

Measurement of Phthalate Acid Esters in Non-alcoholic Malt Beverages by MSPE-GC/MS Method in Tehran City: Chemometrics

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Abstract

The goal of this research was to assess the phthalic acid esters (DEP, DMP, BBP, DBP, DEHP, DnOP and total PAE) in non-alcoholic malt beverages bottled were magnetized with iron (MWCNT-Fe₃O₄) using Mass spectrometry is gas chromatography (GC-MS) in Tehran. The results showed that maximum and minimum of total phthalate esters in samples were 9483.93 and 2412.50 ng/l, respectively. The mean of DEHP (which has also been found to be carcinogenic) in all samples was lower than 5944.73 ng/l. The maximum concentration of DEHP in four samples was upper than 8957.87 ng/l. Multivariate techniques and heat-map visualization were used to assess the correlation among the type and levels of PAEs with brand, color, product date, pH, sugar, volume and gas pressure. Therefore, based on heat-map and principal component analysis (PCA) results, the Bis (2- ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions, indicating that these variables had similar trends. Based on the results, it can be stated that due to the low average of total phthalate esters in non-alcoholic malt beverages, there is no serious health hazard of these compounds for humans.

1. Introduction

Large amounts of phthalic acid esters (PAEs) are used annually to produce a wide variety of common consumer goods around the world (Arfaeinia et al. 2020, Harunarashid et al. 2017). PAEs are used mainly as plasticizers to increase durability, workability, and flexibility of polymeric materials in polyethylene terephthalate (PET) packaging or other plastic packaging, But, in addition, they can be present in many products such as inks, paints, cosmetics and adhesives. (Rafiei Nazari et al. 2018). The phthalates are not covalently bound to the polymer matrix composites, so easily released from the products and then transported to the environment (with migration way), and finally can cause contaminate air, soil, water, and food products. However, other parameters may also be considered in respect to the potential migration such the chemical structure of the additive (crystallinity, molecular weight) and the plastic polymer type, solubility behaviours and various storage conditions (Dobaradaran et al. 2020, Jarošová 2006, Kouhpayeh et al. 2017). In recent years, food contamination with PAEs has become a problem of public concern. PAEs have been reported in beverages such as alcoholic and non-alcoholic malt beverages, soft drinks, meat and meat products, fruits, vegetables, dairy products, cereals, vegetable edibe oils and other food products (Kiani et al. 2018, Liu et al. 2020). PAEs are also widespread pollutants in the environment and appear to be the cause of cancer in humans and animals through defects in fetal, altered hormone levels, uterine damage and reduced sperm production and motility, Sertoli cell damage and Leyding cell tumors, cryptorchidism. The USEPA (Environmental Protection Agency of USA) has listed PAEs as the important contaminant (Jarošová 2006, Moazzen et al. 2019, Shen et al. 2019). The most important PAEs include bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), butylbenzyl phthalate (BBP), dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP). DEHP is one of the most important these compounds, which causes liver cancer in rodents and humans (Liu et al. 2020, Sinha et al. 2019). DBP, BBP, DEHP and some phthalate esters metabolites in animals have shown teratogenic effects. The phthalates are involved in the pathogenesis of asthma, allergic symptoms, contribute to airway remodeling and the premature puberty in young girls (Harunarashid et al. 2017, Kiani et al. 2018). Several analytical techniques have been developed to detect PAE in various samples packaged in plastics (especially PET), such as drinking water, beverages, and cosmetics. High-performance liquid chromatography (HPLC), Gas chromatography (GC) and GC combined with mass spectrometry (GC-MS) are commonly used (Dobaradaran et al. 2020, Moazzen et al. 2019, Wu et al. 2014). Numerous preparation methods can be used to prepare samples for analysis these compounds in different matrixes, including single-drop microextraction (SDME), liquid-liquid extraction (LLE), solvent extraction (SE), liquid-phase microextraction (LPME), LPME method based on the solidification of a floating organic micro drop (LPME-SFO), accelerated solvent extraction (ASE), stir-bar sorptive extraction (SBSE), solid-phase extraction (SPE) and column chromatographic cleanup (CC), but these methods are expensive, insensitive and time-consuming (Kiani et al. 2019, Roudbari et al. 2020, Shariatifar et al. 2020). Recently, a different type for solid-phase extraction (SPE), that called MSPE or magnetic solid-phase extraction has been established (this method based on the using of magnetic adsorbents). Magnetic adsorbents compared with non-magnetic adsorbents, can make separation process faster and easier without any additional procedures such as filtration or centrifugation. The MSPE method can prevent the time-consuming column operations seen in SPE (Kouhpayeh et al. 2017, Moazzen et al. 2018, Shen et al. 2019). The PCA is one of the most important statistical technique used to characterize the interrelationships between variables and visualization of the data patterns (Ghelichkhani et al. 2019, Samiee et al. 2020). Heat-map used to represent the similar or vastly different expression status characteristics values (Arabameri et al. 2019b, Heydarieh et al. 2020). PAEs are phthalic acid esters that are used in beverage packaging bottles as the main plasticizers, so the possibility of releasing phthalate esters into beverages (including malt non-alcoholic beverages) is very high, so there is concern to consumer health and monitoring organizations. Considering the high consumption of these products in the daily diet, it is necessary to investigate the PAEs contaminants in alcoholic malt beverages, since so far, no research has been performed on PAEs contaminants in non-alcoholic malt beverages in Iran. Therefore, this research was conducted to investigate of PAEs in non-alcoholic malt beverages based on MSPE-GC/MS method by using MWCNT-Fe₃O₄ as magnetic adsorbent offered in Tehran, Iran. Also the PCA was applied to characterize the

interrelationships between variables and visualization of the data patterns and Heat-map was applied to represent similar or vastly different expression characteristics values

2. Materials And Methods

2.1. Reagents and Chemicals

Phthalate acid esters (DEHP, BBP, DBP, DEP, DMP and DNOP) and other used chemicals including ethanol, methanol, NaCl and n-hexane were obtained from Sigma-Aldrich Company (St. Louis, MO). $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, Benzyl benzoate (internal standard (IS)), NaBH_4 , HCl, NaOH and 3,5-dinitrosalicylic acid (DNSA) were purchased from Merck Company (Germany). The diameter and length of multi walled carbon nanotube (MWCNTs, Panchkula, India) were 30–60 nm and 5.0–30 mm, respectively. The grade of all the other used chemical and solvents were analytical reagent. The stock solution of analyzed contaminant was ready in methanol (100 mg/mL). Afterward the solutions of phthalate acid esters working standard were prepared by consecutive dilutions of the stock solution (10–10000 ng/L). To prepare I.S., benzyl benzoate (10 μg) was dissolved in ethanol (10 mL), and at the end for preparing samples, 100 μL of prepared I.S. was added. On the same day of the study, the QC (quality control) were prepared from diluted solutions of stock standard. The prepared solutions were retained at 4°C and in the dark place, until analysis. In this research, all the laboratory glass dishes (before using) were washed with an AlO_3 solution, and then they were immersed in acetone for 40 min, and were washed with n-hexane as well as finally were dried (in the oven) for 5 h at 150°C.

2.2. Determination of sugar

In the first, five samples with different dilutions were prepared from sucrose stock solution (1000 mg/dL) as standard. Afterward, 10 mL of non-alcoholic malt beverages samples diluted with distilled water up to the 100 mL as samples. 2 mL of each standard solution was added into a test tube and two mL of distilled water was insert into a separate test tube (blank solution). Then, two mL of HCl hydrochloric acid (6 M) solution was added and placed in boiling water bath for 10 min and then 8 mL of 2.5 M NaOH solution was added to neutralize. Afterward, add 2 mL of 0.05 M 3,5-dinitrosalicylic acid solution and cover the test tube with cover (film) and shake well to mix. Then placed in a boiling water bath for 5 minutes and placed in very cold water for 10 min. (The time between 3,5-dinitrosalicylic acid solution addition and measurements should be same for all test solutions.). Finally, the absorbance of the five standards and references at 580 nm was measured using UV/Vis Spectrophotometer (Roig & Thomas 2003).

2.3. Gas measurement

The gas pressure of the non-alcoholic malt beverages samples were determined using a digital gas meter at room temperature set at 25°C.

2.4. The pH measurement

The pH of the non-alcoholic malt beverages samples were determined using a digital pH meter at room temperature and pressure of 1 atm (Godwill et al. 2015).

2.5. Preparation of adsorbent

Magnetic adsorbents ($\text{MWCNTS-Fe}_3\text{O}_4$) were prepared according our previous study (Kiani et al. 2018, Kouhpayeh et al. 2017, Moazzen et al. 2018).

2.6. Evaluate the properties of the prepared adsorbent

Phase identification of adsorbent was assessed by XRD (X-ray Diffraction) and characterization of elemental was assessed by EDX (energy dispersive X-ray) and morphological analysis was assessed by SEM (scanning electron microscope) (Moazzen et al. 2019). The models of our devices were SEM: PHILIPS, and S360 Mv2300, EDX: PHILIPS, S360, and Mv2300 and XRD: Philips, X'PertPro 2002.

2.7. Sampling, sample preparation

In the beginning, 120 non-alcoholic malt beverages samples were bought with five brand of most commonly used from chain store in Tehran, Iran and all samples were degassed (with a bath of ultrasonic) at temperature of room for 20 minute. Afterward, 10 mg of prepared adsorbent ($\text{MWCNT-Fe}_3\text{O}_4$) were activated with solutions of methanol and water. To the ten milliliter of each degassed non-alcoholic malt beverage samples, 10 mg the activated adsorbent, 0.5 gr of NaCl, and 100 μL I.S. were added. The mixture was mixed strongly (to extract the contaminant compounds) for four minute, after that adsorbent was gathered (with an exterior magnet) to the side of the laboratory

dish (within 90 seconds) and the other compounds in the mixture were discarded. Then, 2 mL of n-hexane was added to the adsorbent and was mixed with blender (2 min) vigorously, to elute contaminant compounds (PAEs) from the adsorbent. After that, by using an external magnet, the adsorbent was collected to the side of the laboratory dish, and the supernatant was moved to a vial. Desorption solvent was dehydrated with a mild stream of N₂ gas (at room temperature) and was maintained in the cold place such as refrigerator. Finally, the dehydrated contents of the previous step was dissolved in one mL solvent (n-hexane), and one µL of the mentioned solution was injected into the gas chromatography (Agilent 7890 N) –mass spectrometry (5975) device.

2.8. Instrument conditions

The column of chromatographic was DB-5–J & W Scientific (30m, 250µm, 0.5µm). Helium (He) was selected as the gas of carrier at one mL/min (ratio of split of 50:1). Splitless was the mode of injection with an inlet temperature of 290-centigrade degree. The program of GC-MS temperature was: 80-centigrade degree, retained for two minute, 80–285 centigrade degree at seven °C/min, retained for ten minute. For the quantitative determination of PAEs compounds, the mode of selective ion monitoring was used. The device outputs (the retention times (RT), quantitative and qualitative ions) of six phthalate acid esters and I.S. are presented in Table 1.

2.9. Optimization of method of the extraction process

The optimization of method was performed according to the 1 factor at a time method. (Kiani et al. 2018, Moazzen et al. 2019).

2.10. Validation of method

The validation of method was performed based on the currently established Guideline of FDA (U.S. Food and Drug Administration) for industries (Kouhpayeh et al. 2017, Moazzen et al. 2018).

2.11. Statistical analysis

The outcomes were statistically analyzed by SPSS version 18 (SPSS Inc, Chicago, IL) for Windows. Data analysis done by test of Kolmogorov–Smirnov and tests of Kruskal-Wallis. Statistical significance was a p-value of <0.05. For a better understanding of the most significant contribution to distribution PAEs levels among the different samples, the PCA was done by the software of SPSS (Arabameri et al. 2019a, Heydari et al. 2020). Multivariate techniques were used to assess the correlation between the amount and type of PAEs levels with properties samples. Heat-map analysis was used to analyze the correlation between samples online at <https://biit.cs.ut.ee/clustvis/>.

3. Results And Discussion

3.1. Quantitative analysis

To draw the calibration curves were prepared five different concentration levels of phthalate acid esters (DEHP, DBP, DEP, DNOP, BBP and DMP) at the range of 10 to 12000 ng/L (including 10, 100, 1000 and 5000 ng/L). The correlation coefficient ranged from 0.9979 to 0.9997 and the LODs (detections limit) and LOQs (quantifications limit) for the target analytes were 13 to 30 ng/L and 39 to 90 ng/L, respectively (Table 2). The recovery values of the 6 PAEs were 94.2% to 104.3% with the RSDs less than 7.6%. To control the quality three levels (50, 500 and 5000 ng/L) from mix of PAEs were prepared and were analyzed duplicate in several days. The inter- and intra- day precision measured for three consecutive days in triplicate analyzes and they were lower than 7.8% and 8%, respectively (Table 3). The selectivity of method was examined by analyzing 25 non-alcoholic malt beverages.

3.3. Images of SEM and analysis of EDX

The SEM image and EDX analysis of the magnetic adsorbents are presented in Figure 1 and 2. The graphs show that the placement of magnetic particle on the surface of multi wall carbon nanotubes is comparatively uniform and after filling with magnetic particles, the surface of the multi wall carbon nanotubes became rougher (Figure 1). Besides, there's no significant modification is detected in the surface of construction of the magnetic adsorbents after extraction procedure. A growth in adsorbent diameter is apparent. In addition, we concluded that the adsorbent exhibited a chain-like morphology without apparent collection. This conducts to a high ability of adsorption of the adsorbent. This results similar to study of the Moazzen et al (Kiani et al. 2018).

The chemical compounds of the prepared adsorbent were assessed by using EDX analysis. The spectrum of EDX displayed oxygen (O), iron (Fe) and carbon (C). The atomic C, Fe, and O ratio (64.6, 22.7 and 12.7, respectively) as the principal elements in the structure of prepared adsorbent confirmed the quantitative representation of the existence of Fe₃O₄ nanoparticles on the MWCNT surface (Figure 2).

3.4. XRD images

By XRD analysis, the construction of multi wall carbon nanotubes and MWCNT-Fe₃O₄ composites were more confirmed. The patterns of XRD of adsorbent were exhibited in Figure 3. The strong diffraction peaks at $2\theta=31/77^\circ$ and $2\theta=45/52^\circ$ were shown MWCNTs and Fe₃O₄, respectively. The get XRD outcomes displayed that the Fe₃O₄ were efficaciously coated on the surface and texture of multi wallet carbon nanotubes using a co-precipitation technique.

3.5. Evaluation of phthalate esters in non-alcoholic malt beverages bottled in PET bottle

Mean concentrations and other statistical analyses of PAEs in all samples were shown in table 4. There was a significant difference between the groups in terms of phthalate esters ($P < 0/05$). According to table 4 from the research mean of all compounds in all the samples were less than the standard defined by the EPA (6000 ng/L) and WHO-EU (8000 ng/L) in drinking water, but maximum of DEHP in sample was upper than the EPA and WHO-EU standards (8957.87 ng/L).

The mean value and range of pH in samples was 3.3 (3.18-3.53) and sugar (per 100g) was 8.7 (4.4-11.9) and gas pressure was 1140 (830-1398) millimeters of mercury (mmHg). There was a significant difference between the groups in terms of sugar content and gas pressure ($P < 0/05$). However, reduce sugar of non-alcoholic malt beverages could imply an undesired impact on physicochemical (optical, rheological properties, total soluble sugars) and sensory properties (good consumer acceptance).

Moazzen, M et al. (2018) showed the highest concentration of PAEs in carbonated soft drinks in PET bottle was DEHP, that it was upper than the standard level (STL) in the 4 samples (14008, 9301, 9201 and 6766 ng/L) and other PAEs compounds were lower than the STL, which was somewhat similar to our study (Moazzen et al. 2018).

Xu, X et al. (2019) showed 3 PAEs compounds (DBP, DMP and DEP) were measured in ten common brands of bottled water (Made of PET) in Beijing (China). The total concentration of PAEs ranged from 0.101ng/L to 0.709ng/L, and DBP contained the highest contents (0.511 ng/L), that was lower than compare our study (Xu et al. 2020).

Vincenzo Russo, M et al in 2014 with the determination of PAEs in soft drinks and alcoholic drinks (light) showed that DEHP (3600–101000 ng/L), DBP (1900–4400 ng/L), DiBP (200–2500 ng/L), DEP (100–1000 ng/L) and BBP (80–800 ng/L), are present in all samples, while iBcEP (80 ng/L) and DMP (1900 ng/L) are present only in 1 beer sample, that was higher than compare our study (Russo et al. 2014).

The important physical and chemical parameters that can influence the migration of PAEs into food products are composite food samples, pH, volume, storage time and temperature (Arfaenia et al. 2020, EFSA Panel on Food Contact Materials et al. 2019, Rafiei Nazari et al. 2018). They reported that migration of PAEs in beverages bottled increased significantly with decrease pH and volume.

Rafiei Nazari, R et al. (2017) with determine the migration modelling of PAEs from non-alcoholic beer bottles showed that storage duration increased and temperature resulted in an increase in migration level ranging from 600 ng/L to 2900 ng/L, which was lower than our study (Rafiei Nazari et al. 2018).

Carnol, L et al. (2017) identified 6 PAEs of Luxembourgish beer stored in various containers (aluminum, glass and can bottle) and total PAEs were found in all samples at levels of 61560 ng /L, which was upper than the present study (Carnol et al. 2017).

Victor E. Balderas-Hernández et al. (2020) identified PAEs in tequila beverage and showed that 22% of samples (65 samples) lacked PAE. DINP (1640000–3430000 ng/L), BBP (50000–2910000 ng/L) and DEP (130000-270000 ng/L) were found in 5 (1.69%), 37 (12.54%) and 11 (3.73%) samples, respectively. However, these levels weren't higher than the maximum standard level of PAEs for alcoholic beverages. DEHP (30000–4640000 ng/L) and DBP (10000–2200000 ng/L) were found in 224 (75.93%) and 96 (32.54%) samples, from them just 15 (5.08%) and 10 (3.39%) samples, respectively, exceeded the maximum standard levels for alcoholic drinks. Bis (2-ethylhexyl) phthalate was the most repetitious PAEs detected in tequila and detected concentrations of DEHP were two-times higher in ultra-aged tequilas compared to those in white tequilas (Balderas-Hernández et al. 2020).

Yang, JF et al. (2016) measured the PAEs in non-alcoholic drinks bottled in PET from China and showed Bis(2-ethylhexyl) phthalate contained the highest median and mean contents (620 ng/L and 1600 ng/L), followed by DBP (270 ng/L and 1340 ng/L). (Yang et al. 2017).

Rodríguez-Ramos, R et al (2020) by evaluating plastic migrants in non-alcoholic and alcoholic drinks showed that there are 4 PAEs in the range of 140-1100 ng /L in some beers, 1 PAE in the range of 1200-1500 ng / L in 3 grape juices and 6 PAE in several cider samples, in the range of 300-2100 ng/L, which was less than the present study (Rodríguez-Ramos et al. 2020).

Wu, PG et al. (2014) with determination of PAEs in non-alcoholic beverages showed a wide variety of PAEs contents was detected in 48 non-alcoholic beverages. Bis(2-ethylhexyl) phthalate was the most abundant PAEs compound followed by DOP, DPP and DBP. Bis(2-ethylhexyl) phthalate was detected in fruit juice samples (22000–126000 ng/L), sport beverages (15000–98000 ng/L), coffee (28000–159000 ng/L) and tea (16000–123000 ng/L), that was higher than our study (Wu et al. 2014).

March, JG et al. (2015) showed low-alcohol beer had DEHP 400 and DBP 2200 ng/L, that DEHP was lower than our study and DBP was higher our study (March & Cerdà 2015).

Heinemeyer, G et al. (2013) showed that the DEHP in Beer (alcoholic) was 0.022 and in nonalcoholic Beverages was 0.020 ng/L (Heinemeyer et al. 2013).

Wang, F et al. (2017) showed DEHP in alcoholic beverages (liquor) was ranged from 618200 to 1089000 ng/L, that was higher than our study (Wang et al. 2017).

Pang, YH et al (2019) showed DEHP in alcoholic carbonated beverage and beer was not found, that was lower than our study (Pang et al. 2020).

The data comparison of this research with other articles shows differences that can be due to reasons such as: alcoholic or non-alcoholic beverage, use of plastic or other containers, contamination of raw materials or secondary contaminants during the production process, carbonated or non-carbonated beverage and also the amount of gas in carbonated beverage (pH), duration Keep in the package as well as the amount of other ingredients in the drink.

3.6. Structural relationship of parameters

Multivariate techniques and heat-map visualization were applied to evaluate the correlation between the type and levels of PAEs with brand, color, product date, pH, sugar, volume and gas pressure. Consequently, based on heat-map and PCA results, the Bis(2-ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions, indicating that the DEHP made up most of the total PAE. Heat-map clustered the 40 non-alcoholic malt beverages using correlation distance and average linkage, reflecting similarities and relationship among the type and levels of PAEs samples. The heat-map clearly grouped samples into two major clusters and two sub clusters (Fig. 4). First cluster includes DEHP and total PAEs, Second cluster contains two sub-groups with brand, color, product date, pH, sugar, volume, gas pressure, DMP, DEP, DBP, BBP and DNOP. In consideration of the different levels of PAEs in samples, these quantities can be expressed individually into several subgroups based on their levels and obtained a linked contribution to simple distinction in non-alcoholic malt beverages. The DEHP and total PAEs were the closest accessions, indicating that these variables had similar trends. Quantitative results obtained for the type and levels of PAEs were used to PCA to investigate the most significant contribution among non-alcoholic malt beverages. The compounds included samples brand, color, product date, pH, sugar, volume, gas pressure and levels of PAEs (DEHP, DMP, DNOP, BBP, DBP and DEP).

The dependence relations between different PAEs can be found from Fig. 4, the graph subset illustrates a visual representation about the relations among different PAEs. The nearest neighbor among the properties, the greater significant relationship existed among dependent variable. As shown in Fig. 5, the first five principal components accounted for 77.86% of the data variance in all samples, and their contribution rates were 26.80%, 15.87%, 14.27%, 11.46% and 9.43%, respectively. The DEHP and total PAEs were the closest accessions, indicating which these variables had similar trends. The components can be used as the correlation of identical each variables. The Total phthalate, DEP, DBP, BEHP had a high positive correlation with PC1, while had negative correlation with sugar content and gas pressure. The results illustrated in Table S1 where the sugar, pH, brand had a positive correlation with PC2, while had negative correlation with gas pressure and DOP. The grouping of item in PCA plots illustrated potential migration, which are important for their potential to health hazard of humans.

4. Conclusion

In first magnetic adsorbent was ready using a simple, sensitive and cost-effective method for measuring 6 PAEs (DEHP, DMP, BBP, DnOP, DEP and DBP) from non-alcoholic malt beverages. Moreover, SEM, EDX, XRD, VSM and TEM analysis were used to describe the generated MWCNT-Fe3O4. Analysis of non-alcoholic malt beverage samples evaluated in Tehran and showed that the concentration of none of the PAEs released from the bottles was upper than the standard levels (6000 ng/L by USEPA). The correlation analysis focuses on the numerical relationship among types and levels of PAEs in all samples. Comparing relationships between PAEs levels from different non-alcoholic malt beverages showed that the Bis (2-ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions. Furthermore, the results of heat-map visualization clearly indicated that relationship between PAEs levels under different samples was properly

diagnosed. Based on the results, it can be stated that due to the low average of total phthalate esters in non-alcoholic malt beverages, there is no serious health hazard of these compounds for humans. However, these levels (DEHP) was in 4 (3.33%) samples higher than the maximum standard level of PAEs for non-alcoholic malt beverages.

Declarations

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Competing interests:

The authors have no conflicts of interest to declare in this research.

Ethical Approval:

"This study does not involve any human or animal testing" or "This study was approved by the School of Public Health of Tehran University of Medical Sciences.

Consent to participate:

All authors participated in this work.

Consent to publish:

All authors agree to publish.

Data availability:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions:

Nabi Shariatifar, Gholamreza Jahed: Conceptualization, Supervision, Design of study, Writing- Reviewing and Editing. **Hana Rezaei and Mojtaba Moazzen:** Data curation, Writing- Original draft preparation. **Mohammad Hadi Dehghani:** Visualization, Investigation. **Mahsa Alikord:** Software, Methodology. **Majd Arabameri :** Software, Validation:

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Tables

Table 1. The RT of each analyst, quantitative and qualitative ions for the GC/MS outputs of 6 phthalate acid esters and internal standard.

Group of ions	Compounds	RT (minute)	Quantitative ions (abundance) (m/z)	Qualitative ions (abundance) (m/z)
1	Di methyl phthalate (DMP)	12.8–13.2	163	76, 134, 162, 195
2	Di ethyl phthalate (DEP)	14.2–14.8	149	121, 149, 177, 222
3	Di butyl phthalate (DBP)	20.1–20.4	149	121, 149, 205, 223
4	Butyl benzyl phthalate (BBP)	25.2–25.6	149	91, 149, 206, 238
5	Benzyl benzoate (IS)	26.1–26.4	105	212 (40), 194 (35)
6	Bis(2- ethyl hexyl) phthalate (DEHP)	27.1–27.6	149	113, 149, 167, 279
7	Di-n-octyl phthalate (DNOP)	29.3–29.5	149	149, 179, 261, 279

Table 2. The linear range, LOD, LOQ and coefficient of estimation of the developed MSPE technique for measurement of phthalate acid esters

Target compound	Linear range (ng/L)	Detections limit (LOD) (ng/L)	quantifications limit (LOQ) (ng/L)	Coefficient of estimation (r2)
Di methyl phthalate (DMP)	10 - 12000	23	69	0.9979
Di ethyl phthalate (DEP)	10 - 12000	15	45	0.9984
Di butyl phthalate (DBP)	10 - 12000	13	39	0.9991
Butyl benzyl phthalate (BBP)	10 - 12000	30	90	0.9990
Di-n-octyl phthalate (DNOP)	10 - 12000	18	54	0.9988
Bis(2-ethyl hexyl) phthalate (DEHP)	10 - 12000	26	78	0.9997

Table 3. Estimated recoveries, precisions and accuracies for determination of the PAEs compounds at three different concentrations (n = 6) in QC samples.

Target compound	Sample	Nominal concentration (ng/L)	Mean of calculated concentration (ng/L)	RSD(%) of calculated concentration (intraday)	RSD(%) of calculated concentration (interday)	RE(%) of calculated concentration	Estimated recoveries (%)	RSD(%) of calculated recovery
Di methyl phthalate (DMP)	QCI	50	52	6.4	7.3	4	95.3	7.2
	QCII	500	503	7.1	6.6	0.6	96.0	6.4
	QCIII	5000	5050	7.8	7.9	1	97.3	7.0
Di ethyl phthalate (DEP)	QCI	50	47	5.3	7.1	-6	99.7	6.9
	QCII	500	510	6.4	8.0	2	96.3	7.6
	QCIII	5000	5100	6.2	5.8	2	94.2	5.7
Di butyl phthalate (DBP)	QCI	50	53	6.3	7.2	6	98.4	7.0
	QCII	500	505	6.8	6.7	1	98.4	5.6
	QCIII	5000	5045	7.0	6.9	0.9	99.8	6.3
Butyl benzyl phthalate (BBP)	QCI	50	56	5.6	5.9	12	104.3	5.8
	QCII	500	512	6.4	7.7	2.4	98.3	7.2
	QCIII	5000	5039	6.5	7.0	0.78	98.6	6.0
Di-n-octyl phthalate (DNOP)	QCI	50	56	5.2	6.3	12	98.2	5.7
	QCII	500	504	7.3	7.2	0.8	98.9	6.9
	QCIII	5000	5105	6.6	5.9	2.1	97.1	5.8
Bis(2-ethyl hexyl) phthalate (DEHP)	QCI	50	49	6.9	7.8	-2	101.3	7.0
	QCII	500	515	6.0	6.7	3	98.8	5.6
	QCIII	5000	5070	6.8	7.3	1.4	98.4	6.9

Table 4. Statistical analysis of phthalate esters in non-alcoholic malt beverages(ng/L)

Target compound	number	mean	SD	min	max
DMP	120	18.31	8.75	8.6	98.1
DEP	120	151.33	31.73	74.83	268.9
DBP	120	496.73	108.99	300.76	803.21
BBP	120	nd	—	nd	nd
DEHP	120	5944.73	2518.14	1897.12	8957.87
DnOP	120	46.16	13.24	15.43	97.13
Total	120	6657.28	1600.9	2412.5	9483.93