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# Membrane assisted solvent extraction coupled to large volume injection-gas chromatography–mass spectrometry for trace analysis of synthetic musks in environmental water samples

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ABSTRACT

This work describes the optimisation, validation and application of membrane assisted solvent extraction (MASE) together with a large volume injection (LVI) in a programmable temperature vaporisation (PTV) injector coupled to gas chromatography–mass spectrometry (GC–MS) for the quantification of ten synthetic musk fragrances (musks) in surface and wastewater samples. Regarding the MASE, musks were extracted from 150 mL of aqueous samples to 200 μL of *n*-hexane hold in home-made low density polyethylene (LDPE) bags. The extraction took 240 min and the performance of the method made possible the direct analysis of the extracts by LVI-PTV-GC–MS without needing any further treatment and avoiding losses of analytes. During the optimisation of LVI-PTV set-up, the response surfaces of every analyte signal against the cryo-focussing temperature, injection speed and vent time were built. Finally, the figures of merit of the whole procedure allowed the analysis of most of the musks owing to the low method detection limits (between 4 and 25 ng $L^{-1}$) and good precisions (<20%). In fact, this method was successfully applied to the analysis of musks in surface and wastewater samples. Galaxolide and tonalide are the main two synthetic musks observed in most of the analysed environmental water samples.



## 1. Introduction

The effects and consequences of chemical contaminants in the environment and in the human health are matters of high concern. The increase of the human population and anthropogenic activities has multiplied the quantity of residues and waste discharges into water bodies, which can be considered one of the main environmental exposure pathways of organisms to toxic contaminants. In fact, most of the compounds included among the lists of priority contaminants are organic pollutants with a significant threaten to the aquatic ecosystems. Persistent organic pollutants, such as hydrocarbons, organochlorine compounds, organic solvents, pesticides or chlorophenols, are fully characterised and mostly integrated in environmental monitoring programs. However, the presence of new substances in water bodies, such as some pharmaceuticals, hormones and endocrine disrupting compounds (EDCs) has gained the attention of environmental regulators and authority bodies and the term emerging pollutants has been coined [1]. As a consequence, many pharmaceuticals (drugs, antibiotics, etc.) and personal-care products (PCPs) (fragrances, UV-filters, cosmetics, etc.) have been included as candidates for monitoring and regulation (Water Framework Directive in the EU and Environmental Protection Agency in the USA) and the need of reliable analytical methods has been highlighted [2].

PCPs are chemical products used in daily human life (e.g. cosmetics, soaps, detergents, lotions or even food) that are released continuously to the environment through down-the-drain disposal [3]. Several synthetic organic chemicals are often added to these products such as synthetic musk fragrances, antimicrobials, sunscreen agents, insect repellents and parabens [4].

Synthetic musk compounds (musks) are among the most important substances used in the fragrance industry and they are added to a wide variety of consumer products to provide odour-enhancing and blending properties and, thus to mask malodours and deliver consumer-preferred odours [5,6]. Synthetic musks mainly include four categories of compounds: *nitro musks* (musk ambrette (MA), musk ketone (MK), musk moskene (MM) and musk xylene (MX)); *polycyclic musks* (galaxolide (HHCB), tonalide (AHTN), traseolide (ATII), celestolide (ADBI) and phantolide (AHMI) and cashmeran (DPMI)); *macrocyclic musks* (ambrettolide, muscone, ethylene brassilate, globalide and thibetholide) and *alicyclic musks* (romandoline and helvetolide). Currently, the most widely used musks are HHCB, AHTN, MX and MK, which account for 95% of the total market volume for polycyclic musks [7]. At the present, the use of polycyclic musks is under discussion in scientific committees advising the European Commission. Regarding to the nitro musks, their use

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is currently banned in cosmetics except for MK and MX, which concentration in these products is limited to around 1% (CE N° 1223/2009). For this reason, the occurrence of nitro musks in the environment has decreased over the last years [8].

Although most PCPs are present in rather low concentrations in consumer products, the large consumption rates give raise to a chronic contamination of water bodies and unknown environmental fate [8]. One of the examples is found in the wastewater treatment plants (WWTP), where the conventional treatments are inefficient to remove many emerging contaminants, and as a consequence, WWTPs act as a collector and a secondary source of many contaminants [9,10].

One of the major challenges of the analysis of organic emerging pollutants in water samples arises from the low concentration found in the environment. Traditional methods, such as solid phase extraction (SPE) or liquid–liquid extraction (LLE), have been successfully applied to the pre-concentration of synthetic musks from environmental water matrices [11–13]. Recently, solventless approaches such as solid phase microextraction (SPME) and, more recently, stir-bar-sorptive extraction (SBSE) have been successfully applied for the analysis of musk fragrances in environmental water samples [14–18]. Additionally, membrane liquid-phase microextraction is also in vogue due to their rapid and inexpensive extraction approaches [19,20].

Membrane based techniques are simple liquid–liquid extraction between the aqueous sample (donor phase) and a microvolume of acceptor phase, protected by a membrane that avoids the mixture of the two phases and acts as a selective barrier in terms of analyte permeation through the membrane. Broadly speaking, two types of techniques can be distinguished based on the characteristics of the membrane: supported liquid membrane and microporous membrane liquid–liquid extraction (MMLLE), which use porous membranes, and membrane assisted solvent extraction (MASE) that uses non-porous membranes [20,21].

MASE, composed of three-phase aqueous polymeric organic system, allows handling very complex matrices especially for the pre-concentration of trace organic compounds from water matrices [22]. Besides, MASE requires low volumes of organic solvents (400–1000 μL) and medium sample volumes (10–150 mL) for achieving a high sensitivity at the ng $L^{-1}$ level.

Furthermore, since synthetic musks are not thermo-labile compounds, most of the current inlet devices for gas chromatographic analysis, such as split/splitless, on-column or programmed temperature vaporiser (PTV) can be used. Additionally, PTV offers the possibility to perform large volume injection (LVI), improving the method sensitivity. Hence, LVI-PTV has become one of the most preferred injection devices in gas chromatography as it is revealed in many recent research works on organic pollutants of environmental concern [23,24].

The main aim of this work was to develop a novel analytical method approach to monitor ten synthetic musk fragrances in environmental water samples using MASE followed by LVI-PTV-GC–MS analysis. The milestones of this method were the optimisation and validation of the extraction and analysis to fulfil the requirements of water analysis. Finally, the applicability of the optimised methodology to determine these compounds in real environmental samples is also evaluated since their continuous determination is desired in many monitoring programs.

## 2. Experimental work

### 2.1. Cleaning procedure

As musk fragrances are ubiquitous, in order to avoid the contamination of samples and laboratory material, some special cares were taken. First of all, a strict cleaning procedure was followed. No detergent was used during the cleaning steps to avoid possible interferences from the detergent residues. All the laboratory material was washed with abundant pure water (<0.2 μS $cm^{-1}$, Millipore, USA) and then sonicated under clean acetone (Q.P., Panreac Química, Spain) during at least an hour or maintained in a clean acetone bath overnight. Afterwards, the material was rinsed with ultrapure water (<0.057 μS $cm^{-1}$, Milli-Q Model 185, Millipore). Finally, the glass material was dried in an oven at 400 °C for at least 4 h. In addition, the use of perfumes by laboratory personnel was restricted.

### 2.2. Reagents and materials

The six polycyclic musks: 4-acetyl-1,1-dimethyl-6-tert-butylindane (ADBI, celestolide®), 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI, phantolide®), 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN, tonalide®), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII, traseolide®), 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI, cashmeran®) and 1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran (HHCB, galaxolide®) were supplied by LGC Standards GmbH (Germany). The nitro musk fragrances, 1-tert-butyl-2-methoxy-4-methyl-3,5-dinitrobenzene (MA, musk ambrette) and 4-aceto-3,5-dimethyl-2,6-dinitrotert-butylbenzene (MK, musk ketone) were obtained from Dr. Ehrenstorfer GmbH (Germany) and 1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM, musk moskene) and 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (MX, musk xilene) from Fluka (Germany). An overview of chemical properties of these compounds is given in Table 1. The surrogate standards: [$^2H_3$] AHTN and [$^2H_{15}$] MX were purchased from Dr. Ehrenstorfer GmbH (Germany) at 100 mg $L^{-1}$ in isooctane and acetone, respectively.

Individual stock solutions from each solid standard were dissolved to prepare ~1000 μg $g^{-1}$ in 2-propanol (HPLC-grade, 99.8%, LabScan, Ireland) with the exception of musk xylene and musk moskene, which were supplied in stock solutions of 100 μg $g^{-1}$ in acetonitrile. All stock solutions were stored in amber vials at −20 °C.

Mixed fresh stock solutions containing 50 μg $g^{-1}$ of all polycyclic and nitro musks (except MX and MM) were prepared monthly in 2-propanol. Intermediate dilutions at lower concentrations of above mentioned stocks were prepared daily, according to the experimentation.

Sodium chloride (NaCl, Merck, Germany) and methanol (MeOH Anhydrous, HPLC-grade, 99.9%, LabScan, Ireland) were used for matrix modification experiments. Humic acids (technical grade) used to study the matrix effect were obtained from Fluka (Sigma–Aldrich, Germany). The solvents, *n*-hexane and ethyl acetate (HPLC-grade), were supplied by LabScan.

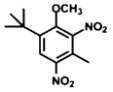
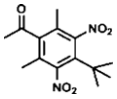
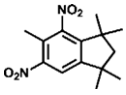
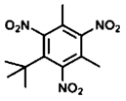
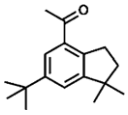
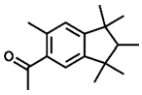
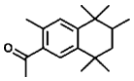
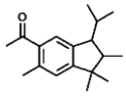
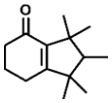
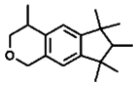
Thin low density polyethylene (LDPE) was obtained from freezing bags for food (with a membrane thickness of 0.02 mm) and thick LDPE from Garciplast (Spain) (membrane thickness of 0.07–0.095 mm). Polyethylene terephthalate (PET) (membrane thickness of 0.05 mm) was purchased from Goodfellow (England).

### 2.3. Sampling procedure

Surface water samples and the influent and effluent of two urban WWTPs (Basque Country, Spain), which collect wastewater from ca. 1 million inhabitants, were analysed in order to test the performance of the method in real environmental waters.

The surface water samples from the estuary of Urdaibai (Bay of Biscay, North of Spain) were collected in May 2011. In the case of WWTPs, 24-h flow proportional composite untreated influent

**Table 1**
Target compounds including chemical structures, CAS number, purity, log $K_{ow}$, vapour pressure and $m/z$ values of fragment ions.

| Compound | Structure | CAS No. | Purity (%) | Log $K_{ow}$ | $P_v$ (Pa) | $m/z$ quantifier and (qualifier) |
|---|---|---|---|---|---|---|
| *Nitro musks* | | | | | | |
| Musk ambrette (MA)[1] |  | 83-66-9 | 99.0 | 3.7 | $3.3 \times 10^{-3}$ | 253(268,254) |
| Musk ketone (MK)[1] |  | 81-14-1 | 98.0 | 4.3 | $4 \times 10^{-5}$ | 279(294,280) |
| Musk mosken (MM)[1] |  | 116-66-5 | 96.0 | 5.8 | $2.3 \times 10^{-4}$ | 263(278,264) |
| Musk xylene (MX)[1] |  | 81-15-2 | 98.0 | 4.8 | $3 \times 10^{-5}$ | 282(297,283) |
| [$^2H_{15}$] Musk xylene (MX) | | | | | | 246(261) |
| *Polycyclic musk* | | | | | | |
| Celestolide (ADBI)[2] |  | 13171-00-1 | 99.8 | 6.6 | $1.92 \times 10^{-2}$ | 229(244,173) |
| Phantolide (AHMI)[2] |  | 15323-35-0 | 93.1 | 6.7 | $1.96 \times 10^{-2}$ | 229(244,187) |
| Tonalide (AHTN)[2] |  | 1506-02-1 | 97.9 | 5.7 | $6.08 \times 10^{-2}$ | 243(258,159) |
| Traseolide (ATII)[1] |  | 68140-48-7 | 83.2 | 6.3 | $9.1 \times 10^{-3}$ | 215(258,173) |
| Cashmeran (DPMI)[2] |  | 33704-61-9 | 89.5 | 4.9 | 5.2 | 191(206,192) |
| Galaxolide (HHCB)[2] |  | 1222-05-5 | 53.5 | 5.9 | $7.3 \times 10^{-2}$ | 243(258,213) |
| [$^2H_3$] Tonalide (AHTN) | | | | | | 294(207) |

[1] Compound corrected with [$^2H_{15}$] Musk Xylene.
[2] Compound corrected with [$^2H_3$] Tonalide.

(upstream) and final treated effluent (downstream) urban wastewater samples were collected at WWTP of Bakio and at WWTP of Galindo in May 2011. Samples were collected in pre-cleaned amber bottles and carried to the laboratory in cooled boxes at 4 °C. After collection, samples were filtered using a 0.45 μm cellulose filters (UK), stored at 4 °C before treatment and analysed within 48 h.

### 2.4. Membrane assisted solvent extraction (MASE)

The extraction procedure was performed through LDPE membrane bags (2.5 cm length and 1 cm i.d. for 200 μL of solvent and 4 cm length and 1 cm i.d for 800 μL of solvent), which were tailor-made using a shrink-wrapping device. After thermally sealing the borders, the overlaying borders were carefully cut in order to minimise the edges where analytes could be absorbed. The home-made membranes were cleaned with *n*-hexane and maintained in clean *n*-hexane before their use in order to minimise the cross-contamination of interfering compounds from the membrane material.

The extraction was carried out using conventional head-space glass vials. LDPE membranes were attached to a metal funnel and fixed with a Teflon ring (Gerstel, Germany). Then, the membranes were filled with 200 μL of *n*-hexane and immersed in the water sample, held by a metal funnel, which was placed in the bottle-neck. Vials were sealed with PTFE septa and aluminium crimp caps.

Extraction vials were stirred using a magnetic 15 position stirring hot-plate from Gerstel at 700 rpm. A water bath was used when extractions were performed at 15 °C and 30 °C. Once the extraction step was accomplished, the extracting phase was transferred to a chromatography vial and weighted. In the case of using higher organic solvent volume, i.e. 800 μL, the extracts were transferred

to a 2 mL amber vial and evaporated to dryness at 11 °C under a low stream of nitrogen in a Turbovap LV Evaporator (Zymark, USA) and the extracts were re-dissolved in 200 μL of *n*-hexane.

### 2.5. GC–MS analysis

The MASE extracts obtained after different optimisation steps were analysed in an Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass spectrometer using an Agilent 7683 autoinjector. 2 μL were injected in splitless mode at 300 °C in a capillary column HP-5MS (30 m × 0.25 mm, 0.25 μm, Agilent) with hydrogen (AD-1020 Hydrogen Generator, Cinel Strumenti Scientifi, Italy) as carrier gas at constant flow (1.3 mL $min^{-1}$). The following oven temperature program was used for the separation of the target analytes: 60 °C (1 min), temperature increase at 30 °C $min^{-1}$ to 200 °C, a second increase of 3 °C $min^{-1}$ up to 240 °C followed by a 30 °C $min^{-1}$ up to 300 °C, where it was finally held for 3 min. The mass spectrometer worked in the electron impact mode with an electron energy of 70 eV. The temperature of the interface between the chromatograph and the detector was kept at 310 °C while the temperature of the ionisation source and quadrupole were maintained at 230 °C and 150 °C, respectively. The measurements were performed both in full scan (50–525 amu) and SIM (selected ion monitoring) modes (see Table 1). The first ion was used as quantifier while the ions in brackets were considered as qualifiers.

### 2.6. LVI-PTV-GC–MS analysis

LVI of the extracts was carried out using a CIS 4 PTV inlet (Gerstel) which consisted of a septumless head and an empty baffled deactivated glass liner cooled with liquid nitrogen. A 45 μL aliquot of sample extract was injected using a 100 μL syringe operated by a multipurpose sampling device (MPS2 autosampler, Gerstel) at 20 °C while the vent valve was opened for 0.5 min at a flow rate of 75 mL $min^{-1}$ and a vent pressure of 5 psi. Then, the vent valve was closed for 1.5 min and the temperature of the PTV injection port was increased at 12 °C $s^{-1}$ to 300 °C and held for 2 min. Finally, the injector was cleaned at a purge flow of 75 mL $min^{-1}$ prior to subsequent injections.

Separation and detection were performed in a 6890N gas chromatograph (Agilent Technologies, USA) equipped with an Agilent 5975N electron impact ionisation mass spectrometer and with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm) from Agilent. The oven temperature was programmed from 60 °C (held 3 min) to 190 °C at 30 °C $min^{-1}$ and then until 290 °C at 5 °C $min^{-1}$, where it was held for 3 min (total analysis time 30.33 min). Helium (99.9995%, Carburos Metálicos, Spain) was used as carrier gas at a constant flow of 1.3 mL $min^{-1}$. The transfer line temperature was maintained at 310 °C and the ion source and the quadrupole at 230 °C and 150 °C, respectively. Detection was carried out both in the scan (50–525 *m*/*z*) and in the SIM modes simultaneously. The *m*/*z* values of the fragment ions monitored in the SIM mode are listed in Table 1.

## 3. Results and discussion

### 3.1. Optimisation of the MASE procedure

There are several variables that can affect the extraction efficiency [25]: the nature and volume of the acceptor phase, the nature of the membrane, the salting out effect, the addition of methanol, the stirring rate, the sample volume, the temperature of extraction and the extraction time. Solutions obtained after MASE procedure were analysed by GC–MS analysis.

#### 3.1.1. Nature and volume of the acceptor phase

In a first approach, both the nature and volume of the acceptor phase were evaluated. The boiling point and the polarity of the solvent were considered in order to choose the solvents to be used. A wide range of solvents with different polarities have been used in the literature in order to extract synthetic musks by LLE (*n*-hexane, ethyl acetate, dichloromethane, chloroform, and toluene) [11,12]. However, ideal organic solvents for MASE have to fulfil several conditions, such as inertness to the membrane or high solubility for the studied analytes [22]. Moreover, due to large volumes of the extract injected in the LVI (45 μL), the studied solvents must be volatile enough in order to be easily eliminated during the vent step. Organic solvents like *n*-hexane, ethyl acetate or chloroform fulfil required conditions but, in order to use more environmental-friendly solvents, the use of chloroform was discarded. Besides, although MASE uses low solvent volumes, due to the high volatility of some musk compounds, possible analyte losses can be observed during the MASE extract evaporation step [26]. Therefore, to study this procedure, it was decided to evaluate the extraction yields using two different solvent volumes, 200 μL and 800 μL.

Thus, 15 mL of Milli-Q water samples, spiked at a concentration of 10 μg $L^{-1}$ of each compound, were extracted using 200 μL and 800 μL of *n*-hexane and ethyl acetate under constant stirring speed (500 rpm), extraction temperature (room temperature) and extraction time (90 min). The results (as chromatographic peak area multiplied by extract weight) obtained throughout the assays performed in triplicate are shown in Fig. 1. On the one hand, *n*-hexane provided better extraction yields than ethyl acetate. The main reason of this difference can be attributed to the permeation of ethyl acetate through the membrane into aqueous sample due to its high water solubility (8.5 g in 100 g water at 20 °C) [27]. As a consequence, the contact and transfer extraction area are reduced, which was reflected both in the little volume of ethyl acetate recovered after extraction step and in the poor extraction yield. On the other hand, the use of 200 μL of solvent volume provided higher recoveries since evaporation was avoided and the analyte losses were minimised. Therefore, 200 μL of *n*-hexane was used as acceptor organic phase during the optimisation of the rest of the variables.

#### 3.1.2. Nature of membrane

Different non-porous membranes were evaluated for the extraction of the target compounds from water samples in order to select the most convenient, i.e. the one providing the highest recoveries and lowest losses. For this purpose, 15 mL of spiked Milli-Q water (10 μg $L^{-1}$) were extracted using home-made membranes with different materials (LDPE and PET) and thicknesses (0.02 mm and 0.05 mm) during 90 min at room temperature. As it is plotted in Fig. 2, both selected material and thickness affect to the response of the extraction (average response of three replicates). LDPE yielded better results when the thinnest membrane was used, due to the faster analyte permeation. Therefore, thin LDPE membranes were chosen for further experiments.

#### 3.1.3. Modifications of the aqueous medium

The characteristics of the aqueous medium (i.e. pH, ionic strength or the addition of organic modifier) are variables to be taken into account in MASE [28]. Thus, 15 mL of Milli-Q water spiked at 10 μg $L^{-1}$ concentration level were extracted at previously established conditions (200 μL *n*-hexane, 90 min, room temperature and 500 rpm) to study the effects when the aqueous matrix is modified. Firstly, the influence of pH was evaluated at three levels: acidic (pH: 2), neutral (pH: 7) and alkaline (pH: 12). Since the responses were equivalent ($p > 0.05$), it was considered unnecessary to adjust the pH of the water samples.

The influence of an inert salt addition (NaCl) and an organic modifier (MeOH) was studied simultaneously by means of a

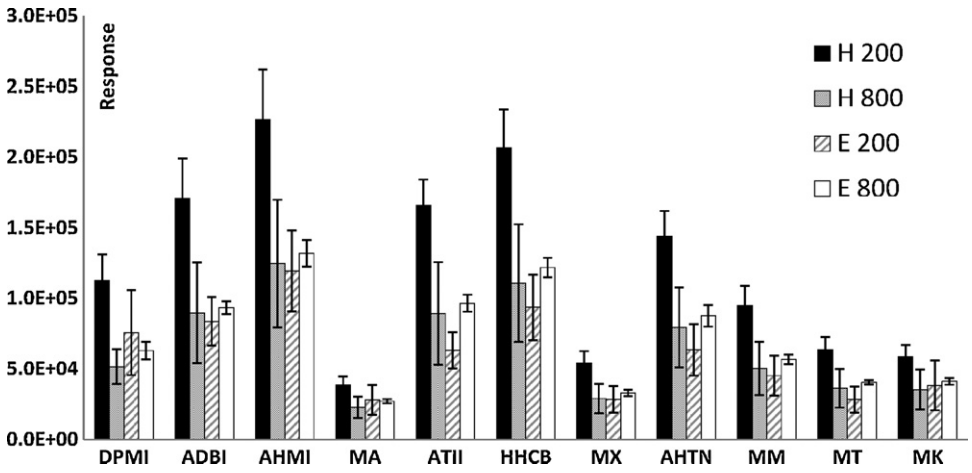



**Fig. 1.** Comparison of the chromatographic average responses ($n = 3$, 95% confidence level) obtained for the different organic solvents (H: *n*-hexane and E: ethyl acetate) and volumes (200 μL and 800 μL).

central composite design (CCD). On the one hand, the addition of salt increases the ionic strength of aqueous samples, decreasing the solubility of the analytes and improving their transference to the organic acceptor. On the other hand, the addition of organic modifier can also improve the extraction yields for non-polar compounds since their adsorption in the walls is avoided, while the solubility of the hydrophobic compounds in the aqueous solution is increased. Thus, the influence of the addition of NaCl and MeOH were studied in the 0–20% and in the 0–10% ranges, respectively. The responses obtained for the CCD were analysed by means of multiple linear regression. Similarly to others works found in the literature [26,29], the addition of MeOH was not significant ($p$-value > 0.05) whereas the addition of NaCl had a negative effect for all the compounds except for DPMI and MK. According to the obtained results (data not shown), the water samples were directly extracted without the addition neither of NaCl nor MeOH.

### 3.1.4. Sample volume

Owing to an improvement in chromatographic responses, extraction of higher sample volumes (higher mass of analytes) was also studied even if the extraction efficiency may be decreased. Three different volumes of Milli-Q water (i.e. 14 mL, 50 mL and 150 mL) were spiked at the same concentration (10 $\mu g\,L^{-1}$) and pre-treated under fixed extraction conditions (200 μL *n*-hexane, 90 min, room temperature and 500 rpm). As it can be observed in Fig. 3, all the analytes showed increased signals for higher sample volumes. Thus, in order to maximise the chromatographic response, 150 mL sample aliquots were selected for further experiments.

### 3.1.5. Stirring rate

Once the sample volume was fixed, the stirring rate was optimised. In most of the cases, when a vigorous mixing of the sample is assured, the extraction efficiency can be enhanced due to a decrease of the thickness of the boundary layers. However, too high agitation speeds may increase the formation of bubbles and reduce the extraction efficiency. Owing to these constraints 150 mL of Milli-Q water spiked at 600 $ng\,L^{-1}$ concentration level were extracted at three different stirring rates, 500 rpm, 700 rpm and 900 rpm in triplicate using 200 μL of *n*-hexane for 90 min at room temperature. Since, for almost all the synthetic musks under study, the intermediate and high stirring rates provided higher responses (data not shown) than the lowest one, agitation speed of 700 rpm was fitted to use in upcoming experiments.

### 3.1.6. Time-profile

Although at elevated temperatures the extraction equilibrium is reached faster, application of high temperatures can increase the losses of volatile components. In order to fix the extraction time to assure the equilibrium between both phases, a kinetic experiment from 5 to 720 min was carried out in triplicate under the previous fixed conditions at different controlled extraction temperatures (15 °C, 22 °C and 30 °C) and at two levels of concentration (600 $ng\,L^{-1}$ and 1300 $ng\,L^{-1}$). In agreement with other authors

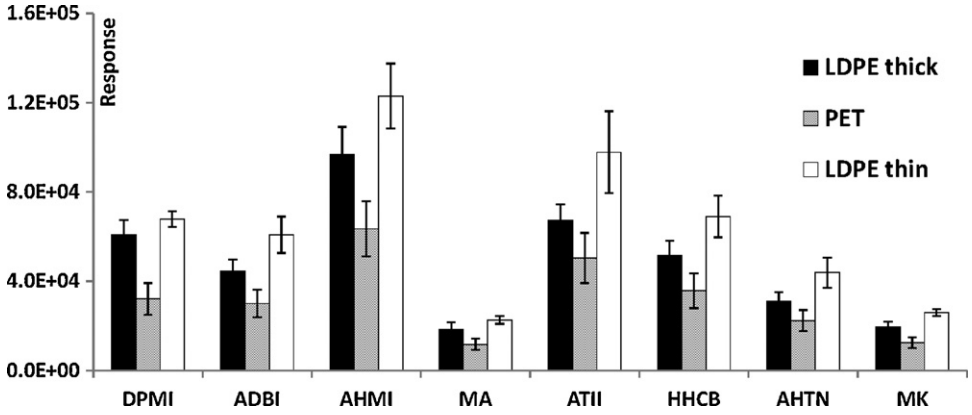



**Fig. 2.** Responses ($n = 3$, 95% confidence level) obtained after the extraction with different membrane materials: thick low density polyethylene, polyethylene terphthalate and thin low density polyethylene.

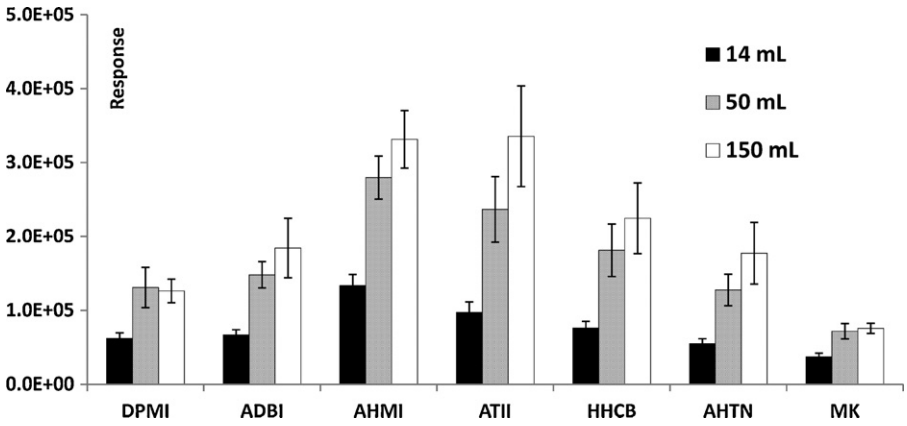



**Fig. 3.** Chromatographic responses ($n = 3$, 95% confidence level) after the extraction of different sample volumes (14 mL, 50 mL and 150 mL).

[27], increasing temperature showed clearly an increase in the response of the studied musks (see Fig. 4). The equilibrium in these conditions was reached after approximately 240 min (4 h). Furthermore, very long extractions (i.e. 720 min) showed a decrease in the response, suggesting an evaporation of the organic solvent and so, a reduction of the absorption capacity. The shape of the kinetic profile of all the target compounds was comparable, regardless the temperature or the concentration of the analytes. Therefore, the minimum stirring time to reach the equilibrium was fixed in 4 h. As expected, the efficiency of the extraction is higher when temperature is increased, but for the routine work it is more convenient to work at room temperature since better reproducibility was obtained (see Fig. 4).

Therefore, for the extraction of 150 mL of water sample, by means of MASE, final extraction conditions were established as it follows: thin LDPE membrane filled with 200 μL of *n*-hexane; no addition of NaCl nor MeOH; no variation of pH; stirring speed 700 rpm and extraction time 240 min at room temperature.

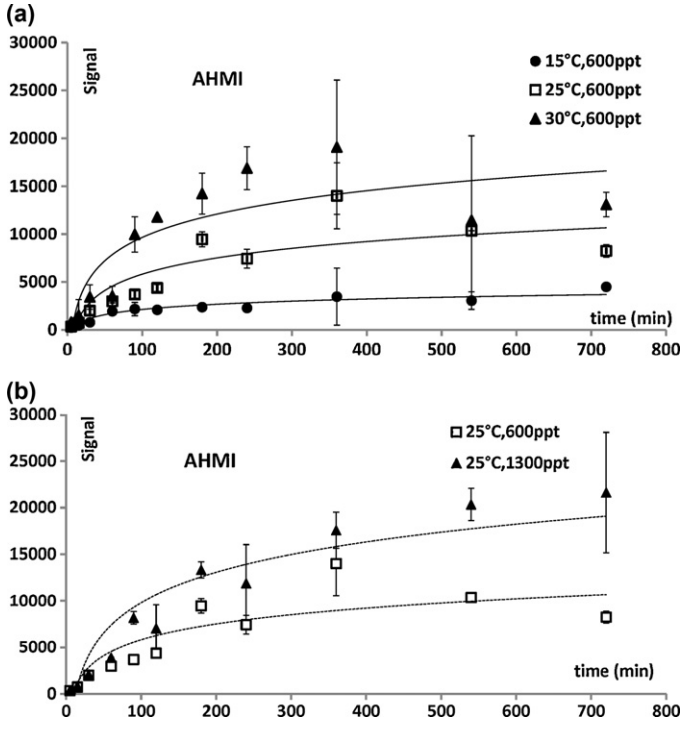



**Fig. 4.** Time profile for AHMI at three different temperatures: 15 °C, 25 °C and 30 °C and at two levels of concentration: 600 ng $L^{-1}$ and 1300 ng $L^{-1}$.

### 3.2. Optimisation of LVI-PTV-GC–MS analysis

In order to increase further on the sensitivity of this method, the optimisation of the LVI-PTV setup was considered. Based on previous studies [23], there are many variables to be considered and some were fixed (vent flow: 75 mL $min^{-1}$, purge flow: 75 mL $min^{-1}$, splitless time: 1.5 min and injection volume: 45 μL) and only the injection speed ($v_{inj}$, μL $s^{-1}$), cryo-focusing temperature ($T_{cis}$, °C) and vent time ($t_{vent}$, min) were studied following a CCD. The ranges of studied variables were: cryo-focusing temperature (15–70 °C), injection speed (2–6 μL $s^{-1}$) and vent time (0.4–5.5 min). The design matrix, involving 18 randomised experiments and the responses (as chromatographic peak areas) are summarised in Table 2. The precision of the measurements was estimated from the four replicates of the central point (RSD values for the all the analytes were between 2 and 4%).

According to MLR models, injection speed was not significant ($p > 0.05$) and, therefore response surfaces were built against the other two variables, as shown in Fig. 5 for ATII and AHMI. As can be seen, lower values of vent time (i.e. 0.5 min) provided the best responses for all the analysed target compounds, whereas the $T_{cis}$ optimum values varied from low temperatures (15 °C for ADBI, AHMI and MA) to medium temperatures (25 °C for AHTN, ATII, HHCB and MK). Therefore, it was decided to fix $T_{cis}$ at a consensus value of 20 °C.

Briefly, according to the optimised values, the LVI-PTV parameters were established as follows: cryo-focussing temperature is maintained at 20 °C in order to inject 45 μL of *n*-hexane extract at 6 μL $s^{-1}$ while the solvent is vented at 75 mL $min^{-1}$ and 5 psi pressure during 0.5 min. Afterwards, the vent valve is closed for 1.5 min while the analytes are quantitatively introduced into the column. After 2 min elapse, the vent valve is re-opened and the injector is purged at 75 mL $min^{-1}$ in order to avoid possible contamination effects.

### 3.3. Figures of merit

The figures of merit of all the analytes are summarised in Table 3. The calibration curves were tested by spiking different amount of standards (from 10 to 200 ng $L^{-1}$) in 150 mL of Milli-Q water and also with a set of 6 standards containing concentrations ranging from LOQ to 100 ng $mL^{-1}$. [$^2H_3$]-AHTN and [$^2H_{15}$]-MX were used as surrogates and they were calibrated in order to know and correct the recoveries of the target compounds. Linearity was good for all the musks obtaining coefficients of determination ($r^2$) higher than 0.999.

Precision in the chromatographic response was determined in terms of repeatability at low (15 ng $mL^{-1}$) and high (75 ng $mL^{-1}$)

**Table 2**
Central composite design matrix and the responses obtained for the target compounds. A: $T_{cis}$ (°C); B: vent time (s); C: injection speed ($\mu L\,s^{-1}$). The replicates of the central point are marked with an *.

| Exp. | Optimised variables | | | Responses $\times 10^5$ (as chromatographic peak areas) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | ADBI | AHMI | AHTN | ATII | HHCB | MA | MK |
| 1* | 42.5 | 3 | 3.5 | 7.31 | 10.54 | 9.31 | 17.78 | 12.06 | 3.13 | 4.76 |
| 2* | 42.5 | 3 | 3.5 | 7.52 | 10.81 | 9.43 | 18.09 | 12.19 | 3.27 | 4.96 |
| 3 | 25 | 4.5 | 2 | 10.07 | 12.73 | 9.36 | 17.57 | 12.27 | 2.94 | 4.24 |
| 4 | 25 | 1.5 | 5 | 14.63 | 17.69 | 12.21 | 23.84 | 16.28 | 4.80 | 5.92 |
| 5 | 42 | 0.5 | 3.5 | 15.00 | 18.27 | 12.34 | 23.86 | 16.58 | 5.11 | 6.12 |
| 6 | 60 | 4.5 | 2 | 2.34 | 3.61 | 4.15 | 8.38 | 5.01 | 0.87 | 2.98 |
| 7* | 42 | 3 | 3.5 | 7.72 | 11.13 | 9.92 | 18.72 | 12.47 | 3.38 | 5.19 |
| 8 | 42 | 3 | 1 | 8.65 | 11.69 | 8.94 | 17.55 | 11.79 | 2.79 | 4.39 |
| 9 | 60 | 1.5 | 5 | 5.69 | 8.96 | 9.33 | 17.66 | 11.33 | 3.04 | 5.59 |
| 10 | 60 | 1.5 | 2 | 6.86 | 10.60 | 10.60 | 20.42 | 12.77 | 3.67 | 6.10 |
| 11 | 42 | 3 | 6 | 7.71 | 11.30 | 9.96 | 19.01 | 12.87 | 2.17 | 5.38 |
| 12 | 25 | 4.5 | 5 | 10.49 | 13.21 | 9.44 | 18.10 | 12.46 | 1.56 | 4.70 |
| 13 | 13 | 3 | 3.5 | 13.00 | 15.67 | 10.87 | 20.46 | 14.17 | 3.80 | 5.31 |
| 14 | 42 | 5.5 | 3.5 | 2.85 | 5.07 | 6.31 | 12.12 | 8.02 | N.A. | 3.82 |
| 15 | 72 | 3 | 3.5 | 1.87 | 2.79 | 3.21 | 6.35 | 3.81 | 0.74 | 2.58 |
| 16 | 60 | 4.5 | 5 | 1.75 | 2.75 | 3.47 | 6.55 | 4.21 | 0.37 | 2.60 |
| 17 | 25 | 1.5 | 2 | 14.24 | 17.18 | 11.62 | 22.38 | 15.58 | 2.46 | 5.80 |
| 18* | 42 | 3 | 3.5 | 7.92 | 11.45 | 9.74 | 18.76 | 12.56 | 2.35 | 5.28 |

calibration levels for 3 replicates analysed within a day. The RSD % values ranged from 9 to 20% and from 6 to 9% for low and high concentration levels, respectively. The precision of the method was evaluated for spiked Milli-Q water at 100 ng $L^{-1}$, obtaining RSD % values between 13 and 22% for all the target compounds.

Limits of detection (LODs) were calculated as the average signal ($n = 5$) of the blank samples plus three times their standard deviation. LODs were obtained in the very low ng $L^{-1}$ range, from 3 ng $L^{-1}$ for ADBI to 8 ng $L^{-1}$ for ATII. The method detection limits (MDLs) were calculated after spiking effluent WWTP water samples at the corresponding LOD for each analyte following the procedure given by US Environmental Protection Agency (EPA). The values obtained were in the range of 4 ng $L^{-1}$ for AHMI and 25 ng $L^{-1}$ for MX, which were in good agreement with those found in the literature [14,16,26,30].

Extraction efficiency and apparent recovery were calculated for spiked Milli-Q water at the 100 ng $L^{-1}$ concentration level. Extraction efficiency was calculated by comparing the spiked concentration with the concentration obtained from external standard calibration. Extraction efficiencies were between 16% for DPMI and 38% for AHTN (see Table 3).

Taking into account the suggested definitions of recoveries and apparent recoveries [31], we have used two experimental approaches. In the first approach, apparent recovery was calculated correcting the extraction efficiency with the extraction efficiency of the corresponding surrogate. The results obtained in this way were acceptable for all target compounds (around 80%, except for DPMI and MA 60%) (see Table 3). It is worth mentioning that in those experiments in which not fresh [$^2H_3$]-AHTN was used, very high recoveries for its analogue AHTN was observed (up to 229% after correcting with [$^2H_3$]-AHTN). [$^2H_3$]-AHTN is produced via proton exchange, however, this reaction may be reversed giving the original undeuterated product [32]. Therefore, this deuterated compound could introduce interferences. In this sense [$^2H_{15}$]-MX does not undergo any reaction itself, so it can be considered as a better surrogate compared to [$^2H_3$]-AHTN.

The second approach consisted on the determination of the apparent recoveries by comparing the spiked concentration of the target compound with the concentration obtained from calibration curve built with Milli-Q spiked water samples. In this case, the recoveries after correction with deuterated analogues were good, even without using deuterated analogues (values between 83% for

**Table 3**
Main method parameters for the MASE-LVI-PTV-GC–MS procedure.

| Analyte | LVI-PTV-GC–MS | | MASE-LVI-PTV-GC–MS | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Repeatability | | LODs (ng $L^{-1}$, $n = 5$) | MDLs(ng $L^{-1}$, $n = 4$) | Repeatability (RSD %, $n = 3$) (100 ng $L^{-1}$) | Extraction efficiency[b] (%) | Apparent recovery (%) | |
| | (RSD %, $n = 3$) (15 ng $mL^{-1}$) | (RSD %, $n = 3$) (75 ng $mL^{-1}$) | | | | | Corrected with surrogate[c] | Procedural calibration[d] |
| ADBI | 9 | 7 | 3 | 4 | 16 | 20 | 81 | 103 |
| AHMI | 9 | 6 | 4 | 4 | 17 | 19 | 79 | 102 |
| AHTN | 2 | 8 | 8 | 24 | 18 | 38 | 80 | 108 |
| ATII | 9 | 8 | 3 | 10 | 13 | 19 | 80 | 98 |
| DPMI | 20 | 9 | 5 | 11 | 21 | 16 | 70 | 83 |
| HHCB | 18 | 8 | 4 | 10 | 22 | 20 | 83 | 90 |
| MA | 13 | 7 | 5 | 7 | 21 | 22 | 64 | 97 |
| MK | 14 | 8 | 4 | 15 | 21 | 28 | 83 | 96 |
| MM | –[a] | –[a] | 3 | 9 | 20 | 41 | 127 | –[a] |
| MX | –[a] | –[a] | 6 | 25 | 19 | 21 | 87 | 89 |

[a] No data available.
[b] Amount extracted to the organic phase during MASE.
[c] Recovery after correction with the corresponding deuterated analogue.
[d] Recovery using calibration curve built with spiked Milli-Q.

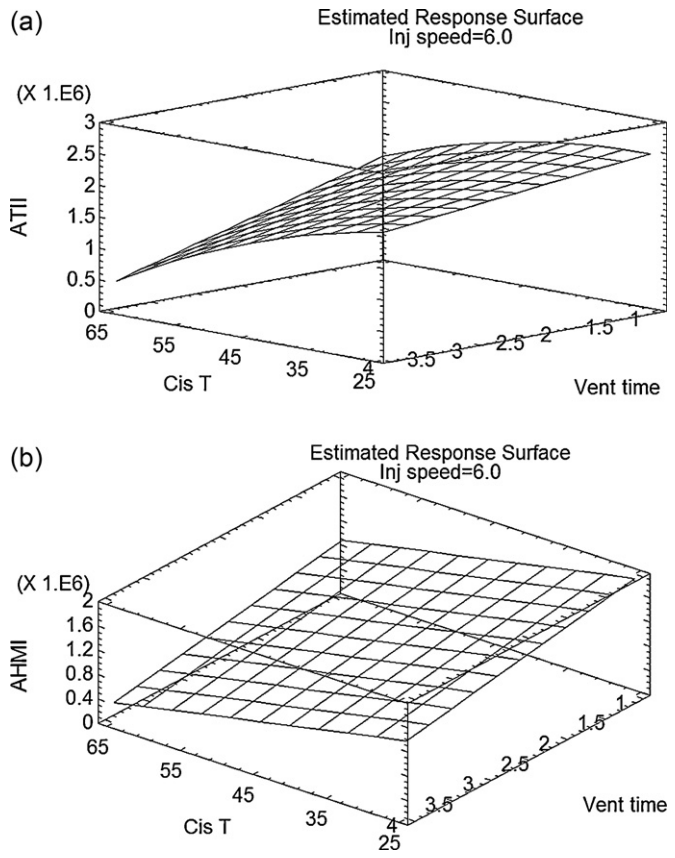



**Fig. 5.** Response surface obtained for ATII and AHMI using significant variables parameters ($p$-value < 0.05): $T_{cis}$ and $t_{vent}$. Injection speed was fixed at 6 μL s$^{-1}$.

DPMI and 108% for AHTN). Thought the procedural calibration may offer slightly higher recoveries than the surrogate corrected one, the latter can be used as a routine basis.

### 3.4. Evaluation of the matrix effect

The influence of the matrix in real water samples, such as suppression or enhancement of analyte signal in matrix solution, must be studied in order to assure the accuracy of the method. In environmental water samples substantial levels of dissolved organic matter (DOM) (e.g. DOM of 125 mg L$^{-1}$ can be often found in effluents of WWTPs) can interfere in the extraction of the compounds to the organic solvent, resulting in poorer extraction yields [27].

Among the different strategies to solve this drawback, several approaches have been suggested in the literature, such as matrix matched calibration, sample dilution, the clean-up of the extracts or the use of deuterated analogues [33], being the last one the most widely accepted. Since high concentration of some musk compounds in WWTPs are expected and, thus the extraction yield may change from sample to sample, the use of matrix matched calibration was initially discarded. Some authors use sample dilution but in order to avoid the loss of sensitivity, the use of isotopically labelled compounds as surrogate standards were evaluated.

Preliminary experiments were carried out analysing the matrix effect with synthetic Milli-Q water samples spiked with different amount of humic acids (0, 50, 100, 250, 500 and 1000 mg L$^{-1}$). The matrix effect was evaluated by comparing the concentrations of analytes in spiked blank water (at 100 ng L$^{-1}$) with those obtained for target compounds in presence of humic acids and without any further correction. The assays were performed in triplicate. To promote the interaction of the target compounds with the synthetic matrix, samples were spiked and stirred for 90 min before performing the extraction. Fig. 6a shows the significant decrease of extraction efficiency of all target compounds in the presence of high concentration of humic acids. However, as shown in Fig. 6b, when [$^2H_3$]-AHTN was used for the correction of ADBI, AHMI, AHTN and HHCB and [$^2H_{15}$]-MX for ATII, DPMI, MA, MK, MM and MX, respectively. The corrected recoveries were within 80–120%, and a more precise way to estimate the concentration can be concluded.

Matrix effect was also evaluated in real environmental samples such as surface water and wastewater in order to evaluate signal suppression or enhancement due to co-eluting matrix constituents also present in the sample extracts. Thus, three replicates of each type of sample were spiked at 100 ng L$^{-1}$ of each compound, and labelled surrogates were also added before the extraction. Extraction efficiency (without correction with surrogates), matrix effect (comparing the concentration of spiked sample with the concentration of spiked Milli-Q at the same concentration level) and corrected recovery (concentration of spiked sample corrected with surrogates) were evaluated.

Table 4 summarises the results obtained for these assays. Matrix effects were not very remarkable in surface water sample, since both Milli-Q and surface water presented comparable extraction efficiencies. A tolerable enhancement was revealed for DPMI (152%), but acceptable recoveries were obtained after correcting with labelled compounds (above 65% for all target compounds). However, the effect of the sample matrix was more evident in wastewater samples, especially in influent water of WWTPs. In the case of effluent water, a clear decrease of extraction efficiency was observed for all the target analytes except for HHCB for which recoveries exceeded 100%. Nevertheless, matrix effect was notably corrected after using deuterated analogues. However, more attention must be paid to samples corresponding to influent waters of WWTPs. The influence of matrix effect was verified in the enhancement of responses and thus, recoveries higher than those observed in spiked Milli-Q. In order to compensate matrix effect, the results were corrected with [$^2H_3$]-AHTN and [$^2H_{15}$]-MX deuterated analogues and acceptable recoveries were obtained (above 70% for all the target analytes).

### 3.5. Application of membrane assisted solvent extraction to real samples

The developed MASE-LVI-PTV-GC–MS method was applied to real samples in order to check its feasibility in the determination of ten synthetic compounds in four different types of water samples (influent and effluent of WWTPs, estuarine water and drinking water) in triplicate ($n$ = 3, 90%).

Two synthetic musks were detected in all the real samples studied: galaxolide (HHCB) and tonalide (AHTN). HHCB was the main musk found in all the cases and its concentration ranged from 41 ± 7 ng L$^{-1}$ in surface water of the estuary of Urdaibai to 295 ± 43 ng L$^{-1}$ in WWTP influent from Galindo, whereas the highest value observed for AHTN was 138 ± 12 ng L$^{-1}$ in WWTP influent from Galindo. These two musk fragrances are described in the literature as the most commonly detected musk compounds in water samples [4,7,11]. Besides these two compounds, ADBI (25 ± 9 ng L$^{-1}$) and MK (24 ± 7 ng L$^{-1}$) were also detected in WWTP influent from Galindo. Regarding to the concentrations of the target compounds found in the effluents from Galindo both HHCB (259 ± 54 ng L$^{-1}$) and AHTN (82 ± 6 ng L$^{-1}$) were also detected. The later results can confirm the fact that most of the WWTPs are not efficient removing synthetic musks as it has been pointed in the literature [4,5,7,34].

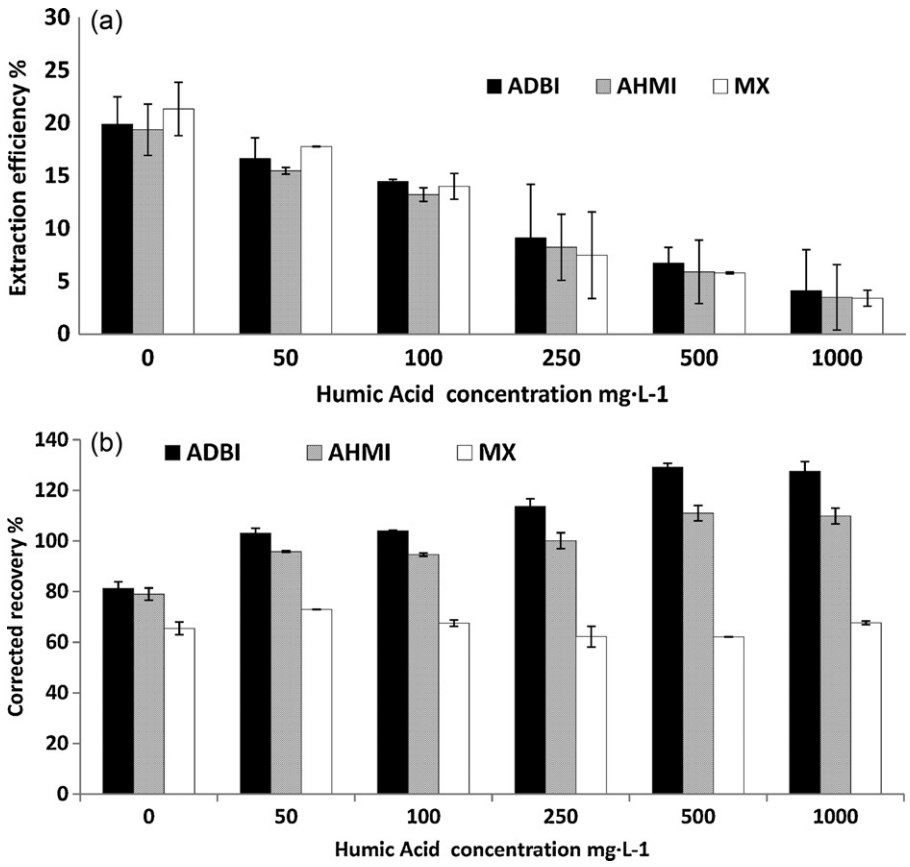



**Fig. 6.** (a) Extraction efficiency ($n = 3$, 95% confidence level) at different concentrations of humic acids. (b) Corrected recoveries ($n = 3$, 95% confidence level) of ADBI, AHMI and MX using corresponding deuterated analogue (see Table 1 for details of deuterated compounds).

**Table 4**
Extraction efficiency, matrix effect and corrected recovery of surface water and WWTP effluent and influent water samples.

| Environmental water sample | Compound | Extraction efficiency (%)[a] | Matrix effect (%)[b] | Corrected recovery (%)[c] |
|---|---|---|---|---|
| Urdaibai estuary | ADBI | 39 | 129 | 69 |
| | AHMI | 37 | 128 | 74 |
| | AHTN | 38 | 112 | 80 |
| | ATII | 32 | 123 | 64 |
| | DPMI | 34 | 152 | 106 |
| | HHCB | 38 | 123 | 70 |
| | MA | 32 | 144 | 138 |
| WWTP effluent | ADBI | 19 | 48 | 126 |
| | AHMI | 23 | 57 | 50 |
| | AHTN | 87 | 75 | 63 |
| | ATII | 12 | 30 | 71 |
| | DPMI | 3 | 16 | 118 |
| | HHCB | 385 | 486 | 85 |
| | MA | 36 | 100 | 76 |
| WWTP influent | ADBI | 64 | 164 | 77 |
| | AHMI | 61 | 156 | 74 |
| | AHTN | 253 | 284 | 80 |
| | ATII | 36 | 110 | 88 |
| | DPMI | 103 | 384 | 124 |
| | HHCB | 420 | 661 | 47 |
| | MA | 56 | 170 | 70 |

[a] Amount of analyte extracted to acceptor organic phase.
[b] Extraction efficiency in real sample/extraction efficiency in Milli-Q water.
[c] Recovery after correction with the corresponding deuterated analogue.

## 4. Conclusions

In order to provide a friendly method to quantify the presence of synthetic musks in water samples a MASE coupled to LVI-PTV-GC–MS has been fully developed for the determination of ten synthetic musk fragrances in environmental water samples (estuarine, influent and effluent water of WWTPs) after optimising several variables affecting both the extraction and analysis steps.

The use of low extractant volumes allows the direct analysis of the extracts avoiding the evaporation step in which volatile analytes can be lost. The easy performance makes the developed method interesting for routine analysis in monitoring programs. Furthermore, the combination of MASE and LVI-PTV-GC–MS provides method detection limits of 4–25 ng $L^{-1}$ which enables detecting analytes at low ng $L^{-1}$ levels. The developed method was applied to real water samples and the matrix effect was evaluated. While estuarine samples are not highly affected, the matrix effect observed in wastewater samples can be corrected using deuterated analogues. Galaxolide and tonalide are the main two synthetic musks observed in most of the analysed samples, even in effluent wastewater samples. This supports that the elimination of this compounds is not effective enough being necessary further monitoring strategies.

## Acknowledgements

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## References

[1] Global assessment of the state-of-science of endocrine disruptors, World Health Organization/International Program on Chemical safety, WHO/PCS/EDC/02.2, http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/.
[2] P. Lepom, B. Brown, G. Hanke, R. Loos, P. Quevauviller, J. Wollgast, J. Chromatogr. A 1216 (2009) 302.
[3] M. Gros, M. Petrovic, D. Barceló, Analysis of Emerging Contaminants of Municipal and Industrial Origin, Springer, Berlin/Heidelberg, 2008.
[4] M.C. Pietrogrande, G. Basaglia, TrAC Trends Anal. Chem. 26 (2007) 1086.
[5] S. Simonich (Ed.), The Handbook of Environmental Chemistry, Springer, Berlin/Heidelberg, 2005, p. 79.
[6] M. Kolossa-Gehring, Environ. Sci. Pollut. Res. 14 (2007) 44.
[7] J. Reiner, J. Berset, K. Kannan, Arch. Environ. Contam. Toxicol. 52 (2007) 451.
[8] J.M. Brausch, G.M. Rand, Chemosphere 82 (2011) 1518.
[9] C. Miège, J.M. Choubert, L. Ribeiro, M. Eusèbe, M. Coquery, Environ. Pollut. 157 (2009) 1721.
[10] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 43 (2009) 363.
[11] J. Reiner, K. Kannan, Water Air Soil Pollut. 214 (2011) 335.
[12] A. Peck, Anal. Bioanal. Chem. 386 (2006) 907.
[13] S.L. Simonich, W.M. Begley, G. Debaere, W.S. Eckhoff, Environ. Sci. Technol. 34 (2000) 959.
[14] N. Ramírez, R.M. Marcé, F. Borrull, J. Chromatogr. A 1218 (2011) 156.
[15] M. Arbulu, M.C. Sampedro, N. Unceta, A. Gómez-Caballero, M.A. Goicolea, R.J. Barrio, J. Chromatogr. A 1218 (2011) 3048.
[16] G. Basaglia, L. Pasti, M. Pietrogrande, Anal. Bioanal. Chem. 399 (2011) 2257.
[17] H. Liu, L. Liu, Y. Xiong, X. Yang, T. Luan, J. Chromatogr. A 1217 (2010) 6747.
[18] T. Heberer, S. Jürgensen, H. Fromme, in: G.G. Rimkus (Ed.), Series Anthropogenic Compounds, Springer, Berlin/Heidelberg, 2004, p. 123.
[19] C. Mahugo-Santana, Z. Sosa-Ferrera, M.E. Torres-Padrón, J.J. Santana-Rodríguez, TrAC Trends Anal. Chem. 30 (2011) 731.
[20] T. Hyötyläinen, M.-L. Riekkola, Anal. Chim. Acta 614 (2008) 27.
[21] S. Almeda, L. Nozal, L. Arce, M. Valcárcel, Anal. Chim. Acta 587 (2007) 97.
[22] B. Hauser, P. Popp, J. Sep. Sci. 24 (2001) 551.
[23] A. Vallejo, L.A. Fernández, M. Olivares, A. Prieto, N. Etxebarria, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1217 (2010) 8327.
[24] E. Hoh, K. Mastovska, J. Chromatogr. A 1186 (2008) 2.
[25] E. Psillakis, N. Kalogerakis, TrAC Trends Anal. Chem. 22 (2003) 565.
[26] A. Silva, J. Nogueira, Anal. Bioanal. Chem. 396 (2010) 1853.
[27] T. Einsle, H. Paschke, K. Bruns, S. Schrader, P. Popp, M. Moeder, J. Chromatogr. A 1124 (2006) 196.
[28] A. Prieto, O. Telleria, N. Etxebarria, L.A. Fernández, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1214 (2008) 1.
[29] C. García-Jares, M. Llompart, M. Polo, C. Salgado, S. Macías, R. Cela, J. Chromatogr. A 963 (2002) 277.
[30] M.J. Gómez, M.M. Gómez-Ramos, A. Agüera, M. Mezcua, S. Herrera, A.R. Fernández-Alba, J. Chromatogr. A 1216 (2009) 4071.
[31] D.T. Burns, K. Danzer, A. Townshend, Pure Appl. Chem. 74 (2002) 2201.
[32] K. Bester, J. Environ. Monit. 7 (2005) 43.
[33] R. Rodil, S. Schrader, M. Moeder, J. Chromatogr. A 1216 (2009) 4887.
[34] V. Matamoros, J. Bayona, Emerging Contaminants from Industrial and Municipal Waste, Springer, Berlin/Heidelberg, 2008, p. 199.