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Short communication

# Optimization of solid-phase microextraction conditions using a response surface methodology to determine organochlorine pesticides in water by gas chromatography and electron-capture detection

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## Abstract

A response surface methodology was applied to optimise the solid-phase microextraction (SPME) conditions using a polyacrylate-coated fiber to determine thirteen organochlorine pesticides from water. Analyses were performed using gas chromatography–electron-capture detection. Variables affecting absorption in both the headspace and immersion extraction were optimised by using a response surface generated with a Doehlert design, and the results were compared. The immersion SPME method was selected since higher recoveries were obtained for most of the compounds studied. The method developed was applied to the analysis of tap and Ebro river water samples. The linear range of most pesticides for real samples was found to be between 0.001 and 2.5  $\mu\text{g l}^{-1}$  and the limits of detection were between 0.15 and 0.35  $\text{ng l}^{-1}$ . The repeatability and the reproducibility between days of the method ( $n=6$ ), expressed as relative standard deviation, for tap water spiked at a level of 1  $\text{ng l}^{-1}$  were between 5.7 and 25.6% and between 7.6 and 26.5%, respectively. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Extraction methods; Water analysis; Environmental analysis; Optimization; Chemometrics; Doehlert design; Organochlorine compounds; Pesticides

## 1. Introduction

Organochlorine pesticides are one of the most persistent organic micropollutants present in water. They are a possible risk to the environment because of their toxicity and ability to bioaccumulate [1] and consequently their presence in water is regulated by legislation [2].

Gas chromatography using electron-capture detection (ECD) [3,4] or mass spectrometry (MS)

detection [3,5] is widely used to determine organochlorine pesticides in water. However, a preconcentration step prior to the chromatographic analysis is necessary in order to achieve the low levels present in real samples. Recently, a new extraction technique, solid-phase microextraction (SPME) has been introduced by Arthur and Pawliszyn [6] as an alternative to conventional techniques. When SPME is applied to the extraction of organochlorine pesticides, poly(dimethylsiloxane) (PDMS) [4,7,8], the first sorbent developed, is the most common fiber coating used.

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In the present paper, the response surface methodology has been used to optimise the immersion and headspace (HS) conditions to determine organochlorine pesticides in real water samples. A polyacrylate fiber for the SPME process, selected according to a previous work [9], was used. The results obtained for both processes, immersion and headspace, are compared and the performance of the developed method at optimum conditions is evaluated with tap and Ebro river water.

## 2. Experimental

### 2.1. Reagents and standards

The organochlorine pesticides studied were:  $\alpha$ -hexachlorocyclohexane (HCH),  $\beta$ -HCH, lindane, heptachlor, heptachlor-endo,  $\alpha$ -endosulfan,  $\beta$ -endosulfan, aldrin, dieldrin, endrin,  $p',p'$ -DDD,  $p',p'$ -DDE and  $p',p'$ -DDT. All pesticides were purchased from Riedel-de Hen (Seelze-Hannover, Germany) with a purity of more than 98% and were dissolved in ethyl acetate. The internal standard was endosulfan sulfate (99.9% purity) which was also supplied by Riedel-de Hen.

Ethyl acetate was of PAR quality (for residue analysis) (Fisher Scientific, Leicestershire, UK). Ultra-pure water was obtained from the Milli-Q (Millipore, Bedford, MA, USA) water purification system. Helium (99.995% quality) and nitrogen (99.995% quality) were supplied by Carbueros Metalicos (Tarragona, Spain). Sodium chloride with a purity of more than 99.5% was supplied by Probus (Barcelona, Spain).

### 2.2. Instrumentation

Chromatographic analyses were performed on a Hewlett-Packard (HP, Palo Alto, CA, USA) 5890 Series II gas chromatograph equipped with a split/splitless injector which was used in the splitless mode and a  $^{63}\text{Ni}$  electron-capture detector. A merlin microseal high-pressure septum from Hewlett-Packard and an insert liner of 0.75 mm I.D. were used. A Hewlett-Packard HP-1 fused-silica capillary column (cross-linked 5% methyl silicone) of 30 m  $\times$  0.25 mm and with a 0.25  $\mu\text{m}$  film thickness separated the

analytes. The injector temperature was set at 250°C and the injection volume was 2  $\mu\text{l}$  when direct injections were made. Chromatographic data were recorded using the HP CHEMSTATION. The carrier gas was helium at a flow-rate of 1.5 ml  $\text{min}^{-1}$  and nitrogen was the make-up gas.

The oven temperature profile was as follows: the initial temperature was 60°C, which was increased to 165°C at 40°C  $\text{min}^{-1}$  and then to 210°C at 1.8°C  $\text{min}^{-1}$ . This temperature was held for 8 min. The total run time was 35.6 min.

### 2.3. SPME procedure

The fiber selected for this study was a fused-silica fiber coated with 85  $\mu\text{m}$  of polyacrylate (PA) and held in an SPME device supplied by Supelco (Bellefonte, PA, USA). The fiber conditioning process and apparatus used for the SPME process were as described in a previous paper [9].

Vials of 4 ml were filled with 3 ml of aqueous samples and the sample was magnetically agitated. The analytes were extracted by immersion SPME directly with the fiber immersed in the aqueous sample for a selected time of 45 min at 60°C. In the HS-SPME extraction, the fiber was suspended above the sample for a selected absorption time of 45 min at 100°C. The last step, the same for both processes, was the thermal desorption of the analytes in the injection port of the gas chromatograph at 250°C for 5 min. The performance of the fiber was checked and at least 30 samples were analysed with the same fiber.

Tap and river water samples were filtered through a 0.45- $\mu\text{m}$  membrane filter (MSI, Westboro, MA, USA) prior to analysis.

### 2.4. Response surface optimization

The effect of absorption temperature ( $T_{\text{abs}}$ ), absorption time ( $t_{\text{abs}}$ ) and salt addition (NaCl) on the immersion SPME and HS-SPME processes was considered in order to obtain the optimal conditions (maximum recovery of each compound with respect to the direct injection) for the extraction of the organochlorine pesticides. Other variables affecting desorption, such as desorption time and temperature,

were the same as in a previous paper [9] for a group of pesticides of diverse nature, since no carryover effect was observed for the organochlorine pesticides studied. Sample pH was not adjusted because preliminary studies showed no differences in recoveries when acidic or basic pH values were used.

For the three factors mentioned, a Doehlert matrix [10] consisting of a set of thirteen experiments was used (see Table 1). Although Doehlert's design may be not used as often as, for example, central composite designs to estimate a 2nd degree polynomial with interactions, Doehlert involves less experiments so it was desirable to show the application of this design in the optimisation of SPME parameters. The design was generated using the NEMROD software [11] and the graphics using MATLAB software [12]. The central point was repeated four times (experiments 13–16) to estimate the experimental repeatability. This matrix is the same for both immersion SPME and HS-SPME and allowed the estimation of coefficients ( $b$ ) of a second degree polynomial model as indicated by the following equation:

$$R = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (1)$$

where  $x_1$ ,  $x_2$ ,  $x_3$  are the values of the three factors studied (absorption temperature ( $T_{\text{abs}}$ ), absorption time ( $t_{\text{abs}}$ ), and salt addition respectively), expressed as coded variables and  $R$  is the sum of the recoveries (%) of the compounds. It is known that the selection of the criterion is a very important step in the optimisation process and in this case we selected the sum of the recoveries in order to obtain the maximum recovery for each compound. This criterion was already been useful in the studies of optimisation of the derivatisation step for carboxylic acids [13].

According to the design (Table 1) each factor  $T_{\text{abs}}$ ,  $t_{\text{abs}}$  and salt addition was studied at different levels. So for  $T_{\text{abs}}$  the variation interval used was 25–100°C at five levels (25, 43.8, 62.5, 81.3 and 100°C), for  $t_{\text{abs}}$  it was 10–90 min at seven levels (10, 23.3, 36.7, 50.0, 63.3, 76.7 and 90 min) and for the NaCl addition it was 0–360 g l<sup>-1</sup> at three levels (0, 180 and 360 g l<sup>-1</sup>, which corresponds to salt saturated solution). These intervals were chosen according to the bibliography [8,9] and preliminary experiments. At temperatures higher than 100°C the vapour pressure gets too high for safe operation and times >90 min involve a long analysis time.

Table 1  
Doehlert experiment matrix planning for the three variables optimized and results

No.	Coded experiments matrix			Experimentation plan			Results	
	Absorption temperature (°C) ( $x_1$ )	Absorption time (min) ( $x_2$ )	NaCl (g l <sup>-1</sup> ) ( $x_3$ )	Absorption temperature (°C) ( $x_1$ )	Absorption time (min) ( $x_2$ )	NaCl (g l <sup>-1</sup> ) ( $x_3$ )	Immersion SPME (R)	HS-SPME (R)
1	1.000	0.000	0.000	100.0	50.0	180.0	1065	1207
2	-1.000	0.000	0.000	25.0	50.0	180.0	572	192
3	0.500	0.866	0.000	81.3	90.0	180.0	988	1170
4	-0.500	-0.866	0.000	43.8	10.0	180.0	268	81
5	0.500	-0.866	0.000	81.3	10.0	180.0	437	363
6	-0.500	0.866	0.000	43.8	90.0	180.0	688	511
7	0.500	0.289	0.816	81.3	63.3	360.0	624	777
8	-0.500	-0.289	-0.816	43.8	36.7	0.0	797	216
9	0.500	-0.289	-0.816	81.3	36.7	0.0	1022	915
10	0.000	0.577	-0.816	62.5	76.7	0.0	1170	944
11	-0.500	0.289	0.816	43.8	63.3	360.0	373	374
12	0.000	-0.577	0.816	62.5	23.3	360.0	210	510
13	0.000	0.000	0.000	62.5	50.0	180.0	788	983
14	0.000	0.000	0.000	62.5	50.0	180.0	836	884
15	0.000	0.000	0.000	62.5	50.0	180.0	838	869
16	0.000	0.000	0.000	62.5	50.0	180.0	854	859

### 3. Results and discussion

#### 3.1. Chromatographic separation

The separation of the organochlorine pesticides by GC–ECD was studied before the SPME process was optimised and the optimal conditions found are indicated in Section 2. An internal standard, endo-sulfan sulfate, was used in order to increase the precision of the method.

The linearity of the response of the chromatographic method was studied in the range between 0.1 and 10  $\mu\text{g l}^{-1}$  by directly injecting 2  $\mu\text{l}$  of standard solutions. Good linearity was obtained for most compounds with coefficients of determination ( $r^2$ ) between 0.995 and 0.999. The limits of detection of the chromatographic method were between 0.03  $\mu\text{g l}^{-1}$  for  $\alpha$ -HCH and 0.7  $\mu\text{g l}^{-1}$  for  $p',p'$ -DDT and they were calculated by the method of Winefordner and Long with a  $K$  value of 6 [14]. The repeatability and reproducibility of the response between days, expressed as relative standard deviation (RSD) ( $n = 3$ ), were between 2.8 and 6.4% and 3.3 and 8.1%, respectively.

#### 3.2. Doehlert optimization

The experiments shown in Table 1 were carried out in a random order with a standard solution containing 7  $\mu\text{g l}^{-1}$  of the pesticides and 2  $\mu\text{g l}^{-1}$  of the internal standard. Therefore two equations were obtained for the model in Eq. (1). The coefficient values for immersion and HS-SPME are shown in

Table 2  
Coefficient values obtained for the equations corresponding to immersion and HS-SPME processes according to the model described

Coefficients	Immersion	HS-SPME
$b_0$	829.0	898.8
$b_1$	241.4	509.1
$b_2$	289.5	331.9
$b_3$	-363.7	-84.5
$b_{11}$	-10.5	-199.3
$b_{22}$	-308.2	-423.6
$b_{33}$	-114.8	-258.4
$b_{12}$	75.6	217.7
$b_{13}$	-10.8	-258.2
$b_{23}$	-85.4	-300.6

Table 2. The negative values for all the coefficients corresponding to factor  $x_3$ , salt addition, suggested that the salt addition decreases the recoveries of organochlorine pesticides although not much can be said due to the presence of interactions in the model. Although the addition of salt to the sample improved recovery values in most of the applications described in the bibliography, the results obtained agree with those already reported when organochlorine pesticides are extracted on a PDMS fiber [15].

The coefficients of multiple regression for the calculated models were 0.9971 and 0.9892 for the immersion and headspace conditions, respectively. These values were considered acceptable taking into account the results of the repetitions in the centre of the domain.

The plot of the response surfaces enabled the experimental conditions to be selected. Figs. 1 and 2 show the evolution of  $R$  throughout the experimental domain at three different salt concentration levels (360  $\text{g l}^{-1}$ , 180  $\text{g l}^{-1}$  and without salt addition) for the immersion and the headspace process.

The optimal conditions tended to 100°C (absorption temperature) and 90 min (absorption time), and no salt addition, for both immersion and headspace processes. From the response surface, the experimental conditions were selected. The final absorption temperature selected in the headspace process was 100°C and in the immersion process was 60°C because at higher absorption temperatures there was a considerable decrease in the recovery of heptachlor, despite the slight improvement in the  $R$  values. On the other hand, the absorption time was shortened to 45 min to minimise the total analysis time because the values of  $R$  at these experimental conditions were acceptable from the surface response obtained.

Several previous experiments were carried out to check whether desorption conditions reported in a previous paper [9] could be applied to the present study. No carryover effect was observed, so these conditions were applied. The two models were validated with standard solutions at a concentration of 7  $\mu\text{g l}^{-1}$  for the pesticides and 2  $\mu\text{g l}^{-1}$  for the internal standard. The experimental values were seen to agree well with the predicted ones.

The results of the recoveries obtained by direct injection for both processes, immersion and headspace, under the conditions selected are shown in

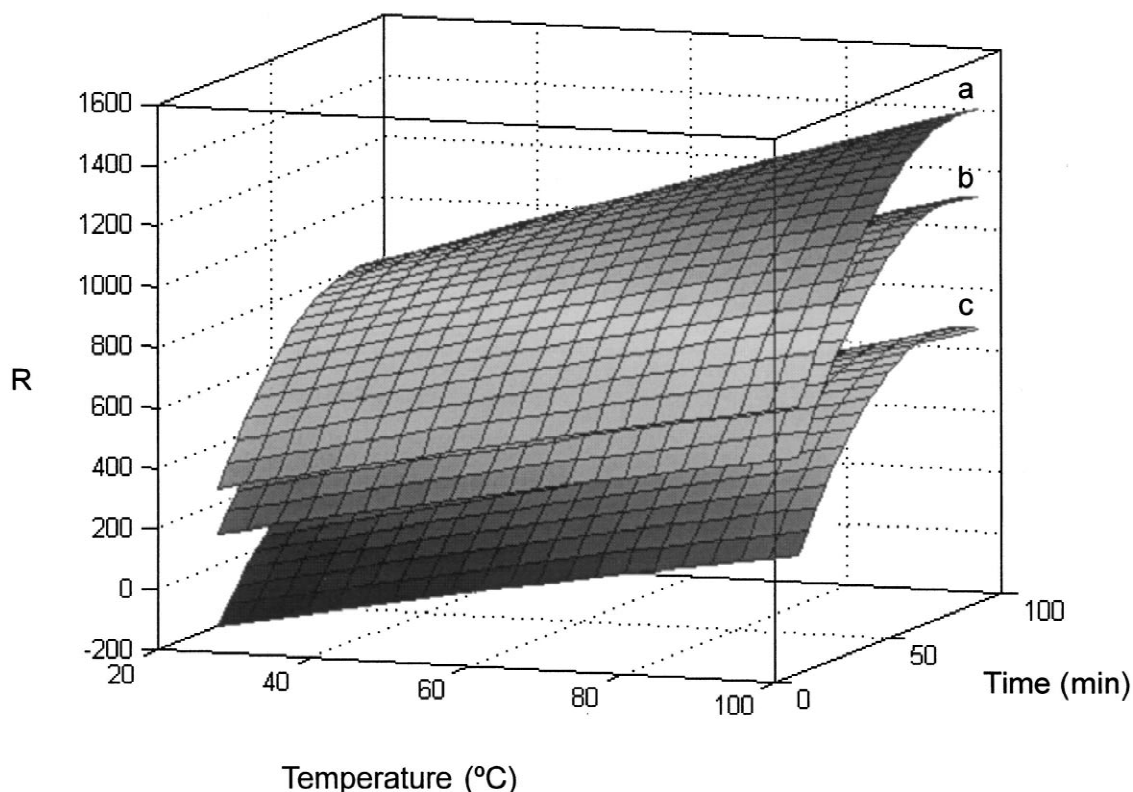


Fig. 1. Response surface for absorption temperature against absorption time in immersion process: (a) without salt addition to the sample; (b) with addition of  $180 \text{ g l}^{-1}$  of NaCl; (c) with addition of  $360 \text{ g l}^{-1}$  of NaCl.

Table 3. The recoveries were similar in both cases for most of the analytes except for  $\beta$ -HCH, lindane,  $\beta$ -endosulfan and the internal standard. Immersion SPME gave better recoveries for these compounds, although the recoveries for heptachlor and  $p',p'$ -DDT were higher with the HS-SPME process. So, immersion SPME was selected for further studies. Recoveries were similar to those reported in the literature [4] with a PDMS fiber.

### 3.3. Application of optimised SPME process to the analysis of real samples

The performance of the method was checked with real samples such as tap and Ebro river water.

The recoveries for tap water were similar to those for Milli-Q water except for  $p',p'$ -DDD. This analyte could not be quantified because an interfering compound eluted at its retention time. Analytical

data were calculated in the same way as for Milli-Q water. Data are collected in Table 4. The organochlorine pesticides studied, except  $p',p'$ -DDD, can be determined in tap water by SPME-GC-ECD at levels set by the European Union. The values of repeatability and reproducibility, which may seem to be high, are values acceptable at such low levels of concentration. For instance, the AOAC accepts values up to 22%, so only some of them are marginal. Fig. 3 shows the chromatograms obtained when both unspiked tap water and tap water spiked with  $1 \text{ ng l}^{-1}$  of each compound were analysed.

The method was also tested by analysing Ebro river water samples and the results were in accordance with those obtained for tap water samples. None of the organochlorine pesticides studied was detected in one of the samples, which was used to obtain the blank chromatogram. The number of non-identified peaks increased compared to the blank

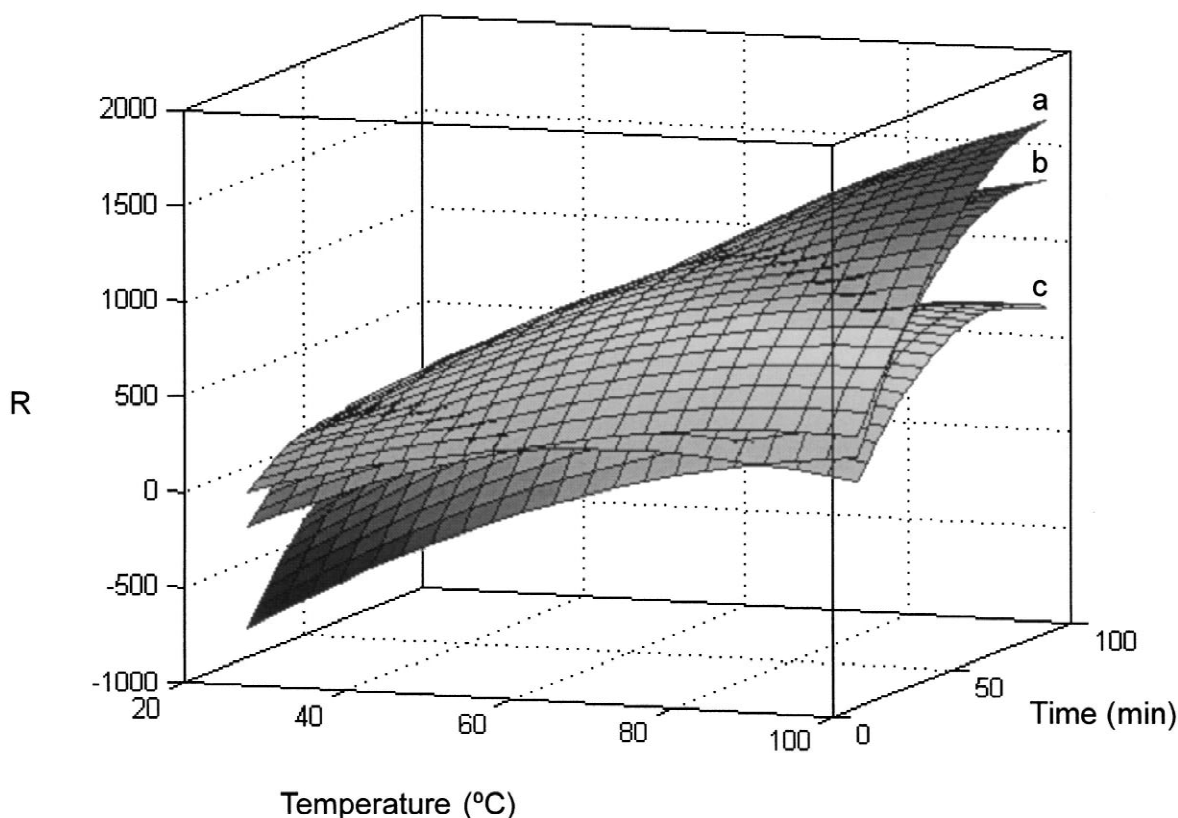


Fig. 2. Response surface for absorption temperature against absorption time in headspace process: (a) without salt addition to the sample; (b) with addition of  $180 \text{ g l}^{-1}$  of NaCl; (c) with addition of  $360 \text{ g l}^{-1}$  of NaCl.

chromatogram obtained for tap water due to the matrix composition. An interfering peak meant that  $\alpha$ -endosulfan could not be quantified, but recoveries for the rest of the organochlorines were similar those for Milli-Q water. Linearity and detection limits were essentially the same as for tap water. The repeatability and reproducibility of the method between days, checked with samples of Ebro river water spiked at a concentration level of  $1 \text{ ng l}^{-1}$  of each compound, were slightly higher than the values for tap water. The RSD values ( $n=6$ ) for repeatability were between 9.6 and 22.1%, and for reproducibility between 14.0 and 27.9%. In another river water sample some peaks appeared at the same retention time of some pesticides being studied: lindane, heptachlor,  $\alpha$ -endosulfan and  $p',p'$ -DDE. Unfortunately, confirmation by solid-phase extrac-

tion-GC-MS was not possible due to the low concentration levels of these compounds.

#### 4. Conclusions

The response surface methodology enables the SPME conditions to be optimised with a reasonable number of experiments, taking into account the interaction between the variables affecting the absorption SPME process. Both headspace and immersion conditions were optimised to extract a group of organochlorine pesticides in a polyacrylate SPME fiber and subsequently determination by GC-ECD. The absorption temperature selected was  $60^\circ\text{C}$  for immersion and  $100^\circ\text{C}$  for HS-SPME. The absorption

Table 3

Comparison between the organochlorine pesticide recoveries and relative standard deviation (RSD) values obtained under immersion and headspace selected conditions

No.	Compounds	Immersion		Headspace	
		Recovery (%)	RSD (%) ( $n=3$ ) <sup>a</sup>	Recovery (%)	RSD (%) ( $n=3$ ) <sup>a</sup>
1	$\alpha$ -HCH	110.6	14	91.9	1
2	$\beta$ -HCH	112.8	6	53.9	1
3	Lindane	100.8	15	43.2	5
4	Heptachlor	53.6	4	87.8	5
5	Aldrin	75.7	26	103.1	20
6	Heptachlor endo	98.9	13	101.9	13
7	$\alpha$ -Endosulfan	117.3	12	109.9	5
8	$p',p'$ -DDE	103.8	23	103.8	11
9	Dieldrin	104.2	24	112.9	9
10	Endrin	113.8	11	118.3	5
11	$\beta$ -Endosulfan	99.4	9	41.2	2
12	$p',p'$ -DDD	113.6	23	109.9	20
13	$p',p'$ -DDT	32.3	23	98.5	27
I.S.	Endosulfan sulfate	106.2	11	18.7	14

<sup>a</sup> Reproducibility between days.

time selected was 45 min for both processes. Desorption was carried out at 250°C for 5 min and no salt was added to the sample prior to the extraction in either immersion or HS-SPME.

Immersion SPME was selected, under the conditions indicated above, since recoveries were higher

than those for HS-SPME for some of the compounds studied.

The method developed by immersion SPME–GC–ECD enabled the organochlorine pesticides studied in tap and Ebro river water to be quantified at 1 ng l<sup>-1</sup> levels.

Table 4

Analytical data for immersion SPME–GC–ECD of organochlorine pesticides in tap water

Compounds	Tap water				
	Linear range ( $\mu\text{g l}^{-1}$ )	$r^2$	Limit of detection (ng l <sup>-1</sup> )	Repeatability (%) ( $n=6$ ) <sup>a</sup>	Reproducibility (%) ( $n=6$ ) <sup>a</sup>
$\alpha$ -HCH	0.0005–2.5	0.9942	0.15	12	19
$\beta$ -HCH	0.0005–1.0	0.9989	0.15	14	23
Lindane	0.0005–1.0	0.9989	0.15	8	15
Heptachlor	0.001–2.5	0.9952	0.30	11	26
Aldrin	0.0005–2.5	0.9970	0.15	16	22
Heptachlor endo	0.0005–2.5	0.9995	0.15	14	22
$\alpha$ -Endosulfan	0.001–2.5	0.9993	0.30	12	24
$p',p'$ -DDE	0.0005–2.5	0.9961	0.20	15	18
Dieldrin	0.0005–2.5	0.9962	0.15	6	11
Endrin	0.001–2.5	0.9987	0.30	7	8
$\beta$ -Endosulfan	0.001–2.5	0.9973	0.30	26	26
$p',p'$ -DDD	N.q. <sup>b</sup>				
$p',p'$ -DDT	0.001–5.0	0.9918	0.30	16	19

<sup>a</sup> Determined at 0.05  $\mu\text{g l}^{-1}$ .

<sup>b</sup> N.q.: not quantified.

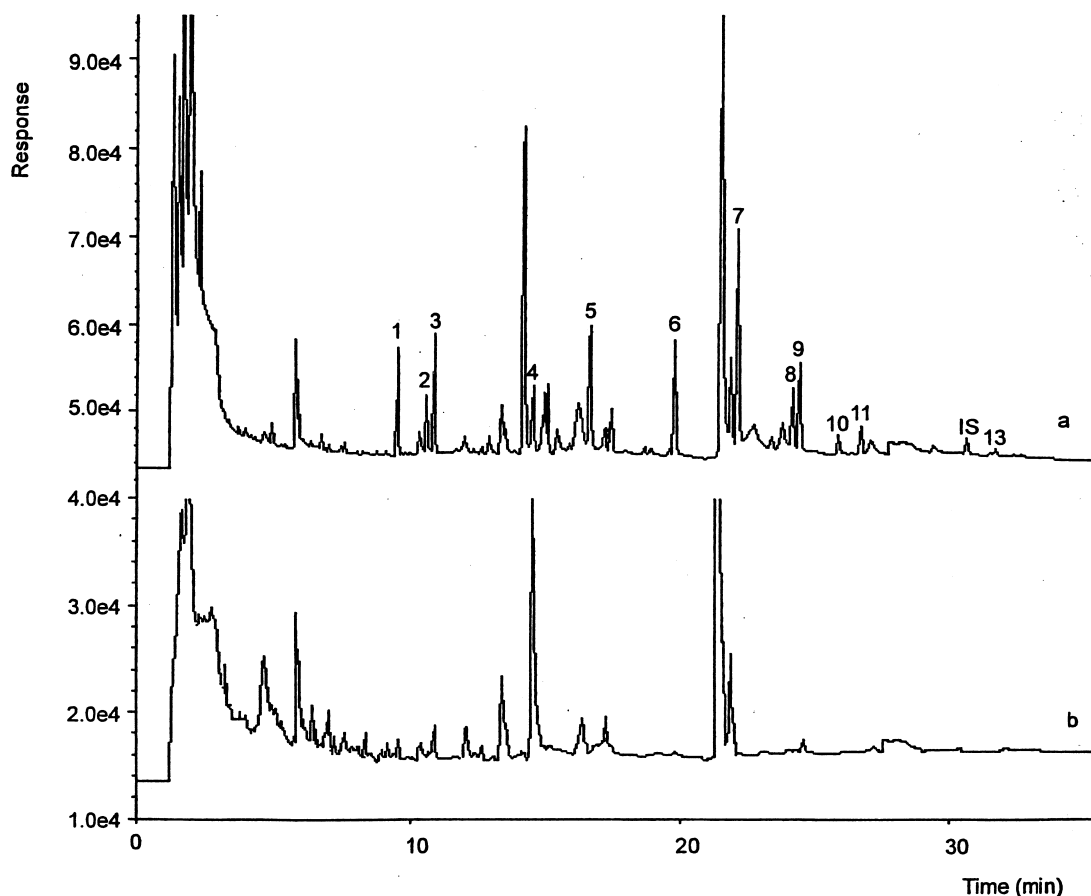


Fig. 3. Chromatograms obtained by immersion SPME–GD–ECD of (a) tap water spiked with a standard solution of  $1 \text{ ng l}^{-1}$  for pesticides and  $2 \text{ } \mu\text{g l}^{-1}$  for internal standard and (b) tap water. Peak assignment: 1= $\alpha$ -HCH, 2= $\beta$ -HCH, 3=lindane, 4=heptachlor, 5=aldrin, 6=heptachlor-endo, 7= $\alpha$ -endosulfan, 8= $p',p'$ -DDE, 9=dieldrin, 10=endrin, 11= $\beta$ -endosulfan, 12= $p',p'$ -DDD, 13= $p',p'$ -DDT.

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