



Ultrasound-assisted emulsification–microextraction of fragrance allergens in water

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ABSTRACT

A method based on ultrasound-assisted emulsification–microextraction (USAEME) and gas chromatography–mass spectrometry (GC–MS) has been developed for the analysis of regulated fragrance allergens in water. Extraction conditions such as the type of solvent, extraction temperature, irradiation time, and salting-out effect were optimized using a multivariate approach. Compounds were extracted during 2 min in an acoustically emulsified media formed by 100 μ L chloroform and 10 mL sample. The USAEME process provided an efficient and exhaustive extraction (enrichment factor $\sim$ 100) and, after centrifugation, the extract was ready for GC analysis.

Validation was performed using spiked ultrapure water as well as other most complex matrices such as sewage water. Recoveries between 75% and 110% were generally obtained, and precision was characterized by RSD values $<10\%$ in most cases. The limits of detection (LODs) were at the sub-nanogram per millilitre level.

The proposed procedure was applied to the determination of allergens in several real samples including tap water, baby bathwater, recreational place water, public washing place water, and sewage water. The presence of some of the target compounds was confirmed in all the samples excluding tap water, demonstrating the ubiquity of this group of cosmetic and personal care products ingredients.

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1. Introduction

Most personal care products (PCPs) as well as many household products contain fragrances among their ingredients. The International Fragrance Association (IFRA) defines fragrances as any basic substance used in the manufacture of fragrance materials for its odorous, odour enhancing or blending properties. Fragrance ingredients may be obtained by chemical synthesis from synthetic, fossil or natural raw materials or by physical operations from natural sources (IFRA, accessed June 2010). The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has selected 26 compounds as likely to cause contact allergies (SCCNFP/0017/98, 1999). In the European Union (EU) these “suspected allergens” require labelling on cosmetic and detergent products (EU, 2009). 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 most of these substances are also restricted by the IFRA (accessed June 2010), with the purpose of ensuring the safety of fragrance materials. Some may have a direct impact on the skin, eyes and mucous membranes, but they may also cross the skin and be

absorbed into the body. The long-term impact due to the possible bioaccumulation in human tissues is also cause of concern.

Although the main route of exposition to these cosmetic ingredients is, in general, the direct application of cosmetics on the skin, the contact with water containing these fragrances should be also considered. A recent study has analyzed the concentration of 15 fragrance allergens in baby bathwater showing quite high levels for some of them (Lamas et al., 2009). These compounds can also be present in recreational waters and so, skin can be extensively exposed to these and other chemicals through these waters (WHO, accessed June 2010).

As they are important components of daily use products, allergen fragrances are continuously introduced into the environment at high quantities, mainly via urban wastewater effluents. Bath waters as well as the residual waters from many residential communities enter the environment via direct spill into superficial waters or through disposal into the sewage treatment system, contributing to water pollution and causing environmental concern.

While analytical methods for fragrance allergens have been developed for cosmetics analysis (Rastogi et al., 1999; Leijds et al., 2005; Niederer et al., 2006; Bassereau et al., 2007) and, recently, to evaluate the levels of several fragrances on the skin treated with fortified cream (Sgorbini et al., 2010), to the best of our knowledge only one method has been reported for the determination of

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fragrance suspected allergens in water samples (Lamas et al., 2009). This method based on solid-phase microextraction (SPME) has been applied to the analysis of several fragrance components in baby bathwaters. SPME is a solvent-free extraction technique that incorporates pre-treatment, concentration and sample introduction into a single procedure but this technique is non exhaustive, and calibration problems and possible matrix effects are drawbacks that must be considered.

The use of ultrasound energy in analytical chemistry has been generally applied to solid matrices (ultrasound-assisted extraction, UAE) (Capelo and Mota, 2005; Bossio et al., 2008; Pumure et al., 2010). Recently, the use of emulsions generated by ultrasound radiation has found interest in analytical chemistry. In this way, a microextraction technique for aqueous samples, known as ultrasound-assisted emulsification–microextraction (USAEME) has been proposed (Regueiro et al., 2008). This approach is based on the emulsification of a microvolume of organic extractant in an aqueous sample by ultrasound radiation and further separation of both liquid phases by centrifugation. The application of ultrasonic radiation accelerate the mass-transfer process between two immiscible phases, which together with the large surface of contact between both phases leads to an increment in the extraction efficiency in a minimum time. Thus, USAEME can be employed as a simple and efficient extraction and preconcentration procedure for organic compounds in aqueous samples. This technique was for the first time applied to the determination of synthetic musk fragrances, phthalate esters and lindane, and then it was reported its successful application to the determination in environmental waters of preservatives (Regueiro et al., 2009), polybrominated diphenyl ethers (Fontana et al., 2009), pesticides (Ozcan et al., 2009a; Chunhong et al., 2010), polychlorinated biphenyls (Ozcan et al., 2009b), and 2,4,6-trichloroanisole (Fontana and Altamirano, 2010). It has also been extended to the inorganic analysis (Ma et al., 2009).

The aim of the present work is to develop a method of USAEME with gas chromatography–mass spectrometry (GC–MS) for the analysis of regulated fragrance suspected allergens in water samples. One of the great advantages of USAEME versus other techniques such as SPME or single-drop microextraction (SDME) is that it can be considered as an exhaustive microextraction technique and, in consequence, quantification can be performed by standard calibration.

Optimization of the extraction conditions is achieved using a multifactorial experimental design approach. Validation of the method is performed in terms of accuracy, linearity, repeatability and limits of detection (LODs). To demonstrate the applicability of the proposed method, several real samples including baby bath waters, recreational pool waters, and waste waters, are analyzed.

2. Materials and methods

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% (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% (citra, 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). The internal standard PCB-30 (2,4,6-trichlorobiphenyl) was purchased as $10 \mu\text{g mL}^{-1}$ solutions in isooctane from Dr. Ehrenstorfer (Augsburg, Germany).

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 CAS registry numbers, molecular weights, and main physicochemical properties of the target compounds.

Ethyl acetate, acetone, chloroform (CHCl_3) and sodium chloride were provided by Merck (Darmstadt, Germany). 1,1,1-Trichloroethane ($\text{C}_2\text{H}_3\text{Cl}_3$) was obtained from Sigma (St. Louis, MO, USA) and carbon tetrachloride (CCl_4) was purchased from Fluka (Buchs, Switzerland). All solvents and reagents were analytical grade.

Individual stock solutions of each compound were prepared in acetone and a standard mixture solution of all target compounds was prepared in acetone at a final concentration of about $100 \mu\text{g mL}^{-1}$. Different standard working solutions were obtained by appropriate dilution and stored in amber colored vials at -20°C .

Different real water samples were analyzed: tap water, baby bathwater, swimming pool and Spa pool water, public clothes washing place water, and residual treatment plant water (STP). STP water was filtrated through a $0.45 \mu\text{m}$ Millipore HA membrane filter (Billerica, MA, USA). The excess of free chlorine in the water samples was removed by addition of sodium thiosulphate (0.1 mg mL^{-1}). All samples were stored in glass bottles at 4°C .

2.2. Ultrasound-assisted emulsification–microextraction (USAEME)

Aliquots of 10 mL sample were placed in 15 mL conical-bottom glass centrifuge tubes, which were previously cleaned. Under final optimized conditions, 2 g of NaCl, 100 μL of chloroform as extractant solvent and 10 μL of a $10 \mu\text{g mL}^{-1}$ solution of PCB-30 in hexane (internal standard) were added. The tube was then immersed into a 5 L-ultrasonic water bath Selecta Ultrasounds (Barcelona, Spain) in such a way that the level of both liquids (bath and sample) was the same. Extractions were performed at 40 kHz of ultrasound frequency and 100 W of power for 2 min at $25 \pm 3^\circ\text{C}$. As a result, oil-in-water (O/W) emulsions of chloroform (dispersed phase) in water (continuous phase) were formed. Emulsions were then disrupted by centrifugation at 3400 rpm for 10 min and the organic phase was sedimented at the bottom of the conical tube. Chloroform was removed by using a 100 μL Hamilton syringe (Reno, NV, USA) and transferred to a 100 μL glass insert located in a 1.8 mL gas chromatography vial. The thus obtained extracts were stored at -20°C until analysis by GC–MS.

2.3. Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (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;

Table 1

Physicochemical properties, retention times and selected ions of the target compounds.

Key	Retention time (min)	Compound	CAS number	Molecular weight	log K_{OW}	Boiling point (°C)	Solubility (mg L ⁻¹)	Quantifier and qualifier (m/z)
1	8.36	Limonene	5989-27-5	136	4.57	176	13.8	67, 93, 121
2	8.72	Benzyl alcohol	100-51-6	108	1.05	205	40 000	77, 79, 108
3	9.72	Linalol	78-70-6	154	3.28	198	1589	43, 93, 121
4	10.99	Methyl-2-octynoate	111-12-6	154	2.60	219	-	67, 79, 95
5	11.38	Citronellol	106-22-9	156	3.38	225	322	69, 81, 95
6	11.42	Citral	5392-40-5	152	3.17	229	590	39, 69, 109
	11.69							
7	11.59	Geraniol	106-24-1	154	3.28	229	531	41, 69, 111
8	11.82	Cinnamaldehyde	104-55-2	132	2.22	252	1420	77, 103, 131
9	11.87	Hydroxy-citronellal	107-75-5	172	1.54	240	23 800	43, 59, 81
10	12.00	Anisyl alcohol	105-13-5	138	1.10	259	2070	77, 109, 138
11	12.21	Cinnamyl alcohol	104-54-1	134	1.93	250	1800	77, 92, 134
12	12.43	Eugenol	97-53-0	164	2.20	256	<1000	103, 131, 164
13	12.71	Methyl eugenol	93-15-2	178	2.9	248	500	147, 163, 178
14	12.80	Isoeugenol	97-54-1	164	2.45	267	984	103, 131, 164
	13.09							
15	13.11	Coumarin	91-64-5	146	1.39	298	2500	89, 118, 146
16	13.21	Ionone	127-51-5	206	4.41	266	16	107, 135, 150
17	13.54	Lilial	80-54-6	204	4.07	279	33	131, 147, 189
18	14.36	Amyl cinnamal	122-40-7	202	4.80	289	8.5	115, 129, 202
19	14.54	Lylal	31 906-04-4	210	2.53	319	185–1045	79, 91, 136
20	14.73	Amyl cinnamyl alcohol	101-85-9	204	4.37	>200	26	91, 115, 133
21	14.54	Farnesol	4602-84-0	222	5.31	283	267	41, 69, 81
22	15.20	Hexylcinnamaldehyde	101-86-0	216	4.82	308	2.8	115, 129, 216
	15.49							
23	15.52	Benzyl benzoate	120-51-4	212	3.97	324	19.8	105, 194, 212
24	16.79	Benzyl salicylate	118-58-1	228	4.31	320	<1000	65, 91, 228
25	20.59	Benzyl cinnamate	103-41-3	238	3.65	371	9	91, 131, 192

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 × 0.25 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 °C min⁻¹, to 200 °C at 25 °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 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 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 was varied from 39 to 400 m/z at 3 µscans, starting at 5 min and ending at 22.50 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 and retention times for each target compound are listed in Table 1.

3. Results and discussion

3.1. Optimization of USAEME process

The chromatographic method for the separation of the target allergens was optimized elsewhere (Lamas et al., 2010) and it is described in the Experimental section. Table 1 summarizes the

retention times as well as the qualification and quantification ions of the target analytes.

Regarding the USAEME process, the selection of a suitable extractant is limited by several characteristics that are necessary for emulsification in the presence of ultrasonic radiation. Some of these characteristics are a higher density than water and low water solubility. The selected solvent must be compatible with the separation and detection technique and, therefore, a good gas chromatographic behavior is another desirable characteristic. Most of heavy solvents are halogenated compounds; several of them, were initially tested in order to evaluate the chromatographic performance, and the possible presence of interferences during mass spectrometry detection. Based on previous studies (Regueiro et al., 2008, 2009), carbon tetrachloride, chloroform, and 1,1,1-trichloroethane were initially selected. All of them demonstrated to be suitable for fragrance allergens GC–MS analysis, showing similar peak resolution. In addition, the responses obtained were equivalent for all target analytes.

First USAEME experiments were performed with aliquots of 10 mL of ultrapure water and 100 µL of solvent. The US irradiation time was 10 min. In all experiments, PCB-30 was used as internal standard to minimize possible problems resulting from lack of repeatability in the sedimented phase volume. Emulsification was observed with all three solvents. Organic phases were then separated by centrifugation and injected into the GC–MS system. Fig. 1 compares the chromatographic responses obtained by each one of the three solvents. As can be seen in the figure, the responses were equivalent for some analytes. Nevertheless, 10 of the target compounds presented very low response with carbon tetrachloride, and, in consequence, this solvent was discarded for further optimization.

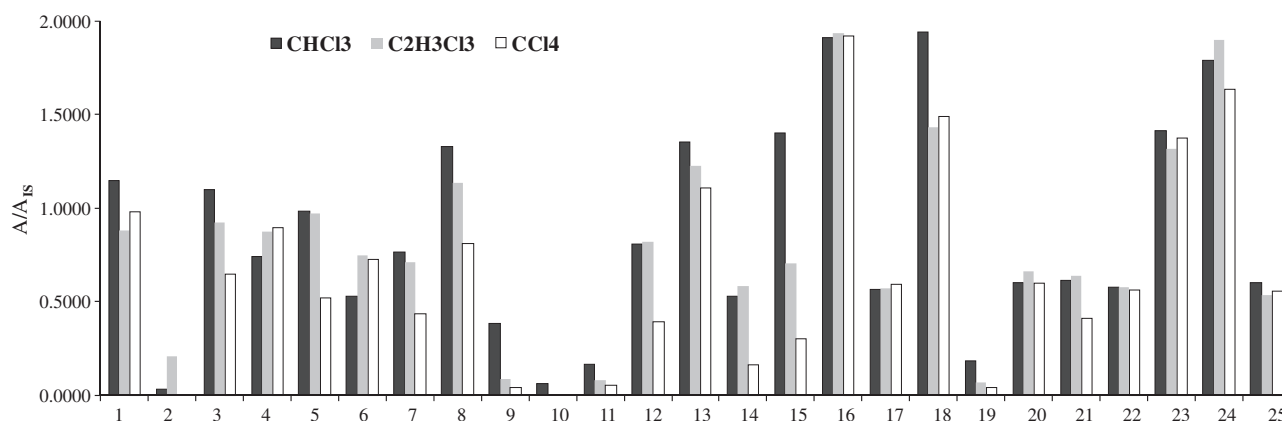


Fig. 1. Comparison of the chromatographic responses obtained by each one of the three solvents: chloroform (CHCl_3); 1,1,1-trichloroethane ($\text{C}_2\text{H}_3\text{Cl}_3$); and carbon tetrachloride (CCl_4).

3.2. Multifactor design study

The influence of the main variables potentially affecting the USAEME efficiency was evaluated by using a multifactorial screening design. The study consisted of a complete factorial 2^4 design, involving 16 randomized experiments and allowing 5 degrees of freedom to estimate the experimental error. This design has resolution V, which means that it is capable of evaluating all main effects and all two-factor interactions. Numerical analysis of data resulting from the experimental design was made with the statistical software package Statgraphics-Plus v5.1 (Manugistics, Rockville, MD, USA). Factorial design optimization was performed using aliquots of water spiked with the analytes at a concentration of 10 ng mL^{-1} .

The selection of an appropriate solvent is essential for all liquid–liquid extraction (LLE) – based processes. Chloroform and trichloroethane, preselected in previous experiments, were included in the experimental design optimization, in an attempt to achieve the highest extraction efficiency for the target compounds. Extraction time is usually an important factor in most extraction procedures. The effect of this factor was examined at 2 and 10 min. Temperature is other frequently important parameter, and was studied at two levels, 25°C and 50°C . Finally, the salting-out effect is frequently used in LLE, SPME and liquid phase microextraction (LPME) procedures. Generally, addition of salt can decrease the solubility of analytes in the aqueous phase, promoting the transfer towards the organic phase. Therefore, the concentration of sodium chloride in the aqueous solution was the last factor evaluated at two levels: 0% (no addition) and 20% (m/V). In brief, four variables were screened in this design, namely: extraction time (A), solvent (B), temperature (C), and sodium chloride concentration (D).

Numerical analysis of the results leads to the ANOVA results shown in Table 2. For the sake of simplicity, only the interaction factors that were significant in any case are included. As can be seen, the most important factors, with statistical significance for many of the target compounds, are the extractant and the concentration of sodium chloride. The temperature and the extraction time were in general non significant with few exceptions. Regarding the two-factor interactions, the only important effect was the one between the solvent and the concentration of sodium chloride (BD), which was statistically significant for 10 compounds.

The information included in the ANOVA can be graphically plotted by means of the Pareto charts. In Fig. 2, some representative graphics are included. In these graphics 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.

Other interesting graphic option provided by the statistic software is the main effects plot. Fig. 3a shows the main effects diagrams for several 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 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. As commented above, solvent and concentration of sodium chloride were the most important factors and its influence is clearly appreciated in the plots. For most compounds higher extraction efficiency was observed when chloroform was used, although trichloroethane extraction was more favourable for six analytes, such as citral (see Fig. 3a). In general, the addition of NaCl affects positively the extraction of analytes (see some examples in the figure), excluding limonene (Fig. 3a), hexylcinnamaldehyde, and benzyl cinnamate. Regarding temperature and extraction time, these factors were not significant with a few exceptions, in which case better extraction was obtained at 25°C and 2 min.

The interaction of the most important main factors, solvent and addition of salt, was also significant for several analytes, being even the most important factor in some cases (e.g. benzyl alcohol, Fig. 2). This interaction is shown in Fig. 3b for some analytes. It can be appreciated how the addition of salt favours chloroform extraction but it does not benefit trichloroethane extraction.

In view of the results of the experimental design, the selected general conditions for the simultaneous ultrasound-assisted emulsification–microextraction of the target compounds from water samples were as follows: an extraction time of 2 min, chloroform as extractant, 25°C , and 20% sodium chloride.

3.3. Method performance: application to real samples

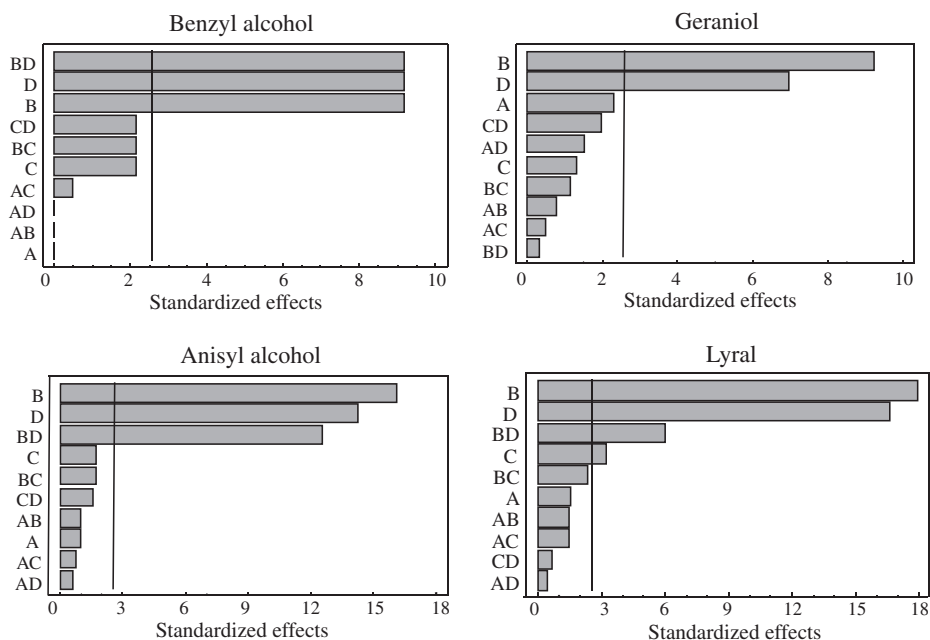
Due to the ubiquity of the target compounds, which are used in all kind of cleansing and cosmetics products, the risk of sample contamination is not negligible, so it is advisable to extreme precautions to avoid sources of interference in the laboratory environment. Blanks must be run very frequently to warranty the absence of contamination.

Analytical quality parameters were evaluated in order to assess the performance of the USAEME method.

Method linearity was tested by injecting chloroform standard solutions between $0.020 \mu\text{g mL}^{-1}$ and $10 \mu\text{g mL}^{-1}$. Determination

Table 2*F* ratios and *p* values obtained in the analysis of variance.

	Main effects								Interactions			
	A: time		B: solvent		C: temperature		D: % NaCl		BC		BD	
	<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value	<i>F</i> ratio	<i>p</i> value
Limonene	22	+	9	+	11	+	271	+	0.2		8	+
Benzyl alcohol	0.0		84	+	4		84	+	4		84	+
Linalol	2		1		1		43	+	2		0.0	
Methyl-2-octynoate	0.03		35	+	5		4		1		18	+
Citronellol	2		6		0.5		3		0.0		0.2	
Citral	0.1		80	+	2		7	+	5		24	+
Geraniol	5		84	+	2		48	+	1		0.1	
Cinnamaldehyde	0.3		19	+	23	+	126	+	0.01		1	
Hydroxycitronellal	0.3		479	+	0.2		1016	+	5		15	+
Anisyl alcohol	1		259	+	3		203	+	3		155	+
Cinnamyl alcohol	0.5		491	+	4		1068	+	10	+	123	+
Eugenol	0.4		8	+	31	+	65	+	0.1		3	
Methyl eugenol	0.5		3		4		1		0.01		0.1	
Isoeugenol	17	+	17	+	2		21	+	16	+	1	
Coumarin	0.3		533	+	97	+	262	+	21	+	0.4	
Ionone	5		83	+	6		1		1		30	+
Lilial	0.3		3		0.3		0.01		0.4		3	
Amyl cinnamal	0.3		5		0.1		6		0.01		0.3	
Lylal	2		320	+	10	+	275	+	5		35	+
Amyl cinnamyl alcohol	2		0.2		2		3		5		10	+
Farnesol	11	+	0.4		0.1		0.2		0.3		1	
Hexylcinnamaldehyde	1		0.4		14	+	7	+	0.0		3	
Benzyl benzoate	0.0		2		0.1		3		0.1		3	
Benzyl salicylate	0.0		27	+	0.1		2		0.03		2	
Benzyl cinnamate	0.1		3		0.4		9	+	0.2		0.3	

+ cell, *p* value < 0.05; empty cell, *p* value > 0.05.**Fig. 2.** Pareto charts showing the significant factors (95%) for some selected fragrance allergens (see factor codes in Table 2).

coefficients (R^2) between 0.9974 and 1.0000 were obtained for all compounds (Table 3).

Recoveries were evaluated using milliQ water spiked at four concentrations from 0.5 ng mL⁻¹ to 100 ng mL⁻¹. They were calculated by dividing the difference between the measured concentrations for spiked and non-spiked samples by the concentrations of the compounds added. The thus obtained values can be considered satisfactory, generally between 70% and 110%, excluding 3 of the 25 target analytes, limonene, benzyl alcohol, and anisyl alcohol. For these last compounds, recoveries were very low, with average

values of 18%, 20% and 27%, respectively (Table 3). Precision of the experimental procedure was also evaluated at several concentration levels by calculating the relative standard deviation (RSD). These results are also shown in the table, and they were lower than 10% in most cases. LODs (signal-to-noise ratio of 3) are also presented, and all of them were below 1 ng mL⁻¹ with the exception of anisyl alcohol.

The aim of the present work is to develop a methodology suitable for the analysis of the target compounds in different kinds of water samples and, consequently, it is necessary to evaluate possible

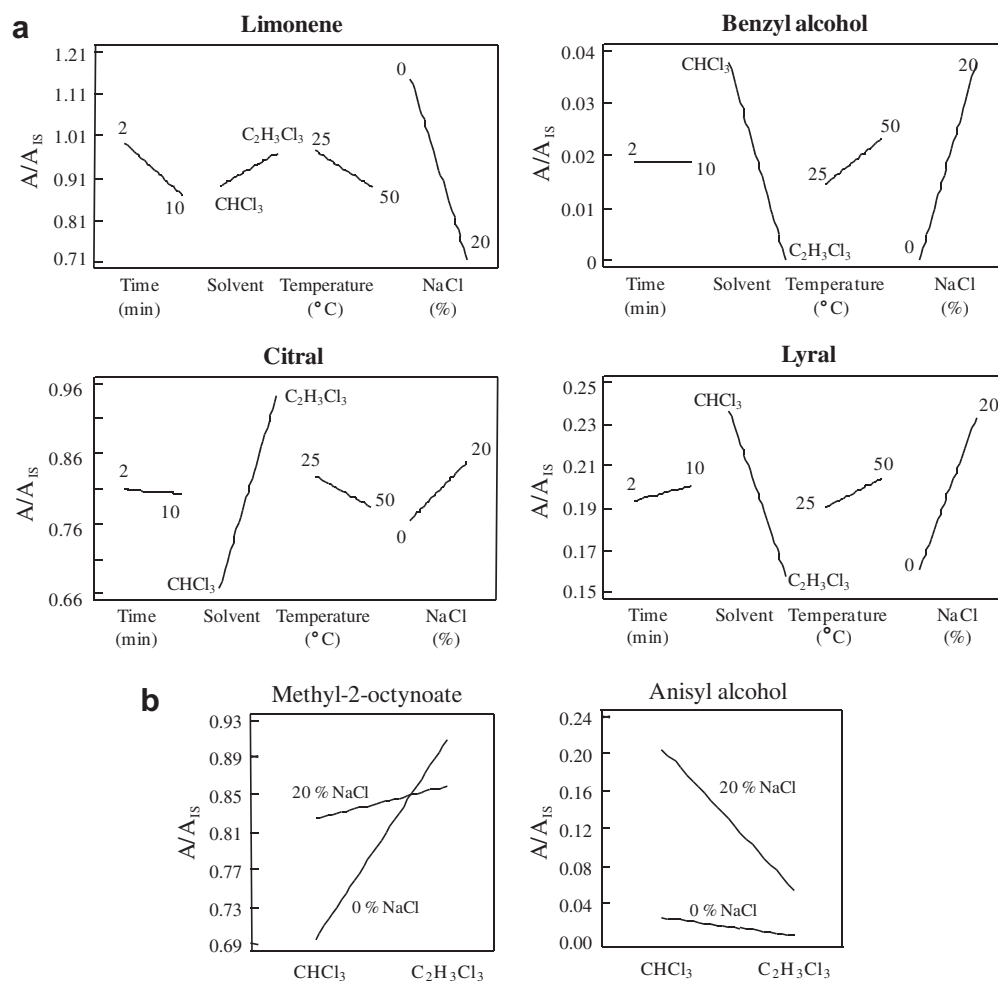


Fig. 3. (a) Main effects and (b) interaction effect (BD, solvent – addition of salt) plots for some selected fragrance allergens.

Table 3

Linearity, limits of detection, recovery and precision of the method.

Compound	Linearity R^2	LOD (ng mL ⁻¹)	Recovery (RSD)%					
			0.5 ng mL ^{-1a}	2 ng mL ^{-1a}	10 ng mL ^{-1a}	100 ng mL ^{-1b}	BBW ^c (10 ng mL ⁻¹)	STP ^c (2 ng mL ⁻¹)
Limonene	0.9986	0.30	25.0 (8.5)	19.0 (6.4)	16.1 (10)	18.2	9.0 (10)	19.9 (3.5)
Benzyl alcohol	0.9995	0.95		21.5 (15)	21.9 (8.3)	19.7	25 (23)	20.9 (4.3)
Linalol	0.9999	0.042	113 (8.1)	89.1 (6.0)	95.4 (2.3)	91.3	87.9 (8.3)	96.3 (1.6)
Methyl-2-octynoate	0.9998	0.055	79.2 (6.8)	81.6 (0.2)	88.7 (4.5)	81.7	81.7 (4.5)	81.8 (10)
Citronellol	0.9997	0.28		91.3 (11)	97.5 (4.3)	86.5	113 (5.3)	94.5 (8.0)
Citral	0.9994	0.064	88.0 (10)	95.6 (6.1)	96.9 (6.5)	85.8	108 (13)	96.4 (3.6)
Geraniol	0.9993	0.45		84.2 (1.9)	95.8 (2.9)	84.7	79.2 (2.9)	91.4 (4.7)
Cinnamaldehyde	0.9999	0.017	99.8 (6.9)	88.7 (5.9)	90.6 (3.7)	81.2	114 (3.1)	88.2 (8.0)
Hydroxycitronellal	0.9999	0.049	88.5 (1.1)	71.8 (2.7)	70.5 (6.9)	63.1	81.4 (3.7)	73.1 (7.0)
Anisyl alcohol	0.9981	1.15		29.4 (3.9)	27.5 (3.9)	26.3	29.1 (14)	22.4 (7.0)
Cinnamyl alcohol	0.9974	0.25		75.8 (8.6)	83.4 (7.5)	70.2	81.5 (6.9)	78.8 (5.8)
Eugenol	0.9998	0.006	105 (6.4)	96.5 (6.6)	96.4 (3.0)	85.1	109 (3.9)	98.6 (5.8)
Methyl eugenol	0.9999	0.0065	105 (8.5)	95.0 (5.1)	96.3 (1.9)	87.8	109 (7.5)	93.3 (3.0)
Isoeugenol	0.9987	0.037	78.0 (8.6)	83.5 (12)	92.6 (5.8)	83.5	82.6 (3.0)	85.5 (9.5)
Coumarin	0.9996	0.011	96.5 (3.6)	86.3 (6.4)	86.6 (4.8)	75.2	98.7 (11)	83.3 (2.1)
Ionone	0.9998	0.012	85.2 (6.7)	77.2 (1.2)	83.3 (7.0)	77.9	90.0 (5.6)	75.4 (0.9)
Lilial	0.9997	0.010	112 (6.7)	89.9 (5.7)	90.2 (7.2)	86.6	88.8 (7.0)	79.5 (1.9)
Amyl cinnamal	0.9999	0.024	102 (7.4)	75.3 (14)	85.7 (3.9)	80.3	72.9 (7.3)	79.1 (4.2)
Lyrall	0.9999	0.063	91.8 (8.9)	76.2 (3.1)	75.4 (11)	69.7	89.2 (5.9)	89.9 (0.5)
Amyl cinnamyl alcohol	0.9999	0.28		96.2 (15)	106 (3.4)	84.8	116 (4.8)	108 (6.4)
Farnesol	0.9999	0.55		81.5 (9.0)	81.8 (7.5)	81.7	113 (9.1)	86.7 (7.2)
Hexylcinnam-aldehyde	0.9999	0.026	79.8 (8.8)	72.0 (0.8)	76.6 (3.8)	77.0	70.0 (6.5)	69.7 (11)
Benzyl benzoate	0.9996	0.014	89.0 (10)	86.8 (4.5)	92.5 (6.8)	83.2	96.3 (3.9)	92.4 (4.5)
Benzyl salicylate	1.0000	0.013	95.0 (7.6)	97.5 (1.4)	95.2 (6.3)	85.8	95.3 (4.5)	106 (4.1)
Benzyl cinnamate	0.9993	0.023	70.3 (6.1)	91.0 (2.0)	85.8 (9.2)	78.1	89.4 (5.3)	90.4 (9.2)

^a $n = 4$.

^b $n = 2$.

^c $n = 3$.

Table 4
Target compounds found in different types of water (ng mL⁻¹).

Compound	BBW1	BBW2	BBW3	BBW4	BBW5	BBW6	BBW7	BBW8	BBW9	BBW10	BBW11	BBW12	BBW13	PWP1	PWP2	PWP3	SPW1	SPW2	SPW3	SPW4	ASP	STP
Limonene ^a	0.620	2.64	128	0.380	4.78	1.15	2.22	7.75	4.23	5.95	3.37	7.75	18.9			0.550	0.765				4.98	0.251
Linalol		19.9	76.3		23.0	9.57	87.4	55.2	32.8	4.15	26.1	30.2	204									
Benzyl ^a alcohol		6.55		3.95		2.74	2.83		3385													
Citronellol		91.8	7.59		5.23	1.69	4.29			1.82		51.1				0.987						
Citral											2.11		5.33				0.190					
Geraniol		23.4	9.25		4.66		11.5	2.50	2.18	5.67		11.4	21.6									
Hydroxy-citronellal													0.444									
Cinnamyl alcohol		1.77			6.02	0.225				0.829	0.706	4.25	6.93		0.093	0.017	0.180				3.75	
Eugenol															>LOD	>LOD					0.146	
Methyl eugenol																						
Ionone			2.48		4.39																	0.121
Isoeugenol																						
Lilial	0.133	0.462		0.040	0.462		0.385	0.378	4.55			0.759	0.417	0.040	0.052	0.053	0.630	0.133	0.183	0.165	0.995	
Lyral																						
Coumarin	0.200	23.2	0.500	0.046	11.5	0.990				0.232		43.4	0.148				0.090		0.101	>LOD		
Hexylcinnam-aldehyde			1.13		0.364	2.13							0.340									
Benzyl benzoate																						
Benzyl salicylate	0.086	2.08	0.364		0.861							8.94	0.247		0.177	>LOD	0.093	0.215	0.077	0.058		
Benzyl cinnamate		0.840		0.089	0.894	0.233	0.979	0.300				0.289	0.556									0.092

Blank spaces mean values below LOD.

^a Values were corrected taking into account the average extraction efficiency.

matrix effects. Samples such as sewage treatment plant (STP) and baby bathwater (BBW), were spiked with the target compounds at different concentrations, and then analyzed by the proposed method. As can be seen in the two last columns of Table 3, recoveries were satisfactory for all compounds, with the exception of the three same compounds with low recovery from milliQ water. These low recoveries were consistent in all samples, and also consistent with the ones obtained from ultrapure water. Therefore, the method can be considered suitable for the determination of 22 of the target compounds in real samples. The concentration of the other three compounds, limonene, benzyl alcohol, and anisyl alcohol, could be estimated introducing a correction factor, although these concentrations should only be considered as estimative values.

Finally, the proposed method was applied to the analysis of several non-spiked water samples, including tap water, baby bathwater, swimming pool and Spa water, public clothes washing place water, and sewage treatment plant water. The presence of some of the target compounds was confirmed in all the samples excluding tap water (see Table 4). In the case of the bathwater samples (BBW), the use of products such as shampoos, bubble baths and moisturising soaps containing fragrances, is the cause of the high levels found for some of the target analytes. The samples from public washing places (PWP), also showed the presence of some allergen fragrances. These samples were taken at evening hours, in the exit collector that directly discharges the wastewater into a river. In this case, the presence of some of the target allergens is due to the use of perfumed clothes cleaning products. Although the number and the concentration of compounds found in winter indoor swimming pool (SPW) and sewage treatment plant (STP) water samples was not very high, the occurrence of fragrance allergens demonstrate the ubiquity of these cosmetic ingredients. A sample collected from an aromatherapy Spa pool (ASP) containing high levels of some of the target allergens is also included.

4. Conclusions

In the present work, a method based on ultrasound-assisted emulsification–microextraction (USAEME) coupled to GC–MS has been developed for the analysis of 25 regulated fragrance allergens in water. USAEME is proposed as an efficient, simple, rapid, environmentally friendly, and non-expensive alternative to other extraction techniques such as solid phase extraction (SPE), SPME and LPME. Using the optimized conditions established after a multivariate study of the USAEME process, good recoveries were obtained for almost all compounds, even in complex samples. Method precision was satisfactory and detection limits at the sub-nanogram per millilitre level were obtained. No matrix effects were observed and, therefore, quantification could be carried out by means of standard calibration. The proposed method was applied to the analysis of several real water samples including tap water, baby bathwater, recreational place water, public washing place water, and sewage water. The presence of fragrance allergens was confirmed in all the samples, demonstrating the ubiquity of the target compounds.

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