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Determination of fragrance allergens in indoor air by active sampling followed by ultrasound-assisted solvent extraction and gas chromatography–mass spectrometry

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ABSTRACT

Fragrances are ubiquitous pollutants in the environment, present in the most of household products, air fresheners, insecticides and cosmetics. Commercial perfumes may contain hundreds of individual fragrance chemicals. In addition to the widespread use and exposure to fragranced products, many of the raw fragrance materials have limited available health and safety data. Because of their nature as artificial fragrances, inhalation should be considered as an important exposure pathway, especially in indoor environments. In this work, a very simple, fast, and sensitive methodology for the analysis of 24 fragrance allergens in indoor air is presented. Considered compounds include those regulated by the EU Directive, excluding limonene; methyl eugenol was also included due to its toxicity. The proposed methodology is based on the use of a very low amount of adsorbent to retain the target compounds, and the rapid ultrasound-assisted solvent extraction (UAE) using a very low volume of solvent which avoids further extract concentration. Quantification was performed by gas chromatography coupled to mass spectrometry (GC–MS). The influence of main factors involved in the UAE step (type of adsorbent and solvent, solvent volume and extraction time) was studied using an experimental design approach to account for possible factor interactions. Using the optimized procedure, 0.2 m^{-3} air are sampled, analytes are retained on 25 mg Florisil, from which they are extracted by UAE (5 min) with 2 mL ethyl acetate. Linearity was demonstrated in a wide concentration range. Efficiency of the total sampling-extraction process was studied at several concentration levels (1, 5 and 125 $\mu g\,m^{-3}$), obtaining quantitative recoveries, and good precision (RSD < 10%). Method detection limits were $\leq$0.6 $\mu g\,m^{-3}$. Finally, the proposed method was applied to real samples collected in indoor environments in which several of the target compounds were determined.

1. Introduction

Indoor air quality has become an important global community concern due to the increased amount of personal time spent in indoor environments. Taking into account that people in developed countries spend up to 90% of their time indoors [1,2], inhalation of indoor air is potentially the most important exposure pathway to many pollutants [2]. The high comfort achieved in developed countries increased the demand and the widespread consumption of fragranced household products, fresheners and cosmetics. Inadequate ventilation, high temperatures and humidity coupled with the slow indoor degradation processes may increase indoor levels of many components of these consumer products [3].

The primary purposes of fragrances are to impart a scent to a product, mask the odor of other materials in the product or, in some cases, alter mood. More than 2600 ingredients have been documented for use in fragrances [4] but many of the raw fragrance materials have little available health and safety data. The potential for exposure to these materials in our society is, therefore, very high. With increased usage and exposure there are increased anecdotal and clinical accounts of fragranced products causing, triggering and exacerbating health conditions. In addition to known dermatological problems [5,6], fragrances can induce or worsen respiratory problems due to their irritant effect. They are thought to trigger asthma, asthmatic exacerbations, and other respiratory conditions [7,8]; headaches [9]; and mucosal symptoms [10]. Those with asthma, allergies, sinus problems, rhinitis and other such conditions are more susceptible to the effects of irritants, often at levels

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that are many times lower than what would cause problems in the general population [11]. The long-term impact due to the possible bioaccumulation in human tissues is also cause of concern. In addition, there are environmental concerns, as fragranced products add to both air and water pollution.

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has identified 26 of these ingredients as likely to cause contact allergies [12]. They have been designated by the European Union (EU) as requiring labeling on cosmetic and detergent products [13,14]. 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. The use of some of the 26 fragrance compounds is already more restricted, i.e. the finished cosmetic product must not contain more than 0.01% of methyl-2-octynoate, 0.02% of isoeugenol and 1.0% hydroxycitronellal. Methyleugenol must not be part of the composition of cosmetic products, although there are some exceptions. The most of these substances are also restricted by the International Fragrance Association (IFRA) [15], the official representative body of the fragrance industry worldwide, with the main purpose of ensuring the safety of fragrance materials. Analytical methods for the determination of this group of substances are mainly based on gas chromatography–mass spectrometry (GC–MS) [16–19]. 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 [20–22]. Recently, a method for the quantification of 15 fragrance allergens in baby bathwaters has been published [23]. The analytical procedure is based on solid phase microextraction (SPME) and GC–MS analysis.

To our knowledge, there are no studies developing analytical methodology for the analysis and quantification of these fragrance allergens in indoor air. Few studies have reported the analysis of synthetic musk compounds in indoor air and suspended particulate matter. In all of them, musks have been collected by active sampling and, in general, reduced flow rates, using polyurethane foam as adsorbent [24–26]. The extraction of musk compounds from this adsorbent is carried out by Soxhlet using different solvent mixtures [25,26] and pressurized solvent extraction (PSE) [24]. To overcome the drawbacks of these methods related to time-consuming steps and large volumes of organic solvents required, Regueiro et al. [27] proposed the use of SPME as an alternative to solvent extraction. In this way, musk compounds are adsorbed onto a small amount of Tenax and analytes are transferred to a SPME fiber in the headspace mode. As an alternative to SPME fiber as the acceptor phase in the desorption of the analytes from the adsorbent, Barro et al. [28,29] proposed a simple method based on the rapid desorption of the analytes adsorbed on Tenax to a small volume of n-hexane for the determination of polychlorinated biphenyls [28] and pyrethroid insecticides [29] in indoor air.

The aim of the present study was to develop a fast, simple and inexpensive method for the determination of 24 fragrance allergens in indoor air based on the use of a very low amount of adsorbent to retain the compounds, which allowed their rapid desorption by UAE in a very low volume of solvent, avoiding further sample manipulation. The optimization of the methodological parameters was carried out using an experimental design approach to study the main factors as well as possible factor interactions. The performance of the method was studied in terms of linearity, precision, accuracy and limits of detection. The application to real samples collected in home and car environments allowed the determination of several of the target compounds at concentrations ranging from <1 to >100 $\mu g\,m^{-3}$.

2. Experimental

2.1. Reagents and materials

3,7-Dimethyl-1,6-octadien-3-ol, 97% (linalool, CAS number 78-70-6); 3,7-dimethyloct-6-en-1-ol, 95% (citronellol, 106-22-9); 2-methoxy-4-prop-2-enyl phenol, 99% (eugenol, 97-53-0); 1,2-dimethoxy-4-(2-propenyl)-benzene, 99% (methyleugenol, 93-15-2); 2H-1-benzopyran-2-one, 99% (coumarin, 91-64-5); 3,7,11-trimethyldodeca-2,6,10-trien-1-ol, 95% (farnesol, mixture of isomers, 4602-84-0); 3,7-dimethylocta-2,6-dienal, 95% (citral, cis/trans, 5392-40-5); 1-methyl-4-prop-1-en-2-yl-cyclohexene 97% (limonene, 5989-27-5); 4-methoxybenzene methanol, 98% (anisyl alcohol, 105-13-5); 2-methoxy-4-(1-propenyl) phenol, 98% (isoeugenol, cis/trans, 97-54-1); 3-phenyl phenylmethyl ester-2-propenoic acid, 99% (benzyl cinnamate, 103-41-3); and 2-(phenylmethylene)-heptanal, 97% (amyl cinnamal, 122-40-7) 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, ≥85% (ionone, 127-51-5); 3,7-dimetil-2,6-octadien-1-ol, ≥96% (geraniol, 106-24-1); 2-(phenylmethylene)-1-heptanol, ≥85% (amyl cinnamyl alcohol, 101,85-9); 3-(4-tert-butylphenyl)-2-methylpropanal, ≥95% (lilial, 80-54-6); 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde, ≥97% (lyral, 31906-04-4); and 2-hydroxy-phenylmethyl ester benzoic acid, ≥99% (benzyl salicylate, 118-58-1) were purchased from Fluka (Fluka Chemie GmbH, Steinheim, Germany).

2-Octynoic acid, methyl ester, ≥ 99% (methyl 2-octynoate, 111-12-6); 7-hydroxy-3,7-dimethyloctanal, ≥95% (hydroxycitronellal, 107-75-5); 3-phenyl-2-propenal, ≥93% (cinnamaldehyde, 104-55-2); 2-(phenylmethylene) octanal, ≥95% (hexyl cinnamaldehyde, 101-86-0), were purchased from SAFC Supply Solutions (St. Louis, USA).

Benzene methanol, 99% (benzyl alcohol, 100-51-6); 3-phenyl-2-propen-1-ol, 98% (cinnamyl alcohol, 104-54-1); phenylmethyl benzoate, 98.5% (benzyl benzoate, 120-51-4) was purchased from Chem Service (West Chester, USA).

n-Hexane, ethyl acetate, and acetone were provided by Merck (Darmstadt, Germany). Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in acetone and then stored in amber glass vials at −20 °C.

2.2. Gas chromatography–mass spectrometry

The GC–MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 280, 150 and 230 °C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a HP5-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). 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 °C min^{-1}, to 200 °C at 25 °C min^{-1} (held 5 min) and a final ramp to 225 °C at 8 °C min^{-1}.

Splitless mode (held 2 min) was used for injection, the split flow was set at 20 mL min^{-1} and the injector temperature was kept at 260 °C.

In the full scan mode the mass range was varied from 39 to 300 *m/z*, starting at 5 min. The analytes were positively identified by comparison of their mass spectra and retention times to those of

Table 1
Quantification ions and performance of the GC–MS method.

Key	Compound	MS detection	Linearity	Precision (% RSD)				
		Quantification Ions	Correlation coefficient (R)	Intra-day ($n=4$)			Inter-day ($n=7$)	
				0.05[a]	0.5[a]	10[a]	0.5[a]	10[a]
1	Limonene	93	1.0000	2.0	1.5	1.9	2.1	2.1
2	Benzyl alcohol	108	1.0000	17	3.9	1.6	10	2.9
3	Linalool	93	0.9997	2.3	2.2	1.7	0.8	1.7
4	Methyl-2-octynoate	95	0.9992	5.4	3.3	1.7	10	1.8
5	Citronellol	69	0.9971	–	3.2	2.0	5.4	1.8
6	Geraniol	69	0.9995	–	4.1	1.4	3.6	1.1
7	Citral	69	0.9999	4.6	2.3	1.8	3.2	1.8
8	Cinnamaldehyde	131	0.9991	3.8	4.1	0.98	3.5	1.7
9	Anisyl alcohol	138	0.9994	6.7	3.4	1.2	5.0	2.0
10	Hydroxycitronellal	59	0.9992	3.0	2.2	1.7	3.3	1.8
11	Cinnamyl alcohol	134	0.9996	–	5.5	2.2	8.3	2.4
12	Eugenol	164	1.0000	4.7	2.1	0.83	1.3	1.4
13	Methyleugenol	178	0.9997	2.8	0.7	1.1	5.7	1.4
14	Coumarin	146	0.9998	1.2	2.8	1.3	10	2.7
15	Isoeugenol	164	1.0000	5.4	2.4	1.1	3.4	1.7
16	Ionone	135	0.9990	1.2	1.4	1.3	1.2	1.1
17	Lilial	189	0.9993	2.3	1.0	1.1	1.1	1.5
18	Amyl cinnamal	129	0.9991	2.3	1.7	1.1	14	1.6
19	Lyral	136	0.9970	8.5	3.9	3.9	10	1.7
20	Amyl cinnamic alcohol	133	0.9988	–	5.5	0.95	12	2.9
21	Farnesol	69	0.9976	–	4.7	4.2	9.6	1.5
22	Hexyl cinnamaldehyde	129	0.9995	5.9	2.9	1.1	6.4	1.6
23	Benzyl benzoate	105	0.9989	4.4	1.1	1.7	4.6	1.8
24	Benzyl salicylate	91	0.9997	2.8	4.5	4.8	9.1	1.6
25	Benzyl cinnamate	131	0.9995	6.3	2.5	1.1	11	2.0

[a] Concentration levels ($\mu g\,mL^{-1}$).

standards. The quantification ions for each target compound are listed in Table 1.

2.3. Ultrasound-assisted extraction

To optimize the UAE of target compounds, a volume of 100 μL of standard mixtures of the analytes in acetone were directly spiked on 25 mg of the adsorbent: activated Florisil of 60–100 μm mesh (Aldrich, Steinheim, Germany) or Tenax TA of mesh size 60–80 (Supelco). Florisil was activated overnight in an oven at 130 °C. The spike was left 2 h at room temperature allowing the evaporation of the solvent, and then the selected volume (1 or 2 mL depending on the experiment) of the extractant organic solvent (ethyl acetate or n-hexane) was added to the glass vial, and sealed with a headspace aluminum cap furnished with PTFE-faced septum. The analytes were extracted from the samples to the organic solvent using an ultrasound bath (Ultrasons Med-II, J.P. Selecta, Barcelona, Spain) at 40 kHz of ultrasound frequency and 200 W power at 25 ± 3 °C or 45 ± 3 °C for 5 or 10 min, depending on the experiment. Afterwards, the extract was filtered through a 0.22 μm Millex®-GV filter (13 mm diameter) (Millipore, Bedford, USA), and injected in the chromatographic system.

In the final optimized conditions, 25 mg of Florisil were sonicated with 2 mL ethyl acetate for 5 min at 25 ± 3 °C. Blanks were periodically run during the analysis to confirm the absence of contamination.

2.4. Air sampling

To collect the target compounds from air, a known volume of air was pumped through a glass tube containing 25 mg of activated Florisil adsorbent by using a S-8 vacuum pump (Telstar, Tarrasa, Spain). Only PTFE tubing was used for all connections to minimize contaminations. Different volumes of air (0.05–1 m^3) were pumped at 0.010 $m^3\,min^{-1}$ through the microfiltration glass funnels containing 25 mg Florisil. The adsorbent with the retained compounds was then simply transferred from the glass funnel into a 10-mL headspace glass vial and the UAE 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.

To detect possible breakthrough of the adsorbent, some experiments required the coupling on-line of a second and a third glass tube filled with 25 mg of non-spiked Florisil to the first spiked one. Each portion of adsorbent was individually extracted.

2.5. Statistical analysis

Basic and descriptive statistics and experimental design analysis were performed using Statgraphics XV Centurion (Rockville, MD) as software package. The experimental design was applied in the optimization of the UAE method, to analyze the simultaneous effect of the main parameters.

3. Results and discussion

Difficulties described in literature dealing with the effective separation of the regulated suspected allergens [20] led to test different oven temperature programs in order to obtain a suitable chromatography of the compounds. First experiments also allowed the selection of the quantification ions to attain the maximum signal-to-noise ratio. In the GC–MS conditions summarized in the experimental section, all compounds could be determined in less than 21 min. Fig. 1 shows the chromatogram of a standard mixture of 25 allergen fragrances at a concentration of 5 $\mu g\,mL^{-1}$, in which the good separation of the compounds can be noticed.

Linearity of the GC–MS method was evaluated in the concentrations range from 0.025 to 20 $\mu g\,mL^{-1}$ (9 levels). The correlation coefficients were higher than 0.997 for all compounds (see Table 1). Intra-day and inter-day precision were evaluated at several concentration levels and both were satisfactory (<5% in most cases).

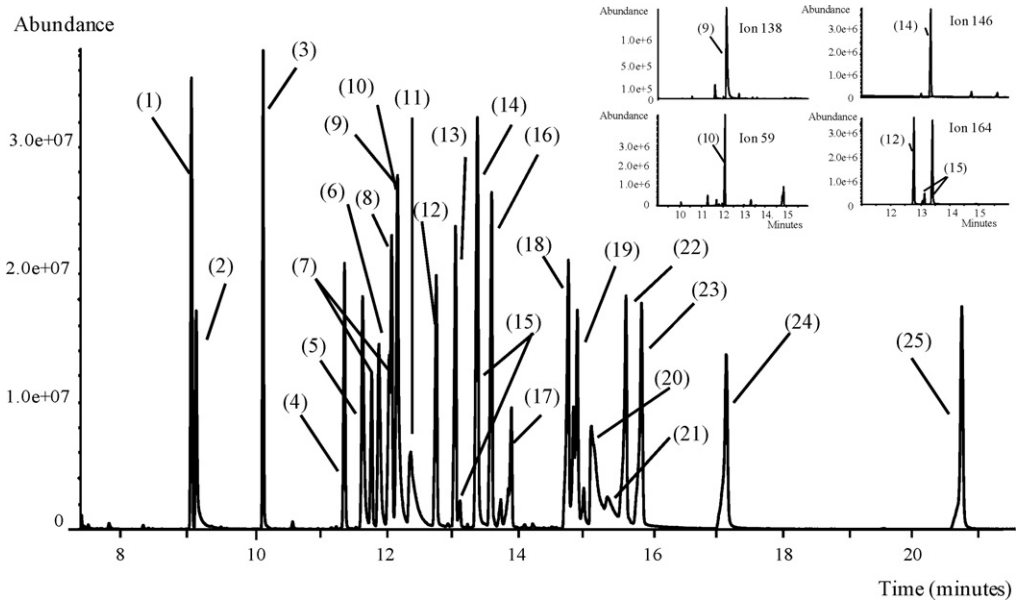

Fig. 1. GC–MS full scan chromatogram of a standard mixture of the fragrance allergens at 5 μg mL^{-1} in ethyl acetate (see number code equivalence in Table 1).

3.1. Optimization of the ultrasound-assisted solvent extraction

Desorption step determines the efficiency of the final method and then, experimental work was initially focused on the optimization of the UAE process using an experimental design approach. Five main factors were selected for this study: type of adsorbent, type and volume of extracting solvent, extraction temperature and ultrasounds application time. Tenax TA and Florisil were the choice for the two levels of factor type of adsorbent. The efficiency of Tenax TA and Florisil in the retention of some organic pollutants in air, even at such little amounts as 25 mg, was previously reported [30–32] and thus, both adsorbents were considered in the present study. Selection of the two solvents was related to the type of adsorbents we intended to check; on one hand, a very low polarity solvent such as n-hexane, and on the other, a medium polarity solvent such as ethyl acetate. This last factor was studied at three levels whereas all the other factors were studied at two levels. The factors selected and their levels are presented in Table 2.

A $3 \times 2^{(4-1)}$ mixed level fraction design was proposed (Statgraphics XV Centurion). The resolution of the design is V, enabling an estimation of all main effects and all two-factor interactions. Two center points were added to increase the degrees of freedom to evaluate the experimental error; thus, 26 experiments were run.

The outcomes of the experimental design can be simply interpreted by visualizing several intuitive software tools provided by Statgraphics. For practical reasons, only some representative examples are illustrated in Figs. 2–4. In the Pareto charts (Fig. 2), the standardized effects are plotted in decreasing order of absolute magnitude, thus making easier to see which are the most important factors and interactions. In addition, the line drawn on the chart indicates if an effect is statistically significant at a specified significance level (in this case, 95%).

Table 2
Factors and levels considered in the experimental design.

Factor	Code	Low level (−)	High level (+)	Continuous
Solvent	A	n-Hexane	Ethyl acetate	Yes
Temperature	B	25 °C	45 °C	Yes
Extraction time	C	5 min	10 min	Yes
Solvent volume	D	0.5 mL	2 mL	Yes
Adsorbent	E	Tenax	Florisil	No

Analyzing the Pareto charts (Fig. 2), it was observed that type of solvent (A), and solvent volume (D) were the most important parameters for the extraction efficiency. Factor A was significant for all analytes excluding limonene, and factor D was significant for half of the target compounds. The type of adsorbent used (factor E) was only significant for limonene and lilial. The standardized effect of the other 2 factors, B and C (temperature and extraction time, respectively) did not reach the significance border line. Fig. 3 shows the main effects plots for some representative compounds. These graphics show how the response varies when each factor is changed from its low level to its high level, while all other factors held at the center of the experimental domain. As can be seen, all analytes were more efficiently extracted from the adsorbent using 100% of ethyl acetate (the high level of this factor). The use of n-hexane provided lower responses than ethyl acetate and for some analytes the lowest results were obtained when a mix of both solvents was used (represented by a central minimum, e.g. linalool and benzyl benzoate, see Fig. 3). This last effect is also showed in the Pareto chart diagram (Fig. 2) with a significant effect for the quadratic term of this factor (AA) (for example, see lilial and hexyl cinnamaldehyde in the figure). Regarding solvent volume, all analytes were better extracted at the high level of this factor, 2 mL. For the other 3 main factors, the differences between the analytical response obtained for the low and the high level of the factor were not important, and so, these factors are represented by a short and almost horizontal line, excluding factor E for limonene and lilial as previously indicated, being the extraction more favorable from Tenax for limonene and from Florisil for lilial (see Fig. 3). Concerning interaction effects, only AD interaction was significant for some analytes such as hexyl cinnamaldehyde and benzyl benzoate (Fig. 2), and this effect is shown in Fig. 4 for some representative compounds. In these plots, the predicted response for each combination of the low and high levels of two factors is displayed at the end of each line segment. As it can be observed, the extraction efficiency using n-hexane is considerably lower than using ethyl acetate, as it was already concluded from the main effects plots (Fig. 3). Using the first solvent, the responses obtained were in general quite similar for 0.5 and 2 mL. Nevertheless, when ethyl acetate was used, higher response and, in consequence, better extraction efficiency was achieved with 2 mL of solvent.

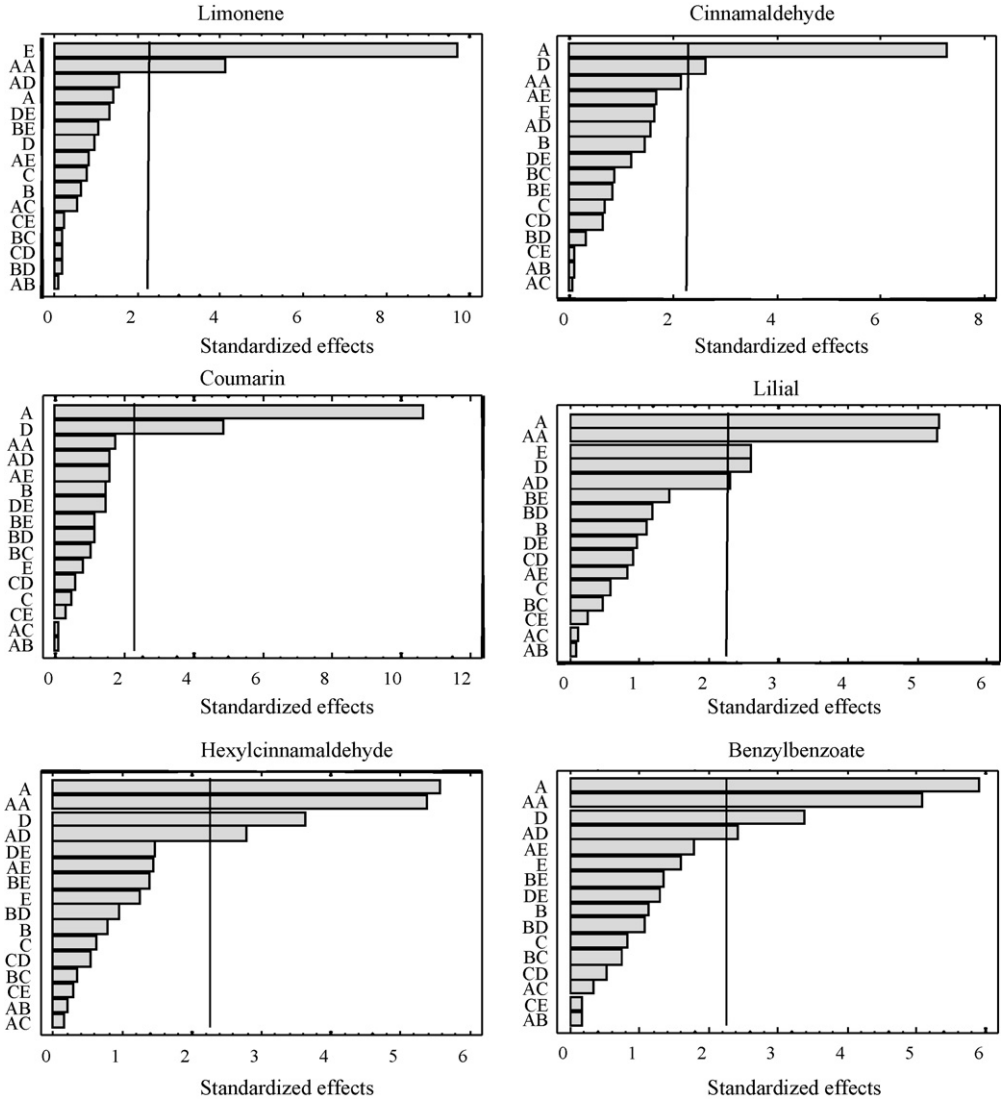

Fig. 2. Pareto charts showing the significant factors (95%) for some selected fragrance allergens (see factor codes in Table 2).

After optimization of the investigated factors, the recommended procedure for the simultaneous UAE of the target analytes was established as follows: temperature 25 °C, 2 mL of ethyl acetate, and 5 min of extraction time using Florisil or Tenax as adsorbent.

Under the experimental conditions selected, extraction efficiency was calculated using Florisil spiked at three levels (0.2, 2, and 25 μg of each compound) and, as can be seen in Table 3, average recoveries were satisfactory for most analytes (>80%), excluding benzyl salicylate for which recovery was about 50%. Anyway, the recovery for this last compound was consistent and equivalent at different concentration levels. The precision was also satisfactory with RSD in general lower than 10% (see Table 3); therefore, the extraction method can be considered suitable for all the target analytes.

The possibility of performing simultaneous extractions was also evaluated and the results obtained (Fig. 5) were equivalent for single and multiple extractions ($n = 6$), allowing in this way to improve the throughput of this method step.

3.2. Optimization of the sampling step

Once optimized the extraction process and confirmed that the allergens could be recovered from the adsorbent, the sampling step was studied. Initial experiments using Tenax and Florisil demonstrated the inefficiency of the Tenax to effectively retain the analytes.

To evaluate the possible breakthrough, portions of 25 mg Florisil were spiked in duplicate with 10 μg of the analytes and then, different volumes of air ranging from 0.05 to 1 m^3 were sampled. The portions of adsorbent were individually extracted under the optimized extraction conditions. Fig. 6 shows the results obtained. As it is clearly appreciated, limonene is almost completely lost in all experiments, even for a sample volume as low as 0.05 m^3. Benzyl alcohol and isoeugenol showed significant breakthrough in the sample range tested and analyte losses are evident above 0.2 m^3 air. Other compounds showed slightly lower responses for higher sample volumes, whereas for some compounds, in general the less volatile ones, no breakthrough was observed in the entire interval.

Some experiments were also run using larger amounts of adsorbent (up to 200 mg) and the results obtained were not improved. With the objective of mainly studying limonene losses and evaluating the possibility of recovering this compound satisfactorily, a series of experiments were carried out with 3 devices, each one containing 25 mg of Florisil, connected in series, and sampling only 0.05 m^3 of air. Limonene was detected and quantified in the three devices (at 1.6, 11 and 18% respectively), but the total recovery was

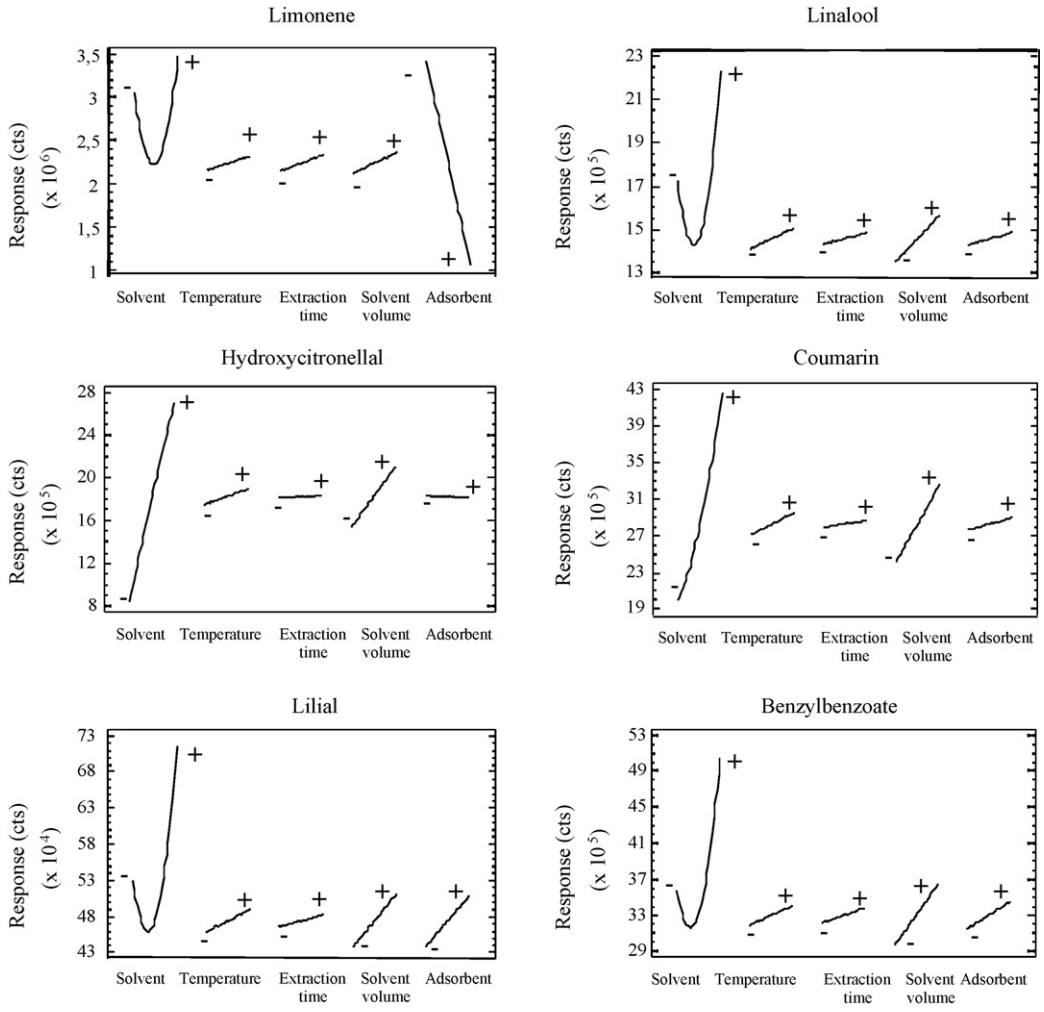

Fig. 3. Main effects plots for some selected fragrance allergens (see levels in Table 2).

only 31%. The other compounds were efficiently retained in the first device and only no significant amounts of some of the most volatile analytes were detected in the additional two Florisil portions.

Some additional UAE experiments performed leaving the spiked Florisil in an open vial for 10 min gave satisfactory recovery values for all analytes but again a very low recovery of limonene (24%), demonstrating the easiness of this compound to be lost either by volatilization, transformation (e.g. oxidation), or both mechanisms [33].

Due to the need of exhaustive cleaning of the glass microfilter samplers to avoid memory effects, and also for other practical reasons (e.g. easiness in transport), the use of disposable SPE cartridges instead of the glass adsorbent supports, was evaluated. No significant differences were found in the obtained results (data

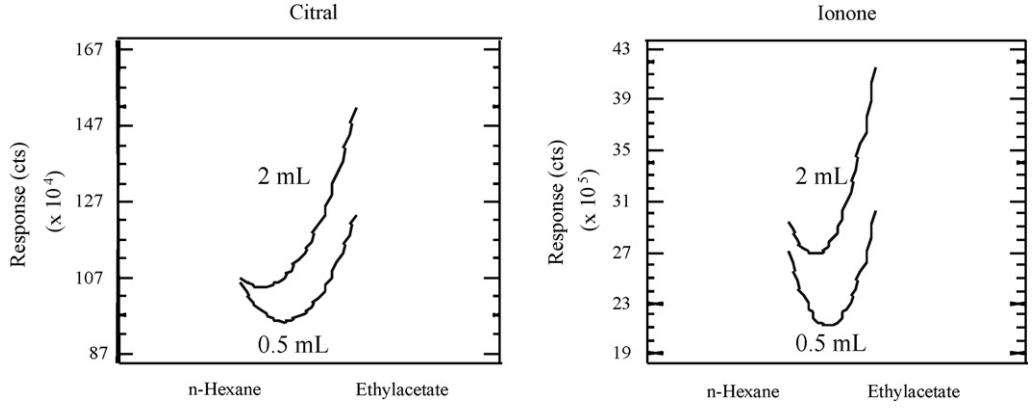

Fig. 4. Combined effect of factors type of solvent (A) and solvent volume (D) for two selected fragrance allergens: citral and ionone.

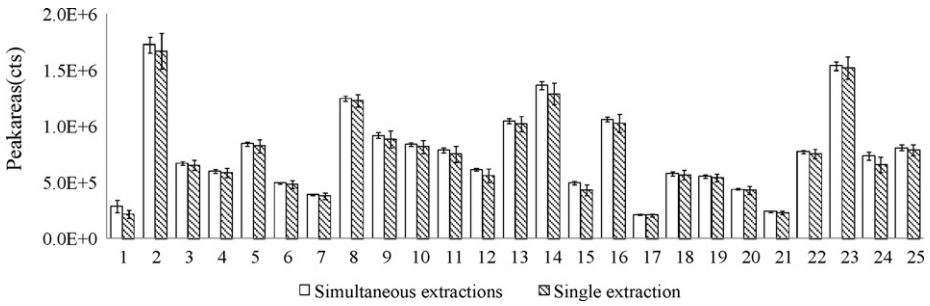

Fig. 5. Comparison of the responses obtained for simultaneous and single extractions (see number code equivalence in Table 1).

Table 3
Extraction efficiency (%) from Florisil at three spiked concentration levels.

Compound	0.2 μg (*n* = 4)		1 μg (*n* = 4)		25 μg (*n* = 4)	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Limonene	96.0	3.0	110	8.6	102	7.7
Benzyl alcohol	84.4	5.6	102	2.0	109	7.6
Linalool	98.9	6.0	105	4.2	105	7.2
Methyl-2-octynoate	88.5	5.6	106	3.1	108	8.3
Citronellol	111	13	105	8.5	108	8.4
Geraniol	98.8	6.5	110	3.2	106	8.2
Citral	90.3	2.7	108	2.8	108	8.1
Cinnamaldehyde	94.4	3.4	106	3.9	112	7.5
Anisyl alcohol	97.2	8.6	109	8.2	112	6.7
Hydroxycitronellal	93.9	5.8	104	4.0	112	6.8
Cinnamyl alcohol	79.2	7.8	101	6.7	114	6.6
Eugenol	90.0	5.5	81.1	5.2	93.4	10
Methyleugenol	89.0	6.5	107	2.8	110	7.7
Coumarin	93.4	5.0	103	4.0	113	3.6
Isoeugenol	104	11	94.2	8.6	86.6	11
Ionone	100	7.5	101	1.9	107	6.6
Lilial	101	2.8	105	3.0	105	8.2
Amyl cinnamal	100	7.1	105	1.5	114	7.8
Lyral	90.4	7.3	111	2.4	104	7.2
Amyl cinnamyl alcohol	100	6.8	102	4.6	114	7.7
Farnesol	104	8.9	111	5.0	118	7.3
Hexyl cinnamaldehyde	99.5	3.5	100	2.1	114	7.5
Benzyl benzoate	94.2	5.9	110	2.1	115	5.1
Benzyl salicylate	44.9	9.6	43.6	8.1	59.8	10
Benzyl cinnamate	110	3.3	120	0.6	96.9	3.6

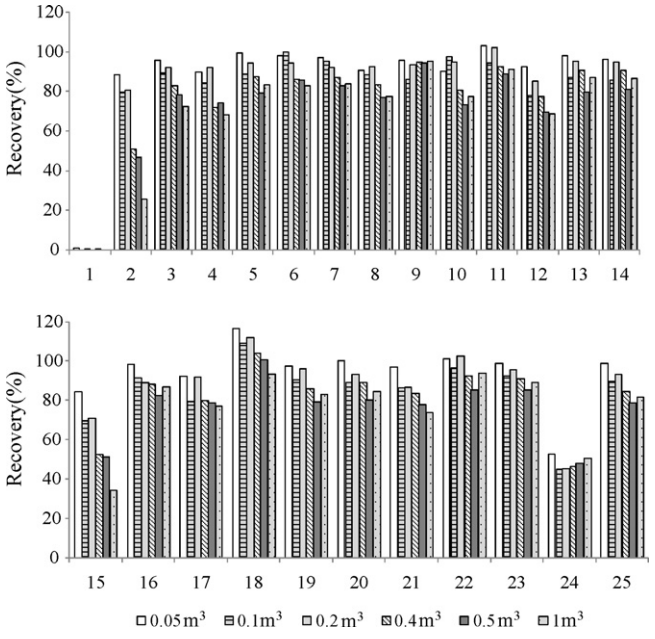

Fig. 6. Variation of the chromatographic response with the volume of air sampled (see number code equivalence in Table 1).

not shown) and thus, both materials could be used in the same way.

In view of the results obtained and with the aim of establishing a general method for the analysis of all target allergens in air excluding limonene, a sampling volume of 0.2 m^3 was selected. If more sensitivity were required larger sample sizes (up to 1 m^3) could be collected assuming important losses only for two analytes (benzyl alcohol and isoeugenol) and slight losses (about 10–20%) for some other analytes.

3.3. Performance of the method

In all validation experiments, results obtained are referred to the sampling of 0.2 m^3 air. With the aim to assure blank samples, air blanks as well as adsorbent blanks were obtained in a clean room provided with a laminar flow system and analyzed before every set of experiments.

Efficiency of the total sampling-extraction process was evaluated at three concentration levels (1, 5, 125 μg m^{-3}). Recovery was satisfactory with values >80% in most cases (see Table 4). Recovery values for benzyl salicylate were corrected according to the extraction efficiency for this compound (see Section 3.1). Precision of the method can be considered good with RSD values generally <10%.

Limits of detection (LOD, S/N = 3) of the proposed method are also included in Table 4, showing values ≤0.6 μg m^{-3}, with the

Table 4
Recovery (%), repeatability (%), and limits of detection of the total sampling-extraction process.

Compound	1 μg m^{-3} (n = 4)		5 μg m^{-3} (n = 4)		125 μg m^{-3} (n = 4)		LOD (μg m^{-3})
	Recovery	RSD	Recovery	RSD	Recovery	RSD	
Benzyl alcohol	98.7	5.8	86.4	5.5	76.5	0.7	0.19
Linalool	77.6	4.1	87.4	3.4	88.7	0.5	0.015
Methyl-2-octynoate	104	9.8	92.1	5.0	92.6	2.4	0.13
Citronellol	116	8.4	89.9	4.4	92.4	0.2	0.36
Geraniol	92.4	7.4	99.4	3.2	93.2	13	0.29
Citral	90.6	3.3	108	4.8	91.3	2.1	0.16
Cinnamaldehyde	94.4	8.2	97.6	6.0	87.6	2.3	0.12
Anisyl alcohol	98.6	9.5	107	11	86.7	1.4	0.23
Hydroxycitronellal	92.4	8.6	97.2	4.5	98.8	0.9	0.038
Cinnamyl alcohol	90.5	6.6	109	5.1	97.9	0.7	0.55
Eugenol	67.2	4.7	76.5	2.7	85.0	1.6	0.041
Methyleugenol	95.9	6.6	97.4	6.9	88.0	3.3	0.027
Coumarin	108	7.5	99.9	6.3	88.4	0.7	0.069
Isoeugenol	90.4	9.1	83.4	4.8	70.6	5.2	0.37
Ionone	91.6	6.4	94.7	6.8	83.6	10	0.017
Lilial	102	7.2	100	3.6	112	1.2	0.019
Amyl cinnamal	105	5.2	105	2.5	112	10	0.17
Lyral	99.3	6.3	111	3.5	96.0	5.9	0.16
Amyl cinnamyl alcohol	102	8.4	106	5.8	85.4	1.0	0.18
Farnesol	101	7.9	114	6.6	85.2	6.4	2.2
Hexyl cinnamaldehyde	87.4	5.5	99.7	4.6	100	4.0	0.15
Benzyl benzoate	95.3	6.2	108	3.3	87.8	1.2	0.037
Benzyl salicylate[a]	111	10	97.4	8.4	90.4	1.5	0.036
Benzyl cinnamate	105	2.6	114	5.8	90.3	0.1	0.15

[a] Recovery values were corrected taking into account the average extraction efficiency for this compound.

Table 5
Compounds found (μg m^{-3}) in indoor air samples.[a].

Compound	S1	S2	S3	S4	S5	S6	S7	S8	S9
Benzyl alcohol	<LOQ		<LOQ			<LOQ		3.9	
Linalool	14	<LOQ	104	136	43	3.08	100	38	<LOQ
Citronellol	<LOQ	1.1	11	3.8	5.7		9.1	9.5	35
Citral		2.9	4.5	10.1	24.3	0.23	0.91	6.1	2.2
Hydroxycitronellal									61
Eugenol			0.42	0.41			3.6	0.34	3.1
Coumarin				0.96					
Ionone	0.54	1.1	0.21	0.76	1.7	<LOQ	5.1	1.8	
Lilial	1.2	1.4	3.1	1.9	15.1	0.33	64	60	194
Lyral									4.6
Hexyl cinnamaldehyde	<LOQ	0.89	<LOQ		0.54		4.9	0.72	
Benzyl benzoate		<LOQ	<LOQ		<LOQ			<LOQ	0.53
Benzyl salicylate		0.16	<LOQ		<LOQ			0.17	

[a] Blank spaces mean values below LOD.

exception of farnesol (2.2 μg m^{-3}). LOD values at the low ng m^{-3} were obtained for several compounds (linalool, hydroxycitronellal, eugenol, methyleugenol, coumarin, ionone, lilial, benzyl benzoate, and benzyl salicylate).

3.4. Application to real indoor air samples

Finally, the proposed method was applied to real samples collected in different home rooms (0.2 m^3, 0.010 m^3 min^{-1}) that had been treated with aerosols, electrical diffusion units, as well as different common cleaning products of general domestic use in Spain. The application of the products was made following the recommendations of the manufacturers regarding the appropriate amounts to be used, when available, and depended on the use of the sampled room and always respected the generalized habits people have in using this kind of products. The air inside of a car was also sampled (sample S9). Concentrations of the compounds are summarized in Table 5. As can be seen, several of the target analytes were present in the indoor air and could be determined. Linalool and lilial were found in all the analyzed samples, whereas citronellol and ionone were present in seven of the nine air samples. The highest found concentrations corresponded to lilial (194 μg m^{-3}) and linalool (136 μg m^{-3}).

4. Conclusions

A very simple and sensitive method to analyze fragrance allergens in indoor air was developed. The active retention of the target compounds on a very small amount of Florisil and the subsequent desorption by application of ultrasounds using only 2-mL ethyl acetate, avoided for the requirements of extract concentration prior to chromatographic analysis. After optimization of the extraction step, the study of the retention efficiency from air demonstrated that for most compounds no breakthrough occurred up to 0.2 m^3. Only limonene was not efficiently retained even sampling very low air volumes. For all the other analytes a general methodology was satisfactorily developed and proposed. The study of method performance demonstrated its linearity, quantitative recoveries, and good sensitivity, with LODs $\leq$0.6 μg m^{-3}. In addition, the method allowed high sample throughput since the total sampling-extraction-analysis process is completed within one hour. The analysis of several air samples demonstrated the validity

of the proposed method for the analysis of the target compounds in indoor environments.

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