



Pressurized liquid extraction-gas chromatography-mass spectrometry analysis of fragrance allergens, musks, phthalates and preservatives in baby wipes[☆]



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ABSTRACT

Baby wipes and wet toilet paper are specific hygiene care daily products used on newborn and children skin. These products may contain complexes mixtures of harmful chemicals. A method based on pressurized liquid extraction (PLE) followed by gas chromatography-mass spectrometry (GC-MS) has been developed for the simultaneous determination of sixty-five chemical compounds (fragrance allergens, preservatives, musks, and phthalates) in wipes and wet toilet paper for children. These compounds are legislated in Europe according Regulation EC No 1223/2009, being twelve of them banned for their use in cosmetics, and one of them, 3-iodo-2-propynyl butylcarbamate (IPBC), is banned in products intended for children under 3 years. Also, propyl-, and butylparaben will be prohibited in leave-on cosmetic products designed for application on the nappy area of children under 3 years from April 2015. PLE is a fast, simple, easily automated technique, which permits to integrate a clean-up step during the extraction process reducing analysis time and stages. The proposed PLE-based procedure was optimized on real non-spiked baby wipe samples by means of experimental design to study the influence on extraction of parameters such as extraction solvent, temperature, extraction time, and sorbent type. Under the selected conditions, the method was validated showing satisfactory linearity, and intra-day, and inter-day precision. Recoveries were between 80–115% for most of the compounds with relative standard deviations (RSD) lower than 15%. Finally, twenty real samples were analyzed. Thirty-six of the target analytes were detected, highlighting the presence of phenoxyethanol in all analyzed samples at high concentration levels (up to 0.8%, 800 $\mu\text{g g}^{-1}$). Methyl paraben (MeP), and ethyl paraben (EtP) were found in 40–50% of the samples, and the recently banned isobutyl paraben (iBuP) and isopropyl paraben (iPrP), were detected in one and seven samples, respectively, at concentrations between 0.093 and 247 $\mu\text{g g}^{-1}$. In the case of phthalates, the forbidden phthalates dibutyl phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) were also found in thirteen samples at low levels. All the samples contained fragrance allergens in many cases at high levels (up to 2400 $\mu\text{g g}^{-1}$) and three musks were detected in the samples. Excluding the banned compounds, all samples complied with the concentration limits established by the European Regulation although 25% of them did not fulfill the labeling requirements for fragrance allergens.

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1. Introduction

In last years, there is an increasing use of disposable wet tissues due to their commodity; makeup remover, suntan cream, deodorants or moisturizing lotions among others, are offered as

wipes. Child specific care products such as baby wipes are usually employed for the cleansing of newborns, babies and children [1–3]. Children under three years are daily exposed to this product (up to sixteen units per day), mainly applied in the sensitive diaper zone including the genital area. This zone has a higher pH and is usually irritated due to the prolonged contact of the skin with urine and feces, which may damage the skin barrier and increase its permeability [4]. The skin barrier development in babies remains incomplete until 12 months of age, and the trans epidermal water loss in an infant is much higher than in an adult [5]. Therefore, skin is more susceptible to microbial and contaminants invasion. It has

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been reported that around 25% of babies develop atopic dermatitis, and 50% show napkin dermatitis [6,7]. In addition, babies and children are most susceptible to exposure to certain chemicals due to the immaturity of their physiological functions. Infants are more vulnerable due to the lack of enzymes to break down and remove toxins, and particularly sensitive to harmful substances that can affect endocrine, immune or nervous systems [8,9].

Fragrance allergens, preservatives, plasticizers, and synthetic musks are common ingredients in personal care products. Fragrances provide nice and attractive scents to make the product more attractive. Preservatives are essential to deliver a safe product to consumers; they are intended to actively prevent microbial growth within cosmetic products. In the case of baby wipes, the wet tissue liquids are aqueous; the storage temperature and the hard surface wipe (commonly cellulose) create an optimal medium for microbial growth. Plasticizers (phthalates and adipates) are mainly employed as fragrance solvents. Synthetic musks and plasticizers do not appear as such in the cosmetics label; musks are present under the terms “fragrance” or “parfum”.

These families of chemicals are regulated in Europe by the Regulation (EC) No 1223/2009 and its subsequent amendments [10]. In this way, 26 fragrances must be monitored, the so-called suspected allergen substances or fragrance allergens. Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products, and 0.001% for leave-on products. One of these fragrances, lylal®, was recently proposed to be transferred from Annex III (list of substances allowed in cosmetics with restrictions) to Annex II (list of substances prohibited in cosmetic products).

Parabens (esters of p-hydroxybenzoic acid) are the preservatives most frequently used due to their broad antimicrobial spectrum and low cost. It is estimated that 75–90% of cosmetics contain parabens at levels between 0.01 and 0.3% [11]. Their maximum permitted concentration is 0.4% for a single ester and 0.8% for mixture of esters. Isopropyl-, isobutyl-, phenyl-, benzyl-, and pentylparaben have been recently banned for their use in cosmetics [10], and ethyl-, methyl-, propyl- and butylparaben are categorized as potential endocrine disrupters. In addition, propyl- and butylparaben have been banned in Denmark in products for children under 3 years [12]; they will be banned in all Europe from April 2015 for their use in the diaper area for children below 3 years, and their maximum permitted concentration in other cosmetic products will be 0.14% (instead 0.8%). Phenoxyethanol is one of the most commonly used preservatives and its maximum permitted concentration is 1%. The France National Agency of Security of Medicaments (ANSM) has recently proposed to avoid the use of phenoxyethanol in products intended for children under 3 years, and to reduce the maximum permitted concentration to 0.4% in other personal care products [13]. Maximum permitted concentration for triclosan, was recently decreased to 0.2–0.3% in several products. 3-Iodo-2-propynyl butylcarbamate (IPBC) is banned in products for children under 3 years, except in bath products, shower gels and shampoos (0.02%); for leave-on products its maximum permitted concentration is 0.01%. The bromine-containing preservative bronidox can also be present in personal care products at a maximum concentration of 0.1%. The antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) can be used without restrictions.

Concerning synthetic musks and phthalates, European legislation on cosmetics banned the use of musk ambrette, moskene and tibetene since 1995 due to their bioaccumulative properties. Other musks (phthalide, tonalide and ketone) are allowed with restrictions. Diethyl phthalate (DEP) is widely used in personal care products; however, the European Commission on Endocrine Disruption has listed DEP as a priority substance. Other six phthalates: dibutyl phthalate (DBP), dimethoxyethyl phthalate (DMEP),

diisopentyl phthalate (DIPP), dipentyl phthalate (DPP), benzyl butyl phthalate (BBP) and di(2-ethylhexyl)phthalate (DEHP) have been linked to endocrine disruption and they are forbidden in cosmetics and personal care products.

In last years, some efforts have been made to determine all these target families of cosmetics ingredients, and several analytical methods based in traditionally procedures (liquid–liquid, and solid–liquid extractions), and current sample preparation (stir-bar sorptive extraction, matrix-solid phase dispersion, or pressurized liquid extraction, among others) have been proposed [14–19]. Nevertheless, there is a lack of studies devoted to the simultaneous determination of several families of cosmetic ingredients, and there are not specific analytical studies for this particular cosmetic product: the baby wipes. Most literature only includes aspects related with allergies [20–22] and only two studies include the determination of a reduced number of parabens [23,24]. Nevertheless, the analytical control of this product is essential to guarantee product safety for babies and children. Pressurized liquid extraction (PLE) is a very suitable technique to extract cosmetic ingredients, being analytes efficiently extracted from the samples at high pressure, minimizing sample preparation time [19,25,26].

The aim of this study is the development of a PLE and gas chromatography-mass spectrometry (GC-MS) method for the rapid (25 min) simultaneous determination of sixty-five chemicals belonging to four families of cosmetic ingredients in baby wipes and wet toilet paper.

2. Experimental

2.1. Chemicals, materials and samples

The studied compounds, their purity, suppliers, CAS numbers, retention time and quantification and identification ions are summarized in Table 1. Also, European legislation restrictions are included. Deuterated methyl-4-hydroxybenzoate-2,3,5,6-d₄ (MeP-d₄; 98atom% D), benzyl-d₇ alcohol (98atom% D) and bis(2-ethylhexyl)phthalate-3,4,5,6-d₄ (DEHP-d₄; 98atom% D) used as surrogate standard, were obtained from C/D/N Isotopes (Quebec, Canada), Aldrich (St. Louis, MO, USA), and Fluka Chemie GmbH (Steinheim, Germany), respectively.

Acetone, ethyl acetate, n-hexane, methanol and acetonitrile were provided by Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Sand (200–300 µm mesh particle size) was provided by Scharlau (Barcelona, Spain). Florisil (60–100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and anhydrous sodium sulphate (99%) from Panreac (Barcelona, Spain).

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

Metallic, and glass material; sorbents (Florisil and sodium sulphate anhydrous) were baked at 230 °C for 12 h before use to eliminate possible phthalate contamination. All materials were allowed to cool down wrapped with aluminum foil and Florisil and sodium sulphate anhydrous in desiccator.

Baby wipes and wet toilet paper samples intended for children under 3 years of age from national and international brands were obtained from local sources. The samples were kept in their original containers at room temperature, until their analysis.

2.2. PLE procedure

Extractions were performed on an ASE 150 (Dionex, Co., Sunnyvale, CA, USA), equipped with 10 mL stainless steel cells and 60 mL collection vials. Two cellulose filters (Dionex, 27 mm) were

Table 1

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

Fragrance allergens ^a	Purity (%)	CAS	Retention time (min)	Quantification and identification ions	Maximum concentration permitted [10]
Pinene	≥99 ^b	80-56-8	5.18	77 (27), 93 (100), 121 (13)	n.r
Limonene	97 ^b	5989-27-5	6.82	68 (100), 93 (76), 121 (25)	n.r
Benzyl alcohol	99 ^b	100-51-6	6.86	77 (73), 79 (115), 108 (100)	1% (as preservative)
Linalool	97 ^b	78-70-6	7.74	71 (100), 93 (84), 121 (24)	n.r
Methyl-2-octynoate	≥99 ^b	111-12-6	8.85	79 (66), 95 (100), 123 (73)	n.r
Citronellol	95 ^b	106-22-9	9.10	69 (100), 95 (49), 109 (18)	n.r
Citral	95 ^b	5392-40-5	9.25/9.48	69 (100), 94 (17), 109 (10)	n.r
Geraniol	≥96 ^b	106-24-1	9.33	69 (100), 93 (18), 111 (6)	n.r
Cinnamal	≥93 ^b	104-55-2	9.54	77 (35), 103 (50), 131 (100)	n.r
Hydroxycitronellal	≥95 ^b	107-75-5	9.60	59 (100), 71 (13), 81 (43)	1%
Anise alcohol	98 ^b	105-13-5	9.62	109 (77), 121 (55), 138 (100)	n.r
Cinnamyl alcohol	98 ^b	104-54-1	9.80	92 (100), 105 (53), 115 (54)	n.r
Eugenol	99 ^b	97-53-0	10.18	103 (28), 131 (27), 164 (100)	n.r
Methyleugenol	99 ^b	93-15-2	10.45	147 (31), 163 (29), 178 (100)	0.01% (fine fragrance); 0.004% (eau de toilette); 0.002% (fragrance cream); 0.0002% (other leave-on products); 0.001% (rinse-off products)
Isoeugenol	98 ^b	97-54-1	10.53/10.79	103 (22), 131 (20), 164 (100)	0.02%
Coumarin	99 ^b	91-64-5	10.79	90 (42), 118 (110), 146 (100)	n.r
α-isomethyl ionone	≥85 ^b	127-51-5	11.02	107 (58), 135 (100), 150 (61)	n.r
Lilial [®]	≥85 ^b	80-54-6	11.33	131 (39), 147 (40), 189 (100)	n.r
Amyl cinnamal	97 ^b	122-40-7	12.28	115 (89), 129 (100), 145 (57)	n.r
Lylal ^{®g}	≥97 ^b	31906-04-4	12.43	79 (74), 93 (78), 136 (100)	n.r
Amylcinnamyl alcohol	≥85 ^b	101-85-9	12.61	91 (88), 115 (60), 133 (100)	n.r
Farnesol	95 ^b	4602-84-0	12.66/12.90	69 (100), 93 (27), 107 (15)	n.r
Hexylcinnamal	≥95 ^b	101-86-0	13.27	129 (100), 145 (51), 216 (40)	n.r
Benzyl benzoate	98 ^b	120-51-4	13.54	77 (28), 91 (47), 105 (100)	n.r
Benzyl salicylate	≥99 ^b	118-58-1	15.04	65 (11), 91 (100), 228 (12)	n.r
Benzyl cinnamate	99 ^b	103-41-3	18.56	91 (100), 131 (90), 192 (63)	n.r
Preservatives	Purity (%)	CAS	Retention time (min)	Quantification and identification ions	Maximum concentration permitted [10]
Bronidox	≥99 ^c	30007-47-7	9.00	85 (27), 106 (49), 137 (100)	0.1%
Phenoxyethanol (PhEtOH)	99 ^c	122-99-6	9.06	77 (28), 94 (100), 138 (31)	1%
Methyl paraben (MeP)	99 ^b	99-76-3	10.76	93 (21), 121 (100), 153 (35)	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Butylated hydroxyanisole (BHA)	98.5 ^c	25013-16-5	11.00	137 (63), 165 (100), 180 (51)	n.r
Butylated hydroxytoluene (BHT)	99 ^c	128-37-0	11.22	177 (8), 205 (100), 220 (25)	n.r
Ethyl paraben (EtP)	99 ^b	120-47-8	11.22	121 (100), 138 (21), 166 (18)	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Isopropyl paraben (iPrP)	≥99 ^b	4191-73-5	11.43	121 (100), 138 (39), 180 (14)	Prohibited
Propyl paraben (PrP) ^h	99 ^b	94-13-3	11.96	121 (100), 138 (58), 180 (7)	0.14% as acid (for single ester) 0.8% as acid (for mixtures of esters) Prohibited in leave-on cosmetics designed for application on the nappy area of children under 3 years.
3-Iodo-2-propynyl butylcarbamate (IPBC)	97 ^c	55406-53-6	12.32	100 (15), 165 (100), 182 (50)	Rinse-off products: 0.02% (not to be used in products for children under 3 years, except in bath products); leave-on products: 0.01% (not to be used in body lotion and body cream); deodorants: 0.0075%
Isobutyl paraben (iBuP)	≥97 ^b	4247-02-3	12.46	93 (12), 121 (100), 138 (58)	Prohibited
Butyl paraben (BuP) ^h	99 ^b	94-26-8	12.90	121 (100), 138 (84), 194 (6)	0.14% as acid (for single ester) 0.8% as acid (for mixtures of esters) Prohibited in leave-on cosmetics designed for application on the nappy area of children under 3 years.
Triclosan	≥97 ^c	3380-34-5	18.75	218 (93), 288 (100), 289 (93)	0.3%
Benzyl paraben (BzP)	99 ^b	94-18-8	18.95	91 (46), 121 (100), 228 (21)	Prohibited

Table 1 (Continued)

Phthalates	Purity (%)	CAS	Retention time (min)	Quantification and identification ions	Maximum concentration permitted [10]
Dimethyl phthalate (DMP)	98 ^c	131-11-3	10.81	77 (13), 194 (66), 163 (100)	n.r
Diethyl phthalate (DEP)	98 ^b	84-66-2	11.79	149 (100), 150 (12), 177 (24)	n.r
Diisobutyl phthalate (DIBP)	99 ^f	84-69-5	14.79	57 (12), 149 (100), 223 (6.8)	n.r
Dibutyl phthalate (DBP)	99 ^b	84-74-2	16.42	149 (100), 150 (9), 223 (4.9)	Prohibited
Dimethoxyethyl phthalate (DMEP)	94 ^f	117-82-8	17.07	59 (100), 104 (18), 149 (29)	Prohibited
Diisopentyl phthalate (DIPP)	99.5 ^b	605-50-5	18.20	71 (23), 149 (100), 237 (10)	Prohibited
Dipentyl phthalate (DPP)	99.2 ^b	131-18-0	19.09	71 (16), 149 (100), 237 (5.6)	Prohibited
Benzyl butyl phthalate (BBP)	98 ^b	85-68-7	20.47	91 (53), 149 (100), 206 (24)	Prohibited
Diisooheptyl phthalate (DIHP)	99 ^b	71888-89-6	21.05	149 (100), 223 (7), 265 (100)	n.r
Di(2-ethylhexyl)phthalate (DEHP)	99.5 ^c	117-81-7	21.51	167 (30), 149 (100), 279 (10)	Prohibited
Diclohexyl phthalate (DCHP)	99 ^b	84-61-7	21.46	55 (19), 149 (100), 167 (31)	n.r
Diphenyl phthalate (DPhP)	98 ^b	84-62-8	21.63	77 (19), 153 (4), 225 (100)	n.r
Di-n-octyl phthalate (DOP)	≥98 ^d	117-84-0	22.68	149 (100), 223 (22), 279 (6.2)	n.r
Diisononyl phthalate (DINP)	99 ^b	28553-12-0	23.43	149 (100), 279 (7), 293 (17)	n.r
Diisodecyl phthalate (DIDP)	99 ^b	26761-40-0	24.30	71 (34), 149 (100), 307 (20)	n.r
Musks	Purity (%)	CAS	Retention time (min)	Quantification and identification ions	Maximum concentration permitted [10]
Cashmeran	≥95 ^f	33704-61-9	11.23	135 (43), 191 (100), 206 (57)	n.r
Celestolide	98 ^f	13171-00-1	12.99	173 (22), 229 (100), 244 (44)	n.r
Phantolide	≥98 ^e	15323-35-0	13.50	187 (11), 229 (100), 244 (24)	n.r
Musk Ambrette	99 ^b	83-66-9	14.49	253 (100), 254 (13), 268 (35)	Prohibited
Traseolide	99 ^e	68140-48-7	14.70	43 (41), 215 (100), 258 (14)	n.r
Galaxolide	55.8 ^b	1222-05-5	14.79	213 (23), 243 (100), 258 (20)	n.r
Tonalide	98 ^f	1506-02-1	14.95	43 (48), 243 (100), 258 (26)	Leave-on products: 0.1% (except hydroalcoholic products:1%; fine fragrance:2.5% and fragrance cream:0.5%); rinse-off products: 0.2%
Musk Moskene	≥99 ^b	116-66-5	13.35	263 (100), 264 (20), 278 (8.9)	Prohibited
Musk Tibetene	≥99 ^b	145-39-1	16.29	43 (33), 251 (100), 266 (28)	Prohibited
Ambrettolide	≥97 ^b	7779-50-2	16.18	67 (100), 81 (98), 96 (89)	n.r
Musk Ketone	≥98 ^b	81-14-1	16.95	191 (24), 294 (26), 279 (100)	Fine fragrance:1.4%; eau de toilette: 0.56%; other products:0.042%

^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 Fluka Chemie GmbH (Steinheim, Germany).

^d Supelco Analytical (Bellefonte, USA).

^e LGC Standards GmbH (Wesel, Germany).

^f Dr Ehrenstorfer (Ausborg, Germany).

^g Is proposed to be excluded completely from cosmetics and personal care products

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

^h The legislation requirements will enter into force from April 2015.

placed at each end of the PLE cell and also, in the bottom, a polyethylene frit (Supelco, 12 mL) was employed at the bottom. An individual baby wipe was weighted (wipes weight range: 2.48–8.03 g), introduced into the cell and spiked with 10 μ L of the surrogate solution (50 μ g mL⁻¹). Previously, 1.5 g of clean sand were placed (different sorbents were tested during the method optimization) in the cell. For the preparation of fortified samples, the baby wipe was spiked with 100 μ L of the corresponding acetone solution of the target compounds to get the desired final concentration. Finally, the dead volume was immediately filled with sand to avoid losses of the most volatiles analytes. The cell was tightly closed and placed into the PLE system. The extraction pressure was 1500 psi, the flush volume was 60%, and the purge time 60 s. Different extraction conditions were tested along the study. Methanol, acetonitrile, ethyl acetate, acetone, and the mixture hexane:acetone (1:1, v/v) were employed as extraction solvent. Three temperatures were studied: 70, 90 and 110 °C, as well as three different times 5, 10 and 15 min. The optimal extraction conditions were: MeOH, 110 °C, and 5 min. In all cases, the extracts were levelled to a final volume of 20 mL with the extraction solvent. Then, PLE extracts were diluted in ethyl acetate (1:5 v/v), filtered through a 0.20 μ m PTFE filters, and directly analyzed by GC-MS, without any pre-concentration or clean-up additional steps.

2.3. GC-MS analysis

The GC-MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975 C 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 SLB™-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 10 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.

2.4. Statistical analysis

Basic and descriptive statistics, as well as experimental design analysis, were performed using Statgraphics-Plus v5.1

Table 2
F ratios and *p* values obtained in the ANOVA study of the sorbents^a

Compounds	F	<i>p</i>
Limonene	27	0.011
Benzyl alcohol	106	0.0015
α-isomethylionone	5.0	0.11
Lilial®	3.5	0.16
Hexylcinnamal	0.71	0.61
DEP	4.1	0.14
DIBP	28	0.010
PhEtOH	0.76	0.59
MeP	2.2	0.26
BHT	1.1	0.47
EtP	2.4	0.24
PrP	11	0.041
BuP	0.18	0.90
Galaxolide	1.3	0.42

^a *p* < 0.05 means statistical significance.

(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 PLE.

3. Results and discussion

The chromatographic conditions were optimized to achieve an efficient separation of the target compounds: 26 fragrance allergens, 13 preservatives, 15 phthalates, and 11 musks (see conditions in the experimental section). For GC-MS analysis, the mass spectra detector (MSD) was operated in selected ion monitoring (SIM) mode, monitoring three ions per compound (Table 1).

3.1. Optimization of the extraction process

The influence of the main variables potentially affecting the PLE procedure must be evaluated to obtain an efficient extraction. In this way, the study of the influence of PLE conditions was accomplished in several steps. First, the sorbent added to the extraction cell, and the extraction solvent were evaluated with the intention of obtaining homogeneous and clean extracts as well as high chromatography response. Once the sorbent was selected, the solvent and other variables, such as the temperature and the extraction time, were optimised by means of a multifactor experimental design.

3.1.1. Preliminary tests

3.1.1.1. Sorbent screening. Different sorbents were tested during the PLE procedure. The effect of sand (A), and mixtures of sand with Florisil (1:1, w/w) (B), and sand with Florisil and anhydrous sodium sulphate (1:1:1, w/w) (C), were compared. Also, the extraction without the inclusion of a sorbent agent in the extraction cell (D) was tested. Two cellulose filters and a polyethylene frit were placed at the cell bottom followed by 0.5 g of sand, 1 g of the corresponding sorbent or mixture of sorbents, and the baby wipe; finally, the cell was completely filled with sand and other cellulose filter was placed on the top. In the case D, a baby wipe was placed into the extraction cell on top of the polyethylene frit. All extractions were performed with MeOH at 90 °C for 10 min. In this study, a real non-spiked baby wipe sample containing 14 target compounds was employed, in this way the real analyte-matrix interactions are considered. A one-way analysis of variance (ANOVA) was carried out and the results are summarized in Table 2. The sorbent was statistically significant for limonene, benzyl alcohol, DIBP and PrP (*p* < 0.05). As can be seen in the mean-value charts showed for limonene and DIBP (Fig. 1), the lowest response was obtained by not using sorbent (D), whereas responses were similar employing only

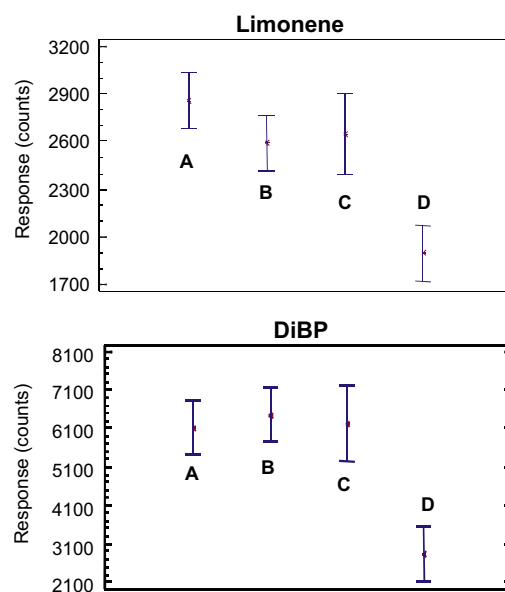


Fig. 1. ANOVA mean charts for the sorbent.

Table 3
Factors and levels considered in the experimental design.

Factor	Key	Low level (–)	Central level	High level (+)
Solvent	A	Methanol	Acetonitrile	Acetone
Temperature (°C)	B	70	90	110
Time (min)	C	5	10	15

sand (A) or the sorbents mixtures: (B), (C). Besides, turbid extracts were obtained when sorbents mixtures were used; therefore, we decided to use sand as sorbent since it offers the cleanest extracts with good extraction response.

3.1.1.2. Solvent screening. Acetonitrile (ACN), methanol (MeOH), ethyl acetate (EtAc), and hexane:acetone (1:1, v/v) were tested. In this case, a real non-spiked baby wipe sample containing 13 target compounds of the four cosmetics families of additives was employed. Extraction conditions were 90 °C and 10 min, taking into account previous results, and the sorbent employed was sand. As can be seen in Fig. 2, MeOH provides the highest responses for the majority of the compounds. When EtAc and the mixture hexane:acetone (1:1, v/v) were used, the formation of two phases and foams were observed and, therefore, these solvents were discarded. MeOH and ACN were included in the next study, and also acetone was incorporated due to its similar chemical properties with the other two preselected solvents.

3.1.2. Experimental design

Once the sorbent was selected (sand) and two solvents (MeOH and ACN) were pre-selected, other two parameters which can drastically affect extraction, PLE temperature and time, were optimized. 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. A real non-spiked baby wipe sample containing 15 target compounds (limonene, benzyl alcohol, α-isomethylionone, lilial®, hexylcinnamaldehyde, DEP, DEHP, DIBP, phEtOH, MeP, BHT, EtP, PrP, BuP, and galaxolide) was employed. The extraction process was optimized by means of a multifactor experimental design 3×2^2 and three factors were analyzed: elution solvent (A), extraction temperature (B), and extraction time (C) (Table 3). The efficiency of three solvents (MeOH, ACN, and acetone)

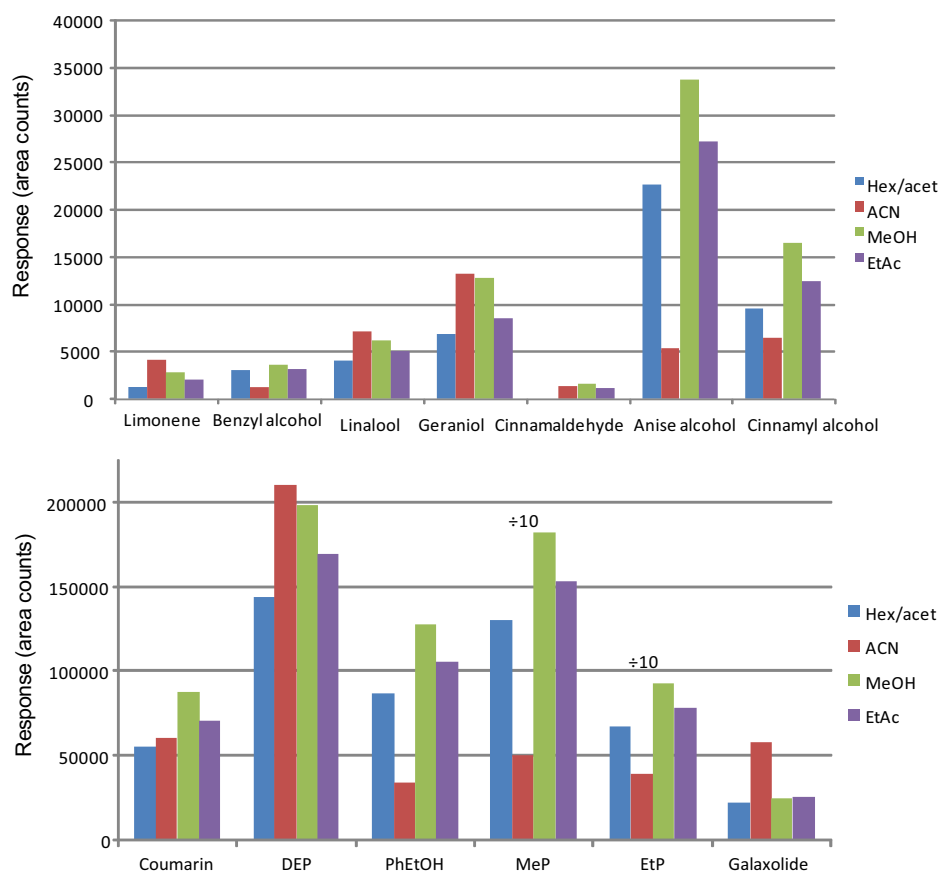


Fig. 2. Comparative responses for different elution solvents ($n = 2$).

was tested. MeOH and ACN were chosen because in preliminary studies showed cleaner extracts and the best extraction efficiency, and acetone was included in the experimental design because it has similar chemical characteristics and it also provides clean extracts. Other two parameters which considerably can affect PLE, temperature (B) and time (C), were studied: 70 and 110 °C (temperature) and 5 and 15 min (extraction time). These levels were chosen based on previous studies [19,25,26]. Also, two intermediate levels 90 °C and 10 min were included.

The results for the experimental design are shown in the ANOVA table (Table 4). As can be seen, the temperature was the most relevant factor being statistically significant or very close to this level for several analytes (α -isomethylionone, linalol®, DIBP, BHT, and galaxolide). Solvent (A) was significant for DEHP. The interactions solvent-temperature (AB) and solvent-time (AC) were not significant in any case, except for galaxolide, and DIBP, respectively. Solvent-solvent (AA) interaction was only significant for these last compounds. Extraction time (C) and its interaction with temperature (BC) were not significant ($p > 0.05$) in any case, therefore BC is not shown in the Pareto charts, and interaction plots (Figs. 3 and 4). 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. Fig. 3 shows the Pareto charts and main effect plots for some representative analytes of the different studied families (linalol®, BHT, galaxolide, and DIBP). As can be seen, temperature (factor B) exceeds the significance limit for these compounds; the main

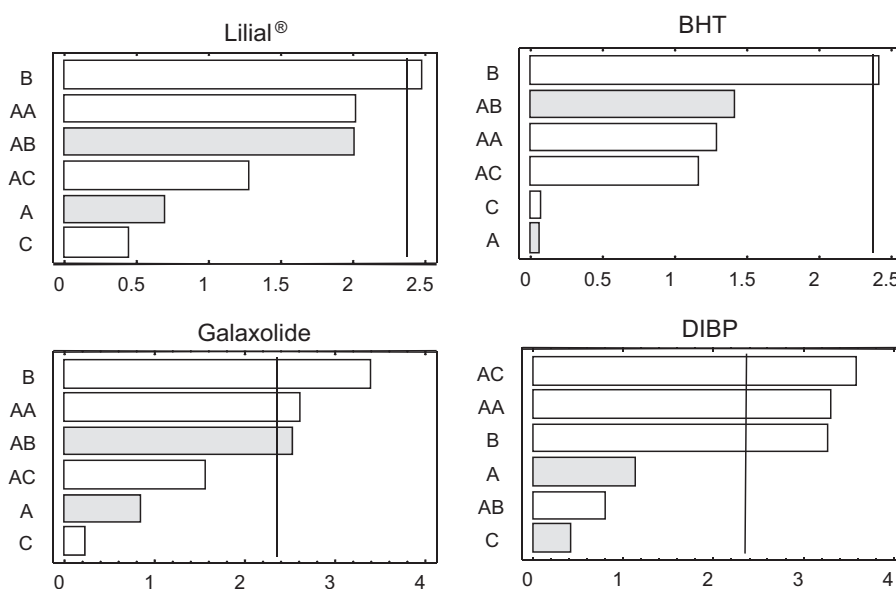
effect plots (Fig. 4a and b) show higher response when extractions are carried out at 110 °C (+) and employing MeOH (–) as extraction solvent. Fig. 4c and d shows interactions solvent-temperature (AB) and solvent-time (AC) for galaxolide and DIBP, respectively. In the first case, higher responses were obtained employing MeOH at 110 °C, for DIBP, higher efficiency was obtained using MeOH with 5 min of extraction time (these trends were also observed for most compounds). Therefore, in view of the results, the selected conditions involve the PLE extraction at 110 °C for 5 min using MeOH as extraction solvent.

3.2. Method performance

The GC-MS method performance parameters were studied for all the target compounds and they are summarized in Table 5. Regarding the instrumental linearity, calibration standards were prepared in ethyl acetate covering a concentration range from 2 to 2000 ng mL⁻¹ (see specific ranges in Table 5). The method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Correlation coefficients $R \geq 0.991$ were obtained in all cases. Instrumental method precision was studied within a day ($n = 5$) and among days ($n = 8$) at 100 ng mL⁻¹ (other concentration levels, 250 and 500 ng mL⁻¹ were also evaluated, data not shown). Relative standard deviation (RSD) values were in all cases below 10% and below 15% for intra-day precision and inter-day precision, respectively. Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of three ($S/N = 3$). IDLs were in the low ng mL⁻¹ for most compounds. For fragrance allergens and preservatives IDLs were below 6 ng mL⁻¹ (excluding farnesol, and IPBC, 70 and 10 ng mL⁻¹, respectively). For phthalates, they were below 1 ng mL⁻¹, excluding DIHP, DINP, and DIDP, which are mixtures of

Table 4F ratios and *p* values obtained in the analysis of variance for the multifactor optimization study^a

Compounds	A: Solvent		B: Temperature		C: Time		AA		AB		AC	
	<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	0.74	0.42	0.39	0.55	0.26	0.63	2.9	0.13	1.1	0.32	3.1	0.12
Benzyl alcohol	0.40	0.55	0.38	0.56	0.06	0.82	1.3	0.29	1.1	0.32	0.91	0.37
α -isomethylionone	0.00	0.98	5.8	0.04	0.14	0.72	2.4	0.16	0.35	0.57	0.15	0.71
Lilial®	0.47	0.51	6.1	0.04	0.19	0.68	4.0	0.08	4.0	0.09	1.6	0.25
Hexylcinnamal	1.6	0.25	2.6	0.16	0.19	0.68	4.2	0.08	2.2	0.19	3.9	0.09
DEP	0.01	0.92	2.2	0.19	0.04	0.85	3.6	0.11	2.0	0.20	1.3	0.29
DEHP	5.9	0.04	0.57	0.46	0.13	0.73	0.83	0.39	0.20	0.66	0.18	0.69
DIBP	1.3	0.30	11	0.01	0.16	0.70	11	0.01	0.62	0.46	13	0.01
PhEtOH	0.42	0.54	0.32	0.59	0.07	0.80	0.95	0.36	1.1	0.32	0.94	0.37
MeP	0.08	0.79	0.54	0.49	0.06	0.82	1.5	0.26	1.4	0.28	1.2	0.30
BHT	0.00	0.96	5.8	0.04	0.00	0.96	1.6	0.24	1.9	0.20	1.3	0.28
EtP	0.00	0.99	1.2	0.32	0.00	0.96	1.5	0.27	1.8	0.23	1.8	0.22
PrP	0.09	0.78	1.9	0.21	0.09	0.78	2.8	0.14	2.6	0.15	2.5	0.16
BuP	0.38	0.56	3.0	0.12	0.06	0.81	3.6	0.10	3.6	0.10	2.9	0.13
Galaxolide	0.69	0.43	12	0.01	0.04	0.84	6.8	0.04	6.3	0.04	2.4	0.16

^a *p* < 0.05 means statistical significance.**Fig. 3.** Pareto charts for lilial®, BHT, galaxolide, and DIBP.

isomers, and their chromatographic signal is composed of several analytical peaks. In the case of musks, their IDLs were in the low ng mL⁻¹ with values between 0.16 and 3.00 ng mL⁻¹.

Method quality parameters for the whole procedure PLE/GC-MS analysis are shown in Table 6, and they were also evaluated using real baby wipe, and wet toilet paper samples. Recovery studies were carried out by applying the optimized PLE method to the extraction of 2 real spiked samples at 10 and 100 µg/wipe. Previous analyses of the samples showed the presence of some of the target compounds, and these initial concentrations were taken into account to calculate the recoveries. Recoveries were higher than 90% for the majority of the studied compounds. These recoveries can be considered quantitative and no matrix effects were observed; therefore, quantification by external calibration can be effectively employed. The absence of matrix effect could be attributed to the fact that the proposed method provides clean extracts, and it does not require concentration (a baby wipe is extracted with 20 mL of solvent and the extract is further diluted 1:5, v/v). Precision was also evaluated (see RSD values in the table) attaining RSD values generally lower than 10%. Therefore, the method can be considered suitable for

the determination of all target fragrance allergens, preservatives, musks, and phthalates in real samples.

Limits of detection and quantification (LODs and LOQs) were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N = 3) and ten (S/N = 10), respectively using spiked samples at low level of concentration. The LOD values are shown in Table 6 and they are expressed in µg g⁻¹ and in µg per wipe. LOD for the fragrance allergens ranged from 0.0011 µg g⁻¹ to 0.031 µg g⁻¹ (0.0044–0.12 µg/wipe) (excluding farnesol). For preservatives, LOD values were between 0.00077 and 0.051 µg g⁻¹ (0.0030–0.200 µg/wipe). For musks and phthalates these values were between 0.00067 and 0.015 µg g⁻¹ (0.0026–0.0600 µg/wipe) (excluding DIHP, DINP and DIDP (0.40–0.80 µg/wipe)). All the obtained LODs and LOQs were several orders of magnitude below the European legal requirements.

3.3. Application to real samples

The validated method was applied to the analysis of twenty real commercial samples (13 baby wipes and 7 wet toilet paper), which

Table 5
Instrumental quality parameters (GC-MS).

Fragrance allergens	Lineal range (ng mL ⁻¹)	Correlation coefficient (R)	IDL (ng mL ⁻¹)	Intra-day precision ^a (RSD, %)	Inter-day precision ^a (RSD, %)
Pinene	2-1000	0.9998	0.22	0.18	6.4
Limonene	2-1000	0.9996	0.41	0.24	1.2
Benzyl Alcohol	5-1000	0.9981	1.2	1.3	0.30
Linalool	5-1000	0.9974	1.5	0.87	2.5
Methyl-2-octynoate	5-1000	0.9970	1.5	1.4	2.8
Citronellol	10-1000	0.9967	2.1	0.28	1.9
Citral	10-1000	0.9969	2.8	0.34	2.3
Geraniol	10-1000	0.9961	2.8	6.3	2.3
Cinnamal	10-1000	0.9988	2.2	0.39	5.0
Hydroxycitronellal	5-1000	0.9952	1.3	0.74	3.0
Anise Alcohol	20-1000	0.9978	6.1	1.3	1.8
Cinnamyl Alcohol	20-1000	0.9982	4.4	2.6	3.2
Eugenol	5-1000	0.9973	1.2	1.3	2.6
Methyleugenol	5-1000	0.9995	1.0	5.2	5.5
Isoeugenol	10-1000	0.9993	2.6	1.1	7.0
Coumarin	10-1000	0.9991	2.2	1.2	14
α -isomethyl ionone	2-1000	0.9983	0.61	0.27	13
Lilial®	2-1000	0.9978	0.44	0.35	5.4
Amyl Cinnamal	20-1000	0.9960	3.2	0.77	12
Lylal®	10-1000	0.9960	3.0	0.01	1.9
Amylcinnamyl Alcohol	20-1000	0.9990	6.0	1.3	3.1
Farnesol	250-1000	0.9988	70	0.20	8.3
Hexylcinnamal	10-1000	0.9979	3.0	0.87	6.4
Benzyl Benzoate	10-1000	0.9990	1.9	0.42	3.2
Benzyl Salicylate	10-1000	0.9951	2.3	1.3	2.2
Benzyl Cinnamate	10-1000	0.9961	2.3	1.8	3.2
Preservatives	Lineal range (ng mL ⁻¹)	Correlation coefficient (R)	IDL (ng mL ⁻¹)	Intra-day precision ^a (RSD, %)	Inter-day precision ^a (RSD, %)
Bronidox	10-1000	0.9971	2.6	1.6	2.8
PhEtOH	5-1000	0.9954	1.1	2.5	2.4
MeP	10-1000	0.9934	3.0	4.9	13
BHA	2-1000	0.9910	0.50	6.0	10
BHT	2-1000	0.9954	0.15	1.3	5.6
EtP	10-1000	0.9954	3.2	3.1	10
iPrP	20-1000	0.9971	3.2	0.24	15
PrP	20-1000	0.9942	3.1	1.7	9.7
IPBC	50-1000	0.9918	10	7.9	8.9
iBuP	20-1000	0.9936	3.9	0.88	11
BuP	10-1000	0.9914	2.8	2.0	9.8
Triclosan	20-1000	0.9934	3.2	2.1	14
BzP	20-1000	0.9965	6.2	5.9	15
Phthalates	Lineal range (ng mL ⁻¹)	Correlation coefficient (R)	IDL (ng mL ⁻¹)	Intra-day precision ^a (RSD, %)	Inter-day precision ^a (RSD, %)
DMP	2-1000	0.9995	0.26	0.06	4.0
DEP	2-1000	0.9995	0.13	0.14	4.7
DIBP	2-1000	0.9966	0.18	0.03	3.6
DBP	2-1000	0.9975	0.44	0.28	4.5
DMEP	2-1000	0.9946	0.59	0.37	9.6
DIPP	2-1000	0.9941	0.66	0.18	3.6
DPP	2-1000	0.9977	0.37	0.29	5.0
BBP	5-1000	1.0000	0.88	0.07	5.2
DIHP	100-2000	0.9970	20	10	15
DEHP	2-1000	0.9957	0.36	0.20	12
DCHP	2-1000	0.9931	0.22	1.6	4.9
DPhP	2-1000	0.9952	0.51	0.60	7.8
DNOP	2-1000	0.9965	0.48	0.69	3.9
DINP	100-2000	0.9992	20	10	13
DIDP	200-2000	0.9998	40	6.4	14
Musks	Lineal range (ng mL ⁻¹)	Correlation coefficient (R)	IDL (ng mL ⁻¹)	Intra-day precision ^a (RSD, %)	Inter-day precision ^a (RSD, %)
Cashmeran	2-1000	0.9969	0.53	0.96	4.1
Celestolide	2-1000	0.9946	0.16	0.17	4.6
Phantolide	2-1000	0.9957	0.16	0.02	3.7
Musk Ambrette	5-1000	0.9903	1.5	0.92	5.9
Traseolide	2-1000	0.9901	0.16	0.54	4.6
Galaxolide	2-1000	0.9954	0.22	0.32	3.3
Tonalide	2-1000	0.9968	0.21	0.55	3.7
Musk Moskene	2-1000	0.9925	0.61	1.0	5.3
Musk Tibetene	2-1000	0.9927	1.5	0.61	5.1
Ambrettolide	10-1000	0.9959	2.0	0.60	3.7
Musk Ketone	10-1000	0.9930	3.0	1.2	5.9

^a For 100 ng mL⁻¹ (excepted DIHP, DINP, DIDP: 200 ng mL⁻¹).

Table 6
Recoveries (%), precision (RSD,%), and LODs for the whole PLE/GC-MS method.

Fragrance allergens	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD ($\mu\text{g g}^{-1}$)	LOD ($\mu\text{g/wipe}$)
	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$		
Pinene	80.2 (2.4)	92.3 (1.5)	84.0 (6.1)	88.6 (4.7)	0.0011	0.0044
Limonene	84.5 (1.9)	88.1 (0.02)	96.9 (2.2)	90.4 (0.15)	0.0021	0.0082
Benzyl Alcohol	100 (2.6)	115 (3.9)	106 (3.6)	107 (2.3)	0.0059	0.023
Linalool	93.6 (15)	105 (8.0)	82.7 (11)	95.8 (1.9)	0.0075	0.029
Methyl-2-octynoate	82.4 (2.2)	92.0 (4.8)	87.3 (15)	81.6 (0.81)	0.0077	0.030
Citronellol	104 (6.3)	107 (7.8)	104 (3.1)	96.6 (6.1)	0.011	0.043
Citral	93.3 (1.7)	81.2 (15)	92.4 (4.5)	97.5 (5.0)	0.014	0.056
Geraniol	89.4 (12)	81.7 (7.0)	101 (4.8)	85.8 (1.9)	0.015	0.057
Cinnamal	92.6 (1.6)	103 (1.8)	92.0 (2.5)	81.3 (1.6)	0.011	0.044
Hydroxycitronellal	86.3 (2.5)	115 (8.3)	82.9 (6.3)	93.6 (5.7)	0.0067	0.026
Anise Alcohol	91.0 (5.5)	115 (11)	96.9 (4.4)	100 (4.5)	0.031	0.12
Cinnamyl Alcohol	92.0 (0.40)	115 (11)	90.0 (5.8)	104 (5.5)	0.023	0.089
Eugenol	95.2 (1.6)	110 (4.1)	104 (4.2)	90.7 (2.6)	0.0063	0.025
Methyleugenol	86.3 (1.7)	95.8 (2.1)	84.6 (3.4)	80.4 (4.6)	0.0054	0.021
Isoeugenol	92.7 (3.6)	108 (1.5)	95.1 (3.9)	90.4 (3.1)	0.014	0.052
Coumarin	84.6 (2.5)	103 (0.80)	82.2 (3.0)	83.2 (0.61)	0.011	0.044
α -isomethyl ionone	99.3 (9.7)	98.1 (0.80)	89.6 (3.2)	80.1 (3.0)	0.0031	0.012
Lilial®	89.5 (2.2)	96.4 (1.4)	88.8 (3.5)	80.1 (1.0)	0.0023	0.0088
Amyl Cinnamal	86.5 (15)	99.5 (0.60)	107 (4.8)	92.1 (5.3)	0.016	0.064
Lyrall®	94.9 (0.60)	113 (4.8)	103 (10)	104 (4.8)	0.015	0.060
Amylcinnamyl Alcohol	103 (1.3)	106 (3.2)	115 (1.9)	92.1 (5.3)	0.031	0.12
Farnesol	100 (12)	96.8 (6.5)	83.7 (1.2)	98.8 (3.5)	0.36	1.4
Hexylcinnamal	100 (4.1)	98.5 (1.2)	84.7 (5.7)	108 (3.4)	0.015	0.060
Benzyl Benzoate	94.3 (3.8)	96.1 (2.4)	85.6 (5.5)	104 (0.42)	0.0097	0.038
Benzyl Salicylate	99.5 (0.40)	119 (1.5)	115 (7.7)	109 (2.6)	0.012	0.047
Benzyl Cinnamate	112 (6.6)	104 (1.0)	95.8 (6.7)	83.4 (1.2)	0.012	0.046

Preservatives	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD ($\mu\text{g g}^{-1}$)	LOD ($\mu\text{g/wipe}$)
	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$		
Bronidox	81.0 (2.5)	114 (5.6)	74.9 (15)	114 (4.8)	0.013	0.052
PhEtOH	–	–	–	–	0.0057	0.022
MeP	84.1 (3.1)	104 (13)	112 (3.9)	100 (4.8)	0.015	0.060
BHA	77.3 (1.0)	104 (15)	76.9 (2.9)	105 (3.7)	0.0026	0.010
BHT	78.4 (6.0)	99.0 (2.7)	75.0 (4.3)	111 (2.0)	0.00077	0.0030
EtP	84.3 (3.4)	103 (0.81)	95.5 (3.6)	94.0 (2.0)	0.017	0.065
iPrP	81.4 (2.8)	107 (4.1)	80.0 (4.6)	111 (0.70)	0.016	0.063
PrP	82.3 (2.7)	102 (3.3)	87.8 (4.5)	83.5 (6.6)	0.016	0.062
IPBC	82.3 (0.30)	109 (11)	82.2 (8.7)	84.8 (1.8)	0.051	0.20
iBuP	82.0 (2.8)	98.9 (2.5)	96.4 (4.9)	95.5 (3.2)	0.020	0.078
BuP	94.6 (1.3)	110 (6.1)	112 (15)	115 (2.6)	0.014	0.056
Triclosan	101 (0.10)	112 (6.6)	120 (4.6)	120 (3.5)	0.017	0.065
BzP	102 (1.0)	96.3 (2.9)	120 (4.1)	90.9 (8.5)	0.032	0.12

Plasticizers	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD ($\mu\text{g g}^{-1}$)	LOD ($\mu\text{g/wipe}$)
	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$		
DMP	89.2 (3.8)	93.3 (1.8)	113 (5.9)	101 (2.0)	0.0013	0.0052
DEP	72.3 (3.0)	92.4 (0.90)	80.2 (4.0)	97.3 (0.34)	0.00067	0.0026
DIBP	95.2 (2.5)	90.1 (0.80)	101 (4.9)	79.3 (0.35)	0.00092	0.0036
DBP	115 (1.5)	91.1 (0.90)	89.3 (4.1)	110 (0.69)	0.0023	0.0088
DMEP	113 (0.80)	102 (3.7)	113 (5.9)	81.8 (1.7)	0.0030	0.012
DIPP	90.2 (1.3)	96.3 (8.3)	110 (2.1)	90.4 (9.8)	0.0034	0.013
DPP	120 (0.90)	91.5 (0.70)	118 (7.1)	84.6 (1.2)	0.0019	0.0074
BBP	114 (3.6)	104 (0.20)	118 (5.7)	90.8 (0.63)	0.0045	0.018
DIHP	118 (4.0)	108 (2.3)	94.6 (0.80)	114 (8.4)	0.10	0.40
DEHP	109 (5.1)	96.8 (0.10)	99.6 (2.6)	83.7 (4.7)	0.0018	0.0072
DCHP	114 (2.4)	104 (13)	120 (3.7)	79.9 (4.9)	0.0011	0.0044
DPhP	104 (5.6)	106 (2.3)	111 (1.8)	91.1 (4.7)	0.0026	0.010
DNOP	120 (0.02)	102 (1.6)	139 (6.1)	86.4 (5.6)	0.0024	0.0096
DINP	110 (9.3)	89.3 (9.8)	87.7 (1.5)	108 (14)	0.10	0.40
DIDP	102 (15)	101 (0.20)	71.5 (15)	97.1 (15)	0.21	0.80

Musks	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD ($\mu\text{g g}^{-1}$)	LOD ($\mu\text{g/wipe}$)
	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$	10 $\mu\text{g/wipe}$	100 $\mu\text{g/wipe}$		
Cashmeran	105 (3.5)	94.3 (2.4)	96.8 (3.9)	82.6 (0.85)	0.0027	0.011
Celestolide	104 (3.6)	87.4 (0.80)	93.9 (3.5)	79.5 (0.05)	0.00082	0.0032
Phantolide	108 (1.5)	87.6 (0.40)	93.6 (6.4)	79.6 (0.74)	0.00082	0.0032
Musk Ambrette	98.8 (4.5)	82.4 (0.10)	92.7 (2.4)	109 (2.8)	0.0075	0.029
Traseolide	109 (0.60)	n.c	99.0 (1.8)	n.c	0.00082	0.0032
Galaxolide	120 (2.3)	86.9 (0.40)	107 (4.7)	82.4 (0.42)	0.0011	0.0044
Tonalide	113 (2.8)	86.3 (0.20)	101 (4.1)	81.6 (1.1)	0.0011	0.0042
Musk Moskene	106 (2.5)	82.6 (0.50)	112 (9.5)	110 (1.5)	0.0031	0.012
Musk Tibetene	115 (1.5)	83.3 (0.90)	113 (2.0)	109 (0.09)	0.0075	0.029
Ambrettolide	102 (12)	86.9 (0.70)	99.8 (5.2)	108 (0.21)	0.010	0.040
Musk Ketone	111 (0.30)	86.2 (2.1)	115 (5.3)	112 (1.3)	0.015	0.060

(–) Not calculated since initial concentration is higher than the spiked level (see Table 7).

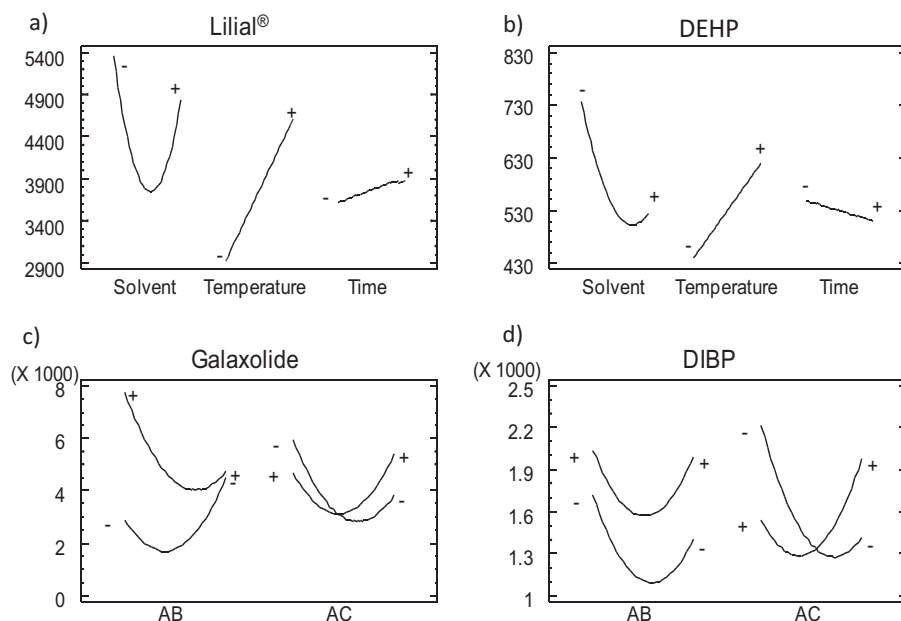


Fig. 4. Main effect plots for (a) lilial® (b) DEHP, and interaction plots for (c) galaxolide, (d) DIBP.

represent a wide variety of these kinds of baby care products. The analysis of the considered groups of cosmetic ingredients or additives is complex due to the own nature of the wipe samples and due to the fact that the analytes can be present from traces concentrations at the sub $\mu\text{g g}^{-1}$ level up to several thousands of $\mu\text{g g}^{-1}$. The inclusion of injection blanks (after each sample analysis) and process blanks is mandatory.

Thirty-six of the 65 targets were found in the analyzed samples. Results are shown in Table 7 (expressed as $\mu\text{g g}^{-1}$) and Table S1 (expressed as μg per wipe). The recoveries of benzyl alcohol- d_7 , MeP- d_4 and DEHP- d_4 (surrogate standards) were satisfactory, with values between 84 and 111%, 85 and 112%, and 74 and 119%, respectively. Fig. 5 shows the chromatograms of two real samples, a baby wipe (S1), and a wet toilet paper (S20), highlighting the presence of PhEtOH, and MeP with concentration levels up to 0.25% (2500 $\mu\text{g g}^{-1}$)

3.3.1. Fragrance allergens

Nineteen of the twenty-six fragrance allergens were detected in the samples at concentrations between 0.0341 and 2446 $\mu\text{g g}^{-1}$, and all samples except S12 (labeled as fragrance free) contained fragrance allergens. Limonene, benzyl alcohol and linalool were the most frequently found (45–70% of the samples) with values up to 2446 $\mu\text{g g}^{-1}$ (0.2%). Also geraniol, and benzyl benzoate were detected in 5 samples at concentration levels below 13 $\mu\text{g g}^{-1}$. Regarding the number of compounds by cosmetic sample, S3 contained 9 of the target fragrance allergens, highlighting benzyl alcohol concentration (711 $\mu\text{g g}^{-1}$), whereas the other samples contained between 1 and 8 target analytes. All samples fulfilled European restrictions regarding this group of ingredients.

3.3.2. Preservatives

In the case of preservatives, phenoxyethanol was found in all samples, at very high concentration in many of them (>1300 $\mu\text{g g}^{-1}$, 0.13%), although these concentration levels do not exceed the maximum concentration permitted by the European legislation (1%). In the case of parabens, 6 of the 7 targets were detected. The most common were MeP and EtP (50 and 40% of the samples, respectively) at concentrations up to 0.3%. PrP and BuP (will be banned from April 2015) were also found in 6 and 4 samples,

respectively. iPrP and iBuP, which have been recently banned for their use in cosmetics and personal care products (the prohibition entered into force in October 2014), have been detected in 7 and 1 samples, respectively, at concentration between 0.0934 and 247 $\mu\text{g g}^{-1}$. IPBC, banned in products intended for children under 3 years was detected in 1 sample (S14) at 12.7 $\mu\text{g g}^{-1}$ (in this case, the label indicates do not use this product for children under 3 years). BHT was detected in eight samples at low levels (<0.694 $\mu\text{g g}^{-1}$). The maximum number of target preservatives per sample was seven (S1), highlighting MeP and phenoxyethanol at concentrations higher than 0.1%. 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.

All samples comply with European legislation in terms of maximum concentration permitted since phenoxyethanol and parabens (in this last case the samples were acquired before October 2014) do not exceed these levels (see Table 1) and the presence of IPBC is indicated in the label. Although European Regulation does not require the inclusion of preservatives in the personal care products list of ingredients, in general, all of them were declared.

3.3.3. Musk

Only 3 of the 10 studied musks were detected in the samples. Galaxolide was the most found, 40% of the samples, at levels below 0.7 $\mu\text{g g}^{-1}$, except S6 and S20 with concentrations of 10.8 and 21.4 $\mu\text{g g}^{-1}$, respectively. Tonalide and cashmeran were detected in 3 samples, at low levels (<1.85 $\mu\text{g g}^{-1}$). Most samples only contain galaxolide, excluding S19 that presents cashmeran, galaxolide and tonalide at concentrations between 0.044 and 1.85 $\mu\text{g g}^{-1}$. Only tonalide presents restrictions about its maximum concentration permitted in personal care products and, in all cases, concentrations were below the established limit.

3.3.4. Phthalates

Five phthalates were detected in 18 samples. The most found was DEP (in 80% of the samples), highlighting its high concentration in S20 (412 $\mu\text{g g}^{-1}$). The banned DEHP, and DBP were also detected

Table 7
Analysis of commercial baby wipes and wet toilet paper ($\mu\text{g g}^{-1}$).

Fragrance allergens	Baby wipes													Wet toilet paper						
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Pinene	7.33			1.09									0.107							
Limonene	180	0.741	12.4	23.9	0.212	1.89			0.0669	0.0360	1.61		0.273		0.239		0.0760	0.262		2.67
Benzyl Alcohol	5.99		711	3.40		3.01	0.160	2446	0.0341	0.0448				1.67	2.81		0.0656		0.172	7.06
Linalool	76.0	32.8		59.9					1.10	1.04	0.810							0.492	0.698	14.6
Citronellol									1.82											
Citral	39.9			6.60																
Geraniol		12.7							0.311	0.250	1.21									11.1
Hydroxycitronellal	200										0.203									
Anise Alcohol																		11.8		56.4
Cinnamyl Alcohol								0.503			0.741						0.207			32.3
Coumarin		14.0																		77.0
α -isomethylionone		5.37				0.292		4.10			0.805									
Lilial®		0.0435	1.51	7.98			0.103													
Lyrall®			0.932																	
Amylcinnamyl alcohol			0.470														1.29			
Farnesol			1.76																	
Hexylcinnamal		0.189	2.25			60.1														
Benzyl Benzoate		0.0613	0.0488					5.92			0.0585					0.0750				
Benzyl Salicylate			0.0617								0.168								0.0491	
Preservatives ^a	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
PhEtOH	2459	1303	587	8368	4743	799	2412	2012	229	664	435	2297	727	2.68	2073	3427	19.5	3779	714	74.3
MeP	1213	11.3	0.230	3017	235	267		0.382							739		2.16			1387
BHT	0.602					0.638	0.0162					0.0302			0.0369	0.126	0.694	0.0311		
EtP	290	4.80		704	286	55.8									7.94				0.132	587
iPrP															8.66					
PrP	126	1.41		315	486	27.0									226					
IPBC														12.7						
iBuP	97.0	0.0934	1.48	247			2.71	2.03						0.113						
BuP	218	2.63		600		54.1														
Phthalates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
DEP	0.770	0.168	0.0285		0.0420	46.9	0.364				55.5	0.842	1.09	1.43	1.09	0.802	2.26	0.471	3.24	412
DIBP		0.0763	0.0404			0.0446	0.189					0.0481		0.102		0.155		0.111	0.0907	
DBP		0.130	0.136	0.176			0.130					0.0556		0.0359	0.132	0.0980		0.111		
DEHP			0.0357		0.161				0.0955				0.0351		0.225	0.126			0.0277	
DCHP									0.108											
Musks	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Cashmeran								0.183								0.480			1.85	
Galaxolide		0.735				10.8						0.332	0.0508		0.0610			0.151	0.0414	21.4
Tonalide											0.865					0.0270			0.0641	

^a Parabens concentration expressed as acid.

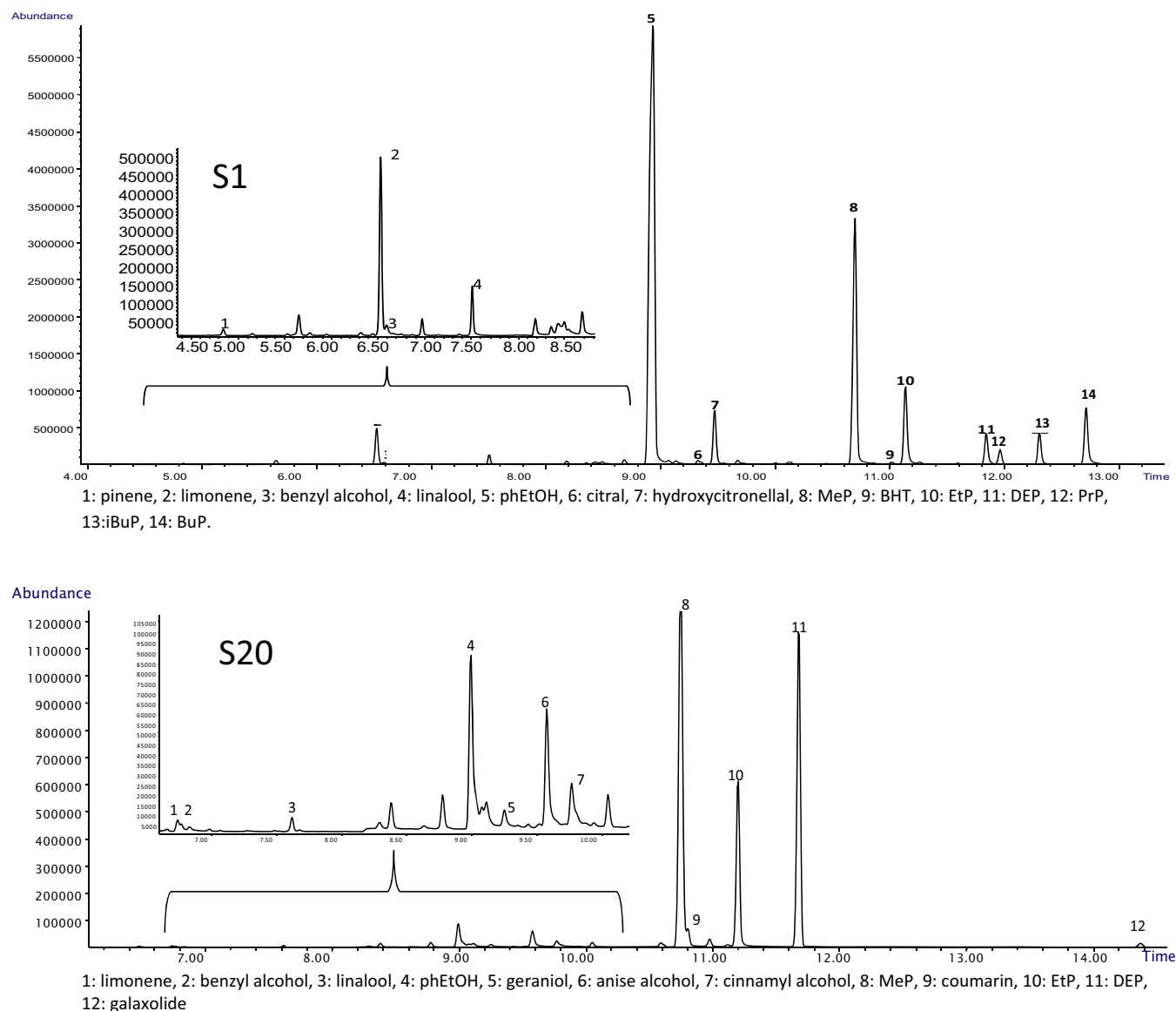


Fig. 5. Chromatograms for samples S1 and S20 (see concentrations in Table 7).

in 13 samples at low levels ($<0.225 \mu\text{g g}^{-1}$). DCHP only was found in one sample at $0.108 \mu\text{g g}^{-1}$.

4. Conclusions

A pressurized liquid extraction followed by gas chromatography–mass spectrometry method has been developed for the determination of fragrance allergens, preservatives, phthalates, and musks in baby wipes and wet toilet paper intended for children. Twenty-five of the 65 target analytes are banned or subjected to restrictions according to European Legislation (EC No 1223/2009). The PLE–GC–MS method was optimized by means of experimental design in order to select optimal PLE conditions (MeOH, 110°C and 5 min). The method exhibited a satisfactory performance in terms of linearity, sensitivity, accuracy and precision, with mean recoveries of 90% and RSD values generally below 10%. IDLs were well below the established regulated limits. Finally, the validated method was applied to a high number of real samples available at the market. Most of the target substances were found in the samples at concentration levels from the sub parts per million to the parts per million. Nineteen of the 26

target fragrance allergens were detected in the analyzed samples. Limonene, benzyl alcohol and linalool were the most frequently found (45–70% of the samples) with values up to 0.2%. In the case of preservatives, phenoxyethanol was detected in all samples at high concentrations in some cases ($>0.2\%$ in 9 samples), MeP, and EtP were found in 40–50% of samples and the recently banned iPrP and iBuP, have been detected in seven and one samples, respectively, at concentration levels between 0.0934 and $247 \mu\text{g g}^{-1}$. In the case of phthalates and musks, five and three target analytes were detected, respectively, including the forbidden phthalates DBP, and DEHP. In addition, 25% of the samples did not fulfill the labeling requirements for fragrance allergens. It is important to note that according to the last updates of the European legislation, 50% of the analyzed commercial samples would be prohibited from April 2015.

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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.2015.01.049>.

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Supporting information

for

**Pressurized liquid extraction-gas chromatography-mass spectrometry analysis of
fragrances allergens, musks, phthalates and preservatives in baby wipes.**

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Table S1. Analysis of commercial baby wipes and wet toilet paper (µg/wipe)

	BABY WIPES												WET TOILET PAPER							
<i>Fragrances allergens</i>	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Pinene	18.2			4.28									0.690							
Limonene	448	3.96	107	93.4	0.777	10.2			0.591	0.201	7.82		1.75		0.740		0.437	0.948		17.0
Benzyl Alcohol	14.9		6135	13.3		16.2	0.957	7164	0.301	0.250				5.85	8.67		0.377		0.502	45.0
Linalool	189	175		235					9.67	5.78	3.92							1.78	2.03	93.1
Citronellol									16.1											
Citral	99.3			25.8																
Geraniol		67.7							2.75	1.40	5.87									70.5
Hydroxycitronellal	498										0.986									
Anise Alcohol																		42.6		359
Cinnamyl Alcohol								1.47			3.61						1.19			206
Coumarin		74.6																		491
α-isomethylionone		28.7				1.57		12.0			3.92									
Lilial®		0.232	13.0	31.3			0.616													
Lyrall®			8.04																	
Amylcinnamyl alcohol													1.65				7.40			
Farnesol			15.2																	
Hexylcinnamal		1.1	19.4			323														
Benzyl Benzoate		0.330	0.421					17.4			0.285					0.291				
Benzyl Salicylate			0.530								0.816								0.143	
<i>Preservatives^a</i>	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
PhEtOH	6116	6961	5065	32770	17392	4292	14464	5893	2026	3705	2117	16500	4664	9.38	6406	13297	111	13673	2079	473
MeP	3017	60.3	1.99	11815	861	1433		1.12							2283		12.4			8837
BHT	1.49					3.43	0.0970					0.234				0.489	3.99	0.112		
EtP	721	25.6		2757	1048	300									24.5				0.384	3738
iPrP															26.7					
PrP	312	7.55		1235	1781	145									697					
IPBC													44.4							
iBuP	240	0.499	12.8	967			22.8	5.96					0.395							
BuP	541	14.0		2350		291														
<i>Phthalates</i>	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
DEP	1.91	0.895	0.246		0.155	252	2.18				270	6.05	7.02	4.99	3.38	3.11	13.0	1.70	9.43	2628
DIBP		0.407	0.349			0.240	1.13					0.350		0.358		0.601		0.400	0.264	
DBP		0.697	1.17	0.689			0.78					0.399		0.126	0.408	0.380		0.421		
DEHP			0.308		0.591				0.843				0.225		0.696	0.489			0.0807	
DCHP									0.952											
<i>Musks</i>	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Cashmeran								0.536							1.48				5.39	
Galaxolide		3.93				57.9						2.39	0.326		0.188			0.546	0.121	137
Tonalide											4.21					0.105			0.187	

^a Parabens concentration expressed as acid.