STUDY ON THE METHOD FOR ANALYSIS OF PARABENS IN SWIMMING POOL WATER SAMPLES

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TÓM TẮT

NGHIÊN CỨU PHƯƠNG PHÁP PHÂN TÍCH CÁC HỢP CHẤT PARABEN TRONG MẪU NƯỚC BỂ BƠI

Nghiên cứu đã phát triển phương pháp xác định đồng thời 7 hợp chất paraben trong mẫu nước bằng phương pháp sắc ký lỏng ghép nối khối phổ (LC-MS/MS) kết hợp với kỹ thuật chiết pha rắn. Paraben được tách sắc ký trên cột C18 (Kinetex: 150 mm x 2,1 mm, 2,6 µm, P/N 00F-4462-AN), với pha động (A): acetic acid (AcOH) 0,05% và (B): Methanol (MeOH). Mẫu nước được axít hóa đến pH~3 bằng axít formic. 100 ng mỗi chất đồng hành (^{13}C-MeP and ^{13}C-BuP) được thêm vào 100 mL nước. Mẫu nước sau đó được cho qua cột SPE (Oasis HLB 500 mg), đã được hoạt hóa trước đó lần lượt bằng 3 mL MeOH và 3 mL nước siêu tinh khiết. Các chất phân tích được rửa giải bằng 5 mL MeOH. Dung dịch sau rửa giải được cô đặc đến 1 mL dưới dòng khí nitrogen. Dung dịch cuối cùng được lọc qua giấy lọc Whatman (kích thước lỗ: 0,2 µm) và tiến hành phân tích trên thiết bị LC-MS/MS. Đường chuẩn của các chất phân tích được xây dựng trong khoảng nồng độ 0,5-200 ng/mL (R^2 > 0,997). Độ thu hồi của các chất đồng hành ^{13}C-MeP và ^{13}C-BuP tương ứng trong khoảng 85,5–105% (RSD < 9,5) và 88,0–110% (RSD < 8,0). Giới hạn phát hiện của phương pháp đối với các chất paraben trong mẫu nước nằm trong khoảng 0,2 đến 1,6 ng/mL. Phương pháp tối ưu đã được áp dụng để xác định nồng độ các paraben trong 24 mẫu nước bể bơi thu tại khu vực Hà Nội và bước đầu đánh giá mức độ phân bố của paraben trong các bể bơi.

Từ khóa: *Paraben; LC-MS/MS; SPE; Nước bể bơi,*

1. INTRODUCTION

Swimming is a globally popular sport and recreational activity, offering benefits for people of all ages and physical conditions [12]. In addition, concerns about emerging organic contaminants in the pool environment are increasing. Parabens, one of the most common endocrine-disrupting chemicals (EDCs) in pool water, have been largely unstudied [20]. Parabens, also known as *p*-hydroxybenzoic acid esters or 4-hydroxybenzoic acid, are mainly produced synthetically by esterification of p-hydroxybenzoic acid with the corresponding alcohol (e.g., methanol, ethanol, or *n*-propanol) in the presence of a suitable catalyst (concentrated sulfuric acid or p-toluenesulfonic acid) [2].

Parabens are widely used in cosmetics, personal care products, foods, and pharmaceuticals, subsequently released

into water, soil, and the air environment. They possess the ability to effectively inhibit microorganisms and yeasts, thus extending the shelf life of products without diminishing their efficacy [4,6]. In 2022, over 22,000 cosmetic products were statistically classified as containing parabens alone or in mixtures as preservatives, a multi-fold increase compared to the previous period [19, 8]. An earlier study reported concentrations of parabens found in several indoor dust samples collected in America, Korea, Japan, and China up to 23,000 ng/g [14]. In 2015, the mean total concentrations of 7 parabens found in swimming pool water samples collected from Beijing were 114 ng/L (outside pool) and 6.78 ng/L (inside pool) [3]. Parabens in swimming pool water originate from many different sources: disinfectants and swimming equipment (e.g., paddleboards and swimsuits), and from the swimmers themselves through personal care products, sweat, urine, and saliva [4, 7]. Once these compounds enter the water, they will participate in reaction and transfer processes, accumulating over time due to the circulating water system with infrequent replenishment. Although these parabens are usually present in water at low concentrations ranging from ng/L to mg/L, prolonged exposure can pose significant health risks [2]. Parabens are considered endocrine-disrupting factors in animals and humans. Humans can absorb them through the skin, respiratory tract, or foods containing this substance. Then, they are rapidly metabolized by the liver and are excreted in the urine within 24 to 48 hours [6], causing infertility in men and, breast cancer in women and skin problems [9, 15].

In 2010, the European Society for the Scientific Study of Consumer Safety (SCCS) recommended a limit concentration of no more than 0.19% for propylparaben (PrP) and butylparaben (BuP) in cosmetic products. The European Union (EU) and the US Food and Drug Administration (FDA) require that the concentration of individual and mixed parabens should not exceed 0.4% [1]. The EU has limited the concentration of ethylparaben (EtP) and BuP in cosmetics from 0.4% to 0.14% since 2014. In Vietnam, the Drug Administration banned five types of parabens (isoPrP, isoBuP, phenylparaben, BzP, and pentylparaben) in July 2015, while also stipulating that propylparaben and butylparaben, along with their related salts, can be used individually at a maximum concentration of 0.14% or in mixtures with a total concentration not exceeding 0.8% [1, 5].

Despite the high demand for paraben consumption and their significant emissions into the environment affecting both environmental and human health, there is still limited research on paraben pollution levels in swimming pool water. To provide more information on the level of pollution as well as a reliable analysis, the aims of this study are (1) optimizing the analysis process of seven typical parabens (MeP, EtP, PrP, isoPrP, BuP, BzP, and HepP) in water samples; (2) monitoring the distribution of parabens in swimming pool water samples collected from Hanoi urban areas, Vietnam.

2. EXPERIMENT

2.1. Chemicals and Materials

Seven native standards (purity ≥99%) including methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), isopropylparaben (isoPrP), butylparaben (BuP), benzylparaben (BzP), heptylparaben (HepP), and two surrogate

standards including ^{13}C-methylparaben (^{13}C-MeP) and ^{13}C-bultylparaben (^{13}C-BuP) were purchased from Sigma-Aldrich (USA). Analytical grade methanol (MeOH) was obtained from Merck KGaA (Germany). The native and surrogate standard solutions were prepared in methanol. HLB Oasis solid-phase extraction (SPE) cartridge (500 mg/5 mL, 30 µm, 60 Å), HLB Oasis (60 mg/3 mL, 30 µm, 60 Å) (Waters Corporation, Milford, America), CNWBOND C18 (45 µm, 60 Å, 500 mg, 3 mL), CHROMABOND C18 (45 µm, 60 Å, 500 mg, 6 mL), 12-position Vacuum Dispenser-Complete (ANPEL Inc., Shanghai, China), and pH meter (HI2215 pH/ORP, Hana, Rumani) were used to investigate sample extraction conditions.

2.2. Sample collection

Twenty-four swimming pool water samples were collected in Hanoi urban areas of Vietnam from October 2023 to October 2024 (the survey results of sampling conditions are described in detail in Section 3.1). All water samples were contained in glass bottles before being transported to the laboratory and prepared immediately for paraben analysis or stored at 5 ^{o}C for no more than one week.

2.3. Sample preparation

The sample was prefiltered through Whatman filter paper (20 µm). 100 µL of ^{13}C–paraben solution (1000 ng/mL) was added to 100 mL of water sample. Formic acid was added until pH~3 by pH meter (pH HI2215 pH/ORP); then, the sample was gently shaken and equilibrated at room temperature for 15 min. The extraction was performed according to the following steps. The sample was passed through an SPE column (HLB Oasis, SPE 500 mg/5 mL) treated with 3 mL of MeOH and 3 mL of UPW using a 12-position Vacuum Dispenser-Complete. The target compounds were eluted with 5 mL of methanol. Finally, the eluted solution was concentrated under a gentle stream of nitrogen to 1 mL, filtered through a Whatman membrane filter (0.2 µm), and transferred to a 1.5-mL vial for LC-MS/MS instrument analysis.

2.4. The optimal conditions of LC analysis

This study used Shimadzu LC-MS/MS 8040 chromatographic conditions to analyze seven parabens. Parabens were separated using a C18 column (Kinetex: 150 mm x 2.1 mm, 2.6 µm, P/N 00F-4462-AN). The analysis procedure was similar to that described earlier [10, 11, 18] with slight modifications. The parameters were set on the instrument as follows: column temperature 40 °C, flow rate 0.3 mL/min, injection volume 5 µL. The mobile phase solvents were (A): AcOH 0.05% in water and (B): MeOH; the mobile phase program was from 0 to 0.5 min 5% (B), from 0.5 to 1.5 min 60% (B), from 1.5 to 20 min 100% (B), and from 20 to 23 min 5% (B). The MS detector was operated in the negative ion selective mode. The fragment ion *m/z* for the quantification of the seven parabens (MeP, EtP, PrP, isoPrP, BuP, BzP, and HepP) were 151, 165, 179, 179, 193, 227, and 235, respectively. The surrogate standards for each standard used carbon 13, fragment ion *m/z* 157 (^{13}C-MeP), and 199 (^{13}C-BuP) for the quantification of the surrogate standards.

3. RESULTS AND DISCUSSION

3.1. Optimization of sample preparation

The chromatograms of all analytes are shown in (Figure 1). The analytes give good analytical signals, indicating that this method is reliable for analyzing seven parabens in water samples.

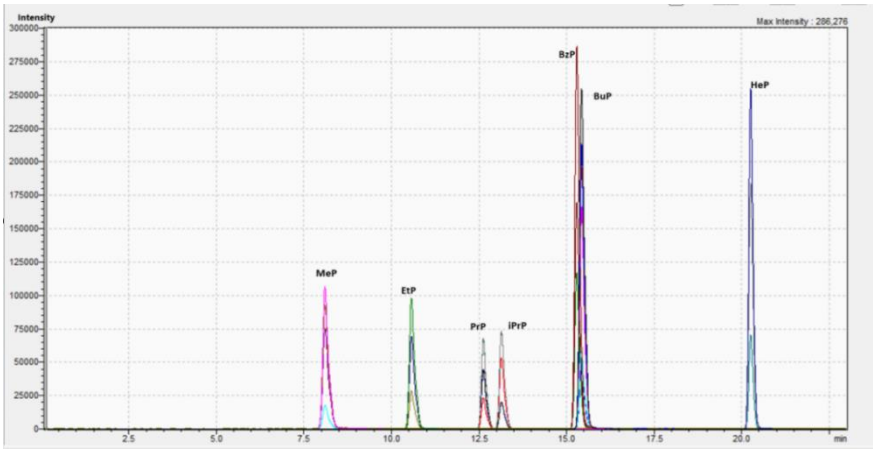

Figure 1. The chromatogram of parabens

The corresponding retention times of 7 parabens (MeP, EtP, PrP, isoPrP, BuP, BzP, and HepP) were 8.3, 10.6, 12.7, 13.2, 15.3, 15.5, and 20.2 min, respectively, and those of 2 surrogate standards, ^{13}C-MeP and ^{13}C-BuP, were 8.2 and 14.4 min. Two substances are separated quite closely: BzP (15.3 min) and BuP (15.5 min); however, they can still be quantified because their fragments are different (*m/z* for BzP: 227 and *m/z* for BuP: 193). The two isomers, PrP and isoPrP, have the same *m/z* fragment of 179, but based on the retention time, we can determine that these two substances are 12.7 min (PrP) and 13.2 min (isoPrP).

The solid-phase extraction procedure was surveyed with four types of columns: Chromabond C18 and CNWBOND C18, Oasis HLB 500 mg, and Oasis HLB 60 mg (2.1). 100 ng of individual surrogate standards (^{13}C-MeP and ^{13}C-BuP) were spiked in 100 mL of ultrapure water. The sample was adjusted to pH~3 with formic acid and then equilibrated for 15 min at room temperature. The sample was loaded onto SPE columns previously activated with 3 mL of MeOH and 3 mL of ultrapure water. The compounds were then eluted with 5 mL of methanol. The eluate was concentrated under a gentle stream of nitrogen to 1 mL and transferred to an LC vial for analysis on the LC-MS/MS instrument. The results showed that the best recovery of the Oasis HLB column (500 mg) for the two surrogate standards was in the range of 91.1–92.2% compared to 53.9–54.5% for the Chromabond C18 column and 61.2–62.2% for the CNWBOND C18 column. The Oasis HLB 60 mg column gave a low result of 30.2–33.4%, which was thought to be due to the small amount of packing material not enough to hold the analyte on the column. Therefore, HLB-SPE (500 mg) was chosen to extract parabens in the swimming pool water sample (Figure 2).

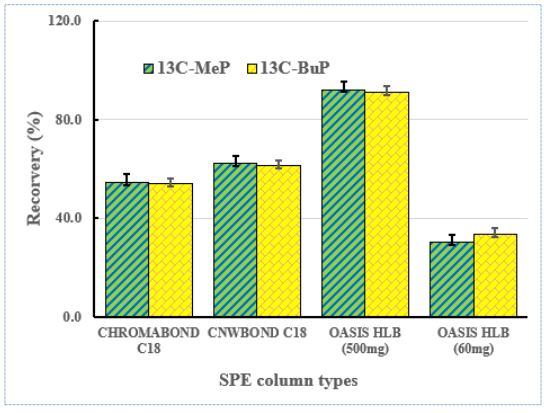

Figure 2. Extraction efficiency depends on the type of SPE column

The type of solvent used to elute from the Oasis HLB 500 mg column was also studied in this report. The kinds of elution solvents, including ACN, MeOH, and MeOH:ACN (1:1), were also investigated. Meanwhile, the factors of sample volume (100 mL), pH (~3), amount of surrogate standards (100 ng), and extraction column were kept constant. The results showed that MeOH still gave the best elution performance compared to other solvents.

The MeOH solvent eluted from the Oasis HLB 500 mg column was also changed to 3 and increased to 10 mL to find the appropriate solvent amount. The results showed that the substances were not completely eluted at 3 mL, resulting in low efficiency; when increased to 10 mL, the efficiency also increased insignificantly. The research team chose the MeOH solvent elution volume of 5 mL to save solvent and drying time and protect the environment.

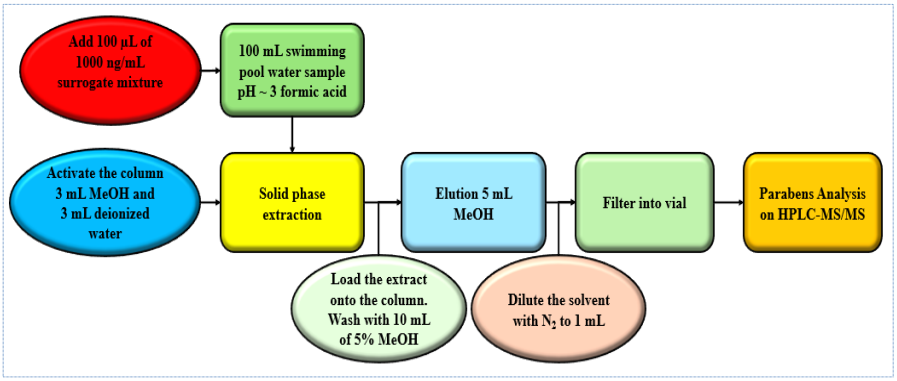

Figure 3. Optimum procedure for water sample treatment

Briefly, the collected pool water samples were prefiltered through Whatman filter paper (20 µm) to remove suspended solids. Then, 100 µL of ^{13}C-MeP and ^{13}C-BuP surrogate standard mixtures corresponding to 1000 ng/mL (100 ng) were added to 100 mL of sample adjusted to pH~3 with formic acid and allowed to stand for 15 min to equilibrate. The Oasis HLB 500 mg column was activated with 3 mL of methanol and 3 mL of ultrapure water; the pool water sample was loaded through the column, washed with 10 mL of 5% methanol, and eluted with 5 mL of pure methanol. The eluate was then concentrated under a gentle stream of nitrogen to 1 mL and transferred to a 1.5 mL vial for analysis (Figure 3).

Each batch of samples was analyzed using a procedural blank. Parabens were not detected in the procedural blank. Observed LOD values of 0.01 ng/mL (MeP, EtP, PrP, isoPrP); 0.006 ng/mL (BuP), and 0.004 ng/mL (BzP and HepP) multiplied by an injected sample volume of 5 µL determined instrumental detection limits (IDLs) of 0.05 (pg) for MeP, EtP, PrP, isoPrP, and 0.03 (pg) for BuP and 0.02 pg for BzP and HeP instrumental quantification limits (IQLs) of 0.15 (pg), 0.09 (pg), and 0.6 (pg), respectively. The method detection limits (MDLs) for each target compound in water were 0.5 (ng/L), 0.3 (ng/L), and 0.1 (ng/L), respectively (Table 1). The recoveries of the analytes and surrogate standards are presented in Table 1. These optimized results demonstrate that the method meets the requirements for trace-level analysis in swimming pool water samples specified by the Association of Official Analytical Chemists (AOAC).

Table 1. Statistical analysis parameters of parabens

Paraben	Recoveries (%)									IDL(pg)	IQL(pg)	MDL(ng/L)	MQL(ng/L)
	1	2	3	4	5	6	7	Mean	RSD				
MeP	96.4	87.2	94.5	89.1	91.1	89.5	93.7	91.6	3.62	0.05	0.15	0.5	1.5
EtP	93.2	87.5	94.6	92.3	90.2	89.5	85.1	90.3	3.68	0.05	0.15	0.5	1.5
PrP	85.2	89.4	83.1	90.5	86.4	93	89.2	88.1	3.85	0.05	0.15	0.5	1.5
isoPrP	87.5	92.1	84.3	82.3	84.9	88.2	92.3	87.4	4.40	0.05	0.15	0.5	1.5
BuP	84.3	82.1	80.4	88.2	84.7	87.1	86.2	84.7	3.26	0.03	0.09	0.3	0.9
BzP	85.1	88.1	81.3	84.9	82.2	88.1	83.0	84.6	3.20	0.02	0.06	0.2	0.6
HepP	83.1	87.2	80.4	81.5	81.1	80.8	84.3	82.5	2.96	0.02	0.06	0.2	0.6
^{13}C-MeP	92.1	90.5	88.6	94.1	89.1	88.2	90.0	90.4	2.33				
^{13}C-BuP	89.3	84	87.6	88.2	90.1	89.9	84.0	87.6	2.97				

The mean recoveries of parabens and their surrogate standards in the aqueous samples diluted in a blank matrix ranged from 82.5 to 91.6%, meeting the AOAC requirements for recovery (80–110% at 10 ng/mL concentrations). The repeatability of the method was also quite good, as assessed by RSD values ranging from 2.3 to 4.4% and meeting the AOAC requirements for repeatability with RSD values <21% at 10 ng/mL concentrations.

3.2. Concentrations of parabens in pool water samples in Hanoi

Eight swimming pools, including indoor and outdoor pools in different districts of Hanoi, Vietnam, were selected for water sample analysis for parabens. The total concentrations of parabens in water samples (1a–8c) collected are shown in Figure 4. There were 8 swimming pools, and each pool collected three samples: a, b, and c. The total levels of parabens in the studied swimming pool water samples ranged from 19.3 to 340 ng/L. MeP, EtP, and PrP accounted for most of the paraben concentrations. Samples collected at location 7 showed high concentrations of 340 ng/L (7a), 210 ng/L (7b), 187 ng/L (7c). The results suggest that this swimming pool recently used detergents containing parabens to clean the pool, leading to significantly high concentrations of these substances. The remaining samples showed relatively low concentrations of Σparabens. However, this suggests that parabens in pool water may be due to the skin care products swimmers use before entering the pool. These results indicate that the distribution of parabens in pool water varies greatly depending on the pool and the number of swimmers. These results may be explained by different habits in using products containing parabens. Environmental factors may also influence the analysis and require further research.

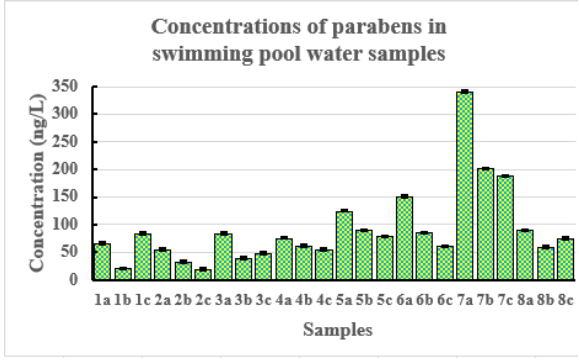

Figure 4. Concentrations of parabens in swimming pool water samples

Previous studies by our group on parabens in dust samples at plastic recycling sites from waste in Hanoi and Hung Yen (52.7–842 ng/g) [10]. Total paraben content in dust samples in Hanoi was 249–513 ng/g [18]. In another report by our group [11], paraben concentrations in water were detectable: wastewater (27.3–1050 ng/L), lake water (18.0–254 ng/L), river water (16.5–52.1 ng/L), tap water (5.01–54.3 ng/L), and bottled water (1.56–39.9 ng/L). The total paraben content is mainly concentrated in MeP and EtP, which can significantly impact the environment, humans, and living animals. Our group also studied the paraben content in fish samples in the coastal waters of Vietnam [16], 6.82–25.3 ng/g for mackerel samples and 6.21–17.2 ng/g for large anchovy samples. The paraben content in water samples collected from the To Lich River ranged from 0.25 ng/mL to 0.84 ng/mL. MeP and PrP were detected at the highest concentrations (mean concentration: 0.21 ng/mL and 0.22 ng/mL) [17]. Various studies have reported the presence of parabens in swimming pool water with significant differences in concentrations. Specifically, in a study of emerging contaminants (including parabens), Li et al. (2015) reported that paraben concentrations range from 0.06 ng/L to 872 ng/L [12]. In addition, MeP was found at the highest level, followed by PrP and EtP [12]. Furthermore, Yue Li et al. [13] recorded concentration values of 178 ng/L for MeP, 13.6 ng/L for EtP, 92.1 ng/L for PrP, and 19.5 ng/L for isoPrP. These results indicate that significant concentrations of parabens were detected in swimming pool water from different regions. Further studies are needed to assess the risks posed by the accumulation of parabens in swimming pool water.

CONCLUSION

This study reports an efficient method for determining parabens in swimming pool water samples using solid-phase extraction combined with the LC-MS/MS technique. The optimized method allows simultaneous measurement of 7 parabens in water with high recovery and stability and low method quantification limit. The method-optimized procedures, such as the Oasis HLB 500 mg solid-phase extraction column and 5 mL methanol elution, meet the requirements for BP monitoring in water samples. The method was applied to evaluate the distribution of 7 parabens in 24 swimming pool water samples collected in Hanoi. The parabens measured in swimming pool water ranged from 19.3 to 340 ng/L. MeP, EtP, and PrP were detected at the higher levels.

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