



Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewater treatment plants by solid phase microextraction coupled to gas chromatography



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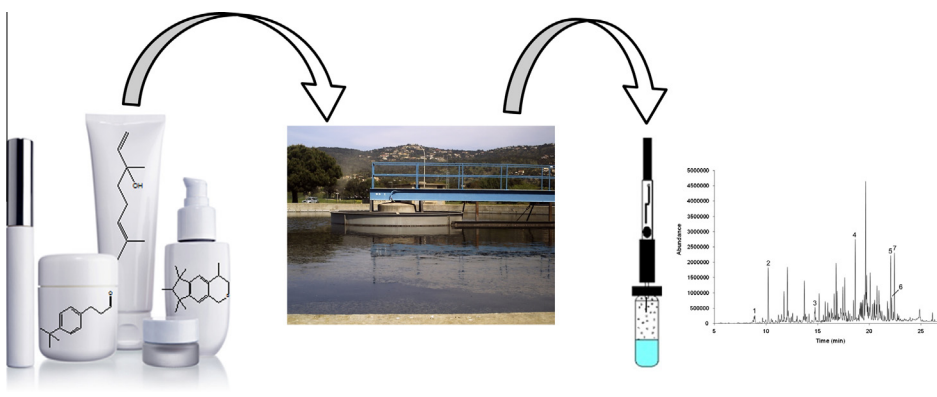
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HIGHLIGHTS

- An SPME/GC–MS procedure is developed for the analysis of fragrance allergens.
- Temperature and time are the most significant factors affecting SPME extraction.
- Five fragrance allergens are systematically detected in primary effluents.
- Four fragrances are not eliminated in the monitored sewage treatment plants.
- Conventional treatments based on activated sludge do not remove some allergens.

GRAPHICAL ABSTRACT



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ABSTRACT

A methodology based on headspace solid phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS) determination was developed for the monitoring and evaluation of the removal efficiency of 16 common fragrance allergens and two polycyclic musks in wastewater treatment plants (WWTPs). An experimental design with a full factorial model was applied to evaluate the effects of the experimental parameters on the extraction (e.g., salt content, time and extraction temperature). After determining the optimum conditions (2.4 g NaCl, 45 min at 90 °C), an external calibration was performed and quality parameters of the proposed method were evaluated. Method detection limits in the range of 0.01–1.7 µg L^{−1} were obtained. Satisfactory inter-day precision values between 4% and 23% (*n* = 5) were obtained for most compounds. The method was applied to the monitoring of the target analytes in samples from two WWTPs. Seven target compounds were detected at the primary effluent of both plants at µg L^{−1} levels. Limonene, linalool and eugenol were quantitatively eliminated during the secondary treatments of both WWTPs, while lilial, benzyl salicylate, galaxolide, and tonalide were still detected at the effluent waters.

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1. Introduction

Personal care products (PCPs) are products used for personal health or cosmetic reasons. PCPs consist of thousands of different chemical substances, including prescription drugs, fragrances, and cosmetics (<http://www.epa.gov/ppcp/>). Fragrances, which are the most extensively used PCPs, are commonly added to soaps, detergents, cosmetics, and other consumer products in order to give a scent to a product or mask the unpleasant odour of other chemical ingredients. However, fragrances in PCPs are one of the most common causes of allergic skin reactions (Klaschka and Rother, 2013). The Scientific Committee on Consumer Safety (SCCS) has identified 26 of these compounds as being fragrance allergens likely to cause contact allergies (SCCS, 2012). Some of these substances have a direct impact on the skin (Bickers et al., 2005), eyes (Elberling et al., 2006) and mucous membranes (Elberling et al., 2005). Moreover, detergents present in consumer products can break down the natural barrier of the skin, allowing allergens and other chemicals to penetrate it (Kownatzki, 2003).

Fragrances have been often found in domestic waters due to their widespread use in everyday products and levels ranging from ng L^{-1} to $\mu\text{g L}^{-1}$ have been detected in WWTPs (Becerril et al., 2010; Becerril-Bravo et al., 2010; Chase et al., 2012; Clara et al., 2011; Eriksson et al., 2003; Escalas et al., 2003; Lee and Rasmussen, 2006; Hernández Leal et al., 2010; Lee et al., 2010; Kameda et al., 2011; Machado et al., 2011; Simonich et al., 2002; Ternes et al., 2003; Stackelberg et al., 2004; Negreira et al., 2010; Silva and Nogueira, 2010; Teijon et al., 2010; Ramírez et al., 2011). The main part of these studies are devoted to the determination of musk fragrances and found that galaxolide and tonalide (two polycyclic musks) are the most abundant detected in WWTPs. It has been found that although the concentrations of polycyclic musks decrease during the different treatments, they are not completely eliminated in conventional treatment plants (Chase et al., 2012; Clara et al., 2011; Hernández Leal et al., 2010; Lee et al., 2010; Machado et al., 2011; Simonich et al., 2002; Ternes et al., 2003; Silva and Nogueira, 2010; Teijon et al., 2010) and, as a result, have been found in rivers (Lee and Rasmussen, 2006; Lee et al., 2010; Kameda et al., 2011; Chase et al., 2012; Machado et al., 2011; Stackelberg et al., 2004; Negreira et al., 2010; Silva and Nogueira, 2010), lakes (Kameda et al., 2011; Chase et al., 2012), and reservoirs (Chase et al., 2012). It has also been reported that these compounds suffer bioaccumulation in aquatic biota (Gatermann et al., 2002; Nakata et al., 2007). However, there is still little information about the elimination of fragrance allergens in WWTPs. It is important to study the behaviour of fragrances that have not been subject to as extensive investigation as musks in treatment plants given that there are more than 40 fragrances, including nine fragrance allergens, that have been identified in the grey wastewaters generated in domestic buildings at levels ranging from 0.1 to $133 \mu\text{g L}^{-1}$ (Eriksson et al., 2003).

Few studies have evaluated a short list of allergens: one specific allergen, such as benzyl salicylate (Simonich et al., 2002; Negreira et al., 2010; Kameda et al., 2011) and limonene (Lee and Rasmussen, 2006); or a set of three, such as limonene, linalool and β -citronellol (Escalas et al., 2003). Only two studies included a list of 24 (Becerril et al., 2010) and 25 allergens (Becerril-Bravo et al., 2010) but a very limited number of WWTP samples were evaluated ($n = 9$ and 1 , respectively).

In the present work we monitored 16 common fragrance allergens and two polycyclic musks in a large set of wastewater samples ($n = 58$) over a three-month period. A method based on solid-phase microextraction (SPME) and GC–MS was optimised and validated before being applied to the monitoring of the target compounds in water samples from two different WWTPs located in

Girona (north-east Spain). Although emerging contaminants are still unregulated in water, their presence is a matter of concern, especially when treated water is used for the drinking water supply. The information obtained here will be of great use in assessing the risk of exposure to these compounds.

2. Experimental

2.1. Chemicals

Fragrance allergens, 1-methyl-4-prop-1-en-2-ylcyclohexene (d-limonene, 99%), 3,7-dimethylocta-1,6-dien-3-ol (linalool, 95%), 3,7-dimethyloct-6-en-1-ol (β -citronellol, 99%), 3,7-dimethylocta-2,6-dienal cis and trans mixture (citral, 96%), 3,7-dimethyl-2,6-octadien-1-ol (geraniol, 99%), 3-phenyl-2-propenal (trans-cinnamaldehyde, 98%), 7-hydroxy-3,7-dimethyloctanal (hydroxycitronellal, 95%), 3-phenyl-2-propen-1-ol (cinnamyl alcohol, 98%), 2-methoxy-4-prop-2-enyl-phenol (eugenol, 99.8%), 2H-1-benzopyran-2-one (coumarin, 99%), 3-(4-tert-butylphenyl)-2-methylpropenal (lilial, 90%), 4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde (lyral, 97%), phenylmethyl benzoate (benzyl benzoate, 98%), 2-hydroxy-phenylmethyl ester benzoic acid (benzyl salicylate, 98%), and 3-phenyl phenylmethyl ester-2-propenoic acid (benzyl cinnamate, 99%) were obtained from Sigma-Aldrich (Steinheim, Germany). Polycyclic musks, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran (galaxolide) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (tonalide), were from LGC Standards (Teddington, UK). Sodium chloride (99.9%) and HPLC-gradient grade methanol were supplied by Carlo-Erba Reagents (Milan, Italy). Sodium thiosulphate 5-hydrate (99.5%) was obtained from Panreac Química S.L.U. (Barcelona, Spain). Milli-Q water from a Milli-Q Plus water purification system (Millipore Ibérica, Barcelona, Spain) was used.

Stock solutions containing about 500 mg L^{-1} were prepared for each analyte by weight in methanol and stored at 4°C for up to a week. Mixed working solutions were made daily by diluting the stock solutions to the required concentration with Milli-Q water.

2.2. Headspace solid-phase microextraction procedure

SPME experiments were performed with a SPME Triplus auto-sampler (Thermo Scientific, Waltham, MA, USA). Two different commercially available fibre coatings were tested: a $65 \mu\text{m}$ PDMS/DVB fibre and a $50/30 \mu\text{m}$ divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre from Supelco (Bellefonte, PA, USA). Before use, each fibre was conditioned according to the manufacturer's instructions to remove contaminants and stabilise the solid phase.

Ten mL of sample solution were placed in 20 mL glass vials containing 2.4 g of NaCl. The vials were then closed with aluminium caps furnished with Teflon-faced septa. Samples were introduced in the oven and the fibre was exposed at 90°C for 45 min to the headspace above the aqueous solution (final conditions). Constant stirring was applied during the extraction process. After the completion of sampling, the fibre was pulled into the needle and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption and analysis.

2.3. Experimental design

A 2^3 full factorial design was used and three variable factors that can affect the extraction yield of the analytes were considered: ionic strength (variable c , quantified as NaCl content, ranging from 1.2 to 2.4 g), temperature (T , 50 – 90°C) and extraction time

(*t*, 15–45 min). The central point (1.6 g, 70 °C, 30 min) was also measured and considered as an experiment. All the experiments were performed in triplicate and in random order. The Minitab v14 computer program was used for data manipulation and calculations. Significance was set at 0.05.

2.4. Equipment and chromatographic conditions

Gas chromatographic analyses were performed with a Trace GC 2000 coupled to a PolarisQ ion trap mass spectrometer detector (Thermo Scientific). A TG-5SIL MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) (Thermo Scientific) was used and the carrier gas was 99.9990% pure helium (Carbueros Metálicos, Barcelona, Spain) at a constant inlet flow rate of 1 mL min⁻¹. The split/splitless injection port was equipped with a 0.75 mm ID SPME liner and operated in splitless mode (maintained for 2 min) at 220 °C.

The oven temperature programme started at 45 °C, held for 2 min; then ramped up to 233 °C at 8 °C min⁻¹, and held for 2 min; total run 27.50 min. MS analyses were conducted in scan mode with an *m/z* interval from 39 to 400 amu. The dwell time of the mass spectrometer was 1.2 ms. Ionisation was performed in the electron impact mode at 70 eV. The transfer line temperature was set at 280 °C and the ion source temperature at 225 °C. The acquisition of chromatographic data was performed using Xcalibur 1.4 software (Thermo Scientific). Table S1 in Supplementary Materials shows the list of the target compounds, their retention times and their qualifier and quantifier ions. Fig. S1 (Supplementary Materials) shows the extracted chromatogram of a standard analysed using the chromatographic conditions described above.

2.5. Sampling of wastewaters

This study was focused on the evaluation of the target fragrances in two WWTPs located in Castell d'Aro and Girona (north-east Spain). The Girona WWTP was designed for an equivalent population of 206250 habitants, with a design flow of 55000 m³ d⁻¹. Its average working flow during the sampling period was between 35000 and 40000 m³ d⁻¹. The Castell d'Aro plant was designed for an equivalent population of 175000 habitants (this plant gives service to a touristic area which had a significantly reduced population during the sampling period), with a design flow of 35000 m³ d⁻¹. Its average working flow during the period monitored was around 15000 m³ d⁻¹. Secondary digestion in both plants consists of activated sludge processes.

Sampling was performed from January to March 2013. Samples from the primary effluent and the biological treatment effluent of both plants were obtained. In the case of the Castell d'Aro WWTP, a tertiary treatment (UV followed by chlorination) is applied when required (e.g., for agricultural purposes), so tertiary effluent samples were also taken when this process was running. 0.5 L amber glass bottles were used for sampling and water samples were stored at 4 °C and analysed before 24 h.

3. Results and discussion

3.1. Selection of SPME extraction conditions

A preliminary study was performed for the selection of the best coating between the PDMS/DVB and the DVB/CAR/PDMS fibers. As can be seen in Fig. S2 in Supplementary Materials, no significant differences (*p* > 0.05, *t*-test) were observed for the studied compounds between the two fibres, except for *t*-cinnamaldehyde (*p* = 0.01), cinammyl alcohol (*p* = 0.007) and coumarin, which

showed lower or no response (coumarin) at the concentrations tested when the DVB/CAR/PDMS coating was used. As a result, the PDMS/DVB fibre was selected for further experiments.

An experimental domain was defined to evaluate the influence of temperature, time of extraction and salt content on the extraction of the target fragrances from aqueous solutions. A full two-level factorial design was used in order to study not only each variable individually, but also the presence of interactions and evidence of curvature effects.

Absolute peak areas were analysed and the results obtained are summarised in Table S2 in Supplementary Materials, where the significances (*p*-values) are given. Temperature has the highest influence on all the target analytes and the optimal temperature level is seen to depend on the volatility of the compounds. For the most volatile compounds (limonene and linalool), the best extraction conditions are obtained at the lowest temperature due to a competitive desorption process of these analytes from the fibre that takes places at high temperatures. For intermediate volatility compounds (β-citronellol, both citrals and geraniol), there is a double interaction between time and temperature showing that using short times, higher temperatures yield better extraction whereas at long times the competitive desorption becomes significant and low temperatures are preferred. For most of the target compounds, 12 out of 18, there is an increase in the response when temperature is set at the highest level.

With regards to the extraction time, only eight of the target compounds were influenced. Lilial, galaxolide and tonalide presented better responses when the time was set at the highest level. For β-citronellol, both citrals, geraniol, and *t*-cinnamaldehyde, the previously mentioned interactions between time and temperature were observed, yielding better extractions when the combination of these two variables decreased (e.g., high *T* and low *t* or low *T* and high *t*).

Finally, in the study of the NaCl content it was observed that the addition of salt improved the extraction of β-citronellol, both citrals, geraniol, *t*-cinnamaldehyde, eugenol, and lilial, with no significant effects on the other compounds.

Taking into account the whole list of target analytes, it can be concluded that the most favourable conditions for SPME extraction are at the highest values for each factor: a temperature of 90 °C, a time of 45 min, and 2.4 g of NaCl. Although these conditions are not the optimum for limonene, linalool, β-citronellol, the two citrals, and geraniol, the selected method allows their quantification with good sensitivity. These main conclusions are best visualised in Pareto graphs (Figs. S3–S20 in Supplementary Materials).

3.2. Method validation

External calibration was performed in the range indicated in Table 1 using five equidistant calibration points (each measured three times) for each compound. Each concentration level was analysed in triplicate. Linearity was confirmed for all compounds from evaluation of the distribution of residuals, with determination coefficients (*r*²) greater than 0.985. The limits of detection (LODs) and the limits of quantification (LOQs) of the method were calculated as three and ten times the signal-to-noise ratio, respectively. The obtained LODs ranged from 0.01 to 1.7 µg L⁻¹.

Inter-day precision and recoveries of the method were also tested. Water samples obtained from the biological treatment effluent of the Castell d'Aro WWTP were spiked at concentrations around the LOQ (*n* = 5, low level) and close to the highest calibration concentration (*n* = 5, high level). For those analytes presenting a short working range only the high concentration level was evaluated.

For precision, the RSD values obtained, which can be considered as acceptable (Taverniers et al., 2004), ranged from 4% to 23%

Table 1
Quality parameters of the developed method. b = Slope, s_b = standard deviation of the slope, r^2 = determination coefficient, LOD = limit of detection. Spiked wastewater samples at concentrations close to LOQ (low level) and close to the highest calibration level (high level) were used for precision and recoveries evaluation ($n = 5$). (–): not determined.

Compound	Calibration range ^a ($\mu\text{g L}^{-1}$)	$b(s_b)$ ($\times 10^5$)	r^2	LOD ($\mu\text{g L}^{-1}$)	Precision ^b (RSD, %)		Recoveries ^b , % (RSD, %)			
					Low level	High level	Low level	High level	Low level ($\text{Na}_2\text{S}_2\text{O}_3$)	High level ($\text{Na}_2\text{S}_2\text{O}_3$)
Limonene	1.0–90	0.67 (0.04)	0.9878	0.3	33	28	60 (20)	90 (30)	80 (30)	80 (20)
Linalool	1.0–50	0.47 (0.03)	0.9902	0.3	22	14	78 (8)	57 (8)	80 (10)	50 (10)
β -citronellol	2.0–160	0.56 (0.02)	0.9937	0.6	17	9	50 (10)	67 (6)	40 (10)	73 (9)
Citral A	2.7–30	0.021 (0.009)	0.9945	0.8	–	13	–	60 (10)	–	56 (6)
Citral B	1.8–30	0.54 (0.02)	0.9974	0.5	–	8	–	60 (10)	–	72 (9)
Geraniol	0.4–110	0.054 (0.008)	0.9989	0.1	10	11	60 (10)	57 (6)	61 (9)	70 (5)
Hydroxycitronellal	5.7–160	0.060 (0.004)	0.9851	1.7	21	9	20 (20)	38 (8)	70 (20)	70 (10)
<i>t</i> -cinnamaldehyde	0.23–14	2.1 (0.1)	0.9934	0.07	–	11	–	90 (5)	–	80 (10)
Cinnamyl alcohol	1.4–90	0.0062 (0.0002)	0.9968	0.4	20	16	70(8)	57 (6)	80 (10)	80 (10)
Eugenol	0.07–7	3.4 (0.2)	0.9936	0.02	–	14	–	47 (9)	–	70 (10)
Coumarin	0.4–65	0.330 (0.009)	0.9980	0.1	23	18	80 (10)	80 (10)	100 (10)	100 (10)
Lilial	0.17–4	12.4 (0.8)	0.9865	0.05	–	16	–	30 (20)	–	45 (7)
Lylal	1.4–140	0.28 (0.01)	0.9923	0.4	–	7	–	20 (10)	–	79 (7)
Benzyl benzoate	0.03–6	14.9 (0.6)	0.9963	0.01	–	8	–	64 (6)	–	89 (5)
Galaxolide	0.13–7	11.8 (0.6)	0.9930	0.04	–	7	–	30 (6)	–	40 (10)
Tonalide	0.13–4	15.6 (0.5)	0.9967	0.04	–	4	–	71 (7)	–	84 (1)
Benzyl salicylate	0.03–3	155 (6)	0.9969	0.01	–	23	–	25 (7)	–	42 (3)
Benzyl cinnamate	0.11–7	15.6 (0.6)	0.9953	0.03	–	17	–	20 (20)	–	60 (10)

^a Quantification limits (LOQs) were fixed as the lowest calibration level.

^b Spiked wastewater from the biologic treatment effluent of Castell d'Aro WWTP was used.

Table 2
Concentrations found ($\mu\text{g L}^{-1}$) at the sampling points from Castell d'Aro and Girona WWTPs. (–): not determined, nd: not detected, sd: standard deviation. Numbers shown in parentheses are the number of samples in which the compound was not detected.

Compound	Castell d'Aro							Girona						
		<i>n</i>	Minimum	Maximum	Median	Mean	sd		<i>n</i>	Minimum	Maximum	Median	Mean	sd
Limonene	Primary	13	nd (6)	3.02	0.31	0.80	0.88	Primary	12	nd (10)	1.39	0.19	0.32	0.35
	Secondary	14	nd	nd	nd	nd	–	Secondary	12	nd	nd	nd	nd	–
	Tertiary	7	nd	nd	nd	nd	–							
Linalool	Primary	13	1.54	20.22	7.65	8.54	5.73	Primary	12	nd (1)	11.62	5	5.37	3.08
	Secondary	14	nd	nd	nd	nd	–	Secondary	12	nd	nd	nd	nd	–
	Tertiary	7	nd	nd	nd	nd	–							
Eugenol	Primary	13	nd (2)	1.8	0.52	0.55	0.48	Primary	12	nd (1)	1.00	0.68	0.56	0.3
	Secondary	14	nd	nd	nd	nd	–	Secondary	12	nd	nd	nd	nd	–
	Tertiary	7	nd	nd	nd	nd	–							
Lilial	Primary	13	nd (2)	0.89	0.27	0.29	0.26	Primary	12	nd (4)	1.58	0.13	0.37	0.47
	Secondary	14	nd(3)	1.18	0.27	0.42	0.38	Secondary	12	nd (9)	0.23	0.03	0.06	0.06
	Tertiary	7	nd (1)	0.81	0.57	0.40	0.31							
Galaxolide	Primary	13	2.31	3.49	2.92	2.92	0.30	Primary	12	1.9	3.43	2.37	2.50	0.53
	Secondary	14	2.87	5.21	3.97	3.94	0.74	Secondary	12	2.5	4.23	3.32	3.36	0.48
	Tertiary	7	2.67	4.58	3.72	3.56	0.78							
Tonalide	Primary	13	nd (2)	0.36	0.22	0.19	0.13	Primary	12	nd (5)	0.48	0.12	0.14	0.15
	Secondary	14	nd (1)	0.94	0.50	0.48	0.31	Secondary	12	nd (2)	1.24	0.28	0.37	0.35
	Tertiary	7	nd (2)	0.91	0.22	0.29	0.32							
Benzyl salicylate	Primary	13	nd (4)	0.86	0.49	0.38	0.32	Primary	12	nd (3)	0.96	0.24	0.28	0.28
	Secondary	14	nd(10)	0.62	0.01	0.07	0.16	Secondary	12	nd	nd	nd	nd	–
	Tertiary	7	nd (6)	0.14	0.01	0.03	0.05							

(Table 1). The worst results were obtained for limonene, whose RSD values were >25% in all concentration levels tested.

Recovery values were <50% for six analytes (hydroxycitronellal, lilial, lylal, galaxolide, benzyl salicylate, and benzyl cinnamate) in spiked wastewaters. Similar results were obtained by Lamas et al. (2009) in spiked bath and swimming pool waters. When they evaluated whether it was possible to avoid the reaction of some analytes during the storage and extraction of samples due to the presence of oxidants in the sample matrix by adding sodium thiosulphate, they found that final recoveries increased to >80% for most compounds. We treated the WWTP samples with $0.1 \mu\text{g L}^{-1}$ of sodium thiosulphate to prevent the oxidation of the analytes and recoveries increased significantly for all the compounds and were satisfactory for most of them (Table 1).

3.3. Occurrence of fragrances

The developed HS-SPME/GC–MS method was applied to the monitoring of 18 fragrances (16 allergens and two polycyclic musks) in two WWTPs located at Castell d'Aro and Girona (Girona province, Spain) over a three-month period. Figs. S21 and S22 in Supplementary Materials illustrate the extracted chromatograms of samples taken at the different stages of the two WWTPs.

Table 2 shows the concentrations of the fragrances detected. Only seven of the target analytes (limonene, linalool, eugenol, lilial, benzyl salicylate, galaxolide, and tonalide) were detected in at least one of the samples. The results obtained are in agreement with those published by other studies (Simonich et al., 2002; Lee and Rasmussen, 2006; Becerril et al., 2010; Becerril-Bravo et al.,

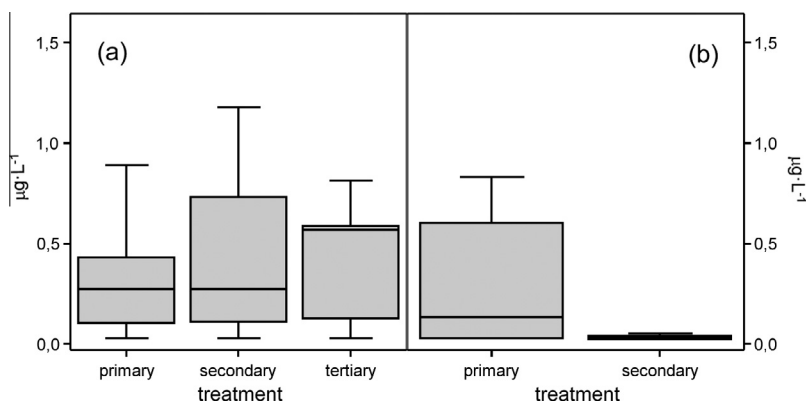


Fig. 1. Box plots of data obtained for linal in (a) Castell d'Aro and (b) Girona. The bottom and top of the box are 25th and 75th percentiles, the line inside the box is the median (50th percentile) and the whiskers indicate the lowest and highest data within 1.5 inter-quartile range.

2010; Hernández Leal et al., 2010; Negreira et al., 2010; Kameda et al., 2011).

For statistical calculations, a value of $\text{LOD}/\sqrt{2}$ was used as the concentration when a compound was not detected. β -citronellol, both citrals, geraniol, *t*-cinnamaldehyde, cinnamyl alcohol, coumarin, lylal, benzyl benzoate, and benzyl cinnamate are not included in the calculations since they were not detected in any of the samples analysed.

First, normality tests were performed and normal distributions were found for all target compounds (Shapiro–Wilk test, $p > 0.05$), except for those analytes which were not detected in >30% of

samples (limonene and benzyl salicylate in Castell d'Aro, and limonene, linal, tonalide, and benzyl salicylate in Girona).

Linalool and galaxolide were the fragrances found at the highest concentrations in both WWTPs, with maximum values of 20.22 and $5.21 \mu\text{g L}^{-1}$ respectively in Castell d'Aro, and 11.62 and $4.23 \mu\text{g L}^{-1}$ in Girona. Linalool was also detected as the allergenic fragrance with the highest content at the primary effluent (mean concentration of $6.4 \mu\text{g L}^{-1}$, $n = 16$) in another study evaluating three allergens at a treatment plant close to the ones evaluated in the present study (Escalas et al., 2003). This compound was also the allergen fragrance detected at the highest levels ($15.4 \mu\text{g L}^{-1}$)

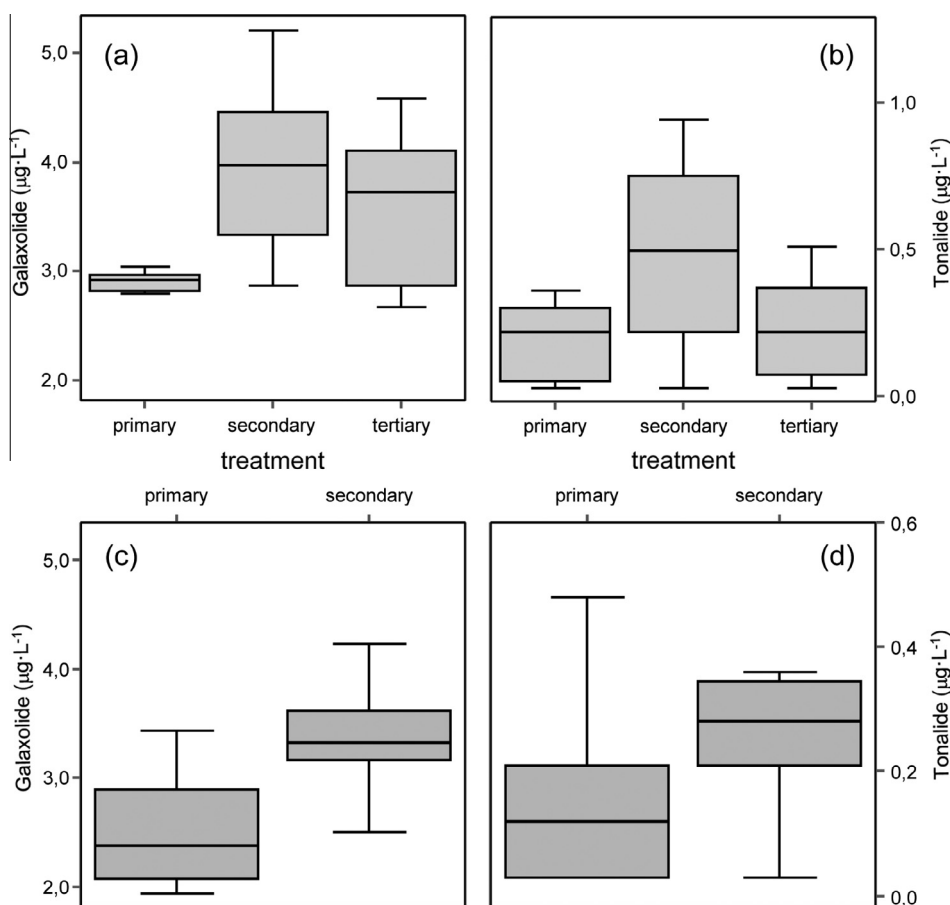


Fig. 2. Box plots of data obtained for (a) galaxolide (Castell d'Aro), (b) tonalide (Castell d'Aro), (c) galaxolide (Girona) and (d) tonalide (Girona). The bottom and top of the box are 25th and 75th percentiles, the line inside the box is the median (50th percentile) and the whiskers indicate the lowest and highest data within 1.5 inter-quartile range.

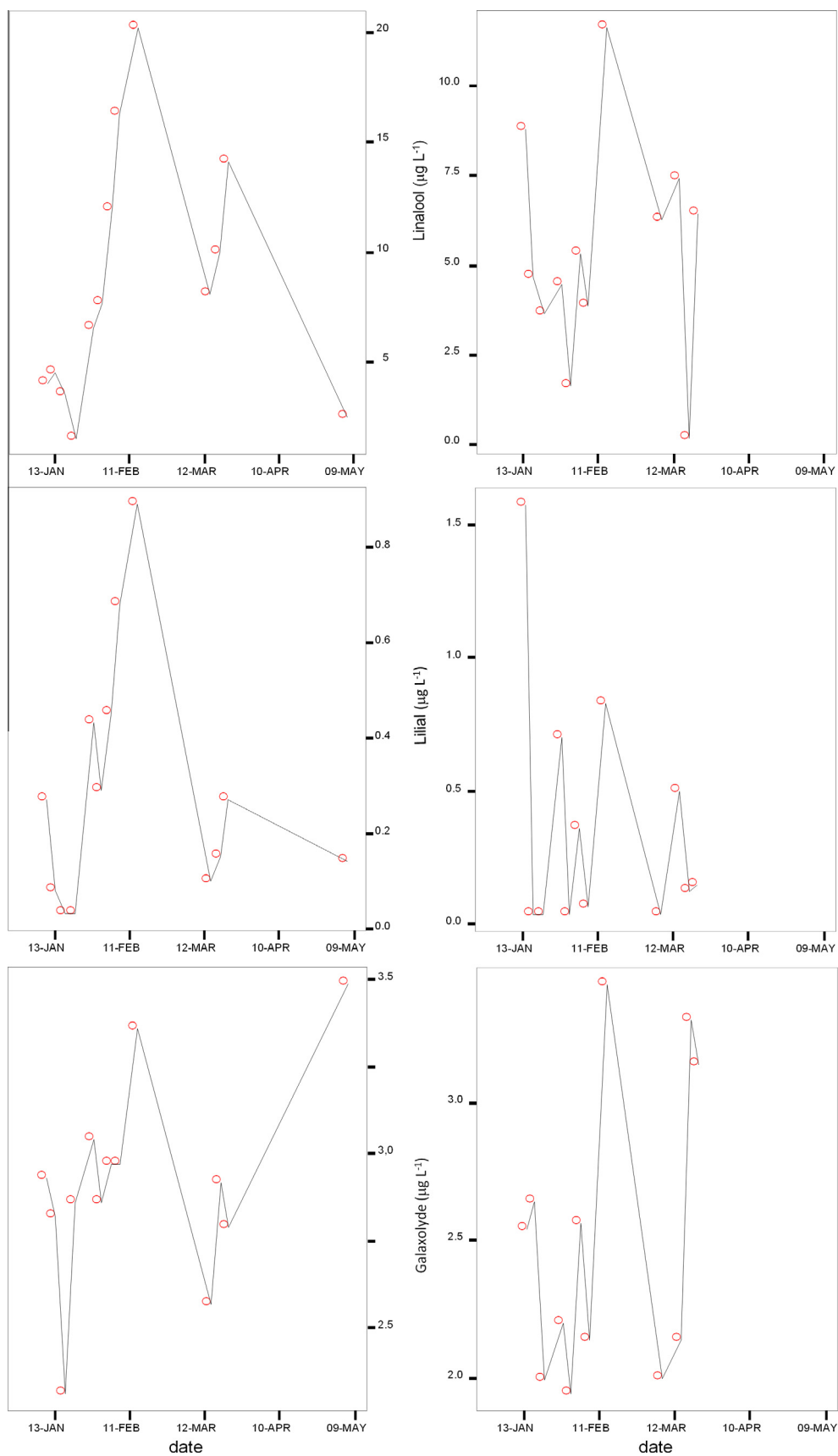


Fig. 3. Plots of the date evolution for three target compounds (lilial, linalool and galaxolide) at the primary effluent of the Castell d'Aro WWTP (left column) and the Girona plant (right column).

in grey wastewaters generated from domestic buildings (Eriksson et al., 2003). In the case of polycyclic musk, levels of galaxolide ranging from 1 to 20 $\mu\text{g L}^{-1}$ have also been reported in many studies (Gatermann et al., 2002; Simonich et al., 2002; Hernández Leal et al., 2010).

Limonene, linalool and eugenol were only detected at the primary effluent of the two monitored WWTPs, which confirms their quantitative elimination during the secondary treatments. Escalas et al. (2003) also found the quantitative elimination of linalool in a WWTP, whereas Lee and Rasmussen (2006) only detected limonene in 4.1% of the effluent samples evaluated ($n = 73$) at levels close to the LOD.

Lilial was found to be the most persistent target allergen and was detected at all sampling points of the two plants, which agrees with the results obtained by Becerril et al. (2010). For Castell d'Aro, statistical analysis showed no significant differences between the values obtained in the three sampling points (ANOVA test, $p = 0.591$), while significant differences were observed between the primary and secondary effluents of the Girona WWTP (t -test, $p = 0.04$) (Fig. 1).

With regards to benzyl salicylate, statistical tests revealed that there were significant differences between the concentrations found at the primary and secondary effluents for both plants (ANOVA test, $p = 0.001$ for Castell d'Aro; t -test, $p = 0.007$ for Girona). This analyte was detected at the primary effluent of both WWTPs (69% of the primary effluent samples in Castell d'Aro and 75% in Girona) and was not detected at the secondary effluent of the Girona WWTP, while at the Castell d'Aro plant was detected in four secondary samples (28.6%). Other studies (Kameda et al., 2011; Negreira et al., 2010; Simonich et al., 2002) also detected the presence of this allergen at the effluent of some WWTPs, which were significantly lower than the levels found at the influents.

The two polycyclic musks, galaxolide and tonalide, were also detected at all sampling points of the two plants (Fig. 2). In the case of Castell d'Aro, significant differences between the three sampling points were obtained for both compounds (ANOVA test, $p = 0.001$ for galaxolide and $p = 0.021$ for tonalide) and musk concentrations were always significantly higher at the secondary rather than the primary effluent. Data obtained from the Girona WWTP show the same behaviour for galaxolide (t -test, $p = 0.044$), whereas no significant differences were observed for tonalide between the two treatments performed at this plant (t -test, $p = 0.058$). Similar behaviour, with an increase of some PCPs during the treatment process, has also been described in other studies (Simonich et al., 2002; Onesios et al., 2009). Simonich et al. (2002) evaluated 17 different treatment plants in UK and USA and found that in the case of activated sludge plants the average relative profile in secondary effluent is enriched in the non-biodegradable, sorptive musks, such as galaxolide and tonalide.

When comparing the values obtained between the two WWTPs, no significant differences were observed between their primary effluents for limonene, linalool, eugenol, lilial, tonalide, and benzyl salicylate (t -test, $p > 0.05$). Galaxolide concentrations were found to be higher at Castell d'Aro primary effluent (t -test, $p = 0.048$). No significant differences were observed between the secondary effluents of the monitored WWTPs for galaxolide (t -test, $p = 0.051$), tonalide ($p = 0.477$) and benzyl salicylate ($p = 0.191$). In the case of lilial, higher concentrations were found at Castell d'Aro secondary effluent (t -test, $p = 0.009$).

A study has been undertaken to assess whether there are time dependent patterns for the compounds evaluated. No significant tendency was found in the case of the Girona WWTP for any of the target compounds evaluated (Fig. 3). It is important to note that the population of the area affected by this plant was relatively constant during the period evaluated. However, some patterns can be detected in the case of the Castell d'Aro plant (Fig. 3). The

maximum levels in the primary effluents for all target compounds in the Castell d'Aro plant were found in the samples obtained on 15th February. As indicated in the experimental section, this plant gives service to a touristic area with significant population increases during holiday periods and long weekends. The peak increase in this plant for all compounds corresponds to the samples obtained after the celebration of an important local festival when there was a significant increase in the population.

In general, few differences were found for the target PCPs between the two studied WWTPs. The results obtained indicate that polycyclic musks (galaxolide and tonalide) are currently the compounds that are detected at the largest concentrations at plant effluents. It has been found, however, that some fragrance allergens (lilial and benzyl salicylate in the present study) cannot be efficiently removed by conventional activated sludge treatments. Therefore, there is a need for more advanced processes in water and sludge treatments in order to avoid their accumulation in the environment. In addition, the occurrence of some of these compounds in surface waters, taken together with the fact that they are highly lipophilic, suggests that bioaccumulation in biota may take place. As a consequence, knowledge about environmental concentrations and the biodegradation pathways of allergens, musks and their metabolites is needed in order to predict the environmental effects and identify the measures that can be taken to limit the risk.

4. Conclusions

A method based on HS-SPME was successfully developed and validated for the simultaneous determination of 16 allergens and two polycyclic musks in aqueous samples from WWTPs. The microextraction procedure was optimised and the final conditions were fixed at 2.4 g of NaCl, 45 min extraction time, and 90 °C extraction temperature. The proposed method was applied to the monitoring of the target fragrances in two WWTPs located in Girona (Spain). Seven of the analytes were detected at the influent of the evaluated plants and limonene, linalool and eugenol were found to be eliminated during their treatments. Lilial, benzyl salicylate, galaxolide, and tonalide were detected at the effluent samples, where galaxolide was the compound detected at the highest concentrations (from 2.50 to 4.58 $\mu\text{g L}^{-1}$). More advanced treatments should therefore be used for a quantitative removal of these analytes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2014.06.072>.

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