



Development of a multianalyte method based on micro-matrix-solid-phase dispersion for the analysis of fragrance allergens and preservatives in personal care products[☆]



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ARTICLE INFO

Article history:

Received 31 January 2014

Received in revised form 26 March 2014

Accepted 27 March 2014

Available online 4 April 2014

Keywords:

Fragrance allergens

Preservatives

Cosmetics

Matrix solid-phase dispersion

Personal care products

GC–MS/MS

ABSTRACT

An effective, simple and low cost sample preparation method based on matrix solid-phase dispersion (MSPD) followed by gas chromatography–mass spectrometry (GC–MS) or gas chromatography–triple quadrupole–mass spectrometry (GC–MS/MS) has been developed for the rapid simultaneous determination of 38 cosmetic ingredients, 25 fragrance allergens and 13 preservatives. All target substances are frequently used in cosmetics and personal care products and they are subjected to use restrictions or labeling requirements according to the EU Cosmetic Directive. The extraction procedure was optimized on real non-spiked rinse-off and leave-on cosmetic products by means of experimental designs. The final miniaturized process required the use of only 0.1 g of sample and 1 mL of organic solvent, obtaining a final extract ready for analysis. The micro-MSPD method was validated showing satisfactory performance by GC–MS and GC–MS/MS analysis. The use of GC coupled to triple quadrupole mass detection allowed to reach very low detection limits (low ng g^{-1}) improving, at the same time, method selectivity. In an attempt to improve the chromatographic analysis of preservatives, the inclusion of a derivatization step was also assessed. The proposed method was applied to a broad range of cosmetics and personal care products (shampoos, body milk, moisturizing milk, toothpaste, hand creams, gloss lipstick, sunblock, deodorants and liquid soaps among others), demonstrating the extended use of these substances. The concentration levels were ranging from the sub parts per million to the parts per mill. The number of target fragrance allergens per samples was quite high (up to 16). Several fragrances (linalool, farnesol, hexylcinnamal, and benzyl benzoate) have been detected at levels $>0.1\%$ ($1000 \mu\text{g g}^{-1}$). As regards preservatives, phenoxyethanol was the most frequently found additive reaching quite high concentration ($>1500 \mu\text{g g}^{-1}$) in five cosmetic products. BHT was detected in eight samples, in two of them (a baby care product and a lipstick) at high concentrations ($>1000 \mu\text{g g}^{-1}$). Methyl paraben was also found at high levels ($>1700 \mu\text{g g}^{-1}$) in three leave-on samples. Finally, triclosan was found at the maximum concentration limit (0.3%) laid down by the European regulation in two deodorant samples, and the total paraben concentration was close to the maximum concentration permitted (0.8%) in one leave-on sample (body milk).

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1. Introduction

The majority of cosmetics and personal care products contain fragrances and preservatives. Fragrances are the most frequent

causes of contact allergy to cosmetics. Allergic reactions and side effects include skin sensitivity, dermatitis, asthma attacks and migraine [1,2]. In order to reduce the risk of skin sensitization, the European legislation [3] requires monitoring 26 volatile compounds used in cosmetics the so-called potentially allergen substances (PAS) or fragrance allergens. Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products (e.g. shampoos), and 0.001% for leave-on products (e.g. lotions, deodorants). Of these 26 substances, 24 are chemically defined volatile compounds whereas the other two are natural moss extracts and do not correspond to defined

[☆] Presented at the XIII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA2013), 8–11 October 2013, Puerto de la Cruz, Tenerife, Canary Islands, Spain.

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chemicals [4]. Methyl Eugenol should also be considered for controlling because recent changes in EU regulations include the transfer of this compound to the Annex II (list of substances prohibited in cosmetic products) to the Annex III (list of substances which cosmetic products must not contain except subject to restrictions) [5].

Preservatives are used in cosmetic formulations to protect them against microbial growth, both to maintain product integrity and to care for consumers [6]. Parabens (alkyl esters of *p*-hydroxybenzoic acid) are the most widely used preservatives in cosmetic products, due to their broad antimicrobial spectrum, relatively low toxicity, and cost [7]. The estimated paraben exposure per person is 77.5 mg day⁻¹ (50 mg via cosmetics and personal care products) [8]. European legislation restricts the preservation of cosmetic products by parabens to a maximum authorized concentration of 0.4% for a single ester or 0.8% for ester mixtures (w/w, calculated as acid) [3]. Although these compounds are not mutagenic agents, recent studies have reported that certain parabens have been associated with genotoxicity, allergies and may also act as antiandrogens [9–11]. Iodopropynyl butylcarbamate (IPBC), 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan) and bromine-containing preservatives such as bronidox are also included in a wide variety of cosmetics and personal care products to prevent or retard bacterial growth. Phenoxyethanol is increasingly turning up in cosmetics as a preservative and as an alternative to parabens; this glycol ether is used as an anti-bacterial as well as perfume stabilizer. Butyl hydroxytoluene (BHT) is frequently used in cosmetics as antioxidant.

In order to guarantee product safety according to regulations and to assess the health risk from the potential exposures, the development of analytical methods for the determination of fragrances and preservatives in cosmetic formulations is mandatory. Moreover, personal care products (PCPs) are included as “emerging organic contaminants” and significant amounts of these products and their metabolites can be present in the environment [12]. The increasing interest on the determination of the levels of both families of these compounds is reflected by the number of recent studies on this matter.

Several analytical methods have been developed for the determination of fragrance allergens in environmental samples, indoor air, and scented toys. In this way, dispersive liquid–liquid microextraction [13], ultrasound-assisted emulsification–microextraction and solid-phase microextraction (SPME) [14,15] have been employed to determine fragrance suspected allergens in natural waters, swimming pool waters, baby bathwaters and waste waters. These volatile substances have been analyzed in indoor air using SPME and solid-phase extraction (SPE) [16,17]. To determine fragrance allergens in scented toys methods using dynamic headspace and SPME followed by GC–MS [18,19] have been reported. Regarding the analysis of fragrances allergens in cosmetics and personal care products, the scientific literature is scarce although some advanced extraction techniques such as pressurized liquid extraction (PLE) [20], and full evaporation dynamic headspace [21], have been used.

Analytical methodologies have also been reported for the determination of preservatives, mostly parabens, in water samples and biological matrices. The extraction of these compounds have mainly been carried out by SPE [22] but in last years, miniaturized techniques such as SPME [23] and hollow fibre liquid-phase microextraction [24] have been employed to determine parabens and triclosan in waters. Dispersive liquid–liquid microextraction has been proposed to determinate parabens in human serum samples [25]. For the determination of preservatives in cosmetics solvent extraction using ultrasonic energy [26] and methods based on PLE [27] have been recently published.

Matrix solid-phase dispersion (MSPD) is as a very suitable analytical tool for the extraction of preservatives and fragrances from

cosmetics, providing efficient and low cost extractions [28–30]. A miniaturized MSPD method has been recently proposed for the determination of plasticizers and synthetic musks in cosmetics [31].

Detection of allergen fragrances and preservatives has mainly been carried out by GC–MS. However, the complexity of the cosmetic matrices which contain many ingredients with similar chemical structures (common ions) can reduce analysis selectivity and reliability. The analysis of allergen fragrances is especially complex due to the difficulties to avoid co-elutions and the similarities in mass spectra [32]. Chaintreau et al. have proposed a chromatographic method based on the use of two different polarity capillary columns together with the use of three ions per fragrance to solve co-elution problems [33]. Tandem mass spectrometry (MS/MS) in combination with GC is a valuable approach that improves selectivity and analyte sensibility, minimising or even avoiding most of matrix interferences. Recently, Qing et al. [34] described the determination of 48 fragrance allergens in toys using GC coupled to ion trap MS/MS demonstrating that the application of MS/MS is an accurate and effective technique for the analysis of fragrance allergens in matrices composed of complex components. To the best of our knowledge, there are not references on the analysis of fragrance allergens in cosmetics or personal care products by GC–MS/MS, and only one reference that includes the determination of only four parabens [26].

The aim of the present study is the development of a MSPD combined with GC–MS and GC–MS/MS method for the simultaneous determination of 25 fragrance allergens and 13 preservatives in cosmetics and personal care products. Miniaturization of the extraction technique will be studied with the purpose of minimizing the consumption of sample, reagents and solvents, meeting “green chemistry” requirements. GC–MS and GC–MS/MS will be compared to determine whether the use of tandem mass spectrometry leads to an improvement in the determination of the target compounds in rinse-off and leave-on cosmetic samples attending both sensitivity and selectivity.

2. Experimental

2.1. Chemicals, materials and samples

The studied compounds, CAS numbers, suppliers, purity of the standards and restrictions according European legislation are summarized in Table 1. Some of the allergen fragrances are commercialized and were acquired as mix of isomers (see Table 1). Deuterated methyl-4-hydroxybenzoate-2,3,5,6-d₄ (MeP-d₄; 98 atom% D) and benzyl-d₇ alcohol (98 atom% D) used as surrogate standard, were obtained from C/D/N Isotopes (Quebec, Canada) and Aldrich (St. Louis, MO, USA), respectively.

Acetone, ethyl acetate and n-hexane were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Florisil (60–100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and anhydrous sodium sulphate (99%) from Panreac (Barcelona, Spain).

Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in acetone, hexane or ethyl acetate. All solutions were stored in amber glass vials at –20 °C. All solvents and reagents were of analytical grade.

All the glass, metallic and ceramic materials, the sorbents (Florisil and sodium sulphate), and the glass wool for laboratory use (Sigma-Aldrich) were baked at 230 °C for 12 h before use to eliminate possible contamination. All materials were allowed to cool down wrapped with aluminum foil. Florisil and sodium sulphate were allowed to cool down in a desiccator.

Table 1

Target compounds: chemical names, suppliers, purity, CAS and EU restrictions (EC No 1223/2009).

Key	Fragrance allergens	Chemical names	Purity (%)	CAS	Maximum concentration permitted [3]
1	Limonene ^a	(4R)-1-Methyl-4-(1-methylethenyl)cyclohexene	97 ^b	5989-27-5	n.r
2	Benzyl alcohol ^a	Benzene methanol	99 ^c	100-51-6	1% (as preservative)
3	Linalool ^a	3,7-Dimethyl-1,6-octadien-3-ol	97 ^b	78-70-6	n.r
4	Methyl-2-octynoate ^a	Methyl heptin carbonate	≥99 ^d	111-12-6	n.r
5	Citronellol ^a	(±)-3,7-Dimethyloct-6-en-1-ol	95 ^b	106-22-9	n.r
6	Citral ^{a,e}	3,7-Dimethyl-2,6-octadienal	95 ^b	5392-40-5	n.r
7	Geraniol ^a	3,7-Dimethyl-(2E)-2,6-octadien-1-ol	≥96 ^f	106-24-1	n.r
8	Cinnamal ^a	3-Phenyl-2-propenal	≥93 ^d	104-55-2	n.r
9	Hydroxycitronellal ^a	7-Hydroxy-3,7-dimethyloctanal	≥95 ^d	107-75-5	1%
10	Anise alcohol ^a	4-Methoxybenzyl alcohol	98 ^b	105-13-5	n.r
11	Cinnamyl alcohol ^a	3-Phenyl-2-propen-1-ol	98 ^b	104-54-1	n.r
12	Eugenol ^a	2-Methoxy-4-(2-propenyl)-phenol	99 ^b	97-53-0	n.r
13	Methyleugenol ^a	1,2-Dimethoxy-4-(2-propenyl)-benzene	99 ^b	93-15-2	0.01% (fine fragrance); 0.004% (eau de toilette); 0.002% (fragrance cream); 0.0002% (other leave-on products); 0.001% (rinse-off products)
14	Isoeugenol ^{a,e}	2-Methoxy-4-(1-propenyl)phenol	98 ^b	97-54-1	0.02%
15	Coumarin ^a	2H-1-benzopyran-2-one	99 ^b	91-64-5	n.r
16	α-Isomethyl ionone ^{a,e}	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	≥85 ^f	127-51-5	n.r
17	Lilial ^a	2-(4-tert-Butylbenzyl)propionaldehyde	≥85 ^f	80-54-6	n.r
18	Amyl cinnamal ^{a,e}	2-Benzylideneheptanal	97 ^b	122-40-7	n.r
19	Lylal ^{a,e}	Hydroxyhexyl-3-cyclohexene carboxaldehyde	≥97 ^f	31906-04-4	n.r
20	Amylcinnamyl alcohol ^a	2-Pentyl-3-phenylprop-2-en-1-ol	≥85 ^f	101-85-9	n.r
21	Farnesol ^{a,e}	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	95 ^b	4602-84-0	n.r
22	Hexylcinnamal ^{a,e}	2-Benzylideneoctanal	≥95 ^d	101-86-0	n.r
23	Benzyl benzoate ^a	Phenylmethyl benzoate	98 ^c	120-51-4	n.r
24	Benzyl salicylate ^a	Benzyl-2-hydroxybenzoate	≥99 ^f	118-58-1	n.r
25	Benzyl cinnamate ^a	3-Phenyl-2-propenoic acid phenylmethyl ester	99 ^b	103-41-3	n.r
Key	Preservatives	Chemical names	Purity (%)	CAS	Maximum concentration permitted [3]
26	Bronidox	5-Bromo-5-nitro-1,3-dioxane	≥99 ^f	30007-47-7	0.1%
27	Phenoxyethanol (phEtOH)	2-Phenoxyethanol	99 ^f	122-99-6	1%
28	Methyl paraben (MeP)	Methyl 4-hydroxybenzoate	99 ^f	99-76-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
29	BHA	Butylated hydroxyanisole	98.5 ^f	25013-16-5	n.r
30	BHT	Butylated hydroxytoluene	99 ^f	128-37-0	n.r
31	Ethyl paraben (EtP)	Ethyl 4-hydroxybenzoate	99 ^f	120-47-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
32	Isopropyl paraben (iPrP)	Isopropyl 4-hydroxybenzoate	≥99 ^d	4191-73-5	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
33	Propyl paraben (PrP)	Propyl 4-hydroxybenzoate	99 ^f	94-13-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
34	IPBC	Carbamic acid, butyl-3-iodo-2-propynyl ester	97 ^f	55406-53-6	0.02% (rinse-off products); 0.01% (leave-on products); 0.0075% (deodorants)
35	Isobutyl paraben (iBuP)	Isobutyl 4-hydroxybenzoate	≥97 ^d	4247-02-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
36	Butyl paraben (BuP)	Butyl 4-hydroxybenzoate	99 ^f	94-26-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
37	Triclosan	2,4,4'-Trichloro-2'-hydroxydiphenyl ether	≥97 ^f	3380-34-5	0.3%
38	Benzyl paraben (BzP)	Benzyl hydroxybenzoate	99 ^b	94-18-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)

^a The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% (leave-on products) and 0.01% (rinse-off products).^b Sigma Aldrich Chemie GmbH (Steinheim, Germany).^c Chem Service (West Chester, USA).^d SAFC Supply Solutions (St. Louis, USA).^e Mixture of isomers.^f Fluka Chemie GmbH (Steinheim, Germany).

n.r.: no restricted by EC No 1223/2009.

Cosmetic samples from national and international brands were purchased from local sources. They included leave-on and rinse-off products such as body milk, moisturizing milk, nail polish remover, toothpaste, hand cream, lipstick, gloss lipstick, sunblock, deodorants, shampoos and liquid soaps, among others. Samples were kept in their original containers at room temperature until their analysis.

2.2. MSPD procedure

0.1 grams of cosmetic were exactly weighted into a 10-mL glass vial and spiked with 10 μ L of benzyl alcohol- d_7 and MeP- d_4 surrogate solution (25 μ g mL $^{-1}$). When it was necessary, the sample was spiked with 10 μ L of the corresponding acetone solution of the target compounds to get the desired final concentration. Then, the sample was gently blended with 0.2 g of a drying agent (anhydrous Na $_2$ SO $_4$) and 0.4 g of dispersing sorbent (Florisil or sand) into a porcelain mortar using a porcelain pestle until a homogeneous mixture was obtained (ca. 5 min). The mixture was transferred into a glass Pasteur pipette (aprox. 150 mm), with a small amount of glass wool at the bottom, containing 0.1 g of Florisil (to obtain a further degree of fractionation and sample clean-up). Finally, a small amount of glass wool was placed on top of the sample before compression with a small metallic spatula. Elution was made by gravity flow with ethyl acetate or hexane:acetone (1:1, v/v), collecting 1 or 2 mL of extract into a volumetric flask. The MSPD extracts, diluted when necessary, were directly analyzed by GC–MS and GC–MS/MS.

2.3. Derivatization procedure

Acetylation was carried out by adding 50 μ L of acetic anhydride containing 2.5% of pyridine to 0.5 mL of the standard or extract solutions. The mixture was maintained at 80 °C for 15 min. The derivatized MSPD extracts, diluted when necessary, were directly analyzed by GC–MS and GC–MS/MS. Optimization of the derivatization conditions to improve the chromatographic analysis of phenolic preservatives was optimized elsewhere [26,27]. Three of the studied compounds (bronidox, BHT and IPBC) did not undergo derivatization. Bronidox and IPBC do not have chemical groups susceptible to acetylation; the acetylation of BHT could not be demonstrated as the highly hindered hydroxyl group with poor nucleophilicity may prevent the acetylation under the studied conditions. For the other preservatives and antioxidants, reaction yield was quantitative and satisfactory.

2.4. GC–MS analysis

The GC–MS analysis was performed using 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 SLBTM-5ms capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness) obtained from Supelco Analytical (Bellefonte, PA, 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 60 °C (held 1 min) to 100 °C at 8 °C min $^{-1}$, to 150 °C at 20 °C min $^{-1}$, to 200 °C at 25 °C min $^{-1}$ to 220 °C at 8 °C min $^{-1}$ and 30 °C min $^{-1}$ to 290 (held 15 min). For the analysis of the derivatized extracts, the GC–MS oven temperature was programmed from 80 °C (held 2 min) to 290 °C at 14 °C min $^{-1}$. Pulsed splitless mode was used for injection (30 psi, held 1.2 min). After 1 min, the split valve was opened (75 mL min $^{-1}$), and the injector temperature was kept at 260 °C. The injection volume was 1 μ L. The electron multiplier was set at a nominal value of 1376 V.

2.5. GC–MS/MS analysis

The GC–MS/MS analysis was performed using a Thermo Trace 1310–Thermo Triple Quadrupole TSQ 8000 with autosampler IL 1310 from Thermo Scientific (San Jose, CA, USA). The temperatures of the transfer line and the ion source were set at 290 and 350 °C, respectively. The system was operated by Xcalibur 2.2 and Trace Finder TM 3.0.

Separation was carried out on a TG-5 SILMS capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness) obtained from Thermo Scientific (San Jose, 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 60 °C (held 1 min) to 100 °C at 8 °C min $^{-1}$, to 150 °C at 20 °C min $^{-1}$, to 200 °C at 25 °C min $^{-1}$, to 220 °C at 8 °C min $^{-1}$, and 30 °C min $^{-1}$ to 290 (held 9.5 min); and from 80 °C (held 2 min) at 14 °C min $^{-1}$ to 290 °C (held 3 min) for the derivatized extracts analysis. Splitless w/Surge mode was used for injection (200 kPa, held 1.2 min). After 1 min the split valve was opened (75 mL min $^{-1}$), and the injector temperature was kept at 260 °C. The injection volume was 2 μ L. The electron multiplier was set at a nominal value of 1638 V.

2.6. Statistical analysis

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

3. Results and discussion

The chromatographic conditions were optimized to achieve an adequate separation of the target compounds, 25 fragrance allergens and 13 preservatives (see conditions in Section 2). For GC–MS analysis, the mass spectra detector (MSD) was operated in selected ion monitoring (SIM) mode, monitoring three ions per compound. For GC–MS/MS analysis, the detector was operated in selected reaction monitoring (SRM) mode selecting three transitions for each compound according to ion abundance. The most intense transition was used for quantification purposes, whereas the second and the third ones were employed for identification/confirmation purposes (Tables S1 and S2). Fig. 1 shows a chromatogram of a 1000 ng mL $^{-1}$ standard solution in ethyl acetate. For some analytes constituted by isomeric mixtures two chromatographic peaks were obtained (citral, farnesol). As can be seen, some of the analytes co-elute. As previously mentioned in the introduction, the chromatographic separation of fragrance allergens is quite complex and some authors have propose the use of two different GC capillary columns and three ions (SIM mode) employing a total of six calibration curves per compound (specially designed software is required) [33]. In our case the co-elution problems (e.g. isoeugenol, coumarin, MeP) could be initially overcome since none of the selected ions were common (see as example, the ion chromatograms in Fig. 1). In addition, the use of MS/MS in the SRM mode improves selectivity by the use of specific transitions (three per compound), which could virtually eliminate matrix background.

3.1. MSPD optimization

All the optimization studies have been developed employing non-spiked real samples. In this way, the real matrix-analyte interactions are considered in the selection of the most suitable extraction conditions. Since the objective was to develop a micro-MSPD method, only 0.1 g of sample were used in all experiments.

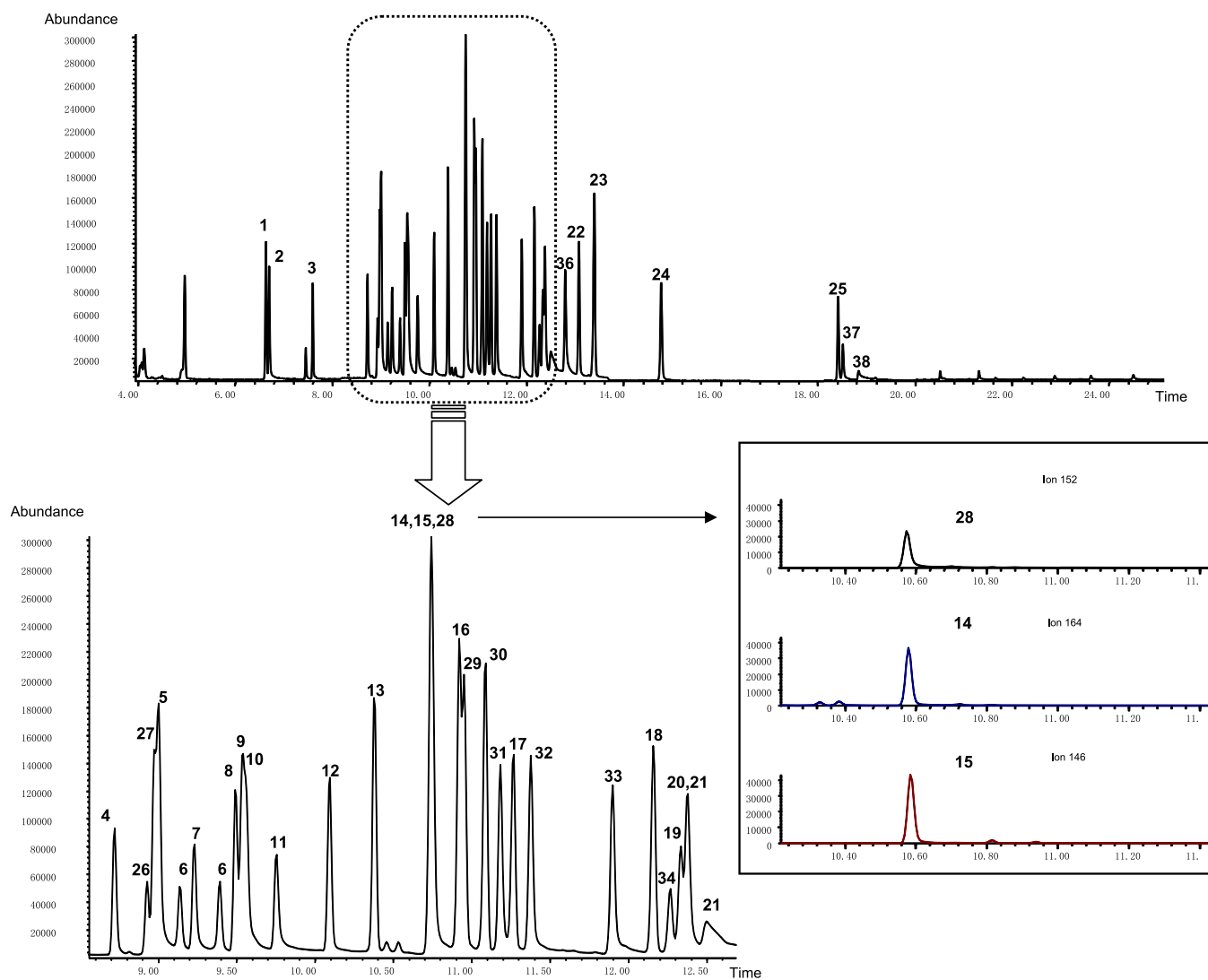


Fig. 1. Chromatogram of a 1000 ng mL⁻¹ standard solution of all the target analytes in ethyl acetate (see compound key in Table 1).

The sample was mixed with the sample drying agent, anhydrous sodium sulphate (1:2, w/w). As described in Section 2, all experiments were performed on 1.8 mL Pasteur pipettes with glass wool stoppers that were discarded after each extraction. Two factorial designs were carried out: for the first one, a leave-on cosmetic (moisturizing cream), and for the second one, a rinse-off cosmetic (shower gel), were employed. In both cases, the factors studied comprised the elution solvent, the dispersant, and the solvent volume (Table 2).

3.1.1. Leave-on sample

The analysis of the sample showed the presence of 13 target analytes. All of them were included in the product ingredients label. In addition, two phthalates (DEP and DBP) and the musk fragrance galaxolide were found in the product.

The ANOVA results are summarized in Table 3. Factor B, the dispersant, was significant for the most volatile analytes (excluding limonene). The other factors, as well as the factor interactions, were not significant ($p > 0.05$). Fig. 2 shows the Pareto charts and the main effect plots for some analytes, benzyl alcohol, geraniol, and phenoxyethanol, obtained in the factorial experiment design. In the Pareto charts, the standardized effects are plotted in decreasing order of absolute magnitude, thus making it easier to see which ones are the most important factors and interactions. In addition, the line drawn on the chart indicates whether an effect is statistically significant at a specified significance level (in this case, 95%). Main effect plots show how the response varies when each factor is changed from its low level to its high level, while all other factors are held at the center of the experimental domain. In the Pareto charts, factor B clearly exceeds the significance limit (vertical line in the graphs) for benzyl alcohol and geraniol and it is close to the

Table 2

Factors and levels considered in the experimental designs.

Factor	Key	Lower level	Upper level
Solvent	A	Hexane:acetone	Ethyl acetate
Dispersant	B	Sand	Florasil
Volume (mL)	C	1	2

Table 3*F* ratios and *p* values obtained in the analysis of variance for the leave-on and rinse-off study^a.

Leave-on		A: Solvent		B: Dispersant		C: Volume		AB		AC		BC	
Compounds		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Limonene		0.06	0.82	3.68	0.15	0.10	0.77	0.06	0.82	0.42	0.56	0.10	0.77
Benzyl alcohol		0.23	0.66	46.9	0.01	0.10	0.77	0.17	0.70	0.03	0.88	0.55	0.51
Linalool		0.20	0.68	41.2	0.01	0.00	0.96	0.19	0.69	0.01	0.91	0.02	0.89
Geraniol		0.11	0.76	12.4	0.03	0.32	0.61	0.36	0.59	0.00	0.98	0.18	0.70
Ionone		0.12	0.75	8.70	0.06	1.03	0.38	0.53	0.51	0.02	0.89	0.30	0.62
Lilial		0.03	0.87	2.13	0.24	0.69	0.46	0.56	0.50	0.05	0.84	0.28	0.63
Hexylcinnamal		0.02	0.89	0.29	0.62	0.51	0.52	0.60	0.49	0.02	0.89	0.31	0.61
Diethyl phthalate		1.29	0.33	6.86	0.07	0.28	0.63	3.85	0.14	0.84	0.42	0.04	0.85
Dibutyl phthalate		1.21	0.35	1.17	0.35	0.32	0.61	0.72	0.45	0.02	0.89	0.71	0.46
Phenoxyethanol		0.03	0.86	6.86	0.07	0.00	0.95	0.48	0.53	0.00	0.96	0.50	0.52
Methyl paraben		0.00	0.99	0.02	0.90	0.19	0.69	0.74	0.45	0.03	0.86	0.13	0.74
Ethyl paraben		0.00	0.97	0.00	0.97	0.50	0.53	0.79	0.43	0.03	0.86	0.11	0.75
Propyl paraben		0.01	0.92	0.00	0.97	0.97	0.39	0.80	0.43	0.04	0.86	0.16	0.71
iButyl paraben		0.01	0.91	0.11	0.76	0.28	0.63	0.73	0.45	0.02	0.89	0.18	0.69
Butyl paraben		0.01	0.92	0.01	0.93	0.60	0.49	0.78	0.44	0.06	0.82	0.11	0.76
Galaxolide		0.03	0.88	0.18	0.69	0.42	0.56	0.64	0.48	0.03	0.86	0.28	0.63
Rinse-off		A: Solvent		B: Dispersant		C: Volume		AB		AC		BC	
Compounds		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Limonene		0.16	0.71	7.39	0.07	0.02	0.90	0.16	0.71	0.06	0.82	0.02	0.90
Linalool		0.06	0.82	189	0.001	4.65	0.12	2.44	0.21	0.02	0.88	1.44	0.31
Citronellol		0.04	0.85	34.8	0.001	9.55	0.05	1.53	0.30	0.12	0.75	0.07	0.80
Geraniol		0.01	0.91	28.8	0.01	13.1	0.03	1.64	0.29	0.01	0.91	0.08	0.79
Eugenol		0.38	0.58	30.8	0.01	13.8	0.03	0.79	0.44	0.07	0.81	0.90	0.41
Hexylcinnamal		1.64	0.29	9.00	0.05	4.42	0.12	0.44	0.55	0.25	0.64	2.34	0.22
Benzyl benzoate		11.9	0.05	5.04	0.11	16.2	0.02	4.89	0.11	10.1	0.05	3.39	0.16
Diethyl phthalate		2.33	0.22	1.48	0.31	26.4	0.01	1.39	0.32	2.75	0.19	1.10	0.37
Dibutyl phthalate		2.48	0.21	0.70	0.46	6.37	0.08	0.21	0.67	0.50	0.53	1.50	0.30
Methyl paraben		0.12	0.75	6.89	0.07	10.2	0.04	0.53	0.51	3.95	0.14	0.18	0.70

^a *p* < 0.05 means statistical significance.

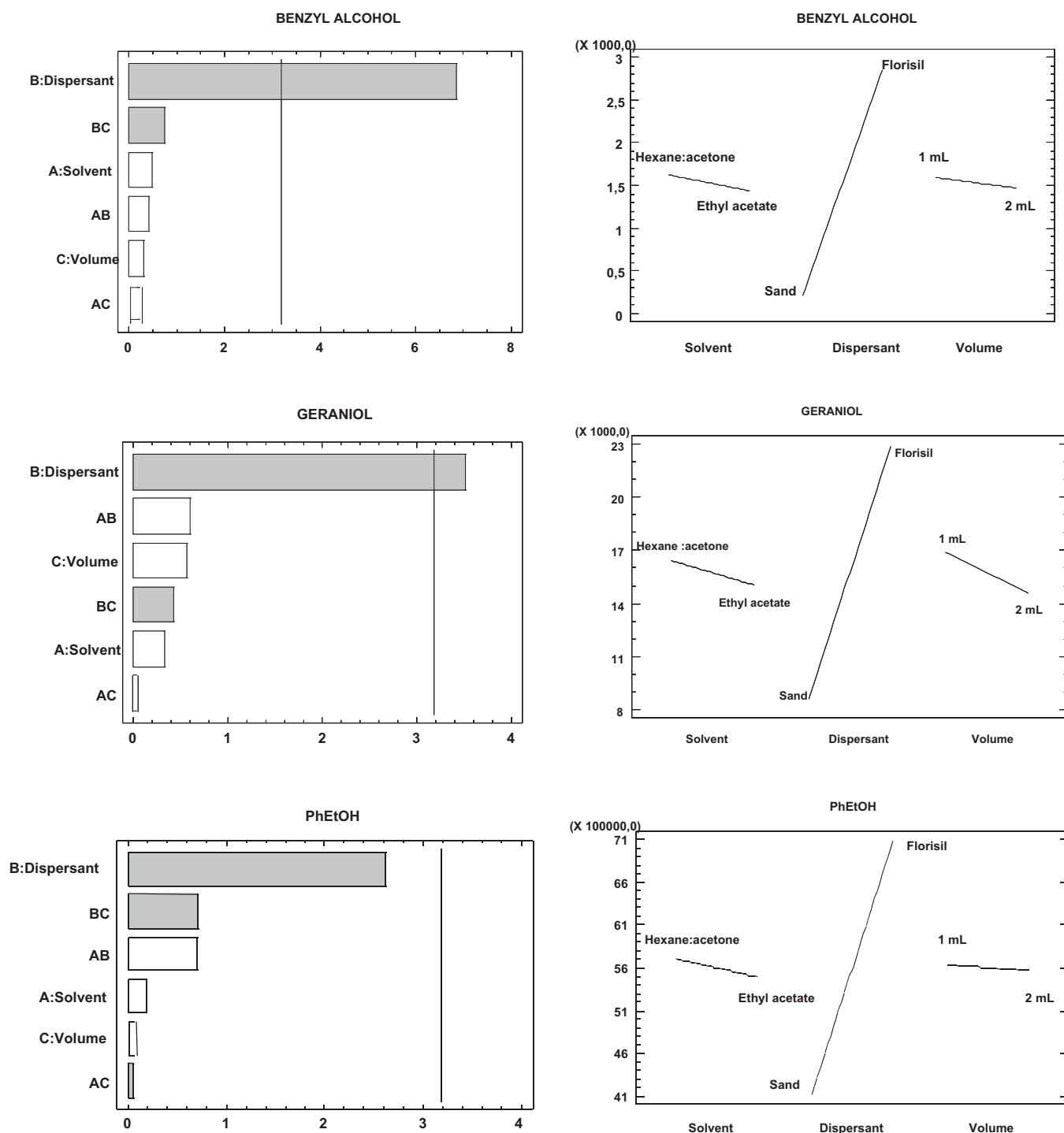


Fig. 2. Pareto charts and main effects plot showing the significant factors (95%) for the leave-on sample.

line for phenoxyethanol whereas the other factors are no significant. The main effects plots show how higher response is achieved when using Florisil instead of sand. The other two factors, solvent and solvent volume do not affect the response obtained.

3.1.2. Rinse-off sample

The analysis of the sample showed the presence of limonene, linalool, citronellol, geraniol, eugenol, hexylcinnamal, benzyl benzoate and methyl paraben. The phthalates DEP and DBP were also found in this sample. The ANOVA study (Table 3) showed the statistical significance of factor B, the dispersant, for the fragrances

(excluding limonene) and factor C, the elution volume, for many of the analytes. The Pareto charts for some analytes are included in Fig. 3. Factors B and C are the most important with the highest standardized effect (larger bar). Also main effect diagrams are included; as can be seen, higher response is again achieved using Florisil instead of sand. Regarding the elution volume, the low level of the factor, 1 mL, was more favorable than 2 mL. Regarding the solvent, both gave similar response.

In view of the results, it is possible to establish a general method for the simultaneous extraction of the fragrance allergens and the preservatives both in leave-on and rinse-off cosmetics. The selected

Table 4
Quality parameters of the method for the GC–MS and GC–MS/MS analysis of fragrance allergens.

Fragrances	GC–MS (SIM)			GC–MS/MS (SRM)			Recoveries (%, RSD) ^b					
	Correlation coefficient (R)	IDL (ng mL ⁻¹)	LOD ^a (% w/w × 10 ⁴)	Correlation Coefficient (R)	IDL (ng mL ⁻¹)	LOD ^a (% w/w × 10 ⁴)	Rinse-off ^c			Leave-on ^c		
							2 µg g ⁻¹	10 µg g ⁻¹	20 µg g ⁻¹	2 µg g ⁻¹	10 µg g ⁻¹	20 µg g ⁻¹
Limonene	0.9993	1.07	0.0107	1.0000	0.13	0.00130	66.1 (8.0)	70.7 (2.1)	65.5 (1.7)	74.2 (7.4)	84.1 (0.53)	77.0 (8.8)
Benzyl Alcohol	0.9980	2.91	0.0291	0.9999	0.10	0.00100	112 (3.2)	106 (12)	106 (4.0)	112 (7.3)	105 (4.4)	112 (1.7)
Linalool	0.9984	2.10	0.0210	0.9999	0.60	0.00600	87.4 (5.8)	115 (6.0)	87.4 (2.7)	106 (11)	91.0 (7.6)	104 (2.5)
Methyl-2-octynoate	0.9969	2.83	0.0283	0.9999	0.62	0.00620	109 (11)	104 (6.1)	87.4 (12)	104 (12)	84.3 (6.1)	105 (2.5)
Citronellol	0.9965	2.97	0.0297	0.9999	2.07	0.0207	103 (17)	108 (4.8)	83.9 (2.3)	106 (9.8)	95.3 (7.6)	106 (1.7)
Citral	0.9973	2.83	0.0283	0.9997	2.12	0.0212	110 (2.9)	113 (8.6)	114 (6.6)	112 (1.1)	98.0 (2.3)	113 (2.4)
Geraniol	0.9969	2.98	0.0298	0.9995	2.51	0.0251	103 (5.0)	86.0 (5.1)	92.8 (6.2)	95.2 (9.1)	99.4 (5.4)	86.4 (3.0)
Cinnamal	0.9984	3.00	0.0300	0.9997	0.23	0.00230	112 (9.3)	98.0 (3.6)	88.1 (3.7)	105 (11)	91.8 (5.6)	102 (1.5)
Hydroxycitronellal	0.9972	2.01	0.0201	0.9999	0.51	0.00510	103 (2.4)	94.3 (5.4)	86.9 (5.1)	109 (8.7)	108 (3.1)	92.5 (2.3)
Anise Alcohol	0.9972	4.00	0.0400	0.9997	2.01	0.0201	114 (15)	88.1 (6.3)	89.2 (4.6)	115 (9.8)	96.0 (0.15)	106 (2.0)
Cinnamyl Alcohol	0.9965	5.08	0.0508	0.9994	1.91	0.0191	112 (13)	96.8 (13)	96.0 (7.1)	114 (2.8)	92.1 (7.7)	108 (2.1)
Eugenol	0.9964	2.99	0.0299	0.9990	0.56	0.00560	104 (4.1)	103 (2.6)	94.0 (5.0)	88.1 (9.9)	89.2 (7.5)	105 (2.2)
Methyleugenol	0.9982	2.00	0.0200	0.9998	0.06	0.00060	97.0 (2.7)	101 (2.6)	88.6 (3.2)	95.4 (8.2)	94.7 (8.2)	102 (2.0)
Isoeugenol	0.9976	2.93	0.0293	0.9987	1.70	0.0170	88.3 (8.3)	89.3 (2.3)	85.2 (4.6)	80.9 (1.3)	80.4 (9.3)	91 (2.9)
Coumarin	0.9997	1.70	0.0170	0.9998	0.25	0.00250	109 (2.4)	93.7 (3.3)	87.5 (2.7)	101 (11)	102 (0.94)	105 (1.7)
α-isomethyl ionone	0.9985	0.81	0.00810	0.9998	0.05	0.00050	93.2 (4.6)	92.3 (5.6)	93.7 (3.3)	94.2 (13)	91.0 (3.3)	101 (1.5)
Lilial	0.9984	0.43	0.00430	0.9999	0.10	0.00100	90.4 (3.3)	83.0 (2.4)	96.5 (3.7)	93.1 (9.2)	91.4 (6.8)	103 (2.2)
Amyl Cinnamal	0.9963	2.04	0.0204	0.9995	1.60	0.0160	105 (5.6)	109 (1.7)	95.9 (3.5)	105 (9.1)	88.0 (6.3)	104 (1.6)
Lylal	0.9937	2.06	0.0206	0.9997	1.91	0.0191	97 (8.1)	115 (17)	100 (8.3)	110 (9.9)	95.2 (6.4)	103 (2.1)
Amylcinnamyl Alcohol	0.9930	6.00	0.0600	0.9989	1.50	0.0150	107 (6.7)	102 (4.5)	107 (5.3)	105 (9.3)	89.5 (10)	108 (2.2)
Farnesol	0.9978	70	0.700	0.9956	52	0.520	n.d	105 (15)	114 (14)	109 (10)	93.1 (12)	109 (4.3)
Hexylcinnamal	0.9954	2.99	0.0299	0.9994	1.32	0.0132	96.0 (15)	102 (14)	111 (2.6)	105 (11)	89.5 (9.9)	103 (14)
Benzyl Benzoate	0.9986	2.53	0.0253	0.9998	0.04	0.00040	113 (6.1)	103 (3.5)	93.3 (3.3)	99.2 (6.0)	93.2 (1.3)	103 (4.3)
Benzyl Salicylate	0.9926	2.62	0.0262	0.9930	0.54	0.00540	114 (3.9)	102 (2.7)	114 (4.9)	114 (10)	103 (4.1)	104 (0.90)
Benzyl Cinnamate	0.9945	2.95	0.0295	0.9963	0.59	0.00590	114 (7.7)	112 (3.5)	105 (3.8)	102 (14)	107 (0.34)	101 (2.5)

^a Equivalent to µg g⁻¹.

^b Calculated by GC–MS/MS.

^c Initial concentration (µg g⁻¹). *Rinse-off*: Limonene: 0.115; benzyl alcohol: 0.932; hexylcinnamal: 1.15; benzyl salicylate: 0.450. *Leave-on*: Lilial: 0.273.

n.d.: not detected.

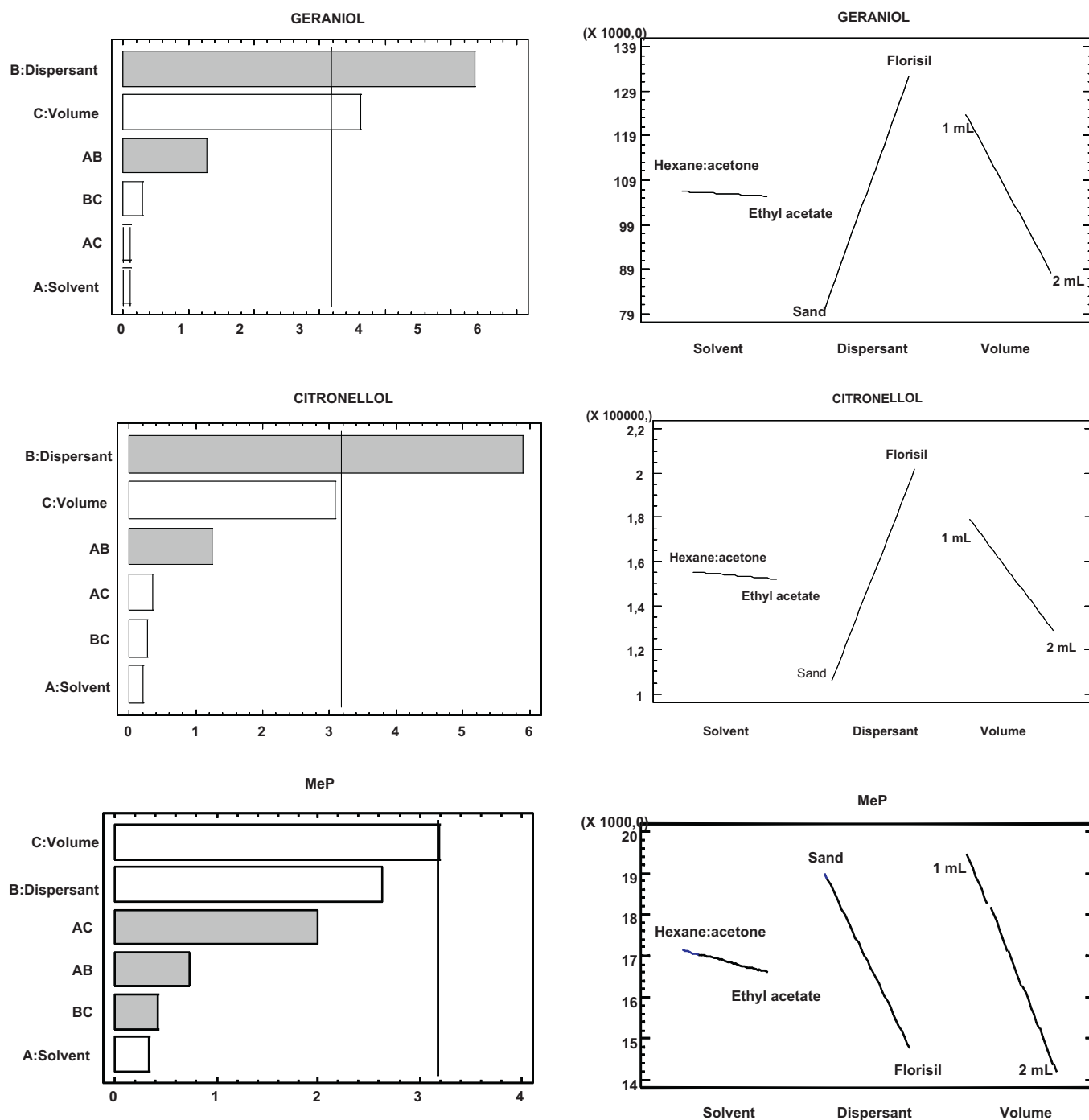


Fig. 3. Pareto charts and main effects plot showing the significant factors (95%) for the rinse-off sample.

conditions involve the use of Florisil as dispersant and the elution with 1 mL of solvent. As the solvent was a non significant factor, both solvents, hexane:acetone and ethyl acetate, could be employed. In next studies, ethyl acetate was chosen.

3.2. Derivatization

For the optimization study, all the analytes were determined in a single GC run. Nevertheless, the chromatographic peak shape as well as the chromatographic response can be improved for most preservatives including a derivatization step. Direct analysis produced peaks with appreciable tailing for some compounds due to the interaction of hydroxyl groups with the chromatographic

system. Therefore, a derivatization step was introduced prior to GC determination to improve preservative chromatographic analysis. In this way, the MSPD extract was divided in two aliquots: one of them was directly analyzed and the other one was analyzed after derivatization. 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 previous work dealing with the acetylation of preservatives and other phenolic species [23,27], and it is described in Section 2. Three of the preservatives (bromidox, IPBC and BHT) did not undergo derivatization. For the other compounds, reaction yield was quantitative and satisfactory, improving significantly the chromatographic analysis of

the target compounds. In any case, preservatives method validation has been evaluated in both cases, with and without the inclusion of an acetylation step. The acetylated derivatives were stable for at least several weeks.

3.3. Method performance

The GC–MS and GC–MS/MS method performance parameters for the 25 fragrance allergens and 13 preservatives (non derivatized) are summarized in Tables 4 and 5, respectively. All 38 targets were simultaneously analyzed. Regarding the instrumental linearity, the method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Calibration standards in ethyl acetate were prepared covering a concentration range from 1 to 1000 ng mL⁻¹. Correlation coefficients $R \geq 0.995$ for GC–MS and GC–MS/MS were obtained in most cases. Method precision was studied within a day ($n=5$) and among days ($n=8$) at two concentration levels, 50 and 500 ng mL⁻¹ (Table S3). RSD values ranged from 0.24–7.1% (intra-day precision) to 0.61–12% (inter-day precision) for GC–MS analysis; for GC–MS/MS analysis, RSD ranged from 0.56–7.1% to 1.8–13% (intra and inter-day precision, respectively). Instrumental detection limits (IDLs) were at the low ng mL⁻¹ or below 1 ng mL⁻¹ (GC–MS/MS); for many analytes, IDLs were between 4 and 10 times lower using GC–MS/MS.

Method quality parameters were evaluated using real cosmetic samples and are also shown in Tables 4 and 5. In this way, recovery studies were carried out by applying the optimized method to the extraction of two real samples spiked at 2, 10 and 20 $\mu\text{g g}^{-1}$: a rinse-off sample (liquid baby soap) and a leave-on sample (regenerating cream). These samples were expressly selected for recovery studies since they were almost free of the target fragrances and preservatives and so, specially suitable for recovery evaluation. The leave-on sample was labeled as perfume free and preservative-free. Additional recovery studies were also performed in other real samples (liquid soap, shower gel, shampoo, sunblock, body milk and lipstick) to demonstrate method suitability and the absence of matrix effects. The results are provided as supplementary material (Tables S4 and S5). Recoveries were calculated as the ratio between the detected amount and the spiked amount ($\times 100$). For samples containing the analyte the initial amount in the sample was substrated from the total amount [35]. If the initial amount in the sample is higher than the spiked level recovery is not calculated since the error associated with estimated value would be high. Recoveries were between 83% and 115% in both samples included in Tables 4 and 6 at the three levels with limonene the only exception. For this compound, slightly lower recoveries were obtained with a mean value of 73%. This lower recovery could be probably due to the high volatility of this compound which could give analyte loss during MSPD treatment, especially during sample disruption. Recoveries were also satisfactory for the other seven samples tested (generally above 90%, see Tables S4 and S5) with the exception of limonene with recoveries about 70% in some cases. Since all samples were analyzed in triplicate, precision was also assessed (see RSD values in the tables) attaining RSD values generally lower than 10%. Therefore, the method can be considered suitable for the determination of all target fragrance allergens and preservatives in real samples.

Limits of detection (LODs) were calculated as the compound concentration giving a signal-to-noise ratio of three ($S/N=3$). As shown in Table 4, LOD values for the fragrance allergens ranged from 0.00430 $\mu\text{g g}^{-1}$ to 0.0600 $\mu\text{g g}^{-1}$, for GC–MS analysis (excluding farnesol). For GC–MS/MS analysis, LOD values ranged from 0.0004 to 0.025 $\mu\text{g g}^{-1}$. For preservatives (Table 5), LOD values were between 0.006–0.100 $\mu\text{g g}^{-1}$ (GC–MS analysis) and 0.00050–0.037 $\mu\text{g g}^{-1}$ (GC–MS/MS analysis).

Table 5
Linearity and limits of detection of the method for the GC–MS and GC–MS/MS analysis of preservatives.

Preservatives	GC–MS (SIM)				GC–MS/MS (SRM)							
	Non derivatized		Derivatized		Non derivatized		Derivatized					
	Correlation coefficient (R)	IDL (ng mL ^{−1})	LOD ^a (% w/w × 10 ⁴)	Correlation coefficient (R)	IDL (ng mL ^{−1})	LOD ^a (% w/w × 10 ⁴)	Correlation coefficient (R)	IDL (ng mL ^{−1})	LOD ^a (% w/w × 10 ⁴)			
Bronidox	0.9977	3.05	0.0305	0.9999	2.24	0.0224	0.9998	0.15	0.00150	0.9999	0.11	0.00110
Phenoxy Ethanol	0.9967	2.81	0.0281	0.9990	1.50	0.0150	0.9996	1.50	0.0150	0.9998	1.21	0.0121
Methyl paraben	0.9972	3.02	0.0302	0.9995	0.58	0.00580	0.9982	0.86	0.00860	1.0000	0.010	0.00010
BHA	0.9984	2.20	0.0220	0.9979	0.98	0.00980	0.9996	0.091	0.00090	0.9993	0.032	0.00032
BHT	0.9990	0.60	0.00600	0.9993	0.57	0.00170	0.9996	0.051	0.00050	1.0000	0.031	0.00031
Ethyl paraben	0.9974	3.00	0.0300	0.9996	0.53	0.00530	0.9978	0.91	0.00910	0.9999	0.059	0.000590
Isopropyl paraben	0.9972	2.90	0.0290	0.9997	0.48	0.00480	0.9989	0.58	0.00580	1.0000	0.018	0.00018
Propyl paraben	0.9956	2.92	0.0292	0.9998	0.46	0.00460	0.9980	0.95	0.00950	1.0000	0.098	0.00100
IPBC	0.9956	10	0.100	0.9984	8.20	0.0820	0.9978	3.73	0.0373	0.9986	2.80	0.0280
Isobutyl paraben	0.9941	2.84	0.0284	0.9995	0.44	0.00440	0.9972	0.58	0.00580	0.9999	0.042	0.00042
Butyl paraben	0.9942	2.92	0.0292	0.9998	0.63	0.00630	0.9979	0.75	0.00750	0.9999	0.098	0.00100
Triclosan	0.9915	5.71	0.0571	0.9997	0.20	0.00200	0.9911	2.04	0.0204	1.0000	0.089	0.00090
Benzyl paraben	0.9942	5.90	0.0590	0.9984	0.46	0.00460	0.9920	2.90	0.0290	0.9997	0.12	0.00120

^a Equivalent to $\mu\text{g g}^{-1}$.

Table 7Analysis of rinse-off and leave-on samples^a (% w/w × 10⁴)^b.

Fragrances	Rinse-off samples					Leave-on samples											
	Sh1	Sh2	Too	SG	LS	BML	SB	LP	GL	Deo1	Deo2	Deo3	Deo4	NPR	BM1	BM2	BM3
Limonene	0.098	0.093	30.3	88.0	0.260	65.3	0.465	0.717	0.080	200	396	204	1.12	104	9.20	0.092	2.76
Benzyl Alcohol		7.01			5.27		4.36	2.93	1.31			11.2	6.24		41.4	0.084	
Linalool	8.02		12.9	544		360	39.3	101		1019	2028	185	316	0.643	39.6		167
Methyl-2-octynoate		2.24															
Citronellol		50.1		20.2		6.60		1.45	1.00	18.3	278		262	20.9	18.6		
Citral						7.29		6.28			17.8	61.3		4.77			
Geraniol		3.47		111	0.280	39.7		2.83					22.9	57.7	27.4		
Cinnamal										4.81				0.106			
Hydroxycitronellal	3.08				0.226		0.427			1.55					1.73	0.877	
Anise Alcohol							0.288								0.155	43.6	
Cinnamyl Alcohol							0.698								8.34	0.204	
Eugenol			1.44				3.48			49.5	0.182	45.8	3.45	43.8	0.135		
Methyl Eugenol															0.075		
Coumarin	9.82	10.1		26.3		6.17	0.986			566	50.0	26.5	168		31.8	0.041	
α-Isomethylionone					0.024					1.20	171		145		48.2		
Lilial	0.844				0.069	357	1.24			4.49	675	598	476			0.038	
Lylal	1.76	1.56		3.33											19.0		
Farnesol												2119	2800				
Hexylcinnamal	14.5	233			33.3	0.631	19.4			7.43	2627	1147	638		135		1.12
Benzyl Benzoate				2.21	0.121	1.88	0.515		807	0.685	1313			0.587	11.5	0.054	
Benzyl Salicylate		0.271		1.65		2.10	18.3			1.01	781	887	154		18.6	0.179	
Preservatives ^c	Sh1	Sh2	Too	SG	LS	BML	SB	LP	GL	Deo1	Deo2	Deo3	Deo4	NPR	BM1	BM2	BM3
Phenoxy Ethanol	24.6	1.32	0.425	9.85	0.915	30.1	2351	1.45	1557				0.292		2973	2125	2037
Methyl paraben	5.45	0.212		2.31		7.45	1782		589				1.05		2079	3.34	2929
BHA							3.27										
BHT				0.160	0.130	1057	14.5	2996	9.63	6.23	174	13.4	29.4				
Ethyl paraben				0.824		2.31	200		149						36.7		2267
Propyl paraben	0.986					2.76	635	1494	283						884	0.474	2574
IPBC													17.8				
Isobutyl paraben	0.888						94.9									0.508	
Butyl paraben	1.70					0.662	177									1.10	
Triclosan			0.370							6.66	5763	3271					
Benzyl alcohol.d ₇ ^d	119	104	94	106	83	91	114	113	91	96	95	108	101	99	101	105	115
Methyl paraben.d ₄ ^d	95	82	91	84	105	86	96	78	83	95	83	79	89	99	94	102	115

^a Sh: shampoo, Too: toothpaste; SG: shower gel; LS: liquid soap; BML: baby moisturizing lotion, SB: sunblock, LP: lipstick, GL: gloss lipstick, Deo: deodorant, NPR: nail polish remover, BM: body milk.^b Equivalent to μg g⁻¹.^c Parabens concentration expressed as acid (% w/w × 10⁴).^d Surrogate recovery, (%).

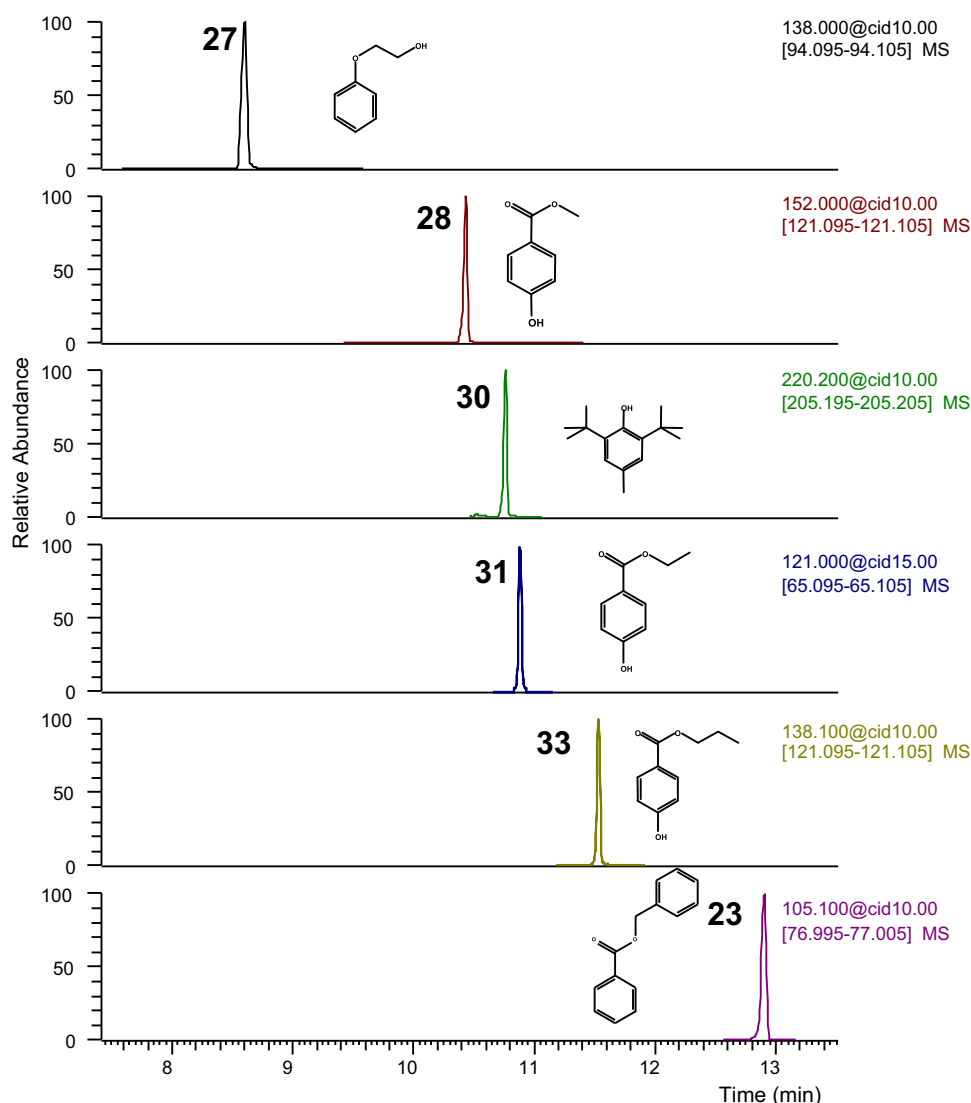


Fig. 4. GC-MS/MS transition chromatograms for a gloss lipstick (GL) (see compound key in Table 1 and sample concentrations in Table 7).

while methyl-2-octynoate and methyl eugenol were found in a shampoo and a body milk at low levels ($<2.3 \mu\text{g g}^{-1}$). Regarding the number of compounds by cosmetic sample, a body milk (BM1) sample contained sixteen of target fragrance allergens, while in the other cosmetic samples, the number of these compounds was between 3 and 12, highlighting a deodorant (Deo2) with high concentrations ($>0.1\%$) of linalool, hexylcinnamal and benzyl benzoate.

EU Regulation limits, established for benzyl alcohol, hydroxycitronellal, methyleugenol and isoeugenol, were fulfilled in all cases. Nevertheless, labeling requirements (see introduction) were not complied in 25% of the samples.

3.4.2. Preservatives

In the case of preservatives, they were present in all samples being phenoxyethanol the most frequently founded (76% of the samples) at very high concentration in four leave-on samples ($>1500 \mu\text{g g}^{-1}$, 0.15%), including a gloss lipstick (GL), although these levels do not exceed the maximum concentration permitted by European legislation (1%). Fig. 4 shows the GC-MS/MS transition chromatograms for this last sample. BHT was detected in eight samples, in same of them at high levels (baby care product BML, $1057 \mu\text{g g}^{-1}$, lipstick, LP 0.3%). This substance does not present any

restriction of use in cosmetics. Five of the 7 studied parabens were found in the samples. The most common were methyl paraben (59%) with high levels ($>1700 \mu\text{g g}^{-1}$) in three leave-on samples (SB, BM1, BM3) and propyl paraben (47%). Other parabens, ethyl, butyl, and isobutyl, were found in 6, 4 and 3 cosmetics, respectively. The total paraben concentration was very high in one leave-on sample (BM3), close to the legal limit of 0.8%. Triclosan was detected in 4 samples, at very high concentration in two deodorants, reaching the limit established by the European regulation (0.3%) [3]. BHA and IPBC were detected at low concentration levels in one sample. The maximum number of target preservatives, eight, was found in a sunblock sample (SB), containing very high concentrations of phenoxyethanol ($2350 \mu\text{g g}^{-1}$), and methylparaben ($1780 \mu\text{g g}^{-1}$). Other cosmetic and personal care samples contained between 2 and 6 of the target preservatives. Trace levels of preservatives at the low and sub $\mu\text{g g}^{-1}$ have been detected. Their presence may be caused by their use during manufacture of other cosmetics or as an impurity of other cosmetic ingredients, since those very low concentrations do not have antimicrobial effect.

Although regulation is not clear regarding preservatives labeling requirements, in general, parabens were declared when their concentration exceed 0.01%. Phenoxyethanol was included in the product label for four of the samples (concentrations above 0.1%)

and triclosan appeared in the label of the two products containing the highest levels.

4. Conclusions

A micro-MSPD method followed by gas chromatography–mass spectrometry/gas chromatography–triple quadrupole–mass spectrometry (GC–MS/MS) has been optimized for the determination of two groups of cosmetic additives, fragrance allergens and preservatives. This study included a total of thirty eight target substances subjected to restrictions or requirements according to the EU Cosmetic Directive. Multivariate optimization by means of experimental design was carried out in real non-spiked rinse-off and leave-on personal care products. The micro-MSPD procedure involved the use of only 0.1 g of sample as well as very low amounts of sorbents and since it was performed in Pasteur glass pipettes it could be implemented in any laboratory at very low cost. The method followed by GC–MS and GC–MS/MS analyses was validated in real samples showing satisfactory performance in terms of linearity, sensitivity, accuracy and precision with mean recoveries of 90% and RSD values generally below 10%. The use of GC coupled to triple quadrupole mass detection (MS/MS) instead of single quadrupole (MS), enabled reaching very low detection limits (low ng g^{−1}). The inclusion of a derivatization step allowed improving the chromatographic peak shape as well as the chromatographic response for the phenolic preservatives. The validated method was applied to real cosmetic samples, with the aim of demonstrating the method suitability for a wide variety of the most common personal care products. Most of the target substances were found in the samples at concentration levels from the sub parts per million to the parts per mill. Several fragrances and preservatives have been detected at levels above 0.1% (1000 µg g^{−1}). Regarding compliance with the EU regulation, maximum concentration limits were satisfied although two deodorants and a baby care product presented concentration of triclosan (0.3%) and parabens (0.8%) at the legal limits, respectively. In addition, several samples did not fulfill the labeling requirements for fragrance allergens.

Acknowledgments

This research was supported by FEDER funds and projects CTQ2010-19831 (Ministerio de Ciencia e Innovación, Spain) and CN 2012/299 (Research group's consolidation program, Xunta de Galicia, Spain).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2014.03.070>.

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Supporting information

for

Development of a multianalyte method based on micro-matrix-solid phase dispersion for the analysis of fragrance allergens and preservatives in personal care products

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Table S1. Retention time, selected MS ions (SIM) and transitions (SRM) for fragrance allergens

Compound	GC-MS (SIM)		GC -MS/MS (SRM)	
<i>Fragrance allergens</i>	Retention time	Quantification and identification ions ^a	Retention time	Precursor ion → Product ion (Collision energy, eV)
Limonene	6.65	68 , 93,121	6.08	67.9 → 53.0 (10) 92.8 → 77.0 (15) 92.8 → 91.0 (10)
Benzyl Alcohol	6.72	77, 79, 108	6.13	79.0 → 77.0 (10) 108.2 → 77.0 (25) 108.2 → 79.1 (15) 71.0 → 43.0 (10)
Linalool	7.61	71 , 93, 121	7.14	92.9 → 77.0 (10) 92.9 → 91.0 (10)
Methyl-2-octynoate	8.74	79, 95 , 123	8.34	79.0 → 77.0 (10) 94.9 → 55.1 (10) 94.9 → 67.0 (10)
Citronellol	9.02	69 , 95, 109	8.63	67.1 → 41.0 (15) 68.9 → 39.0 (15) 68.9 → 41.1 (10)
Citral	9.16/9.41	69 , 94, 109	8.76/9.03	39.0 → 38.2 (15) 68.9 → 39.1 (15) 93.9 → 79.0 (10)
Geraniol	9.25	69 , 93, 111	8.87	68.9 → 39.0 (15) 68.9 → 41.0 (10) 92.9 → 77.1 (10)
Cinnamal	9.51	77, 103, 131	9.11	103.1 → 77.0 (10) 131.1 → 51.0 (40) 131.1 → 77.0 (25) 59.0 → 31.0 (10)
Hydroxycitronellal	9.55	59 , 71, 81	9.18	59.0 → 43.0 (25) 71.0 → 43.0 (10)
Anise Alcohol	9.57	109, 121, 138	9.19	109.1 → 77.0 (15) 109.1 → 94.0 (10) 137.0 → 77.0 (20)
Cinnamyl Alcohol	9.77	92 , 105, 115	9.39	92.1 → 91.0 (10) 134.1 → 78.0 (15) 134.1 → 91.2 (20)
Eugenol	10.10	103,131, 164	9.75	131.1 → 103.0 (10) 164.2 → 103.0 (20) 164.2 → 149.1 (10) 178.1 → 77.0 (35)
Methyleugenol	10.39	147, 163, 178	10.05	178.1 → 147.1 (10) 178.1 → 163.1 (10)
Isoeugenol	10.75	103, 131, 164	10.12/10.41	77.0 → 50.9 (15) 103.0 → 77.0 (10) 164.1 → 149.1 (10)
Coumarin	10.76	90, 118, 146	10.40	118.0 → 89.0 (20) 118.0 → 90.1 (10) 146.1 → 118.1 (10) 107.0 → 91.0 (10)
α-isomethyl ionone	10.93	107, 135 , 150	10.59	150.1 → 91.0 (20) 150.1 → 135.1 (10) 189.2 → 131.1 (10)
Lilial	11.28	131, 147, 189	10.92	204.2 → 147.2 (10) 204.2 → 189.2 (10)
Amyl Cinnamal	12.18	115, 129 , 145	11.75	116.8 → 115.1 (10) 128.9 → 128.0 (20) 202.0 → 129.1 (10)
Lyrall	12.35	79, 93, 136	11.91	92.9 → 77.0 (15) 92.9 → 77.0 (10) 136.1 → 79.0 (10) 91.1 → 65.0 (15)
Amylcinnamyl Alcohol	12.47	91, 115, 133	12.04	133.1 → 55.0 (10) 133.1 → 115.1 (10)
Farnesol	12.47/12.70	69 , 93, 107	12.06/12.27	69.0 → 39.0 (15) 69.0 → 41.0 (10) 108.9 → 67.0 (10) 116.8 → 91.0 (15)
Hexylcinnamal	13.08	129 , 145, 216	12.59	129.0 → 127.0 (20) 216.3 → 129.1 (10)
Benzyl Benzoate	13.41	77, 91, 105	12.86	90.9 → 65.0 (15) 105.1 → 77.0 (10) 194.1 → 165.1 (20)
Benzyl Salicilate	14.78	65, 91 , 228	14.09	91.0 → 39.0 (30) 91.0 → 65.0 (15) 228.1 → 91.1 (10)
Benzyl Cinnamate	18.43	91 , 131, 192	17.74	131.1 → 77.0 (20) 131.1 → 103.0 (10) 192.0 → 191.2 (15)

Table S2. Retention time, selected ions (SIM) and transitions (SRM) for preservatives.

	GC-MS (SIM)				GC-MS/MS (SRM)			
	Non derivatized		Derivatized		Non derivatized		Derivatized	
	Retention Time	Quantification and identification ions	Retention Time	Quantification and identification ions	Retention Time	Precursor ion → Product ion (Collision Energy, eV)	Retention time	Precursor ion → Product ion (Collision Energy, eV)
Bronidox	8.95	85.0, 106.9, 136.9	8.95	85.0, 106.9, 136.9	8.52	134.9 → 107.0 (5) 137.0 → 109.0 (5)	6.59	134.9 → 107.0 (5) 137.0 → 109.0 (5)
Phenoxyethanol	8.99	77.0, 94.0 , 138.0	10.28	77.0, 87.0 , 94.0	8.58	94.0 → 61.1 (15) 94.0 → 65.8 (10) 138.0 → 94.1 (5)	8.30	87.0 → 85.1 (5) 93.9 → 65.7 (10)
Methyl paraben	10.74	93.0, 121.0 , 152.0	10.92	93.0, 121.0 , 152.0	10.41	121.0 → 65.1 (15) 121.0 → 93.1 (10) 152.0 → 121.1 (10)	9.22	121.0 → 65.1 (15) 121.0 → 93.1 (10) 152.0 → 121.1 (10)
BHA	10.96	137.0, 165.1 , 180	11.51	137.0, 165.1 , 180.1	10.63	137.1 → 77.1 (20) 165.2 → 137.1 (10) 180.1 → 165.1 (10)	9.30	137.1 → 77.1 (20) 165.2 → 137.1 (10) 180.1 → 165.1 (10)
BHT	11.10	177.0, 205.1 , 220.2	11.10	177.0, 205.1 , 220.2	10.76	205.1 → 145.1 (15) 205.1 → 177.2 (10) 220.2 → 205.2 (10)	9.48	205.1 → 145.1 (15) 205.1 → 177.2 (10) 220.2 → 205.2 (10)
Ethyl paraben	11.19	121.0 , 138.0, 166.0	11.41	121.0 , 138.0, 166.0	10.85	121.0 → 65.1 (15) 121.0 → 93.1 (10) 138.0 → 121.0 (10)	9.88	121.0 → 65.1 (15) 121.0 → 93.1 (10) 138.0 → 121.0 (10)
Isopropyl paraben	11.39	121.0 , 138.0, 180.1	11.63	121.0 , 138.0, 180.1	11.04	121.0 → 65.1 (15) 121.0 → 93.0 (10) 138.1 → 121.1 (10)	10.16	121.0 → 65.1 (15) 121.0 → 93.0 (10) 138.1 → 121.1 (10)
Propyl paraben	11.90	121.0 , 138.0, 180.0	12.18	121.0 , 138.0, 180.0	11.52	121.0 → 65.1 (15) 121.0 → 93.0 (10) 138.1 → 121.1 (10)	10.75	121.0 → 65.0 (15) 121.0 → 93.1 (10) 138.1 → 121.0 (10)
IPBC	12.28	100.0, 164.9 , 181.9	12.28	100.0, 164.9 , 181.9	11.85	164.9 → 126.9 (35) 181.9 → 153.9 (10)	10.88	164.9 → 126.9 (35) 181.9 → 153.9 (10)
Isobutyl paraben	12.39	93.0, 121.0 , 138.0	12.69	93.0, 121.0, 138.0	11.96	121.1 → 65.1 (15) 121.1 → 93.1 (10) 138.0 → 121.1 (10)	11.22	121.1 → 65.1 (15) 121.1 → 93.1 (10) 138.0 → 121.1 (10)
Butyl paraben	12.80	121.0 , 138.0, 194.1	13.16	121.0, 138.0 , 194.1	12.34	121.1 → 65.1 (15) 121.1 → 93.1 (10) 138.0 → 121.1 (10)	11.59	121.1 → 65.1 (15) 121.1 → 93.1 (10) 138.0 → 121.1 (10)
Triclosan	18.53	218.0, 287.9 , 289.9	19.20	218.0, 287.9 , 289.9	17.84	218.0 → 126.9 (30) 218.0 → 155.1 (20) 288.0 → 218.0 (15)	14.62	218.0 → 126.9 (30) 218.0 → 155.1 (20) 288.0 → 218.0 (15)
Benzyl paraben	18.84	91.0, 121.0 , 228.0	19.16	91.0, 121.0 , 228.0	18.26	121.0 → 65.1 (20) 121.0 → 93.1 (10) 228.2 → 121.1 (10)	14.58	121.0 → 65.1 (20) 121.0 → 93.1 (10) 228.2 → 121.1 (10)

Table S3. Intra-day and inter-day method precision (RSD, %).

GC-MS (SIM)									GC-MS/MS (SRM)								
Intra-day precision					Inter-day precision				Intra-day precision					Inter-day precision			
<i>Fragrances</i>	50 ng mL ⁻¹		500 ng mL ⁻¹		50 ng mL ⁻¹		500 ng mL ⁻¹		50 ng mL ⁻¹		500 ng mL ⁻¹		50 ng mL ⁻¹		500 ng mL ⁻¹		
Limonene	1.2		1.8		3.4		3.1		1.3		1.2		3.7		7.2		
Benzyl Alcohol	0.30		2.5		4.1		1.3		4.9		1.8		9.8		6.2		
Linalool	0.39		2.8		4.1		2.5		1.6		1.3		3.4		6.4		
Methyl-2-octynoate	1.9		1.9		10		3.2		2.5		0.75		4.5		6.1		
Citronellol	4.6		2.3		7.7		5.6		3.4		0.96		7.3		9.5		
Citral	6.2		3.0		9.1		3.6		3.7		0.98		6.4		5.8		
Geraniol	2.7		5.0		9.6		8.4		1.8		0.71		11		7.4		
Cinnamal	0.90		1.8		8.5		3.4		2.8		1.9		2.8		2.8		
Hydroxycitronellal	2.0		2.6		7.1		3.3		3.7		1.1		5.7		7.8		
Anise Alcohol	3.2		2.5		7.8		1.0		3.1		1.8		5.3		3.7		
Cinnamyl Alcohol	5.5		2.2		11		0.96		1.9		1.9		4.8		5.1		
Eugenol	7.1		2.3		5.2		0.61		2.4		2.1		10		4.8		
Methyleugenol	1.2		1.4		3.0		1.8		3.6		1.1		4.1		5.6		
Isoeugenol	7.3		2.0		4.6		0.63		5.2		3.6		7.7		7.7		
Coumarin	5.4		1.3		6.7		2.6		2.2		3.6		5.2		3.9		
α-isomethyl ionone	0.83		1.9		6.7		2.7		1.9		0.56		1.8		3.7		
Lilial	0.46		2.0		5.4		2.1		4.8		1.3		5.5		4.2		
Amyl Cinnamal	1.6		1.9		8.9		4.1		1.7		1.4		4.3		2.5		
Lylal	3.4		2.8		8.5		3.1		4.9		1.7		5.9		6.6		
Amylcinnamyl Alcohol	8.3		2.1		12		1.2		4.5		3.1		9.7		13		
Farnesol	-		0.5		-		9.2		7.0		6.1		12		10		
Hexylcinnamal	3.2		1.9		8.2		4.4		3.8		1.8		2.9		2.8		
Benzyl Benzoate	0.24		1.4		6.2		2.8		4.0		1.5		6.6		3.0		
Benzyl Salicilate	1.3		2.1		8.2		1.6		5.7		7.1		9.8		10		
Benzyl Cinnamate	3.2		1.7		8.4		3.5		5.9		5.7		5.5		8.5		
Intra-day precision					Inter-day precision				Intra-day precision					Inter-day precision			
	50 ng mL ⁻¹		500 ng mL ⁻¹		50 ng mL ⁻¹		500ng mL ⁻¹		50 ng mL ⁻¹		500 ng mL ⁻¹		50ng mL ⁻¹		500 ng mL ⁻¹		
<i>Preservatives^a</i>	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	
Bronidox	2.8	5.9	1.9	0.67	3.8	8.0	1.7	3.7	2.2	0.84	1.8	1.7	6.6	6.5	1.7	3.6	
Phenoxy Ethanol	0.23	1.8	2.4	0.56	6.2	7.7	3.1	4.7	2.6	3.8	1.6	1.1	3.6	6.5	4.5	1.4	
Methyl paraben	0.16	3.8	1.7	4.4	6.3	10	0.73	8.4	6.9	1.0	4.8	2.3	9.1	5.3	7.4	5.3	
BHA	0.05	5.1	2.0	2.6	4.1	6.5	1.3	4.8	1.9	1.5	1.1	3.4	7.7	3.7	4.8	4.9	
BHT	5.5	6.2	1.4	3.2	2.9	8.2	1.4	3.7	1.9	1.4	1.1	1.2	3.6	4.3	5.4	7.7	
Ethyl paraben	1.6	3.2	2.0	0.46	5.5	8.2	0.73	5.4	5.2	1.5	5.0	2.4	7.8	5.4	4.9	4.4	
Isopropyl paraben	0.92	3.9	1.7	1.8	5.6	5.5	0.81	8.6	4.3	0.91	4.3	2.1	9.7	5.1	6.0	4.5	
Propyl paraben	1.2	2.0	2.0	0.19	7.9	5.7	1.1	5.0	7.1	1.3	6.3	1.1	5.6	2.1	7.1	8.3	
IPBC	0.91	5.2	1.7	7.5	5.9	3.0	2.9	6.5	9.9	4.1	5.9	6.3	8.1	7.7	4.4	5.0	
Isobutyl paraben	0.74	2.6	1.6	0.40	6.4	5.6	1.0	4.8	5.4	1.2	6.0	1.6	5.3	6.9	8.5	7.8	
Butyl paraben	0.86	1.6	1.8	0.60	8.9	6.7	2.0	4.5	5.6	1.1	7.3	1.5	5.8	6.4	8.9	4.6	
Triclosan	1.3	0.7	2.2	1.2	13	3.0	12	1.9	2.1	0.45	5.9	3.5	8.9	4.3	13	3.9	
Benzyl paraben	1.9	1.3	3.1	1.9	7.9	5.3	11	2.6	15	1.6	10	1.8	15	5.2	15	3.1	

^a ND: Non derivatized; D: Derivatized

Table S4. Recoveries for fragrance allergens in cosmetics and personal care products (% , RSD)^a

	BM2		LS			SG		Sh	SB	BM3	LP
<i>Fragrances</i>	2 µg g ⁻¹	20 µg g ⁻¹	2 µg g ⁻¹	10 µg g ⁻¹	20 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹
Limonene	71.2 (7.2)	78.6 (6.7)	69.1 (7.5)	75.2 (7.2)	72.0 (12)	-	90.1 (1.7)	77.2 (2.0)	93.1 (0.87)	96.2 (4.2)	
Benzyl Alcohol	114 (2.8)	92.5 (5.7)	-	98.1 (1.9)	107 (5.6)	115 (5.0)	109 (0.19)	91.2 (10)	116 (0.38)	113 (12)	
Linalool	97 (1.4)	96.3 (3.9)	81.4 (5.8)	80.2 (2.3)	95.3 (9.5)	-	117 (0.28)	-	-	-	
Methyl-2-octynoate	100 (5.0)	94.8 (5.2)	93.5 (5.7)	84.3 (0.69)	97.2(9.2)	102 (5.7)	93.1 (3.2)	81.2 (9.7)	113 (6.1)	96.2 (6.3)	
Citronellol	102 (1.8)	108 (4.0)	91.8 (5.0)	85.5 (2.3)	109 (9.8)	-	105 (6.7)	100 (2.2)	100 (2.8)	98.1 (12)	
Citral	112 (4.3)	108 (4.5)	112 (7.3)	83.4 (5.1)	102 (4.6)	113 (5.3)	116 (7.2)	94.5 (4.0)	108 (8.1)	107 (13)	
Geraniol	103 (6.9)	97.0 (4.7)	108 (8.8)	88.0 (0.98)	114 (0.27)	-	93.4 (1.9)	92.3 (4.1)	97.8 (7.3)	111 (14)	
Cinnamal	105 (1.9)	101 (5.9)	97.6 (4.4)	82.8 (1.5)	101 (3.6)	95.0 (7.8)	103 (2.8)	97.8 (4.6)	108 (2.5)	100 (9.5)	
Hydroxycitronellal	111 (2.5)	93.2 (4.7)	89.1 (10)	72.4 (2.8)	95.0 (3.9)	93.1 (5.2)	102 (6.8)	81.2 (7.4)	111 (6.8)	93.1 (9.1)	
Anise Alcohol	-	-	99.1 (9.5)	89.3 (4.2)	111 (1.6)	98.3 (7.9)	101 (4.2)	96.3 (6.1)	104 (0.041)	96.0 (12)	
Cinnamyl Alcohol	110 (6.2)	101 (7.9)	100 (13)	89.2 (4.2)	103 (1.3)	98.9 (8.7)	97.2 (6.7)	90.9 (8.8)	109 (3.5)	83.5 (12)	
Eugenol	87.1 (3.5)	101 (10)	88.2 (4.6)	90.2 (2.4)	100 (1.6)	96.5 (7.8)	95.3 (4.2)	92.3 (3.6)	109 (5.9)	100 (1.2)	
Methyleugenol	88.1 (2.5)	95.0 (5.6)	99.1 (5.7)	88.7 (1.6)	98.2 (3.5)	92.1 (5.7)	110 (0.45)	96.0 (4.9)	106 (3.7)	93.0 (8.1)	
Isoeugenol	91.3 (14)	89.1 (11)	90.0 (1.5)	85.2 (3.3)	98.1 (3.8)	100 (9.2)	93.5 (2.9)	82.4 (5.3)	95.2 (0.45)	113 (3.2)	
Coumarin	101 (2.1)	103 (6.5)	96.1 (1.3)	85 .2 (0.74)	96.4 (2.5)	-	118 (0.62)	81.4 (1.5)	97.3 (0.52)	108 (7.1)	
α-isomethyl ionone	85.1 (2.6)	98.3 (5.4)	91.2 (4.0)	86.2 (1.0)	105 (4.3)	86.4 (5.9)	104 (1.9)	93.9 (0.24)	103 (2.5)	108 (8.1)	
Lilial	91.6 (3.3)	97.4 (6.1)	86.8 (4.6)	78.2 (1.4)	99.3 (1.6)	90.0 (5.3)	98.1 (1.2)	85.2 (0.10)	102 (4.2)	93.5 (9.0)	
Amyl Cinnamal	98.7 (3.4)	100 (6.5)	87.4 (6.9)	87.2 (3.5)	99.4 (2.8)	101 (9.0)	107 (0.82)	96.2 (3.6)	106 (4.2)	114 (13)	
Lylal	108 (3.1)	102 (6.7)	104 (2.6)	76.3 (5.1)	98.0 (2.6)	76.1 (7.3)	98.2 (1.5)	82.5 (11)	114 (7.5)	114 (12)	
Amylcinnamyl Alcohol	109 (13)	105 (6.7)	90.9 (8.8)	98.1 (4.2)	109 (3.1)	85.5 (9.9)	98.2 (6.7)	96.2 (9.4)	111 (9.1)	113 (13)	
Farnesol	n.d	94.3 (5.1)	n.d	89.0 (4.9)	98.3 (4.7)	112 (15)	95 (0.70)	96.0 (13)	106 (12)	89.2 (15)	
Hexylcinnamal	93.3 (3.3)	97.4 (6.4)	-	-	-	94.4 (8.7)	-	-	112 (5.9)	100 (5.8)	
Benzyl Benzoate	93.8 (1.0)	91.2 (6.3)	87.3 (4.6)	85.1 (2.1)	100 (3.5)	95.8 (8.7)	105 (0.43)	95.5 (1.2)	113 (5.1)	97.2 (7.4)	
Benzyl Salicilate	110 (3.7)	80.4 (7.3)	100 (13)	109 (3.7)	102 (2.9)	115 (10)	115 (6.9)	-	114 (4.3)	94.3 (15)	
Benzyl Cinnamate	109 (3.7)	91.5 (7.5)	103 (5.5)	115 (7.5)	113 (3.2)	107 (12)	116 (0.14)	112 (0.40)	115 (4.8)	108 (12)	

BM: Body milk, LS: liquid soap, SG: shower gel, Sh: shampoo, SB: sunblock, LP: lipstick
* See initial concentrations in Table 7
n.d: Not detected
Blank cells: Not calculated since initial sample concentration is higher than the spiked level

Table S5. Recoveries for preservatives in cosmetics and personal care products (% , RSD)

	BM2				LS						SG	Sh	SB	BM3	LP
<i>Preservatives</i>	2 µg g ⁻¹	2 µg g ^{-1b}	20 µg g ⁻¹	20 µg g ^{-1b}	2 µg g ⁻¹	2 µg g ^{-1b}	10 µg g ⁻¹	10 µg g ^{-1b}	20 µg g ⁻¹	20 µg g ^{-1b}	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹	10 µg g ⁻¹
Bronidox	95.1 (1.2)	96.2 (2.9)	100 (6.6)	113 (6.2)	91.1 (4.3)	107 (9.5)	91.1 (1.4)	83.1 (2.6)	101 (5.9)	96.0 (8.6)	89.1 (7.0)	101 (2.8)	108 (0.90)	95.1 (5.2)	86.0 (4.1)
Phenoxy Ethanol	-	-	-	-	90.2 (10)	88.9 (7.6)	93.0 (1.5)	80.4 (4.9)	101 (4.0)	100 (9.9)	85.0 (5.0)	-	-	-	101 (4.7)
Methyl paraben	-	-	107(6.5)	110 (4.9)	110 (4.3)	103 (9.7)	90.0 (6.3)	89.5 (5.5)	98.0 (0.74)	100 (113)	109 (7.6)	100 (7.7)	-	-	110 (5.9)
BHA	88.3 (0.70)	106 (11)	95.7 (8.4)	103 (11)	85.1 (8.3)	107 (5.7)	85.1 (1.1)	93.1 (3.0)	100 (0.96)	93.4 (7.7)	95.4 (7.3)	94.1 (0.89)	83.2 (0.91)	102 (2.7)	108 (3.6)
BHT	75.2 (4.5)	86.3 (10)	91.0 (7.7)	102 (8.9)	75.2 (5.2)	90.9 (0.22)	77.4 (0.70)	80.4 (3.6)	101 (3.5)	105 (9.2)	114 (15)	92.1 (0.10)	-	100 (3.2)	-
Ethyl paraben	104 (5.6)	97.1 (2.9)	108 (6.3)	109 (7.8)	97.3 (8.6)	95.6 (3.1)	89.0 (5.6)	87.2 (4.9)	98.3 (0.33)	95.3 (13)	102 (10)	92.2 (9.8)	-	-	91.5 (5.4)
Isopropyl paraben	108 (8.3)	105 (2.6)	105 (3.1)	113 (11)	106 (8.9)	85.5 (13)	94.2 (4.3)	94.2 (0.10)	97.2 (2.0)	100 (12)	106 (10)	114 (8.5)	115 (1.2)	103 (3.8)	106 (6.5)
Propyl paraben	110 (6.6)	91.4 (13)	104 (9.2)	108 (10)	87.1 (3.6)	104 (9.3)	100 (5.6)	89.1 (6.3)	101 (1.4)	1004(11)	102 (11)	115 (1.2)	-	-	-
IPBC	91.2 (10)	92.1 (4.8)	100 (7.7)	108 (8.0)	97.0 (4.3)	92.8 (6.3)	94.0 (12)	94.7 (0.43)	97.9 (1.3)	100 (12)	96.0 (0.42)	84.3 (10)	113 (15)	114 (10)	105 (7.1)
Isobutyl paraben	109 (8.9)	93.9 (8.8)	105 (7.2)	107 (8.7)	92.5 (12)	115 (6.8)	98.3 (5.3)	96.9 (4.8)	102 (2.5)	1004(8.7)	85.2 (10)	111 (5.3)	-	106 (1.2)	103 (2.9)
Butyl paraben	107 (15)	99.1 (1.4)	101 (9.3)	107 (6.9)	104 (12)	107 (8.1)	104 (6.1)	91.9 (3.4)	106 (4.8)	103 (9.7)	114 (15)	107 (0.79)	-	103 (3.8)	106 (5.3)
Triclosan	115 (4.6)	103 (6.9)	111 (11)	100 (11)	103 (13)	103 (11)	95.2 (2.5)	95.2 (3.5)	115 (6.7)	92.5 (5.1)	86.4 (4.2)	96.2 (3.0)	106 (13)	110 (3.5)	113 (1.3)
Benzyl paraben	107 (7.8)	106 (10)	94.5 (11)	104 (9.1)	114 (15)	111 (9.9)	109 (13)	109 (1.7)	109 (6.8)	92.9 (15)	87.5 (5.1)	108 (7.9)	116 (9.0)	102 (3.5)	113 (4.3)

BM: body milk, LS: liquid soap, SG: shower gel, Sh: shampoo, SB: sunblock, LP: lipstick

^a See initial concentrations in Table 7.

^b Derivatized

Blank cells: Not calculated since initial sample concentration is higher than the spiked level