Supercritical fluid extraction of organochlorine pesticides and some metabolites in frogs from National Park of Ordesa and Monte Perdido

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Abstract

The optimization of supercritical fluid extraction (SFE) with CO₂ for the extraction of a series of organochlorine pesticides (OCPs) and some metabolites in biota samples (frogs) from the National Park of Ordesa and Monte Perdido (Spain) and further analysis by GC–electron capture detector (ECD) is carried out. An experimental design is applied to the optimization of SFE. The Soxhlet extraction of the same samples is optimized too, together with the necessary clean-up step using four different solid cartridges. The analytical procedures as well as the results obtained are compared. Seven in sixteen OCPs under study showed recovery values above 80%, when using Soxhlet while nine OCPs were over 80% in the case of SFE. The analysis of frog samples showed values below 7 ng/g on the basis of dry matter for all the OCPs under study. The analytical procedures as well as the results obtained in real samples are discussed.

Keywords: Organochlorine pesticides; Metabolites; Analysis; Biota; SFE

1. Introduction

Since 1940, organochlorine pesticides (OCPs) have been applied to protect crops against the pests, allowing a spectacular increase of production as well as the eradication of illness such as malaria and typhus. The use of pesticides has been assumed as something necessary and it demands well established rules to prevent the increasing contamination. Although the restrictions that most of the pesticides have around the world, OCPs have caused the lost of equilibrium of those ecosystems in which they are introduced [1]. OCPs are accumulative and persistent compounds in both biota and the environment and as liposoluble compounds they are difficult to be metabolized by living beings [2]. It has been also demonstrated that some species have developed new protection mechanisms which make them resistant to further applications of OCPs although such application affects the trophic chain [3].

When an OCP is applied, it is distributed into the different environmental compartments such as the atmosphere, water, soil and biota, depending on the physical conditions. For all these reasons, most of OCPs are now banned in most of the developed countries. However, developing countries still use some OCPs and as a result of the atmospheric

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transport, these pollutants are found everywhere, even in protected areas. This is the case of National Park of Ordesa and Monte Perdido, a natural protected and beautiful area of Spanish Pyrenees, far from both agricultural and industrial uses, in which some polluted living species were found in 1993 [4]. Previous studies in the area showed an atmospheric transport which through the rain, wind and snow [5,6], carried some pesticides to this area.

In order to study the influence of this transport on the trophic chain, a research work was designed which involves the determination of the concentration of several organochlorine compounds and some metabolites in the trophic chain starting with a kind of grass-eater, such as the locust Orthopter orthopter [7], and then frogs, birds and mammals living in the area. The present paper shows the study carried out in frogs.

As very low concentrations are expected, their analysis required a very sensitive and accurate procedure. A quite extensive literature about the analytical procedures for OCPs has been reviewed but none of the papers deal with a sample similar to frogs. On the other hand, the analytical procedures usually involve an extraction step with solvent, a clean-up system and a final concentration step of the extract, previous to the GC–electron capture detector (ECD) analysis. In the last few years, the trends for simplifying the analytical procedures have driven to the development of new sample treatment techniques which avoid the clean-up step. In this context, supercritical fluid extraction (SFE) appears as one of the most appropriate technique for this type of analysis in different solid matrices [8–11]. It allows to do selective extractions of different chemicals without additional clean-up steps as well as to use little sample amounts which in this case, working with small animals or protected species, is a very important feature. In contrast, Soxhlet extraction has been widely used for OCPs [12–16]. It requires high volume of organic solvents and a high handling degree and time consuming.

This paper shows the SFE optimization for the determination “off-line” of some OCPs and their metabolites by GC–ECD in frogs, as a potential bioindicators of the pollution. The analytical procedure as well as the results of OCPs in frogs from the Parque Nacional de Ordesa y Monte Perdido (Spain) are discussed.

2. Experimental

2.1. Reagents and solutions

Heptachlor, heptachloroepoxide, α-endosulphan and endosulphan-sulphate were from Riedel-de Häen (Seelze, Germany). α-Hexachlorocyclohexane (HCH), β-hexachlorocyclohexane, γ-hexachlorocyclohexane (lindane), dieldrin, endrin, aldrin, endrin-aldehyde, β-endosulphan, p,p'-DDE, p,p'-DDT, p,p'-DDE and PCB-52 were from Dr. Ehrenstorfer (Augsburg, Austria). PCB-52 from Chem Service (West Chester, USA) was used as internal standard.

Dichloromethane, n-hexane, isopropanol and toluene of residue analysis quality and silica gel 60 (70–230 mesh ASM) for chromatography were from Merck (Merck, Darmstadt, Germany). Diethyl-ether and anhydrous sodium sulphate for analysis quality were from Panreac (Panreac Química, Barcelona, Spain). Florisil (60–100 mesh) was from Fluka.

2.2. Selection of the pesticides

Previous studies carried out on wild mammals in the same National Park [4] showed the presence of OCPs residues in serum. Also the analysis of snow, air and water as well as of locust captured in the area revealed that the concentration of pesticides increased from snow and water to biota. The appearance in the later samples of some metabolites of organochlorine pesticides suggested the inclusion of these compounds in the list of pollutants under study. For this reason, the following compounds were considered: α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, heptachloroepoxide, heptachlor, aldrin, dieldrin, endrin, endrin-aldehyde, endosulphan-sulphate, α-endosulphan, β-endosulphan, p,p'-DDT, p,p'-DDE and p,p'-DDE.

2.3. Samples

The frogs were captured in 1996 in the Pirineo Aragonés (Spain). Two different groups of frogs were available. The first one corresponded to those captured far from the Parque Nacional de Ordesa y Monte Perdido and they were used as blank samples. Spiked samples were prepared from these blank samples for optimization. The second group was captured in the mentioned National Park. All of them belong the
autotypous specie *Rana pirenaica* of a high ecological value and for this reason a special permission was necessary. Once in the laboratory, the samples were grinded to make an homogeneous paste and frozen for storage at 20 °C below cero. Skin and bones as well as the head were included in the mixture to avoid the use of a higher number of animals. The samples were defrozen just before the analysis. The analysis were performed in 1997. According to the literature[3], no degradation was expected of the residue pesticides during the storage time and conditions.

2.4. Apparatus

Soxhlet and thermostatic bath (Precis Pat S-48200) from Selecta (Selecta, Abera, Spain) were used. Supercritical fluid extractor was from Varian Star SFE and it was equipped with a Premaster Star SFE extractor, a Modifier Pump Star SFE and the Accu-trap Star SFE collector (Suprex Corporation, Pittsburg, USA). Gas chromatograph Varian Star 3400 Cx equipped with a capillary split-splitless injector as well as a septum equipped programmable injector (SPI), an autosampler Varian 8200 Cx and electron capture detector (ECD) of 63Ni was used. The column used was SGL-5 60 m × 0.25 mm i.d. and 0.25 μm of film thickness of 5% diphenyl, 95% dimethylpolysiloxane copolymer (Sugelabor, Madrid, Spain). The program used was as follows: initial temperature 50 °C, hold for 2 min, rate at 25 °C/min till 180 °C and then hold for 15 min, rate at 25 °C/min till 290 °C and then hold for 2 min. Detector temperature was 300 °C. Carrier gas used was hydrogen-C50 (Carburos Metalicos, Spain) at 1 ml/min and N2 at 30 ml/min was used as make up gas. Star Chromatography Workstation 4.51 version (Varian, Texas, USA) and a personal computer were used to obtain the data. Fig. 1 shows a chromatogram with the retention time of the compounds under study.

3. Soxhlet extraction

Soxhlet extraction with dichloromethane as extracting solvent was applied for 8 h at 40 °C. The cycling rate was about 12 cycles per hour. The solid sample was mixed with anhydrous sodium sulphate at the rate 1:5 (w/w) and it was introduced into a cellulose cartridge covered by silanized glass wool. After 8 h, the extract was cleaned-up in a minicolumn containing 2 g of 3% deactivated silica gel, which was then eluted with 40 ml of hexane. The final extract was concentrated to about 1 g of solution and filtered through a syringe filter of 0.22 μm of pore size. PCB-52 was added as internal standard before the GC injection. All the solutions were gravimetrically controlled.
4. SFE

The extraction cell of 3 ml was filled with the mixture of sample and anhydrous sodium sulphate (1:5) in sandwich mode using silanized glass wool at both the bottom and the top of the cell to protect the cell seals. A pressure of 425 atm and 35 °C of temperature with a flow of 2 ml/min of CO₂ for 5 min of static extraction and then 10 min of dynamic extraction were applied to the extraction module. The adsorption temperature in the collection module (Accutrap) was −15 °C and the desorption temperature was 30 °C using 1.7 ml of n-hexane as eluting solvent. The final weight of the extract was considered instead of the volume in order to be consistent with the gravimetric control, according to the Quality Control requirements. PCB-52, used as internal standard, was added to the final n-hexane extract before the GC–ECD analysis.

5. Results and discussion

5.1. Soxhlet extraction and clean-up

The main variables affecting the Soxhlet extraction are the extraction time, the temperature and the extraction solvent. According to the previous studies [7] dichloromethane at 40 °C for 8 h was shown to be the best extraction agent for these analytes in biota samples. However, in these conditions, fat molecules as well as other interferences present in the samples are co-extracted and a clean-up step is necessary. The use of a solid bed of an appropriate adsorbent such as silica, alumina or florisor or even a combination of several adsorbents in a minicolumn, which is further eluted with a solvent, has been widely proposed in the literature [17–20].

As the analytes under study cover a wide range of polarity, several adsorbents as well as different combinations of them were tested, each one having a different elution solvent in both volume and nature. The following systems were considered:

(A) a 3% deactivated silica (2 g) + 5% deactivated florisor (2 g) using two sequential steps of elution, the first one with 20 ml of n-hexane and the second one with 20 ml of n-hexane diethylether (70:30 v/v) mixture;  
(B) a 3% deactivated silica (2 g) eluted with 15 ml of n-hexane diethylether (70:30 v/v) mixture;  
(C) a 5% deactivated florisor (2 g) eluted as mentioned in B;  
(D) a 3% deactivated silica (2 g) eluted with 40 ml of n-hexane.

In all cases the adsorbents were previously activated at 450 °C for 5 h and further deactivated with distilled water. They were stored in a desicator 24 h before using. This procedure was repeated weekly.

The recovery studies were carried out by spiking the adsorbent with 300 µl of a solution containing 800 ng/g of each pesticide and then applying the elution system and analysis before described. The results obtained are shown in Table 1. Both the recovery values and the chromatogram obtained in each case were taken into account for the final selection. It was observed that the presence of diethyl ether in the elution solvent carried a higher amount of fat molecules as well as other molecules which were co-eluted with the compounds under study and interfered the analysis. For this reason, the use of diethyl ether is not recommended even though the recovery values were very high.

As can be seen in Table 1, β-HCH and p,p′-DDE gave values higher that 100% in all cases. This fact could be attributed to several causes. First, the poor separation of the pairs β- and γ-HCH and also p,p′-DDE and dieldrin in the chromatographic column used. This would provide inadequate quantitative results. The second reason could be the decomposition of parent compounds in the case of p,p′-DDE in the injection port, as other authors mention. Although the different studies carried out changing the injector port and improving the analytical conditions, no clear evidence was found to justify the high results of p,p′-DDE. Based on these experimental data, the selected clean-up system was D.

5.2. Recovery study using the Soxhlet extraction

Several frog samples were spiked with 300 µl of a standard solution containing 800 ng/g of each OCP under study and 24 h after being fortified the samples were analyzed following the optimum procedure described under the experimental section. The recovery values as well as the RSD expressed as (%) are shown
Table 1

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>$t_R$ (min)</th>
<th>3% Silica–5% Florisil–Hexane ether</th>
<th>3% Silica–Hexane ether</th>
<th>5% Florisil–Hexane ether</th>
<th>3% Silica–Hexane ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-HCH</td>
<td>17.24</td>
<td>96.3 ± 26</td>
<td>97.1 ± 29</td>
<td>92.1 ± 23</td>
<td>99.2 ± 20</td>
</tr>
<tr>
<td>2</td>
<td>β-HCH</td>
<td>19.28</td>
<td>135.8 ± 11</td>
<td>98.3 ± 26</td>
<td>120.9 ± 5.9</td>
<td>102.8 ± 13</td>
</tr>
<tr>
<td>3</td>
<td>γ-HCH</td>
<td>19.57</td>
<td>93.4 ± 32</td>
<td>99.5 ± 28</td>
<td>91.0 ± 15</td>
<td>97.6 ± 21</td>
</tr>
<tr>
<td>4</td>
<td>δ-HCH</td>
<td>21.74</td>
<td>96.3 ± 31</td>
<td>97.8 ± 26</td>
<td>93.8 ± 13</td>
<td>99.0 ± 12</td>
</tr>
<tr>
<td>5</td>
<td>Heptachlor</td>
<td>25.08</td>
<td>95.0 ± 50</td>
<td>98.3 ± 19</td>
<td>91.8 ± 41</td>
<td>99.8 ± 28</td>
</tr>
<tr>
<td>6</td>
<td>PCB-52</td>
<td>26.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Aldrin</td>
<td>26.78</td>
<td>82.4 ± 27</td>
<td>88.5 ± 14</td>
<td>82.8 ± 20</td>
<td>93.1 ± 12</td>
</tr>
<tr>
<td>8</td>
<td>Hexachlorophosphate</td>
<td>28.78</td>
<td>90.3 ± 24</td>
<td>96.7 ± 8.3</td>
<td>90.7 ± 18</td>
<td>93.8 ± 5.9</td>
</tr>
<tr>
<td>9</td>
<td>α-Endosulphan</td>
<td>30.44</td>
<td>96.6 ± 31</td>
<td>97.7 ± 4.0</td>
<td>97.7 ± 27</td>
<td>86.5 ± 11</td>
</tr>
<tr>
<td>10</td>
<td>p,p' -DDE</td>
<td>31.80</td>
<td>120.9 ± 2</td>
<td>132.1 ± 14</td>
<td>108.5 ± 16</td>
<td>121.4 ± 8.3</td>
</tr>
<tr>
<td>11</td>
<td>Dieldrin</td>
<td>31.90</td>
<td>85.3 ± 27</td>
<td>93.3 ± 10</td>
<td>88.5 ± 13</td>
<td>90.3 ± 18</td>
</tr>
<tr>
<td>12</td>
<td>Endrin</td>
<td>33.50</td>
<td>90.2 ± 30</td>
<td>98.2 ± 8.6</td>
<td>90.9 ± 18</td>
<td>92.3 ± 15</td>
</tr>
<tr>
<td>13</td>
<td>β-Endosulphan</td>
<td>33.85</td>
<td>66.7 ± 17</td>
<td>74.3 ± 18</td>
<td>67.6 ± 9.4</td>
<td>99.1 ± 7.6</td>
</tr>
<tr>
<td>14</td>
<td>p,p' -DDT</td>
<td>34.54</td>
<td>85.9 ± 34</td>
<td>95.6 ± 19</td>
<td>90.3 ± 11</td>
<td>96.1 ± 10</td>
</tr>
<tr>
<td>15</td>
<td>Endrin-aldehyde</td>
<td>35.11</td>
<td>90.5 ± 42</td>
<td>99.7 ± 26</td>
<td>88.5 ± 7.0</td>
<td>74.1 ± 16</td>
</tr>
<tr>
<td>16</td>
<td>Endosulphan-sulphate</td>
<td>36.65</td>
<td>84.5 ± 45</td>
<td>94.6 ± 29</td>
<td>90.0 ± 21</td>
<td>98.3 ± 26</td>
</tr>
<tr>
<td>17</td>
<td>p,p' -DDE</td>
<td>36.88</td>
<td>87.3 ± 39</td>
<td>97.0 ± 21</td>
<td>86.4 ± 30</td>
<td>99.1 ± 27</td>
</tr>
</tbody>
</table>

$s \pm f \sigma (n = 3; f = 4.3; 95\%$ confidence limit).

As can be seen, the recovery of some compounds is below 70% and even lower than 30% for some of them, such as dieldrin, endrin, β-endosulphan, endrin-aldehyde and endosulphan-sulphate, which means that a clear interaction of the added compounds with the frog sample occurs. On the other hand, p,p'-DDE recovery is considerably higher than 100% while dieldrin recovery is very low, which could be attributed to either the poor separation between them, as was earlier mentioned, or to the presence in Fig. 2. Recovery studies by Soxhlet procedure.
of \( p,p' \)-DDE in the blank sample. Direct analysis of blank samples was always below the detection limit, so this attempt could not be confirmed. These results show that the sample matrix strongly affects the behaviour of these OCPs in the extraction and clean-up procedures used. Other authors [19,20] found similar results in biota samples, which means that other extraction procedure is required.

5.3. Supercritical fluid extraction (SFE); optimization based on an experimental design

The variables involved in the SFE can be classified in two groups: (a) those affecting the extraction step and (b) those related to the collection of the analytes. Both groups of variables were independently optimized using a factorial design in which all the likely combinations of factors affecting the experiment, including the crossed interaction of variables, are considered.

To optimize the collection step, the recommendations from Snyder et al. [8] were taken into account, and the extreme values used are shown in Table 2. Each experiment was carried out as follows: The extraction cell was filled with silanized glass wool on the bottom and then anhydrous sodium sulphate to which 500 µl of a standard solution containing 800 ng/g of each pesticide was added. The percentage of total recovery of pesticides was considered as criterion of optimization. The only relevant variable found in the collection step, after the statistical evaluation of the data, was the volume of solvent for elution, which was 1.7 ml. The other two variables were no significant and for this reason, the middle point was adopted in each one.

In the extraction step, the following variables were optimized: the pressure and temperature in the cell and \( \text{CO}_2 \) flow during the dynamic extraction. The experimental design used, based on previous studies [8,21] is shown in Table 3. For this study, the optimum values for the collection step optimized earlier were applied, but the sample preparation was different.

Some frog samples were spiked with 80 µl of a standard solution of pesticides containing 800 ng/g of each compound. The 0.5 g of this spiked sample were mixed with anhydrous sodium sulphate (1:5 w/w) and then the 3 ml extraction cell was filled, adding silanized glass wool on the bottom and on the top. The same criterion as that mentioned in the previous studies was used for optimization. The optimum values obtained were the following: 2 ml/min of \( \text{CO}_2 \) flow, 425 atm and 35 °C in the extraction cell. Table 4 lists the optimum values found

5.3.1. Recovery studies by SFE–GC–ECD

Fig. 3 shows the plot of recoveries obtained. As can be seen, all the values are higher than 40%, being the behaviour of dieldrin, endrin, \( \beta \)-endosulphan, endrin-aldehyde and endosulphan-sulphate much better than in the Soxhlet extraction. On the other hand, \( p,p' \)-DDE recovery is also higher than 100% as it was in Soxhlet extraction, which could be attributed to the presence of this compound in the blank sample. The direct analysis of this compound in the sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption ( T_a ) (°C)</td>
<td>-30</td>
<td>0</td>
</tr>
<tr>
<td>Desorption ( T_d ) (°C)</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Elution volume (hexane)</td>
<td>0.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 2

Table 3

Limit values applied to the experimental design for optimizing the collection step in SFE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (atm)</td>
<td>200</td>
<td>425</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35</td>
<td>75</td>
</tr>
<tr>
<td>Flow of ( \text{CO}_2 ) (ml/min)</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Conditions of the collection step: adsorption \( T_a \) -15 °C; desorption \( T_d \) 30 °C; volume of hexane (elution solvent): 1.7 ml; \( t \) (static): 5 min; \( t \) (dynamic): 10 min.

Table 4

Optimum values found for the extraction of OCPs from frog samples by SFE

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (atm)</td>
<td>425</td>
</tr>
<tr>
<td>Adsorption temperature (°C)</td>
<td>-15</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35</td>
</tr>
<tr>
<td>Desorption temperature (°C)</td>
<td>30</td>
</tr>
<tr>
<td>Flow of ( \text{CO}_2 ) (ml/min)</td>
<td>2</td>
</tr>
<tr>
<td>Volume of solvent (ml)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

\( t \) (static): 5 min; \( t \) (dynamic): 10 min.
gave values under the detection limit and for this reason, this attempt could not be confirmed. The obtained differences between Soxhlet and SFE are not only due to the extraction itself, but SFE does not require a further clean-up step and it is well known that the clean-up steps are usually the most likely sources of error in the quantitative determination of pesticides [22].

5.4. Comparison of Soxhlet and SFE techniques

Recovery efficiency is an important factor when an analytical method is evaluated. It can be considered that a method is acceptable when the recoveries are over 80% [17]. Figs. 2 and 3 clearly show that SFE provides a higher recovery in all cases with the only exception of \( p,p'-\text{DDT} \) whose values are 77% in Soxhlet extraction and 47.3% in SFE. Seven compounds in 16 gave values higher than 80% in Soxhlet extraction whereas nine compounds were recovered over the recommended value in SFE, being only three compounds with values around 50%. The explanation for this low values is not very clear. Reimer et al. [23] described that the mixture of the samples with the drier agent such as \( \text{Na}_2\text{SO}_4 \) decreases the recovery of organochlorine compounds and they suggest to introduce both the drier agent and the sample in sandwich beds instead of mixing them. This fact is specially critic in \( \alpha-\text{and } \beta-\text{endosulphan} \) and could be the explanation in this case. In contrast, Valverde et al. [24] insist of using an homogeneous mixture of the sample and the drier which is essential to get accurate values. However, Echarri et al. [25] and Wells and Echarri [26] worked with anhydrous sodium sulphate as drier agent for biota samples and no adsorption effect was observed. Also the lyophilization process before the extraction has been proposed as a good approach.

The main problem of the presence of water is its solubility in the supercritical \( \text{CO}_2 \) which is approximately 0.3%. This fact not only affects the extraction itself but the ice which is formed in the restrictor can block the outlet and consequently the \( \text{CO}_2 \) flow is not constant. Also in this case the water drops in the final extract can be demixed producing two phases, which do not provide quantitative values [27].

\( \beta\)-HCH and \( p,p'-\text{DDE} \) are always recovered over 100%. As was mentioned earlier, in the case of \( p,p'-\text{DDE} \) it could be attributed to a poor separation of dieldrin, whose recovery is always quite poor. \( p,p'-\text{DDE} \) is also a metabolite from DDT and other authors have described the decomposition of DDT compounds in the injection port at high temperature. This is the reason why on column cold injectors are preferred for this type of analysis. In fact, DDT recoveries are lower than 100% in both Soxhlet and SFE procedures. Unfortunately, on column injection couldn’t be used in this study. On the other hand, no correlation was found between the compound properties (structure, volatility, etc.) and recovery by either method.
Another factor in favour with the SFE extraction is its better precision which reduces the number and magnitude of errors. This is again because the SFE procedure is more automatic and has a lower number of steps. Figs. 2 and 3 show the RSD values and in general those corresponding to SFE are lower than in Soxhlet. The variation coefficient is quite high for $p,p'$-DDE and dieldrin in SFE and this fact could be attributed to the chromatographic separation.

Concerning the environmental factors, SFE is much more convenient than Soxhlet, as the former does not require the use of organic solvents (only 1.7 ml of hexane) while the later employs 210 ml of them, and among them 150 ml are of dichloromethane, a chlorinated solvent, expensive and toxic.

The comparison of total time of the analytical procedure, a more and more important factor, shows that SFE is considerably faster than Soxhlet extraction, as high volumes of organic solvents are handled and a clean-up step is necessary when using Soxhlet while it does not in SFE.

SFE is also environmental friendly whereas Soxhlet is not, due to the high consumption of organic solvents.

5.5. Analysis of pesticides in frogs

Four different samples of frogs “Rana pirenaica” captured in the National Park of Ordesa and Monte Perdido (Spain) were analyzed. The direct analysis using an internal standard showed that the real values were below the quantification limit (between 7.39 and 30.28 ng/g) and for this reason, the standard addition procedure was applied. Six different concentration values from 0 to 202.24 ng/g of pesticides were used and in all cases the gravimetric control was applied to the solutions. The results obtained are shown in Fig. 4.

It is interesting to point out that all the pesticides and metabolites were found in the four samples at very low concentration level, with the only exception of $\beta$-HCH which was no present in two of the samples. The trends of concentration values are similar in all samples, which show higher concentration of the most persistent compounds such as $\beta$-HCH and $p,p'$-DDT [28]. On the other hand, it can be observed that metabolites such as $p,p'$-DDD, $p,p'$-DDE, endrin-aldehyde, endosulphan-sulphate and heptachlor are present at

![Fig. 4. Concentration of OCPs in frog samples from National Park of Ordesa and Monte Perdido.](image-url)
higher concentration than their parents, which confirms a strong interaction of these pesticides with biota.

These results agree with those obtained in previous studies carried out in the same area with other biota samples (locust Orthopter orthopter) [7]. Also is important to consider the values obtained for these compounds in the atmosphere and in the water system in the same area [5,6]. Fig. 5 shows the comparison. The bioaccumulation and bioconcentration factors obtained are similar to those described in the literature and suggests that the frogs could be used as bioindicators of the contamination by these compounds.

6. Conclusions

SFE has been shown as a powerful technique for the extraction of organochlorine compounds in biota samples, with clear advantages such as efficiency (higher recoveries), time consuming, cost and environmentally friendly, versus Soxhlet procedures. However, it requires the optimization in depth since the extraction behaviour is strongly affected by the type of sample.

From the environmental point of view, the frog samples under study showed the presence of organochlorine pesticides and some metabolites, which confirms once again the atmospheric transport of pesticides everywhere and their persistence in biota. On the other hand, frogs seem to be good candidates to be used as bioindicators of air pollution since the samples are not very difficult to handle and these animals are present in a wide variety of environments.

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References