ANALYSIS OF PHTHALATE PLASTICIZER IN JORDANIAN BOTTLED WATERS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROPHOTOMETRY (LC-MS/MS)

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Abstract

A promising method for the quantitative determination of phthalate esters was optimized in nine different Jordanian commercial bottled water samples using liquid chromatography tandem -mass spectrometry (LC-MS/MS). The present study described an efficient method for the determination of this compound using LC-MS/MS as the analysis technique. The compound was separated with C18 Thermo fisher gold column (100mm x 5µm) through a flow rate of 1ml/min using isocratic mobile phase). The method was validated using triplicate analyses, recovery experiment and statistical analysis. Liquid- liquid extraction was employed as a pre-concentration step with methanol as the extraction solvent and methanol: ammonium acetate: de-ionized water: formic acid (68%:30%:2%:0.1%) as the mobile phase. The molecular ion was found to be m/z 390.56 and the base peak was found to be m/z 149. The correlation coefficient (R^2) was found to be 0.9996. The LOD and LOQ were found to be 0.02ng/ml and 0.06 ng/ml respectively. This method showed satisfactory recovery of 98.08%, CV of 0.960%, SD of 0.006, error of 0.929 and 95% CI of 0.960 ± 0.007 . The ranges of the calculated concentrations of the commercial mineral bottled waters were found to be from 0.729-1.26ng/ml.

Keywords: Phthalate esters, plasticizer, LC-MS/MS

Introduction

The main components of plastic bottles are plasticizers (Coltroa et al., 2014). The addition of plasticizers offers plastic strength and flexibility (Rahman and Brazel, 2004). Plastic involved in plastic water bottles has two major forms: Polyvinyl chloride plastics (PVC) and polycarbonate plastics. PVC is mainly consisted of commercial plasticizer known as phthalates, while polycarbonate plastics are composed of a chemical called bisphenol-A (BPA) (Ventrice et al., 2013).

Several studies have suggested that both phthalates and bisphenol-A are associated with various health impacts including hormone disrupting chemicals, infertility, premature puberty, asthma, allergies, menstrual cycle irregularities and breast cancer and prostate cancer (Martino-Andrade and Chahoud, 2010; Singh and Li, 2012).

In their study, Goulas *et al* (1998) reported that there is a possibility for materials stored in plastic including water, dairy products, foodstuffs and beverages to be affected by plastic additives such as antioxidants, plasticizers residual monomers and stabilizers from the plastic packaging materials into the contained foodstuffs through a phenomenon of immigration.

According to Agency for Toxic Substances and Disease Registry (2000) and David *et al* (2001), phthalate esters are considered as the main plasticizers that have been approved by FDA to be utilized in food-contact plastics.

From a chemical point of view, phthalate esters are either mono or dialkyl aryl esters of phthalic acid (also called 1,2-benzenedicarboxylic acid). It has been shown that the addition of phthalate esters to plastics, they work to make the long polyvinyl molecules sliding against each another (Baloyi, 2012). Phthalate esters are also colourless, odourless liquids produced by reacting phthalic anhydride with an appropriate alcohol (Kembra et al., 2008).

it has been indicated that phthalates to be a continuous environmental harmful because of their widespread use. Furthermore, their physical properties help in immigration into beverages , foods, and drinking water, dairy products from the packaging or bottling material or manufacturing processes (Balafas *et al*., 1999; Wahl *et al*., 1999). Other studies such as that conducted by Schettler (2006) pointed to accumulation of phthalates in fatty foods including milk and dairy products, fish, meat and vegetable oils. It has also been suggested by the same study that the major source of phthalate to due to dietary intake from adulterated food.

Phthalates are viewed as endocrine disrupting chemicals that have the ability to show carcinogenic effects (McKee *et al*., 2004). It has been realized that phthalate esters are potentially hazardous for human health and environment and have been listed as priority substances by many national and international regulatory organizations. As an example, the European Union (EU) issued a list of substances with evidence or potential endocrine disrupting action including di-n-butyl phthalate, butyl benzyl phthalate and Di-2-Ethylhexyl Phthalate (DEHP). Furthermore, because DEHP is the most widespread phthalate produced and used, it has been included in the list of priority substances in the field of water policy established by EU (European Union Council, 2001). According to the World Health Organization (WHO), a guideline has been established to consider limit value of 8.0 μg L^{-1} phthalate esters in fresh and drinking waters (WHO, 2003).

Materials and Methods

Apparatus Assembly

A chromatographic system of model API 300 Applied Biosystem consisted of an Agilent stable-bond C18 Thermo fisher gold column (100mm x 5μm) with in-built detector was used. The guard and analytical columns were mounted in a thermo stated column compartment set at 30 °C. The peak areas were integrated automatically by computer using Analyst software version 1.4 (AB Sciex) software program. Other apparatus used included a centrifuge 230VAC/3A, centrovap-consol 1011, water bath 1083 and vortex.

Chemicals used

Pure dioctyl phthalate plasticizer with analytical grade methanol, ammonium formatted, formic acid and acetone were purchased from Sigma –Aldrich Chemicals, in Jordan. All reagents were analytical grade. All reagents and water were checked for contamination with phthalates before use.

Procedure for high-performance liquid chromatography

Chromatographic conditions

Solutions and mobile phases were prepared at the time of use. The mobile phases used were methanol:ammonium acetate:deionized water: formic acid (68:30:2:0.1 v/v/v/v) (pH 3.5). The analytical column used was C18 Thermo gold (100mm x 5μm) . All analysis were done under isocratic conditions at a flow-rate of 1.0 ml min^{-1} and at room temperature.

Standard Solutions

Fifty mg DOP reference standard were accurately weighed and transferred to 50-mL volumetric flask. It was then diluted to volume with methanol to obtained 1mg/ml. 0.25 ml of standard stock solution was then transferred into a 50-mL volumetric flask and diluted to volume with methanol as mixed stock solution. Further dilution with methanol was done and the following concentration range 0.00 ~ 50.00 ng/ml was obtained and used as working standard solutions.

Sample Solution Preparation

Five ml of the sample was accurately weighed and transferred to a 50-mL volumetric flask and 40 mL of methanol was added, sonicated for 30 minutes and then cooled to room temperature. The mixture was diluted with methanol to volume and shaked vigorously to ensured thorough mixing. It was then allowed to stand for ten minutes, 5ml aliquot was transferred to a glass tube and centrifuged at 3500 rpm for 10 min. The filtered supernatant was used as the sample solution.

Calibration and linearity

Calibration curve was constructed in the range 0.00–50.0 ngml^{-1} to encompass the expected concentrations in measured samples. Curve was obtained by plotting the peak area of the pure phthalate against concentrations of the phthalates. Linear calibration curve was generated by linear regression analysis and obtained over the respective standard concentrations ranges.

Analytical recovery

Absolute recoveries of 5 different concentrations of phthalate (0.00–50.0 ngml^{-1}) in commercial bottled waters were determined by assaying the samples as described above and comparing the peak areas of both phthalates with those obtained from direct injection of the compounds dissolved in the processed blank sample.

Precision and accuracy

The precision and accuracy of the assay was ascertained based on analysis of quality control samples. Five replicate quality control samples at each concentration were analyzed and the means, standard deviations (SD) and coefficients of variation (C.V.) were calculated by standard methods.

Study Findings

Linearity, LOD, and LOQ

Standard solution series with concentration range of 0.00 to 50 ng/mL were prepared by diluting the stock solutions in methanol. The standard solutions were directly injected in triplicate. Peak areas were plotted against the amount of the analyte injected, and linear regression equation was obtained. The linear ranges was ~ 0.00–0.50 ng/ml, and the correlation coefficient was found to be 0.9996, as showing in figure 1.

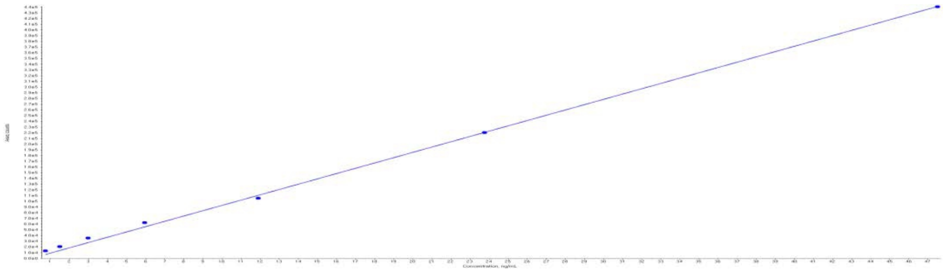


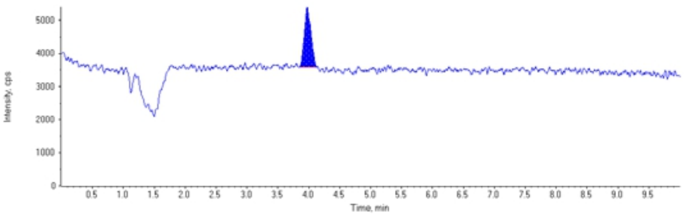
Fig 1. Calibration Graph of Di-n-Octyl Phthalate
y = 9.29e+003 x (r = 0.9996)

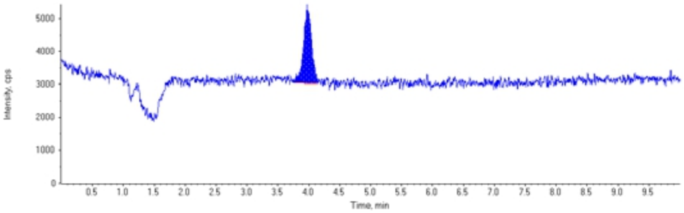
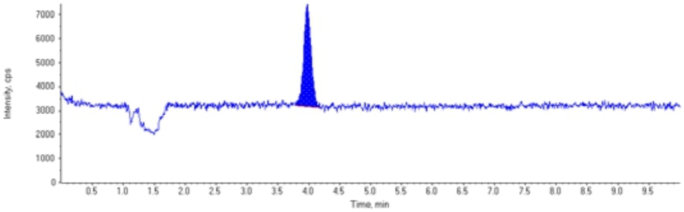
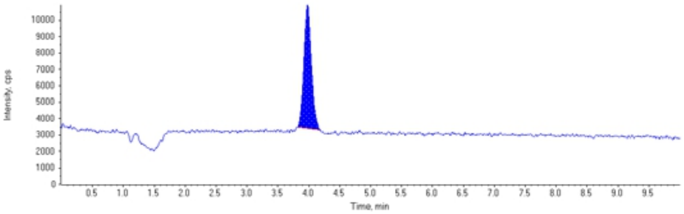
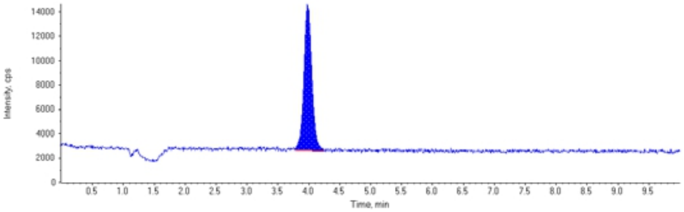
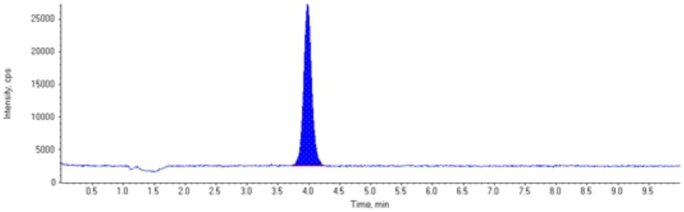
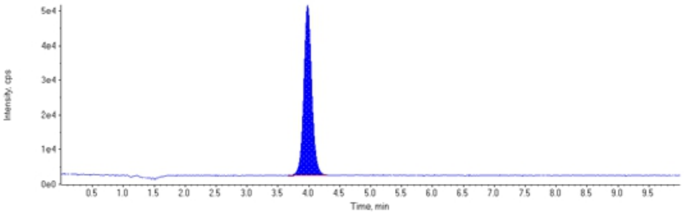
The results obtained for LOD and LOQ are listed in Table 1. The LOD was found to be 0.02ng/ml where as the LOQ was found to be 0.06ng/ml.

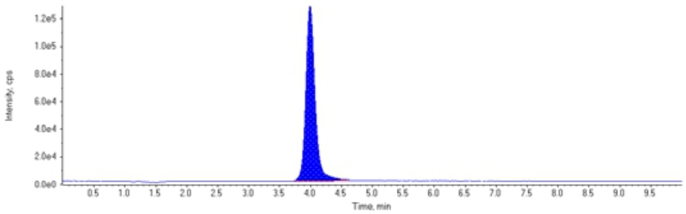
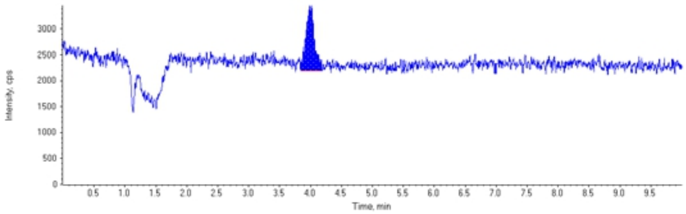
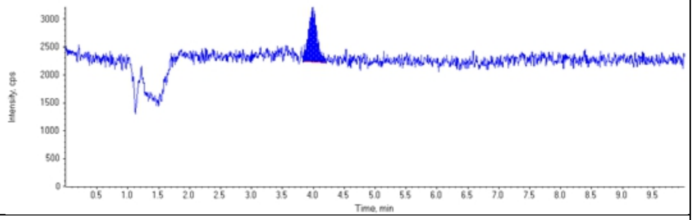
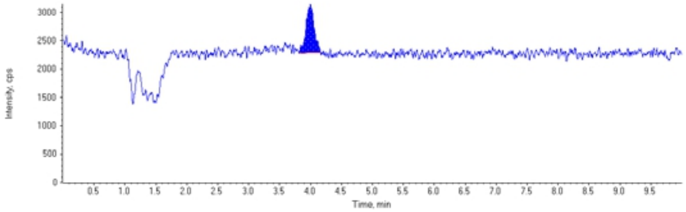
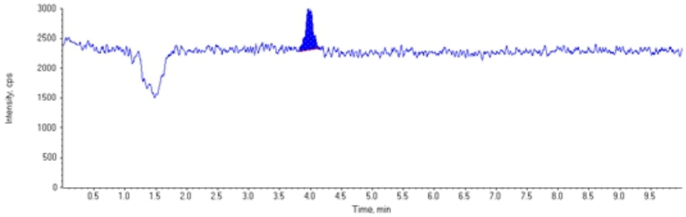
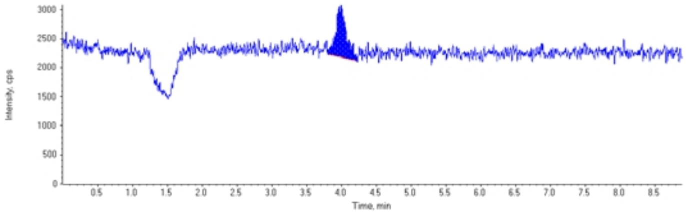
Table 1: Recoveries, CV, SD, LOD, LOQ, CV and Error Values Obtained from the Standard Addition Method in Commercial Bottled Water Samples.

Replicate Samples	Conc. Of DOP (ng/ml)	Amount added (ng/ml)	Amount found (ng/ml)	Recovery %
1	0.965	50	49.700	99.40
2	0.965	50	49.000	98.00
3	0.95	50	48.400	96.80
4	0.96	50	48.800	97.60
5	0.96	50	49.300	98.60
Average	0.960	50.000	49.040	98.080
SD	0.006	0.000	0.493	0.986
CV (%)	0.63	0.00	1.01	1.01
LOD	0.02			
LOQ	0.06			
Error	0.929			
95% CI	0.96_0 ± 0.007			

Total ion chromatograms obtained from the LCMS/MS was shown in Figure 2. The peaks exhibited sharp and symmetrical peak shapes.

<table>
<tr><td><i>STD1</i></td><td></td><td rowspan="5">
</td></tr>
<tr><td><i>RT (Exp. RT):</i></td><td><i>3.97 (3.98) min</i></td></tr>
<tr><td><i>Calculated Conc:</i></td><td><i>1.44 ng/mL</i></td></tr>
<tr><td><i>Area:</i></td><td><i>1.34e+004</i></td></tr>
<tr><td><i>Sample Type:</i></td><td><i>(Standard)</i></td></tr>
</table>

STD2		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***2.22 ng/mL***	
Area:	***2.07e+004***	
Sample Type:	***(Standard)***	
STD3		
RT (Exp. RT):	***3.98 (3.98) min***	
Calculated Conc:	***3.84 ng/mL***	
Area:	***3.57e+004***	
Sample Type:	***(Standard)***	
STD4		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***6.73 ng/mL***	
Area:	***6.26e+004***	
Sample Type:	***(Standard)***	
STD5		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***11.4 ng/mL***	
Area:	***1.06e+005***	
Sample Type:	***(Standard)***	
STD6		
RT (Exp. RT):	***3.98 (3.98) min***	
Calculated Conc:	***23.7 ng/mL***	
Area:	***2.21e+005***	
Sample Type:	***(Standard)***	
STD7		
RT (Exp. RT):	***3.98 (3.98) min***	
Calculated Conc:	***47.4 ng/mL***	
Area:	***4.41e+005***	
Sample Type:	***(Standard)***	

STD8		
RT (Exp. RT):	***3.99 (3.98) min***	
Calculated Conc:	***141. ng/mL***	
Area:	***1.31e+006***	
Sample Type:	***(Unknown)***	
AL-FANNAZ		
RT (Exp. RT):	***4.02 (3.98) min***	
Calculated Conc:	***1.26 ng/mL***	
Area:	***1.17e+004***	
Sample Type:	***(Unknown)***	
ABU-RASHED		
RT (Exp. RT):	***3.99 (3.98) min***	
Calculated Conc:	***0.931 ng/mL***	
Area:	***8.65e+003***	
Sample Type:	***(Unknown)***	
VIVIAN		
RT (Exp. RT):	***4.00 (3.98) min***	
Calculated Conc:	***0.838 ng/mL***	
Area:	***7.78e+003***	
Sample Type:	***(Unknown)***	
ULTRA		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***0.633 ng/mL***	
Area:	***5.88e+003***	
Sample Type:	***(Unknown)***	
AL-MAS		
RT (Exp. RT):	***4.00 (3.98) min***	
Calculated Conc:	***0.984 ng/mL***	
Area:	***9.15e+003***	
Sample Type:	***(Unknown)***	

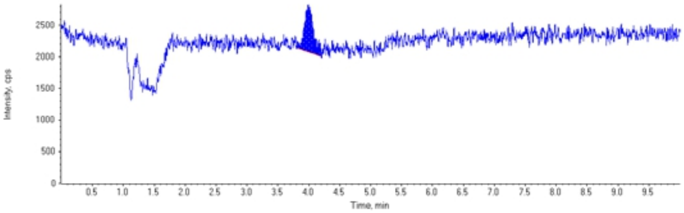
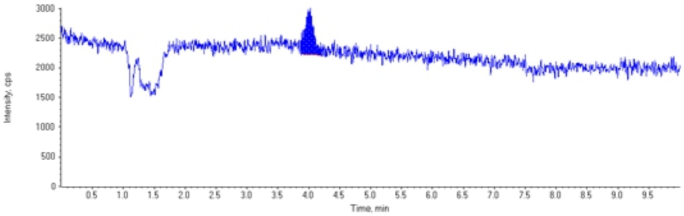
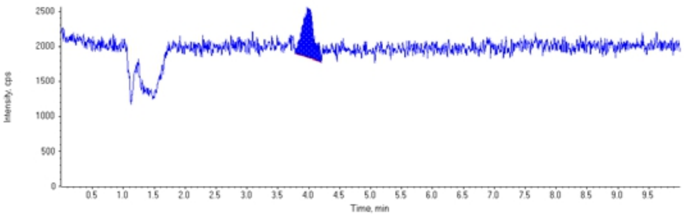
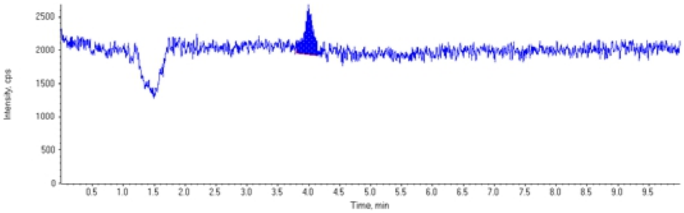
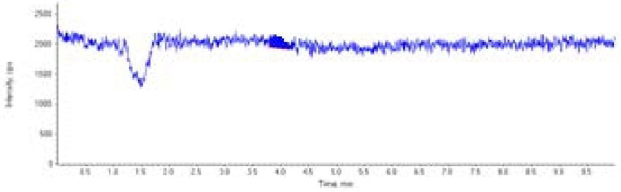
AQUAFINA		
RT (Exp. RT):	***3.99 (3.98) min***	
Calculated Conc:	***0.803 ng/mL***	
Area:	***7.46e+003***	
Sample Type:	***(Unknown)***	
GHADEER		
RT (Exp. RT):	***4.02 (3.98) min***	
Calculated Conc:	***0.781 ng/mL***	
Area:	***7.25e+003***	
Sample Type:	***(Unknown)***	
AL-JOUD		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***0.965 ng/mL***	
Area:	***8.96e+003***	
Sample Type:	***(Unknown)***	
AL-RUFEI		
RT (Exp. RT):	***3.99 (3.98) min***	
Calculated Conc:	***0.729 ng/mL***	
Area:	***6.77e+003***	
Sample Type:	***(Unknown)***	
De-Ionized		
RT (Exp. RT):	***3.97 (3.98) min***	
Calculated Conc:	***0.00 ng/mL***	
Area:	***0.00***	
Sample Type:	(Unknown)	

Figure 2: Total ion chromatograms of the pure DOP phthalate and the samples

Recovery and accuracy

The absolute recovery was calculated by comparing the areas under the peaks obtained from standard working solutions with the peak-areas from standard samples. The replicate samples were analyzed and the results shown in Table 2 demonstrated that the average recovery of phthalate was 98.08%. The replicability of the analytical method was excellent and the coefficient of variation was 0.63%.

Analytical applications

The recommended analytical method has been applied to the determination of nine commercial brands of bottled waters in order to identify this phthalate, and to validate the applicability and reliability of this method with real samples. The results were listed in Table 2. The results show a remarkable presence of phthalates in all the commercial brands of bottled waters analyzed.

Table 2: Concentrations of Di-n-Octyl phthalate in the pure phthalate and bottled water samples

Sample Name	**Sample Type**	**Area (cps)**	**RT (min)**	**Target [Conc]. (ng/ml)**	**Calculated Conc. (ng/ml)**
STD1	*Standard*	*1.340e+04*	*3.97*	*0.743*	*0.743*
STD2	*Standard*	*2.070e+04*	*3.97*	*1.49*	*1.55*
STD3	*Standard*	*3.570e+04*	*3.98*	*2.97*	*3.1*
STD4	*Standard*	*6.260e+04*	*3.97*	*5.94*	*6.43*
STD5	*Standard*	*1.060e+05*	*3.97*	*11.9*	*11.4*
STD6	*Standard*	*2.210e+05*	*3.98*	*23.8*	*23.7*
STD7	*Standard*	*4.410e+05*	*3.98*	*47.5*	*47.4*
STD8	*Unknown*	*1.310e+06*	*3.99*	*N/A*	*141.*
AL-FANNAZ	*Unknown*	*1.170e+04*	*4.02*	*N/A*	*1.26*
ABU-RASHED	*Unknown*	*8.650e+03*	*3.99*	*N/A*	*0.931*
VIVIAN	*Unknown*	*7.780e+03*	*4.00*	*N/A*	*0.838*
ULTRA	*Unknown*	*5.880e+03*	*3.97*	*N/A*	*0.633*
AL-MAS	*Unknown*	*9.150e+03*	*4.00*	*N/A*	*0.984*
AQUAFINA	*Unknown*	*7.460e+03*	*3.99*	*N/A*	*0.803*
GHADEER	*Unknown*	*7.250e+03*	*4.02*	*N/A*	*0.781*
AL-JOUD	*Unknown*	*8.960e+03*	*3.97*	*N/A*	*0.965*
AL-RUFEI	*Unknown*	*6.770e+03*	*3.99*	*N/A*	*0.729*
De-IONIZE	*Unknown*	*0.00*	*3.97*	*N/A*	*0.00*

Discussion

The purpose of this research work is to develop an accurate, precise, cheaper, simpler, reproducible and sensitive LCMS/MS method for the quantitative analysis of di-n-octyl phthalate in Jordanian bottled waters. The method was found to be promising without addition of any internal standard. The analyte was ionized by positive mode. Fragmentation of the molecular ions produced fragment ions which were suitable for quantification. A full scan ms protocol was used. The parent mass was found to be 390.56 m/z, scan range of m/z 50-450 and the base peak was found to be m/z 149**.**

Extraction solvents used were methane, ammonium acetate, de-ionized water and formic acid. The formic acid added yielded a better resolution far better than its absence.

The correlation coefficient was found to be 0.99996 and the regression equation was found to be y = 9.29e+003x as shown in figure 1. The respective chromatograms for both pure phthalate and samples were listed in figure 2. The mass spectrum was also shown in figure 2. The achieved limit of detection (LOD, based on SD of 5 measurements of replicate samples x 3) and the limit of quantification (LOQ, based on SD of 5 measurements of replicate samples x 10) as shown in Table 1 were found to be 0.02 and 0.06ng/ml respectively. The confidence interval was computed to be $0.965_0 \pm 0.007$. The absolute recovery was calculated by comparing the areas under the peaks obtained from standard working solutions with the peak-areas from standard samples. The method shows a satisfactory average recovery of 98.08±0.063 in the commercial bottled waters. The concentration of each sample was measured in triplicate. The range of average calculated concentrations was 0.00ng/ml – 50.00 ng/ml as listed in Table 2. The present study revealed that there is little or no interference, hence no endogenous or exogenous substances were observed during the analysis. The retention times range were 3.97-4.02 min as listed in Table 2. The range of concentrations of the pure anayte concentrations in the commercial bottled waters was analyzed to be 0.743-47.4ng/ml as listed in Table 1. Also the range of concentrations of anayte in the commercial bottled waters were analyzed to be 0.729-1.26ng/ml as listed in Table 1. From the results, it was found out that, the sample with the least amount of di-n-octyl phthalate was Al-Rufei bottled water with concentration of 0.729ng/ml while the sample with the highest concentration of di-n-octyl phthalate was Al-Fannaz bottled water. From Table 1 and figure 1 above, it was found out that the de-ionized water did not contain any amount of phthalate

In comparison with the work of Lin et al (2011), on Simultaneous Determinaton of Nine Types of Phthalate Residues in Commercial Milk Products Using HPLC-ESI-MS-MS. The linear range used was 0.02-0.40µg/ml, correlation coefficient of 0.9913, average recoveries of 65.8%, RSD of 8.2% , LOD of 15 µg/kg and LOQ of 50 µg/kg were obtained for di-n-octyl phthalate also using liquid-liquid extraction with similar mobile phases. Therefore the analysis employed for di-n-octyl phthalate in this research work was found to be better in terms of recovery, LOD, LOQ, RSD and correlation coefficient (R^2) in comparison with the work of Lin et al (2011) mentioned above.

Conclusion

The newly developed method of liquid chromatography tandem mass spectrophotometry (LC-MS/MS) using the MRM mode has proved to be a sensitive, selective and reliable approach for the quantitative determination of di-n-octyl phthalate in commercial brands of bottled waters . The method was found to be easier, cheaper, simpler, faster, precise, accurate , sensitive and reliable for the quantitative determination of di-n-octyl phthalate in commercial brands of bottled waters in comparison with the work of Lin et al (2011). This shows that the most portable commercial bottled water amongst the samples analyzed was Al-Rufei bottled water.

From the results obtained we can deduce that the commercial brands of bottled waters contain moderate amount of di-n-octyl phthalate and the de-ionized water that was used as control contain zero amount of phthalate**.** It can also be deduced that the amount of di-n-octyl phthalate present was found to be below the U.S. ATSDR Minimal Risk Level (MRL) of Oral intermediate exposure of 0.4 mg/kg/day, based on hepatic effects.

References:

Agency for Toxic Substances and Disease Registry (2000). Toxicological profile for di (2-ethylhexyl) phthalate (DEHP) Final Report. ATDSR/TP-9, Public Health Service, US Department of Health and Human Services, Atlanta.

Balfas, D., k.J. Shaw, F.B. Whitefield (1999). Phthalate and adipate esters in Australian packaging materials. Food Chem., 65 (3): 297-288.

David, R.M., R.H. Mckee,. J.H. Butala, R.A. Barter, M. Kayser, E. Bingham, B. Cohrssen, C.H. Powell (2001). Patty's Toxicology. Wiley, New York, pp: 635 (Chapter 802).

European Union Council (2000). Decision No. 2455/2001/60/EC. Off. J. Eur. Commun, pp:3311.

Goulas A.E., K.A. Riganakos, D.A.E. Ehlermann, P.G. demertzis and M.G. Kontominans (1998). Effect of high-dose electron beam irradiation on the migration of DOA and ATBC plasticizers from food grade PVC and PVD/PVC films, respectively, into olive oil. J. Food Prot., 61 (6): 720-724.

Kembra, L.H., Cynthia, V.R., Vickie, S.W. and Earl Gray L (2008). Mechanism of action of phthalate esters, individually and in combination, to induce abnormal reproductive development in male laboratory rats. Environmental Research.102 (2): 168-176.

Leda Coltro, Joyce Borghetti Pitta, Paulo Afonso da Costa Mary Ângela Fávaro Perez, Vanessa Aparecid de Araújo, Rafaela Rodrigues (2014). Migration of conventional and new plasticizers from PVC films into food simulants: A comparative study. Food Control, 44: 118-129.

Lin S, Ku HY, Su PH, Chen JW, Huang PC, Angerer J, Wang SL (2011). Phthalate exposure in pregnant women and their children in central Taiwan. Chemosphere, 82:947-955.

Martino-Andrade, A.J., Chahoud, I. (2010). Reproductive toxicity of phthalate esters. Mol. Nutr. Food Res. 54, 148–157.

McKee, R., J. Butala, R. David, G. Gan (2004). NTP center for the evaluation of risks to human reproduction reports on phthalates: Addressing the data gaps. Rep. Toxicol., 18 (1): 1-22.

Mustafizur Rahman, Christopher S. Brazel (2004). The plasticizer market: an assessment of traditional plasticizers and research trends to meet new challenges Review Article. Progress in Polymer Science, 29 (12): 1223-1248.

Ntsako Dellas Baloyi (2012). prevalence of endocrine disrupting phthalate esters in selected foods and food wrappers from some supermarkets around Pretoria, South Africa. Master thesis, environmental science, University Of South Africa (UNISA).

Pasquale Ventrice, Domenica Ventrice, Emilio Russo (2013). Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. Environmental Toxicology and Pharmacology, 36 (1): 88–96.

Schettler, T (2006). Human exposure to phthalates via consumer products. Int. J. Androl., 290 (10: 134-139.

Sher Singh, Steven Shoei-Lung Li (2012). Bisphenol A and phthalates exhibit similar toxicogenomics and health effects. Gene, 494: 85–91.

Wahl, H., A. Hoffmann, H. Harring and H.M. Liebich (1999). Identification of platicizers in medical products by a combined direct thermodesportion-cooled injection system and gas chromatography-mass spectrometry. J. Chromatogr. A., 847 (1-2): 1-7.

WHO (2003). Chapter 8: Chemical aspects, Guidelines for drinking water quality. 3rd Edn. World health Organization, Geneva.