Microwave Extraction of Phthalate Esters from Marine Sediment and Soil

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Key Words

Summary

As part of an on-going ASEAN[+)]-Canada Cooperative Programme on Marine Science, microwave-assisted solvent extraction has been employed for the extraction of six phthalate esters from marine sediment and soil samples. Five of the six esters studied are among the United States Environmental Protection Agency's list of top priority pollutants. The effects of extraction solvent, extraction temperature, duration of extraction and extraction volume on the mean recoveries of the six phthalate esters were quantitatively evaluated by means of an analysis of variance, followed by testing the differences among the level means for each condition with least significant difference method. Microwave-assisted solvent extraction allowed comparable or higher recoveries of the six phthalate esters (70.1–91.0 %) in comparison with conventional soxhlet (65.5–89.5 %) and sonication (64.6–88.6 %). The precision of results by microwave-assisted solvent extraction was improved significantly compared to the conventional techniques. The microwave extraction system has many advantages over the soxhlet and sonication extraction, e.g., no laborious clean-up procedure, lower usage of hazardous organic solvent, and larger sample throughput. The technique has been employed for the analysis of native marine sediment and soil samples in Singapore.

Introduction

One class of organic pollutants which has been extensively studied for their levels in the environment is phthalate esters [1, 2]. They are one of the most widely distributed chemicals owing to their high rate of production and application as plasticisers in the formulation of polymers [3–7]. Carcinogenicity [8], hepatoxicity [9] and mutagenicity [10] have been associated with phthalate esters. Of the six target analytes studied in the present work, five have been listed by USEPA (United States Environmental Protection Agency) as top priority pollutants [11].

Two solvent extraction techniques commonly used for phthalate esters in suspended particulate matter, sediment and sewage sludge samples are soxhlet and sonication [11–13]. The former is time-consuming and requires large volumes of possibly toxic and hazardous organic solvents. In the latter technique, several successive extractions have to be carried out in order to achieve quantitative extraction of organic compounds from solid matrices. In both cases, laborious clean-up procedures may be necessary to remove co-extractives before the final analysis.

The use of microwave irradiation in the presence of solvents for the extraction of different types of organic compounds (polynuclear aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls, organophosphorus pesticides and other semi-volatile compounds listed in USEPA Method 8250) from various solid matrices has been reported [14–16]. Recently, this technique has been automated in the form of a closed vessel microwave extraction system which can extract 12 samples in one run. It also has several control and safety features which further enhance the ease of operation. This extraction technique can reduce the sample preparation time to less than 30 min and the volume of solvent required to under 50 mL.

GC with electron-capture detection (ECD) and flame-ionization detection (FID) are two techniques for the analysis of phthalate esters which have the disadvantage of requiring several clean-up procedures to eliminate interferences [12]. Selective gas chromatographic detection with a mass spectrometer in the selected ion monitoring mode (GC-MS-SIM) allows the analysis of phthalate esters in sediment [12], water [17, 18] and air particulate [19] without an elaborate clean-up procedure.

[+)] ASEAN refers to the group of South-east Asian countries (Singapore, Malaysia, Thailand, Brunei, Indonesia and the Philippines).

In this paper, microwave-assisted solvent extraction (MASE) was employed to extract phthalate esters from marine sediment and soil, and comparison with conventional soxhlet and sonication extraction was also carried out. The optimum MASE conditions were established for the quantitative recoveries of phthalate esters from environmental samples. The results reported here were part of an on-going ASEAN-Canada Cooperative Programme (CPMS) on Marine Science (Phase II).

Experimental

Reagent

All organic solvents (dichloromethane, hexane, acetone, 30–60 °C petroleum ether and methanol) used were of pesticide grade and obtained from Fischer Scientific (Fair Lawn, NJ, USA). Dibutyl phthalate (DBP), diethyl phthalate (DEP) (purity both > 98 %), bis (2-ethylhexyl) phthalate (BEHP) (purity > 97 %) and dimethyl phthalate (DMP) (purity > 99 %) were obtained from Fluka (Buchs, Switzerland). Both diallyl phthalate (DAP) and benzyl n-butyl phthalate (BBP) with purity above 98 % were obtained from Tokyo Kasei Kyogo Co. Standard stock solutions of 1000 µg mL^{-1} of each phthalate ester were prepared in acetone, and further dilution with hexane to give a final concentration of 10 µg mL^{-1} as a working standard solution.

Sample Preparation

The sediment samples collected along the eastern coastal region of Singapore (with minimal industrial activity) with moisture content of 2.5 % (w/w) and organic content of less than 0.8 % were used as a fortified sample matrix (FSM) during the recovery studies. They were checked for the background phthalate ester level. All FSMs used in the recovery studies were spiked with phthalate ester working standard solution at the 2.0 mg kg^{-1} level. An equilibrium period of about 1 h is required to allow the evaporation of the spiking solvent (dichloromethane) under room temperature, and also for the mixing of the analytes with the matrix.

Three native marine sediment samples were collected within the Tuas Bay and three soil samples were also collected in the vicinity of the Tuas/Jurong industrial park. All the marine sediment and soil samples collected were preserved immediately at 4 °C in a refrigerator and analysed within a week. Prior to extraction, all sediment and soil samples were homogenized until fine and regular-size solid particulates were obtained.

To control contamination by phthalate esters from glasswares and other apparatus, thorough cleaning of the apparatus is necessary prior to use. All glassware is cleaned by soaking in 5 % nitric acid followed by drying in an oven at 200 °C and consecutive rinsing using pesticide-grade acetone just before use. Repetition of the cleaning procedure may be necessary to ensure low blanks.

The three extraction techniques, soxhlet, sonication and MASE, were employed for the extraction of phthalate esters from marine sediment and soil samples. For soxhlet extraction, a 5 g solid sample was accurately weighed out and transferred quantitatively into a pre-rinsed (with dichloromethane) extraction thimble. The sample was extracted with 300 mL of dichloromethane with a soxhlet apparatus for 16 hours. After the extraction is complete, the extract was preconcentrated, the solvent changed to hexane, and the final volume reduced to 1 mL with evaporation in a rotary evaporator with a stream of nitrogen. For sonication, 50 mL of dichloromethane were added to 5 g of the sediment and the sample was then subjected to sonication in an ultrasonic water bath for 15 min. The extraction procedure was repeated twice with the same volume of fresh dichloromethane. After a series of filtrations and washings, all the combined extracts of dichloromethane were preconcentrated, solvent changed to hexane and the final volume reduced to a 1 mL hexane extract with evaporation in a rotary evaporator under a stream of nitrogen. For GC analyses, the clean-up procedure used in this study was similar to that described by Zurmühl [13].

In the case of MASE, 5 g of sediment sample was weighed out and transferred quantitatively to the Teflon-lined extraction vessels. After extraction by a MES-1000 microwave extraction system (CEM, Matthews, NC, USA), the vessels were allowed to cool down to room temperature before opening the caps. After decantation, centrifugation and preconcentration, a 1 mL extract was obtained for analysis by either GC-ECD or GC-MS.

For optimization of the MASE conditions, some previously published information was available [15, 16]. Three different extracting solvents (variable A), dichloromethane, (1:1, v/v) acetone/hexane and (1:1, v/v) acetone/petroleum ether (30–60 °C) were evaluated in this study. Extractions were performed at three different temperatures (variable B), 80, 115 or 145 °C for 5, 10 or 15 min duration (variable C) with varying solvent volume (variable D) of 25, 30 or 35 mL at 50 % power (based on six extraction vessels). The differences among the level means for each variable were then tested by comparison of mean percent recoveries using a least significant difference (LSD) method, followed by analysis of variance (ANOVA).

Analysis of Extracts

A Hewlett-Packard model 5890A gas chromatograph equipped with an electron capture detector (ECD) was used for GC analysis. The column used as an HP Ultra-2 column (30 m × 0.32 mm, i.d., 0.32 µm). The injector and detector temperatures were maintained at 270 °C and 300 °C respectively. The oven temperature program was as follows: 140 °C for 1.5 min; increased to 280 °C at a rate of 15 °C min^{-1}; final holding time 10 min. Nitrogen was used as the carrier gas and the flow rate was set at 1.0 mL min^{-1}. For gas chromatographic-mass spectrometric analysis, a Hewlett-Packard 5988A GC-

MS equipped with an HP-5 column (30 m × 0.25 mm, i.d., 0.25 μm film thickness) with helium carrier gas at a linear velocity of 39 cm s^{-1} was employed. The instrument was tuned daily with PFTBA (perfluorotributylamine). The temperature program was similar to that described for GC-ECD. Quantitative analyses were based on the integrated SIM peaks of phthalate esters which have the characteristic dominant m/z 149 (phthalic anhydride base peak) except for dimethyl phthalate (m/z 163); these peaks are particularly well-suited for GC-MS-SIM. Splitless injection of 2 μL of the standards or extract was used for both GC-ECD and GC-MS.

Results and Discussion

During the optimization of the MASE conditions, all the extracts were analyzed by GC-ECD. Figure 1a gives the GC-ECD chromatogram of the phthalate ester standard mixture, and Figure 1b shows an extract of an FSM extracted by MASE. The concentration of the blank obtained during the preparation of the FSM was about 1 μg kg^{-1} of BEHP which is comparable to those reported in previous work [12, 20].

The effect of extracting solvent on the MASE recoveries of phthalate esters is presented in Table I. The mean percent recovery (y_i) obtained by (1:1, v/v) acetone/hexane was significantly better at $P < 0.05$ for DAP and DBP compared to (1:1, v/v) acetone/ petroleum ether, as determined by ANOVA and LSD. The mean recoveries of individual phthalate esters with (1:1, v/v) acetone/hexane and dichloromethane, showed no significant difference (Table I). However, since no solvent exchange is required for (1:1, v/v) acetone/ hexane, the losses of analytes during the sample preparation can be reduced. Moreover, dichloromethane is not a suitable solvent for GC-ECD analysis. Thus, (1:1, v/v) acetone/hexane was selected as the extracting solvent and was employed for the rest of the recovery studies. The same observation was also reported by Lopez-Avila et al. [15, 16].

Table I. Mean recovery of individual phthalate esters from a fortified sample matrix as a function of different extracting solvents[a].
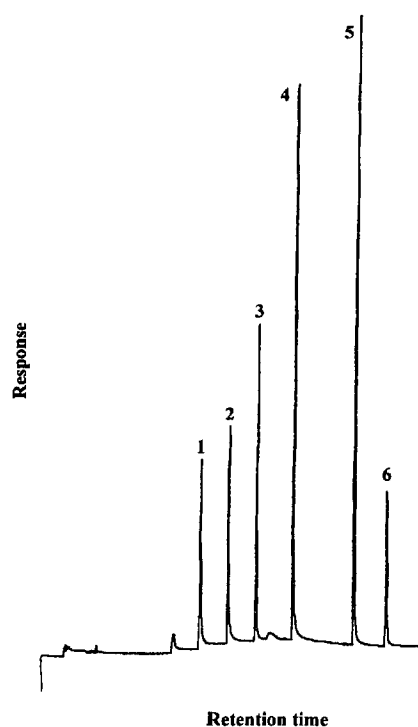
Phthalate esters	Extracting Solvents		
	1[b]	2	3
DMP	65.1 (3.4)	67.6 (4.1)	60.1 (3.1)
DEP	72.2 (6.1)	74.0 (4.0)	71.1 (5.0)
DAP	77.9 (2.5)	78.1 (3.0)	70.1 (3.6)
DBP	85.9 (3.4)	86.4 (5.0)	73.0 (4.0)
BBP	79.4 (2.1)	80.9 (3.9)	78.4 (3.6)
BEHP	89.9 (4.5)	91.0 (5.0)	83.9 (5.9)

[a] Mean deviation in parentheses; n = 3.

[b] Extracting solvent (1 = dichloromethane, 2 = acetone/hexane, 3 = acetone/petroleum ether).

Retention time

1a

Retention time
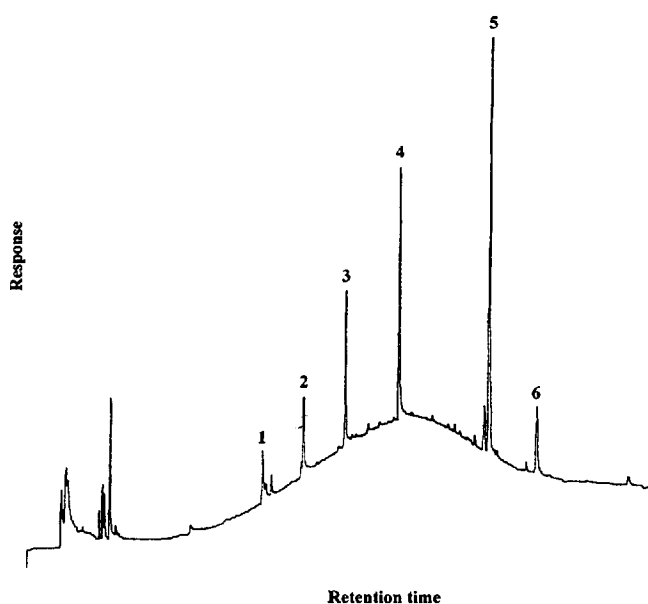
1b

Figure 1

GC-ECD chromatograms of (a) 6 phthalate ester standard mixture; (b) sediment samples spiked with phthalate esters and extracted by optimum MASE conditions. The assignment of peaks are as follow: 1 = Dimethyl phthalate; 2 = Diethyl phthalate; 3 = Diallyl phthalate; 4 = Dibutyl phthalate; 5 = Benzyl n-Butyl phthalate; 6 = Bis (2-ethylhexyl) phthalate.(Conditions, see Experimental).

The effects of varying extraction temperatures, duration of extraction and solvent volume on the mean recoveries of the esters are illustrated in Table II. Generally, an increase in the extraction temperatures resulted in an increase in the recoveries of the esters (Table II). The difference was statistically significant at $P < 0.05$ for DMP, DEP and DAP when tested for comparison of means by the LSD, following ANOVA when the temperature was raised from 80 °C to 115 °C. No significant difference was observed on the mean recoveries of the analytes between 115 °C and 145 °C. As the time required to reach 115 °C during microwave irradiation was only half that required to reach 145 °C, the optimum extraction temperature was selected at 115 °C. Generally, an increase in extraction time from 5 to 10 min gave a considerable increase in the mean recoveries of all esters except for DMP. The differences in mean recoveries were insignificant for all compounds when the duration of extraction was increased from 10 to 15 min as determined by ANOVA and LSD. Thus, on the basis of the results obtained, a 10 min extraction time at 115 °C was selected. For solvent volume, the difference in the mean recoveries was statistically insignificant for all compounds when the solvent volume was increased from 25 to 35 mL (Table II). However, an average recovery of 77.9 % were obtained using a 30 mL solvent volume which was higher than that for 25 mL (73.4 %) and 35 mL (76.6 %). The overall optimum MASE conditions for the extraction of phthalate esters from marine sediment or soil samples were 30 mL of (1:1, v/v) acetone/hexane as the extracting solvent, and an extraction temperature of 115 °C for a 10 min. The conditions described here are quite similar to those reported in previous work [15, 16] except for the duration of extraction.

In general, MASE gave comparable or higher recoveries for all the six phthalate esters from FSM (dry matrix) compared to soxhlet and sonication extraction. The range of mean recoveries of the phthalate esters using MASE, soxhlet and sonication were 70.9–91.0 %, 65.5–89.5 % and 64.6–88.6 % respectively. The precision for MASE was also better. In the case of soxhlet extraction, a much higher matrix blank (100 µg kg^{-1} for BEHP) was obtained compared to MASE (1 µg kg^{-1} for BEHP) and sonication (50 µg kg^{-1} for BEHP). Thus, soxhlet extraction is very sensitive to clean-up procedure as the amount of interferents has a significant impact on the determination of phthalate esters from solid matrices. In contrast, no laborious clean-up procedure is required when MASE is coupled to GC-MS analysis.

The effect of moisture content on the recoveries of the phthalate esters was also studied and the results are presented in Table III. The differences in mean recoveries were significant for all compounds except for DMP and DEP when the moisture content was increased from 0 to 10 % (w/w), but not significant for most of the compounds except for DBP when the moisture content was increased from 10 to 20 % (w/w). Thus, it is recommended to extract environmental samples in field-wet conditions when recovering phthalate esters by MASE.

Since FSM cannot really reproduce the influence of different matrix effects on the recoveries of phthalate esters, a comparative study on the extraction efficiency between sonication and the MASE technique using environmental samples was carried out. Figure 2 shows the location of these sampling sites for sediment samples (Tuas Bay) and soil samples (Tuas/Jurong Industrial Park) in Singapore island. The distribution of the water contents and organic contents present in these sediment and soil samples were 2.1–6.8 % (w/w) and 1.5–5.7 % (w/w) respectively. Two phthalate esters were consistently found in all the sediment and soil samples being analyzed. Generally, lower recoveries of DBP and

Table II. Mean recovery of individual phthalate esters from a fortified sample matrix as a function of extraction temperature, duration of extraction and volume of solvent[a].

Phthalate esters	Temperature (°C)			Duration of Extraction (min)			Solvent Volume (ml)		
	80	115	145	5	10	15	25	30	35
DMP	59.1 (3.5)	69.1 (5.1)	70.1 (4.3)	61.0 (3.0)	62.1 (4.5)	67.5 (4.1)	60.6 (5.1)	66.9 (4.4)	65.7 (5.0)
DEP	66.1 (1.3)	75.3 (4.9)	76.0 (4.5)	68.5 (3.6)	74.5 (3.1)	73.5 (5.6)	66.9 (4.3)	72.1 (4.5)	69.1 (6.9)
DAP	70.1 (5.2)	81.2 (3.3)	82.4. (2.4)	71.0 (3.1)	80.9 (4.1)	80.0 (5.6)	72.9 (5.3)	82.1 (4.9)	80.1 (4.8)
DBP	83.0 (6.2)	88.9 (6.1)	88.0 (5.7)	79.9 (4.7)	85.6 (4.4)	85.9 (3.7)	78.0 (5.5)	81.1 (3.6)	80.1 (4.2)
BBP	72.1 (6.1)	78.6 (5.0)	77.4 (4.9)	70.5 (6.8)	79.0 (5.3)	78.2 (3.8)	74.2 (4.8)	75.9 (5.1)	74.9 (7.0)
BEHP	86.1 (4.5)	90.1 (3.4)	89.9 (4.5)	83.1 (5.6)	84.9 (4.3)	85.0 (5.2)	88.0 (2.3)	89.0 (2.1)	89.9 (3.7)

[a]Mean deviation in parentheses; n = 3.

Table III. Comparison of mean recovery of individual phthalate esters (spiked at 2.0 mg kg^{-1} level) from a fortified sample matrix (dry and wet) after extraction by soxhlet, sonication and MASE techniques.

Phthalate ester	Extraction Techniques				
	Soxhlet	Sonication	MASE		
			0 % (w/w)[b]	10 % (w/w)	20 % (w/w)
DMP	65.5 (7.0)	64.6 (9.0)	70.9 (4.0)	72.4 (5.0)	74.6 (5.2)
DEP	73.4 (4.9)	73.0 (7.3)	73.6 (2.9)	74.9 (6.1)	75.6 (5.3)
DAP	75.0 (7.5)	74.6 (9.2)	76.2 (5.1)	85.9 (5.7)	87.8 (6.2)
DBP	85.1 (6.6)	86.3 (10.1)	87.7 (4.9)	90.2 (6.8)	97.4 (4.5)
BBP	80.5 (7.1)	79.2 (7.4)	85.7 (3.7)	95.3 (4.3)	99.6 (5.2)
BEHP	89.5 (8.0)	88.6 (9.7)	91.0 (5.0)	97.6 (5.3)	100.3 (4.1)

[a] Mean deviation in parentheses; n = 3.
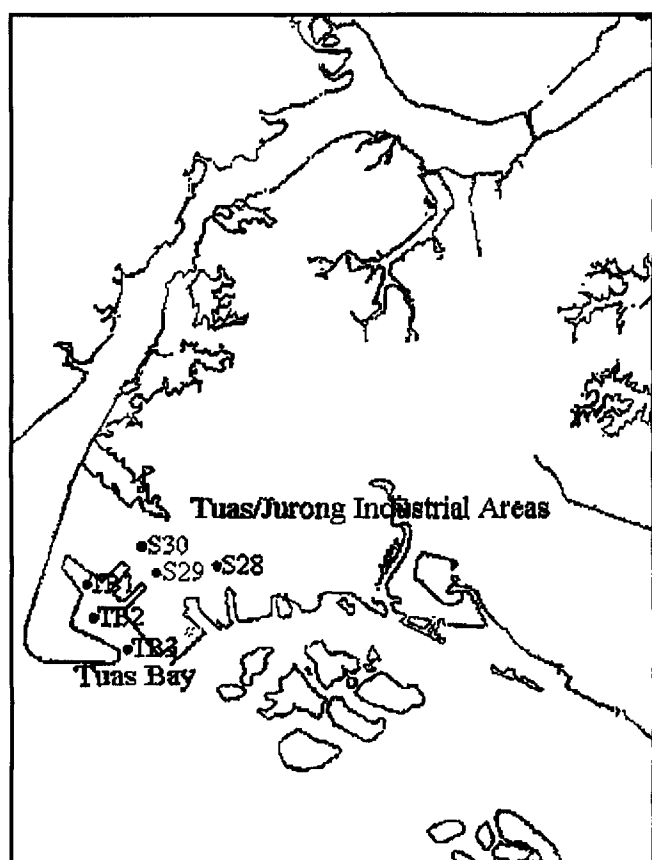[b] Weight percentage of moisture content in the FSM.

Figure 2

Map of Tuas/Jurong Industrial Area in Singapore showing the sampling sites.
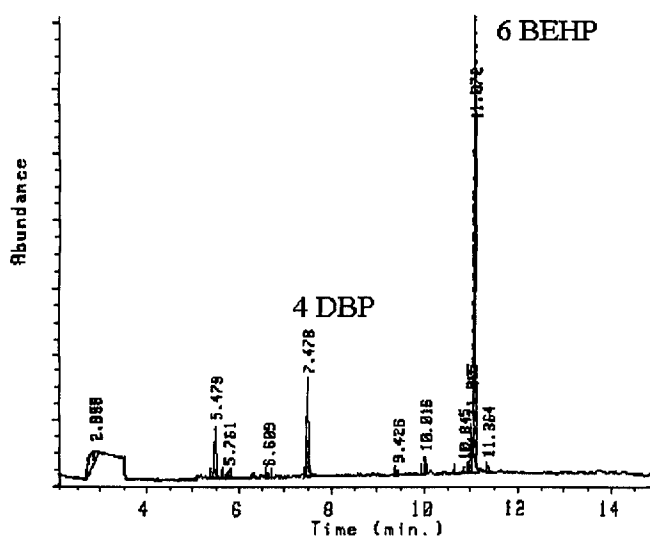

Figure 3

GC-MS-SIM chromatogram for the analysis of a real marine sediment from Tuas Bay (TB2). The assignment of peaks are as follow: 4 = Dibutyl phthalate; 6 = Bis (2-ethylhexyl) phthalate.(Conditions, see Experimental).

BEHP in four out of the six sediment and soil samples (TB1, TB2, S28 and S30) were obtained by sonication compared to MASE as shown in Table IV. The concentration ranges of DBP obtained by MASE were between 0.74 and 1.60 mg kg^{-1} for Tuas Bay sediment (TB1, TB2 and TB3), while the concentration ranges were between 0.68 and 0.98 mg kg^{-1} for Tuas/Jurong In-

dustrial park soil samples (S28, S29 and S30). In the case of BEHP, the concentration obtained by MASE were between 0.94 and 2.79 mg kg^{-1} in sediment and between 0.16 and 1.06 mg kg^{-1} for soil samples. Similarly, the precisions of the survey results were better for MASE (at $P < 0.05$ confidence level) compared to sonication for most of the environmental samples. The chromatograms for a real sample of marine sediment collected from Tuas Bay and a soil sample collected from Tuas/Jurong Industrial park are shown in Figures 3 and 4. Higher levels of DBP and BEHP were found in TB1 (upper bay) compared to TB2 (lower bay) and TB3 (mouth of bay). Generally, the levels of phthalate esters found in sediment were higher than those found on land.

Table IV. Concentration of phthalate esters (mg kg^{-1}) in marine sediment and soil samples during the analysis of real samples by MASE (A) and Sonication (B).

Phthalate Esters	Concentration (mg kg^{-1})[a]											
	TB1		TB2		TB3		S28		S29		S30	
	A	B	A	B	A	B	A	B	A	B	A	B
DBP	1.60 (8.9)	1.35 (10.1)	0.80 (7.0)	0.56 (9.7)	0.74 (6.2)	0.69 (7.7)	0.95 (8.0)	0.80 (12.2)	0.68 (7.2)	0.60 (5.7)	0.98 (4.2)	0.79 (9.7)
BEHP	2.79 (5.1)	2.60 (9.0)	1.96 (7.2)	1.51 (13.4)	0.94 (3.0)	0.89 (9.0)	0.99 (4.4)	0.76 (11.0)	0.16 (7.9)	0.15 (8.1)	1.06 (6.3)	0.88 (11.1)

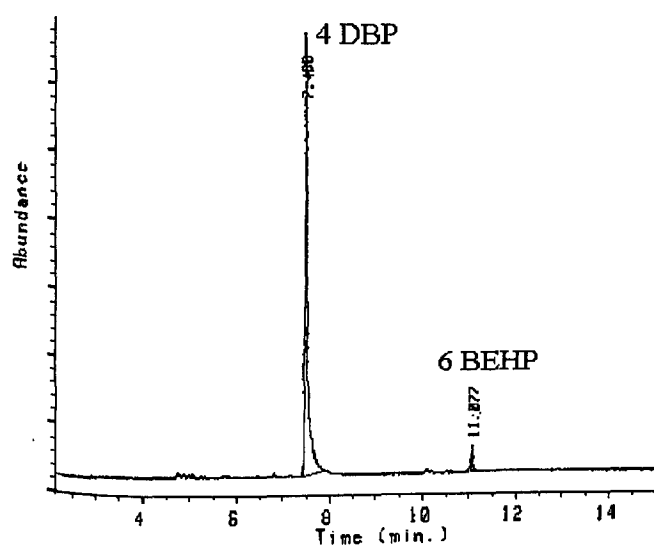[a] Mean deviation in parentheses; n = 3.

Figure 4

GC-MS-SIM chromatogram for the analysis of a real soil sample (S29) from Tuas/Jurong Industrial Park. The assignment of peaks are as follow: **4** = Dibutyl phthalate; **6** = Bis (2-ethylhexyl) phthalate. (Conditions, see Experimental).

The survey results obtained here were also compared with other studies. The phthalate ester concentrations in the sediment collected from the River Rhine varied from 3 to 70 mg kg^{-1} for BEHP and 0.1 to 2 mg kg^{-1} for DBP [20, 21]. However, in Swedish rivers, a concentration range of BEHP from 1 to 1500 mg kg^{-1} was reported [23]. In the studies reported by Ritsema et al., high values of BEHP between 12 and 105 mg kg^{-1}, and concentration of DBP between 0.3 and 0.9 mg kg^{-1} were also obtained from the River Rhine (location: Lobith, The Netherlands) [11]. In general, the level of DBP and BEHP in all the environmental samples studied were lower than the levels reported for the European countries. Thus, the enrichment of these pollutants in our local environment especially in the major industrial areas is not considered to be a serious problem.

Conclusions

The MASE technique using microwave extraction coupled to GC-MS is suitable for the routine analysis of marine sediment for the presence of phthalate esters. As compared with conventional techniques, MASE does not require the consumption of large amounts of organic solvents, and a larger sample throughput can also be achieved. Only low levels of phthalate ester contamination were present in the native environmental samples when compared to those found in sediment and soil of some European countries.

Acknowledgements

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