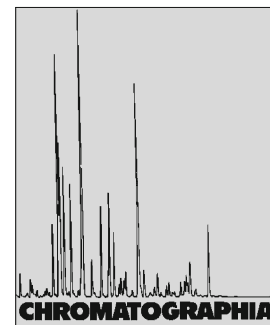


Determination of Two Pesticides in Soils by Dispersive Liquid-Liquid Microextraction Combined with LC-Fluorescence Detection



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Abstract

In the present work, a simple, rapid and sensitive sample pre-treatment technique, dispersive liquid-liquid microextraction (DLLME) coupled with liquid chromatography-fluorescence detection (LC-FLD), has been developed to determine carbamate (carbaryl) and organophosphorus (triazophos) pesticide residues in soil samples. Methanol was first used as extraction solvent for the extraction of pesticides from the soil samples and then as dispersive solvent in the DLLME procedure. Under the optimum extraction conditions, the linearity was obtained in the concentration range of 0.1–1,000 ng g⁻¹ for carbaryl and 1–5,000 ng g⁻¹ for triazophos, respectively. Correlation coefficients varied from 0.9997 to 0.9999. The limits of detection (LODs), based on signal-to-noise ratio (S/N) of 3, ranged from 14 to 110 pg g⁻¹. The relative standard deviation (RSDs, for 20.0 ng g⁻¹ of each pesticide) varied from 1.96 to 4.24% (*n* = 6). The relative recoveries of two pesticides from soil A1, A2 and A3 at spiking levels of 10.0, 20.0 and 50.0 ng g⁻¹ were in the range of 88.2–108.8%, 80.8–110.7% and 81.0–111.1%, respectively. The results demonstrated that DLLME was a sensitive and accurate method to determine the target pesticides, at trace levels, in soils.

Keywords

Dispersive liquid-liquid microextraction
Liquid chromatography-fluorescence detection
Organophosphate and carbamate in soil

Introduction

Carbamate and organophosphate pesticides, as substitute of the organochlorine compounds, are widely used in China for

agricultural activities due to their lower environmental persistence and highly effective ability to control pests, weeds and diseases. However, since they are inhibitor of acetyl-cholinesterase, many of these

compounds display high acute toxicity [1, 2] and are suspected for mutagenic [3], carcinogenic and endocrine disruptor effects [4]. Thus, the increasing use of these pesticides constitutes a great threat to the environment and human health. There is an increasing demand for developing a simple and sensitive method for the determination of such contaminations, at trace levels, in environmental matrices, especially in soils.

Sample preparation plays an important role in the field of pesticide residue analysis. Traditionally, Soxhlet extraction and ultrasound-assisted extraction are the conventional pretreatment methods to extract pesticides from soil and sediment samples [5–7]. However, these methods usually are laborious, time-consuming and need large volumes of toxic organic solvents. In the latest decade, the more efficient and environmentally friendlier techniques such as supercritical fluid extraction (SFE) [8], microwave-assisted extraction (MAE) [9] and accelerated solvent extraction (ASE) [9] have been developed to extract pesticides from soil samples. The main advantages of these techniques are low consumption of organic solvents, short extraction time and high recovery [11]. Nevertheless, the instruments required for these techniques are relatively expensive and cannot be accessed in

many ordinary laboratories. In the past few years, simple and miniaturized sample preparation techniques such as solid phase microextraction (SPME) [12, 13], stir bar sorptive extraction (SBSE) [14, 15] and liquid-phase microextraction (LPME) [16,18] have been reported as alternatives to conventional sample preparation procedures. However, these microextraction methods are primarily focused on liquid samples such as water and fruit juices.

Recently, a novel microextraction technique termed as dispersive liquid–liquid microextraction (DLLME) has been developed by Assadi and coworkers [19]. It is based on a ternary component solvent system like homogeneous liquid–liquid extraction (HLLE) and cloud point extraction (CPE). Since its introduction, this technique has been mostly applied to analyze trace organic pollutants and metal ions in liquid samples [19–25]. To the best of our knowledge, only a few papers reported these microextraction methods to be used in solid samples such as soils, vegetables and tea leaves [26–28]. So far, none of the literature reported DLLME combined with LC-FLD to simultaneously determine carbamate and organophosphorus pesticides in soil samples. The aim of this study is to assess DLLME suitability for the determination of two pesticides in soil samples.

Experimental

Standards and Reagents

Carbaryl (purity 99.5%) and triazophos (purity 99.0%) were purchased from Agro-Environmental Protection Institute of Agricultural Ministry (Tianjin, China). The individual stock standard solution of 100 $\mu\text{g mL}^{-1}$ was prepared in LC-grade methanol. Working standard solution was prepared by appropriate dilution of the stock solution with deionized water. The stock and working standard solutions were stored at 4 °C until use.

Chloroform (CHCl_3), carbon tetrachloride (CCl_4), tetrachloroethane ($\text{C}_2\text{H}_2\text{Cl}_4$), chlorobenzene ($\text{C}_6\text{H}_5\text{Cl}$) and acetone were all analytical grade and redistilled four times to remove any trace impurities. Acetonitrile and methanol (LC-grade)

were obtained from Tedia (Fair lawn, NJ, USA). Sodium chloride was of the highest purity available from Merck (Darmstadt, Germany). Deionized water was purified with a Mill-Q purification System (Millipore, Bedford, MA, USA).

Sample Preparation

Soil samples A1, A2 and A3 were collected from the suburb of Wenzhou City, China. The soil was air-dried, pulverized, sieved to a grain size of 250 μm sequentially. The extraction procedures were as follows: accurately weighing 1.0 g soil sample into a 50 mL conical flask, and then 10 mL of methanol were added. The flask was vigorously shaken for 30 min at 250 rpm on a mechanical shaker. The supernate (methanol extract) was filtered through a 0.45- μm membrane filter (Scharlau, Barcelona, Spain) and transferred into a test tube, then an aliquot of 1.0 mL of methanol extract was subjected to the DLLME procedure.

Instrumentation

Chromatographic analysis was carried out on an Agilent 1200 LC system equipped with a manual injector and fluorescence detection (FLD). An Agilent offline ChemStation was used to process chromatographic data. A Zorbax Eclipse XDB-C₈ column (4.6 mm \times 150 mm, 5- μm particle size) from Agilent Company (Wilmington, DE, USA) was used for separation and thermostated at 25 ± 1 °C. The mobile phase was a mixture of methanol/water (70/30, v/v) with a flow rate of 0.8 mL min^{-1} . The excited and emitted wavelengths for fluorescence detection were set at 274 and 335 nm, respectively. All injections were performed manually with 20.0 μL sample loop. The temperature-controlling centrifuge (model 80-2, Changzhou, China) was used for centrifugation.

DLLME Procedures

5.0 mL deionized water was placed into a 15-mL screw-cap glass centrifuge tube with conic bottom. 1 mL of methanol extract (as dispersive solvent) containing 50.0 μL

tetrachloroethane (as extraction solvent) was rapidly transferred into the above-mentioned centrifuge tube. After gently shaking, the mixture was then centrifuged for 3 min at 3,500 rpm, causing the dispersed fine droplets of the extraction phase sedimented at the bottom of the conical test tube. The volume of the sedimented phase was determined using a 50.0- μL microsyringe (zero dead volume, Shanghai, China), and was completely transferred to another 100 μL centrifuge tube. After evaporation of the solvent ($\text{C}_2\text{H}_2\text{Cl}_4$) under a gentle nitrogen flow, the residue was redissolved in 25 μL LC-grade methanol and injected into LC for analysis.

Results and Discussion

Selection of Organic Solvent for the Extraction of Pesticides from Soils

In this study, the extraction solvent from soil was further used as the dispersive solvent in the DLLME step. The solvent is selected on the basis of its miscibility in the organic phase (extraction solvent) and aqueous phase (sample solution), and its high extraction capability from soil. Therefore, acetonitrile, acetone and methanol were chosen for this purpose. In order to achieve the constant volume of sedimented phase (25.0 μL), a series of experiments were conducted by using 1.0 mL acetonitrile extract, acetone extract and methanol extract containing 25.0, 30.0, and 50.0 μL of $\text{C}_2\text{H}_2\text{Cl}_4$ (as extraction solvent), respectively. The result showed that the variations of recoveries using methanol (99.1–108.0%), acetonitrile (94.7–96.9%) and acetone (91.2–109.0%) were not remarkable. Additionally, the peak of impurity of the methanol extract was much less in comparison with acetonitrile and acetone extract. Thus, methanol was selected as extraction solvent in soil samples and dispersive solvent in DLLME step.

Selection of Extraction Solvent in DLLME Process

The extraction solvent has to meet four requirements: (i) higher density than

water; (ii) good chromatographic behavior; (iii) high extraction capability of interested compounds and (iv) low water solubility. Therefore, CHCl_3 (density; 1.47 g mL^{-1}), CCl_4 (density; 1.59 g mL^{-1}), $\text{C}_2\text{H}_2\text{Cl}_4$ (density; 1.60 g mL^{-1}) and $\text{C}_6\text{H}_5\text{Cl}$ (density; 1.16 g mL^{-1}) were examined in this research. A series of sample solutions were studied by using 1.0 mL methanol containing different volumes of extraction solvent (50.0 μL $\text{C}_2\text{H}_2\text{Cl}_4$, 115.0 μL CHCl_3 , 40.0 μL CCl_4 and 33.0 μL $\text{C}_6\text{H}_5\text{Cl}$) to achieve 25.0 μL of settled phase. As shown in Fig. 1, $\text{C}_2\text{H}_2\text{Cl}_4$ gave the best extraction efficiency for carbaryl, CHCl_3 and $\text{C}_6\text{H}_5\text{Cl}$ were the most suitable extraction solvents for triazophos. But CCl_4 showed the poorest extraction efficiency for carbaryl and triazophos. Although $\text{C}_2\text{H}_2\text{Cl}_4$ was not the best extraction solvent for triazophos, its extraction efficiency was comparable with that of CHCl_3 and $\text{C}_6\text{H}_5\text{Cl}$. Therefore, $\text{C}_2\text{H}_2\text{Cl}_4$ was selected as the extraction solvent in the subsequent experiments.

Effect of Extraction Solvent Volume in DLLME

To evaluate the effect of extraction solvent volume on extraction efficiency, 1.0 mL of methanol, containing different volumes of $\text{C}_2\text{H}_2\text{Cl}_4$ (50–90 μL at 10 μL interval) was subjected to the same DLLME procedure. It was obvious that by increasing the volume of $\text{C}_2\text{H}_2\text{Cl}_4$ from 50.0 to 90.0 μL , the volume of the sedimented phase increased from 25.0 to 67.7 μL in concomitant with the sharply decreasing of the analyte signals. Thus, 50.0 μL $\text{C}_2\text{H}_2\text{Cl}_4$ were selected in the subsequent experiments.

Effect of Dispersive Solvent Volume in DLLME

To obtain the optimum volume of methanol, various experiments were performed by using different volumes of methanol (0.25, 0.50, 1.0, 1.5 and 2.0 mL) containing 38.0, 40.0, 50.0, 54.0 and 65.0 μL $\text{C}_2\text{H}_2\text{Cl}_4$, respectively. The

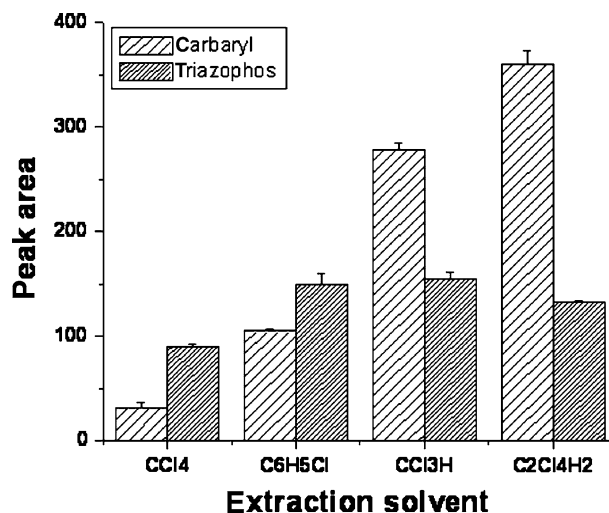


Fig. 1. Effects of different extraction solvents on extraction efficiency of carbaryl and triazophos. Extraction conditions: water sample volume, 5.00 mL; dispersive solvent (methanol) volume, 1.0 mL; extraction solvent volumes, 50.0 μL $\text{C}_2\text{H}_2\text{Cl}_4$, 115.0 μL CHCl_3 , 44.0 μL CCl_4 and 33.0 μL $\text{C}_6\text{H}_5\text{Cl}$; sedimented phase volume, $25.0 \pm 1.0 \mu\text{L}$; concentration of each pesticides, 4 ng mL^{-1} for carbaryl and triazophos

Table 1. Quantitative results of two pesticides by LC-FLD–DLLME from soil sample

Pesticides	RSDs(%) $n = 6$	LR ^a (ng g^{-1})	R^2	LOD ^b (ng g^{-1})
Carbaryl	4.2	0.1–1,000	0.9997	0.014
Triazophos	2.0	1–5,000	0.9999	0.11

result showed that the extraction efficiency increased at first by increasing the methanol volume up to 1.0 mL, and then decreased with further increase of the methanol volume. This possible explanation may be that the cloudy solution is not formed well at low methanol volumes, while the solubility of pesticides in water increases at high volumes, which both results in the decrease in extraction efficiency. Therefore, 1.0 mL methanol was chosen for the subsequent experiments.

Effect of Salt Concentration

The effect of salt addition on extraction efficiency was examined by adding a different amount of NaCl (0–10%, w/v) under other constant experimental conditions. By increasing the ionic strength from 0 to 10%, the volume of the sedimented phase increased from 25.0 to 32.5 μL due to the decrease in aqueous solubility of the extraction solvent in the presence of salt, which consequently

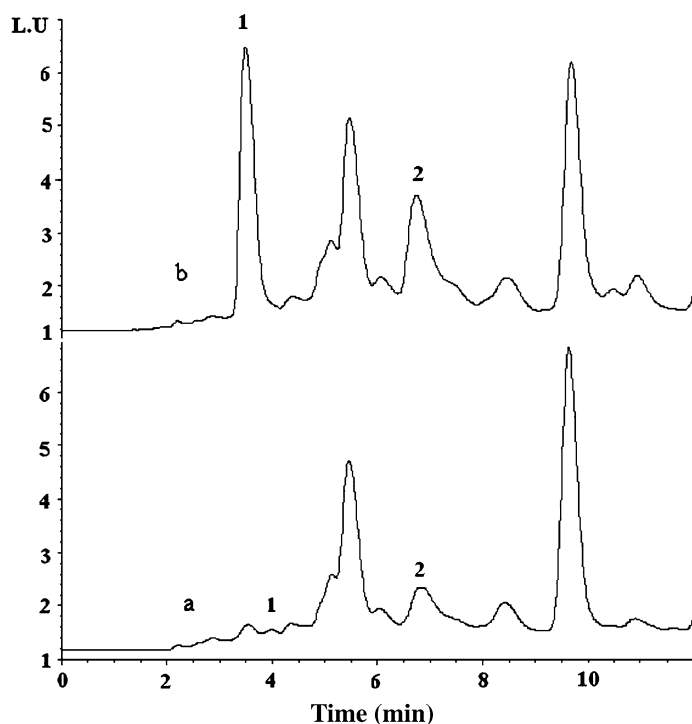
resulted in the decrease of extraction efficiency. Therefore, all the extraction experiments were carried out without addition of salt.

Effect of Extraction Time

In this research, a series of extraction times ranging from 0 to 60 min were studied with other experimental conditions remaining constant. The results showed that the variations of peak area versus extraction time were not remarkable. It could be explained that the surface area between the extraction solvent and the aqueous phase is infinitely large when the cloudy solution was formed. Thereby, the transfer of analytes from aqueous phase (sample) to extraction phase is fast and equilibrium state is achieved quickly, which results in the short extraction time. This is the remarkable advantage of DLLME technique over other extraction techniques, such as single-drop microextraction, solid phase microextraction, stir-bar adsorption extraction and so on.

Table 2. Analytical results in soil samples

Analytes	Soil	Real (ng g ⁻¹)	Added (ng g ⁻¹)	RSDs (%) (n = 3)	Relative recovery (%)
Carbaryl	A1	0.89	10.0	5.66	108.8
			20.0	7.30	88.2
			50.0	3.69	97.9
	A2	0.73	10.0	7.70	95.4
			20.0	7.53	94.8
			50.0	5.91	103.9
	A3	1.68	10.0	3.47	100.7
			20.0	4.24	96.3
			50.0	5.27	94.9
Triazophos	A1	35.10	10.0	3.94	88.8
			20.0	5.87	107.6
			50.0	4.72	97.3
	A2	50.65	10.0	2.26	90.4
			20.0	4.34	110.7
			50.0	4.74	80.8
	A3	28.21	10.0	3.14	111.1
			20.0	1.96	96.7
			50.0	2.56	81.0

**Fig. 2.** Chromatogram of soil A2 from blank (a) and after spiking at concentration level of 20.0 ng g⁻¹ of each pesticide (b) by DLLME under the optimum conditions. Peak identification: 1 = carbaryl; 2 = triazophos

Quantitative Analysis

Under the optimum conditions, analytical characteristics of the method, including linear range, limits of detection and reproducibility are summarized in Table 1.

Linearity was observed in the concentration range of 0.1–1,000 ng g⁻¹ for

carbaryl and 1–5,000 ng g⁻¹ for triazophos, respectively. Correlation coefficients varied from 0.9997 to 0.9999. The relative standard deviation (RSDs, for 20.0 µg kg⁻¹ of each pesticide) varied from 1.96 to 4.24% (*n* = 6). The limits of detection (LODs) of carbaryl and triazophos, based on signal-to-noise ratio (S/N) of 3, were 14 and 110 µg g⁻¹, respectively.

Real Samples Analysis

Three soil samples, which were collected at upper, middle and lower reaches of Ao River (Wenzhou, China.), respectively, were analyzed using the proposed method. The soil samples were first extracted with methanol, then concentrated by DLLME technique and finally analyzed by LC-FLD. The detection results showed that all the soil samples were contaminated by the two pesticides. The soil samples were spiked with two pesticides at concentration levels of 10.0, 20.0 and 50.0 ng g⁻¹ to assess the matrix effect on the proposed method. Each treatment was in triplicate, and the results are summarized in Table 2. The relative recoveries of soil A1, A2 and A3 were in the range of 88.2–108.8%, 80.8–110.7% and 81.0–111.1%, respectively, with RSDs ranging from 1.96 to 7.70%. These results demonstrated that the soil matrices, used in this experiment, had little effect on DLLME efficiency. Chromatograms of soil A2 for blank and spiking at 20.0 ng g⁻¹ of each analyte are shown in Fig. 2.

Conclusions

In the present study, an efficient DLLME–LC–FLD method for the simultaneous determination of carbaryl and triazophos at trace levels in soils was developed and validated. The technique provides good repeatability, good recovery and low LODs. Besides, no matrix effect was observed when the proposed DLLME technique was applied to real soil samples spiked with the analytes. In comparison with conventional method, DLLME had the advantage of lower consumption of organic solvents (about 10 mL) and simplicity of operation. In a word, the proposed method is very rapid, simple, low cost and environment-friendly.

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