

Full Length Research Paper

Analysis of phthalate esters contamination in drinking water samples

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Phthalate esters are widely used as plasticizer. They can migrate from plastic materials to the environment. Exposure to some of these compounds may increase the risk of getting cancer. Phthalate esters: dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and bis(2-ethylhexyl) phthalate (DEHP) were extracted simultaneously using a solid-phase extraction and compared with liquid-liquid extraction and analyzed by a gas chromatograph. The optimum condition method was successfully applied to the analysis of phthalate esters contamination in bottled drinking water samples. The concentration of DMP, DEP and DBP in drinking water samples were below allowable levels, while the DEHP concentration in three samples was found to be greater than the regulatory, maximum admissible concentration.

Key words: Phthalate esters, drinking water, sample extraction, gas chromatography.

INTRODUCTION

Phthalate esters are widely use as additives in the manufacture of plastics, paint and cosmetics (Cai et al., 2003; Penalver et al., 2000) to increase their flexibility, transparency, durability and longevity. Most phthalate esters are used in the manufacturing of polyvinyl chloride (PVC). Dibutyl phthalate (DBP) is used in epoxy resins, while dimethyl (DMP) and diethyl phthalate esters (DEP) are typically used in cellulose ester-based plastics (Stales et al., 1997). Phthalate esters can migrate from plastic materials to the environment. They are often found in water, soil, air, food products and the human body (Castillo et al., 1998). The fact that some phthalates: DEP, DBP and bis(2-ethylhexyl phthalate (DEHP) have been found in environmental samples, shows they can be

released during use and migrate from the packaging to contaminate the contents such as foods and beverages (Feng et al., 2005; Holadova et al., 2007; Prokupkova et al., 2002; Guo et al., 2010). Some of these compounds are carcinogen and estrogenic (Holadova et al., 2007). The US Environmental Protection Agency (EPA) has set the maximum admissible concentration (MAC) or maximum contamination level (MCL) of DEHP at $6 \mu\text{gL}^{-1}$ (US EPA, 1991), the threshold limit value (TLV) of DEP, DBP, DMP and DEHP are 0.55, 0.45, 5.0 and 5.0mgL^{-1} , respectively. In order to detect phthalate esters at sub mgL^{-1} levels, a preconcentration step is necessary before analysis. Various methods have been used for extraction of phthalate esters such as solid-phase extraction (Holadova et al., 2007), solid-phase microextraction (Feng et al., 2005; Holadova et al., 2007; Prokupkova et al., 2002) and dispersive liquid-liquid microextraction have gained importance for determination of phthalate esters in water samples (Liang et al., 2008). Liquid-liquid extraction is a simple method for extraction of phthalate esters (Yasuhara et al., 1997). However, the conventional liquid-liquid extraction method needs large quantities of sample and organic solvents (Farahani et al., 2007), the liquid-liquid extraction has been modified so that it can be

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Abbreviations: PVC, Polyvinyl chloride; DBP, dibutyl phthalate; DMP, dimethyl phthalate; DEP, diethyl phthalate; DEHP, bis(2-ethylhexyl phthalate; MAC, maximum admissible concentration; MCL, maximum contamination level; PET, polyethylene terephthalate; PE, polyethylene.

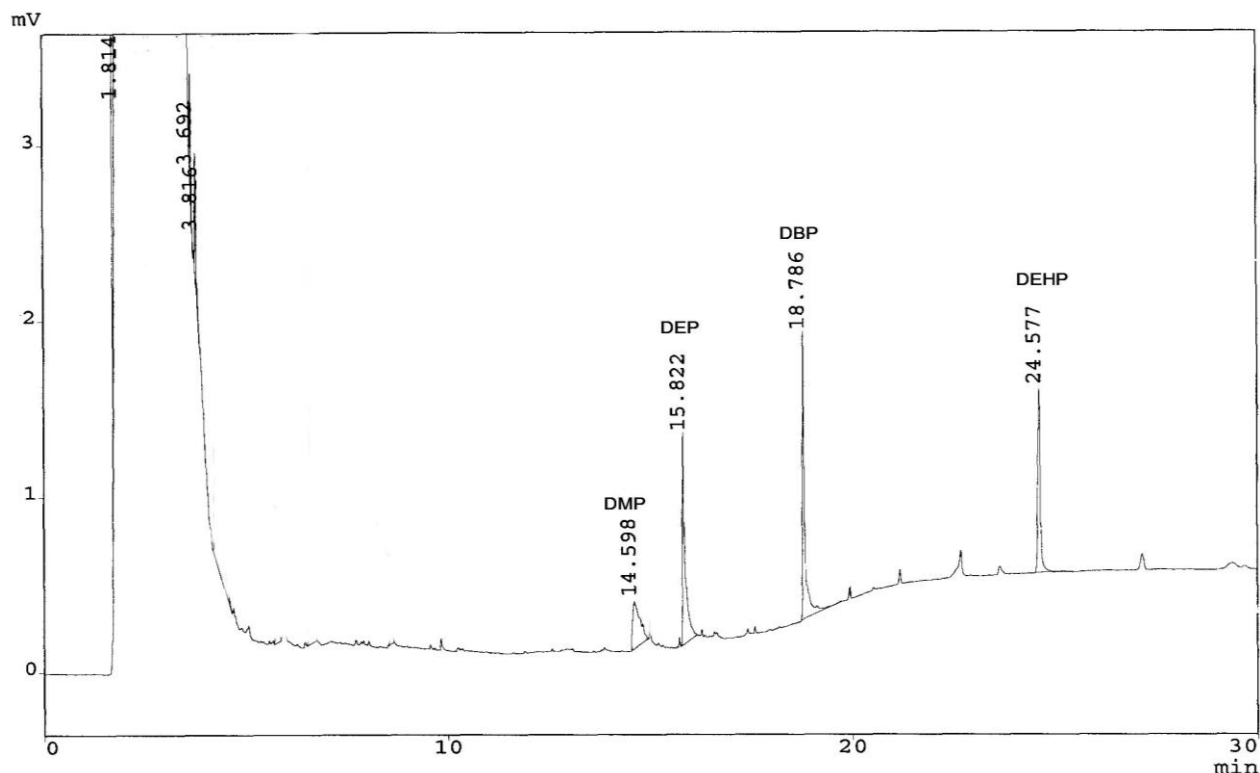


Figure 1. Chromatogram of DMP, DEP, DBP and DEHP with retention times of 14.48, 15.76, 18.72 and 24.41 min, respectively, by gas chromatograph using DB-5 fused silica capillary column (30 m x 0.25 mm id., 0.25 μ m film thickness).

performed using a small amount of solvent. The aim of this study was to analyze phthalate esters contamination in bottled drinking water.

MATERIALS AND METHODS

Instrumentation

A gas chromatograph model GC17 (Shimadzu, Japan) with a DB-5 fused silica capillary column (30 m x 0.25 mm id., 0.25 μ m film thickness) was used. The injector and detector temperature were 270°C with a flame ionization detector. The temperature program was started at 60°C and held for 5 min then ramp to 270°C at a rate of 15°C/min and held for 11 min.

Chemicals

Dimethyl phthalate, diethyl phthalate, dibutyl phthalate and bis(2-ethylhexyl) phthalate, all analytical grade reagents were obtained from Fluka (Switzerland). The individual standard stock solutions were prepared in methanol and stored at 4°C. All organic solvent were HPLC grade obtained from Labscan (USA).

Solid-phase extraction (SPE)

The 3.0 ml LC-18 (500 mg) and 6.0 ml Florisil (1g) columns were obtained from Supelco (USA). Prior to extraction, the SPE columns

were conditioned with 5.0 ml methanol under vacuum, followed by 5.0 ml of deionized water. Deionized water (100 ml) was spiked with the standard solution of phthalate esters (20 mgL⁻¹ of each) and subsequently passed through the column with the flow rate of 1.0 mLmin⁻¹. The phthalate esters were eluted with various kinds of organic solvents. The percentage recovery was carried out by gas chromatography.

Liquid-liquid extraction

The bottled drinking water sample (100 ml) was spiked with the standard solution of phthalate ester (20 mgL⁻¹ of each), 1.0 g of sodium chloride and 1.0 ml of extracting solvent were added and then shaken for 10, 30 or 60 min in a 10 ml of glass vial. The solvent layer (1.0 μ l) was directly injected into the gas chromatograph.

Analysis of phthalate esters contamination in water samples

Phthalate esters in drinking water which was contained in polyethylene terephthalate (PET) and polyethylene (PE) bottles were analyzed by using the optimum conditions.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of DMP, DEP, DBP and DEHP with retention times of 14.48, 15.76, 18.72

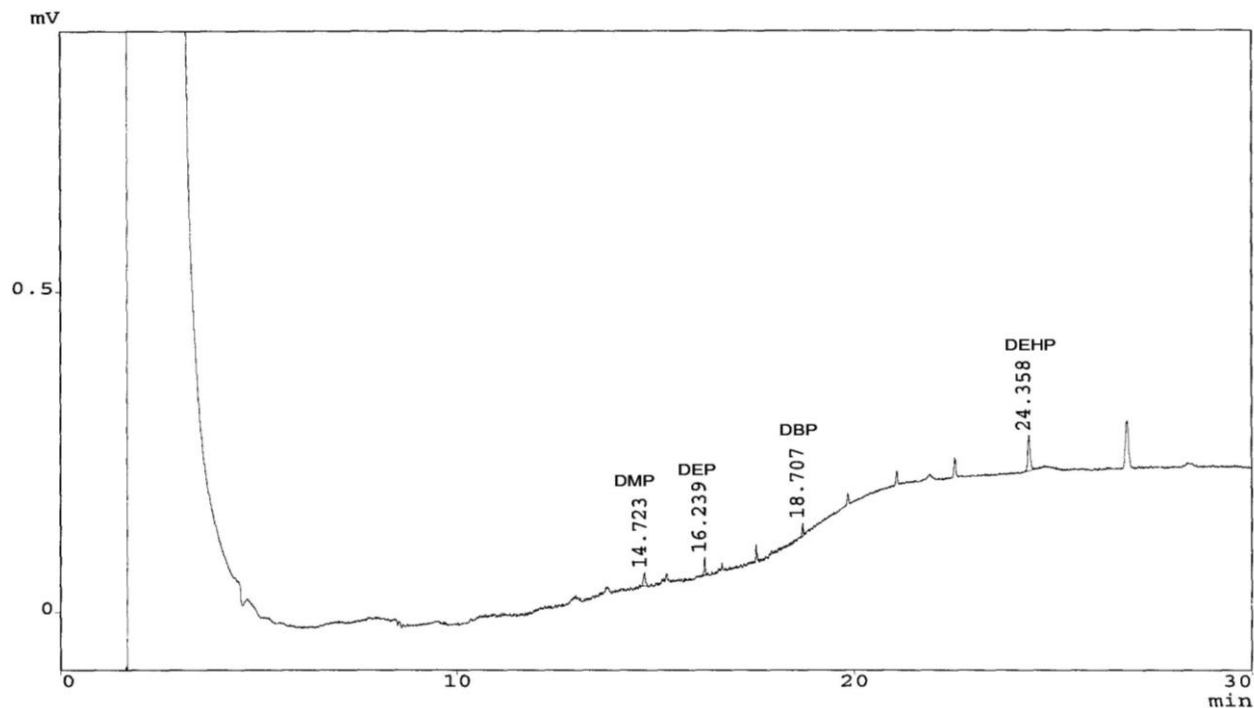


Figure 2. Chromatogram of mineral water no. 1 by solid-phase extraction and gas chromatograph using DB-5 fused silica capillary column (30 m x 0.25 mm id., 0.25 μm film thickness).

and 24.41 min, respectively. The linearity ranges were 0.5 to 100 mgL^{-1} for DMP and DEP, 0.25 to 100 mgL^{-1} for DBP and DEHP with correlation coefficients in the ranges of 0.9935 to 0.9972, as summarized in Table 1.

Optimization of solid-phase extraction

The percentage recoveries were determined from spiked sample (20 mgL^{-1} of each) using LC-18 and Florisil cartridges with various eluting solvents. Florisil was suitable for extraction of phthalate esters, as Florisil was in the form of a powdered magnesium-silica gel which used the normal phase. The interaction is adsorption, the elution step is usually carried out with a polar solvent. Ethyl acetate (5.0 ml) showed good recoveries in the range of 99.1 to 103.7%, as shown in Table 2. The results agreed with the previous study, where ethyl acetate was successfully used as the elution solvent (Holadova et al., 2007).

Optimization of liquid-liquid extraction

Selection of extracting solvent

The percentage recoveries were determined when various extracting solvent were used with an extraction time of 30 min and 1.0 g of sodium chloride added, as

shown in Table 3. The polar analytes, DMP and DBP showed low extraction efficiency when using a non polar solvent. The long alkyl chain DEHP was obtained at a high recovery when using a moderate solvent. Dichloromethane (1.0 ml) showed good recoveries of 4 phthalate esters in the ranges of 79.1 to 100.8%.

Effect of extraction time

The extraction time profiles of analytes were determined at 10, 30 and 60 min. The equilibrium for extraction was obtained quickly within 30 min. The results are shown in Figure 3.

Sodium chloride addition

Sodium chloride was added to the sample to increase the ionic strength and enhance the extraction. The results show that the peaks were highest when 1 g was added, as shown in Figure 4.

Comparison of solid-phase extraction and liquid-liquid extraction

The limit of determination (LOD) and limit of quantitation (LOQ) for extraction of phthalate esters by solid-phase

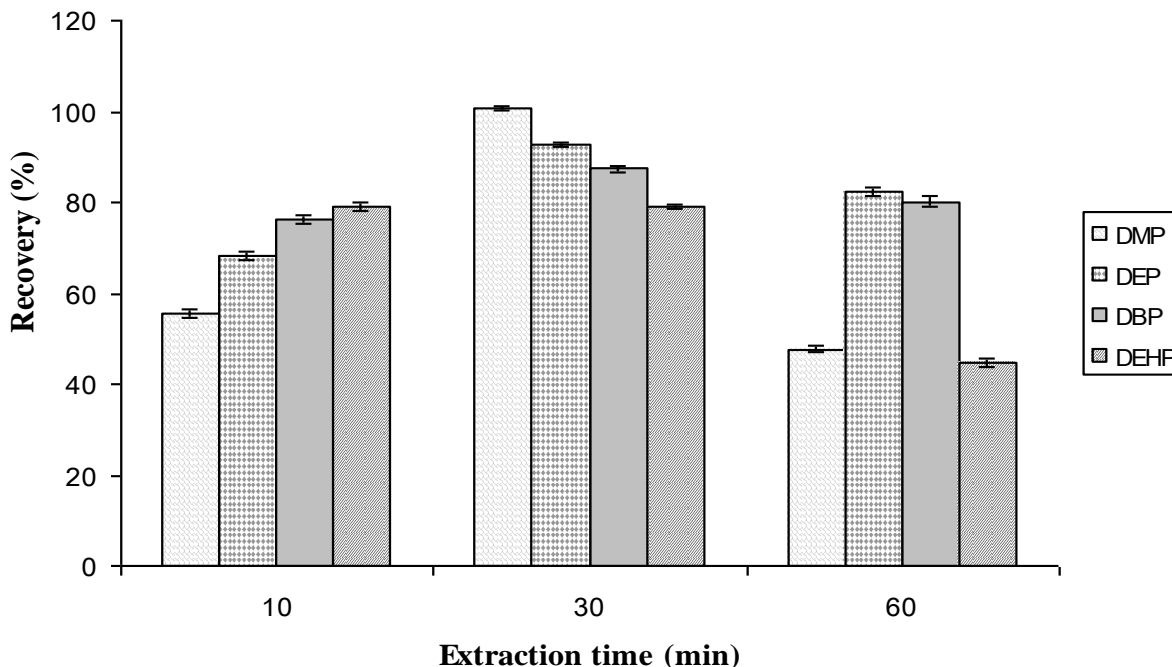


Figure 3. The effect of extraction time by liquid-liquid extraction using 1.0 ml of dichloromethane and 1.0 g of sodium chloride.

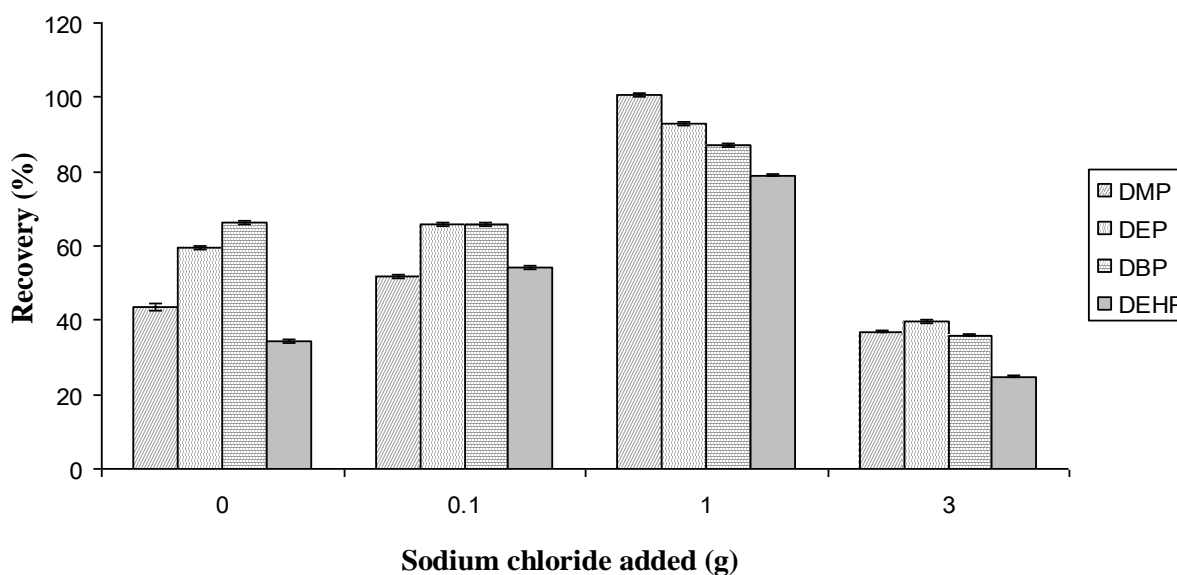


Figure 4. The effect of sodium chloride addition by liquid-liquid extraction using 1.0 ml of dichloromethane and extraction time OF 30 min.

extraction (Florisil) using ethyl acetate (5.0 ml) as eluting solvent were in the ranges of 0.3 to 1.0 mgL⁻¹ and 0.8 to 3.3 mgL⁻¹, respectively. The reproducibilities were in the ranges of 1.2 to 2.2% RSD, as shown in Table 4. The LOD and LOQ by liquid-liquid extraction using dichloromethane (1.0 ml) as extracting solvent (1.0 g

sodium chloride and 30 min extraction time) were in the ranges of 5.0 to 7.0 and 17 to 23 mgL⁻¹, respectively. Solid-phase extraction showed great potential for 4 phthalate esters with the high recoveries, low limit of determination and reduced interferences. The results agree with the work of Prokupkova et al. (2002).

Table 1. Linearity range, calibration data of phthalate ester using DB-5 fused silica capillary column (30 m x 0.25 mm id., 0.25 μm film thickness).

Phthalate esters	Linearity (mgL^{-1})	Linear equation	R ²	LOD (mgL^{-1})
DMP	0.5-100	Y=16X+184	0.9935	0.50
DEP	0.5-100	Y=29X+200	0.9937	0.50
DBP	0.25-100	Y=39X+96	0.9961	0.25
DEHP	0.25-100	Y=58X-181	0.9972	0.25

Table 2. The percentage recoveries of phthalate ester by solid-phase extraction with various eluting solvent (mean \pm SD, n=3).

Phthalate ester	LC-18			Florisil	
	5 ml Methanol	5 ml Acetonitrile	5 ml (1:1) Methanol + acetonitrile	5 ml Ethyl acetate	(1:1) Ethyl acetate + methanol
DMP	58.1 \pm 5.6	60.1 \pm 0.7	60.2 \pm 1.4	99.1 \pm 1.1	109.0 \pm 1.7
DEP	96.2 \pm 0.7	96.5 \pm 0.7	118.4 \pm 2.1	103.7 \pm 0.3	57.7 \pm 1.4
DBP	96.3 \pm 0.7	89.2 \pm 0.7	88.5 \pm 0.7	99.4 \pm 0.7	97.5 \pm 0.7
DEHP	55.6 \pm 7.0	47.3 \pm 0.7	74.0 \pm 1.4	99.8 \pm 0.5	90.0 \pm 1.4

Table 3. The percentage recoveries of phthalate ester by liquid-liquid extraction with various extracting solvent (mean \pm SD, n=3), 1.0 g sodium chloride added.

Extracting solvent	Recovery \pm SD			
	DMP	DEP	DBP	DEHP
Hexane	11.8 \pm 0.2	87.7 \pm 0.1	93.9 \pm 0.1	18.7 \pm 0.1
Toluene	69.9 \pm 0.1	84.4 \pm 0.7	92.6 \pm 0.1	32.8 \pm 0.6
Cyclohexane	61.4 \pm 0.1	91.1 \pm 0.2	95.7 \pm 0.1	62.9 \pm 0.1
Dichloromethane	100.8 \pm 0.1	92.7 \pm 0.1	87.3 \pm 0.1	79.1 \pm 0.1
Dichloromethane + cyclohexane (1:1)	70.9 \pm 0.4	98.7 \pm 0.1	90.3 \pm 0.1	48.4 \pm 0.1

Table 4. Recovery, limit of determination (LOD), limit of quantitation (LOQ) and reproducibility (RSD%) for determination of phthalate esters by solid-phase extraction and liquid-liquid extraction.

Phthalate ester	Solid-phase extraction				Liquid-liquid extraction			
	Recovery (%)	LOD (mgL^{-1})	LOQ (mgL^{-1})	RSD (%)	Recovery (%)	LOD (mgL^{-1})	LOQ (mgL^{-1})	RSD (%)
DMP	99.1	1.0	3.3	1.3	100.8	7.0	23	1.4
DEP	103.0	1.0	3.3	1.2	92.7	7.0	23	1.9
DBP	99.4	0.5	1.7	2.0	87.3	5.0	17	2.1
DEHP	99.8	0.3	0.8	2.2	79.1	5.0	17	2.8

Analysis of phthalate esters in drinking water samples

To achieve the optimum SPE procedure, the technique was used to determine phthalate esters in drinking water samples, purchased from a supermarket in Maha-Sarakham Province, Thailand. Figure 2 shows the

chromatogram of mineral water sample no 1, the results showed DMP, DBP and DEHP. Table 5 shows the concentration of phthalate esters in drinking water and mineral water produced by different brands and in different bottles. The results found that the phthalate esters content depended on the sample brand. DMP was found in drinking water sample no. 2 (PET bottle), mineral

Table 5. Phthalate esters concentration (mgL^{-1}) in drinking water sample (mean \pm SD, n=3).

Water sample	No	DMP	DEP	DBP	DEHP
Drinking water in PET bottle	1	Nd	Nd	0.17 \pm 0.14	0.50 \pm 0.02
	2	0.38 \pm 0.01	Nd	Nd	Nd
	3	Nd	Nd	Nd	Nd
	4	Nd	Nd	Nd	0.28 \pm 0.07
	5	Nd	0.54 \pm 0.03	Nd	Nd
Mineral water in PET bottle	1	0.16 \pm 0.01	Nd	0.33 \pm 0.01	0.49 \pm 0.07
	2	0.53 \pm 0.04	Nd	Nd	Nd
	3	Nd	Nd	Nd	Nd
	4	0.46 \pm 0.05	Nd	Nd	Nd
	5	0.27 \pm 0.01	0.11 \pm 0.01	Nd	Nd
Drinking water in PE bottle	1	0.27 \pm 0.02	0.11 \pm 0.01	Nd	Nd
	2	0.27 \pm 0.01	0.11 \pm 0.01	Nd	Nd

*Nd = Not detected, lower than LOD.

water samples no. 1, 2, 4 and 5, and drinking water in PE bottle no. 5 in the ranges of 0.16 to 0.53 mgL^{-1} . DEP was found in drinking water sample (PET bottle) no. 5 and mineral water sample no 5 with the values of 0.54 and 0.11 mgL^{-1} , respectively (TLV 0.55 mgL^{-1}). DBP was found in drinking water sample (PET bottle) no. 1, mineral water sample no. 1 with the values of 0.17 and 0.33 mgL^{-1} , respectively (TLV 0.45 mgL^{-1}). The DEHP content in drinking water sample no. 1 and 4 and mineral water sample no. 1 were found in the ranges of 0.28 to 0.50 mgL^{-1} , and were not found in drinking water from PE bottles. The results are at levels which are greater than the regulatory maximum admissible concentration of 6 μgL^{-1} . The results agree with previous studies, where DEP, DBP and DEHP were found in the water samples from PET bottles. Prokupkova et al. (2002) reported that DBP and DEHP were found in mineral water at concentrations higher than that in the other water samples. Phthalate esters may migrate from the plastic bottle and the cap into the contents of bottle. People who drink water containing DEHP in excess of the maximum permitted contamination level for many years may have problems with their liver and may be at an increased risk of getting cancer (Prokupkova et al., 2002).

Conclusions

The DMP, DEP and DBP contents in drinking water samples were found to be at safe levels, while the DEHP concentration in three samples was found to be higher than the regulated maximum admissible concentration.

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