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Determination and Distribution of Phthalate Diesters in Plastic Bottled Beverages Collected in Hanoi, Vietnam

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Abstract: Phthalates, industrial synthetic chemicals, are widely used to improve the flexibility of plastics. The more plastic products are used, the more phthalates are found in the environment. In spite of their significant benefits to our lives and industry, their bad effects on laboratory animals have been recorded in some studies. Nevertheless, until now the understanding of the occurrence, distribution, and risk of human exposure to phthalates has been limited in Vietnam. This study introduces a gas chromatography system in combination with mass spectrometry for analyzing nine phthalates in the beverage samples collected in Hanoi (Vietnam). The linearity ranged from 1.00 to 1,000 ng/mL with $R^2 \geq 0.998$. The method detection limits (MDLs) and method quantitative limits (MQLs) of phthalates in the beverage ranged from 0.01 to 0.05 ng/mL and 0.03 to 0.15 ng/mL, respectively. The recoveries of surrogate compounds ranged from 73.4 ± 5.5% (d_4-DPP) to 91.6 ± 9.9% (d_4-BzBP), with RSD≤10.7%. The total concentrations of phthalates in several brands of beverage collected in Hanoi were found ranging from 18.2 to 86.0 ng/mL. Among the studied phthalates, the concentrations of di(2-ethylhexyl)phthalate (DEHP) and di(*n*-octyl)phthalate (DnOP) were found much higher than the others'. However, the concentrations of phthalate in all the studied samples were below the maximum residual level specified in the relevant Vietnamese regulations.

Keywords: Phthalates, DEHP, beverage, plastic bottle, GC/MS.

1. Introduction

Phthalates (acid phthalatic diester) have been widely used in many commercial opproducts. Phthalates play asignificant role in giving plastic more and more flexible [1-3]. Moreover, phthalates are beneficial in stabilizing the color and scent in cosmetic where they decrease evaporation rate and create

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long-lasting aroma, reduce brittleness in nail polish, and help hair gel and skin softener, smoother [1, 4, 5]. Each year, over 470 million pounds of phthalates are produced in the world [6]. However, the effects of phthalate exposure on both animals and human body have been reported in several previous studies from the United State and European countries [6, 7]. Some experiments with rats have shown that the toxicities of phthalates can decrease testicular weight semi ferrous tubular atrophy then damage the male reproductive system of offspring [2, 8].

Because of spread use in our lives, phthalates can easily leach out into the environment over time from products and also migrate to body through dietary intake, air inhalation, and dermal absorption [9, 10, 11]. Although phthalates exist naturally in di-ester form, only mono-ester form was found in humans [12]. After absorption, diester phthalates are rapidly hydrolyzed to the corresponding mono-esters [7]. In 2007, di(2-ethyl)hexyl phthalate (DEHP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BzBP) weregiven in the list of restricted substancesof European Council (section 52, Annex XVII) [13]. In 2009, eight phthalates including di-*n*-butyl phthalate (DBP), di-*iso*butyl phthalate (DiBP), butyl benzyl phthalate (BzBP), di-(2-ethylhexyl) phthalate (DEHP), di-*n*-octyl phthalate (DnOP), di-*iso*decyl phthalate, di-*n*-phenyl phthalate and di-*iso*nonyl phthalate were added to the list of chemicals of concern by the United States Environmental Protection Agency [6, 9]. Among nine phthalates selected to analyze in this study, there were five typical phthalates in the list above, including DBP, DiBP, BzBP, DEHP, and DnOP.

2. Materials and methods

2.1. Chemicals

Nine standard compounds containing Diethyl phthalate (DEP), Dipropyl phthalate (DPP), Di*iso*butyl phthalate (DiBP), Dibutyl phthalate (DBP), Dihexyl phthalate (DnHP), Dicyclohexyl phthalate (DCHP), Di-(2-ethylhexyl) phthalate (DEHP) and Di-*n*-octyl phthalate (DnOP) with their purities ≥ 98% were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Benzyl butyl phthalate (BzBP) with purity 99.9% was purchased from Supelco (Bellefonte, PA, USA).

Sixd_4(deuterated) surrogate standards containingd_4-DEP, d_4-DPP, d_4-DiBP, d_4-DnHP, d_4-BzBP, and d_4-DEHP with purity of >99% corresponding with above standard compounds were purchased from Dr. Ehrenstorfer GmbH (Wesel, Germany). Each surrogate standard was used for calculation of own target compounds. Besides, DBP, DnOP, and DCHP were calculated based on d_4-DiBP, d_4-DEHP, and d_4-DnHP, respectively.

Solvents (acetone and *n*-hexane), sodium sulfate anhydrous and silica-gel were purchased from Merck KGaA (Darmstadt, Germany). All standards and surrogate standards were dissolved in *n*-hexane. Silica-gel and sodium sulfate anhydrous were heated 400 ^oC for five hours before using.

2.2. Sample preparation

Eight different widely brand of beverage samples were purchased from a local supermarket in Hanoi. All selected beverage samples were packaged in plastic bottles. The samples were stored at room temperature until analyzing (no over expiry date). In this study, all real samples were named by using A, B, C… H. The amounts of 500 ng of individual surrogate standard compounds were spiked into 50 mL of beverage. The extraction was repeated three times with 15 mL *n*-hexane by shaking by handing 20 minutes. The extracted solution was concentrated to approximately 5 mL in a rotary evaporator (EVISA, Germany) at 40 ^oC. The solution was then transferred into a packed column (1.5 g of Silica-gel) for cleaning. Na_2SO_4 layers were added under and above of the Silica-gel layer for drying. The target compounds were eluted with 15 mL *n*-hexane. Finally, the eluted solution was concentrated by

using a gentle stream of nitrogen to exactly 1 mL and then transferred to a GC vial.

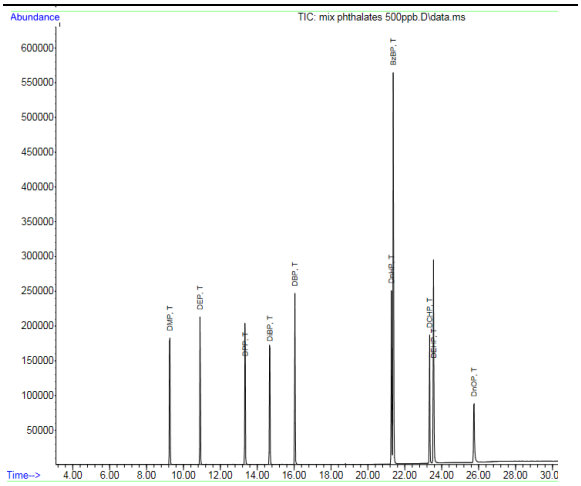

Figure 1. The chromatograph of standards.

2.3. The optimal conditions of GC analysis

Analysis was performed on a gas chromatography Agilent Technologies 7890B system coupled with a mass spectrometer Agilent Technologies 5977A MSD. Separation of nine phthalates was achieved by a fused-silica capillary column (DB-5MS (5% diphenyl 95% dimethylpolysiloxane) from Agilent; 30 m x 0.25 mm i.d.; 0.25 µm film thickness). The volume of injection with splitless mode was 1 µL via autosampler with the splitless mode. The chromatographic analysis was similar to that described in earlier reports [4, 11]. The temperature of the injector and ion source were set at 280 ^{o}C and 230 ^{o}C, respectively. The oven temperature programmed was started from 80 ^{o}C (held for 1.0 min), raised to 180 ^{o}C at 12 ^{o}C/min (held for 1.0 min), increased to 230 ^{o}C at 6 ^{o}C/min, then to 270 ^{o}C at 8 ^{o}C/min (held for 2.0 min), and finally to 300 ^{o}C at 30 ^{o}C/min (held for 10 min).

The MS detector was operated in the selective ion monitoring mode. Ion fragments *m/z* 149 was monitored for quantification phthalates [4, 11]. Ion fragments *m/z* 177 for DEP, *m/z* 233 for DiBP and DBP, *m/z* 279 for DnHP were used for confirmation of the target compounds. BzBP was quantified by ion fragments *m/z* 223 and *m/z* 206. Both ion fragments *m/z* 167 and *m/z* 279 were used to confirm DEHP [5, 6, 19]. Deuterated surrogate standards for each standard were used for quantification. Ion fragments *m/z* 153 was monitored for allsurrogate standards. The chromatograph of all standards is shown on Figure 1.

2.4. Quality Assurance and Quality control

The trace levels of phthalates in laboratories and equipment have been reported in several previous studies [9, 12, 14]. Therefore, the experimental process was performed carefully with clean equipment and it is important to consider the amount of phthalates in equipment and in solvent used in extraction. Procedural blanks were analyzed for each batch of samples. The trace levels of DEP (0.27-0.32 ng), DPP (0.47-0.54 ng), DiBP (3.33-6.40 ng), DBP (3.11-5.93 ng), DnHP (0.33-0.39 ng), DCHP (0.33-0.78 ng), DEHP (1.06-1.42 ng), and DnOP (0.98-1.12 ng) were measured in procedural blanks involving solvents. BzBP was not found in blank samples. All reported concentrations in real samples were subtracted from the mean value found in the blank procedures. In this method, the linearity ranged from 1.0 to 1000 ng/mL with relative coefficient $R^2 \geq 0.998$.

The method detection limit (MDL) is the lowest amount of analyzed compound in sample passing through sample preparation that gives signal as three times as noise and the method quantitative limit(MQL) is the lowest amount of analyzed compound in sample to quantify by analytical method. In this work, MDLs and MQLs of nine phthalates were ranging of 0.01 – 0.05 ng/mL and 0.03 – 0.15 ng/mL, respectively. The MQLs in this study were comparable to that of previous study on GC/MS equipment.

The recoveries of surrogate standards in samples ranged from 73.4 ± 5.5% (d_4-DPP)-

91.6 ± 9.9% (d_4-BzBP) were followed process mentioned in section 2.2.

Table 1. Recoveries of surrogate standards

Surrogate standards	**Re (%) ± rsd (%)**
d_4-DEP	75.9± 8.0
d_4-DPP	73.4± 5.5
d_4-DiBP	87.8± 4.3
d_4-DnHP	79.1± 10.4
d_4-BzBP	91.6± 9.9
d_4-DEHP	85.2± 10.7

3. Results and discussion

3.1. Concentrations of phthalates in beverages

In this study, we focused on the analytical method optimization of several typical phthalates in water samples. The optimal process was shown in section 2.2. The analytical procedures were applied for measurement of target compounds in some kind of beverages collected in Hanoi, Vietnam. The concentrations of phthalates in beverage samples were calculated based on the volume of sample and recoveries of individual corresponding surrogate standards. The analysis was processed at least three times for each brand of samples and the report concentrations were the mean values. The samples were named from A to H letter. The results of analysis were shown in Table 2 and the total levels of phthalates in beverage samples were illustrated in Figure 2.

Total phthalate concentrations present in investigated beverage collected from Hanoi ranged from 18.0 to 87.5 ng/mL. The highest concentration of phthalates was measured in sample named F (86.0 ng/mL), followed by sample named G (72.0 ng/ml), and sample named D (69.8 ng/mL). In contrast, total phthalates were found at the low levels in some kind of samples such as sample named C (18.2 ng/mL) and H (42.0 ng/mL). This difference can be explained by the phthalates contamination in the beverage production process, or by the dispersion of bottles derived from various types of plastic products.

Table 2. Concentrations of phthalates in some brands of beverages (ng/mL)

Sample	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
DEP	0.22	0.43	0.09	2.08	1.34	2.68	1.78	0.08
DPP	0.45	ND	0.18	ND	0.08	0.56	ND	0.19
DiBP	ND	0.25	ND	0.27	ND	1.16	ND	3.96
DBP	ND	0.24	0.89	1.86	ND	1.69	0.93	1.77
DCHP	ND	ND	0.09	ND	ND	0.17	0.14	ND
DnHP	ND	ND	ND	ND	ND	ND	ND	ND
BzBP	ND	ND	ND	ND	ND	ND	ND	ND
DnOP	29.9	19.9	6.65	24.2	37.2	37.4	33.3	12.0
DEHP	14.7	23.3	10.3	41.3	28.4	42.3	35.9	23.8
Sum	**44.3**	**44.0**	**18.2**	**69.8**	**67.0**	**86.0**	**72.0**	**42.0**

A-H: refer to the symbols of the samples; ND: no detection; sum: total concentrations

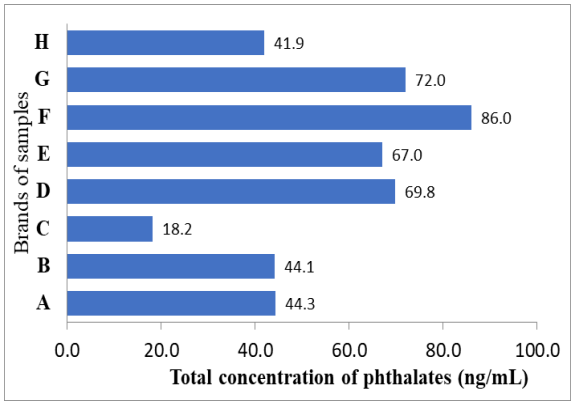

Figure 2. Total concentrations of phthalates in beverages

There are few studies reported the occurrence of phthalates in beverages. Mohammed et al., (2014) reported the total concentrations of phthalates in bottled water were ranged from 8.1 to 19.8 ng/mL [12], which were lower than the values in this study. These results were similar to the earlier report of phthalate concentrations in plastic bottled waters collected from Riyadh city, Saudi Arabia [15].

3.2. Distribution of phthalates in beverage

In general, three phthalates including DEP, DnOP, and DEHP were found in all investigated samples. DBP, DIBP, DBP, and DCHP were presented with lower frequencies (from 37.5 to 75%). Meanwhile, DnHP and BzBP were not detected in all brands of samples. DEHP and DnOP concentrations were significantly higher than the other phthalates. DEHP was distributed of total concentrations of nine considered phthalates about 33.2-59.2%, followed by DnOP (28.7-65.3%). Meanwhile, the other detectable phthalates accounted for 2.94% the amount of phthalates in all brands of samples (Figure 3).

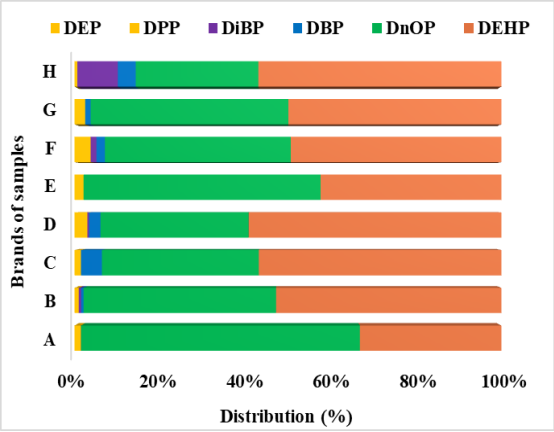

Figure 3. Distribution of individual phthalate in beverage

Several studies reported the presence of phthalates residues in food and drinking water. Concentrations of phthalates in this study were lower than in an earlier survey. The presence of DEP, DBP, DEHP, and DnOP were found in 45 soft drinks and mineral waters available on the Croatian market [16]. DEP, DBP, BzBP, and DEHP were measured up to 200, 133, 27.0, and 136 ng/mL, respectively. Another report was performed by Schecter et al. (2013) to determine the occurrence of phthalates in beverage from Albany, New York [17]. Unlike in Croatia, there was no detection for DEP, DBP, DnHP, BzBP and DnOP, whereas, the detection frequencies of DiBP, and DEHP were 38.0% and 13.0%, respectively. Those results were different from in this report that DEHP was found most often. However, similar to the finding in the USA, DEHP was quantified concentration of the phthalates tested at the highest level. Facts have shown that phthalates have been controlling strictly in the USA. Moreover, a research analyzing phthalates in food products and packaging material sold on the Belgian market gave information that the phthalate levels in beverage were much lower than in Vietnam [18]. That study reported the concentration of eight phthalates in beverage and their levels fluctuated from no detection to 2.50 ng/mL. Samples included the maximum DEHP in a concentration of 11.0 ng/mL as a quarter as this figure in this measurement. Further, Mohammedet.al. (2014) has showed about the dependence of phthalate concentration on temperature: increasing the storage temperature of the bottled water increased the content of leached phthalates in the water [19]. DEP value in bottled water stored at 4 ^{0}C (1.78 ng/mL) and at room temperature (0.34 ng/mL) [15], higher than in this study. Besides, BzBP highest value measured by Bosnir et al (2007) was 4.59 ± 3.08 ng/mL), much higher than BzBP concentration followed by our experiments [16].

DEHP is the most common phthalates produced and used. The highest levels of DEHP were found in this research with a maximum value of 44.3 ng/mL. Our value was higher than the maximum values of 1.25 ng/mL [15] and not as high as maximum value of 98 ng/mL in sport drinks and 134 ng/mL in fruit juices [20]. However, these results weremuch lower compared withthepermissible limit of phthalates for food at the liquid form byVietnam Ministry of Health. The permissible limit of DEHP in beverage is 1.5 mg/L (or 1500 ng/mL) [14]. Now, Vietnam has no regulations for other phthalates in food. In spite of mentioned results, it is important to controlphthalates limitation in consumer products in Vietnam.

3.3. Correlation of phthalates

The correlation of concentrations for several phthalate couples in beverage samples have been considered in this study (Figure 4). A significant correlation existed between some couples such as DEP-DEHP (r=0.957); DEP-DnOP (r=0.724); DnOP-DEHP (r=0.622), and DBP-DEHP (r=0.549). Meanwhile, some concentration couples have no significant coefficient. There are also compounds found at low concentrations in beverage.

Although the number of samples was limited but this is one of the few studies on correlation of phthalates in beverage. Other studies on the distribution of phthalates in environmental samples indicated that the couple of target compounds existed high correlation can be diffused from the same source [4, 9, 11]. Accordingly, these results suggest that some phthalates in this study were distributed into the beverage from the same source such as from water source or contained bottles. Additionally, further researches are needed to clarify this issue.

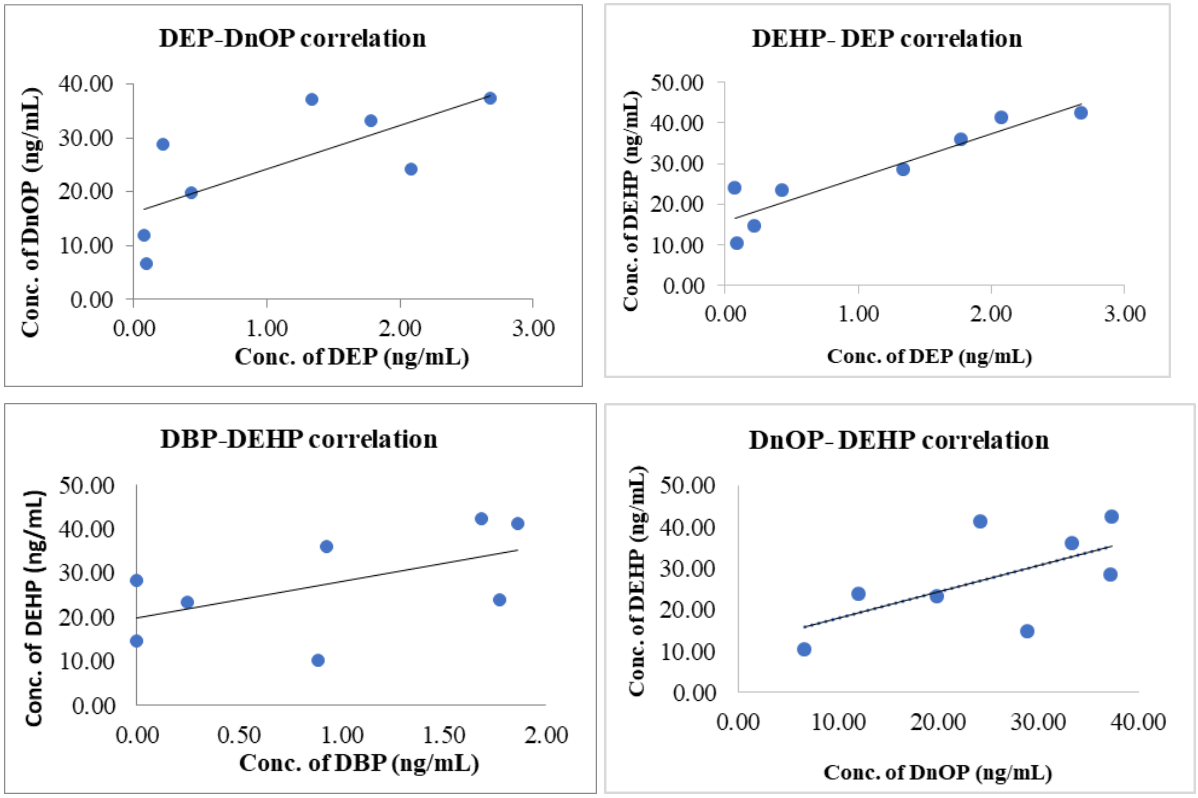

Figure 4. Correlations of some phthalate couplesin beverage samples (DEP-DnOP: r = 0.724; DEHP-DEP: r = 0.957; DBP-DEHP: r = 0.549; DnOP-DEHP: r = 0.622)

4. Conclusions

This is the first study to report the occurrence of phthalates in beverages collected from Vietnam. The method quantitative limits of nine phthalates ranged from 0.03 – 0.15 ng/mL. The recoveries of surrogate compounds ranged from 73.4 to 91.6%, with RSD < 10.7%. The measured concentrations of phthalates in several investigated beverages were compared toother reports. However, the amount of phthalates measured real samples in this study has been below maximum residual level allowed in Vietnam.

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Xác định và sự phân bố của Phthalate Diester trong một số loại đồ uống đóng chai nhựa thu tại Hà Nội, Việt Nam

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Tóm tắt: Phthalate là các hóa chất tổng hợp công nghiệp được sử dụng rộng rãi trong các chế phẩm bằng nhựa với vai trò làm tăng tính dẻo. Ngày nay, các sản phẩm bằng nhựa đã trở nên rất phổ biến và chúng được sử dụng với nhiều mục đích khác nhau như các chai nhựa đựng đồ uống, sản phẩm chăm sóc cá nhân và vật liệu xây dựng.Mặc dù, chưa có những bằng chứng cụ thể về những tác hại của phthalate đối với sức khỏe của con người nhưng đã có nhiều nghiên cứu chỉ ra những ảnh hưởng xấu của phthalate đối với động vật phòng thí nghiệm.Nhưng cho đến nay thì những hiểu biết về lớp hợp chất này vẫn còn rất hạn chế.Trong nghiên cứu này, chúng tôi đã khảo sát điều kiện tối ưu trong phòng thí nghiệm để xác định đồng thời chín phthalate trong nước bằng phương pháp sắc kí khí khối phổ. Giới hạn định lượng của phương pháp đạt 0,03 đến 0,15 ng/mL. Độ thu hồi của các chất đồng hành đạt 73,4 đến 91,6% với độ lệch chuẩn <10,7%. Phương pháp đã được áp dụng để xác định các phthalate trong một số đồ uống đóng chai nhựa thu mua tại Hà Nội, Việt Nam.Tổng nồng độ của chín phthalate trong các mẫu đồ uống xác định được nằm trong khoảng 18.2 to 86.0 ng/mL. Di (2-ethylhexyl)phthalate (DEHP) và di(n-octyl)phthalate (DnOP) được tìm thấy với trong tất cả các mẫu nghiên cứu và ở nồng độ cao hơn nhiều so với các phthalate khác. Tuy nhiên, giá trị này thấp hơn nhiều so với quy định tạm thời của Bộ Y tế, năm 2011, giới hạn cho phép chỉ riêng với DEHP trong thực phẩm dạng lỏng là 1,5 mg/L (1500 ng/mL).

Keywords: Phthalates, DEHP, đồ uống, chai nhựa, GC/MS.