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Optimisation of a solid-phase microextraction method for synthetic musk compounds in water

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Abstract

A solid-phase microextraction method (SPME) for determining trace levels of synthetic musk fragrances in residual waters has been developed. Six polycyclic musks (cashmeran, phantolide, celestolide, traseolide, galaxolide and tonalide), and a macrocyclic musk (ambrettolide) have been analysed. A detailed study of the different parameters affecting the extraction process is presented. The main important factors affecting the microextraction process have been studied and optimised by means of a categorical factorial design. Two extraction modes (direct SPME and headspace SPME) were tried at different extraction temperatures using four different fiber coatings [polydimethylsiloxane (PDMS), Carboxen (CAR)–PDMS, PDMS–divinylbenzene (DVB) and Carbowax (CW)–DVB]. An extraction temperature of 100 °C sampling the headspace over the sample using CAR–PDMS or PDMS–DVB as fiber coatings were found to be the experimental conditions that lead to a more effective extraction. The method proposed is very simple and yields high sensitivity, with detection limits in the low pg/ml, good linearity and repeatability for all the target compounds. The total analysis time, including extraction and GC analysis, was only 45 min. The optimised method performed well when it was applied to waste water from an urban treatment plant.

Keywords: Solid-phase microextraction; Water analysis; Headspace analysis; Factorial design; Musk compounds

1. Introduction

Personal care products (PCPs) are composed of a wide variety of active ingredients, including fragrance components. Musks are a class of fragrance ingredient that not only contribute to the distinctive odours of consumer products, but they also serve to maintain the integrity of the product's scent. The natural (animal or botanical) sources of musk compounds have been mostly replaced by synthetic musks with a variety of chemical structures [1]. Basically, three families of musks, nitromusks, polycyclic musks and macrocyclic musks, may be potentially added to PCPs such as detergents, soaps, perfumes, creams, etc. In recent years, growing environmental presence of musk compounds has attracted the attention of several authors [2–10]. These compounds are not considered among the priority persistent pollutants and long-term toxicity was not fully evaluated. Nevertheless, musk compounds need not be persistent if they are continuously introduced in the environment mainly via the urban wastewater effluents. Due to their lipophyllic nature, these compounds are known to bioaccumu-

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late in fatty tissues similarly to chlorinated pesticides.

Several analytical methods were developed for the identification and quantification of musk compounds in a variety of environmental samples. Most methods are based on gas chromatography (GC) coupled to low-resolution mass spectrometry (MS), working in the electron ionisation mode to achieve accurate and sensitive identification of species.

In spite of the detection technique, several approaches are possible for the extraction of musk compounds depending on the sample matrix. Pressurized liquid extraction (PLE) and liquid–liquid extraction were applied to determine some musks in water, sludge, fish, etc. [5–7]. Solid-phase microextraction (SPME) is a relatively new technique that was successfully applied to determine a wide range of water pollutants such as polychlorinated biphenyls (PCBs) or phenols [11–13]. For the analysis of musk compounds in water samples, SPME was recently proposed as an extraction technique due to its inherent advantages [9,14]. In these studies, SPME is performed by directly immersing the fiber into the liquid sample. However, when direct SPME is applied to waste waters, several problems may arise such as irreversible adsorption of major components leading to fiber deterioration.

The aim of the present paper is to provide a method based on SPME–GC–MS that rapidly permits the analysis of synthetic musk compounds in water. Full discussion on the factors influencing the extraction of seven synthetic musk compounds (including one macrocyclic compound) most used as ingredients of PCPs in Spain is also provided. Among these factors, temperature, sampling type or fiber type have been extensively studied. Optimisation was performed by means of experimental design. Data for linearity, precision, and accuracy are provided for the method. Limits of detection (LODs) and quantification (LOQS) were found at the ppt level.

2. Experimental section

2.1. Reagents and materials

The musk compounds, 6,7-dihydro-1,1,2,3,3-pentamethyl-4-(5H)-indanon (cashmeran, DPMI, [33704-61-9]), 4-acetyl-1,1-dimethyl-6-*tert*.-butylindan (celestolide, ADBI, [13171-00-1]), 1,3,4,6,7,8-hexahydro-4, 6, 6, 7, 8, 8-hexamethylcyclopenta[*g*]-2-benzopyran (galaxolide, HHCB, [1222-05-5]), 7-acetyl-1,1,3,4,4,6-hexamethyltetralin (tonalide, AHTN, 1506-02-1]), oxacycloheptadec-10-en-2-one (ambrettolide, [28645-51-4]) were kindly supplied by Ventós (Cornella de Llobregat, Barcelona, Spain); 6-acetyl-1,1,2,3,3,5-hexamethylindan (phantolide, AHMI, [15323-35-0]), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindan (traseolide, ATII, [68140-48-7]), were supplied by Promochem Iberia (Barcelona, Spain). Isooctane, acetone, methanol, NaCl, were all purchased from Merck (Mollet del Vallés, Barcelona, Spain). All the solvents and reagents were analytical grade. The SPME manual holders and fibers were obtained from Supelco (Bellefonte, PA, USA). In this work four fibers were used: 85 μm polyacrylate (PA), 100 μm polydimethylsiloxane (PDMS), 75 μm Carboxen–polydimethylsiloxane (CAR–PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB) and 65 μm Carbowax–divinylbenzene (CW–DVB). The fibers were conditioned as recommended by the manufacturer.

2.2. Experimental set-up

The samples were placed in headspace vials which total volume depending upon the experiment performed. Vials were sealed with a headspace aluminium cap furnished with a PTFE-faced septum and immersed in a water bath maintained at 100 °C. Samples were let to equilibrate for 5 min before SPME. Afterwards, the fiber was exposed to the headspace over the sample (or immersed into the sample) for 25 min, depending on the experiment. Once finished the exposition period, the fiber was immediately inserted into the GC injector and the chromatographic analysis was carried out. Desorption time was set at 2 min although an extra period of 5 min for desorption was considered to avoid carryover effect after the analysis of more concentrated samples. As some of the experimental parameters were optimised by factorial design, further discussion about them will be furnished in subsequent sections.

2.3. Gas chromatography–mass spectrometry

GC–MS analysis were performed in a Varian 3400 CX chromatograph equipped with a Saturn 3 ion trap mass detector and a split/splitless injector, operated by Saturn version 5.4 software. Musk compounds were separated on a 25 m×0.25 mm I.D., Varian VA-5MS column coated with a 0.25 μm film. The GC oven temperature program was: 60 °C hold 2 min, rate 10 °C/min to 250 °C, rate 20 °C/min to final temperature 280 °C hold for 4.5 min (total analysis time, 27 min). Helium was employed as carrier gas, with a column initial head pressure of 8 p.s.i. (1 p.s.i.=6894.76 Pa). Injector was operated in the splitless mode for 2 min and its temperature was set at 250 or 270 °C (depending on the type of fiber coating being desorbed). Transfer line temperature was maintained at 280 °C. The ion-trap mass spectrometer was operated in the electron ionisation mode (70 eV), and the trap temperature was set at 230 °C. Other experimental parameters influencing the acquisition and analysis of mass spectra were the following: full scan mass acquisition range, 35–300 amu; scan rate, 1 s/scan; multiplier voltage, 2100 V; axial modulation voltage, 4 V; filament emission current, 23 μA; ionisation control, automatic mode; filament/multiplier delay, 12 min.

3. Results and discussion

The first experiments were conducted to achieve good chromatographic separation of the target analytes. The mass spectrum for each musk compound was also obtained at the experimental conditions, and the most adequate ions for quantification were selected. In Table 1, the retention times at the optimised chromatographic conditions are shown, as well as the quantification and identification ions. It must be noticed that the quantification ion is not always the base peak of the mass spectrum. The criteria followed in the selection of quantification ions were (1) maximum sensibility, (2) minimum possible interference of fragments from other sample components. Fig. 1 shows the mass chromatograms of a 500 pg/ml standard solution containing the seven musks.

Due to their occurrence as ingredients of all kind of cleansing products and cosmetics, the risk of sample contamination with musks when they are manipulated in the laboratory is not negligible, so it is advisable to extreme precautions to avoid sources of interference in the laboratory environment. In fact, galaxolide and tonalide were found in blanks of unspiked reagent water analysed at the beginning of the work. To avoid sample contamination, appropriate steps should be emphasised.

Table 1
Retention times and selected ions for the analysis of the target musk compounds

Compound	Retention time (min)	Quantification ion	Identification ion
Cashmeran	13.89	163	191.206
Celestolide	16.46	229	173.244
Phantolide	16.96	229	244.187
Traseolide	17.96	215	173.258
Galaxolide	18.04	213	243.258
Tonalide	18.11	159	187.243
Ambrettolide	19.03	109	67.81

3.1. Factorial design

Once the chromatographic conditions were selected, a factorial design was run to evaluate the main parameters affecting the extraction efficiency. For this study, 10 ml water samples in 22 ml vials were spiked with a standard solution of the musks in acetone to give a final concentration of 10 ng/ml. Samples were not stirred during extraction and the exposition time was 25 min. Three parameters were studied in this design: the type of fiber coating, the extraction mode and the temperature. Four different fibers were used in this study: PDMS, PDMS–DVB, CAR–PDMS and CW–DVB. PA fiber was not included in this design because earlier investigations showed poor musk extraction efficiencies. The extraction mode was studied at two levels: direct sampling (SPME) and headspace sampling (HS-SPME). The extraction temperature was tested at three levels (25, 60 and 100 °C).

A multifactor categorical 4*3*2 type V resolution design, which involves 24 runs, was selected [15]. This design enables the study of the main effects and two-factor interactions. The analysis of the data obtained led to the analysis of variance (ANOVA)

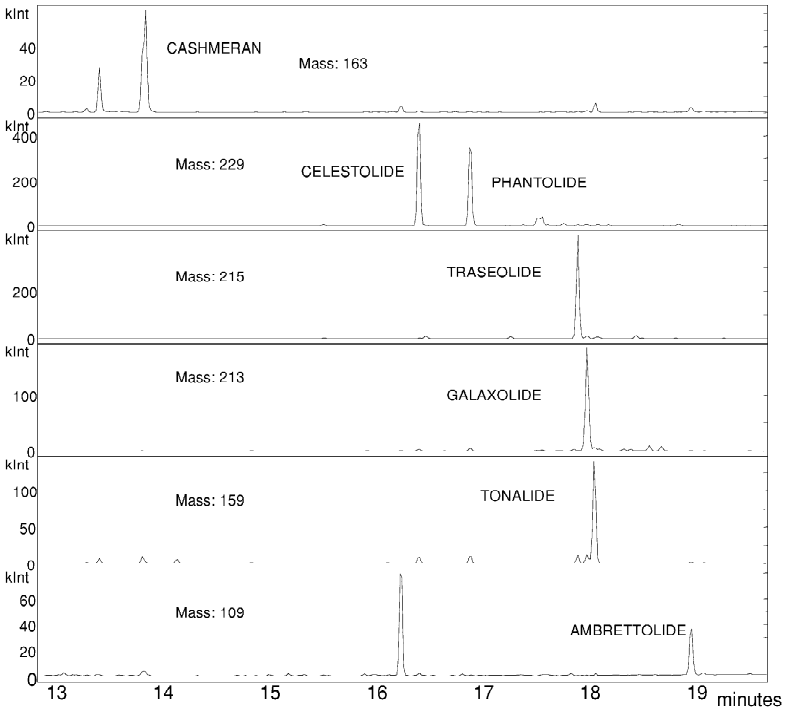

Fig. 1. GC–MS selected ion chromatograms for a water sample containing 500 pg/ml of each musk compound.

results shown in Table 2. In this table, and for the sake of simplicity, only the *F*- and *P*-values are given. The *P*-values test the statistical significance of each of the factors. When *P*-value is less than 0.05, the factor has a statistically significant effect at the 95% confidence level. As can be seen, temperature (B) and extraction mode (C) were found to be significant, as well as the interaction between these

Table 2
ANOVA results showing the significance of main effects and interactions

Compound	Source	Main effects			Interactions		
		A: Fiber	B: Temperature	C: Extraction mode	AB	AC	BC
Cashmeran	*F*-ratio	5.46	4.19	2.83	1.01	0.37	3.46
	P-value	0.04	0.07	0.14	0.49	0.78	0.10
Celestolide	*F*-ratio	4.66	14.96	20.27	1.62	1.60	8.90
	P-value	0.05	0.00	0.00	0.29	0.29	0.02
Phantolide	*F*-ratio	5.00	17.05	13.79	1.85	1.21	9.58
	P-value	0.05	0.00	0.01	0.24	0.38	0.01
Traseolide	*F*-ratio	3.78	26.55	30.88	1.58	1.91	14.27
	P-value	0.08	0.00	0.00	0.30	0.23	0.01
Galaxolide	*F*-ratio	3.73	23.28	22.16	1.47	1.32	12.73
	P-value	0.08	0.00	0.00	0.33	0.35	0.01
Tonalide	*F*-ratio	4.06	27.64	21.18	1.73	1.62	14.54
	P-value	0.07	0.00	0.00	0.26	0.28	0.01
Ambrettolide	*F*-ratio	2.91	41.07	62.70	1.58	2.17	2.68
	P-value	0.12	0.00	0.00	0.30	0.19	0.00

Factors are significant when *P*-value$<$0.05.

two factors (BC) for all the compounds, with the exception of cashmeran. Surprisingly, the factor fiber (A) was only significant for cashmeran, celestolide and phantolide. Nevertheless, with the exception of ambrettolide this main effect was quite close to the significance level for the other musks.

Table 3 shows the interaction of factors fiber and temperature on the response for the target compounds. It must be pointed out that for all the compounds, the best responses were achieved working at the highest temperature (100 °C) for all of the fibers, with the exception of cashmeran. The lowest responses were attained when working at 25 °C. CAR–PDMS and DVB–PDMS fibers gave the best extraction efficiencies at 100 °C. For cashmeran, the maximum extraction was obtained at two different experimental conditions: at 60 °C with CAR–PDMS and at 100 °C with DVB–PDMS. Considering that 100 °C was the best extraction temperature for the other musks, this temperature was selected as the optimum value.

In Table 4, the interaction of fiber and extraction mode on the extraction efficiency was considered for the analytes. HS-SPME is more efficient than direct SPME for all of the fibers. Table 5 shows the results of the interaction temperature–extraction mode for the compounds. In this table, the significant interaction between the two factors can be seen. At 25 °C, the extraction efficiency is higher when direct SPME is performed, but at higher temperatures (60 or 100 °C) just the opposite occurs; the efficiency of HS-SPME is significantly higher.

For all the compounds considered, response increased as temperature increased both with direct SPME and with HS-SPME. The only exception was cashmeran, for which a decreasing response was evident at 100 °C. Moreover, in direct SPME the extraction efficiency at 100 °C is worse than at 25 °C for this compound.

After analysing all these data, a general method can be established for the extraction of musks in water. The method uses HS-SPME at 100 °C with CAR–PDMS or PDMS–DVB fiber and 10 ml sample.

3.2. Evaluation of other experimental parameters

Other factors not included in the design have been

Table 3
Fiber–temperature interaction (results are expressed as area counts)

Compound	Temperature (°C)	Fiber			
		PDMS	PDMS–DVB	CAR–PDMS	CW–DVB
Cashmeran	25	32679	62328	44013	23563
	60	63616	126071	170524	48440
	100	29174	149206	96562	31146
Celestolide	25	45717	51480	41828	32557
	60	154389	161019	271358	111846
	100	151568	310054	350482	101434
Phantolide	25	33265	38973	33070	24154
	60	104704	108494	180766	79007
	100	101798	229550	248453	82076
Traseolide	25	25342	23738	21576	18543
	60	83487	86946	141984	69550
	100	132579	212117	227139	88867
Galaxolide	25	41334	44138	36627	26517
	60	139360	137349	209723	98237
	100	193851	308000	275996	112199
Tonalide	25	40321	39804	35094	27456
	60	125865	120803	196292	95586
	100	188307	309831	330675	128669
Ambrettolide	25	44486	29553	27449	29252
	60	129765	132526	167689	106393
	100	207981	272988	242300	124828

Table 4
Fiber–extraction mode interaction (results are expressed as area counts)

Compound	Extraction mode	Fiber			
		PDMS	PDMS–DVB	CAR–PDMS	CW–DVB
Cashmeran	HS	67271	143104	111686	44826
	Direct	32214	81966	95713	23939
Celestolide	HS	157076	264813	320323	108946
	Direct	77373	83556	122122	54945
Phantolide	HS	99571	179302	206293	76426
	Direct	60274	72043	101900	47065
Traseolide	HS	110208	168553	195945	80505
	Direct	50730	46648	64521	37467
Galaxolide	HS	163384	242351	242335	102956
	Direct	86311	83974	105896	55011
Tonalide	HS	149772	232641	263371	106404
	Direct	86556	80984	111335	61403
Ambrettolide	HS	179792	232411	226174	123290
	Direct	75029	57634	65451	50359

considered: sample volume, agitation, salting-out and carryover.

Sample volume can increase the total amount of musks extracted by the fiber. To check the effect of sample volume on the amount of musks extracted by the HS-SPME method, a set of experiments was carried out using the PDMS–DVB fiber. Samples of 10, 30, 60 and 100 ml were introduced in 120 ml vials and HS-SPME was carried out. As can be seen in Table 6, increasing the volume led to higher responses for all the target compounds [16]. Thus, a sample volume of 100 ml is recommended for analysis requiring high sensitivity.

The possible influence of agitation on the efficiency when HS-SPME is performed was also evaluated by exposing the PDMS–DVB fiber to the headspace of magnetically stirred samples. Results showed that extraction efficiency was not affected by stirring.

Salting-out was evaluated analysing water samples without salt and with 20% NaCl in the HS-SPME mode. No differences in response were found in these experiments.

Carryover was evaluated for the PDMS–DVB and CAR–PDMS fibers working in the HS-SPME mode after the extraction of water samples spiked with 500 pg/ml. After desorption, the fiber was reinserted in the GC injector and a blank analysis was run. This

Table 5
Temperature–extraction mode interaction (results are expressed as area counts)

Compound	Extraction mode	Temperature (°C)		
		25	60	100
Cashmeran	HS	24540	123389	127238
	Direct	56752	80936	37686
Celestolide	HS	24982	260568	352819
	Direct	60809	88738	103950
Phantolide	HS	14049	159388	247757
	Direct	50682	77098	83181
Traseolide	HS	11457	146463	258490
	Direct	33143	44521	71861
Galaxolide	HS	15989	209385	337896
	Direct	58319	82949	107127
Tonalide	HS	13322	186025	364795
	Direct	58015	83248	113946
Ambrettolide	HS	25696	217179	328375
	Direct	39674	51007	95673

Table 6
Effect of sample volume on the efficiency of the SPME (expressed as area counts)

Compound	Sample volume (ml)			
	10	30	60	100
Cashmeran	7061	71234	96631	141324
Celestolide	33628	381298	557916	838700
Phantolide	34384	311506	404534	543526
Traseolide	42557	377437	544807	786854
Galaxolide	63229	482575	632160	835668
Tonalide	67527	490390	619408	805002
Ambrettolide	89498	600448	836276	1177926

process was carried out with each fiber. The response recorded for the seven target musks, was compared to the response obtained for the previously analysed water samples. In all cases, carryover for the CAR–PDMS fiber was higher than for the other fiber. For PDMS–DVB, carryover increased as did the retention time of the compounds. Nevertheless, it was lower than one thousandth (1‰), even for the macrocyclic musk, ambrettolide. When CAR–PDMS was used, the carryover found was about 3–5‰, with the exception of ambrettolide (25‰). This could be expected due to its more highly lipophyllic nature.

3.3. Performance evaluation of the proposed method

Linearity, repeatability and detection limits were evaluated in order to assess the performance of the HSSPME method. Results are shown in Table 7.

The calibration studies were performed with PDMS–DVB and CAR–PDMS fibers, using 100 ml spiked water samples. These samples were analysed in duplicate or triplicate at the optimal extraction conditions established by the design and the complementary experiments discussed above. Calibration curves were linear in the concentration range studied: from 2 to 2000 pg/ml. As can be seen in Table 7, the r^2 values were higher than 0.990 for all of the compounds so, a directly proportional relationship between the extracted amount of musks and its initial concentration in the sample at the tested concentration range was demonstrated. No differences were found in linearity when considering PDMS–DVB or CAR–PDMS fibers. Linearity of cashmeran could not be evaluated at the total concentration range considered when using CAR–PDMS fiber, due to interference by a major unknown coeluting substance (most probably a polysubstituted phenolic compound).

The precision of the experimental procedure was also evaluated at two different concentration levels by calculating the relative standard deviation (RSD) of three replicates of each level (Table 7). The RSD values were between 7.7% and 21.0% with PDMS–DVB, and between 2.2% and 17.4% with CAR–PDMS. Highest values were obtained for the less volatile and more lipophyllic musk, the ambrettolide.

Detection limits (based on a signal-to-noise ratio = 3) are shown in Table 7. These were below 1 pg/ml for celestolide, phantolide and traseolide with both fibers; 1–2 pg/ml for cashmeran, galaxolide and tonalide; and somewhat higher for ambrettolide. Nevertheless, these limits of detection must always be checked by analysing blank samples daily in order to update signal-to-noise ratios.

A real contaminated water sample taken from the effluent of an urban treatment plant was also analysed to check the method performance. Fig. 2 shows the total ion chromatogram. Concentration of the identified compounds was evaluated by external calibration and by standard addition. Both the PDMS–DVB and the CAR–PDMS fibers were used again. Results are presented in Table 8. These were very consistent using both fibers and both quantification methods. It must be pointed out that concentrations lie in the low ppt for the most of the musks, and only galaxolide and tonalide appear at higher

Table 7
Linearity, limit of detection and repeatability for the target musk compounds considering the PDMS–DVB and the CAR–PDMS fibers

Compound	Correlation factor (r^2)		Detection limit ($S/N = 3$ pg/ml)		Repeatability (RSD, % $n = 3$)			
	PDM–DVB	CAR–PDMS	PDM–DVB	CAR–PDMS	PDM–DVB		CAR–PDMS	
					10 pg/ml	2000 pg/ml	10 pg/ml	2000 pg/ml
Cashmeran	0.9989		1.7	1.5	9.1	13.2	3.9	7.9
Celestolide	0.9990	0.9985	0.1	0.1	8.4	7.9	3.7	6.5
Phantolide	0.9990	0.9991	0.2	0.3	9.1	11.1	2.4	0.96
Traseolide	0.9977	0.9991	0.3	0.3	8.9	13.5	6.9	14.1
Galaxolide	0.9986	0.9989	1.2	1.0	7.7	10.9	2.2	17.4
Tonalide	0.9999	0.9999	1.8	1.0	9.9	15.1	7.2	9.1
Ambrettolide	0.9968	0.9973	4.6	9.0	18.8	21.0	17.9	15.1

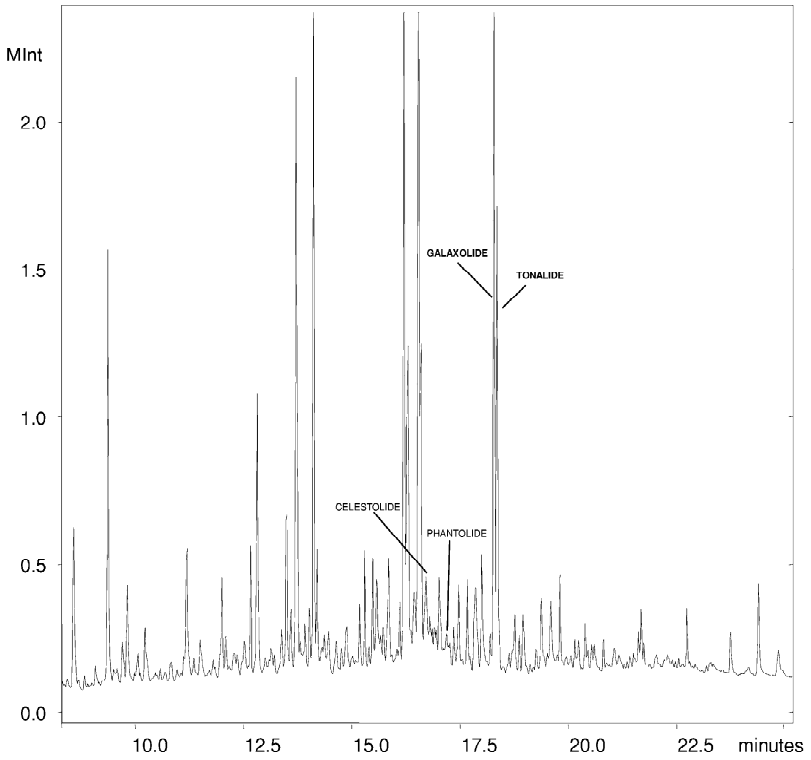

Fig. 2. Total ion chromatogram of a real water sample taken at the effluent of an urban treatment plant.

Table 8
Concentration (pg/ml) of the target musk compounds found in a real contaminated sample from an urban treatment plant

Compound	Standard addition		External standard	
	PDMS–DVB	CAR–PDMS	PDMS–DVB	CAR–PDMS
Cashmeran	nd	nd	nd	nd
Celestolide	12.3±0.8	9.8±1.1	9.1±0.6	10.7±1.2
Phantolide	6.8±0.7	4.9±0.7	5.3±0.6	4.5±0.6
Traseolide	15.8±0.3	12.3±1.2	12.9±0.3	12.8±1.3
Galaxolide	478±33	507±5	372±26	514±5
Tonalide	125±1	153±3	102±1	136±3
Ambrettolide	nd	nd	nd	nd

Results were obtained for the fibers PDMS–DVB and CAR–PDMS by two calibration methods: standard addition and external standard.

levels (about 500 and 120 ppt, respectively). As can be seen, a slight matrix effect was found for PDMS–DVB and so, the results obtained by this fiber using the external standard procedure were lower.

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