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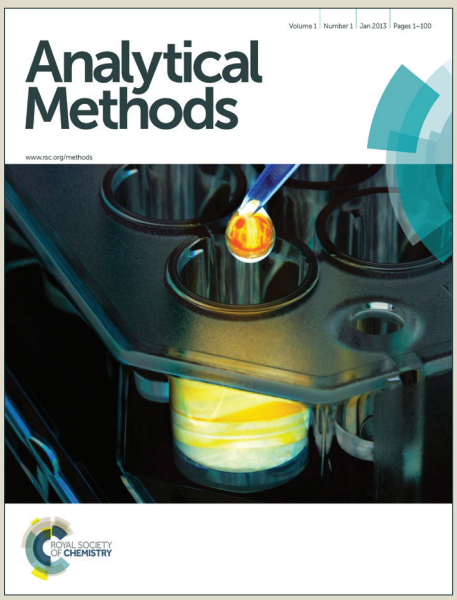

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A novel method based on combining ultrasonic-assisted dispersive solid-phase extraction and low-density solvent dispersive liquid-liquid microextraction (UA-DSPE-LDS-DLLME) for the determination of organophosphorus pesticides in soil

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Abstract:

A new method has been developed to determine trace levels of organophosphorus pesticides (OPPs) in soil samples by using dispersive solid-phase extraction (DSPE) combined with dispersive liquid-liquid microextraction (DLLME), followed by gas chromatography pulsed-flame photometric detection (GC-PFPD) analysis. In this approach, the OPPs were first extracted from the soil sample into acetone, and a DSPE purification step was performed using PSA and GCB as the sorbent material. Next, 1 mL of cleaned acetone (dispersive solvent-containing analytes) and 100 μL of 2-ethyl hexanol (extraction solvent) were rapidly injected into a 5-mL soft polyethylene Pasteur pipette containing 5 mL of doubly distilled water (aqueous phase), thus ending the pre-concentration step of the DLLME procedure. The upper solvent was collected and analysed by GC-PFPD after centrifugation. The advantage of the procedure was the use of a less toxic, low-density solvent and the use of a pipette as the extraction device. This method broadens the applicability of DLLME to a wider range of solvents. The ultrasound technique was applied to accelerate the emulsification and increase the extraction efficiency. Furthermore, the technique combining DSPE with DLLME not only pre-concentrates the analytes from environmental matrices, but it also reduces the matrix effects. Additionally, the critical parameters affecting the extraction efficiency were systematically evaluated. Under optimum conditions, the proposed method performed with good linearity in the range of 5 to 200 ng g^{-1} with a correlation coefficient between 0.9910 and 0.9967. The enrichment factors (EF) varied from 22- to 35-fold. The limit of detection (LOD) (S/N=3) and the limit of quantification (LOQ) (S/N=10) were 0.2-0.5 ng g^{-1} and 0.5-1.2 ng g^{-1}, respectively. The relative recoveries at the two spiking levels of 10.0 and 50.0 ng g^{-1} were in the range of 79.6% to 106.8%, and the relative standard

deviations (RSDs) were less than 8.0% (n = 5). The proposed method provides a sensitive, convenient, and eco-friendly process for determining OPPs in soil samples.

Key words:

Organophosphorus pesticides, Low-density organic solvent, Dispersive solid-phase extraction, Dispersive liquid-liquid microextraction, Gas chromatography-pulsed flame photometric detection, Soil analysis

Introduction

Organophosphorus pesticides (OPPs) are widely used in agriculture for pest control and to obtain high yields. Although most of them are easily degradable and less toxic to organisms than organochlorine pesticides (OCPs), OPPs are an important source of environmental contamination because they can move and be absorbed by the soil, sediment and water. Finally, pesticides and their derivatives accumulate in the human body and cause several conditions, such as acute anaemia, nervous-system disorders and teratogenic effects.[1] Therefore, it is necessary to develop fast, sensitive and selective analytical methods to regularly monitor their residues in the environment.

Sample preparation is the key step in an analytical procedure. As a novel, miniaturised sample pre-treatment technique, dispersive liquid-liquid micro-extraction (DLLME) was first introduced by Assadi and co-workers in 2006.[2] It is based on a ternary-component solvent system consist of extraction solvent, dispersing solvent and aqueous phase. The conventional process of DLLME is as follows: a few microliters of an organic extraction solvent and a small volume of a dispersing solvent are mixed together and injected rapidly into an aqueous sample; then a cloudy solution forms, and extraction equilibrium is quickly achieved. The analytes present in the aqueous phase are rapidly extracted into the extraction solvent. After centrifugation, the extract can be sedimented at the bottom of the vial and subsequently analysed using the appropriate instrumental method.[3,4] To this point, DLLME has been widely applied to the determination of organic and inorganic substances due to its advantages of simplicity, rapidity, low cost and a high enrichment factor.[3-5]

However, like other analytical methods, DLLME also has its drawbacks. The typically used extraction solvents in DLLME are high-density organic solvents, such

as chlorobenzene, chloroform and carbon tetrachloride, which are halogenated hydrocarbons and are highly toxic and environmentally unfriendly. This type of solvent also has limitations.[3,6] Later, as a solution to this issue, DLLME based on the solidification of a floating organic drop (DLLME-SFO) was developed by using an extraction solvent lighter than water. The procedure of DLLME-SFO is the same as that of conventional DLLME except for the last step: after centrifugation, the floating organic phase at the top of the aqueous phase is put in an ice bath for cooling to ensure its solidification.[7] Solvents that are commonly used in this method are: 1-dodecanol, 1-undecanol and hexadecane, which have low volatility, dissolve in water and have melting points near room temperature.[6] DLLME-SFO has been applied to identify several organic compounds, including polycyclic aromatic hydrocarbons (PAHs),[8] polychlorinated biphenyls (PCBs) [9] and OCPs.[10] To expand the applicability of DLLME, the technique of low-density solvent-based solvent emulsification DLLME (LDS-SD-DLLME) was recently introduced. As the typical extraction solvents for this method, *n*-hexane, toluene and isooctane were collected by the use of special extraction devices.[11-13] Each of these devices works on a similar principle: after the extraction procedure, the extraction solvent of low density accumulates at the top of the aqueous phase and is then elevated to the narrow part of the device, followed by withdrawal for subsequent analysis.[6] These improved DLLME methods use halogen-free extraction solvents of low toxicity and retain the advantages of conventional DLLME.

Another disadvantage of DLLME is that its anti-interference ability is not satisfactory. This is the main reason that the majority of the research on DLLME has been focused on simple, aqueous samples.[3,4] Therefore, it is necessary to extend the applicability of DLLME to sample preparation for complex matrices. The improved

methods of DLLME combined with other techniques have been reported to pre-treat the samples matrices. Supercritical-fluid extraction followed by dispersive liquid-liquid microextraction (SFE-DLLME) has been developed for the extraction and determination of polycyclic aromatic hydrocarbons (PAHs) [14] and OPPs in soil and marine sediment samples.[15] Solid-phase extraction combined with dispersive liquid-liquid microextraction (SPE-DLLME) was established to determine polybrominated diphenyl ethers (PBDEs) in water and plant samples.[16] Additionally, much of the research has focused on the QuEChERS-DLLME technique and successfully applied it to analyse organic compounds in fruits and vegetables,[17,18] herbs,[19] nuts and seeds.[20] Only a few articles report an approach using dispersive solid-phase extraction combined with dispersive liquid-liquid microextraction (DSPE-DLLME) to analyse the pesticides in soil, grains and the polybrominated diphenyl ethers (PBDEs) in sediment.[21-23]

DSPE is used as an efficient purification technique for the removal of interfering substances from the matrix after solvent extraction. The choice of an appropriate adsorbent is a critical factor to obtain high recoveries in the DSPE procedure. Numerous sorbents, such as primary-secondary amine (PSA), octadecyl-bonded silica (C_{18}), graphitised carbon black (GCB) and even multi-walled carbon nanotubes (MWCNTs), [24,25] have been applied for the removal of various natural pigments, organic acids and sugars from the matrix. The main advantages of this method include low operating expenses, lowered solvent consumption and high recovery.

The aim of this study was to combine ultrasonic-assisted dispersive solid-phase extraction with low-density solvent, dispersive liquid-liquid microextraction (UA-DSPE-LDS-DLLME) in sample preparation, followed by gas chromatography equipped with a pulsed-flame photometric detector to quantitatively analyse. The

developed method will increase the selectivity of DLLME and extend its application to more complex matrix samples. To the best of our knowledge, this is the first time that this technique has been used in the determination of OPPs in the soil. Various parameters affecting the extraction and enrichment efficiency were evaluated and optimised. A special device mentioned by Guo and Lee[26,27] was used to accumulate the upper-layer solvent, which is a commercially available polyethylene plastic Pasteur pipette and has the advantage of being simple, cheap, flexible and disposable. The pipette makes it easy to retrieve the low-density extraction solvent in tiny volume. The established methods not only can pre-concentrate the analytes from environmental matrices but can also reduce the matrix effects.

Experimental

Reagents and solutions

Six pesticide standards (diazinon, disulfoton, chlorpyrifos, fenthion, parathion and quinalphos) were obtained from the Agro-Environmental Protection Institute, Ministry of Agriculture (China). Each standard was diluted in acetone to prepare a mixed standard solution of 100 mg L^{-1}. A fresh standard solution of OPPs (1.00 mg L^{-1}) was prepared in acetone and stored at 4°C.

The solvents 2-ethyl hexanol, cyclohexane, *n*-hexane, toluene, acetonitrile, acetone and methanol were commercially available in analytical reagent grade and were obtained from the Beijing Chemical Factory (Beijing, China). Primary-secondary amine (PSA, 40-60 μm), C_{18} (50 μm, 60 Å) and graphitised carbon black (PestiCarb, 120-400 mesh) were purchased from Agela Technologies Company (Tianjin, China), Doubly distilled water was filtered with a 0.45 μm membrane filter before use.

Instrumentation

A gas chromatograph (CP-3800, Varian, U.S.) equipped with a split/splitless injector system and a pulsed-flame photometric detector was used for analysis. Pesticide separation was conducted with an Rtx-1701 capillary column (30 m × 0.25 mm i.d., 0.25 m film thickness, Restek, U.S.). A vortex agitator (Jiangsu, China) was used for vortex. An ultrasonic cleaner with temperature control (Shanghai, China) was used for ultrasonic extraction. A centrifuge (Shanghai, China) was used for the complete phase separation and the polyethylene Pasteur pipette (5 mL) was used in the DLLME procedure.

Sample preparation

The soil samples were collected from the farmland of the Chenggong District (Kunming, China) and dried at room temperature. All of the samples were ground and sifted with a 200-mesh sieve.

UA-DSPE-LDS-DLLME procedure

Crude extraction: A 5.00-g soil sample was weighed into a 50-mL Teflon centrifuge tube. Next, 10 mL of acetone was added into the tube and vigorously vortexed for 1 min, followed by sonication (40 kHz) for 15 min and centrifugation for 5 min at 4000 rpm; afterward, the supernatants were collected. The process was repeated, after which the extract (twice-extracted) was transferred into a pear-shaped flask and concentrated by a rotary evaporator at 40°C until near dryness; it was then diluted with 2 mL acetone.

DSPE procedure: Two-mL portions of the acetone solution were transferred to 10-mL

screw-cap glass tubes with a conical bottom, containing 120 mg PSA and 25 mg graphitised carbon black. The solution was vortex for 2 min and centrifuged at 4000 rpm for 5 min.

DLLME procedure: A mixture of 1 mL of purified acetone extract and 100 µL of 2-ethyl hexanol was injected rapidly to a 5-mL of soft polyethylene Pasteur pipette containing 5 mL of doubly distilled water. A fine, cloudy solution resulted, and the analytes were extracted into 2-ethyl hexanol. This was followed by sonication (40 kHz) for 5 min. After centrifugation for 5 min at 4000 rpm, the upper layer, comprising the pesticides, moved into the narrow stem of the pipette by squeezing slightly. Finally, it was withdrawn by microsyringe, and 1 µL of the extract was directly injected for GC-PFPD analysis.

The procedure was show in Fig. 1.

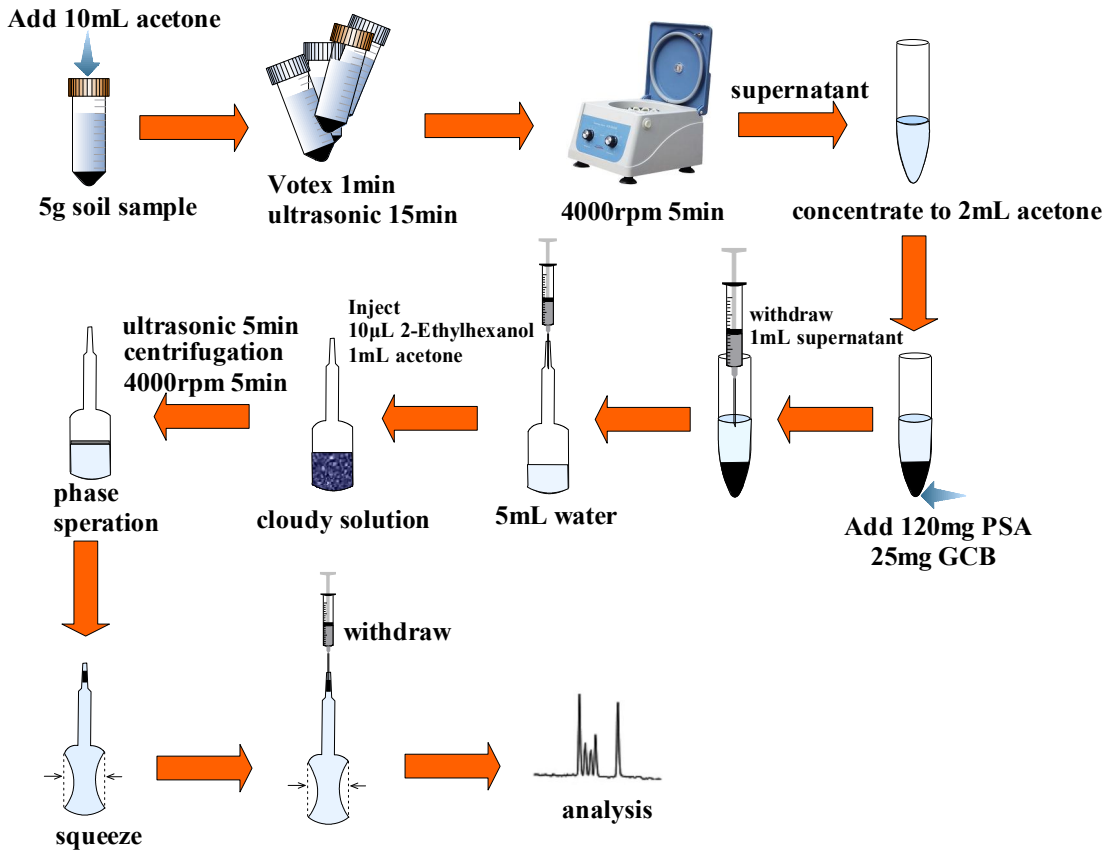


Figure 1. The procedure of UA-DSPE-LDS-DLLME

GC conditions

The operating conditions for the gas chromatograph were as follows: the injection port temperature was 250°C, and 1.0 μL of the sample was injected in the splitless mode. The detector temperature was held at 300°C. High-purity nitrogen (99.999%) was used as the carrier gas with a flow rate of 1 mL min^{-1}, hydrogen and zero air (99.999%) as an oxidant gas for PFPD. All the gas was obtained from Kunming Messer Gas Products Co., LTD, (Kunming, China).

The temperature program of the Rtx-1701 capillary column commenced at 120°C and was then raised by 30°C min^{-1} to 180°C and was held at that temperature for 0 min, after which it was raised by 10°C min^{-1} to 220°C and held for 2 min. Finally, the temperature was increased by 10°C min^{-1} to 270°C and kept at this temperature for 2 min. The total time required for one GC run was 15 min.

Results and discussion

A 5.00-g sample of soil free of pesticides and spiked with 50 ng of each OPP was used to study the extraction performance under various conditions. All of the experiments were performed in triplicate. The recovery was the parameter used to evaluate the influence of these variables on the extraction efficiency.

Optimisation of the crude-extraction condition

Effect of the extraction solvent type

There are two principles for the selection of the extracting solvent for soil samples: (1) it must have the proper polarity to match the analytes and be able to extract the

pesticides from the soil samples efficiently, and (2) it can be a dispersing solvent in the DLLME that is miscible both in the extraction solvent and water. Based on the criteria above, methanol, acetone and acetonitrile were studied in our experiments. For methanol, the recoveries of the six pesticides are lower than for the other two solvents. Acetonitrile extraction showed only 64.2% and 50.8% recoveries for disulfoton and diazinon, respectively. The recoveries of acetone extraction are in the range of 85.2-95.4%. As a result, acetone was selected as the extraction solvent in soil samples and the dispersive solvent in the DLLME step.

Effect of the extraction time

Sonication will accelerate the transfer of analytes from the sample to the extraction solvent, and so extraction equilibrium can be reached quickly. In this study, sonication times of 10 min, 15 min, 20 min, 25 min and 30 min were investigated. Considering that most of the pesticides exhibit satisfactory recovery at 15 min (75.6%-92.8%), 15 min was chosen as the sonication time for the crude extraction.

Optimisation of DSPE

As a purification technique, DSPE may reduce the matrix interferences of soil samples before pre-concentration. The efficiency of DSPE depends on the type and quantity of the sorbent. Several materials commonly used, including C_{18}, PSA and GCB, are evaluated in this experiment. C_{18} is known for its strong ability to reserve water-soluble alkaline compounds and remove protein interferences. However, in the assay, the recovery changed little by adding C_{18} to a concentrated acetone solution in the range of 50 to 200 mg. GCB usually exhibits a strong retaining activity for pigments and steroids. In our experiment, 25 mg of GCB shows an excellent effect on pigment movement, indicating that GCB will absorb the pesticides and influence the

recovery. PSA is used to remove sugars, fatty acids and other co-extractive interferences based on hydrogen-bond formation.[28] Due to its ability to increase the recovery of diazinon, disulfoton and chlorpyrifos, 120 mg of PSA was selected. Finally, a mixture of 120 mg of PSA and 25 mg of GCB was selected as the final sorbent composition for DSPE.

Optimisation of DLLME

In this experiment, DLLME was applied as a pre-concentration technique due to the low concentration of pesticides in the soil samples after the DSPE purification step. To optimise the critical variables of the DLLME procedure, 1 mL of acetone spiked with 50 ng mL^{-1} of each of the six pesticides was used. The parameters include the type and the volume of the extraction solvent, the volume of doubly distilled water, ultrasonic time, the pH value and the salt addition.

Effect of the type and the volume of the extraction solvent

Selection of the extraction solvent is a key step affecting DLLME efficiency. The extraction solvent must have specific properties, such as a high extraction capability for the analytes, low solubility in water and high solubility in the dispersive solvent, and it must be compatible with the analytical instrumentation. Considering these requirements, 2-ethyl hexanol, cyclohexane, n-hexane and toluene were tested in this study. The density of the selected organic solvents at 20°C are 0.833 g mL^{-1} (2-ethyl hexanol), 0.865 g mL^{-1} (toluene), 0.660 mg mL^{-1} (*n*-hexane), and 0.779 mg mL^{-1} (cyclohexane). They are all solvents with densities lower than that of water.

As seen in Fig. 2, better extraction ability for the OPPs was achieved by using both 2-ethyl hexanol and cyclohexane. However, phase separation is not obvious, and it is easy to form an emulsion when cyclohexane is used as the extraction solvent after

centrifugation. Therefore, 2-ethyl hexanol was selected as the optimum extraction solvent.

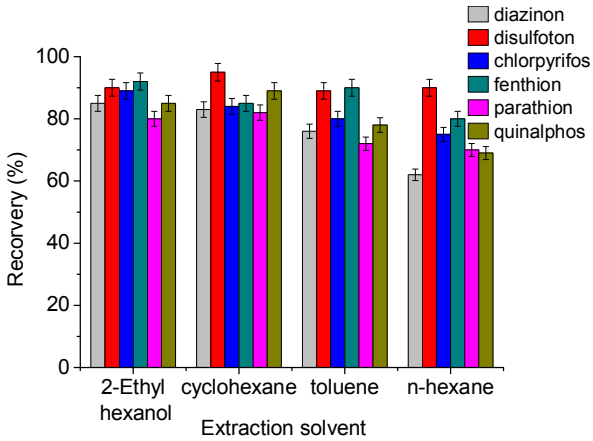


Figure 2. Effect of the extraction solvent type on the extraction efficiency of OPPs
Extraction conditions: doubly distilled water volume, 5.0 mL; dispersive solvent, 1.0 mL acetone; extraction solvent volume, 100 µL; sonication time, 5 min.

To study the effect of the volume of the extraction solvent, 2-ethyl hexanol was investigated within a volume range of 50-150 µL. For all of the target analytes, the result indicated (Fig. 3) that, when the volume of 2-ethyl hexanol was increased from 50-100 µL, the extraction recovery was increased and then remained almost constant between 100 and 150 µL. Thus, 100 µL 2-ethyl hexanol was selected as the volume of the extraction solvent.

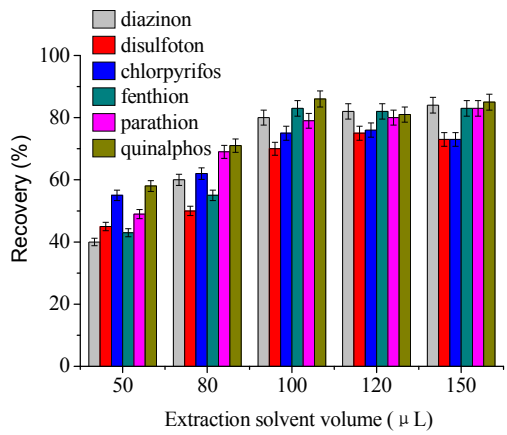


Figure 3. Effect of the extraction solvent volume on the extraction efficiency of OPPs
Extraction conditions: doubly distilled water volume, 5.0 mL; dispersive solvent, 1.0 mL acetone; extraction solvent, 2-ethyl hexanol; sonication time, 5 min.

Effect of the volume of the aqueous phase

DLLME is a ternary system containing an extraction solvent, a dispersive solvent and an aqueous phase. The aim of our experiment is the extraction and pre-concentration of pesticides from acetone (the dispersive solvent). It was important to optimise the volume of the aqueous phase (doubly distilled water) because it is the factor affect the formation of fine cloudy solution. Therefore, the volume of the water was investigated in the range of 3-7 mL. For this, 1 mL acetone including the target analytes was mixed with 100 µL 2-ethyl hexanol and injected into various volumes of water. The effect of this parameter on the extraction performance of DLLME is shown in Fig. 4. The results revealed that the recovery of OPPs was improved by increasing the double distilled water volume up to 5 mL. At higher volumes, the extraction efficiency decreased. This may be explained as the extraction solvent (2-ethyl hexanol) dissolving in the excess aqueous phase. Therefore, 5 mL is the proper volume of water under these experimental conditions.

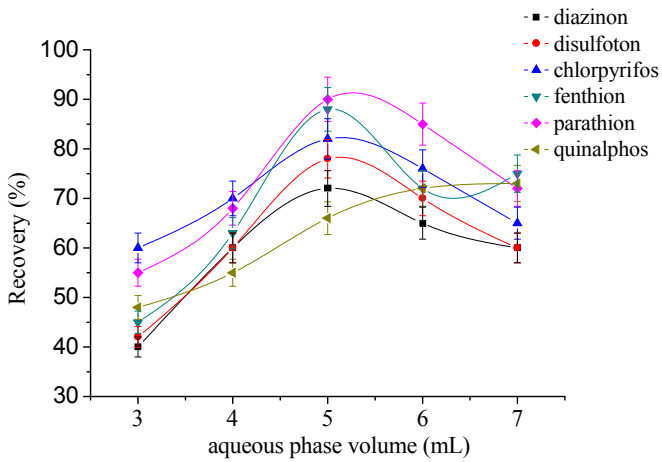


Figure 4. Effect of the aqueous phase volume on the extraction efficiency of OPPs
Extraction conditions: dispersive solvent, 1.0 mL acetone; extraction solvent, 100 µL 2-ethyl hexanol; sonication time, 5 min.

Effect of the sonication time

Ultrasonication was an auxiliary technique to improve the efficiency of DLLME via its effects on both the emulsification and the mass-transfer processes. A sonication time in the range of 0-10 min was adopted to evaluate the effect on the extraction ratio. Fig. 5 demonstrates that the recovery increased gradually in the first 6 min, but a prolonged extraction time did not contribute significantly to an increase in the extraction recovery. Thus, the ultrasonic extraction time of 6 min was selected.

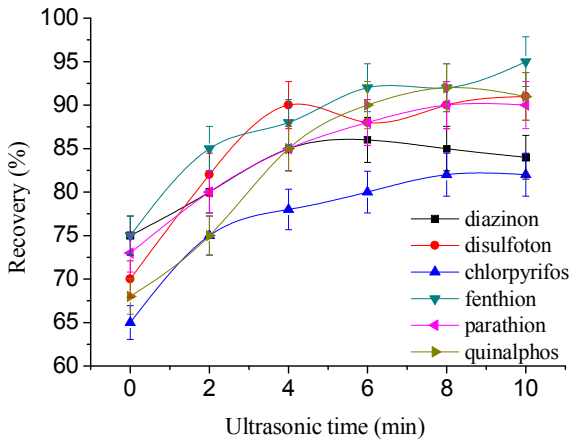


Figure 5. Effect of the sonication time on the extraction efficiency of OPPs
Extraction conditions: doubly distilled water volume, 5.0 mL; dispersive solvent, 1.0 mL acetone; extraction solvent, 100 μL 2-ethyl hexanol.

Effect of the pH and salt addition

A pH adjustment may change the analytes into their neutral forms that can be easily extracted into the hydrophobic phase. Most of the OPPs are less stable in alkaline solutions than in acid ones. Thus, in the present study, the pH was adjusted in the range of 2.0-6.0 using acetic acid (1 mol L^{-1}). The results showed that the pH value has no significant effect on the extraction performance for most of the analytes. A pH value that is too low may induce degradation of the pesticides. As a result, maintaining the initial pH level was suitable for the DLLME procedure.

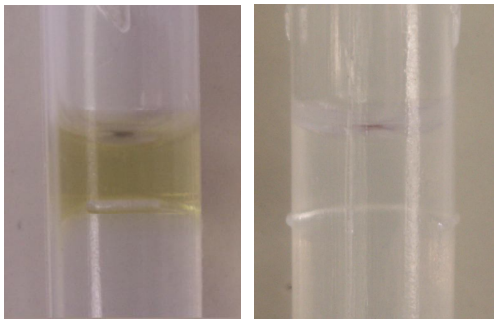
The ionic strength of the aqueous medium will affect the analyte transfer and the extraction efficiency. Salt was added to the samples to examine the influence of the

salting-out effect. Various amounts of sodium chloride (NaCl, 0-10%, w/v) were added to the aqueous phase in the experiment. The results indicated that there was no significant influence on the recoveries and EFs. Therefore, salt was not added in the proposed method.

All of the parameters discussed above can affect extraction efficiency, but selection of an appropriate extracting solvent is the major parameter for DLLME process. Extractive capability of extraction solvent for the interesting compounds is the main factor to decide the extraction ratio. The volume of extracting solvent has important effect on the enrichment factor (EF). Thus, the optimal extracting solvent volume should ensure both high EF and enough volume of the separated phase for the subsequent determination after centrifugation[3]. Dispersive solvent enabled the extraction solvent to be dispersed as fine particles in aqueous phase by enlarge the surface area between extraction solvent and aqueous phase. In this work, the volume of aqueous phase affects the formation of cloudy solution and upper phase volume when the dispersive solvent volume is fixed. So, aqueous phase volume is still important for extraction performance in our experiment. The value of pH and ionic strength is chosen so as to make the analyte less soluble in the water phase, but not all the target analyte would affect, they are not as important as other factors mentioned before for contribute to extraction efficiency.

Under the optimal conditions, we compare the difference between the UA-LSD-DLLME (Fig. 6 a) and the UA-DSPE-LDS-DLLME (Fig 6 b) procedures. Fig. 6 b shows that the extraction phase became clear after purification, and the pigment and interferences were removed from the acetone solution. The results indicated that the DSPE step is necessary to reduce the matrix interference in soil samples. The pigment and other impurities would contaminate the capillary column

and affect the analytical efficiency if no purification step was used.



a b

Figure 6. Photographs of the result of: (a) the UA-LDS-DLLME process and (b) the UA-DSPE-LDS-DLLME process.

Methods validation

Soil samples free of pesticide, spiked at six concentration levels (5, 10, 20, 50, 100 and 200 ng g^{-1}) of target OPPs, were used to prepare a series of matrix-matched calibration curves. The samples were measured with the optimised UA-DSPE-LDS-DLLME procedures established above. The validation parameters of the methods, including the linearity, precision, repeatability, enrichment factors were investigated and summarised in Table 1. The limits of detection (LOD) were determined by considering a value three times the background noise of the blank sample at the retention time of each pesticide (S/N = 3), whereas the limits of quantification (LOQ) (S/N = 10) were calculated by considering a value ten times that background noise. The linear range of the pesticide was from 5-200 ng g^{-1}, and the correlation coefficients (r) and the enrichment factors were 0.9910-0.9967 and 22-35, respectively. The LOD values obtained were in the range of 0.2-0.5 ng g^{-1}, and the LOQ values were from 0.5-1.2 ng g^{-1}. The relative standard deviations (RSD) are 6.3%-8.0%.

Table 1. Parameters of the UA-DSPE-LSD-DLLME-GC-PFPD method (n=3).

OPPs	LR (ng g^{-1})	*r*	LOD (ng g^{-1})	LOQ (ng g^{-1})	EF	RSD (%)
Diazinon	5-200	0.9967	0.2	0.6	35	7.2
Disulfoton	5-200	0.9910	0.2	0.5	26	7.9
Chlorpyrifos	5-150	0.9938	0.4	1.0	22	8.0
Fenthion	5-150	0.9943	0.5	1.2	25	6.5
Parathion	5-200	0.9955	0.5	1.2	30	6.3
Quinalphos	5-200	0.9921	0.5	1.0	30	6.8

LR: Linear range
r: Correlation coefficient
LOD: Limit of detection
LOQ: Limit of quantification
EF: Enrichment factors
RSD: Relative standard deviations

Application to real sample analysis

To evaluate the performance of the presented method, ten portions of the soil samples were analyzed under the optimum conditions. The results revealed that they were free of OPP contamination. It may explain that the concentration of the residue was below the LOD of the proposed method, the tested soil sample have indeed low level of OPPs and lost in the sample preparation procedure due to matrix interference. The evaluation of the recoveries was performed by adding OPP standard solutions at levels of 10 and 50 ng g^{-1} into the blank soil samples. As can be seen in Table 2, the recoveries were within the acceptable range of 79.6-106.8%, and the RSD varied from 4.8% to 7.8%. The typical chromatograms of the spiked sample are shown in Fig. 7. The results prove that the method established has a high sensitivity and repeatability. It can be used for the trace analysis of OPP residues in soil samples.

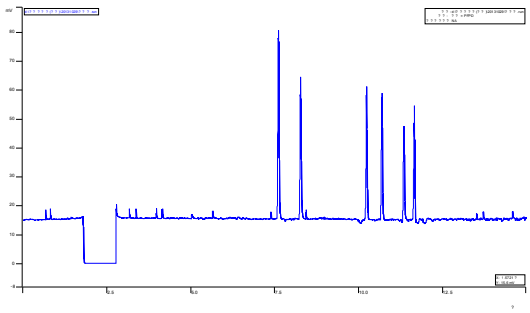


Figure.7. The chromatogram of a soil sample using the UA-DSPE-LDS-DLLME procedures adding a standard solution at the concentration of 50 ng mL^{-1}. The order is as follows: diazinon (t_R=7.76), disulfoton (t_R=8.40), chlorpyrifos (t_R=10.37), fenthion (t_R=10.84), parathion (t_R=11.49), and quinalphos (t_R=11.79)

Table 2. Average recovery and RSD for OPP-spiked soil sample (n=5).

OPPs	Spiked(ng g^{-1})	Found(ng g^{-1})	Recovery (%)	RSD (%)
	0	ND		
Diazinon	10	10.21	102.1	6.9
	50	44.95	89.9	7.2
	0	ND		
Disulfoton	10	10.68	106.8	5.3
	50	51.70	103.4	4.8
	0	ND		
Chlorpyrifos	10	9.08	90.8	7.8
	50	45.60	91.2	6.8
	0	ND		
Fenthion	10	7.96	79.6	6.5
	50	42.50	85.0	7.3
	0	ND		
Parathion	10	9.15	91.5	5.1
	50	46.15	92.3	5.4
	0	ND		
Quinalphos	10	8.36	83.6	6.4
	50	43.70	87.4	5.7

ND: not detected;

Comparison of the proposed method with other methods

A comparison of the proposed UA-DSPE-LDS-DLLME method with other reported techniques for the determination of OPPs in complex matrices is summarised in Table 3. The developed method has comparable LOD, RSD and recovery values to those of

other extraction methods. Furthermore, 2-ethyl hexanol was selected as the extraction solvent because it has the advantage of lower toxicity than the chlorinated solvents widely used in conventional DLLME. Additionally, its low cost, simple operation and high sensitivity are advantages of the proposed method. It can be concluded that UA-DSPE-LDS-DLLME combined with GC-PFPD exhibits strong purification and pre-concentration abilities, and it may be an alternative method to analyse OPPs in soil samples.

Table 3. Comparison of proposed UA-DSPE-DLLME-GC-PFPD method with other methods of determination of OPPs in various matrices

Methods	Matrix	Microextraction solvent	Linearity ($ng\ g^{-1}$) or ($ng\ mL^{-1}$)	LOD ($ng\ g^{-1}$)	RSD (%)	References
SDME-GC–FPD	water	toluene	0.50-50	0.21-0.56	1.7-10	29
DSPE-GC-MS	soybean oil	-	10-500	20-250	<20	30
DSPE-GC-MS	Peanuts oil	-	5-200	0.7-1.6	<8.25	31
DLLME-GC-FPD	Cucumber and watermelon	chlorobenzene	-	0.8-2.0	3-7	32
UASE-DLLME-GC-FPD	tomato	chlorobenzene	0.5-1000	0.1-0.5	<10	33
UASE-DLLME-SFO-HPLC-UV	Ribbed melon	1-undecanol	5-800	1-4	<9	34
UA-DSPE-LSD-DLLME-GC-PFPD	soil	2-ethyl hexanol	5-200	0.2-1.5	4.8-8.0	This work

Conclusions

In this work, a novel method of UA-DSPE-LDS-DLLME followed by GC-PFPD was proposed for the extraction and determination of OPPs in soil samples. A low-toxicity extraction solvent was used to reduce the risk for human health and the environment. An ultrasound-assisted process was applied to accelerate the formation of a fine cloudy solution and to increase the extraction efficiency. The method performance under optimal conditions was proven to show strong purification and

pre-concentration ability in soil samples. The DSPE procedure combined with the DLLME method increases the selectivity and sensitivity of the method and makes it possible to determine the trace analytes in complex-matrix samples. Compared with other conventional methods, the new method employs simple and inexpensive equipment and shows the advantages of low limits of detection, good repeatability and high recovery.

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