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New developments in the analysis of fragrances and earthy–musty compounds in water by solid-phase microextraction (metal alloy fibre) coupled with gas chromatography–(tandem) mass spectrometry

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ABSTRACT

Fragrances are widespread aquatic contaminants due to their presence in many personal care products used daily in developed countries. Levels of galaxolide and tonalide are commonly found in surface waters, urban wastewaters and river sediments. On the other hand, earthy–musty compounds confer bad odour to drinking water at levels that challenge the analytical capabilities. The combined determination of earthy–musty compounds and fragrances in water would be a breakthrough to make the traditional organoleptic evaluation of the water quality stricter and safer for the analyst. Two approaches were attempted to improve the analytical capabilities: analyte pre-concentration with a newly developed PDMS-DVB solid-phase microextraction fibre on metal alloy core and sensitive detection by tandem mass spectrometry (MS/MS). The optimization of SPME parameters was carried out using a central composite design and desirability functions. The final optimum extraction conditions were: headspace extraction at 70 °C during 40 min adding 200 g L^{-1} of NaCl. The detection limits in tandem MS (0.02–20 ng L^{-1}) were marginally lower compared to full scan except for geosmin and trichloroanisol which go down to 0.1 and 0.02 ng L^{-1}, respectively.

The analysis of different water matrices revealed that fragrances and earthy–musty compounds were absent from ground- and drinking waters. Surface waters of river Leça contained levels of galaxolide around 250 ng L^{-1} in the 4 terminal sampling stations, which are downstream of WWTPs and polluted tributaries. Geosmine was ubiquitously distributed in natural waters similarly in rivers Leça and Douro at concentrations <7 ng L^{-1}.

1. Introduction

Fragrances are synthetic chemicals widely employed in developed countries to improve the olfactory properties of many personal care products (soaps, detergents, deodorants, perfumes and creams) [1–3]. There are two main groups of synthetic fragrances: polycyclic musks (the most consumed being galaxolide (GAL) and tonalide (TON)) and nitroaromatic musks (mainly musk xylene (MX) and musk ketone (MK)) [1]. Especially the polycyclic musks are used in huge quantities so their residues may easily reach the water compartments via domestic wastewaters [4,5]. Typically, these substances are very lipophilic and hardly degraded in the aquatic environment [1,6–8]. Nevertheless, the simple feature of being continuously discharged into the aquatic media explains their environmental significance and supports their inclusion in the group of emerging pollutants. The use of some fragrances was prohibited due to their potential to bioaccumulate in the fat tissue of wildlife and their endocrine disruptive activity [9,10]. Neurotoxicity has also been reported [2]. The Oslo and Paris Commission (OSPAR) has included musk xylene in the list of priority substances [2,11,12]. In the preparatory stage of the Directive “Environmental Quality Standards in the field of Water Policy” the European Union has evaluated four fragrances as candidate priority substances [9]; however the final version (Directive 2008/105/EC) only includes musk xylene in Annex III [13].

On the other hand, earthy–musty compounds bestow bad odour to drinking water at excessively low levels (at sub- or a few ng L^{-1}) [14–16]. Additionally, they are difficult to remove by conventional chemical drinking water treatment [17,18]. The most important earthy–musty compounds are: geosmin (GSM), isoborneol (IB) and 2-methylisoborneol (MIB) produced by Actinomycetes bacteria, fungi and algae; and 2,4,6-trichloroanisole (TCA) formed by biomethylation of trichlorophenol [14,15,19]. Some evidences indicate that GSM and MIB can lead to mutagenicity and hepatotoxicity [20].

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Several authors have reported the presence of fragrances in surface waters, urban waste waters and river sediments [2,3,7,21–24]. Polycyclic musks occur at concentrations up to several $\mu g L^{-1}$ while the nitroaromatic musks are present in the $ng L^{-1}$ range. Earthy–musty odours have been found in surface and drinking waters in concentrations of a few $ng L^{-1}$ [16,18,25,26].

The combined determination of earthy–musty compounds and fragrances in water would be highly convenient since it could replace the traditional and highly subjective organoleptic evaluation of water quality, besides avoiding risks for the operator. The available methods focus only in one of the groups [1,4,6–8,14,15,20,25], therefore joint determination would also significantly reduce the analysis time and costs.

Two approaches were used to improve the analytical capabilities of the proposed method based on solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS): a recently developed polydimethylsiloxane-divinylbenzene (PDMS-DVB) SPME fibre on metal alloy core was evaluated as preconcentration media and a highly selective and sensitive detection by tandem mass spectrometry (MS/MS) was adopted. The metal alloy fibres are more mechanical resistant than the fused-silica making them last longer [27]. The PDMS-DVB adsorbent also has different thickness and diameter compared to the conventional fibres. Setkova et al. have investigated the reproducibility of different coatings on the metal alloy and conventional cores for the analysis of pump oil samples (benzene, 2-pentanone, 1-nitropropane, pyridine and toluene). The metal alloy fibres were generally more precise, the durability went beyond 200 extraction cycles without any significant loss in sensitivity but no comparison of the extraction efficiency and selectivity was given [27].

The developed method was optimised through an experimental design approach and multiresponse evaluation and then applied to the analysis of several water samples with the aim to screen odours and fragrances in as much as possible water sources (ground waters, surface waters, waste water and drinking waters).

2. Experimental

2.1. Chemicals and reagents

Isoborneol, 2-methylisoborneol, geosmin, 2,4,6-trichloroanisole, musk xylene and musk ketone standards were purchased from Sigma–Aldrich (Madrid, Spain). Galaxolide and tonalide were purchased from LGC Standards (Barcelona, Spain). Their chemical structure, molecular formula and important physico-chemical parameters for the SPME process are given in Table S1, as supplementary material. Individual stock standard solutions were prepared in methanol and stored at −18 °C protected from light. A mixture solution at $1 mg L^{-1}$ was then obtained. Daily working solutions were prepared in ultra-pure Milli Q water (Millipore, Molsheim, France). Sodium chloride p.a. was used to adjust the ionic strength of aqueous standards and samples.

2.2. Solid-phase microextraction

Six SPME fibres on conventional fused silica core supplied by Supelco (Bellefonte, PA, USA) were tested: 85 μm polyacrylate (PA), 100 μm polydimethylsiloxane (PDMS), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS), 60 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm PDMS/DVB and 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Two additional fibres on metal alloy core were compared for their analytical advantages: 65 μm PDMS/DVB and 85 μm CAR/PDMS. The fibres were conditioned in the GC injector as recommended by the manufacturer. The samples (10 mL) were placed in headspace vials of 20 mL capacity. The extraction was carried out at a stirring rate of 250 rpm. The extraction time, temperature and ionic strength were optimised through a multifactorial design to reach a compromise of extraction efficiency and practicality for both chemical families.

2.3. Gas chromatography–mass spectrometry analysis

Chromatographic analyses were carried out in a Varian CP 3800 (Walnut Creek, CA, USA) gas chromatograph equipped with a fused-silica capillary column coated with 5% diphenylmethylsiloxane, VF-5 MS (30 m × 0.25 mm I.D., 0.25 μm film thickness) from Varian. High-purity helium (99.9999%) at a constant flow rate of $1 mL min^{-1}$ was used as the carrier gas and also as the collision gas at the ion trap chamber. Samples were analysed using the following oven temperature programme: initial temperature 60 °C, increased by $40 °C min^{-1}$ to 100 °C (held for 1 min), increased by $15 °C min^{-1}$ to 200 °C, increased by $1 °C min^{-1}$ to 205 °C and finally increased by $40 °C min^{-1}$ to 260 °C. The analytes were desorbed from the fibre at an injector temperature of 250 °C for 2 min in splitless mode followed by 3 min in split mode. Compounds were detected by a 4000 GC–MS ion trap mass spectrometer from Varian Instruments (Walnut Creek, CA, USA) operated in full scan and MS/MS modes. The trap and transfer line temperatures were set at 180 and 280 °C, respectively. The emission current of the ionisation filament was set at 50 μA. The electron multiplier voltage was 1400 V. The GC–MS system was mounted with a CombiPal autosampler (CTC-Analytics, AG, Switzerland) that allowed controlling all SPME variables.

2.4. Multifactorial design

A multifactorial experimental design – central composite design (CCD) – was employed with multiple objectives: to evaluate the main variables that affect the extraction of earthy–musty odours and fragrances; to disclose interactions between variables; to determine the optimum extraction conditions; and to evaluate the robustness of the method in the vicinity of that optimum. The CCD was established for the following variables: extraction time, temperature and ionic strength at 5 levels for each factor ($-\alpha$, −1, 0, +1, $+\alpha$, with $\alpha = 1.6818$). The experimental conditions evaluated were: 10, 22.16, 40, 57.8 and 70 min of extraction time; 30, 42.4, 60, 77.8 and 90 °C for temperature and 0, 40.54, 100, 159.46 and $200 g L^{-1}$ of NaCl concentration for ionic strength. The border values were chosen taking into account previous studies in the literature. The CCD was run with genuine duplicates for each experiment. The experiments were divided in 3 blocks with 2 central points in each block, in a total of 34 experiments. For optimizing the response of multiple dependent variables (8 compounds) we have used a desirability function considering a linear and “the highest the best” desirability factor. A quadratic model for fitting desirability surfaces/contours was employed. The data was previously normalized to the maximum of each compound along the experimental series (by variable). The experimental design was produced and analysed using the programme Statistica 6.0 (Statsoft, Tulsa, USA).

3. Results and discussion

3.1. Detailed optimisation of the chromatographic separation and detection

The initial experiments were performed with the aim to obtain full resolution of the analytes. Chromatographic separation and quantification of galaxolide and tonalide is particularly challenging since the two compounds have the same molecular weight (Mr 258.403) and some common important fragment ions (m/z

Table 1
Chromatographic and detection parameters in full scan and MS/MS modes.

Compound	Retention time (min)	Full scan	MS/MS			
		Quantification ion (m/z)	Segment (min)	Parent ion (m/z)	Quantification ion (m/z)	CID excitation amplitude (V)
IB	4.000	95	3.0–4.8	95	67	40
MIB	4.281	95				
TCA	5.380	197	4.8–5.8	197	167 + 169	80
GSM	6.182	112	5.8–8.0	112	97	40
GAL	9.900	213	8.0–10.8	213	171	1[a]
MX	9.920	282		282	161	94
TON	9.990	159		159	131	62
MK	11.629	279	10.8–15.0	279	160	96

[a] Resonant excitation mode. The remaining are in non resonant excitation mode.

243, 213, 187), despite their rather different structure. Thus, a very smooth increase of the column temperature was attempted giving a satisfactory analyte separation. The quantification ions in full scan mode were selected as presented in Table 1, to minimize interferences.

MS/MS was also used in order to achieve better sensitivity and selectivity. To achieve these goals, it is required to adjust the detector operation conditions for each analyte, in particular the selection of the parent ion, RF storage voltage and collision induced dissociation (CID) voltage. The final instrumental conditions are displayed in Table 1. Although typically MS/MS allows quantifying separately coeluted analytes, galaxolide and tonalide have common MS/MS fragment ions, just differing in intensity. Therefore, separate quantitation of both compounds relies mostly on chromatographic resolution although MS/MS detection allows minimizing mutual interference. Tonalide interference in galaxolide response is 8.86% in full scan further reduced to 0.86% in MS/MS mode. This strategy does not produce any benefits in the case of tonalide since galaxolide always interfere about 8–9%. Nevertheless, reasonable chromatographic resolution of the two compounds was achieved with the proposed GC conditions ($\alpha = 1.0$).

3.2. SPME fibres evaluation

Firstly we have evaluated the extraction performance of 6 fibres with different coating. The headspace was let equilibrate with the sample at the extraction temperature for at least 20 min. As shown in Fig. 1, the best coating for the overall compounds was the PDMS/DVB (best for 5 out of 8 analytes). Analysing each group individually, the earthy–musty odours are better extracted with the DVB/CAR/PDMS fibre, except TCA, while the fragrances can be analysed either with the PDMS/DVB or PDMS fibres. The results are in agreement with the feature that fragrances are more lipophilic ($\log K_{\mathrm{ow}}$ 4.3–5.9) than the earthy–musty odours, therefore they are better extracted with more apolar coatings conversely to the latter that have higher affinity for an intermediate polarity coating. The aim of this work was to analyse all compounds at once, therefore we selected the PDMS/DVB fibre to continue our study. It showed also the best precision (average RSD 8.8%). For the same coating (PDMS/DVB) there are 3 different types of fibres available on the market: 60 μm PDMS/DVB (fused silica), 65 μm PDMS/DVB (fused silica, FS) and 65 μm PDMS/DVB (metal alloy, MA), so they were directly compared to conclude about their efficiency in the analysis of the target analytes. The results are displayed in Fig. 2. The new fibre with metal alloy core proved to be similar or better than the remaining. It is the most consistent across the two groups of substances although significant differences are only seen individually to any one of the other fibres (6 out of 8 cases). This fact can be explained in part by its structural features since it has a bigger coating volume (0.440 vs 0.418 mm^3 of the PDMS/DVB 65 μm FS) as well as a larger diameter (0.270 vs 0.260 mm), due to its internal metallic core. Nevertheless, the special HPLC fibre of 60 μm has the biggest coating volume (0.459 mm^3) and the largest diameter (0.290 mm) and in some instances rivalled with the PDMS/DVB metal alloy fibre, despite its thinner coating.

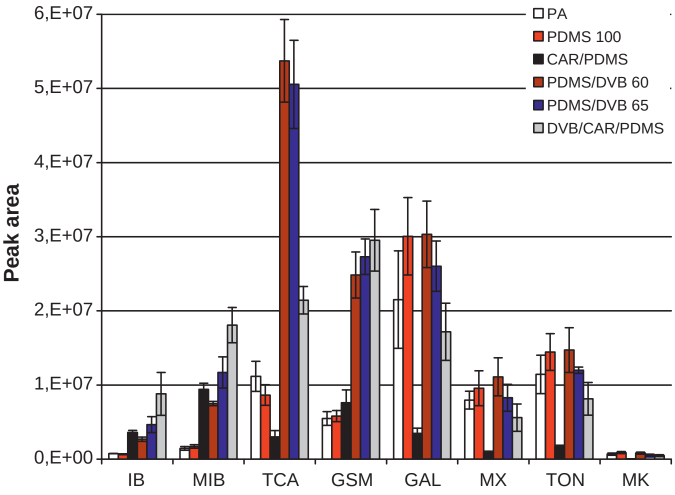

Fig. 1. Comparison of 6 SPME fibres of different coating nature and thickness for the extraction of earthy–musty odours and fragrances in water. The error bars represent the confidence interval ($\alpha = 0.05$) of the mean with $n = 4$.

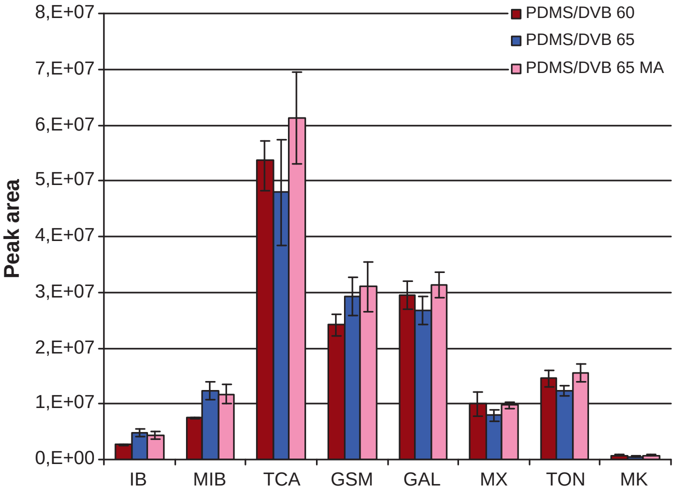

Fig. 2. Direct comparison of the 3 PDMS/DVB fibre types available on the market including the new metal alloy core. The error bars represent the confidence interval ($\alpha = 0.05$) of the mean with $n = 6$.

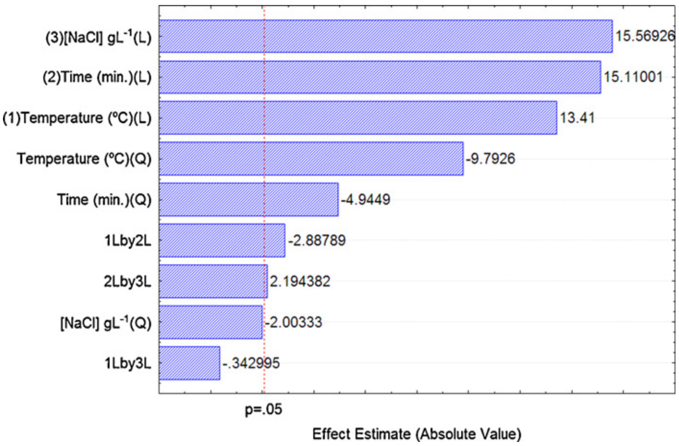

Fig. 3. Pareto chart of standardized effects taking into consideration the joint analysis of fragrances and earthy–musty odours (3 factors, 3 blocks, 34 runs; MS residual = 0.0910077).

The metal alloy fibres are advised to be used with mechanic septa in the injector as the Merlin microseal. Although more costly, it eliminates the presence of septa debris in the liner that forces to periodic maintenance and is source of chromatographic abnormalities. The flexibility of these fibres is remarkable nevertheless, the adsorbing section cannot be seriously injured with the risk of damaging the coating. The PDMS/DVB metal alloy fibre fits best the method's purpose thus it was selected for further optimization.

3.3. Optimisation by multifactorial design

After the selection of the best SPME fibre for the simultaneous analysis of fragrances and earthy–musty compounds, a central composite experimental design was implemented and analysed with desirability functions to determine the optimal values of three variables: extraction time, temperature and ionic strength. Other variables such as pH, stirring rate, sample volume and extraction mode were not considered important for the analysis of non-ionisable and volatile compounds. The experiments and calculations were conducted as described in Section 2. To assess the statistical significance of the variables two estimates of the analytical error could be considered: MS residual and MS pure error. The MS residual was used since it represented the worst case scenario. The Pareto chart of main effects presented in Fig. 3 allows visualizing the statistical significance ($p = 0.05$) of the variables and their relative importance in the extraction process, analysing their linear (L) and quadratic (Q) effects as well as the interaction between the variables. Considering all the compounds under study, the Pareto chart shows that the ionic strength (L), the time (L and Q) and the temperature (L and Q) have a significant effect in the SPME extraction efficiency. The interactions between the variables: temperature and time; and time and ionic strength are also significant, which means that particularly extraction time can be reduced if the temperature is raised. Ionic strength has the highest influence in SPME efficiency, followed by time and temperature (linear effects). The Pareto chart also shows that the temperature has a negative quadratic effect on the extraction, meaning that its beneficial role diminishes as temperature is increased. This is in perfect accordance with the SPME concepts since temperature has the double effect of raising the vapour pressure of the analytes, which is favourable, but it also decreases their partition coefficient to the fibre coating, which is counter productive [28]. A compromise should be found. Likewise the temperature, extraction time also has a negative quadratic behaviour which is due to the equilibrium process that characterizes SPME [28].

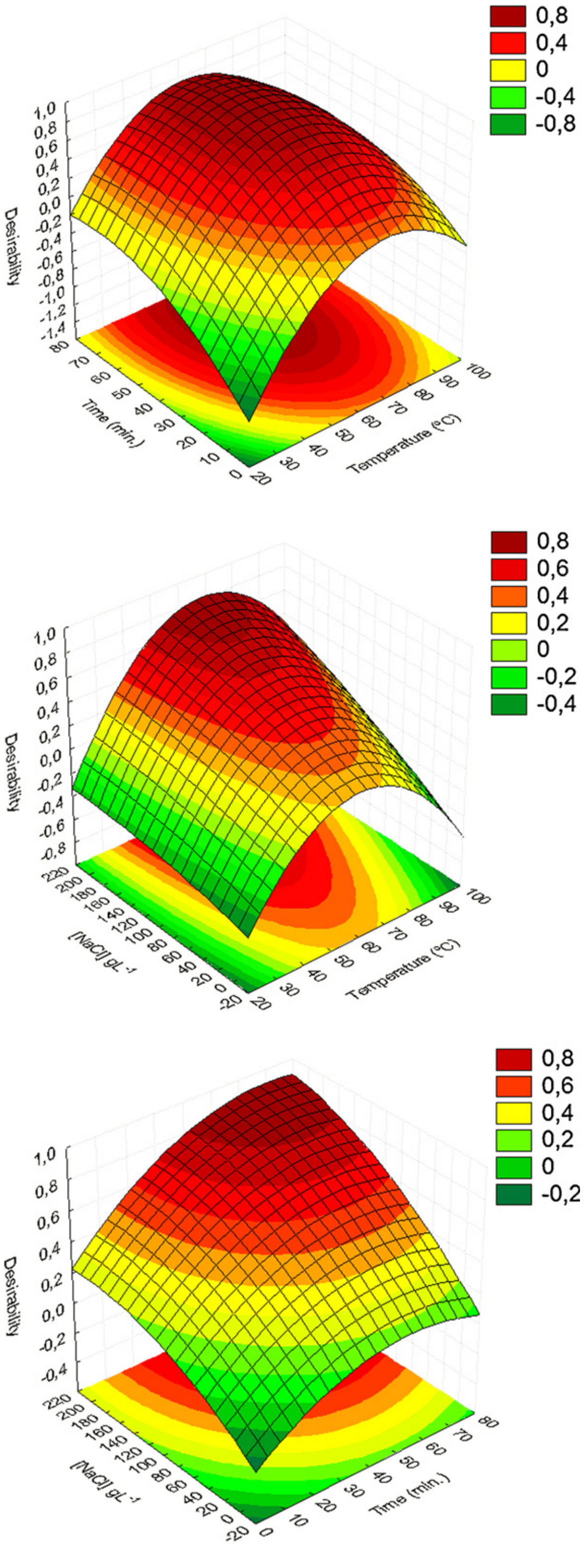

Fig. 4. Desirability surfaces of the three factors under study considering the analysis of all compounds. The third factor in the surface representation is placed at the optimum value.

After the confirmation that the three factors have an important impact in the extraction efficiency, we have inspected the response surfaces (desirability surfaces) to locate the optimum coordinates of these variables (see Fig. 4). [NaCl] has a positive nearly linear effect on the extraction efficiency thus we have established the ionic strength at its highest value of 200 g L^{-1}. Since the

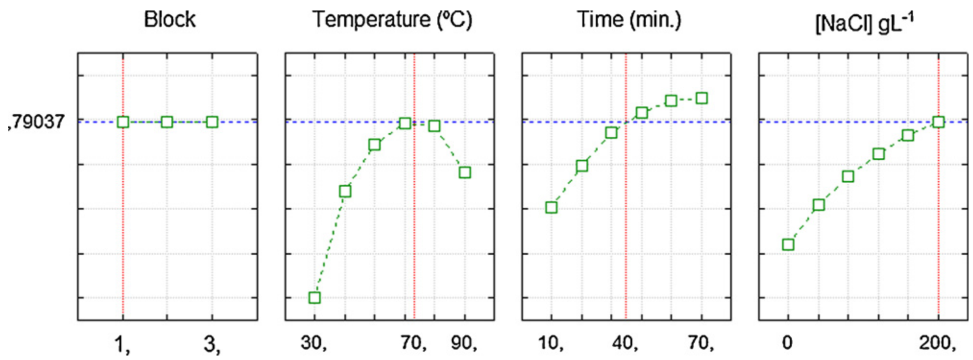

Fig. 5. Profiles of predicted values according to the variables and trace of the selected optimum extraction conditions (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

chromatographic run only takes 15 min, ideally the extraction time should not be longer than that, this way the throughput of the method is maximized. From the desirability surface of time vs temperature depicted in Fig. 4 it can be seen that to maximize the response the extraction should last for at least 40 min. The corresponding temperature for this setting is 70 °C. This way, the best conditions for the analysis of fragrances and earthy–musty compounds were established as follows: PDMS-DVB metal alloy fibre extracting the analytes during 40 min at 70 °C with 200 g L^{-1} [NaCl]. The desirability profiles of the tested variables presented in Fig. 5 confirm that the values chosen allow the highest extraction efficiency, except the extraction time which, as already mentioned, was balanced with productivity considerations. The selected conditions compared to the strictly optimum conditions proposed by the model (70 min, 68 °C and 200 g L^{-1} NaCl) entail an efficiency loss not higher than 4.4% (desirability of 0.79 vs 0.83) but allow almost the double productivity. The burden of combined analysis, earthy–musty compounds and fragrances, is 17%. Fig. 5 also shows that the method is robust to small variations in extraction temperature close to the optimum but extraction time and ionic strength should be precisely controlled. Analysing the graph of predicted vs. observed values (Fig. S1) it is possible to conclude that the model showed very good adequacy to the experimental results. The intercept does not statistically differ from zero, at the 95% confidence level, and the slope does not statistically differ from one, with a good determination coefficient. Also, the errors have a normal distribution proving that they are simply random (see Fig. S2). Both these aspects apportion confidence to the conclusions extracted from the central composite model.

The experimental design adopted in this study is also very versatile; it allows determining the optimum experimental conditions for the analysis of fragrances and earthy–musty odours individually, should anyone be interested in it, or just to infer about their different behaviour under SPME. The Pareto chart of main effects (Fig. S3) shows that ionic strength (L), time (L and Q) and temperature (Q) affect significantly the extraction of earthy–musty odours. These compounds benefit from the salting out effect, so the [NaCl] should also be adjusted to 200 g L^{-1}. The analysis of the desirability surface of temperature vs time (Fig. 6A) reveals that these compounds are better extracted at lower temperatures. Adopting an equal extraction time of 40 min, the optimum temperature is about 50–60 °C. Performing simulations of the analytical response we concluded that 50 °C should be used (8% efficiency loss) and temperatures above 60 °C already give place to a notable decrease in the response. The volatile character of these compounds is clearly reflected in the shape of the response surface since the temperature has essentially a quadratic behaviour with a maximum at a moderate temperature.

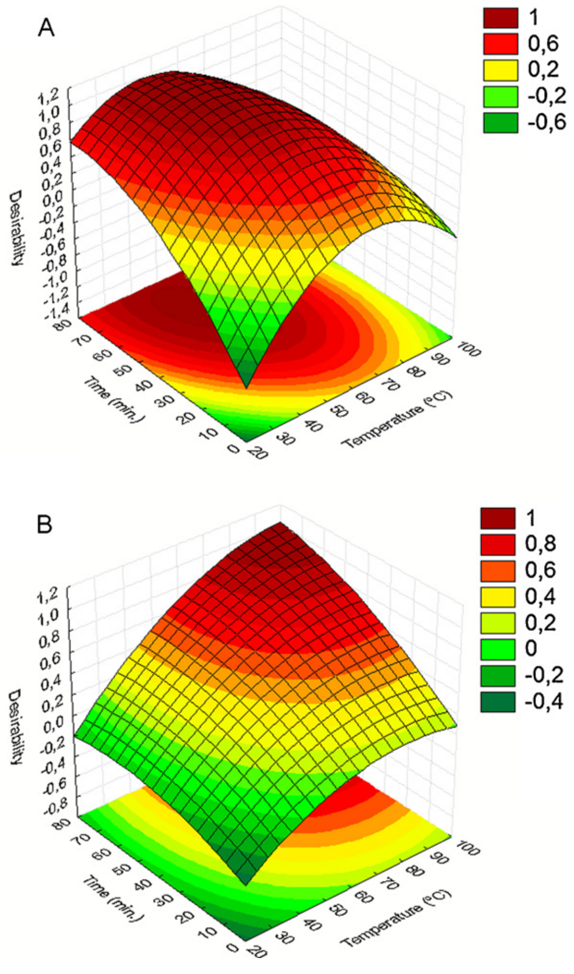

Fig. 6. Desirability surfaces of temperature (°C) vs time (min) with NaCl concentration adjusted to 200 g L^{-1}, considering individually the earthy–musty compounds (A) and the fragrances (B).

Regarding the analysis of fragrances (Fig. S4), all factors have a significant influence in the extraction by SPME, ranked in the following order: temperature (L), time (L), [NaCl] (L), temperature (Q), time (Q) and [NaCl] (Q). The linear effects are all positive whereas the quadratic are smaller and negative. No significant interaction between the three variables is observed. The response surface of time vs [NaCl] is similar to the previous ones, so the value of [NaCl] was settled to 200 g L^{-1}. The desirability surface of the temperature vs time (Fig. 6B) shows that the optimal response is obtained with the highest values for the two parameters (90 °C and 70 min). Since to reach the equilibrium a long extraction time is required

Table 2
Validation data: limits of detection (LOD) and quantification (LOQ), repeatability and intermediate precision, and linearity of the method running in full scan and MS–MS modes.

Compound	LOD S/N = 3 (ng L^{-1})		LOQ S/N = 100 (ng L^{-1})		Repeatability (n = 6, RSD %)		Intermediate precision (n = 18, RSD %)		Determination coefficient (r^2)	
	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS	Full scan	MS/MS
IB	5	4	17	13	12.3	9.4	11.2	9.9	0.993	0.995
MIB	8	20	26	66	14.3	9.3	17.1	21.1	0.992	0.992
TCA	2	0.02	7	0.07	13.1	10.0	13.3	11.1	0.996	0.991
GSM	2	0.1	7	0.3	6.4	5.9	10.2	9.6	0.996	0.996
GAL	10	2	33	7	9.4	11.3	12.6	30.7	0.998	0.989
MX	4	2	13	7	9.0	8.4	11.7	18.5	0.991	0.991
TON	3	4	10	13	10.7	9.3	17.1	16.8	0.997	0.987
MK	5	3	17	10	14.5	19.6	19.5	23.6	0.991	0.995

we have chosen 40 min and the forecasted efficiency loss is 22%. Once again the shape of the response surfaces is modelled by the physico-chemical properties of the fragrances relevant for SPME. Despite their volatility, fragrances are characterized by high apolarity (log K_{ow} 4.3–5.9) and higher molecular weight (MW 258–297) thus, raising the extraction temperature is required to increase their vapour pressure and favour the headspace SPME extraction. Conversely, the earthy–musty compounds are much more polar thus the ionic strength of the sample has an important role to promote their affinity to the extraction coating.

From the discussion above it is clear that balanced extraction conditions are required to analyse this set of odorous compounds in water by SPME, both in the polarity of the fibre coating (the best of which is PDMS-DVB) and the temperature of the sample. Besides being theoretically a more judicious design, the adopted optimisation methodology surely was faster than the univariate approach [29] since just 34 experiments were needed in 3 blocks.

3.4. Validation of the method

To assess the fitness for purpose of the developed method for the analysis of fragrances and earthy–musty compounds we have determined the following figures of merit: detection (LODs) and quantitation limits (LOQs), repeatability, intermediate precision, calibration parameters and matrix effect. The method validation was carried out at the optimal conditions determined previously: 40 min of headspace extraction with a PDMS-DVB metal alloy fibre at 70 °C and 200 g L^{-1} NaCl concentration. Typical chromatograms of a blank and a standard at 50 ng L^{-1} level are shown in Fig. S5. As can be seen, blank subtraction is needed for the quantification of GAL since there is an interference that could never be eliminated, prejudicing also the respective LOD.

The precision was evaluated as repeatability, performing repeated determinations on the same day (n = 6), and as intermediate precision (n = 18), extending the analysis for three different days. The concentration used in these assays was 0.1 μg L^{-1} in both acquisition modes. The results can be seen in Table 2. The repeatability in both modes is quite good, with values of relative standard deviation (RSD) between 5.9 and 19.6%. The repeatability in MS/MS is slightly better, except for galaxolide and musk ketone. The intermediate precision is also typical of an SPME procedure with RSD values ranging from 10.2 to 19.5% (14.1% on average) in full scan mode and between 9.6 and 30.7% (17.7% on average) in MS/MS mode. In MS/MS mode the RSDs for earthy–musty odours are noticeably better than for fragrances. At least in part, this aspect can be explained by the fact that three fragrances are monitored in the same acquisition segment, because of their very close retention times, this way precision is prejudiced due to the lower number of points defining the chromatographic peak.

The LODs were calculated based on a signal-to-noise ratio equal to three, latter on confirmed analysing low concentrations of the analytes. In full scan mode the LODs for earthy–musty odours vary between 2 and 8 ng L^{-1}, while for fragrances they go from 3 to 10 ng L^{-1}. In MS/MS mode the LODs are lower, except for 2-methylisoborneol and tonalide. It should be pointed out that the LODs for geosmin and trichloroanisole in MS/MS mode are in the range of sub-ppt, respectively 20 and 100 times lower than in full scan mode and also lower than those obtained by the purge and trap technique [14] and ultrasound assisted emulsification microextraction [19]. Overall, the precision of fragrance analysis was similar to that reported by Garcia-Jares et al. [1] however our LODs are slightly more conservative to account blank variability. MS/MS operation, however, allows unequivocal confirmation of concentrations at LOQ level conversely to full scan operation. Although exceptionally low LODs (and good precision) could be achieved with the PTV technique, the purpose of the present work was to analyse several odorous substances together and the achieved LODs reach the odour threshold for earthy–musty compounds in water, which can be as low as 0.03–50 ng L^{-1} for TCA [16,30].

The calibration curves (full scan and MS/MS modes) were linear in the concentration range studied: from LOQ to 1 μg L^{-1}. The determination coefficients (r^2) were higher than 0.991 with the exception of galaxolide and tonalide in MS/MS mode. This less than optimal fit of two linear regressions was punctual since better calibration fit was obtained in latter experiments. The matrix effect was also tested by a recovery assay analysing one ground water sample, three very distinct surface waters and a wastewater effluent all spiked at 0.1 μg L^{-1} concentration. The results are displayed in Table 3. The water matrix might influence the extraction efficiency especially the most loaded samples as the wastewaters (EfPar) and surface water receiving WWTP effluents (Teofilo). IB and MIB are susceptible to matrix composition in these latter samples while TCA and GSM are quite tolerant. GAL and TON might suffer a reduction in extraction efficiency of about 20–30% in dirty samples while MX is not much affected and MK shows a trend to increased recoveries in real samples. Due to the different type of matrix effect, correction of results by recovery experiments, or use of deuterated internal standards for the most ubiquitous compounds, is recommended. No carry over with analytical significance was observed even for the most lipohilic analyte, as reported before by Garcia-Jares et al. [1].

3.5. Application to real samples

The developed method was applied to screen the presence of fragrances and earthy–musty odours in several waters samples: 13 surface waters, 8 groundwaters, 1 wastewater and 8 drinking waters collected in the northern region of Portugal in November and December 2008 and January 2009. The analyses were per-

Table 3
Matrix effects measured as the relative recoveries in the analysis of earthy-musty compounds and fragrances in different environmental matrices (GW – groundwater; Douro, Reg(uenga), Teofilo – surface waters, EfPar – wastewater effluent) Rec. – recoveries, C.I.–confidence interval).

Compound	Rec. GW	C.I. GW	Rec. Douro	C.I. Douro	Rec. Reg.	C.I. Reg.	Rec. Teofilo	C.I. Teofilo	Rec EfPar	C.I. EfPar
IB	94.0	10.0	102.6	16.4	99.9	11.4	81.8	5.3	59.6	16.8
MIB	119.6	5.2	105.5	26.1	103.2	12.6	96.5	6.5	69.5	24.0
TCA	102.2	17.2	105.4	2.2	106.0	12.1	90.3	20.6	96.0	49.9
GSM	102.9	3.9	102.6	21.5	102.0	11.0	98.2	11.3	89.6	15.8
GAL	95.4	20.7	106.0	7.2	72.2	5.1	71.5	15.9	67.0	4.5
MX	104.7	37.6	97.0	8.1	78.3	12.5	98.4	10.7	109.9	19.5
TON	95.3	20.9	81.4	10.5	69.5	17.0	96.1	8.2	82.1	5.2
MK	84.7	36.1	133.8	11.0	104.7	18.7	134.4	17.6	119.8	8.5
Average	99.8	19.0	104.3	12.9	92.0	12.5	95.9	12.0	86.7	18.0

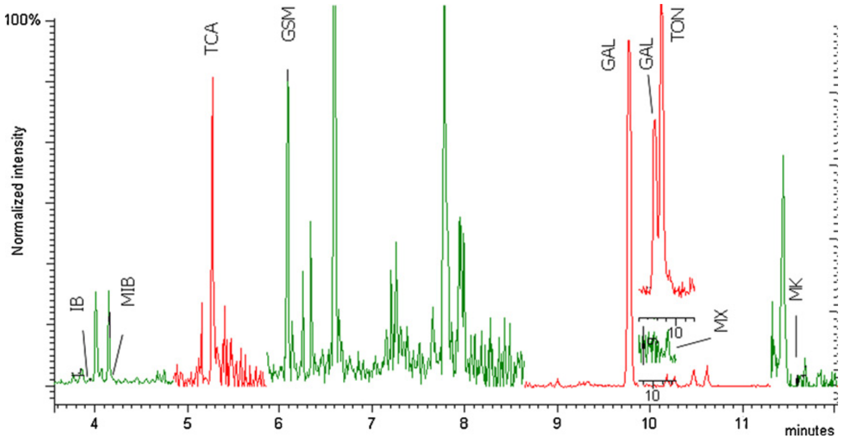

Fig. 7. Extracted ion chromatogram obtained from a Leça river water sample collected in Pte Pedra. Concentrations: IB – nd; MIB – nd; TCA – nd; GSM – <2; GAL – 248 ng L^{-1}; MX – nd; TON – not confirmed; MK – nd. Note: nd – not detected.

formed in full scan mode and the positives were further confirmed by MS/MS. None of the samples of drinking, surface and groundwater contained any of the compounds analysed. On the other hand, we have found isoborneol (471 ng L^{-1}) and galaxolide (444 ng L^{-1}) in the waste water sample. Later on, we have analysed fragrances and earthy–musty compounds in 10 sampling stations all along the river Leça, a small river of just 45 km path and 3.4 m^3 s^{-1} flow highly contaminated in its terminal path. A similar study was performed in river Douro, in 12 selected sampling stations downstream the confluence of river Paiva. The sampling took place in the 23rd of February and 04th of March 2009, respectively. River Leça receives wastewaters from Valongo (1 WWTP) and Maia (2 WWTPs) cities whereas Douro receives wastewaters from Porto (2 WWTPs) and Gaia (4 WWTPs) cities. In the 4 terminal sampling stations of river Leça we have found the following concentrations of galaxolide, respectively: 225, 248, 287 and 222 ng L^{-1}. Later results showed that the WWTPs are not the only source of galaxolide, which come also from some contaminated tributaries, probably with untreated domestic effluents. A chromatogram of Pte Pedra sample is given in Fig. 7. Geosmine was present in all except the first sampling station at the Leça riverhead but the concentrations were always below the LOQ, although the analyte was confirmed by MS/MS. In river Douro, geosmine seems to be widespread at trace levels nevertheless it could not be quantitated (<LOQ in 10 stations). These results are in agreement with Wang et al. and Filho and Alves in which geosmine was one of the most important off-flavours in natural waters [18,31]. Galaxolide was detected at <LOQ concentration in 9 stations. The stream of river Douro is huge enough to dilute any fragrances that might come from the WWTPs, conversely to that happening in river Leça. From our analysis it is safe to collect water for production of drinking water in river Douro, besides, all the WWTPs are downstream of the abstraction dam. Eight more samples were collected in several rivers (Ovil, Cávado, Lima and Neiva) where geosmine was detected at <LOQ in all samples and galaxolide at <LOQ in two samples. Additionally, nine drinking water samples collected from the distribution system of Porto metropolitan area were analysed but none contained any trace of these compounds.

4. Conclusions

The PDMS-DVB coating in the metal alloy configuration is the most adequate to analyse the totality of odorous compounds (fragrances and earthy–musty) in water. The best extraction conditions obtained through an experimental design, were the following: 40 min of headspace extraction at 70 °C with the addition of 200 g L^{-1} of NaCl. Individually, the earthy–musty odours require lower temperatures (50 °C) while for fragrances a higher temperature should be used (90 °C). All three variables tested: time, temperature and ionic strength have a significant impact in the extraction efficiency. Temperature and time also exhibit a quadratic behaviour while the ionic strength is essentially linear.

The LODs in MS/MS are generally similar to full scan except for geosmin and trichloroanisole which are remarkably lower reaching the sub-ng L^{-1} range. Such levels are needed to measure the odour threshold concentrations. The confirmation capability in MS/MS mode at LOQ level is an additional advantage.

Geosmine was found in surface waters at trace levels while galaxolide reached concentrations around 250 ng L^{-1} especially after discharge of effluents from waste water treatment plants. Ground- and drinking waters were absent of these compounds. Natural waters were affected by biogeochemical contamination with an earthy–musty compound at levels not causing concern but a synthetic fragrance (galaxolide) was found at significant levels in a wastewater and a polluted stream, although it has been phased out.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.03.014.

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