Study on determination of PAEs in water samples by using solid phase extraction combined with gas chromatography-mass spectrometry

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Abstract

Phthalic acid diesters (PAEs) are industrial chemicals used as plasticizers in a variety of commercial products. Due to wide usage, PAEs have been found in various environments and their human exposure risks have also attracted a lot of concerns. However, little information about the distributions of PAEs in the water samples collected from Vietnam was reported. In this study, the method for the determination of nine typical PAEs in water samples by using the solid phase extraction (SPE) combined with gas chromatography-mass spectrometry (GC-MS) technique was optimized. The method detection limits (MDLs) and method quantitation limits (MQLs) of PAEs in water samples were in ranges of 0.2–0.4 ng/L and (0.6-1.2) ng/L, respectively. The recoveries of surrogate compounds in blank and real samples ranged from (74.8 to 93.9) %, with RSD < 9.2 %. The calibration curve for all standards was linear over a concentration range of (1.0-1,000) ng/mL (with $R^2 \geq 0.998$). The total concentrations of PAEs measured in bottled water of six brands were ranged from 10.0 ng/L to 6,690 ng/L. Among the studied PAEs, di-(2-ethylhexyl) phthalate (DEHP) was found at the highest frequencies and concentrations. Overall, the distribution of PAEs in plastic bottled water is significantly lower than the permitted regulations (only with DEHP) in drinking water by the Ministry of Health of Vietnam in 2011.

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1 Introduction

Phthalic acid diesters (PAEs) are widely used in consumer products such as flexible polyvinyl chloride (PVC) products, perfumes, hair sprays, adhesives and glues, floor and wall coverings, cable jacketing, automotive products, toys, medical tubing, and blood storage bags, and food packaging materials [1]. Globally, approximately 8.4 million tonnes of plasticizers are produced and consumed every year and most of them are PAEs [2].

Due to the wide use of plastic products containing PAEs, they can easily leach into the environment. The concentrations of PAEs measured in dust samples collected from a private home in Vietnam ranged from 3,440 ng/g to 106,000 ng/g (median: 22,600 ng/g) [3]. The total concentrations of 10 PAEs in particulate and gas phases ranged from 95.2 µg/g to 13,100 µg/g and from 57.0 ng/m^3 to 14,900 ng/m^3, respectively [4]. Furthermore, the concentration of DEP measured in air samples was in a range of (20.4-134) ng/m^3 [5].

Human exposure to PAEs through various pathways such as dietary intake, inhalation, and dermal absorption was reported [6,7,8]. Tran et al. (2017) reported the human exposure doses to PAEs through inhalation for infants, toddlers, children, teenagers, and adults were (780, 485, 416, 292, and 213) ng/kg-bw/day, respectively [4]. Meanwhile, the estimated exposure doses to PAEs through dust ingestion were ranged from 19.4 to 90.4 ng/kg-bw/day [3]. In addition, the metabolite form of PAEs (i.e. mono-ester PAEs) was found in urine, blood, and mother's milk samples [9].

The aim of this study is to optimize the method for the determination of PAEs in water samples by using solid-phase extraction combined with gas chromatography-mass spectrometry (GC-MS) technique. The optimal method was applied to measure the distribution of PAEs in several plastic bottled water samples collected from supermarkets in Hanoi. The human exposure doses to PAEs through the consumption of drinking water were estimated.

2 Materials and methods

2.1 Materials

Nine standard compounds, including diethyl phthalate (DEP), dimethyl phthalate (DMP), di-n-propyl phthalate (DPP), diisobutyl phthalate (DiBP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BzBP), di-n-hexyl phthalate (DnHP), dicyclohexyl phthalate (DCHP), and di-(2-ethylhexyl) phthalate (DEHP) with a purity of ≥ 98 %, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and benzyl butyl phthalate (BzBP) with a purity of > 99.9 % was purchased from Supelco (Bellefonte, PA, USA). Seven d4 (deuterated) surrogate standards including d4-DMP, d4-DEP, d4-DPP, d4-DiBP, d4-DnHP, d4-BzBP, and d4-DEHP with a purity of > 99 %, were purchased from Dr. Ehrenstorfer GmbH (Wesel, Germany). Each surrogate standard was used for the calculation of its target compounds, while d4-DiBP and d4-DEHP were used for the calculation of DBP and DnHP, respectively.

Acetone, dichloromethane (DCM), and *n*-hexane were purchased from Merck KGaA (Darmstadt, Germany). All standards and surrogate standards were dissolved in *n*-hexane. Solid-phase extraction columns (CNWBOND HC-C18) were obtained from ANPEL Laboratory Technologies Inc. (Shanghai, China).

2.2. Sample collection

Plastic bottled water samples (S1-S6) of six brands were collected from supermarkets in Hanoi, Vietnam in December, 2020. Water samples were prepared immediately for PAEs analysis or stored at room temperature until before the expiry date.

2.3. Sample preparation

Two hundred µL of seven d4-PAEs solutions (1000 ng/mL) were spiked into 1500 mL of water samples. Then, samples were gently shaken and equilibrated at room temperature for 15 min. Extraction was performed according to the following steps. The sample was passed through a SPE column (CNWBOND HC-C18, ANPEL Inc., Shanghai, China) which had been conditioned with 5 mL *n*-hexane by using 12 Position Vacuum Manifold Set-Complete (ANPEL Inc., Shanghai, China). The SPE column absorbed PAEs was dried under a gentle stream of nitrogen. The target compounds were eluted with 20 mL of a mixture (*n*-hexane/DCM, 3/1, v/v). Finally, the eluted solution was concentrated under a gentle stream of nitrogen to 1 mL and transferred into a GC vial for analysis.

2.4. GC analysis

A gas chromatography coupled with a mass spectrometer (MSD) (7890B/5977A, Agilent Technologies, Santa Clara, CA, USA) was used to perform analysis. Separation of nine PAEs was achieved by a fused-silica capillary column (DB-5MS) (5 % diphenyl 95 % dimethylpolysiloxane) from Agilent; 30 m × 0.25 mm i.d.; 0.25 µm film thickness). The volume of injection was 2 µL via autosampler with the splitless mode. Moreover, the chromatographic analysis is described in a similar way as in previous reports [8, 9]. In brief, the temperature of the injector and ion source was set at 280 °C and 230 °C, respectively. The temperature program of column oven was started from 80 °C (held for 1.0 min), raised to 180 °C at 12 °C/min (held for 1.0 min), increased to 230 °C at 6 °C/min, then to 270 °C at 8 °C/min (held for 2.0 min), and finally to 300 °C at 30 °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 PAEs [10-12]. 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. In addition, both ion fragments m/z 167 and m/z 279 were used to confirm DEHP [4, 13]. Deuterated surrogate standards for each standard were used for quantification. Ion fragments m/z 153 was monitored for all surrogate standards. The chromatogram of all standards is shown in Figure 1.

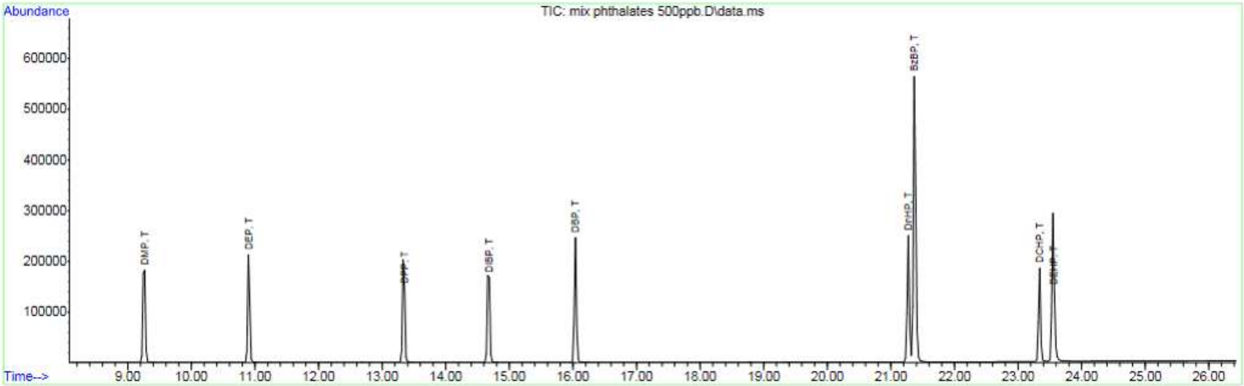

Fig. 1 The chromatogram of nine PAEs

3 Results and discussion

3.1 Optimization of sample preparation

The effects of the trace levels of PAEs in laboratories have been reported in several previous studies [7,10, 13]. The experimental process was performed carefully with clean equipment and it is important to consider the amount of PAEs from equipment, glassware, and solvents. All glassware was baked at 450 °C for 20 h and placed in an oven at 100 °C until use. In addition, the GC vials were capped with aluminum foil, and the solvents were dispensed directly from new glass bottles (i.e., a solvent bottle that was kept open for more than a day was not used). Furthermore, *n*-hexane was injected into the GC-MS until the background levels of PAEs became stable. *n*-hexane also was injected before every sample as a check for background contamination and carry-over.

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), DMP (3.11-5.93 ng), DnHP (0.33-0.39 ng), DCHP (0.33-0.78 ng), DEHP (1.06-1.42 ng), and DBP (0.98-1.12 ng) were found in procedural blanks. All reported concentrations in real samples were subtracted from the mean values found in the procedural blanks. The calibration curve was linear over a concentration range of (1.0-1000) ng/L, with a relative coefficient $R^2 \geq 0.998$. The recoveries of surrogate standards in blank samples (n = 8) and all real samples (spiked surrogate standardsspiked) ranged from (76.7 ± 6.4) % (for d4-DEP) to (93.9 ± 9.2) % (for d4-DEHP). These results suggest that the method meets the requirements of the analysis of trace levels in environmental samples specified by the Association of Official Analytical Chemists (AOAC).

Table 1 Recoveries of surrogate standards

Surrogate standards	**Recoveries (%) ± RSD**
d_4-DMP	79.8 ± 7.5
d_4-DEP	84.8 ± 5.5
d_4-DPP	82.3 ± 4.5
d_4-DiBP	92.1 ± 8.8
d_4-DnHP	76.7 ± 6.4
d_4-BzBP	85.7 ± 6.2
d_4-DEHP	93.9 ± 9.2

The method detection limit (MDL) is the lowest concentration of the analyte in the sample that can be detected. The MDL was determined based on the instrument detection limit (which was reported in our previous studies [4,12]), sample volume (1500 mL), final solution volume (1 mL), and mean surrogate recovery. The method quantification limit (MQL) is assigned with a value equal to three times the MDL. In this work, MDLs and MQLs of nine PAEs were ranged from (0.2-0.4) ng/L and from (0.6-1.2) ng/L, respectively.

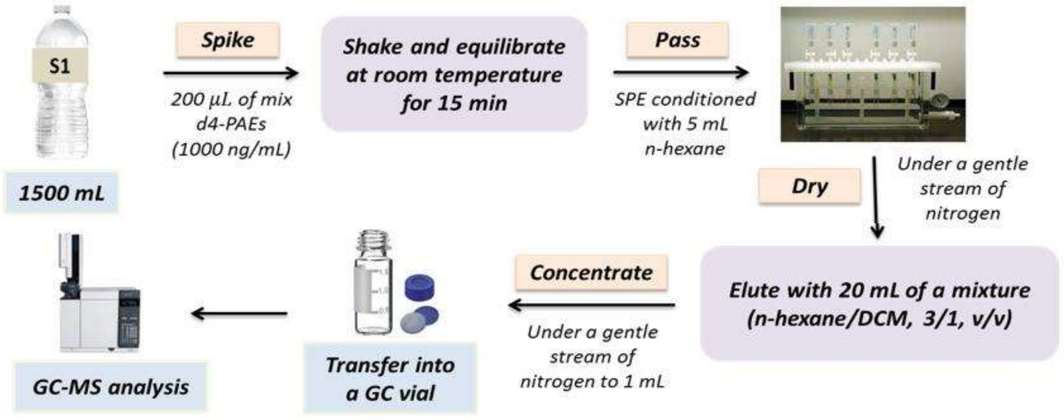

Fig. 2 The diagram of sample preparation

3.2 Concentrations of PAEs in bottled water

The samples were named from S1 to S6. The results of the analysis were shown in Table 2 and the total levels of PAEs in bottled water samples were illustrated in Figure 3.

Table 2 The concentration of PAEs in some bottled water (ng/L)

Compounds	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
DMP	ND	10	20	10	ND	10
DEP	10	10	20	10	20	10
DPP	ND	ND	ND	ND	ND	10
DiBP	60	40	300	150	470	630
DBP	310	80	370	260	620	890
DnHP	10	10	60	ND	70	40
BzBP	50	40	290	ND	410	1150
DCHP	130	110	ND	ND	ND	ND
DEHP	1140	1260	6690	840	1150	2800
Total PAEs	1710	1550	7750	1270	2740	5550
ND: no detection						

Overall, the total PAEs concentrations present in investigated bottled water collected from Vietnam's market ranged from 1,710 ng/L to 7,750 ng/L. In addition, the highest concentration of PAEs was measured in the S3 sample (7,750 ng/L), followed by S6 sample (5,550 ng/L) and S5 sample (2,740 ng/L). By contrast, total PAEs were found at low levels in S4 (1,270 ng/L) and S2 (1,550 ng/L). The evidence for different contributions of PAEs to bottled water is still unclear. However, PAEs were found in polyethylene terephthalate (PET) bottles packaged drinking water [14]. Therefore, Le at al. (2021) suggested that the sources of PAEs in bottled water might be from releasing of plastic container, water source, or/and production and bottling [12].

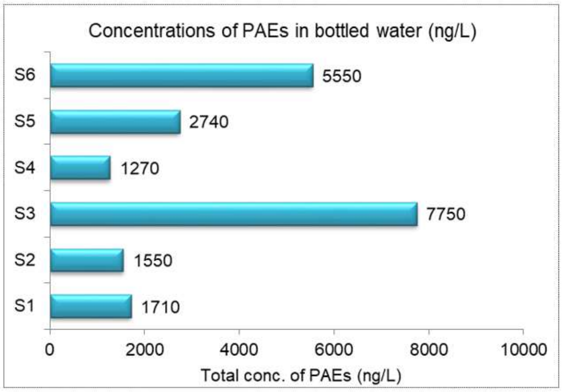

Fig. 3 Total concentrations of PAEs (ng/L)

Few studies monitored the occurrence of PAEs in beverages. Zaater et al. (2014) reported the total concentrations of PAEs in bottled water in a range of 810–19800 ng/L [13]. Overall, these results were similar to the earlier report of PAEs concentrations in plastic bottled waters collected from Riyadh city, Saudi

Arabia [1]. However, the PAE concentrations measured in plastic bottled water in this study were significantly lower than the reference levels. According to the US EPA [15] and the World Health Organization [16], the allowable maximum concentrations of DEHP only in drinking water are 6,000 ng/L and 8,000 ng/L, respectively. The DEHP level in drinking water is regulated by the Vietnamese Ministry of Health with a maximum permissible limit of 1,500 µg/L [17].

3.3 Distribution of PAEs in bottled water

In general, four PAEs including DEP, DiBP, DBP, and DEHP were found in all investigated samples. Among PAEs, DMP, DPP, DnHP, BzBP, and DCHP were detected with lower frequencies (from 16.67 to 83.3%). DEHP and DBP concentrations were significantly higher than the other PAEs. The distribution of DEHP in the total concentration of nine PAEs accounted for (42.0-86.3) %, followed by DBP (4.77-22.6%). Meanwhile, the other detectable PAEs accounted for a small amount of PAEs in all samples (Figure 4).

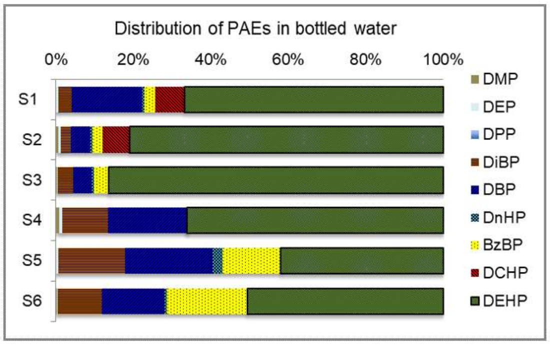

Fig. 4 The distribution of PAEs in bottled water samples

The patterns of PAEs in bottled water samples collected from Egypt are similar to those in this study. In general, the distribution of DEHP and DBP was significantly higher than other PAEs [18]. Similar to previous studies, DEP was also found at the highest concentration in other environments such as air [19,11,4] and dust [3]. These suggest that DEHP, DBP, and DEP are used in significantly higher levels than other PAEs in commercial products [10,20].

3.3 Human exposure doses to PAEs through the consumption of bottled water

Several previous studies reported the human exposure to PAEs through various pathways such as inhalation [4,8], dust ingestion [3], and dermal absorption [10,11]. However, very few studies estimated the human exposure doses to PAEs through the consumption of drinking water. Jeddi et al. (2016) reported the exposure doses to PAEs via drinking water pathway for Iranians in a range of 21–70 ng/kg-bw/day [21]. In this study, human exposure doses to PAEs were estimated based on the measured concentration of PAEs in bottled water; the consumption rate of drinking water; and the average body weights:

$$ED = \frac{C.f}{M}$$

Where:

- ED: the exposure dose (ng/kg-bw/day)
- C: the concentrations of PAEs measured in a water sample (ng/L)
- f: the consumption rate of drinking water per day (L/day)
- M: the body weight (kg)

The estimated exposure doses to PAEs through the consumption of drinking water pathways were 200 and 267 ng/kg-bw/day for children and adults, respectively. The exposure doses to PAEs through the drinking water consumption were significantly lower than the inhalation pathway [19,4]. However, PAEs exposure doses via the drinking water were higher than the dust ingestion [3] and dermal absorption pathways [10]. Therefore, the human exposure doses to PAEs through the consumption of bottled water in this study are still lower than the oral reference doses recommended by the US FDA (100 and 10 µg/kg/day for DBP and DEHP, respectively) [18].

4 Conclusions

This study has reported the method for the determination of PAEs in water samples by using the solid-phase extraction combined with the GC-MS technique. The optimized method allows simultaneous determination of 9 typical PAEs in the aqueous phase with the high recovery and stability that meet the requirements of traceability analysis. The distribution of PAEs in several plastic bottled water samples collected from Hanoi (Vietnam) were assessed. Based on the measured concentrations, the human exposure doses to PAEs through the consumption of drinking water were also estimated in this work.

Acknowledgement

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Nghiên cứu xác định paes trong mẫu nước bằng cách sử dụng kĩ thuật chiết pha rắn kết hợp với sắc ký khí ghép nối khối phổ

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Tóm tắt Phthalic acid diesters (PAEs) là các hóa chất tổng hợp được sử dụng làm tác nhân dẻo hóa nhựa trong nhiều sản phẩm thương mại khác nhau. Vì được sử dụng rộng rãi nên PAEs được tìm thấy trong nhiều môi trường khác nhau và ảnh hưởng xấu đến sức khỏe con người là điều rất đáng quan tâm. Tuy nhiên, rất ít thông tin về sự phân bố của PAEs trong môi trường nước được báo cáo tại Việt Nam. Trong nghiên cứu này, phương pháp xác định 9 hợp chất PAEs điển hình trong mẫu nước đã được tối ưu bằng sự kết hợp giữa kĩ thuật chiết pha rắn (SPE) với sắc kí khí ghép nối khối phổ. Giới hạn phát hiện của phương pháp (MDLs) và giới hạn định lượng của phương pháp (MQLs) đối với các PAEs trong mẫu nước nằm trong khoảng (0,2-0,4) ng/L và (0,6-1,2) ng/L, tương ứng. Độ thu hồi của các chất đồng hành trong mẫu trắng và mẫu thực nằm trong khoảng từ 74,8 % đến 93,9 %, với RSD < 9,2 %. Phương trình đường chuẩn đối với tất cả các chất chuẩn được xây dựng trong khoảng nồng độ (1,0-1.000) ng/mL (với $R^2 \geq 0,998$). Tổng nồng độ của PAEs đo được trong mẫu nước đóng chai thu được từ 6 hãng sản xuất bán trên thị trường nằm trong khoảng từ (10,0-6.690) ng/L. Trong số các PAEs nghiên cứu, di-(2-ethylhexyl) phthalate (DEHP) được tìm thấy với tần suất và nồng độ lớn nhất. Nhìn chung, sự phân bố của PAEs trong nước đóng chai thấp hơn đáng kể so với tiêu chuẩn cho phép (chỉ đối với DEHP) trong nước uống được quy định bởi Bộ Y tế Việt Nam, năm 2011.

Từ khóa PAEs, DEHP, nước đóng chai, GC-MS, SPE.