, the fibers used in SPME are expensive and the lifetime of the fibers is short, which limits the application of this method.

In recent years, current trends are directed toward miniaturization by using microextraction techniques, which consume less toxic organic solvent than the conventional techniques. Magnetic

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solid-phase extraction (MSPE) has been developed as a fast, simple, cost effective and versatile extraction method based on the use of magnetic or magnetizable adsorbent [20–28]. In MSPE, magnetic adsorbent is added into a sample solution containing the target analytes. The analytes are adsorbed onto the magnetic adsorbent and then the adsorbent is isolated from the sample solution using an appropriate magnetic separator. The analytes are consequently eluted from the adsorbent and analyzed. The main advantages of this method are that the phase separation could be conveniently performed by applying an external magnetic field and the adsorbent does not need to be packed into the SPE cartridges. It greatly shortens the time of preparation. This method can be applied for the adsorption and separation of analytes from large volume of environment samples in a short period. However, MSPE is mainly applied to the extraction of the analytes from liquid matrices [23–28]. The extraction of analytes from solid matrices based on MSPE has been rarely reported. As a matter of fact, extraction of analytes in very complex samples, such as vegetable matrices could be carried out by organic solvent based liquid–solid extraction.

Recently, magnetic adsorbents used in MSPE are usually nanometer-sized particles (NPs). Among the magnetic NPs, Fe_3O_4 NPs appear the interesting advanced composite materials [29,30]. The advantage of Fe_3O_4 NPs is that their surface can be easily modified to achieve the extraction of selective analytes. Wang et al. synthesized Fe_3O_4 NPs with diameter of several hundred nanometers by solvent-thermal method and coated magnetic core Fe_3O_4 NPs with carbon [31]. SiO_2 is usually used to modify Fe_3O_4 NPs, due to its stability under acidic condition, high thermal resistance and versatility in surface modification [32]. The adsorption ability of magnetic NPs could be improved by surface modification of the magnetic NPs with alkyl chain [33,34]. Ultrafine Fe_3O_4 NPs used in this study are prepared by chemical coprecipitation method and their diameters are about 15 nm. They have larger surface area and could be more easily dispersed in solution compared with those prepared by solvent-thermal method. C_{18}-functionalized ultrafine magnetic silica nanoparticles (C_{18}-UMS NPs) were synthesized by coating ultrafine Fe_3O_4 NPs with silica and subsequently modified with chlorodimethyl-*n*-octadecylsiane, which is according to our previous studies [35]. These C_{18}-UMS NPs were used as magnetic adsorbent to extract six kinds of pyrethroid residues in vegetable samples based on LSE-MSPE. To our best knowledge, the application of LSE coupled with MSPE to extraction of pyrethroid residues from vegetable samples was reported first time.

2. Experimental

2.1. Chemicals

The standards of lambda-cyhalothrin, cypermethrin, deltamethrin and esfenvalerate were purchased from National Research Center (NRC, China), permethrin and bifenthrin were obtained from National Institute of Metrology (NIM, China). The purities of the pyrethroids (Table S1) are ≥99.6% (w/w). Stock solutions of each compound at the concentration of 200 μg mL^{-1} were prepared in acetonitrile. Then the mixed stock solution containing all compounds (10 μg mL^{-1}) was prepared from individual stock standard solution by diluting with acetonitrile and stored at 4 °C in the dark. Chromatographic grade methanol and acetonitrile were purchased from Fisher (New Jersey, USA). Chlorodimethyl-*n*-octadecylsiane was supplied by Alfa Aesar (USA). $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, NaCl and NaOH were supplied by Guangfu Fine Chemical Research Institute (Tianjin, China). Analytical grade acetone, toluene, triethylamine, *n*-hexane, ethanol, isopropanol, tetraethyl orthosilicate (TEOS), ammonia, hydrochloric acid were obtained from Beijing Chemical Works (Beijing, China). The deionized water was prepared with Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Preparation of C_{18}-functionalized ultrafine magnetic silica NPs

The magnetic nanoparticles, C_{18}-UMS NPs, were synthesized by chemical coprecipitation, silanization and alkylation method similar to previous method [35]. Firstly, $FeCl_2 \cdot 4H_2O$ (2.0 g), $FeCl_3 \cdot 6H_2O$ (5.2 g) were dissolved in deionized water (25 mL) and 12 mol L^{-1} HCl (850 μL) was added under stirring. Then the mixture was slowly added into a four-neck flask containing NaOH solution (1.5 mol L^{-1}, 250 mL) under vigorous stirring with nitrogen gas passing continuously through the solution. Subsequently the mixture was stirred vigorously for 3 h and reacted at 80 °C. The obtained Fe_3O_4 NPs was separated from the solution under the magnetic field and washed with 100 mL deionized water 4 times. Then, the newly prepared Fe_3O_4 NPs were homogeneously dispersed into a mixture of deionized water (12 mL), isopropanol (86 mL) and ammonia (25 wt%, 2.5 mL). After stirring for 15 min with nitrogen gas passing through the solution, TEOS (250 μL) was added into the beaker. The resulting solution was then stirred for 4 h at room temperature. Then the magnetic silica nanoparticles were washed with deionized water 3 times and dried in a vacuum oven at 60 °C. The dried magnetic silica microspheres (0.6 g) were added into anhydrous toluene (30 mL). The resulting mixture was heated to boiling point, and then triethylamine (0.6 mL) and chlorodimethyl-*n*-octadecylsiane (0.9 g) were added into the mixture. The mixture was then refluxed for 5 h. The obtained C_{18}-UMS NPs were washed and dried.

2.3. Sample collection

Fresh vegetables (Chinese cabbage, sample 1 and 2 and celery, sample 3 and 4) were purchased from a local market in Changchun, China. Hundred grams of each vegetable sample was cut out and put into the food disintegrator. The disintegrator was operated for 3 min. The homogenized samples were obtained.

2.4. Preparation of spiked sample

A 5.0 g of homogenized sample was accurately weighed and transferred into a 50 mL beaker. The spiked sample was prepared by adding 50 μL of standard solution containing analytes at the concentration of 10 μg mL into the beaker. Then the sample was stored in the dark at 4 °C standing overnight in order to obtain a homogeneous spiked sample.

2.5. LSE-MSPE procedure

The fresh vegetable samples was crushed completely with disintegrator for three minutes, and then the pyrethroid residues could be extracted using 10 mL of acetone by vigorously shaking the sample by hand for 4 min. The extract was filtered with Advantec 5 A filter paper and placed into an evaporation flask. The filtrate was evaporated under vacuum at 50 °C until dryness.

The residue was redissolved with deionized water. The resulting solution was transferred to a beaker and diluted to a total volume of 10 mL. 1 g of NaCl was added and then, 30 mg of C_{18}-UMS NPs was added into the solution. The mixture was sonicated for 10 min. Subsequently, the C_{18}-UMS NPs adsorbing pyrethroid residues were isolated with a strong magnet at the bottom of the beaker and the supernatant was poured out. The C_{18}-UMS NPs were washed with 1 mL of deionized water. After magnetic separation, C_{18}-UMS NPs adsorbing the analytes were placed in

2 mL of acetone, and the resulting mixture was sonicated for 45 s and the pyrethroid residues were dispersed in acetone. After magnetic separation, the eluate was dried under a stream of nitrogen at 50 °C and dissolved in 0.2 mL of acetonitrile, which was referred to as the analytical solution. C_{18}-UMS NPs can be recycled by washing with methanol for 3 min, deionized water for 3 min and methanol for 2 min.

2.6. Apparatus

The ultra fast liquid chromatographic (UFLC) system (Shimadzu Corporation, Kyoto, Japan) consisted of two LC-20AD pumps, a SIL-20A automatic sample injector, a CTO-20A column oven and a SPD-20A UV–vis detector. Relevant data acquisition and processing were performed with the LC-solution software (Shimadzu, Japan). The separation of pyrethroid residues was performed on a Shim-pack VP-ODS column (150 mm × 4.6 mm, 4.6 μm particle size). The mobile phase consisted of a mixture of acetonitrile and water (83:17, v/v) and the flow-rate was set at 1.0 mL min^{-1}. The monitoring wavelength was 210 nm. The temperature of column was controlled at 30 °C. Injection volume was 5 μL.

The characterization of the C_{18}-UMS NPs was carried out based on Fourier transform-infrared (FT-IR) spectra, transmission electron microscope (TEM) images and magnetization curves of both the C_{18}-UMS NPs and their semi-products. FT-IR was performed on a Nicolet FT-IR 360 spectrometer (Nicolet, USA). A Hitachi H-800 transmission electron microscope (Hitachi, Japan) was used to observe the morphology and particle size of the materials. A magnetic property measurement system (MPMS) vibrating sample magnetometer (Quantum Design, USA) was used to study the magnetic properties of the C_{18}-UMS NPs.

3. Results and discussion

3.1. Characterization of magnetic nanoparticles

To ascertain the formation of SiO_2 layer and alkyl layer on the MNPs, FT-IR spectra of the silica-MNPs and the C_{18}-UMS NPs were obtained. Fig. 1 exhibits these spectra. The FT-IR spectrum of the silica-MNPs (Fig. 1a) shows typical Fe-O-Fe vibration of magnetite at 580 cm^{-1}. The absorption peak at 1110–1000 cm^{-1} results from the Si-O-Si group stretching vibration of silica layer formed on the surface of magnetite nanoparticles. The absorption peak at ~1640 cm^{-1} can be assigned to the adsorbed water on the silica shell or the silanol groups of the silica. After surface modification, the new emergence of absorption peak at ~2960 cm^{-1} (Fig. 1b) is ascribed to CH_2 originated from silane coupling agent, suggesting that the alkyl groups have been successfully grafted onto the surface of silica-MNPs.

Fig. 2 displays the TEM images of Fe_3O_4 NPs and C_{18}-UMS NPs. As is shown in Fig. 2a, the Fe_3O_4 NPs dispersed evenly with a mean size of around 10 nm. In Fig. 2b, the C_{18}-UMS NPs exhibit changes in diameter compared with Fe_3O_4 NPs, which implies that there are a thin SiO_2 coating and alkyl coating on the Fe_3O_4 NPs.

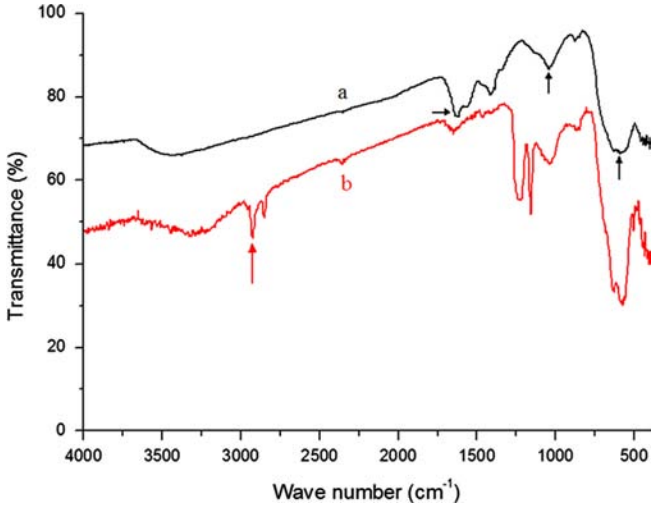

Fig. 1. FT-IR spectra of (a) silica-MNPs and (b) C_{18}-UMS NPs.

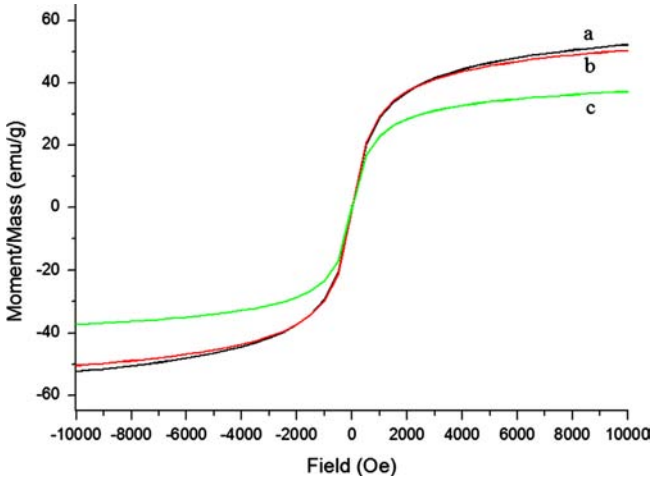

Fig. 3. Room-temperature magnetization curves of (a) Fe_3O_4 NPs, (b) silica-MNPs, and (c) C_{18}-UMS NPs.

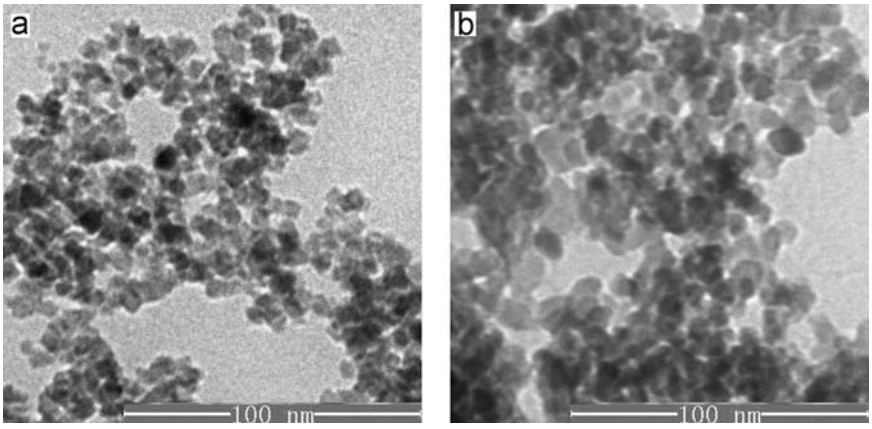

Fig. 2. TEM images of (a) Fe_3O_4 NPs and (b) C_{18}-UMS NPs.

Fig. 3 exhibits the room temperature-magnetization curves of Fe_3O_4 NPs, silica-MNPs, and C_{18}-UMS NPs. None of the magnetization curves has hysteresis, indicating that they are all superparamagnetic. Moreover, satisfactory magnetic property of C_{18}-UMS NPs is proved with saturation intensities of 38.36 emu g^{-1}, which is obviously lower than that of Fe_3O_4 NPs (60.49 emu g^{-1}), and sufficient for magnetic separation with a conventional magnet.

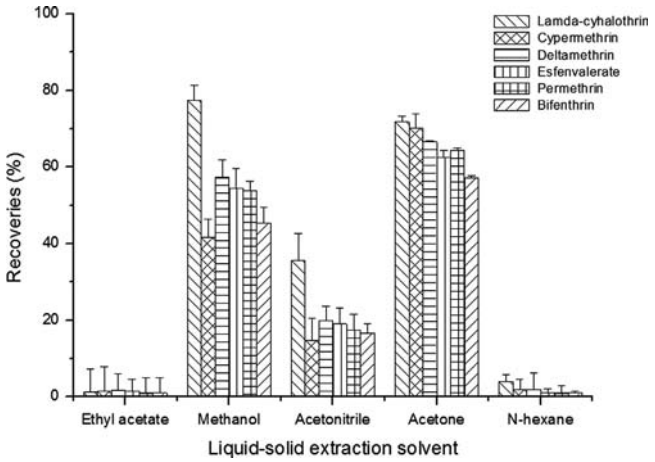

Fig. 4. Effect of the liquid–solid extraction solvent on recoveries of pyrethroids. Liquid–solid extraction time, 4 min; the amount of C_{18}-UMS NPs, 40 mg; magnetic solid-phase extraction time, 15 min; desorption solvent, methanol; desorption time, 60 s.

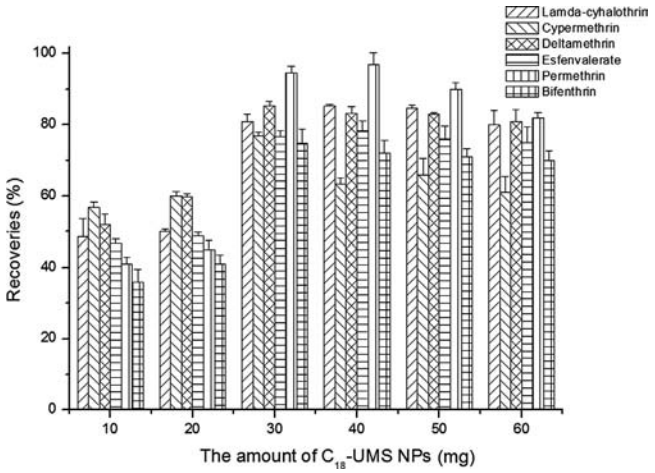

Fig. 5. Effect of the amount of C_{18}-UMS NPs on recoveries of pyrethroids. Liquid–solid extraction solvent, acetone; liquid–solid extraction time, 4 min; the amount of NaCl, 1.0 g; magnetic solid-phase extraction time, 15 min; desorption solvent, methanol; desorption time, 60 s.

3.2. Optimization of LSE-MSPE conditions

The major parameters that influence the extraction of pyrethroid residues were optimized by analyzing the spiked sample 1 containing the pyrethroids at concentration of 100 ng g^{-1}. All the experiments were performed in triplicate.

3.2.1. Optimization of LSE conditions

In order to avoid loss of pyrethroids and improve the extraction efficiency, the selection of the extraction solvent is indispensable. The extraction solvent used for LSE should have a suitable solubility in water, and high extraction capability for the target analytes. Based on these criteria, methanol, acetone, acetonitrile, *n*-hexane and ethyl acetate are used as the extraction solvents. As can be seen in Fig. 4, recoveries obtained with methanol and acetone are much higher than those obtained with other extraction solvents. Acetone is easier to be evaporated in the following step than methanol and chosen to extract pyrethroid residues from vegetable samples.

When the extraction was completed with 10 mL of acetone once or 5 mL of acetone each time twice, no significant difference in recoveries is observed (Fig. S1). So the extraction is completed with 10 mL of acetone once.

Effect of the liquid–solid extraction time on the recoveries of six pyrethroids was studied. The liquid–solid extraction equilibrium can be obtained in 4 min (Fig. S2). No significant increase in recoveries is observed when the liquid–solid extraction time increase from 4 to 10 min. The liquid–solid extraction time is set at 4 min in further experiments.

3.2.2. Optimization of MSPE conditions

Ionic strength could play a decisive role to enhance extraction efficiency by decreasing the affinity of the pyrethroids to the aqueous matrix rather than to C_{18} coating of the magnetic adsorbent. Thus, the effect of ionic strength was studied by changing the amount of sodium chloride in the sample solution from 0 to 3.0 g (Fig. S3). An increase in extraction recoveries with the increase of the salt amount from 0 to 1.0 g is observed. However, when the salt amount is larger than 1.5 g, there is sodium chloride sediment at the bottom of the beaker and the pyrethroid recoveries decrease. Based on such an observation, 1.0 g of NaCl is added.

The amount of magnetic adsorbent is known to be related to the amount of analytes adsorbed in MSPE. Thus, the effect of the amount of C_{18}-UMS NPs was examined. Fig. 5 shows the changes of the extraction recoveries with the C_{18}-UMS NPs amount ranging from 10 to 60 mg. The highest extraction recoveries for pyrethroids are

Table 1
Experimental results of the orthogonal test ($n=3$).

Design ID number	Factor			Lambda-cyhalothrin	Cypermethrin	Deltamethrin	Esfenvalerate	Permethrin	Bifenthrin
	(A) Extraction solvent	(B) Amount of C_{18}-UMS NPs	(C) Solid-phase extraction time	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)
1	A_1	B_1	C_1	88.39	65.66	73.29	77.36	79.34	71.21
2	A_1	B_2	C_2	101.8	87.58	99.06	96.72	87.50	90.13
3	A_1	B_3	C_3	90.51	81.67	95.53	93.94	96.93	88.47
4	A_2	B_1	C_3	86.85	90.05	90.78	86.80	83.12	82.02
5	A_2	B_2	C_1	96.52	90.72	94.91	89.30	99.44	86.35
6	A_2	B_3	C_2	101.4	92.60	101.5	99.94	101.8	93.13
7	A_3	B_1	C_2	77.57	72.98	78.07	75.63	67.24	61.62
8	A_3	B_2	C_3	85.09	77.24	83.79	81.55	84.07	79.78
9	A_3	B_3	C_1	85.71	67.72	78.73	75.35	84.58	71.96

Table 2
Analysis of orthogonal test results.

Analyte	Factor	K_1[a]	K_2	K_3	R[b]	Optimal level
Lambda-cyhalothrin	A	93.57	94.92	82.79	12.13	A_2
	B	84.27	94.47	92.54	10.20	B_2
	C	90.21	93.59	87.48	6.11	C_2
Cypermethrin	A	78.30	91.12	72.65	18.47	A_2
	B	76.23	85.18	80.66	8.95	B_2
	C	74.70	84.39	82.99	9.69	C_2
Deltamethrin	A	89.29	95.73	80.19	15.54	A_2
	B	80.71	92.59	91.92	11.88	B_2
	C	82.31	92.88	90.03	10.57	C_2
Esfenvalerate	A	89.34	92.01	77.51	14.50	A_2
	B	79.93	89.19	89.74	9.81	B_3
	C	80.67	90.76	87.43	10.09	C_2
Permethrin	A	87.92	94.79	78.63	16.16	A_2
	B	76.57	90.34	94.44	17.87	B_3
	C	87.78	85.51	88.04	2.53	C_3
Bifenthrin	A	83.27	87.17	71.12	16.05	A_2
	B	71.62	85.42	84.52	13.80	B_2
	C	76.51	81.63	83.42	6.91	C_3

[a] $K_i^F = (1/3)\sum$ the recoveries of target analytes at F_i
[b] $R_i^F = \max\{K_i^F\} - \min\{K_i^F\}$, here F and i mean factor and setting level, respectively.

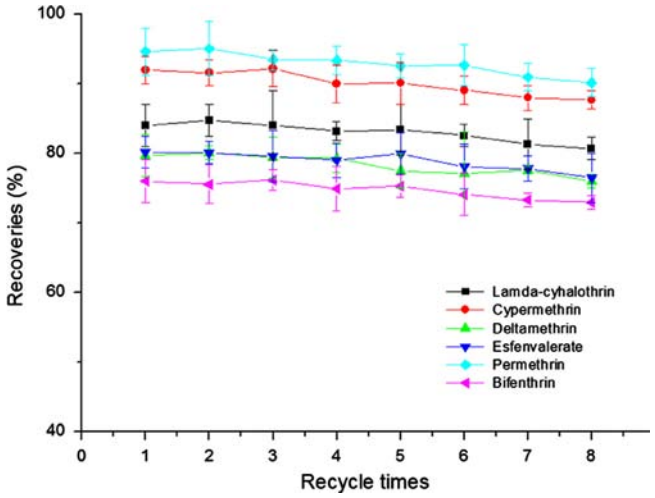

Fig. 6. Effect of recycling times on the recoveries of pyrethroids.

obtained when the amount of magnetic adsorbent is between 30 and 60 mg. Thus, 30 mg is adopted as the amount of magnetic adsorbent in the following studies.

Generally, sufficient magnetic solid-phase extraction time was required to attain adsorption equilibrium for target analytes on magnetic adsorbent. The effect of magnetic solid-phase extraction time was studied in the time range of 3–20 min (Fig. S4). The extraction recoveries increase significantly when extraction time rise from 3 to 10 min. No significant increase in recoveries is observed when the magnetic solid-phase extraction time increase from 10 to 20 min. Therefore, magnetic solid-phase extraction time of 10 min is selected.

The desorption of pyrethroids from C_{18}-UMS NPs was studied with different organic solvents, including acetone, methanol and acetonitrile (Fig. S5). We can find that all the three desorption solvents can desorb pyrethroids effectively. Due to the boiling point of acetone is lower than those of methanol and acetonitrile, acetone is chosen as the desorption solvent. Last but not least, the adsorbed analytes are easily desorbed with acetone, and no analyte is observed in the eluate after washing 3 times.

The effect of the desorption time (15, 30, 45, 60, 75 s) was investigated (Fig. S6). The highest recoveries of six pyrethroids are obtained when the desorption time is 45 s. No significant increase in recoveries is observed when the desorption time increase from 45 s to 75 s. Finally, 45 s is finally chosen as the desorption time.

3.3. Orthogonal experiment

Based on the previous experimental results obtained by univariate method, orthogonal experiment (L9 (3^3)) was carried out in order to determine the optimum operating conditions. The orthogonal screening was completed in pyrethroid-free vegetable sample (sample 1). The effects of LSE extraction solvent (A_1, methanol; A_2, acetone; A_3, acetonitrile), the amount of C_{18}-UMS NPs (B_1, 20 mg; B_2, 30 mg; B_3, 40 mg) and MSPE extraction time (C_1, 5 min; C_2, 10 min; C_3, 15 min) on the recoveries are shown in Table 1. K_n and R values are calculated and listed in the Table 2. K_n is the mean effect of each factor at the different levels and R is the range. The experimental results indicate that the LSE extraction solvent plays an important role in the extraction, followed by the amount of C_{18}-UMS NPs and MSPE extraction time. Based on the experimental results, LSE extraction solvent, the amount of C_{18}-UMS NPs, MSPE extraction time were selected as acetone, 30 mg and 10 min, respectively.

3.4. Reusability of the magnetic adsorbent

In order to investigate the effectiveness of the magnetic adsorbent, the C_{18}-UMS NPs were reused in LSE-MSPE. The C_{18}-UMS NPs

Table 3
Analytical performances.

Analyte	Linear range (ng g^{-1})	Chinese cabbage				Celery			
		Calibration equations	Correlation coefficient (r)	LOD (ng g^{-1})	LOQ (ng g^{-1})	Calibration equations	Correlation coefficient (r)	LOD (ng g^{-1})	LOQ (ng g^{-1})
Lambda-cyhalothrin	5.0–500.0	$y=(323.6\pm4.8)x+(638.5\pm97.9)$	0.9995	0.74	2.5	$y=(349.4\pm9.4)x+(287.6\pm74.6)$	0.9996	0.79	2.6
Cypermethrin	5.0–500.0	$y=(63.6\pm1.8)x+(2511.7\pm406.5)$	0.9991	1.2	4.0	$y=(60.99\pm1.0)x+(457.6\pm261.7)$	0.9997	1.2	4.2
Deltamethrin	5.0–500.0	$y=(133.0\pm1.3)x+(167.9\pm287.8)$	0.9998	0.87	2.9	$y=(102.3\pm4.5)x+(217.2\pm167.0)$	0.9995	0.77	2.6
Esfenvalerate	5.0–500.0	$y=(188.7\pm3.2)x+(581.7\pm126.1)$	0.9994	0.82	2.7	$y=(179.5\pm3.3)x+(438.5\pm347.7)$	0.9996	0.83	2.7
Permethrin	5.0–500.0	$y=(174.2\pm2.9)x+(1553.3\pm775.3)$	0.9993	0.63	2.1	$y=(117.0\pm3.0)x+(413.4\pm204.3)$	0.9993	0.69	2.3
Bifenthrin	5.0–500.0	$y=(247.2\pm1.8)x-(186.2\pm212.7)$	0.9998	1.1	3.8	$y=(237.0\pm2.5)x-(256.8\pm63.0)$	0.9998	1.1	3.6

were recycled by washing with methanol for 3 min, deionized water for 3 min and methanol for 2 min before the reuse. The experimental results shown in Fig. 6 indicate that the recoveries of analytes decrease only slightly when the adsorbent is reused 8 times.

3.5. Validation of the method

Pyrethroid-free vegetable samples (samples 1 and 3) were used as blanks for LSE-MSPE standard calibrations. An appropriate amount of the mixture standard solution of the target analytes was added into the samples. A series of working samples containing the pyrethroids at six concentration levels (5.0, 10.0, 20.0, 100.0, 200.0, and 500.0 ng g^{-1}) were prepared for the establishment of the calibration curves. The calibration curves are applied to evaluating the limits of detection (LODs) and quantification (LOQs). The LODs and LOQs were calculated by the following equations:

$$LODs = 3\frac{s}{k};\ LOQs = 10\frac{s}{k};$$

where s is the standard deviation of blank signal and k is the slope of the working curve. The LOQs is obtained by calculating based on the noise for analyzing the blank samples and the slope of the working curve. The lower concentration limit in the linear range is obtained based on the experimental determination. The characteristic calibration data are listed in Table 3. When the concentration of the analytes in Chinese cabbage is 5.0 ng g^{-1}, the peak areas of lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, permethrin and bifenthrin were 2317, 2954, 810, 1472, 2410 and 1042 μv·s and RSDs were 2.5, 4.1, 2.8, 3.3, 1.9 and 1.6%, respectively. Fig. 7 shows liquid chromatograms of spiked vegetable samples at the analytes concentration of 5.0 ng g^{-1}. The calibration curves exhibit good linearity with correlation coefficients above 0.999. The LODs were between 0.63 and 1.2 ng g^{-1}, which had enough sensitivity for the determination of pyrethroids in vegetable samples. The precision was evaluated by determining the relative standard deviations (RSDs) of intra- and inter-day tests. The intra-day precision was determined by analyzing a sample in six replicates in one day. The inter-day precision was determined by analyzing a sample once per day in six consecutive days. The results are listed in Table 4. The results demonstrated that the present method was feasible for the determination of pyrethroids in vegetable samples.

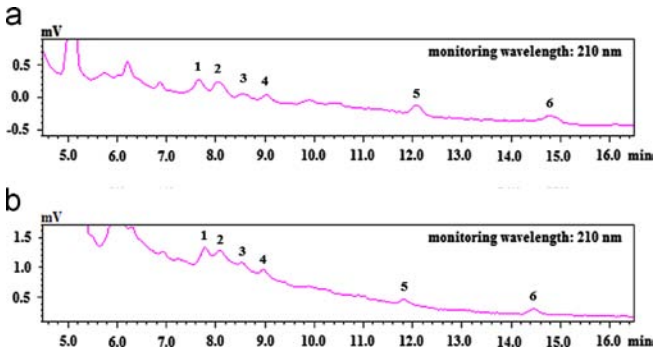

Fig. 7. Liquid chromatograms of spiked vegetable samples at the analytes concentration of 5.0 ng g^{-1}.

The spiked sample 1 and sample 3 were analyzed to compare the recoveries obtained with magnetic silica NPs (not functionalized), solely C_{18} SPE and C_{18}-UMS NPs. Not functionalized magnetic silica NPs could hardly adsorb pyrethroid residues. The recoveries obtained with C_{18} SPE and C_{18}-UMS NPs are shown in Table 5. From Table 5, it can be seen that the recoveries obtained with C_{18}-UMS NPs are slightly higher than those obtained with C_{18} SPE. Compared with C_{18} SPE, when C_{18}-UMS NPs were used the extraction time is shorter. Considering the results, the C_{18}-UMS NPs should be best choice to work with.

3.6. Determination of pyrethroids in vegetable samples

The present method was applied to the determination of pyrethroid residues in Chinese cabbage and celery. Permethrin is detectable in sample 2 and the concentration of the pyrethroid is 19.5 ng g^{-1}. The recoveries of pyrethroids were studied by adding the pyrethroids at three concentrations (10, 20 and 200 ng g^{-1})

Table 4
The recoveries of the analytes and inter-day and intra-day precision in Chinese cabbage (n=6).

Analyte	Concentration (ng g^{-1})	Chinese cabbage				Celery			
		Intra-day		Inter-day		Intra-day		Inter-day	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Lambda-cyhalothrin	10.0	105.0	2.8	98.3	1.6	89.5	2.7	90.5	1.8
	20.0	92.6	3.0	99.0	4.1	91.4	1.6	89.7	1.2
	200.0	101.4	1.5	87.6	6.5	100.4	4.9	92.2	2.9
Cypermethrin	10.0	89.2	2.1	106.5	4.1	91.2	4.0	88.7	2.5
	20.0	96.9	1.9	91.7	4.9	95.3	3.2	84.6	1.7
	200.0	102.4	3.6	93.9	1.7	86.7	2.1	81.0	5.3
Deltamethrin	10.0	96.3.	4.2	91.4	3.0	88.4	2.3	85.6	2.7
	20.0	95.0	1.7	97.6	1.3	95.8	1.9	87.2	4.7
	200.0	92.5	3.4	103.0	3.7	84.3	2.2	92.8	4.1
Esfenvalerate	10.0	95.4	2.2	94.3	2.9	101.9	3.5	95.4	2.8
	20.0	90.0	0.9	99.5	2.1	97.5	2.1	90.8	1.6
	200.0	94.4	3.8	104.1	3.1	90.8	0.9	92.0	4.0
Permethrin	10.0	99.7	2.6	96.6	6.2	95.4	3.7	88.9	6.1
	20.0	100.2	4.1	89.4	3.3	81.5	2.0	101.2	4.1
	200.0	94.1	3.7	100.1	2.9	85.3	2.6	91.3	2.9
Bifenthrin	10.0	102.6	1.0	87.8	1.5	99.8	3.1	81.7	2.3
	20.0	100.1	3.2	95.6	3.8	93.4	1.8	95.4	1.9
	200.0	91.4	4.9	94.5	5.6	94.1	4.7	93.6	3.0

Table 5
The recoveries obtained with magnetic silica NPs, C_{18} SPE and C_{18}-UMS NPs.

Analyte	Concentration (ng g^{-1})	Chinese cabbage				Celery			
		C_{18} SPE		C_{18} UMS NPs		C_{18} SPE		C_{18} UMS NPs	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Lambda-cyhalothrin	10.0	81.4	1.9	92.3	3.0	72.6	2.4	80.6	2.1
	20.0	79.4	2.4	89.8	1.4	71.4	1.9	85.6	1.7
	200.0	73.8	2.8	95.4	3.3	80.5	3.7	94.2	3.3
Cypermethrin	10.0	69.4	3.4	85.1	2.6	74.9	3.4	86.1	2.4
	20.0	75.1	2.5	89.6	1.8	70.3	4.2	96.4	3.7
	200.0	79.9	2.6	92.3	4.2	71.4	1.4	81.0	3.4
Deltamethrin	10.0	76.2	4.3	90.9	3.7	81.7	2.0	86.9	1.9
	20.0	75.5	3.7	91.3	1.4	74.6	1.8	90.2	2.5
	200.0	71.6	1.8	87.6	3.0	72.8	4.0	87.3	4.7
Esfenvalerate	10.0	75.2	3.6	85.9	4.1	76.1	1.8	95.8	2.8
	20.0	70.1	2.3	86.4	1.6	75.4	3.4	90.6	3.5
	200.0	74.5	4.2	91.7	2.5	79.7	3.9	91.7	2.4
Permethrin	10.0	78.8	1.5	94.6	3.9	80.0	2.7	87.4	3.6
	20.0	80.6	3.8	92.5	2.4	71.9	2.0	86.4	1.7
	200.0	74.9	3.4	88.6	2.3	70.5	3.6	90.2	2.1
Bifenthrin	10.0	69.4	4.0	85.3	3.1	76.3	1.0	82.5	4.5
	20.0	78.6	2.9	86.7	4.5	72.2	3.1	89.8	2.7
	200.0	71.4	1.7	81.3	4.0	68.4	2.3	84.6	1.6

Table 6
Analytical results of vegetable samples, mean ± SD (n=3).

Vegetable sample	Added (ng g^{-1})	Lambda-cyhalothrin		Cypermethrin		Deltamethrin		Esfenvalerate		Permethrin		Bifenthrin	
		Found (ng g^{-1})	Recovery (%)	Found (ng g^{-1})	Recovery (%)	Found (ng g^{-1})	Recovery (%)	Found (ng g^{-1})	Recovery (%)	Found (ng g^{-1})	Recovery (%)	Found (ng g^{-1})	Recovery (%)
Sample 1	0.0	0.0		0.0		0.0		0.0		0.0		0.0	
	10.0	9.5	94.6 ± 1.8	7.9	79.5 ± 4.1	9.2	91.8 ± 3.0	9.0	89.6 ± 3.4	8.4	83.8 ± 0.9	8.0	80.2 ± 3.1
	20.0	19.9	99.5 ± 3.6	17.5	87.6 ± 2.3	17.8	88.9 ± 4.1	17.7	88.3 ± 2.8	19.8	99.1 ± 4.5	16.9	84.6 ± 5.3
	200.0	187.1	93.6 ± 2.5	192.2	96.0 ± 4.7	169.5	84.7 ± 1.1	184.4	92.2 ± 3.2	156.7	78.4 ± 2.1	194.3	87.2 ± 2.8
Sample 2	0.0	0.0		0.0		0.0		0.0		19.5		0.0	
	10.0	8.9	89.5 ± 5.6	8.7	87.4 ± 6.0	7.7	76.6 ± 2.6	9.2	91.7 ± 4.6	28.5	89.7 ± 2.3	8.7	87.1 ± 3.6
	20.0	19.3	96.4 ± 4.4	18.3	91.3 ± 1.8	15.6	78.1 ± 4.7	15.7	78.7 ± 1.5	34.7	76.0 ± 6.0	18.4	92.1 ± 5.4
	200.0	179.6	89.8 ± 1.6	176.6	88.3 ± 3.2	165.3	82.7 ± 1.6	174.2	87.1 ± 4.3	178.8	79.6 ± 4.5	160.3	80.2 ± 1.2
Sample 3	0.0	0.0		0.0		0.0		0.0		0.0		0.0	
	10.0	9.3	92.7 ± 1.6	8.8	88.1 ± 2.5	9.4	93.7 ± 2.1	9.6	95.7 ± 3.9	9.8	98.1 ± 2.7	8.8	78.3 ± 2.8
	20.0	18.7	93.3 ± 1.7	17.1	85.7 ± 4.3	16.8	83.8 ± 2.6	17.5	87.9 ± 0.8	18.0	90.1 ± 1.3	16.0	76.1 ± 1.6
	200.0	185.3	92.7 ± 4.2	166.9	83.4 ± 2.5	175.3	87.7 ± 3.5	181.1	90.6 ± 4.5	177.6	88.8 ± 5.6	166.1	83.0 ± 3.1
Sample 4	0.0	0.0		0.0		0.0		0.0		0.0		0.0	
	10.0	8.9	89.9 ± 3.7	8.3	83.3 ± 1.9	8.1	80.9 ± 1.7	7.8	78.3 ± 2.9	9.7	97.3 ± 4.7	9.0	89.7 ± 3.0
	20.0	19.0	95.2 ± 2.9	18.0	90.2 ± 4.5	18.4	91.8 ± 5.1	16.7	83.6 ± 3.6	17.5	87.6 ± 3.7	19.7	78.4 ± 3.2
	200.0	191.5	95.7 ± 1.9	176.5	88.2 ± 3.6	173.6	86.8 ± 2.7	180.0	90.0 ± 2.4	186.2	93.1 ± 2.4	179.4	89.7 ± 1.4

into vegetable samples. The recoveries for the target compounds are listed in Table 6. The recoveries of lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, permethrin and bifenthrin are in the range of 76.0 ± 6.0 to 99.5 ± 3.6%. The results indicate that the recoveries for the analytes are satisfactory.

3.7. Comparison of the present method with other methods

A comparison of the present method and other methods [3,9,16,36] reported in literature was made, and the results are shown in Table 7. The results indicate that there is no significant

Table 7
Comparison with other methods for pyrethroids determination.

Analytes	Sample preparation step	Extraction time (min)	LOD (ng g^{-1})	Recy-cled	Recoveries (%)	Ref.
Organochlorine Pyrethroid	Fruit/vegetables —(ethyl acetate, extraction)→ extract —(ethyl acetate, re-extraction)→ extract —(N_2, evaporation)→ residue —(acetone/hexane, extraction)→ solution —(centrifugation)→ supernatant —(SAX/PSA, clean-up)→ adsorbate —(acetone/hexane, elution)→ eluate —(dissolution)→ analytical solution	--	3-15	No	54.0-104.1	[3]
Pyrethroid	Vegetables —(hexane/acetone, H_2O, NaCl, extraction)→ extract —(PDMS/DVB fiber, extraction)→ adsorbate —(hot split-splitless injector, desorption)→ gaseous analytes	35	3-25	No	--	[9]
Pyrethroid	Porcine tissue —(alumina, grind)→ solid mixture —(sodium sulfate, diatomaceous earth, packed)→ mixture —(hexane, elution)→ eluate —(N_2, evaporation)→ residue —(acetonitrile, dissolution)→ analytical solution	--	10-56	No	85.8-105.3	[16]
Neonicotinoid	Vegetables —(acetonitrile, $MgSO_4$, NaCl, extraction)→ extract —(centrifugation)→ supernatant —(MWCNTs, PSA, clean-up)→ eluate —(centrifugation)→ supernatant —($CHCl_3$, NaCl, H_2O, extraction)→ extract —(centrifugation)→ sedimented phase —(N_2, evaporation)→ residue —(methanol, dissolution)→ analytical solution	17	0.5-1.0	No	84.6-97.5	[36]
Pyrethroid	Vegetables —(acetone, extraction)→ extract —(evaporation)→ residue —(C_{18}-UMS NPs, H_2O, NaCl, extraction)→ adsorbate —(acetone, elution)→ eluate —(N_2, evaporation)→ residue —(acetonitrile, dissolution)→ analytical solution	14	0.69-1.2	Yes	76.0-99.5	This method

difference in the recoveries obtained by these methods. However, the present method is simpler compared with other methods and has some advantages in extraction time.

In recent reports, MSPE has been developed as a fast, simple, cost effective and versatile technique. In this work, MSPE coupled with liquid–solid extraction was developed innovatively. After LSE, the extraction and clean-up steps could be fulfilled synchronously by mixing the magnetic adsorbents and matrix sample. The operation procedure is simplified and the analytical cost is reduced. Meanwhile, the magnetic adsorbents possessed superparamagnetism properties, which enabled them to be completely isolated from matrix in a short period (less than 1 min) with a strong magnet.

4. Conclusion

In this investigation, LSE coupled with MSPE has been developed for the extraction of pyrethroid residues in vegetable samples. The combination of LSE and MSPE makes it possible to determine the trace analytes in solid matrix samples. C_{18}-functionalized ultrafine magnetic silica NPs were successfully synthesized and used as magnetic adsorbent. The pyrethroids could be greatly adsorbed by the hydrophobic C_{18} group of the magnetic adsorbent. The C_{18}-UMS NPs adsorbed pyrethroids could be separated from the matrix solution easily owing to the superparamagnetic Fe_3O_4 core. The desorption was easily done with acetone. The present method was proved to provide good recoveries and precision for the determination of pyrethroids in vegetable samples. We believe that this method will be potentially useful for the determination of other similar analytes in solid matrices.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.04.004.

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