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Tracking Down of a Selected Panel of Parabens: A Validated Method to Evaluate Their Occurrence in Skin Layers

Ritamaria Di Lorenzo [1,†], Ilaria Neri [1,†], Giacomo Russo [2], Sonia Laneri [1,*] and Lucia Grumetto [1,*]

[1] Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via D. Montesano 49, I-80131 Naples, Italy
[2] School of Applied Sciences, Sighthill Campus, Edinburgh Napier University, 9 Sighthill Ct, Edinburgh EH11 4BN, UK
* Correspondence: slaneri@unina.it (S.L.); lucia.grumetto@unina.it (L.G.)
† These authors contributed equally to the work.

Abstract: A method was set up and validated to identify and quantify seven parabens in each of the three skin layers, i.e., Stratum Corneum, Epidermis, and Dermis, because, even if only some analogues are legally allowed in Europe, forbidden parabens are also detected in many personal care products and therefore can be absorbed by the skin. A solid/liquid extraction followed by a gradient elution chromatographic separation method was performed and validated according to European guidelines. Our validated method afforded the detection of all seven parabens with limit of detection values ranging from 0.026 to 0.090 μg mL^{-1} and recoveries ranging from 61.80 to 105.73 μg mL^{-1} at high and low concentration values (50.0–5.0 μg mL^{-1}), respectively. The proposed method can help assess paraben's skin bioaccumulation since people are repeatedly exposed to consumer goods containing parabens in their daily routine, posing a chronic risk to human health.

Keywords: parabens; chemical analysis; extraction method; skin barrier; penetration

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1. Introduction

Well-being is nowadays regarded as a priority in everyday life. Personal care products (PCPs) involve a vast number of consumer goods offering benefits by increasing the aesthetic appeal of individuals and, therefore, their self-confidence. Most PCPs are cosmetics, lipsticks, eye and facial makeup preparations, or hygiene products such as shampoos, bath soaps, antiperspirants, and dental-care products. Such products are not required to be sterile, but they must not contain levels of microorganisms high enough to reduce shelf life or to pose any health risks to consumers.

Several chemicals can be employed to preserve and prolong PCPs' shelf life, even if the latter are formulated for external use.

The skin, the largest organ of the human body, is one of the major routes of exposure to toxic substances that can be absorbed. This depends on contact time [1] and skin integrity.

Parabens (PBs) are esters of p-hydroxybenzoic acid (pHBA), acting against several microorganisms, especially mold and yeast. They are widely used as preservatives mainly in cosmetics and PCPs, but also in pharmaceuticals and foodstuffs [1], and their primary uptake from the human body is the dermal route [2]. Moreover, PBs can irritate or sensitize the normal human skin when patients are patch-tested to a parabens' mix [3]. Humans are exposed through cosmetics to PBs to an extent accountable to 76 mg pro die, and once in contact with the skin, these can be metabolized by the carboxylesterase in dermal keratinocytes [4].

PBs are also considered endocrine disruptors due to their estrogenic or androgenic activity. Indeed, they can be toxic to male reproductive organs and exert a proliferative

effect on the human breast [5]. In fact, the European Union (EU) limited short-chained parabens, such as Methyl 4-hydroxybenzoate (MP) and Ethyl 4-hydroxybenzoate (EP), to 0.4% w/w of a total product, lowering the concentration limits of Propyl 4-hydroxybenzoate (PrP) and Butyl 4-hydroxybenzoate (BuP) to 0.14% in cosmetics [6] and prohibiting their use in children's products, as already established in Denmark since 2010 (SCCS/1348/10) [7]. Concerning long-chained parabens, such as PrP, the EU set limits to 0.19% w/w and banned isopropyl, isobutyl, phenyl, benzyl, and pentyl parabens in PCPs [6].

The assessment of ex vivo permeation assists in the evaluation of the overall safety of the products containing PBs. Generally, excised human or animal skin is recommended for ex vivo permeation studies. However, due to the limited availability of human skin and ethical issues surrounding the use of animal skins, the permeation studies are hardly performed. In addition, the fact of their being time-consuming and poorly inter-lab reproducible motivated the development of a wide variety of alternatives in the last three decades [8]. Unfortunately, however, alternative methods such as in silico prediction or biomimetic liquid chromatography, alone, can occasionally be misleading in the permeability potential assessment of these preservatives [9]. For this reason, the need to correlate these methods with classical ex vivo methods emerged.

Our work aims at setting up an ad hoc and validated suitable method to simultaneously identify and quantify seven PBs in the skin matrix. The selected panel of compounds includes MP, EP, PrP, BuP, Isopropyl 4-hydroxybenzoate (iPrP), Isobutyl 4-hydroxybenzoate (iBuP), and Benzyl 4-hydroxybenzoate (BzP). The dataset was assembled according to several criteria: these are (i) ample range of lipophilicity, offering wide applicability of this protocol; (ii) established occurrence in PCPs, even if misleadingly labelled as preservative free; (iii) authorization status, assembling both allowed and forbidden PBs [10].

PBs quantification in the skin matrix remains challenging due to the high degree of complexity of the skin layers, composed mainly of ceramides, fatty acids, and cholesterol [11], which can severely interfere with chemicals' quantification [12,13]. To the best of our knowledge, most research works report analytical methods to evaluate the concentration levels of PBs but of a limited number of analogs along with other chemicals [11,14] or, in most cases, the study aims to follow the uptake of PBs from skin explants by Franz Diffusion Cell (FDC) experiments, applying the most varied topic formulations. Instead, we propose and validate an analytical method to simultaneously determine up to seven PBs in each skin layer, Stratum Corneum, Epidermis, and Dermis, studying the diffusion of a neat analyte.

2. Materials and Methods

2.1. Chemicals and Reagents

The purity of all of the PBs as primary standard (Methyl 4-hydroxybenzoate (MP), Ethyl 4-hydroxybenzoate (EP), Propyl 4-hydroxybenzoate (PrP), Butyl 4-hydroxybenzoate (BuP), (purchased from Merck & Co., Poole, UK), Isopropyl 4-hydroxybenzoate (iPrP) (purchased from Fluorochem, Glossop, UK), Benzyl 4-hydroxybenzoate (BzP) (purchased from Sigma Aldrich, Milan, Italy), and Isobutyl 4-hydroxybenzoate (iBuP) (purchased from J&K, San Jose, CA, USA) was equal to or higher than 98%. Stock solutions (2 mg mL^{-1}) were obtained by dissolving each PBs in ethanol. Chromatographic-grade solvents were used, and ultra-purified water Milli Q was produced in house (conductivity 0.055 $\mu S\ cm^{-1}$ at 25 °C, resistivity equals 18.2 MΩ·cm). For the differential tape-stripping process, 3M™ Scotch®, Milan, Italy, was used. The skin of porcine ears was obtained from a local slaughterhouse (Avellino, Italy).

2.2. Skin Sample Preparation and Extraction Method

According to the Organization for Economic Co-operation and Development (OECD) guidelines, the skin samples were excised from a male pig ear, post-sacrifice,

obtained from a local slaughterhouse (Avellino, Italy) within 3 h from the animal's death. The outer part of the ear was used. Subcutaneous fat was removed, skin samples were processed at room temperature (22 ± 2 °C) and hydrated in saline solution (NaCl 0.9%) for 5 min, then were placed on a filter paper (Fisherbrand™ Grade 601; Fisher Scientific, Leicestershire, UK) and cut into pieces of about 2 cm^2 size before the extraction procedure. The stripping technique was used to obtain isolated Stratum Corneum (SC) through one piece of adhesive tape, cut into 2 cm × 2 cm, applied and removed fifteen times with the same pressure. After stripping, the skin was submitted to a heat separation process. Skin was heated with a hair dryer for 30 secs, and then the Epidermis was separated from the Dermis by scraping with a scalpel. Tape strips (SC), Epidermis, and Dermis were placed in individual glass test tubes and kept in contact with 1 mL of an ethanolic solution containing all seven PBs for 1 h. Subsequently, the solvent was gently evaporated under N_2 flow. The experiments were carried out employing two different concentrations, the higher of 50 μg mL^{-1}, out of iBuP, which was 100 μg mL^{-1}, and the lower of 5 μg mL^{-1}, out of iBuP, which was 10 μg mL^{-1}, respectively. Extraction from each skin layer (SC, Epidermis, and Dermis) was performed using 1 mL of water:ethanol sol. 50/50 (v/v) at room temperature overnight, keeping samples under magnetic stirring. The day after, the skin samples were discharged, the extraction solvent was transferred to plastic vials, sonicated for 30 min, and then centrifuged at 6000 rpm for 25 min. The supernatant was filtered using nylon syringe filters 0.22 μm (Phenomenex, Bologna, Italy) and analyzed using high-performance liquid chromatography (HPLC). The experiments were performed in triplicate.

2.3. Liquid Chromatography Method

A reversed-phase HPLC-UV technique was used for the development and validation of the analytical method using an LC-20 VP apparatus (Shimadzu Corp., Kyoto, Japan); the stainless-steel column was a silica-based Cholesterol-bonded reversed-phase column (150 mm × 4.6 mm, 5 μm, Cosmosil (Nacali Tesque, Kyoto, Japan). The seven PBs were separated, under the following chromatographic conditions: eluent A (aqueous added of 0.01% v/v Trifluoroacetic acid (TFA)) and eluent B (acetonitrile (ACN), added of 0.01% v/v TFA), flow rate set to 1.0 mL min^{-1}, column temperature 22 ± 2 °C. Then, 60 μL of standard and matrix solutions, i.e., three times the loop volume, were injected, and the signals from the UV detector were recorded at 254 nm. The separation was performed on a multi-step gradient elution: starting from 30% eluent B, a sequential linear gradient to 35% eluent B in 10 min, then 38% B in 20 min, and finally 95% B in 1 min. The post-run equilibration time was 20 min. Data acquisition and integration were accomplished by Cromatoplus software.

2.4. Chromatographic Method Validation

Method was validated by testing the parameters of selectivity, linearity, precision, accuracy, matrix effect, carry-over effect, robustness, limit of detection (LOD), and limit of quantification (LOQ) according to the European validation guidelines [15].

2.5. Calibration Curve and Linearity

Calibration curves were constructed using different concentrations of a mixed solution of the seven analytes. The linearity ranges were tested based on the average peak areas versus the concentration (μg mL^{-1}) of PBs; ethanolic standard solutions were prepared and added to the matrix to final concentrations from 50.0 to 0.5 μg mL^{-1}. Linear regression analysis and calibration curve parameters (correlation, coefficient, slope, and intercept) were back-calculated from the peak areas using the regression line by the method of least squares, and the mean accuracy values were determined.

2.6. Limits of Detection and Quantification

LOD and LOQ were estimated to be the concentrations providing signals equal to 3 and 10 times, respectively. They were calculated based on the following equations: LOD = SD·3/S and LOQ = SD·10/S, [13], where SD is the standard deviation of the intercept response with the y-axis of the calibration curves, and S is the slope of a calibration curve. The spike level is in the appropriate range using a concentration of 0.5 μg mL^{-1} five times.

2.7. Precision and Accuracy

Accuracy and precision can be evaluated independently. The method's precision was evaluated by running five replicates of the sample repeated in the same day and in two different days to cover both intra-day and inter-day precision, expressed as relative standard deviation (RSD). Repeatability was assessed using the nominal concentration of PBs mixed solution (0.5 μg mL^{-1}). The accuracy of this method was determined considering the recovery of all seven PBs from the different skin layers (Stratum Corneum, Epidermis, and Dermis) in triplicate, spiking at high and low concentration values (50.0 and 5.0 μg mL^{-1}), and reported as a percentage of the nominal value (percent recovery).

2.8. Selectivity

Selectivity, i.e., the ability to discriminate the investigated analytes from other chemicals possibly interfering with their signals, was assessed by utilizing blank samples of each skin layer. SC, Epidermis, and Dermis samples were kept in contact with 1 mL of ethanol for 1 h and extracted with the same procedure indicated in the skin sample preparation and extraction method section. They were used as blank to verify the occurrence of interfering peaks eluting at the same retention time of each analyte.

2.9. Carry-Over

The carry-over effect of the method was evaluated by injecting ethanol solvent after running the highest concentrated samples of PBs spiked in the skin (three times) and observing the occurrence of signals within the retention windows of the target chemicals.

2.10. Matrix Effect

The matrix effect was investigated by calculating the ratio of the peak area in the presence of matrix (matrix spiked with PBs-mixed solution) to the peak area in the absence of matrix (PBs-mixed pure solution), i.e., determined by comparing the analyte response in the presence of matrix with that in the absence of matrix. The spiking was executed at 50.0 μg mL^{-1}:

$$Matrix\ effect = \frac{peak\ area\ in\ presence\ of\ matrix}{peak\ area\ in\ absence\ of\ matrix}$$

2.11. Robustness

Robustness of the analytical method was tested throughout the study period, applying changes in selected analysis parameters, including different batches and qualities of solvents (ACN, EtOH), reagents (TFA, water), equipment, sonication, and centrifugation time, and finally the operator over short (days), medium (weeks), and long (months) time windows.

3. Results and Discussion

3.1. Optimization of Extraction and Chromatographic Method

Our proposed method of extraction and quantification of PBs in the different skin layers resulted in being easy to apply and sensitive. Indeed, although several scientific works have already determined some PBs, to the best of our knowledge these research

papers described the simultaneous detection of up to four PBs [16], or of some permitted PBs along with compounds belonging to different chemical classes [16,17]. The extraction from the skin was performed by using two organic solvents, methanol, and ethanol, at different percentages with water. The highest recovery was obtained with ethanol: water 50:50 v/v.

The effective separation of the seven studied chemicals was optimized after the modulation of several parameters, such as: (i) stationary reversed-phases (LC column Supelco Ascentis C18, 250 mm × 4.6 mm, 5.0 µm i.d; Kinetex phenyl-hexyl 100 Å 150 mm × 4.6 mm, 5.0 µm particle size); (ii) the mobile phase composition (organic solvents: methanol and acetonitrile); (iii) flow rate (range 0.5–1.0 mL min^{-1}); and (iv) gradient elution program, to achieve a satisfactory resolution for all the PBs. The best separation conditions were achieved with the replacement of methanol with acetonitrile because the use of methanol resulted in substandard peak symmetry, and the separation of iBuP and BuP to a degree <50%. Moreover, the best resolution of all chemicals was achieved using a Cholesterol-bonded reversed-phase column, where this cholesterol derivative is attached to a propyl spacer through an ether bond. The stationary phase features a rigid scaffold that allows shape recognition forces to a greater extent than the ODS phases when free-branched moieties can accommodate analytes of different shapes, being less selective. The cholesterol-bonded phase has similar hydrophobicity to C18 (ODS) but is better suited for compounds with similar hydrophobicity (carbon content approx. 10%) and a slightly different molecular shape, such as closely related PBs.

3.2. Method Validation

All method validation parameters are shown in Table 1.

Table 1. Summary of method validation parameter results.

Calibration Parameters	**MP**	**EP**	**iPrP**	**PrP**	**iBuP**	**BuP**	**BzP**
Linear range µg mL^{-1}	50.0–0.5	50.0–0.5	50.0–0.5	50.0–0.5	100.0–1.0	50.0–0.5	50.0–0.5
Slope	4836.6	4256.8	4657.3	4512.7	2133.7	6501.9	3177.8
Intercept	7668.8	5554.6	2590.7	1816.1	4877.7	450.06	2853.4
R^2	0.986	0.990	0.997	0.998	0.997	0.995	0.997
Repeatability (n = 5) RSD%	4.908	7.480	6.480	5.518	8.977	3.351	20.155
Intermediate precision (n = 10) RSD%	4.492	7.581	7.518	8.173	10.193	7.919	30.041
LOQ µg mL^{-1}	0.087	0.161	0.244	0.197	0.301	0.121	0.216
LOD µg mL^{-1}	0.026	0.048	0.073	0.059	0.090	0.036	0.065
Matrix effect SC	0.92	0.83	0.85	0.86	1.01	0.81	0.80
Matrix effect Epidermis	0.92	0.84	0.87	0.88	0.98	0.80	0.89
Matrix effect Dermis	0.89	0.81	0.83	0.84	0.98	0.82	0.78

R2 coefficient of determination; RSD % Relative Standard Deviation %; LOQ: limit of quantification; LOD: limit of detection; SC: stratum corneum; Methyl 4-hydroxybenzoate (MP), Ethyl 4-hydroxybenzoate (EP), Isopropyl 4-hydroxybenzoate (iPrP), Propyl 4-hydroxybenzoate (PrP), Isobutyl 4-hydroxybenzoate (iBuP), Butyl 4-hydroxybenzoate (BuP), Benzyl 4-hydroxybenzoate (BzP).

The linear R-squared values (R^2) of all the calibration curves ranged from 0.986 to 0.998 for all PBs. The sensitivity of the developed method is appreciable from the listed LOD and LOQ parameters, with values ranging from 0.026 μg mL^{-1} to 0.090 μg mL^{-1} and from 0.087 μg mL^{-1} to 0.301μg mL^{-1}, respectively. The RSD% of within-run precision was in the range of 4.908 and 20.155, while the RSD% between-run precision ranged from 4.492 to 30.041. The selected panel of analytes have different lipophilicity, ranging from 1.96 (MP) to 3.56 (BzP) log P values and different solubility, from 2.50×10^3 (MP) to 92 (BzP) mg L^{-1} that can cause the different recovery values. Recovery from SC, Epidermis, and Dermis, respectively, was evaluated at high and low spiking concentrations (50.0 and 5.0 ug/mL): (a) for the highest spiked concentrations, the SC values ranged from 109.90 to 62.27, Epidermis ranged from 106.85 to 63.69, and Dermis ranged from 109.52 to 66.07; (b) for the lower spiked concentrations, SC ranged from 100.08 to 61.80, Epidermis ranged from 100.72 to 64.94, and finally Dermis ranged from 105.73 to 65.34. For each skin layer, the extraction recovery decreased with solubility increasing. Table 2 summarizes the recovery values for each of the seven PBs.

Table 2. Recovery of Parabens (PBs) from each isolated skin layers: Stratum corneum, Epidermis, and Dermis at the two concentration levels (50.0 * and 5.0 ** μg mL^{-1}).

Recovery μg mL⁻¹	***MP***	***EP***	***iPrP***	***PrP***	***iBuP***	***BuP***	***BzP***
Stratum Corneum *	90.45 ± 1.75	82.19 ± 1.74	84.21 ± 1.52	84.91 ± 1.42	109.90 ± 3.34	62.27 ± 0.59	78.77 ± 1.59
Stratum Corneum **	100.08 ± 5.06	92.10 ± 4.70	74.93 ± 4.86	72.19 ± 5.33	76.05 ± 2.88	61.80 ± 2.96	76.60 ± 2.90
Epidermis *	90.92 ± 0.47	83.53 ± 0.39	86.09 ± 0.90	87.37 ± 1.03	106.85 ± 0.50	63.69 ± 0.62	87.53 ± 3.05
Epidermis **	100.72 ± 4.43	99.26 ± 1.31	82.52 ± 2.50	77.15 ± 2.92	79.44 ± 1.61	64.94 ± 2.78	76.97 ± 1.85
Dermis *	79.78 ± 4.78	73.37 ± 3.37	76.02 ± 4.11	76.00 ± 5.01	109.52 ± 2.32	68.89 ± 0.42	66.07 ± 3.68
Dermis **	105.73 ± 1.65	99.46 ± 0.28	82.72 ± 0.28	77.08 ± 0.10	79.66 ± 0.57	65.34 ± 0.57	70.98 ± 1.47

These results support that the developed analytical method offers a reliable response which is crucial in such a complex biological matrix. Chromatograms obtained for the analytes dissolved in ethanol (Figure 1a) and the skin matrix (Figure 1b–d) with respective PBs retention time, under the selected chromatographic conditions and without interfering peaks, are shown. The table in Figure 1a also shows the resolution ± SD of each peak pair calculated as the following equation formula:

$$Resolution = \frac{t_{RefPeak} - t_R}{W_{50\%\,RefPeak} + W_{50\%\,R}} \quad (1)$$

where $t_{RefPeak}$ is the retention time of the reference peak, which by default is the peak after the current peak; t_R is the retention time of the current peak; and $W_{50\%RefPeak}$, and $W_{50\%R}$ are the width of these two peaks at 50% of the peak height. After running concentrated samples of PBs, ethanol injections did not show any interfering signals.

Peak pairs	*Resolution*
MP-EP	5.24 ± 0.36
EP-iPrP	6.16 ± 0.42
iPrP-PrP	1.66 ± 0.09
PrP-iBuP	8.83 ± 0.45
iBuP-BuP	1.02 ± 0.07
BuP-Bz	3.08 ± 0.25

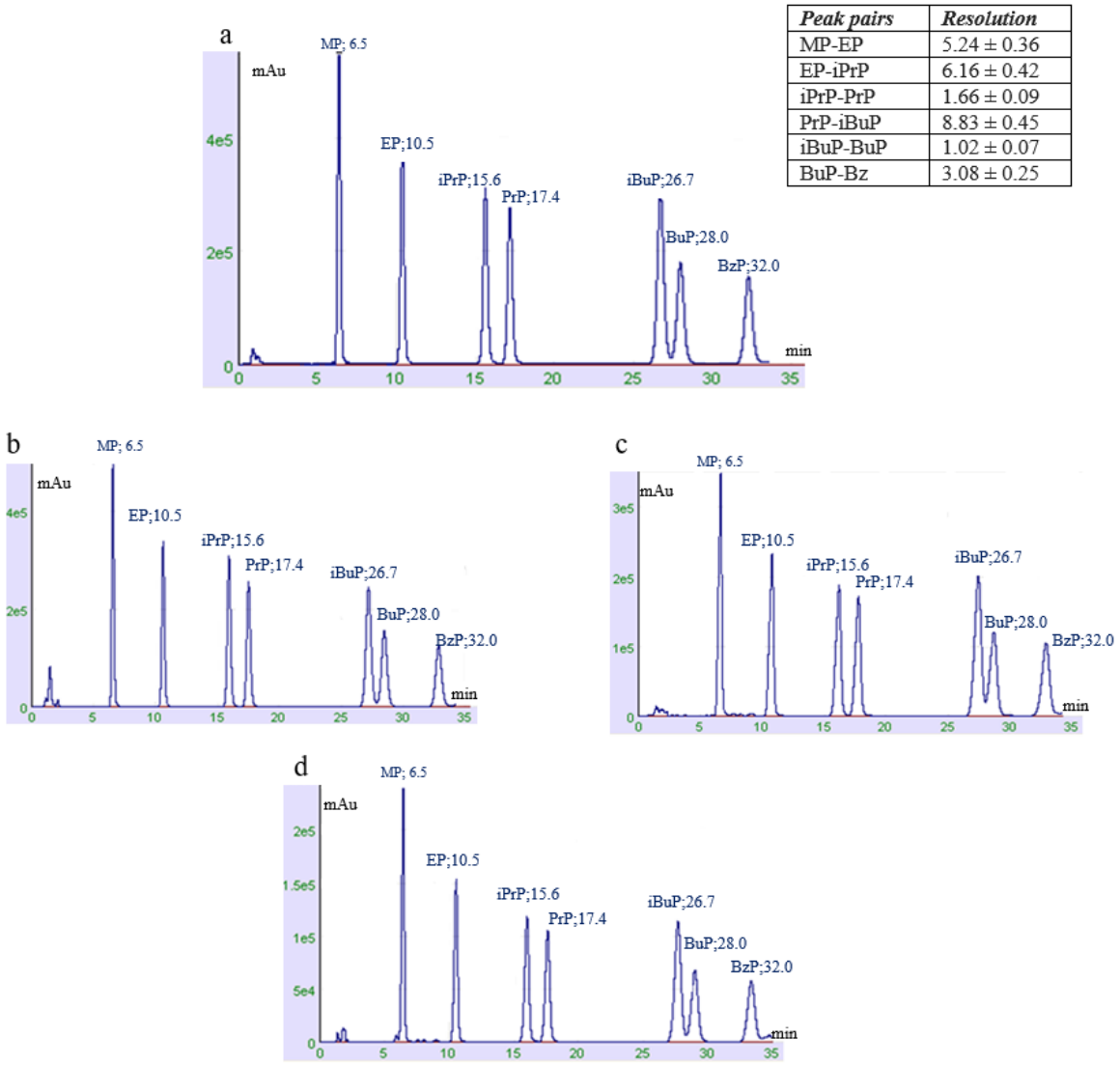

Figure 1. Chromatograms obtained for a standard mixture of the Parabens in ethanol (**a**) and of a 50 μg mL^{-1} extracted from Stratum Corneum (**b**); Dermis (**c**); Epidermis (**d**).

Matrix effect values ranged from the lowest value of 0.78 (Dermis) to 1.01 (Stratum Corneum). Concerning the robustness, minor variations in the experimental parameters did not cause any appreciable change in the method performance. For the extraction procedures, distinct organic solvents in various percentages with water were tested. The spike levels (50.0 μg mL^{-1} and 5.0 μg mL^{-1}) were in the recommended range, i.e., calculated LOD < spike level < 10 × calculated LOD. The literature reports several analytical methods for the determination of PBs, but (a) alone but up to four analogs, (b) in combination with molecules belonging to other chemical classes that could interfere in the diffusion, and (c) formulated in topical pharmaceutical and cosmetic formulations, such as ointment, sunscreen, body lotion, and so on (references in Table 3). Table 3 summarizes several studies concerning PBs analysis performed with LC_UV detection, along with LOD parameters.

Table 3. Comparison of present study with the literature's analytical methods for the estimation of MP, PrP LOD values. * PBs along with compounds of other chemical classes. # not reported.

Analytes	Vehicle	Method	LOD μg mL^{-1}	LOQ μg mL^{-1}	Ref
MP, PrP	Ethanol	LC_UV	0.003–0.053	0.087–0.197	Present study
MP, PrP	Water: acetonitrile (50:50)	LC_UV	0.026–0.026	n.r. #	[18]
MP, PrP	Pharmaceutical formulation	LC_UV	0.110–0.090	1.85–0.06	* [19]
MP, and PP	Cosmetic products	UPLC_DAD	0.068–0.064	0.02 and 0.04	* [20]
MP and PP	Pharmaceutical formulation	LC_UV	0.025–0.029	0.076–0.088	* [21]

LC_UV Liquid Chromatography_Ultraviolet Detection; UPLC_DAD UltraPerformance Liquid Chromatography_ Diode-Array Detection.

We reported a comparison only for MP and PrP because they are the most frequently investigated PBs mixture. Usually, scientific works reported LODs for permitted preservatives, but not for the forbidden ones. Indeed, as also reported recently in the literature, low concentrations of iPrP, iBuP, and BzP were detected in 70% of PCPs, labeled as "green" or "natural," despite their being banned from the EU [10]. The LOD values in the present study are similar to those in Table 3. Other works, not reported in Table 3, afford lower LOD values but use different detection systems such as mass spectrometry (MS) [22,23]. MS is indeed more sensitive, but the concentration levels of PBs in the marketed formulation are in the mg range and, therefore, sensitively detectable with a more cost-effective piece of equipment such as UV detectors, which are standard for routine laboratory use. Furthermore, these studies assess PBs in other matrices, such as blood serum, cosmetic, and pharmaceutical samples [18,20]. The proposed method allows for the simultaneous analysis of as many as seven PBs analogues in a relatively short time, extracting them from each skin layer. Lastly, our experiments were run using neat ethanol as the vehicle, evaluating only the passage through the skin [24] and its retention therein.

4. Conclusions

Considering that approximately 90% of personal care products, such as toothpaste, shampoo, shower gel, body cream, and antiperspirants, contain one or more parabens [25], it is clear that demands for robust and flexible methods aimed at investigating PBs' skin uptake are emerging. In fact, many cosmetics are applied to the whole body, therefore, suspected, or hazardous preservatives could accumulate in the skin and constantly be released into the bloodstream. This scientific work proposes an analytical method for the simultaneous identification and quantification of seven PBs, based on a cost-friendly and efficient sonication-assisted solvent extraction in each of the three skin layers, i.e., SC, Epidermis, and Dermis, with reasonable accuracy. This protocol does not require large amounts of organic solvent and affords very little interference from the matrix. This method might be suitable for large-scale cosmetic and pharmacological assessment of these preservatives in each skin layer and to be used routinely in analytical laboratories with relatively simple and cost-effective equipment.

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References

1. Al-Halaseh, L.K.; Al-Adaileh, S.; Mbaideen, A.; Hajleh, M.N.A.; Al-Samydai, A.; Zakaraya, Z.Z.; Dayyih, W.A. Implication of parabens in cosmetics and cosmeceuticals: Advantages and limitations. *J. Cosmet. Dermatol.* **2022**, *21*, 3265–3271. https://doi.org/10.1111/jocd.14775.
2. Błędzka, D.; Gromadzińska, J.; Wąsowicz, W. Parabens. From environmental studies to human health. *Environ. Int.* **2014**, *67*, 27–42. https://doi.org/10.1016/j.envint.2014.02.007.
3. Ma, X.; Wang, H.; Song, Y.; Pan, Y. Skin irritation potential of cosmetic preservatives: An exposure-relevant study. *J. Cosmet. Dermatol.* **2021**, *20*, 195–203. https://doi.org/10.1111/jocd.13502.3.
4. Soni, M.G.; Carabin, I.G.; Burdock, G.A. Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem. Toxicol.* **2005**, *43*, 985–1015. https://doi.org/10.1016/j.fct.2005.01.020.
5. Pollock, T.; Weaver, R.E.; Ghasemi, R.; deCatanzaro, D. Butyl paraben and propyl paraben modulate bisphenol A and estradiol concentrations in female and male mice. *Toxicol. Appl. Pharmacol.* **2017**, *325*, 18–24. https://doi.org/10.1016/j.taap.2017.04.001.
6. EU. *Regulation (EC) No 1004/2014 of the European Parliament and of the Council on Cosmetic Products*. Official Journal of the European Union Luxembourg, 2014.
7. Bodin, L.; Rogiers, V.; Bernauer, U.; Chaudhry, Q.; Coenraads, P.J.; Dusinska, M.; Ezendam, J.; Gaffet, E.; Galli, C.L.; Granum, B.; et al. Opinion of the Scientific Committee on Consumer Safety (SCCS)—Final Opinion on propylparaben (CAS No 94-13-3, EC No 202-307-7). *Regul. Toxicol. Pharmacol.* **2021**, *125*, 105005. https://doi.org/10.1016/j.yrtph.2021.105005.
8. Neupane, R.; Boddu, S.H.S.; Renukuntla, J.; Babu, R.J.; Tiwari, A.K. Alternatives to Biological Skin in Permeation Studies: Current Trends and Possibilities. *Pharmaceutics* **2020**, *12*, 152. https://doi.org/10.3390/pharmaceutics12020152.
9. Neri, I.; Laneri, S.; Di Lorenzo, R.; Dini, I.; Russo, G.; Grumetto, L. Parabens Permeation through Biological Membranes: A Comparative Study Using Franz Cell Diffusion System and Biomimetic Liquid Chromatography. *Molecules* **2022**, *27*, 4263. https://doi.org/10.3390/molecules27134263.
10. van der Schyff, V.; Suchánková, L.; Kademoglou, K.; Melymuk, L.; Klánová, J. Parabens and antimicrobial compounds in conventional and "green" personal care products. *Chemosphere* **2022**, *297*, 134019. https://doi.org/10.1016/j.chemosphere.2022.134019.
11. Piao, C.; Chen, L.; Wang, Y. A review of the extraction and chromatographic determination methods for the analysis of parabens. *J. Chromatogr. B* **2014**, *969*, 139–148. https://doi.org/10.1016/j.jchromb.2014.08.015.
12. Ferreira-Nunes, R.; Angelo, T.; da Silva, S.M.M.; Magalhaes, P.O.; Gratieri, T.; da Cunha, M.S.S.; Gelfuso, G.M. Versatile chromatographic method for catechin determination in development of topical formulations containing natural extracts. *Biomed. Chromatogr.* **2018**, *32*, e4062. https://doi.org/10.1002/bmc.4062.
13. Pereira, M.N.; Matos, B.N.; Gratieri, T.; Cunha-Filho, M.; Gelfuso, G.M. Development and validation of a simple chromatographic method for simultaneous determination of clindamycin phosphate and rifampicin in skin permeation studies. *J. Pharm. Biomed. Anal.* **2018**, *159*, 331–340. https://doi.org/10.1016/j.jpba.2018.07.007.
14. Özcan, S.; Levent, S.; Can, N.; Kozanli, M. A Novel HPLC Method for Simultaneous Determination of Methyl, Ethyl, n-propyl, Isopropyl, n-butyl, Isobutyl and Benzyl Paraben in Pharmaceuticals and Cosmetics. *Comb. Chem. High Throughput Screen.* **2021**, *24*, 352–365. https://doi.org/10.2174/1386207323999200728121657.
15. European Union Guidelines. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf (accessed on 20 September 2022).
16. Shen, H.-Y.; Jiang, H.-L.; Mao, H.-L.; Pan, G.; Zhou, L.; Cao, Y.-F. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. *J. Sep. Sci.* **2007**, *30*, 48–54. https://doi.org/10.1002/jssc.200600215.
17. Khesina, Z.B.; Iartsev, S.D.; Revelsky, A.I.; Buryak, A.K. Microextraction by packed sorbent optimized by statistical design of experiment as an approach to increase the sensitivity and selectivity of HPLC-UV determination of parabens in cosmetics. *J. Pharm. Biomed. Anal.* **2021**, *195*, 113843. https://doi.org/10.1016/j.jpba.2020.113843.
18. Katakam, L.N.R.; Ettaboina, S.K.; Dongala, T. A simple and rapid HPLC method for determination of parabens and their degradation products in pharmaceutical dosage forms. *Biomed. Chromatogr.* **2021**, *35*, e5152. https://doi.org/10.1002/bmc.5152.
19. Dewani, A.P.; Bakal, R.L.; Kokate, P.G.; Chandewar, A.V.; Patra, S. Development of a Single Ion Pair HPLC Method for Analysis of Terbinafine, Ofloxacin, Ornidazole, Clobetasol, and Two Preservatives in a Cream Formulation: Application to In Vitro Drug Release in Topical Simulated Media-Phosphate Buffer Through Rat Skin. *J. AOAC Int.* **2015**, *98*, 913–920. https://doi.org/10.5740/jaoacint.14-189.

20. Qadir, M.A.; Ahmed, M.; Shafiq, M.I.; Ali, A.; Sadiq, A. Analytical Method for the Identification and Assay of Kojic Acid, Methylparaben, and Propylparaben in Cosmetic Products Using UPLC: Application of ISO 12787:2011 Standard. *J. AOAC Int.* **2016**, *99*, 1191–1196. https://doi.org/10.5740/jaoacint.16-0026.
21. Abd El-Hay, S.S.; Mohram, M.S. Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation. *Chromatogr. Res. Int.* **2016**, *2016*, 4909547. https://doi.org/10.1155/2016/4909547.
22. Vela-Soria, F.; Ballesteros, O.; Rodríguez, I.; Zafra-Gómez, A.; Ballesteros, L.; Cela, R.; Navalón, A. A new treatment by dispersive liquid-liquid microextraction for the determination of parabens in human serum samples. *Anal. Bioanal. Chem.* **2013**, *405*, 7259–7267. https://doi.org/10.1007/s00216-013-7181-x.
23. Lecce, R.; Regazzoni, L.; Mustazza, C.; Incarnato, G.; Porrà, R.; Panusa, A. Screening of preservatives by HPLC-PDA-ESI/MS: A focus on both allowed and recently forbidden compounds in the new EU cosmetics regulation. *J. Pharm. Biomed. Anal.* **2016**, *125*, 260–269. https://doi.org/10.1016/j.jpba.2016.03.044.
24. Mansour, R.S.H.; Hamdan, I.I.; Salem, M.S.H.; Khalil, E.A.; Sallam, A.A. HPLC method development/validation and skin diffusion study of caffeine, methyl paraben and butyl paraben as skin-diffusing model drugs. *PLoS ONE* **2021**, *16*, e0247879. https://doi.org/10.1371/journal.pone.0247879.
25. Andersen, A. Final Amended Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben as used in Cosmetic Products. *Int. J. Toxicol.* **2008**, *27*, 1–82. https://doi.org/10.1177/109158180802704s01.