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Original Article

Development of an Effective Method for the Determination of *p*-Hydroxybenzoic Acid Esters (Parabens) Surface Sediment in To Lich River

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Abstract: This study developed a method for the simultaneous determination of seven paraben compounds in sediment samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with solid-phase extraction (SPE). Parabens were separated on a C_{18}-column (Kinetex: 150 mm x 2.1 mm, 2.6 µm), with mobile phases (A): 0.05% acetic acid and (B): methanol. One hundred nanograms of each surrogate standard, ^{13}C-methylparaben (^{13}C-MeP) and ^{13}C-butylparaben (^{13}C-BuP), were added to 500 mg of freeze-dried sediment sample. Parabens were then ultrasonically extracted for 10 min with 5 mL of methanol/acetonitrile (1/1, v/v) acidified to pH~3 by formic acid. The sample was centrifuged at 4500 rpm for 5 min, and the supernatant was transferred to a glass tube. The extraction procedure was repeated twice. The extracted solution was then concentrated to ~1.5 mL under a gentle stream of nitrogen. The sample was then added with 10 mL of ultra-pure water and passed through an SPE column (Oasis HLB 500 mg), which had been activated respectively with 3 mL of methanol and 3 mL of ultra-pure water. The target compounds were eluted with 5 mL of methanol. The eluted solution was concentrated to 1 mL under a stream of nitrogen. The final solution was filtered through a Whatman filter paper (pore size: 0.2 µm) and analyzed on an LC-MS/MS instrument. Calibration curves of the native compounds were constructed in the concentration range of 0.5–200 ng/mL ($R^2 > 0.997$). The recoveries of the surrogate standards ^{13}C-MeP and ^{13}C-BuP were in the range of 85.5–105% (RSD < 9.5%) and 88.0–110% (RSD < 8.0%), respectively. The method detection limits of parabens in sediment samples ranged from 0.027 to 0.067 ng/g, corresponding to a quantification limit value of 0.07 to 0.19 ng/g. The standardized method was applied to determine the distribution of parabens in sediment samples collected from To Lich River, Hanoi. Total concentrations of parabens in sediment samples ranged from 12.5 to 81.9 ng/g.

Keywords: LC-MS/MS; SPE; Sediment; Parabens.

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1. Introduction

Parabens are endocrine disruptors widely used in various foods, personal care products, and industrial products [1]. These include plastics, foods, detergents, building materials, soaps, and toothpaste [2, 3]. Many worldwide countries have not yet paid much attention to the possible ecological impacts of this endocrine disruptor, although the World Health Organization highlighted these concerns in 2013 [4].

Since the beginning of 2020, the increasing use of pharmaceutical and personal care products containing parabens may have put additional pressure on the environment [5]. Therefore, the impact of this compound on the environment cannot be ignored. In recent years, these compounds have been frequently reported in aquatic environments around the world, including groundwater [8], lakes [5, 10], rivers [6, 7], oceans [11], wastewater [9], and wetlands [3, 12]. In previous studies by our group, we detected parabens in dust samples from major cities in Vietnam [13] and collected at waste plastic recycling sites in Hanoi and Hung Yen [14]. The total concentrations of paraben in dust samples collected from urban areas in Hanoi ranged from 249 to 513 ng/g [15]. Paraben concentrations found in another report by our group [16] included 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 paraben content, mainly methylparaben and ethylparaben, can significantly affect living animals, humans, and the environment. In addition, our group tested the paraben content in fish samples in the coastal waters of Vietnam [17]. The paraben content in mackerel samples was 6.82–25.3 ng/g; in large anchovy samples, it was 6.21–17.2 ng/g.

Continuous exposure to parabens has been associated with adverse effects on human health [18]. Even low concentrations of these compounds can harm aquatic organisms [19]. According to research, parabens can alter animal and human hormones [7]. Manufacturers may gradually stop using these substances in food contact products [2]. On the other hand, many manufactured and used products continue to be manufactured and used. Water sources often contaminate these pollutants because they are discharged directly into the environment or not adequately removed in wastewater treatment plants [20]. Therefore, understanding the occurrence and fate of these compounds in lakes is essential for maintaining aquatic ecosystems and human health. However, few reports have been published on the occurrence of parabens in freshwater lakes and rivers across Vietnam, leading to a lack of information on the occurrence of parabens and their environmental risks.

According to the Hanoi Department of Natural Resources and Environment, the To Lich river is over 14 km long, flowing through 6 densely populated districts of Hanoi, including Cau Giay, Dong Da, Thanh Xuan, Ba Dinh, Hoang Mai, and Thanh Tri. The To Lich River receives about 160,000 m^3 of domestic wastewater daily. Most of this wastewater is not treated or is not treated to standards and is discharged directly into the river. This is also the main reason the river is heavily polluted: the water is black and smells bad. Due to urbanization and industrialization in recent decades, the water quality of this river is deteriorating, and pollutants are often condensed under the sediment layer. This is the final reservoir of organic pollutants and is a suitable matrix for assessing the level of pollution in the environment. Therefore, the level of paraben pollution in sediments must be analyzed to determine the potential risks they may pose to the environment. This will allow for quick solutions to be developed.

The objectives of this study were to (1) optimize the analytical procedure for seven typical parabens in sediment samples and 2) analyze and evaluate the distribution of parabens in sediment samples collected from the To Lich River, Hanoi, Vietnam.

2. Materials and Methods

2.1. Materials

Seven native standards 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) and acetonitrile (ACN) were obtained from Merck KGaA (Germany). The native and surrogate standard solutions were prepared in methanol. Various types of solid-phase extraction (SPE) columns including HLB Oasis cartridge (500 mg/6 mL, 30 µm, 60 Å), HLB Oasis (60 mg/3 mL, 30 µm, 60 Å) (Waters Corporation, Milford, America), CNWBOND C_{18} (45 µm, 60 Å, 500 mg, 3 mL) (Bonna-Agela Technologies, China); CHROMABOND C_{18} (45 µm, 60 Å, 500 mg, 6 mL) (Macherey-Nagel, Germany) were used to investigate sample cleanup efficiency.

2.2. Sample Collection

Fifteen surface sediment samples from (0-4cm) depth (TT1–TT15) were collected along the To Lich River, Hanoi, Vietnam using a Van-Veen Grab-250 cm^2 (KC Denmark). The collected sediment samples were covered in aluminum foil and sealed in polypropylene bags. The sediment samples were kept in a refrigerator and then freeze-dried before analysis for no more than one week. Samples were collected at locations near sewage drains and the bridge pier.

2.3. The Optimal Conditions of LC-MS/MS Analysis

In this study, the appropriate chromatographic conditions of Shimadzu LC-MS/MS 8040 were used to analyze seven parabens. Parabens were separated using a C_{18} column (Kinetex: 150 mm x 2.1 mm, 2.6 µm, P/N 00F-4462-AN). 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): acetic acid 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). MS/MS mass spectrometry program: MRM observation mode was used, ionization mode was negative ion (-), nebulization gas flow was 0.3 mL/min, solvent reduction line temperature was 250 °C, heating block temperature was 400 °C, and drying gas flow was 15 mL/min. The fragment ion *m/z* for the quantification of the seven parabens (MeP, EtP, PrP, isoPrP, BuP, BzP, and HepP) were 151/136, 165/137, 179/137, 179/137, 193/136, 227/136, and 235/137, respectively. The surrogate standards for each standard used ^{13}C-labeled-paraben, fragment ion *m/z* 157/142 (^{13}C-MeP), and 199/142 (^{13}C-BuP) for the quantification of the surrogate standards.

The method validation was based on the results of sediment blank samples spiked with target and surrogate standards, followed by the sample treatment under optimal conditions. The sediment blank sample was prepared by extracting 5 g of the actual sample 5 times with 10 mL of a MeOH/ACN (1/1) solvent mixture.

The instrumental detection limit (IDL) was determined by injecting 7 times the lowest concentration that the instrument could detect giving a signal S/N~3. The method detection limit (MDL) and method quantification limit (MQL) of each target compound were determined based on the IDL, sample weight and recovery.

2.4. Sample Preparation

The SPE column and MeOH elution solvent were investigated previously and the results showed that using Oasis HLB 500 mg column and MeOH 5 mL elution were the most optimal. In addition, this report also surveyed the extraction solvent system without pH adjustment and the extraction solvent system with pH~3 using formic acid.

In this work, the types of elution solvents were surveyed and selected. Firstly, the type of solvent and the specific process were investigated. A sediment blank sample was prepared by ultrasonic extraction with MeOH/ACN (1/1, v/v) to remove parabens. 500 mg of the blank sample was added with 100 µL of a 1000 ng/mL surrogate mixture. Then, the sample was ultrasonically extracted for 10 min with 5 mL of the following solvents: ACN, MeOH, and MeOH/ACN (1/1, v/v), which had been adjusted to pH~3 with formic acid before. The sample was then centrifuged at 4500 rpm for 5 min. The supernatant was transferred to a glass tube. The extraction process was repeated twice (with 3 mL of extraction solvent mixture per each). The extracted solution was concentrated to 1.5 mL under a gentle stream of nitrogen. Then, 10 mL of ultrapure water was added to the samples for diluting before SPE. The Oasis HLB column was activated with 3 mL of MeOH and 3 mL of ultrapure water; the sample was then loaded through the column and eluted with 5 mL of pure MeOH. The eluted solution was further concentrated to 1 mL under a gentle stream of nitrogen and filtered through a 0.2 µm Whatman filter before being transferred into a 1.5 mL vial and analyzed on the LC-MS/MS instrument.

500 mg of freeze-dried sediment sample was weighed into a polypropylene tube. 100 ng of each surrogate (100 µL of 1000 ng/mL solution) was added to the sample. The sample was ultrasonically extracted with 5 mL of MeOH/ACN (1/1, v/v) solvent mixture (to which formic acid had been added until pH~3) for 10 min; then centrifuged at 4500 rpm for 5 min, and the supernatant was transferred to a glass tube. The extraction was repeated twice. Then, the extracted solution was concentrated to 1.5 mL under a gentle stream of nitrogen. The sample was diluted with 10 mL of deionized water. The SPE process specifically as follows: the sample was passed through an SPE column (HLB Oasis 500 mg/6 mL, Waters Corporation, Milford, USA), which was pre-treated with 3 mL of methanol and 3 mL of ultra-pure water using a 12-position Vacuum Dispenser-Complete (ANPEL Inc., Shanghai, China). 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 analysis by an LC-MS/MS instrument.

3. Results and Discussion

3.1. Optimization of LC-MS/MS Conditions

The chromatograms of all analytes are shown in Figure 1.

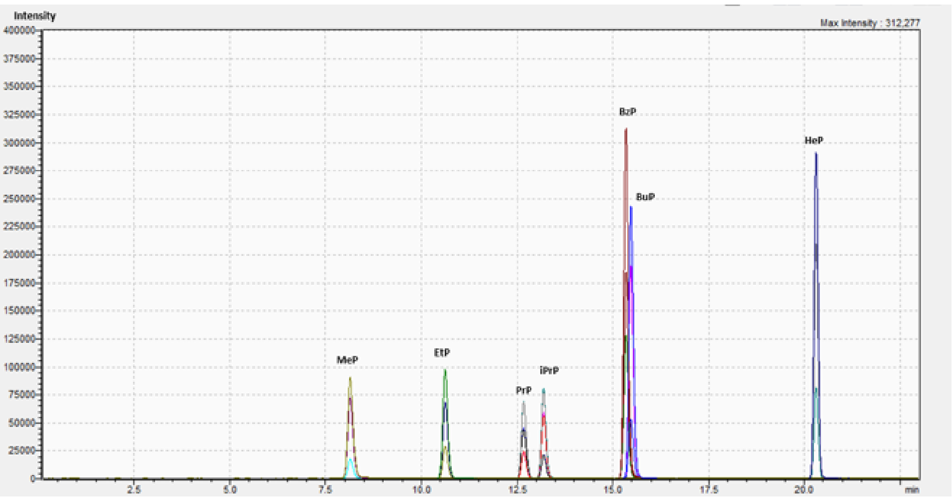

Figure 1. The chromatogram of parabens.

The analytes give good analytical signals, indicating that this method is reliable for analyzing seven parabens in sediment samples. The equivalent retention durations of two surrogate standards, ^{13}C–MeP and ^{13}C–BuP, were 8.2 and 14.4 min, respectively, while those of seven 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. Because their fragments differ (*m/z* for BzP: 227 and *m/z* for BuP: 193), two compounds that are separated very closely BzP (15.3 min) and BuP (15.5 min), can nevertheless be quantified. Although the *m/z* fragment of 179 is the same for both PrP and isoPrP, they were inferred from the retention time (12.7 and 13.2, respectively).

3.2. Optimization of Sample Preparation Procedure

The results showed that the above solvents can separate parabens from the sediment sample matrix (Figure 2a).

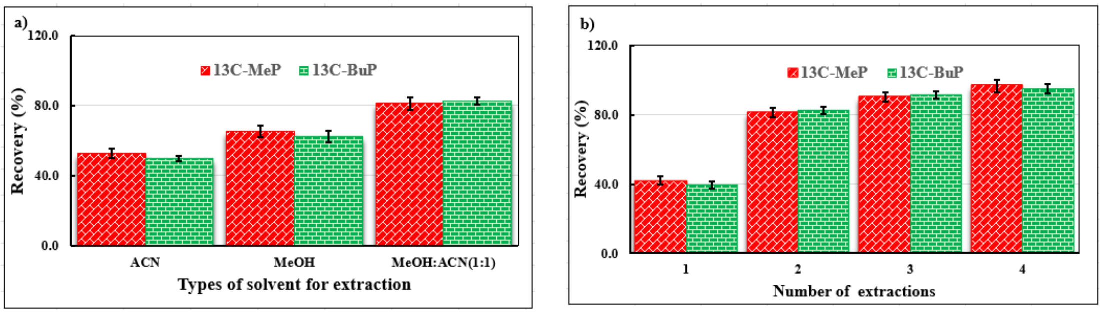

Figure 2. Extraction efficiency depends on (a) Types of solvent for extraction and (b) Number of extractions.

The two solvents, MeOH and ACN, gave relatively low efficiencies of 53.1 and 50.3% (ACN) and 65.4 and 62.4% (MeOH). The reason is that the MeOH solvent has too high polarity, and ACN has a weak polarity, so separating parabens from the sample matrix is not good. However, with two solvents in a 1/1 (v/v) ratio, the efficiency increased significantly to 81.3 and 82.4%. The reason suggested that the solvent mixture should be used for paraben extraction. In addition, the polarity was adjusted to match the polarity of parabens, thereby separating them well and effectively.

The results showed that the ratio of 1/1 was still the most suitable for separating paraben from the sediment background compared to the ratios of 1/2 and 1/5. This was explained by the fact that the mobile phase of the solvent system used was MeOH, so when the amount of MeOH was reduced, and the amount of CAN was increased, the signal was significantly reduced.

The number of times the sample was extracted using the solvent mixture MeOH/ACN (1/1, v/v) was also investigated (Figure 2b). The results showed that when extracting once with 5 mL of the solvent mixture, the efficiency was not high (42.1-39.3%), and the parabens were not completely separated from the sediment sample. When adding 3 mL for the second extraction, the efficiency improved significantly (81.3-82.4%) and continued to increase (90.4–91.5%) third extraction, and (95.2–96.6) fourth extraction. It was found that the results of 3 extractions were better than 1 and 2 extractions and did not change much compared to 4 extractions. To save solvents and protect the environment, three-time extractions (respectively, a solvent volume of 5 mL, 3 mL, and 3 mL) were chosen to extract parabens from the sediment sample matrix

The results showed that the solvent system with low pH adjustment gave significantly higher efficiency (89.2%) than the extraction solvent system without pH adjustment (65.4%). This is explained by the fact that in low pH environments, parabens exist in the HA state and are easily separated from the sample matrix. In neutral or high pH states, parabens exist in the salt form, making it difficult to separate from the sample matrix. The amount of extraction solvent added was also investigated. The amount of extraction solvent was changed to 3, 5, 7, and 10 mL of extraction solvent. The results showed that at 3 mL, the efficiency was low; the substances seemed to have not been separated from the sample matrix. The efficiency increased significantly when the extraction solvent was increased to 5 mL. However, the efficiency increased slightly when the volume of the extraction solvent increased to 7 and 10 mL. Therefore, to save solvent and protect the environment, the research team chose 5 mL of extraction solvent mixture as the optimal condition.

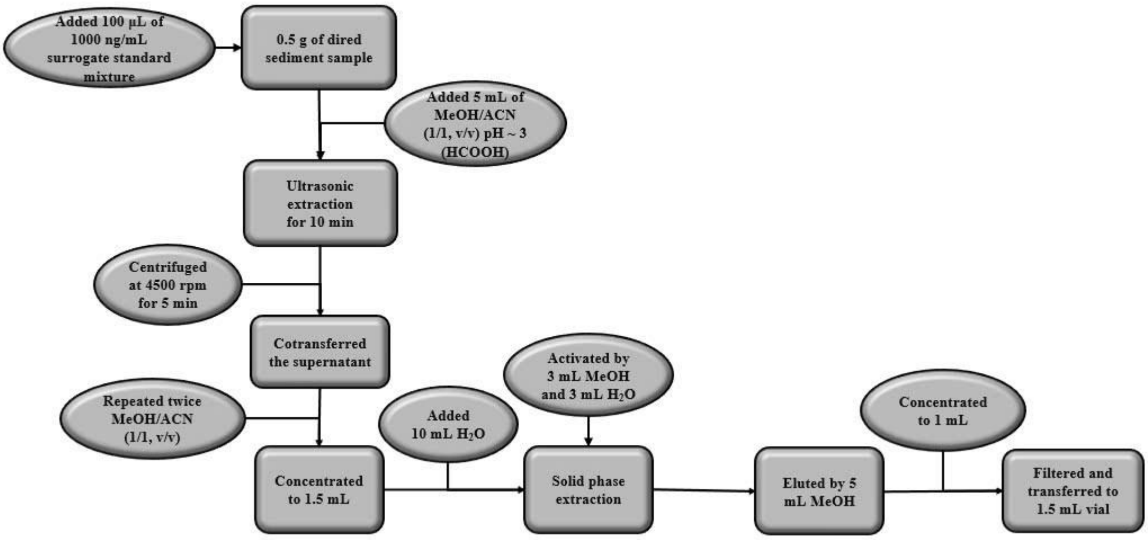

Figure 3. The optimized procedure for sediment sample treatment.

Briefly, 100 ng of ^{13}C–MeP and ^{13}C–BuP surrogate standard (100 µL of 1000 ng/mL solution) were added to 500 mg of freeze-dried sediment sample and kept for 15 min to equilibrate. Then, 5 mL of MeOH/CAN (1/1, v/v) (adjusted to pH~3 with formic acid) was added to the sample. The extraction was performed using an ultrasonic for 10 min and centrifuged at 4500 rpm for 5 min. The supernatant was transferred to a glass tube. The extraction was repeated twice with a volume of extraction solvent of 3 mL. The combined supernatant was concentrated to 1 mL under a gentle stream of nitrogen. A further 10 mL of water was added to the sample to dilute it before solid-phase extraction. The Oasis HLB 500 mg column was activated with 3 mL of methanol and 3 mL of deionized water. The sample was loaded through the column, and the target compounds were eluted with 5 mL of methanol. The eluted solution was concentrated to 1 mL under a gentle stream of nitrogen. Then the sample was filtered and transferred to 1.5 mL vial for analysis (Figure 3).

Method validation was performed using sediment samples repeatedly extracted and spiked with native and surrogate standards for quantification control. The limit of detection (LOD) of 0.01 ng/mL (for MeP, EtP, PrP, and isoPrP); 0.006 ng/mL (BuP), and 0.004 ng/mL (BzP and HepP) multiplied by an injection volume of 5 µL to determine the instrumental detection limits (IDLs). The IDLs were 0.05 (pg) for MeP, EtP, PrP, and isoPrP, 0.03 (pg) for BuP, and 0.02 (pg) for BzP and HeP. The method detection limits (MDLs) for each target compound in sediments were 0.03 (ng/g), 0.02 (ng/g), and 0.01 (ng/g), respectively. The recoveries of the native 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 by LC-MS/MS

Parabens	Recoveries (%)									IDL (pg)	IQL (pg)	MDL (ng/g)	MQL (ng/g)
	1	2	3	4	5	6	7	Mean	RSD				
MeP	97.4	88.2	92.5	87.1	91.4	87.5	93.7	91.1	4.15	0.05	0.15	0.022	0.067
EtP	92.2	86.5	92.6	94.3	90.5	88.5	85.1	90.0	3.77	0.05	0.15	0.022	0.067
PrP	83.3	87.4	85.1	91.5	86.4	93.1	89.2	88.0	3.97	0.05	0.15	0.022	0.067
isoPrP	86.5	91.1	83.3	84.3	84.7	88.2	92.3	87.2	3.99	0.05	0.15	0.022	0.067
BuP	85.3	83.3	82.4	86.2	84.3	87.1	86.2	85.0	2.01	0.03	0.09	0.013	0.040
BzP	84.1	87.1	82.3	85.9	82.5	88.1	83.0	84.7	2.75	0.02	0.06	0.009	0.027
HepP	82.2	87.2	81.4	83.5	81.8	82.8	84.3	83.3	2.38	0.02	0.06	0.009	0.027
^{13}C-MeP	91.1	92.6	88.6	90.1	89.1	88.2	90.0	89.9	1.70	-			
^{13}C-BuP	87.3	83.7	87.6	87.2	90.1	89.9	84.0	87.1	2.90	-			

The average recoveries of parabens and their surrogate standards in sediment samples diluted in a blank matrix ranged from 83.3 to 91.1%, meeting the AOAC requirements for recovery (80–110% at a concentration of 10 ng/mL). The method's repeatability was also quite good, as assessed by RSD values ranging from 1.7 to 4.2% and meeting the AOAC requirements for repeatability with RSD values <21% at 10 ng/mL concentrations.

3.3. Concentrations of Parabens in Surface Sediment Samples Collected from the To Lich River

The parabens were quantified in all sediment samples (TT1–TT15) collected from the To Lich River, Hanoi, Vietnam, as shown in Figure 4. The total mean paraben level was 42.4 ng/g (range: 12.5–81.9 ng/g). MeP, EtP, and BuP accounted for the majority of the total paraben concentration, of which MeP accounted for the majority at about 80%, followed by EtP at 10-15% and finally BuP at 5% (Figure 4). The collected samples that showed higher concentrations than the remaining samples were TT-6 (76.9 ng/g), TT-7 (71.2 ng/g), TT-8 (81.9 ng/g), TT-9 (79.0 ng/g), and TT-10 (77.4 ng/g). These samples were collected in the areas near the discharge sewers. These are also places where densely populated residential areas can cause parabens to be released into the river and settle into surface sediments over time. Meanwhile, the low concentrations of parabens were measured in other samples. The results in this report indicate that the distribution of parabens in surface sediments varies greatly depending on location. Samples TT6-TT10 showed higher concentrations than samples TT1-TT5 or samples TT11-TT15. This is the first study on the distribution of parabens in sediment samples in Vietnam, so there is a lack of information to compare with other studies in the same area. However, these results are also consistent with the reports from previous studies [1, 6, 11]. An earlier study on emerging contaminants reported that the paraben concentrations in Baiyang Lake, China, ranged from 2.42 to 18.37 ng/g, with MeP found at the highest level (2.42–13.6 ng/g), followed by PrP (not detection [nd]–4.01 ng/g), EtP (nd–2.16 ng/g), and BuP (nd–0.17 ng/g) [1]. In addition, Feng et al., (2019) also reported paraben concentrations in sediments in the Yellow River (13.3–37.2 ng/g) and Huai River (16.1–31.6 ng/g) [6]. These results indicate that paraben concentrations were detected in sediment samples from different areas.

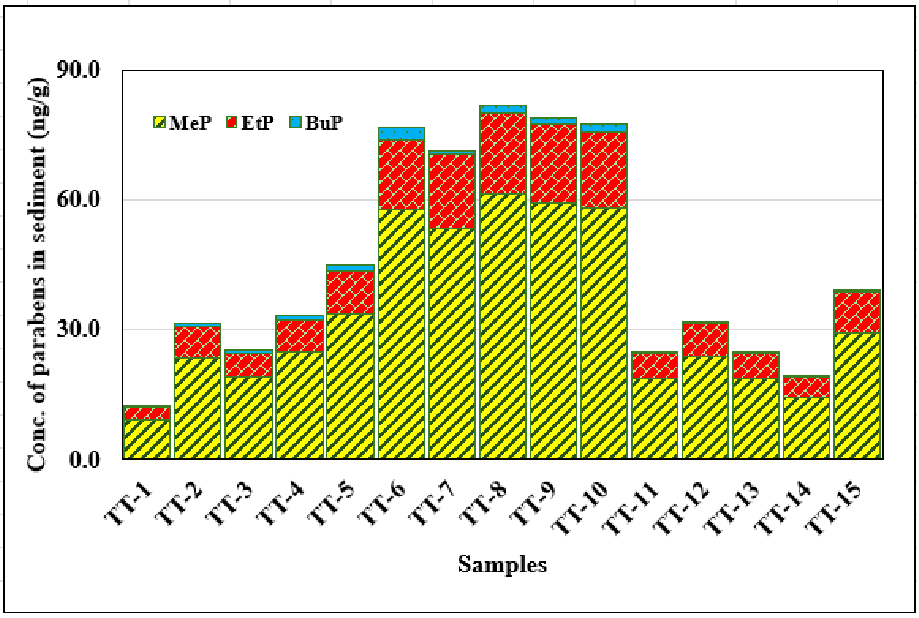

Figure 4. Concentrations of parabens in sediment samples.

4. Conclusions

This study is the first report on the distribution of parabens in sediment collected from Hanoi, Vietnam. In this work, the efficient method for determining parabens in sediment samples using ultrasonic extraction (MeOH/ACN; 1/1, v/v) and solid-phase extraction combined with LC-MS/MS techniques. The optimized method allows simultaneous measurement of 7 parabens in sediments with high recovery (83.3-91.9%) and stability (RSD: 1.7-4.15%), and a low method quantification limit (0.027-0.067 ng/g). The analytical procedure was applied to evaluate the distribution of 7 parabens in 15 sediment samples collected in the To Lich River, Hanoi. Overall, the level of parabens contamination (mean: 42.4 ng/g; range: 12.5–81.9 ng/g) in sediment samples collected from the To Lich River was slightly higher than in previous studies worldwide. The results of this study contribute additional data to the understanding of the level of paraben contamination in the environment in Vietnam.

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