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Multivariate optimization of a solid-phase microextraction method for the analysis of phthalate esters in environmental waters

Maria Polo, Maria Llompart*, Carmen Garcia-Jares, Rafael Cela

Departamento de Quimica Analitica, Nutricion y Bromatologia, Facultad de Quimica, Instituto de Investigacion y Analisis Alimentario, Universidad de Santiago de Compostela, E-15782 Santiago de Compostela, Spain

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Abstract

A solid-phase microextraction method (SPME) coupled to gas chromatography–mass spectrometry (GC–MS) has been developed for the determination of the six phthalate esters included in the US Environmental Protection Agency (EPA) Priority Pollutants list in water samples. These compounds are dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP) and di-*n*-octyl phthalate (DOP). Detailed discussion of the different parameters, which could affect the extraction process, is presented. Main factors have been studied and optimized by means of a multifactor categorical design. Different commercial fibers, polydimethylsiloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS–DVB), polyacrylate (PA), Carboxen–polydimethylsiloxane (CAR–PDMS) and Carbowax–divinylbenzene (CW–DVB), have been investigated, as well as the extraction mode, exposing the fiber directly into the sample (DSPME) or into the headspace over the sample (HS-SPME), and different extraction temperatures. The use of this experimental design allowed for the evaluation of interactions between factors. Extraction kinetics has also been studied. The optimized microextraction method showed linear response and good precision for all target analytes. Detection limits were estimated considering the contamination problems associated to phthalate analysis. They were in the low pg mL^{-1}, excluding DEHP (100 pg mL^{-1}). The applicability of the developed SPME method was demonstrated for several real water samples including mineral, river, industrial port and sewage water samples. All the target analytes were found in real samples. Levels of DEP and DEHP were over 1 ng mL^{-1} in some of the samples.
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1. Introduction

Phthalic acid esters (dialkyl or alkylaryl esters of 1,2-benzenedicarboxylic acid), also known as phthalates, are a class of chemicals that are produced at large scale due to the wide variety of uses. World production of these compounds is estimated to be several million tonnes per year. Significant migration of them into the environmental compartments is possible during their production, manufacture, use and disposal [1,2]. Certain phthalates and/or their metabolites are suspected human cancer-causing agents, and endocrine disruptors [3]. Due to their potential risks for human health and environment, several of them have been included in the priority list of pollutants of different national and supranational organizations. In this way, up to 12 PAEs, including di-*n*-butyl phthalate (DBP), butylbenzyl phthalate (BBP), and di-2-ethylhexyl phthalate ester (DEHP), are in the list of the proposed substances suspected to produce endocrine alterations published by European Union (EU) [4]. According to Section 307 of the US Clean Water Act, diethyl phthalate (DEP), dimethyl phthalate (DMP), DEHP, BBP, DBP and di-*n*-octyl phthalate (DOP) should be considered Priority Toxic Pollutants [5]. DEHP is the most prevalent phthalate used and, thus, the most regulated. The EU has included it in the list of 33 substances of priority or possibly priority substances in the field of water policy [6]. The World Health Organization (WHO) has established a guideline value of 8 ng mL^{-1} for DEHP for fresh and drinking water [7], which is similar to the maximum contaminant level (MCL) for DEHP set by the Environmental Protection Agency (EPA) (6 ng mL^{-1}). This

* Corresponding author. Tel.: +34 981563100x14387; fax: +34 981595012.
E-mail address: qblvrlgb@usc.es (M. Llompart).

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agency recommends the closely monitoring of concentrations above 0.6 ng mL^{-1} [8]. Other institutions as the Netherlands National Institute of Public Health and Environment [3] and the Danish Environmental Protection Agency [9] have also established some limits.

Taking into account all these considerations, the development of sensitive and reliable analytical methods to analyze phthalates from different water samples is necessary. Considerable care must be taken to avoid sample contamination, which is the main problem associated to phthalate analysis [10].

Extraction and pre-concentration techniques, such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are widely applied to determine phthalates in water samples [10–13]. The EPA has published analytical procedures dealing with the determination of phthalate esters in drinking water and in municipal and industrial wastewater [14,15] based on these pre-concentration techniques. Nevertheless, these methods are expensive, time-consuming, and employ different sorbent materials and solvents, enhancing contamination risks. In the last years, solid-phase microextraction (SPME) has acquired an increased importance in the analysis of semivolatile compounds [16–18] including phthalates [19–23]. This technique is an interesting alternative for the determination of phthalates in aqueous samples, because the risk of contamination during sample handling can be significantly reduced. In addition, the elimination of organic solvents in the sample preparation process could reduce phthalate background levels. Nevertheless, the main problem for applying SPME to phthalate analysis is the levels of phthalates found in blanks of laboratory purified water and even commercial water (especially for DBP and DEHP).

In these studies, some factors affecting the extraction efficiency are evaluated. Nevertheless, most papers consider optimization strategies based in the study of one factor at a time. This approach can lead to erroneous conclusions about the importance of certain factors on the extraction process, due to the fact that interactions between factors are not being considered. A multivariate approach to the optimization of the SPME process allows the simultaneous study of various factors and then, it is more advantageous than univariate. To the knowledge of the authors, up to now only one study using a multivariate strategy was applied to the problem of phthalate determination by SPME [23]. In this interesting study, a screening method for the analysis of 16 PAHs, 6 PCBs and 6 phthalate esters has been developed using multisimplex optimization. Due to blank problems, detection limits for some phthalates were quite high, especially for DEHP (3.15 μg L^{-1}). In addition, due to inherent difficulties of simplex with categorical variables only one fiber (PDMS) was studied, although many others are potentially applicable to phthalate analysis.

In the present work, a SPME method followed by gas chromatography–mass spectrometry (GC–MS) analysis was developed for the determination of phthalate esters in water samples following a multivariate optimization strategy. A multifactor categorical design was selected to study and optimize main experimental factors affecting SPME. This kind of experimental design allows the study of main effects as well as second order interactions. The factors included in this design were type of fiber, extraction mode and extraction temperature, and from the result obtained, it could be demonstrated that these three factors are essential to achieve high sensitivity. It could be also demonstrated that some interaction effects between factors must be considered.

Bearing in mind that the main problem for applying SPME to phthalate analysis are the levels of phthalates found in blanks of purified water, detailed discussion about methodological aspects of the analysis (such as the precautions to minimize contamination) is provided. Finally, the optimized method is evaluated in terms of linearity and precision. Limits of detection (LODs) are found at the pg mL^{-1} level and the applicability of the proposed method to real water samples is demonstrated.

2. Experimental

2.1. Reagents and materials

Dimethyl phthalate (>98%) and diethyl phthalate (>98%) were purchased from Fluka Chemika (Buchs, Switzerland); di-*n*-butyl phthalate (>98%) and di-2-ethylhexyl phthalate (>99%) were from Sigma (St. Louis, MO, USA); and benzyl butyl phthalate (97.2%) and di-*n*-octyl phthalate (99.7%) were supplied by Riedel-de Häen (Seelze-Hannover, Germany) and Supelco (Bellefonte, PA, USA), respectively.

Isooctane, acetone, and NaCl were all purchased from Merck (Mollet del Valles, Barcelona, Spain). All the solvents and reagents were analytical grade. Ultrapure (resi-analyzed) water for environmental inorganic and organic trace analysis was supplied by J.T. Baker (Phillipsburg, NJ, USA).

Individual stock solutions of each phthalate ester (20 mg mL^{-1}) were prepared in acetone. A standard mixture of the target analytes was prepared at a final concentration of about 200 μg mL^{-1} in acetone. From this solution, several standard working solutions were prepared. Solutions were stored at −20 °C and working solutions were prepared weekly.

Different real water samples were analyzed: bottled mineral water, river water, industrial harbour water, influent and effluent from a sewage treatment plant (corresponding to a population of approximately 100,000 inhabitants located in Galicia, Spain), and wastewater from an urban collector.

Commercially available 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 85 μm polyacrylate (PA), 74 μm Carboxen–polydimethylsiloxane (CAR–PDMS) and 65 μm Carbowax–divinylbenzene (CW–DVB) fibers housed in manual SPME holders were obtained from Supelco (Bellefonte, PA, USA).

Special care was taken to avoid the contact of reagents and solutions with plastic materials. Laboratory glassware was washed prior to use with ultrapure water and dried at 250 °C. This material was stored in aluminium foil to avoid adsorption of phthalates from the air.

2.2. Experimental set-up

Aliquots of 10 mL sample were placed in headspace vials of 22 mL, which were cleaned according to the procedure described earlier. Stir bars (also previously cleaned) were introduced into the samples, and then, vials were sealed with a headspace aluminium cap furnished with a PTFE-faced septum, and immersed in a water bath maintained at the temperature of the experiment. Samples were let to equilibrate for 5 min before analysis. SPME fibers were re-conditioned at 260–290 °C (depending on the fiber used) for at least 3 min and then, exposed to the headspace over the sample or immersed into the sample for 5–80 min, depending on the experiment. During all the sampling process, samples were magnetically stirred. Once finished the exposition period, the fiber was immediately inserted into the GC injector and chromatographic analysis was carried out. Considering the thermal stability of phthalates, we selected the maximum possible desorption temperatures for each fiber (without exceeding 290 °C) in order to achieve maximum response; thus, desorption temperature was 260 °C for CW–DVB, 270 °C for PDMS and PDMS–DVB, and 290 °C for PA and CAR–PDMS fibers. Desorption time was set at 5 min.

The wastewater samples analyzed were previously filtered through glass fiber filters (Millipore, Madrid, Spain). All the filtration process was performed using glass material which was cleaned following the procedure indicated in Section 2.1.

2.3. Gas chromatography–mass spectrometry

The GC–MS analyses were performed on a Varian 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) equipped with an ion-trap mass detector Varian Saturn 2000 (Varian Chromatography Systems, Walnut Creek, CA, USA). The system was operated by Saturn GC–MS Workstation v5.4 software. Phthalate esters were separated on a 25 m length × 0.25 mm i.d., CP-Sil8 CB Low-bleed/MS column (Varian Chromatography Systems, Walnut Creek, CA, USA) coated with a 0.25 μm film. The GC oven temperature program was: 60 °C hold 2 min, rate 20 °C min^{-1} to 190 °C, rate 10 °C min^{-1} to 280 °C, hold for 5 min. Helium (purity 99.999%) was employed as carrier gas, with a constant column flow of 1.2 mL min^{-1}. Injector was operated in the splitless mode and programmed to return to the split mode after 2 min from the beginning of a run. Split flow was set at 50 mL min^{-1}. Injector temperature was between 260 and 290 °C depending on the fiber used. The ion-trap mass spectrometer was operated in the electron ionisation mode (70 eV). The mass range was scanned from 80 to 300 amu.

Table 1
Retention time and selected ions for the analysis of the target phthalates

Compound	Retention time (min)	Quantification ions	Identifications ions
DMP	8.31	163, 164	163, 164
DEP	9.21	149, 177	149, 176, 177, 222, 223
DBP	11.93	149	149, 205
BBP	15.06	149, 206	91, 104, 149, 206
DEHP	16.15	149, 167	149, 167
DOP	17.90	149, 279	149, 279

Experimental parameters for ionisation were: multiplier voltage, 1750 V; filament emission current, 30 μA; axial modulation voltage, 4 V; ionisation control, automatic mode; filament/multiplier delay, 6 min. Trap, manifold and transfer line temperatures were 250, 50 and 280 °C, respectively.

3. Results and discussion

First experiments were conducted to optimize the chromatographic separation of the target analytes and the optimal conditions are described in Section 2. In these conditions, all analyzed phthalate esters were adequately resolved avoiding interferences with siloxane peaks coming from the chromatographic column and/or the coating of SPME fibers. In Table 1, the retention times at the optimized chromatographic conditions, as well as, the identification and quantification ions (based on best signal-to-noise criteria) are shown.

It is well known that the most important problem concerning phthalate analysis is the risk of contamination, resulting in false positive results and over-estimated concentrations. The sources of contamination can be present in any step of the analytical procedure. To check the presence of phthalates in the chromatographic system (in the inlet and the gas supply system), blank runs of the chromatograph and direct injections of isooctane were made. The presence of phthalate esters was not detected. None of the target phthalates was present in the chromatograms.

Before starting SPME optimization, blank chromatographic injections of the SPME device using different coatings were made. In these analyses, the presence of phthalates was detected. To avoid this background problem, the SPME fibers were desorbed at 270 °C just before injection. In this way, consistent blanks were attained. So this pre-processing step was applied systematically in all experiments described.

3.1. Optimization of microextraction process: multifactor categorical design

A factorial design was carried out to evaluate the influence of main factors affecting the microextraction process in order to obtain the optimal values. The experimental parameters studied were: type of fiber, extraction mode and extraction temperature. The fibers included in the design were: 100 μm PDMS, 65 μm PDMS–DVB fiber, 75 μm CAR–PDMS,

Table 2
ANOVA results showing the significance of main effects and interactions

Compound	Source	Main effects			Interactions		
		Fiber (A)	Temperature (B)	Extraction mode (C)	AB	AC	BC
DMP	*F*-value	10.31	0.65	26.48	1.62	5.57	0.84
	p-ratio	*0.00*	0.55	*0.00*	0.25	*0.02*	0.47
DEP	*F*-value	14.99	5.49	44.38	1.76	8.10	1.08
	p-ratio	*0.00*	*0.03*	*0.00*	0.22	*0.01*	0.38
DBP	*F*-value	20.09	44.33	90.63	3.16	6.41	8.59
	p-ratio	*0.00*	*0.00*	*0.00*	0.06	*0.01*	*0.01*
BBP	*F*-value	8.15	2.29	77.44	1.14	4.47	0.92
	p-ratio	*0.01*	0.16	*0.00*	0.43	*0.01*	0.44
DEHP	*F*-value	3.33	35.13	5.83	1.35	0.55	20.96
	p-ratio	0.07	*0.00*	*0.04*	0.34	0.71	*0.00*
DOP	*F*-value	6.66	40.57	0.11	3.17	0.54	10.93
	p-ratio	*0.01*	*0.00*	0.74	0.06	0.71	*0.01*

Italized numbers are used to denoted a significant effect.

65 μm CW–DVB and 85 μm PA. In principle, all these fibers could be adequate for phthalate extraction. Extraction temperature was set at three levels: 25, 60 and 100 °C and the extraction mode was direct sampling (DSPME) and headspace sampling (HS-SPME), depending on the experiment.

A multifactor categorical $5 \times 3 \times 2$ type V resolution design, which involves 30 runs, was selected [24]. This design is a standard factorial, consisting of all combinations of the levels of the factors, that enables the study of main effects, as well as two-factor interactions. The design was carried out with 10 mL aliquots of ultrapure water spiked at 4 ng mL^{-1} of each target analyte. Sampling time was set at 20 min to achieve maximum throughput considering GC run time.

One of the statistical options of the proposed design is the analysis of variance (ANOVA) which measures whether a factor contributes significantly to the variance of the response. The results of the ANOVA are shown in Table 2. In this table, and for the sake of simplicity, only the *F*-ratios and *p*-values are given. The *F*-ratios measure the contribution of each factor or interaction on the variance of the response. The *p*-values test the statistical significance of each of the factors and interactions. When *p*-value is less than 0.05, the factor has a statistically significant effect at the 95% confidence level. As can be seen, all three main factors were found to be significant for most compounds. Furthermore, interaction between type of fiber and extraction mode (AC), and interaction between temperature and extraction mode (BC) were significant for some compounds. Considering *F*-ratio values, it is evident that temperature plays a very important role in the extraction of DBP, DEHP and DOP, and extraction mode in the extraction of DMP, DEP, DBP and BBP. An adequate selection of the optimal conditions requires a deeper look at the results of the design by means of the graphic options.

Fig. 1 shows the response plots for type of fiber and extraction temperature. As can be seen, the most efficient extraction conditions for DMP and DEP were obtained using CAR–PDMS at 100 °C. Nevertheless, for the remaining compounds, other fibers perform better. That is, their response increases while the response for CAR–PDMS decreases. The highest microextraction response is reached using PDMS–DVB fiber at 100 °C (for DEHP, responses with PDMS–DVB and PA were almost identical). CAR–PDMS, however, presented very low efficiency for the extraction of BBP, DEHP and DOP. For this last compound, the CAR–PDMS response was almost negligible. On the other hand, PDMS–DVB yielded the highest microextraction efficiency for DBP, BBP, DEHP and DOP, and was the second most efficient fiber for the extraction of DMP and DEP. Both fibers (CAR–PDMS and PDMS–DVB) have an intermediate polarity but they differ in the pore size. Carboxen coating has a micropore size ideal to extract small molecules and PDMS–DVB mainly presents mesopore size best suited to extract medium molecular sized compounds. The relation between molecular size and extraction efficiency using CAR–PDMS fiber was clearly appreciated in our study, where CAR–PDMS was only suitable for the extraction of the two compounds with the smallest molecular size (DMP and DEP). Regarding the type of fiber other aspect can be pointed out. For DOP and especially for DEHP, the responses with all fibers excluding CAR–PDMS were quite similar and, initially, all these fibers would be suitable for the extraction of these two compounds. As it has been already mentioned, the most favourable extraction temperature for all compounds was 100 °C but the influence of this factor is more pronounced for the two least volatile compounds, DEHP and DOP. For these compounds, responses at 25 and 60 °C are considerably lower (see Fig. 1).

Fig. 2 shows the response plots for the factors temperature and extraction mode. No significant interaction was found for DBP, DMP, DEP and BBP, and the most suitable extraction mode at any temperature is direct sampling

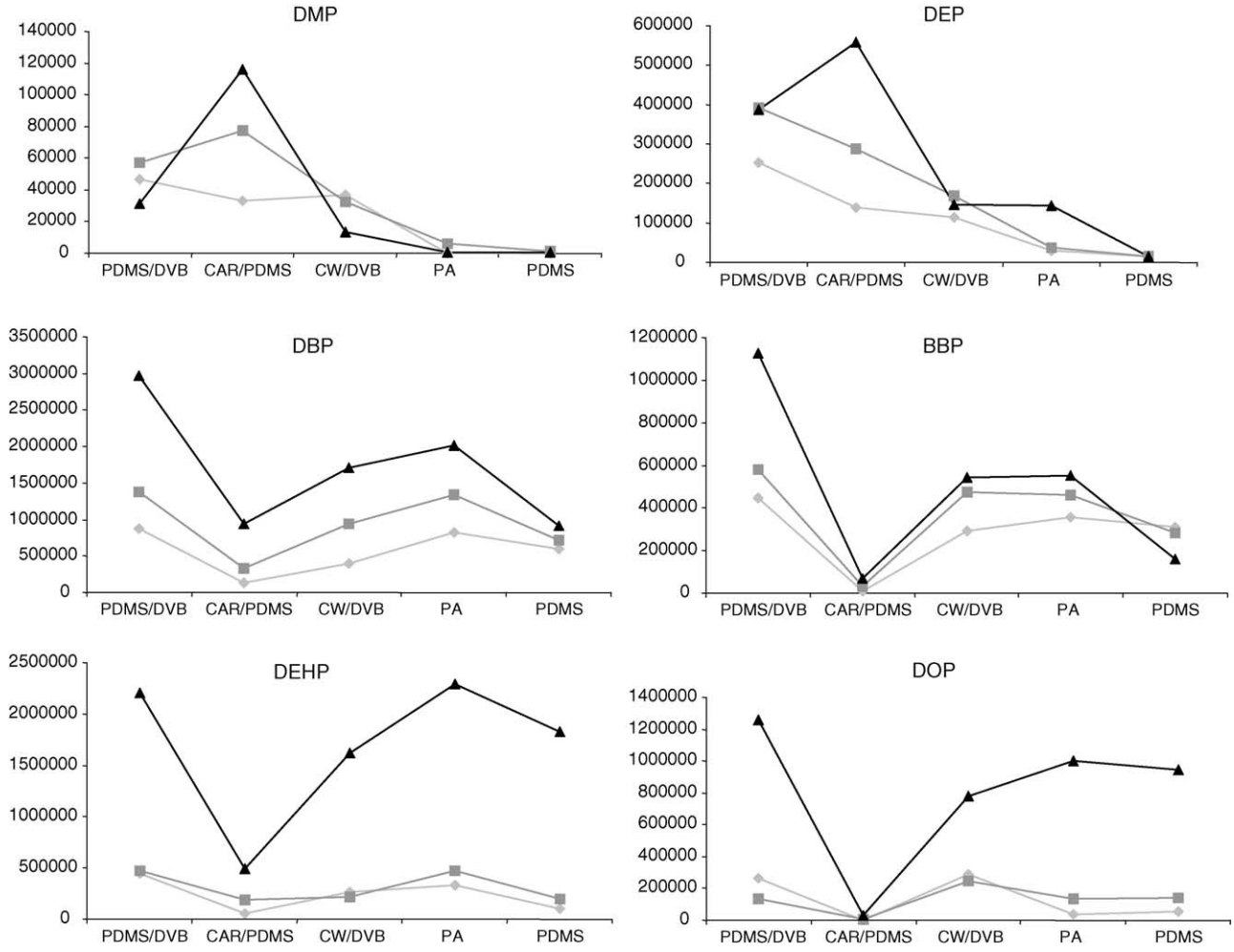

Fig. 1. Fiber–extraction temperature interaction plots for all target phthalates (response in area counts): (◆) 25 °C, (■) 60 °C and (▲) 100 °C.

(DSPME), although, the highest microextraction response was generally obtained by direct sampling at 100 °C. However, for DEHP and DOP, a significant interaction between these two factors was, in fact, observed. At 25 and 60 °C, HS-SPME response was very low and, therefore, DSPME is recommended at this temperature interval. However, at 100 °C, the response obtained by HS-SPME increases markedly, and maximum response is achieved under these conditions. Initially, it might appear strange that the apparently less volatile analytes are more efficiently extracted by HS-SPME and the least volatile ones by DSPME. It is true that the lower molecular weight phthalate esters are quite volatile, but because of their moderate water solubility they have a very low Henry law constant (*H*) [25]. In consequence, they volatilise very slowly from aqueous solutions. On the other hand, the higher molecular weight phthalate esters are less volatile, but because of their very low water solubility they have a considerably higher Henry law constant (*H*). Thus, the higher molecular weight phthalate esters will potentially evaporate more rapidly from water, especially at high temperature and this phenomena might be the cause of the behavior observed.

Finally, Fig. 3 shows the response plot for the factors type of fiber and extraction mode. Only the diagram corresponding to DOP has been included since the graphs for the remaining compounds do not add more information to the results already commented. In this figure, HS-SPME appears as the most convenient sampling mode for all the fibers but PDMS–DVB and, although responses for all fibers and for both sampling modes are quite similar (excluding CAR–PDMS responses) maximum response was achieved by PDMS–DVB and direct sampling.

As concluded from these observations, the optimal extraction conditions are presented in Table 3. These conditions were different depending on the considered compounds, so the final selection should consider the purpose of the study. If the objective is mainly to analyze DEHP, best conditions would include HS-SPME sampling mode. On the other hand, to analyze the most volatile phthalate esters, such as DMP and DEP, CAR–PDMS would be the most suitable fiber. If simultaneous analysis of all compounds is required; the most favourable conditions are DSPME at 100 °C using PDMS–DVB fiber. In fact, these conditions were employed for the rest of experiments in this study.

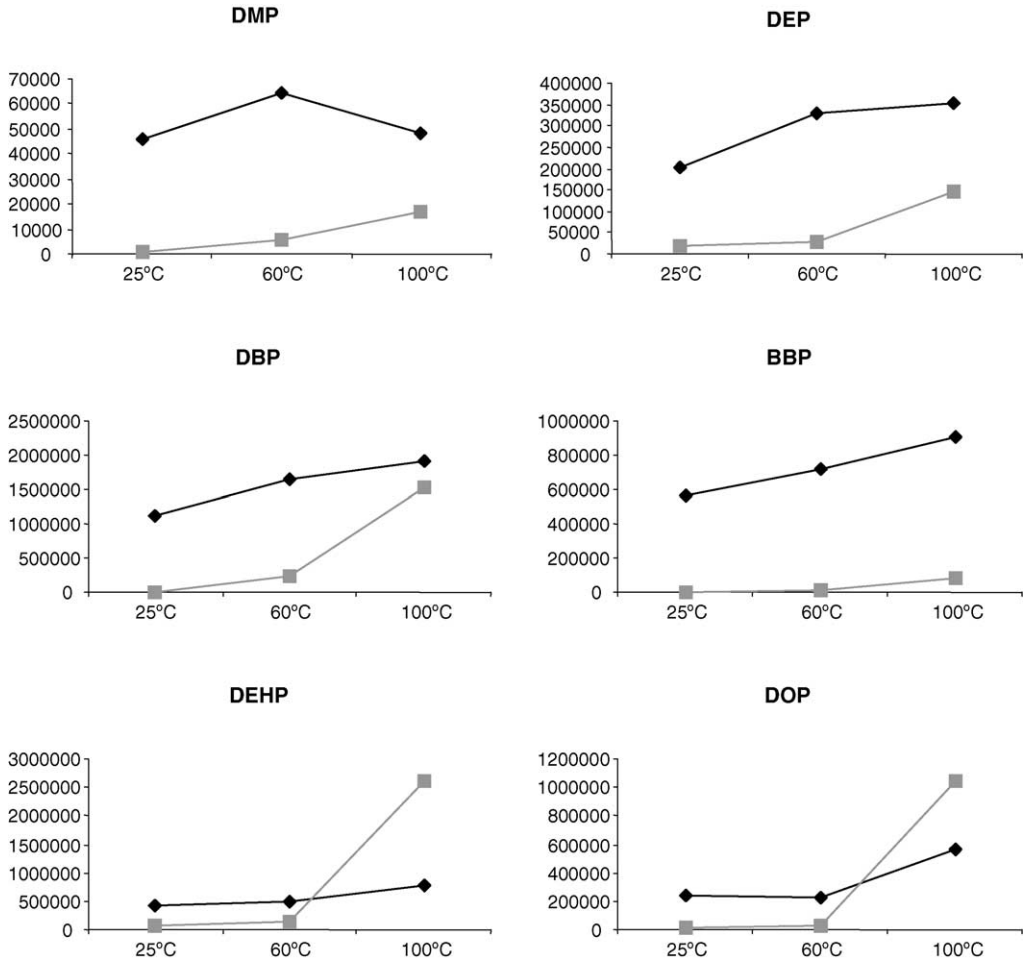

Fig. 2. Extraction temperature–extraction mode interaction plots for all compounds (response in area counts): (▲) DSPME and (◆) HS-SPME.

The addition of salt was initially not considered to avoid additional sources of possible phthalate contamination that could distort the results of the design. In fact, in our first experiments to study the influence of this factor, we had serious contamination problems with NaCl bottled in a plastic container. So, we decided to purchase high purity reagent in a glass bottle. This reagent was suitable for phthalate analysis and did not required previous clean-up, since solvent extraction of this salt did not show the presence of phthalates in the GC–MS analysis. Under these conditions, the salting effect was evaluated by analysing water samples with 0 and 20% NaCl, in the experimental conditions indi-

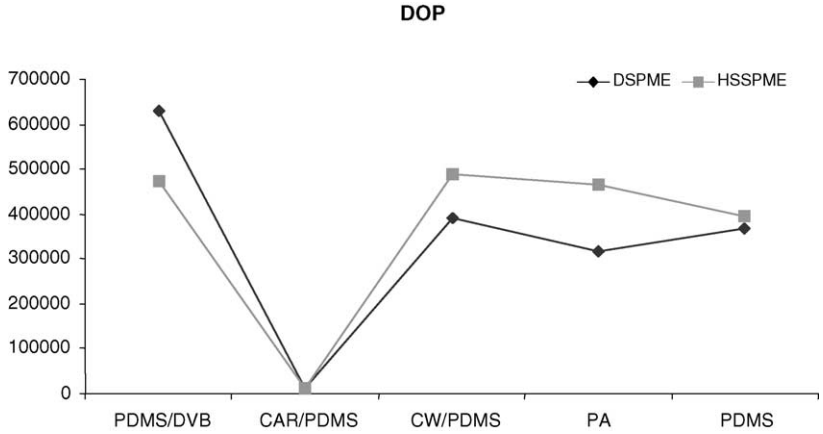

Fig. 3. Fiber–extraction mode interaction plot for DOP (response in area counts).

Table 3
Optimal conditions for each compound given by multifactor categorical 5 × 3 × 2 design

	DMP	DEP	DBP	BBP	DEHP	DOP
Fiber	CAR–PDMS	CAR–PDMS	PDMS–DVB	PDMS–DVB	PA	PDMS–DVB
Temperature (°C)	100	100	100	100	100	100
Sampling mode	SPME	SPME	SPME	SPME	HS-SPME	SPME

cated earlier (DSPME, PDMS–DVB, 100 °C). Addition of salt produced a noticeable increase in the response obtained for DMP and DEP, while for the rest of compounds the response decreased, especially for DOP. As is known, the addition of salt increases the ionic strength of the water sample and in this way, it can favour the transfer of neutral analytes from the sample to the fiber. This effect is evident for DMP and DEP, the two analytes with the highest water solubility and the lowest molecular size. For the rest of compounds, analytes with very low water solubility and quite slow SPME kinetics (see Section 3.2), kinetic aspects could be responsible of the observed decrease in response. Similar behavior has been observed for other organic pollutants [26,27].

The influence of extraction time was also studied and the results are shown in Fig. 4. As can be seen, the time required for reaching equilibrium is, in general, directly related to the molecular weight of the phthalate. For DMP and DEP, the extraction kinetics is quite fast and equilibrium is achieved in 20 and 40 min, respectively. After 80 min of sampling, a decrease in DMP response is observed; this behavior might be due to competitive adsorption that could produce the displacement of more volatile analytes form the fiber surface [28]. For the rest of compounds, equilibrium is not reached even within 80 min of exposure.

3.2. Performance evaluation of the proposed method

For the following studies, the optimal SPME experimental design conditions (DSPME, PDMS–DVB coating, 100 °C extraction temperature) were employed. Extraction time was 20 min.

To evaluate the performance of the method in terms of linear range and detection limits, it is necessary, or at least convenient, to have water free of analytes to establish the background of the method. Nevertheless, one of the most important problems in the analysis of phthalates in water samples is the detection of these compounds in the samples used as blanks. Phthalates have been detected in purified water commonly used in laboratories, including water distilled in a glass distillation apparatus, Milli-Q water, and commercially available water special for VOC determination. Some authors have reported the levels of phthalate esters found in purified water employed in their studies [23,29,30]. The concentrations found are frequently high considering the levels of concentration at which these compounds must be controlled in the environment [3,7–9]. These blank signals forced the limits of detection achieved, mainly for DBP and DEHP, the most ubiquitous phthalate esters. In the present study, blank SPME analyses were initially carried out with Milli-Q water, and the presence of phthalates was detected being BBP and DEHP the compounds found at the highest level. Analyses of commercial ultrapure water (see Section 2) shown the presence of DEP and DBP at very low levels and DEHP at higher level although lower than in our laboratory Milli-Q water. The estimated concentrations of DEP, DBP and DEHP were 5, 14 and 550 pg mL^{-1}, respectively. Contamination of commercial ultrapure water by DEHP was further evidenced because other real mineral and river water samples analyzed in identical conditions produced 10-fold lower results for DEHP. However, regarding DEP and DBP, it is difficult to accurately assign the origin of the detected levels. These results may be attributed to the presence of low levels of these compounds in the commercial ultrapure water or due to contamination during the analytical procedural stages. In spite of these results, because the unavailability of a perfect blank water sample, the commercial ultrapure water was adopted for further performance studies.

To evaluate linearity of the SPME method, calibration studies were performed using multilevel spiked samples. The concentration range tested was from 80 to 8000 pg mL^{-1} for DMP, DEP, DBP, BBP and DOP, and from 500 to 8000 pg mL^{-1} for DEHP. Background levels were subtracted from the results. Coefficients of determination (R^2) are given in Table 4. They were equal or higher than 0.998, demonstrating a directly proportional relationship between the extracted amount of phthalate esters and their initial concentration in the sample.

Precision of the experimental procedure was also evaluated at two different concentration levels by calculating the relative standard deviation (RSD) of three replicates of each level. These results are shown in Table 4. RSD values were between 3.4% for DEP and 16% for DEHP, and between 7.3% for BBP and 12% for DEP, for the low and the high concentration level, respectively.

Estimates of detection limits (LOD = blank signal + 3SD) take into account the background levels measured in the commercial ultrapure water. Obviously, this approach cannot be applied to DEHP because the important contamination detected in the performance sample. Because we tested several different water samples including natural and drinking ones as well as ultrapure from different origins, the background level used to calculate LOD for DEHP was that found in the sample giving the lowest signal. In this way, the estimated

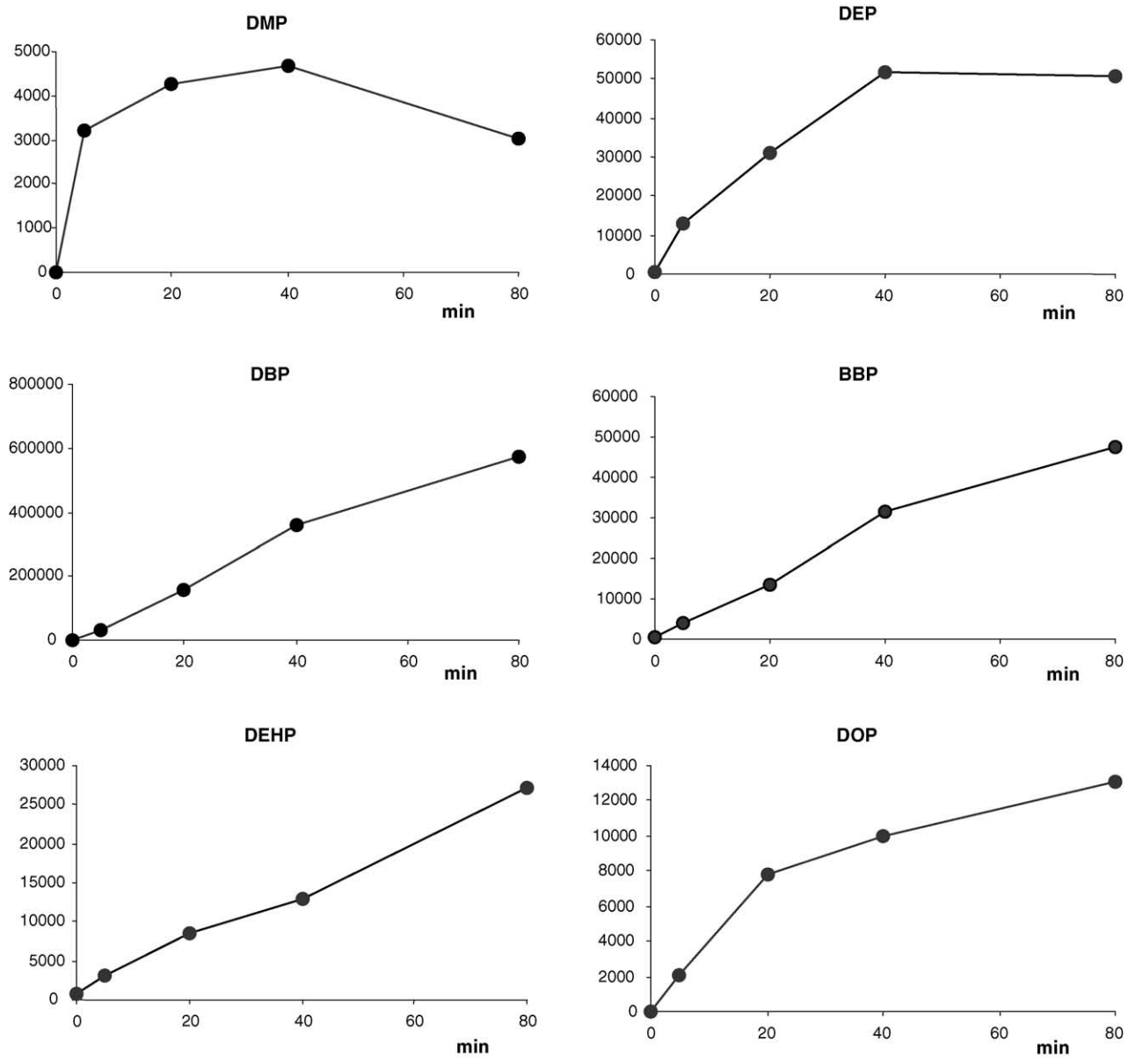

Fig. 4. Extraction times profiles (response in area counts). Extraction conditions: PDMS–DVB, 100 °C, DSPME.

LODs are summarised in Table 4. These estimates maybe considered as conservative. If the levels of phthalates found in blank sample analyses might be attributed to real sample contamination instead of process contamination, the estimated LODs for DEP, DBP and DEHP would be considerably lower, probably below 2 pg mL^{-1}.

When filtration is carried out before SPME (e.g. wastewater samples), it must be considered in the estimation of LODs as a possible source of contamination. The current study evaluated this effect by analysing blanks of ultrapure water before and after filtration. The responses obtained were equivalent in both cases, with the exception of DEP and DBP. In consequence, estimated LODs for these compounds are higher when a filtration step is included (37 and 60 pg mL^{-1} for DEP and DBP, respectively).

Table 4
Linearity, limit of detection (LOD) and repeatability of the proposed method

Compound	Coefficient of determination (R^2)	LOD (pg mL^{-1})	Repeatability (RSD, %)	
			500 pg mL^{-1}	2500 pg mL^{-1}
DMP	0.9989	8	5.2	8.2
DEP	0.9991	7	3.4	12
DBP	0.9990	26	9.7	9.3
BBP	0.9985	2	8.0	7.3
DEHP	0.9994	103	16	6.0
DOP	0.9980	16	11	11

3.3. Analysis of real samples

Due to their widespread applications, phthalates were found in all examined samples. Real samples analyzed in-

cluded: mineral bottled water, industrial harbour water, river water, urban collector water, and influent and effluent waters from an urban wastewater treatment plant. Influent sample is the most complex matrix of all them, so this sample was selected to study possible matrix effects. This sample was spiked with the target analytes and analyzed by the proposed procedure. The amounts of analytes found were in good agreement with the amount of analyte added obtaining recoveries from 87 to 110% (RSD = 3–10%). Therefore, no significant matrix effects were found, which makes possible quantification by external standard calibration. Table 5 shows the phthalate concentrations found in the different water samples. As indicated in Section 2, wastewater samples were filtered and filtration blanks were considered for quantification. DEP, DBP and DEHP were the compounds present in more extent, especially in the urban wastewater samples. These high concentrations could be expected since DEHP is the most used plasticizer and DEP and DBP are quite common components in personal care and pharmaceutical products (PPCPs). In Fig. 5, the chromatogram obtained for the influent wastewater plant sample is shown. In the industrial harbour water sample, DEP appeared at high level of concentration; surprisingly, DBP and DEHP were under LOD. DMP was detected in the mineral and river water samples at lev-

Table 5
Concentration (pg mL^{-1}) of the target phthalates found in real samples

Water samples	Concentration (pg mL^{-1})					
	DMP	DEP	DBP	BBP	DEHP	DOP
Bottled mineral	26	<LOD	<LOQ	<LOQ	<LOD	<LOD
Industrial harbour	<LOD	1606	<LOD	19	<LOD	<LOD
River	28	30	<LOQ	11	<LOD	<LOD
Effluent	<LOD	116	303	<LOD	859	<LOD
Influent	<LOD	2917	405	21	3280	<LOD
Urban collector	<LOD	460	866	127	6172	270

LOD: detection limit; LOQ: quantification limit.

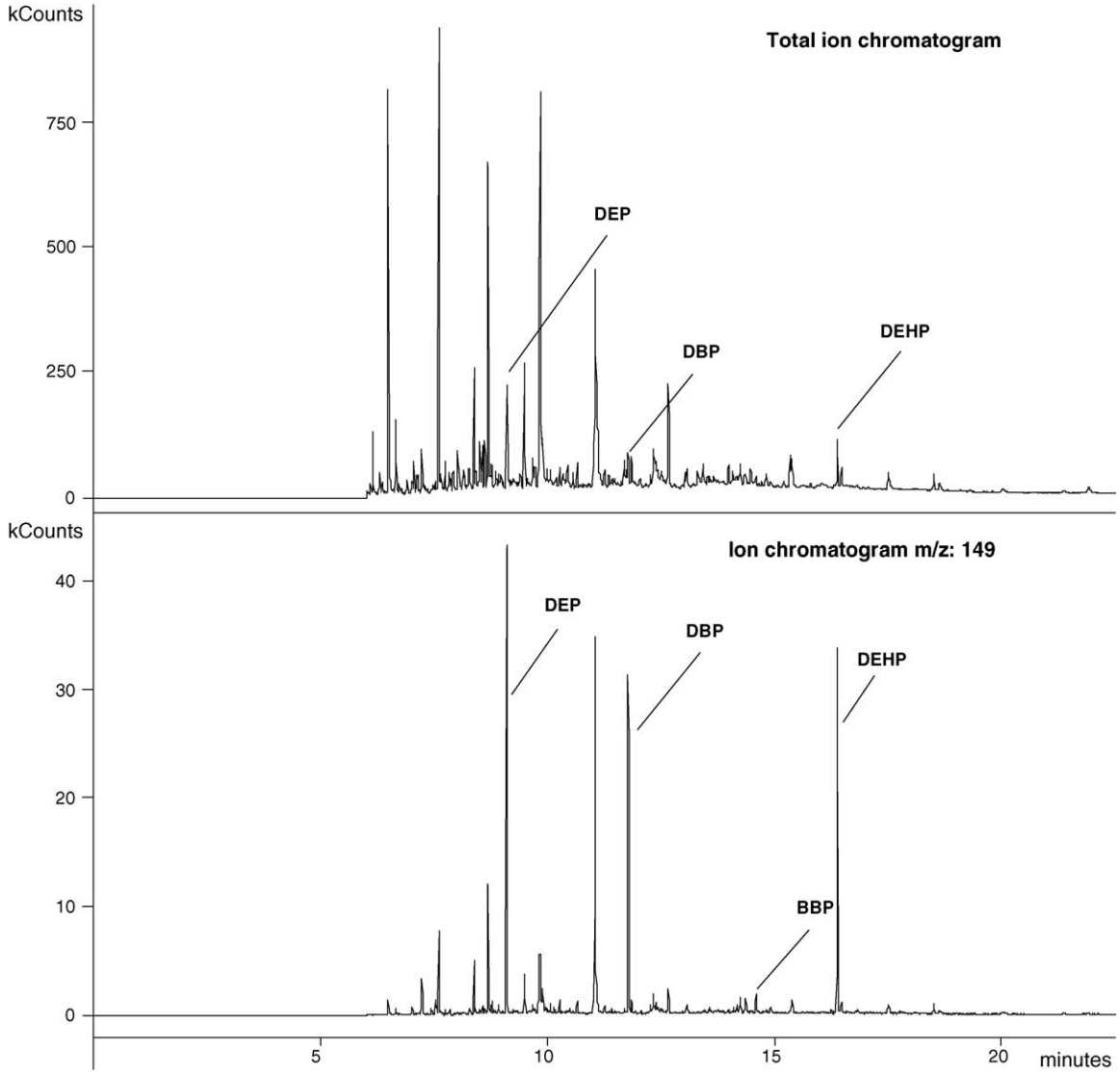

Fig. 5. Chromatograms of an influent wastewater plant sample. Extraction conditions: PDMS–DVB, 100 °C, DSPME, 20 min.

els of 25–30 pg mL^{-1}. DOP was only detected in an influent wastewater sample.

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