chemosensors

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Analysis of Parabens and Bisphenol A in Female Hair via LC-MS/MS and Its Application to a Biomonitoring Study in Southern Brazil

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Abstract: Parabens (PBs) and bisphenols are endocrine disruptors (EDs) widely used in everyday products and associated with health issues, such as reproductive disorders, breast cancer, obesity, hypertension, and asthma. Hair has been proposed as an alternative matrix due to its ability to reflect prolonged exposure while being less affected by short-term fluctuations. This study developed a rapid and sensitive analytical method for the determination of PBs (butylparaben, methylparaben, ethylparaben, and propylparaben) and bisphenol A in hair samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Hair sample preparation included acid hydrolysis, extraction with ethyl acetate, and derivatization with dansyl chloride. The chromatographic run time was 5.50 min. The method presented acceptable precision (CV < 9.09%) and accuracy (100.71–108.58%), meeting validation guidelines. The validated method was applied to hair samples from 101 volunteers, demonstrating its reliability as a biomonitoring tool for assessing long-term exposure to PBs and bisphenol A in human populations.

Keywords: parabens; bisphenol A; biomonitoring; LC-MS/MS

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Received: 13 February 2025
Revised: 14 March 2025
Accepted: 19 March 2025
Published: 22 March 2025

Citation: Peteffi, G.P.; Loredo, C.D.; de Souza, C.F.; Hahn, R.Z.; Bondan, A.P.; Linden, R. Analysis of Parabens and Bisphenol A in Female Hair via LC-MS/MS and Its Application to a Biomonitoring Study in Southern Brazil. *Chemosensors* **2025**, *13*, 118. https://doi.org/10.3390/chemosensors13040118

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1. Introduction

In daily life, parabens (PBs) and bisphenols are families of endocrine disruptors (EDs) widely used in consumer products [1]. In 2002, the World Health Organization defined EDs as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or (sub)populations" [2]. The biological effects of EDs are associated with their ability to interfere with the synthesis and metabolism of endogenous hormones, as well as their capacity to mimic or antagonize hormonal activity [3].

PBs are commonly used as antimicrobial preservatives due to their antibacterial and antifungal properties, particularly in pharmaceutical products, cosmetics, beverages, and foods. They are present in numerous everyday items, including personal care products, shampoos, food packaging, lipsticks, medications, and baby wipes [4]. The widespread use of PBs as preservatives is attributed to their low toxicity, chemical stability, broad-spectrum antimicrobial activity, and global regulatory acceptance [5,6]. Their antifungal and antimicrobial efficacy increases with longer alkyl chain lengths; however, this also reduces their solubility in water, limiting their applications. Methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP) are the most frequently used PBs, often

in combination to enhance their effectiveness at low concentrations [7]. However, several studies have demonstrated that PBs can be absorbed by the human body through both oral and dermal exposure [8], potentially leading to reproductive disorders [9], increased obesity risks [10], genotoxicity [11], allergic sensitivity [7], and breast cancer [12].

Bisphenols, characterized by the presence of two hydroxyphenol functional groups, include bisphenol A (BPA) as the most prevalent compound [13]. BPA is used in the production of polycarbonate plastics and epoxy resins and is found in various consumer goods, such as food and beverage cans, thermal paper receipts, dental sealants, food packaging, and personal care products (PCPs) [14]. BPA has been implicated in multiple health conditions, including asthma, reproductive disorders, hypertension, endometriosis, behavioral abnormalities, birth weight anomalies, preterm birth, diabetes, and obesity [15]. Both PBs and BPA can enter the human body not only through dermal or oral exposure but also via breast milk and perinatal transmission through transplacental passage [16].

Human biomonitoring of PBs and BPA is typically conducted using blood and urine samples [17]. However, due to the low water solubility of long-chain PBs in urine, their quantification is challenging. Additionally, PBs are rapidly metabolized into p-hydroxybenzoic acid and its sulfate and glucuronide conjugates, which are excreted in urine within 24 h. Furthermore, blood is not an ideal matrix for evaluating long-term exposure. To overcome these limitations, non-invasive matrices, such as hair, nails, saliva, and breast milk, have been explored for biomonitoring purposes [18]. Hair serves as a valuable analytical matrix for assessing the long-term exposure to various chemicals, including EDs [19]. It enables retrospective investigations of chronic exposure [20] and offers several advantages, such as non-invasive sampling, long-term storage stability, minimal sample degradation, and a low risk of contamination [21]. Given the widespread use of PBs and BPA and their potential risks to human health, hair biomonitoring is a valuable tool for assessing human exposure to these chemicals [1,17,19,22–24].

Due to the complexity of biological matrices and the low concentrations of most EDs, an initial sample preparation step is required before the chromatographic analysis to clean the matrix and concentrate the analytes [25]. Most chemicals are transferred in urine within a few hours after exposure, and their concentration may become undetectable rapidly after exposure stops. Conversely, hair can hold chemicals that have been in the body (even briefly) for a period ranging from weeks to months, depending on the length of the hair [23].

Several methods aiming the analysis of these EDs in hair, combining gas [26] or liquid chromatography with mass spectrometry [1,17,19,22,27,28], have been described, with adequate sensitivity, precision, and accuracy. However, monitoring PBs and BPA in hair represents an analytical challenge that requires a sensitive method capable of detecting multiple compounds.

This study describes the validation of a sensitive method for the simultaneous determination of MeP, EtP, PrP, BuP, and BPA in human hair using LC-MS/MS, following hydrolysis, liquid extraction, and derivatization with dansyl chloride. In addition, this method was applied to a cohort of Southern Brazilian volunteers to evaluate exposure to these compounds.

2. Materials and Methods

2.1. Study Population and Sample Collection

Hair samples were collected from 101 healthy adult volunteers, including 3 men and 98 women, aged 17 to 57 years, residing in the southern Brazilian cities of Novo Hamburgo, São Leopoldo, and Porto Alegre, between September 2023 and May 2024. Volunteers completed a questionnaire regarding the frequency of cosmetic use. Participant selection was non-discriminatory, ensuring data confidentiality, anonymity, and ethical compliance.

The study was approved by the institutional review board of Feevale University (protocol number 6.024.337), and all participants provided written informed consent. For hair collection and pre-treatment, hair was cut at the posterior vertex as close as possible to the scalp. Only the first proximal centimeter was analyzed to represent the month prior to sampling. The hair was wrapped in aluminum foil, and variations in length were due to different hairstyles among the volunteers. The samples were dried at room temperature and stored in the dark at room temperature to prevent photodegradation.

2.2. Chemicals, Reagents, and Solutions

EtP (≥99%) and BuP (≥99%) were obtained from Chem Service (West Chester, PA, USA), while MeP (≥99%), PrP (≥99%), sodium dodecyl sulfate (SDS), BPA, and BPA-d_{16} were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The deuterated analogs MeP-d_4, EtP-d_4, and BuP-d_9, used as internal standards (IS), were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Acetic acid, acetone, dichloromethane, ammonium formate, dansyl chloride, methanol, sodium bicarbonate, sodium hydroxide, and formic acid were also purchased from Sigma-Aldrich. Ethyl acetate was supplied by Honeywell (Morris Plains, NJ, USA), and activated charcoal was obtained from Dinâmica Química (Indaiatuba, Brazil). Stock solutions of each compound were prepared in methanol at concentrations of 1 mg/mL. Stock solutions were stored at −20 °C. All organic solvents and reagents were of analytical grade. Purified water was produced using a Purelab Ultra system (Elga, High Wycombe, UK). Stock solutions of the target compounds were prepared at 1 mg/mL by dissolving the powders in methanol. Methanolic working solutions of the analytes were prepared in methanol at different concentration ranges: 25, 50, 100, 500, 1000, 2500, 5000, and 10,000 ng/g for MeP; 2.5, 5, 10, 50, 100, 250, 500, and 1000 ng/g for BuP and EtP; 5, 10, 20, 100, 200, 500, 1000, and 2000 ng/g for PrP; and 2, 4, 8, 40, 80, 200, 400, and 800 ng/g for BPA.

These solutions were stored at −20 °C. IS working solutions were also prepared in methanol at the following concentrations: BPA-d_{16} at 500 ng/mL, BuP-d_9 at 50 ng/mL, EtP-d_4 at 50 ng/mL, and MeP-d_4 at 250 ng/mL. The ISs used for each analyte were MeP-d_4 for MeP, EtP-d_4 for EtP, BuP-d_9 for PrP and BuP, and BPA-d_{16} for BPA.

A 10 mM sodium bicarbonate buffer at pH 10.5 was prepared by dissolving 0.42 g of sodium bicarbonate in 50 mL of ultrapure water, with pH adjustment using a 1 M sodium hydroxide solution. A 0.1% SDS solution was prepared in ultrapure water. For hair sample hydrolysis, a 1.5% HAc solution was freshly prepared by diluting 1 mL of HAc in 9 mL of ultrapure water, followed by a second dilution, in which 6 mL of the first solution was mixed with 34 mL of ultrapure water.

2.3. Method Development and Validation

2.3.1. Hair Washing (Volunteers' Hair, Curves, and Controls)

The hair strand was washed in an ultrasonic bath with ultrapure water for 5 min, followed by washing with a 0.1% (*w*/*v*) SDS solution in an ultrasonic bath for another 5 min, and then rinsed again with ultrapure water for an additional 5 min to remove residual chemicals. After washing, the hair was dried at 80 °C in a laboratory oven.

2.3.2. Preparation of Blank Hair

Washed hair was cut into small pieces (2–3 mm) with stainless steel scissors and divided into 50 mg aliquots. Each aliquot was transferred to a polypropylene microtube and mixed with 250 µL of a 1:1 (*v*/*v*) methanol:dichloromethane solution, followed by sonication for 30 min. Afterward, the tube was centrifuged at 4500 rpm for 5 min, and the supernatant was discarded. This procedure was repeated twice. Finally, the hair was dried at room temperature. After the acidic hydrolysis step, described below, the supernatant

was transferred to a 2 mL polypropylene tube and treated with 60 mg of activated charcoal under agitation at 1000 rpm at 25 °C for 1 h. Charcoal treatment was performed only for the blank samples used in calibration and control curves and was not applied to volunteer samples. Following charcoal treatment, the hydrolysate was centrifuged at 15,000 rpm for 5 min, and the supernatant was collected in another tube. Centrifugation was repeated twice until all activated charcoal was visually removed.

2.3.3. Extraction of Hair Samples

Washed hair was cut into small pieces (2–3 mm) with stainless steel scissors. An aliquot of 50 mg of minced hair was weighed into a 5 mL polypropylene tube and incubated with 1 mL of 1.5% glacial acetic acid at 38 °C for 12 h. After acidic hydrolysis, the samples were cooled to room temperature and centrifuged at 15,000 rpm for 5 min. An aliquot of 800 µL of the supernatant was transferred to another tube and was enriched with 40 µL of either spiking solutions (calibrator and quality control) or pure methanol (for blank samples), along with 100 µL of the internal standard (IS) working solution. Two liquid–liquid extraction cycles were performed using 1 mL of ethyl acetate, with vortex stirring for 1 min per cycle, followed by centrifugation at 15,000 rpm for 5 min. In each extraction step, 700 µL of the supernatant was collected in a 2 mL polypropylene tube, totaling 1.4 mL, which was then evaporated to dryness using a vacuum concentrator at 60 °C. The dry residues were reconstituted in 50 µL of a 10 mM sodium bicarbonate buffer and 50 µL of dansyl chloride in acetone (1 mg/mL), vortexed, incubated at 60 °C for 15 min, cooled to room temperature, and evaporated again at 60 °C. The final dry residues were reconstituted in 75 µL of methanol and 75 µL of a 10 mM ammonium formate aqueous solution and filtered through a 0.22 µm polytetrafluoroethylene hydrophobic filter, and 10 µL was injected into the LC-MS/MS for analysis.

2.3.4. LC-MS/MS Analysis

Analysis was performed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system composed of an Acquity I-Class chromatography coupled to a XEVO TQS Micro triple quadrupole mass spectrometer (Waters, Milford, CT, USA). The chromatographic column used was an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 µm, Waters), maintained at 40 °C, while the samples were kept at 10 °C with an injection volume of 10 µL. The mobile phase flow rate was 350 µL/min, using two mobile phases: (A) ultrapure water with 0.1% formic acid and (B) methanol with 0.1% formic acid. The initial gradient started at 10% phase B, increasing to 95% within 1.9 min, maintained until 3.6 min, and returning to the initial conditions after 3.7 min, with a total run time of 5.5 min. The mass spectrometric conditions included positive electrospray ionization, a capillary voltage of 3.0 kV, a desolvation temperature of 500 °C, a desolvation gas flow of 1000 L/h, and a source temperature of 150 °C. The compound-specific acquisition parameters are presented in Table 1.

Table 1. Retention times and mass spectrometric acquisition parameters of the target compounds in hair.

Analytes	Parent (*m*/*z*)	Quantification ion (*m*/*z*)	Qualification ion (*m*/*z*)	Collision Voltage (V)	Cone Voltage (V)	Rt (min)	Dwell (s)
MeP	386.2	171.2	156.1	23	30	2.79	0.022
EtP	400.2	171.2	156.1	23	30	2.87	0.022
PrP	414.2	171.2	156.1	27	30	2.95	0.022
BuP	428.3	171.2	156.1	27	30	3.05	0.022
BPA	695.2	171.2	156.1	50	56	3.26	0.022
MeP-d_4	390.3	171.2	-	23	30	2.79	0.022

Table 1. *Cont.*

Analytes	Parent (*m*/*z*)	Quantification ion (*m*/*z*)	Qualification ion (*m*/*z*)	Collision Voltage (V)	Cone Voltage (V)	Rt (min)	Dwell (s)
BuP-d_9	437.4	171.2	-	28	30	3.04	0.022
EtP-d_4	404.3	171.2	-	26	30	2.86	0.022
BPA-d_{16}	711.2	171.2	-	50	56	3.24	0.022

All of the source and instrument parameters were optimized by infusing with the derivatized; Rt = retention. time; *m*/*z* = mass/charge; s = seconds; V = voltage.

2.3.5. Evaluation of Hair Washing

The effectiveness of the hair washing procedure was evaluated by testing control samples fortified with low (QCL) and high (QCH) concentrations, in quintuplicate. These samples were fortified before the washing procedure and were washed and extracted as described above. The peak area ratios of the extracts were compared to a control sample at the lower limit of quantification of the method.

2.3.6. Method Validation

The method was developed and validated according to the International Conference on Harmonization (ICH) bioanalytical method validation [29].

The linearity of the calibration models was evaluated at eight concentration levels in sextuplicate. Blank hair was spiked with combined calibrator solutions to obtain analyte concentrations of 25, 50, 100, 500, 1000, 2500, 5000, and 10,000 ng/g for MeP; 2.5, 5, 10, 50, 100, 250, 500, and 1000 ng/g for BuP and EtP; 5, 10, 20, 100, 200, 500, 1000, and 2000 ng/g for PrP; and 2, 4, 8, 40, 80, 200, 400, and 800 ng/g for BPA. Linearity was considered acceptable if the back-calculated concentrations of calibration samples were within ±15% of their nominal values, with correlation coefficients above 0.99 [30].

Calibration curves were constructed by comparing the ratios of analyte peak areas to internal standard peak areas (y) against nominal concentrations (x). Homoscedasticity was assessed using the *F*-test at a 95% confidence level, and linear regression models were adjusted using weight factors ($1/x$, $1/x^{0.5}$, $1/x^2$, $1/y$, $1/y^{0.5}$, $1/y^2$). Calibration models were evaluated based on correlation coefficients (*r*) and the cumulative percentage error ($\sum$ %ER) [31].

Precision and accuracy were also assessed at the LQ, MeP 25 ng/g, BuP and EtP 2.5 ng/g, PrP 5 ng/g, and BPA 2 ng/g tested in triplicate on three different days. The acceptance criteria were accuracy within 100 ± 20% of the nominal concentration and a maximum intra- and inter-assay CV% of 20%.

Aliquots of blank hair were enriched with PBs to obtain quality control (QC) samples at low concentrations (QCL; MeP 40 ng/g, BuP and EtP 4 ng/g, PrP 8 ng/g, BPA 3.2 ng/g), medium concentrations (QCM; MeP 250 ng/ g, BuP and EtP 25 ng/g, PrP 50 ng/g, BPA 20 ng/g), and high concentrations (QCH, MeP 7500 ng/g, BuP and EtP 750 ng/g, PrP 1500 ng/g, BPA 600 ng/g). QC samples were analyzed in quintuplicate on three different days, and intra-assay and inter-assay precision were expressed as the CV%. The acceptance criterion for precision was a maximum CV% of 15%, while accuracy was considered acceptable if values fell within ±15% of the theoretical concentration [29].

Stability studies were conducted at QCL and QCH levels under autosampler conditions, with extracted QC samples injected into the LC-MS/MS system at 1 h intervals over 12 h. The peak area ratios of parabens and BPA to ISs from the first injection were compared to the final injection, and deviations below 15% were considered acceptable [29].

The extraction yield (EY) and matrix effects (MEs) were assessed using three sets of QC samples (QCL and QCH). Set A contained analyte solutions derivatized and reconstituted

in methanol and ammonium formate. Set B consisted of post-extraction spiked hair extracts, while set C involved pre-extraction spiked samples. The extraction yield was calculated as EY = (C/B) × 100%, and matrix effects were determined as ME = 100 − (B/A)%. A CV% below 15% was required for acceptance [32].

In the dilution integrity test, due to the impracticality of diluting a hair sample, we evaluated the processing of a reduced amount of hair containing the target compounds at above-curve concentrations. In this evaluation, samples of 10 mg of hair containing PBs and BPA with QCH in triplicate were processed and analyzed as described above. These samples were quantified in triplicate on three different days, allowing for a calculation of the accuracy and precision for this experiment. The acceptance criterion for the dilution integrity was accuracy between 80 and 120% [33].

In the routine analysis, concentrations below the LQ are generally not reported and are instead left censored as "below the limit of quantification" (BLOQ). Laboratories may also use "not detectable" (ND) instead of BLOQ when no analyte is detected at all. Several methods have been proposed to handle values below the LQ. Our method applies one of the most commonly used approaches, which includes either discarding values below the quantification limit (BLQ) or replacing BLQ values with LQ/2 [34].

3. Results

The analytes exhibited short chromatographic retention times: MeP (2.82 min), EtP (2.90 min), PrP (2.98 min), BuP (3.07 min), and BPA (3.29 min), differing from previous studies (Figure 1). An ion chromatogram of a blank hair sample is presented in Figure S1 (Supplementary Materials). Chromatograms obtained at the LQ (1), IS (2), and volunteer samples (3) displayed high-resolution peaks for each target analyte (Figure 1), using the quantification transitions shown in Table 1.

Sample preparation was simple, based on liquid extraction with ethyl acetate, yielding mean recoveries of 83.83% for MeP, 58.54% for EtP, 64.67% for PrP, 70% for BuP, and 100.71% for BPA after two extraction cycles. Ethyl acetate was chosen as the extraction solvent due to its safety profile, making it one of the least harmful organic solvents [35]. Activated charcoal was used to remove trace amounts of PBs and BPA from hair, ensuring a blank sample. Acid hydrolysis with 1.5% glacial acetic acid at 38 °C for 12 h was performed to extract the target analytes following the decomposition of the capillary matrix.

Prior to LC-MS/MS analysis, derivatization parameters, including the incubation time and temperature, were optimized to enhance the analytical signals of PBs and BPA from hair samples while minimizing variability. Peak areas were similar for the evaluated conditions, but with differences in variability. For the derivatization optimization, the following conditions were evaluated: 15 min at 60 °C, resulting in coefficient of variation (CV%) values of 17.19 for MeP, 16.48 for EtP, 11.62 for PrP, 8.28 for BuP, and 44.86 for BPA, with a mean CV% of 19.69 across all analytes. Under the condition of 30 min at 60 °C, the CV% values increased to 30.43 for MeP, 18.23 for EtP, 14.92 for PrP, 17.84 for BuP, and 113.48 for BPA, with a mean CV% of 38.98. Finally, at 15 min and 70 °C, the CV% values increased significantly, with 105.59 for MeP, 118.76 for EtP, 116.74 for PrP, 114.93 for BuP, and 71.42 for BPA, resulting in a mean CV% of 105.49 across all analytes. The optimal derivatization conditions were established at 15 min of incubation at 60 °C, leading to acceptable peak areas and a lower CV% in peak-area ratios for all analytes.

The hair-washing procedure effectively removed the analytes. The peak area ratios of the added analytes (QCL and QCH, respectively) after washing were less than 20% of the peak area ratios found in the LLOQ samples: MeP (17.89% and 18.92%), EtP (18.70% and 16.78%), PrP (14.54% and 16.25%), BuP (16.39% and 11.94%), and BPA (17.17% and 19.93%).

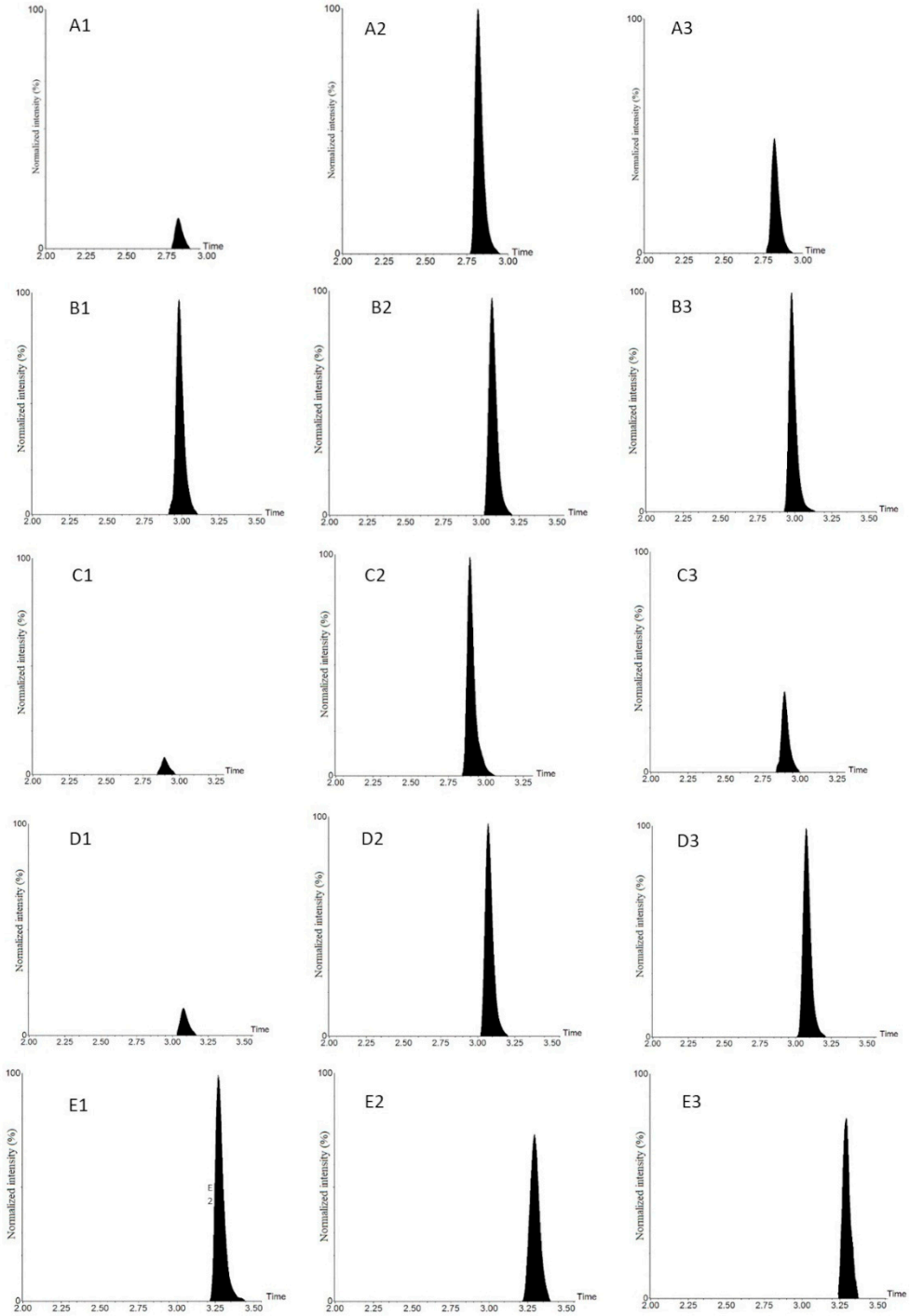

Figure 1. LLOQC sample, normalized intensity (%), and retention time. (**A1**): MeP 25 ng/g, 1.119×10^7, 2.82; (**A2**): IS MeP-d_4 250 ng/mL, 3.70×10^6, 2.82; (**A3**): volunteer: 508.86 ng/g, 9.39×10^6, 2.82. (**B1**): PrP 5 ng/g, 2.52×10^5, 2.98; (**B2**): IS BuP d9 250 ng/mL, 6.02×10^5, 3.07; (**B3**): volunteer: 1660.81 ng/g, 2.71×10^7, 3.08. (**C1**): EtP 2.5 ng/g, 1.31×10^6, 2.90; (**C2**): IS EtP-d_4 250 ng/mL, 1.01×10^6, 2.89; (**C3**): volunteer: 35.25 ng/g, 1.66×10^6, 2.89. (**D1**): BuP 2.5 ng/g, 3.21×10^5, 3.08; (**D2**): IS BuP d9 250 ng/mL, 6.02×10^5, 3.07; (**D3**): volunteer: 53.61 ng/g, 1.24×10^6, 3.08. (**E1**): BPA 2 ng/g, 1.17×10^4, 3.29; (**E2**): IS BPA d16 250 ng/mL, 4.53×10^5, 3.27; (**E3**): Volunteer: 3.77 ng/g, 1.56×10^4, 3.29.

Calibration samples for each analyte were prepared at eight concentrations and analyzed in sextuplicate. Calibration curves were generated using $1/x^2$ weighted least-squares linear regression for BPA, BuP, and EtP, with the smallest $\sum$ % relative errors being 2.13×10^{-14}, 2.75×10^{-14}, and -2.98×10^{-14}, respectively. For MeP and PrP,

1/x weighted least-squares linear regression provided the smallest ∑ % relative errors of -2.52×10^{-13} and 2.22×10^{-14}, respectively. The method exhibited satisfactory linearity, with all correlation coefficients exceeding 0.99.

Assay validation data are presented in Table 2. The precision and accuracy of the method were determined by analyzing QC samples at different concentrations of individual PBs and BPA. The intra-day (n = 5) precision (% CV) ranged from 1.60% to 9.09%, while the inter-day (n = 5) precision ranged from 1.68% to 8.75%. The accuracy ranged from 100.71% to 108.58%. The method demonstrated satisfactory sensitivity, with LQs of 25 ng/g for MeP, 2.5 ng/g for EtP, 5 ng/g for PrP, 2.5 ng/g for BuP, and 2 ng/g for BPA. The intra-day and inter-day precision at the LQ were as follows: MeP (4.61% and 3.25%), EtP (5.75% and 4.93%), PrP (5.76% and 6.21%), BuP (4.38% and 4.98%), and BPA (4.87% and 1.86%).

Table 2. General method validation parameters for PBs and BPA determination: precision, accuracy, extraction yield, matrix effect, and processed sample stability at the autosampler (AS).

Analyte	QC Sample	Nominal Concentration (ng/g)	Precision (CV %)		Accuracy (%)	Extraction Yield (%)	Matrix Effect (%)	Processed Sample Concentration Change After 12 h in AS (%)
			Intra-Assay	Inter-Assay				
MeP	LQ	25	4.61	3.25	104.38	-	-	-
	QCL	40	8.09	4.78	102.63	88.13	−3.95	0.26
	QCM	250	7.40	5.72	103.82	-	-	-
	QCH	7500	1.60	1.68	101.68	87.13	−1.32	0.34
EtP	LQ	2.5	5.75	4.93	103.90	-	-	-
	QCL	4	5.69	5.87	104.94	58.54	−9.43	−6.23
	QCM	25	6.09	5.38	106.75	-	-	-
	QCH	750	4.20	2.73	102.00	48.38	−7.49	−0.05
PrP	LQ	5	5.76	6.21	105.15	-	-	-
	QCL	8	8.94	7.84	104.64	64.97	9.62	0.38
	QCM	50	9.09	5.05	107.58	-	-	-
	QCH	1500	4.55	5.47	102.90	66.30	−3.36	−0.45
BuP	LQ	2.5	4.38	4.98	108.58	-	-	-
	QCL	4	7.04	6.01	103.18	70.00	−0.26	−1.45
	QCM	25	3.56	2.02	106.43	-	-	-
	QCH	750	2.80	2.68	101.83	79.55	−0.72	0.55
BPA	LQ	2	4.87	1.86	107.11	-	-	-
	QCL	2	7.58	8.75	100.71	83.74	4.78	10.37
	QCM	20	4.02	3.38	107.29	-	-	-
	QCH	800	3.78	2.88	104.18	95.35	1.47	−1.56

CV = coefficient of variation; BPA = bisphenol A; MeP = methylparaben; EtP = ethylparaben; PrP = propylparaben; BuP = butylparaben; LQ = limit of quantification; QCL = quality control at low concentration; QCM = quality control at medium concentration; QCH = quality control at high concentration. Precision and accuracy n = 45, processed sample stability n = 24, matrix effect n = 36, extraction yield n = 36.

The stability of the extracts was confirmed after 12 h in the autosampler, with the maximum response differences from the start of the analysis series recorded as 10.79% (BPA), 6.18% (BuP), 3.88% (PrP), −6.23% (EtP), and 1.58% (MeP). The EY ranged from 88.13% to 87.13% for MeP, 58.54% to 48.38% for EtP, 64.97% to 66.30% for PrP, 70.00% to 79.55% for BuP, and 83.74% to 95.35% for BPA. Internal standard-corrected MEs were −3.95% to −1.32% for MeP, −9.43% to −7.49% for EtP, 9.62% to −3.36% for PrP, −0.26% to −0.72% for BuP, and 4.78% to 1.47% for BPA.

Since the hair matrix did not allow for the standard dilution integrity evaluation typically performed in liquid specimens, a smaller amount of hair (10 mg) from highly concentrated QC specimens was analyzed. The accuracy, intra-assay, and inter-assay precision of these QC samples were deemed acceptable, with values as follows: MeP (103.61%, 4.49%, 7.37%), EtP (103.91%, 3.58%, 6.36%), PrP (107.26%, 6.25%, 8.81%), BuP (102.13%, 4.52%, 7.30%), and BPA (105.43%, 7.42%, 4.50%).

A brief overview of this method, along with other LC-MS/MS-based methods available for the determination of BPA, MeP, PrP, BuP, and EtP in hair, is provided in Table 3. Relevant articles for the comparison were identified by searching the bibliography in PubMed and Google Scholar using the keywords ‘hair’, ‘LC-MS/MS’, ‘biomonitoring’,

'parabens', 'bisphenol A', 'endocrine disruptors', and 'analytical method' for studies published between 2016 and 2022.

Table 3. Short overview of the available LC-MS/MS methods for the determination of BPA, MeP, PrP, BuP, and EtP in hair.

Hair Amount (mg)	Extraction Solvent	Incubation Time	Derivatization	Use of Charcoal	Analyte	Extraction Yield (%)	Column	LQ (ng/g)	Retention Time (min)	Matrix Effect (%)	Article (Ref.)
100	2 × 2 mL of methanol	4 h	No	No	MeP EtP PrP BuP	125 90.7 99.7 107.0	Supelco Discovery column C18	1.4 3.3 2.2 0.8	Na	Na	[22]
50	2 × 1 mL of ethyl acetate	Overnight	No	Yes	MeP EtP PrP BuP BPA	90 (for LQ 0.25 ng/g) 91 (for LQ 0.25 ng/g) 94 (for LQ 0.25 ng/g) 96 (for LQ 0.25 ng/g) 156 (for LQ 0.25 ng/g)	Kinetex® Polar C18	0.25 0.25 0.25 0.25 0.25	3.02 3.92 4.73 5.42 4.89	LQ 110 LQ 107 LQ 96 LQ 101 LQ 203	[1]
50	2 × 2 mL of acetone	Overnight	No	No	MeP EtP PrP BPA	Na	Acquity UPLC BEH C18 column	10 2 10 10	Na	Na	[32]
100	2 mL of methanol	4 h	No	No	MeP EtP BuP	112.1 ± 27.6 110.7 ± 41.1 81.62 ± 48.1	Supelco Discovery column C18	12.08 1.81 0.94	13.07 14.97 17.93	Na	[19]
100	3 mL of acetone	Overnight	No	No	MeP EtP PrP BPA	120 ± 13 for 0.25 µg/g 100 ± 1 for 0.25 µg/g 99 ± 2 for 0.25 µg/g 77 ± 5 for 0.25 µg/g	Agilent Zorbax Eclipse XDBeC18 Rapid Resolution HT	5.2 2.6 2.6 6.1	6.2 8.9 11.4 12	11 −11 −4 −4	[17]
50	1 mL of acetonitrile	Overnight	No	No	MeP EtP PrP BuP BPA	107.2 103.5 109.7 97.8 105.9	Acquity UPLC BEH C18	0.5 2 1 1 7	Na	Na	[33]
50	2 × 1 mL of ethyl acetate	Overnight	Yes	Yes	MeP EtP PrP BuP BPA	QCL 83.13 QCL 58.54 QCL 64.97 QCL 70.00 QCL 100.71	Acquity UPLC BEH C18	25 2.5 5 2.5 2	2.82 2.90 2.98 3.07 3.29	QCL 3.95 QCL 9.43 QCL 9.62 QCL 0.26 QCL 4.78	This study

Comparison made only with the same analytes that we used in our study. Na: not available; ref = reference, LQ = limit of quantification; CV = coefficient of variation; BPA = bisphenol A; MeP = methylparaben; EtP = ethylparaben; PrP = propylparaben; BuP = butylparaben; QCL = quality control at low concentration.

The applicability of the proposed method was assessed by determining the target compounds in hair samples from 101 volunteers between September 2023 and May 2024. For MeP, 14 volunteers had concentrations above the calibration curve limit of 10,000 ng/g, while 7 were below the LQ of 25 ng/g. For EtP, 1 volunteer had concentrations above 1000 ng/g, while 25 were below the LQ of 2.5 ng/g. For PrP, 20 volunteers had concentrations exceeding 2000 ng/g, while 6 were below the LQ of 5 ng/g. For BuP, 6 volunteers had concentrations above 500 ng/g, while 47 were below the LQ of 5 ng/g. For BPA, no samples exceeded the calibration curve limit of 800 ng/g, while 61 were below the LQ of 2 ng/g. The mean, median, standard deviation, and coefficient of variation for MeP, EtP, PrP, BuP, and BPA concentrations in the hair samples of the 101 volunteers are presented in Table 4.

Table 4. Volunteers for MeP, EtP, PrP, BuP, and BPA, the mean, medians, standard deviations and coefficient of variation.

Descriptive Measures	MeP (ng/g)	EtP (ng/g)	PrP (ng/g)	BuP (ng/g)	BPA (ng/g)
Mean	3647.76	386.97	1080.65	220.49	6.05
Median (ng/g)	687.68	14.34	109.15	4.97	1 *
Standard deviations (ng/g)	8584.92	1850.98	2296.24	1018.49	20.34
Coefficient of variation (%)	0.42	0.21	0.47	0.22	0.30

LQ = limit of quantification. * Concentration lower than LQ (LQ/2).

A correlation was observed between the natural logarithm of MeP (Ln MeP) and PrP (Ln PrP) in the scatter plot, with the equation $y = 0.8469x - 0.6235$ ($r^2 = 0.5781$). This indicates that the linear model explains 57.81% of the variation in the data. Pearson's correlation coefficient (r = 0.78) confirmed a strong positive linear relationship between the two variables (Figure 2).

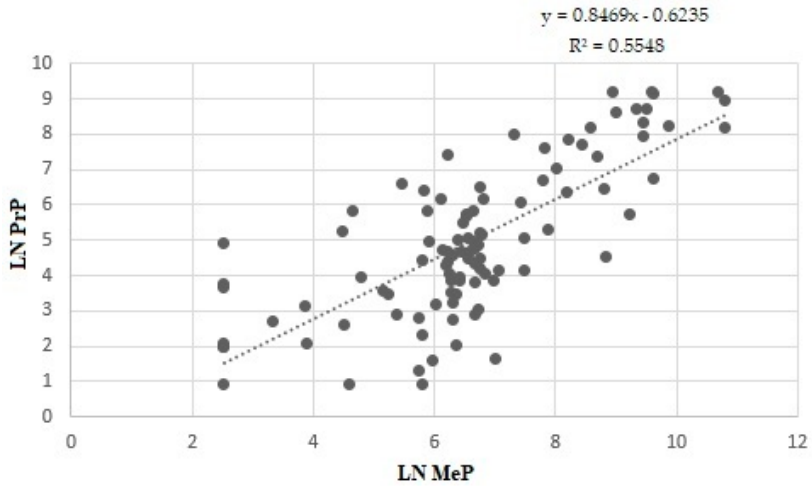

Figure 2. Relationship between LN of MeP and PrP concentrations (y = 0.84692x − 0.6235, r^2 = 0.554, r = 0.78).

4. Discussion

The present study was conducted at the Integrated Center for Health Specialties at Feevale University, in southern Brazil, where most students, professors, and workers are female. Consequently, the sample consisted of 97 women and 3 men, reflecting the demographic profile of the institution's patient population. Although the male sample was small, it was included as part of the general sampling rather than for a comparative analysis between sexes. Therefore, this study primarily focuses on evaluating female exposure to parabens and bisphenol A, with male participants representing a minor proportion of the total sample. In this context, it is important to note that female volunteers may have distinct lifestyle habits that influence their exposure to parabens and bisphenol A, potentially differentiating them from male individuals.

One of the main challenges in hair analysis is distinguishing between external contaminants and chemicals incorporated into the human body. Therefore, before initiating the extraction process, it is necessary to wash the hair for the following reasons: analytes may adhere in the hair surface from the individual's environment, potentially leading to erroneous results, and residues from hair care products, as well as sebum, sweat, and dust, can contribute to increased analytical noise/background [20]. The optimal washing process should remove external impurities without extracting incorporated substances or damaging the sample. We followed the washing optimization proposed by Martin et al. (2016), who evaluated four solvents—water, isopropanol, acetone, and 0.1% aqueous SDS solution—on both blank and volunteer samples [17]. SDS 0.1% was selected as the most effective option, avoiding organic solvents that might extract the analytes of interest. Subsequently, blank samples were extracted using dichloromethane and methanol. Before extraction, hair is typically cut into 2–3 mm segments using scissors, though it can also be processed by grinding. However, grinding does not improve the extraction process and may result in sample loss [17,20]. The target analytes must be extracted after decomposing the hair matrix. Acidic hydrolysis was chosen for this purpose, as it provides better extraction results for BPA and preserves the stability of the ester functions of PBs. Glacial acetic acid was selected for acid hydrolysis due to its ability to enhance the BPA extraction efficiency while maintaining the stability of PBs [18].

Hair samples were used to prepare calibration standards and quality controls (QCs). The results revealed significant baseline contamination with BPA and PBs, leading to large intercepts that hindered the generation of ideal calibration curves. According to ICH (2022) bioanalytical validation guidelines, calibration standards should be prepared

using the same biological matrix as the study samples [29]. While synthetic hair matrices produced industrially are a potential alternative, they do not guarantee the complete absence of analytes and are expensive [1,29]. The use of activated charcoal for adsorption is considered one of the most competitive methods due to its cost-effectiveness, simplicity, and ease of handling, without requiring high temperatures or operating pressures [36]. When applied after the hydrolysis step, activated charcoal yielded the best results, producing linear calibration curves with low intercepts for all target analytes [1]. According to other studies [1,37], we tested the use of charcoal to remove trace analytes from blank samples. Vandenberg et al. (2014) treated human serum with charcoal three times to eliminate steroids and BPA [37]. Robin et al. (2022) developed an analytical method for biomonitoring bisphenols and PBs in human hair using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in France [1]. To generate a blank hair matrix, they employed charcoal after the hydrolysis stage, which yielded optimal results. In their study, hair samples were washed with 0.1% SDS, and decontamination was performed using methanol. In contrast, our study utilized a dichloromethane/methanol mixture (1:1, v/v) for decontamination.

Claessens et al. (2022) highlighted the lack of reference materials, such as blank matrices, as a limitation in environmental pollutant studies, which can result in variability between laboratory results and explain discrepancies in reported contamination levels. In our study, PBs and BPA were removed from the matrix to construct calibration and control curves [27].

Wojtkiewicz et al. (2021) analyzed human exposure to PBs in northeastern Poland through hair sample analysis but did not mention the use of blank samples for calibration curves and controls [22]. Rodríguez-Gómez et al. (2017) injected procedural blanks to assess background contamination but did not describe the process for obtaining blank samples [28].

The analytical validation of our method confirmed the linearity, accuracy, limits of quantification, autosampler stability for 12 h, dilution integrity, and detection capability. The findings reinforce previous evidence that hair analysis is an effective method for assessing human exposure to PBs and serves as an alternative to traditional matrices, such as urine and blood.

No previous studies have employed derivatization to enhance the sensitivity of PB analysis in hair. Dansyl chloride, due to its reactivity with the phenolic portion of all target compounds, was used for derivatization [38]. The derivatization reaction was performed following the methodologies described by Sosvorova et al. (2017) [38], Vitku et al. (2015) [39], and Anari et al. (2002) [40] for serum analysis, with modifications optimized for time and temperature.The derivatization with dansyl chloride enhanced electrospray ionization, contributing to the development of a highly sensitive and specific method for rapid and accurate quantification of the target compounds [40,41], and was essential for enabling the measurement of EDs at the ng/g level [39].

The results clearly indicate that individuals from Southern Brazil are exposed to several parabens (PBs). The highest mean concentrations, along with the highest and lowest values, were observed for MeP (3647.76 ng/g, 48,768.17 ng/g, and <LQ) and PrP (1080.65 ng/g, 9853.66 ng/g, and <LQ), respectively. Both MeP and PrP were detected at high frequencies in hair samples, with MeP showing the highest mean concentration. BuP (220.49 ng/g, 6683.32 ng/g, and <LQ) and EtP (386.97 ng/g, 16,452.19 ng/g, and <LQ) were found to have lower mean concentrations and frequencies (Table 4). The results clearly indicate that individuals from Southern Brazil are exposed to several parabens (PBs). The highest mean concentrations, along with the highest and lowest values, were observed for MeP (3647.76 ng/g, 48,768.17 ng/g, and <LQ) and PrP (1080.65 ng/g, 9853.66 ng/g, and <LQ), respectively. Both MeP and PrP were detected at high frequencies in hair samples,

with MeP showing the highest mean concentration. BuP (220.49 ng/g, 6683.32 ng/g, and <LQ) and EtP (386.97 ng/g, 16,452.19 ng/g, and <LQ) were found to have lower mean concentrations and frequencies (Table 4). As noted by Martin et al. (2019), the concentration trend follows the order MeP > PrP > EtP. MeP and PrP are among the most used PBs and are frequently found together in cosmetic products [17]. They are the most widely used PBs, and except for water, they are the most common ingredients in cosmetic formulations [42]. MeP was detected in at least 93.7% of samples, PrP in 97.03%, BuP in 62.38%, EtP in 78.22%, and BPA in 42.57%.

Among the volunteers who quantified MeP above the mean (3647.76 ng/g), 94.21% used perfumes more than three times a week, 63.16% used lotions and creams more than three times a week, and 73.78% used leave-in products more than three times a week. For those who quantified EtP above the mean (386.97 ng/g), 80% used perfumes more than three times a week, 60% used lotions and creams more than three times a week, and 60% used leave-in products more than three times a week. In the group of volunteers who quantified PrP above the mean (1080.65 ng/g), 83.33% used perfumes more than three times a week, 72.22% used lotions and creams more than three times a week, and 77.78% used leave-in products more than three times a week. Finally, for the volunteers who quantified BuP above the mean (220.49 ng/g), 83.33% used perfumes more than three times a week, 66.67% used lotions and creams more than three times a week, and 33.33% used leave-in products more than three times a week.

Additionally, for those who quantified BPA above the mean (6.05 ng/g), only 26.67% consumed canned foods and drinks more than three times a week. Nearly all volunteers who quantified PBs above the average used these personal care products more than three times a week, with the exception of the BuP group, which reported the lowest frequency of use among the PBs. Regarding BPA, other potential sources of exposure, such as thermal paper, should be considered additional contributors.

It is important to highlight that, unlike in other countries, such as Korea [24], Belgium [27], Luxembourg [23], Greece [19], Spain [16,17,28], France [1], and Poland [22], there was no assessment of PBs and BPA in hair in Brazil before this study. The following mean concentrations were reported in previous studies: Claessens et al. (2022) [22] found 104.2 ng/g MeP, 46.1 ng/g BPA, 8.7 ng/g EtP, and 22.9 ng/g PrP; Cho et al. (2018) [24] reported 123.6 ng/g MeP, 64.5 ng/g EtP, 136.9 ng/g PrP, and 74.2 ng/g BuP; Rodríguez-Gómez et al. (2017) [27] measured 20.7 ng/g MeP, 66.9 ng/g PrP, 9.0 ng/g EtP, and 5.76 ng/g BuP; and Wojtkiewicz et al. (2021) found 4302.0 ng/g MeP, 704.0 ng/g EtP, 825.7 ng/g PrP, and 154.5 ng/g BuP [28]. The only study that reported MeP concentrations as high and similar to those observed in our study was conducted by Karzi et al. (2019), which found 4501.2 ng/g for MeP, 510.1 ng/g for EtP, and 237.1 ng/g for BuP [19]. Two studies conducted on populations in Spain and Poland [16,43] reported significantly higher median BPA concentrations in hair compared to the present study, with values of 200 ng/g in Spain, 411.2 ng/g in Poland, and 6.05 ng/g in our study. However, a study on BPA concentrations in the hair of adults from northeastern Poland reported results that were more comparable to ours, ranging from 3.6 to 52.9 ng/g [44].

5. Conclusions

A new LC-MS/MS method was developed, optimized, and validated for the determination of BPA and PBs in human hair. The method involves incubation with 1.5% acetic acid, derivatization with dansyl chloride, extraction with ethyl acetate, and subsequent analysis via LC-MS/MS. Significant correlations were observed between the logarithmic values of MeP and PrP concentrations, with MeP and PrP being detected at higher frequencies and concentrations, providing insights into the combined exposure to these compounds.

The validated method demonstrated high sensitivity and accuracy, making it suitable for long-term exposure assessments and biomonitoring. It holds potential for use in epidemiological studies to assess exposure levels and investigate possible associations with health outcomes. This method was applied for the first time to a Brazilian cohort of volunteers, with MeP being the most frequently detected compound. Future research should focus on further integrating the hair analysis into biomonitoring, thereby strengthening its role as a tool for public health policies aimed at reducing human exposure to EDs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors13040118/s1, Figure S1: Ion chromatogram of a blank hair extract. 1: MeP, 2: EtP, 3: PrP, 4: BuP. Background peaks of the target analytes are indistinguishable from instrumental noise.

Author Contributions: G.P.P. was responsible for conceptualization, validation, investigation, and writing (review and editing); C.D.L. and C.F.d.S. for sample collection; A.P.B. and R.Z.H. for validation and investigation; and R.L. for conceptualization, resources, validation, and writing (review and editing). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAPES 16/2022 PDPG—Pós-doutorado estratégico.

Institutional Review Board Statement: Held at Feevale University, authorized by the Ethics and Research Committee with opinion no. 6,024,337.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for providing financial support for this study.

Conflicts of Interest: The authors declare that they have no competing interests.

Abbreviations

The following abbreviations are used in this manuscript:

Parabens	PBs
Endocrine disruptors	EDs
Ultra-performance liquid chromatography-tandem mass spectrometry	LC-MS/MS
Methylparaben	MeP
Propylparaben	PrP
Ethylparaben	EtP
Butylparaben	BuP
Bisphenol A	BPA
Personal care products	PCPs
Methylparaben-d_4	MeP-d_4
Ethylparaben-d_4	EtP-d_4
Butylparaben-d_9	BuP-d_9
Bisphenol A-d_{16}	BPA-d_{16}
Acid glacial solution	Hac
Sodium dodecyl sulfate	SDS
Retention time	Rt
Mass/charge ratio	m/z
Voltage	V
Internal standard	IS
Quality control	QC
Quality control samples at low concentrations	QCL

Quality control samples at medium concentrations	QCM
Quality control samples at high concentration	QCH
Coefficient of variation	CV %
Cumulative percentage error	∑ %ER
Extraction yield	EY
Matrix effect	ME
Limit of detection	LOD
Below the limit of quantification	BLOQ
Limit of quantification	LQ
Correlation coefficients	r
Determination coefficient	r^2
Standard deviation	SD
Bisphenol S	BPS
Bisphenol F	BPF
Natural logarithm	LN

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