



Sorbent trapping solid-phase microextraction of fragrance allergens in indoor air

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ABSTRACT

Exposure to fragrance substances is exponentially increasing in our daily life due to the enhanced use of scented products. Some fragrances are known to be important sensitizers, inhalation being an important exposure pathway in indoor environments. A simple and sensitive method based on solid-phase enrichment and solid-phase microextraction (SPME) followed by gas chromatography–mass spectrometry (GC–MS) has been developed for the analysis of 24 volatile fragrance allergens in indoor air. Suspected allergens present in the air (0.2 m^3) were adsorbed onto a very small quantity of florisisil (25 mg) and then transferred to a SPME fiber in the headspace mode (HS). To the best of our knowledge, this paper describes the first application of SPME for the determination of these compounds in air samples. The experimental parameters affecting the microextraction process have been optimized using a multifactor experimental design strategy. Accuracy, linearity, precision and detection limits (LODs) were evaluated to assess the performance of the proposed method. External calibration, using spiked sorbent standards, and not requiring the complete sampling process (only the SPME step), demonstrated to be suitable for the quantification of all suspected allergens. Recovery studies were performed at three concentration levels (0.04, 1.00 and $50 \mu\text{g m}^{-3}$), obtaining quantitative recoveries ($\geq 85\%$) in most cases. LOD values at the low ng m^{-3} level were achieved for all the target compounds. The application of the method to daily home air samples demonstrated the ubiquity of this kind of fragrance ingredients in quotidian indoor environments, finding 18 of the 24 considered compounds in concentrations ranging from 0.01 to $56 \mu\text{g m}^{-3}$. Benzyl alcohol, linalool, citronellol, ionone and linal were found in most analyzed samples.

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1. Introduction

Fragrance containing products are part of our daily life. The majority of personal-care products, household and laundry products on the market contain fragrances and, consequently, the exposure to these substances in our society is very high due to our lifestyle [1,2]. In addition to known dermatological problems [3,4], fragrances can induce or worsen respiratory problems due to their irritant effect. These product ingredients are thought to trigger asthma, asthmatic exacerbations, and other respiratory illness; headaches; and mucosal symptoms [5–9]. Indoor air quality is an increasingly important problem worldwide and home exposure to allergens and pollutants represents one of the major public health issues of the moment [10].

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has identified 24 fragrance compounds as likely to cause contact allergies [11]. The most of these substances are also restricted by the International Fragrance Association (IFRA) [12], the official representative body of the fragrance industry world-

wide, with the main purpose of ensuring the safety of fragrance materials since many of the raw fragrance materials have little available health and safety data [13].

According to the European Union (EU) regulation on cosmetic products [14], the presence of these fragrances must be indicated in the list of ingredients when its concentration exceeds the 0.001% in leave-on products and 0.01% in rinse-off products. For benzyl alcohol the maximum concentration in ready for use preparation should not exceed the 1.0%; and for methyleugenol the maximum allowed concentration is 0.01% in fine fragrance, 0.004% in eau de toilette, 0.002% in fragrance cream, 0.0002% in other leave-on products and oral products, and 0.001% in rinse-off products.

In the last years, several analytical methods for determining musk compounds in air have been developed. Samples have typically been collected by active sampling of high volumes using different sorbents [15–17]. The samples were then solvent extracted and, usually, a clean-up step is needed, which entails time-consuming and tedious methods. Regueiro et al. [18] proposed a very simple method for the analysis of synthetic musks in indoor air, in which musk compounds are adsorbed onto a small amount of Tenax and, then, analytes are transferred to a solid-phase microextraction (SPME) fiber. Ramirez et al. [19] developed a rapid and straightforward method for determining synthetic

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musks, parabens, and insect repellents in air samples, based on thermal desorption-gas chromatography–mass spectrometry (TD-GC–MS).

Very recently we presented a simple methodology for the analysis of 24 fragrance allergens in indoor air [20]. The procedure is based on the use of a very low amount of sorbent to retain the target compounds, and the rapid ultrasound-assisted solvent extraction (UAE) with only 2 mL of solvent, avoiding further concentration. As far as we know, this is the only work related to the determination of fragrance allergens in indoor air.

Regarding solid-phase microextraction (SPME) of fragrance allergens, Lamas et al. have developed a method for the quantification of 15 of these cosmetic ingredients in baby bathwaters [21], and Masuck et al. [22] proposed a sensitive method to determine and quantify the emission of 24 allergens from scented toys.

In the present study, we developed a simple and highly sensitive method based on solid-phase extraction followed by solid-phase microextraction (SPE–SPME) for the analysis of 24 volatile fragrance allergens in indoor air. To the best of our knowledge, this paper describes the first application of SPME for the determination of these compounds in air samples. To study and optimize main experimental parameters affecting the microextraction process, a multifactor experimental design is employed. Accuracy, linearity, precision and detection limits (LODs) are evaluated to assess the performance of the proposed method. Finally, several indoor air samples are analyzed in order to demonstrate the applicability of the method, and the high exposure levels to fragrance allergens in quotidian home indoor environments.

2. Experimental

2.1. Reagents and materials

3,7-Dimethyl-1,6-octadien-3-ol, 97% (linalool); 3,7-dimethyloct-6-en-1-ol, 95% (citronellol); 2-methoxy-4-prop-2-enyl phenol, 99% (eugenol); 1,2-dimethoxy-4-(2-propenyl)-benzene, 99% (methyleugenol); 2H-1-benzopyran-2-one, 99% (coumarin); 3,7,11-trimethyldodeca-2,6,10-trien-1-ol, 95% (farnesol, mixture of isomers); 3,7-dimethylocta-2,6-dienal, 95% (citral, *cis/trans*); 1-methyl-4-prop-1-en-2-yl-cyclohexene 97% (limonene); 4-methoxybenzene methanol, 98% (anisyl alcohol); 2-methoxy-4-(1-propenyl) phenol, 98% (isoeugenol, *cis/trans*); 3-phenyl phenylmethyl ester-2-propenoic acid, 99% (benzyl cinnamate); and 2-(phenylmethylene)-heptanal, 97% (amyl cinnamal) were purchased from Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, $\geq 85\%$ (ionone); 3,7-dimethyl-2,6-octadien-1-ol, $\geq 96\%$ (geraniol); 2-(phenylmethylene)-1-heptanol, $\geq 85\%$ (amyl cinnamyl alcohol); 3-(4-tert-butylphenyl)-2-methylpropanal, $\geq 95\%$ (lilial); 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde, $\geq 97\%$ (lyral); and 2-hydroxy-phenylmethyl ester benzoic acid, $\geq 99\%$ (benzyl salicylate) were purchased from Fluka (Fluka Chemie GmbH, Steinheim, Germany).

2-Octynoic acid, methyl ester, $\geq 99\%$ (methyl 2-octynoate); 7-hydroxy-3,7-dimethyloctanal, $\geq 95\%$ (hydroxycitronellal); 3-phenyl-2-propenal, $\geq 93\%$ (cinnamaldehyde); 2-(phenylmethylene)octanal, $\geq 95\%$ (hexyl cinnamaldehyde) were purchased from SAFC Supply Solutions (St. Louis, USA).

Benzene methanol, 99% (benzyl alcohol); 3-phenyl-2-propen-1-ol, 98% (cinnamyl alcohol); phenylmethyl benzoate, 98.5% (benzyl benzoate) were purchased from Chem Service (West Chester, USA).

Ethyl acetate and acetone were analytical grade and were provided by Merck (Darmstadt, Germany). Individual stock solutions of each compound and a mixture of them were prepared in ace-

tone. Working solutions were made by appropriate dilution in ethyl acetate and acetone and then stored in amber glass vials at -20°C . The latter were employed for spiking samples. Water (50–100 μL , Milli-Q grade) was used to favour sorbent desorption.

SPME manual holders and fibers were supplied by Supelco (Bellefonte, PA, USA). Two different commercial fiber coatings were used throughout the present work: 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Prior to first use, fibers were conditioned according to the manufacturer's recommendations.

2.2. Air sampling: solid-phase extraction

Air samples were collected in several indoor environments using an active sampling procedure. A known volume of air was pumped ($0.010\text{ m}^3\text{ min}^{-1}$) through a microfiltration glass funnel containing 25 mg of sorbent by using a S-8 vacuum pump (Telstar, Tarrasa, Spain). Florisil of 60–100 μm mesh (Aldrich, Steinheim, Germany) and Tenax TA of mesh size 60–80 (Supelco) were the sorbents of choice since they were successfully used in several studies employing similar setup. Florisil was activated overnight in an oven at 130°C . Only PTFE tubing was used for all connections to minimize contaminations. The sorbent with the retained compounds was then simply transferred from the glass funnel into a 10-mL headspace glass vial and sealed with an aluminum cap furnished with PTFE-faced septum. Finally, SPME was carried out under the optimized conditions.

For method validation experiments, the sampler was placed in a clean room provided of a laminar flow system in order to avoid external contamination.

2.3. Solid-phase microextraction

The headspace vial containing the enriched sorbent was placed into a water bath thermostated at the temperature of the experiment (50 or 100°C). Then, the compounds retained by the sorbent were extracted by exposing the SPME fiber to the headspace of the vial (HS–SPME) for 20 min. To favour desorption of the analytes from the sorbent to the fiber, the effect of adding a small volume (50–100 μL) of solvent to the sorbent before thermostatzation was tested. Once SPME was completed, the fiber was inserted into the GC injection port and desorbed for 5 min.

For method optimization, as well as in validation studies, the sorbent was spiked with the analytes at different concentrations and subjected to the SPME step or to the whole SPE–SPME process.

2.4. Gas chromatography–mass spectrometry

The GC–MS analysis was performed using a Varian 450-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to an ion trap mass spectrometer Varian 240-MS (Varian Chromatography Systems) with a waveboard for multiple MS (MS^n) analysis, equipped with an automatic injector CP-8400. The system was operated by Varian MS workstation v6.9.1 software.

Separation was carried out on a HP5 capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1} . The GC oven temperature was programmed from 45°C (held 2 min) to 100°C at 8°C min^{-1} , to 150°C at $20^\circ\text{C min}^{-1}$, to 200°C at $25^\circ\text{C min}^{-1}$ (held 5 min) and a final ramp to 225°C (held 1 min) at 8°C min^{-1} .

The injector was operated in the splitless mode and programmed to return to the split mode after 2 min from the beginning

Table 1
Main properties and quantification ions of the studied allergens.

Key	Retention time (min)	Compound	CAS number	IUPAC name	Molecular weight	log K_{OW}	Boiling point (°C)	Solubility ^a (mgL ⁻¹)	Quantifier and qualifier (m/z)
1	9.29	Limonene	5989-27-5	1-Methyl-4-prop-1-en-2-yl-cyclohexene	136	4.57	176	13.8	67, 93, 121
2	9.46	Benzyl alcohol	100-51-6	Benzene methanol	108	1.05	205	40000	77, 79, 108
3	10.36	Linalool	78-70-6	3,7-Dimethylocta-1,6-dien-3-ol	154	3.28	198	1589	43, 93, 121
4	11.53	Methyl-2-octynoate	111-12-6	2-Octynoic acid, methyl ester	154	2.60	219	-	67, 79, 95
5	11.80	Citronellol	106-22-9	3,7-Dimethyloct-6-en-1-ol	156	3.38	225	322	69, 81, 95
6	11.93	Citral	5392-40-5	3,7-Dimethylocta-2,6-dienal	152	3.17	229	590	39, 69, 109
	12.19								
7	12.02	Geraniol	106-24-1	3,7-Dimetil-2,6-octadien-1-ol	154	3.28	229	531	41, 69, 111
8	12.31	Cinnamal-dehyde	104-55-2	2-Propenal, 3-phenyl-	132	2.22	252	1420	77, 103, 131
9	12.31	Hydroxy-citronellal	107-75-5	7-Hydroxy-3,7-dimethyloctanal	172	1.54	240	23800	43, 59, 81
10	12.38	Anisyl alcohol	105-13-5	Benzene methanol, 4-methoxy-	138	1.10	259	2070	77, 109, 138
11	12.57	Cinnamyl alcohol	104-54-1	2-Propen-1-ol, 3-phenyl-	134	1.93	250	1800	77, 92, 134
12	12.89	Eugenol	97-53-0	2-Methoxy-4-prop-2-enyl-phenol	164	2.20	256	<1000	103, 131, 164
13	13.17	Methyleugenol	93-15-2	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	178	2.9	248	500	147, 163, 178
14	13.25	Isoeugenol	97-54-1	Phenol, 2-methoxy-4-(1-propenyl)-	164	2.45	267	984	103, 131, 164
	13.54								
15	13.57	Coumarin	91-64-5	2H-1-Benzopyran-2-one	146	1.39	301	2500	89, 118, 146
16	13.70	Ionone	127-51-5	4-(2,6,6-Trimethyl 2-cyclohexen-1-yl)-3-methyl-3-buten-2-one	206	4.41	266	16	107, 135, 150
17	14.08	Lilial	80-54-6	3-(4-tert-Butylphenyl)-2-methylpropanal	204	4.07	279	33	131, 147, 189
18	15.03	Amyl cinnamal	122-40-7	Heptanal, 2-(phenylmethylene)-	202	4.80	289	8.5	115, 129, 202
19	15.24	Lylal	31906-04-4	4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde	210	2.53	319	185-1045	79, 91, 136
20	15.38	Amyl cinnamyl alcohol	101-85-9	1-Heptanol, 2-(phenylmethylene)-	204	4.37	>200	26	91, 115, 133
21	15.61	Farnesol	4602-84-0	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	222	5.31	111	267	41, 69, 81
22	16.05	Hexylcinna-maldehyde	101-86-0	Octanal,2-(phenylmethylene)-	216	4.82	174	2.8	115, 129, 216
	16.35								
23	16.43	Benzyl benzoate	120-51-4	Phenylmethyl benzoate	212	3.97	324	19.8	105, 194, 212
24	17.99	Benzyl salicylate	118-58-1	Benzoic acid, 2-hydroxy-, phenylmethyl ester	228	4.31	208	<1000	65, 91, 228
25	21.57	Benzyl cinnamate	103-41-3	2-Propenoic acid, 3-phenyl-, phenylmethyl ester	238	3.65	198	9	91, 131, 192

^a Water, 25 °C.

of a run. Split flow was set at 20 mL min^{-1} and the injector temperature was kept at 260°C .

The ion trap mass spectrometer was operated in the electron impact (EI) ionization mode ($+70 \text{ eV}$) using an external ionization configuration. Manifold, ion trap, ion source and transfer line temperatures were maintained at 40, 150, 200 and 280°C , respectively.

In the full scan mode the mass range varied from 39 to 400 m/z at $3 \mu\text{s}$ scans, starting at 5 min and ending at 22.50 min. The filament emission current was $25 \mu\text{A}$. The analytes were positively identified by comparison of their mass spectra and retention times to those of standards. The identification and quantification ions and retention times for each target compound are listed in Table 1, as well as their main properties.

The chromatographic method was evaluated by direct injection of solutions in ethyl acetate of different concentrations of the target analytes. Linearity was good in the studied concentration range ($0.025\text{--}25 \mu\text{g mL}^{-1}$, 10 points) and linear regression coefficients were ranged from 0.990 to 0.999. Repeatability ($n=5$) in terms of relative standard deviation (RSD) was lower than 7.9%.

3. Results and discussion

3.1. Method development

The most relevant step for the application of the SPE-SPME procedure is the transfer of the analytes from the sorbent to the headspace of the vial and then to the SPME fiber. In this way, the optimization of the SPME process is clearly necessary to improve the amount of compound adsorbed by the fiber and hence the limits of detection and quantification of the method. A multivariate optimization that allows the simultaneous study of main variables potentially affecting SPME was performed.

In order to define the experimental domain, previous experiments were carried out. These experiments demonstrated the importance of the use of high temperature as well as the addition of a small volume of solvent to favour, or make even possible, the extraction of the analytes through the fiber coating (data not shown). Therefore, these two factors were included in the experimental design: the temperature at 50 and 100°C , and the addition of solvent at 50 and $100 \mu\text{L}$. Other three factors were also included in this study: sorbent type, fiber coating, and type of solvent. Florisil and Tenax TA were the sorbents selected for the study due to their suitability to combine with SPME as it has been previously demonstrated in the analysis of several classes of pollutants in indoor air [18,23,24]. The addition of a small volume of solvent to the sorbent previously to the SPME has demonstrated an improvement of the extraction of some kinds of compounds. Regarding the fiber, DVB/CAR/PDMS and PDMS/DVB coatings were selected. This last fiber has recently been employed for the extraction of suspected allergens from baby bathwater, leading to excellent results [21]. On the other hand, the presence of CAR in the fiber DVB/CAR/PDMS and the double length of this fiber could improve the results of the PDMS/DVB for some analytes, and it was considered for study.

All these factors were then included in a screening study by means of a 2^{5-1} half fraction factorial design, involving a total of 16 randomized runs, with each parameter evaluated at two levels. Table 2 summarizes the factors and levels considered. All experiments were performed using a small amount of sorbent (25 mg) spiked with the target analytes at a level of $4 \text{ ng per milligram}$. The design has resolution V, which means that it is capable of evaluating all main effects and all two-factor interactions. Numerical analysis of data was made with the statistical software package Statgraphics Plus 5.1 (Manugistics, Rockville, MD, USA). The selected design allows studying the results using various statistical tests and graphic tools. Table 3 summarizes the analysis of variance (ANOVA) obtained. For simplicity, only *F*-ratios and *p*-values

Table 2

Factors and levels considered in the screening experimental design.

Factor	Key	Low level (–)	High level (+)
Solvent volume	A	$50 \mu\text{L}$	$100 \mu\text{L}$
Extraction temperature	B	50°C	100°C
Fiber	C	PDMS/DVB	DVB/CAR/PDMS
Sorbent	D	Tenax	Florisil
Solvent	E	Water	Acetone

are given. The *F*-ratio measures the contribution of each factor or interaction on the variance of the response. The *p*-value tests the statistical significance of each factor and interaction. When *p*-value is less than 0.05, the factor has a statistically significant effect at the 95% confidence level. As can be seen, three of the main factors, type of sorbent (D), type of solvent added (E), and extraction temperature (B), as well as the interaction effect DE, were significant for most analytes. The other main factors were nonsignificant for almost all analytes but some other interaction effects such as AC, BD and BE were important in some cases. This information can clearly be visualized by means of the Pareto charts.

Fig. 1 shows the Pareto charts including the main factors and two-factor interactions for several selected compounds. 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. Vertical line in the graphs represents the statistically significant bound at the 95% confidence level. Pareto charts clearly show that the most relevant factors are the sorbent (D) and the type of solvent (E). The extraction temperature (B) was also significant in many cases, although the associated effect is low (see the corresponding bars in the figure). In addition, the fiber coating (C) and the solvent volume (A) were non-significant for most analytes. Second order factors were in general very important and in some cases more relevant than main factors. DE (sorbent-type of solvent) was significant for most of compounds, while other factor interactions such as BD (extraction temperature-sorbent), BE (extraction temperature-type of solvent), and AC (solvent volume-type of fiber) were also significant for some of the analytes.

Fig. 2 shows the main effects plots for several compounds of different chemical structures, representing general behaviours. This kind of plots 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 effect magnitude of each factor in the extraction process, and the sign of the slope indicates the level of the factor that produces the highest response. These graphs again show the high influence on response of the sorbent and the type of solvent added. In all cases, higher responses were obtained selecting florisil and water. Regarding temperature, 100°C favours, in general, SPME extraction, excluding some of the most volatile analytes that prefer 50°C (e.g. citronellol). In the few cases in which the fiber type was significant (only for four compounds, see Table 3), higher responses were obtained using the DVB/CAR/PDMS coating (e.g. methyleugenol, Fig. 2).

The interaction graphics (Fig. 3) are very useful and help to visualize optimal conditions. Only the most relevant second order effects (see Table 3) and those showing general behaviours will be commented here. The most strong interaction effect is the sorbent-type of solvent (DE) one. This effect was significant for almost all compounds (see Table 3). We can clearly appreciate in Fig. 3a that the response obtained using florisil is considerably improved when water instead of acetone is added, whereas the solvent has little effect in the case of Tenax, although the responses with this sorbent are very low. Other important interaction is BE (extraction temperature-type of solvent) (see Fig. 3b). For the most volatile compounds, the most favourable conditions

Table 3F-ratios and *p*-values obtained in the analysis of variance.

	Main effects										Interactions							
	A: Volume		B: Temperature		C: Fiber		D: Sorbent		E: Solvent		AC		BD		BE		DE	
	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value	F-ratio	<i>p</i> -value
Limonene	0.2		3		3		9	+	9	+	4		4		4		9	+
Benzyl alcohol	8	+	4		3		5		2		2		0.0		0.0		9	+
Linalool	0.3		7		2		14	+	14	+	8	+	10	+	9	+	14	+
Methyl-2-octynoate	10		98	+	9		364	+	364	+	138	+	180	+	132	+	370	+
Citronellol	0.1		18	+	4		56	+	61	+	27	+	39	+	30	+	65	+
Citral	0.2		20	+	4		62	+	67	+	24	+	35	+	24	+	70	+
Geraniol	0.1		13	+	6		70	+	75	+	23	+	39	+	26	+	81	+
Cinnamaldehyde	7		5		5		10		12	+	2		0.0		1		17	+
Hydroxycitronellal	0.4		0.2		2		1		1		0.2		1		1		2	
Anisyl alcohol	24	+	138	+	43	+	4		1		42	+	0.2		1		43	+
Cinnamyl alcohol	2		4		2		3		3		2		0.3		11		5	
Eugenol	1		0.1		3		20	+	25	+	0.1		2		0.4		29	+
Methyleugenol	4		41	+	41	+	361	+	246	+	8		1		1		331	+
Isoeugenol	2		6		3		14	+	16	+	3		0.1		1		24	+
Coumarin	16		1892	+	265	+	241	+	9		120		47		29		215	+
Ionone	1		15	+	9	+	339	+	203	+	2		3		3		330	+
Lilial	1		9		4		493	+	449	+	8		0.2		1		534	+
Amyl cinnamal																		
Lylal	0.3		51		4		35		4		33		4		1		49	
Amyl cinnamyl alcohol	1		289	+	3		322	+	219	+	217	+	214	+	156	+	288	+
Farnesol	3		84	+	2		80	+	77	+	84	+	67	+	67	+	93	+
Hexylcinnamaldehyde	9		178	+	2		359	+	204	+	89	+	117	+	51	+	277	+
Benzyl benzoate	6		895	+	17		1544	+	1202	+	650	+	778	+	568	+	1330	+
Benzyl salicylate	2		87	+	4		131	+	123	+	81	+	76	+	71	+	136	+
Benzyl cinnamate	1		3		2		5		3		1		3		1		4	

+ cell, *p*-value < 0.05; empty cell, *p*-value > 0.05.

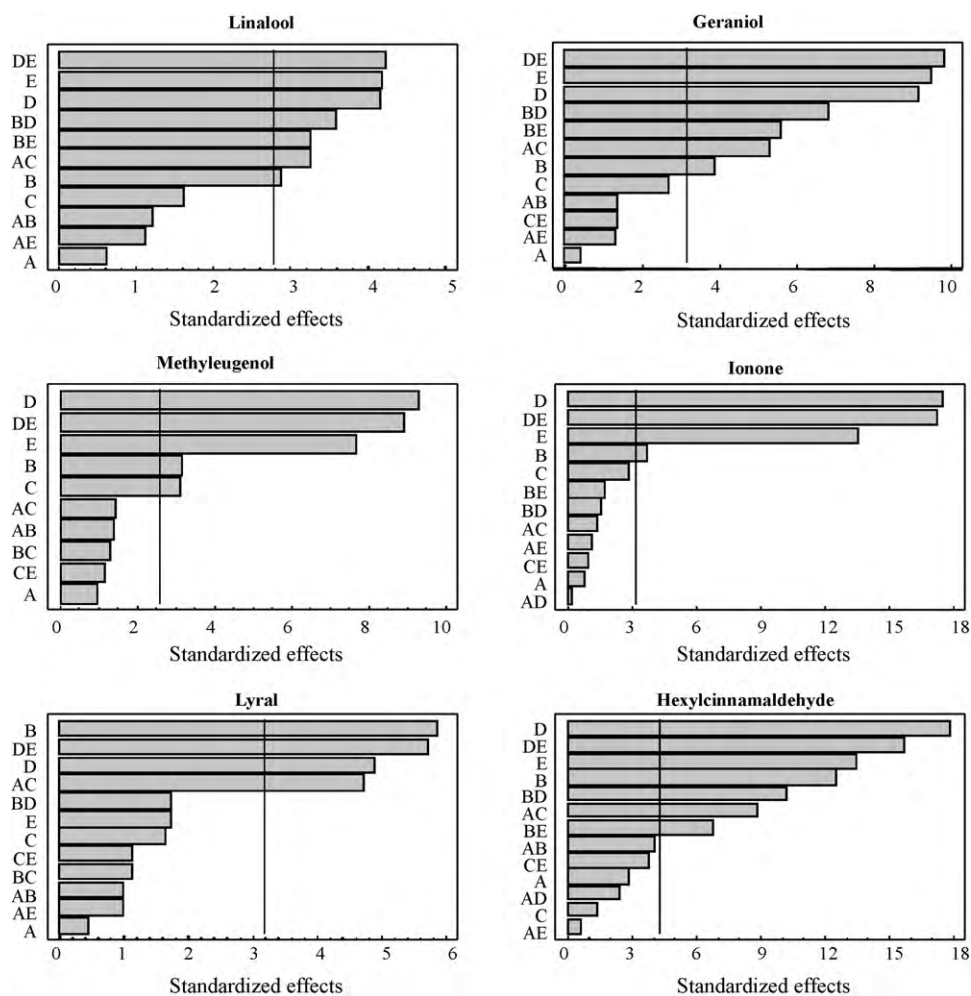


Fig. 1. Pareto charts showing the significant factors (95%) for some selected fragrance allergens (see factor codes in Table 2).

are 50 °C and water; nevertheless, for the rest of compounds, the responses at 50 °C are quite low and similar independently of the solvent employed, but, at 100 °C, extraction efficiency is considerably improved using water. BD (extraction temperature-sorbent)

effect also shows the improvement in the response obtained using florisl at 100 °C for the less volatile compounds (see Fig. 3c). Finally, and although the main factors A and C were mostly not significant, the interaction effect AC was significant for half of the compounds

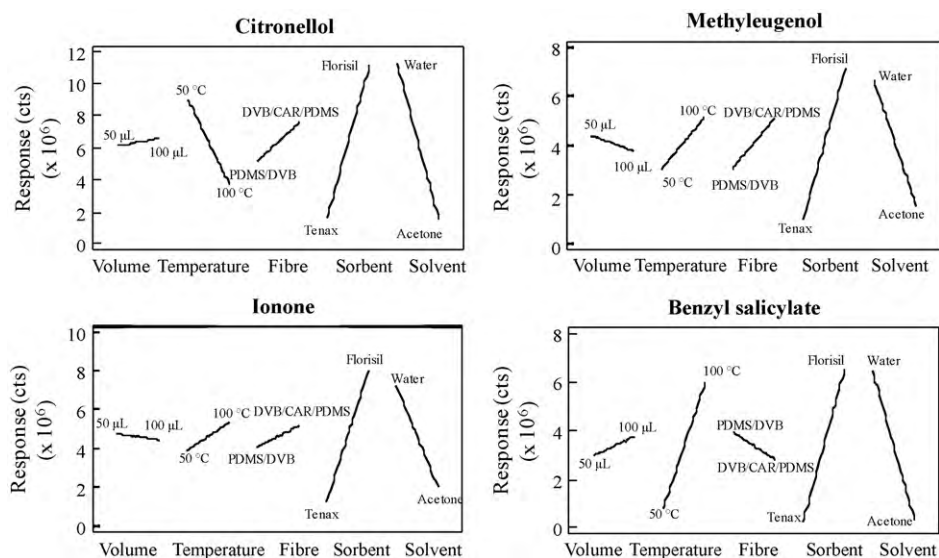


Fig. 2. Main effect plots for some selected fragrance allergens.

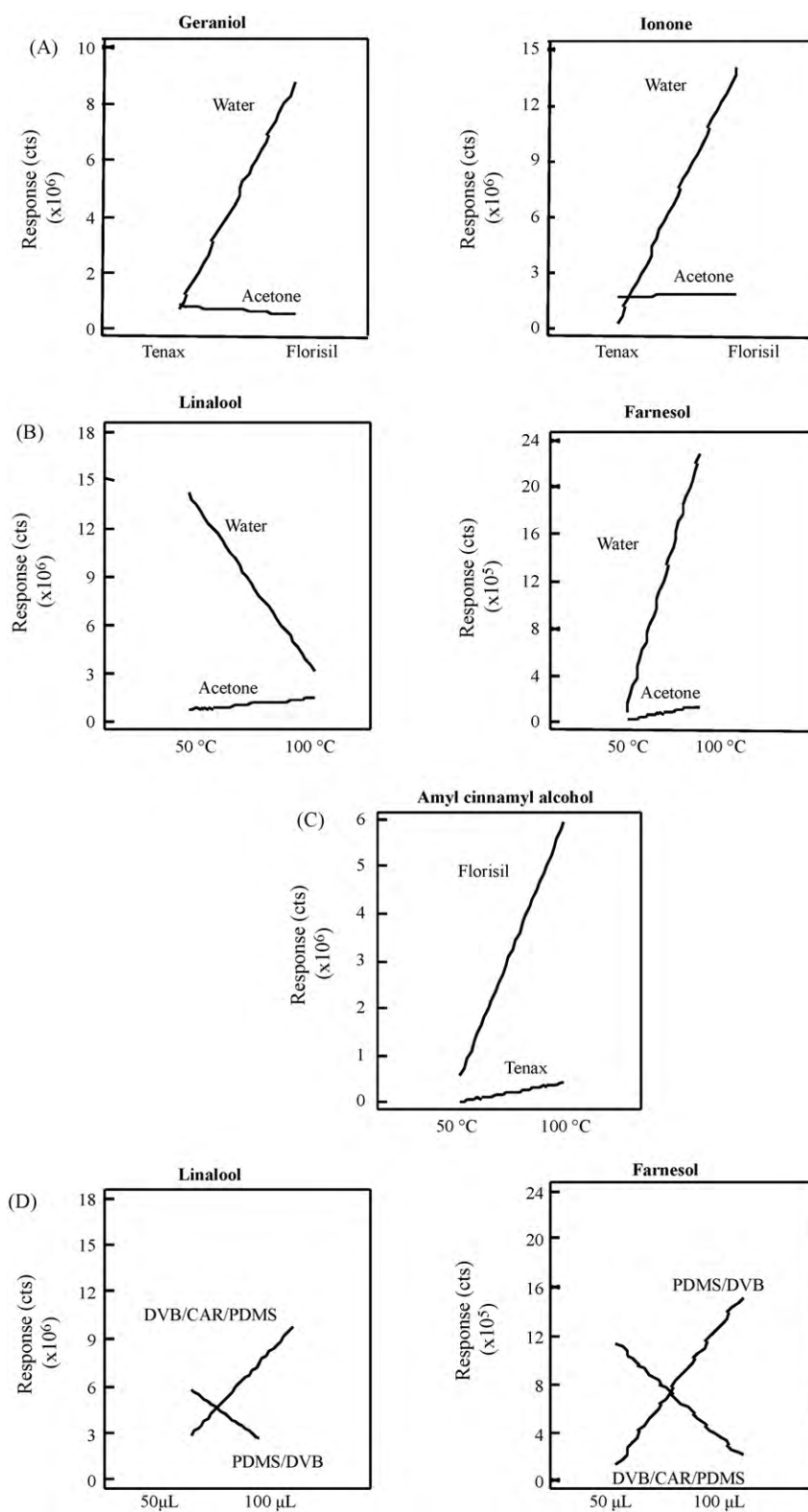


Fig. 3. Combined effect of factors for some selected fragrance allergens: A, type of sorbent and type of solvent; B, extraction temperature and type of solvent; C, extraction temperature and type of sorbent; D, solvent volume and type of fiber.

(see Table 3), showing as most favourable conditions the use of DVB/CAR/PDMS and 100 μL of solvent for the most volatile compounds and PDMS/DVB and 100 μL of solvent for the less volatile ones (see Fig. 3d).

In summary, after optimization, the experimental conditions selected for the SPME of allergenic compounds involve the use of

florisil as sampling sorbent, the addition of 100 μL water to the sorbent, and the extraction at 100 °C using a DVB/CAR/PDMS fiber, although PDMS/DVB coating would also be suitable.

Regarding SPE air enrichment, breakthrough volume was studied in a previous work [20], demonstrating that air volumes of 0.2 m³ could be sampled without significant losses of any com-

Table 4

Linearity, limits of detection, recovery and precision of the method (20 min, 10 L/min).

Compound	Linearity		LOD (ng m ⁻³)	Recovery (RSD) % (n = 3)		
	R ²	LOF test (p)		0.04 µg m ⁻³	1.00 µg m ⁻³	50 µg m ⁻³
Benzyl alcohol	0.9997	0.216	2.5	90.3 (5.7)	110 (9.8)	99.9 (8.0)
Linalool	0.9984	0.406	3.1	85.9 (15)	98.9 (5.5)	95.2 (5.4)
Methyl-2-octynoate	0.9992	0.180	2.5	103 (6.4)	128 (7.3)	109 (8.3)
Citronellol	0.9972	0.164	2.3	89.4 (11)	106 (8.7)	105 (4.1)
Citral	0.9947	0.738	1.9	102 (3.5)	115 (3.0)	93.0 (11)
Geraniol	0.9992	0.143	2.5	89.6 (5.2)	110 (5.0)	117 (11)
Cinnamaldehyde	0.9988	0.748	3.6	72.5 (6.9)	98.7 (5.3)	107 (3.6)
Hydroxycitronellal	0.9984	0.459	12	110 (5.1)	127 (2.0)	99.0 (1.0)
Anisyl alcohol	0.9995	0.967	1.6	105 (12)	112 (9.6)	88.2 (14)
Cinnamyl alcohol	0.9995	0.881	1.8	80.6 (4.6)	110 (8.7)	99.0 (3.7)
Eugenol	0.9999	0.840	0.72	70.0 (2.6)	80.4 (1.9)	104 (10)
Methyleugenol	0.9977	0.072	0.047	72.3 (2.7)	107 (3.1)	109 (15)
Isoeugenol	0.9954	0.523	2.0	46.0 (4.2)	34.0 (11)	65.0 (10)
Coumarin	0.9974	0.431	1.5	73.1 (1.8)	79.0 (4.2)	92.0 (3.9)
Ionone	0.9959	0.168	0.50	116 (2.9)	105 (0.7)	111 (9.0)
Lilial	0.9996	0.262	10	112 (14)	105 (2.3)	107 (6.8)
Amyl cinnamal	0.9994	0.839	2.5	83.8 (5.4)	73.3 (4.0)	108 (2.7)
Lylal	0.9999	0.727	1.3	95.0 (9.4)	76.9 (4.9)	103 (1.1)
Amyl cinnamyl alcohol	0.9999	0.934	1.9	85.7 (5.4)	99.2 (7.6)	96.1 (11)
Farnesol	0.9948	0.741	12	102 (4.6)	92.4 (7.1)	110 (13)
Hexylcinnamaldehyde	0.9999	0.759	2.5	91.5 (11)	78.0 (4.0)	112 (3.6)
Benzyl benzoate	0.9997	0.839	1.1	96.0 (1.8)	103 (6.1)	120 (7.9)
Benzyl salicylate	0.9996	0.828	2.5	88.6 (5.4)	76.7 (6.5)	115 (10)
Benzyl cinnamate	0.9976	0.184	2.7	108 (0.5)	75.9 (10)	124 (8.0)

pond, excluding limonene (almost completely lost even with lower sampling volumes), and isoeugenol (with recoveries about 70%). In the present study, this same volume was selected for method validation.

3.2. Method performance

In order to assess the performance of the proposed method, main analytical quality parameters were evaluated. Linearity was tested using sorbent portions spiked in the range from 0.08 to 40 ng mg⁻¹ at 9 levels of concentration, each one analyzed in duplicate or triplicate. Determination coefficients (R²) for the calibration curves are shown in Table 4. All compounds showed good correlation with R² values higher than 0.9947. To validate the regression data, an analysis of variance (ANOVA) was performed. The lack-of-fit (LOF) test is designed to determine whether the selected model is adequate to describe the experimental data. The test compares the variability of the proposed model residuals to the variability between observations (area counts) at replicate values of the independent variable. Results of the LOF test for the calibration range considered at a confidence level of 95% are also shown in Table 4.

Since *p*-values for LOF test are greater than 0.05 for all compounds, the linear regression models appear to be adequate for the experimental data. For highly contaminated atmospheres, calibration in split mode might be necessary, and linearity in a higher range (20–800 ng mg⁻¹ at 6 levels) was also proven.

Due to the general use of these fragrance compounds in all classes of personal-care products and cleaning products, trace analysis demands special precautions during all analytical procedures to minimize contamination risk, and blank samples must be daily run. To estimate the detection limits (LODs) of the method, blank air samples were collected in a clean room provided of a laminar flow system. None of the compounds with the exception of lilial was detected in blank air samples. LODs were calculated as the average amount of analyte giving a response that is the blank signal plus three times the standard deviation (LOD = blank signal + 3SD). Thus, the estimated LODs considering a sample volume of 0.2 m³ are shown in Table 4. Values ranged from 0.05 to 12 ng m⁻³ and are 1–2 orders of magnitude lower than the ones achieved by the previously proposed SPE-UAE method [20].

Recoveries using the proposed SPE-SPME procedure were estimated sampling 0.2 m³ air at three different concentration levels:

Table 5Compounds found (µg m⁻³) in indoor air samples^a under normal daily conditions (WR, washroom; LR, laundry room; C, corridor; L, living room; K, kitchen; BR, bedroom; SR, storage room).

Compound	WR1	WR2	LR1	LR2	C1	C2	C3	L1	K1	BR1	SR
Benzyl alcohol	0.583	2.67	0.170	0.392	0.238	0.043	0.312	1.37	1.02	0.049	0.343
Linalool	2.11	6.11	0.153	2.43	0.629	0.185	0.863		2.85	0.231	0.980
Citronellol	0.192	0.342	0.171	0.144				0.476	0.926	0.017	0.307
Citral								0.642	0.447		
Geraniol		0.491						0.394			0.208
Cinnamaldehyde	0.020										0.631
Hydroxycitronellal	0.793		0.329			0.094			0.991		
Eugenol	0.036								0.018		0.028
Ionone	0.187	0.193	0.055	0.113	0.031	0.017	0.043	0.765	0.478	0.015	0.032
Lilial	1.09	0.898	0.224	0.116	0.343		0.364	0.310	0.714		0.254
Amyl cinnamal											
Farnesol		0.543			0.052		0.122	0.294			
Hexylcinnamaldehyde	0.020	0.035	0.081		0.024		0.011	0.189	0.057		
Benzyl benzoate	0.012	0.013						0.015	0.013		0.017
Benzyl salicylate								0.014	0.014		0.018

^a Blank spaces mean values below LOD.

Table 6

Compounds found ($\mu\text{g m}^{-3}$) in indoor air samples^a after the application of different aerosol products (CAR, car; WR, washroom; C, corridor; L, living room; K, kitchen; BR, bedroom).

Compound	CAR	WR3	WR4	C4	L2	K2	K3	BR2	L3	K4	BR3
Benzyl alcohol	6.71	3.30	6.21	2.04	6.18	6.18	2.36	3.06	1.38		1.80
Linalool	30.8	53.6	23.7	15.9	31.6	30.8	26.2	20.9	7.54		6.84
Methyl-2-octynoate		7.33									
Citronellol	9.97	20.8	6.25	5.75	10.6	12.2	7.52	7.18	0.483		
Citral				3.19	1.50		1.12				
Geraniol	4.75	15.8	1.83		7.65	5.97	7.38	3.76			
Cinnamaldehyde		0.472		0.918				0.103			
Hydroxycitronellal					4.33	2.38	4.28		0.384		
Cinnamyl alcohol		4.32									
Eugenol		3.48	0.916	4.08	2.32	1.78	2.24	0.172			
Isoeugenol ^b			0.943	1.90	1.80	1.78	1.28				
Coumarin							0.692			0.204	
Ionone	1.54	7.57	0.577	1.19	0.274		0.523	1.69	0.144	0.008	0.423
Lilial	7.71	13.8	2.70	6.92	6.99	5.35	7.41	9.78	1.30		0.169
Amyl cinnamal								0.088	3.87	0.051	
Farnesol											
Hexylcinnamaldehyde	1.07	14.7	1.44	0.111	0.911	0.136	0.271	3.84	0.216		0.213
Benzyl benzoate	0.511	0.599	0.202	0.976	0.521	0.184	0.329		1.23	0.235	
Benzyl salicylate	0.251	55.8	0.793	0.389	0.983	1.52		3.58	0.600	0.033	0.099

^a Blank spaces mean values below LOD.

^b Values were corrected taking into account the average extraction efficiency for this compound.

0.04, 1.00 and $50 \mu\text{g m}^{-3}$. Recoveries were calculated as the ratio of the measured concentration to the spiked concentration and expressed as a percentage. Table 4 shows that recoveries were satisfactory, between 80 and 115% in most cases. Therefore, quantification by external calibration using directly spiked sorbent can be employed. Only one compound, isoeugenol, gave low recoveries

(about 50%), and the concentration estimate for this compound in real samples can be only considered as semi-quantitative.

The precision of the method was evaluated at the three same concentration levels and results are also shown in Table 4. Repeatability can be considered good with RSD values below 10% in many cases.

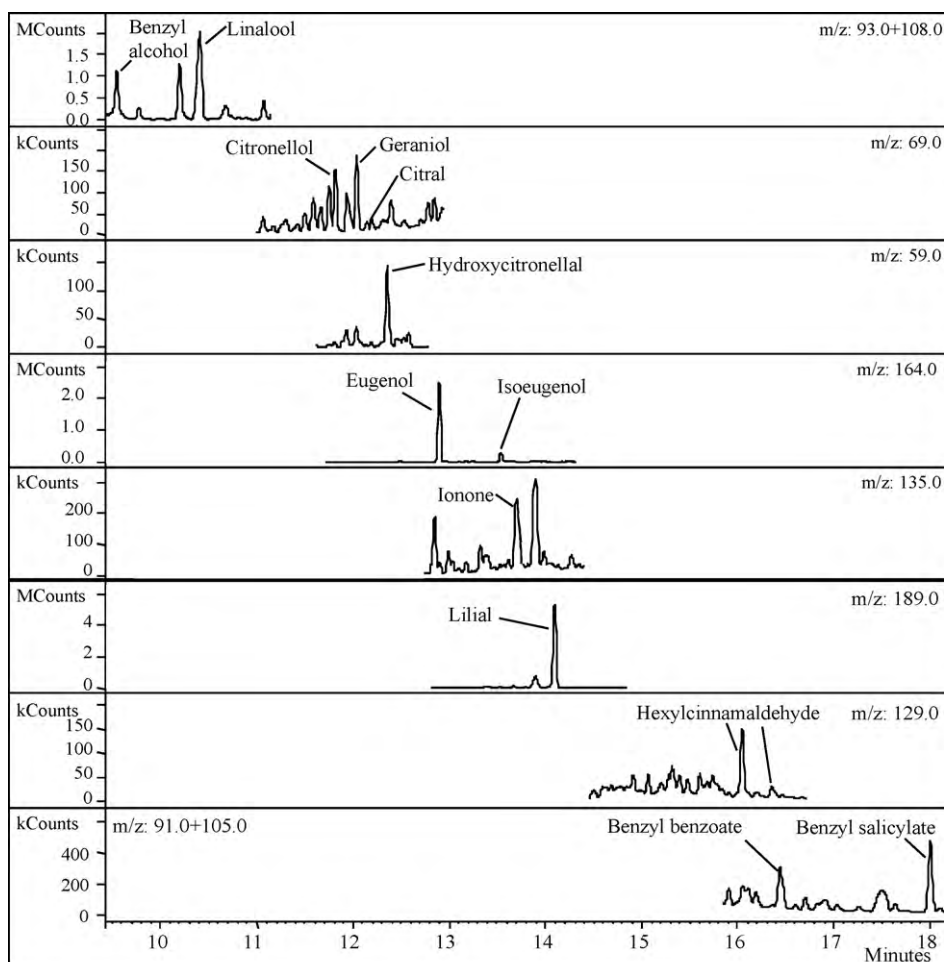


Fig. 4. Extracted ion chromatograms of sample L2 (see concentrations in Table 6).

3.3. Method application: determination of fragrance allergens in indoor air

The SPE-SPME method was applied to the analysis of indoor air samples collected in different home places (0.2 m^3 , $0.010 \text{ m}^3 \text{ min}^{-1}$ including kitchen (code K in Table 5), living room (L), washroom (WR), corridors (C), bedrooms (BR), storage room (SR) and laundry room (LR) samples, at normal daily conditions (Table 5). Other air room samples and a car sample (CAR) were taken after the application of different aerosol products such as insecticides and freshener aerosols or diffusers (Table 6). All samples were collected in duplicate and initially analyzed by GC–MS in the splitless mode; if analyte concentration exceeds the linear calibration range, the sample is also analyzed in the split mode. As can be seen in Table 5, 14 of 24 target analytes were present in the daily indoor air samples, at concentrations reaching $\mu\text{g m}^{-3}$ in some cases. Four of them, benzyl alcohol, linalool, ionone, and linal, were found in almost all environments. This fact should attract the attention regarding the ubiquitous presence of this kind of compounds in quotidian indoor environments.

When different air fresheners (10 commercial brands were tested) were applied following the recommendations of the manufacturers (when available) into the home places (see eight first columns in Table 6), the measured concentrations of the studied allergenic fragrances were markedly high. Under these conditions, 18 of the 24 allergens were found in some samples, and 10 of them in almost all the samples. The highest concentrations were due to linalool (from 6.8 to $53.6 \mu\text{g m}^{-3}$) but high concentrations above $10 \mu\text{g m}^{-3}$ were also found for citronellol, geraniol, linal and benzyl salicylate in some cases. L3, K4 and BR3 (Table 6) are samples taken into three different rooms after the application of different insecticides. Higher concentrations and higher number of allergen fragrances were found in L3 and BR3. The insecticides applied into these two rooms were aerosols labelled as “perfumed”, whereas K4 corresponds to an insecticide for surface application.

The extracted ion chromatograms for one of the indoor air samples (L2) are shown in Fig. 4.

4. Conclusions

A method based on sorbent trapping combined with SPME followed by GC–MS was optimized for the rapid analysis of 24 suspected allergens in indoors air samples. To the best of our knowledge, this is the first method based on SPME for the analysis of these fragrance compounds in air. Compounds in the pumped air could be retained on a very small amount of florasil (25 mg) and then extracted in the headspace mode by SPME. The SPME step was optimized by means of a factorial design demonstrating the importance of the interaction effects. The best conditions involve the use of florasil and the extraction at 100°C , after the addition of $100 \mu\text{L}$ of water to promote desorption, using DVB/CAR/PDMS

or PDMS/DVB as fiber coating. One of the best attainments of the proposed method is the external calibration, which can be performed by direct spike of the sorbent with the target compounds. The performance of the method has been demonstrated in terms of linearity, accuracy (recoveries $>85\%$ in most cases) and precision ($\text{RSD} < 15\%$). The sensitivity of the method is very high; limits of detection below 4 ng m^{-3} were obtained for the majority of the target compounds. In addition, the proposed SPE-HS-SPME method is simple, fast, cheap, and easy to implement in non-specialized laboratories. The application of the method to daily home air samples demonstrates the ubiquity of this kind of fragrance ingredients in quotidian indoor environments, which were found in all the samples, and frequently at quite high levels.

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