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Simultaneous In-Cell Derivatization Pressurized Liquid Extraction for the Determination of Multiclass Preservatives in Leave-On Cosmetics

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An effective one-step sample preparation methodology for the determination of multiclass preservatives in cosmetics has been developed, applying, for the first time to this kind of matrix, pressurized liquid extraction (PLE) and a very simple, cheap, and fast derivatization procedure: acetylation with acetic anhydride and pyridine. A multifactorial experimental design has been used to evaluate and optimize the main experimental parameters potentially affecting the extraction process. In the final conditions the sample was mixed with Florisil as the dispersing sorbent and extracted with ethyl acetate for 15 min at 120 °C. One of the main goals of this work was to demonstrate the possibility of carrying out direct cosmetic preservative acetylation by simply adding the derivatization reagents into the PLE cell. The extract was then analyzed by GC/MS without any further cleanup or concentration step. The accuracy, precision, linearity, and detection limits (LODs) were evaluated to assess the performance of the proposed method. Quantitative recoveries were obtained, and relative standard deviation values were lower than 10% in all cases. The obtained LODs ranged from 0.000004% to 0.0001% (w/w), values far below the established restrictions in the European Cosmetics Regulation, making this multicomponent analytical method suitable for routine control. Finally, several cosmetic products such as moisturizing and antiwrinkle creams and lotions, hand creams, sunscreen and after-sun creams, baby lotions, and hair care products were analyzed. All the samples contained several of the target cosmetic ingredients, in some cases at quite high concentrations, although the actual European Cosmetics Regulation was fulfilled in all cases.

Preservatives are substances added to cosmetics for the primary purpose of inhibiting the development of microorganisms (antimicrobial function), but may also be added to protect such products against damage and degradation caused by the exposure to oxygen (antioxidant function).

The esters of *p*-hydroxybenzoic acid (parabens), iodopropynyl butylcarbamate (IPBC), 2,4,4′-trichloro-2′-hydroxydiphenyl ether (triclosan, TCS), and bromine-containing preservatives such as 5-bromo-5-nitro-1,3-dioxane (Bronidox) and 2-bromo-2-nitropropane-1,3-diol (Bronopol) are included in a wide variety of cosmetics and personal-care products to prevent or retard bacterial growth. Parabens are the most widely used antimicrobial preservatives in cosmetic products. Their antimicrobial activity is generally selective, so their mixtures or mixtures with other classes of preservatives offer powerful antimicrobial activity against an extremely broad spectrum of microorganisms.[1]

2-*tert*-Butyl-4-methoxyphenol (BHA) and 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) are antioxidant preservatives frequently used to prevent oxidation in foods and cosmetics. The use of mixtures of both of them is very common since there is a synergic increase of their antioxidant power.[2]

Together with the positive protective effects of cosmetic preservatives, unintended possible side effects of these ingredients are a matter of concern, because exposure to some of these compounds could have harmful effects on human health. Some of these ingredients, such as parabens and BHA, may modulate and disrupt the endocrine system,[3] IPBC could cause acute inhalation toxicity,[4] and some compounds such as BHA or some transformation products of triclosan, Bronidox, and Bronopol are even suspected carcinogenics.[5−7] There is also current scientific evidence that indicates that the use or misuse of biocidal products may contribute to the increased occurrence of antibiotic-resistant bacteria, both in humans and in the environment.[8]

To ensure a high level of protection of human health, cosmetic products are regulated and controlled worldwide. The new European Union (EU) Cosmetic Products Regulation[9] (which is,

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to a great extent, a recast of the previous Cosmetics Directive[10] and its successive amendments and adaptations), the federal Food, Drug and Cosmetic Act (FD&C Act) and the Fair Packaging and Labeling Act (FPLA) drawn up by the Food and Drug Administration (FDA) in the United States, and, finally, the Pharmaceutical Affairs Law (PAL) adopted in Japan constitute the three main regulatory systems on cosmetic products. The preservatives allowed in the EU context are listed in Annex VI of the EU Cosmetics Regulation,[9] where limitations, requirements, label warnings, and the maximum permissible concentrations are indicated (see Table S-1 (Supporting Information) for the target preservatives of this study). In Japanese legislation there is also a positive list of preservatives, but the allowed substances and authorized contents are quite different.[11] In the U.S. framework there is not a positive list of preservatives, although there is a short list of substances, published by the FDA, banned or restricted in cosmetics, including different compounds formerly used as preservatives.[1]

Thus, to protect consumer health and ensure compliance to existing government regulations, there is a need for the development of effective and convenient methodologies to identify and determine preservatives in cosmetics both accurately and sensitively.

A great part of the analytical effort has been focused on paraben determination,[12−15] while methods for the determination of other preservatives in cosmetic formulations are very limited or inexistent. However, multicomponent analytical methods are required since cosmetic products very often contain mixtures of preservatives belonging to different chemical classes. Simultaneous analysis of more than one class of preservatives is scarce and mainly based on liquid chromatography (LC)[16−18] and capillary electrophoresis (CE).[19,20] Flow injection analysis (FIA) has also been employed, enhancing sample throughput.[21]

In most of these procedures, sample preparation is usually performed through several steps which can include solvent extraction or dilution, mixing, sonication, heating, addition of acids or bases, centrifugation, and filtration. These procedures are frequently tedious and time-consuming, and the use of hazardous solvents is usually required. In addition, the possible presence of interferences that could distort the results is not rejectable. To overcome some of these drawbacks, supercritical fluid extraction (SFE),[16,22] solid-phase extraction (SPE),[12] and solid-phase microextraction (SPME)[23] have been recently applied for the determination of different additives in cosmetics.

Pressurized liquid extraction (PLE) has been applied for the analysis of cosmetic ingredients (parabens and TCS, among them) in environmental matrixes, such as sewage sludge.[24,25] PLE is fast, increases automation, decreases the amount of organic solvents, and offers the possibility of controlling the selectivity of the extraction by loading different sorbents instead of inert materials into the extraction cell.

Due to the polar nature of most preservatives, a derivatization step previous to gas chromatography (GC) analysis is highly recommended to reduce adsorption in the chromatographic system and improve sensitivity, peak separation, and peak symmetry.[14,22] Acetylation is one of the most common derivatization procedures for phenolic compounds,[26,27] and it has been applied for the determination of parabens and triclosan in water,[27,28] but to our knowledge, this derivatization procedure has never before been employed for cosmetic samples. The advantages of acetylation are the high efficiency obtained using low-cost reagents, especially compared with silylation agents.

The aim of this work is to develop a method based on PLE with acetylation followed by gas chromatography/mass spectrometry (GC/MS) for the simultaneous determination of different classes of preservatives including two bromine-containing preservatives, seven parabens, IPBC, TCS, and the antioxidant preservatives BHA and BHT in multimatrix cosmetic samples. The possibility of performing simultaneous derivatization and extraction by adding the acetylation reagents into the PLE cell will be evaluated. To our knowledge, both acetylation and PLE are applied for the first time to the analysis of cosmetics.

MATERIALS AND METHODS

Chemicals. Bronidox (≥99.0%) was acquired from Fluka (Buchs, Switzerland). Bronopol (98%), methylparaben (99%, MeP), ethylparaben (99%, EtP), propylparaben (99%, PrP), butylparaben (99%, BuP), benzylparaben (99%, BzP), butylated hydroxyanisole (≥98.5%, BHA), butylated hydroxytoluene (99%, BHT), IPBC (97%), and triclosan (≥97.0%, TCS) were purchased from Aldrich (Milwaukee, WI). Isopropylparaben (≥99%, iPrP) and isobutylparaben (≥97%, iBuP) were purchased from TCI Europe (Belgium). Table S-1 (Supporting Information) shows the IUPAC names and chemical structures of the studied compounds.

Deuterated methyl 4-hydroxybenzoate-*2,3,5,6-d*$_4$ (MePd_4, 98.3 atom % D) was obtained from C/D/N Isotopes (Quebec, Canada). The internal standard PCB-30 (2,4,6-trichlorobiphenyl) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetone, ethyl acetate, *n*-hexane, pyridine, and acetic anhydride (Ac_2O) were provided by Merck (Darmstadt, Germany). Florisil (60−100 mesh) and C18 (70−230 mesh) were obtained from

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Aldrich (Milwaukee, WI). Before being used, Florisil was activated at 130 °C for 12 h and then allowed to cool in a desiccator. Sodium sulfate anhydrous (99%) was purchased by Panreac (Barcelona, Spain).

Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in acetone, hexane, hexane/acetone (1:1, v/v), and ethyl acetate. All solutions were stored in amber glass vials at −20 °C. All solvents and reagents were of analytical grade.

Acetylation was carried out by adding 100 μL of acetic anhydride containing 2.5% pyridine to 1 mL of the standard or extract solutions. The mixture was then maintained at 80 °C for 30 min and then allowed to cool to room temperature.

Cosmetic Samples. Different cosmetics from national and global companies were purchased from local stores. They included moisturizing and antiwrinkle creams and lotions, hand creams, sunscreen and after-sun creams, and baby lotions. Two products for hair care were also considered. Samples were kept in their original containers at room temperature until their analysis.

A 0.5 g portion of cosmetic sample was weighed exactly into a 10 mL glass vial. When it was necessary, the sample was spiked with 50 μL of the corresponding acetone solution of the target compounds to get the desired final concentration in the cosmetic sample. The sample was then mixed with 1 g of a drying agent (anhydrous Na_2SO_4) and 1 g of dispersing sorbent (C18 or Florisil).

PLE and Derivatization Procedures. Extractions were performed on an ASE 200 system (Dionex Co., Sunnyvale, CA) equipped with a 24-sample carousel, 11 mL stainless steel cells, and 40 mL collection vials. Two cellulose filters (Dionex) were placed at each end of the PLE cell. The sample, mixed with the drying agent and the dispersing sorbent, was introduced into the cell, where previously 1 g of clean sand (50−70 mesh particle size, Sigma-Aldrich) was placed. In all experiments, 20 μL of $MePd_4$ surrogate solution (2500 μg mL^{-1}) was added to each sample before extraction. Finally, the dead volume of the cell was filled with sand. The cell was tightly closed and placed into the carousel of the ASE system. Extractions were performed by preheating the cell before filling with solvent (preheat method). The extraction pressure was set to 1500 psi, the flush volume was 60%, and the purge time was set to 60 s. Hexane/acetone (1:1, v/v) or ethyl acetate was employed as the extraction solvent, depending on the experiment. The extraction temperature and extraction time varied during the optimization of the method. After extraction, 20 μL of PCB 30 (100 μg mL^{-1}) was added to the final extract (∼15 mL) to correct possible variations of the extract volume. Then PLE extracts were derivatized and analyzed by GC/MS.

In the simultaneous derivatization−extraction experiments, 100 μL of acetic anhydride containing 2.5% pyridine was added to the cosmetic sample before the addition of the drying agent and the dispersing sorbent. Then the PLE procedure previously described was carried out. Finally, the extracts were directly analyzed since in-cell derivatization was accomplished during extraction.

GC/MS Analysis. Analyses were performed on a Varian CP 3900 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) equipped with a 1079 split/splitless injector and an ion trap spectrometer, Varian Saturn 2100 (Varian Chromatography Systems). Separation was carried out on an HP5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA). Helium (purity 99.999%) was employed as the carrier gas at a constant column flow of 0.8 mL min^{-1}. Two different GC oven temperature programs were tested. The first was used for the derivatization studies, and it consisted of the following: 45 °C (held 2 min) to 100 °C at 8 °C min^{-1}, to 150 °C at 20 °C min^{-1}, to 200 °C at 25 °C min^{-1} (held 5 min), to 220 °C (held 1 min) at 8 °C min^{-1}, and a final ramp to 260 °C (held 7 min) at 30 °C min^{-1}. The second program was optimized to keep good resolution of the target compounds, increasing the sample throughput: 60 °C (held 2 min) to 200 °C at 30 °C min^{-1} and a final ramp to 260 °C (held 4 min) at 40 °C min^{-1} (total analysis time 15 min).

The injector was programmed to return to the split mode after 2 min from the beginning of a run. The split flow was set at 20 mL min^{-1}. The injector temperature was held constant at 260 °C. The trap, manifold, and transfer-line temperatures were 220, 120, and 280 °C, respectively.

The GC/MS system was operated by Saturn GC/MS Workstation v5.52 software. In the full scan mode the mass range was varied from 50 to 320 m/z at 0.6 s $scan^{-1}$, starting at 4 min and ending at 15 min. The filament emission current was 15 μA. The analytes were positively identified by comparison of their mass spectra and retention times to those of the standards.

Statistical Analysis. Basic and descriptive statistics and experimental design analysis were performed using Statgraphics-Plus v5.1 (Manugistics, Rockville, MD) as the software package. The experimental design was applied in the optimization of the extraction method to analyze the simultaneous effect of the main parameters affecting PLE.

RESULTS AND DISCUSSION

Derivatization and GC/MS Analysis. Optimization of the chromatographic conditions was accomplished using a standard mixture solution of all target compounds in *n*-hexane. Direct analysis produced peaks with appreciable tailing for most compounds due to the interaction of hydroxyl groups with the chromatographic system. Therefore, a derivatization step was introduced prior to GC determination to improve the chromatographic analysis. Acetylation with acetic anhydride is one of the most simple and cheap derivatization procedures for phenolic compounds. The procedure to obtain standard solutions of the corresponding acetylated compounds was based on a previous work dealing with the acetylation of other phenolic species[26] and a recent study including some of the target compounds,[28] and it is described in the Materials and Methods.

Different families of preservatives are studied in this work (Table S-1, Supporting Information), and for some of these compounds no previous studies on their acetylation reaction were found (e.g., Bronopol). It is necessary to ensure which compounds undergo derivatization and to demonstrate the chromatographic benefits of this reaction. Figure 1 shows the extracted ion chromatograms before (A) and after (B) acetylation, and Figure S-1 (Supporting Information) compares the chromatographic responses obtained. The retention times and the quantification and identification ions for the nonderivatized and derivatized analytes are included in Table S-2 (Supporting Information).

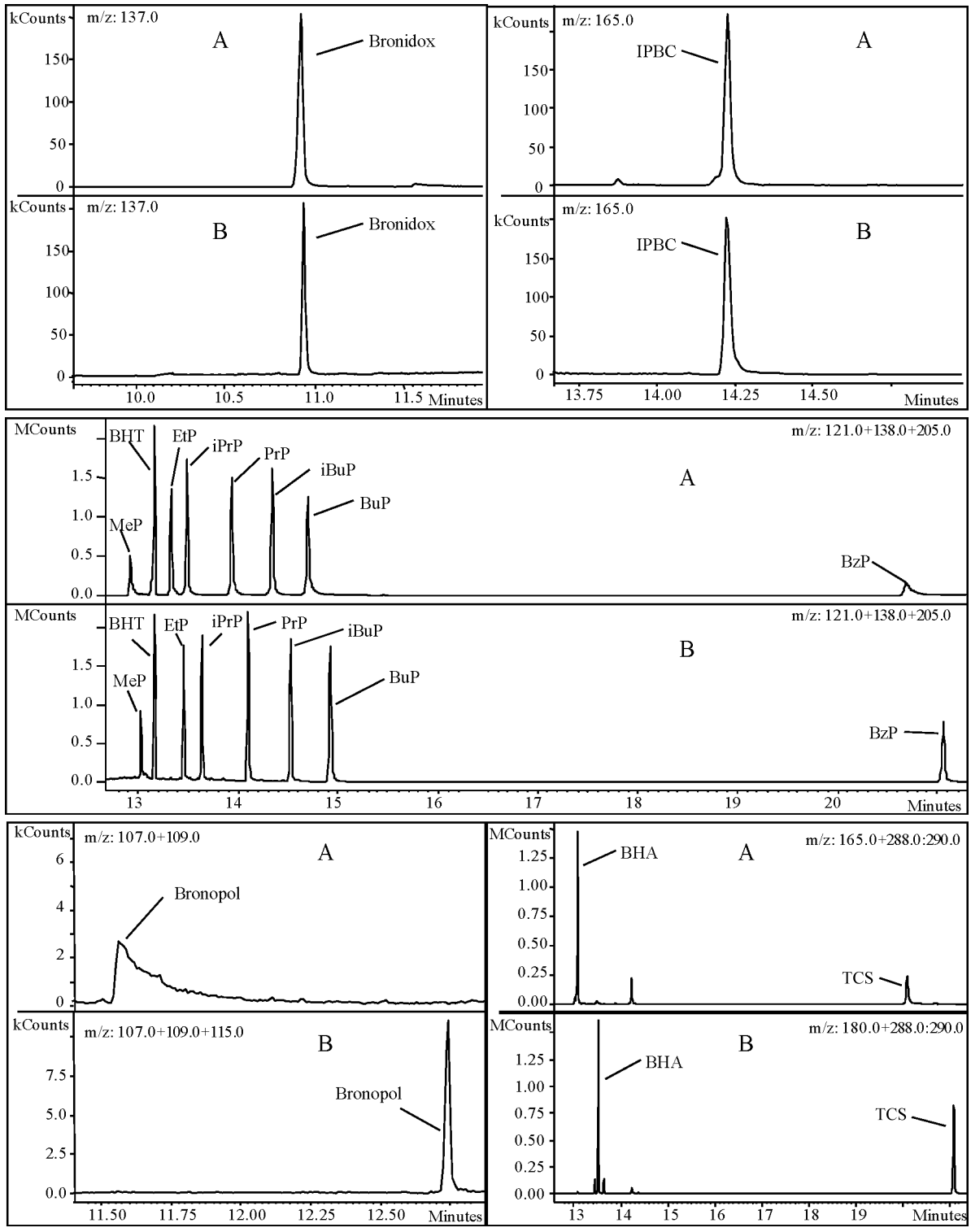

Figure 1. Extracted ion chromatograms corresponding to a 10 μg mL^{-1} solution of the target analytes before (A) and after (B) derivatization.

Bronidox and IPBC do not undergo derivatization since these compounds do not have chemical groups susceptible to acetylation; the retention times are not modified, and neither are their chromatographic responses (Figures 1 and S-1, Supporting Information).

On the contrary, parabens and triclosan are acetylated under selected conditions. This fact is confirmed because of the displacement of the retention times (see Figure 1), as well as the improvement in the peak shapes, since the tailing observed in the nonderivatized species disappears and peaks completely symmetric are obtained. This improvement is especially noticeable for MeP, BzP, and TCS; for these compounds, responses are also significantly higher (Figure S-1, Supporting Information). Additionally, small differences can also be observed in the obtained mass spectra. The ratio of ion intensities is slightly modified when the derivatization takes place (see as an example MeP in Figure S-2, Supporting Information). The molecular ions corresponding to the acetylated derivatives were not present in the mass spectra

in most cases. This absence has been previously reported as a result of the loss of the acetyl group upon ionization.[28,29] Complete acetylation can be assured since nonderivatized species were not detected.

Regarding Bronopol, the effect of the derivatization on the peak shape and chromatographic response is much more evident as can be seen in Figures 1 and S-1 (Supporting Information). The retention time was also considerably modified (more than 1 min). In addition, the mass spectrum of the acetylated derivative differs significantly from the spectrum of Bronopol (see both spectra in Figure S-2). In this compound two hydroxyl groups are present (Table S-1), which means that the acetylation can take place in two reaction centers. In fact, in this case, the molecular ion corresponding to the doubly acetylated compound was identified in the mass spectrum (m/z 283), confirming the above hypothesis. In addition, a cluster of ions, typical of bromine-containing compounds, around m/z 195 and 197 corresponding to the loss of two CH_3CO groups is present. The base peak (m/z 115) was formed by the subsequent loss of the bromine atom.

BHA also undergoes derivatization (see Figure 1). The most intense fragment ions for the acetylated derivative (see Figure S-2, Supporting Information) were also formed by the loss of CH_3CO, in such a way that the mass spectrum of the derivative was similar to that of the nonderivatized compound, with the exception of the ratio of ion intensities and the presence of the acetylated BHA molecular ion at m/z 222.

In the case of BHT, the acetylation could not be demonstrated since the retention time, peak shape, chromatographic response (Figure 1), and mass spectra were equivalent before and after the addition of the acetylation reagents. The highly hindered hydroxyl group with poor nucleophilicity (see the structure in Table S-1, Supporting Information) may prevent the acetylation under the studied conditions. This is in agreement with the study of Monsef-Mirzai,[30] who demonstrated that very hindered phenols, such as BHT, remain unacetylated. Anyway, the underivatized BHT peak shape and chromatographic response are both satisfactory.

In summary, three of the compounds (Bronidox, IPBC, and BHT) did not undergo derivatization. For the other compounds, the reaction yield was quantitative, since we could not find any trace of the underivatized analytes, and satisfactory, improving significantly the chromatographic analysis of the target compounds both qualitatively and quantitatively. The reaction was also carried out with standard solutions in ethyl acetate and hexane/acetone (1:1, v/v), demonstrating the suitability of these solvents to accomplish derivatization. The acetylated derivatives were stable for at least several weeks.

PLE Optimization. The influence of the main variables potentially affecting the PLE process must be evaluated to obtain an efficient extraction. In the usual working range for this technique, the pressure generally has a negligible effect on the extraction yield,[31,32] so we decided to conduct the experiments at 1500 psi, which is the standard operating pressure in PLE extractions.[33] The flush volume and purge time were set at 60% and 60 s, respectively. The influence of the remaining variables was studied using a multifactor strategy. The study consisted of a complete factorial 2^4 design, involving 16 randomized experiments and allowing 5 degrees of freedom to estimate the experimental error. This design has resolution V, which means that it is capable of evaluating all main effects and all two-factor interactions. Numerical analysis of data resulting from the experimental design was made with the statistical software package Statgraphics-Plus v5.1. The experiments were performed using 0.5 g of a real moisturizing cream sample initially labeled as containing some of the target compounds (Bronopol, MeP, BHT, and PrP) and fortified with all compounds at 100 $\mu g\ g^{-1}$. Since drying of the sample is essential for an efficient PLE, in all experiments 1 g of anhydrous sodium sulfate was added. Sand was employed to avoid dead volume. The studied factors were the extraction temperature (A), extraction solvent (B), dispersing sorbent (C), and extraction time (D).

The temperature factor (A) was studied at 80 and 120 °C. The choice of an appropriate solvent is another essential aspect in the development of extraction methods. For an efficient extraction, the solvent must solubilize the target compounds while leaving the sample matrix as intact as possible.[33] Two solvents (factor B) were investigated: hexane/acetone (1:1, v/v) and ethyl acetate.

The inclusion of an in situ cleanup step by adding certain sorbents to the PLE cell contributes to obtaining clean extracts. In this way, lipids and other coextractable matrix materials are prevented from coming out to the extract. In addition, these sorbents can act as a dispersing phase, contributing to the execution of a more efficient extraction. Thus, 1 g of dispersing sorbent (factor C), C18 or Florisil, was mixed with the sample and packed in the PLE cell. The last factor considered was the extraction time (factor D), and it was assessed at 5 and 15 min.

The 16 experiments were carried out; after extraction, the extracts were acetylated at 80 °C for 30 min before GC/MS analysis (see the Materials and Methods). Numerical analysis of the results obtained leads to the analysis of variance (ANOVA) results shown in Table 1. As can be seen, the most important factor, with statistical significance for most of the target compounds, is the extraction solvent. The extraction time was also significant for many analytes, whereas the temperature and the dispersing sorbent were each significant for five compounds. Some second-order effects are also important, especially interactions AB (temperature−solvent) and BD (solvent−time).

The information included in the ANOVA can be graphically plotted by means of the Pareto charts. In Figure S-3 (Supporting Information), some representative graphics are shown. The length of each bar is proportional to the absolute value of its associated standardized effect. The vertical line in the graphs represents the statistically significant bound at the 95% confidence level.

Figure 2 shows the main effects diagrams for several representative compounds. This kind of plot shows the main effects with a line drawn between the low and the high levels of the

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Table 1. *F* Ratios and *p* Values[a] Obtained in the Analysis of Variance

	main effects								interactions									
	(A) temperature		(B) solvent		(C) dispersant		(D) time		AB		AC		AD		BC		BD	
	F ratio	*P* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value
Bronidox	20	+	11	+	8	+	1		18	+	8	+	22	+	3		21	+
Bronopol	5		15	+	0.3		4		6		5		1		0		6	
MeP	5		19	+	0.1		14	+	5		4		2		2		5	
BHA	16	+	28	+	2		40	+	10	+	9	+	0.01		3		13	+
BHT	1		1		0		2		5		0.2		1		1		1	
EtP	12	+	19	+	2		28	+	13	+	3		6		0.01		9	+
iPrP	7	+	10	+	1		17	+	7	+	2		3		0.01		8	+
PrP	0		31	+	4		6		1		0.2		1		0.1		1	
IPBC	8	+	40	+	37	+	19	+	12	+	4		5		0.01		10	+
iBuP	5		54	+	7	+	35	+	11	+	3		0.1		0.1		5	
BuP	6		36	+	9	+	41	+	12	+	6		2		0.3		7	+
BzP	1		0.5		7	+	11	+	1		0.3		0.2		0.2		11	+
TCS	1		1		6		3		0		1		1		0.4		12	+

[a] Key: + cell, p value <0.05; empty cell, p value >0.05.

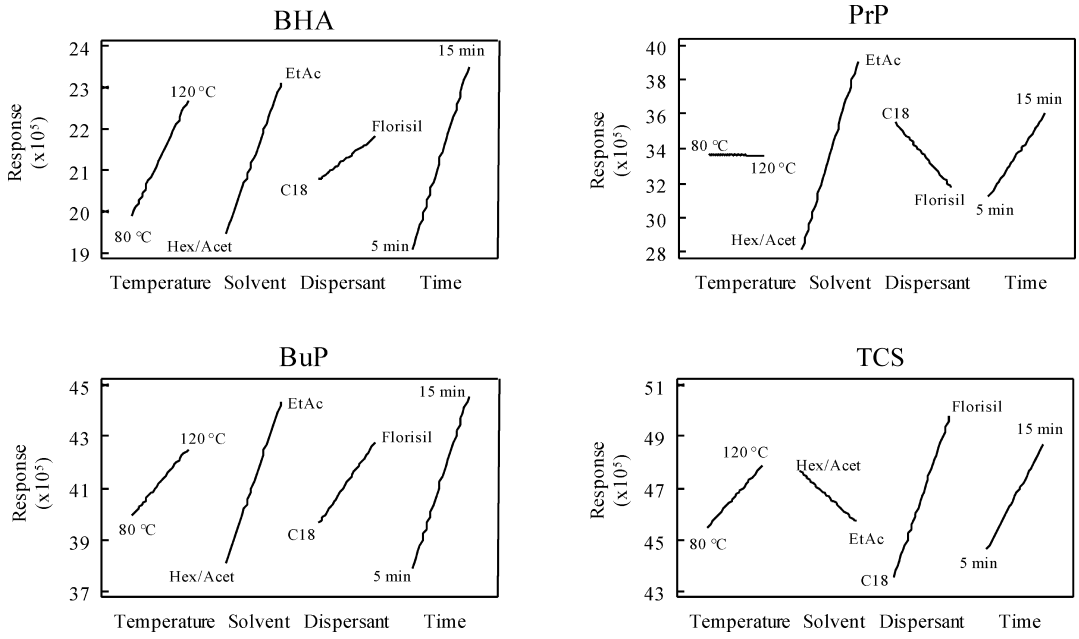

Figure 2. Main effects plots for some representative compounds (EtAc = ethyl acetate; Hex/Acet = hexane/acetone).

corresponding factors. The length of the lines is proportional to the effect magnitude of each factor in the extraction process, and the sign of the slope indicates the level of the factor that produces the highest response. Regarding the significant factors B and D (see the ANOVA in Table 1), the best extractions were obtained at the high level of the factors for all compounds, which means ethyl acetate and 15 min. The other two main factors A and C were significant for less compounds (Table 1) but, in those cases, were also characterized by a positive slope, so better extractions were also achieved at the high level of the factors, 120 °C and Florisil.

Before a general method for the simultaneous extraction of the 13 target compounds is proposed, it is necessary to examine the interaction effects, since some of them, especially AB and BD (see Table 1), were significant for several analytes. These second-order effects are shown in Figure 3 for some analytes, as an example, since the trends were equivalent in all cases. Although the slopes of the lines are quite different, the lines do not intercept, so the general conditions established after analysis of the main effects do not change. Interaction AB shows again as the most favorable conditions the extraction at 120 °C using ethyl acetate. Regarding the BD effect, the most favorable conditions were ethyl acetate and 15 min, although it is interesting to notice that in general the time is only significant when hexane/acetone is used. An exception to this behavior was BzP and TCS (see the BzP plot in Figure 3). For these two compounds, the most favorable conditions would involve the extraction with hexane/acetone for 15 min.

In view of the results of the experimental design, the selected general conditions for the simultaneous extraction of the target preservatives and antioxidants were established as follows: extraction temperature of 120 °C, ethyl acetate as solvent, Florisil as dispersing sorbent, and extraction time of 15 min.

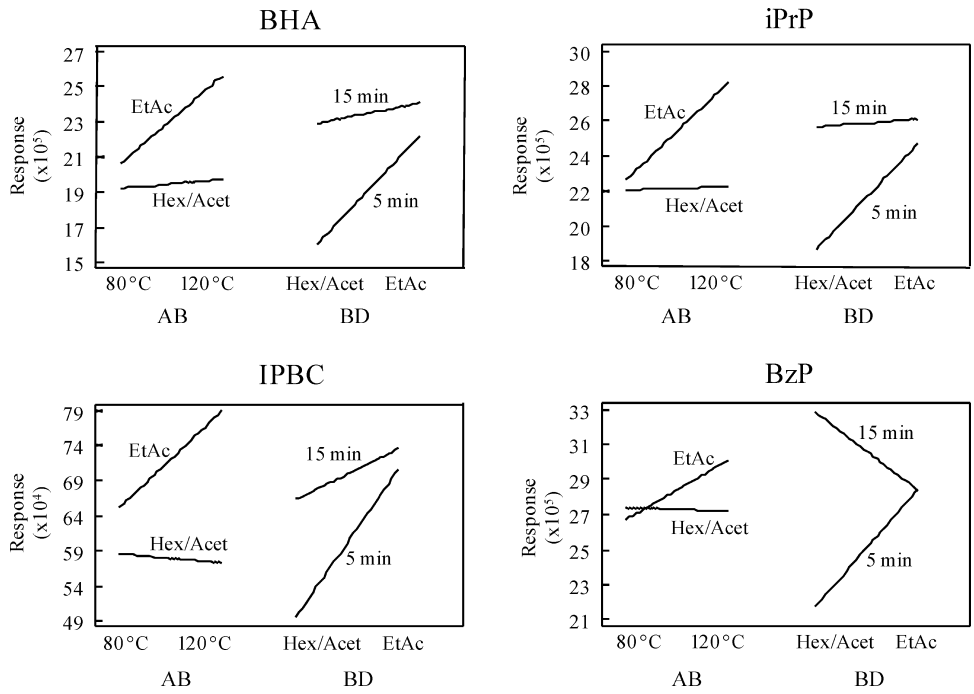

Figure 3. Interaction effects plots: AB (temperature−solvent) and BD (solvent−time).

Table 2. Quality Parameters of the Proposed Method[a]

			recovery[b] (RSD) (%) (n = 3)					
	instrumental parameters		derivatization after extraction		in-cell derivatization			
compd	R^2	IDL (ng mL^{-1})	20 μg g^{-1}	100 μg g^{-1}	20 μg g^{-1}	100 μg g^{-1}	LOD (%, w/w)	LOQ (%, w/w)
Bronidox	0.9971	5.6	73.7 (1.5)	98.3 (2.7)	97.9 (1.5)	85.7 (9.0)	0.000094	0.00031
Bronopol	1.0000	18	nc	98.2 (7.3)	83.5 (3.7)	88.4 (1.9)	0.00015	0.00051
MeP	0.9991	1.0	nc	94.8 (9.5)	nc	113 (4.1)	0.0000053	0.000018
BHA	0.9996	1.2	110 (0.6)	93.0 (2.3)	87.9 (8.0)	90.1 (4.1)	0.0000081	0.000027
BHT	0.9994	0.41	91.0 (5.8)	98.1 (0.5)	107 (4.0)	105 (0.3)	0.0000041	0.000013
EtP	1.0000	1.4	95.5 (2.2)	101 (0.4)	109 (8.7)	111 (0.9)	0.0000080	0.000027
iPrP	0.9992	1.7	100 (7.0)	101 (0.8)	95.4 (8.1)	104 (1.8)	0.0000098	0.000033
PrP	0.9965	1.0	99.3 (8.4)	107 (0.1)	nc	89.7 (6.8)	0.0000058	0.000019
IPBC	0.9946	2.3	94.5 (5.3)	90.9 (5.2)	90.6 (7.9)	100 (2.7)	0.000085	0.00028
iBuP	0.9971	0.86	104 (6.0)	96.5 (1.6)	102 (6.4)	97.0 (4.8)	0.0000065	0.000022
BuP	0.9988	0.64	101 (4.2)	97.2 (2.3)	108 (1.1)	106 (5.1)	0.0000060	0.000020
BzP	0.9998	2.0	95.5 (7.2)	99.1 (9.1)	88.9 (4.1)	104 (1.3)	0.000068	0.00023
TCS	0.9977	0.73	109 (4.5)	110 (5.3)	93.6 (8.4)	111 (2.9)	0.000040	0.00013

[a] nc = not calculated since the concentrations in the sample were much higher than the added concentration. [b] Real sample MC1 (Table 3) was employed in the recovery studies.

Experiments were also run with the objective of studying the possibility of performing in-cell derivatization of the target compounds in the PLE cell. In the simultaneous derivatization−extraction experiments, 100 μL of acetic anhydride containing 2.5% pyridine was added to the cosmetic sample and the PLE procedure was carried out in the selected conditions indicated above. The initial results were fully satisfactory, obtaining equivalent extracts, and as a consequence, both processes, PLE followed by derivatization, as well as the simultaneous pressurized liquid derivatization−extraction, were considered for method validation.

Method Performance. Application to Real Samples. Method quality parameters were evaluated (Table 2). The instrumental linearity was proved at a concentration range between 0.05 and 10 μg mL^{-1} (including six concentration levels) using derivatized standard solutions prepared in ethyl acetate (see the Materials and Methods). Each concentration level was injected in triplicate, and the response function was found to be linear with determination coefficients (R^2) higher than 0.9946.

Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of 3 (S/N = 3). Values ranged from 0.41 to 18 ng mL^{-1}, as can be seen in Table 2.

The other figures of merit were calculated using real cosmetic samples.

Recovery studies were carried out by applying the optimized PLE method to the extraction of a real cream sample spiked at two different concentrations, 20 and 100 μg g^{-1}. Previous analyses of this sample showed the presence of some of the target compounds (see sample MC1 in Table 3), and these initial concentrations were taken into account to calculate the recoveries.

Table 3. Analysis of Real Cosmetic Samples (%, w/w)[a]

	MC1	MC2	MC3	MC4	MC5	ML1	ML2	AW1	AW2	HC1	HC2	SC	AS	BL1	BL2	CO	SH
$MePd_4$[b]	103	92.0	100	115	91.5	106	94.0	109	88.9	96.8	107	83.7	109	97.7	105	95.1	94.5
Bronidox						0.000934											
Bronopol	0.007032																
MeP	0.054677	0.050380	0.064103	0.170330	0.001340	0.062912	0.302970		0.004412	0.042237	0.102072	0.168082		0.004880	0.252910	0.238926	
BHA					0.000310				0.001520		0.000145		0.000473				
BHT	0.000027	0.000012	0.000015				0.000010		0.002681	0.035424	0.000905	0.000109		0.034710	0.000070	0.000316	
EtP		0.015187	0.018247		0.000623					0.024454	0.000337	0.069245			0.104400		
iPrP																0.001954	
PrP	0.008843	0.006379	0.008243	0.022110	0.001120	0.007470	0.130250	0.000111		0.007858	0.011480			0.158300	0.240740	0.122902	
IPBC																	0.001450
iBuP		0.006031	0.006648							0.008113	0.000073					0.000211	
BuP		0.009487	0.011481	0.029380						0.007631	0.000232			0.000191	0.012930	0.000222	
BzP			<LOQ														
TCS																	
total parabens as acid	0.056421	0.074294	0.092583	0.192492	0.002077	0.062844	0.374923	0.000085	0.004006	0.075900	0.101970	0.210166		0.125930	0.510171	0.312951	

[a] MC = moisturizing cream, ML = moisturizing lotion, AW = antiwrinkle cream, HC = hand cream, SC = sunscreen cream, AS = after-sun cream, BL = baby moisturizing lotion, CO = hair conditioning lotion, SH = shampoo. [b] The recovery of the surrogate (%) is given in the first row. Blank spaces mean the values are below the LOD.

As can be seen in Table 2, the recoveries were between 74% and 110% in all cases. The precision was also evaluated, and the relative standard deviation (RSD) values were lower than 10% with an average value of 4.2%.

As was commented, the possibility of performing simultaneous derivatization−extraction by adding the acetylation reagents in the PLE cell was also evaluated. Recoveries are also given in Table 2. As can be seen, the recoveries were satisfactory, with values ranging from 84% to 111%. The precision of the method expressed as the RSD was between 1% and 9%. Performing the combined derivatization−extraction process, the method quality parameters are equivalent and the method is even more simple and time saving.

The limits of detection (LODs) and quantification (LOQs) of the overall method were calculated as the compound concentration giving a signal-to-noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively. These values are shown in Table 2, expressed as a percentage (w/w) to be equivalent with the units used in the European Cosmetics Regulation.[9] The obtained limits are much lower than the established restrictions (see Table S-1, Supporting Information), and it is important to emphasize that, if necessary, these limits could be easily reduced (by at least 1 order of magnitude) by concentrating the PLE extract (~15 mL).

Finally, the method was applied to the analysis of real cosmetic samples including moisturizing creams (MCs) and lotions (MLs), antiwrinkle (AW) creams, hand creams (HCs), sunscreen creams (SCs), after-sun (AS) creams, baby lotions (BLs), and hair conditioning (CO) and shampoo (SH) products. The results are shown in Table 3. The extracted ion chromatogram of sample HC1 is shown in Figure S-4 (Supporting Information). For all the samples, the recoveries of $MePd_4$ (surrogate standard) were satisfactory, with values ranging from 83.7 to 115 (see the first row, Table 3). As commented in the first section of this paper, the presence of these ingredients must be included in the cosmetic label, and these levels cannot exceed the regulated limit in each case. Regarding parabens, the compounds mainly found were MeP and PrP; both compounds are usually associated with an increase in the preservative activity. EtP, iBuP, and BuP were also found in the samples but much less frequently. The maximum allowed concentration of parabens in ready for use preparations is 0.4% for a single ester and 0.8% for mixtures of esters, expressed as acid (see the European Cosmetics Regulation limits in Table S-1). For this reason, the total content of parabens in the samples was determined and expressed as a percentage (w/w) of acid, being included in the last row of Table 3. All samples presented paraben concentrations below the legal limits, although one of the samples, a baby moisturizing lotion (BL2), was close to the total paraben maximum concentration limit. Most of the samples were correctly labeled with the exception of EtP, iBuP, and BuP in HC2, PrP in AW1 and MC5, and iPrP in CO, which were not included in the label. The antioxidant BHT was found in most of the samples, whereas BHA was found in four samples, in two of them associated with BHT, which increases the antioxidant power due to the synergism. Although there is some concern about the safety of both compounds, there are no restrictions about their use in cosmetic formulations. The presence of BHT and BHA was not indicated in the label with the exception of HC2 and BL1. IPBC was found in one rinse-off product (SH), and it was included in

the product label. Finally, Bronopol was detected in one leave-on cosmetic (MC1), and in this case, it was also listed as an ingredient.

CONCLUSIONS

A method based on acetylation PLE followed by GC/MS for the simultaneous determination of different classes of preservatives, including two bromine-containing preservatives, seven parabens, IPBC, TCS, and the antioxidant preservatives BHA and BHT, in multimatrix cosmetic samples has been developed. To our knowledge, both acetylation and PLE are applied for the first time to the analysis of cosmetics. We have demonstrated the possibility of performing simultaneous in situ derivatization by adding the acetylation reagents directly on the cosmetic sample into the PLE cell, making possible the GC/MS analysis of the extract without any further step. The obtained LODs are far below the established restrictions in the European Cosmetics Regulation, making this multicomponent analytical method suitable for routine control. The reliability of the method was demonstrated through a broad range of cosmetic products showing compliance with the actual European Cosmetics Regulation.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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