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Solid-phase microextraction as a clean-up and preconcentration procedure for organochlorine pesticides determination in fish tissue by gas chromatography with electron capture detection

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Abstract

The feasibility of developing a single-step clean-up, enrichment procedure for organochlorine pesticides (OCPs) in fish tissue samples based on solid-phase microextraction (SPME) was investigated. The general analytical methodology developed combines conventional solid-liquid extraction of the OCPs from the sample using an organic solvent with SPME over the organic extract followed by gas chromatography–electron-capture detection (GC–ECD) analysis. Experimental SPME conditions such as extraction time, temperature and matrix effects were optimised. Under optimised conditions, precision, linearity range, detection limits and accuracy were evaluated. Detection limits obtained for fish tissue samples were in the range of 0.1–0.7 ng g^{-1}. Good recoveries (over 70% in all cases) were achieved from samples spiked at a concentration level of 10 ng g^{-1}. The accuracy of the developed SPME–GC–ECD procedure in real samples has been verified by analysing, using the standard addition method, a certified reference material (CRM 430, OCPs in pork fat) with satisfactory results.

Keywords: Fish; Environmental analysis; Solid-phase microextraction; Pesticides; Organochlorine compounds

1. Introduction

The widespread use of organochlorine pesticides (OCPs) has created significant environmental concern. The hazardous nature of OCPs is a result of their toxicity in combination with high chemical and biological stability and a high degree of lipophilicity. The two latter characteristics make OCPs prone to bioaccumulation along the food chain involving a wide range of trophic levels [1,2]. As a consequence, although the use of most OCPs has been restricted or even banned in many countries, they continue to be found widespread in the environment especially in biological matrices [1,2].

The determination of OCPs in biota samples usually comprises three steps: extraction, clean-up and chromatographic analysis. The clean-up is the most laborious step in most analytical procedures since the OCPs have to be accurately separated from the

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coextracted bulk fatty matrix material in this step. Therefore, a variety of different clean-up procedures to remove lipids have been studied in the literature [3,4] including destructive methods such as sulphuric acid [5] or sodium hydroxide treatment [6] and non-destructive methods, including column chromatography with gel permeation [7], Florisil [8], alumina, silica or a combination of both [9–11] and supercritical fluid extraction (SFE) [12]. However, the destructive methods will degrade some OCPs [13], while non-destructive methods generally require large volume of solvents and multiple operation steps rendering them too time- and labour-consuming for the analysis of large amounts of samples. In brief, there is still a need to develop simple (preferably one step) clean-up procedures that could be automated and coupled on-line with the final analytical measurement.

Solid-phase microextraction (SPME) [14,15] is a novel solvent-free analytical technique, able to integrate extraction, concentration and sample introduction in a single step. Thus, it has proved to offer a significantly more rapid, simple and easy to automate extraction approach than traditional extraction techniques [16–18].

Usually the SPME technique employs a coated fiber to extract and concentrate non-polar analytes which are then desorbed in the injection port of a gas chromatograph for analysis. By now SPME methods have been developed for a variety of applications including the determination of OCPs in different types of samples (water, soil, food and biological fluids) [19].

While application of SPME to water samples can be easily achieved, SPME from solid samples, such as biota samples, is more difficult. In fact, no studies have been found in the available literature concerning the use of SPME for the analysis of OCPs in biota samples. However, SPME of solid samples can still be achieved as a way to develop a simple clean-up procedure, if the sample is first extracted with a suitable organic solvent via a conventional liquid–solid extraction method. The organic extract can then be diluted with water to carry out further SPME sample treatment.

Therefore, the aim of this work was to investigate the feasibility of developing a single-step clean-up enrichment procedure for OCPs extracted from animal tissue samples based on SPME prior to gas chromatography–electron-capture detection (GC–ECD) analysis. Sixteen OCPs, belonging to different chemical groups: hexachlorobenzene (HCB), hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH and δ-HCH), cyclodienes (aldrin, dieldrin, isodrin, endrin, heptachlor, endosulfan α and endosulfan β) and diphenyl aliphatics (*p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD and methoxychlor) pesticides, were selected as the model compounds because residues of these compounds are very often detected in environmental samples. The animal tissue selected was salmon trout muscle because the consumption of contaminated fish is considered to be are important route of exposure of humans to OCPs.

Thus, the SPME process was studied in detail in salmon trout (muscle tissue) samples and important variables involving temperature effect, extraction time and sample matrix were optimised. In addition a certified reference material was analysed for OCPs to validate the SPME clean-up procedure developed.

2. Experimental

2.1. Reagents and samples

The sixteen organochlorinated pesticides (OCPs) selected in this work were purchased from Riedel de Haën (Seelze, Germany). Stock standard solutions of each OCPs at a concentration of 1000 $\mu g\,ml^{-1}$ were prepared in methanol and stored at −20 °C. These solutions were used for the preparation of working standard mixtures of the 16 OCPs in methanol or hexane. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Methanol for ultratrace analysis was obtained from Merck (Darmstadt, Germany). Hexane and acetone were for pesticides residue analysis (Riedel de Haën). Anhydrous sodium sulphate (analytical–reagent grade) from Fluka (Buchs, Switzerland) was purified by overnight heating at 300 °C.

The certified reference material CRM 430 (Organochlorine pesticides in pork fat) was from the Institute for Reference Materials and Measurement (Geel, Belgium). Salmon trouts (*Salmo trutta*) of average weight 65 g (lipid content 2–6% and water content 75%) were fished using electrofishing equipment from a river (Pigüeña river, Asturias, Spain) located

in an area without agricultural or industrial activities. The fish samples were wrapped in hexane-washed aluminium foil and stored at −20 °C until analysis. Preliminary analysis of these samples by a chromatographic method based on Soxhlet extraction with hexane–acetone [20,21] and clean-up the extract on a Florisil column [11,22] before GC–ECD analysis showed no detectable concentrations of the OCPs under study demonstrating that they are suitable for spiking experiments.

All glassware was washed with detergent (Mucasol, Brand, Germany) rinsed with Milli-Q water and finally rinsed with acetone. Clean glassware was stored wrapped in hexane-washed aluminium foil.

2.2. Chromatographic equipment and experimental conditions

Gas chromatography was carried out with a HP-5890 Series II gas chromatograph equipped with an electron-capture detector (Hewlett-Packard, Avondale, PA, USA). A, 30 m × 0.32 mm i.d., 0.25 μm film thickness, RTX.CL Pesticides two fused-silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used with helium as carrier gas at a pressure of 10 p.s.i. The injector was equipped with thermogreen LB-2 pre-drilled septa of 11 mm from Supelco (Bellefonte, PA, USA) and an injection liner of 0.75 mm i.d. (Supelco) special for SPME. The injector was operated in a split–splitless mode, with a splitless injection time of 2 min.

The temperature of the injector was maintained at 260 °C and the temperature of the detector at 300 °C. The column temperature was held at 50 °C for 1 min, then raised to 170 °C at a rate of 30 °C min^{-1} and finally raised to 300 °C at 7 °C min^{-1}.

2.3. Soxhlet extraction procedure

Fish muscle tissue (10 g wet weight) was ground with four-fold excess of activated anhydrous sodium sulphate until a fine powder was obtained. This mixture was Soxhlet extracted with 300 ml of hexane:acetone (1:1) for 16 h [20,21]. The extract was concentrated under vacuum rotatory evaporation to 100 ml. Aliquots of 1 ml were then taken for SPME–GC–ECD analysis.

2.4. SPME equipment and experimental conditions

The fiber selected for this study was a fused-silica fiber coated with 100 μm of polydimethylsiloxane (PDMS) and held in a SPME device supplied by Supelco (Madrid, Spain). Prior to use the fibers were conditioned by heating them in the injection port of the gas chromatograph for 1 h at 260 °C.

The SPME extraction was carried out as follows: 1 ml aliquots of the fish tissue organic extract from the Soxhlet were placed into a 10 ml Teflon-lined screw-capped vials and evaporated just to dryness under a gentle stream of nitrogen. The residue was redisolved in 10 ml of 5% (v/v) methanol/water. The vial was placed in a thermostatic water bath and the fiber was immersed into the solution and kept there for 30 min at ambient temperature (25 ± 1 °C). During extraction the solution was vigorously stirred with an unused PTFE-coated small magnetic stir bar. After extraction, the fiber was thermally desorbed at 260 °C for 5 min into the glass liner of the chromatograph injection port.

Possible carryover was removed by keeping the fiber in the injector port for an additional period of time of 5 min with the injector in the split mode.

Procedure blanks were run periodically during the analysis to check possible fiber contaminations.

3. Results and discussion

3.1. SPME optimisation

In order to develop a SPME procedure for clean-up and concentration of OCPs from fish tissue extracts a step-by-step optimisation study of the parameters influencing the SPME process such as the extraction time, the extraction temperature and the effect of the sample matrix was carried out.

A 100 μm PDMS fiber was selected for this study according to the fiber/water partition coefficients reported in the literature [23] and the general usage data available [19].

The ionic strength of the samples was not adjusted before SPME because our preliminary studies showed that the addition of salt (NaCl) to the sample did not

significantly affect the extraction of pesticides by the PDMS fiber and the effect of sample pH in the SPME efficiency was not taken into account, according to scientific-literature results [24].

First, optimum desorption conditions were determined by testing various temperatures and heating times. The time and temperature required to successfully desorb all the OCPs from the fiber coating with minimal carryover in a subsequent analysis (fiber blank) were considered as the more suitable desorption conditions. Such conditions were stablished as 5 min at 260 °C.

As a second step, SPME extraction-time profiles for each OCP under study were generated by extracting aqueous standards mixtures, with the same concentration, for increasing exposure times between 15 and 60 min. The results obtained are given in Fig. 1 showing that more than 60 min are necessary to reach the equilibrium between the fiber stationary phase and the aqueous sample for all the OCPs, except for the HCHs compounds showing a shorter equilibrium time of 15 min.

These results are consistent with previously reported data [23,25]. Since an equilibrium time longer than 60 min was considered too long from a practical point of view, non-equilibrium conditions work was considered. In this vein, a time of 30 min was selected for OCPs extraction in order to obtain a

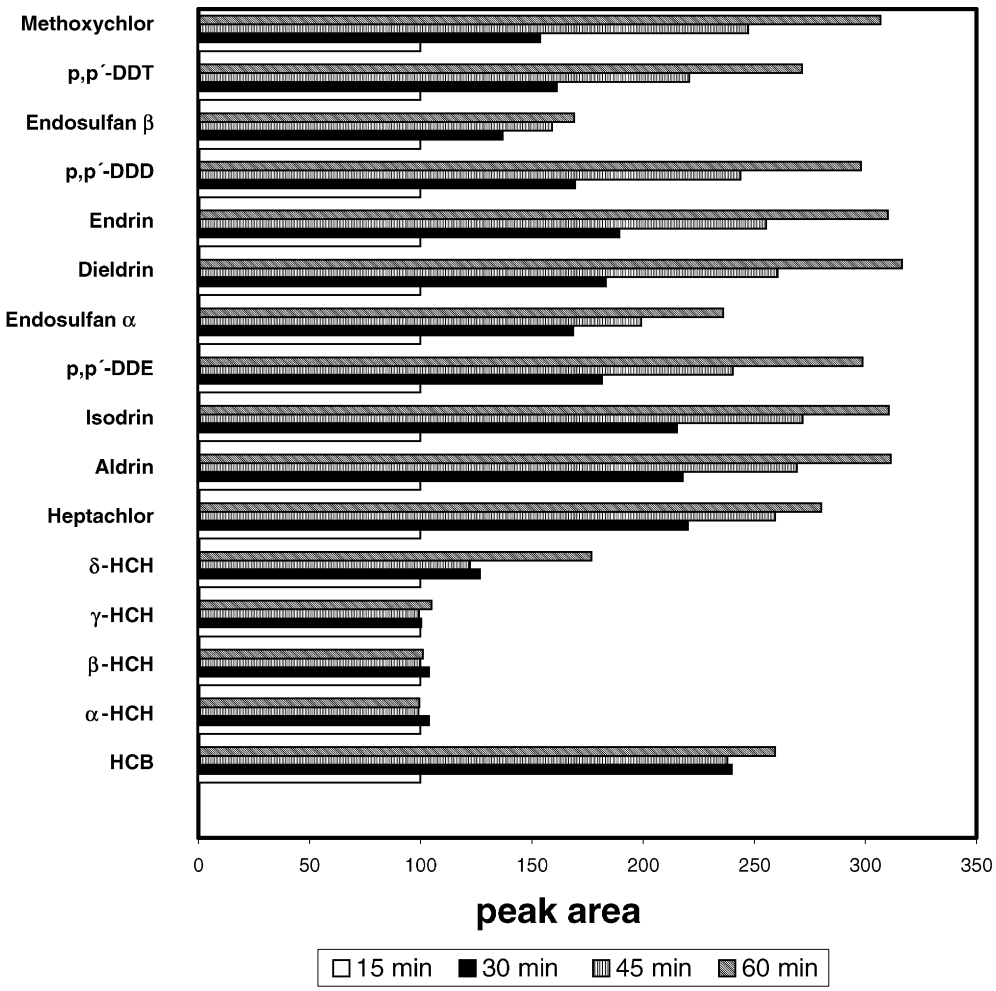

Fig. 1. Effect of extraction time on SPME efficiency (expressed by peak area).

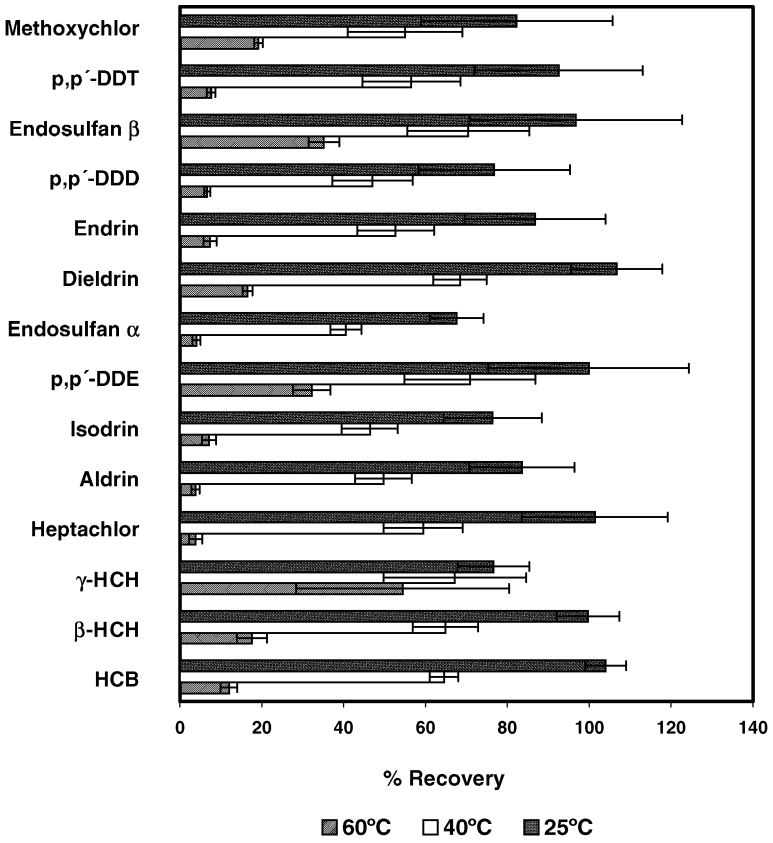

Fig. 2. Effect of the extraction temperature on SPME efficiency (expressed by percent relative recovery ± S.D.).

good compromise between sensitivity and analysis time.

The SPME temperature effect was also tested using fish tissue organic extracts (*OCPs free*) prepared as described in the experimental section and spiked with 1 ng ml^{-1} OCPs (final concentration in the SPME vial of 0.1 ng ml^{-1}). Fig. 2 illustrates the percent recoveries obtained at 25 °C, 40 °C and 60 °C for all the selected OCPs, except α-HCH and δ-HCH (the recovery for α-HCH and δ-HCH cannot be calculated a this concentration level because these compounds elute very close to other unknown compounds present in the fish tissue sample). The relative recovery that is determined as the peak area ratio of fish sample and ultrapure water sample spiked with analytes at the same level was applied. As can be seen in Fig. 2 when the temperature was increased from 25 to 60 °C an important decrease in the relative recovery for all the OCPs was observed. Thus, a temperature of 25 °C was chosen for further work.

Finally, in order to evaluate the effect of sample matrix (lipid content) on the SPME extraction efficiency, aliquots of spiked fish tissue organic extracts ranging from 1 to 3 ml were submitted to the SPME procedure. In all the samples, extracted by SPME the final concentration of OCPs in the vial was 0.25 ng ml^{-1} but the content in lipids ranged from 3–5 mg (corresponds to an 1 ml aliquot of the fish tissue extract) to 9–15 mg (corresponds to a 3 ml aliquot of the fish tissue extract). As can be seen in Fig. 3, all the pesticides studied showed a dramatic decrease in the relative recovery when the lipid content in the sample increases. Fig. 3 also shows that a satisfactory recovery (>70%) of all OCPs can be obtained only for samples with low lipid contents (3–5 mg,

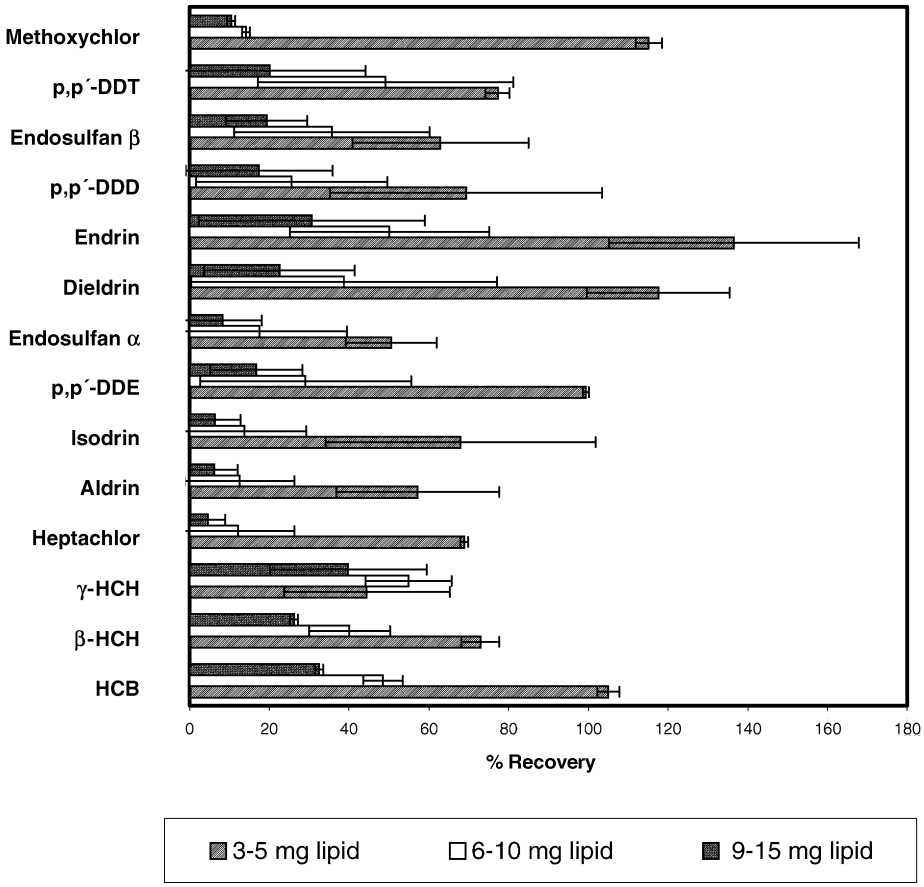

Fig. 3. Effect of the sample matrix (lipid content) on the SPME efficiency (expressed by percent relative recovery ± S.D.).

which corresponds to a 1 ml aliquot of the fish tissue extract).

In order to correct the influence of the amount of fat on the SPME efficiency the use of internal standards or the standard addition method is required. It is important to note, that for internal standards to work well in SPME their partition coefficients between the sample and the fiber coating must be very similar to those of the target analytes, and this fact is very difficult to achieve unless the expensive and not always available isotope-labelled analogues are used. Moreover, due to the wide range of physical–chemical properties of the OCPs studied, it is not feasible to obtain satisfactory matrix effect corrections for all compounds when only one internal standard is used. Therefore, quantitative analysis using a standard addition procedure was selected in order to reduce matrix influence on the SPME efficiency.

3.2. Method evaluation

Once established the experimental SPME conditions, *evaluation* of the developed SPME–GC–ECD methodology was carried out in terms of linearity range, precision, detection limits and accuracy.

The linearity of the method was tested in 5% (v/v) methanol/ultrapure water samples. The calibration curves (peak area versus concentration) were linear over the whole concentration range tested (25 to 500 ng l^{-1}) for all the OCPs with correlation coefficients (R^2) higher than 0.99 in almost all cases (Table 1).

Table 1
Linear range precision and detection limit data for the organochlorine pesticides

Compound	Linear range ($ng\,l^{-1}$)	Correlation coefficient (R^2)	R.S.D. (%)[a,b]	LOD ($ng\,l^{-1}$)	LOD[c] ($ng\,g^{-1}$)
HCB	25–500	0.999	28	0.5	0.2
α-HCH	25–500	0.998	20	0.8	–
β-HCH	25–500	0.998	16	0.8	0.1
γ-HCH	25–500	0.994	24	2.3	0.2
δ-HCH	25–500	0.999	18	21	–
Heptachlor	25–500	0.988	18	1.6	0.7
Aldrin	25–500	0.992	13	0.9	0.2
Isodrin	25–500	0.994	15	0.9	0.2
p,p′-DDE	25–500	0.999	22	0.7	0.2
Endosulfan α	25–500	0.992	17	1.2	0.3
Dieldrin	25–500	0.996	10	0.6	0.2
Endrin	25–500	0.999	6	1.1	0.2
p,p′-DDD	25–500	0.986	10	0.6	0.3
Endosulfan β	25–500	0.995	13	0.9	0.4
p,p′-DDT	25–500	0.995	16	1.6	0.2
Methoxychlor	25–500	0.954	19	1.5	0.4

R.S.D., relative standard deviation; LOD, limit of detection.
[a] Test concentration $100\,ng\,l^{-1}$ ($n = 3$).
[b] Precision observed indicate that no significant thermal degradation seems to take place.
[c] Limit of detection referred to the fish sample.

The SPME–GC–ECD precision was determined by three replicate analysis of a 5% (v/v) methanol/ultrapure water sample at a concentration level of $100\,ng\,l^{-1}$. As shown in Table 1 the repeatability in terms of percent relative standard deviation (R.S.D.) varied between 6% (endrin) and 28% (HCB). These latter R.S.D. values are quite high but within the ranges observed with SPME [19].

Table 1 also shows the detection limits calculated as the lowest concentration of an analyte giving a signal of three-times the base line noise of the chromatogram. As can be seen, the values obtained ranged from 0.5 to $2.3\,ng\,l^{-1}$ (except for δ-HCH). The detection limits were also evaluated using spiked fish tissue organic extracts. The obtained results are given in Table 1.The data of the Table 1 show that the proposed method allows detection of all the pesticides in fish tissue samples at concentrations lower than $0.7\,ng\,g^{-1}$ (except for α-HCH and δ-HCH which elute too close to other unknown compounds present in the fish tissue sample). Fig. 4 shows a representative chromatogram corresponding to the SPME–GC–ECD analysis from a fish tissue organic extract spiked at $1\,ng\,ml^{-1}$ level in the extract (final concentration in the SPME vial of $0.1\,ng\,ml^{-1}$) and its respective blank (unspiked fish tissue extract).

The accuracy (expressed as percent relative recovery) of the proposed method was also investigated by analysing fish tissue organic extracts spiked with all the OCPs at a concentrations level of $1\,ng\,ml^{-1}$ level in the extract (final concentration in the SPME vial of $0.1\,ng\,ml^{-1}$). The results obtained are collected in Table 2, which shows that the percentages recoveries (mean ± standard deviation, $n = 3$) ranged between 70 ± 7 (for endosulfan α) and 104 ± 5 (for HCB). In many cases, the standard deviation was much greater than for these two compounds. As a "worst case" example, the recovery (mean ± standard deviation, $n = 3$) for dieldrin was 88 ± 34.

3.3. Method validation

Finally, in order to fully validate the method the proposed SPME–GC–ECD procedure was applied to the analysis of a reference material, since a fish tissue reference material certified only for OCPs is not available, the reference material, CRM 430

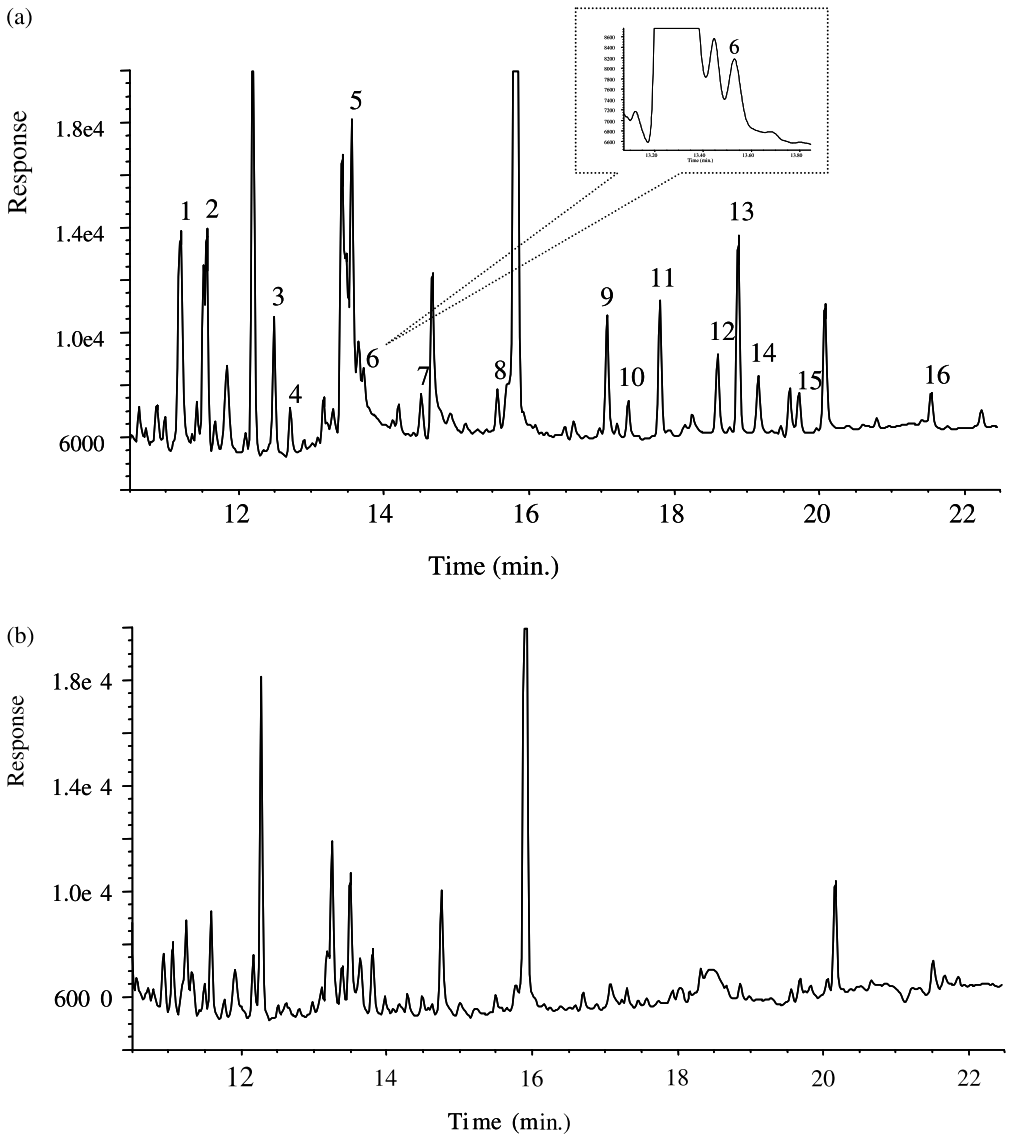

Fig. 4. Chromatograms obtained by SPME–GC–ECD of: (a) fish tissue extract spiked with OCPs (final concentration in the SPME vial of 100 ng l^{-1}) and (b) unspiked fish tissue extract. Peak assignment: (1) HCB, (2) α-HCH, (3) β-HCH, (4) γ-HCH, (5) δ-HCH, (6) heptachlor, (7) aldrin, (8) isodrin, (9) *p,p′*-DDE, (10) endosulfan α, (11) dieldrin, (12) endrin, (13) *p,p′*-DDD, (14) endosulfan β, (15) *p,p′*-DDT, (16) methoxychlor.

(OCPs in pork fat) was analysed. The concentrations of eight OCPs (HCB, α-HCH, β-HCH, γ-HCH, dieldrin, endrin, *p,p′*-DDE and *p,p′*-DDT) are certified in this CRM 430 [26] material and indicative concentration is given for *p,p′*-DDD [26]. Determination was carried out by addition of standards to 0.5 ml aliquots of a solution of the CRM 430 reference material (0.1 g dissolved in 10 ml of hexane) before SPME-CG-ECD analysis. The results obtained are given in Table 3. Good agreement was obtained between the certified and the obtained values for all OCPs indicating that the SPME–GC–ECD

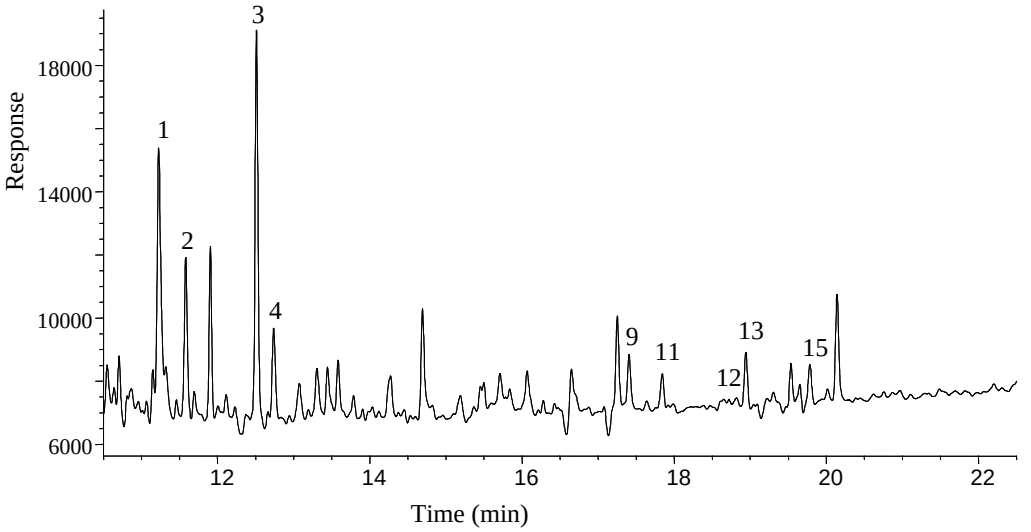

Fig. 5. Chromatogram obtained by SPME–GC–ECD of the Pork Fat, Community Bureau of Reference (CRM 430) reference material. Peak identities as in Fig. 4.

methodology developed here can be used reliably for the determination of OCPs in fatty samples. Fig. 5 shows a representative chromatogram corresponding to the SPME–GC–ECD analysis of the pork fat reference material.

Table 2
Recoveries (mean ± S.D.) of the selected OCPs in spiked fish tissue extracts by using SPME

Compound	Recovery[a] (%)
HCB	104 ± 5
α-HCH	–
β-HCH	100 ± 8
γ-HCH	77 ± 9
δ-HCH	–
Heptachlor	84 ± 32
Aldrin	84 ± 13
Isodrin	76 ± 12
p,p′-DDE	100 ± 12
Endosulfan α	70 ± 7
Dieldrin	88 ± 34
Endrin	87 ± 17
p,p′-DDD	77 ± 19
Endosulfan β	97 ± 25
p,p′-DDT	93 ± 21
Methoxychlor	82 ± 23

[a] Spiked level 1 ng ml^{-1} ($n = 3$).

Table 3
SPME–GC–ECD determination of OCPs in (Pork Fat, Community Bureau of Reference) CRM 430

Compound	Certified value (mean ± S.D. (ng/g))	Found value (mean ± S.D. (ng/g))
HCB	392.4 ± 33.6	394.1 ± 80.3
α-HCH	139.8 ± 14.5	136.7 ± 18.8
β-HCH	259.4 ± 24.7	259.0 ± 38.5
γ-HCH	499.1 ± 39.8	489.4 ± 42.5
Dieldrin	123.6 ± 12.4	122.7 ± 9.4
Endrin	20.2 ± 3.2	13.8 ± 8.2
p,p′-DDT	3401.1 ± 235.9	3419.9 ± 83.1
p,p′-DDD[a]	766 ± 108	741.5 ± 100.9
p,p′-DDE	818.8 ± 74.9	795.9 ± 65.2

[a] Indicative concentration only.

4. Conclusions

The successful development of a procedure, based on the SPME technique, for the clean-up of OCPs extracts from fish tissue samples prior to GC-ECD analysis has been outlined. The use of SPME allows sample manipulation to be substantially reduced and offers significant savings of glassware, solvents and time compared to more conventional techniques.

A control of the lipid content in the sample extract prior to SPME is necessary in order to improve the

SPME efficiency; in this vein, the amount of extract that can be taken for SPME is limited (1 ml equivalent to 3–5 mg lipid content). This fact determines that LODs obtained by the overall procedure are relatively high (between 0.1 and 0.7 ng g^{-1}). The developed SPME–GC–ECD method shows adequate analytical performance in terms of linearity range and precision (RSD between 6 and 28%) with recoveries higher than 70% for most of the pesticides investigated. However, quantitative analysis using a standard addition procedure is recommended in order to reduce matrix influence on the SPME efficiency.

Successful application of the developed SPME–GC–ECD method to the analysis of OCPs in CRM 430 (matrix of pork fat) proves that it can be a suitable approach for the clean-up of OCPs extracts from fatty samples.

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