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Determination of suspected fragrance allergens in cosmetics by matrix solid-phase dispersion gas chromatography–mass spectrometry analysis

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ABSTRACT

An effective low cost sample preparation methodology for the determination of regulated fragrance allergens in leave-on and rinse-off cosmetics has been developed applying, for the first time, matrix solid-phase dispersion (MSPD) to this kind of analytes and samples. The selection of the most suitable extraction conditions was made using statistical tools such as ANOVA, as well as a factorial multifactor experimental design. These studies were carried out using real cosmetic samples. In the final conditions, 0.5 of sample, previously mixed with 1 g of anhydrous Na_2SO_4, were blended with 2 g of dispersive sorbent (Florisil), and the MSPD column was eluted with 5 mL of hexane/acetone (1:1). The extract was then analyzed by GC–MS without any further clean-up or concentration step. Accuracy, precision, linearity and detection limits (LODs) were evaluated to assess the performance of the proposed method. Quantitative recoveries (>75%) were obtained and RSD values were lower than 10% in all cases. The quantification limits were well below those set by the international cosmetic regulations, making this multi-component analytical method suitable for routine control. In addition, the MSPD method can be implemented in any laboratory at low cost since it does not require special equipment. Finally, a wide variety of cosmetic products were analyzed. All the samples contained several of the target cosmetic ingredients, with and average number of seven. The total fragrance allergen content was in general quite high, even in baby care products, with values close to or up to 1%, for several samples, although the actual European Cosmetic Regulation was fulfilled.

1. Introduction

Some of the fragrance chemicals, widely used in every day products, have been shown to cause various side effects, like skin sensitivity, rashes, dermatitis, coughing, asthma attacks, migraine, etc. [1–4]. Legislations in the European Union [5], the United States (US) [6] and Japan [7], establish that all the components of cosmetics should be included on the label. Two different restrictions are applied to several suspected fragrance allergens in the Annex III of the EU Cosmetics Directive [5], i.e. substances that can be included up to a maximum allowed concentration, and substances for which their presence must be indicated in the list of ingredients when their concentrations exceed the 0.001% (w/w) in leave-on products and 0.01% (w/w) in rinse-off products (see in Table 1 the compounds considered in this study and their limitations). The confirmed and suspected negative effects on the health of such substances may drive in the future to lower these limits and even to establish maximum allowed concentration for many of these substances. In fact, it has been already observed the inclusion of the term "fragrance free" in several cosmetic products as a positive characteristic. These requirements imply reliable procedures to detect and quantify low levels of these ingredients in highly complex mixtures. These procedures must be versatile considering the wide variety of cosmetic products and the range of fragrance allergen concentrations [8]. Sample preparation is an essential step since the direct analysis of cosmetic samples, such as creams, lotions, etc. is quite problematic due to the difficulty to obtaining homogeneous solutions, the coelution of the matrix components, and the contamination of the chromatographic system [9–12]. Therefore, the development of analytical methods for the determination of fragrance allergens in leave-on as well as rinse-off cosmetics is as challenging as necessary; moreover, considering that the literature is somewhat scarce in this subject. Given the highly complex mixtures of fragrances and raw materials used in cosmetics, and to prevent false positives and false negatives, the GC–MS determination in full scan mode is recommended to accomplish the analysis of fragrance allergens in cosmetics [10–12]. Recently, the authors have developed a pressurized solvent extraction (PSE) procedure followed by GC–MS analysis for the determination of regulated fragrance allergens in

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Table 1
Target fragrance allergens: CAS numbers, purity, chromatographic retention times, and qualification and quantification ions.

Common name	Chemical name	CAS number	Purity	Retention time (min)	Qualifiers and quantifiers
Limonene[a]	(4R)-1-Methyl-4-(1-methylethenyl)cyclohexene	5989-27-5	97%[d]	4.55	68,93,121,136
Benzyl alcohol[b]	Benzene methanol	100-51-6	99%[e]	4.66	77,79,107,108
Linalool[a]	3,7-Dimethyl-1,6-octadien-3-ol	78-70-6	97%[d]	5.67	71,93,121,136
Methyl-2-octynoate[a]	Methyl heptin carbonate	111-12-6	≥99%[f]	6.90	79,95,123,139
Citronellol[a]	(±)-3,7-Dimethyloct-6-en-1-ol	106-22-9/26489-01-0	95%[d]	7.16	69,81,95,123
Citral[a]	3,7-Dimethyl-2,6-octadienal	5392-40-5	95%[d]	7.32	69,84,94,109
				7.59	
Geraniol[a]	3,7-Dimethyl-(2E)-2,6-octadien-1-ol	106-24-1	≥96%[g]	7.43	69,93,111,123
Cinnamal[a]	3-Phenyl-2-propenal	104-55-2	≥93%[f]	7.63	77,103,131
Anise alcohol[a]	4-Methoxybenzyl alcohol	105-13-5	98%[d]	7.70	94,109,121,138
Hydroxycitronellal[a]	7-Hydroxy-3,7-dimethyloctanal	107-75-5	≥95%[f]	7.73	43,59,71
Cinnamyl alcohol[a]	3-Phenyl-2-propen-1-ol	104-54-1	98%[f]	7.91	92,105,115,134
Eugenol[a]	2-Methoxy-4-(2-propenyl)-phenol	97-53-0	99%[d]	8.31	131,149,164
Methyleugenol[c]	1,2-Dimethoxy-4-(2-propenyl)-benzene	93-15-2	99%[d]	8.61	147,163,178
Isoeugenol[a]	2-Methoxy-4-(1-propenyl)-phenol	97-54-1	98%[d]	8.67	131,149,164
				8.98	
Coumarin[a]	2H-1-Benzopyran-2-one	91-64-5	99%[d]	8.92	89,118,146
α-Isomethyl ionone[a]	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5	≥85%[g]	9.16	107,135,150
Lilial®[a]	2-(4-tert-Butylbenzyl) propionaldehyde	80-54-6	≥95%[g]	9.45	131,147,189
Amyl cinnamal[a]	2-Benzylideneheptanal	122-40-7	97%[e]	10.30	115,129,202
Lyral®[a]	Hydroxyhexyl-3-cyclohexene carboxaldehyde	31906-04-4	≥97%[g]	10.42	93,105,136,192
Amylcinnamyl alcohol[a]	2-Pentyl-3-phenylprop-2-en-1-ol	101-85-9	≥85%[g]	10.58	91,115,133,204
Farnesol[a]	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	4602-84-0	95%[d]	10.63	69,81,93,133
				10.84	
Hexyl cinnamal[a]	2-Benzylideneoctanal	101-86-0	≥95%[f]	11.15	115,129,145,216
Benzyl benzoate[a]	Phenylmethyl benzoate	120-51-4	98%[e]	11.38	77,91,105,212
Benzyl salicylate[a]	Benzyl-2-hydroxybenzoate	118-58-1	≥99%[g]	12.63	65,91,228
Benzyl cinnamate[a]	3-Phenyl-2-propenoic acid phenylmethyl ester	103-41-3	99%[d]	16.20	91,103,131,192

[a] According to REGULATION (EC) No 1223/2009, the presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products.
[b] Maximum allowed concentration 1% (for use other than as a preservative).
[c] Maximum allowed concentration in fragrance cream: 0.002%, rinse-off products: 0.001%; and other leave-on products: 0.0002%.
[d] Purchased from: Sigma–Aldrich Chemie GmbH (Germany).
[e] Purchased from: Chem Service (West Chester, USA).
[f] Purchased from: SAFC Supply Solutions (St. Louis, USA).
[g] Purchased from: Fluka Chemie GmbH (Steimheim, Germany).

leave-on cosmetics [13]. The PSE method has proved to be an efficient and rapid technique for the extraction of this kind of targets from cosmetic samples. Method performance was fully satisfactory in terms of LODs, recoveries, repeatability, and reproducibility. Nevertheless, one of the main drawbacks of this methodology is the high cost of the instrumentation compared to the low cost of other techniques such as matrix solid-phase dispersion (MSPD), which does not require special equipment. MSPD was introduced by Barker et al. [14]. MSPD involves blending a viscous, solid or semisolid sample with a solid support. The shearing forces of blending with a mortar and pestle disrupt the gross architecture of the sample, breaking the material in smaller pieces. At the same time, sample components dissolve and disperse into the bound organic phase on the surface of the particle, leading to complete disruption of the sample and its dispersion over the surface. The possibility of performing extraction and clean-up at the same time is one of the main advantages of this technique, which reduces sample contamination during the procedure and decreases the amount of solvent required [15,16]. MSPD developments and applications are compiled in several reviews [15–19]. This technique has been applied for the isolation of a wide variety of analyte classes, such as drugs, pesticides, polychlorinated biphenyls, antibiotics and antibacterial, surfactants and naturally occurring compounds, in several matrices (food, biota, vegetables and environmental samples) [20–22], but, up to know, it has not been applied to personal care products and cosmetics.

The aim of this work is to develop a method based on MSPD followed by gas chromatography–mass spectrometry (GC–MS) to simultaneously identify and quantify 25 fragrances in multi-matrix cosmetic samples, including both products designed to remain on the skin and rinse-off products. To our knowledge, MSPD is applied for the first time to the analysis of cosmetics, and it is also the first time that it is applied to the analysis of suspected fragrance allergens.

2. Experimental part

2.1. Reagents and materials

The 25 fragrance allergens considered in this study are listed in Table 1, where their common and chemical name, CAS number, purity and supplier are included.

Internal standard PCB-30 (2,4,6-trichlorobiphenyl) was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Acetone, ethyl acetate, and *n*-hexane were provided by Merck (Darmstadt, Germany). Florisil (60–100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA). Neutral alumina, C_{18}, and sand (50–70 mesh) were achieved from Sigma–Aldrich (St. Louis, MO, USA). Silica gel 60 (230–240 mesh) was obtained from Merck KGaA (Darmstadt, Germany). Before being used, Florisil, alumina and silica were activated at 130 °C for 12 h and then allowed to cool down in a desiccator. Sodium sulphate 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/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.

Table 2
F-ratios and *p*-values obtained in the analysis of variance study.

	Limonene	Benzyl alcohol	Linalool	Geraniol	Coumarin	α-Isomethyl ionone	Lilial®	Hexyl cinnamal	Benzyl benzoate	Benzyl salicylate
F-ratio	86.75	6.51	10.33	6.06	6.89	5.95	3.63	2.70	3.69	2.3
p-value	**0.0001**	**0.0322**	**0.0124**	**0.0371**	**0.0288**	**0.0385**	0.0950	0.1522	0.0925	0.1933

p-values lower than 0.05 indicate statistical significance.

2.2. Cosmetic samples

Different cosmetics from national and international brands were purchased from local sources. They included leave-on and rinse-off products such as moisturizing creams and lotions, anti-cellulite creams, hand creams, shampoos and gels, hair conditioners, and hand soaps. Samples were kept in their original containers at room temperature until their analysis.

The sample was then mixed with 1 g of a drying agent (anhydrous Na_2SO_4) and 2 g of dispersive sorbent.

2.3. MSPD procedure

0.5 grams of cosmetic sample were exactly weighted 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. The sample was gently blended with 1 g of a drying agent (anhydrous Na_2SO_4) and 2 g of the dispersive sorbent into a glass mortar using a glass pestle, until a homogeneous mixture was obtained (ca. 5 min). Then, the mixture was transferred into a column with a polypropylene frit at the bottom containing 0.5 g of Florisil (to obtain a further degree of fractionation and sample clean-up). A second frit was placed on top of the sample before compression with a syringe plunger. Elution was made by gravity flow with ethyl acetate or hexane/acetone (1:1, v/v), depending on the experiment. 5 mL of eluents were collected into a graduated conical tube and 50 μL of PCB 30 solution (200 $\mu g\,mL^{-1}$) were finally added. The MSPD extracts, diluted when necessary (dilution factors of 1:10 to 1:1000), were directly analyzed by GC–MS.

2.4. GC–MS analysis

Analyses were performed on an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230 °C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a HP5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 $mL\,min^{-1}$. The GC oven temperature was programmed from 80 °C (held 2 min) to 100 °C at 8 $°C\,min^{-1}$; to 150 °C at 20 $°C\,min^{-1}$; to 200 °C (held 5 min) at 25 $°C\,min^{-1}$; to 220 °C at 8 $°C\,min^{-1}$; and a final ramp to 290 °C (held 6 min) at 30 $°C\,min^{-1}$ (total analysis time = 25 min). Pulsed splitless mode was used for injection (30 psi, held 1.2 min). After 1 min the split was opened at a flow of 75 $mL\,min^{-1}$ and the injector temperature was kept at 220 °C. The injection volume was 2 μL.

The mass spectra detector (MSD) was operated in the scan mode and the mass range was varied from 40 to 300 *m*/*z*, starting at 4 min and ending at 25 min. The electron multiplier was set at a nominal value of 1300 V. The analytes were positively identified by comparison of their mass spectra and retention times to those of the standards. Table 1 summarizes the retention times as well as the qualification and quantification ions of the target analytes.

3. Results and discussion

The chromatographic method for the separation of the target fragrance allergens was optimized elsewhere [23,24] and it is described in Section 2.

3.1. MSPD optimization

One of the most important steps in the development of an efficient MSPD method is the selection of the dispersive phase. First experiments were carried out to study the influence of this parameter, using a real non-spiked cosmetic sample. Five sorbents were considered: alumina, Florisil, silica, sand and C_{18}. The sample consisted of a leave-on cosmetic (a body milk), containing 10 of the target compounds. We chose a sample with a high number of fragrance allergens since we wanted to work with the sample as it is (without spiking), to really evaluate the capability of MSPD to break the analyte–matrix interactions. MSPD was conducted applying the most usual sample/solid support material ratio (1 to 4), blending 2 g of solid support with 0.5 g of sample [17]. Since drying of the sample is essential for an efficient extraction, 1 g of anhydrous sodium sulphate was added in all experiments. The MSPD column was eluted with two fractions of 5 mL of hexane/acetone that were analyzed separately by GC–MS. All experiments were performed twice. The 10 fragrances included in the cosmetic label were extracted, and they were detected in the first fraction, independently of the sorbent used. Regarding the second fraction, only three of the compounds (lilial®, hexyl cinnamal and benzyl salicylate) were detected; in those cases, the chromatographic response was lower than 0.2% compared with the first fraction, which may indicate that a solvent volume of 5 mL is sufficient to elute the MSPD column.

The results obtained for the first fraction were analyzed by ANOVA. For most compounds, the sorbent used was statistically significant (Table 2). Analyzing the multiple range tests, we could realize that, in many cases, the results with the different sorbents were equivalent excluding sand, which gave lower general results (Fig. 1).

To extent the study to all the 25 compounds considered (see Table 1), we perform an experimental design using, in this case, a moisturizing lotion originally containing a low number (only 2) and low concentration of fragrance allergens. The sample was spiked with the target compounds (100 $\mu g\,g^{-1}$). Although considering the previous ANOVA the sand could be discarded, it would not significantly reduce the number of experiments, so we maintained all 5 sorbents (factor A). The second factor considered was the elution solvent (factor B), that it was studied at two levels: hexane/acetone (1:1, v/v), and ethyl acetate. Both solvents have intermediate polarity, which should favour the simultaneous extraction of all the analytes. In both cases, the solvent volume was 5 mL. The study consisted of a multifactor categorical 5 × 2 design, involving 10 randomized experiments.

The results obtained are represented in the two factor plots in Fig. 2. For simplicity, only 8 of the 25 compound graphics are included. As can be seen, the sand was once again not a suitable sorbent, displaying the lowest extraction efficiency. On the other hand, we can observe that hexane/acetone is the most suitable solvent in all cases excluding sand, which gave higher response in combina-

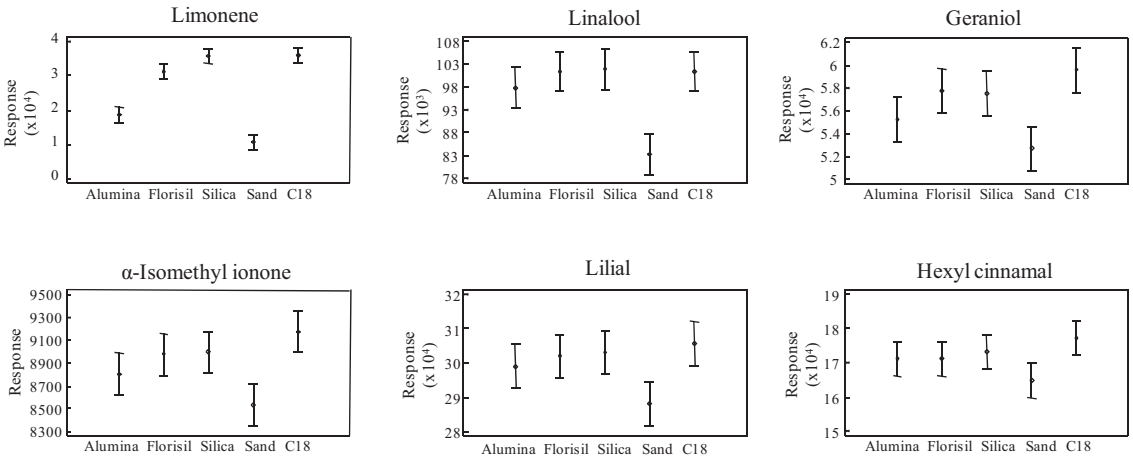

Fig. 1. Mean plots for several representative allergens obtained in the one-way ANOVA study.

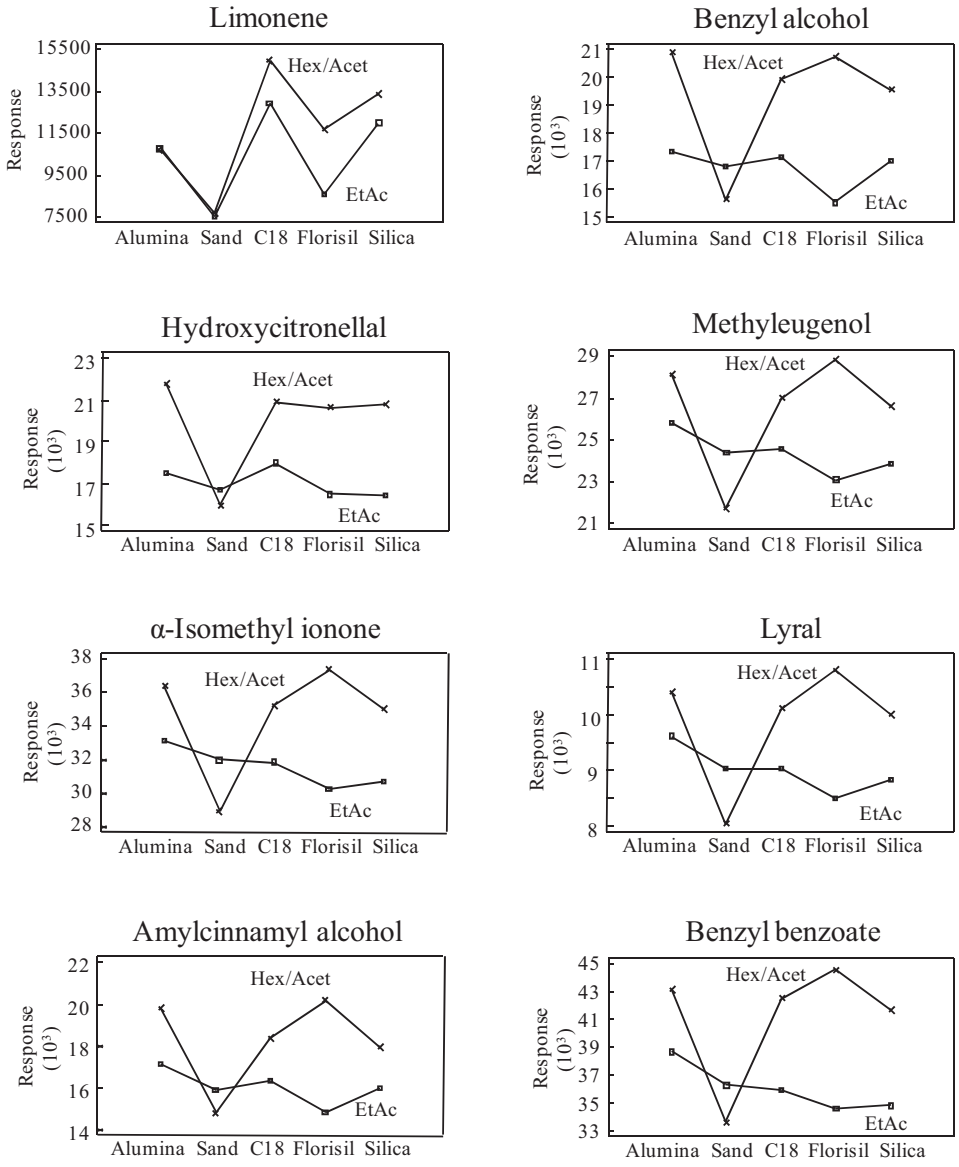

Fig. 2. Interaction plots for some representative fragrance allergens (Hex/Acet: hexane/acetone; EtAc: ethyl acetate).

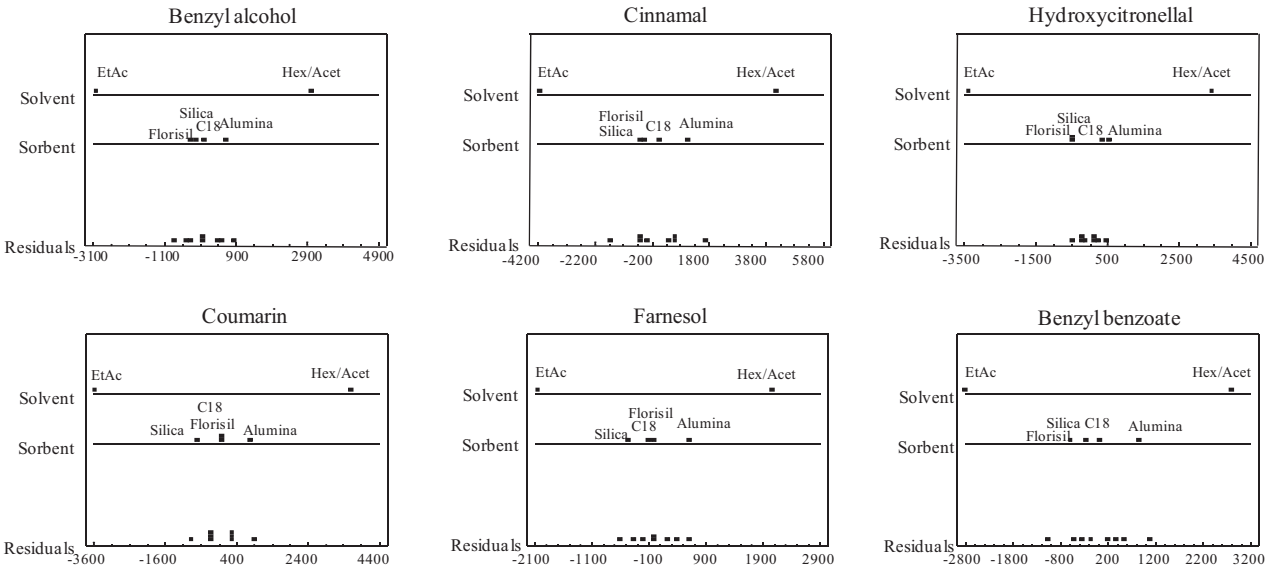

Fig. 3. ANOVA plots for several fragrance allergens.

tion with ethyl acetate. ANOVA analysis of the design results was performed excluding the sand experiments. The ANOVA results are graphically displayed in Fig. 3, which includes some representative examples, since the general behaviour was equivalent for all analytes. This kind of plots shows the scaled effects of each factor so that the natural variance of the points in the diagram can be compared to that of the residuals, displayed at the bottom of the plot. By comparing the variability amongst the factors to that of the residuals, it is easy to identify those showing differences of a greater magnitude than could be solely accounted by the experimental error. It can be clearly observed that the solvent is a significant factor, and higher efficiency is achieved using hexane/acetone (on the right of the graphics). On the other hand, the sorbent is not significant for all the compounds.

After optimization, the proposed MSPD conditions include the dispersion of the sample with Florisil (2 g), although other three sorbents (silica, C_{18}, and alumina) are also suitable, and the elution with 5 mL hexane/acetone (1:1, v/v).

3.2. Method performance

Method quality parameters were evaluated and they are included in Table 3. The instrumental linearity was proved at a concentration range between 0.05 and 10 $\mu g\,mL^{-1}$ (including seven concentration levels) using standard solutions prepared in hexane/acetone (see Section 2). Each concentration level was injected in triplicate or duplicate and the response function was found to be linear with correlation coefficients (R) higher than 0.9995.

Instrumental detection limits (IDL) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3). Values ranged from 1 to 12 $ng\,mL^{-1}$ in most cases (Table 3). The other figures of merit were calculated using real cosmetic samples.

Recovery studies were carried out by applying the optimized method to the extraction of three real samples, including both leave-on and rinse-off samples spiked at 20 $\mu g\,g^{-1}$ and 100 $\mu g\,g^{-1}$. Previous analyses of the samples showed the presence of some of the target analytes and these initial concentrations were taken into account to calculate the recoveries. As can be seen in Table 3, recoveries were between 75 and 118% in all cases. Precision was also evaluated and RSD values were in most cases lower than 10% with an average value of 1.7% and 5.1% for intra- and inter-day precision, respectively.

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 three (S/N = 3) and ten (S/N = 10), respectively. These values are shown in Table 3, expressed as percentage (%, w/w) in order to be consequent with the units used in the European Cosmetics Regulation [5]. The obtained limits are much lower than the established restrictions, and it is important to emphasize that, if necessary, these limits could be reduced by concentrating the extract (5 mL).

3.3. Application to real samples

The method was applied to the analysis of real leave-on and rinse-off cosmetic samples. Leave-on cosmetics included: moisturizing creams (MC) and lotions (ML), an anti-cellulite cream (AC), moisturizing lotions for babies (MLB), and a hand cream (HC). As regards rinse-off products, three different shampoos (Sh), a hair conditioner (HC), gels (G) and hand soap (HS), and baby gels (BG) were analyzed.

Found concentrations in rinse-off products are included in Table 4 and they ranged from 0.000057 (eugenol in sample G) to 0.47% (w/w) (anise alcohol in sample Sh2), with an average value of 6 fragrance allergens per sample, and an average concentration of 0.025% (w/w). All the samples contained at least three different targets. 9 out of 25 fragrance allergens were not detected in any sample, whereas the most common ones were linalool (identified in 7 out of 8 samples) and limonene and benzyl alcohol (both found in 6 out of 8 samples). The most of the samples were properly labelled (the component was indicated on the label when its concentration exceeds the 0.01% (w/w)) with the exception of Sh1 and Sh2. For these samples, lilial® was present in the shampoo at higher concentrations than 0.01% (w/w) (Table 4) and their presence was not included on the label. It is noticeable the quite high total fragrance allergen content in a baby gel (BG1), 0.21% (w/w), the second highest value of the analyzed samples.

In the case of leave-on cosmetics (Table 5), found concentrations ranged from 0.000076 (benzyl alcohol in sample MC1) to 1.01% (w/w) (lilial® in sample ML), with an average value of 5 fragrance allergens per sample and an average concentration of 0.064% (w/w). The most frequently found was linalool, identified in all the analyzed samples, followed by limonene, present in 7 out of 9 samples. All samples were properly labelled, and the component was

Table 3
Quality parameters of the method.

Compound	Correlation coefficient (R)	IDL ($ng\,mL^{-1}$)	Intra-day precision (%)[a]	Inter-day precision (%)[b]	Recovery (RSD) (%)[a]				LOD (%, w/w)	LOQ (%, w/w)
					"Leave-on"		"Rinse-off"			
					$20\,\mu g\,g^{-1}$	$100\,\mu g\,g^{-1}$	$20\,\mu g\,g^{-1}$	$100\,\mu g\,g^{-1}$		
Limonene	0.9997	5.5	0.19	5.9	85.4 (5.3)	98.3 (9.1)	79.3 (8.0)	75.3 (2.5)	0.000021	0.000070
Benzyl alcohol	1.0000	6.0	1.5	3.0	95.0 (3.3)	101 (3.7)	98.9 (1.0)	111 (6.1)	0.0000060	0.000020
Linalool	1.0000	5.4	2.0	5.5	98.2 (6.8)	92.8 (6.6)	105 (5.5)	105 (3.9)	0.0000081	0.000027
Methyl-2-octynoate	0.9999	11	0.8	4.1	88.0 (11)	107 (2.7)	100 (0.7)	101 (4.2)	0.000011	0.000037
Citronellol	1.0000	12	0.3	10	89.1 (2.3)	101 (6.2)	108 (1.3)	106 (3.4)	0.000042	0.00014
Citral	0.9999	12	3.9	4.6	98.5 (6.8)	99.3 (3.6)	83.5 (11)	87.3 (7.0)	0.000095	0.00032
Geraniol	0.9999	14	3.0	6.5	91.1 (8.2)	106 (6.7)	110 (2.8)	108 (4.7)	0.000071	0.00024
Cinnamal	0.9999	2.0	1.1	4.0	93.3 (12)	101 (0.9)	92.8 (9.8)	75.9 (9.9)	0.0000020	0.0000067
Anise alcohol	1.0000	11	3.1	5.8	83.5 (10)	95.8 (7.6)	104 (1.5)	103 (5.0)	0.000011	0.000037
Hydroxycitronellal	0.9999	3.0	4.1	5.3	93.5 (8.0)	103 (0.9)	92.1 (9.0)	77.2 (2.2)	0.000015	0.000050
Cinnamyl alcohol	0.9999	11	3.3	8.4	100 (13)	106 (3.9)	116 (3.1)	105 (9.2)	0.000011	0.000037
Eugenol	1.0000	2.0	0.84	2.0	112 (2.1)	99.7 (7.0)	93.9 (5.1)	104 (4.3)	0.0000020	0.0000067
Methyleugenol	0.9998	1.5	1.4	3.7	109 (3.8)	91.0 (6.2)	103 (8.5)	106 (4.1)	0.0000015	0.0000050
Isoeugenol	0.9995	12	1.9	8.9	108 (4.4)	110 (4.7)	115 (1.3)	102 (4.7)	0.000012	0.000040
Coumarin	0.9998	12	2.5	3.5	102 (3.0)	99.7 (2.2)	96.8 (9.3)	114 (8.1)	0.000012	0.000040
α-Isomethyl ionone	0.9998	3.8	0.80	4.7	112 (2.2)	94.2 (4.9)	109 (7.3)	102 (4.2)	0.0000038	0.000013
Lilial®	1.0000	12	3.3	4.0	103 (11)	115 (3.5)	n.c.	n.c.	0.000012	0.000040
Amyl cinnamal	0.9998	13	0.21	5.3	105 (3.7)	96.8 (1.2)	113 (7.2)	100 (5.3)	0.000013	0.000043
Lyral®	0.9999	11	0.52	3.5	94.8 (1.4)	112 (2.3)	97.9 (2.4)	77.6 (5.3)	0.000011	0.000037
Amylcinnamyl alcohol	0.9999	12	1.2	4.7	93.5 (6.3)	96.5 (4.0)	113 (4.0)	109 (1.9)	0.000012	0.000040
Farnesol	0.9999	60	1.5	8.3	94.5 (5.8)	90.4 (1.7)	99.9 (5.1)	99.0 (3.2)	0.00006	0.00020
Hexyl cinnamal	0.9997	7.9	0.94	4.8	106 (1.8)	101 (3.0)	106 (6.0)	100 (6.2)	0.0000079	0.000026
Benzyl benzoate	0.9996	4.2	1.5	4.4	95.2 (4.9)	101 (1.9)	102 (2.6)	104 (4.3)	0.0000042	0.000014
Benzyl salicylate	0.9999	11	3.1	3.4	102 (7.5)	118 (1.7)	109 (4.2)	88.7 (7.2)	0.000011	0.000037
Benzyl cinnamate	0.9999	11	1.6	3.6	112 (1.6)	98.1 (4.9)	115 (3.3)	108 (3.5)	0.000011	0.000037

n.c.: not calculated, lilial was present in the sample at high concentration.

[a] $n = 3$.

[b] $n = 5$.

Table 4
Analysis of real rinse-off cosmetic samples (Sh: shampoo; HC: hair conditioner; G: gel; HS: hands soap; BG: baby gel).

% (w/w)	Sh1	Sh2	Sh3	HC	G	HS	BG1	BG2
Limonene	0.000096	0.000545	0.051106		0.000629		0.034125	0.000229
Benzyl alcohol	0.001055	0.002264		0.008715	0.016589	0.001457		0.013072
Linalool	0.039104	0.012726	0.062486		0.011862	0.000212	0.056376	0.001119
Methyl-2-octynoate								
Citronellol	0.036997							
Citral				0.009415				
Geraniol			0.010466		0.002922	<LOQ		0.000380
Cinnamal								
Anise alcohol		0.466929						
Hydroxycitronellal								
Cinnamyl alcohol								
Eugenol					0.000057			
Methyl eugenol			0.000305					
Isoeugenol								
Coumarin	0.001558		0.003668			0.008033		
α-Isomethyl ionone	0.019767	0.004758						
Lilial®	0.017002	0.046826				0.082463	0.105540	0.002492
Amyl cinnamal								
Lyral®		0.009352						
Amylcinnamyl alcohol								
Farnesol								
Hexyl cinnamal		0.021230		0.000274	0.034138		0.010947	0.000513
Benzyl benzoate		0.000441						0.001751
Benzyl salicylate		0.007574	0.003752		0.000367			0.000185
Benzyl cinnamate								
Total fragrance allergen content	0.116	0.573	0.132	0.0184	0.0666	0.0922	0.207	0.0197

Blank cells mean values below LODs.

Table 5
Analysis of real leave-on cosmetic samples (MLB: moisturizing lotion for babies; MC: moisturizing cream; AC: anti-cellulite cream; ML: moisturizing lotion; HC: hands cream).

% (w/w)	MLB1	MLB2	MLB3	MC1	MC2	MC3	AC	ML	HC
Limonene		0.024311	0.101416	0.000932	0.004773		0.001352	0.011883	0.000422
Benzyl alcohol				0.000076		0.001015		0.000625	0.002337
Linalool	0.006570	0.031511	0.104257	0.013790	0.004191	0.000617	0.011808	0.070680	0.005137
Methyl-2-octynoate									
Citronellol									0.001166
Geraniol		0.007229		0.000258					0.000798
Citral		0.010814	0.007833						
Cinnamal									
Anise alcohol									
Hydroxycitronellal			0.000428						0.000737
Cinnamyl alcohol									
Eugenol									
Methyl eugenol									
Coumarin								0.020720	
Isoeugenol									
α-Isomethyl ionone				0.002953				0.002470	0.000703
Lilial®		0.481264	0.678693	0.051238				1.010490	0.006109
Amyl cinnamal									
Lyral®				0.006212					
Amylcinnamyl alcohol									
Farnesol									
Hexyl cinnamal			0.029158	0.007360				0.087747	0.003606
Benzyl benzoate	0.000259			0.000502				0.002845	
Benzyl salicylate					0.012318			0.228331	0.010465
Benzyl cinnamate									
Total fragrance allergen content	0.0068	0.530	0.820	0.0824	0.0165	0.00163	0.0118	1.42	0.0311

Blank cells mean values below LODs.

indicated on the label when its concentration exceeds the 0.001% (w/w). The highest total fragrance allergen contents were found in the moisturizing lotions, ML with the value of 1.42% (w/w) and two lotions for babies, MLB2 and MLB3 with contents of 0.530 and 0.820% (w/w), respectively.

4. Conclusions

A fast, efficient, and cheap method based on MSPD followed by GC–MS for the simultaneous determination of fragrance allergens (including the 24 regulated in the EU Cosmetics Directive) in multi-matrix cosmetic samples has been developed. Optimization was carried out using real cosmetic samples and several statistical tools. Recovery studies were performed on leave-on and rinse-off samples, demonstrating the reliability of the optimized procedure.

The method was applied to a broad range of cosmetics. Target ingredients were present in all the analyzed samples and, in most cases, a quite high number of fragrance allergens were detected, although compliance with the actual European Regulation in terms of labelling was observed in most cases. To our knowledge, this is

the first application of MSPD to the analysis of cosmetics, as well as to the analysis of fragrance allergens.

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