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Determination of Phthalate Esters in Soil Samples by Microwave Assisted Extraction and High Performance Liquid Chromatography

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Abstract A method was developed for the determination of phthalate esters (dimethyl phthalate, diethyl phthalate, benzyl butyl phthalate, di-n-butyl phthalate, di-n-octyl phthalate and di-(2-ethylhextyl) phthalate) in soil samples. The method was based on microwave-assisted extraction of soil using acetonitrile as extractant. Phthalate esters in the extract were determined by high performance liquid chromatography with variable wavelength detector. Microwave-assisted extraction operational parameters, such as the solvent type, extraction temperature and time, were studied and optimized. Under the resultant conditions, recoveries of phthalate esters from spiked soil samples were in the range from 84 to 115% for two different spiking levels (0.1 and 0.5 μg g^{-1}), and relative standard deviations of the recoveries were below 8%. The limits of detection ranged from 1.24 to 3.15 μg L^{-1}. The method did not require clean-up or preconcentration steps. The obtained results showed that microwave-assisted extraction combined with high performance liquid chromatography was a fast and simple method for the determination of phthalate esters in soil samples.

Keywords Microwave-assisted extraction · High performance liquid chromatography · Phthalate esters · Soil samples

Phthalate esters (PAEs) are used primarily as plasticizers in polymeric materials to increase their flexibility through weak secondary molecular interactions with polymer chains. Since they are not chemically but only physically bound to the polymer chains, the release of phthalate esters to the environment may occur during the production and distribution of these compounds, and during incineration and migration of plasticizers from the materials containing them (Staples et al. 1997; Balafas et al. 1999). Worldwide production of phthalate esters and their frequent application in different products of everyday use has resulted in their widespread presence in all parts of the environment. Certain phthalate esters, as well as their metabolites and degradation products, can cause adverse health effects (in particular on liver, kidney and testicles) (Arcadi et al. 1998). Potential endocrine disrupting properties were also reported (Petrovic et al. 2001). Due to their potential risks for human health and environment, several PAEs have been included in the priority list of pollutants of different national and supranational organizations (US Environmental Protection Agency 1999). The accumulation of PAEs in the soils may lead to contamination of vegetables and food chains, with consequent direct or indirect human exposure. Moreover, leaching, evaporation and migration of PAEs from soil are possible sources of atmospheric and groundwater contamination (Zeng et al. 2009). Therefore, evaluation and monitoring of PAEs in soils is imperative for human health protection and environmental control.

Analysis of PAEs in solid environmental matrices by gas chromatography or high performance liquid chromatography requires sample preparation to extract the analytes from the sample. Conventional extraction techniques, such as Soxhlet extraction, sonication and mechanical shaking, are laborious, time-consuming and need large volumes of toxic organic solvents (Wang et al. 2007). New extraction techniques are mainly instrumental and performed under elevated pressure and/or temperature. These

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techniques comprise supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE) (Dean and Xiong 2000; Andreu and Pico 2004). However, SFE and PLE bear the drawbacks such as high equipment cost, the large number of parameters to optimize and possible restrictor blockage.

Microwave-assisted extraction is well suitable for routine analysis and offers a great reduction in time and solvent consumption, and a high throughput of samples. Since its first application in the extraction of organic compounds from solid matrices, many investigations have been concerned with the applicability of MAE as a powerful alternative technique to other conventional methods in recent years (Camel 2000; Eskilsson and Bjorklund 2000). The technique has been successfully applied to the simultaneous extraction of toxic organic contaminants from different solid matrices, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenols and pesticides (Basheer et al. 2005; Cheng et al. 2007; Itoh et al. 2008; Garcia-Lopez et al. 2009). The purpose of this study was to investigate the feasibility of using microwave energy for the efficient extraction of PAEs from soil and develop a simple and rapid method for the determination of PAEs in soil based on MAE and high performance liquid chromatography with variable wavelength detection.

Materials and Methods

Dimethyl phthalate (DMP, 99.5%), diethyl phthalate (DEP, 99.0%), di-n-butyl phthalate (DnBP, 99.0%), benzyl butyl phthalate (BBP, 99.5%), di-n-octyl phthalate (DnOP, 99%) and di-2-ethylhextyl phthalate (DEHP, 99%) were obtained from Accustandard Inc (New Haven, MI, USA). The individual stock standard solution were prepared in acetonitrile at a concentration of 100 μg mL^{-1} and stored at 4°C. The standard working solutions were daily prepared by dilution of stock standard solution with acetonitrile to the required concentrations. Acetone, dichloromethane and methanol were all of analytical grade and purchased from Shanghai Chemistry Reagent Company (Shanghai, China). These solvents were redistilled in all-glass system to remove trace impurities. The HPLC-grade acetonitrile was obtained from TEDIA Company (Fair lawn, NJ, USA). The water used was purified on an MYQ sub-boiling distilling water purification system (Changsha, China).

A soil sample collected from local site of our university was used during the optimization steps. The developed method was applied to other soil samples that were collected from different sampling points in Hubei province of China. Once in the laboratory, the soil samples were frozen and lyophilized at low temperatures (−20°C) and pressures (10 Pa) in a LGJ-12 freeze-drier (Beijing, China). The dried samples were ground and sieved through a 60-mesh sieve, stored in glass vials and kept in the fridge at 4°C until analysis. To reduce the background contamination, glass materials were used instead of plastic materials. Prior to analysis, all glassware was washed with water and soap, then rinsed with ultrapure water, thoroughly rinsed with acetone and finally thermally treated at 400°C.

An initiator microwave system (Biotage, Uppsala, Sweden) equipped with 10 Teflon vessels was used for closed-vessel microwave-assisted extraction of PAEs from soils. The chromatographic analysis was performed with an Agilent 1100 HPLC system equipped with a automatic injector, 20 μL injection loop and variable wavelength detector (VWD). A personal computer equipped with Agilent ChemStation software for LC was used to process chromatographic data. The analytes were separated on a Zorbax Eclipse XDB-C_8 column. Sample injection volume was 20 μL, column temperature was set at 25°C and the VWD was set at 254 nm. The mobile phase consists of a gradient mixture of acetonitrile and water and the flow rate was set at 1.0 mL min^{-1}. The gradient elution was carried out as follows: 70% acetonitrile from the beginning to 5 min, then increased to 100% acetonitrile and kept until 20 min, then decreased to 70% acetonitrile and kept until 30 min for next analysis.

Acetonitrile (5 mL) as extraction solvent was added to the MAE extraction vessel, which contained 1.0 g soil sample. Each vessel was closed gas-tight and shaken vigorously for several seconds, then the vessel was placed into the microwave sample preparation system. Extraction was carried out at 100°C for 30 min, pre-stirring for 10 s. After extraction, the vessels were removed from the microwave sample preparation system and allowed to cool down to the room temperature. The extract was filtered through a 0.45 μm membrane. The residue and the filter were washed three times with little acetonitrile per time. These aliquots of acetonitrile were collected together with acetonitrile extract after filtration. Subsequently, all of the acetonitrile extract was evaporated to near dryness with a gentle stream of nitrogen under low temperature. The residue was redissolved with 300 μL of acetonitrile for subsequent analysis with HPLC.

Results and Discussion

A series of preliminary experiments were conducted to select the optimum operation conditions of the MAE procedure. Some parameters, such as the solvent type, extraction temperature and time, were studied and optimized. The HPLC peak area of the analytes was used to evaluate the extraction efficiency under the different conditions.

The selection of an appropriate organic solvent is a critical step in the MAE process. Acetonitrile, acetone/

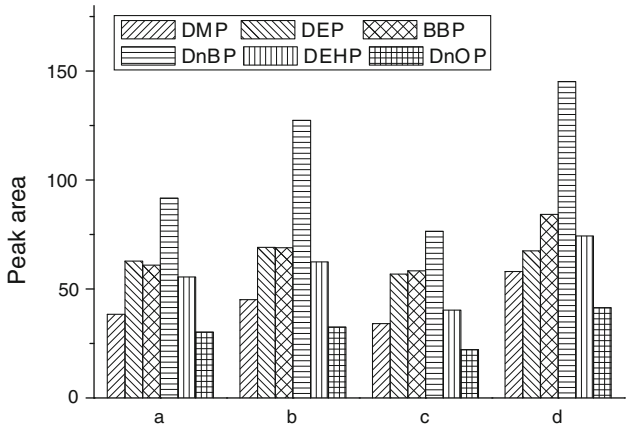

Fig. 1 Effect of solvent type on the microwave-assisted extraction efficiency of phthalate esters. **a** acetone/dichloromethane (1:1); **b** acetone/dichloromethane (1:4); **c** acetone/dichloromethane (4:1); **d** acetonitrile

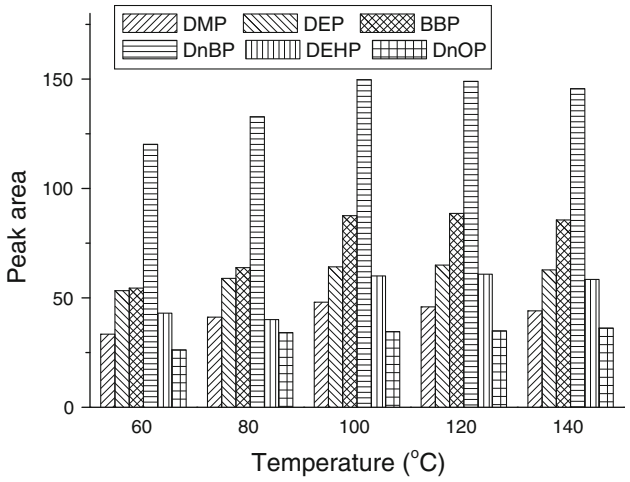

Fig. 2 Effect of extraction temperature on the MAE extraction efficiency of phthalate esters

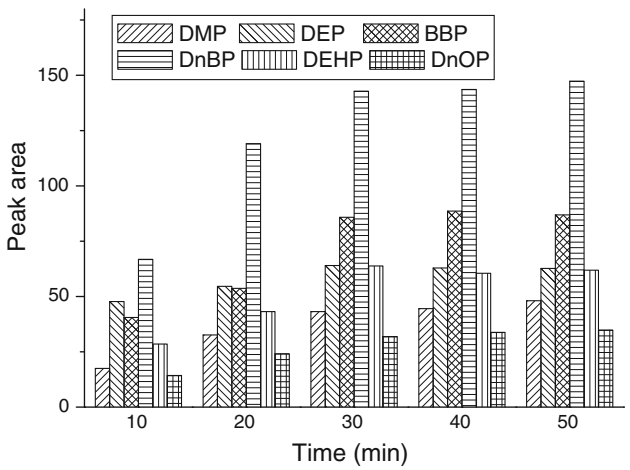

Fig. 3 Effect of extraction time on the MAE extraction efficiency of phthalate esters

dichloromethane (1:1, v/v), acetone/dichloromethane (1:4, v/v), and acetone/dichloromethane (4:1, v/v) were selected as extraction solvents. The soil sample used was spiked with PAEs standard solution to a final concentration of 0.1 μg g^{-1}. The extraction was carried out using 5 mL solvent at 100°C for 30 min. Comparing the extraction efficiencies of PAEs with each of the investigated solvents (Fig. 1), it can be observed that acetonitrile is the best choice and thus it was used as the extraction solvent.

The volume of acetonitrile used for the extraction in this work was not optimized, as the minimum amount of solvent of the MAE system was 5 mL. It was sufficient for complete immersion of the soil sample in the extraction solvent. Increasing the solvent volume could complicate the extraction procedure especially that lower recovery rates were obtained with increased solvent volumes as reported previously (Eskilsson et al. 1999; Carro et al. 1997). Based upon the above considerations, the minimum amount of solvent was used for extraction in this method.

Temperature of microwave irradiation affects interactions and equilibrium rate and controls partition of analytes between sample and solvent. To investigate the effect of extraction temperature on the extraction efficiency, the extractions were performed at 60, 80, 100 and 120°C for 30 min, respectively. The extraction efficiency is shown in Fig. 2. It can be seen that the extraction efficiency for most PAEs increase up to 100°C. However, above 100°C a slight decrease in the extraction efficiency was observed. So a temperature of 100°C was selected for further optimization of the method.

The time required for MAE is short compared with conventional soxhlet extraction and mechanical shaking. To investigate the influence of extraction time, the extractions were carried out at 100°C for 10, 20, 30, 40 and 50 min using acetonitrile as the extraction solvent. A time of 30 min was found to be optimal, a longer extraction time did not result in any considerable increase in the extraction efficiency (Fig. 3). Therefore, 30 min was selected as extraction time in this method.

Calibration curves were constructed in the concentration range from 0.01 to 5 μg mL^{-1} by HPLC. The linear relationships between the peak area and the concentration of PAEs are in good agreement with a correlation coefficient above 0.999. All method parameters are shown in Table 1. The limits of detection (LODs) were calculated based on a signal-to-noise ratio of 3 times. The precision of the method was estimated with seven replicate analyses of the same soil sample spiked at 0.1 μg g^{-1}, and the relative standard deviations (RSDs) obtained were all within 8%.

The developed method was applied to the soil samples that were collected from different sampling points in Hubei province of China. Figure 4 shows the chromatograms of a

Table 1 Method performance parameters

Compound	Calibration curve	Linear range (μg mL^{-1})	*R*	LOD (μg L^{-1})	R.S.D (%)
DMP	A = 140.07751C + 2.19692	0.01–5	0.9994	2.32	3.98
DEP	A = 108.60486C + 1.82553	0.01–5	0.9998	2.15	3.12
BBP	A = 137.19615C − 1.17088	0.01–5	0.9995	1.92	5.88
DnBP	A = 88.50557C + 6.83759	0.01–5	0.9999	1.24	3.29
DEHP	A = 61.14408C − 0.47162	0.01–5	0.9998	2.03	5.24
DnOP	A = 92.92835C + 0.71529	0.01–5	0.9992	3.15	7.73

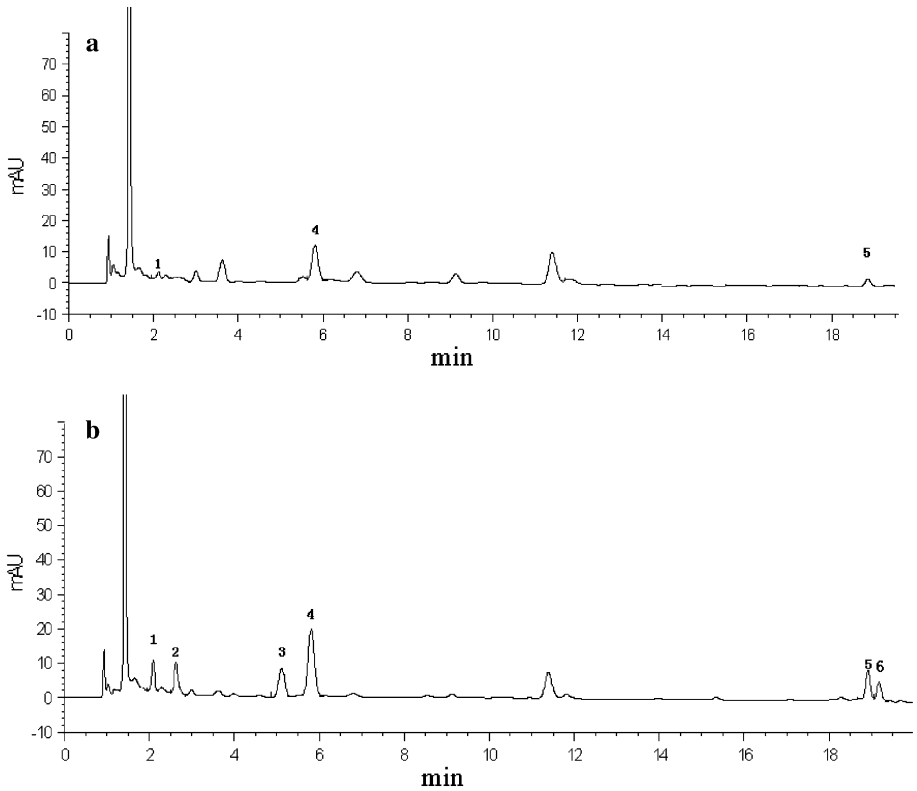

Fig. 4 Chromatograms obtained for a real soil sample (**a**) and spiked sample with 0.1 μg g^{-1} of phthalate esters (**b**) after MAE extraction under the optimum conditions. Peak: (*1*) DMP, (*2*) DEP, (*3*) BBP, (*4*) DnBP, (*5*) DEHP and (*6*) DnOP

Table 2 Analytical results of phthalate esters in real soil samples (n = 5)

Compound	Found (μg g^{-1})	Average recovery (%)		R.S.D (%)	
		0.1 μg g^{-1}	0.5 μg g^{-1}	0.1 μg g^{-1}	0.5 μg g^{-1}
DMP	0.048	104.5	109.5	7.65	4.88
DEP	0.029	91.6	88.9	6.54	3.72
BBP	ND	107.4	114.9	4.12	5.94
DnBP	ND	96.6	97.8	4.63	2.70
DEHP	0.144	83.9	86.2	5.32	4.58
DnOP	ND	102.2	99.5	8.63	7.28

ND not detected

real soil sample (Fig. 4a) and then spiked with 0.1 μg g^{-1} of PAEs (Fig. 4b). There were only DMP, DnBP and DEHP detected in the soil sample before spiking, and two level (0.1 and 0.5 μg g^{-1}) spike experiments were performed. As can seen from Table 2, the recoveries were from 84% to 115%, and acceptable for the trace analysis.

In conclusions, microwave-assisted extraction using acetonitrile as extractant combined with high performance

liquid chromatograph has been developed to determine PAEs in soil sample in this study. The results indicate that this extraction procedure is feasible and efficient, and provides a labor and time-saving method for the determination of PAEs in solid samples.

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