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Analysis of regulated suspected allergens in waters

Elias Becerril, J. Pablo Lamas, Lucia Sanchez-Prado, Maria Llompart, Marta Lores, Carmen Garcia-Jares*

Departamento de Quimica Analitica, Nutricion y Bromatologia, Facultad de Quimica, Universidad de Santiago de Compostela, E-15782 Santiago de Compostela, Spain

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ABSTRACT

Fragrance suspected allergens including those regulated by the EU Directive 76/768/EEC have been determined in different types of waters using solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS). The procedure was based on headspace sampling (HS-SPME) using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers and has been optimized by an experimental design approach. The method performance has been studied showing good linearity ($R \geq 0.994$) as well as good intra-day and inter-day precision (RSD $\leq$ 12%). Detection limits (S/N = 3) ranged from 0.001 to 0.3 ng mL^{-1}. Reliability was demonstrated through the quantitative recoveries of the compounds in real water samples, including baby bathwaters, swimming pool waters, and wastewaters. The absence of matrix effects allowed quantification of the compounds by external aqueous calibration. The analysis of 35 samples of different types of waters showed the presence of suspected allergens in all the analyzed samples. All targets were found in the samples, with the exception of methyl eugenol and amyl cinnamic alcohol. Highest concentrations of suspected allergens were present in baby bathwaters, containing from 5 to 15 of the compounds at concentrations ranging from few pg mL^{-1} to several hundreds of ng mL^{-1}.

1. Introduction

Fragrances are a group of chemicals incorporated in most cosmetic and other personal care products including baby care ones. The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP), currently known as the Scientific Committee on Consumer Products (SCCP), has identified 26 of these ingredients as likely to cause contact allergies [1,2]. Twenty-four of these suspected allergens are able to be analyzed by GC, whereas the other two are not single compounds but very complex natural extracts (oak moss and tree moss) unsuitable for GC. The European Cosmetic Directive requires an indication of the presence of potential fragrance allergens in cosmetic products if the limits of 0.01% and 0.001% for rinse-off and leave-on products, respectively, are exceeded [3].

Among products for baby care, those intended for the bath such as shampoos, bubble baths, shower gels, and soaps, contain detergents that can break down the natural barrier of the skin, allowing other irritants and allergens to penetrate. In the developed countries the daily baby bath is a common practice, and babies and kids usually expend long time in the bath playing with toys. During bath, the exposure of children to chemicals is not only through dermal absorption, but also inhalation and ingestion can play an important role.

Swimming pools have been recently recognized as important sources of exposure to pollutants. Epidemiologic studies have shown increased risk of asthma both in indoor and outdoor pools [4]. In the case of suspected allergens, they can be easily transferred to the pool water since they are present in all kind of cosmetics and sun creams.

Some of the suspected allergens do not only pose the risk of causing contact allergies, but also systemic effects [5]. On the contrary, methyl eugenol that had been included in 2002 in the list of forbidden substances in the EU Cosmetic Directive [6] due to the potential risk of inducing cancer [7] has been recently incorporated to the regulated list of compounds to be used as fragrance components [3].

Analytical methods for the determination of this group of substances are mainly based on gas chromatography–mass spectrometry (GC–MS) [8–11]. To overcome difficulties on obtaining good resolution between compounds and with other matrix components, especially in cosmetic samples, several methods based on multidimensional chromatography have been proposed [12,13].

The suitability of solid-phase microextraction (SPME) for the analysis of suspected allergens has been recently demonstrated [14]. The proposed procedure allowed the reliable determination of 15 selected fragrance ingredients frequently found in baby bath-

* Corresponding author. Tel.: +34 981563100x14394; fax: +34 981595012.
E-mail addresses: JBecerrilB@iingen.unam.mx (E. Becerril), juanpablo.lamas@usc.es (J.P. Lamas), lucia.sanchez@usc.es (L. Sanchez-Prado), maria.llompart@usc.es (M. Llompart), marta.lores@usc.es (M. Lores), carmen.garcia.jares@usc.es (C. Garcia-Jares).

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waters. A SPME followed by GC–MS method was also developed by Masuck et al. [15] to determine the emission of several fragrance allergens released from scented toys into ambient air.

The aim of the present study is to develop a general procedure based on solid-phase microextraction (SPME) with gas chromatography–mass spectrometry (GC–MS) for the analysis of fragrance suspected allergens in water samples that include all the regulated compounds amenable by GC. Since these compounds belong to diverse chemical families with a broad range of polarities and volatilities, optimization of the main variables affecting the SPME process has been performed using an experimental design approach. The SPME-GC–MS method has then been validated for the identification and quantification of 24 fragrance suspected allergens regulated in the EU Directive [3], as well as pinene and methyl eugenol. The method has been applied to baby bathwaters, swimming pool waters and wastewaters. Results demonstrated the presence of several of the target compounds in all samples and, in the particular case of baby bathwaters at concentrations of several hundreds of ng mL^{-1} in some of the samples.

2. Experimental methods

2.1. Reagents and materials

3,7-Dimethyl-1,6-octadien-3-ol, 97% (linalool); 3,7-dimethyloct-6-en-1-ol, 95% (β-citronellol); 2-methoxy-4-prop-2-enyl phenol, 99% (eugenol); 1,2-dimethoxy-4-(2-propenyl)-benzene, 99% (methyl eugenol); 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% ((R)-(+)-limonene); 4-methoxybenzene methanol, 98% (anis alcohol); 2-methoxy-4-(1-propenyl) phenol, 98% (isoeugenol, cis/trans); 2-(phenylmethylene)-heptanal, 97% (amyl cinnamaldehyde); and 3-phenyl phenylmethyl ester-2-propenoic acid, 99% (benzyl cinnamate) were purchased from Aldrich (Sigma–Aldrich Chemie GmbH, Steinheim, Germany).

2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene, ≥99% ((−)-α-pinene); 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, ≥85% (ionone); 3,7-dimetil-2,6-octadien-1-ol, ≥96% (geraniol); 2-(phenylmethylene)-1-heptanol, ≥85% (amyl cinnamic alcohol); 3-(4-tert-butylphenyl)-2-methylpropanal, ≥95% (lilial); 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde, ≥97% (lyral); and 2-hydroxy-phenylmethyl ester benzoic acid, ≥99% (benzyl salicylate) were purchased from Fluka (Fluka Chemie GmbH, Steinheim, Germany).

2-Octynoic acid, methyl ester, ≥99% (methyl-2-octynoate); 7-hydroxy-3,7-dimethyloctanal, ≥95% (hydroxycitronellal); 3-phenyl-2-propenal, ≥93% (cinnamaldehyde); 2-(phenylmethylene) octanal, ≥95% (hexylcinnamaldehyde), 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) was purchased from Chem Service (West Chester, USA).

Table 1 shows the chemical abstract service (CAS) registry numbers, IUPAC names, molecular formula, as well as the main physicochemical properties of the target compounds. Molecular structures are depicted in Fig. 1.

Table 1
CAS number, IUPAC names, molecular formula; and main properties of the studied allergens.

Key	Compound	CAS number	IUPAC name	Molecular formula	Molecular weight	log K_{OW}	Boiling point (°C)	Solubility[a] (mg/L)
1	Pinene	80-56-8	2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136	4.37	155	18
2	Limonene	5989-27-5	1-Methyl-4-prop-1-en-2-yl-cyclohexene	$C_{10}H_{16}$	136	4.57	176	13.8
3	Benzyl alcohol	100-51-6	Benzene methanol	C_7H_8O	108	1.05	205	40000
4	Linalool	78-70-6	3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$	154	3.28	198	1589
5	Methyl-2-octynoate	111-12-6	2-Octynoic acid, methyl ester	$C_9H_{14}O_2$	154	2.60	219	–
6	Citronellol	106-22-9	3,7-Dimethyloct-6-en-1-ol	$C_{10}H_{20}O$	156	3.38	225	322
7	Citral	5392-40-5	3,7-Dimethylocta-2,6-dienal	$C_{10}H_{16}O$	152	3.17	229	590
8	Geraniol	106-24-1	3,7-Dimethyl-2,6-octadien-1-ol	$C_{10}H_{18}O$	154	3.28	229	531
9	Cinnamaldehyde	104-55-2	2-Propenal, 3-phenyl-	C_9H_8O	132	2.22	252	1420
10	Hydroxycitronellal	107-75-5	7-Hydroxy-3,7-dimethyloctanal	$C_{10}H_{20}O_2$	172	1.54	240	23800
11	Anis alcohol	105-13-5	Benzene methanol, 4-methoxy-	$C_8H_{10}O_2$	138	1.10	259	2070
12	Cinnamyl alcohol	104-54-1	2-Propen-1-ol, 3-phenyl-	$C_9H_{10}O$	134	1.93	250	1800
13	Eugenol	97-53-0	2-Methoxy-4-prop-2-enyl-phenol	$C_{10}H_{12}O_2$	164	2.20	256	<1000
14	Methyl eugenol	93-15-2	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_2$	178	2.9	248	500
15	Isoeugenol	97-54-1	Phenol, 2-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O_2$	164	2.45	267	984
16	Coumarin	91-64-5	2H-1-Benzopyran-2-one	$C_9H_6O_2$	146	1.39	301	2500
17	Ionone	127-51-5	4-(2,6,6-Trimethyl 2-cyclohexen-1-yl)-3-methyl-3-buten-2-one	$C_{14}H_{22}O$	206	4.41	266	16
18	Lilial	80-54-6	3-(4-tert-Butylphenyl)-2-methylpropanal	$C_{14}H_{20}O$	204	4.07	279	33
19	Amylcinnamaldehyde	122-40-7	Heptanal, 2-(phenylmethylene)-	$C_{14}H_{18}O$	202	4.80	289	8.5
20	Lyral	31906-04-4	4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde	$C_{13}H_{22}O_2$	210	2.53	319	185–1045
21	Amyl cinnamic alcohol	101-85-9	1-Heptanol, 2-(phenylmethylene)-	$C_{14}H_{20}O$	204	4.37	>200	26
22	Farnesol	4602-84-0	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	$C_{15}H_{26}O$	222	5.31	283	267
23	Hexylcinnamaldehyde	101-86-0	Octanal, 2-(phenylmethylene)-	$C_{15}H_{20}O$	216	4.82	308	2.8
24	Benzyl benzoate	120-51-4	Phenylmethyl benzoate	$C_{14}H_{12}O_2$	212	3.97	324	19.8
25	Benzyl salicylate	118-58-1	Benzoic acid, 2-hydroxy-, phenylmethyl ester	$C_{14}H_{12}O$	228	4.31	320	<1000
26	Benzyl cinnamate	103-41-3	2-Propenoic acid, 3-phenyl-, phenylmethyl ester	$C_{16}H_{14}O_2$	238	3.65	371	9

[a] Water, 298 K.

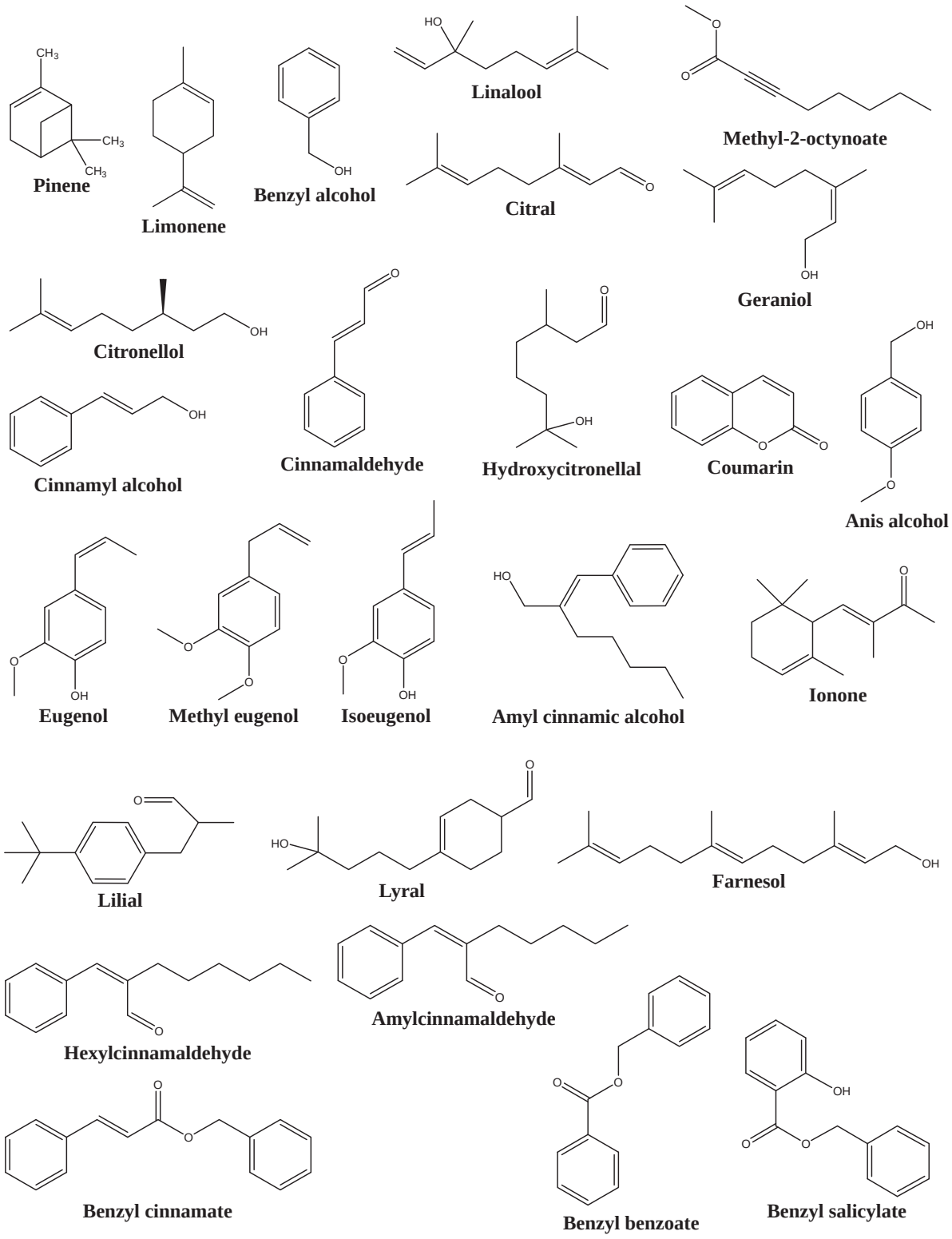

Fig. 1. Chemical structures of the considered fragrance compounds.

Methanol, ethyl acetate, and acetone were provided by Merck (Darmstadt, Germany). Individual stock solutions of each compound were prepared in methanol. Further dilutions and mixtures were prepared in acetone. The latter were employed for spiking water samples. Working solutions were made by appropriate dilution and then stored in amber glass vials at −20 °C.

Sodium chloride 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).

The SPME manual holders and 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers were supplied by Supelco

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

Key	Ret. time (min)	Compound	Quantification ions	Identification ions
1	6.66	Pinene	77,93	41,77,93,121
2	8.65	Limonene	67,93	39,67,93,136
3	8.95	Benzyl alcohol	77,79,108	77,79,108
4	9.84	Linalool	43,71,93	43,71,55,93
5	11.08	Methyl-2-octynoate	67,79,95	67,79,93,95
6	11.36	Citronellol	41,67,69	41,67,69,81
7	11.50	Citral	39,41,69	39,41,69,84
	11.77			
8	11.59	Geraniol	41,69	41,69,93,123
9	11.87	Cinnamaldehyde	77,103,131	51,77,103,131
10	11.92	Hydroxycitronellal	43,59,71	41,43,59,71
11	11.97	Anis alcohol	77,109,138	77,109,137,138
12	12.14	Cinnamyl alcohol	78,91,92	78,91,92,134
13	12.47	Eugenol	164	103,131,164
14	12.78	Methyl eugenol	178	147,163,178
15	12.85	Isoeugenol	164	77,103,164
	13.12			
16	13.16	Coumarin	118,146	89,118,146
17	13.28	Ionone	93,121,136	93,121,135,136
18	13.62	Lilial	189	131,147,189
19	14.16	Amylcinnamaldehyde	129	117,129,202
20	14.62	Lyral	79,91,93	79,91,93,136
21	14.74	Amyl cinnamic alcohol	91,133	91,115,129,133
22	14.94	Farnesol	41,69	41,69,81,121
23	15.32	Hexylcinnamaldehyde	129,216	117,129,216
	15.57			
24	15.64	Benzyl benzoate	105,194	77,91,105,194
25	16.94	Benzyl salicylate	91	39,65,91
26	20.75	Benzyl cinnamate	91,131	91,131,192

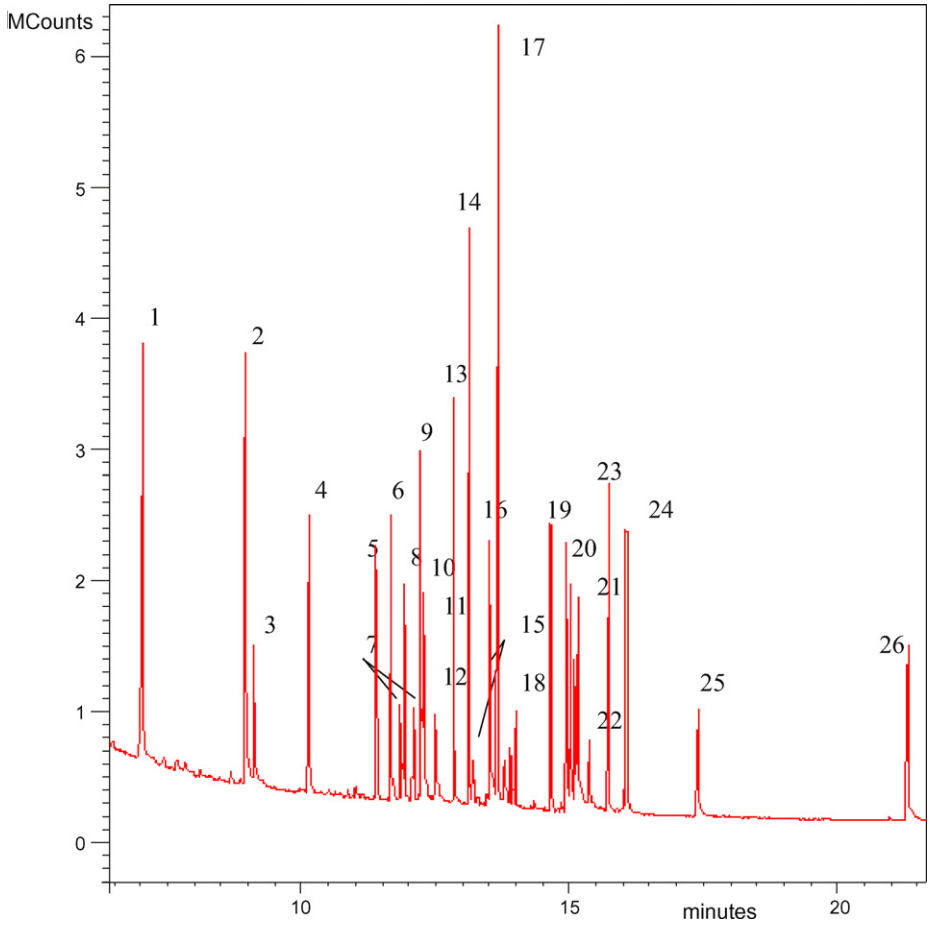

Fig. 2. GC–MS chromatogram obtained by direct injection of a standard mixture of the fragrance compounds at 10 μg mL^{-1} in ethyl acetate (see number code equivalence in Table 1).

Table 3
Optimal conditions for the microextraction mode and temperature (HS, headspace, IM, immersion).

Key	Compound	Temperature (°C)	Extraction mode
1	Pinene	50.0	HS
2	Limonene	50.0	HS
3	Benzyl alcohol	50.0	IM
4	Linalool	66.8	HS
5	Methyl-2-octynoate	55.9	HS
6	Citronellol	86.3	HS
7	Citral	89.5	HS
8	Geraniol	92.8	HS
9	Cinnamaldehyde	57.4	IM
10	Hydroxycitronellal	53.7	IM
11	Anis alcohol	100	HS
12	Cinnamyl alcohol	50.0	IM
13	Eugenol	75.9	IM
14	Methyl eugenol	100	HS
15	Isoeugenol	81.7	HS
16	Coumarin	50.0	IM
17	Ionone	81.7	HS
18	Lilial	100	HS
19	Amylcinnamaldehyde	100	HS
20	Lyral	100	IM
21	Amyl cinnamic alcohol	100	HS
22	Farnesol	100	HS
23	Hexylcinnamaldehyde	100	HS
24	Benzyl benzoate	100	HS
25	Benzyl salicylate	81.7	HS
26	Benzyl cinnamate	100	IM

(Bellefonte, PA, USA). Prior to first use, fibers were conditioned as recommended by the manufacturer.

Water samples were collected in amber glass containers. Sodium thiosulphate (0.1 mg mL^{-1}) was added to remove possible free chlorine. Samples were stored in the dark at 4 °C until analysis.

2.2. 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 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 × 0.32 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 °C min^{-1}; to 200 °C at 25 °C min^{-1}; (held 5 min); and a final ramp to 233 °C at 8 °C min^{-1} (total analysis time = 22.5 min). The splitless mode (held 2 min) was used for injection, with the split flow at 20 mL min^{-1}. The injector temperature was kept at 220 °C.

The ion trap mass spectrometer was operated in the electron impact (EI) ionization mode (+70 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.

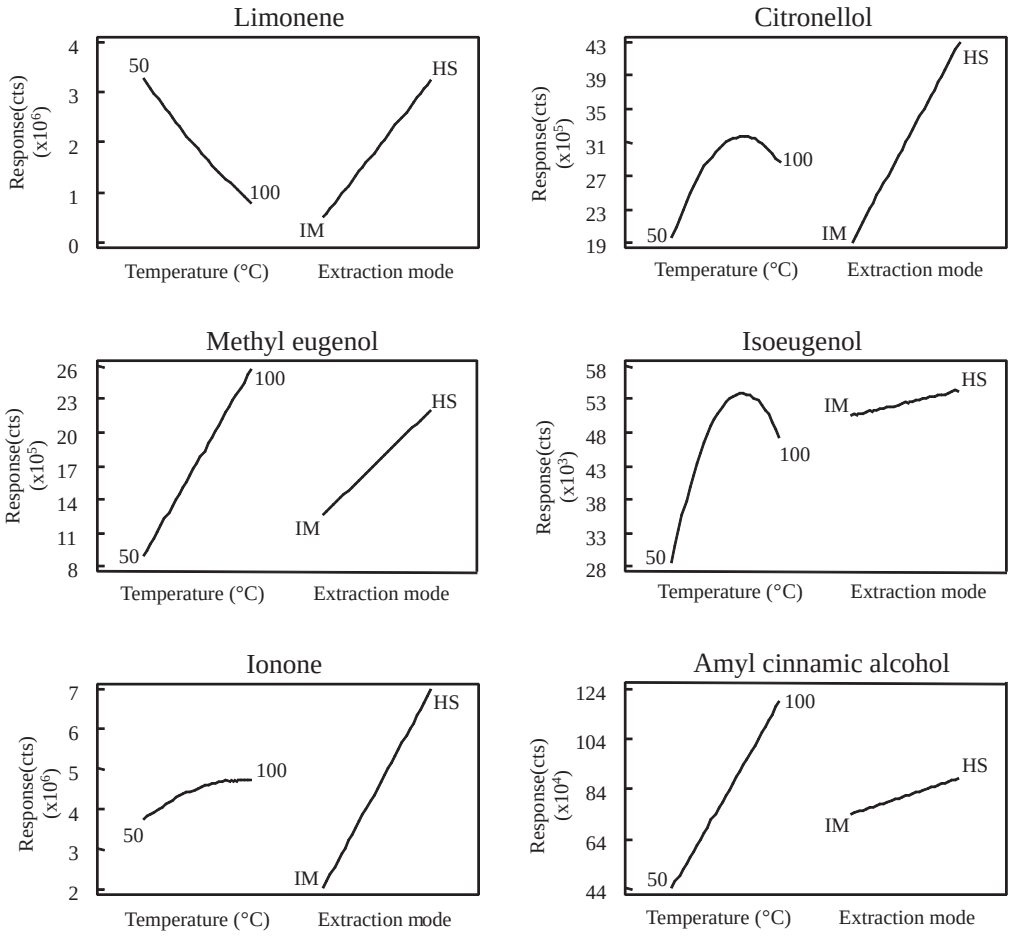

Fig. 3. Main effects plots for some selected compounds.

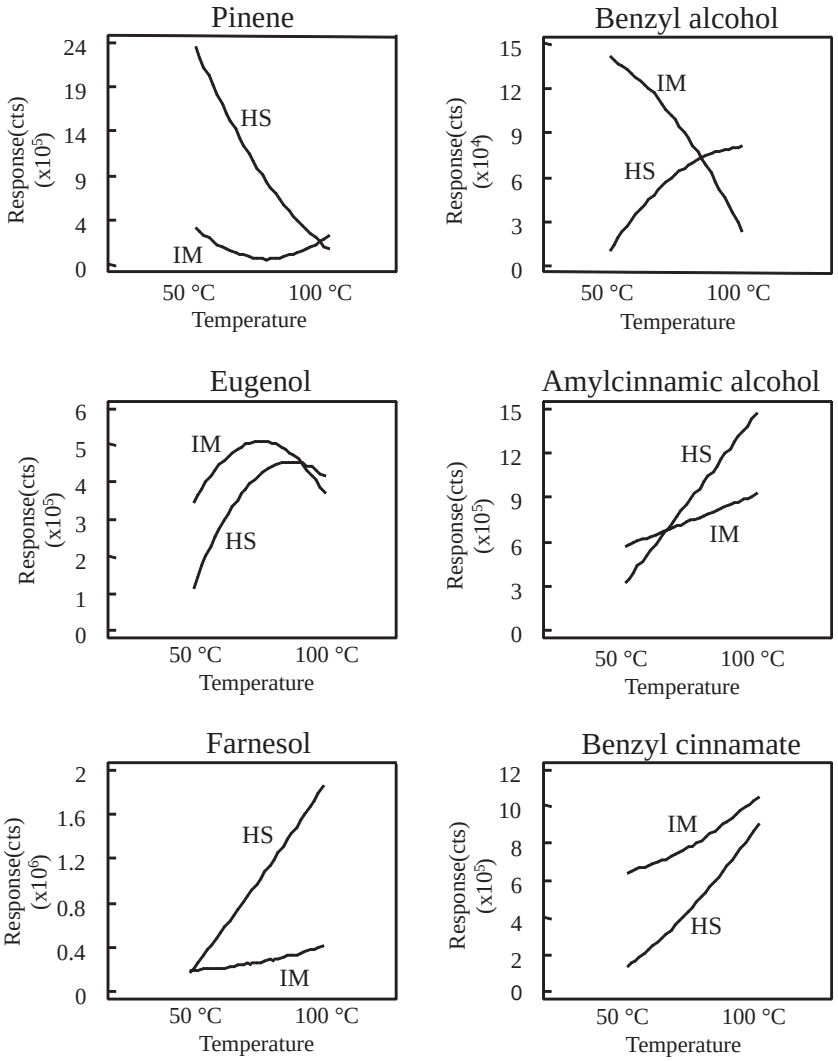

Fig. 4. Combined effect of factors for some selected compounds.

In the full scan mode the mass range was varied from 39 to 400 m/z at 3 μscans, starting at 5 min and ending at 22.5 min. The filament emission current was 25 μA. The analytes were positively identified by comparison of their mass spectra and retention times to those of standards. The identification and quantification ions, as well as the retention times for each target compound are listed in Table 2.

2.3. Solid-phase microextraction

Initial SPME conditions were optimized elsewhere [14]. In summary, aliquots of 10 mL water sample are placed in 22 mL headspace vials containing sodium chloride (2 g). Then, vials are sealed with aluminium caps furnished with Teflon-faced septa and immersed in a thermostatized water bath. Samples are let to equilibrate for 5 min before the exposition of a DVB/PDMS fiber for 20 min. In the optimized procedure, samples are heated at 100 °C and extracted in the headspace mode. Magnetic stirring was performed during the extraction process. Once finished the exposition period, the fiber was retracted into the needle of the holder syringe and immediately inserted into the GC injector. Desorption was carried out at 220 °C for 5 min. 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.

3. Results and discussion

3.1. Optimization of the analytical procedure

Difficulties described in literature due to the complexity of fragrance mixtures dealing with the effective separation and accurate determination of the 24 regulated suspected allergens [9,12,16] 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 Section 2, as well as in Table 2, all compounds could be determined in less than 21 min. The GC–MS instrumental linearity was evaluated by direct injection of the target analytes at different concentrations in ethyl acetate (1–50 μg mL^{-1}, 2 μL injection volume, 6 calibration levels). Linearity was good in the studied concentration range, with linear regression coefficients ranging from 0.993 to 0.999. Repeatability (n = 5) expressed in terms of relative standard deviation (RSD) was lower than 4.4%. Fig. 2 shows the chromatogram of a standard mixture of the 26 allergen fragrances at a concentration of 10 μg mL^{-1}.

The suitability of the SPME technique for the determination of 15 fragrance allergens had been previously demonstrated, highlighting the importance of extraction mode and temperature in the

Table 4
Precision, linearity, and limits of detection and quantification of the method.

Compound	Intra-day precision (%RSD, $n=3$)			Inter-day precision (%RSD, $n=5$)			Linearity (R)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
	1 ng mL^{-1}	5 ng mL^{-1}	20 ng mL^{-1}	5 ng mL^{-1}	10 ng mL^{-1}	20 ng mL^{-1}			
Pinene	3.6	3.8	9.7	4.0	11	8.6	0.9963	0.10	0.35
Limonene	1.7	4.2	4.1	6.2	5.1	9.7	0.9993	0.006	0.021
Benzyl alcohol	2.8	5.7	1.0	9.7	13	11	0.9974	0.19	0.63
Linalool	2.2	7.8	1.0	6.4	12	4.6	0.9990	0.009	0.031
Methyl-2-octynoate	5.2	4.1	9.6	8.6	12	8.6	0.9960	0.13	0.42
Citronellol	4.2	5.4	0.8	8.7	7.9	6.5	0.9988	0.021	0.069
Citral	0.7	0.7	0.6	1.2	3.4	2.9	0.9982	0.042	0.14
Geraniol	3.5	8.9	0.7	6.6	5.3	2.5	0.9981	0.048	0.16
Cinnamaldehyde	2.1	4.7	1.0	7.7	12	4.3	0.9995	0.060	0.20
Hydroxycitronellal	8.9	5.7	3.6	4.3	11	3.7	0.9944	0.20	0.67
Anis alcohol	n.a.	4.7	7.1	3.6	11	6.0	0.9961	0.94	3.1
Cinnamyl alcohol	7.3	3.8	5.9	5.3	5.4	7.5	0.9997	0.14	0.46
Eugenol	8.9	5.5	2.8	6.5	6.6	3.9	0.9994	0.012	0.040
Methyl eugenol	0.9	1.4	2.7	2.5	2.7	7.3	0.9995	0.003	0.010
Isoeugenol	12	4.7	3.0	6.3	0.6	2.4	0.9963	0.038	0.13
Coumarin	2.7	4.6	4.3	7.1	7.7	3.7	0.9991	0.19	0.63
Ionone	1.9	3.8	0.9	7.0	2.5	0.8	0.9974	0.001	0.003
Lilial	2.4	5.3	1.6	4.8	4.8	3.0	0.9988	0.004	0.013
Amyl cinnamaldehyde	0.7	0.5	2.5	4.6	8.9	3.7	0.9998	0.001	0.004
Lyral	n.a.	7.0	1.5	11	17	3.2	0.9998	1.1	3.6
Amyl cinnamic alcohol	4.0	4.6	0.8	5.9	5.5	4.3	0.9965	0.055	0.18
Farnesol	10	4.5	1.4	7.1	6.3	1.9	0.9996	0.30	1.0
Hexylcinnamaldehyde	7.8	5.2	2.5	4.0	8.8	3.3	0.9991	0.002	0.007
Benzyl benzoate	5.3	5.8	3.6	8.9	7.5	2.9	0.9989	0.003	0.010
Benzyl salicylate	6.3	3.2	7.6	11	3.2	9.2	0.9983	0.009	0.029
Benzyl cinnamate	2.3	1.3	6.8	12	15	5.3	0.9993	0.008	0.025

extraction efficiency [14]. Thus, the optimization of the SPME conditions for the simultaneous extraction of the 26 targets has been focused on these both parameters, and a multivariate strategy has been applied to account for possible second order effects. An experimental screening design 3×2, which allowed to study temperature at three levels (50, 75 and 100 °C) and the two extraction modes (HS and direct SPME), has been selected. Experiments were performed using 10 mL aliquots of water spiked with the analytes at a concentration of 10 ng mL^{-1}. Sampling time was set at 20 min, sodium chloride was added in a proportion of 20%, and magnetic stirring was used to favour mass transfer in the aqueous media. Numerical analysis of data resulting from the experimental design was made with the statistical software package Statgraphics Centurion XV (Manugistics, Rockville, MD, USA).

The optimal conditions obtained for the 26 target compounds are summarized in Table 3. It can be seen that the extraction of 17 of the 26 compounds was favoured by high temperatures (>75 °C). The headspace extraction conditions are best suited for the most compounds, with only eight showing higher responses in the direct SPME mode.

Fig. 3 shows the main effect plots for several representative compounds. 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 line is proportional to the effect magnitude of the factor in the extraction process, and the sign of the slope indicates the level of the factor that produces the highest response. The high influence on response of both extraction mode and temperature is clearly appreciated for most of compounds.

The most volatile compounds, pinene and limonene, are better extracted at 50 °C, while higher temperatures are needed as the boiling point of the compounds increases (see Table 3). This can be seen in Fig. 3, in which limonene, citronellol, isoeugenol, and methyl eugenol are examples of this tendency. Several exceptions are found regarding the extraction temperature: compounds such as benzyl alcohol, cinnamaldehyde, or coumarin are better extracted at the lowest temperature. Headspace extraction mode was generally better for the most of compounds, with some exceptions that are better extracted in the immersion mode (see Table 3).

Concerning the interaction between temperature and extraction mode, some results can be outlined (Fig. 4). Very high volatile compounds (pinene and limonene) must be extracted in the HS mode, and for these two compounds responses obtained at 100 °C are clearly low and independent of the extraction mode. A very different behaviour has been followed by benzyl alcohol or cinnamaldehyde, which are better extracted in the immersion mode but only if temperature is kept at 50 °C. If 100 °C is the temperature selected for the extraction, then the efficiency of the extraction is higher in the HS mode. Clear examples of compounds requiring the

Table 5
Percent recovery of the compounds from three different water samples spiked at 20 ng mL^{-1} (baby bathwater), 5 ng mL^{-1} (swimming pool water) and 10 ng mL^{-1} (wastewater).

Compound	Bathwater	Swimming pool	Wastewater
Pinene	116 ± 11	100 ± 6	113 ± 11
Limonene	100 ± 7	85 ± 15	112 ± 7
Benzyl alcohol	106 ± 7	90 ± 12	78 ± 11
Linalool	90 ± 1	90 ± 3	81 ± 10
Methyl-2-octynoate	87 ± 3	115 ± 4	88 ± 7
Citronellol	83 ± 6	90 ± 3	85 ± 10
Citral	85 ± 2	101 ± 4	89 ± 5
Geraniol	104 ± 5	106 ± 6	96 ± 7
Cinnamaldehyde	88 ± 6	100 ± 4	103 ± 13
Hydroxycitronellal	99 ± 8	136 ± 8	110 ± 5
Anis alcohol	121 ± 5	117 ± 14	120 ± 10
Cinnamyl alcohol	103 ± 13	78 ± 1	106 ± 1
Eugenol	103 ± 5	107 ± 2	106 ± 15
Methyl eugenol	79 ± 5	104 ± 2	95 ± 7
Isoeugenol	96 ± 9	85 ± 3	80 ± 7
Coumarin	116 ± 1	107 ± 2	87 ± 10
Ionone	73 ± 1	91 ± 3	92 ± 1
Lilial	87 ± 2	83 ± 10	92 ± 3
Amyl cinnamaldehyde	86 ± 4	n.a.	85 ± 8
Lyral	96 ± 4	107 ± 7	124 ± 17
Amyl cinnamic alcohol	88 ± 5	109 ± 9	98 ± 3
Farnesol	120 ± 12	78 ± 4	106 ± 15
Hexylcinnamaldehyde	88 ± 3	105 ± 2	84 ± 2
Benzyl benzoate	103 ± 6	103 ± 3	100 ± 2
Benzyl salicylate	122 ± 12	107 ± 9	122 ± 10
Benzyl cinnamate	79 ± 4	101 ± 7	108 ± 7

Table 6
Concentration (ng mL^{-1}) of the suspected fragrance allergens in water samples.

Compound	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22
Pinene													8.6							37	1.1	
Limonene	0.57	0.10	0.03		2.6	0.41	0.24	0.08	0.55		1.1	1.8	56	0.42			5.5	1.7	28	152	12	0.07
Benzyl alcohol		1.3		14	43						845							30		361		
Linalool	8.6	0.28			1.4	0.38	0.76		12		13		47	4.5			12	8.0	6.7	59	6.4	0.15
Methyl-2-octynoate	2.0	1.8	3.1																3.5	1.8		
Citronellol	0.45		0.09	0.07		0.25		0.11	8.5	1.7	3.5		34	1.5	0.10		29	2.3		2.5	13	1.5
Citral	1.9		1.4	8.9		8.8	3.0	4.8	1.0	0.40	0.50	0.82		0.65	0.64	41	1.4	4.0		6.3	2.1	1.0
Geraniol	2.4								4.5		2.8		25				28	2.4	1.1	7.2	7.38	
Cinnamaldehyde			0.28	0.35									1.5									
Hydroxycitronellal			4.4	1.7	6.5	16	8.7	12	2.5				103	20	31	177		5.1	25	4.5	29	
Anis alcohol							8.3	13														
Cinnamyl alcohol								129	7.1				122								31	
Eugenol									3.8				32	0.72			1.7	0.80		6.1	7.0	
Isoeugenol		0.82																0.54				
Coumarin									6.1				157				43				25	
Ionone	0.13				0.61			0.02	4.1				11								5.9	
Lilial			0.07	0.12	0.09	0.14	0.61	0.58	0.38	0.91		1.1		0.26	0.43	0.26	2.8	0.65	0.20	0.25		6.3
Lyral																	42					
Farnesol		2.3			2.0	11	6.2	9.7	5.8	49	25	4.8	27	28	24	27	74	17	28	51	66	2.8
Hexylcinnamaldehyde	0.19				3.0		0.34			0.50		0.22	0.21	29			0.21			0.11	0.12	
Benzyl benzoate					0.12				1.2				12		0.04	0.05	11	0.05	0.04	0.06	4.5	0.36
Benzyl salicylate						0.12	5.9	0.40	0.19		0.81		4.1				1.7	0.71	0.38	0.42	1.2	0.07
Benzyl cinnamate	0.04									0.03												

Compound	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	W34	W35
Pinene													
Limonene	0.03	0.03		0.86	0.16	0.23					0.078	0.36	0.23
Benzyl alcohol													
Linalool						0.051					0.21		
Methyl-2-octynoate													
Citronellol					0.044[a]	0.032[a]		0.64					
Citral				0.19									
Geraniol													
Cinnamaldehyde													
Hydroxycitronellal					0.68	1.80							
Anis alcohol													
Cinnamyl alcohol					1.1	0.49			1.1				
Eugenol						0.051							
Isoeugenol							0.19		0.74				
Coumarin													
Ionone	0.003	0.004				0.031	0.032	0.36	0.032	0.024	0.10	0.20	0.19
Lilial	0.097	0.033	0.19	0.69	0.035	0.037	0.041	0.23	0.031	0.066	0.11	0.40	0.20
Amyl cinnamaldehyde				0.050		0.022							
Lyral													
Farnesol					0.33[a]								
Hexylcinnamaldehyde	0.051		0.071	0.087		0.028							
Benzyl benzoate	0.070		0.033	0.074		0.18							
Benzyl salicylate													
Benzyl cinnamate													

Empty cell: not detected compound (<LOD).
[a] Detected at concentration <LOQ.

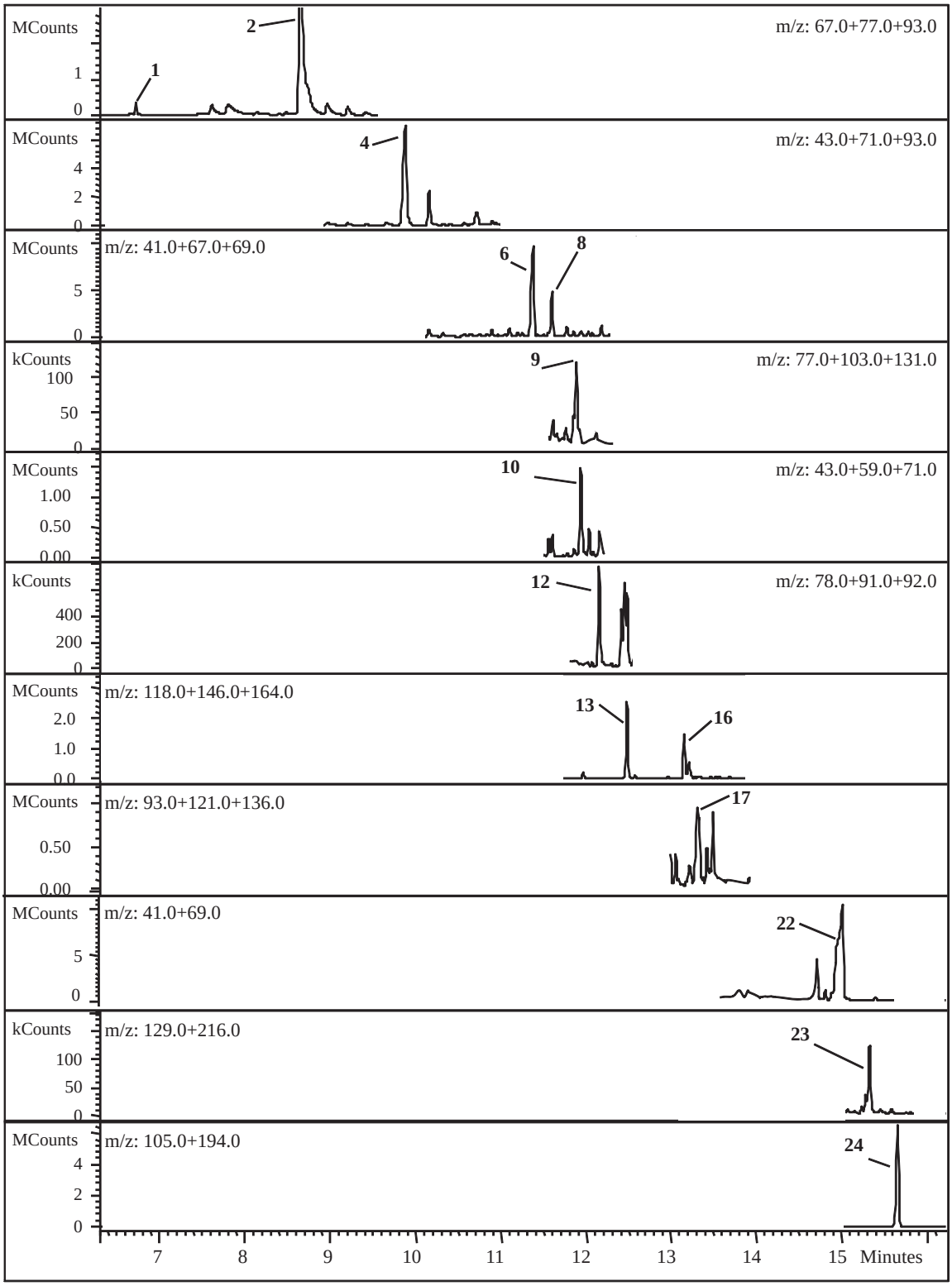

Fig. 5. HS-SPME-GC–MS extracted ion chromatograms for a baby bathwater sample (W13). For compound concentrations, see Table 6.

highest temperatures are amyl cinnamic alcohol and farnesol (see Fig. 4). This kind of compounds is extracted with very low efficiency at 50 °C independently of the extraction mode. It can be appreciated that for certain compounds the difference in efficiency between the direct and HS mode may be very short depending on temperature. Some examples are also depicted in Fig. 4. The optimal extraction conditions for eugenol were 75.9 °C and immersion (Table 3). Nevertheless, its interaction graph shows that at higher temperatures the extraction efficiency is similar for both extraction modes. In the same way, benzyl cinnamate showed optimal conditions at 100 °C and immersion (Table 3), but the analysis of the interaction plot shows that the use of both extraction modes gives the best and quite similar responses at 100 °C; direct SPME is only better than HS-SPME if extraction is performed at 50 °C.

These all results allow concluding that the best conditions for the simultaneous determination of fragrance suspected allergens including all the regulated ones in waters imply HS-SPME at 100 °C.

3.2. Method performance study

Method linearity has been evaluated performing a calibration study in the experimental conditions. The calibration range was established from 0.01, 0.1 or 0.5 ng mL^{-1} (depending on the individual limits of quantification) to 50 ng mL^{-1}, with 6–7 calibration levels. The method exhibited a direct proportional relationship between the extracted amount of each fragrance allergen and their initial concentration in the sample, with correlation coefficients (R) ranging from 0.994 to 1.000 (Table 4).

Precision of the experimental procedure was assessed at four concentration levels: 1, 5, 10, and 20 ng mL^{-1}. Results showed good intra-day and inter-day precisions, with relative standard deviation (RSD) values in general lower than 10% (see Table 4).

Limits of detection (LOD, signal-to-noise ratio of 3) and limits of quantification (LOQ, signal-to-noise ratio of 10) of the method are also presented in Table 4. These limits are at the sub-ng mL^{-1}, with two exceptions (anis alcohol and lyral), and therefore, the sensitivity of the proposed method can be considered satisfactory.

3.3. Application to water samples

The method was applied to the analysis of 35 water samples including 22 baby bathwater samples (W1–W22), 4 indoor swimming pool water samples (W23–W26), and 9 wastewaters (W27–W35). Three samples, a baby bathwater, a swimming pool water, and a wastewater, were selected for matrix effect and recovery studies.

Apparent 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 percentage. Concentrations were calculated by external calibration using ultrapure water standards. Recoveries are shown in Table 5, and ranged from 80 to 120% for most compounds. These recoveries can be considered quantitative, and thus, no matrix effects were observed, allowing quantification by external water calibration.

The levels of the target compounds were then determined in a wide range of water samples. Baby bathwaters were obtained at homes from Galicia (Northwestern Spain) during the daily bath of children aged from few months to three years. The products employed for bath preparation, as well as the quantities were the usual and included shampoos, bubble baths and moisturising soaps all intended for babies. Wastewaters include two samples taken from the spill of a collective washing place (W27, W28), samples taken at the influent (W29–W31) and at the effluent (W32, W33) of sewage treatment plants, all in Galicia (Spain), as well as two samples taken at the central emissary of Mexico city (W34) and at Cerro Colorado (W35), both in Mexico, respectively.

Results of the analysis are shown in Table 6, and as can be seen, all target compounds were detected in the samples with the exception of amyl cinnamic alcohol and methyl eugenol. Compounds have been found at concentrations ranging from 0.003 to 845 ng mL^{-1}. It should be noticed that several bathwater samples showed high concentrations of several compounds, reaching values even greater than 100 ng mL^{-1}. The concentration levels found in wastewaters are in the range of those found for other widespread used fragrance compounds such as polycyclic musks in this kind of waters [17,18]. In swimming pool water samples, suspected allergens were found at concentrations generally below 0.1 ng mL^{-1}, although these levels can be expected to increase in summertime. Fig. 5 shows the HS-SPME-GC–MS extracted ion chromatograms obtained for a baby bathwater sample.

The SPME-GC–MS proposed method showed good performance characteristics for the analysis of fragrance allergens, with low LODs allowing the sensitive determination in waters of 26 fragrance components including the 24 EU regulated suspected allergens, with low cost, simplicity and time-saving.

4. Conclusions

The combination of SPME and GC–MS was shown to be a simple and effective procedure for the determination of fragrance compounds including 24 regulated suspected allergens in waters.

The optimization of the extraction was carried out using experimental design showing that sampling mode and temperature were variables that highly influenced extraction efficiency. The optimal experimental conditions implied the use of PDMS/DVB coating for the extraction in the headspace mode at 100 °C.

The method was validated and demonstrated to be reliable and linear in the concentration range of interest. LODs were satisfactory (0.001–1.1 ng mL^{-1}) as well as reproducibility (RSD < 12%). Quantitative recoveries were obtained for targets in waters including wastewater (>80% for most compounds). The application of the method to water samples including baby bathwater, swimming pool waters and wastewaters, demonstrated the presence of suspected allergens in all samples. In baby bathwater some compounds were found at concentrations of several hundreds of ng mL^{-1}, while in wastewaters the concentration levels were in the range of those reported for other widespread fragrance components such as musks.

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