

DETERMINATION OF PHTHALATES FROM BOTTLED WATER BY GC-MS

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ABSTRACT- Determination of phthalates from bottled water by GC-MS. Phthalates are ubiquitous pollutants in the environment, due to their widespread use in the last years. These compounds are used principally as plasticizers, to impact flexibility, workability and durability to polymers but they can also be found in products such as paints, adhesives, inks and cosmetics. Phthalates are not chemically bounded to polymers; hence they are easily released and migrate into foods, beverages and drinking water from the packaging or bottling materials or manufacturing processes. This process accelerates as plastic products age and break down. With respect to their endocrine disrupting potential, phthalates such as benzyl butyl phthalate (BBP), di-butyl phthalate (DBP) and di-isobutyl phthalate (DIBP) have been found to elicit estrogenic responses in *in vitro* assays. It is possible that phthalates are a contributory factor to endocrine-mediated adverse effects observed in wildlife and humans over the past few decades. In this experiment we have analyzed the phthalates from different bottled waters purchased from the market. Determination by gas chromatography in combination with mass spectrometry detector (GC–MS) in electronic ionization mode (EI) with selected-ion monitoring (SIM) acquisition method (GC–MS (EI–SIM)) has been carried out. Methods have been developed for both qualitative and quantitative analysis of phthalates. The base peak ($m/z = 149$) of all the phthalates was selected for the screening studies. The characteristic ions of each compound were chosen for quantitative studies.

Keywords: phthalates, DBP, DiBP, BBP, bottled water

1. INTRODUCTION

Phthalates are a group of aromatic chemicals containing a phenyl ring with two attached and extended acetate groups. It is used to keep plastics soft or more flexible. They are typically colorless liquids, man-made substance, used to make plastics more flexible, soft and resilient. Because they are not a part of the chain of chemicals (polymers) that makes up plastics, they can be released fairly easily from these products. These plastics are found in products such as toothbrushes, automobile parts, tools, toys, and food packaging. Some are also used in cosmetics,

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insecticides, medical tubing, aspirin, blood storage bags and adhesives [ATSDR, 2008; Kotowska et al, 2006].

Phthalates are easily released and migrate into foods, beverages and drinking water from the packaging or bottling materials or manufacturing processes. This process accelerates as plastic products age and break down. With respect to their endocrine disrupting potential, phthalates such as benzyl butyl phthalate (BBP), di-butyl phthalate (DBP) and di-isobutyl phthalate (DIBP) have been found to elicit estrogenic responses in in vitro assays. It is possible that phthalates are a contributory factor to endocrine-mediated adverse effects observed in wildlife and humans over the past few decades [Amiridou and Voutsas, 2011].

The consumption of bottled water in Romania has been growing up the last decades similarly to the trend observed worldwide.

In this paper, the studies of gas chromatography in combination with mass spectrometry detector (MSD) for the determination of diethyl phthalate (DEP); dibutyl phthalate (DBP); di-iso-butyl phthalate (DiBP); benzylbutyl phthalate (BBP) and di-2-ethylbutyl phthalate (DEHP) have been reported.

2. EXPERIMENTAL

2.1. Reagents and materials

Phthalate esters were supplied from Cambridge Isotope Laboratories and Supelco. Dichlorometane was supplied from Labscan and anhydrous sodium sulfate was purchased from Merck.

2.2. Sample preparation

Commercially available bottled waters from randomly chosen brands were purchased from local markets. The water sample from each brand was divided in three parts. Characteristics of the examined bottled waters as shown in their labels are presented in Table 1. The first was analyzed immediately upon purchase. The other two parts of samples were stored in the fridge (at approximately 4°C degrees) and respectively at room temperatures (at approximately 24°C degrees), near heater and directly exposed to sunlight, for 20 days during the period December 2011–January 2012.

Table 1. Characteristics of the examined bottled waters

	Bottled waters				
	1	2	3	4	5
Bottle type	PET	PET	PET-1	PET	PET
Bottle volume (l)	2	2	2	2	2
Water type	natural mineral water	carbonated mineral water	natural mineral water	carbonated mineral water	carbonated mineral water
pH	7.91	5.79	7.38	5.48	4.87
Na ⁺	0.98	245	1,2	20,24	1,2
Mg ²⁺	2.88	45,7	4,7	48,51	4,23
Ca ²⁺	66.68	261,2	15,51	163,5	15,51
K ⁺	0.45	-	0,7	4,6	0,67
HCO ₃ ⁻	-	1589	72,0	762,5	69,98

Sample analysis was performed following the method of Amiridou and Voutsas, 2011. Water sample (500 mL) was transferred from the bottle to a separating glass funnel. Liquid–liquid extraction with dichloromethane (3×50 mL) was employed for the isolation of phthalates. The combined extracts were poured through a chromatographic column filled with anhydrous sodium sulfate and concentrated in the evaporator and further under a gentle stream of nitrogen to 1 mL.

2.3. Analytical determination

The analysis was performed using a gas chromatograph (Shimadzu GC-2010) coupled with a mass spectrometer (Shimadzu QP 2010) and an autosampler (AOC 20i, Shimadzu Corporation). Compounds were separated on a TraceGold TG-5MS 5% diphenyl–95% dimethyl polysiloxane capillary column (30m length, 0.25mm i.d., 0.25mm film thickness) from Thermo Scientific.

The compounds were separated using the following oven program: the column temperature was initially set at 80 °C for 2 min, then increased at a rate of 17 °C/min up to 320 °C which was maintained for 5 min. Helium carrier gas (99.9999% purity) was maintained at a constant rate of 1.2 mL/min. The temperature at the injector was 150 °C. The ion source and transfer line temperature was set at 280 °C and at 320 °C, respectively. Mass spectra were obtained using electron impact ionization (70 eV).

The identification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. Three ions were chosen to be monitored by mass spectrometer detector with selected-ion monitoring (MS-SIM) mode according to the mass spectra characteristic features obtained in the full-scan mode and by comparison with the NIST05 library reference spectral bank. To evaluate the mass spectral fragmentation pattern of each compound, a standard solution (1000 µg/L) of each compound was analyzed by capillary GC–MS in the full-scan mode, for which the target (base peaks) and qualifier ions were chosen to attain the best response in the SIM mode acquisition. By monitoring those selected ions, good sensitivity and selectivity as well as symmetrical peak shape could be achieved under the optimized capillary GC–MS(EI-SIM) conditions in a suitable analytical time (< 22 min), using locking conditions for precise retention times [Serodio and Nogueira, 2006].

The instrumental calibration was performed with standard mixtures ranging from 0.01 to 100 µg/L for the five phthalates, using the corresponding target ion abundances. An instrumental LOD of 0.05 µg/L was found for DEP, DEHP and 0.01 µg/L for DBP, DiBP and BzBP. Table 2 summarize the SIM ions selected, the retention times under lock conditions, the linear dynamic range and instrumental LODs.

The eventual laboratory contamination was monitored by analysing blank samples. They were obtain from distilled water treated in the same manner as the water samples.

Phthalates	SIM ions	Retention time (min)	Linear range ($\mu\text{g/L}$)	LOD ($\mu\text{g/L}$)
Diethyl phthalate (DEP)	149/177/65	9.413	0.01 - 100	0.05
Dibutyl phthalate (DBP)	149/73/147	11.23	0.01 - 100	0.01
Di-iso-butyl phthalate (DiBP)	149/57/41	11.81	0.01 - 100	0.01
Benzyl butyl phthalate (BBP)	149/91/65	14.003	0.01 - 100	0.01
Di(2-ethylhexyl) phthalate (DEHP)	149/167/57	15.063	0.01 - 100	0.05

Table 2. SIM ions, retention time under lock conditions, linear dynamic range and instrumental LODs achieved for the five phthalates by GC-MS(EI-SIM).

The instrumental calibration was checked with standard solution analyzed in each batch of 6 samples.

3. RESULTS AND DISCUSSION

The concentration range for phthalates in the water samples was between 7.85 to 62.1 (fig. 1).

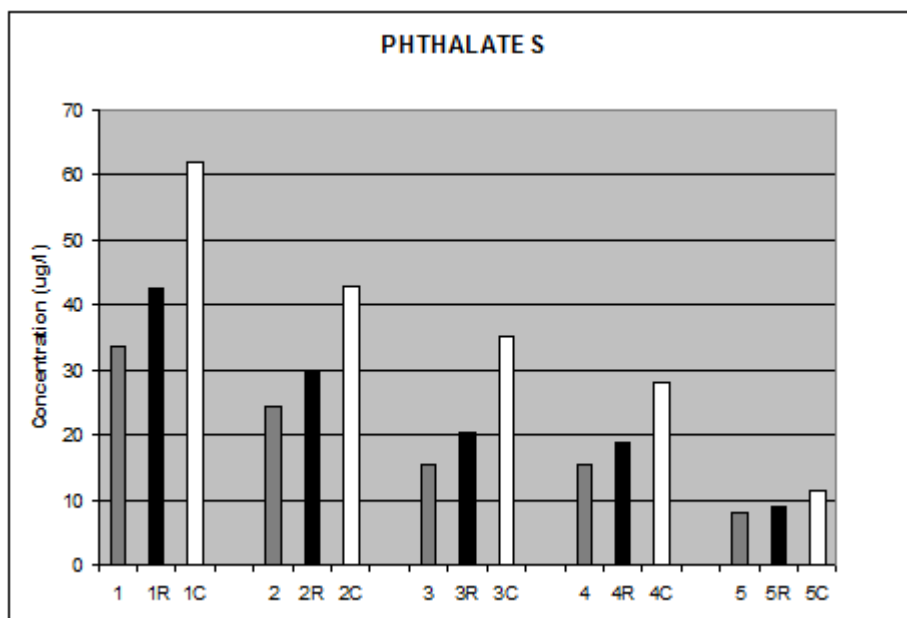


Fig. 1. Phthalates concentration under different condition; 1,2,3,4,5 are the number of analyzed sample water upon purchase; R – water was stored in the fridge at 4°C ; C- water was stored at room temperature (24°C), near a source of heat and sunlight.

In natural mineral water samples growth rate of phtahalates concentration is higher than the carbonated water samples which means that pH has an important part in phtahalates transfer from plastic to water.

DBP, DiBP and DEHP are found in high concentration (fig.2, fig.3, fig.4, fig.5). The concentration of benzyl butyl phtalate was lower than the detection limit of the metod. DEHP was found in concentrations between 1.04 for carbonated water number 5 to 17.6 in mineral water number 1 kept at room temperature (fig. 2).

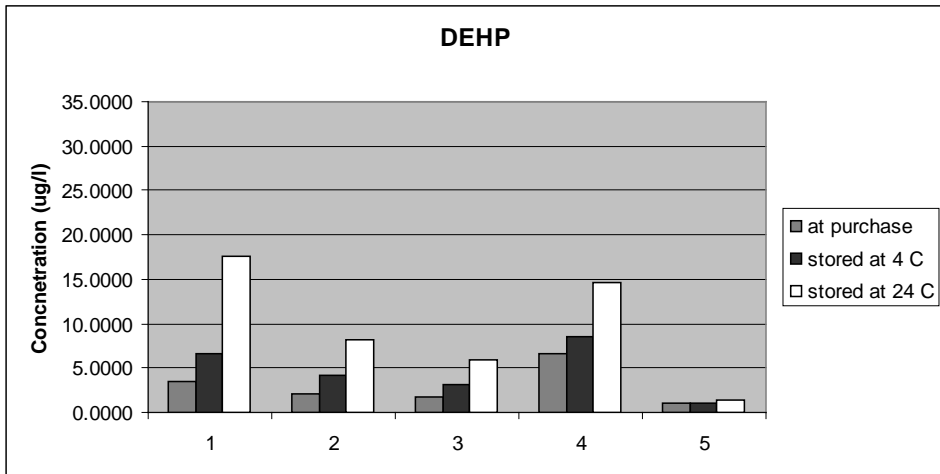


Fig. 2. DEHP concentration under different condition; 1,2,3,4,5 are the number of analyzed sample water; 4 C – water was stored in the fridge at 4° C; 24 C- water was stored at room temperature (24° C), near a source of heat and sunlight.

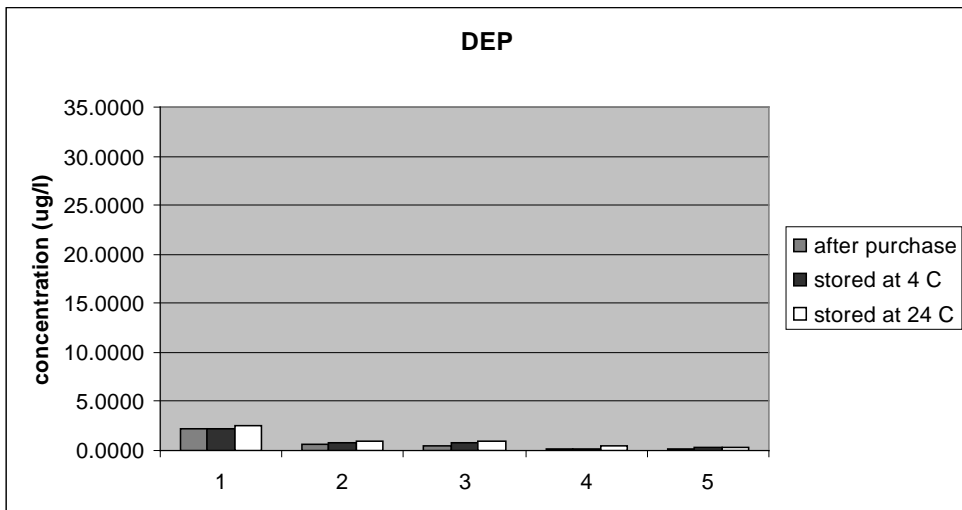


Fig. 3. DEP concentration under different condition; 1,2,3,4,5 are the number of analyzed sample water; 4 C – water was stored in the fridge at 4° C; 24 C- water was stored at room temperature (24° C), near a source of heat and sunlight.

All water samples had low concentrations for DEP ranging from 0.2 for carbonated water number 4 to 2.47 in mineral water number 1 kept at room temperature (fig. 2).

DBP and DiBP were found in high concentration in all water samples from 1.67 for carbonated water number 5 to 32.01 in mineral water number 1 kept at room temperature (fig. 2).

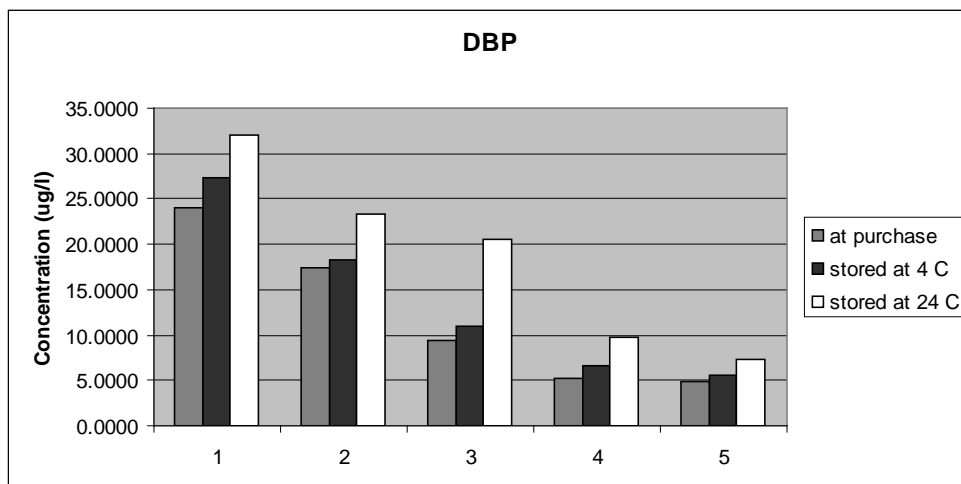


Fig. 4. DBP concentration under different condition; 1,2,3,4,5 are the number of analyzed sample water; 4 C - water was stored in the fridge at 4°C; 24 C - water was stored at room temperature (24°C), near a source of heat and sunlight.

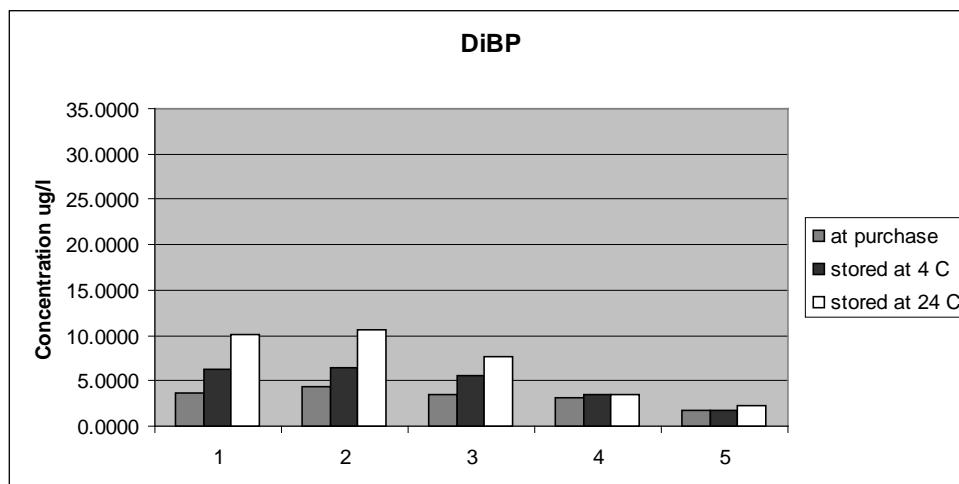


Fig. 5. DiBP concentration under different condition; 1,2,3,4,5 are the number of analyzed sample water; 4 C - water was stored in the fridge at 4°C; 24 C - water was stored at room temperature (24°C), near a source of heat and sunlight.

Our results for DBP, DEP are significantly higher than those found in Greece by Amiridou and Voutsas (2011). Also for DEHP found in Greece by Amiridou and Voutsas (2011) and Levaidara et al (2007). The results found in Canada by Cao (2007) are lower than ours for all five compounds. This can result from different kind of plastic used to fabric bottles and also the manufacturing process can increase the contamination with phthalates (Levaidara, 2007).

DEHP is the only compound regulated in United States of America by US EPA (2009) at a maximum level of 6,0 µg/l. Most of the samples analysed are below that limit at purchase, except sample number 4 which has 6,53 µg/l, but after two weeks of storage at room temperature only carbonated water number 5 is below the regulation. If we consider the limit of World Health Organisation (WHO), which is 8,0 µg/l, all the water samples at purchase time were below regulation for DEHP concentration.

In the present Romania doesn't have a regulation for phthalates in bottled or drinking water to compare the results with. European Commission has a regulation for phthalates in plastics used for food packaging, but doesn't have one for them in drinking water.

4. CONCLUSIONS

Phthalates mobilisation from water decrease by pH.

Concentration of the phthalates increased by time and temperature. Even the samples are stored at low temperature it appears a slightly increasing in each compound concentration.

The concentration of phthalates found in this study shown the necessity of further investigation on influence of storage condition and type of bottle.

Future studies are needed to establish regulation for health effect prevention in chronic exposure considering the growth of bottled water consumption.

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