

Solid-phase microextraction gas chromatography-mass spectrometry determination of fragrance allergens in baby bathwater

J. Pablo Lamas · Lucia Sanchez-Prado ·
Carmen Garcia-Jares · Maria Llompарт

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Abstract A method based on solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) has been optimized for the determination of fragrance allergens in water samples. This is the first study devoted to this family of cosmetic ingredients performed by SPME. The influence of parameters such as fibre coating, extraction and desorption temperatures, salting-out effect and sampling mode on the extraction efficiency has been studied by means of a mixed-level factorial design, which allowed the study of the main effects as well as two-factor interactions. Excluding desorption temperature, the other parameters were, in general, very important for the achievement of high response. The final procedure was based on headspace sampling at 100 °C, using polydimethylsiloxane/divinylbenzene fibres. The method showed good linearity and precision for all compounds, with detection limits ranging from 0.001 to 0.3 ng mL⁻¹. Reliability was demonstrated through the evaluation of the recoveries in different real water samples, including baby bathwater and swimming pool water. The absence of matrix effects allowed the use of external standard calibration to quantify the target compounds in the samples. The proposed procedure was applied to the determination of allergens in several real samples. All the target compounds were found in the samples, and, in some cases, at quite high concentrations. The presence and the levels of these chemicals in baby bathwater should be a matter of concern.

Keywords Fragrance allergens · Allergens · Cosmetics · Personal care products · Solid-phase microextraction · Water analysis · Multifactor optimization · Factorial design

Introduction

Personal care products play an intimate role in our daily routines [1, 2]. They are complex formulations that contain a whole host of chemicals. Baby products are not different from this. The average baby hygiene product and especially baby bath products and bubble baths contain a complex mix of chemicals, including artificial dyes and colours, fragrances, phthalates, preservatives, harsh chemicals and sodium lauryl sulphate (used to promote lathering).

While children have never been better protected in some ways, mounting evidence suggests they have never been more vulnerable to things we have always considered safe: air, water and consumer products such as bubble bath. Children inhale more air than adults do, their skin is about five times thinner and is significantly more permeable: what goes on it, in general, goes into the child's body in some form. And once in there, it may have a surprisingly strong effect [3]. According to one briefing on baby toiletries from the Women's Environmental Network [4], infants lack a blood-brain barrier to prevent blood-borne toxins entering the brain until they are 6 months old and, in consequence, low-level exposures that would have little or no effect on an adult brain can sabotage a fetal one.

Most of us assume that the chemicals we put on our babies and children daily have been thoroughly tested and regulated, and are safe. This is technically correct: the ingredients within any toiletry and cosmetic product are subject to EU regulations and testing [5, 6]. But some toxicologists, and many environmental groups, believe such

J. P. Lamas · L. Sanchez-Prado · C. Garcia-Jares ·
M. Llompарт (✉)
Departamento de Quimica Analitica, Nutricion y Bromatologia,
Facultad de Quimica, Instituto de Investigacion y Analisis
Alimentario, Campus Sur, Universidad de Santiago de
Compostela,
15782 Santiago de Compostela, Spain
e-mail: maria.llompарт@usc.es

tests are inadequate. Even when the product is correctly labelled indicating the presence of those regulated ingredients, there will be no guaranties it has been tested for safety by an independent regulatory panel. In addition, of chemicals that have been tested, few have been examined for their effects on children's health [3, 7].

In the opinion of some experts, the escalating use of unnecessary products in the name of hygiene could certainly be doing our children's skin more harm than good [8]. Our consumption of products such as bubble baths, lotions, oils, talcs, wipes and even baby perfumes has shot up in recent decades. The number of children affected nowadays by eczema and asthma has increased significantly.

This problem has transcended the scientific world and is a subject of social concern [9]. The most used baby products are those intended for use in the bath, such as shampoos, bubble baths, shower gels and soaps. These products also contain some detergents that can break down the skin's natural barrier, and so it allows other irritants and allergens to penetrate it. In developed countries, the daily baby bath is a common practice. Some babies and children spend a long time playing in the bath. The exposure to these chemicals is not only through dermal absorption, but is also by inhalation and ingestion (it is quite common to swallow bathwater either intentionally or unintentionally). In this sense, some experts recommend considering bathing babies a little less often and using soap only when it is truly required.

A group of these hazardous chemicals included in most personal care products are the allergen fragrances. The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has identified 26 of these ingredients as likely to cause contact allergies [5, 10], and the EU Cosmetic Directive [5] requires an indication of the presence of potential fragrance allergens in cosmetic products if a limit of 0.01% for rinse-off and 0.001% for leave-on products is exceeded [5].

It is important to underline that some of these substances not only pose the risk of causing allergies, but there is also evidence of brain and nervous system effects at low doses, as well as immunotoxicity and broad systemic effects in animal studies [11, 12]. In fact, one of these ingredients, methyleugenol, was included in 2002 in the list of forbidden substances in the EU Cosmetic Directive [5], owing to the potential risk of inducing cancer [13].

Analytical methods for the determination of this group of substances are mainly based on gas chromatography-mass spectrometry (GC-MS) [14–17]. Most of these methods are focused on the determination of these compounds in cosmetics. Owing to the difficulty of obtaining a good compound resolution as well as with other matrix components, advanced methods based on multidimensional chromatography have been proposed [18, 19].

In the present study, we developed a method for the identification and quantification of 15 of the most common fragrance allergens [5, 10] in baby bathwaters (see Table 1). We have not found other studies on the determination of these fragrances in water. The analytical procedure is based on solid-phase microextraction (SPME) followed by GC-MS analysis. This is also the first application of SPME specifically devoted to the determination of fragrance allergens. The proposed method was finally applied to a variety of baby bathwaters collected at the end of the bath. All the target compounds were found in the samples, and, in some cases, at quite high concentrations.

Experimental

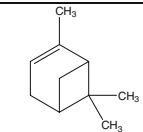
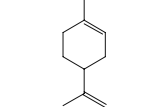
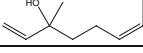
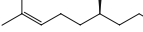
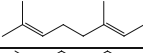
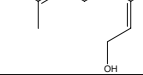
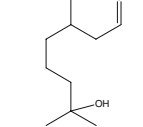
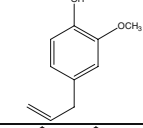
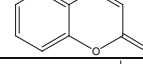
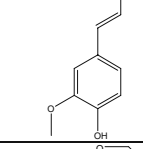
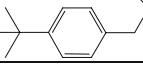
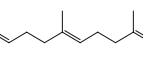
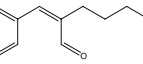
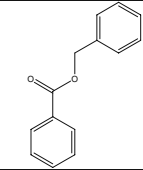
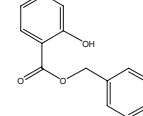
Reagents and materials

3,7-Dimethyl-1,6-octadien-3-ol (linalol; 97%), 3,7-dimethyloct-6-en-1-ol (β -citronellol; 95%), 2-methoxy-4-prop-2-enyl phenol (eugenol; 99%), 2*H*-1-benzopyran-2-one (coumarin; 99%); 3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol, mixture of isomers; 95%), 3,7-dimethylocta-2,6-dienal (cital, *cis/trans*; 95%), 1-methyl-4-prop-1-en-2-yl-cyclohexene [(*R*)-(+)-limonene; 97%) and 2-methoxy-4-(1-propenyl) phenol (isoeugenol, *cis/trans*; 98%) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene [(–)- α -pinene; $\geq 99\%$], 3,7-dimethyl-2,6-octadien-1-ol (geraniol; $\geq 96\%$), 2-hydroxyphenylmethyl ester benzoic acid (benzyl salicylate; $\geq 99\%$) and 3-(4-*tert*-butylphenyl)-2-methylpropanal (lilial; $\geq 95\%$) were purchased from Fluka Chemie (Steinheim, Germany). 7-Hydroxy-3,7-dimethyloctanal (hydroxycitronellal; $\geq 95\%$) and 2-(phenylmethylene)octanal (hexylcinnamaldehyde; $\geq 95\%$), were purchased from SAFC Supply Solutions (St. Louis, USA). Phenylmethyl benzoate (benzyl benzoate; 98.5%) was purchased from Chem Service (West Chester, USA). Table 1 shows the Chemical Abstract Service registry numbers, the physicochemical properties and the chemical structures of the target compounds.

Methanol, ethyl acetate and acetone were provided by Merck (Darmstadt, Germany). Individual stock solutions of each compound ($2,000 \mu\text{g mL}^{-1}$) were prepared in methanol. Further dilutions and mixtures were prepared in ethyl acetate and acetone. The latter were employed for spiking water samples. Working Milli-Q water solutions with concentrations from 0.1 to 50 ng mL^{-1} were made by appropriate dilution and were then stored in amber glass vials at -20°C .

NaCl was provided by VWR Prolabo (Fontenay-sous-Bois, France). All solvents and reagents were of analytical grade. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA).

Table 1 Chemical Abstract Service (CAS) number, IUPAC names, molecular structures and formulas, and main properties of the allergens studied

Compound	CAS number	IUPAC name	Molecular structure	Molecular formula	MW (g/mol)	Log K _{OW}	Boiling Point (°C)	Solubility (mg/L)
Pinene	80-56-8	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene		C ₁₀ H ₁₆	136	4.37	155	18
Limonene	95327-98-3	1-methyl-4-prop-1-en-2-yl-cyclohexene		C ₁₀ H ₁₆	136	4.57	176	13.8
Linalol	78-70-6	3,7-dimethylocta-1,6-dien-3-ol		C ₁₀ H ₁₈ O	154	3.28	198	1589
Citronellol	106-22-9	3,7-Dimethyloct-6-en-1-ol		C ₁₀ H ₂₀ O	156	3.38	225	322
Citral	5392-40-5	3,7-dimethylocta-2,6-dienal		C ₁₀ H ₁₆ O	152	3.17	229	590
Geraniol	106-24-1	3,7-dimethyl-2,6-octadien-1-ol		C ₁₀ H ₁₈ O	154	3.28	229	531
Hydroxy-citronellal	107-75-5	7-hydroxy-3,7-dimethyloctanal		C ₁₀ H ₂₀ O ₂	172	1.54	240	23800
Eugenol	97-53-0	2-methoxy-4-prop-2-enyl-phenol		C ₁₀ H ₁₂ O ₂	164	2.20	256	<1000
Coumarin	91-64-5	2H-1-Benzopyran-2-one		C ₉ H ₆ O ₂	146	1.39	301	2500
Isoeugenol	97-54-1	Phenol, 2-methoxy-4-(1-propenyl)-		C ₁₀ H ₁₂ O ₂	164	2.45	267	984
Lilial	80-54-6	3-(4-tert-Butylphenyl)-2-methylpropanal		C ₁₄ H ₂₀ O	204	4.07	279	33
Farnesol	4602-84-0	3,7,11-trimethyldodeca-2,6,10-trien-1-ol		C ₁₅ H ₂₆	222	5.31	111	267
HCA	101-86-0	Octanal, 2-(phenylmethylene)		C ₁₅ H ₂₀ O	216	4.82	174	2.8
Benzyl benzoate	120-51-4	Benzyl benzoate		C ₁₄ H ₁₂ O ₂	212	3.97	323.5	19.8
Benzyl salicylate	118-58-1	phenylmethyl 2-hydroxybenzoate		C ₁₄ H ₁₂ O	228	4.31	208	<1000

The SPME manual holders and fibres were supplied by Supelco (Bellefonte, PA, USA). Five different commercial fibre coatings were used throughout the present work: 85 μm polyacrylate (PA), 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS) and 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Prior to first use, fibres were conditioned as recommended by the manufacturer.

Different real baby bathwater samples as well as an indoor swimming pool water sample were collected in amber glass containers. The products for daily baby care, including shampoos and bubble baths, were among the most frequently used in Spain. The excess of free chlorine in the water samples was removed by addition of sodium thiosulphate (0.1 mg mL⁻¹). Samples were stored in the dark at 4 °C until analysis.

Gas chromatography-mass spectrometry

The GC-MS analysis was performed using a Varian 3800-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to a Varian Saturn 2000 ion trap mass spectrometer (Varian Chromatography Systems) equipped with a 1079 split/splitless injector. The system was operated by Saturn GC-MS workstation version 5.4 software.

Separation was carried out on a J&W DB-WAX capillary column (50 m $\times$ 0.20-mm inner diameter, 0.20- μm film thickness) from Agilent Technologies (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as the

carrier gas at a constant column flow of 1.0 mL min⁻¹. The gas chromatograph oven temperature was programmed from 45 °C (held for 2 min) to 230 °C at 15 °C min⁻¹ (held for 16 min) (total analysis time 30.33 min).

Splitless mode (maintained for 2 min) was used for injection; the split flow was set at 50 mL min⁻¹ and the injector temperature was kept at 260 °C.

The ion trap mass spectrometer was operated in the electron impact ionization mode (+70 eV). Manifold, ion trap and transfer line temperatures were maintained at 110, 200 and 220 °C, respectively.

In the full-scan mode the mass range was varied from 39 to 250 m/z at 0.7 s per scan, starting at 4 min and ending at 15 min. From 15 min until the end of the analysis (30.33 min), the mass range of the full-scan mode was varied from 50 to 250 m/z at 0.7 s per scan. The filament emission current was 10 μA . The analytes were positively identified by comparison of their mass spectra and retention times with those of standards. The identification and quantification ions and retention times for each target compound are listed in Table 2.

Solid-phase microextraction

Aliquots of 10 mL water sample were placed in 22-mL headspace vials, into which 0, 1 or 2 g NaCl, as necessary for the experiment, had previously been added. Then, the vials were sealed with aluminium caps furnished with Teflon-faced septa and immersed in a water bath maintained at the selected extraction temperature (50 , 75 or 100 °C). Samples were allowed to equilibrate for 5 min

Table 2 Retention times, quantification and identification ions of the target compounds

Retention time (min)	Compound	Quantification ions	Identification ions
7.96	Pinene	93, 77	93, 77, 41, 121
8.41	Limonene	67, 93	67, 93, 39, 136
11.61	Linalol	93, 71, 43	93, 71, 43, 55
12.84	Citral	39, 41, 69	39, 41, 69, 84
13.33			
13.41	Citronellol	67, 69, 41	67, 69, 41, 81
14.05	Geraniol	41, 69	41, 69, 123, 93
14.79	Hydroxycitronellal	43, 59, 71	43, 59, 71, 41
15.79	Lilial	189	189, 147, 131
16.79	Eugenol	164	164, 131, 103
18.10	Farnesol	69, 41	69, 41, 81, 121
18.22			
18.57			
18.74	Isoeugenol	164	164, 77, 103
19.00	Hexylcinnamaldehyde	129, 216	129, 216, 117
20.81	Coumarin	146, 118	146, 118, 89
23.93	Benzyl benzoate	105, 194	105, 194, 91, 77
28.28	Benzyl salicylate	91	91, 65, 39

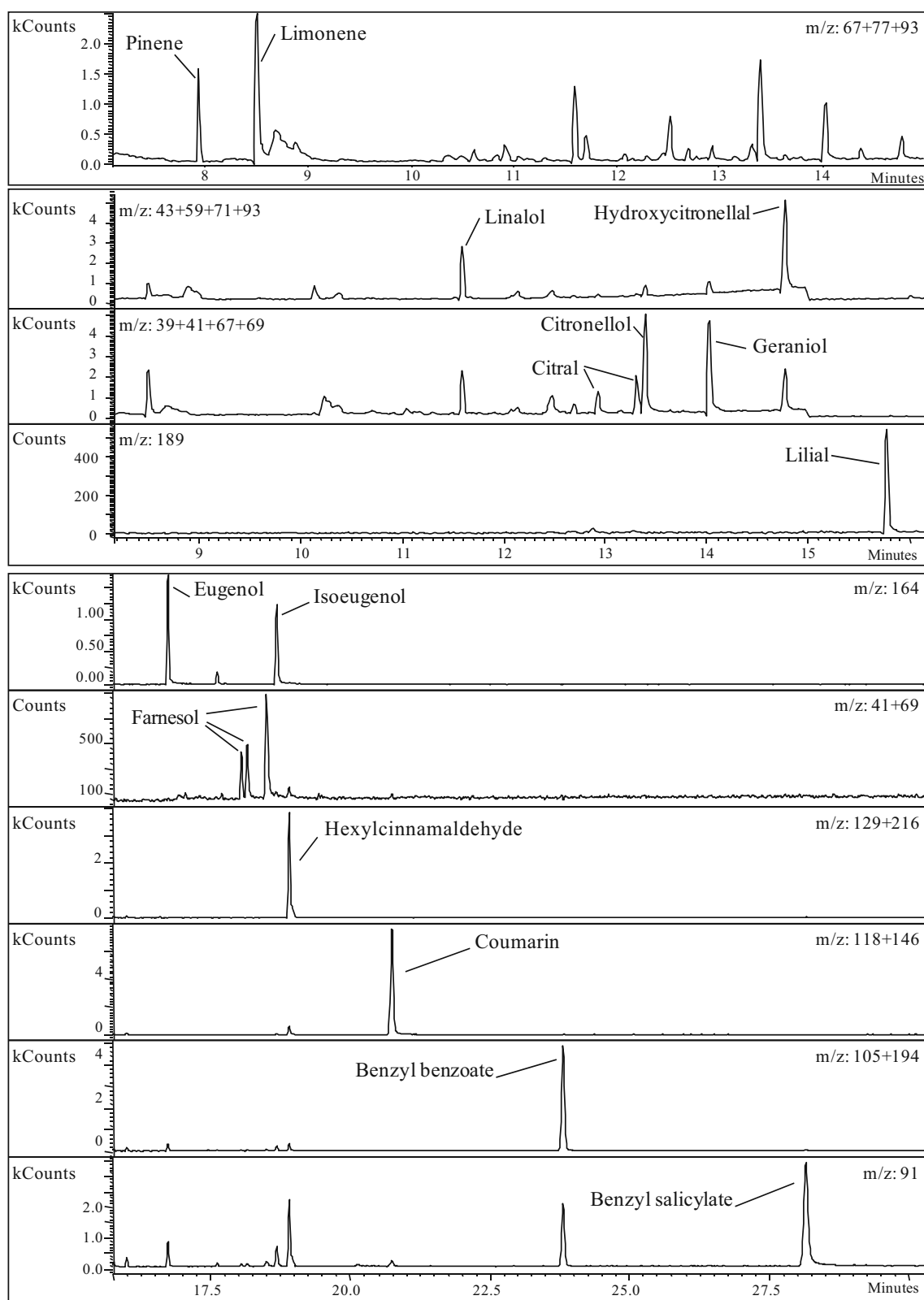
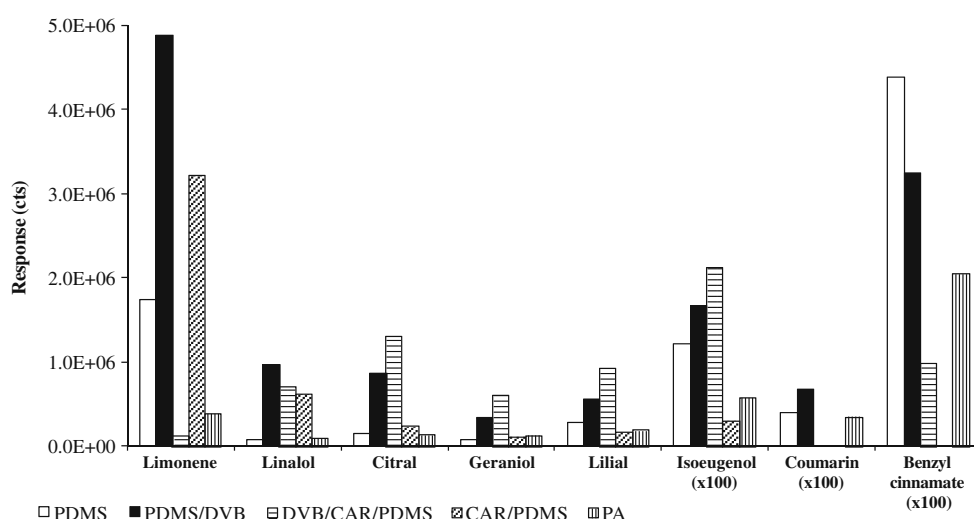


Fig. 1 Gas chromatography-mass spectrometry (GC-MS) ion chromatograms obtained from the direct injection of an ethyl acetate solution containing $50 \mu\text{g mL}^{-1}$ of each fragrance allergen

Fig. 2 Influence of the fibre coating on the headspace solid-phase microextraction (HS-SPME) of some selected allergens. *PDMS* polydimethylsiloxane, *DVB* divinylbenzene, *CAR* Carboxen, *PA* polyacrylate



before placement of the SPME device. Afterwards, the fibre was exposed to the headspace over the sample (headspace SPME, HS-SPME) or immersed into the sample (SPME) for 20 min, depending on the experiment. A stir bar was introduced into the sample vial and magnetic stirring was performed during all the extraction process. Once the exposure period had finished, the fibre was retracted into the needle of the holder syringe and immediately inserted into the gas chromatograph injector. Desorption was carried out at 220 or 260 °C depending on the experiment.

Under the final optimized conditions, the target compounds were extracted at 100 °C for 20 min in the headspace mode using a PDMS/DVB fibre over the stirred samples with addition of 2 g NaCl. Fibre desorption was performed at 220 °C. Possible carryover was checked and, under the selected conditions, it was not observed. Blanks were periodically run during the analysis to confirm the absence of contamination.

Results and discussion

Multivariate optimization of the SPME process

First, experiments were conducted to optimize the chromatographic separation of the target analytes as well as to select the quantification ions to obtain the maximum signal-to-noise ratio. The chromatographic conditions were summarized in “Experimental” and the identification and

quantification ions are included in Table 2. Figure 1 shows the ion chromatograms of an ethyl acetate standard mixture of allergen fragrances at a concentration of 50 µg mL⁻¹.

The analytes included in this study comprise alcohols, aldehydes, esters, ethers and one lactone, compounds with very different physicochemical properties, so an appropriate optimization is essential for the development of a suitable method for the simultaneous extraction of all analytes (see Table 1). It is important to study not only the individual effects of the factors affecting the SPME process, but also the possible interaction effects between the main factors. Thus, the optimization was carried out with the use of an experimental design strategy.

Initial SPME experiments were carried out with the purpose of selecting the factors as well as the factor levels to be considered in the multivariate experimental approach. In these experiments, five fibres were tested (PDMS, PDMS/DVB, DVB/CAR/PDMS, CAR/PDMS, PA) with sampling in the headspace mode (HS-SPME) at 50 °C for 20 min. The results obtained for some of the target analytes are represented in Fig. 2. Three of the fibres were not satisfactory, giving, in general, low responses. The most appropriate fibres were PDMS/DVB and DVB/CAR/PDMS; nevertheless, the latter fibre was not adequate for the extraction of limonene and coumarin. Therefore, PDMS/DVB was selected as the most suitable fibre for the simultaneous determination of all analytes.

Some previous tests were also performed at different extraction temperatures in both direct (SPME) and head-

Table 3 Factors and levels considered in the experimental design

Factor	Code	Low level (–)	High level (+)	Continuous
Extraction temperature (°C)	A	50	100	Yes
Sampling mode	B	SPME	HS-SPME	No
NaCl (% w/v)	C	0.0	20.0	Yes
Desorption temperature (°C)	D	220	260	Yes

HS headspace SPME solid-phase microextraction

space (HS-SPME) sampling modes, demonstrating that both factors, extraction temperature and sampling mode, were very important and interrelated, and that both deserved their inclusion in the factorial design optimization. Room temperature was inappropriate for HS-SPME, giving lower results than 50 °C for SPME; therefore, this temperature value was discarded for further optimization.

A multivariate strategy of optimization was carried out to assess the influence of the main factors on the microextraction process, to select the optimal working conditions. The factors included in the factorial design were as follows: extraction temperature (A), at three levels (50, 75 and 100 °C); sampling mode (B), at two levels (SPME and HS-SPME,); salt addition (NaCl) (C), at two levels (0 and 20%), and desorption temperature (D), at two levels (220 and 260 °C). The factors and levels considered, as well as the corresponding codes, are summarized in Table 3.

A $3 \times 2^{3-1}$ mixed-level multifactor design, which involved 12 runs and two central points, was selected, allowing two degrees of freedom to estimate the experimental error. This design has resolution V, which means that it is capable of evaluating all main effects and all two-factor interactions. Numerical analysis of data resulting from the experimental design was performed with the statistical software package

Statgraphics Centurion XV (Manugistics, Rockville, MD, USA). The experiments were carried out with 10-mL aliquots of Milli-Q water spiked at 25 ng mL⁻¹ with the target analytes. Samples were magnetically stirred and the sampling time was set at 20 min to allow maximum throughput.

The selected design allows us to interpret the results using statistical tests and graphic tools to determine which factors and interactions have a statistically significant effect.

Pareto charts of the standardized effects for the main factors and two-factor interactions are shown in Fig. 3. The length of each bar is proportional to the absolute value of its associated standardized effect. The standardized effect is obtained by dividing the estimated effect of each factor or interaction by its standard error. The vertical dotted line in the graph represents the statistically significant bound at the 95% confidence level. As can be seen in the figure, desorption temperature (D) was not statistically significant for any of the target compounds, excluding benzyl benzoate; in contrast, the other three main factors were, in general, quite important, although their effects varied depending on the compound. The interactions AB and BC (see the factor codes in Table 3) were also significant in many cases. The quadratic term AA was also significant for some analytes,

Fig. 3 Pareto charts for some selected allergens (see the factor codes in Table 3)

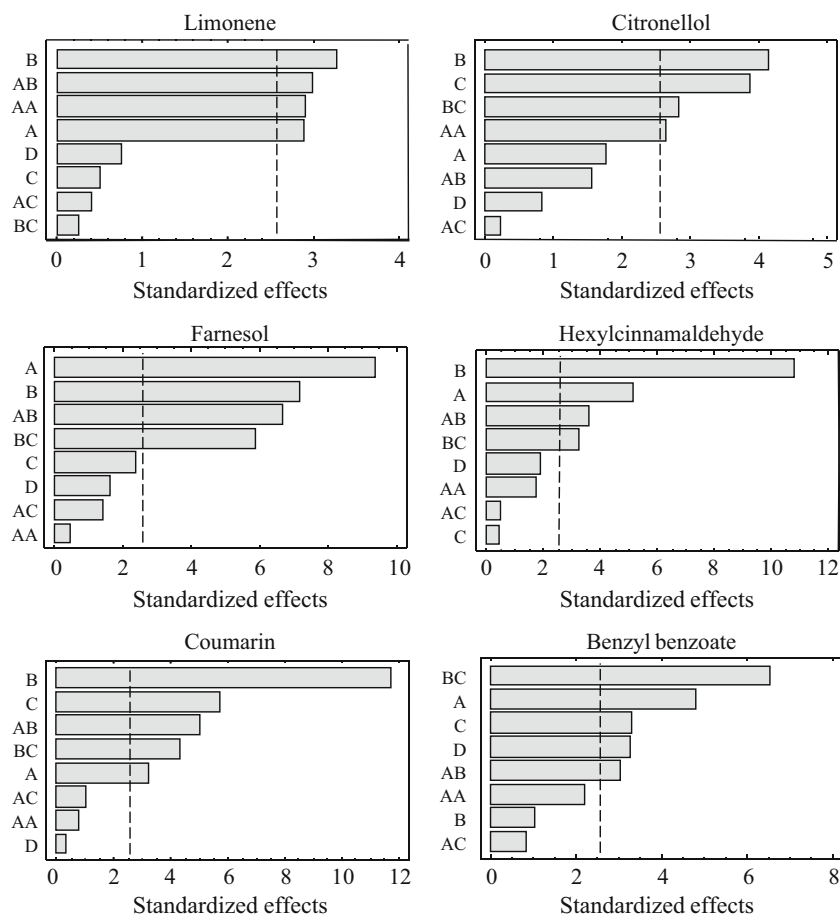
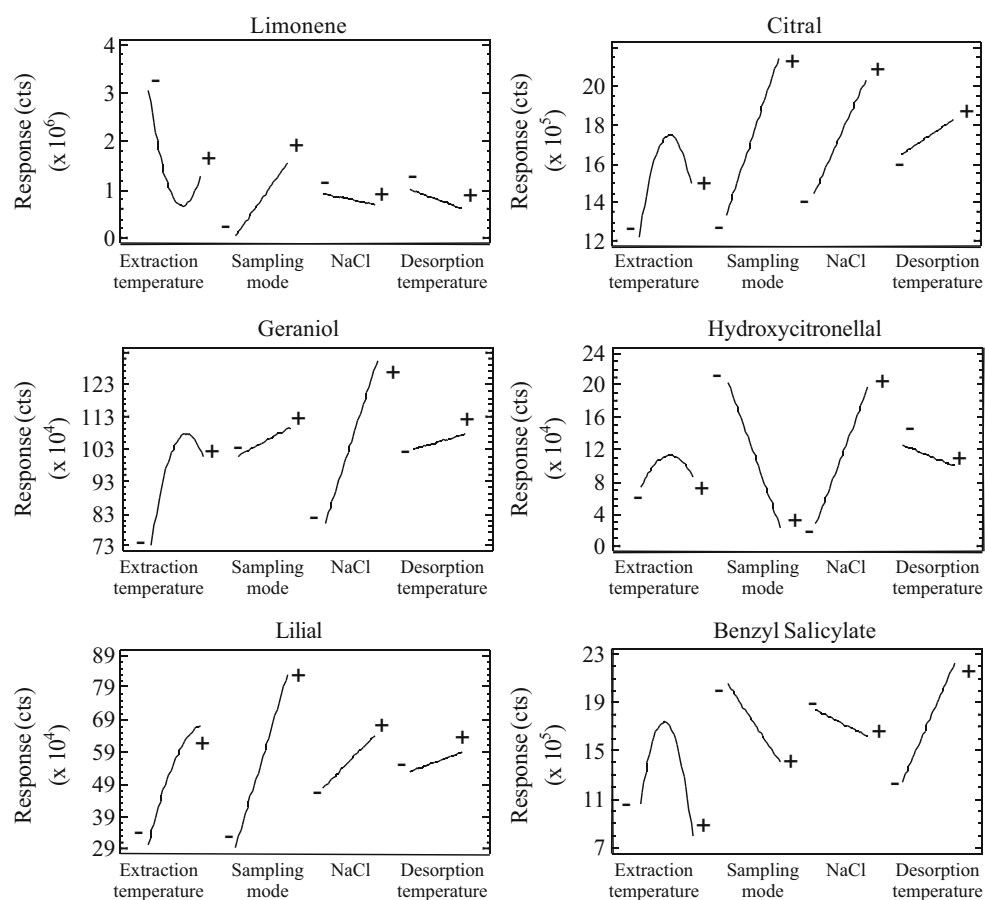


Fig. 4 Main effects plots for some selected allergens (see the factor codes and levels in Table 3)

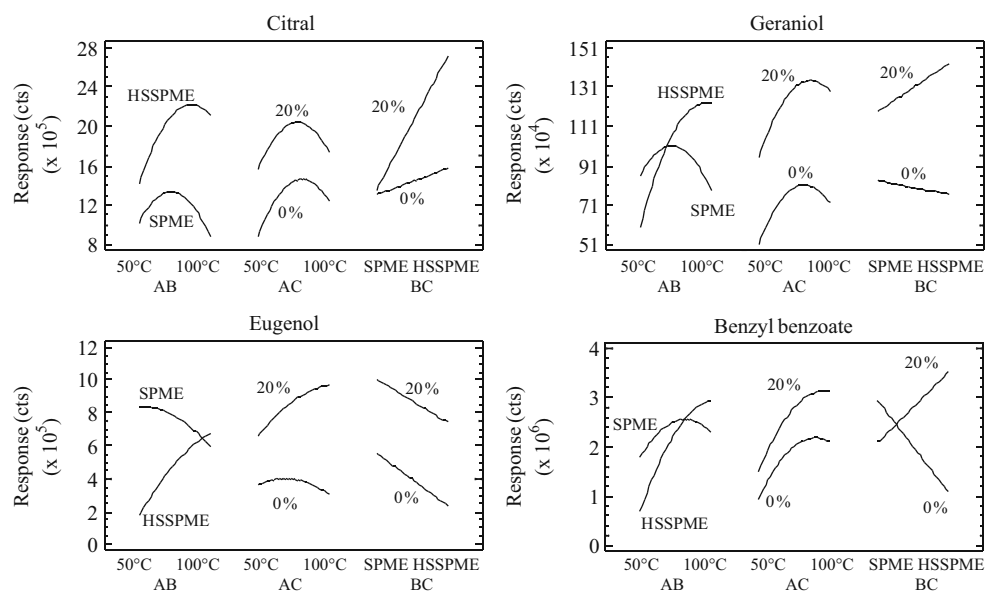


which means that the factor extraction temperature presents a minimum or a maximum in the domain studied.

Figure 4 shows the main effect plot for various selected allergens. This kind of plot shows the main effects with a line drawn between the low and the high level of the corresponding factors. The length of the lines is proportional

to the magnitude of the effect of each factor, and the sign of the slope indicates the level of the factor that produces the highest response. The influence of the extraction temperature, as well as the quadratic term of this factor (AA) in some cases, is clearly appreciated in the figure. The optimal temperature varies depending on the compound. For the

Fig. 5 Interactions plots for some selected allergens (see the factor codes in Table 3)



most volatile analytes, such as pinene and limonene, the most favourable temperature was 50 °C (see the last one in the figure). Most of the other compounds presented optimal response at intermediate temperatures, which is represented in the graphic by a maximum (factor AA statistically significant; see also Fig. 3). Finally, some compounds, such as linal, showed better extraction efficiency at the highest level of this factor, i.e. at 100 °C.

Regarding the sampling mode, the analyte behaviour was also quite different. Nine of the compounds presented better responses in the headspace mode, whereas five of them preferred direct extraction (see some examples in Fig. 4). Finally, the influence of the addition of salt was positive for most of the compounds.

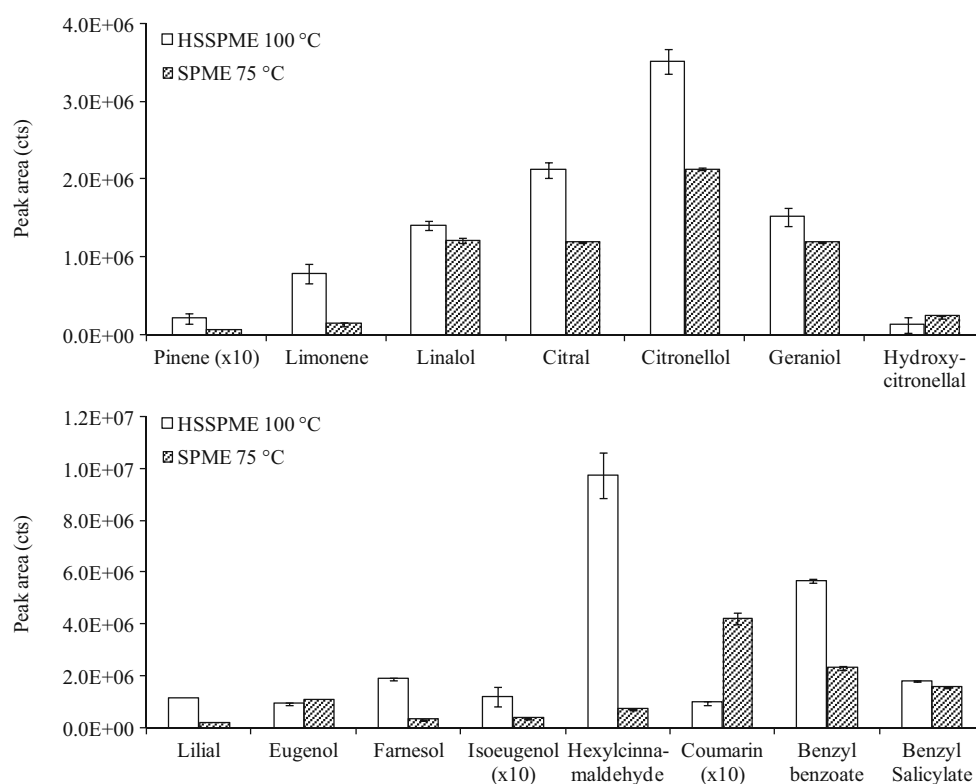
As mentioned above, interaction factors such as BC, and especially AB, were also significant for many compounds and, consequently, they must be analysed. Interaction graphs for the relevant two-factor interactions of several representative allergens are depicted in Fig. 5. Especially interesting is the AB interaction effect, which can be clearly observed in the figure. As previously discussed, many of the compounds showed the most favourable extraction at intermediate temperature (see Fig. 4). Nevertheless, analysing the AB factor, we can observe that the maximum appears shifted towards the high level (100 °C) when sampling was performed in the headspace mode. In fact, for most analytes HS-SPME gives much higher responses at 100 °C or very close temperatures. On the other hand, the direct sampling

mode SPME does not show such an important extraction efficiency variation with temperature although, usually, the optimal extraction temperature was around 75 °C (e.g. citral and geraniol, Fig. 5). It is also important to note that for most of the compounds that showed more favourable extraction by SPME (e.g. eugenol, Fig. 5), analysing the second-order factor AB, we can clearly see that the response achieved by SPME is much higher than that obtained by HS-SPME at low temperature values, but it is also very close to the HS-SPME responses at 100 °C. Regarding the BC interaction, we can also observe in the graphs that the addition of salt clearly benefits HS-SPME, whereas its effect on SPME is not so important, and even in some cases is negative.

Therefore, after analysing the interaction effects, we can conclude that the most favourable general conditions for the simultaneous extraction of the target analytes are HS-SPME at 100 °C, after the addition of salt. As mentioned above, the desorption temperature was not significant, so we decided to set this parameter to 220 °C, with the aim of achieving more selectivity in the analysis of real samples, since other compounds requiring higher desorption temperatures will not be introduced into the gas chromatography system. These general conditions are only unfavourable for four compounds: limonene and pinene, which will be better extracted at lower temperature, and coumarin and hydroxycitronellal, which would give higher responses by SPME.

In Fig. 6, the responses achieved by the selected conditions (HS-SPME, 100 °C, 20% NaCl) and by direct

Fig. 6 Comparison between HS-SPME (100 °C) and SPME (75 °C) (concentration 20 ng mL⁻¹)



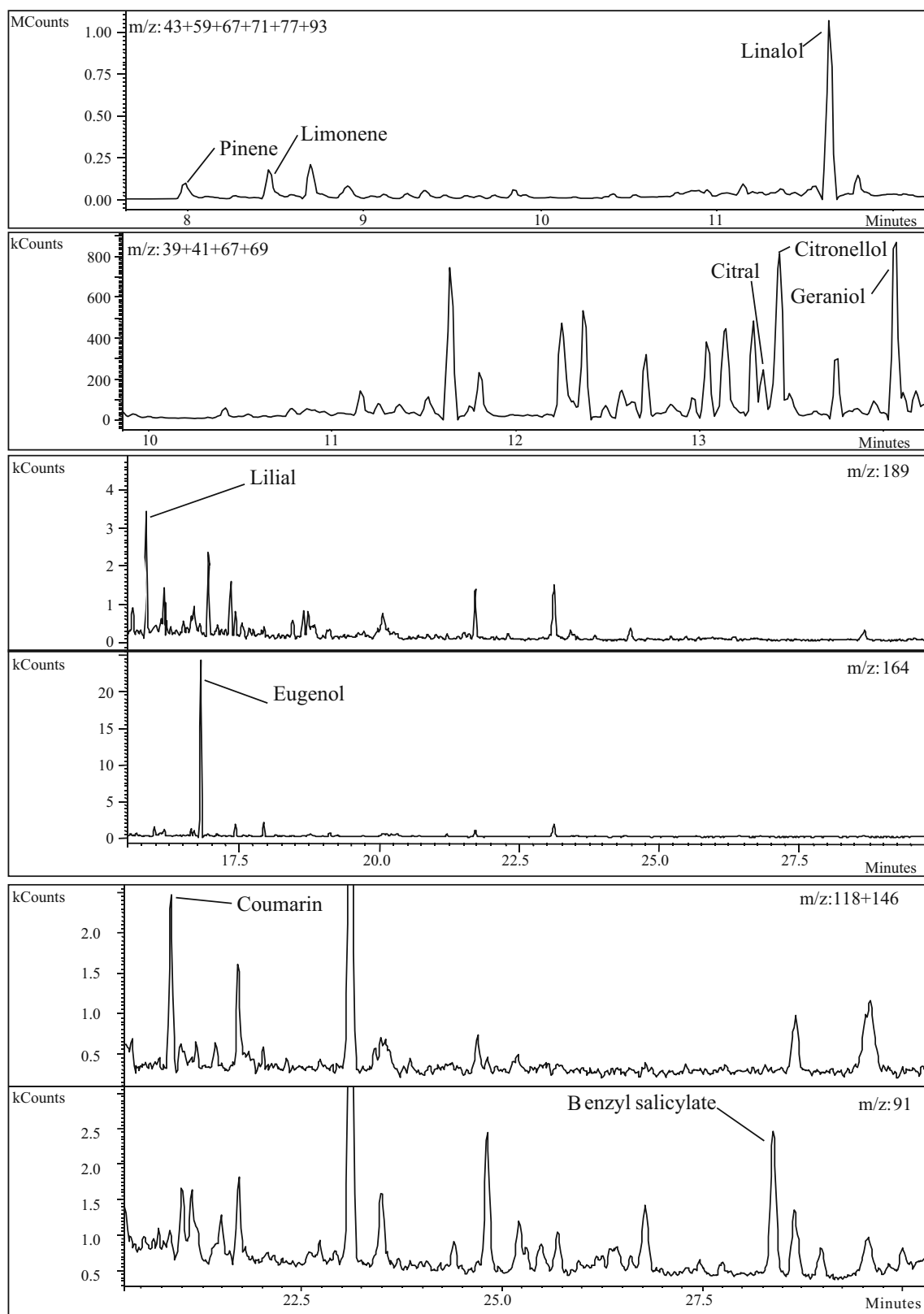


Fig. 7 HS-SPME-GC-MS ion chromatograms obtained for a real non-spiked baby bathwater sample (BBW1)

Table 4 Precision (relative standard deviation, %), linearity, limit of detection (*LOD*) and limit of quantification (*LOQ*) of the proposed method

Compound	Intraday precision ^a			Interday precision ^b 20ng mL ⁻¹	Linearity ^c (<i>R</i>)	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)
	1ng mL ⁻¹	5ng mL ⁻¹	20ng mL ⁻¹				
Pinene	16	3.8	13	10	0.994	0.188	0.625
Limonene	17	12	1.3	10	0.996	0.097	0.323
Linalol	5.1	3.0	5.7	7.7	0.993	0.115	0.385
Citral	3.9	9.7	4.9	8.9	0.997	0.273	0.909
Citronellol	4.0	3.3	2.9	2.8	0.995	0.176	0.588
Geraniol	9.7	10	2.5	5.2	0.998	0.250	0.833
Hydroxycitronellal	0.8	5.8	13	10	0.993	0.273	0.909
Lilial	4.8	4.4	1.0	8.2	0.995	0.012	0.041
Eugenol	3.6	7.9	0.9	5.0	0.996	0.021	0.070
Farnesol	5.0	14	4.4	21	0.999	0.055	0.182
Isoeugenol	17	9.7	8.6	12	0.996	0.300	1.000
Coumarin	8.7	5.6	4.8	8.7	0.998	0.136	0.455
Hexylcinnamaldehyde	5.6	9.2	2.2	2.0	0.999	0.001	0.004
Benzyl benzoate	5.6	1.4	0.4	5.6	1.000	0.002	0.008
Benzyl salicylate	16	3.9	5.5	5.7	0.999	0.013	0.042

^a *n*=3^b *n*=5^c Concentration range 0.1–50 ng mL⁻¹

SPME at 75 °C (the general most favourable extraction temperature for direct sampling) and 0% NaCl are compared. The improvement achieved by HS-SPME is very clear, excluding the two exceptions mentioned above: coumarin and hidroxicitronellal. In both cases (HS-SPME and SPME), method repeatability was satisfactory.

Performance study of the HS-SPME method

To evaluate method linearity, a calibration study was performed by selecting the optimal conditions indicated above. The calibration range was from 0.1 to 50 ng mL⁻¹ and the number of calibration levels was six. The

Table 5 Recovery of the target analytes from two different samples spiked at 5 ng mL⁻¹ (swimming pool water) and 20 ng mL⁻¹ (baby bathwater)

Compound	Swimming pool water	Bathwater	Swimming pool water with addition of sodium thiosulphate	Bathwater with addition of sodium thiosulphate
Pinene	36	116	100	109
Limonene	44	35	85	76
Linalol	72	29	75	75
Citronellol	81	60	74	79
Citral	85	70	90	85
Geraniol	87	48	75	80
Hydroxycitronellal	100	102	90	94
Eugenol	20	68	107	86
Coumarin	102	105	111	116
Isoeugenol	37	9	85	96
Lilial	104	92	80	90
Farnesol	85	94	105	124
Hexylcinnamaldehyde	99	87	91	81
Benzyl benzoate	109	117	92	103
Benzyl salicylate	129	146	105	99

calibration solutions were prepared in Milli-Q water. The method exhibited a directly proportional relationship between the extracted amount of each fragrance allergen and its initial concentration in the sample, with correlation coefficients (*R*) ranging from 0.993 to 1.000 (Table 4).

The precision of the experimental procedure was assessed at three concentrations: 1, 5 and 20 ng mL⁻¹. The results showed good intraday and interday precision, with relative standard deviation, in general, lower than 10% (see Table 4).

Limits of detection (signal-to-noise ratio of 3) and limits of quantification (signal-to-noise ratio of 10) are also presented in Table 4. These limits are at the subnanogram per millilitre level, and, therefore, the sensitivity of the proposed method can be considered satisfactory.

Application to real baby bathwater samples

The reliability of the proposed method was demonstrated by analysing real baby bathwater samples (BBW1–BBW9) and also an indoor swimming pool water sample (SPW). Two of the samples, one baby bathwater and the swimming pool water, were selected for matrix effect and recovery studies. These samples were spiked at 20 and 5 ng mL⁻¹, respectively.

Recoveries were calculated as the ratio of the measured concentration, after subtracting the initial concentration in the non-spiked sample, to the spiked concentration, and expressed as a percentage. Concentrations were calculated by external calibration using ultrapure water standards. Recoveries were satisfactory for eight of the target allergens, but they were low for six compounds: pinene,

limonene, linalool, geraniol, eugenol and isoeugenol. On the other hand, recoveries were too high for benzyl salicylate (see Table 5). The samples were then treated with sodium thiosulphate (0.1 mg mL⁻¹), to remove the excess of free chlorine, and, in this way preventing analyte oxidation, and the recovery studies were performed again. All the results were satisfactory and the values were in most cases over 80% (see Table 5, last two columns). These recoveries can be considered quantitative and, in consequence, after the addition of sodium thiosulphate no matrix effects were observed. Therefore, quantification by external calibration using ultrapure water standard solutions is suitable.

The method was finally applied to determine the levels of the target analytes in baby bathwater samples (BBW1–BBW9). Additionally, the levels found in the swimming pool water sample (SPW) were also calculated. In the case of the bathwater samples, the products used included shampoos, bubble baths and moisturizing soaps intended for babies. Sodium thiosulphate was added to all the samples after the sampling step. The results are shown in Table 6. Figure 7 shows the HSPME-GC-MS ion chromatograms obtained for a real baby bathwater sample containing ten of the target analytes (BBW1, see concentrations in Table 6). As can be seen, all the target compounds were detected in at least two of the samples. It is interesting to underline the high concentrations found for some compounds in several samples, reaching values even greater than 100 ng mL⁻¹ in some cases. Taking into account that it is not infrequent for babies to spend 15 min or even longer times in the bath every day, and that the exposure pathway to these and other ingredients is not only dermal absorption but

Table 6 Levels found (ng mL⁻¹) in real samples

Compound	BBW1	BBW2	BBW3	BBW4	BBW5	BBW6	BBW7	BBW8	BBW9	SPW
Pinene	39.0		8.6		354		5.5			
Limonene	5.4		56.3	0.4	568	0.3	1.7		3.0	
Linalol	11.9		47.3	2.1	108	0.6	26.2	2.3	19.3	
Citral	1.1			11.7		2.3	38.2	6.8	1.8	
Citronellol	4.3		34.0	1.7	14.8	3.3	5.7	6.5	13.8	
Geraniol	9.6		25.3	2.6	40.5		11.3		10.8	
Hydroxycitronellal							103		88.5	
Lilial	0.4	0.7		0.5	0.8	0.6	3.0	0.7		7.9
Eugenol	2.6	0.1	31.9		40.7					
Farnesol		2.9	27.4		62.5	2.2	17.7	1.6	2.7	
Isoeugenol										
Hexylcinnamaldehyde		0.2	0.21		0.2	0.01	3.1			0.1
Coumarin	2.9	10.0	157	2.7						1.4
Benzyl benzoate		2.3	12.1	0.03	0.3	0.03		0.03		0.3
Benzyl salicylate	0.4	3.5	4.1	3.3	1.6	0.4			0.06	0.1

Empty cells indicate concentrations lower than LODs.

also inhalation, and frequently ingestion, the presence and the levels of these chemicals in bathwater should be a matter of special concern.

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