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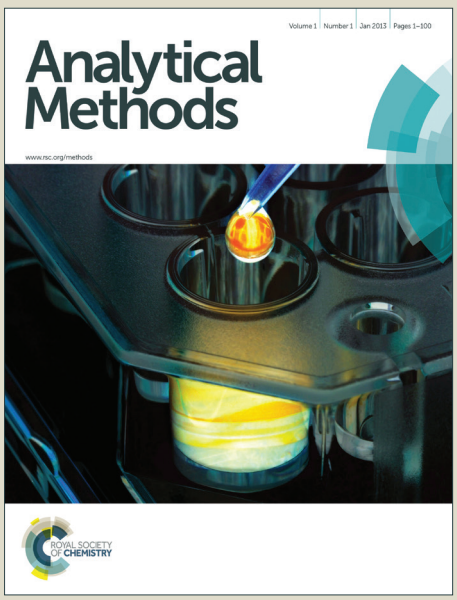

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Trace determination of five organophosphorus pesticides by using QuEChERS coupled with dispersive liquid–liquid microextraction and stacking before micellar electrokinetic chromatography

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Abstract

A procedure involving QuEChERS sample extraction combined with dispersive liquid–liquid microextraction (DLLME) and stacking as off-line and on-line preconcentration techniques followed by micellar electrokinetic chromatography (MEKC) has been developed for the determination of five organophosphorus pesticides (dimethoate, phosphamidon, paraoxon-methyl, paraoxon and fensulfothion). The important parameters that influence the stacking and DLLME efficiency were evaluated. The RSDs of migration time ranged from 0.06% to 1.03% and the peak area ranged from 1.12% to 5.15% for the five analytes, indicating the good repeatability of the method. The method was extensively validated by evaluating the linearity ($r^2 \geq 0.9956$), LODs (0.010–0.018 μg mL^{-1}) and recovery (78.75–118.15%). The QuEChERS-DLLME-stacking-MEKC method has been successfully applied to assay the five organophosphorus pesticides in *Astragalus membranaceus*. Under the optimized conditions, the proposed method provided a 90.0- to 167.3-fold enrichment of the five pesticides compared with the normal MEKC method, which offers an ideal solution in the determination of some trace pesticides in real samples with complex matrices.

1. Introduction

Recently, various kinds of pesticides have been used to kill or control unwanted insects, fungi or other pests in the process of herbal production. Because of better insecticidal effect and faster degradation in the environment, organophosphorus pesticides (OPPs) are widely used in agriculture instead of organochlorine pesticides (OCPs). However, trace amounts of OPPs can be transferred to humans via the food chain, then result in reducing the activity of neurotransmitters and irreversible effects on the nervous system.[1,2] Therefore, it is necessary to develop sensitive and selective methods for monitoring of trace levels of these pesticides in agricultural samples.

OPPs have been analyzed by gas chromatography (GC) with nitrogen phosphorus detector (NPD), electro capture detector (ECD), flame photometric detector (FPD) or MS detector[3-6] and liquid chromatography (LC) with various detectors.[7-10] Nowadays, as a powerful and environmentally friendly technique, capillary electrophoresis (CE) has been widely used to detect the pesticide residues due to minimal reagent consumption and high separation efficiency compared with GC and LC methods.[11-13] Micellar electrokinetic chromatography (MEKC) is an important branch of CE by adding surfactants into the electrolyte solution to form micelles. The separation mechanism is based on differential partitioning of analytes between micelle phase and surrounding aqueous phase. Due to the powerful ability for the separation of both charged and neutral substances with either hydrophobic or hydrophilic properties, MEKC has attracted much attention in the determination of pesticide residues in recent years.[13]

However, CE has a relatively low sensitivity as compared to LC technique resulting from the low sample volume injected and short optical path employed for most commonly used UV detection. In order to overcome this limitation, on-line and off-line sample preconcentration strategies have been developed.[14,15] As useful tools for on-line preconcentration in MEKC, sample stacking and sweeping are applied individually or simultaneously for the separation of analytes. Field-amplified sample stacking (FASS),[16] isotachophoretic (ITP) stacking,[17] sweeping[18,19] have been successfully applied to simultaneously detect pesticide residues in environmental water sample or soil sample. Among of them, ITP stacking is applicable only to ionic or ionizable analytes. Sweeping was first introduced by Quirino et al,[20] and it had been effectively used to enrich the analytes into narrow band within the capillary by pseudostationary phase. The enrichment of the analytes is largely dependent on the interaction of the analytes with the pseudostationary phase.[21] The difference between sweeping and FASS is that no field enhancement in the sample zone is required in sweeping with a field strength equal to or lower than that of background electrolyte solution (BGS).[22] Moreover, another stacking method which namely as "reversed electrode polarity stacking mode", was practiced by Quirino et al.[23] Compared with normal stacking mode, this method affords larger volume injection and significantly enhances the sensitivity of neutral analytes in MEKC. To the best of our knowledge, this online stacking mode has been developed for the trace determination of pesticides in water sample or wines, and provided hundred-fold sensitivity enhancement in compare with normal MEKC method.[24-26] However, there are few literatures on

application of this stacking mode for analysis in Chinese medicine.

Liquid–liquid extraction (LLE),[27] solid–phase extraction (SPE),[28] solid–phase microextraction (SPME),[29] and liquid–phase microextraction (LPME)[30] are the most common off-line preconcentration methods widely used for residue analysis. In 2006, dispersive liquid–liquid microextraction (DLLME) was proposed based on LPME, with the advantages of simplicity of operation, short extraction time, small amount of solvents used and high enrichment factor.[31-35] However, the lack of purification for more complex matrix samples, such as food and soil, has caused this method to be limited to those with simpler matrices.[4] Fortunately, as the most common technique for multi-residue pesticides analysis in the samples with complex matrices, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method can make up for this deficiency.[36] This method provides a great clean-up effect on the extract using dispersive solid-phase extraction (d-SPE) by several kinds of sorbents. Generally, primary secondary amine (PSA) sorbent could remove various sugars, pigments, and polar organic acids while graphitized carbon black (GCB) sorbent was used for removing sterols and pigments.[36,37] Moreover, the main limitation of QuEChERS, i.e. the poor enrichment factor, can be overcome by coupling some off-line or on-line sample preconcentration approaches after this technique.

In the present study, a procedure involving QuEChERS extraction combined with DLLME and stacking as off-line and on-line preconcentration techniques followed by MEKC has been developed to determinate five OPPs (dimethoate, phosphamidon, paraoxon-methyl, paraoxon and fensulfothion) in herbal medicine *Astragalus*

membranaceus. The important experimental parameters that influence the stacking and DLLME efficiency were evaluated. To our knowledge, this may be the first report about the application of QuEChERS-DLLME-stacking-MEKC for the analysis of pesticides in real samples.

2. Experimental

2.1 Reagents, chemicals and standards

Five OPPs standards including dimethoate, phosphamidon, paraoxon-methyl, paraoxon and fensulfothion (Fig. 1) were purchased from Ehrensdorfer (Germany). Individual stock solutions of the pesticides at a concentration of 100.0 mg L^{-1} were prepared in acetonitrile and stored at 4℃. The mixed standard solution containing 10.0 mg L^{-1} of each pesticide (except paraoxon-methyl at 20.0 mg L^{-1}) was prepared in acetonitrile and stored at 4℃. Standard working solutions at various concentrations were prepared daily by an appropriate dilution of the stock solutions with deionized water after dryness under a stream of nitrogen.

Sodium tetraborate decahydrate, sodium dodecyl sulphate (SDS), sodium hydroxide (NaOH), sodium chloride (NaCl), PSA (sorbent, 50 μm) and GCB (sorbent, 45 μm) were analytical reagents from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), chlorobenzene (C_6H_5Cl), methanol and acetonitrile were HPLC-grade solvents from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water (Millipore, MA, USA) was used for the preparation of all aqueous solutions.

2.2 Apparatus and materials

All CE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, USA), equipped with an auto sampler and a diode array detector (DAD). A SevenEasy conductivity meter (Mettler-Toledo, USA) was used for measuring the conductivity of the buffer solutions. An uncoated fused-silica capillary with 75 μm i.d (Beckman Coulter, USA) was used throughout the experiments. The total length was 57 cm (50 cm effective length). All experiments were thermostat at 25 ℃.

2.3 Sample preparation

The dried *Astragalus membranaceus* sample was sieved through a 20 mesh to obtain fine, uniform powder. The optimized and validated sample preparation procedures entailed the following steps: (1) 1.0 g of dry powders was accurately weighted and transferred to a 15 mL glass centrifuge tube; (2) 10 mL acetonitrile was added in to the tube, then the tube was vortexed for 5 min by vortex mixer and subsequently centrifuged at 3000 g for 5 min; (3) 4.0 mL of the upper acetonitrile extract was transferred in to a 15 mL glass centrifuged tube containing 400 mg PSA, 60 mg GCB; (4) the tube was vortexed for 1 min and centrifuged at 3000 g for 5min. Then a DLLME procedure was carried out; (5) 1.5 mL of the upper acetonitrile extract was mixed with 300 μL of chloroform and transferred in to a 15 mL tube; (6) the mixture was added quickly into 5.0 mL of deionized water with the salt concentration of 4% to form cloudy solution; (7) the tube was vortexed for 1 min and centrifuged at 3000 g for 5 min; (8) the sedimented phase was transferred to another tube completely and evaporated to dryness under a stream of nitrogen; (9) the residue

was re-dissolved in 200 μL of 5 mM borate buffer for CE analysis.

2.4 Genera electrophoresis procedure

Before first use, capillary was conditioned with methanol (10 min), water (10 min), 0.1 M NaOH (5 min), and water (20 min). Between consecutive analysis the capillary was conditioned by flushing with 0.1 M NaOH (3 min), then with water (3 min), and finally with the running buffer for 5 min at 30 psi. In the stacking procedure, the capillary was first filled with the running buffer (10 mM borate buffer containing 40 mM SDS and 20% methanol at pH 9.3). Then the large plug of sample was hydrodynamic injected for 90 s at 0.5 psi (1 psi = 6,895 pa). Electrophoresis was performed at a reverse voltage (-20 kV) firstly and the voltage was turned off when the current became 95~99% of the value obtained with running buffer. Finally, the separation was carried out at a direct voltage (+20 kV) at 25 ℃ with diode array detection at 200 nm.

3. Results and discussion

3.1 Optimization of electrophoretic conditions

An aqueous mixture solution of five pesticide standards (10 mg L^{-1}, except paraoxon-methyl at 20.0 mg L^{-1}) was used to study the analytical parameters, including buffer concentration, SDS concentration, pH, organic solvent content, under different conditions by direct hydrodynamic injection (5 s, 0.5 psi).

3.1.1 Effect of the buffer and SDS concentrations. In order to find the suitable BGS for the analysis of the pesticides, the separation was optimized using different mixture of 5 to 40 mM borate buffer containing 10 to 60 mM SDS. The result

demonstrated that the migration time was increased with increased concentration of borate. Both the peak area and migration time of the pesticides were increased with the increase of SDS concentration. When the concentration of SDS was increased to 60 mM, the peaks became overlaped and the migration sequences were changed. Considering analysis time and resolution, 10 mM and 40 mM were chosen as the optimum concentrations of borate and SDS, respectively.

3.1.2 Effect of the buffer pH. Since the acidity of the running buffer could affect the migration time and separation efficiency of the analytes, the running buffers of 10 mM borate containing 40 mM SDS at different pH values varying from 7 to 10 were tested. The result indicated that the migration time of pesticides was increased with the decrease of pH value. Interestingly the BGS with no pH adjustment at the value of 9.3 showed the best separation efficiency and relatively short analysis time in this study, which was chosen for further studies.

3.1.3 Effect of the organic solvent. The organic solvents could cause a difference in affinity between micelles and analytes due to the decreasing of the aqueous phase polarity. In this study, the effect of methanol addition was investigated with the concentration in the range of 5 to 25% (v/v). The separation performance between paraoxon-methyl and phosphamidon was worse with methanol content varying from 5% to 15%. With the methanol concentration increased, the migration time was extended. Given an overall consideration of resolution and migration time, 20% of methanol was selected in the study.

Considering the above-mentioned procedure, the optimum BGS was the mixture

of 10 mM borate buffer and 40 mM SDS containing 20% methanol at pH 9.3.

3.2 Optimization of stacking-MEKC procedure

In order to increase the sensitivity of the determination of the analytes, important parameters of stacking-MEKC procedure were optimized including the sample solvent and the sample injection time.

3.2.1 Effect of sample solvent. The proper sample solvent normally should possess lower conductivity than that of the BGS. Different solvents (water, 5 mM, 10 mM, and 15 mM borate buffer without SDS or organic solvent) were explored in this study. The results indicated that 5 mM borate buffer as the sample solvent could provide the highest sensitivity and the best separation efficiency, which had lower conductivity (0.98 mS cm^{-1}) than that of the BGS (3.38 mS cm^{-1}).

3.2.2 Effect of sample injection time. For the stacking technique, prolonging the sample injection time would theoretically increase the amount of sample introduced into the capillary and then enhance the detection sensitivity for the analytes. In this study, the injection time was optimized varying from 20 to 150 s at 0.5 psi. With increasing injection times, the peak area of all analytes was increased while the migration time had no significant change. However, the longer injection times resulted in peak distortion. When the injection time was more than 90 s, the resolution between the peaks of phosphamidon and paraoxon-methyl greatly deteriorated. Therefore, as a compromise between the sensitivity and the resolution, the sample could be injected in the capillary up to 90 s at 0.5 psi.

In the present study, the reversal time (the time need to pump out sample matrix

by applying negative voltage at inlet) was 0.4 min. In comparison with the non-preconcentration procedure, the current method provided an approximately 20-fold enrichment of the pesticides under the optimum conditions.

3.3 Optimization of DLLME procedure

In order to evaluate the extraction efficiency under different parameters in the DLLME procedure, extraction recovery (ER%) was used according to the following equation.

$$ER\% = \frac{C_{rec}V_{rec}}{C_0V_{aq}} \times 100 \quad (1)$$

Where C_{rec}, V_{rec}, C_o and V_{aq} were the mixed standard concentration in the final reconstituted solution, the volume of the final reconstituted solution, the initial mixed standard concentration in the aqueous samples and the volume of the aqueous sample, respectively.[31] The different experimental parameters that can affect the extraction efficiency, including type of extraction and dispersive solvents, volume of extraction and dispersive solvents, and salt addition have been optimized in this study.[35]

3.3.1 Selection of extraction solvent and dispersive solvent. Selection of the extraction solvents was important for the extraction of pesticides in DLLME procedure. CH_2Cl_2, $CHCl_3$ and C_6H_5Cl were selected as potential extraction solvents, while all the solvents had a higher density than water and low solubility in water. Extraction efficiency was evaluated by comparing the recoveries of the pesticides. As indicated in Fig. 2, $CHCl_3$ achieved the best extraction efficiency for most of the analytes with acetonitrile as dispersive solvent, which was chosen as the extraction solvent for further study.

In this study, acetonitrile and acetone were selected to evaluate the effect of dispersive solvents on the performance of DLLME. The result showed that the best extraction recoveries were obtained when acetonitrile was used as a dispersive solvent. Therefore, acetonitrile was chosen for the further study.

3.3.2 Volume of dispersive solvent and extraction solvent. In order to evaluate the effect of dispersive solvent volume on the performance of the DLLME procedure, different volumes of acetonitrile (0.5 mL, 1.0 mL, 1.5 mL, and 2.0 mL) mixed with 200 µl of $CHCl_3$ were added into 5 mL of deionized water with 4% of sodium chloride. For this ternary solvent system, the analytes existed in the acetonitrile extract at first. When the volumes of acetonitrile increased from 0.5 to 1.5 mL, more analytes could be extracted from acetonitrile to chloroform phase. In other words, the extraction efficiency could increase continuously with the increase volume of acetonitrile extract when the volumes of chloroform and 4% NaCl solution were kept at 200 µL and 5 mL, respectively. However, no two-phase system was observed when the volume of acetonitrile was up to 2 ml. Consequently, 1.5 mL of acetonitrile was selected for the experiment.

In this experiment, different volumes of $CHCl_3$ (100 to 500 µl in 100 µl intervals) were investigated while the dispersive solvent acetonitrile (containing same amount of analytes) was maintained at 1.5 mL. When the volume of $CHCl_3$ was less than 300 µl, the recoveries of all pesticides increased with the increase of the $CHCl_3$ volume. While the $CHCl_3$ volume increased from 300 to 500 µl, the extraction efficiency of all pesticides remained constant or slightly fluctuated. Therefore, 300 µl was selected as

the optimum volume of $CHCl_3$.

3.3.3 Effect of salt addition. The salting-out effect is a significant parameter for DLLME procedure. Generally, the addition of salt could increase the ionic strength of the aqueous phase and then decrease the solubility of the analytes in it. As a result, the partitioning of the analytes into the organic phase would enhance.[35] Among the different kinds of salts that can be selected, sodium chloride was the most commonly used for exploring the effect of ionic strength.[34,35,38] Therefore, in the present study, sodium chloride with the concentrations from 0 to 10% (w/v) was investigated to examine the effect of salt on the performance of DLLME,. As shown in Fig. 3, the extraction efficiency for most of the investigated analytes (except paraoxon-methyl) increased with the increasing of NaCl concentration up to 4%, which could be explained by the salting-out effect. However, with the salt concentration continued to increase, the extraction efficiency showed slight decrease. In this process polar molecules in the solution may participate in electrostatic interaction with the salt ions, which will reduce their ability to move into the extraction phase.[39] Especially, when the salt concentration was higher than 8%, the sedimented phase could not be formed at the bottom of the centrifuge tube. Based on the results, 4% of NaCl was used in further experiments.

3.4 Analytical characteristics and method validation

The analytical performance of the QuEChERS-DLLME-stacking-MEKC method was validated by measurement of the linearity of matrix-matched calibration, the limit of detection (LOD), the intra-day and inter-day precision. A series of working

samples containing each of the pesticides at five concentration levels of 0.05, 0.1, 0.5, 1.0, and 2.0 μg mL^{-1} (except paraoxon-methyl at 0.1, 0.2, 1.0, 2.0, and 4.0 μg mL^{-1}) were prepared for the establishment of the calibration curves. As shown in Table 1, most analytes exhibited excellent linearity with correlation coefficient (r^2) ranging from 0.9956 to 0.9999. Under the optimized conditions, the LODs of the proposed method were calculated at a signal-to-noise ratio (S/N) of about 3. The LODs for the studied pesticides were in the ranges of 0.010–0.018 μg mL^{-1}, which were lower than the maximum residue limits (MRLs) regulated by the FDA (Food and Drug Administration in United States) and EU (European Union). The precisions were determined by analyzing replicate of mixed standard (2 μg mL^{-1}, except paraoxon-methyl at 4 μg mL^{-1}) and expressed as the relative standard deviation (RSD) of peak area and migration time in the same day (n=5) and on the different days (n=5 ×3 day). The developed method showed good repeatability with RSD lower than 5.15 % and 1.03 % for peak area and migration time, respectively.

Moreover, Fig. 4 shows the electropherograms for the separation of the five pesticides obtained by the normal MEKC (without preconcentration) method, the stacking–MEKC method and the DLLME-stacking-MEKC method, respectively. In the stacking–MEKC procedures (Fig. 4B and 4C), electrophoresis was firstly performed at a reverse voltage (-20 kV) after a large hydrodynamic injection of the sample solution. The analytes in sample solution with different retention factors would be concentrated with the help of SDS and located at the different positions of capillary comparing to the inlet region under normal MEKC mode (Fig. 4A)[23]. On the

other hand, although anionic micelles would migrate from the BGS region to the sample solution region driven by electroosmotic flow (EOF), the SDS concentration of the sample solution in the stacking–MEKC mode was relatively lower than the SDS concentration in the normal MEKC mode due to the short time of reversed electrode polarity stacking. As discussed in section 3.1.1, the migration time of the analytes would decrease with the decrease of SDS concentration. Therefore, the peaks in Fig. 4A were eluted slower than the corresponding peaks in Fig. 4B and 4C.

The enrichment factor was calculated as a ratio of peak area obtained using the method with preconcentration step to those obtained by normal injection. As shown in Table 2, after combined with off-line and on-line preconcentration procedures, the proposed method could produce a 90.0- to 167.3-fold enrichment of the five pesticides in comparison to the non-preconcentration MEKC. The results also demonstrated that the DLLME-stacking-MEKC method greatly improved the sensitivity compared with stacking–MEKC without DLLME procedure.

3.5 Real samples analysis

In the present study, the proposed QuEChERS-DLLME-stacking-MEKC method was applied to determine the five organophosphorus pesticides in *Astragalus membranaceus*. In order to evaluate the applicability of the proposed method, the spiked two known concentrations of pesticide (0.05 and 0.2 μg mL^{-1}; except paraoxon-methyl at 0.1 and 0.4 μg mL^{-1}) mixture in sample were used in the present study. As shown in Table 3, the sample was contaminated by paraoxon at the concentration of 187 ng mL^{-1} (1.87 mg kg^{-1}), which was higher than the MRLs

standard, i.e., 0.5 mg kg^{-1} set by European Commission. However, the MRL of this pesticide has not been regulated by CFDA (China Food and Drug Administration). The proposed method showed satisfactory recoveries for most of the analytes (in the range 78.75 to 118.15%), which indicated that this method was suitable for the trace analysis of these pesticides in complex real samples like herbal medicine.

4. Concluding remarks

In this experiment, a QuEChERS-DLLME-stacking-MEKC method has been developed to determine five organophosphorus pesticides and characterized by good resolution, great repeatability, and satisfactory recovery. The proposed method has been validated for the clean-up and preconcentration effects in herbal medicine *Astragalus membranaceus*. Due to the combination with off-line and on-line preconcentration procedures, the enrichment factor of this method was up to 167.3-fold compared with the normal MEKC method. Therefore, the current QuEChERS-DLLME-stacking-MEKC method offered a substantial improvement in the detection of the trace organophosphorus pesticides in some real samples with complex matrices.

Conflict of interest

The authors have no competing financial interests to declare.

Acknowledgements

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Figure Legends

Fig. 1. The chemical structures of the studied pesticides.

Fig. 2. Effects of different extraction solvents on the recovery of the studied pesticides in DLLME. Extraction conditions: extraction solvent volume, 200 μL; dispersive solvent, 1.0 mL acetonitrile; salt solution, 5 mL 4% NaCl solution.

Fig. 3. Effects of salt addition on the recovery of the studied pesticides in DLLME. Extraction conditions: salt solution volume, 5 mL; extraction solvent, 300 μL $CHCl_3$; dispersive solvent, 1.5 mL acetonitrile.

Fig. 4. Electropherograms of the standard mixture at 2.0 μg mL^{-1} (except paraoxon-methyl at 4.0 μg mL^{-1}) in 5 mM borate solution obtained by (A) normal MEKC method (sample injection at 0.5 psi for 5 s; separation voltage at +20 kV), (B) stacking–MEKC method (sample injection at 0.5 psi for 90 s; reversed voltage at -20 kV for 0.4 min; separation voltage at +20 kV) and (C) DLLME-stacking-MEKC method (after DLLME procedure, sample injection is the same as described in B).
Peak assignment: 1. Dimethoate; 2. Phosphamidon; 3. Paraoxon-methyl; 4. Paraoxon; 5. Fensulfothion. The BGS consisted of 10 mM borate buffer containing 40 mM SDS and 20% methanol at pH 9.3. DAD monitoring wavelength: 200 nm.

Figure Legends

Fig. 1. The chemical structures of the studied pesticides.

Fig. 2. Effects of different extraction solvents on the recovery of the studied pesticides in DLLME. Extraction conditions: extraction solvent volume, 200 μL; dispersive solvent, 1.0 mL acetonitrile; salt solution, 5 mL 4% NaCl solution.

Fig. 3. Effects of salt addition on the recovery of the studied pesticides in DLLME. Extraction conditions: salt solution volume, 5 mL; extraction solvent, 300 μL $CHCl_3$; dispersive solvent, 1.5 mL acetonitrile.

Fig. 4. Electropherograms of the standard mixture at 2.0 μg mL^{-1} (except paraoxon-methyl at 4.0 μg mL^{-1}) in 5 mM borate solution obtained by (A) normal MEKC method (sample injection at 0.5 psi for 5 s; separation voltage at +20 kV), (B) stacking–MEKC method (sample injection at 0.5 psi for 90 s; reversed voltage at -20 kV for 0.4 min; separation voltage at +20 kV) and (C) DLLME-stacking-MEKC method (after DLLME procedure, sample injection is the same as described in B).
Peak assignment: 1. Dimethoate; 2. Phosphamidon; 3. Paraoxon-methyl; 4. Paraoxon; 5. Fensulfothion. The BGS consisted of 10 mM borate buffer containing 40 mM SDS and 20% methanol at pH 9.3. DAD monitoring wavelength: 200 nm.

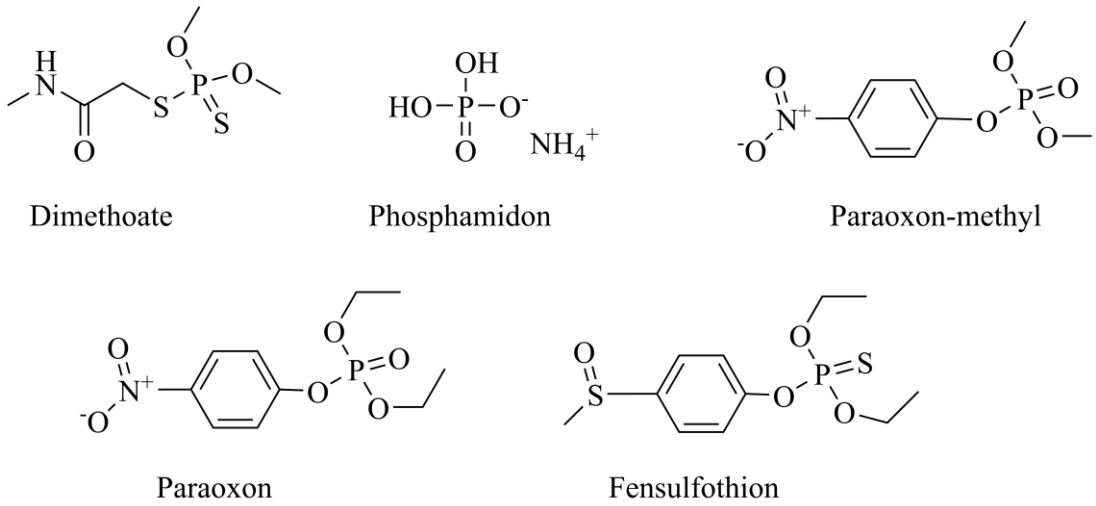

Fig. 1

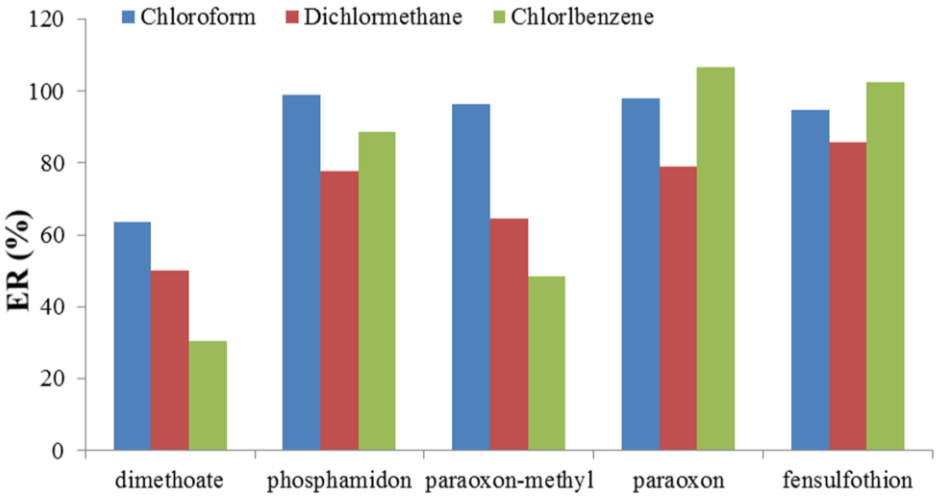

Fig. 2

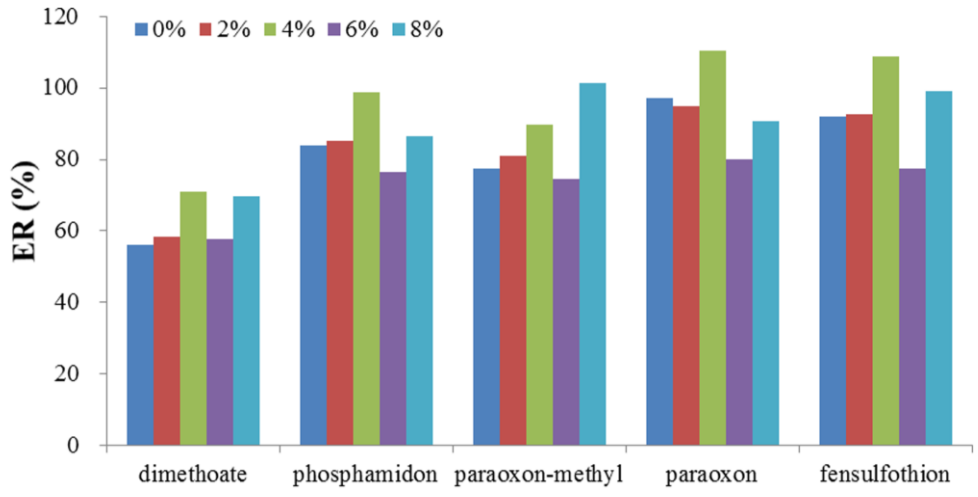

Fig. 3

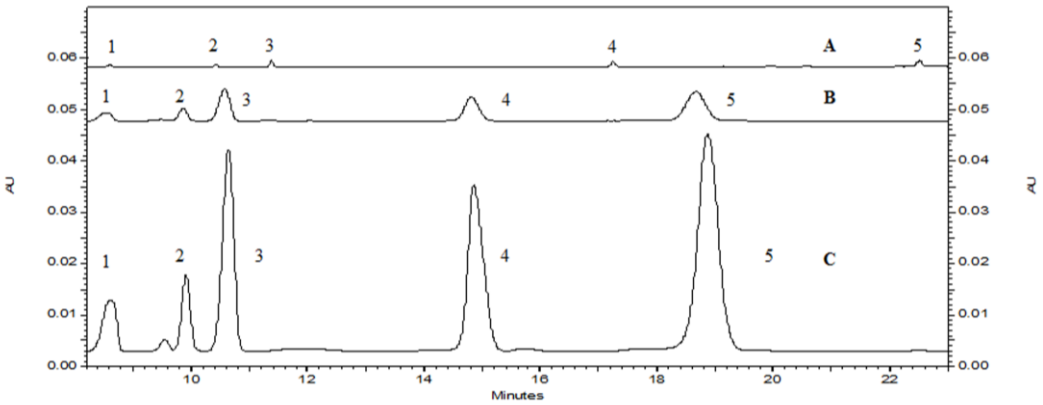

Fig. 4

Table 1 Analytical performance of the QuEChERS-DLLME-stacking-MEKC method.

OPPs	Linearity ($\mu g\ mL^{-1}$)	r^2	LOD ($\mu g\ mL^{-1}$)	Intra-day (n=5), %RSD		Inter-day (n=5×3 day), %RSD	
				t_R, min	Peak area	t_R, min	Peak area
dimethoate	0.05-2	0.9956	0.018	0.20	5.15	0.80	4.23
phosphamidon	0.05-2	0.9980	0.012	0.12	1.66	0.55	5.02
paraoxon-methyl	0.1-4	0.9999	0.016	0.10	1.12	0.63	2.21
paraoxon	0.05-2	0.9994	0.010	0.06	2.41	0.73	3.25
fensulfothion	0.05-2	0.9967	0.010	0.07	4.10	1.03	3.34

Table 2 Enrichment factors (EF) obtained by on-line and off-line preconcentration method.

	dimethoate	phosphamidon	paraoxon-methyl	paraoxon	fensulfothion
t_R, min	9.37	11.20	12.15	18.02	23.29
EF of stacking-MEKC	13.9	16.5	19.3	19.3	21.6
EF of DLLME-stacking-MEKC	90.0	110.9	122.7	144.4	167.3

Table 3 Recoveries obtained from the determination of OPPs in spiked *Astragalus membranaceus* sample.

Pesticide	Spiked ($\mu g\ mL^{-1}$)	Measured ($\mu g\ mL^{-1}$)	Recovery (%)
dimethoate	0	ND[a]	
	0.05	0.048	95.46
	0.2	0.224	112.10
phosphamidon	0	ND	
	0.05	0.039	78.75
	0.2	0.190	95.12
paraoxon-methyl	0	ND	
	0.1	0.092	91.67
	0.4	0.398	99.57
paraoxon	0	0.187	
	0.05	0.228	81.30
	0.2	0.388	100.35
fensulfothion	0	ND	
	0.05	0.058	115.48
	0.2	0.236	118.15

[a] ND: not detected.