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Simple one-step preconcentration and cleanup with micellar system for high performance liquid chromatography determination of pyrethroids in traditional Chinese medicine

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Abstract

A novel but simple one-step preconcentration and cleanup by micellar system is developed to extract and preconcentrate four pyrethroids including beta-cyfluthrin, cyhalothrin, cyphenothrin and permrthin in traditional Chinese medicine with the use of the nonionic surfactant Triton X-114. Before high performance liquid chromatography analysis, a cleanup stage by adding an appropriate sorbent to remove the interfering components from the extracts was optimized. Based on the analysis, the best recoveries (86.8%-94.3%) were obtained by preconcentration with 15% (m/v) NaCl, 2/1 (g/g) of activated carbon/sample, and equilibration at 60 °C for 40 min, which was after a micellar extraction with 7.0% (v/v) Triton X-114, liquid/solid ratio of 20/1 (mL/g), ultrasonic extraction time of 20 min at pH of 7.0. Results of the four pyrethroids obeyed linearity within the range of 0.06-6.00 μg g^{-1}. Detection limits of beta-cyfluthrin, cyhalothrin, cyphenothrin and permrthin were 0.0108, 0.0086, 0.0083 and 0.0092 μg g^{-1}, respectively. Consequentially the method was applied for extraction and determination of the four pyrethroids in different traditional Chinese medicines (Rhubarb, Herba lysimachiae, Ardisia japonica and Camptotheca acuminate fruit). Based on these results, the proposed method was a simple, effective and environmental-friendly technique for the analysis of pyrethroid residues.

Keywords: Micellar extraction; Pyrethroids; Cleanup sorbent; HPLC; Traditional Chinese medicine

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1. Introduction

Traditional Chinese medicine has been widely used to treat many common diseases, such as cardiovascular diseases, respiratory diseases and infectious diseases all over the world in recent years.[1,2] It is well known that traditional Chinese medicine would be attacked by several pests and diseases, normally, some pyrethroids including beta-cyfluthrin, cyhalothrin, cyphenothrin and permrthin pesticides were often used to prevent and control mites, leafhoppers, plant bugs. [3,4] So it is very important to make sure the safety of the traditional Chinese medicine.

The pyrethroids represent a relatively new group of synthetic insecticides,[5] and they have been widely used ranging from agriculture to public health in the world because of their high insecticidal activity, low toxicity to mammalian cells, and adequate stability upon exposure to light and air.[6-10] Although pyrethroids are thought to be safe for humans for a long time, it was reported that high exposure to pyrethroids might cause lymph node suppressive effects on the immune system, endocrine disruption and splenic damage, even carcinogenesis.[10-12] With the public concern on possible health risks increasing in recent years, residue analysis of pyrethroids in traditional Chinese medicine sample becomes increasingly necessary.

There are many methods to determine pyrethroid residues such as high performance liquid chromatography (HPLC) with ultraviolet detection and gas chromatography (GC) with electron capture detector or mass spectrometric detection.[13-19] However, it is difficult to directly monitor the residues of pyrethroids because of the complexity of samples, especially samples of traditional Chinese medicine, and the relatively low concentrations of pyrethroids. Hence, sample pretreatment prior to instrumental analysis is the most important and crucial steps in a whole analytical process.[20, 21] Presently, several conventional methods have been used for the extraction and detemination of pyrethroids residues in different samples. Some frequently used methods for extraction of pyrethroids are solid-phase extraction (SPE),[22] liquid-liquid extraction (LLE),[23] solid-phase microextraction (SPME)[24-27] and liquid-phase microextraction (LPME).[28] Although these methods have their advantages, they also have some disadvantages. For example, SPME is expensive because the fiber it used is fragile and has a limited lifetime, LLE requires a large amount of high-purity organic solvent, and SPE and LPME are time-consuming.

Over the past few years, micellar extraction has been successfully applied as a promising extraction and preconcentration process.[29] Surfactants are well known for their capability to enhance the solubility of hydrophobic materials.[30] Several nonionic or zwitterionic surfactants tend to

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separate into two liquid phases (a surfactant rich-phase and aqueous phase) above a certain temperature which is known as the cloud-point temperature.[31-34] During the process of micellar extraction, the hydrophobic species will be solubilized and incorporated in the nonionic micellar assembly efficiently and concentrated into this surfactant-rich phase.[35] The surfactant-rich phase can be separated by centrifugation.[36] Micellar extraction as an effective extraction method which only requires a very small amount of relatively nonflammable and nonvolatile surfactant. It was thought to be an efficient, low-cost and environmentally-friendly process with high extraction efficiency and a high preconcentration factor.[37,38]

This is the first study focused on developing a one-step preconcentration and cleanup by micellar extraction for analyzing pyrethroids residues in traditional Chinese medicine. Firstly, the pyrethroids were extracted from samples into aqueous Triton X-114 solution assisted with ultrasonic. Then the pyrethroids were preconcentrated by phase separation based on the cloud point phenomenon of Triton X-114. However, during the first step of micellar extraction, lots of interfering components can also be co-extracted from samples with the target pesticides simultaneously. In order to improve the detection sensitivity and selectivity of the target analytes, an appropriate sorbent was added to the system to absorb some interfering compounds during the second step. Finally the obtained purified extract was analyzed by HPLC. Various of experimental conditions, including types of extraction solvent, Triton X-114 concentration, extraction solvent pH, liquid/solid ratio, types of sorbent, proportion of the sorbent and sample, ultrasonic extraction time, NaCl concentration, equilibration time and equilibration temperature were investigated to evaluate and optimize this method.

2. Experimental

2.1 Samples and reagents

Nonionic surfactant Triton X-114 and the standards of pyrethroids including beta-cyfluthrin, cyhalothrin, cyphenothrin and permethrin were purchased from Sigma (St. Louis, MO, USA). The traditional Chinese medicine samples including Rhubarb, Herba lysimachiae, Ardisia japonica and Camptotheca acuminate fruit were purchased from Huqiao (Haozhou, China). Sodium chloride, sodium hydroxide, hydrochloric acid, methanol, ethanol, acetone, chloroform, and acetonitrile were of analytical grade and purchased from Kermel (Tianjin, China). Neutral alumina, diatomaceous earth and florisil were purchased from Kermel. Activated carbon was purchased from Cnbest Teck

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(Beijing, China). Silica gel was purchased from Sinopharm (Beijing, China). The chromatographic grade acetonitrile was purchased from Fisher (Pittsburgh, PA, USA). The water used was purified with a Milli-Q water purification system made by Millipore (Billerica, MA, USA). The laboratory glassware was soaked in washing liquid for several minutes and rinsed with distilled water at least three times prior to use.

The dry samples of traditional Chinese medicine were powdered using a cyclone mill into a homogeneous size and then sieved (60 mesh). The stock standard solutions of different pyrethroids (0.5 mg mL^{-1}) were prepared by dissolving an appropriate amount of this compound into acetonitrile solution. The solution was stored in a refrigerator at 4 ^{o}C. The working standard solution was prepared daily by diluting the stock standard solution. Various concentrations of aqueous surfactant solutions were prepared by dissolving appropriate amounts of the surfactant into water.

2.2 Apparatus

Chromatographic analysis was performed on a LC-15C HPLC with a UV detector (Shimadzu, Kyoto, Japan). A Zorbax SB-C18 column (150 mm × 4.6 mm I.D., 5μm) was used as analytical column (Palo Alto, CA, USA). A SX-4-10 muffle furnace (Tianjin, China) was used to activate the sorbents. A KQ5200E ultrasonic apparatus (Kunshan, China) at a constant power of 200 W and a frequency of 40 KHz was used for assisting the extraction of pyrethroid. A TG 16-WS centrifuge (Changsha, Chain) was used to accelerate the phase separation process. Besides, a SH-36 vortex mixer (Jintan, China) and a DZKW-C thermostatic bath (Shanghai, China) were also used.

2.3 Sorbent conditioning

According to previous studies,[39,40] the sorbents used in this work were activated. Activated carbon was filtered after being soaked overnight in 3 mol • L^{-1} HCl aqueous solution and was washed with water until pH value reaches 7.0, then heated at 120 ^{o}C for 2 h. Diatomaceous earth was soaked in 5% HCl aqueous solution and boiled for 1 h. After being cooled, the diatomaceous earth was filtered and washed with water until pH value reaches 6.0, and was reheated in a muffle furnace at 500 ^{o}C for 2 h. Florisil was heated in a muffle furnace at 550 °C for 6 h and cooled in a desiccator, then was added with purified water (equivalent to 5% w/w) and homogenized by rotation for 2 h. After that, the Florisil was left in a closed container to equilibrate for 48 h before use. Silica gel was heated in a muffle furnace at 110 ^{o}C for 1 h. Neutral alumina was heated in a muffle furnace at 450 ^{o}C for 4 h. All of those sorbents were needed to be cooled in a desiccator after

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heating.

2.4 Micellar extraction procedure

The powdered traditional Chinese medicine sample (1.0 g) was accurately weighed and put into a 50 mL centrifuge tube. Then 20 mL of 7.0% (v/v) aqueous Triton X-114 solution were added into the system. The tube was then placed in an ultrasonic bath for 20 min. The supernatant was obtained by centrifuging at 5000 rpm for 5 min.

2.5 One step preconcentration and cleanup procedure

The supernatant obtained in procedure as above was transferred into another 50 mL centrifuge tube. NaCl (3.0 g) was added into the tube and mixed vigorously using a vortex mixer. Then 2.0 g of the appropriate sorbent was added into the tube. Then the resultant cloudy sample solution was incubated in a water bath at 60 ^{o}C for 40 min. After that, separation of the aqueous and surfactant-rich phase was accomplished by centrifuging at 5000 rpm for 5 min. Finally, the sticky surfactant-rich phase was separated by syringing from the system and diluted to 3 mL with methanol to reduce its viscosity.

2.6 HPLC-UV analysis

All pyrethroids were identified by their chromatogram and retention times compared with those of the standards. UV wavelength was set at 234 nm. The mobile phase was 80% acetonitrile aqueous solution. The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was room temperature and the injection volume was 20 μL. The extraction solutions were finally filtered through 0.22 μm filter papers before HPLC analysis.

3. Results and discussion

The diagram of extraction process was shown in Fig. 1. During the extraction procedure, the cell walls of the samples were ruptured by ultrasonic, and the inside target analytes and interfering compouds dissolved and dispersed into the extraction solvent. Then the target analytes were transfered immediately into the middle of the micelle system due to the unique structure of micelle. After adding the sorbent, the interfering compounds are adsorbed by the sorbent while the target analytes remain in the extraction solvent, which is finally collected in the surfactant-rich phases. Finally, the surfactant-rich phase was diluted by methanol before HPLC analysis.

3.1 Optimization of extraction conditions

In this section, the effects of various operating conditions including the extraction solvents,

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Triton X-114 concentration, extraction solvent pH, liquid/solid ratio and ultrasonic extraction time have been discussed. The extraction process was evaluated by the recoveries of the pyrethroids.

3.1.1 Effect of extraction solvent

The extraction solvents tested in this study were methanol, ethanol, acetonitrile, chloroform, acetone and Triton X-114. Table 1 showed the influence of extraction solvents on the recoveries of pyrethroids. Compared to the results obtained by other organic solvents, the recoveries achieved by acetonitrile as extraction solvent were higher. It was probably caused by the higher solubility of the pyrethroids in acetonitrile than in other organic solvents. However, the recoveries of pyrethroids achieved by Triton X-114 were similar or even higher than that reached by acetonitrile. Triton X-114 molecule consists of two distinct chemical moieties: the hydrophilic and the hydrophobic. When the concentration of Triton X-114 molecules dissolved in water over a certain threshold, aggregate structures known as micelles are formed spontaneously. In a micelle, the hydrophobic tails all flock inside to minimize the unfavorable contact with water, while the hydrophilic heads remain in the outer surface in order to maximize contact with the aqueous medium. Due to this unique structure, and assisted with ultrasonic, the target analytes pyrethroids were extracted fully into the micelle system. In this case, Triton X-114, which is less toxic than organic solvents and capable to preconcentrate the target analytes pyrethroids, was chosen as the extraction solvent for further application.

3.1.2 Effect of Triton X-114 concentration

Triton X-114 was chosen for the extraction of the pyrethroids from the traditional Chinese medicine as it has both relatively low cloud point temperature (24 ^{o}C) and critical micelle concentration (2.1×10^{-4} mol L^{-1}).[41] The concentration of surfactant must be sufficient for the formation of micelle aggregates and quantitative extraction of the target analytes. There is a narrow range to achieve easy phase separation and maximum extraction efficiency. Beyond this range, the preconcentration factor would decrease or the accuracy and reproducibility would most likely suffer.[42] So the effect of surfactant concentration on pyrethroids recoveries was evaluated by extracting the pesticide from traditional Chinese medicine samples at different Triton X-114 concentrations, varied from 1.0% to 9.0% (v/v). As shown in Fig. 2a, the pyrethroids recoveries increased with the increasing of the concentration of Triton X-114 from 1.0% to 7.0%, while did not significantly increase from 7.0% to 9.0%. 7.0% (0.23 mol L^{-1}) was sufficient to achieve satisfactory extraction for the recovery of pyrethroids.

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3.1.3 Effect of pH

The pH of the sample solution also plays an important role in the extraction of the target analytes. Depending on the pH of the extract solvent, some organic compounds can assume an ionizable form, resulting in a lower solubility inside the micelle, and consequently lower the recoveries.[33] Pyrethroids recoveries were evaluated from pH 2.0 to 10.0. The pH of water samples were adjusted with HCl or NaOH diluted solutions. It can be seen from the Fig. 2b that the pyrethroids recoveries increased rapidly when pH of solution increased from 2.0 to 7.0 and decreased slightly from 7.0 to 10.0. Therefore, the value of 7.0 was chosen as the optimum extraction solvent pH.

3.1.4 Effect of liquid/solid ratio

The liquid/solid ratio is the ratio of solvent volume to the amount of crude material. A smaller amount of solvent could result in incomplete extraction, while the chance of bioactive components coming into contact with the solvent would increase with the increasing of the amount of solvent. But a larger volumes of solvent would decrease the economic feasibility and create unnecessary waste.[43] Therefore the effect of different liquid/solid ratios (5/1, 10/1, 15/1, 20/1 and 25/1) on the extraction of pyrethroids was subsequently studied. As can be clearly seen in Fig. 2c, the recoveries of the four pyrethroids increased by increasing the liquid/solid ratio up to 20/1 and remained constant for ratios above 20/1. So the best choice of the liquid/solid ratio was 20/1 (mL/g).

3.1.5 Effect of ultrasonic time

During this study, ultrasonic was chosen to enhance the extraction of pyrethroids from traditional Chinese medicine. The bubbles create and collapse when the amplitude of ultrasonic waves traveling through the solvent, which can disrupt the cell walls of traditional Chinese medicine. This significantly makes the penetration of solvent into cells and the release of the target analytes from cells into the solvent easier.[44] In order to insure the mass transfer was finished completely, the extraction time was the key factor to test the proposed method. The influence of ultrasonic time on the ability of Triton X-114 to extract pyrethroids from traditional Chinese medicine was examined in the range of 10 to 60 min. As shown in Fig. 2d, the recovery for each pyrethroid increases rapidly when the ultrasonic time increases from 10 to 20 min. After 20 min, the extraction recovery makes no significant change. Meanwhile, the amount of the interfering compounds extracted from samples increased with the increasing of the ultrasonic time. The recoveries of four pyrethroids were all achieved over 85% at 20 min.

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After extraction, the extract and solid residues were separated by centrifugation. The centrifugation time was studied by varying the time from 5 to 20 min. The results showed that the satisfied phase separation and extraction recoveries were obtained with 5 min.

3.2 Optimization of preconcentration and cleanup conditions

The following parameters were taken into consideration in the optimization of the preconcentration and cleanup procedure: the types of sorbent used for the cleanup step, proportion of the sorbent and sample, NaCl concentration, equilibration temperature and equilibration time.

3.2.1 Effect of different sorbents

After the micellar extraction, lots of interfering components were also co-extracted from a solid herbal matrix together with the target pesticides. In order to absorb those interfering compounds to improve the detection sensitivity and selectivity of the target analytes, a comparative study on five common sorbents (silica gel, activated carbon, neutral alumina, diatomaceous earth and florisil) was conducted. In order to perform a comparison of all sorbents, 2.0 g different sorbents were added into the supernatant obtained in the first step. As shown in Fig. 3, compared with the result obtained without sorbent, the color of the solution became obviously shallow by different degrees with the addition of different sorbents. The results showed that the cleanup abilities of the sorbents decreased in the following order: Florisil > activated carbon > neutral alumina > silica gel > diatomaceous earth. The cleanup abilities of diatomaceous earth, silica gel and neutral alumina were extremely insignificant that the analytes were severely interfered during the analysis. The result indicated that both activated carbon and florisil were suitable for cleanup procedure. However, florisil as sorbent could not only absorb the interfering compounds but also absorb certain amount of the target analytes, which resulted in a low recovery of pyrethroids. So activated carbon was selected for further experiments as the sorbent to achieve the best compromise between cleanup ability and extraction recovery. At the same time, the chromatograms in Fig. 4 showed the results obtained with and without the cleanup by activated carbon as sorbent, and the results indicated that activated carbon played a capable role in cleaning up which lead to a good pyrethroids recovery and less interfering components.

3.2.2 Effect of the proportion of the sorbent and sample

As the activated carbon was selected as the most appropriate sorbent for the preconcentration procedure, the effect of the proportion of the sorbent and sample ranging from 1/1 to 5/1 (the amount of activated carbon/the amount of sample, g/g) was investigated and the results were shown

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in Fig. 5a. The recoveries of four pyrethroids increased as the proportion of the sorbent and sample decreased. This was caused by the sorbent's adsorption of certain amount of target analytes despite it has been pretreated before the experiment. So the less sorbent used, the higher recoveries of four pyrethroids can be obtained. However, some interference occurred in HPLC analysis when the proportion decreased to 1/1. Consequently, 2/1 was selected as the optimized proportion of the sorbent and sample for the following experiments.

3.2.3 Effect of NaCl concentration

The addition of electrolytes to the surfactant micellar solution can often modify its cloud-point temperature. This phenomenon can be possibly explained by the changes of intermicellar repulsions or of the sizes of the micelles due to the adsorptions of ions to these micelles.[45] In most cases of nonionic surfactants, the presence of salt may facilitate phase separation since it increases the density of the aqueous phase.[46] So the effect of the concentration of NaCl on the perconcentraction of pyrethroids was investigated in the range of 0-25% (m/v), and the phenomenon was observed. When the concentration of NaCl was within 0-5%, the surfactant-rich phase was not steady in the lower part of the aqueous solution. When the concentration of NaCl was higher than 5%, the surfactant-rich phase was suspended on the top of the aqueous solution. Fig. 5b exhibits that the recoveries of four pyrethroids all increased with the increasing of NaCl concentration from 0% to 15%. Then the recoveries of four pyrethroids kept constant when the concentration of NaCl was over 15%. Therefore, 15% was selected to aid the separation of the surfactant-rich phase from the aqueous phase.

3.2.4 Effect of equilibration temperature

In general, the optimal incubation temperature of cloud point extraction (15-20 ^{o}C) is higher than the cloud point temperature of the surfactant.[47] Too high temperatures are not suitable in the proposed analytical method since they could create stability problems for target analytes. While at higher temperature, critical micelle concentration of nonionic surfactants decreases.[48] Thus, equilibration temperatures ranging from 40 to 80 ^{o}C were investigated. It is evident from Fig. 5c that the recoveries of the pyrethroids increased rapidly when temperature was raised from 40 to 50 ^{o}C, then the recoveries of the pyrethroids increased dilatorily and reached the maximum values at 60 ^{o}C. The cause of this result could be the number of hydrophobic micelles in the surfactant-rich phase increased correspondingly with the rise of temperature, causing the strengthen of extraction ability of preconcentration for pyrethroids.[49] When the temperature was elevated over 60 ^{o}C, the

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recoveries of the pyrethroids almost kept constant. Thus, 60 °C was chosen as the working temperature.

3.2.5 Effect of equilibration time

As the extraction recovery depends on the time that the analytes need to interact with the micelles and get into their cores,[49] the shortest equilibration time is very desirable for compromise between completion of extraction and efficient separation of phases. The effect of equilibration time upon extraction recovery was studied by varying the equilibration time between 10 and 60 min. It can be seen from the Fig. 5d, the recoveries of the pyrethroids increased from 10 to 40 min, then kept almost constant. So 40 min was chosen as the optimal equilibration time.

The separation of the aqueous and surfactant-rich phase was accomplished by centrifuging at 5000 rpm for 5 min. The analytical results were not improved with longer centrifugation time. Otherwise, the phase separation was not completely if the centrifugation time is less than 5 min.

3.3 Analytical characteristics

Table 2 summarizes the analytical characteristics of the optimized method, such as linear range, regression equation, R^2 and limits of detection. The linearity of the four pyrethroids was within the range of 0.06-6.00 μg g^{-1}. The limits of detection (LODs) defined as three times the ratio of signal to noise for beta-cyfluthrin, cyhalothrin, cyphenothrin and permethrin were 0.0108, 0.0086, 0.0083 and 0.0092 μg g^{-1}, respectively.

3.4 Comparison of the proposed method with other methods reported in literatures

The analytical results obtained by the proposed method were compared with those obtained by the methods reported in the literatures for analyzing pyrethroids in herb samples. Data in Table 3 indicates that the LOD, recovery and RSD obtained from the proposed method are comparable to those reported in other methods. However, the proposed method is time-saving, simple-stepped and eco-friendly whereas the methods reported in literatures used one or more kinds of toxic organic solvents.

3.5 Application to the analysis of real samples

In order to validate the viability of the proposed method, the proposed method was applied to extract and determine four pyrethroids (beta-cyfluthrin, cyhalothrin, cyphenothrin and permethrin) in other different traditional Chinese medicines. The insets and the chromatograms in the Fig. 6 indicates the cleanup ability of activated carbon for extraction and preconcentration of four pyrethroids from Herba lysimachiae, Ardisia japonica and Camptotheca acuminate fruit,

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respectively. The recovery of four pyrethroids from traditional Chinese medicine samples was investigated. No pyrethroids residues at detectable level were found in these samples. The recovery study was then carried out by spiking the traditional Chinese medicine samples with pyrethroids. Based on Table 4, all pyrethroids obtained satisfying percentage of recovery varied from 72.4% to 94.3% with relative standard deviations (RSD) less than 6.7%.

4. Conclusion

One-step preconcentration and cleanup with micellar system, a novel selective extraction technique, was successfully developed in this study for the extraction and determination pyrethroids from traditional Chinese medicine. The capability of five types of commercially available sorbents used for removing the interfering components were determined, and the activated carbon was proved to have extraordinary cleanup ability with satisfying recoveries of four pyrethroids under the optimized experimental conditions. This method was successfully validated by extracting the four pyrethroids from three kind of different traditional Chinese medicines. The recoveries were 72.4%-94.3% and the RSDs were 4.2% - 6.7%. Moreover, this procedure is very simple, sensitive, effective, selective, precise, low cost, and less toxic to the environment. This work demonstrates an effective usage of micellar extraction for the multiresidue analysis of pyrethroids in traditional Chinese medicines. Since this report is a preliminary study for the proposed new system, further studies are necessary to expand target pesticides and applicable samples.

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Figure captions:

Fig. 1. The schematic diagram of extraction and cleanup process of the method.

Fig. 2. The influence of several parameters on the micellar extraction: Triton X-114 concentration (a), pH (b), liquid/solid ratio (c), ultrasonic time (d). The preconcentration and cleanup conditions: proportion of the sorbent and sample, 2/1 (g/g); NaCl concentration, 15% (m/v); equilibration temperature, 60 ^{o}C; equilibration time, 40 min.

Fig. 3. Effect of different sorbents on the cleanup of Rhubarb sample.

Fig. 4. The chromatograms of standard pyrethroids (a), and pyrethroids in Rhubarb sample with (b) and without (c) adding activated carbon. The spiked pyrethroids concentration: 0.2 μg g^{-1}. HPLC conditions: mobile phase, 80% acetonitrile aqueous solution; flow rate,1.0 mL min^{-1}; UV wavelength, 234 nm.

Fig. 5. The influence of several parameters on the preconcentration and cleanup: proportion of the sorbent and sample (a), NaCl concentration (b), equilibration temperature (c), equilibration time (d). The micellar extraction conditions: Triton X-114 concentration, 7.0% (v/v); pH, 7.0; liquid/solid ratio, 20/1 (mL/g); ultrasonic time, 20 min.

Fig. 6. Chromatograms of different traditional Chinese medicine samples of Herba lysimachiae (a), Ardisia japonica (b) and Camptotheca acuminate fruit (c) without and with adding activated carbon. The spiked pyrethroids concentration: 0.2 μg g^{-1}. HPLC conditions: mobile phase, 80% acetonitrile aqueous solution; flow rate,1.0 mL min^{-1}; UV wavelength, 234 nm.

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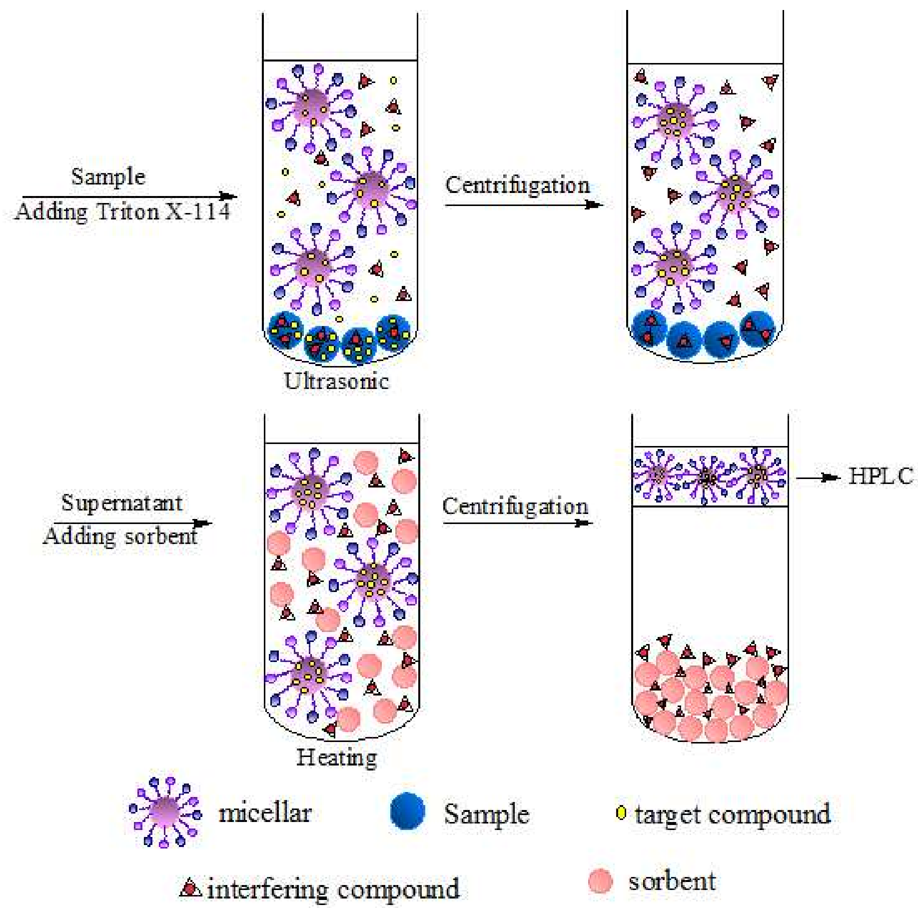

Fig. 1

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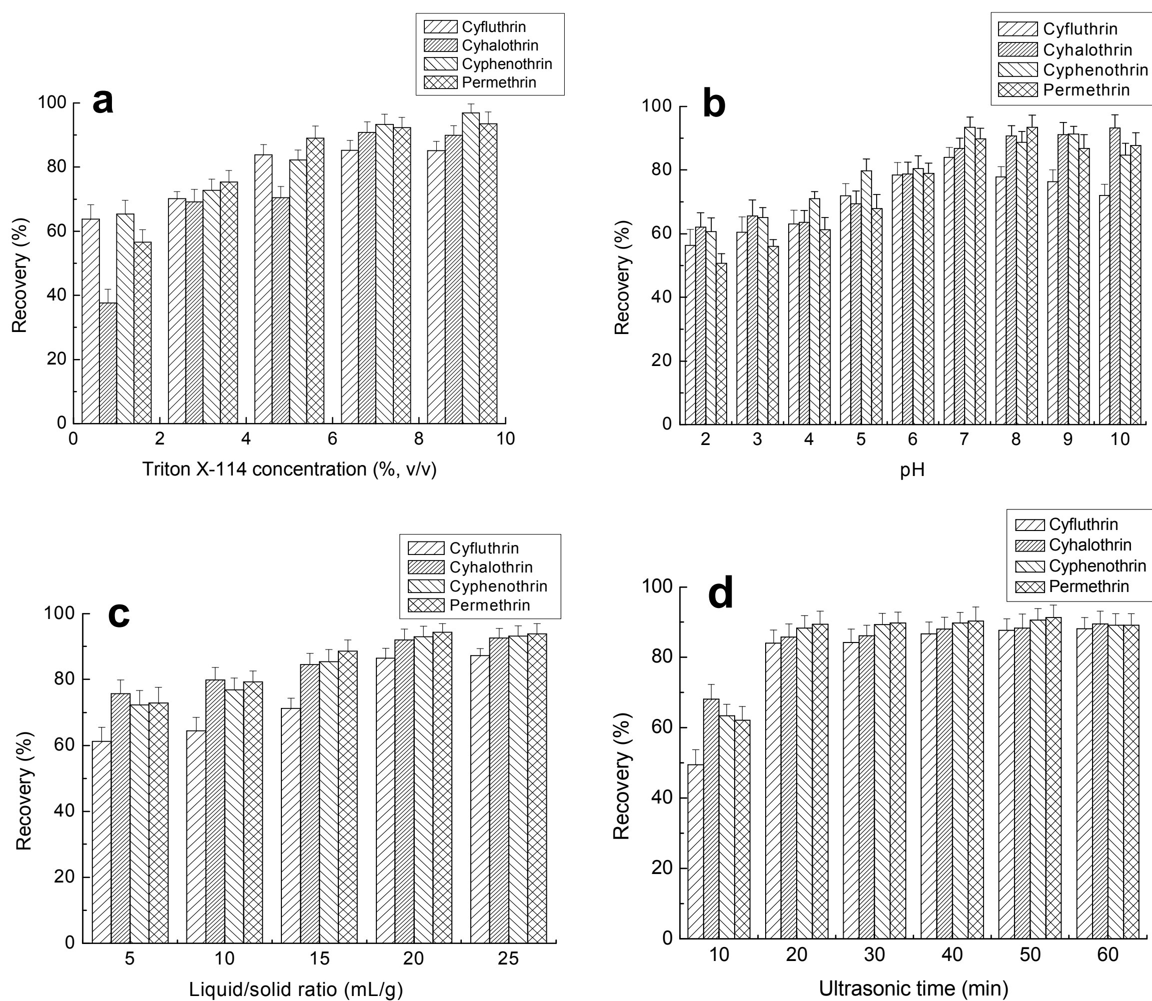

Fig. 2

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Fig.3

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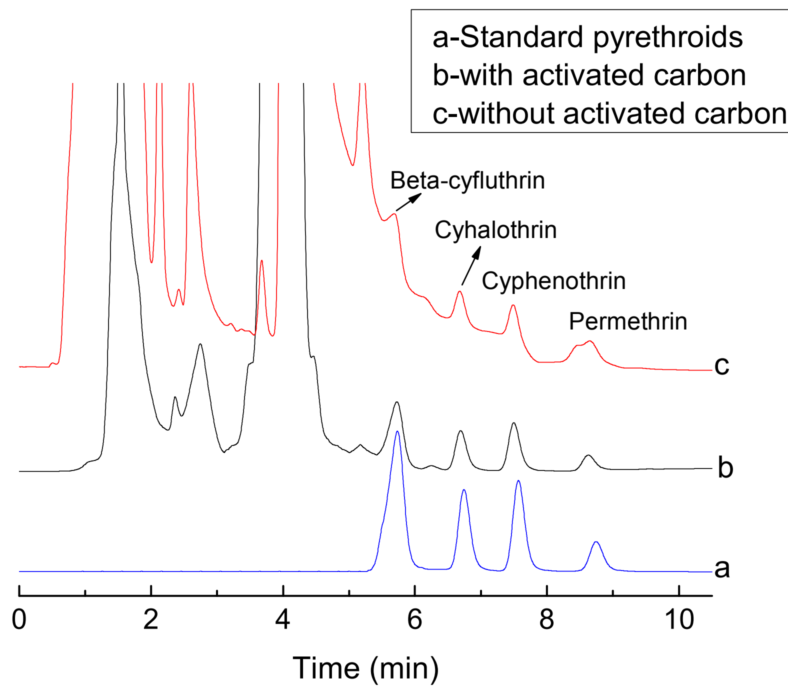

Fig. 4

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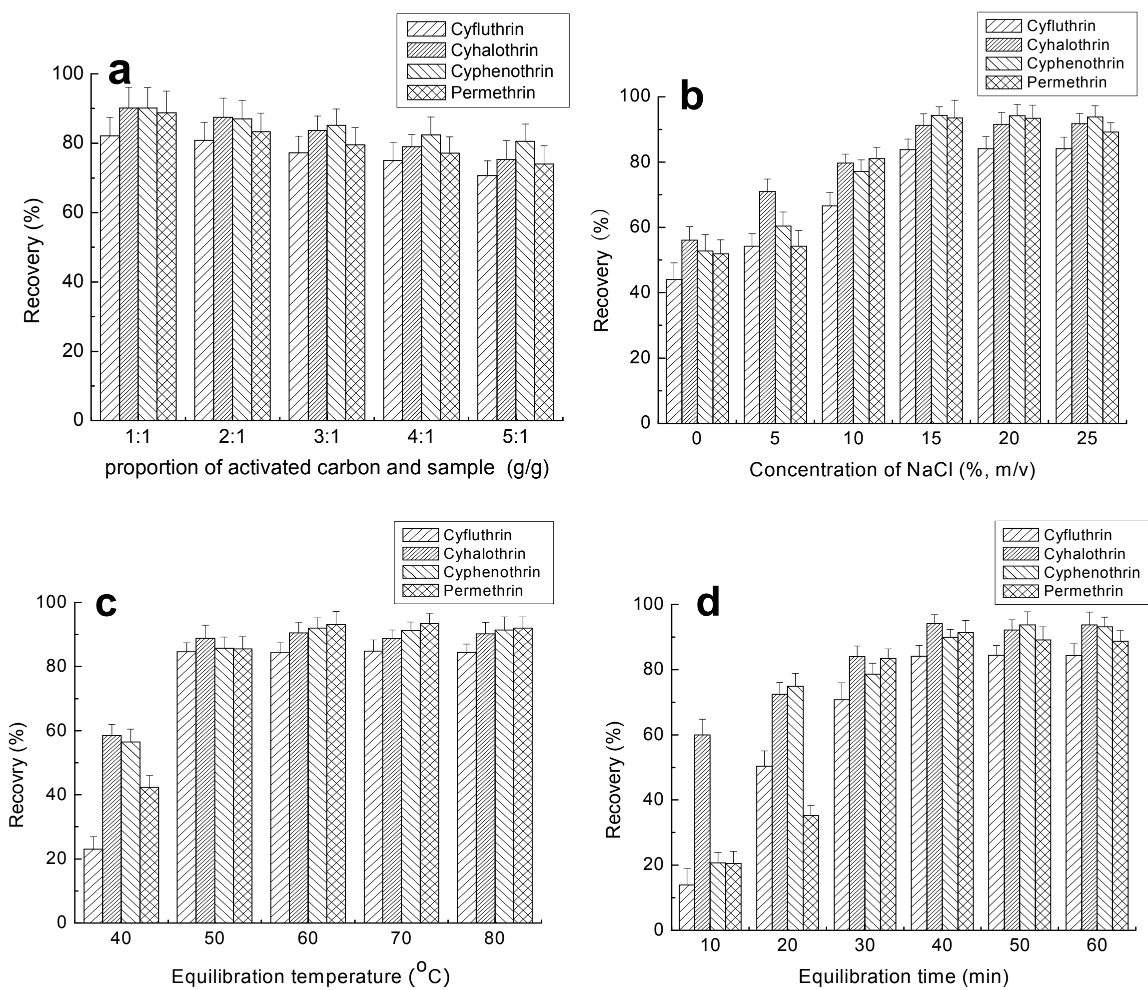

Fig. 5

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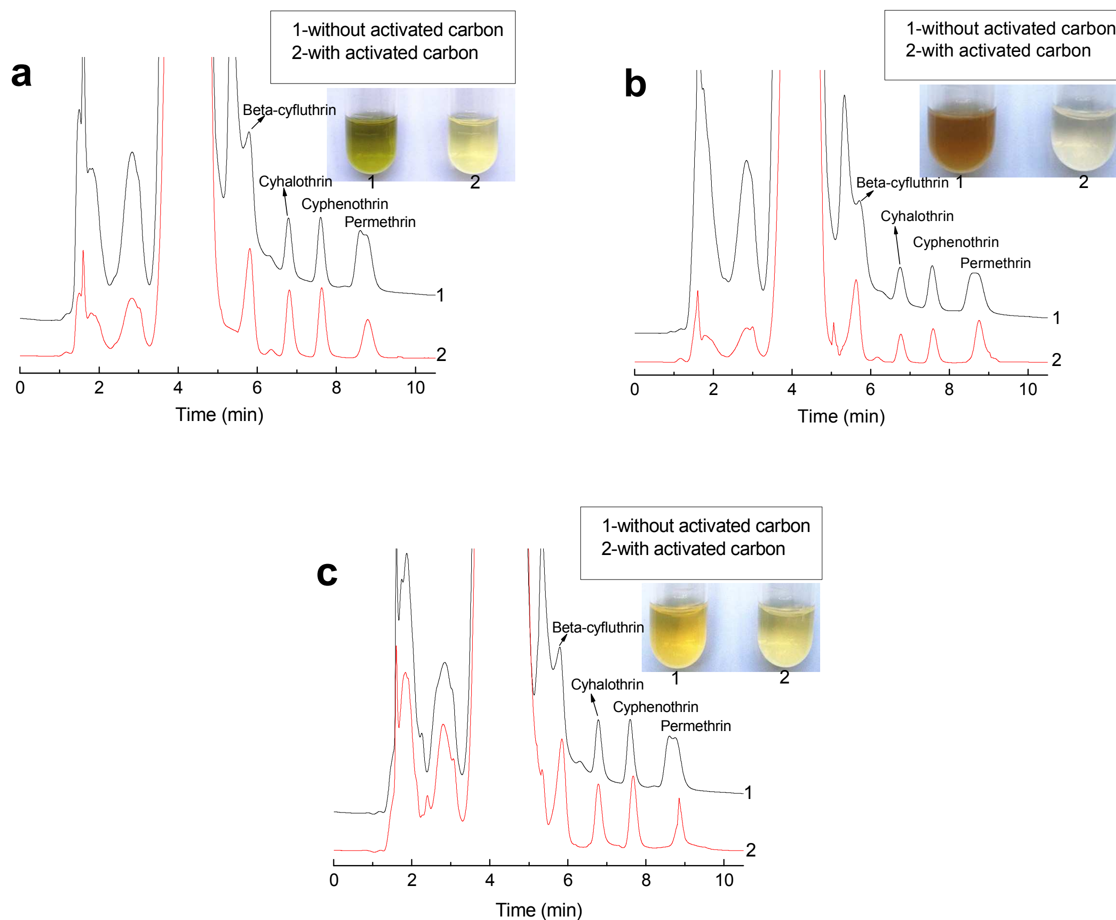

Fig. 6

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Table 1

Comparison of different solvents used for extraction of four pyrethroids (n=5)

Extraction solvents	Beta-cyfluthrin		Cyhalothrin		Cyphenothrin		Permrthin	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Methanol	73.82	4.9	79.02	5.1	82.45	5.4	73.01	6.3
Ethanol	70.12	6.8	75.52	6.3	79.39	5.2	88.29	5.8
Acetonitrile	89.61	7.2	78.58	4.9	79.38	6.1	92.20	4.9
Acetone	62.73	5.7	80.25	4.7	79.37	4.9	81.52	6.8
Chloroform	14.95	7.3	12.45	5.5	9.18	6.3	9.46	5.6
Triton X-114	96.28	4.2	80.53	4.6	84.54	5.0	94.79	5.2

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Table 2

Analytical characteristics of the proposed method

Chemical name	Linear range ($\mu g\ g^{-1}$)	Linear equation	R^2	LOD ($\mu g\ g^{-1}$)
Beta-cyfluthrin	0.06-6.00	$y = 2.68\times10^5x + 1.99\times10^3$	0.9982	0.0108
Cyhalothrin	0.06-6.00	$y = 2.29\times10^5x + 2.73\times10^3$	0.9964	0.0086
Cyphenothrin	0.06-6.00	$y = 1.63\times10^5x + 0.19\times10^3$	0.9973	0.0083
Permrthin	0.06-6.00	$y = 1.49\times10^5x + 0.31\times10^3$	0.9998	0.0092

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Table 3

Comparison of the proposed method with other methods reported in literatures

Extraction methods	Detection methods	Extraction solvents	LOD ($\mu g\ kg^{-1}$)	Recovery (%)	Precision (RSD, %)	Peference
Accelerated solvent extraction	GC-ECD	Acetone and n-hexane	0.15-3.00	74.1-109	0.62-8.98	50
Accelerated solvent extraction	GC-ECD	Ethyl acetate and hexane	3-5.9	69.6-113.2	2.0-14.6	51
Solvent extraction	GC-MS	Acetonitrile	0.05-1.7	82.4-117.9	1.3-9.6	52
QuEChERS	GC-ECD	Acetonitrile	2.2-21.7	70-120	1-22	53
Micellar- assisted extraction	HPLC	7% Triton X-114	8.3-10.8	72.4-94.3	3.2-6.7	The proposed method

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Table 4

Recovery of pyrethroids in spiked traditional Chinese medicine samples (n=5)

Compounds	Herba lysimachiae		Ardisia japonica		Camptotheca acuminate fruit	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Beta-cyfluthrin	91.7	4.6	90.6	5.5	87.7	4.7
Cyhalothrin	86.2	6.7	81.8	4.9	84.8	6.1
Cyphenothrin	83.4	6.3	72.4	4.6	83.3	5.3
Permrthin	79.3	5.4	86.1	6.2	78.0	5.6

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