



# A retention time locked gas chromatography–mass spectrometry method based on stir-bar sorptive extraction and thermal desorption for automated determination of synthetic musk fragrances in natural and wastewaters

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## ABSTRACT

A stir-bar sorptive extraction (SBSE) method followed by automated thermal desorption (ATD) coupled to gas chromatography–mass spectrometry was optimized for determining trace levels of 18 synthetic fragrances (musks). Using the method developed a retention time locked library is created and converted to a screening database. This homebuilt database can be combined with deconvolution software for the identification of musks. A factorial design was provide to evaluate the main parameters and interactions between the factors affecting the process of SBSE. Operating with de MS-detector in the full-scan mode, high sensitivity with detection limits in the low  $\text{ng L}^{-1}$  range, and good linearity and repeatability were achieved for all musks. The applicability of the method developed was tested in natural waters (surface and groundwater) and wastewater of a plant treatment (WWPT). The results obtained confirmed the usefulness of the proposed method for the determination and unequivocal identification of musks. This approach enables the developed method to be used for routine screening of environmental samples and posterior rapid quantitation of the positive samples.

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## 1. Introduction

Emerging organic contaminants (EOCs) have long been present in the environment, however until recently they have not aroused scientific or public attention. The focus for water pollution research has nowadays been extended from the conventional “priority” pollutants to the so-called “emerging contaminants”, many of which have been unknown until recently and, therefore, are generally not included in the environmental legislation. The European Commission established a Water Framework Directive [1] to reduce chemical pollution of surface waters and defined a list of substances presenting a significant risk to the aquatic ecosystem [2]. European Directive 2000/60/EC has identified some personal care products (PCPs) as future emerging priority candidates for monitoring and regulation.

PCPs represent a group of interest [3–5] since the endocrine-disrupting effects of certain compounds have been drawn attention to, furthermore, most of these compounds are very lipophilic and tend to accumulate in the environment having adverse effects on aquatic ecosystems. This group of emerging contaminants consti-

tutes a broad class of chemicals widely used in daily life, such as synthetic fragrances, UV-filters, antiseptics, antioxidants or insect repellents [6]. These compounds are continuously introduced into the environment mainly via urban wastewater effluents. Wastewater treatment plants (WWTPs) have been identified as a major point source of synthetic fragrances entering the environment since they receive continuous inputs of these compounds [7,8]. However, the efficiencies in WWTPs are often low and compounds which are not removed are released to the surface waters [9], groundwater [10], air and biota [11]. Their presence in remote regions of the Arctic suggests that atmospheric transport plays an important role in dispersing these fragrances throughout the global environment [12].

Although synthetic fragrances mainly contain nitro musks and polycyclic musks, four different musk families exist according to their physico-chemical properties: nitro musks (musk ketone, musk ambrette, musk xylene, musk tibeten and musk moskene), polycyclic musks (galaxolide, tonalide, celestolide, phantolide, cashmeran, and traseolide), macrocyclic musks (ambrettolide, muscone, ethylene brassilate, globalide and thibetolide) and alicyclic musks (romandolide and helvetolide). The four major synthetic musk fragrances are musk xylene, musk ketone, galaxolide and tonalide which account for 95% of those used [13]. Several analytical methods based on GC–MS were developed for the iden-

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tification and quantification of musks in a variety of environmental samples: superficial or natural waters [14–16], sediments [17,18], biota [19] and air [20]. Recent trends in extraction procedures, solventless and pre-concentration sample techniques based on adsorption of organic compounds into polymer coated, solid phase micro extraction (SPME) [21,22] and stir-bar sorptive extraction (SBSE) [23], have been applied to the extraction of a wide range of water pollutants from aqueous matrices. SPME has been used by directly immersing the fiber into the liquid samples to extract this kind of compounds [21], and by headspace sampling (HS-SPME) [24].

The robustness of the stir-bar and physical difference between SPME (volume of fiber coated is usually 0.5  $\mu\text{L}$ ) and SBSE (smallest stir-bar is 24  $\mu\text{L}$ ) made this technique the most sensitive [25] and suitable for the present study. On the other hand, due to the musk values of octanol–water distribution coefficient ( $K_{ow} > 2.5$ ), the polydimethylsiloxane (PDMS) coated used for SBSE is adequate for this kind of compounds. Recently, Silva and Nogueira [23] developed an analytical approach to determine four synthetic musks in environmental waters using SBSE and liquid desorption combined with large volume injection in GC–MS. In this paper, the SBSE technique is coupled with an automated thermal desorption (ATD) system, that from the practical point of view, seems to be a more attractive approach for the desorption of the analytes in combination with GC.

In contrast to other existing devices which need to use a transfer line with a special injector, the ATD does not use the GC injector port and has its own carrier gas control modules, thereby enormously improving reproducibility [26]. Therefore, carrier and split flow stability and retention time reproducibility are superior to systems using the electronic control modules or the manual pressure control systems of the GC. This has enabled the use, with complete guarantee, of the retention time locked systems (RTL) [27], designed to reproduce retention times in long-term despite system maintenance or other perturbations by adjusting the carrier gas flow. Retention time is a critical component of sample identification, and the mass spectra of the compounds in the database libraries may be acquired using retention time locking (RTL) [28].

The aim of this study is to combine the aforementioned elements, SBSE–ATD/RTL–GC–MS, to develop a method for the sensitive and specific quantification of 18 synthetic fragrances listed in a homebuilt RTL musk database in water samples. To confirm its practical application, natural waters and wastewater samples were analyzed with the proposed method.

## 2. Experimental

### 2.1. Chemicals and reagents

GC residue analysis grade cyclohexane and 2-propanol used to prepare stock solutions were purchased from Scharlab (Barcelona, Spain). Sodium chloride from Panreac (Barcelona, Spain) was used for the SBSE procedure. The water used in this work was of Milli-Ro Plus quality obtained by purifying demineralized water in a Milli-Q water filtration system (Millipore, Milford, MA, USA).

The standards of synthetic fragrances 2,6-dinitro-3-methoxy-4-tert-butyltoluene (musk ambrette), 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (musk xylene), 4-aceto-3,5-dimethyl-2,6-dinitro-tert-butylbenzene (musk ketone), 3-methylcyclopentadecanone (musk muscone), oxacyclohexadecan-2-one (globalide), 1-oxacyclohexadecan-2-one (thibetolide), 1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta[g]-2-benzopyran (galaxolide), 1,1,3,3,5-pentamethyl-4,6-dinitroindane (musk moskene), 17-oxacycloheptadec-6-en-1-one (musk ambret-

tolide) and 1,4-dioxacycloheptadecane-5,17-dione (musk ethylene brassilate) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) all of them with a certified purity higher than 99%. 7-acetyl-1,1,3,4,4,6-hexamethyltetraline (tonalide), 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (cashmeran), 6-acetyl-1,1,2,3,3,5-hexamethylindane (phantolide), 4-acetyl-1,1-dimethyl-6-tert-butylindane (celestolide), 1-[(2R,3R)-2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl]-(traseolide) and 1-(tert-butyl)-3,4,5-trimethyl-2,6-dinitrobenzene (tibeten) were obtained from LGC Standards GMBH (Barcelona, Spain) all of them with a certified purity higher than 99.9%. 1-[(3',3'-dimethyl-1'-cyclohexyl)-ethoxycarbonyl]methyl propanoate (romandolide) and (3',3'-dimethyl-1'-cyclohexyl)-2,2-dimethyl-3-oxapentyl propanoate (helvetolide) was supplied by Firmenich (Barcelona, Spain). The surrogate standard [ $^2\text{H}_6$ ] fenitrothion was supplied by Cambridge Isotope Lab (Andover, MA, USA).

### 2.2. Preparation of standard solutions and spiked water samples

Most of the standards were purchased as solid standards, and individual stock solutions containing 1000  $\text{mg L}^{-1}$  of each analyte were prepared in 2-propanol. Musk ethylene brassilate and musk moskene were purchased as 10  $\text{mg L}^{-1}$  in cyclohexane solutions. The individual stock solutions were stored at  $-42^\circ\text{C}$  and preserved at  $4^\circ\text{C}$  in everyday use.

Mixed stock solutions of each family were prepared in 2-propanol containing 0.5  $\text{mg L}^{-1}$  in the case of nitro musks and musk polycyclic, and 1  $\text{mg L}^{-1}$  in the case of musk alicyclic and musk macrocyclic families. These solutions were used to obtain the mass spectra and qualitative information of the analytes. Working solutions were prepared daily at a required concentration by diluting the stock mixed standard in water.

For the method evaluation, the water samples (30 mL) were spiked in 40 mL screw-cap vials with 0.5  $\mu\text{g L}^{-1}$  of fenitrothion deuterated as internal standard and were shaken and left to stand for 30 min to allow the solution to stabilize. Then, the samples were spiked with aliquots (100  $\mu\text{L}$ ) of the working standard mixture at different concentration levels.

### 2.3. Natural water and wastewater real samples

The proposed method was applied to the analysis of surface and ground water samples and influents and effluents of a wastewater treatment plant (WWTP) with a capacity of 100,000 population-equivalents, which are located in Alava province (north of Spain).

The surface water samples from the Alegría river were obtained from 7 sampling points previously established according to the hydrogeological characteristics of the area. Six sampling campaigns were performed between January 2009 and June 2010. The groundwater was sampled in July 2009 at six points in a network of piezometric control within the vulnerable area of the quaternary aquifer of Vitoria-Gasteiz (Alava). In the case of the WWTP, 24-h flow-proportional composite untreated influent (upstream) and final treated effluent (downstream) urban wastewater samples were collected on five days in October 2009.

Samples were collected in 250 mL glass bottles supplied by Scharlab (Barcelona, Spain), transported to the laboratory under refrigeration and stored at  $-42^\circ\text{C}$  until the analysis. To remove particles, centrifugation of the wastewater at 420  $\text{rad s}^{-1}$  was performed on the same day of sampling, using a centrifuge Orto Alresa Model Unicen (Madrid, Spain).

## 2.4. Instrumentation

To perform sorptive extraction, commercial polydimethylsiloxane (PDMS) coated stir bars (twister) of 10 mm length and 0.5 mm film thickness (Gerstel, Mülheim and der Ruhr, Germany) were used. Stirring and heating was carried out using a nine position Multimix Heat D (Ovan, Spain).

After the extraction step, the twisters were introduced into a stainless steel tube for thermal desorption in an automated thermal desorber (ATD) turbo-Matrix 650 from PerkinElmer (Shenton, CT, USA). The analytes were trapped in a cold trap at  $-5^{\circ}\text{C}$  controlled by the Peltier cooling system [29].

The musk compounds were desorbed prior to transfer to the gas chromatography column through a heated transfer line connected to the analytical column with a column ultimate union assembly. The heated line transfer can be heated to between  $50^{\circ}\text{C}$  and  $300^{\circ}\text{C}$ . A GC system coupled to a 5973 inert mass-selective detector, both of Agilent Technologies (Palo Alto, CA, USA) was provided with a HP-5ms column ( $30\text{ m} \times 0.25\text{ mm}$ ,  $0.2\ \mu\text{m}$ ) from Agilent Technologies.

A constant pressure of 14.1 psi was selected by the ATD injector, using helium as a carrier gas. Temperature programming of the column was set to  $120^{\circ}\text{C}$  for 2 min and then heated to  $200^{\circ}\text{C}$  at  $2^{\circ}\text{C min}^{-1}$ , then at  $40^{\circ}\text{C min}^{-1}$  to  $280^{\circ}\text{C}$  and maintained for 15 min. The transfer line was set to  $280^{\circ}\text{C}$  guaranteeing the correct transfer of analytes. Solvent delay was 3.5 min, ion source and quadrupole temperatures were  $230^{\circ}\text{C}$  and  $150^{\circ}\text{C}$ , respectively, and the MS-detector acquired in full-scan mode (SCAN) operating at an electron impact energy of 70 eV.

## 2.5. SBSE and thermal desorption procedure

Before each use, stainless steel TD tubes were thermally cleaned at  $300^{\circ}\text{C}$  for 15 min under a helium flow rate of  $75\text{ mL min}^{-1}$ . Prior to use, stir bars were conditioned in a thermal desorption tube at  $300^{\circ}\text{C}$  for 90 min with helium at  $75\text{ mL min}^{-1}$ . Stir bars were stored in cleaned twister vials (Gerstel) until their use to prevent contamination.

Two different methodologies were used: direct immersion SBSE (DI-SBSE) and headspace-SBSE (HS-SBSE). Using DI-SBSE, the PDMS stir bars were directly immersed in 40 mL screw-cap vials (Supelco, Bellefonte, PA, USA) and the samples were stirred at the selected temperature and time. When HS-SBSE was used, the stir bar was placed in a 20 mL twister-headspace vial with insert (Gerstel), which allows to hold the stir bar in the top of the vial before sealing the vial with a crimp cap. In both cases, the SBSE procedure was carried out by the addition of sodium chloride to water samples. After adsorption step, stir bars were removed from samples, rinsed with milli-Ro water and dried with a lint-free tissue. The twisters were placed in a stainless steel desorption tube and were desorbed in an ATD applying the selected conditions of temperature ( $290^{\circ}\text{C}$ ), time (5 min) and flow desorption ( $75\text{ mL min}^{-1}$ ). Then the analytes were trapped in a cold trap at  $-5^{\circ}\text{C}$  for 5 min and instantaneously desorbed by applying  $290^{\circ}\text{C}$ .

## 2.6. Data evaluation

RTL methodology allowed the creation of a specific GC-MS method, which may be linked to a library of retention times and mass spectra, in order to allow the analyst to screen real samples. In this case, the obtained chromatograms were evaluated using a homebuilt RTLmusk database library, which has been associated to the AMDIS 2.1 (Agilent) deconvolution software for unambiguous identification of the target analytes. The RTLmusk database library must be transferred to an “.msl” extension to obtain a proper RTL-AMDIS association.

All the process is managed by the Deconvolution Reporting Software (Version A. 03.0.84), which results from a combination of the three different software packages: the Agilent GC/MS ChemStation, the homebuilt RTLmusk database library and the automated mass spectral deconvolution and identification system (AMDIS).

A factorial design was provided to evaluate the main parameters affecting the SBSE direct immersion efficiency. The Multivariate Data Analysis and Design of Experiments Package Unscrambler (CAMO Software AS, USA) was used to obtain the relations between chromatographic responses and SBSE parameters.

## 3. Results and discussion

### 3.1. SBSE procedure

Some authors [12,30] have developed and compared immersion and headspace SPME extraction methods to determine musk compounds. However, a comparative study between SBSE direct immersion (DI-SBSE) and headspace-SBSE (HS-SBSE) has not been reported. Prior to apply multivariable design to SBSE extraction parameters, preliminary experiments to decide the extraction mode were carried out. To study the extraction efficiency of both modes (HS and DI-SBSE), two sets of assays with 20 mL of spiked waters at  $60^{\circ}\text{C}$  and at  $90^{\circ}\text{C}$ , in stirring conditions were run, varying the extraction time from 5 to 120 min. Both values of temperature were fixed based on headspace literature [31].

The obtained results revealed that HS-SBSE mode was more efficient than DI-SBSE working at the highest temperature ( $90^{\circ}\text{C}$ ) for all compounds except for nitro musks. The nitro musk family presents no response at this temperature. This behavior can be explained because of an increment of nitro musk solubility in water thus hindering their adsorption in the stir bar [11]. In addition, HS-SBSE requires large extraction times working at low values of temperature to obtain the same extraction efficiency than the direct mode, for this reason DI-SBSE mode was selected for this study.

### 3.2. Experimental designs

The experimental screening Full Factorial Design (FFD) was carried out to identify those factors that had significant effects on the extraction and later to apply other designs such as Central Composite Design (CCD) to obtain the best extraction conditions. For this study, extractions of aqueous standards to a level of concentration  $2\ \mu\text{g L}^{-1}$  were performed. Samples were taken in the direct immersion mode stirring rate sufficient to create the vortex in the solution ( $31.4\text{ s}^{-1}$  for 3 mL to  $125.7\text{ s}^{-1}$  for 30 mL).

Four parameters that could potentially affect the SBSE extraction, such as extraction temperature, extraction time, effect of ionic strength and sample volume were selected. In assessing the effect of temperature, the range was from  $30^{\circ}\text{C}$  up to  $70^{\circ}\text{C}$  due to the limitations of the nitro musk at high temperatures. The extraction time was tested from 15 to 240 min. In order to not increase excessively the total analysis time, the SBSE time was selected without having reached the steady state [20]. The obtained response for every compound exhibited a good reproducibility and it was considered suitable to achieve the required LOD. The influence of the ionic strength was studied by modifying samples containing salt with concentrations ranging from 10% to 30% and the influence of the sample volume was studied in the range from 3 to 30 mL.

The chromatographic response was not significantly affected by the temperature and salting-out effects, except in the case of cashmeran. In agreement with other authors [22], high temperatures showed a decrease in the cashmeran response, so that for next design the extraction temperature was performed for  $30^{\circ}\text{C}$ .

In the case of the ionic strength factor, the adding of 10% NaCl increased the signal of this analyte. So for further experiments it was fixed to 10% NaCl. The measures to establish whether a factor contributes significantly to the response were proposed with the analysis of variance in CCD. The best results of signal measured were obtained with a volume of 30 mL and an extraction time of 240 min. The response obtained for each compound suggested that, using high sample volumes, the signal of non-polar analytes increased, but the time needed to reach equilibrium also increased, while more hydrophilic compounds ( $K_{ow} < 3.5$ ) the signal decreased dramatically when sample volume increased [32]. Some authors [23,33] indicated that, in addition to  $K_{ow}$  parameter, the total amount extracted also depends on the phase ratio, which is the quotient of the volume of the water sample and the volume of the PDMS sorbent. A smaller phase ratio provided practical benefits such as faster equilibration, and a good effectiveness of the extraction in combination with an acceptable extraction time.

After evaluating all these data, an SBSE method was able to be established for the extraction of 18 musks in water samples. The developed method uses direct SBSE at 30 °C for 30 mL of sample containing 10% NaCl, stirring at 125.5 s<sup>-1</sup> for 240 min.

### 3.3. Thermal desorption conditions

The desorption step is accomplished with thermal desorption under helium flow. Desorption temperatures up to 300 °C and desorption flows up to 75 mL min<sup>-1</sup> are not recommended by the manufacturer. To ensure the total desorption of all analytes, the thermal desorption conditions of stir bars located in a stainless steel tube were as follows: temperature at 290 °C for 5 min and helium flow of 75 mL min<sup>-1</sup>. Then the analytes were trapped in a cold trap at -5 °C for 5 min and instantaneously desorbed by applying 290 °C. The transfer line was set to 280 °C guaranteeing the correct transfer of analytes.

Blank runs of twisters were made before and after each analysis and no carry-over effect occurred for the target analytes. In this way, the stir bars and the desorption stainless steel tubes can be used again after each analysis up to 10–15 times, without a washing stage.

### 3.4. Creating a musk retention time locked screener library

Methods based on retention time locking (RTL) are generated from already developed methods to accurately reproduce the retention times in chromatographic development.

To this end, the developed SBSE online ATD coupled RTL–GC–MS method was tested for extraction and measurement of target synthetic musks. Then, the creation of the mass spectral library from samples acquired by the RTL GC/MS method was necessary and finally the conversion of the library to a screening database was carried out.

Prior to validation assays, the proposed method should be correctly locked to ensure unequivocal compound identification in a screening of real samples. GC–MS system was automatically locked using ChemStation's Retention Time Locking software (Agilent) providing a reference value (benchmark) for testing head pressure in the ATD.

Musk ambrette was selected as an appropriate RT locking compound to be easily identifiable and elutes toward the middle of the chromatographic run. Calibration data from RTL runs were obtained by thermal desorption of five twisters with ambrette extracted at five different head pressures. The retention time of the target analyte was determined for each calibration run and the corresponding set of retention times and head pressures were fitted with a polynomial of degree 2. The retention time precision was achieved at a

head pressure of 14.10 psi in ATD unit. This value was determined from the retention time calibration (in ATD) and relocking process to be appropriate to provide the target retention time of 24.06 min for the musk ambrette locking compound.

A spiked water sample containing all target synthetic musks, was analyzed with the method locked for musk ambrette. Using mass spectra, target ion and up to three selected qualifiers, and retention times of each compound (Table 1), a retention time locked library is created and converted to a screening database. This homebuilt database was combined with the AMDIS deconvolution database. Thus, the scan data were analyzed using deconvolution reporting software to identify the compounds in the studied matrices. Fig. 1 shows a full scan chromatogram of an urban wastewater sample (Effluent 4) and the unmodified tonalide mass spectrum (upper) and the deconvoluted tonalide spectrum overlaid with homebuilt library compound spectrum. The software marked tonalide as probable hits and AMDIS qualitative data allowed confirmation.

### 3.5. Method evaluation

Method quality parameters were estimated (Table 2). The linearity of the SBSE–ATD/RTL–GC–MS method developed was evaluated with a set of eight standards containing concentrations ranged from LOQ to 8000 ng L<sup>-1</sup> for all compounds. All levels were spiked with 0.5 µg L<sup>-1</sup> of fenitrothion deuterated as an internal standard. This deuterated compound was used in order to evaluate and, if necessary, compensate the possible variations in real samples extraction procedure. For each level, duplicate analysis under selected conditions were performed. The results obtained, correlation coefficients  $r$  between 0.992 and 0.999 were indicative of excellent linear dynamic ranges.

LOQs were estimated as minimal concentrations that could be quantified with RSD at 25%. LOQs were necessarily obtained in full scan mode due to the use of RTLmusk database library associated to the AMDIS deconvolution software. Even better LOQs could be obtained by operating in selected ion monitoring (SIM) mode [14]. This, however, would lead to losing the structural information necessary for unambiguous identification. In order to check the precision of the proposed method, the RSD was studied in ten replicate samples at LOQ concentration and at ten LOQ concentration levels.

Precision in the chromatographic response was determined in terms of repeatability and reproducibility at a low level (80 ng L<sup>-1</sup>). As can be observed in Table 2 the RSDs obtained ranged from 3.7% to 23.5% and 16.2–27.5% for intra- and inter-day studies, respectively.

The influence of the matrix in real water samples must be evaluated in order to ensure the correct quantification. Because of the impossibility of obtaining blank samples, the samples were previously analyzed using the SBSE–ATD/RTL–GC–MS developed method, to check the presence of the target compounds. In this case, the matrix-matched calibration curves are discouraged, because of high concentrations of some compounds in the water samples and for the variability of the matrix (the extraction yield might drastically change from sample to sample). The best approach to achieve proper quantification would be to use a labelled isotopic standard for each analyte. This is not always possible because of financial constraints and availability of labelled compounds. Therefore, in order to avoid the influence of the matrix, especially in WWPT wastewater, and assuming its existence which has been referenced by many authors [34], the real samples were analyzed using the standard addition method (SAM). The SBSE–ATD/RTL–GC–MS developed method is especially indicated for the automatic screening and unequivocal qualification of the target compounds, which makes quantification easier using SAM and the choice of the corresponding spiking levels for each detected analyte.

**Table 1**  
Locked retention times, qualifier ions and quantification mass (in bold) of the target compounds.

Compound	Locked retention time (min)	Qualifiers ions ( <i>m/z</i> ) (relative abundance, %)
Cashmeran	10.060	<b>191</b> (100), 206 (48), 135 (43), 41 (28)
Helvetolide	18.821	<b>129</b> (100), 57 (75), 139 (41), 210 (10)
Celestolide	18.883	<b>229</b> (100), 244 (43), 173 (21), 230 (17)
Phantolide	20.630	<b>229</b> (100), 244 (24), 230 (18), 43 (11)
Globalide	23.041	<sup>a</sup> <b>238</b> (12), 68 (100), 81 (65), 207 (59)
Romandolide	23.589	<b>55</b> (100), 240 (5), 41 (83), 69 (59)
Thibetolide	23.607	<sup>a</sup> <b>180</b> (44), 222 (25), 240 (15), 138 (100)
Ambrette	24.057	<b>253</b> (100), 268 (40), 254 (12), 91 (35)
Muscone	24.248	<b>238</b> (100), 209 (67), 223 (43), 180 (37)
Traseolide	24.734	<b>215</b> (100), 258 (18), 173 (15), 216 (18)
Galaxolide	24.788	<b>243</b> (100), 258 (20), 213 (24), 155 (17)
Musk xylene	25.326	<b>282</b> (100), 283 (7), 297 (8), 43 (6)
Tonalide	25.390	<b>243</b> (100), 258 (31), 244 (19), 159 (17)
Moskene	26.343	<b>263</b> (100), 278 (8), 264 (13), 128 (14)
Ambrettolide	28.056	<b>67</b> (100), 81 (88), 55 (69), 252 (15)
Tibetene	28.628	<b>251</b> (100), 266 (27), 252 (17), 91 (16)
Musk ketone	30.881	<b>279</b> (100), 294 (29), 280 (15), 43 (67)
Ethylene brassilate	31.992	<sup>a</sup> <b>227</b> (70), 98 (100), 55 (92), 86 (64)

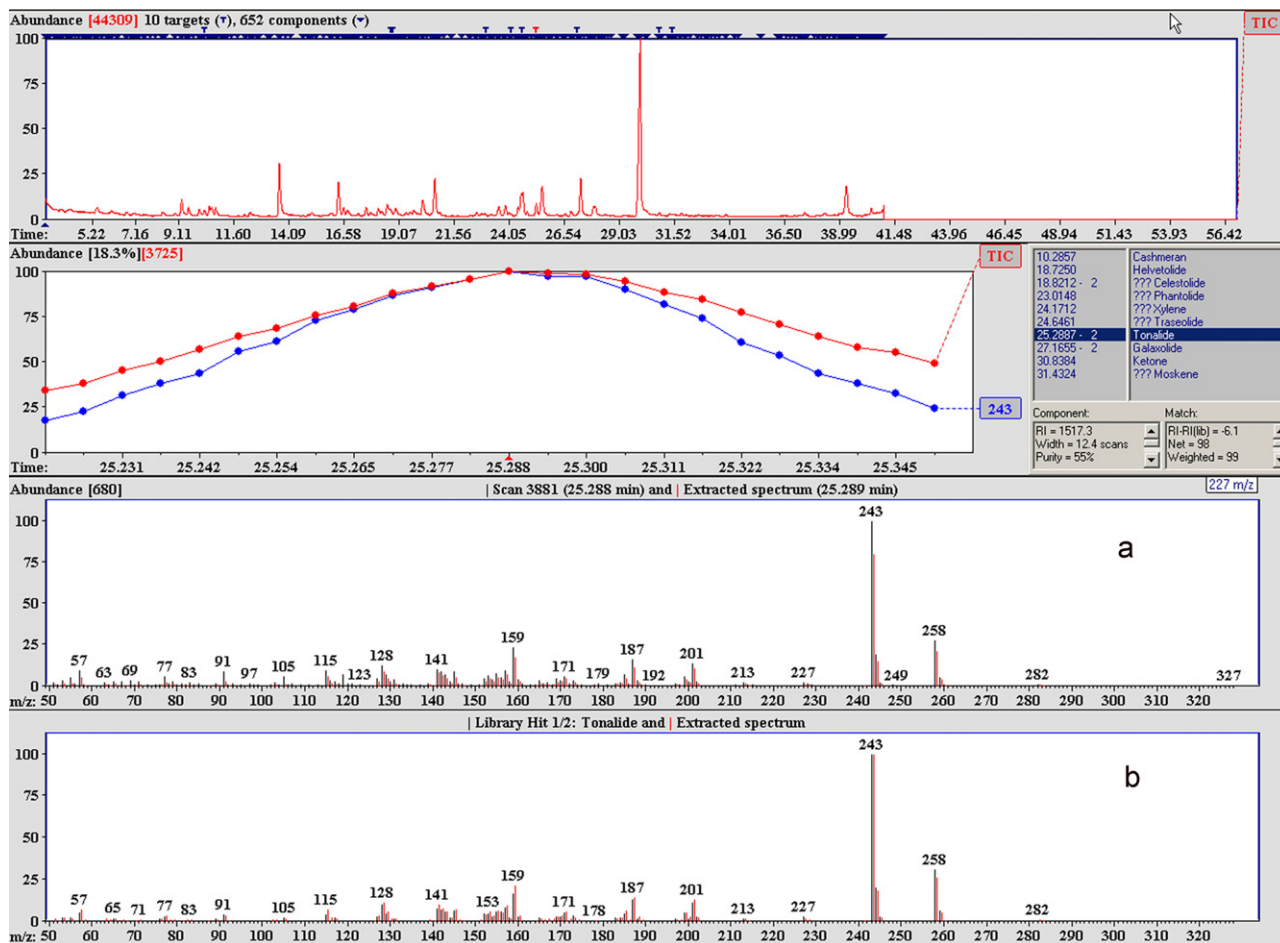
<sup>a</sup> Target ions with abundances less than 100%, in order to differentiate between different qualifications musks.

### 3.6. Screening of synthetic fragrances in natural water and wastewater real samples

An SBSE-ATD/RTL-GC-MS method was applied to 42 surface water samples, 6 groundwater and 10 wastewater samples to check its practicability and feasibility for the screening of synthetic musks. The results obtained confirmed the usefulness of the pro-

posed method for determination and unequivocal identification and quantitation of musks present in water samples.

Chromatograms were evaluated using the homebuilt RTL Musk Database. This software applies two annotations for the possible hits depending on the matching quality [35]. If the match is low, the compound is marked as possible hits with “?”, but if the match is satisfactory, it is indicated as probable hits with “x” (Fig. 2).



**Fig. 1.** Full scan chromatogram of an urban wastewater sample (Effluent 4), and the AMDIS representation of (a) the unmodified tonalide mass spectrum and (b) the AMDIS deconvoluted tonalide spectrum overlaid with homebuilt library compound spectrum.

**Table 2**  
Method quality parameters.

Synthetic fragrances	Correlation coefficient ( <i>r</i> )	LOQ (ng L <sup>-1</sup> )	Repeatability RSD % ( <i>n</i> = 5)	Reproducibility RSD % ( <i>n</i> = 5)
Cashmeran	0.998	20	9.6	25.9
Helvetolide	0.993	10	21.7	26.1
Celestolide	0.996	20	3.7	19.4
Phantolide	0.999	5	9.4	27.5
Globalide	0.999	80	22.4	24.4
Romandolide	0.992	40	11.5	22.0
Thibetolide	0.993	80	20.4	25.4
Ambrette	0.997	80	21.2	23.5
Muscone	0.997	80	11.0	23.8
Traseolide	0.996	20	23.5	27.0
Musk xylene	0.992	10	22.6	16.2
Tonalide	0.997	5	18.6	20.6
Galaxolide	0.994	80	23.0	24.7
Moskene	0.994	80	23.3	26.6
Ambrettolide	0.996	80	14.2	24.3
Tibetene	0.996	80	8.6	23.5
Musk ketone	0.993	20	18.9	24.7
Ethylene brassilate	0.999	20	17.5	24.0

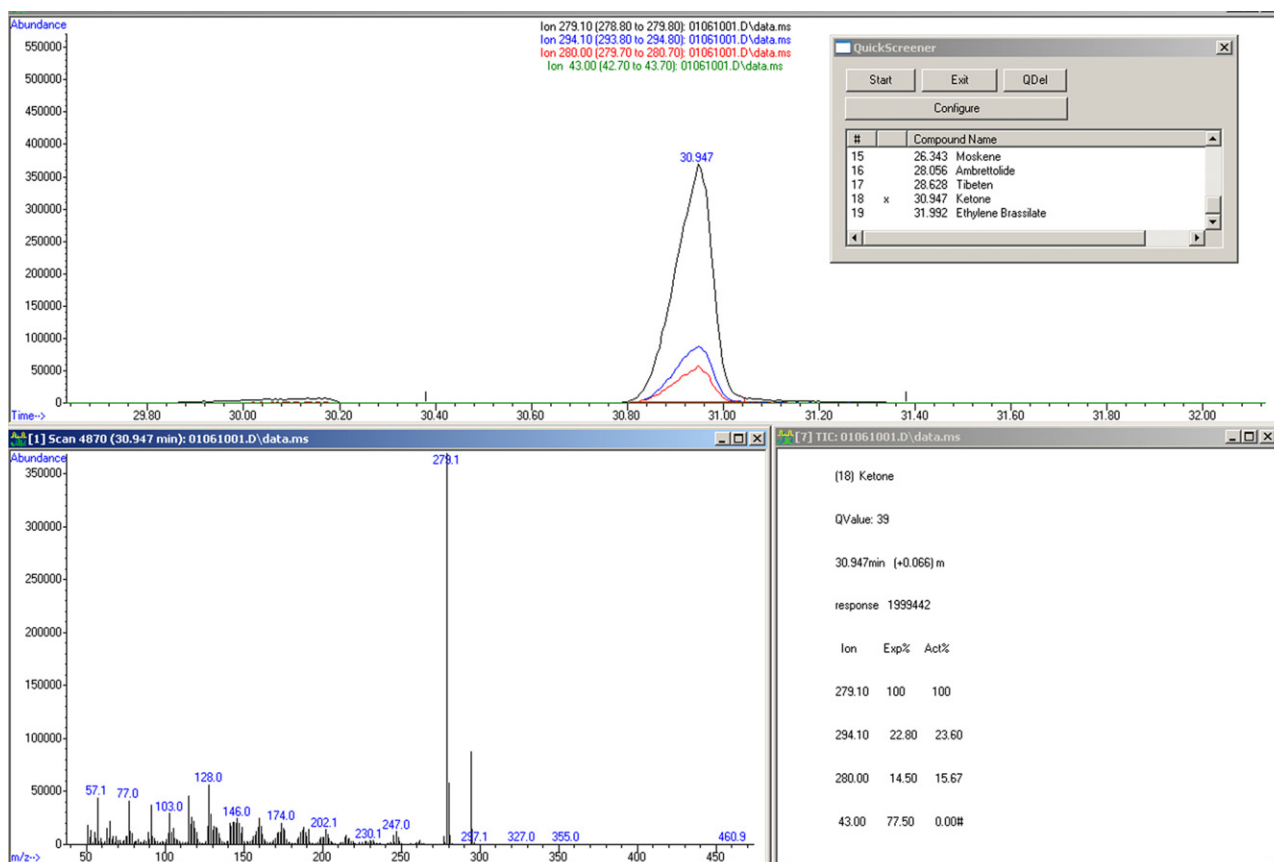
The quantitative data results for the positive identifications were calculated using the SAM (Tables 3 and 4). For this, positive samples were fortified with the working standard mixture selected to produce the corresponding spiking level for each identified compound.

Ten different musks of the four different families of synthetic fragrances were detected in real samples, of which galaxolide was found in all the samples analyzed. The Alegría river presented five musks with concentrations ranging from 41 ng L<sup>-1</sup> for celestolide, to 2544 ng L<sup>-1</sup> for thibetolide. Cashmeran and galaxolide polycyclic musks were present in groundwater in the same range of concentrations found in surface waters. This may indicate that musks

present in groundwater samples may be related to natural recharge of the aquifer for the Alegría river.

Eight musks were detected in the influent and effluent urban wastewaters from the WWTP. Galaxolide was the main musk found with concentrations varying from 689 to 3568 ng L<sup>-1</sup>, cashmeran and ketone were the other compounds found in all wastewater samples although their levels of concentration were lower than galaxolide. These results showed that the elimination in the WWTP of galaxolide, cashmeran and ketone is not effective, which matches with the results obtained by other authors [36,37].

The wide variation of concentrations of musks in different WWTPs might depend on usage, amount of fragrance materials,



**Fig. 2.** RTL selected ion chromatogram of musk ketone found in an urban wastewater sample (Effluent 4) and the experimental and reference mass spectra.

**Table 3**  
Occurrence of synthetic fragrances in superficial and groundwaters (units in ng L<sup>-1</sup>).

	January 2009			February 2009		May 2009		Thibetolide	July 2009		January 2010			July 2010			
	Galaxolide	Cashmeran	Romandolide	Galaxolide	Cashmeran	Galaxolide	Cashmeran		Galaxolide	Cashmeran	Galaxolide	Cashmeran	Thibetolide	Celestolide	Romandolide	Galaxolide	Romandolide
Alegria river																	
A0	353	n.d.	n.d.	291	n.d.	517	350	178	241	318	538	n.d.	n.d.	n.d.	n.d.	835	n.d.
A1	790	n.d.	n.d.	257	n.d.	308	n.d.	n.d.	273	589	929	368	n.d.	41	n.d.	220	271
A2	482	n.d.	n.d.	n.d.	277	342	342	n.d.	226	358	n.d.	1377	2544	63	n.d.	804	n.d.
A3	424	454	n.d.	n.d.	n.d.	543	n.d.	181	309	539	2184	n.d.	228	96	73	376	n.d.
IL	419	n.d.	306	294	n.d.	359	n.d.	n.d.	1197	595	1394	476	n.d.	n.d.	n.d.	243	n.d.
J1	414	424	n.d.	n.d.	279	389	302	n.d.	n.d.	340	n.d.	n.d.	n.d.	n.d.	n.d.	1226	n.d.
J2	430	455	n.d.	n.d.	n.d.	518	365	220	244	337	653	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Groundwater <sup>a</sup>																	
1						338	573										
2						270	424										
3						295	438										
4						287	413										
5						n.d.	354										
6						310	530										

<sup>a</sup> Groundwater samples were sampled at a single sampling campaign (May 2009).

**Table 4**  
Occurrence of synthetic fragrances in urban wastewaters (influent and effluent of a WWTP). Samples were collected on five days in October 2009. The rest of the musks were not detected in any sample (units in ng L<sup>-1</sup>).

	Galaxolide	Cashmeran	Romandolide	Celestolide	Musk xylene	Tonalide	Helvetolide	Musk ketone
Influent 1	2580	370	n.d.	n.d.	50	57	n.d.	58
Influent 2	3568	438	45	n.d.	40	n.d.	58	60
Influent 3	900	70	n.d.	68	n.d.	79	n.d.	59
Influent 4	1150	470	n.d.	70	n.d.	57	45	61
Influent 5	3543	530	n.d.	n.d.	91	74	n.d.	59
Effluent 1	906	400	n.d.	60	31	24	21	67
Effluent 2	3021	350	56	n.d.	45	n.d.	n.d.	41
Effluent 3	800	100	n.d.	40	n.d.	60	n.d.	55
Effluent 4	1341	400	n.d.	80	n.d.	59	70	69
Effluent 5	689	250	n.d.	n.d.	n.d.	n.d.	n.d.	32

size of treatment plant, the population served, the types of wastewater (domestic, industrial and commercial), as well as the treatment technique [38]. Some musks showed effluent concentrations higher than the influents, which could be explained either by inaccurate sampling or the erroneous estimate of hydraulic retention time [10,36,39].

#### 4. Conclusions

The direct SBSE–ATD/RTL–GC–MS method can be used to detect 18 synthetic musks providing a good effectiveness in identifying and quantifying synthetic fragrances in natural and wastewater samples. The proposed method minimizes laborious sample preparation procedures and requires low-sample volume, shows excellent linear dynamic range and detection limits at the  $\text{ngL}^{-1}$  levels using full-scan mode with MS-detector.

River water samples presented five musks, being thibetolide the most abundant one. Cashmeran and galaxolide polycyclic musks were present in groundwater in the same range of concentrations found in surface waters. Eight musks were detected in the influent and effluent urban wastewaters from the WWTP. Galaxolide was the main musk found and their elimination in the WWTP seemed to be non effective.

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