PHTHALATES RELEASED FROM PLASTIC BOTTLES TO INNER DRINKING WATER WHICH THREATEN FOOD SAFETY AND PUBLIC HEALTH

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ABSTRACT

This work is an attempt to monitor the most recent hazards of phthalate(s) in bottled drinking water recently consumed in Egyptian markets. A simple and reliable method using GC-MS was validated and applied to detect the most common phthalate esters used in the manufacturing of different sizes of bottles of drinking water. Data of method validation showed that the linear range of the GC-MS calibration of the concentrations of 25, 50 & 100 ug/ ml, had a mean correlation coefficient of 0.991 ± 0.016. The detection limit was < 25 ng./ ul. and the recovery percentages were 90.6 ± 6.9 depending upon the type of phthalate congener. An experimental nested classification design, include the variables of 3 different commercial brands of bottled water in 3 different bottle sizes (1, 3 & 5 liters) stored at 3 different time that, all the random collected samples of the 3 different brands were proved to be phthalate(s)-free at the intervals of 1 and 3 months of production date. By advancing storage time, traces of di-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DOP) were determined in small and medium bottled samples of 2 of the tested brands at the interval of 6 month storage. It's worthy to mention that the common used plasticizers had only commercial names with no reference to the contents and/ or the percentages of its components of phthalate esters, which makes the process of inspection or tracing the contaminants very difficult. Thus, further work is recommended to confirm the relation between storage period, temperature and the released phthalate congeners.

KEYWORDS: Plasticizers–phthalate congeners–recovery percentage–validation–Linear range.

INTRODUCTION

Plastic products showed common and wide use in the daily life and many consumed products of all people in both developed and developing countries. It's well established that, the intentionally added plasticizers to enhance the industrial and technical properties of the plastic products were proved to be chemical hazards. Such chemical hazards are mainly phthalate esters with different degrees of toxic effects threaten human health (Saad, 2014). Phthalate plasticizers (PAE's) are defined as benzene dicarboxylic acid esters (C_6H_4-$(COO)_2$ RR') with high boiling point (280 – 400 C), low volatility and limited water solubility (Tzung et al., 2011). As an environmental chemical pollutant, phthalates are degraded slowly by the hydrolysis process of an ester bond to corresponding monoester, followed by hydroxylation of the alkyl moiety and finally mineralization (Paris et al., 2003). Many reports exhibited the increased quantities of phthalates consumed in both developed and developing countries (Saad, 2014). The European Union (EU) determined the annual production of phthalates with 6 million tons, unfortunately no data is available concerning the consumed amounts in most countries including Egypt. The wide usage of plastic products in the processes of food and bottled water packaging and filling suggest the problem of phthalate residues in packaged food and bottled water. Considering that, the history of a particular food samples and the extent of contact between the food packaging material(s) influenced the level of contamination during food shelf-life (Chen, 2007). Food inspection for the residues of phthalate congeners needs validated, accurate and reliable analytical method(s), but so far there are no available official or recommended methods to determine the residues of the different phthalate esters in the different types and kinds of foods (Saad, 2014). Only, some trials with different degrees of recovery percent and limit(s) of detection. Phthalate congeners are easily enter the environment during production and manufacturing (minor pathway), and by leaching, migration and volatilization (major pathway) during use and after disposal of the products (Chen, 2007). As plasticizers are only physically bounded to plastic products, they are easily able to migrate within the polymer and also to leave and thus enter the surrounded media or environment (Gurusakar et al., 2013). This work aims to establish and optimize a validated convenient method to determine the residues of the most common 6 phthalate esters of DMP, DEP, DBP, BBP, DEHP and DOP in the commercial bottled water marketed in great Cairo of Egypt.

MATERIALS AND METHODS

An accurate and sensitive method was validated for both qualitation and quantitation of the most 6 common and commercially used phthalates esters. These phthalate esters are dimethyl phthalate (DMP), diethyl phthalate (DEP), dibropyl phthalate (DBP), benzyl-bropyl (BBP), diethyl -hexyl phthalate (DEHP) and di-n-octyl phthalate (DOP). A nested classification experimental design involved 3 commercial brands of bottled water (A, B & C), each of 3 different sizes (≥1, 3 & 5 liters) were stored at room temperature (20 C) and withdrawn to analyze at 3 interval times (1, 3 & 6 months) of production date were adopted in this study.

MATERIALS

An individual standard and mixture of the 6 studied phthalate esters were obtained from Restek Corp., United Kingdom. The mixture and/ or each of DMP, DEP, DBP, BBP, DEHP and DOP were dissolved in methanol at concentrations of 2 mg/ ml., each. All the adopted solvents in the techniques of liquid/ liquid partitioning; methanol, dichloromethane, ethyl acetate, acetone and hexane were of chromatographic grade (PA-ACS-Areca-EU). Other reagents include florisil, anhydrous sodium sulphate and other salts were obtained from Sigma-Aldrich Co., USA.

Gas Chromatography with Mass Spectrum detection (GC-MS) was the instrument of choice applied in this work as recommended by previous studies (Mohammed et al., 2013). The column used was TG-5MS fused silica capillary dimensioned (30 m x 0.25 mm x 0.1 mm-film thickness). The carrier gas was helium at a constant flow rate of 1 ml./ min. The temperature program is initiated at 40 C (hold 3 min.) to 280 C as a final temperature, at 5 C (hold 5 min.) and the injection temperature was 280 C and electron ionization energy of 70 eV was used for GC-MS detection.

METHODS

Referring to the available published methods which were based on the chemical and physical properties of the determined phthalates and the nature of food matrices. So, the adopted technique in this current work is liquid/ liquid partitioning using initially 3 different solvent systems; ethyl acetate, dichloromethane and hexane-acetone (80: 20 v/v). 200 ml. of bottled drinking water samples were evaporated in rotary apparatus at 55 C up to reach 10 ml. Replicates of evaporated samples were mixed with 20 ml. each of, ethyl acetate, dichloromethane and hexane-acetone (80:20 v/v) in separatory funnels. Then the solvents of each system were evaporated under stream of nitrogen at 50 C. Clean-up, if necessary, was

conducted using florisil in suitable glass columns. Three sets of spicked water samples with 25, 50 & 100 ug./ ml of each of the 6 studied phthalates were employed with each of the 3 different systems of extracts.

Validation of the method

Data revealed that the solvent polarity of hexane-acetone (80:20 v/v) is the best solvent system for the extraction of the 6 studied phthalate residues. Such solvent system is capable to extract more than 96% of the added concentrations of phthalate esters up to 25 ug./ml. So, the mixture of hexane-acetone (80:20 v/v) was used in this study as the solvent system of choice to extract phthalate residues from bottled water samples.

The adopted method of analysis were previously validated for linearity, accuracy, precision, detection limit and the percentages of recovery as recommended by (ICH, 2005).

Linearity was evaluated via the different injected volumes, each of 1 ul. at 3 different successive concentrations of 25, 50 and 100 ng/ ml. of the 6 studied phthalates. The plotted peak height of the standards was evaluated using the regression equation and correlation coefficient as presented in Table 1.

The values of the calibration linear regression curve equivalent to $y = \sqrt{ax + b}$, where (y) is the response peak height, (x) is the mass of the studied phthalates. The precision is indicated as relative standard deviation or the ratio of (SD) to the mean calibration factor (RSD = SD/CF. The detection limit(s) were estimated based on a signal to noise ratio of 3 successive injections. Both the accuracy (R), and repeatability (r) were determined in conjunction with the calibration study, as follows (r = $\sqrt{b\,x.y\;x\;b\,y.x}$) , where b x.y = $\sum x.y/\,SS\,x$ and b y.x = $\sum y.x/SS\,y.$ The percentages of recovery were evaluated by spiking blank water samples with the 6 studied phthalates and comparing the relative peak height(s) with the adopted standard chromatogram.

RESULTS AND DISCUSSION

Table 1 show the calibration data of spiked blank water samples with the 6 phthalate esters at 3 different concentrations of 25, 50 & 100 ng./m. (n = 3).

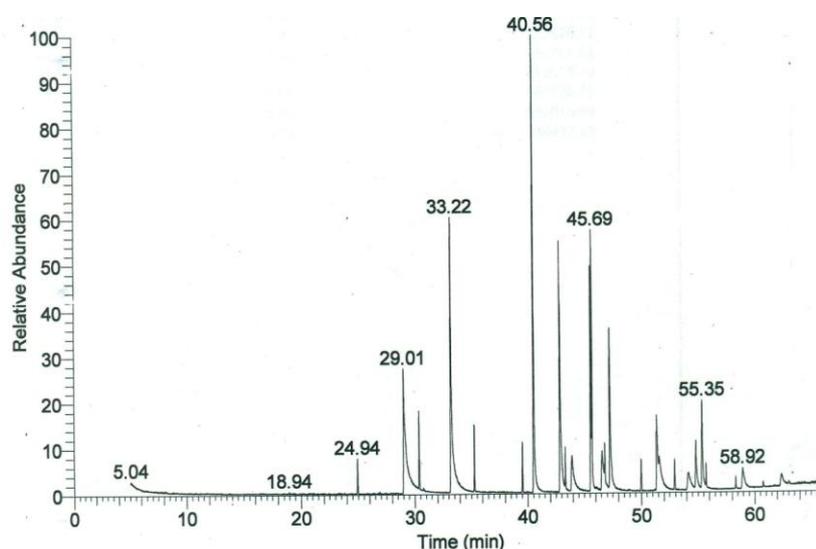| Phthalate esters	Retention time(Rt)	Regression Curve	Accuracy (R)	Less Detect. concent.
DMP	29.10	Y = 1.9 x + 0.3	0.993	25 ng/ ml.
DEP	33.22	Y = 3.1 x + 0.4	0.991	25 ng./ ml
DBP	40.56	Y = 8.2 x + 1.4	0.989	25 ng./ ml
BBP	45.69	Y = 6.3 x + 1.2	0.998	25 ng./ ml
DEHP	55.35	Y = 3.6 x + 0.7	0.990	25 ng./ml
DOP	58,92	Y = 2.7 x + 0.6	0.988	25 ng./ ml
Mean			0.991±0.016	

Figure 1.The retention time (min) versus the relative abundance of 6 phthalates by GC-MS. Injected volume 1 ul containing 25 ng, each of DMP, DEP, DBP, BBP, DEHP & DOP.

The validity and suitability of this current method was evaluated and applied to determine DMP, DEP, DBP, BBP, DEHP & DOP residues in bottled water. Full scan acquisition and selected ion monitoring were applied to detect phthalate residues with accepted accuracy and sensitivity of both qualitative and quantitative estimations. Individually, the studied phthalates were determined by comparing their retention time (qualitative) and mass fragments versus peak area and peak height of each in the mixture at the m/z range of 50-400. Calibration curves from GC-MS were studied for each target phthalate and exhibited accepted linearity over the studied concentration levels within a mean concentration coefficient of 0.991 ± 0.016.

Table 2 show the accuracy and precision of the studied 6 phthalates standards at 25, 50 & 100 ng./ ul. (n= 3).

Phthalates	Concentrations (ng./ ul)			Av. Ac.%	Av.Pr. %
	25	50	100		
DMP	0.222	0.470	0.910	1.18 %	3.12 %
DEP	0.314	0.612	1.150	2.71 %	3.22 %
DBP	0,266	0.488	0,870	3.10 %	3.74 %
BBP	0.315	0.590	1.100	3.07 %	3.56 %
DEHP	0.360	0.710	1.310	2.96 %	3.02 %
DOP	0.284	0.530	1.101	3.11 %	3.11%
Overall mean ± SD				2.69 ± 0.96	3.29 ± 0.36

Table 3 show the percentages of recovery of spiked blank water samples at 25, 50 & 100 ng./ ul. (n = 3).

Phthalates	Concentrations (ng./ ul)			Average Recovery %	Coeffic. Of Var. %
	25	50	100		
DMP	84.6	86.5	88.1	86.4 %	4.8 %
DEP	83.7	81.4	84.6	83.2 %	5.3 %
DBP	95.7	96.7	97.1	96.5 %	4.3 %
BBP	96.8	97.4	96.8	97.0 %	3.9 %
DEHP	91.4	95.3	98.4	95.0 %	4.2 %
DOP	83.6	87.1	86.3	85.7 %	4.7 %
Overall mean ± SD				90.6 ± 6.9	4.5 ± 0.7

Data exhibited that exclusively the analyzed samples of the 3 different brands of the 3 different bottle sizes were phthalates-free after one and 3 months of production. By advancing storage time, traces of DEHP and/ or DOP were observed in small and medium bottled water samples of 2 brands after 6 months of production. The positive samples showed low concentrations ≤ 25 ng./ ul (the less detectable amount of this method). Simultaneously, the 4 phthalate esters of DMP, DEP, DBP and BBP did not detect in any of the 81 analyzed samples.

The current data exhibited that all samples were DMP, DEP, DBP and BBP- free, while the positive samples were only contaminated with traces of DEHP and/ or DOP. Thus, the short alkyl chain phthalate esters were not detected in this current study. Contrary, Staples et al. (1997) reported that the short alkyl chain phthalates show the highest solubility of up to 4000 mg/ L. (DMP), while long alkyl chain phthalates are rarely water soluble. They added that, the degradation rates of phthalates influenced by the environmental media. In acidic media, phthalates are so stable and degradation seemed to be limited by accessibility of oxygen, as

well. Besides, temperature and nutrient content are significantly influenced the degradation rate of phthalates (ECPI, 2003).

Table 4. show phthalate residues in 3 brands of bottled drinking water samples with 3 different bottle sizes at 1, 3 & 6 months of production (n = 3).

Phthalate esters		Brands								
		A			**B**			**C**		
		1	3	6	1	3	6	1	3	6
DMP	S	---	---	---	---	---	---	---	---	---
	M	---	---	---	---	---	---	---	---	---
	L	---	---	---	---	---	---	---	---	---
DEP	S	---	---	---	---	---	---	---	---	---
	M	---	---	---	---	---	---	---	---	---
	L	---	---	---	---	---	---	---	---	---
DBP	S	---	---	---	---	---	---	---	---	---
	M	---	---	---	---	---	---	---	---	---
	L	---	---	---	---	---	---	---	---	---
BBP	S	---	---	---	---	---	---	---	---	---
	M	---	---	---	---	---	---	---	---	---
	L	---	---	---	---	---	---	---	---	---
DEHP	S	---	---	---	---	---	-++	---	---	--+
	M	---	---	---	---	---	--+	---	---	--+
	L	---	---	---	---	---	---	---	---	---
DOP	S	---	---	---	---	---	--+	---	---	--+
	M	---	---	---	---	---	--+	---	---	---
	L	---	---	---	---	---	---	---	---	---

Brands: (A, B, & C). Bottle sizes: small (S) ≤ 1, medium (M) ≤ 3, Large (L) = 5 liters.

Plastic bottles are widely used as containers of drinking water in Egypt. Such growing industry based on the cheap manufacturing materials and process of plastic bottles. The plasticizers of phthalate esters introduce this industry at concentrations could reach up to 60% of the total mass of the end product (Tzung et al., 2011). Sure, plasticizers enhanced the technical and industrial properties of the product including hardness, transparency, flexibility and tightness. But such phthalate esters are easily able to migrate within the polymer and thus enter the surrounded area and environment because of the weak physical bond between esters and the plastic products (Gurusankar et al., 2013). The rapid growing and wide spreading of the usage of plastic.

Products in the different processing steps and packaging of food, beside the unavoidable leached amounts resulted from our modern life products, alarming the scientists and decision makers to give more attention to the problem of phthalates-contamination. The problem of

phthalates determination is still facing all authorities and concerned researchers dealing with food control and environment protection, so there is an urgent need to more efforts aiming to establish an accurate and validated method(s) to determine phthalate residues in different food samples and matrices (Saad, 2014).

CONCLUSION

Although, the presented data showed negligible levels of phthalates-contamination with no significant occurrence in bottled drinking water, there is an urgent need to; firstly, develop more reliable and validated methods of analysis suites the different matrices of foods. Secondly, to determine and establish the relation between the concentration of the released phthalates, temperature and storage time of packaged foods during their shelf life. Thirdly, to initiate extensive studies of risk assessment dealing with phthalate(s) hazards and considering the unavoidable multi-sources of exposure to such hazards.

ACKNOWLEDGEMENT

REFERENCES

1. Chen, M. L., et al. The internal exposure of Taiwanese to phthalate esters - An evidence of intensive use of plastic materials. Envir. Int., 2007; 34: 79-85.

2. ECPI (2003). European Council for plasticizers and intermediates. www.ecpi.org.

3. Gurusankar, S. et al. Biomonitoring of phthalate metabolites in The Canadian population through the Canadian health Measures survey. Int. J. of Hyg. And Envi. Health, 2013; 216: 652 -661.

4. International Conference on Harmonization "ICH" (2005). Validation of Analytical procedures. Text and methodology. www.ich.org/fileadmin/public_web_site/ICH.

5. Mohammed, F.Z. et.al. (2013). Determination of phthalates in Jordanian Bottled water using GC-MS and HPLC-UV. J. of Chromatog. Sci., Adv. Access Published June 5, 2013.

6. Saad, M. M. The most recent hazards of phthalates that threaten Food safety and human health. Int. J. of Med. Sci. & Clinical Inventions, 2014; 1(10): 527-535.

7. Staples, C. et al. The environmental fate of phthalate esters. Chemosphere, 1997; 35: 667 – 749.

8. Paris, I. et al. Simultaneous determination of dimethylhexyl Phthalate and mono ethyhlhexyl phthalate in human Plasma by HPLC. Anal. Let., 2003; 36: 2649 – 2658.

9. Tzung, Hai et al. Food safety involving ingestion of foods and Beverages prepared with phthalate plasticizers containing. Clouding agents. J. of the Formosan Med. Ass., 2011; 110: 671 684.