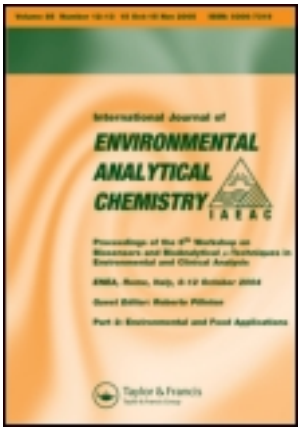

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Response surface optimisation applied to a headspace-solid phase microextraction-gas chromatography-mass spectrometry method for the analysis of volatile organic compounds in water matrices

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Response surface optimisation applied to a headspace-solid phase microextraction-gas chromatography-mass spectrometry method for the analysis of volatile organic compounds in water matrices

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A new method for the simultaneous determination of 12 volatile organic compounds (*trans*-1,2-dichloroethene, 1,1,1-trichloroethane, benzene, 1,2-dichloroethane, trichloroethene, toluene, 1,1,2-trichloroethane, tetrachloroethene, ethylbenzene, *m*-, *p*-, *o*-xylene) in water samples by headspace solid phase microextraction (HS–SPME)–gas chromatography mass spectrometry (GC–MS) was described, using a 100 µm PDMS (polydimethylsiloxane) coated fibre. The response surface methodology was used to optimise the effect of the extraction time and temperature, as well as the influence of the salt addition in the extraction process. Optimal conditions were extraction time and temperature of 30 min and −20°C, respectively, and NaCl concentration of 4 mol L^{-1}. The detection limits were in the range of 1.1×10^{-3}–2.3 µg L^{-1} for the 12 volatile organic compounds (VOCs). Global uncertainties were in the range of 4–68%, when concentrations decrease from 250 µg L^{-1} down to the limits of quantification. The method proved adequate to detect VOCs in six river samples.

Keywords: volatile organic compounds (VOCs); SPME; GC–MS; experimental design (DoE)

1. Introduction

The compounds considered as volatile organic compounds (VOCs) have a high vapour pressure, low water solubility, boiling point up to 200°C and molecular masses between 16 and 250 Da [1]. However, in the literature a wide range of VOC definitions can be found. According to EC Directive 1999/13/EC (Solvent Emissions Directive), they are defined as organic compounds having at 293.15 K a vapour pressure of 10 Pa, at least [2]. This definition is the most usual in Europe, but there are others. In Portugal, the Decree n.° 181/2006 defines VOC as an organic compound, having an initial boiling point lower than or equal to 250°C at a normal pressure of 101.3 kPa [3].

VOCs may be emitted by biogenic or anthropogenic sources. Biogenic emissions contribute to the local input of these compounds (volcanoes, bacteria, marine organisms, as macro- and microalgae). A large fraction is produced by anthropogenic sources, mainly

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Table 1. Physical properties of the studied VOCs: molecular weight (MW), boiling point (T_b), octanol/water partition coefficients (K_{ow}), water solubility (S), density and vapour pressure (P) [9,10].

Compound	Abbreviation	MW ($g\,mol^{-1}$)	T_b (°C)	$\log K_{ow}$	S ($mg\,L^{-1}$ at 25°C)	Density ($g\,cm^{-3}$ at 20°C)	P (Pa at 20°C)
Benzene	BENZ	78	80.1	2.13	1780	0.88	10100
1,2–Dichloroethane	12DCA	99	83.5	1.48	8600	1.25[a]	8280
trans–1,2–Dichloroethene	tDCE	97	48.7	1.93	4500	1.26	36660
Ethylbenzene	ETBENZ	106	136.2	3.15	161	0.86[a]	958
Tetrachloroethene	PCE	166	121.3	2.88	210	1.62	1878
Toluene	TOL	92	110.6	2.73	519	0.86[a]	2913
1,1,1–Trichloroethane	111TCA	133	74.1	2.49	1290	1.34	12890
1,1,2–Trichloroethane	112TCA	133	113.8	2.38	4590	1.44	2347
Trichloroethene	TCE	132	87.2	2.53	1280	1.46	7785
o–Xylene	*o*XY	106	144.5	3.12	171	0.88[a]	638
m–Xylene	*m*XY	106	139.1	3.20	161	0.86[a]	833
p–Xylene	*p*XY	106	138.2	3.15	181	0.86[a]	895

[a]Density in $g\,cm^{-3}$ at 25°C

through several industrial processes and human activities (industrial effluents, fuels, solvents, paints, herbicides, fumigants and disinfection processes) [4]. They have extremely adverse effects on human health and global environment, even at low concentrations, because most of them are toxic, carcinogenic and/or mutagenic, persistent and exhibit bioaccumulation effects. They also contribute to the global warming and stratospheric ozone depletion [5,6]. Owing to their high volatility, VOCs are potential soil and groundwater contaminants. Typically they are found in water matrices at $ng\,L^{-1}$ to $\mu g\,L^{-1}$ levels [5].

For that reason, the European Union has defined suitable legislation, in order to protect the human health and environment. In 2007, the Portuguese government made a review of existing legislation on the potable water quality, creating a new law in accordance with the European Directive no. 98/83/CE [7]. The concentration limits of some VOCs were fixed in the national Decree no. 306/2007: $1.0\,\mu g\,L^{-1}$ to benzene, $3.0\,\mu g\,L^{-1}$ to 1,2–dichloroethane and $10\,\mu g\,L^{-1}$ to the sum of tetrachloroethene with trichloroethene [8].

In this work, 12 volatile organic compounds were studied (Table 1). They were selected taking into account that a strong industrial pole exists in the northern region of Portugal and therefore a monitoring plan was set by the Northern Regional Coordination and Development Commission (CCDR–N). This organisation has the task of defining plans for monitoring, sampling and choosing which contaminants will be monitorised. Concerning VOCs analysis, gas chromatography followed by mass spectrometry (GC–MS) is an appropriate technique for water analysis, considering its high sensitivity [11,12]. However, the low concentrations found in natural waters require a preconcentration step before such analysis. The techniques used for sample preconcentration include gas extraction (static and dynamic headspace (HS) techniques), distillation (steam and vacuum distillation) and more recently, membrane extraction, solid–phase microextraction (SPME) and stir bar sorptive extraction (SBSE). The choice of the technique should be

adequate to the characteristics of the compounds to be studied, particularly their boiling points.

Since its introduction in early 1990s, by Pawliszyn and co-workers, SPME has been successfully applied to the sample preparation and analysis of environmental matrices, food and pharmaceuticals [13]. The SPME technique combines sampling with preconcentration in a single step and is a rapid, easily automated, selective, sensitive and solvent free technique. In this procedure, a fibre of fused-silica coated with a stationary phase is exposed to an aqueous solution for a given period of time to extract the organic compounds from the matrix. The adsorption is based on equilibrium partioning between the coated fibre and the sample. After the adsorption step, the fibre is retracted, inserted directly into the injector of a GC and then exposed for thermal desorption. For the reasons mentioned above, SPME is increasingly used for trace analysis of VOCs in water samples [4,6,11]. The efficiency of the extraction process depends on the fibre type, extraction time intervals and temperatures, sample amounts, desorption time and temperature. In order to achieve higher performances, these conditions must be optimised. The most common approach is to change one-factor-at-a-time, keeping all the other parameters constant. However, this method does not take into account cross-effects from the factors considered, leading to a poor optimisation. Therefore, the application of experimental design (DoE) becomes an essential tool, allowing the reduction in the amount of required experiments, without loss of information. DoE has already been used to optimise the experimental conditions of the SPME procedure in VOCs analysis [14–16]. However, none of them refers to VOCs detection by SPME in aqueous matrices and therefore this was set as one of the objectives of this work.

SPME applications for VOCs analysis are described in Table 2, where the compounds included in this study are signalled. SPME-cryotrap method [19], using a carboxen/polydimethylsiloxane (CAR/PDMS) fibre presents the advantage of achieving the lowest detection limits (at the level of 0.01 $ng\,L^{-1}$), but on the other hand, requires expensive and not commonly used equipment.

Another important feature of Table 2 is the great variety of experimental conditions used as well as equipments with different capabilities, leading to similar detection limits for the VOCs of interest in this study. Some authors used mixed composition of fibres, as DVB/CAR/PDMS [11,12] or CAR/PDMS [4,19,20] because their purpose is to analyse compounds with a large range of polarities, which is not the case of our study. Concerning those studies that use PDMS fibres, it is interesting to notice the significant difference of the extraction temperature used in the experiments, 60°C for [17] and −20°C for [18]. Considering so different approaches, this study was addressed to obtain the optimal conditions for VOCs analysis, using a response surface design of experiments.

Besides that, the present work was intended to display a complete set of validation of the analytical methodology, especially in the vicinity of the limits of detection, which is the region of concentration where most of samples lie, and where there is unexpected uncertainty associated of the results.

2. Experimental

2.1 *Chemicals*

Ethylbenzene (5000 $\mu g\,mL^{-1}$ in methanol), trichloroethene (neat), tetrachloroethene (neat), *trans*–1,2–dichloroethene (neat), 1,2–dichloroethane (neat), 1,1,1–trichloroethane

Table 2. SPME studies to determine VOCs in water samples.

		Analytical conditions									
Technique	Fibre	V sample (mL)	NaCl addition	Agitation	t_{ads} (min)	T_{ads} (°C)	t_{dess} (min)	T_{dess} (°C)	Note[a]	DL ($\mu g\,L^{-1}$)	Ref.
HS–SPME–GC–MS **(SIM mode)**	100 μm	10	300 $g\,L^{-1}$	?	30	60	1	270	tDCE	0.03	[17]
									12DCA	0.02	
									111TCA	0.01	
									112TCA	0.02	
									BENZ	0.01	
	PDMS								TCE	<0.01	
									TOL	<0.01	
									PCE	<0.01	
									*o*XY	<0.01	
									*m*XY + *p*XY	<0.01	
HS–SPME–GC–MS **(Full Scan)**	100 μm	4	no	no	30	−20	10	260	BENZ	0.125	[18]
									TOL	0.025	
									ETBEZ	0.025	
									*m*XY + *p*XY	0.025	
	PDMS								*o*XY	0.025	
									111TCA	0.125	
									TCE	0.125	
									PCE	0.500	
HS–SPME–cryotrap–GC–MS **(SIM mode)**	75 μm	15	267 $g\,L^{-1}$	yes	15	25	2	290	BENZ	0.00004	[19]
									TOL	0.00002	
									ETBENZ	0.00005	
	CAR/PDMS								*m*XY + *p*XY	0.00001	

(*continued*)

Table 2. Continued.

Technique	Fibre	Analytical conditions							Note[a]	DL ($\mu g\,L^{-1}$)	Ref.
		V sample (mL)	NaCl addition	Agitation	t_{ads} (min)	T_{ads} (°C)	t_{dess} (min)	T_{dess} (°C)			
									*o*XY	0.00002	
HS–SPME–GC–MS **(SIM mode)**	85 μm	110	no	no	20	40	5	310	12DCA	0.01	[4]
									BENZ	0.01	
									TCE	0.01	
	CAR/PDMS								112TCA	0.02	
									PCE	0.01	
DI–SPME–GC–MS **(Full scan)**	50/30 μm	1.3	no	yes	15	25	2	260	TCE	0.02	[12]
									PCE	0.1	
	DVB/CAR/PDMS								BENZ	0.04	
									12DCA	0.008	
HS–SPME–GC–MS **(Full Scan)**	DVB/CAR/PDMS	10	0.01 $g\,L^{-1}$	yes	2	35	0.75	270	BENZ	0.066	[11]
									TCE	0.044	
									12DCA	0.065	
									TOL	0.038	
									111TCA	0.071	
									112TCA	0.038	
									PCE	0.044	
									ETBENZ	0.022	
HS–SPME–GC–MS **(Full Scan)**	75 μm	10	no	no	15	20	5	250	BENZ	0.06	[20]
									12DCA	0.07	
									TCE	0.12	
									TOL	0.14	
	CAR/PDMS								PCE	0.16	
									ETBENZ	0.07	
									*o*XY	0.09	
									*m*XY + *p*XY	0.17	

[a]Coincident VOCs between bibliographic source and our study.

(neat), 1,1,2–trichloroethane (neat) were purchased from Supelco (Bellefonte, USA). Benzene (>99.9%) was purchased from Romil Chemicals (Cambridge, UK). Toluene (OEKANAL®, analytical standard), *o*–xylene (OEKANAL®, analytical standard), *m*–xylene (OEKANAL®, analytical standard), *p*–xylene (OEKANAL®, analytical standard) were obtained from Riedel–de Haën (Seelze, Germany). The internal standard, 1–bromo–2–chloroethane (98%) was from Aldrich Chemicals (Milwaukee, USA). The added salt, NaCl (>99.5%), was obtained from Sigma–Aldrich (Milwaukee, USA).

2.2 *Samples collection and preparation*

Six samples, collected from six rivers from the northern region of Portugal, were used for the determination of the matrix effect/accuracy. The samples were collected from the following rivers: Onda, Este, Cávado, Vizela, Ave and Leça. The samples were stored at a maximum of eight weeks in amber glass bottles, kept at −20°C and protected from light until they were processed.

2.3 *Standards preparation*

Individual stock solutions of each VOC at a concentration of 100 mg L^{-1} were prepared in distilled water. These solutions were used to prepare individual standards with 100 μg L^{-1} in distilled water, which were directly injected in the chromatograph in order to determine the retention time for each compound. Standards mixtures of the twelve VOCs were also prepared from the individuals stock solutions for calibration purposes. The internal standard of 1–bromo–2–chloroethane with a concentration of 150 mg L^{-1} was prepared from the commercial solution in distilled water.

All solutions were stored at 4°C. To avoid losses owing to volatilisation, individual stock solutions were stored at a maximum of six weeks.

2.4 *SPME extraction procedure*

A 100 μm PDMS fibre (Supelco, Bellefonte, USA), a non polar fibre for volatile and relatively apolar compounds with a low–mean boiling point (<220°C) [21], was used for extraction. The fibre was thermally conditioned at 250°C for 30 min.

After the optimisation step, the process was validated at the optimal conditions. A 10 mL of water sample was transferred into a 15 mL amber vial, to which 100 μL of internal standard and 2.3 g of NaCl were added. The fibre was exposed in the headspace of the sample for 30 min at −20°C in a static mode. To achieve this temperature the extraction was carried out inside a freezer (Princess HC 120). Finally, the fibre was removed from the vial and immediately inserted into the GC injector for thermal desorption at 260°C for 5 min. In order to obtain more accurate results, avoiding random and systematic errors, 1–bromo–2–chloroethane was added to the samples as an internal standard.

2.5 *Instrumentation*

A Varian 3800 gas chromatograph (Walnut Creek, CA, USA) equipped with an ion trap mass detector Varian Saturn 2000 mass spectrometer was used. The injection port was kept at 260°C. The injection was conducted in splitless mode, using a SPME liner for

Table 3. Quantification and qualifier ions of each individual compound studied and respective retention times.

Compound	Retention time (min)	Quantification ion (m/z)	Qualifier ions (m/z)
trans–1,2–Dichloroethene	2.991	61	96, 98
1,1,1–Trichloroethane	4.275	99	61, 63, 97, 101
Benzene	4.601	78	77
1,2–Dichloroethane	4.834	64	62, 63
Trichloroethene	5.234	132	130, 134
Toluene	6.625	91	92
1,1,2–Trichloroethane	7.146	97	83, 85, 98, 99
Tetrachloroethene	7.225	166	164, 168, 170
Ethylbenzene	8.483	91	65, 106
m–Xylene	8.664	106	65, 91
p–Xylene	8.664	106	65, 91
o–Xylene	9.164	106	91

Varian 1078 injector, ref. 2637805 (Supelco Bellefonte, USA). For the separation of the analytes, the gas chromatograph was equipped with a Factor Four VF–624 ms 30 m × 0.25 mm i.d. × 1.40 µm film thickness column (Walnut Creek, CA, USA). Helium (purity 99.9999%) was employed as carrier gas with a constant flow of 1 mL min^{-1}. The column temperature was held at 50°C for 2 minutes, then programmed to increase at 10°C min^{-1} to 130°C, ramped at 50°C min^{-1} up to 250°C, and held for 3 min. The MS transfer line temperature was kept at 250°C. The mass spectrometer was operated in the electronic impact ionisation (EI) mode. Monitoring ions in the selected ion–monitoring mode (SIM) are listed in Table 3.

2.6 *Optimisation of the SPME conditions*

As an initial step, a central composite design (CCD) was performed to evaluate the variables with more influence in the SPME process. The parameters evaluated were the extraction temperature, the extraction time and the NaCl concentration. Values of these independent variables were chosen according to the preliminary runs. Experimental data analyses were developed using the JMP 5.0.1 software and the statistical support was achieved by ANOVA tests. Each variable was studied at the low ($x_1 = -1$), central ($x_2 = 0$) and high ($x_3 = +1$) levels: extraction temperature at −20, 0 and 20°C, extraction temperature at 15, 30 and 45 min and sodium chloride concentration at 0, 2 and 4 mol L^{-1}. Those levels were set based on preliminary runs, as well as on information from literature.

3. Results and discussion

3.1 *Preliminary runs*

Two critical conditions were a starting point for the study and they are still controversial for different authors: the low temperature of extraction and the choice of PDMS fibres for VOCs extraction. This work intended to compare the effect of temperature in the extraction yield for a specific fibre (PDMS) and the scientific basis for the extraction at low temperatures is discussed in the topic 3.1.1.

The other condition was the use of PDMS. Although a study of different fibres should be included, this was not an initial objective but, instead, the authors intended to optimise the applicability of a standard fibre to the analysis of VOCs. Some authors recommended CAR/PDMS to extract VOCs [4,19,20], but others also used PDMS [17,18]. The sorption mechanism of CAR/PDMS fibres is different than that of PDMS ones. Adsorption is the governing mechanism in a mixed coating as CAR/PDMS and competitive adsorption for active sites may occur in multicomponent extraction. Therefore, although they have been referred as excellent fibres for VOCs, their performance may decrease significantly when large number of analytes is to be extracted at the same time. CAR/PDMS showed less reproducibility and required higher extraction times. PDMS fibres are governed by absorption processes. Because PDMS fibre proved also effective for VOCs and are cheaper than the others, they were selected in this study.

In order to better understand the effect of some operating variables (extraction temperature and time, desorption time and salting effect) in the SPME process, some preliminary runs were performed. On the contrary a high number of assays would be required if these four variables are considered in the experimental design. Therefore, these runs were also used to find a suitable desorption time and to set the ranges for the variables studied in the CCD. In all these experiments a concentration of $500\,\mu g\,L^{-1}$ for toluene, ethylbenzene, *o*/*m*/*p*–xylene, and $5000\,\mu g\,L^{-1}$ for the other VOCs were employed.

3.1.1 *Effect of extraction temperature*

The analytical responses for the VOCs were obtained at −20, 0, 3, 20 and 45°C to evaluate the effect of the extraction temperature (Figure 1). The assays obtained inside the

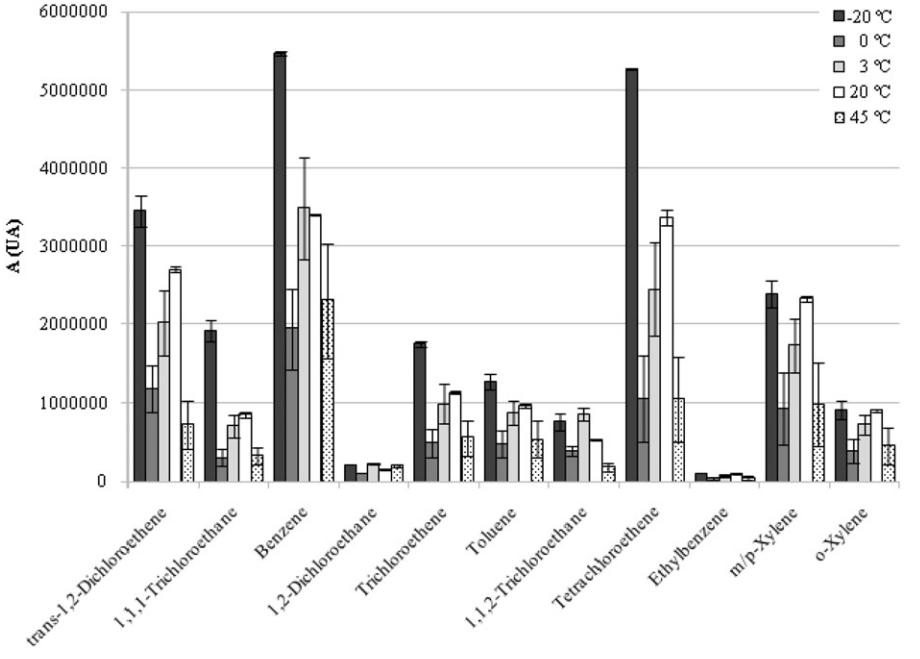

Figure 1. Effect of extraction temperature ($t_{extraction} = 30\,min$, $t_{desorption} = 5\,min$, $[NaCl] = 0\,mol\,L^{-1}$).

refrigerator/freezer were performed in static mode owing to the impossibility of maintaining the equipment closed, avoiding temperature fluctuations. In other situations a magnetic stirrer was used.

The SPME is based on the partition of analytes between an aqueous sample and polymeric film on the fibre. Cooling the fibre increases the partition coefficient between it and headspace, promoting the adsorption of the compounds on the fibre coating (exothermic process) [18]. However, if the sample temperature is decreased, the mass-transfer process is hampered by the decrease in the partition coefficient between the sample and headspace and in the vapour pressure of the analytes [11]. When the results obtained at 20°C are compared with those at 3°C and 0°C, a consecutive decrease in the value of areas was verified. Despite the increase in adsorption capacity of the fibre, the amount adsorbed has not increased. Therefore, in this case, the volatilisation of the compounds to the headspace seems to be the limiting step. When the extraction temperature was increased from 20 to 45°C, the compound areas decreased. This behaviour is predictable because, as mentioned above, increasing temperature originates an increase in the partition coefficient between sample and headspace and in the vapour pressure of the compounds. However, the partition coefficient between fibre and headspace decreases, while desorption of the compounds from the coating increases [11]. Therefore, it is expected that from a certain temperature the total amount of VOCs gradually decreases [19].

In this experiment the best results were achieved at −20°C. At this temperature it was supposed that the compounds concentration in the headspace was the lowest, although the adsorption of the analytes on the fibre increased. Despite the lower vapour pressures, in this situation an enhancement can result from the transfer of the analytes from the solution to the gas phase during the freezing process – freezing-out effect [18]. When the sample begins to freeze (from the sides inwards and from the bottom upwards), a concentration of the analytes in the residual unfrozen solution occurs. So, according to Raoult's law the analytes vapour pressure will increase, allowing their gradual transfer from the solid–liquid interface into the gas phase [18,22].

3.1.2 *Effect of extraction time*

Because SPME is based on the equilibrium, the effect of the time of extraction is an important variable. The extraction time was studied for 15, 30 and 45 min, at −20°C and 20°C. Figure 2 shows a comparison between the different extraction times studied. As can be seen, generally the higher response was obtained for 30 min of extraction time. For the extraction time of 15 min the responses were about 50% lower than for 30 min. When using 45 min of extraction time it was found a decrease in the response for almost all compounds at 20°C and only a few at −20°C. This was an unexpected result that could be possibly explained by the existence of overloading of the analytes for higher extraction times or an unaccounted effect of displacement of all the analytes from the fibre by the internal standard.

3.1.3 *Effect of desorption time*

The influence of desorption time on the response was studied at 5 and 10 min either for −20°C or 20°C. As it is shown in Figure 3, in some cases slightly higher responses for 10 min of desorption time were obtained. Although better responses were obtained for the

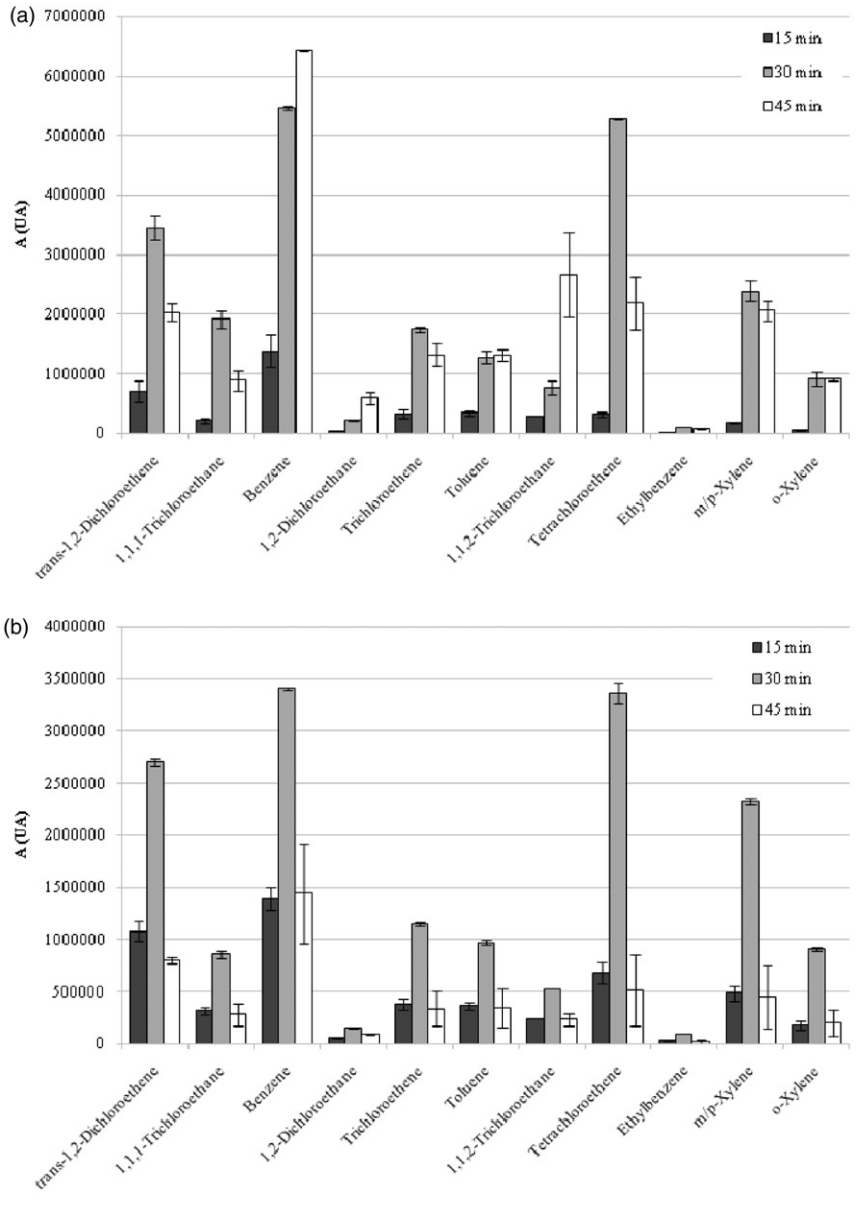

Figure 2. Effect of extraction time ($t_{desorption} = 5$ min, [NaCl] = 0 mol L^{-1}, (a) $T_{extraction} = -20°C$, (b) $T_{extraction} = 20°C$).

longer desorption time, it was considered that this increase was not significant enough. Besides that, for longer desorption times the peak shape was negatively affected. As such, 5 min was the period of desorption of the fibre chosen for further study, and this variable was not optimised. In order to guarantee the complete desorption from the fibre, a reconditioning was performed after each analysis.

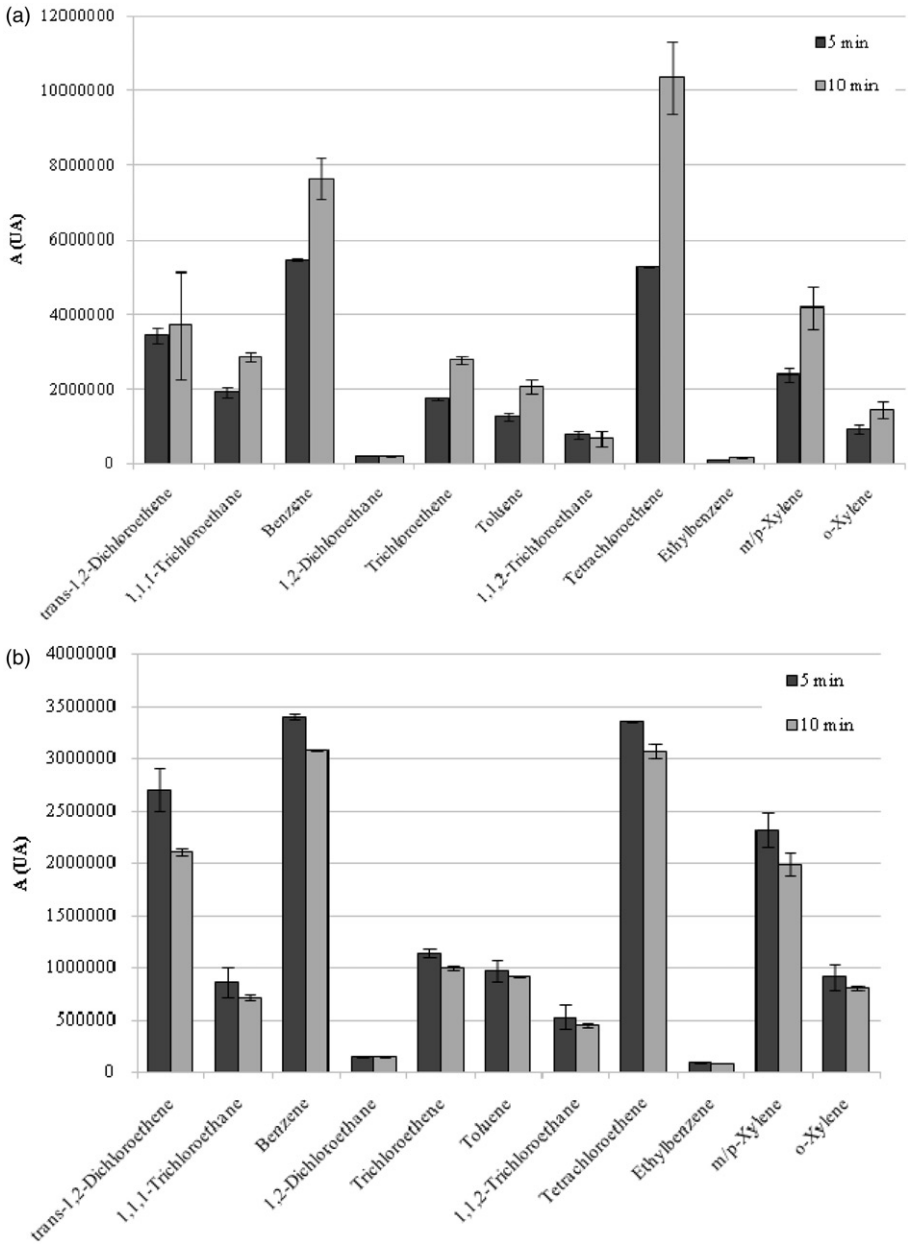

Figure 3. Effect of desorption time ($t_{extraction} = 30$ min, [NaCl] = 0 mol L^{-1}, (a) $T_{extraction} = -20°C$, (b) $T_{extraction} = 20°C$).

3.1.4 *Salting effect*

In order to study the effect of salt addition, experiences with different levels of concentration of NaCl at −20°C and 20°C were carried out (Figure 4).

The addition of inorganic salts has often been used in SPME in order to increase the ionic strength of the aqueous solution and enhance the activity coefficients of the analytes

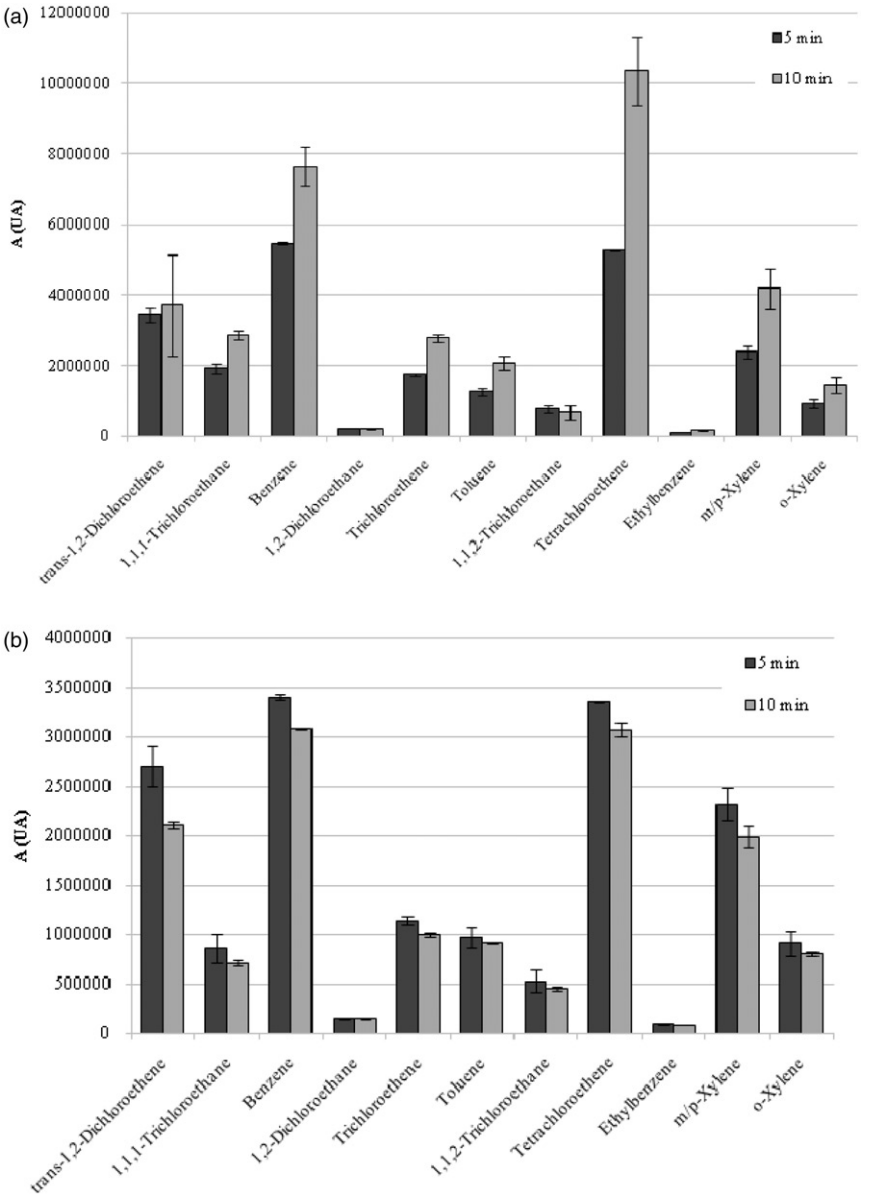

Figure 4. Salting effect ($t_{extraction} = 30\,min$, $t_{desorption} = 5\,min$, (a) $T_{extraction} = -20°C$, (b) $T_{extraction} = 20°C$).

in the aqueous phase. Actually, the change in ionic strength could decrease the solubility of the organic compounds through the arrangement of water molecules in the hydration spheres around the ionic salt, reducing the water amount available to dissolve the analyte molecules [23,24]. On the other hand, the increase in the activity coefficients conducts to the raise of the analytes concentration in the headspace vapour [24,25]. The results obtained shows that an increase in the NaCl concentration from 0 to 4 mol L^{-1} improves

the extraction efficiency. Nonetheless, for saturated solutions a decrease in the response was verified. This reduction may result from the salt crystals suspended in the sample that interfere with the extraction process. In fact, for concentrations above 4 mol L^{-1} the VOCs extraction is usually not improved [25,26]. The addition of salt also increases the viscosity and density of the aqueous phase and may influence the absorption kinetics of some compounds, usually those who already have low diffusion rates, and thus worsen their extraction efficiency [24].

It is important to notice that the NaCl addition in the experiments tested at −20°C will decrease the melting point of the analytes and internal standard. Therefore, the freezing of the solution may not occur during the extraction time tested (e.g. for 4 mol L^{-1} NaCl).

In both cases, it can be seen that the best response was obtained for the concentration of 4 mol L^{-1}.

3.2 *SPME optimisation using experimental design*

As mentioned above, the preliminary tests helped to understand the effect of some important variables in the SPME extraction process. However, to achieve high performances, these parameters should be optimised. In this study instead of using a single-factor-at-a-time approach, a response surface methodology was used as experimental design approach.

3.2.1 *Experimental design*

In this case, a response surface methodology was applied and a central composite design (CCD) selected. Table 4 summarises the experiments performed, as well as the responses based on the experimental runs and the predicted values. As can be seen, the model predictions matched the experimental response (Area) satisfactorily. Using the surface response methodology, a mathematical relationship between dependent and independent variables was established. In this case the experimental data were fitted to a second–order polynomial equation as follows (Equation (1)) [27]:

$$Y = b_0 + \sum_{i}^{n} b_i x_i + \sum_{i}^{n} b_{ii} x_i^2 + \sum_{j>i}^{n} \sum_{i=1}^{n} b_{ij} x_i x_j \quad (1)$$

where Y refers to the response (Area), x_i the codified independent variables, b_0 in the interception term, b_i determines the influence of the variable i in the response (linear term), b_{ii} is a parameter that determines the shape of the curve (quadratic effect) and b_{ij} corresponds to the effect of the interaction among variable i and j. The coefficients of the quadratic model were calculated by a least–squares regression analysis, using coded variables, which allows the comparison of different factors and minimises the errors in the polynomial fit. To convert the natural variables (X_i) in dimensionless codified values (x_i) it was necessary to use the Equation (2):

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (2)$$

where X_0 denotes the value of variable i in the centre of the domain ($x_i = 0$) and ΔX refers to the difference of that variable between $x_i = +1$ and $x_i = 0$.

Table 4. Experimental design matrix and response (Area) based on the experimental runs and predicted values (±standard deviation) proposed by CCD (x_1 – extraction temperature, x_2 – extraction time, x_3 – NaCl concentration, desorption time = 5 min).

					A_{rea}																	
					tDCE		111TCA		BENZ		TCE		TOL		112TCA		PCE		ETBENZ		*m*/*p*XY		*o*XY	
Run	Code	x_1	x_2	x_3	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^6$)	Pred ($\times 10^6$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^4$)	Pred ($\times 10^4$)	Exp ($\times 10^5$)	Pred ($\times 10^5$)	Exp ($\times 10^4$)	Pred ($\times 10^4$)
1	−++	−20	45	4	138	124 ± 12	75	63 ± 7	35	31 ± 3	69	58 ± 5	70	64 ± 5	37	40 ± 4	93	91 ± 9	30	27 ± 2	81	73 ± 5	343	287 ± 2
2	++−	20	45	0	8	14 ± 12	3	1 ± 7	1	2 ± 3	3	6 ± 5	3	5 ± 5	2	2 ± 4	5	11 ± 9	2	4 ± 2	5	8 ± 5	22	34 ± 2
3	+−−	20	15	0	11	14 ± 12	3	11 ± 7	1	3 ± 3	4	6 ± 5	4	7 ± 5	2	3 ± 4	7	11 ± 9	3	4 ± 2	5	8 ± 5	18	34 ± 2
4	+00	20	30	2	17	14 ± 8	6	4 ± 5	3	2 ± 1	8	5 ± 3	8	5 ± 3	6	5 ± 2	12	8 ± 6	6	4 ± 2	11	7 ± 2	51	28 ± 2
5	00−	0	30	0	12	−10 ± 23	3	−7 ± 6	2	−2 ± 2	5	−5 ± 4	5	−5 ± 4	4	1 ± 2	11	−8 ± 7	4	−1 ± 2	9	−6 ± 2	39	−22 ± 2
6	+++	20	45	4	19	13 ± 12	5	−3 ± 7	3	2 ± 3	6	4 ± 5	7	4 ± 5	8	7 ± 4	8	5 ± 9	4	4 ± 2	7	5 ± 5	30	22 ± 2
7	−−−	−20	15	0	7	28 ± 12	2	6 ± 7	1	1 ± 3	3	12 ± 5	3	3 ± 5	3	8 ± 4	3	23 ± 9	2	7 ± 2	2	14 ± 5	6	55 ± 2
8	+−+	20	15	4	13	13 ± 12	3	8 ± 7	2	3 ± 3	4	4 ± 5	5	5 ± 5	6	7 ± 4	6	5 ± 9	3	4 ± 2	5	5 ± 5	22	22 ± 2
9	0+0	0	45	2	10	14 ± 8	2	4 ± 5	2	5 ± 2	3	6 ± 3	3	10 ± 3	4	9 ± 2	2	7 ± 6	1	4 ± 2	2	7 ± 4	8	33 ± 2
10	−00	−20	30	2	116	76 ± 8	49	34 ± 5	17	16 ± 1	43	35 ± 3	36	34 ± 3	20	24 ± 2	83	57 ± 6	21	17 ± 2	44	43 ± 4	160	171 ± 2
11	00+	0	30	4	26	37 ± 10	6	14 ± 6	6	8 ± 2	11	16 ± 4	14	18 ± 4	15	12 ± 2	14	23 ± 7	8	9 ± 2	16	21 ± 4	74	88 ± 2
12	000	0	30	2	9	14 ± 8	3	4 ± 5	2	3 ± 1	4	6 ± 3	5	6 ± 3	4	6 ± 2	5	7 ± 6	3	4 ± 1	5	7 ± 4	26	33 ± 2
13	−+−	−20	45	0	20	28 ± 12	9	17 ± 7	6	10 ± 3	13	12 ± 5	13	18 ± 5	27	21 ± 4	22	23 ± 9	8	7 ± 2	21	14 ± 5	91	55 ± 2
14	−−+	−20	15	4	100	124 ± 12	36	51 ± 7	19	22 ± 3	47	58 ± 5	45	49 ± 5	32	27 ± 4	82	91 ± 9	24	27 ± 2	69	73 ± 5	256	287 ± 2
15	0−0	0	15	2	12	14 ± 8	3	4 ± 5	2	1 ± 2	5	6 ± 3	5	3 ± 3	4	3 ± 2	5	7 ± 6	3	4 ± 2	5	7 ±	18	33 ± 2
Confirmatory assays																								
16	−0−	−20	30	0	34	28 ± 12	19	6 ± 7	5	5 ± 3	17	12 ± 5	13	10 ± 5	8	14 ± 4	53	23 ± 9	10	7 ± 2	24	14 ± 5	92	55 ± 2
17	−0+	−20	30	4	273	124 ± 12	135	63 ± 7	39	27 ± 3	94	58 ± 5	100	56 ± 5	26	33 ± 4	180	91 ± 9	54	27 ± 2	103	73 ± 5	400	287 ± 2
18	+0−	20	30	0	27	14 ± 12	9	11 ± 7	3	3 ± 3	11	6 ± 5	10	6 ± 5	5	3 ± 4	34	11 ± 9	10	4 ± 2	23	8 ± 5	91	34 ± 2
19	+0+	20	30	4	31	13 ± 12	9	8 ± 7	5	2 ± 3	12	4 ± 5	12	5 ± 5	11	7 ± 4	20	5 ± 9	7	4 ± 2	18	5 ± 5	72	22 ± 2

Table 5 shows the quadratic coefficients and the model suitability verified by the ANOVA test. The mean squares were calculated as the ratio of the sum of squares of each variation source and their degrees of freedom. The model F–ratio was obtained by dividing the model mean square by the residual mean square. To evaluate if the model variance is higher than that of the experimental error, the F–ratio should be higher than the F–value (theoretical value, $F_{9,5} = 4.8$ at 95% confidence level), which depends on the degrees of freedom and the confidence level. Besides that, the ANOVA test uses other statistical criteria to evaluate the model fitting, the F–probability (Prob > F). Usually, for values of Prob > F less than 0.05 the model is considered significant, while for values above 0.10 are considered negligible. As can be seen in Table 5, only for 12DCA the Prob > F was significantly higher than 0.10 (Prob > F = 0.538). For this reason, the optimisation of the extraction conditions for this compound was not done using this methodology.

After the analysis of the second order model suitability, the statistically significant variables and interactions were studied, using the Student's *t*–test (*data not shown*). It was verified that in most cases, the extraction temperature and the NaCl concentration were the most significant variables. At this point, the variables and/or interactions that do not affect significantly the model were removed (Table 5) and after that the response surfaces were represented.

3.2.2 *Response surfaces and counter plots*

Three–dimension response surface and two-dimension counter plots of the predicted response of the model were drawn using the JMP software. The model equations, on which the authors were based to plot response surface curves, contain cross-interaction terms, so the effect of each independent variable in the process response depends on the value of the others. However, analysing the response surfaces, it was found that in all cases studied, the best extraction time ranged between 25 and 40 min. So, in order to simplify and save space, only the variation of the response with the salt concentration and with extraction temperature has been presented below. The extraction time was set in 30 min, assuming a compromise between the optimum values for each compound. In Figure 5 the response surfaces are shown. As can be seen from the three-dimensional plot, the maximum response for all the compounds studied was obtained for the minimum temperature value (−20°C) and maximum NaCl concentration (4 mol L^{-1}) of the space domain analysed. Nevertheless, Figure 5 shows that good extraction performances can also be attained at lower salt concentration, although requiring slightly higher temperatures.

This optimisation was developed taking into account the highest response of analytes (achieving the highest sensitivity of the method). However, there is not an exclusive form of developing a SPME-based method. The authors choose an approach based on the highest sensitivity, but other important factors like peak shape, chromatographic resolution, separation efficiency and selectivity can be considered.

3.2.3 *Model verification*

After the extraction process modelling, it was necessary to test its suitability. In this way, the validity of the RSM–predicted process optimums could be verified by applying the empirical model to the preliminary runs. The results are also expressed in Table 5.

As can be seen, the model predicts quite reasonably the experimental results.

Table 5. Second-order polynomial equation and ANOVA test for the response functions.

	Source	Sum of squares	DF	Mean square	F–ratio	Prob > F
tDCE	Model	2.52×10^{14}	9	2.80×10^{13}	7.45	0.020
	Error	1.88×10^{13}	5	3.75×10^{12}		
	$R^2 = 0.931$					
	$Y = 2.28 \times 10^6 - 3.13 \times 10^6 x_1 + 2.37 \times 10^6 x_3 - 2.46 \times 10^6 x_1 x_3 + 4.01 \times 10^6 x_1^2$					
111TCA	Model	6.14×10^{13}	9	6.83×10^{12}	6.64	0.025
	Error	5.14×10^{12}	5	1.03×10^{12}		
	$R^2 = 0.922$					
	$Y = 7.39 \times 10^5 - 1.50 \times 10^6 x_1 + 1.06 \times 10^6 x_3 - 1.21 \times 10^6 x_1 x_3 + 1.92 \times 10^6 x_1^2$					
BENZ	Model	1.23×10^{15}	9	1.37×10^{14}	11.79	0.007
	Error	5.79×10^{13}	5	1.16×10^{13}		
	$R^2 = 0.955$					
	$Y = 3.30 \times 10^6 - 6.73 \times 10^6 x_1 + 2.22 \times 10^6 x_2 + 5.31 \times 10^6 x_3 - 2.51 \times 10^6 x_1 x_2 - 5.46 \times 10^6 x_1 x_3 + 6.71 \times 10^6 x_1^2$					
12DCA	Model	1.85×10^{11}	9	2.05×10^{10}	0.99	0.538
	Error	1.04×10^{11}	5	2.08×10^{10}		
	$R^2 = 0.639$					
TCE	Model	5.59×10^{13}	9	6.21×10^{12}	12.81	0.006
	Error	2.42×10^{12}	5	4.85×10^{11}		
	$R^2 = 0.958$					
	$Y = 7.80 \times 10^5 - 1.50 \times 10^6 x_1 + 1.08 \times 10^6 x_3 - 1.20 \times 10^6 x_1 x_3 + 1.67 \times 10^6 x_1^2$					

(*continued*)

Table 5. Continued.

	Source	Sum of squares	DF	Mean square	F–ratio	Prob > F
TOL	Model	5.25×10^{13}	9	5.84×10^{12}	15.09	0.004
	Error	1.93×10^{12}	5	3.87×10^{11}		
	$R^2 = 0.964$					
	$Y = 7.21 \times 10^5 - 1.41 \times 10^6 x_1 + 3.42 \times 10^5 x_2 + 1.12 \times 10^6 x_3 - 4.12 \times 10^5 x_1 x_2 - 1.18 \times 10^6 x_1 x_3 + 1.40 \times 10^6 x_1^2$					
112TCA	Model	1.81×10^{13}	9	2.01×10^{12}	11.88	0.007
	Error	8.47×10^{11}	5	1.69×10^{11}		
	$R^2 = 0.955$					
	$Y = 5.34 \times 10^5 - 9.35 \times 10^5 x_1 + 3.08 \times 10^5 x_2 + 5.89 \times 10^5 x_3 - 3.42 \times 10^5 x_1 x_2 - 3.68 \times 10^5 x_1 x_3 + 7.38 \times 10^5 x_1^2$					
PCE	Model	1.39×10^{14}	9	1.55×10^{13}	8.07	0.017
	Error	9.59×10^{12}	5	1.92×10^{12}		
	$R^2 = 0.936$					
	$Y = 1.38 \times 10^6 - 2.45 \times 10^6 x_1 + 1.55 \times 10^6 x_3 - 1.85 \times 10^6 x_1 x_3 + 3.13 \times 10^6 x_1^2$					
ETBENZ	Model	1.11×10^{11}	9	1.24×10^{10}	14.38	0.005
	Error	4.31×10^9	5	8.61×10^8		
	$R^2 = 0.963$					
	$Y = 5.28 \times 10^4 - 6.60 \times 10^4 x_1 + 5.04 \times 10^4 x_3 - 5.33 \times 10^4 x_1 x_3 + 7.58 \times 10^4 x_1^2 - 3.52 \times 10^4 x_2^2$					
m/*p*XY	Model	8.55×10^{13}	9	9.50×10^{12}	13.24	0.006
	Error	3.59×10^{12}	5	7.18×10^{11}		
	$R^2 = 0.960$					
	$Y = 8.33x10^5 - 1.84 \times 10^6 x_1 + 1.38 \times 10^6 x_3 - 1.56 \times 10^6 x_1 x_3 + 1.87 \times 10^6 x_1^2$					
*o*XY	Model	1.34×10^{13}	9	1.49×10^{12}	13.38	0.005
	Error	5.57×10^{11}	5	1.11×10^{11}		
	$R^2 = 0.960$					
	$Y = 3.49 \times 10^5 - 7.14 \times 10^5 x_1 + 5.50 \times 10^5 x_3 - 6.12 \times 10^5 x_1 x_3 + 6.84 \times 10^5 x_1^2$					

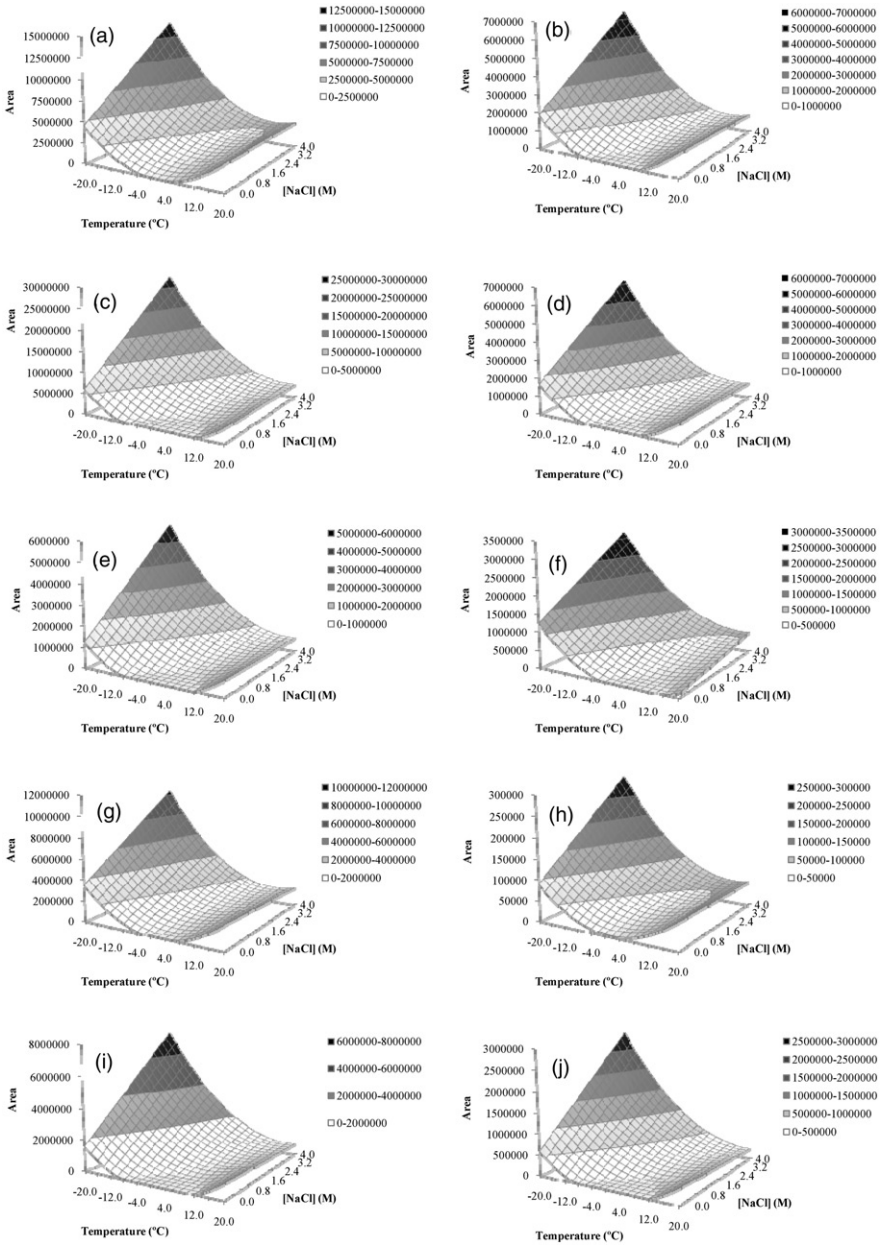

Figure 5. Response surface from (a) tDCE, (b) 111TCA, (c) BENZ, (d) TCE, (e) TOL, (f) 112TCA, (g) PCE, (h) ETBENZ, (i) *m*/*p*XY, (j) *o*XY as a function of extraction temperature and NaCl concentration ($t_{extraction} = 30$ min, $t_{desorption} = 5$ min).

3.3 *Method validation*

3.3.1 *SPME linearity, detection and quantification limits, precision and accuracy*

In order to evaluate the developed SPME–GC–MS methodology, the detection limit, precision and accuracy were evaluated. Also the linearity was studied in the 0.5–500 $\mu g\,L^{-1}$

Table 6. Linearity results, detection and quantification limits for each compound studied.

Compound	Equations	R^2	Calibration range ($\mu g\,L^{-1}$)	DL ($\mu g\,L^{-1}$)	QL ($\mu g\,L^{-1}$)
tDCE	$A/A_{IS} = 0.0052C - 0.4926$	0.987 ($N = 8$)	5–4000	2.3	7.7
111TCA	$A/A_{IS} = 0.0049C - 0.5000$	0.991 ($N = 8$)	5–4000	0.51	1.7
BENZ	$A/A_{IS} = 0.0086C + 0.0468$	0.997 ($N = 9$)	5–5000	0.049	0.16
12DCA	$A/A_{IS} = 0.00005C + 0.0038$	0.989 ($N = 6$)	5–2000	0.14	0.46
TCE	$A/A_{IS} = 0.0016C + 0.0240$	0.997 ($N = 9$)	5–5000	0.091	0.30
TOL	$A/A_{IS} = 0.0196C + 0.1579$	0.997 ($N = 10$)	0.5–500	0.0011	0.0036
112TCA	$A/A_{IS} = 0.0005C - 0.0378$	0.993 ($N = 9$)	5–5000	0.021	0.070
PCE	$A/A_{IS} = 0.0059C - 0.2477$	0.997 ($N = 9$)	5–5000	0.11	0.36
ETBENZ	$A/A_{IS} = 0.0454C - 0.2428$	0.997 ($N = 8$)	0.5–400	0.021	0.069
m/*p*XY	$A/A_{IS} = 0.0228C + 0.2294$	0.990 ($N = 10$)	1–1000	0.012	0.041
*o*XY	$A/A_{IS} = 0.0215C - 0.0196$	0.995 ($N = 10$)	0.5–500	0.0059	0.020

R^2: determination coefficient; N: number of calibration standards; DL: detection limit; QL: quantification limit.

range for ETBENZ, TOL, *o*/*m*/*p*XY and from 5 to 5000 $\mu g\,L^{-1}$ for the other compounds. The squared correlation coefficients (R^2) obtained indicates good linearity for the volatile compounds studied, as shown in Table 6. Detection limits (Table 6) were calculated considering that the signal to noise ratio equals 3 ($S/N = 3$) and for the quantification limits it was assumed that the signal to noise ratio equals 10 ($S/N = 10$). The detection limits of 0.049 $\mu g\,L^{-1}$ for benzene, 0.14 $\mu g\,L^{-1}$ for 12DCA, 0.11 $\mu g\,L^{-1}$ for PCE and 0.091 $\mu g\,L^{-1}$ for TCE (0.12 $\mu g\,L^{-1}$ for the sum of the last two compounds), show that this method can be used for the determination of these compounds in water matrices, since the limits achieved allow the detection of the compounds for which threshold levels have been established in the National Decree n.° 306/2007.

Precision was evaluated by repeatability (4 repeated analyses of the same sample in the same day), at four different levels of concentration. 500, 300, 100 and 1 $\mu g\,L^{-1}$, were the concentration levels studied for ETBENZ, TOL and *o*/*m*/*p*XY and 5000, 3000, 1000 and 10 $\mu g\,L^{-1}$ were the concentration levels used for the other VOCs. The coefficients of variation for each concentration level, for the different compounds, are shown in Table 7. The precision of the proposed method ranges from 1.02% (5000 $\mu g\,L^{-1}$ for BENZ) to 42.6% (10 $\mu g\,L^{-1}$ for tDCE).

In order to evaluate the method accuracy, six different matrices were analysed. All six samples were spiked, so that the final concentration was 2.5 $\mu g\,L^{-1}$ for ETBENZ, TOL and *o*/*m*/*p*XY, and 25 $\mu g\,L^{-1}$ to the other VOCs. Accuracy results, expressed through analytical recovery tests (the observed value divided by the expected value) are shown in Table 8. The average recovery values for all compounds ranged between 71% and 123%, with the exception of tDCE, whose accuracy values were quite different. Actually, this compound has the highest standard deviation, which can be due to its high vapour pressure. Therefore, this analytical method might not be the most adequate for the analysis of this compound.

From the studied compounds, TOL, *o*/*m*/*p*XY, PCE and ETBENZ were the most frequently detected VOCs. The presence of these compounds in real water samples shows the importance of the quality control of water in industrial areas.

Table 7. Precision (% CV) for the different concentration levels, for each compound.

	% CV			
Compound	5000 ($\mu g\,L^{-1}$)	3000 ($\mu g\,L^{-1}$)	1000 ($\mu g\,L^{-1}$)	10 ($\mu g\,L^{-1}$)
tDCE	8.85	18.8	6.50	42.6
111TCA	8.38	13.2	8.16	23.2
BENZ	1.02	9.15	6.39	30.0
12DCA	31.2	21.0	8.36	6.06
TCE	5.54	18.5	8.68	37.7
112TCA	7.62	15.8	4.23	24.6
PCE	8.67	22.6	12.9	37.8
	% CV			
	500 ($\mu g\,L^{-1}$)	300 ($\mu g\,L^{-1}$)	100 ($\mu g\,L^{-1}$)	1 ($\mu g\,L^{-1}$)
TOL	3.49	20.0	6.09	22.9
ETBENZ	3.13	20.3	4.73	21.9
m/*p*XY	2.84	14.7	9.49	25.8
*o*XY	4.13	15.5	10.5	21.5

Table 8. Accuracy (% Rec) for each compound, in the six recovery assays.

	% Rec						
Compound	Sample 1 (Onda river)	Sample 2 (Este river)	Sample 3 (Cávado river)	Sample 4 (Leça river)	Sample 5 (Vizela river)	Sample 6 (Ave river)	% Rec (average ± s.d.)
tDCE	60	48	81	21	94	153	76 ± 45
111TCA	99	93	92	92	95	103	96 ± 4
BENZ	91	102	93	81	97	113	96 ± 11
12DCA	99	84	101	82	95	108	95 ± 10
TCE	91	109	91	89	99	100	97 ± 8
TOL	113	97	104	118	93	88	102 ± 12
112TCA	113	92	88	71	123	117	101 ± 20
PCE	109	101	89	114	88	90	99 ± 11
ETBENZ	114	95	89	103	93	94	98 ± 9
m/*p*XY	107	99	94	104	95	96	99 ± 5
*o*XY	111	97	105	106	103	99	103 ± 5

3.3.2 *Estimation of uncertainty*

The uncertainty arising from sampling seriously limits the reliability of many investigations based upon measurements. The *bottom–up* approach/EURACHEM was the chosen procedure to estimate the uncertainty associated to this study. This approach relies on quantifying all the individual component sources of the uncertainty and then summing them to give an overall estimate. In this study, four major contributions were considered, that is the uncertainty associated to standard preparation (U_1), the uncertainty associated to the calibration curve (U_2), the uncertainty associated to precision (U_3) and the

Table 9. Limit values of the global uncertainty for each compound.

Compound	% *U* at maximum concentration
tDCE	19
111TCA	7
BENZ	6
12DCA	9
TCE	6
TOL	5
112TCA	8
PCE	7
ETBENZ	5
m/*p*XY	4
*o*XY	4

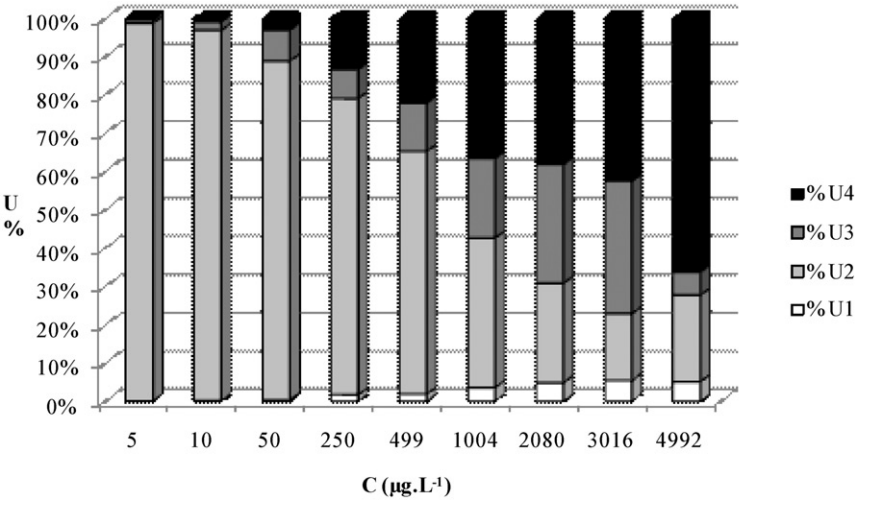

Figure 6. Relative weight of each individual source of uncertainty (*bottom–up* approach/ EURACHEM) for benzene analysis by SPME/GC–MS for all standard concentrations. (U_1: uncertainty associated to standard preparation; U_2: uncertainty associated to the calibration curve; U_3: uncertainty associated to precision; U_4: uncertainty associated to accuracy.)

uncertainty associated to accuracy (U_4). The calculation procedure for the estimation of the global uncertainty (U), following the *bottom–up* approach was the described by Ratola *et al.* [28]. In the Table 9 the variation of the global uncertainty with concentration levels, for each compound is shown. As expected, a constant uncertainty was achieved for the upper and intermediate levels of the calibration ranges. However, when the standard concentrations are lowered, approaching the detection limits, the global uncertainty rises exponentially.

Figure 6 represents the variation of the relative weight of each individual source of uncertainty, for benzene. Owing to the high number of VOCs studied, it was decided to

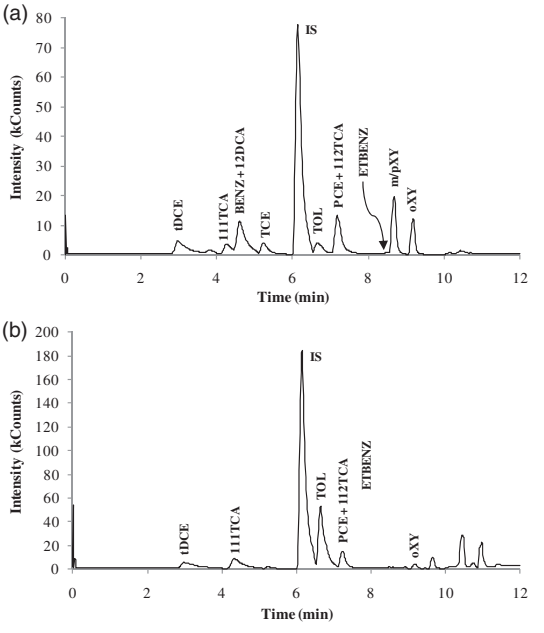

Figure 7. Total ion chromatograms obtained in SIM mode for (a) real sample 5 and (b) standard mixture solution (50 μg L^{-1} for tDCE, 111TCA, BENZ, 12DCA, TCE, 112TCA and PCE; 5 μg L^{-1} for TOL, ETBENZ, *m*/*p*XY and *o*XY).

present only the graph for this compound because it is representative of the group behaviour. As it can be seen, the relative contribution of the uncertainty of standard preparation (U_1) decreases when the concentration diminishes. However, its relative weight to the global uncertainty is always below 2% (this is verified for every compound). Clearly, the importance of the calibration curve uncertainty (U_2) increases as it reaches towards the lower concentrations, and for standards it is almost the only source of associated uncertainty. The contribution of the uncertainty related to precision (U_3) decreases as the lower concentrations are reached, with the exception of the highest standard. The uncertainty associated to accuracy (U_4) has an important relative contribution for the global uncertainty, at the highest concentrations, decreasing as it reaches the lowest ones.

3.3.3 *Application to real samples*

Six river samples were collected in the northern region of Portugal, where proliferate small and medium industrial units, some of them with few resources to carry out an appropriate waste management. The paint, varnishes and polymers plants are the principal sources of VOCs in this region.

The effectiveness of the SPME method in determining volatile organic compounds was tested for these river samples. Figure 7 illustrates and compares the total chromatograms obtained in the SIM mode for the real sample 5 and for a standard mixture solution (50 μg L^{-1} for tDCE, 111TCA, BENZ, 12DCA, TCE, 112TCA and PCE; 5 μg L^{-1} for TOL, ETBENZ, *m*/*p*XY and *o*XY). Table 10 displays the results for the quantitative analysis in the six water samples. As can be seen, PCE, ETBENZ and *o*XY were detected

Table 10. Quantitative analysis of VOCs in water river samples after HS-SPME-GC-MS.

	Concentration ($\mu g\,L^{-1}$)					
Compound	Sample 1 (Onda river)	Sample 2 (Este river)	Sample 3 (Cávado river)	Sample 4 (Leça river)	Sample 5 (Vizela river)	Sample 6 (Ave river)
tDCE	n.d.	100	n.d.	105	107	95.8
111TCA	106	105	n.d.	105	105	n.d.
BENZ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12DCA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TCE	n.d.	n.d.	n.d	n.d.	n.d.	n.d.
TOL	59.7	36.8	n.d.	60.1	49.7	11.2
112TCA	n.d.	n.d.	n.d.	n.d.	75.7	n.d.
PCE	56.1	61.2	42.5	85.8	67.2	44.4
ETBENZ	8.97	7.86	6.22	73.6	8.48	23.2
m/*p*XY	n.d.	n.d.	n.d.	4.73	n.d.	n.d.
*o*XY	1.67	1.53	1.44	4.39	1.63	3.62

in all the samples. PCE was the only compound detected that has a legal limit ($10\,\mu g\,L^{-1}$ for the sum of PCE with TCE), which was exceeded in all the samples.

4. Conclusions

A HS–SPME–GC–MS method was optimised for the determination of 12 volatile organic compounds (defined by the Portuguese entity CCDR–N monitoring plan) in water matrices using an experimental design approach. The effects of time and temperature of extraction and salt addition were the significant parameters in the SPME process. The optimisation process indicated, for the majority of the compounds, that a low temperature of extraction ($-20^{\circ}C$) along with the addition of NaCl ($4\,mol\,L^{-1}$) improves the overall sensitivity of the extraction method. The extraction time of 30 min showed to be sufficient for the adsorption process of the compounds studied. The detection limits achieved with this method were in the range of 0.001–2.30 $\mu g\,L^{-1}$ for the 12 VOCs. It should be noted that the detection limits of $0.049\,\mu g\,L^{-1}$ for benzene, $0.14\,\mu g\,L^{-1}$ for 12DCA, $0.11\,\mu g\,L^{-1}$ for PCE and $0.091\,\mu g\,L^{-1}$ for TCE ($0.12\,\mu g\,L^{-1}$ for the sum of the last two compounds), demonstrate that this method allows the detection of the compounds for which threshold levels have been established in the National Decree n.° 306/2007.

Comparing this analytical methodology with other studies present in literature, it can be seen that some methods are applied to a small range of compounds. Those papers that present coincident VOCs with this study reach similar levels of detection limits. Besides that, the authors could not found in these papers studies in which the experimental design is applied for extraction conditions optimisation, neither a complete set of validation of the analytical methodology, especially the evaluation of the uncertainty associated to the results.

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References

[1] P. Kuráň and L. Soják, J. Chrom. A **733**, 119 (1996).
[2] Council Directive 1999/13/EC, of 11 March 1999, on the limitation of emissions of volatile organic compounds due to the use of organic solvents in certain activities and installations (Official Journal L 085).
[3] Decreto–Lei n° 181/2006 de 6 de Setembro, Diário da República, 1ª. série–N°. 172–6 de Setembro de 2006.
[4] G. Pecoraino, L. Scalici, G. Avellone, L. Ceraulo, R. Favara, E.G. Candela, M.C. Provenzano, and C. Scaletta, Water Res. **42**, 3563 (2008).
[5] T. Huybrechts, J. Dewulf, O. Moerman, and H. Van Langenhoven, J. Chromatogr. A **893**, 367 (2000).
[6] K. Demeestere, J. Dewulf, B. De Witte, and H. Van Langenhove, J. Chromatogr. A **1153**, 130 (2007).
[7] Council Directive 98/83/CE, of 3 November 1998, on the quality of water intended for human consumption (Official Journal L 330).
[8] Decreto–Lei n° 306/2007 de 27 de Agosto, Diário da República, 1ª. série–N°. 164–27 de Agosto de 2007.
[9] D.R. Lide, *CRC Handbook of Chemistry and Physics*, 88th ed. 2007–2008 (Taylor & Francis Books, 2007).
[10] C.L. Yaws, *Chemical Properties Handbook* (McGraw-Hill, New York, 1999).
[11] V.H. Niri, L. Bragg, and J. Pawliszyn, J. Chromatogr. A **1201**, 222 (2008).
[12] A. Lara-Gonzalo, J.E. Sánchez-Uría, E. Segovia-García, and A. Sanz-Medel, Talanta **74**, 1455 (2008).
[13] G. Ouyang and J. Pawliszyn, Trends Anal. Chem. **25**, 692 (2006).
[14] F. Pellati, S. Benvenuti, F. Yoshizaki, D. Bertelli, and M.C. Rossi, J. Chromatogr. A **1087**, 265 (2005).
[15] J. Larreta, A. Vallejo, U. Bilbao, A. Alonso, G. Arana, and O. Zuloaga, J. Chromatogr. A **1136**, 1 (2006).
[16] E.T. Sousa, F.M. Rodrigues, C.C. Martins, F.S. Oliveira, P.A.P. Pereira, and J.B. Andrade, Microchem. J **82**, 142 (2006).
[17] S. Nakamura and S. Daishima, Anal. Chim. Acta **548**, 79 (2005).
[18] E. Fries and W. Püttmann, Anal. Bioanal. Chem. **386**, 1497 (2006).
[19] M.R. Lee, C. M. Chang, and J. Dou, Chemosphere **69**, 1381 (2007).
[20] A.D. Guimarães, J.J. Carvalho, C. Gonçalves, and M.F. Alpendurada, Int. J. Environ. Anal. Chem. **88**, 151 (2008).
[21] R. Eisert and K. Levsen, J. Chromatogr. A **733**, 143 (1996).
[22] M. Elsner, G.L. Couloume, and B.S. Lollar, Anal. Chem. **78**, 7528 (2006).
[23] A. Sarafraz-Yazdi, H.P. Moghadam, Z. Es'haghi, and S. Sepehe, Anal. Methods **2**, 746 (2010).
[24] M. Polo, G. Gómez-Noya, J.B. Quintana, M. Llompart, G. García-Jares, and R. Cela, Anal. Chem. **76**, 1054 (2004).
[25] M.A. Farajzadeh, K. Farhadi, A.A. Matin, P. Hashemi, and A. Jouyban, Anal. Sci. **25**, 875 (2009).
[26] J.-H- Lee, R. Diono, G.-Y. Kim, and D.B. Min, J. Agric. Food Chem. **51**, 1136 (2003).
[27] C.S.D. Rodrigues, L.M. Madeira, and R.A.R. Boaventura, J. Hazard. Mater. B **164**, 987 (2009).
[28] N. Ratola, L. Santos, P. Herbert, and A. Alves, Anal. Chim. Acta **573–574**, 202 (2006).