

# Determination of parabens in breast milk using stir bar sorptive extraction coupled with UHPLC-UV

Xue Ge<sup>a</sup>, Senwei Feng<sup>a</sup>, Linlin Bian<sup>a</sup>, Mingjuan Wang<sup>b, \*\*</sup>, Kefeng Li<sup>c, \*\*\*</sup>, Xu Wang<sup>a, \*</sup>

<sup>a</sup> State Key Laboratory of Food Nutrition and Safety, College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin, 300457, China

<sup>b</sup> Beijing Sun-Novo Pharmaceutical Research Company Ltd, Beijing, 102200, China

<sup>c</sup> Centre for Artificial Intelligence Driven Drug Discovery, Faculty of Applied Sciences, Macao Polytechnic University, Macao SAR, China

## ARTICLE INFO

### Keywords:

Parabens  
Human breast milk  
Infants  
Stir bar sorptive extraction  
Adsorption  
Ultra-high performance liquid chromatography

## ABSTRACT

We developed an analytical method based on ultra-high performance liquid chromatography with UV detection, using a stir bar coated with amino/hydroxyl bifunctional microporous organic network (B-MON), for the analysis of parabens in breast milk samples. B-MON demonstrated superior performance with maximal methylparaben adsorption of 112.15 mg/g. Kinetic fitting revealed that outer diffusion was the key limiting step, and the adsorption was chemisorption. The thermodynamic analysis demonstrated that increased methylparaben adsorption was found at higher temperatures in spontaneous processes. The developed approach showed excellent linearity ( $R^2 \geq 0.9964$ ) and a low detection limit (0.01  $\mu\text{g/L}$ ). Recoveries ranged from 85.8 to 105.5 % and the relative standard deviation was lower than 9.2 %. Based on the daily exposure assessment, these pollutants do not pose unacceptable health hazards to babies. However, the high detection frequencies (41.9%–93.5 %) suggest that breast milk still should be monitored.

## 1. Introduction

Parabens have become commonly used as preservatives in cosmetic products, food, and personal care products in recent years due to their advantages of high chemical stability, non-volatility, broad antimicrobial spectra, low cost, and lack of considerable odour [1]. Methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP) are among the most commonly used parabens, often utilized in combination to achieve greater antimicrobial activity. However, previous studies have shown that parabens possess estrogenic activity and can raise the risk of breast tumors. In addition, parabens exposure may also be associated with a number of human health problems: such as myocardial infarction, reproductive disorders, obesity, genotoxicity and central nervous system malfunction [2–4]. An acceptable daily intake (ADI) of 0–10 mg/kg-bw/day for MeP and EtP has been established by European Food Safety Authority (EFSA) [5], while PrP and BuP are prohibited in food due to their endocrine-disrupting actions. As a result of the widespread usage of parabens, researchers have found them in various environments, including water [6], foodstuff [7], and indoor

dust [8].

Parabens can enter the human body through skin absorption, inhalation and ingestion [9]. Biomonitoring studies of parabens have utilized urine, blood [10], and adipose tissue [11]. Breast milk is known to be a potential medium for the exposure of chemicals commonly found in the environment to the infant. Since newborns have poorly developed enzymatic systems for biotransformation and removal of these compounds, this exposure could be detrimental their health. It is practical to use human milk for biomonitoring research because it provides information on the mother's and the breastfed child's exposure to endocrine disruptors during a time of high sensitivity [12]. Parabens are present in human milk, according to reports by Kim et al. and Zhang et al. [13,14]. Breast milk is the most ideal food for infants, which can provide the required nutrients for their growth and development, therefore, it is scientifically important to monitor parabens in breast milk.

The determination of trace amounts of parabens in complex matrices like human breast milk represents significant challenges, requiring the use of a sensitive and selective analytical procedure. Previous studies have attempted to quantify parabens in breast milk mainly using

\* Corresponding author. State Key Laboratory of Food Nutrition and Safety, College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin, 300457, China.

\*\* Corresponding author.

\*\*\* Corresponding author.

E-mail addresses: [wangmingjuan@sun-novo.com](mailto:wangmingjuan@sun-novo.com) (M. Wang), [kefengl@mpu.edu.mo](mailto:kefengl@mpu.edu.mo) (K. Li), [xuwang@tust.edu.cn](mailto:xuwang@tust.edu.cn) (X. Wang).

<https://doi.org/10.1016/j.talanta.2023.125609>

Received 5 October 2023; Received in revised form 14 December 2023; Accepted 27 December 2023

Available online 28 December 2023

0039-9140/© 2024 Elsevier B.V. All rights reserved.

advanced and expensive instruments such as mass spectrometry (MS) and tandem MS to achieve lower detection limits. However, these instruments are not available in all labs. In contrast, liquid chromatography with UV detection (LC-UV) is a common and important alternative for the determination of compounds in complex matrices [15]. Therefore, other researchers have explored the use of LC-UV in combination with various sample pretreatment methods. Unfortunately, the LC-UV coupled with solid phase extraction (SPE) approach failed to achieve satisfactory detection limits in previous studies [16–18]. To address this issue, a magnetic solid phase extraction (MSPE) pretreatment method was developed to improve sensitivity by Abdolmohammad-Zadeh et al. [19]. However, for the separation of adsorbent and sample solution, this method requires the application of an external magnetic field. In contrast, stir bar sorptive extraction (SBSE) enables instantaneous separation of the extracted sample from the solution without the need for an external magnetic field.

The coating of the stir bar is key to evaluating the extraction performance of SBSE, however, the commercially available coatings lack specificity for the analytes, resulting in limited extraction ability. Therefore, the development of customized coatings tailored to specific analytes of interest is necessary to expand the application of SBSE. As a class of novel materials, microporous organic networks (MONs) have a large area of surface, good stabilities, and a variety of structures and pores [20,21]. Recently, our group discovered a novel amino/hydroxyl bifunctional microporous organic network (B-MON) coated fiber for solid phase microextraction (SPME) of the triclosan and bisphenols [22]. The advantages of SBSE over SPME include a larger adsorption capacity and recovery, as the stir bar is coated 50–250 times more than the SPME fiber [23]. Moreover, SBSE can increase sensitivity and is more robust than SPME [24]. To our knowledge, paraben levels in human breast milk have not yet been determined using SBSE based on a stir bar coated with B-MON.

Developing a method to determine the four parabens in breast milk is the main objective of this research, which combines SBSE coated with B-MON, followed by ultra-high performance liquid chromatography with UV detection (UHPLC-UV) analysis. The adsorption behavior of MeP on B-MON, including adsorption kinetics, isotherms, and thermodynamics was extensively investigated. Additionally, the SBSE settings were optimized, including pH, extraction time, temperature, and desorption time. The developed procedure was validated in the optimized conditions. The potential of using customized MON as SBSE coating in sample treatment was also assessed by applying the proposed approach to quantify low levels of parabens in human breast milk. Finally, an exposure and risk assessment of parabens for infants was conducted.

## 2. Experimental

### 2.1. Reagents and materials

The standards of MeP ( $\geq 98\%$ ), EtP ( $\geq 98\%$ ), PrP ( $\geq 98\%$ ) and BuP ( $\geq 99\%$ ) were provided by Alta Scientific Co., Ltd. (Tianjin, China). Each analyte's stock solutions for 1000 mg/L were made in methanol and kept at 4 °C in which they maintained stable lasting up to four months. The reagents and materials for synthesizing B-MON and stir bar are shown in the Supplementary Materials.

### 2.2. Sample collection

A total of thirty-one (31) samples of human breast milk were obtained from the local mothers. Prior to sampling, all participants provided informed consent. All samples were kept in a  $-20\text{ }^{\circ}\text{C}$  freezer before analysis. Ethics Committee of Tianjin University of Science and Technology has approved the research.

### 2.3. B-MON production and stir bar creation

According to the previously reported method [25], the B-MON was synthesized. The detailed preparation method of B-MON is described in the Supplementary Materials. A 10-mm glass bar containing iron wire was fused into dumbbell-shape and activated with 1 mol/L NaOH and 0.1 mol/L HCl for 2 h, respectively, and then washed to neutrality with ultrapure water. The produced glass bar was allowed to dry at 60 °C. The activated glass bar was then immersed in the PDMS sol and a thin layer of glue was uniformly coated on its surface. The stir bar followed by rolling in a glass-surface vessel containing B-MON until the powder was evenly attached to it. Finally, the prepared stir bar was dried at 70 °C for 24 h and washed with methanol.

### 2.4. Adsorption behavior

The adsorption capacity of the B-MON was evaluated using adsorption kinetic, isotherm, and thermodynamic methods. MeP was chosen as the model pollutant to assess the adsorption efficiency.

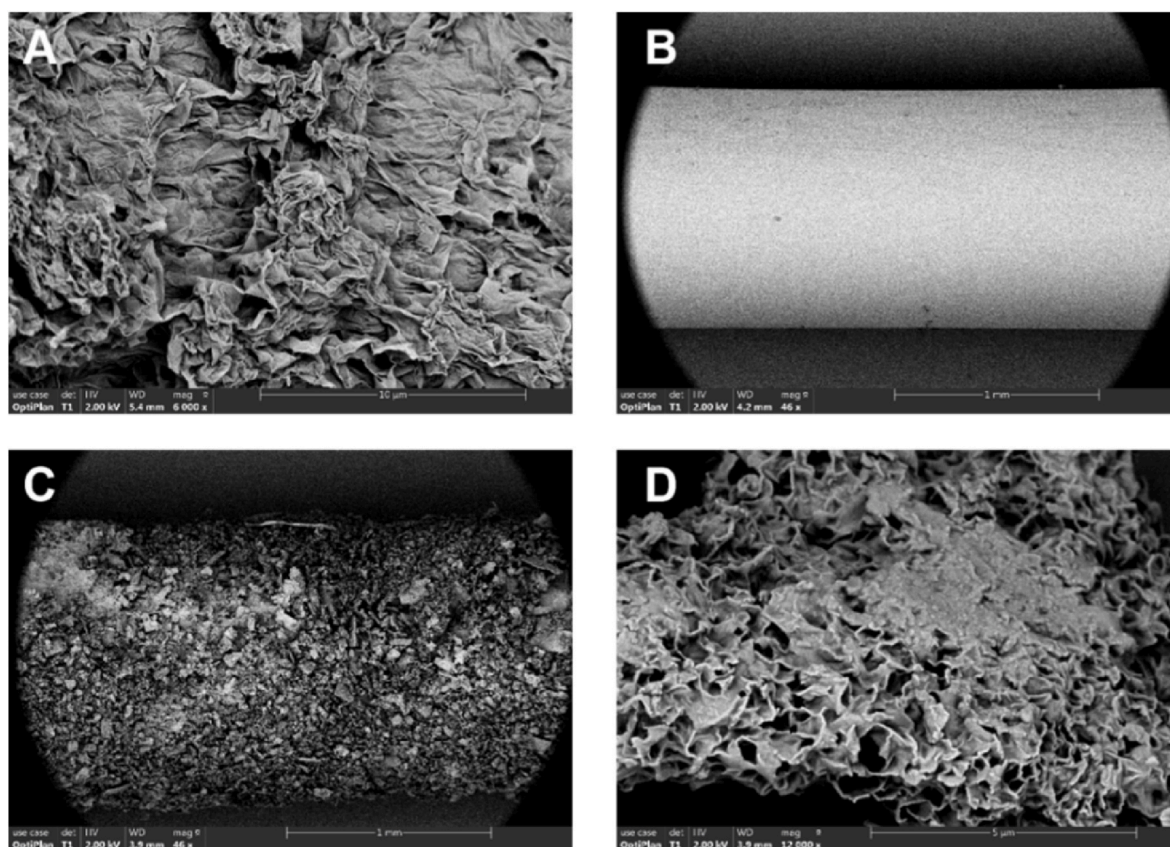
To determine the equilibrium time, we conducted kinetic adsorption experiments using MeP at a concentration of 200 mg/L. 5 mg of B-MON was immersed in the solution and then shaken it for varying times (15–180 min) [26]. The residual MeP in the supernatant was analyzed by UHPLC-UV. The kinetic data were evaluated using the pseudo-first-order model, pseudo-second-order model, intra-particle diffusion model, and Body plot model. The adsorption isotherms and thermodynamics behavior of the system were studied by mixing MeP solution (10–600 mg/L) with 5 mg of B-MON and shaking for 2 h at 25, 35, and 45 °C [25]. The mixture was then separated by centrifugation and the residual MeP in the supernatant was analyzed by UHPLC-UV. For analyzing the isotherm data, the Langmuir, Freundlich, and Temkin models were used. For estimating the adsorption process's spontaneity, the standard Gibbs free energy ( $\Delta G$ ), one of the adsorption thermodynamic factors, is crucial. The degrees of the molecules in the system described by their entropy ( $\Delta S$ ), and the enthalpy ( $\Delta H$ ) can be used to determine whether a process is exothermic or endothermic. The expressions for all models are shown in the Supplementary Materials.

### 2.5. Sample pretreatment

Four mL of acetonitrile was added to the 2 mL of each breast milk sample after being defrosted to precipitate fat and protein. Following a 2-min vortex, the sample went through a 10-min centrifugation at 8000 rpm. The obtained supernatant was received and evaporated under a nitrogen stream to dryness. Afterwards, 2 mL water was used to redissolve the analytes, and then the B-MON stir bar was submerged in the water, and stirred at 600 rpm for 60 min to extract the targets. Then it was put into an Eppendorf tube with 0.4 mL of acetonitrile after extraction to desorb the compounds for 12 min. After filtering, the eluent was transferred to the UHPLC-UV apparatus for detection.

### 2.6. Instrument and analysis conditions

A scanning electron microscope (SEM) (HITACHI, Japan) was used to characterize the morphology of B-MON and stir bar. Separation and determination of the parabens were carried out on a UHPLC system (Agilent, USA), equipped with a thermostated column compartment, a binary pump, an automatic injector, and a UV-Vis detector. 2  $\mu\text{L}$  of samples were loaded into an Agilent ZORBAX C<sub>18</sub> column (2.1 mm  $\times$  100 mm; 1.8  $\mu\text{m}$ ) working at 40 °C. At a flow rate of 0.2 mL/min, an isocratic program was applied to elute parabens. Acetonitrile and water (40:60) composed the mobile phase. The running time of the detection program was 10.0 min in total.



**Fig. 1.** The morphology of the acquired B-MON at magnification of  $6000 \times$  (A), bare glass bar at magnification of  $46 \times$  (B), and the stir bar coated with B-MON at different magnifications of  $46 \times$  (C), and  $12000 \times$  (D) by SEM.

## 2.7. Method validation

Several parameters, including linearity, detection limits (LODs), quantification limits (LOQs), recoveries and precisions (relative standard deviations, RSDs) were used to validate the developed approach. The LOD and LOQ were determined by monitoring the signal-to-noise ratios (S/N) of 3 and 10, accordingly, and were defined as the lowest spiking concentration in the blank breast milk sample. Utilizing matrix-matched calibration curves, which involved spiking the blank breast milk samples with the compounds' standard solutions at concentrations ranging from LOQ-200  $\mu\text{g/L}$ , linearity was assessed. Breast milk samples were spiked with three concentrations of all analytes, at 0.5, 5, and 50  $\mu\text{g/L}$ , to evaluate the method's precision and accuracy. By analyzing three levels of spiked samples with five duplicates for each level of extraction, the intra-day precision was evaluated.

## 2.8. Risk assessment

Eq. (1) was used to derive the estimated daily intakes (EDI) of four parabens for babies:

$$\text{EDI } (\mu\text{g/kg-bw/day}) = C \times M \quad (1)$$

where C is the concentration of parabens, M is the daily intake of breast milk. In this research, we used the EPA's suggested daily consumption of human milk [27]. In accordance with their ages, infants younger than one month, one to three months, three to six months, and six to twelve months consumed daily amounts of breast milk of 150, 140, 110, and 83 mL/kg-bw/day, accordingly.

**Table 1**

Repeatability and reproducibility of B-MON stir bars.

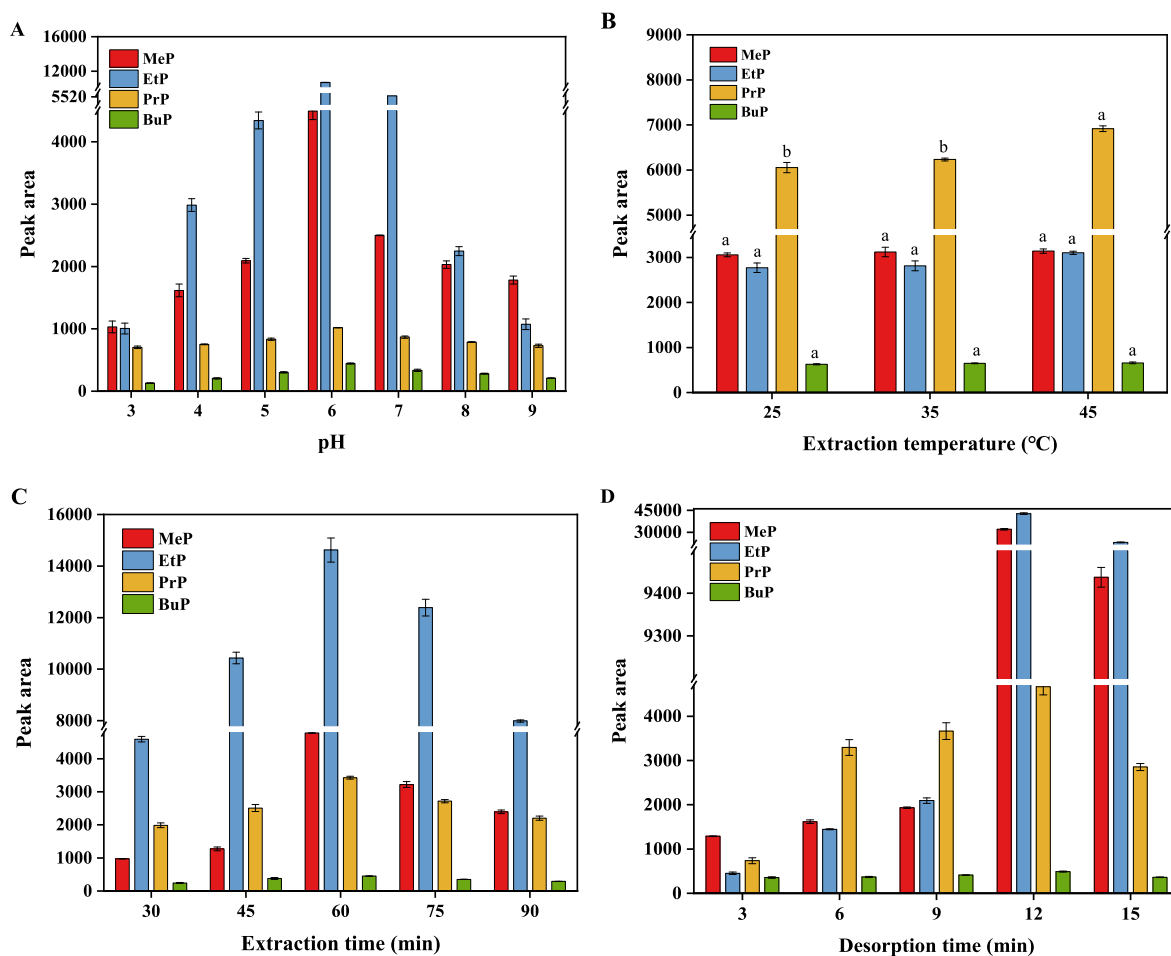
Analytes	RSD (%)					
	Intra-batch			Inter-batch		
	0.5 ( $\mu\text{g/L}$ )	5 ( $\mu\text{g/L}$ )	50 ( $\mu\text{g/L}$ )	0.5 ( $\mu\text{g/L}$ )	5 ( $\mu\text{g/L}$ )	50 ( $\mu\text{g/L}$ )
MeP	1.4	4.0	2.3	3.1	5.4	6.5
EtP	3.8	4.8	3.1	7.1	6.1	4.8
PrP	4.0	5.0	1.6	9.5	8.6	4.1
BuP	3.6	4.2	5.1	5.8	7.3	8.5

## 3. Results and discussion

### 3.1. Morphology and reproducibility of the stir bar

The morphology of the acquired B-MON (Fig. 1A), bare glass stir bar (Fig. 1B) and B-MON coated stir bar (Fig. 1C–D) was examined using a SEM. According to the results, the B-MON was firmly attached to the glass stir bar's surface. Compared with the bare glass stir bar, the B-MON coating displayed a rough, porous and dense surface, which could increase the stir bar's surface area and provide abundant extraction sites.

The reproducibility of the stir bars was evaluated by producing three parallel stir bars, and their repeatability was assessed using the same stir bar for three replicates at 0.5, 5, and 50  $\mu\text{g/L}$ . Table 1 shows that the relative standard deviations obtained for intra-batch and inter-batch were 1.4%–5.0 % and 3.1%–9.5 %, respectively, demonstrating the repeatability and reproducibility of the method used for making the stir bar coated with B-MON.



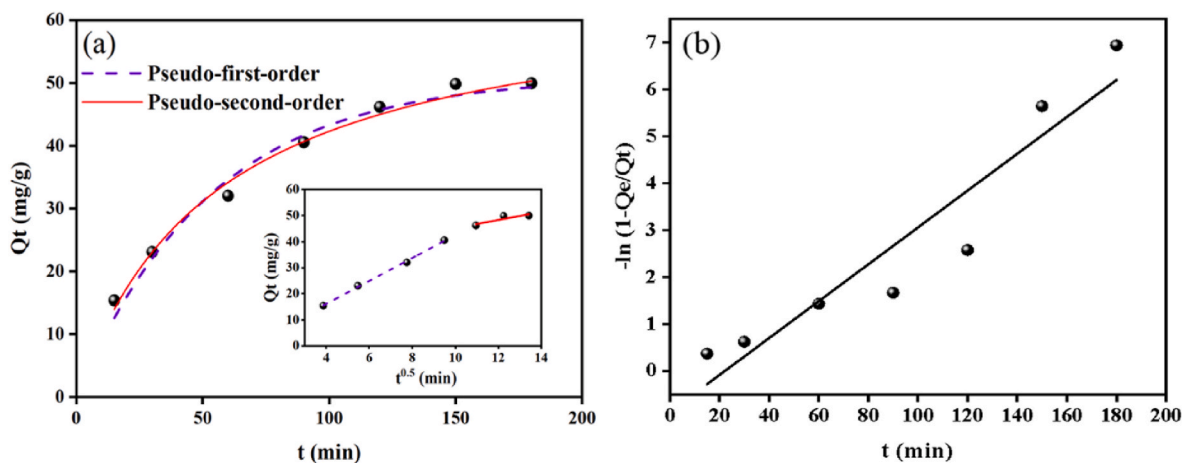
**Fig. 2.** Effect of different parameters on the extraction and desorption efficiency: pH (A), extraction time (B), extraction temperature (C), desorption time (D). The concentration of analyte was 10 µg/L.

### 3.2. Conditions for SBSE optimization

The parameters of pH, extraction temperature, extraction time, and desorption time were optimized to enhance the extraction efficiency of the stir bar coated with B-MON.

pH is a critical factor affecting the chemical properties of compounds and the surface charge of sorbents during the extraction process.

Therefore, a pH range of 3.0–9.0 was used to study its impact on extraction efficiency of parabens. As shown in Fig. 2A, a pH of 6 provided the optimal conditions for the extraction of parabens compounds. Following that, temperatures varying from 25 °C to 45 °C were used to evaluate the impact of extraction temperature on the SBSE of parabens (Fig. 2B). There was no significant different in peak area for MeP, EtP, and BuP. Thus, the extraction temperature of 25 °C was chosen. The



**Fig. 3.** The fitting graphs of adsorption kinetics models of MeP on B-MON: (a) Pseudo-first-order kinetic model, Pseudo-second-order kinetic model, Intra-particle diffusion model and (b) Boyd plot model.



**Table 2**

Kinetic model parameters for the adsorption of MeP on B-MON.

Kinetic models	Parameters	
Pseudo-first-order	$k_1$ (1/min)	$0.0189 \pm 0.0021$
	$Q_{e1}$ (mg/g)	$51.0304 \pm 1.9264$
	$R^2$	0.9771
Pseudo-second-order	$k_2$ (g/mg·min)	$0.0003 \pm 0.00005$
	$Q_{e2}$ (mg/g)	$65.8782 \pm 3.1938$
	$R^2$	0.9845
Intra-particle diffusion	$K_{i1}$ (mg/g·min <sup>1/2</sup> )	$4.4075 \pm 0.1342$
	$c_1$	$-1.5296 \pm 0.9368$
	$R^2$	0.9972
	$K_{i2}$ (mg/g·min <sup>1/2</sup> )	$1.5513 \pm 0.7773$
	$c_2$	$29.7193 \pm 9.5202$
Boyd plot	$R^2$	0.5986
	$R^2$	0.8752

impact of extraction time on SBSE of parabens was investigated for durations of 30–90 min (Fig. 2C). The peak areas gradually increased from 30 min to 60 min. The peak areas decreased after 60 min of extraction, most likely as a result of the targets dissolving in the solvent again during the longer extraction time. Therefore, an extraction time of 60 min was determined to be optimal. Desorption time was also explored with a time range of 3–15 min (Fig. 2D). The desorption amounts of parabens increase steadily from 3 to 12 min, indicating that when the desorption duration was short, the desorption solvent's contact with the targets on the adsorbent material was insufficient, and lengthening the desorption period was advantageous for paraben extraction. However, the recovery of parabens decreased when the desorption duration was increased to 15 min, suggesting that the desorption performance was not improved by increasing the extraction time. Consequently, a desorption time of 12 min was selected for subsequent processes.

### 3.3. Adsorption behavior

#### 3.3.1. Adsorption kinetics

As shown in Fig. 3 (a) and Table 2, the pseudo-second-order model performed better in describing the adsorption kinetics of MeP on B-MON owing to a higher correlation coefficient ( $R^2$ ) than the pseudo-first-order model. This suggests that the adsorption mechanism involved chemisorption significantly. The synthesized B-MON had characteristic peaks for  $-NH_2$  and  $-OH$ , which were characterized by fourier transform infrared in our previous research [22]. Therefore, the hydrogen bonding interactions between  $-OH$  on parabens and  $-NH_2/-OH$  on B-MON may play an important role in the extraction process. To further understand the adsorption mechanism, both the intraparticle diffusion model and

the Body plot model were utilized, as the kinetic models mentioned above could not identify the diffusion mechanism.

The linear fit of the intra-particle diffusion model (Fig. 3 (a)) stayed away from the origin, indicating that the adsorption rate was regulated by multiple steps. The linear curve was divided into two segments, where the first linear segment described the boundary layer diffusion of MeP molecules through the bulk solution to B-MON's exterior surface, and the second segment described the movement of MeP molecules from the surface into B-MON's internal pores. Thus, it can be inferred that the adsorption rate was controlled by both intraparticle diffusion and outer diffusion [28]. The effects of these two diffusion mechanisms on the adsorption process were distinguished using the Boyd plot model (Fig. 3 (b)). The linear fit stayed away from the origin, indicating that the outer diffusion was primarily dominant in the MeP adsorption, which is consistent with the chemisorption process [29].

#### 3.3.2. Adsorption isotherms and thermodynamics

To further understand the adsorption behavior of MeP on B-MON, the adsorption isotherms and thermodynamics were studied. The Freundlich, Langmuir, and Temkin models' applicability to the MeP adsorption process was evaluated, as shown in Fig. 4 (a) and Table S1. A higher  $R^2$  indicated the better fit of the Langmuir models, indicating a monolayer adsorption mechanism containing a finite number of adsorption sites and a uniform surface of the adsorbent. This conclusion

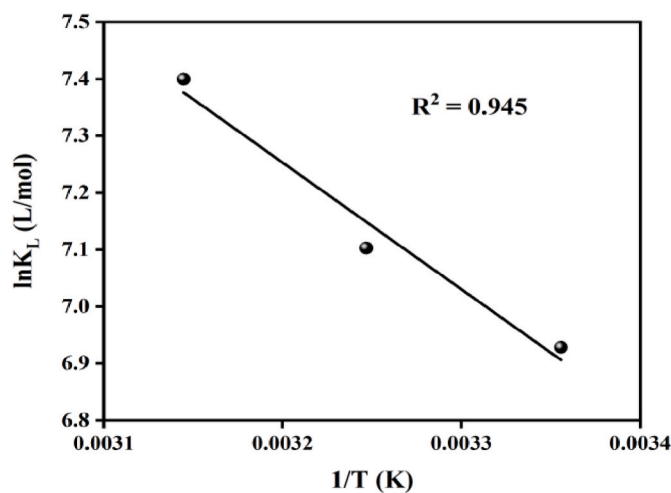
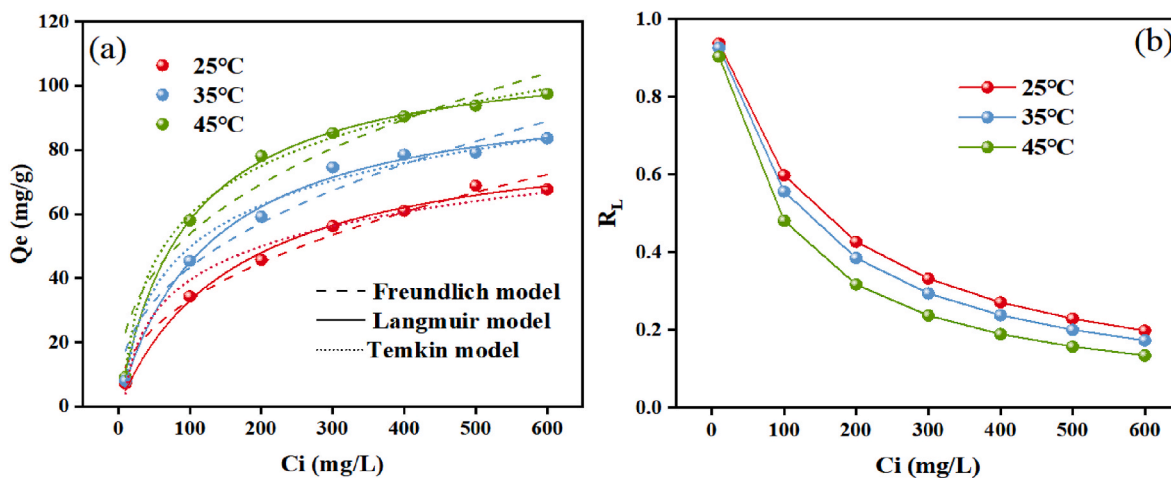
Fig. 5. Plots of  $\ln K_L$  versus  $1/T$  for adsorption of MeP.

Fig. 4. The nonlinear fitting graphs of adsorption isotherms of MeP on B-MON: (a) Freundlich model, Langmuir model, Temkin model and (b) the dimensionless constant  $R_L$  trend curves in the temperature range 25–45 °C.

**Table 3**

Linearity, detection and quantification limits of the proposed method.

Analytes	Linear range (µg/L)	R <sup>2</sup>	LOD (µg/L)	LOQ (µg/L)
MeP	0.03–200.0	0.9992	0.01	0.03
EtP	0.03–200.0	0.9998	0.01	0.03
PrP	0.03–200.0	0.9998	0.01	0.03
BuP	0.03–200.0	0.9964	0.01	0.03

**Table 4**

Accuracy and precision of the proposed method.

Analytes	Spiked (µg/L)	Recovery (%)	RSD (%)
MeP	0.5	103.5 ± 1.8	6.9
	5	87.4 ± 6.0	9.2
	50	92.4 ± 2.2	1.2
EtP	0.5	95.2 ± 3.1	5.4
	5	88.1 ± 1.2	3.2
	50	105.5 ± 1.9	1.6
PrP	0.5	85.8 ± 4.6	8.8
	5	98.1 ± 5.3	1.5
	50	95.4 ± 5.2	4.4
BuP	0.5	104.8 ± 3.7	7.9
	5	88.5 ± 5.9	5.6
	50	92.2 ± 3.4	2.9

was supported by the  $R_L$  values, which were between 0 and 1 [30]. Furthermore, the  $R_L$  values decreased with increasing MeP concentration (Fig. 4 (b)), indicating an irreversible adsorption process at higher concentrations [31]. When the temperature was increased from 25 to 45 °C, the absorption capacity of MeP on B-MON increased, showing that the combining processes were endothermic (Table S1). The values of constant  $n$ , with values of  $1/n$  are less than 1, indicated a simple absorption process.

For MeP adsorption on B-MON, the simulated plot of  $\ln K_L$  versus  $1/T$  is shown in Fig. 5. Table S2 shows the calculated thermodynamic parameters, including  $\Delta G$ ,  $\Delta S$  and  $\Delta H$ . A spontaneous process of MeP adsorption on B-MON was revealed by the negative  $\Delta G$  values. The  $\Delta G$  values increased with increasing temperatures, suggesting a more spontaneous adsorption at high temperature conditions. During the adsorption process, the increase in randomness at the solid-liquid interface was indicated by the positive  $\Delta S$  value, indicating that MeP

molecules were not excessively restricted in the adsorbent [32]. The positive value of  $\Delta H$  indicated the adsorption process has endothermic character, which was consistent with the increase in adsorption capacity with temperature.

### 3.4. Method validation

The analytical performance of the suggested B-MON-SBSE-UHPLC-UV approach was investigated. As shown in Table 3, the LOD and LOQ of the method are 0.01 µg/L and 0.03 µg/L, respectively. In the concentration range of 0.03–200 µg/L, linearity for the target compounds was attained with determination coefficients ( $R^2$ ) better than 0.9964. According to Table 4, the recoveries for human milk ranged from 85.8 % to 105.5 %. By using several replicate assays on the same day, the precisions, expressed as RSDs, were found to range from 1.2 % to 8.8 %. The research results demonstrated that the suggested approach was reliable for determining the levels of parabens in breast milk. As shown in Table 5, the developed method is compared to other reported approaches, which were applied for analyzing parabens in breast milk. The results revealed that the suggested method has lower LOD values compared to most of previous methods.

### 3.5. Method application and risk assessment

The established B-MON-SBSE-UHPLC-UV technique was employed to determine the levels of analytes in various human breast milk samples. Additionally, a preliminary assessment of the exposure and risk of parabens for infants was conducted.

As shown in Table S3, the highest amount of EtP was detected in breast milk in our study, with higher detection frequencies (DF) and EtP concentrations compared to other studies [33–35], but lower than earlier studies conducted in Iran [16,17]. Apart from a Brazilian study [36], the DF of MeP is similar to or higher than in other reports [16,17, 19,33–35]. The DF of PrP is comparable to earlier researches from Iran and Spain [33–35]. In comparison to earlier investigations, the mean levels of PrP are lower [16,17,33,34]. Compared to prior studies, the DF of BuP is lower than in Spain and higher than in Brazil and the mean levels are lower than in other reports [33,34,36], except for a report in Spain [35].

The mean and 95th EDI of MeP and EtP for breastfed infants younger

**Table 5**

Comparison of the established method with analytical procedures previously described for the determination of parabens in breast milk.

Analytes	Extraction Technique	Instrumental technique	LODs (µg/L)	RSD ( % )	Reference
MeP, EtP, PrP	MISPE <sup>a</sup>	LC-UV	10–20 (LOQ)	2.6–12	[18]
MeP, EtP, PrP, BuP	SBSE	GC-MS/MS	0.1	1.7–6.7	[33]
		UHPLC-MS/MS	0.1–0.2	3.1–15	
MeP, EtP, PrP, BuP	SM-SLLME <sup>b</sup>	LC-MS/MS	0.1–0.2	1.1–8.0	[40]
MeP, EtP, PrP, BuP	QuEChERS	UHPLC-MS/MS	0.03	2.0–12.4	[41]
MeP, EtP, PrP, BuP	UAE	UHPLC-MS/MS	0.1–0.2	2.4–11.1	[34]
MeP, EtP, PrP, BuP	SPE	GC-MS	0.008–0.009	4.9–7	[10]
MeP, EtP, PrP, BuP	in-tube SPME	UHPLC-MS/MS	3–10 (LOQ)	1.0–14.8	[36]
MeP, EtP, PrP, BuP	DLLME	LC-MS/MS	0.1	6.2–14.6	[42]
MeP, EtP, PrP	MA-MSPD <sup>c</sup>	LC-UV	25	5.1–11.3	[17]
		LC-MS/MS	0.5		
MeP, EtP, PrP, BuP	SPE	LC-MS/MS	0.1	3.8–7.2	[43]
MeP, EtP, PrP, BuP	QuEChERS	UHPLC-MS/MS	0.1–0.2	5–13	[44]
MeP, EtP, PrP, BuP	QuEChERS	LC-APCI-MS/MS	0.1 (LOQ)	1–16 %	[35]
MeP, PrP	MSPE	HPLC-UV	0.0025–0.0028	1.7–4.9	[19]
MeP, EtP, PrP	µSPE	HPLC-UV	3.0–5.0	4.2–9.5 %	[16]
MeP, EtP, PrP	QuEChERS	LC-MS/MS	0.04–0.13	< 10 %	[13]
MeP, EtP, PrP, BuP	QuEChERS	HPLC-MS/MS	0.1	< 20 %	[39]
MeP, EtP, PrP, BuP	QuEChERS	LC-MS	0.02	1.0–4.5	[38]
MeP, EtP, PrP, BuP	SPE	HPLC-MS/MS	0.001–0.008	< 10 %	[14]
MeP, EtP, PrP, BuP	SBSE	UHPLC-UV	0.01	1.2–9.2 %	This work

<sup>a</sup> Molecularly imprinted solid phase extraction.

<sup>b</sup> Stir-membrane solid–liquid–liquid microextraction.

<sup>c</sup> Magnetically assisted matrix solid phase dispersion.

than 12 months are included in Table S3, and both of them are significantly lower than the ADI of 10 mg/kg-bw/day established by the regulatory agencies for the combined intake of Mep and EtP. EFSA has not established an ADI for PrP and BuP, but non-observed effect levels (NOELs) of 6500 mg/kg-bw/day and 700 mg/kg-bw/day, respectively [37]. In our study, the EDI values of PrP and BuP were several orders of magnitude lower than the NOELs/UF (an "uncertainty factor, UF" of 100 is utilized to ensure a conservative result because of the differences among and within species). Therefore, the dietary intake of four parabens from human breast milk for infants in our study was significantly below their hazard thresholds, which is consistent with other risk assessment studies [38,39].

#### 4. Conclusion

Parabens, as widely used preservatives, can pose health risk for human body. Existing mass spectrometry-based detection methods are sensitive but costly. In this study, we have developed and validated an analytical method based on B-MON coated SBSE for the detection and quantification of four parabens in human breast milk by UHPLC-UV. The B-MON material exhibited excellent extraction efficiency for parabens, which made it possible to enrich and analyze trace analytes in human milk samples. The kinetic fitting results indicate that the adsorption fitted pseudo-second-order kinetic model and the adsorption rate of analytes was dominated by outer diffusion. The results of the isothermal curve fitting indicate that the Langmuir model is a more appropriate fit for the adsorption of parabens, suggesting monolayer adsorption. Meanwhile, the developed method has relatively high sensitivity compared with most existing methods for detecting parabens in breast milk based on LC. Our results indicate that there is no apparent risk of paraben exposure to breastfed infants based on estimated daily intake. Nevertheless, the high detection frequencies highlight the need for continued monitoring of these compounds in human milk to assess their potential risks.

#### CRedit authorship contribution statement

**Xue Ge:** Formal analysis, Investigation, Writing - original draft. **Senwei Feng:** Investigation, Methodology. **Linlin Bian:** Validation. **Mingjuan Wang:** Resources. **Kefeng Li:** Writing - review & editing. **Xu Wang:** Supervision, Writing - review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that has been used is confidential.

#### Acknowledgements

This work was supported by the Open Project Program of State Key Laboratory of Food Nutrition and Safety, China (NO. SKLFNS-KF-201820) and a grant from Macao Polytechnic University (RP/FCSD-02/2022).

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2023.125609>.

#### References

- [1] M. Demirkurt, Y.A. Olcer, M.M. Demir, A.E. Eroglu, Electrospun polystyrene fibers knitted around imprinted acrylate microspheres as sorbent for paraben derivatives, *Anal. Chim. Acta* 1014 (2018) 1–9, <https://doi.org/10.1016/j.aca.2018.02.016>.
- [2] A. Eshraghi, A.H. Talasaz, J. Salamzadeh, M. Salarifar, H. Pourhosseini, Y. Nozari, M. Bahremand, A. Jalali, M.A. Boroumand, Evaluating the effect of Intracoronary N-Acetylcysteine on Platelet activation Markers after primary Percutaneous Coronary Intervention in Patients with ST-Elevation myocardial infarction, *Am. J. Therapeut.* 23 (2016) e44–e51, <https://doi.org/10.1097/mjt.0000000000000309>.
- [3] T. Yin, X. Zhu, I. Cheang, Y. Zhou, S. Liao, X. Lu, Y. Zhou, W. Yao, X. Li, H. Zhang, Urinary phenols and parabens metabolites associated with cardiovascular disease among adults in the United States, *Environ. Sci. Pollut. Res. Int.* 30 (2023) 25093–25102, <https://doi.org/10.1007/s11356-021-15589-5>.
- [4] N.B. Bolujoko, E.I. Unuabonah, M.O. Alfred, A. Ogunlaja, O.O. Ogunlaja, M. O. Omorogie, O.D. Olukanni, Toxicity and removal of parabens from water: a critical review, *Sci. Total Environ.* 792 (2021), 148092, <https://doi.org/10.1016/j.scitotenv.2021.148092>.
- [5] EFSA, Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to para hydroxybenzoates (E 214–219), EFSA J. (2004), <https://doi.org/10.2903/j.efsa.2004.83>.
- [6] M.M. Ariffin, A.H.M. Azmi, N.M. Saleh, S. Mohamad, S.K.M. Rozi, Surfactant functionalisation of magnetic nanoparticles: a greener method for parabens determination in water samples by using magnetic solid phase extraction, *Microchem. J.* 147 (2019) 930–940, <https://doi.org/10.1016/j.microc.2019.04.017>.
- [7] G. Yusakul, C. Thammakhet-Buranachai, S. Poorahong, S. Sakamoto, F. Makkliang, An eco-friendly method using deep eutectic solvents immobilized in a microcrystalline cellulose-polyvinyl alcohol sponge for parabens analysis in food samples, *Microchem. J.* 191 (2023), 108758, <https://doi.org/10.1016/j.microc.2023.108758>.
- [8] W.-H. Chung, J.-S. Lin, W.-H. Ding, Dual-vortex-assisted matrix solid-phase dispersion coupled with isotope-dilution ultrahigh-performance liquid chromatography-high resolution mass spectrometry for the rapid determination of parabens in indoor dust samples, *J. Chromatogr. A* 1605 (2019), 460367, <https://doi.org/10.1016/j.chroma.2019.460367>.
- [9] S. Seidi, E. Sadat Karimi, A. Rouhollahi, M. Baharfar, M. Shanehsaz, M. Tajik, Synthesis and characterization of polyamide-graphene oxide-polypyrrole electrospun nanofibers for spin-column micro solid phase extraction of parabens in milk samples, *J. Chromatogr. A* 1599 (2019) 25–34, <https://doi.org/10.1016/j.chroma.2019.04.014>.
- [10] A. Azzouz, A.J. Rascón, E. Ballesteros, Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry, *J. Pharmaceut. Biomed. Anal.* 119 (2016) 16–26, <https://doi.org/10.1016/j.jpba.2015.11.024>.
- [11] F. Artacho-Cordón, M.F. Fernández, H. Frederiksen, L.M. Iribarne-Durán, I. Jiménez-Díaz, F. Vela-Soria, A.M. Andersson, P. Martín-Olmedo, F.M. Peinado, N. Olea, J.P. Arrebola, Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain, *Environ. Int.* 119 (2018) 203–211, <https://doi.org/10.1016/j.envint.2018.05.052>.
- [12] G.M. Lehmann, M.-A. Verner, B. Luukinen, C. Henning, S.A. Assimon, J.S. LaKind, E.D. McLanahan, L.J. Phillips, M.H. Davis, C.M. Powers, E.P. Hines, S. Haddad, M. P. Longnecker, M.T. Poulsen, D.G. Farrer, S.A. Marchitti, Y.-M. Tan, J.C. Swartout, S.K. Sagiv, C. Welsh, J.L. Campbell Jr., W.G. Foster, R.S.H. Yang, S.E. Fenton, R. Tornero-Velez, B.M. Francis, J.B. Barnett, H.A. El-Masri, J.E. Simmons, Improving the risk assessment of lipophilic persistent environmental chemicals in breast milk, *Crit. Rev. Toxicol.* 44 (2014) 600–617, <https://doi.org/10.3109/10408444.2014.926306>.
- [13] J.H. Kim, D. Kim, S.-M. Moon, E.J. Yang, Associations of lifestyle factors with phthalate metabolites, bisphenol A, parabens, and triclosan concentrations in breast milk of Korean mothers, *Chemosphere* 249 (2020), 126149, <https://doi.org/10.1016/j.chemosphere.2020.126149>.
- [14] D. Zhang, J. Xiao, Q. Xiao, Y. Chen, X. Li, Q. Zheng, J. Ma, J. Xu, J. Fu, J. Shen, L. Xiao, S. Lu, Infant exposure to parabens, triclosan, and triclocarban via breastfeeding and formula supplementing in southern China, *Sci. Total Environ.* 858 (2023), 159820, <https://doi.org/10.1016/j.scitotenv.2022.159820>.
- [15] N. Parham, H.A. Panahi, A. Feizbakhsh, E. Moniri, Synthesis of high generation thermo-sensitive dendrimers for extraction of rivaroxaban from human fluid and pharmaceutical samples, *J. Chromatogr. A* 1545 (2018) 12–21, <https://doi.org/10.1016/j.chroma.2018.02.050>.
- [16] M. Manouchehri, S. Seidi, A. Rouhollahi, H. Noormohammadi, M. Shanehsaz, Micro solid phase extraction of parabens from breast milk samples using Mg-Al layered double hydroxide functionalized partially reduced graphene oxide nanocomposite, *Food Chem.* 314 (2020), 126223, <https://doi.org/10.1016/j.foodchem.2020.126223>.
- [17] M. Fotouhi, S. Seidi, M. Shanehsaz, M.T. Naseri, Magnetically assisted matrix solid phase dispersion for extraction of parabens from breast milks, *J. Chromatogr. A* 1504 (2017) 17–26, <https://doi.org/10.1016/j.chroma.2017.05.009>.
- [18] L.P. Melo, M.E.C. Queiroz, A molecularly imprinted polymer for microdisc solid-phase extraction of parabens from human milk samples, *Anal. Methods* 5 (2013) 3538–3545, <https://doi.org/10.1039/c3ay00025g>.
- [19] H. Abdolmohammad-Zadeh, A. Zamani, Z. Shamsi, Extraction of four endocrine-disrupting chemicals using a Fe<sub>3</sub>O<sub>4</sub>/graphene oxide/di-(2-ethylhexyl) phosphoric acid nano-composite, and their quantification by HPLC-UV, *Microchem. J.* 157 (2020), 104964, <https://doi.org/10.1016/j.microc.2020.104964>.

- [20] J.S.M. Lee, A.I. Cooper, Advances in Conjugated microporous Polymers, *Chem. Rev.* 120 (2020) 2171–2214, <https://doi.org/10.1021/acs.chemrev.9b00399>.
- [21] L. Zhou, Y. Hu, G. Li, Conjugated microporous Polymers with Built-in magnetic nanoparticles for excellent Enrichment of trace Hydroxylated Polycyclic Aromatic Hydrocarbons in human urine, *Anal. Chem.* 88 (2016) 6930–6938, <https://doi.org/10.1021/acs.analchem.6b01708>.
- [22] S. Li, S. Feng, A. Van Schepdael, X. Wang, Hollow fiber membrane-protected amino/hydroxyl bifunctional microporous organic network fiber for solid-phase microextraction of bisphenols A, F, S, and triclosan in breast milk and infant formula, *Food Chem.* 390 (2022), 133217, <https://doi.org/10.1016/j.foodchem.2022.133217>.
- [23] Y. Lei, B. Chen, L. You, M. He, B. Hu, Polydimethylsiloxane/MIL-100(Fe) coated stir bar sorptive extraction-high performance liquid chromatography for the determination of triazines in environmental water samples, *Talanta* 175 (2017) 158–167, <https://doi.org/10.1016/j.talanta.2017.05.040>.
- [24] S. Marín-San Román, P. Rubio-Bretón, E.P. Pérez-Álvarez, T. Garde-Cerdán, Advancement in analytical techniques for the extraction of grape and wine volatile compounds, *Food Res. Int.* 137 (2020), 109712, <https://doi.org/10.1016/j.foodres.2020.109712>.
- [25] J.H. Han, Y.Y. Cui, C.X. Yang, Tailored amino/hydroxyl bifunctional microporous organic network for efficient stir bar sorptive extraction of parabens and flavors from cosmetic and food samples, *J. Chromatogr. A* 1655 (2021), 462521, <https://doi.org/10.1016/j.chroma.2021.462521>.
- [26] S. Li, L. Bian, C. Yang, A. Van Schepdael, X. Wang, Migration study of phenolic endocrine disruptors from pacifiers to saliva simulant by solid phase microextraction with amino-functionalized microporous organic network coated fiber, *J. Hazard Mater.* 438 (2022), 129505, <https://doi.org/10.1016/j.jhazmat.2022.129505>.
- [27] EPA, Exposure Factors Handbook, National Center for Environmental Assessment, 2011. <http://www.epa.gov/ncea/efh>.
- [28] S.P. Hooshyar, H.A. Panahi, E. Moniri, M. Farsadrooh, Tailoring a new hyperbranched PEGylated dendrimer nano-polymer as a super-adsorbent for magnetic solid-phase extraction and determination of letrozole in biological and pharmaceutical samples, *J. Mol. Liq.* 338 (2021), 116772, <https://doi.org/10.1016/j.molliq.2021.116772>.
- [29] H. Wang, S. Wang, S. Wang, L. Fu, L. Zhang, The one-step synthesis of a novel metal-organic frameworks for efficient and selective removal of Cr(VI) and Pb(II) from wastewater: kinetics, thermodynamics and adsorption mechanisms, *J. Colloid Interface Sci.* 640 (2023) 230–245, <https://doi.org/10.1016/j.jcis.2023.02.108>.
- [30] Y.X. Wang, Y.Y. Cui, Y. Zhang, C.X. Yang, Synthesis of reusable and renewable microporous organic networks for the removal of halogenated contaminants, *J. Hazard Mater.* 424 (2022), 127485, <https://doi.org/10.1016/j.jhazmat.2021.127485>.
- [31] A. Morovati, H. Ahmad Panahi, F. Yazdani, Grafting of allylimidazole and n-vinylcaprolactam as a thermosensitive polymer onto magnetic nano-particles for the extraction and determination of celecoxib in biological samples, *Int. J. Pharm.* 513 (2016) 62–67, <https://doi.org/10.1016/j.ijpharm.2016.09.006>.
- [32] N.S.M. Tahiruddin, R.A. Aziz, R. Ali, N.I. Taib, Potential of using jackfruit peel (*Artocarpus heterophyllus*) as green solution for removal of copper (II) and zinc (II) from aqueous solution: adsorption kinetics, isotherm and thermodynamic studies, *J. Environ. Chem. Eng.* 11 (2023), 109953, <https://doi.org/10.1016/j.jece.2023.109953>.
- [33] R. Rodríguez-Gómez, A. Zafra-Gómez, F.J. Camino-Sánchez, O. Ballesteros, A. Navalón, Gas chromatography and ultra high performance liquid chromatography tandem mass spectrometry methods for the determination of selected endocrine disrupting chemicals in human breast milk after stir-bar sorptive extraction, *J. Chromatogr. A* 1349 (2014) 69–79, <https://doi.org/10.1016/j.chroma.2014.04.100>.
- [34] R. Rodríguez-Gómez, N. Dorival-García, A. Zafra-Gómez, F.J. Camino-Sánchez, O. Ballesteros, A. Navalón, New method for the determination of parabens and bisphenol A in human milk samples using ultrasound-assisted extraction and clean-up with dispersive sorbents prior to UHPLC-MS/MS analysis, *J. Chromatogr. B* 992 (2015) 47–55, <https://doi.org/10.1016/j.jchromb.2015.04.022>.
- [35] P. Dualde, O. Pardo, F. Fernández, S. A. Pastor, V. Yusà, Determination of four parabens and bisphenols A, F and S in human breast milk using QuEChERS and liquid chromatography coupled to mass spectrometry, *J. Chromatogr. B* 1114–1115 (2019) 154–166, <https://doi.org/10.1016/j.jchromb.2019.03.004>.
- [36] I.D. Souza, L.P. Melo, I.C.S.F. Jardim, J.C.S. Monteiro, A.M.S. Nakano, M.E. C. Queiroz, Selective molecularly imprinted polymer combined with restricted access material for in-tube SPME/UHPLC-MS/MS of parabens in breast milk samples, *Anal. Chim. Acta* 932 (2016) 49–59, <https://doi.org/10.1016/j.aca.2016.05.027>.
- [37] J. Boberg, C. Taxvig, S. Christiansen, U. Hass, Possible endocrine disrupting effects of parabens and their metabolites, *Reprod. Toxicol.* 30 (2010) 301–312, <https://doi.org/10.1016/j.reprotox.2010.03.011>.
- [38] B. Czarczyńska-Gosińska, T. Grześkowiak, R. Frankowski, J. Lulek, J. Pieczak, A. Zgoła-Grześkowiak, Determination of bisphenols and parabens in breast milk and dietary risk assessment for Polish breastfed infants, *J. Food Compos. Anal.* 98 (2021), 103839, <https://doi.org/10.1016/j.jfca.2021.103839>.
- [39] P. Dualde, O. Pardo, F. Corpas-Burgos, J. Kuligowski, M. Gormaz, M. Vento, A. Pastor, V. Yusà, Biomonitoring of parabens in human milk and estimated daily intake for breastfed infants, *Chemosphere* 240 (2020), 124829, <https://doi.org/10.1016/j.chemosphere.2019.124829>.
- [40] R. Rodríguez-Gómez, M. Roldán-Pijuán, R. Lucena, S. Cárdenas, A. Zafra-Gómez, O. Ballesteros, A. Navalón, M. Valcárcel, Stir-membrane solid-liquid-liquid microextraction for the determination of parabens in human breast milk samples by ultra high performance liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A* 1354 (2014) 26–33, <https://doi.org/10.1016/j.chroma.2014.05.071>.
- [41] R. Rodríguez-Gómez, I. Jiménez-Díaz, A. Zafra-Gómez, O. Ballesteros, A. Navalón, A multiresidue method for the determination of selected endocrine disrupting chemicals in human breast milk based on a simple extraction procedure, *Talanta* 130 (2014) 561–570, <https://doi.org/10.1016/j.talanta.2014.07.047>.
- [42] F. Vela-Soria, I. Jiménez-Díaz, C. Díaz, J. Pérez, L.M. Iribarne-Durán, L. Serrano-López, J.P. Arrebola, M.F. Fernández, N. Olea, Determination of endocrine-disrupting chemicals in human milk by dispersive liquid-liquid microextraction, *Bioanalysis* 8 (2016) 1777–1791, <https://doi.org/10.4155/bio-2016-0073>.
- [43] M. Fisher, S. MacPherson, J.M. Braun, R. Hauser, M. Walker, M. Feeley, R. Mallick, R. Berube, T.E. Arbuckle, Paraben concentrations in Maternal urine and breast milk and its association with personal care product Use, *Environ. Sci. Technol.* 51 (2017) 4009–4017, <https://doi.org/10.1021/acs.est.6b04302>.
- [44] F. Vela-Soria, L.M. Iribarne-Durán, V. Mustieles, I. Jiménez-Díaz, M.F. Fernández, N. Olea, QuEChERS and ultra-high performance liquid chromatography-tandem mass spectrometry method for the determination of parabens and ultraviolet filters in human milk samples, *J. Chromatogr. A* 1546 (2018) 1–9, <https://doi.org/10.1016/j.chroma.2018.02.060>.