



Development of a stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry method for determining synthetic musks in water samples

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ARTICLE INFO

Article history:

Received 7 September 2010
Received in revised form 26 October 2010
Accepted 1 November 2010
Available online 9 November 2010

Keywords:

Stir bar sorptive extraction (SBSE)
Thermal desorption (TD)
Gas chromatography–mass spectrometry (GC–MS)
Synthetic musks
Water samples

ABSTRACT

This study presents the development of an analytical method for determining 9 synthetic musks in water matrices. The developed method is based on stir bar sorptive extraction (SBSE), coated with polydimethylsiloxane, and coupled with a thermal desorption–gas chromatography–mass spectrometry system (TD–GC–MS). SBSE can efficiently trap and desorb the analytes providing low limits of detection (between 0.02 ng L⁻¹ and 0.3 ng L⁻¹). Method validation showed good linearity, repeatability and reproducibility for all compounds. Furthermore, the limited manipulation of the sample required in this method implies a significant decrease of the risk of external contamination of the samples. The performance of the method in real samples was evaluated by analysing biological wastewater treatment plant (WWTP) influent and effluent samples, reverse osmosis treatment plant effluents and river waters. The most abundant musk was galaxolide with values up to 2069 ng L⁻¹ and 1432 ng L⁻¹ in the influent and effluent of urban WWTP samples, respectively. Cashmeran, Pantolide and Tonalide were also detected in all the matrices with values up to 94 ng L⁻¹, 26 ng L⁻¹ and 88 ng L⁻¹, respectively. Although in Europe the use of nitromusks in cosmetics is prohibited, musk xylene and musk ketone were detected both in the WWTP and in the river samples. As far as we know, this is the first time that a SBSE method coupled with TD is applied for the determination of synthetic musks in water samples.

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1. Introduction

Synthetic musks are commonly used as fragrances in a wide range of consumer products such as detergents, cosmetics and other personal care products. These fragrances comprise a broad range of different compounds, including the polycyclic, nitro and heterocyclic musks. Most commonly used are the polycyclic musks, especially galaxolide (HHCB) and tonalide (AHTN), added to the majority of household and cosmetic products. However, polycyclic and nitro musks are not structurally or chemically similar to the natural ones and have a lipophilic nature, causing them to bioaccumulate in sediments, sludge and biota and biomagnify throughout the food chain [1,2]. Consequently, these compounds have even been found in human tissues, such as adipose tissue, breast milk and blood [3–5].

The main route of exposure of synthetic musks into the environment is through wastewater effluent [6]. Since these fragrances are only partially biodegradable and are not completely eliminated by wastewater treatment plants (WWTPs) determination

in wastewaters and in natural water samples is of major importance [7]. The long-range transport and persistence of synthetic musks, as well as, their exchange between air and water matrices, has caused them to be found in remote non-anthropogenic areas such as the Great Lakes [8,9] and Arctic waters [10]. Their presence has also been reported in fish, mussel and crustacean [11,12]. Furthermore, due to their widespread use and the detection of these compounds in natural waters, several authors have suggested the use of polycyclic musks as chemical markers of anthropogenic pollution [13,14].

Several analytical methods for determining synthetic musk compounds in water samples have been developed in the last years, most of them based on GC–MS analysis. One of the most commonly used sample preparation technique is solid-phase extraction (SPE), for which different sorbents, have been successfully tested in previous studies [1,15]. However, due to the widespread use of synthetic musks they can be found in solvents and laboratory equipment, which can contaminate the samples. Therefore, analytical methods that can reduce the risk of the background musk contamination should be developed. In this respect, the use of sorptive techniques coupled with thermal desorption – such as solid-phase micro extraction (SPME) and stir bar sorptive extraction (SBSE) – drastically reduces the risk of contamination. These techniques are

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Table 1Formula name, log K_{OW} , retention times t_R and quantifier and qualifier ions of the target musk compounds.

No.	Compound	Formula name	Log K_{OW} ^a	t_R (min)	Quantifier ion	Qualifiers
1	Cashmeran (DPMI)	6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone	4.9	5.12	191	206(60), 192(14)
2	Celestolide (ADBI), Crisolide	4-acetyl-1,1-dimethyl-6- <i>tert</i> -butylindane	6.6	7.84	229	244(45), 173(19.5)
3	Phantolide (AHMI)	6-acetyl-1,1,2,3,3,5-hexamethylindane	6.7	8.75	229	244(24), 187(9.5)
4	Traseolide (ATII), Fixolide	5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-indane	6.7	10.02	215	258(15), 173(16)
5	Galaxolide (HHCB), Abbalide, Pearlide	1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran	5.9	10.25	243	213(15), 258(10)
6	Tonalide (AHTN)	7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene	5.7	10.38	243	258(27.5), 159(20)
7	Musk Xylene (MX)	2,4,6-trinitro-1,3-dimethyl-5- <i>tert</i> -butylbenzene	4.8	11.22	282	283(15), 297(10)
8	Musk Moskene (MM)	1,1,3,3,5-pentamethyl-4,6-dinitroindane	5.8	11.26	263	264(15), 278(10)
9	Musk ketone (MK)	4-aceto-3,5-dimethyl-2,6-dinitro- <i>tert</i> -butylbenzene	4.3	12.83	279	294(20), 280(15)

^a Log K_{OW} values predicted from SRC-KowWin software.

solventless sample enrichment methods, which allow the direct extraction of the analytes, implying the minimal manipulation of the sample and avoiding the use of organic solvents, with enhanced sensitivity. Furthermore, the combination of the extraction and the concentration of the analytes in one step also reduces the time of sample preparation. Indeed, SPME methods have been previously developed for the detection of fragrances in aquatic matrices [16–18]. However, SBSE is a more powerful extraction technique, with higher preconcentration capacity as the amount of sorbent is 50–250-fold higher than in SPME fibre. Since the development of SBSE in 1999 by Baltussen et al. [19], this extraction technique has been successfully applied for the analysis of trace environmental pollutants in different matrices [20–22].

As far as we know, only one SBSE method with liquid desorption has been recently developed for determining four synthetic musks in water [22]. Therefore, the aim of this study is to propose a method based on SBSE followed by TD–GC–MS for determining nine synthetic musks in water samples. After the extraction step, stir bars were placed in empty stainless-steel tubes and thermally desorbed in a TD equipment designed to desorb sorbent tubes for air analysis. To the best of our knowledge, a SBSE method is for the first time combined with a thermal desorption system for determining these fragrances in water samples. The applicability of the method to real samples was tested by analysing biological wastewater treatment plant influent and effluent samples, reverse osmosis treatment plant effluents and river waters.

2. Experimental

2.1. Chemical standards

The six polycyclic musks: 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI, cashmeran), 4-acetyl-1,1-dimethyl-6-*tert*-butylindane (ADBI, celestolide), 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI, phantolide), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII, traseolide), 1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran (HHCB, galaxolide) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN, tonalide), were supplied by Promochem Iberia (Barcelona, Spain). The nitro musk fragrances 2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene (musk xylene), 1,1,3,3,5-pentamethyl-4,6-dinitroindane (musk moskene) were purchased as 100 mg L⁻¹ solutions in acetonitrile from

Sigma-Aldrich (Steinheim, Germany) and Riedel de Haën (Seelze, Germany), respectively, and 4-aceto-3,5-dimethyl-2,6-dinitro-*tert*-butylbenzene (musk ketone) from Fluka (Buchs, Switzerland). Table 1 shows the formula name of the target compounds and the logarithm of the octanol–water constant (K_{OW}) predicted from the software SRC-KowWin (Syracuse Research Corp., Syracuse, New York, USA).

Individual standard solutions of synthetic musks were prepared in acetone and the mixtures prepared in methanol. The solvents were GC grade with purity >99.9% (SDS, Peypin, France). Helium gas and nitrogen gas of purity 99.999% (Carbueros Metálicos, Barcelona, Spain) were used for the thermal desorption and the chromatographic analysis. Ultra-pure water was obtained using a Mili-Q water purification system (Millipore, Bedford, MA, EEUU).

2.2. Sample collection

Four representative water types were sampled to test the performance of the method in real samples: the influent and effluent of two urban wastewater treatment plants (WWTPs), which collect wastewater from ca. 120,000 inhabitants, respectively; the effluent of a reverse osmosis plant (RO) located after the secondary treatment of one of the studied urban plants; and samples from the Ebro River. Samples were collected in amber glass bottles, pre-cleaned overnight with chromic mixture and subsequently rinsed with Milli-Q water and HPLC grade isopropanol. After collection, samples were filtered using a 0.45 μ m nylon filter (Whatman, Maidstone, UK), stored at 4 °C and analysed within a week.

2.3. Stir bar sorptive extraction

Extractions were carried out with polydimethylsiloxane (PDMS) coated stir bars (20 mm length \times 0.5 mm film thickness, from Gerstel (Mülheim an der Ruhr, Germany)), which correspond to approximately 48 μ L of PDMS phase. Before each use, stir bar were conditioned by thermal cleaning at 300 °C for 3 h, in a pure Helium stream of 100 mL min⁻¹. Stir bars were stored in cleaned 2 mL vials until their use.

For the extraction procedure, a cleaned stir bar was placed in a 250 mL vial containing 100 mL of water, immediately capped and stirred at 900 rpm for 4 h at 25 °C and pH 7. One hundred millilitres of river and reverse osmosis effluent samples were directly

extracted, whereas urban WWTP influent and effluent samples were diluted with Milli-Q water before extraction (5 mL of sample into 100 mL of total volume).

After 4 h of extraction, stir bars were magnetically removed, rinsed with ultra-pure water, dried with a lint-free tissue and placed inside a thermally cleaned stainless-steel tube for the thermal desorption.

2.4. Thermal desorption GC–MS analysis

Thermal desorption of the musks retained on the stir bars was performed in a Unity Thermal Desorption system combined with a Ultra A autosampler (both from Markes International Limited, Llantrisant, UK). Stir bars were placed in empty stainless-steel tubes for thermal desorption (9 cm length \times 6.35 mm o.d. \times 5 mm i.d., also from Markes). Prior to the analysis, the empty tubes were thermally cleaned at 300 °C for 15 min and then stored in a hermetical glass jar under nitrogen atmosphere. The optimised thermal desorption conditions for the stir bar were: pre-purge for 1 min at room temperature, stir bar desorption at 300 °C for 15 min using helium carrier gas at 100 mL min⁻¹, in splitless mode, trapping at 0 °C. The trap was then desorbed at 320 °C for 10 min with a split of 5 mL min⁻¹.

Separation and detection were performed in a 6890N gas chromatograph and 5973 inert mass spectrometer (Agilent Technologies, Palo Alto, USA), using a Zebron ZB- 50 capillary column (30 m \times 0.25 mm \times 0.25 μ m) provided by Phenomenex (Le Pecq Cedex, France). For the GC–MS analysis, the helium carrier gas flow was 2 mL min⁻¹ and the temperature program was as follows: initial temperature 100 °C, 30 °C min⁻¹ to 170 °C, then at 5 °C min⁻¹ to 190 °C and then at 15 °C min⁻¹ to 290 °C and held for 4 min. The GC–MS interface was set at 290 °C. The MS-detector acquired in the selective ion monitoring mode (SIM) operating at an electron impact energy of 70 eV. The GC–MS parameters of the target musks were optimised in a previous paper [23]. Table 1 shows the retention times and the quantifier and qualifier ions used for the SIM detection.

3. Results and discussion

3.1. Method optimisation

As previously stated, the GC–MS parameters for these 9 synthetic musks were optimised in a prior study [23]. It is worth mentioning that the use of a midpolarity phase capillary column (a 50% diphenyl/50% dimethyl polysiloxane) provided the resolution of the compounds in about 13 min, even with the separation of the 4 HHCb diastereoisomers. HHCb was quantified by integrating the signals of 4S and 7R/S isomer peaks, which are the diastereoisomers responsible of the characteristic musky odour [24]. Following the previous optimisation of the GC–MS conditions, this study has focused on the optimisation of the SBSE parameters.

3.1.1. SBSE extraction

In the SBSE technique the extraction is an equilibrium process dependent on the amount of the PDMS phase, the volume of aqueous sample and the partitioning coefficients of the compounds between the PDMS phase and the aqueous phase that are correlated with the octanol–water distribution coefficients (K_{OW}) [19,21]. Therefore, the first parameters fixed were the size of the stir bar and the volume of sample. In order to have high sensitivity, the sample volume was fixed at 100 mL. Therefore, a suitable size of stir bar (20 mm length \times 0.5 μ m film thickness, with ca. 48 μ L of PDMS coating) was selected for this study. The calculated theoretical recoveries for 100 mL of sample using

this stir bar ranged from 90.5% for musk ketone to 100% for the most apolar synthetic musks, such as AHMI and ATII. Regarding the stirring rate, although the increase of the stirring speed can accelerate the extraction, it also reduces the lifetime of the stir bar [25]. Hence, a medium level stirring rate of 900 rpm was used.

Once the amount of organic phase, the volume of sample and the stirring rate were fixed, the influence of other factors, which play an important role in the efficiency of the SBSE extraction, was studied. A screening 2³ factorial design was used to study the influence of the salting-out effect, the time and the temperature on the SBSE extraction, involving 8 randomised experiments performed in duplicate. Statistical analysis was carried out with Statgraphics-Plus 5.1 (Magnugistic, Rockville, MD, USA). All experiments were run using 100 mL solutions of the target musks at a concentration of 70 ng L⁻¹ in Milli-Q water.

The influence of inert salt addition (NaCl) at 0% (no addition), and 20% levels was studied. Salt increases the ionic strength of the solution, which can decrease the solubility of the analytes in the aqueous phase and promote their transference to the organic phase [22]. Although in general for polar analytes with $K_{OW} > 3.5$ the addition of salt reduces the extraction efficiency [26,27], the opposite effect for apolar compounds, such as polycyclic aromatic hydrocarbons, has been also described [28].

Extraction temperature was studied at 25 °C and 60 °C. A high temperature helps to reach the equilibrium faster but also decreases the K_{OW} partition coefficient and the lifetime of the PDMS extraction phase [25]. However, other studies have reported opposite results [29,30]. Finally, the SBSE extraction time was studied at 3 h and 12 h. Extraction time is one of the most important parameters and should be optimised in every application. The optimal extraction time can vary from several minutes to hours or 1–2 days depending on the physical and chemical properties of the analytes, the partition coefficient between the volume of sample and the volume of the organic phase (β) and the experimental conditions [21].

As an example, the calculated standardised effects for the three factors and the two-factor interactions for DPMI, HHCb and MK are shown in the Pareto charts in Fig. 1. The standardised effect is obtained by dividing the estimated effect by its standard error. The vertical line indicates the statistically significant bound at the 95% confidence level. As it can be seen in Fig. 1, temperature was the most important factor for the musks. Moreover, temperature was the only significant factor for MK (Fig. 1C). For all the target musks, increasing the temperature from 25 °C and 60 °C had a negative effect in the extraction. Possible explanations can be the vaporisation of part of the analytes at high temperatures (vapour pressures from 2.69×10^{-3} for DPMI to 5.23×10^{-6} for MX) and/or the decrease of the partition coefficient at high temperatures. Furthermore, the addition of 20% NaCl had a negative effect for all the musks except DPMI and MK whose salting-out effect was not significant. The decrease of extraction efficiency with the addition of NaCl can be explained by the increase of the solution viscosity, which slows down the interaction kinetics of the analytes. Finally, the extraction time did not have an important role at the two levels studied (3 and 12 h), being statistically significant only for DPMI although it has a slightly positive effect for most compounds except for HHCb, which had reduced extraction at the higher time level.

Concerning the two-factor interactions, the effect of NaCl–temperature interaction (AB) was significant for all the target compounds. Therefore, the effect of increasing the temperature in the extraction process is different depending on the amount of NaCl added. However, the effect of NaCl–time interaction (AC) was only important for ADBI and the temperature–time interaction (BC) for DPMI and HHCb.

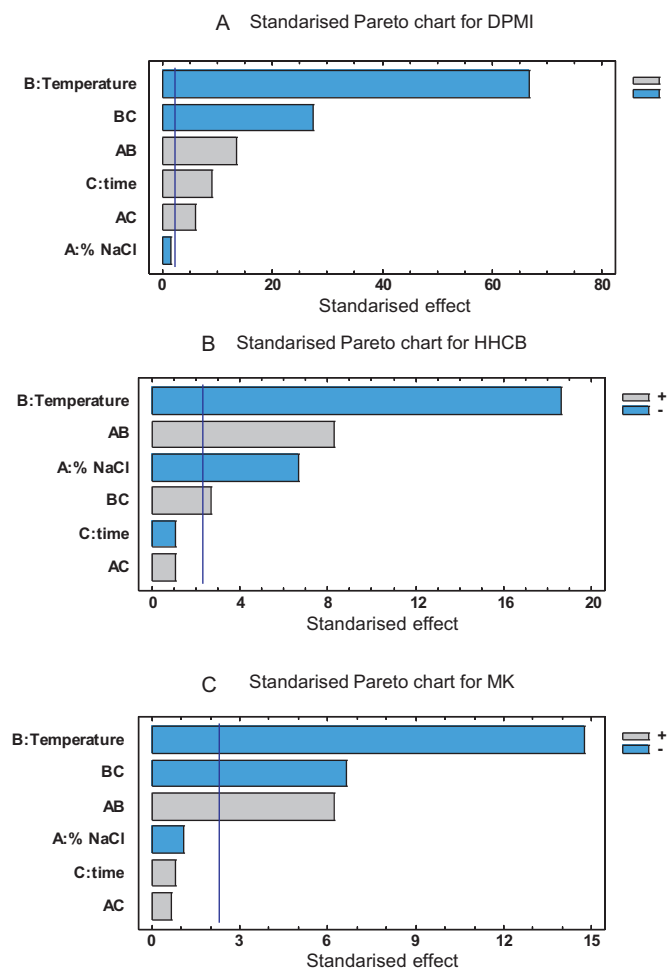


Fig. 1. Standardised Pareto charts for the mean effects and two-factor interactions of the factorial design for three representative synthetic musks: (A) DPML, (B) HHCB and (C) MK.

Taking into account the results of the screening multifactorial design, the subsequent tests were done at 25 °C without the addition of salt in the solution over 3 h of extraction.

Next, the influence of pH was evaluated, at pH 2.0, 7.0 and 10.0. As for previous studies [31,32], the efficiency of the extraction decreased under acidic conditions but no significant effect of sample pH was observed at neutral and basic pH (results not shown). Therefore, neutral pH was selected in order to avoid the PDMS-phase degradation.

The addition of organic modifiers, such as MeOH, can also favour the extraction of apolar analytes by reducing their adsorption to the vial glass walls. However, the presence of the organic modifier also increases the solubility of the apolar compounds in the solution, decreasing the extraction efficiency. In order to determine this effect, 10% MeOH was added to the samples. No significant variations in the analytes responses were observed, hence, the addition of MeOH was rejected in the sample analysis.

After the rest of the conditions were fixed, different extraction times were studied in order to find the best compromise between sample preparation time and extraction efficiency. Results showed that the equilibrium was reached between 4 and 5 h for most compounds (4 h for the polycyclic musks and 5 h for the nitro musks). Consequently, as a compromise 4 h extraction time was selected for the musks determination.

Table 2
Optimised conditions for the stir bar extraction and thermal desorption.

Stir bar extraction	
Sample volume	100 mL
Stirring speed	900 rpm
Temperature	25 °C
% NaCl	0
Time	4 h
Use of organic modifier	No
pH	7
Stir bar thermal desorption	
Temperature	300 °C
Time	15 min
Flow	100 mL min ⁻¹
Trap temperature	0 °C
Split	Splitless

The final conditions for the stir bar extraction of the synthetic musks in the samples are summarised in Table 2.

3.1.2. Stir bar thermal desorption

Polycyclic musks are semi-volatile compounds with relatively high boiling points (between 286 and 392 °C). A previous study showed that relatively high temperatures (320 °C), longer times of desorption (15 min) and high helium flows (100 mL min⁻¹) were needed for the quantitative recovery of these analytes in Tenax TA [23]. PDMS is a weaker sorbent than Tenax TA and desorption temperatures up to 300 °C and desorption flows up to 100 mL min⁻¹ are recommended by the manufacturer in order to avoid the degradation of the stir bar coating. Hence, in this study different desorption temperatures (from 275 to 300 °C), times (from 5 to 20 min) and flows (from 30 to 100 mL min⁻¹) were tested for recovery of analytes from the PDMS stir bar. Carry-over of all analytes was under 1% when applying the maximal recommended desorption temperature (300 °C) and flow rate (100 mL min⁻¹) for 15 min. Higher desorption times did not show significant improvement in the recoveries. Once desorbed from the stir bar, analytes were focused in a Tenax TA cryogenic trap at 0 °C. The optimised stir bar thermal desorption conditions are summarised in Table 2. The conditions of the trap desorption step were optimised in a previous work [23].

3.1.3. Control of blanks

The extensive use of synthetic musks as fragrances in a wide range of consumer products means a high risk of contamination. Therefore, special precautions are required through the whole analytical procedure. The SBSE procedure developed in this study reduces the potential contamination risk because the aqueous sample is directly extracted by the stir bar, which is then analysed by thermal desorption. Even so, further precautions were taken. In this respect, all the glassware used for the sampling and the extraction step was cleaned overnight with chromic mixture and then rinsed five times with Milli-Q water and three times with HPLC grade isopropanol. Furthermore, musk-free gloves were used and the samples were prepared in a fume cupboard. In addition, the thermal desorption tubes were conditioned at 300 °C for 10 min and then stored in sealable glass jars under nitrogen atmosphere, as described above (Section 2.4). Despite these precautions, signals of HHCB corresponding to 0.010 ± 0.007 ng ($n=5$) were found in blanks of 100 mL of pure Milli-Q water.

It should be also taken into account that the thermal desorption of semi-volatile compounds can cause memory effects in the instrumentation, either in the cryogenic trap (due to the partial desorption of the analytes) or in the transfer lines (whose temperature can only be raised to 200 °C and may cause the accumulation of the less volatile compounds) [23,33]. To avoid

Table 3
Method parameters in Milli-Q water: experimental recovery, repeatability, reproducibility, and method detection and quantification limits expressed in ng L⁻¹.

No.	Musk	Recovery (%; n = 3)		Repeatability (%RSD; n = 5)		Reproducibility (%RSD; n = 5)		MDL (ng L ⁻¹)	MQL (ng L ⁻¹)
		5 ng L ⁻¹	100 ng L ⁻¹	5 ng L ⁻¹	100 ng L ⁻¹	5 ng L ⁻¹	100 ng L ⁻¹		
1	DPMI	85.3	84.7	10.1	3.6	14.8	5.9	0.10	0.30
2	ADBI	93.0	94.1	9.6	4.1	11.6	6.9	0.06	0.20
3	AHMI	94.6	94.3	6.5	4.2	6.1	5.7	0.02	0.10
4	ATII	91.7	90.5	9.5	3.7	12.3	4.8	0.05	0.15
5	HHCB	92.1	93.3	10.3	8.2	12.1	6.8	0.30	1.0
6	AHTN	91.2	92.4	7.9	4.2	8.1	4.7	0.03	0.10
7	MX	85.4	87.3	9.8	3.3	10.1	3.4	0.05	0.15
8	MM	88.4	90.8	4.4	3.2	4.3	3.9	0.06	0.20
9	MK	81.6	81.7	6.3	2.2	6.5	2.3	0.05	0.15

these memory effects, in this study the influent and effluent samples from the WWTPs were diluted before the extraction (5 mL of sample diluted to 100 mL with Milli-Q water) and the maximal calibration level was fixed at 20 ng of each compound per sample.

3.2. Method validation

The main method parameters for the optimised SBSE method were tested by spiking different amounts of the standards (from 0.001 to 200 ng L⁻¹) in 100 mL of Milli-Q water and in each kind of sample. Linearity was good for all the target musks with coefficient of determination (r^2) values higher than 0.999.

Table 3 shows the main method parameters in 100 mL of Milli-Q water. Experimental recoveries of the synthetic musks were analysed at low and midpoint calibration levels (5 ng L⁻¹ and 100 ng L⁻¹, respectively, n = 3). To calculate recoveries the response obtained by the SBSE method was compared with the response obtained by spiking the same amount of standard in a tube filled with thermally cleaned deactivated glass wool. Recoveries were similar for both calibration levels ranging between the 82% and the 95%. As expected, the lower recoveries corresponded to the musks with lower K_{OW} such as DPMI, and the three nitromusks.

The limits of detection (MDL) were calculated as the concentration corresponding to three times the noise signal of the target ion of each compound except for HHCB which was present in the blanks of the Milli-Q water. For HHCB the MDL was determined as the average of the blank signal of the target ion plus three times the standard deviation of the signal (n = 5). The MDLs ranged from 0.02 ng L⁻¹ for AHMI to 0.3 ng L⁻¹ for HHCB (see Table 3). The limit of quantification (MQL), which was fixed as the lowest calibration level of each compound, ranged from 0.1 ng L⁻¹ for AHMI and AHTN to 1 ng L⁻¹ for HHCB. It is worth mentioning that the MDLs and MQLs for HHCB, AHTN and MK obtained in a recent study by SBSE followed by liquid desorption [31] ranged from 12 to 19 ng L⁻¹ and from 41 to 62 ng L⁻¹, respectively, which are higher than those found in the present study.

Repeatability and reproducibility between days were also measured at two calibration levels, 5 ng L⁻¹ and 100 ng L⁻¹, respectively. %RSDs for the tests in the same day ranged from 4.4% and 10.3% for the low calibration level and from 2.2% to 8.2% for the midpoint calibration level. The reproducibility was tested by analysing five replicates of each calibration point on different days and ranged from 4.3% to 14.8% for the 5 ng L⁻¹ level to 2.3% to 6.9% for the 100 ng L⁻¹ level.

The method was also validated in the real samples. As mentioned before, in order to avoid matrix effects and to not overload the TD-GC-MS system, the influent and effluent samples were diluted with Milli-Q water (5 mL of sample to 100 mL). The RO effluent samples and the river samples were not diluted because of the lower expected concentrations of the musks. Taking into account these precautions, recoveries of the synthetic musks in the real

samples were similar to those obtained in Milli-Q water at low and midpoint calibration levels (5 ng L⁻¹ and 100 ng L⁻¹, respectively), therefore no matrix effects were observed and quantification was performed by external calibration spiking the standards in Milli-Q water. Similarly, repeatability and reproducibility presented similar results in the four different samples (%RSD between 2.2% and 10.4%, n = 3).

3.3. Analysis of real water samples

The developed SBSE-GC-MS method was used to determine the presence of nine synthetic musks in four different kind of aqueous matrices (urban WWTP effluent and influent, effluent of a RO treatment plant and river water). Three different samples of each matrix were analysed by triplicate. Fig. 2 shows examples of SIM chromatograms from two real samples: the chromatogram of an urban WWTP effluent (diluted in a factor of 1–20, Fig. 2A) and of a river Ebro sample (Fig. 2B).

Table 4 shows the concentration of the synthetic musks detected in the samples. HHCB was the most abundant compound in all the water matrices, with values up to 2069 ng L⁻¹ and 1432 ng L⁻¹ in the influent and the effluent of urban WWTP, respectively. DPMI, AHMI and AHTN were also detected in all the matrices with values up to 94 ng L⁻¹, 26 ng L⁻¹ and 88 ng L⁻¹, respectively. However, ADBI was not detected in the river samples and ATII could only be quantified in some of the influent samples.

Regarding the nitromusks, MM was not found in any sample, which agrees with the prohibition of their use in cosmetics in European countries [34]. However, MX and MK were detected in the influents and effluents of the urban WWTP as well as in some river samples with values up to 126 and 53 ng L⁻¹, respectively. This fact demonstrates that, even though use of nitromusks is prohibited in cosmetics, they are still present in other consumer products and therefore they can be found in wastewaters and environmental matrices.

Finally, it is worth mentioning that the low levels of the compounds detected in the analysed effluents of the RO treatment plant (HHCB up to 1.45 ng L⁻¹) indicate that this kind of plants can

Table 4
Concentrations of the synthetic musks in real samples, expressed in ng L⁻¹ (n = 3).

Musk	Urban WWTP influent	Urban WWTP effluent	Effluent RO	River
DPMI	15.7–87.7	29.8–43.3	<MQL–0.67	0.49–1.72
ADBI	3.6–35.4	<MQL–4.56	n.d.–0.24	<MQL
AHMI	<MQL–25.6	<MQL–4.15	<MQL–0.17	n.d.–0.27
ATII	n.d.–8.1	n.d.	n.d.	n.d.
HHCB	476–2069	233–1432	n.d.–1.45	1.40–26.2
AHTN	17.7–78.7	25.4–93.6	n.d.–0.35	0.34–0.37
MX	22.0–29.1	13.1–126	n.d.–0.3	n.d.–0.55
MM	n.d.	n.d.	n.d.	n.d.
MK	<MQL–20.3	29.2–53.5	n.d.	n.d.–0.80

n.d., values not detected. <MQL, values under the method quantification limit of each sample.

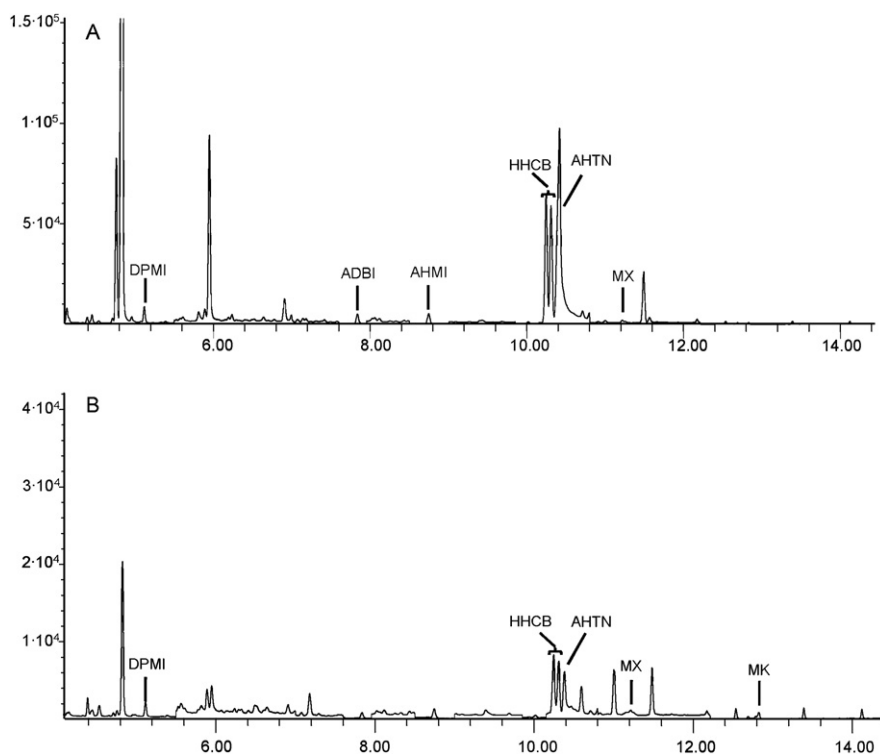


Fig. 2. Chromatograms of two real samples: the influent of a urban WWTP diluted in a factor of 1–20 (A) and the Ebro river (B).

efficiently remove semi-volatile apolar compounds from complex aqueous matrices.

4. Conclusions

A method based on SBSE coupled with a conventional TD–GC–MS system was successfully developed for determining 9 synthetic musks in water samples. The developed SBSE method can efficiently trap and desorb these apolar and semivolatile musks, providing limits of quantification at low ng L^{-1} levels. Furthermore, the limited manipulation of the sample required in this method implies a significant decrease of the risk of external contamination of the samples. The method was applied in real water matrices, such as wastewater and river water. The most abundant musk was HHCB, with also considerable amounts of AHTN, DPME and AHMI. Although nitromusks are prohibited in cosmetics, musk moskene and musk ketone were found both in the WWTP and in the river samples.

Acknowledgments

The authors wish to acknowledge the financial support provided to this study by the Spanish Ministry of Science and Innovation through projects CTM-2008-06847-C02-01 and the Department of Innovation, Universities and Enterprises of the Generalitat de Catalunya through project 2009 SGR 223.

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