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Fully automated ionic liquid-based headspace single drop microextraction coupled to GC–MS/MS to determine musk fragrances in environmental water samples

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ABSTRACT

A fully automated ionic liquid-based headspace single drop microextraction (IL-HS-SDME) procedure has been developed for the first time to preconcentrate trace amounts of ten musk fragrances extensively used in personal care products (six polycyclic musks, three nitro musks and one polycyclic musk degradation product) from wastewater samples prior to analysis by gas chromatography and ion trap tandem mass spectrometry (GC–IT-MS/MS). Due to the low volatility of the ILs, a large internal diameter liner (3.4 mm i.d.) was used to improve the ILs evaporation. Furthermore, a piece of glass wool was introduced into the liner to avoid the entrance of the ILs in the GC column and a guard column was used to prevent analytical column damages. The main factors influencing the IL-HS-SDME were optimized. For all species, the highest enrichments factors were achieved using 1 μL of 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]) ionic liquid exposed in the headspace of 10 mL water samples containing 300 g L^{-1} of NaCl and stirred at 750 rpm and 60 °C for 45 min. All compounds were determined by direct injection GC–IT-MS/MS with a chromatographic time of 19 min. Method detection limits were found in the low ng mL^{-1} range between 0.010 ng mL^{-1} and 0.030 ng mL^{-1} depending on the target analytes. Also, under optimized conditions, the method gave good levels of intra-day and inter-day repeatabilities in wastewater samples with relative standard deviations varying between 3% and 6% and 5% and 11%, respectively ($n=3$, 1 ng mL^{-1}). The applicability of the method was tested with different wastewater samples from influent and effluent urban wastewater treatment plants (WWTPs) and one potable treatment plant (PTP). The analysis of influent urban wastewater revealed the presence of galaxolide and tonalide at concentrations of between 2.10 ng mL^{-1} and 0.29 ng mL^{-1} and 0.32 ng mL^{-1} and < MQL (Method Quantification Limit), respectively; while the remaining polycyclic musks concentrations were below the method quantification limits and two of the nitro musks (musk xylene and musk moskene) were not detected. The analysis of effluent urban wastewater showed a decrease in galaxolide and tonalide concentrations while the other target analytes were not detected. In waters from PTP only galaxolide was found at a concentration higher than MQL.

1. Introduction

Personal care products (PCPs) include a broad range of compounds widely used as additives in cosmetics, flavourings, body oils, soaps, foods and drinks: in short, in a broad range of daily products. They are included in the so-called emerging organic contaminants, which have been of increasing interest to scientists in recent years [1–8].

The musk fragrances are a family of cyclic PCPs which include polycyclic musks, nitro musks and macrocyclic musks. Discussions on the toxicology of nitro musks emerged very early on because of the presence of a nitro-aromatic compound in their structure, and it has been demonstrated, that these compounds can be transformed in both wastewater treatment and vertebrate physiology into aniline transformation products [9,10]. These transformation products can be even more problematic than the parent compounds and this has led to a significant decrease in the use of these compounds and an increase in the production of polycyclic and macrocyclic musks. Nowadays polycyclic musks have a greater presence in environmental matrices than do nitro musks and two of them, galaxolide and tonalide, are included in the US Environmental Protection Agency's (EPA) High Production Volume (HPV) list [11]. In contrast, macrocyclic musks are not as widely used as polycyclic musks because of they are more expensive to

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synthesize, although they are becoming more readily available [2]. Macrocyclic musks seem to have a more intense smell and so less mass is needed to gain the same performance in perfumery. Also, these compounds seem to be more easily degradable in the environment [4,12].

Several analytical methods have been developed for identifying and quantifying of musk compounds in a variety of environmental sample. Available methods are based on gas chromatography (GC) using electron capture detection [13], or GC coupled to mass spectrometry (MS), in either the electron ionization mode [14,15] or in the negative chemical ionization mode [16], and tandem MS [16].

Due to the low concentrations at which musk fragrances are found in environmental water samples, some preconcentration techniques such as liquid–liquid extraction [17], solid phase extraction [18–20] and semipermeable membrane devices [21] have been reported. In any case, any approaches based on liquid–liquid extraction and solid-phase extraction involves the use of organic solvents, which constitutes a pollution problem in itself. To solve this, new microextraction techniques have recently been developed to reduce or eliminate the use of organic solvents during the preconcentration steps and to obtain more environmentally friendly analytical methods [22,23]. Dispersive liquid–liquid extraction (DLLME) [24–26], ultrasound-assisted emulsification–microextraction (USAEME) [27], solid phase microextraction (SPME) [28], single drop microextraction (SDME) [29], microextraction by packed sorbents (MEPs) [15] and hollow fibre membrane solid phase microextraction (HFM-SPME) [30], are only a few examples. However, although fully automated SDME have been used previously for the determination of alkaloids with micellar electrokinetics chromatography [31] or for the determination of phenols with capillary electrophoresis [32], not reports were found with fully automated SDME applied to the determination of musk fragrances.

The main shortcoming of SDME is the instability of the drop when an organic solvent is used as extractant. This limits the usable volume of the extracting medium and directly affects the precision and also the sensitivity of the determinations. This limitation is more marked when headspace single-drop microextraction (HS-SDME) is performed at high temperature because of the evaporation of the organic solvent during the extraction [29,33]. To solve the problem of drop volume repeatability, ionic liquids (ILs) have been proposed as an alternative to organic solvents because their low vapour pressure and high viscosity, which allows the use of larger and more reproducible extracting volumes [34,35].

Ionic liquids, which are ionic media resulting from the combination of organic cations and various anions, are gaining an important recognition as novel solvents in chemistry due to some unique properties, such as dual natural polarity, good thermal stability even at high temperatures and miscibility with water and organic solvents. Additionally, they are easily synthesized and commercially available [36]. These characteristics have led to an extensive range of applications in analytical chemistry as recently reviewed [37–39], which supports their consideration as very potential extractants for liquid phase microextraction (LPME). However, when ILs are employed as extractants in SDME, liquid chromatography [35,40–42] is preferred to GC as separation technique since the low volatility of the ILs. Thus, to the best of our knowledge, the combination of IL-SDME and GC has been described before with some modifications in the injector port [43], modifying the liner [44] and using thermal desorption tubs [45] but not reports were found by direct injection in the GC injector port.

The aim of this study is to develop for the first time a sensitive, environmental friendly and fully automated method to determine ten synthetic musks (polycyclic and nitro musks) in wastewater samples using ionic liquid-based headspace SDME followed by GC–IT-MS/MS.

2. Experimental

2.1. Chemical standards

The six polycyclic musks were supplied by Promochem Iberia (Barcelona, Spain) and were the following: 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5*H*)-indanone (DPMI, cashmeran), 4-acetyl-1,1dimethyl-6-*tert*-butyllindane (ADBI, celestolide), 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI, phantolide), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII, traseolide), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(*g*)-2-benzopyran (HHCB, galaxolide), 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN, tonalide). International Flavors & Fragances Inc. (Barcelona, Spain) provided 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[*g*]-2-benzopyran-1-one (HHCB-lactone, galaxolidone). The nitro musk fragrances 2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene (MX, musk xylene) and 1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM, musk moskene) were purchased as 100 $\mu g\,mL^{-1}$ solutions in acetonitrile from Sigma-Aldrich (Steinheim, Germany) and Riedel de Haën (Seelze, Germany), respectively. The standard 4-aceto-3,5-dimethyl-2,6-dinitro-tertbutylbenzene (MK, musk ketone) was provided by Fluka (Buchs, Switzerland) and d15-Musk xylene (internal standard) was supplied as a 100 $\mu g\,mL^{-1}$ solution in acetone by Symta (Madrid, Spain). Table 1 shows the main characteristics (formula name, boiling point, vapour pressure and molecular structure) of the target compounds.

Individual standard solutions of the synthetic musks were prepared in acetone at concentrations of 4000 $\mu g\,mL^{-1}$ for polycyclic musks and 1000 $\mu g\,mL^{-1}$ for musk ketone and HHCB-lactone. A standard mixture solution of 100 $\mu g\,mL^{-1}$ was prepared in methanol. MX and MM standards were supplied directly at a concentration of 100 $\mu g\,mL^{-1}$ and used as received. Acetone and metanol were GC grade with purity $>99.9\%$ (SDS, Peypin, France).

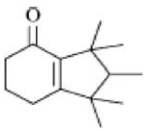
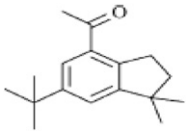
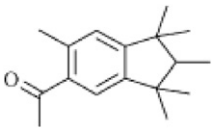
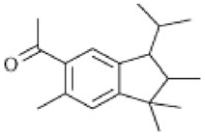
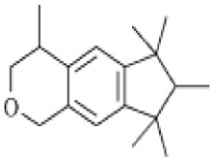
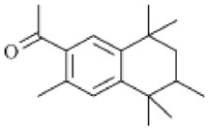
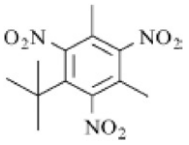
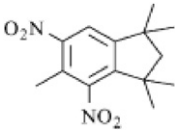
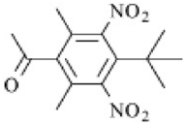
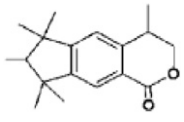
The extraction solvents, toluene and *n*-heptane (both with $>99.9\%$ purity) were purchased from SDS and ionic liquids, 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]) and 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]) with purities of 99% and 98%, respectively were provided by ACROS Organics (Geel, Belgium).

Sodium chloride (ACS reagent $\geq 99\%$) was supplied by Sigma-Aldrich. Ultrapure water was obtained using a purelab ultra purification system (Veolia Water, Barcelona, Spain). Helium gas with a purity of 99.999% was used for the chromatographic analysis (Carburos Metálicos, Tarragona, Spain).

2.2. Instrumentation

The GC–IT-MS/MS analyses were performed using a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA, USA) connected to a Varian 4000 ion trap mass detector. The GC was equipped with a 1079 programmable vaporizing temperature injector and a 3.4 mm i.d. insert liner (Varian) with a piece of glass wool. A fused silica capillary column (3 m × 0.25 mm i.d.) from Micron Phenomenex (Torrance, California, USA) was used as a guard column connected to a ZB-50 analytical column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from Micron Phenomenex. Helium was used as a carrier and collision gas at a flow rate of 1 mL min^{-1}. Varian MS Workstation software v.6.9 was used for instrument control and data processing.

Table 1
Main characteristics of the target compounds.

Compound	Formula name	Boiling point (°C)	Vapor pressure (mmHg)	Molecular structure
Cashmeran, DPMI	6,7-dihydro-1,1,2,3,3-pentamethyl-4(5*H*)-Indanone	286.1	2.69×10^{-3}	
Celestolide, ADBI	4-acetyl-1,1dimethyl-6-*tert*-butyllindane	309	6.5×10^{-4}	
Phantolide, AHMI	6-acetyl-1,1,2,3,3,5 hexamethylindane	336.6	1.11×10^{-4}	
Traseolide, ATII	5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane	350	4.56×10^{-5}	
Galaxolide, HHCB	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(*g*)-2-benzopyran	326	4.14×10^{-4}	
Tonalide, AHTN	7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene	356.8	2.86×10^{-5}	
Musk xylene, MX	2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene	392.3	5.23×10^{-6}	
Musk Moskene, MM	1,1,3,3,5-pentamethyl-4,6-dinitroindane	351.1	8.49×10^{-5}	
Musk ketone, MK	4-aceto-3,5-dimethyl-2,6-dinitro-*tert*-butylbenzene	369	1.22×10^{-5}	
HHCB-Lactone	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[*g*]-2-benzopyran-1-one	*	*	

* Information not found at the bibliography.

Table 2
Retention times and MS conditions.

Compound	Retention time (min)	Parent ion (m/z)	CID Amplitude (V)	CID Storage level (m/z)	Products ions[a] (m/z)	m/z range	Scan time (s/scans)
DPMI	5.39	191	0.82	84.1	**135**, 107, 173	94–201	1.08
ADBI	7.63	229	0.92	100	**173**, 187, 131	110–239	1.01
AHMI	8.48	229	0.92	100	145,131, **187**	110–239	1.01
ATII	9.64	215	0.88	94.7	131,171, **173**	104–225	1.01
HHCB [b]	9.87	243	0.96	122	171, **213**	132–253	0.53
AHTN [b]	10.02	243	0.96	103	159, **145**, 187	113–253	0.53
MX [c]	11.10	282	1.08	124.2	**265,** 281, 266	134–292	0.59
MM [c]	11.18	263	1.02	115.9	**211**, 187, 201	125–273	0.59
MK	13.34	279	1.07	122.9	**191**, 247, 280	132–289	1.05
HHCB-Lactone	14.16	257	1.00	113.2	201, 183, **239**	123–267	1.03
d15-MX (IS)[c]	10.69	294	1.11	129.5	170, **276**, 295	139–304	1.04

[a] Quantification ions (m/z) are shown in bold type.
[b,c] Compounds were separated using a multiple reaction monitoring.

A CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 10 μL, fixed needle, 26 gauge bevel tip syringe (Agilent Technologies, San Jose, CA,USA) and a single Magnet Mixer and controlled by the Cyclo Composer MacroEditor 1.4 Software was used for the fully automated IL-HS-SDME. Due to the high viscosity of the ILs, to make easy take 1 μL of ILs, the fill and ejection speed of the syringe during all the HS-SDME procedure was 1 μL s^{-1}.

2.3. Sampling

All samples were collected from treatments plants located in Catalonia (NE Spain). The urban WWTPs (A and B) are located in two cities with populations of about 120,000 inhabitants and the potable water plant (C) is situated near the Ebro River. For each sample 200 mL was collected in a glass bottle, filtered through a 0.45 μm nylon filter (Scharlab, Barcelona, Spain) and stored at 4 °C until analysis.

2.4. Control of blanks

The extensive use of synthetic musks as fragrances in a wide range of consumer products means there is a high risk of sample becoming contaminated. Therefore, special precautions were required through the whole analytical procedure. Even so, further precautions were taken in this regard. All the glassware used for the sampling and the extraction steps and the stir bars used for stirring the solution during single drop microextraction were cleaned overnight with chromic mixture and then rinsed five times with ultrapure water and five times with HPLC grade isopropanol. Furthermore, musk-free gloves were used and the samples were prepared in a fume cupboard. In the same way, the piece of glass wool which was placed inside the GC liner was removed each 10 analysis and then the liner was cleaned with acetone and methanol to eliminate ionic liquid residues and to prevent blank signals.

2.5. IL-HS-SDME and GC–IT-MS/MS analysis

The fully automated headspace single drop microextraction was optimized to work with the ionic liquid [OMIM][PF_6]. The general HS-SDME procedure was as follows: 10 mL of 1:2 diluted sample or standard solution containing sodium chloride (at a final concentration of 300 g L^{-1}) was placed in a 20 mL glass vial which was tightly sealed with a teflon septum. When the temperature of the Magnet Mixer reached 60 °C, the vial was automatically transported there and stabilized for 1 min. Later on the GC syringe, previously filled with 1 μL of [OMIM][PF_6], was inserted in the vial through the septum until its needle tip was located about 1 cm above the surface of the stirred solution. The plunger was depressed and a microdrop of the acceptor phase was exposed on the headspace above the aqueous solution at 60 °C for 45 min. After the extraction, the drop was retracted and injected into the GC. Both procedures, SDME extraction and GC injection, were performed by the CombiPal autosampler.

For the chromatographic analysis, the injector was operated in splitless mode at 280 °C. The oven temperature program was as follows: initial temperature 100 °C, 30 °C min^{-1} to 170 °C, 5 °C min^{-1} to 210 °C then 20 °C min^{-1} to 290 °C and held for 4 min. All the compounds were separated within 19 min. The transfer line, manifold and trap temperatures were 280 °C, 50 °C, and 200 °C, respectively. A filament-multiplier delay of 3 min was established in order to increase filament's service life. The analytes were ionized by EI. The EI-MS/MS process was carried out by CID using a resonant waveform type. Table 2 summarizes the retention time and the optimal MS parameters for each compound.

3. Results and discussion

3.1. GC–IT-MS/MS

A mixed solution of 10 μg mL^{-1} of 10 musk fragrances and 1 μg mL^{-1} of d15-musk xylene as internal standard was prepared in methanol and 1 μL of this solution was directly injected into the GC–MS, using electron ionization fragmentation in full scan mode. All the compounds were identified by their molecular ion and afterwards the chromatographic separation was optimized by testing several oven temperature programs. All compounds were separated in just 19 min using the chromatographic conditions described in Section 2.5. In order to achieve maximum sensitivity/selectivity of the compounds, the MS/MS method was carried out by selecting appropriate precursor/product ions and then optimizing the IT-MS/MS parameters. Table 2 summarizes the parent ion selected for each compound. All the parent ions were submitted to a CID with a resonant excitation waveform and the isolation window was 3 m/z for all of the compounds. The EI-MS/MS fragmentations were optimized for each compound by selecting an amplitude excitation voltage that gave the maximum abundance of one of the product ions (100%) and a relative abundance of the parent ion between 10% and 20%. The range of the CID amplitude voltage tested for each compound was between 0 V and 1.11 V. Table 2 also summarizes, for each compound, the optimum amplitude excitation voltage, the CID storage level, the product ions (quantifiers and qualifiers), the m/z range of ions analyzed by

EI-MS/MS, and the scan time. Each compound was acquired separately in one segment, except ATII, HHCB and AHTN (segment 4), and d15-MX, MX and MM, (segment 5); because of this, the scan time of these compounds was shorter than the others.

3.2. HS-SDME

The performance of HS-SDME is influenced by several parameters such as extraction solvent, drop volume, stirring rate, extraction temperature, salt concentration, sample volume and extraction time. Of these, the first four variables are strongly correlated and affect drop stability. Thus, they were optimized first.

The HS-SDME was optimized using standard solutions containing all the analytes at a concentration of 1 ng mL^{-1} in ultrapure water ($n=3$). The best extraction conditions were those that provided the highest analyte signal.

3.2.1. Drop stability

Extraction solvent, drop volume, stirring rate and extraction temperature were the four variables studied to obtain the best drop stability.

Four extractants were provided to optimize the single drop microextraction; two conventional organic solvents (toluene and *n*-heptane) and two ionic liquids ([OMIM][PF_6] and [HMIM][PF_6]). The organic solvents were selected on the basis of two requirements: that they should have low volatility in order to be stable during the extraction period; and that they should have affinity with the analyte so as to facilitate the extraction. The ionic liquids were selected on the basis of their extraction capacity and their chemical properties such as hydrophobicity and viscosity. ILs that had high miscibility with water were not used because they can introduce moisture into the GC system and increase the drop volume, thus causing excessive humidification of the drop. Furthermore, ILs with a low viscosity were excluded since they would be easily removed from the glass wool piece of the liner damaging the GC column. To work with ionic liquids some preventive steps needed to be taken regarding the GC. A liner with a 3.4 mm i.d. was chosen to improve the ILs evaporation into which a piece of glass wool was introduced to avoid the entrance of the ILs in the GC column and a guard column was installed before the analytical column to ensure column protection.

In the HS-SDME process, a large microdrop volume may affect both the precision for sampling and the stability of the microdop suspended in the needle. To optimize the drop volume a test was carried out that consisted of exposing 1 μL, 2 μL or 3 μL drops of the extractants in the headspace above 10 mL of water at different agitation intensities between 0 rpm and 750 rpm and at a room temperature for 15 min. The results showed that drops stability was independent of agitation intensity for both kinds of solvents. For organic solvents drops were stable under all the conditions tested while the ionic liquid drop was only stable up to 2 μL. Nevertheless, it was decided to handle only 1 μL of ionic liquid because a non-modified GC injector port was used. Recently some scientists have used laboratory modified injectors to improve the evaporation of the ionic liquid and thus be able to inject larger volumes [34,46]. However, our aim was to use non-modified equipment to make compatible the use of the CombiPal autosampler with the GC to be able to do all the extraction and injection steps automatically.

The drop stability was then tested at temperatures between 25 °C and 80 °C, with the other variables fixed at 15 min extraction time, 750 rpm and 3 μL and 1 μL drop sizes for organic solvents and ionic liquids, respectively. The results showed that the drop volume of the organic solvents decreased when the extraction temperature was higher than 30 °C. On the basis of this, 30 °C was chosen as the optimum extraction temperature for organic solvents. However, when the ionic liquids were used musks peak areas increased at temperatures higher than 45 °C because an increase in temperature improves the evaporation of the target compounds from the sample to the headspace. Fig. 1 therefore shows that the polycyclic musk extraction for [OMIM][PF_6] ionic liquid were more sensitive to extraction temperature than nitro musks and HHCB-lactone because of the polycyclic musk's higher slopes. Although higher peaks areas were obtained at 80 °C for all the analytes, 60 °C was selected as the optimum extraction temperature to avoid introducing trace levels of water into the MS detector.

Under the conditions selected above, peaks areas were compared to select the best extractant. The results shown that [OMIM][PF_6] and toluene gave the highest peak areas. However, [OMIM][PF_6] was chosen as the extracting solvent because its low volatility compared with toluene permitted the extraction temperature to be increased from 30 °C to 60 °C, thus stimulating the presence of the target analytes in the vapour phase and increasing extraction efficiency. At the same time, intra-day repeatability values for toluene and [OMIM][PF_6] were calculated and RSD (1 μg mL^{-1}, $n=3$) decreased significantly from 7% and 24% to 4% and 9% respectively, which confirmed that the ionic liquids were more stable than the organic solvents. Thus the optimal conditions selected were 1 μL of [OMIM][PF_6] as the extraction solvent, 60 °C and 750 rpm.

3.2.2. Salt concentration

To study the influence of adding salt on the efficiency of HS-SDME, the ionic strength of the standard solutions was modified by adding sodium chloride in the range 0 g L^{-1} to 360 g L^{-1}. The other experimental conditions were the same as before: 10 mL volume of the standard solution and the HS-SDME was tested at 60 °C, during 15 min, 1 μL [OMIM][PF_6] and at a 750 rpm stirring rate. Plots of the peak area versus NaCl concentration are shown in Fig. 2. 300 g L^{-1} was selected as the optimal salt concentration because maximal peak areas were obtained for most of the analytes. Celestolide, HHCB-lactone, musk ketone and cashmeran showed maximal peak areas at 200 g L^{-1} NaCl but only a slight decrease in those analytical signals was observed at 300 g L^{-1} NaCl.

It is clear that the addition of NaCl increased the ionic strength and thus promotes the transport of the analytes to the headspace and hence to the extracting drop. This tendency can be explained by the engagement of more water molecules in the hydration sphere around the ionic salt. These hydration spheres reduce the concentration of water available to dissolve the analyte molecules [47]. Hence, it is to be expected that this will drive additional analytes into the headspace or gaseous phase and extractant.

3.2.3. Sample volume

Sample volume plays an important role in HS-SDME analysis. According to the Pawliszyn equation [48], which explain the steady-state mass transfer that is established in the HS-SDME analysis, an increase in sample volume and consequently a decrease in headspace volume, enhances the extracted amount of analyte, and thus improves the sensitivity [49,50]. The effect of sample volume on the extracted amount of musks was investigated as follows; a set of experiments were performed using three 20 mL vials each containing a different volume of the aqueous phase, these being 5 mL, 10 mL and 15 mL while the analytes concentration remain constant at 1 ng mL^{-1}. When the sample volume was increased from 5 mL to 10 mL a nearly linear increase in response was observed for all these compounds except for cashmeran, which presented the highest peak area at 5 mL. At higher volumes (15 mL) a slightly decrease in the signal was observed. This can be explained by the fact that the convection is

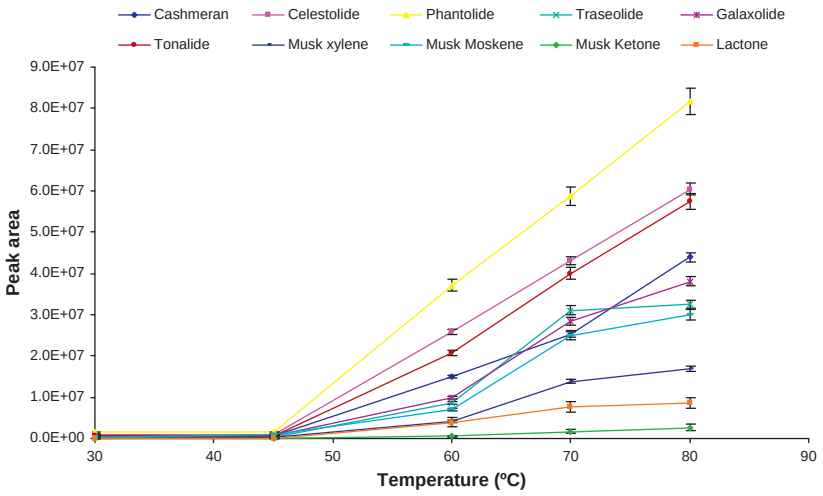


Fig. 1. Effect of temperature on the extraction efficiency of the HS-SDME used to determine musks. Experimental conditions: 1 ng mL^{-1} concentration level, 10 mL standard solution in 20 mL glass vial; 1 μL [OMIM][PF_6]; 750 rpm stirring rate, 15 min sampling time and not salt addition.

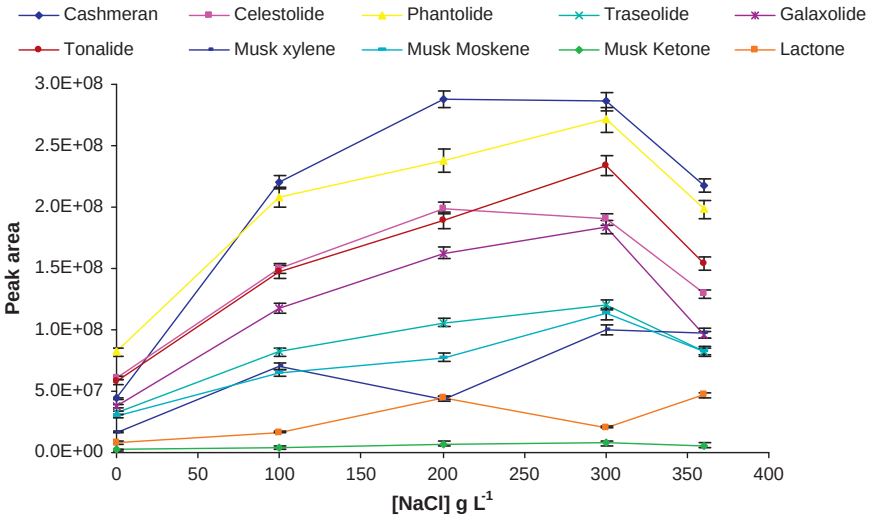


Fig. 2. Effect of salt concentration on the extraction efficiency of the HS-SDME used to determine musks. Experimental conditions: 1 ng mL^{-1} concentration level, 10 mL standard solution in 20 mL glass vial; 1 μL [OMIM][PF_6]; 750 rpm stirring rate; 15 min sampling time and 60 °C temperature.

not as good in the aqueous phase when the solution is stirred at a fixed rate with larger volume which in turn results in less extraction. Therefore, 10 mL was chosen as the optimum sample volume.

3.2.4. Extraction time

In the HS-SDME method, the amount of extracted analyte is expected to increase in line with the amount of time that the stirred sample solution is exposed to the microdrop in the headspace. However, the HS-SDME is not an exhaustive extraction method, so for optimum repeatability of the extraction, it is necessary to choose a time in which equilibrium between the extracting microdrop, the headspace and the sample solution is reached: that is, the equilibrium time.

To test the effect of extraction time on extraction efficiency, we worked at the following different extraction times: 15 min, 30 min, 45 min and 60 min. The other HS-SDME variables were fixed at the values already mentioned above. A progressive increase in peak areas was observed for all the analytes up to an optimum time of 45 min after which there was a decrease when the extraction was extended up to 60 min. The probable reason is that at 45 min the analytes achieved the equilibrium between gas phase and liquid phase while the increasing of extraction time at the given extraction temperature, more water vapour will present in the headspace and the amount of analytes in IL drop decreased due to the distribution of analytes between the IL and the water vapour phase.

To summarize, the optimal conditions for working with HS-SDME were: 1 μL [OMIM][PF_6] used as the extraction solvent suspended in the headspace of a 20 mL vial filled with a 10 mL sample containing 300 g L^{-1} NaCl and stirred at 750 rpm for 45 min at 60 °C.

3.3. Method validation

Before validating the method with a sample from a WWTP, the matrix effect was studied by statistically comparing the slopes of the calibration curves for influent and effluent WWTPs samples with that obtained using ultrapure water. As expected, the matrix effect was observed in both kinds of water, especially influent water. In order to reduce the matrix effect, an internal standard (IS) d15-MX was used but no differences were observed between the external calibration curve and that obtained with IS. Then, the sample dilution was tested and it was found that a 1:2 dilution with ultrapure water eliminated the matrix effect in both matrices. Nevertheless d15-MX was added to the ionic liquid (1 μg mL^{-1}) to

validate the method because its presence reduces the RSD and gives better values of intra-day and inter-day repeatabilities.

The method was then analytically validated with a diluted effluent water sample from WWTP A by establishing the linear range, method detection limits (MDLs), method quantification limits (MQLs), intra-day and inter-day repeatabilities.

Diluted samples from the effluent WWTP A mentioned above were analyzed in triplicate to determine if any analyte was present, and the results revealed peaks of HHCB and AHTN in the chromatogram. The averaged peak area of each detected compound was subtracted from the peak area of each spiked sample.

The linear range of the method was obtained by analysing the WWTP A effluent sample spiked with musks at concentrations of between 0.025 ng mL^{-1} and 10 ng mL^{-1}. The compounds showed a good linear range between 0.050 ng mL^{-1} and 10 ng mL^{-1} (polycyclic musks) or between 0.100 ng mL^{-1} and 10 ng mL^{-1} (nitro musks and HHCB-lactone) with determination coefficients (r^2) higher than 0.994 (Table 3).

The method detection limits (MDLs) for each compound were calculated depending on their presence in the blank analysis. For target compounds without blank signals, the MDLs were calculated as concentrations that give a signal of the quantifier ion three times higher than the noise signal, whereas for target compounds with a blank signal (HHCB and AHTN), the MDLs were estimated as three times the standard deviation of the blank signal of each target compound ($n=3$). In all cases the method quantification limits (MQLs) were fixed at the lowest calibration level. As can be seen in Table 3, the MDLs and MQLs ranged from 0.01 ng mL^{-1} to 0.03 ng mL^{-1} and 0.05 ng mL^{-1} to 0.1 ng mL^{-1}, respectively.

The intra-day and inter-day repeatabilities were determined by spiking three replicates of the effluent WWTP A at 1 ng mL^{-1}. The results obtained (Table 3), expressed as %RSD, were lower than 7% for intra-day repeatability and 12% for inter-day repeatability.

The validation parameters obtained using IL-SDME for HHCB and AHTN were compared with those obtained using other microextraction techniques such as MEPs [15], SPME [28] or DLLME [25] followed by GC–MS. Similar MDLs values for HHCB and AHTN were obtained with IL-SDME (5 mL sample volume), DLLME (5 mL sample volume) and MEPs (800 μL), with values ranging between 24 ng L^{-1} and 53 ng L^{-1} for HHCB and between 21 ng L^{-1} and 49 ng L^{-1} for AHTN. On the other hand, working with SPME significantly lower MDLs, 0.4 ng L^{-1} for HHCB and 0.5 ng L^{-1} for AHTN, were achieved. Apart from that, the automation of the entire IL-SDME avoid all the repeatability problems associated with the SDME microextraction technique with intra-day and inter-day repeatability values slightly better that those obtained with the compared microextraction techniques.

Other parameters related to promoting green chemistry play an important role in this method; for example, it uses ionic liquids whereas the MEPS and MASE use organic solvents. Also the IL-SDME reduces the extractant solvent from millilitres to 1 microliter.

3.4. Method application

The method developed was applied to determine the presence of musk fragrances in different kinds of water: influent and effluent samples collected from urban WWTPs and influent and efffluent samples taken from a PTP over a period of six months (March–August) (Section 2.3).

Table 4 summarizes the results of the average concentrations of the ten musk fragrances found in each type of sample ($n=8$). As expected, the influent musk concentrations were higher than the effluent ones.

An analysis of the results shows that HHCB was the most abundant compound and appeared in all influent water matrices and at the highest concentrations, ranging from 0.29 ng mL^{-1} in the PTP to 2.06 ng mL^{-1} in the WWTP A. AHTN was also present in all the influent samples with a maximum concentration of 0.32 ng mL^{-1} in the WWTP B. The remaining musk fragrances, except MX and MM, were detected in influent samples but the average concentrations were lower than the quantification limit. Fig. 3 shows a chromatogram of a non-spiked influent WWTP B water sample.

In effluent waters only HHCB was detected at values higher than the quantification limit and ranged from 0.09 ng mL^{-1} in the PTP to 0.70 ng mL^{-1} in the WWTP A. The remaining compounds detected in the influent waters were eliminated during the WWTP process, only HHCB-lactone remained as a result of the degradation of the HHCB to HHCB-lactone during the WWTP treatment [51,52].

Previous works [18,53–55] that have focused on the determination of musk fragrances in wastewater samples confirm the findings of the present study, i.e., that the most abundant polycyclic musks are HHCB and AHTN, although other polycyclic musks such as ADI, AHMI or DPMI can also be present in water samples in minor concentrations. A decrease in the concentrations of all polycyclic musks was observed when these results were compared with those obtained in influent and effluent WWTP water samples after the wastewater treatment plant depuration process. Only the HHCB-lactone concentration remains constant. On the other hand, the nitro musks (MX, MM or MK) show important differences in their concentrations depending on the location of the water sample and on if the study was done before they became subject to regulation [18,53–55].

Table 3
Method liner ranges, determination coefficients, MDLs, MQLs, intra-day and inter-day repeatabilities (%RSD, $n=3$, 1 ng mL^{-1}) for effluent WWTP A.

Compound	Linear range* (ng mL^{-1})	Determination coefficient (r^2)	MDLs (ng mL^{-1})	Intra-day repeatability (%RSD)	Inter-day repeatability (%RSD)
DPMI	0.050–10	0.999	0.010	3	7
ADBI	0.050–10	0.994	0.010	2	5
AHMI	0.050–10	0.995	0.010	5	7
ATII	0.050–10	0.997	0.010	3	7
HHCB	0.070–10	0.998	0.024	4	8
AHTN	0.070–10	0.996	0.021	6	7
MX	0.100–10	0.997	0.030	5	9
MM	0.100–10	0.997	0.030	6	9
MK	0.100–10	0.996	0.030	3	9
HHCB-Lactone	0.100–10	0.994	0.030	4	11

* MQLs (ng mL^{-1})=were fixed as the lowest calibration level.

Table 4
Concentrations of the target musks found in the water samples ($n=8$), expressed in ng mL^{-1}.

Compound	WWTP A		WWTP B		PTP C	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
DPMI	< MQL	n.d.- < MQL	< MQL	< MQL	n.d.	n.d.
ADBI	< MQL	n.d.	< MQL	n.d.- < MQL	< MQL	n.d.
AHMI	< MQL	n.d.	< MQL	n.d.- < MQL	< MQL	n.d.
ATII	n.d.	n.d.	n.d.-0.65	n.d.- < MQL	n.d.	n.d.
HHCB	0.33–2.06	0.13–0.70	0.39–1.03	0.10–0.09	0.29	0.09
AHTN	< MQL-0.10	n.d.- < MQL	< MQL-0.32	< MQL-0.14	< MQL	n.d.
MX	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MK	n.d.- < MQL	n.d.	n.d.- < MQL	n.d.	n.d.	n.d.
HHCB-Lactone	< MQL	< MQL	n.d.- < MQL	< MQL	< MQL	n.d.

n.d.; values < MDL.
< MQL: method quantification limit.

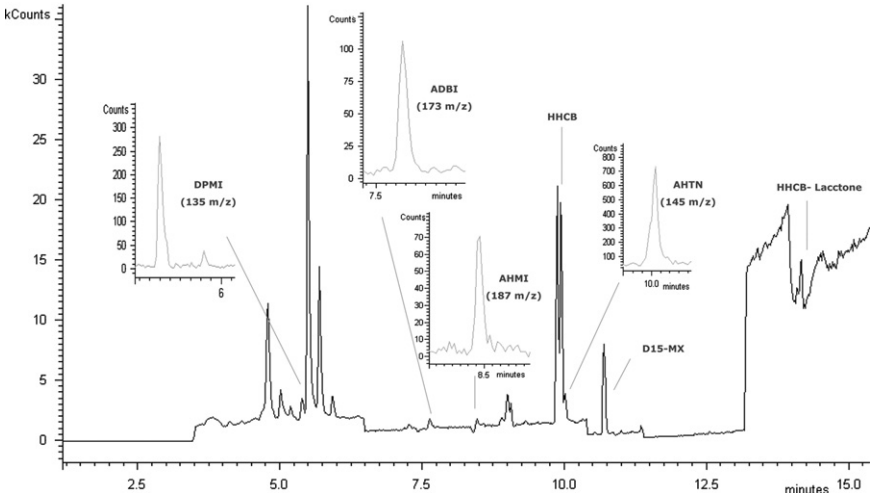


Fig. 3. Chromatogram of a non-spiked influent water sample from WWTP B and analytical signals enlargements.

4. Conclusions

In this study, an automated ionic liquid-based headspace single drop microextraction followed by a GC–IT-MS/MS procedure was developed for determining 10 musk fragrances in water samples.

To adapt the ionic liquid (viscous solvent) to the GC a large internal diameter (3.4 mm) liner was used to improve the ILs evaporation into which a piece of glass wool was introduced to avoid the entrance of the ILs in the GC column and a guard column was used to prevent analytical column damages. The non-modification of the GC injector permitted the development of a completely automated, simple, and environmentally friendly method. Under optimized conditions the method also provide good linearity, acceptable MDLs and MQLs ranging between 0.010 ng mL^{-1} and 0.030 ng mL^{-1}, respectively, and intra-day and inter-day repeatability values below 10% for most of the target musks.

Also the applicability of the method was tested with water samples from influent and effluent WWTPs and a PTP. The most abundant musk fragrances in the samples were HHCB and AHTN.

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