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MultiSimplex optimisation of the solid-phase microextraction–gas chromatographic–mass spectrometric determination of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and phthalates from water samples

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Abstract

Solid-phase microextraction coupled to GC–MS was optimised for the determination of polycyclic aromatic hydrocarbons (PAHs), phthalate esters and polychlorinated biphenyls (PCBs) in water samples. A 30-μm polydimethylsiloxane fiber was immersed in a 30-ml water sample that contained the analytes of interest (PAHs, PCBs and phthalate esters) and the variables studied were extraction time (15–60 min), extraction temperature (30–90 °C), desorption time (1–5 min), desorption temperature (220–270 °C) and the addition of sodium chloride (0–9 g). The MultiSimplex programme based on the simplex algorithm was used to establish the optimal conditions. MultiSimplex allowed the simultaneous study of the variables mentioned above and considered the answers of all types of compounds studied in this work. Thus, the optimal conditions obtained allowed the simultaneous determination of PAHs, phthalate esters and PCBs. Furthermore, the accuracy and repeatability of the developed method were calculated from water samples spiked at known concentrations of the analytes. Finally, the optimised method was used to analyse water samples from different sampling points of the Urdaibai and Nerbioi-Ibaizabal estuaries (Biscay, Spain).

Keywords: Water analysis; Environmental analysis; Optimization; MultiSimplex optimization; Polynuclear aromatic hydrocarbons; Polychlorinated biphenyls; Phthalates

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and phthalate esters are among the different organic compounds that can be found in polluted waters and sediments due to their widespread use over the last 100 years [1]. The concern to handle these anthropogenic compounds has increased in the last decades since several of these families of compounds have turned out to show mutagenic and carcinogenic effects [1,2].

From the analytical point of view, the development of new methods that are capable of analysing this sort of compounds at the levels found in

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environmental samples is essential. The analysis of organic compounds in water samples is usually carried out using both liquid–liquid extraction (LLE) or solid-phase extraction (SPE). LLE is a very useful technique but it is tedious, time consuming and requires large amounts of solvents. SPE is a less time-consuming method but still requires toxic solvents for the elution step [3]. In the last few years, solid-phase microextraction (SPME) has been developed for the analysis or organic and organometallic compounds in water and air samples. SPME is also a sorbent extraction technique similar to SPE but in this case the sorbent material is attached to the surface of a fiber rather than in a cartridge. Besides, in spite of the exhaustive extraction carried out in SPE, SPME only extracts the analyte amount that has reached equilibrium between the two phases [4]. SPME makes no use of extracting organic solvents, smaller sample volumes can be used and fibers can be reused [5].

Many works that optimise SPME for the analysis of PAHs [3,6,7], PCBs [8] and phthalate esters [9,10] can be found in the literature. However, in most of these works the optimisation process is carried out one-factor-at-a-time. Generally speaking, optimisation procedures that alter all variables at the same time are more advantageous since interactions among them are considered. In this sense, the MultiSimplex programme [11] based on the simplex algorithm [12] was used in this work since it allowed the simultaneous study of different variables and different responses. This way, if the analytes studied did not behave in the same manner, MultiSimplex would try to find the best common conditions for all of the analytes studied.

MultiSimplex optimisation is very easy to follow. First of all, the variables, the range of each variable and the responses that are going to be followed are defined. Then, MultiSimplex suggests a $k+1$ number of experiments, where k is the number of variables to be studied. Once the experiments are carried out, the answers of the experiments are introduced and MultiSimplex suggests one new experiment. And the process goes on until the optimum conditions are reached. In order to measure the closeness to the optimum, MultiSimplex makes use of the ''membership value'' [12]. This value ranges from 0 to 1 and takes into account the responses of all the responses considered in the optimisation. Optimised conditions are achieved when the membership value is close to 1. The optimisation procedure includes a re-evaluation rule that means that, for every certain number of experiments, a previous trial is repeated experimentally [11].

In this work, the SPME of PAHs (16), PCBs (6) and phthalate esters (6) was optimised using the MultiSimplex programme. The compounds chosen allowed the study of a wide range of polarities and molecular masses and, in this sense, the use of an optimisation tool such as MultiSimplex to establish the optimal determination procedure of such a variety of compounds was very helpful since such a programme takes into account the answer of every compound. No other works could be found in the literature that studied the simultaneous extraction and determination of the compounds mentioned above.

One of the disadvantages of the MultiSimplex if compared with other multivariate optimisation tools such as experimental designs is that the results of one experiment are necessary before the performance of the next experiment. This fact can be a disadvantage when the procedure to optimise is long and tedious (optimisation of microwave-assisted extraction or pressurised fluid extraction of solid samples, for instance) but this is not the case for SPME since the response is obtained by the time the fiber is ready for the following experiment and this way there are no time lapses were no experimentation is being carried out.

The developed method was tested against synthetic samples prepared in our laboratory and was finally applied to environmental water samples from the Urdaibai and Nerbioi-Ibaizabal estuaries (Biscay, Spain).

2. Experimental

2.1. Reagents and materials

US Environmental Protection Agency (EPA) phthalate esters mix (2000 $\mu g\ ml^{-1}$), PCB congener mix (10 $\mu g\ ml^{-1}$) and SS TCL polynuclear aromatic hydrocarbons mix (2000 $\mu g\ ml^{-1}$) were purchased from Supelco (Walton-on-Thames, UK). A 40 $\mu g\ ml^{-1}$ stock standard solution of PAHs and phthalate esters was prepared in methanol. A 0.2 $\mu g\ ml^{-1}$ stock standard solution of PAHs and PCBs and a

5 μg ml^{-1} stock standard solution of phthalate esters in methanol were daily prepared. These last standards were used for the preparation of the calibration points for the standard addition method.

Methanol was purchased from LabScan (Dublin, Ireland) and sodium chloride from Merck (Darmstadt, Germany).

Polydimethylsiloxane (PDMS) fibers (30 μm) were obtained from Supelco.

Extraction vials (40 ml) and their caps with PTFE septa were purchased from Supelco (Walton-on-Thames, UK).

The MultiSimplex software was obtained from Bergström and Öberg [11].

Environmental water sample were collected from two different estuaries in Biscay: Urdaibai reserve (a natural biosphere, Gernika, North of Spain) and Nerbioi-Ibaizabal (a heavily industrialised area, Bilbao, North of Spain). Water samples were collected in cleaned glass bottles, filtered through 0.45-μm cellulose nitrate filters (Whatman, Kent, UK) and kept in the refrigerator until analysis.

2.2. MultiSimplex optimisation of SPME

In this work, the extraction time (15–60 min) and temperature (30–90 °C), the desorption time (1–5 min) and temperature (220–270 °C) and the addition of sodium chloride (0–9 g) were the variables studied and the response of acenaphthylene, phenanthrene, chrysene, 2,6-dichlorobiphenyl, 2,2′,4,4′-tetrachlorobiphenyl, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl, diethyl phthalate, butyl-benzyl phthalate and *n*-octyl phthalate were followed in the optimisation study. The compounds mentioned above were chosen for the optimisation so that a wide range of volatilities and molecular masses were considered for each family of compounds studied. The experiments were performed as follows.

Milli-Q water (30 ml; Millipore, Bedford, MA, USA), the adequate mass of sodium chloride (according to MultiSimplex) and a magnetic stirring bar were introduced in a 40-ml extraction vial. The vial was closed and magnetically stirred until the sodium chloride was completely dissolved. Then, the cap was opened and the appropriate amount of analytes (1 μg l^{-1} for PAHs and PCBs and 10 μg l^{-1} for phthalate esters) was added. The vial was closed again and the 30-μm PDMS fiber was dipped in the solution while it was gently stirred and the extraction was carried out under the time and temperature conditions suggested by MultiSimplex (see Table 1). Samples were heated using the pocket heater of a purge and trap concentrator (Agilent Technologies, Avondale, PA, USA). Once the extraction period was over, the fiber was desorbed in the injection port for the time (splitless time) and at the temperature fixed by the optimisation programme. Once the desorption time under the splitless injection was over, the fiber was kept in the hot injection-port for another 10 min and the injector was vented. This way, it could be assured that all the analytes were completely desorbed and the fiber was ready for the following analysis.

The stirring speed was also studied but once the extraction and desorption steps had been optimised. Two different agitation speeds (slow and fast) were studied and the average of three experiments were compared in order to establish the best agitation.

2.3. Analysis of the extracts

The extracted compounds were analysed on a 5989 Hewlett-Packard gas chromatograph coupled to a Hewlett-Packard MS Engine mass spectrometer (Agilent Technologies). The fibers were injected in a hot injection port (270 °C) for 2 min. The 30 m× 0.32 mm, 0.25 μm HP-5 capillary column was held at 70 °C for 2 min, increased at 6 °C min^{-1} to 130 °C where it was held for 2 min and increased again at 10 °C min^{-1} to 280 °C where it was finally held for 7 min. The carrier gas was helium (N-50) at a linear velocity of 61.7 cm s^{-1}. The mass spectrometer was operated in the electron impact ionization mode and the energy of the electrons was kept at 70 eV. The interface was kept at 300 °C and the ionization source and the quadrupole at 250 and 100 °C, respectively.

Measurements were performed in the single ion monitoring (SIM) mode, the ions followed have been included in Tables 2–4 and dwell time was set at 100 ms in all cases.

2.4. Estimation of the accuracy, precision and limit of detection

Since low concentrations of the analytes studied were expected in water samples, it was decided to

Table 1
Experimental conditions, responses and membership values for each of the experiments performed for the MultiSimplex optimisation of simultaneous SPME of PAHs, PCBs and phthalate esters

No.	Variables					Responses									Membership
	Extraction		Desorption		NaCl (g)	Acy	DEP	2,6-Dichloro	Phe	2,2′-4,4′-Tetrachloro	BBP	Chry	2,2′,3,4,4′,5,5′-Heptachloro	DOP	
	t (min)	*T* (°C)	*t* (min)	*T* (°C)											
1	15	90	1	270	8	1 461 461	225 736	1 855 595	1 840 650	859 677	249 629	2 191 173	421 282	290 611	0.621
2	45	90	5	270	0	59 165	297 752	495 186	298 147	834 308	311 819	1 970 485	386 639	393 445	0.535
3	15	30	5	220	8	1 382 191	189 796	1 243 189	1 138 336	313 203	163 954	891 057	147 863	198 253	0.551
4	45	30	1	270	0	427 450	4 285 773	4 139 571	4 139 571	2 657 157	1 041 598	9 236 540	1 390 781	450 184	0.874
5	45	90	1	220	8	808 848	80 304	1 309 663	1 670 209	1 674 616	503 528	1 962 297	532 237	509 960	0.648
6	15	90	1	220	0	149 319	33 528	696 206	662 898	232 536	285 156	1 894 221	122 656	40 282	0.434
7	51	42	4	270	9	1 182 682	163 059	2 034 573	1 686 015	694 839	207 059	813 878	101 830	108 468	0.561
8	23	25	1	270	9	2 057 327	969 658	1 541 088	1 706 947	477 990	306 266	1 715 905	276 998	194 344	0.650
9	57	81	1	270	9	910 196	194 493	1 715 190	2 333 920	1 858 493	852 875	3 937 033	716 026	634 551	0.727
10	23	84	5	270	5	712 740	85 326	2 145 508	2 217 291	1 167 602	773 034	2 730 786	375 693	300 977	0.653
11	60	34	3	270	9	368 7633	351 320	3 479 750	4 516 231	1 174 256	1 161 807	4 375 405	652 183	617 921	0.800
12	39	25	3	270	5	1 116 336	61 959	1 492 833	1 335 586	378 888	257 440	765 581	138 254	197 020	0.571
13	44	70	2	270	7	569 080	65 342	1 925 295	1 913 151	1 198 140	536 752	1 703 271	1 536 623	247 874	0.682
14	60	95	4	270	3	690 913	16 336	1 359 231	2 046 568	510 907	725 091	1 795 560	261 936	411 820	0.580
15	35	42	2	270	8	788 191	72 159	1 193 659	995 710	321 395	210 673	630 849	137 887	94 057	0.508
16	60	25	1	270	8	780 703	61 160	930 890	1 125 385	425 998	278 321	992 135	193 762	192 543	0.537

Acy, acenaphthylene; DEP, diethyl phthalate ester; 2,6-dichloro, 2,6-dichlorobiphenyl; Phe, phenanthrene; 2,2′,4,4′-tetrachloro, 2,2′,4,4′-tetrachlorobiphenyl; BBP, butyl, benzyl phthalate ester; Chry, chrysene; 2,2′,3,4,4′,5,5′-heptachloro, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl; DOP, di-*n*-octyl phthalate ester.

Table 2
m/z values, repeatability (RSD, %), accuracy (A, %), squared correlation coefficient (r^2) and limits of detection (L.D. in μg l^{-1}) obtained for the PAHs studied in this work

	PAHs														
	Nap	Acy	Ace	Flu	Phe	Ant	Fluor	Pyr	Benzo[*a*]	Chry	Benzo[*b*]	Benzo[*k*]	Benzo[*a′*]	[*ah*]+[*ghi*][d]	Ind
m/z	128,129	152,153	154,153	166,165	178,179	178,179	202,203	202,203	228,229	228,229	252,253	252,253	252,253	276,277	276,277
RSD %[a]	27	11	5	5	7	3	4	3	13	4	11	15	4	14	15
A %[b]	54	29	6	4	10	19	16	14	29	19	38	27	37	36	23
r^2	0.995	0.992	0.993	0.990	0.991	0.992	0.990	0.998	0.992	0.991	0.990	0.995	0.991	0.991	0.996
L.D.[c]	0.08	0.06	0.05	0.04	0.03	0.10	0.12	0.04	0.09	0.09	0.10	0.07	0.09	0.09	0.06

Nap, naphthalene; Acy, acenaphthylene; Ace, acenaphthene; Flu, fluorene; Phe, phenanthrene; Ant, anthracene; Fluor, fluoranthene; Pyr, pyrene; Benzo[*a*], benzo[*a*]anthracene; Chry, chrysene; Benzo[*b*], benzo[*b*]fluoranthene; Benzo[*k*], benzo[*k*]fluoranthene; Benzo[*a′*], benzo[*a*]pyrene; [*ah*]+[*ghi*], dibenzo[*a*,*h*]anthracene+dibenzo[*g*,*h*,*i*]perylene; Ind, indene(1,2,3-*cd*)pyrene.

[a] Repeatability studied for a 0.4 μg l^{-1} solution.

[b] $A = \frac{(\mu - \bar{x})}{\mu} \times 100.$

[c] L.D., limit of detection estimated as the offset plus three times the standard deviation of the offset.

[d] Dibenzo[*a*,*h*]anthracene and dibenzo[*ghi*]perylene could not be chromatographically separated.

Table 3
m/z values, repeatability (RSD, %), accuracy (A, %), squared correlation coefficient (r^2) and limits of detection (L.D. in μg l^{-1}) obtained for the PCBs studied in this work

	PCBs					
	2,6-Dichloro	2,4,4′-Trichloro	2,2′,4,4′-Tetrachloro	2,2′,4,4′,5,5′-Hexachloro	2,2′,3,4,4′,5′-Hexachloro′	2,2′,3,4,4′,5,5′-Heptachloro
m/z	222,224	256,258	292,290	362,360	362,360	396,394
RSD (%)[a]	9	10	13	8	8	14
A (%)[b]	19	29	12	8	8	9
r^2	0.994	0.990	0.999	0.992	0.992	0.992
L.D.[c]	0.05	0.10	0.03	0.11	0.11	0.08

2,6-Dichloro, 2,6-dichlorobiphenyl; 2,4,4,′-trichloro, 2,4,4′-trichlorobiphenyl; 2,2′,4,4′-tetrachloro, 2,2′,4,4′-tetrachlorobiphenyl; 2,2′,4,4′,5,5′-hexachloro, 2,2′,4,4′,5,5′-hexachlorobiphenyl; 2,2′,3,4,4′,5′-hexachloro′, 2,2′,3,4,4′,5′-hexachlorobiphenyl; 2,2′,3,4,4′,5,5′-heptachloro, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl.

[a] Repeatability studied for a 0.4 μg l^{-1} solution.

[b] $A = \frac{(\mu - \bar{x})}{\mu} * 100$.

[c] L.D., limit of detection, estimated as the offset plus three times the standard deviation of the offset.

study the limit of detection of the developed method and, in this sense, three blank samples were analysed. Thirty ml of Milli-Q water, approximately 9.0 g of sodium chloride and a magnetic bar were added to the extraction vial. The vial was capped and magnetically stirred until the salt was completely dissolved. Then, the samples were extracted for 50 min at 50 °C with the 30-μm PDMS fiber. Finally, the fiber was desorbed in the injection port for 2.0 min at 270 °C. The limit of detection was estimated as the signal of the blank plus three times the standard deviation of this signal or as the offset plus three times the deviation of the offset when no peak was found for the compound in blank sample [13].

Table 4
m/z values, repeatability (RSD, %), accuracy (A, %), squared correlation coefficient (r^2) and limits of detection (L.D. in μg l^{-1}) obtained for the phthalate esters studied in this work

	Phthalate esters				
	DEP	DBP	BBP	DEHP	DOP
m/z	149,177	149,104	149,91	149,167	149,279
RSD (%)[a]	9	2	6	23	5
A (%)[b]	30	46	26	34	2
r^2	0.993	0.993	0.990	0.994	0.994
L.D.[c]	0.11	0.09	0.07	3.15[c]	0.84

DEP, diethyl phthalate; DBP, di-*n*-butyl phthalate; BBP, butyl, benzyl-phthalate; DEHP, bis(2-ethylhexyl) phthalate; DOP, di-*n*-octyl phthalate.

[a] Repeatability studied for a 3.0 μg l^{-1} solution.

[b] $A = \frac{(\mu - \bar{x})}{\mu} \times 100$.

[c] L.D., limit of detection estimated as the signal of the blank plus three times the standard deviation of the blank.

Since di-*n*-butyl phthalate (DBP) ester and bis(2-ethylhexyl) phthalate (DEHP) ester were found in rather high concentrations (~0.09 and 2.0 μg l^{-1}, respectively) in the blank samples, standard addition calibrations were performed for the analysis of the rest of spiked and natural water samples.

For the estimation of the precision of the method, Milli-Q water was spiked at 0.4 μg l^{-1} for PAHs and PCBs and 3 μg l^{-1} for phthalate esters. The sample was then analysed by standard addition calibration. Standard additions at 0, 0.2, 0.4 and 0.6 μg l^{-1} (PAHs and PCBs) and 0, 3, 6 and 9 μg l^{-1} (phthalate esters) were prepared and treated as mentioned above for blank samples.

Four samples at 0.4 μg l^{-1} for PAHs and PCBs and 3 μg l^{-1} for phthalate esters were analysed for the determination of repeatability.

2.5. Analysis of samples from the Urdaibai and Nerbioi-Ibaizabal estuaries

Samples from two different estuaries of Biscay (Urdaibai and Nerbioi-Ibaizabal) were analysed under the conditions mentioned above. Samples from three different stations of the Urdaibai estuary (Gernika, North of Spain) and one from the Nerbioi-

Ibaizabal estuary (Bilbao, North of Spain) were analysed.

Four-point standard addition calibrations were prepared for the analysis of the samples in the range 0–1.2 μg l^{-1} for PAHs and PCBs and 0–9 μg l^{-1} for phthalate esters.

3. Results and discussion

3.1. MultiSimplex optimisation of the simultaneous SPME of PAHs, PCBs and phthalate esters

Extraction time, extraction temperature, desorption time, desorption temperature and the addition of sodium chloride were optimised for the common SPME of PAHs, PCBs and phthalate esters. The experiments proposed by the MultiSimplex programme and the membership value of each of the experiments are summarised in Table 1.

Since the last three experiments gave a similar membership value (~0.5), it was decided to stop the optimisation experiments after the 16th run. As can be seen from data in Table 1, most of the experiments suggested by MultiSimplex recommended high desorption temperatures (sometimes temperatures higher than 270 °C were suggested but such experiments were not performed since the maximum desorption temperature suggested for the 30-μm PDMS fiber was 280 °C) and high concentrations of sodium chloride (masses higher than 9.0 g were not added since it was more difficult to dissolve all the sodium chloride). Other works [9] have also shown an improvement of the signal when inert salts, such as sodium chloride, were added to the extraction vial but this is not always a rule since some pesticides have been best extracted without the addition of sodium chloride [16]. With respect to the other three variables, the experiments that showed the highest membership values were the following: 4, 9, 11, 13. In all of these four experiments, high extraction times (extraction times ranged from 44 to 60 min) were suggested. Approximately the average extraction time, 50 min, was considered as the optimum. In the case of desorption time, it was not very clear whether this variable had a real effect on the process since values ranging from 1 to 3 min were included in the best four experiments. Thus, desorption time was fixed at 2 min. Finally, a wide range of extraction temperatures (30–81 °C) were suggested in the four experiments with the highest membership values. In this case, it was also decided to take the average value for temperature (~50 °C). As mentioned by Peñalver et al. [3], increasing the adsorption temperature increases the diffusion of the analytes from the solution to the fiber but high temperatures may decrease the signal since the adsorption is an exothermic process.

Therefore, 16 experiments were enough to optimise five variables that affected the SPME–GC–MS determination of three different families of compounds in a wide range of polarities and volatilities. This could hardly be done if ''one-factor-at-a-time'' approach had been followed because more experiments would be needed since the optimisation could not be carried out simultaneously.

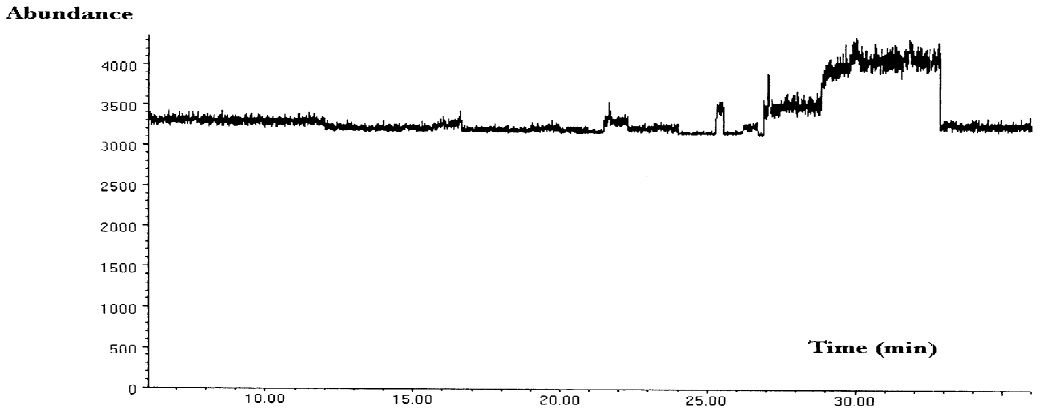
According to the stirring of the solution, comparable responses and repeatabilities were obtained at the two agitation speeds studied in this work.

Therefore, in the rest of the extractions the fibers were exposed for 50 min at 50 °C to a water sample that contained 9 g of sodium chloride. Once the exposure time was over, the fiber was injected in a hot injection port at 270 °C for 2 min. The fiber was kept in the hot injection port for another 10 min so that any possible contamination was desorbed from the fiber.

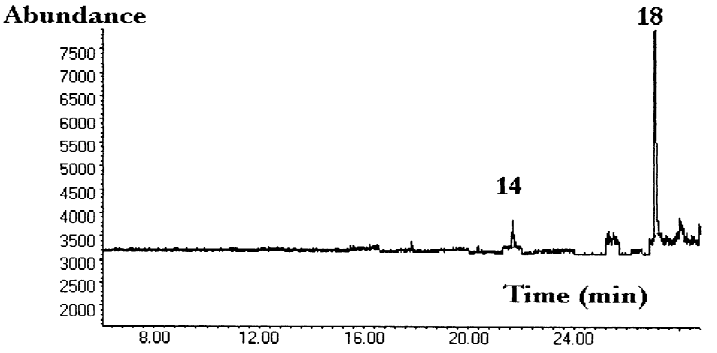
3.2. Estimation of the limits of detection

Three blank samples were run in order to estimate the limit of detection of the developed method. Fig. 1 shows the chromatogram of a blank of the fiber (a) and a blank sample (b). As it could be observed, some phthalate esters [di-*n*-butyl (DBP), and bis(2-ethylhexyl) (DEHP)] could be identified in the chromatogram and especially high signals were observed in the case of DEHP in comparison with the signal obtained when the fiber had not been exposed to a sample. For the rest of the compounds no peak could be integrated at their retention time. Tables 2–4 summarises the limits of detection (in μg l^{-1}) obtained for all the compounds studied in this work.

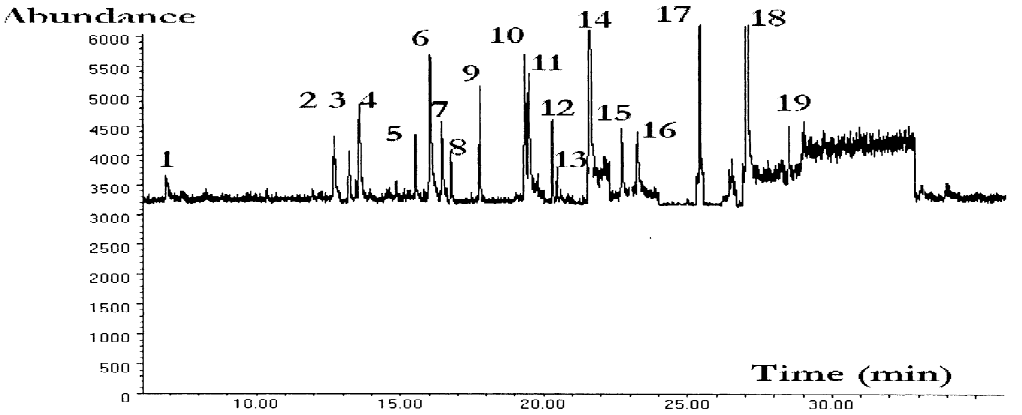
The source of DBP and DEHP has not been determined and even if new vials, magnetic bars,



(a)



(b)



(c)

Fig. 1. SIM chromatograms of (a) the fiber, (b) a blank and (c) a natural water sample from Nerbioi-Ibaizabal. (1) Naphthalene; (2) acenaphthylene; (3) DMP; (4) acenaphthene; (5) fluorene; (6) DEP; (7) 2,6-dichlorobiphenyl; (8) unknown; (9) unknown; (10) phenanthrene; (11) anthracene; (12) 2,4,4′-trichlorobiphenyl; (13) 2,2′,4,4′-tetrachlorobiphenyl; (14) DBP; (15) fluoranthene; (16) pyrene; (17) BBP; (18) DEHP; (19) DOP.

clean vessels, etc., were used, DBP and DEHP signals could be observed in all cases. Glassware is cleaned with different organic solvents [10,14] and dried at high temperatures (300 °C) [10] in the literature. Other authors have silanized their glassware [15]. In our case, extraction vials were ultrasonicated with HPLC-grade acetone and ethyl acetate and dried at 150 °C overnight but still a high signal for DEHP was obtained. Thus, it remained unclear whether the quantities of DBP (not quantified since the results obtained were close to the limit of detection of this compound, 0.09 μg l^{-1}) and DEHP (2.0±0.5 μg l^{-1}) determined by standard addition calibration were due to contamination or were in the Milli-Q water or the glassware used in our laboratory. Contamination due to phthalates is ubiquitous [17] but since the method developed in this work was simple (30 ml of sample were extracted on 30-μm PDMS fiber and desorbed onto a gas chromatograph), the contamination sources were not clear. Possible contamination due to the GC–MS system was discarded since no signal of these compounds was observed when only the fiber was injected. The other possible source of error could be the septum of the vials where the extraction was performed.

According to the above mentioned and taking into account that the concentrations suspected for PAHs and PCBs were low, it was decided to apply the standard addition calibration method for all the samples (spiked or natural) studied in this work.

3.3. Estimation of the repeatability and accuracy

Three Milli-Q water samples were spiked at 0.4 μg l^{-1} level for PAHs and PCBs and 3 μg l^{-1} level for phthalate esters to study the repeatability of the developed method. Tables 2–4 summarise the repeatability obtained for each compound. The repeatabilities obtained were comparable to those obtained in other works [7,8]. The lack of repeatability can be attributed to the SPME but in some cases the integration of peaks, since small peaks were integrated in many cases due to the low concentrations used, should also be considered.

A four-point standard addition calibration was carried out and the concentration was calculated for each of the compounds. The concentration value obtained was compared to the spiking level and the accuracy was estimated as follows:

$$A = \frac{(\mu - \bar{x})}{\mu} \times 100 \quad (1)$$

where A is the accuracy, μ the spiking level and $\bar{x}$ the concentration estimated from the standard addition calibration. The values obtained are summarised in Tables 2–4. The accuracies obtained were not very good (>30% in some cases), especially for some PAHs and phthalate esters. The results obtained could not be compared with other works since we did not find any other work where accuracy was estimated. For compounds of high volatility, it could be possible that losses occurred due to sublimation. In case of the largest PAHs and phthalate esters, they may have been sorbed onto the glass walls of the vials. Thus, it could be possible that the actual concentrations of those analytes differed from those expected.

No statistical information was given for dimethyl phthalate ester (DMP) since this compound was not always recovered. Thus, even if the compound was sometimes detected, it was never quantified due to the high uncertainty in the measurements.

3.4. Analysis of natural water samples from the Urdaibai and Nerbioi-Ibaizabal estuaries

The developed method was applied to the analysis of natural water samples from three different sampling points (URD1, URD2 and URD3) from the Urdaibai estuary and one (GAL1) from the Nerbioi-Ibaizabal estuary. Chromatogram (c) in Fig. 1 shows an extract from the Nerbioi-Ibaizabal estuary. Table 5 summarises the results obtained in the four sampling points. The standard deviations included in Table 5 were estimated by the propagation of errors of the slope and the offset of the calibration curve. The compounds not present in the table were not detected in any of the samples. The Nerbioi-Ibaizabal estuary was the most polluted of the three sampling points and this fact is quite obvious since, while the Nerbioi-Ibaizabal estuary is an industrialised area, the Urdaibai estuary belongs to a protected biosphere reserve. Naphthalene, acenaphthylene, acenaphthene, phenanthrene and anthracene could be

Table 5
Concentrations (in μg l^{-1}) and standard deviation (calculated by the propagation of errors of the slope and the offset of the calibration curve) obtained in natural waters from the Urdaibai and Nerbioi-Ibaizabal estuaries

	GAL1	URD1	URD2	URD3
Naphthalene	0.19±0.05	n.d.[b]	n.d.	–
Acenaphthylene	0.15±0.06	n.d.	–	–
DMP	–[a]	–	–	–
Acenaphthene	–	n.d.	–	–
Fluorene		–	–	–
DEP	0.8±0.2		–	–
2,6-Dichlorobiphenyl	–	n.d.	–	–
Phenanthrene	0.12±0.07	–	–	–
Anthracene	0.10±0.07	–	–	–
2,4,4′-Trichlorobiphenyl	–	n.d.	n.d.	n.d.
2,2′,4,4′-Tetrachlorobiphenyl	–	n.d.	n.d.	n.d.
DBP	1.9±0.7	1.7±0.6	0.9±0.4	0.8±0.4
Fluoranthene	–	n.d.	–	–
Pyrene	–	n.d.	–	–
BBP	–	n.d.	–	–
DEHP	10±1	3.0±0.6	4±2	2.5±0.4
DOP	–	n.d.	n.d.	n.d.

[a] The compound was detected but quantification was not possible since the concentration was below the detection limit.
[b] n.d., the compound was not detected.

measured in GAL1 (Nerbioi-Ibaizabal) although the concentrations were very close to the limits of detection of those analytes. The concentrations obtained were lower than those obtained by Pörschmann et al. [15] for wastewaters. In that work, concentrations that ranged from 0.35 to 3.4 μg l^{-1} were found for different PAHs. In the case of phthalate esters, DBP was found in similar concentrations in all of the sampling points. In the case of DEHP, similar concentrations were found in all the sampling points of the Urdaibai estuary (close to the limit of detection or concentration estimated in the Milli-Q water from our laboratory) but slightly higher concentrations of this compound were found in the Nerbioi-Ibaizabal estuary. Lower concentrations (0.02–0.06 μg l^{-1}) were obtained for DBP and DEHP in drinking waters from Poland and Germany [14] and the River Ebro (0.7 μg l^{-1}) (Spain) although concentrations ranging from 1.62 to 2.12 μg l^{-1} of DEHP were found in industrial ports in Spain [3].

4. Conclusions

Further work should be done to improve the developed method. Appropriate internal standards should be added so that better repeatability and accuracy results were obtained and still further research should be done to really find out the source of DBP and DEHP. However, the method optimised is good as a screening method and the presence of 16 PAHs, six PCBs and six phthalate esters can easily be monitored. It should be noted that no other works on the optimisation of the simultaneous determination of these compounds can be found in the literature.

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