

## Supercritical fluid extraction as a clean-up method for the extraction of pesticides from wool wax. A preliminary approach

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### RESUMEN

**Extracción con fluidos supercríticos como método de limpieza para la extracción de pesticidas de la cera de lana cruda. Un estudio preliminar.**

Este trabajo es un estudio preliminar para el desarrollo de un método de extracción de pesticidas directamente de las muestras de cera mediante extracción con fluido supercrítico. La novedad del estudio consiste en la extracción directa de los pesticidas de la cera de lana cruda sin ninguna limpieza previa, cuando muchos autores han considerado casi imposible la extracción directa.

*PALABRAS-CLAVE: Cera de lana – Dióxido de carbono – Extracción con fluido supercrítico – Pesticidas organofosforados – Pesticidas piretroides sintéticos.*

### SUMMARY

**Supercritical fluid extraction as a clean-up method for the extraction of pesticides from wool wax. A preliminary approach.**

The work presented is a preliminary study for the development of a method for the extraction of pesticides from wax samples by means of superficial fluid extraction. The novelty of the study is the direct extraction of the pesticides from raw wool wax without any cleanup, as direct extraction has been considered almost impossible by many authors.

*KEY-WORDS: Carbon dioxide – Organophosphorous pesticides – Supercritical fluid extraction – Synthetic pyrethroids pesticides – Wool wax.*

### 1. INTRODUCTION

Wool wax is a complex matrix of a mixture of wax esters, sterol esters, triterpene alcohols, free acids and sterols, which are secreted by the sebaceous glands of sheep (Motiuk, K., 1979 a, b, 1980). It is obtained as a cream from raw wool fibers after a scouring process. After purification of the cream, lanolin, a highly valued product, is obtained.

Because sheep are treated with pesticides for parasitic control, traces can remain on wool fibers and may be extracted with the wool wax due to their

lipophilic character. Since lanolin is considered a product of human consumption, the pesticide content must be determined.

Pesticide analysis is traditionally done following a three-step procedure. First, the wax is completely removed from the sample by Soxhlet extraction. Removal of the total amount of wax is the only way to ensure the total removal of pesticides due to their lipophilic character. Second, the pesticides have to be extracted from the wax using a cleanup technique. The last step, the identification and quantification of pesticides is usually made by gas chromatography (López-Mesas et al, 2000, b). The direct extraction of the pesticides from the sample, which would simplify the procedure to a two-step method which would involve a reduction in time, solvents and manipulation, has been considered difficult or even impossible (López-Mesas et al, 2000, a).

The most commonly used extraction technique has been Soxhlet extraction, which consumes a high amount of solvent (about 125 ml) and has an serious environmental impact because of the amount of organic waste it generates. In contrast, extraction carried out by the Supercritical Fluid technique leads to a faster and less solvent consuming technique. It has been developed for the extraction of pesticides from different kinds of samples followed by a cleanup technique to purify the extracted analytes for their further analysis by gas chromatography (Motohashi et al., 2000, Lang et al., 2001, Poustka et al. 2003). The US Department of Agriculture's Food Physical Chemistry has investigated the direct use of supercritical fluid as a technique for the clean up of organochlorine pesticides from greasy samples (France et al., 1991), but to the knowledge of the authors, no studies have been performed on the direct extraction of a mixture of organophosphorous and synthetic pyrethroids pesticides.

The most frequently applied supercritical fluid is carbon dioxide, a non-polar solvent. If polar compounds as pesticides have to be extracted, the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction

because of the increase in the polarity of the supercritical fluid and thus the recovery of pesticides (Snyder et al., 1993, Alzaga et al., 1995, Abaroudi et al. 2002, King et al. 2006). The cosolvent may be added in two ways, directly into the cartridge, at the same time the sample is loaded, or can be pumped, if the equipment allows it. Both configurations have been shown to obtain good results (Reindl et al., 1994).

In this work, an approach of a method for the extraction of the pesticides by supercritical fluids and its direct analysis by gas chromatography from a spiked wool wax is developed with no further cleanup, which reduces time, cost of analysis and minimizes waste.

## 2. EXPERIMENTAL

### 2.1. Reagents

Nine pesticides were selected, three synthetic pyrethroids ( $\lambda$ -cyhalothrin, cypermethrin and deltamethrin) and six organophosphorous (chlorpyrifos, chlorfenvinphos, coumaphos, diazinon, ethion and phosalone). Those pesticides were chosen because they are the most frequently used for the parasite control of sheep.

Raw wool wax, free of pesticides (previously analyzed), and obtained from an Australian wool scouring plant, was spiked with the pesticides for their extraction.

Solvents used were trace organic residue.

### 2.2. Supercritical Fluid Extraction

Supercritical fluid extractions were performed using an ISCO SFX2-10. An ISCO 100DX syringe pump supplied the carbon dioxide. The sample to be extracted was placed into a 10 ml stainless steel cartridge, which contained an inert support, and if necessary, cosolvent was added to the top. The cartridge was closed and placed into the extractor chamber, the temperature and pressure were programmed and the fluid was allowed to enter into the cell. After the equilibration time, 45 minutes, the temperature of the restrictor was selected, the valve was opened and the extracting fluid was delivered into a vial containing 6 ml of toluene. Hexane was evaporated to near dryness under a nitrogen flux, reconstituted to 1 ml and injected into the gas chromatograph. All extractions were done in triplicate. The initial procedure had been optimized for the extraction of wool wax in previous works (Jones, 1997, López-Mesas et al., 2005).

### 2.3. Gas Chromatograph

The equipment used was a gas chromatograph, Varian 3400GC, fitted with a septum-equipped programmable injector (Varian 1093). The column was a BPX-5 (15mx2500  $\mu$ m i.d. x 0.25  $\mu$ m film, SGE), helium was used as the carrier gas and detection was made by ECD. The operating

conditions were set as follows: injector temperature ranged from 65 °C to 280 °C at 100 °C/min and held at 280 °C during the rest of the analysis. The oven temperature ranged from 100 °C to 150 °C at 20 °C/min, and then at 4 °C/min up to 295 °C where was held for 1 min. The detector was set at 350 °C. The pesticide tetradifon was used as internal standard and is not expected to be present in the samples analyzed due to the different application (mainly used for plant protection). All injections were carried out in triplicate.

## 3. RESULTS AND DISCUSSION

To make the extraction of the pesticides without extraction of the wax, an inert support, which retains the wax and produces no retention of pesticides would be desirable.

For this purpose, wax was mixed with different polymeric supports and loaded into the cartridge. In a previous work, the best conditions for the extraction of wool wax were found (López-Mesas et al., 2005) so for the present work, chamber conditions were set at those found to be the less favorable for the extraction of wax, 80 °C and 250 atm. The supports selected for the study were Chromosorb, Acid Alumina, Silica-Gel and activated Florisil, because these polymeric materials are the most commonly used when the purification of a mixture pesticide-grease has to be made. As the size of the polymeric material was smaller than the pore filter size, some material enters into the equipment and a destabilization of the flow of the supercritical fluid, CO<sub>2</sub> occurs. To eliminate the problem, additional filters were added to the cartridge to ensure that the only extracted material was the wax or the pesticides.

With the established parameters, extractions were performed and the extracted material was collected in a collecting vial. The collecting vial, located at the end of the restrictor (a stainless steel depressurization capillary), was changed after 3 ml of liquid carbon dioxide passed through the restrictor. The vials were previously weighed and filled with 6 ml of toluene. For the analysis of wax, toluene was evaporated to dryness under a nitrogen flow and by difference the amount of extracted wax was calculated.

As can be seen in Figure 1, activated Florisil and Acid Alumina are the supports that retain more wax. Activated Florisil was the polymer selected for subsequent trials and the cartridge was always filled with 4 g of the support.

To evaluate the interaction of the pesticides with the selected supporting material, 1 ml of a sample of 8 ppm of the mixture of the nine pesticides was directly added to the Florisil. As a blank, Florisil was substituted with filter paper because it is known that there are no interactions between pesticides and this kind of support [Wuchner et al., 1993, Andersen et al., 1990]. For the extraction of the pesticides from the Florisil, recoveries for all pesticides are under

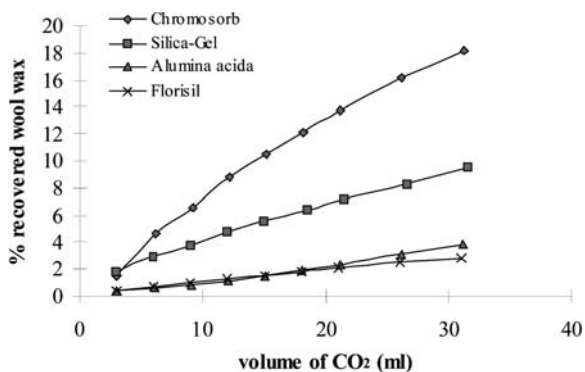


Figure 1  
Effect of the polymeric support on the retention of wool wax.

20%, while for the filter paper, they were higher than 80%. This result shows that the poor extraction is only due to the interaction between the chosen support and the pesticides (individual data for each pesticide not shown). Nevertheless, this support is one of the most commonly used for cleanup purposes with the adequate extracting solvents. Recoveries below 100% of the pesticides extracted through filter paper showed that an optimization of the extraction parameter conditions was necessary, thus, the temperatures of the restrictor and chamber of extraction, the pressure of the chamber and the addition of a cosolvent to the cartridge were evaluated.

### 3.1. Influence of the amount of cosolvent added to the cartridge

Ethyl acetate was chosen as cosolvent because it has been the most frequently used solvent for the elution of pesticides from activated Florisil columns (Robards et al., 1994, Fernández et al., 2002).

Parameters of the equipment were selected as follows, 80 °C and 85 °C for the chamber and restrictor temperatures respectively and 250 atm for the pressure of the chamber. Pesticides were added to the Florisil support and the required amount of ethyl acetate was then added. The extract was collected in 6 ml of toluene and evaporation and reconstitution was analyzed by gas chromatography. Figure 2 shows the obtained results.

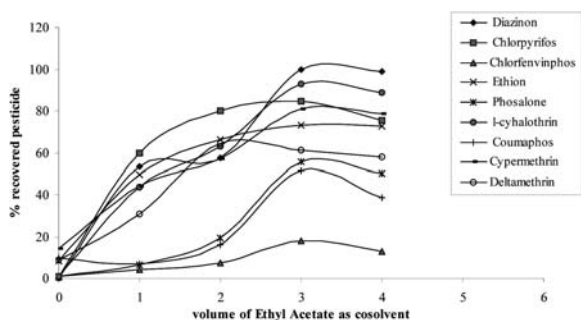


Figure 2  
Effect of the amount of ethyl acetate added into the cartridge as cosolvent.

As previously mentioned, for the extraction of the pesticides without the addition of the ethyl acetate, the recovery was lower than 20% and even for some of the pesticides lower than 10%. Increasing the amount of the ethyl acetate increases the recovery rate of the pesticides with a maximum found when 3 ml were added. A higher volume of cosolvent overloads the cartridge and a leakage was observed at the bottom (which probably drags part of the pesticides, thus decreasing the recovery).

With 3 ml of the cosolvent, all recoveries were found to be between 50 and 100% except for Chlorfenvinphos, which showed a recovery of less than 20%.

### 3.2. Effect of temperature of the restrictor and pressure and temperature of the extraction chamber

The restrictor is a stainless steel depressurization capillary. Pressure in the chamber is delivered to atmosphere pressure through this capillary and the supercritical fluid changes from its supercritical state to a gas state. If the capillary is not heated the CO<sub>2</sub> loses its solvating power and a Joule-Thompson effect is produced with a consequent cooling (Porter et al., 1992). Usually, the analytes are collected at the end of the restrictor in a vial containing a chosen solvent.

The recovery of the pesticides was studied for two different temperatures of the restrictor and of the extraction chamber and at two different pressures for the last one. 3 ml of ethyl acetate were always directly added to the cartridge. Results are shown in Table 1.

For the restrictor, 35 °C and 85 °C, were the chosen temperatures. The temperature and pressure of the chamber were kept constant at 80 °C and 250 atm respectively. At the highest temperature a decrease in the recoveries was seen probably due to the evaporation of the collecting solvent with the consequent loss of pesticides.

Keeping constant the temperatures of the restrictor and of the chamber, 35 °C and 80 °C respectively, the pressure was selected at 150 atm and 250 atm.

When pressure increases, recoveries were increased only for the pesticides belonging to the organophosphorous family. For the pesticides belonging to the synthetic pyrethroids family there was little or no variation. Usually, when pressure increases, solubility of the compounds increases, keeping constant the temperature of the chamber, due to the increase in fluid density.

The temperature of the chamber changed from 40 °C to 80 °C while maintaining a constant pressure, 250 atm, and restrictor temperature, 35 °C. As before, 3 ml of cosolvent were added.

By increasing the temperature of the extraction chamber, the diffusion coefficient in the fluid increases, increasing the volatilization of the fluid and increasing the recoveries. As expected, recoveries

Table 1  
**MPercentage of pesticide recovered under the specified conditions  
 (always S.D. lower than 15%)**

Chamber and restrictor conditions				
<i>Organophosphorous</i>	P = 250, Tc = 80, Tr = 35	P = 150, Tc = 80, Tr = 35	P = 250, Tc = 80, Tr = 85	P = 250, Tc = 40, Tr = 35
Diazinon	100	100	100	73
Chlorpyrifos	100	86	84	97
Chlorfenvinphos	65	19	19	36
Ethion	100	80	77	100
Phosalone	94	65	65	43
Coumaphos	100	89	59	85

Chamber and restrictor conditions				
<i>Synthetic pyrethroids</i>	P = 250, Tc = 80, Tr = 35	P = 150, Tc = 80, Tr = 35	P = 250, Tc = 80, Tr = 85	P = 250, Tc = 40, Tr = 35
Cypermethrin	77	81	82	77
Deltamethrin	96	100	69	96
lambda-cyhalothrin	83	88	96	90

were increased when temperature was high but as occurred with the variation in pressure, only organophosphorous pesticides showed this behavior and synthetic pyrethroids showed little or no variation.

### 3.3. Extraction of pesticides from a spiked wool wax

1 ml of 25% wool wax was added to the Florisil placed into the cartridge, and spiked with a mixture of pesticides. Extraction was carried out at the best conditions found for the extraction of pesticides with a minimum extraction of the wax. The sample collected at the end of the restrictor in the collecting vial filled with toluene, was evaporated, the internal standard was added and it was reconstituted and directly injected into the GC. The chromatogram obtained is shown in Figure 3. Apparently there were no peaks coming from the wax and an acceptable base line was found.

To test if any residual wax remained in the sample with the eluted pesticides, in which case a further cleanup would be necessary in order to preserve the chromatographic system, another collected sample from the supercritical fluid equipment was passed through an alumina cartridge (Varian, ALN 1210-2049, small syringes) and then injected into the chromatographic system (Jones, 1996). Comparing the results with those obtained previously, no differences were observed in relative peak intensity, base line or ratio signal/noise (chromatograms not shown).

Extractions of the pesticides directly added to the Florisil, with and without wax, were performed.

Results are shown in Table 2. The second column shows the results for the extraction of the pesticides without wax, which shows interactions between Florisil and pesticides. Column four shows the recoveries of the pesticides when they were extracted with the addition of wax to the Florisil. The last column shows the rate between both recoveries, expressed as f factor, which has been found to be higher than 0.75 for all pesticides except for Phosalone and Coumaphos with factor f higher than 0.50. The reduction in recoveries was due to the interaction between the wax and the pesticides.

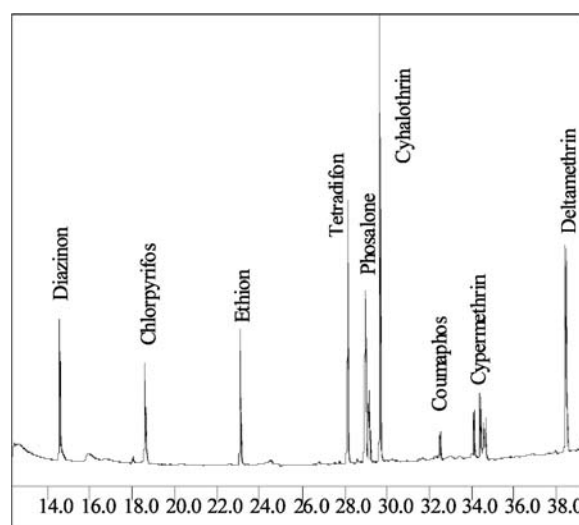


Figure 3  
 Chromatogram for the pesticide mixture extracted by SFE from a spiked wool wax sample with no further clean up.



Table 2  
Recoveries of the pesticides extracted from the spiked Florisil  
or the spiked wax added to the Florisil (f: ratio between recoveries)

Pesticide	Recoveries with no wax added	Standard deviation	Recoveries with wax added	Standard deviation	f
Diazinon	111.0	9.0	110.5	5.4	1.00
Chlorpyrifos	107.0	1.3	109.6	3.5	1.02
Chlorfenvinphos	67.2	0.8	53.3	2.7	0.79
Ethion	105.9	1.7	79.2	2.6	0.75
Phosalone	91.7	3.2	50.2	4.7	0.55
$\lambda$ -Cyhalothrin	87.6	0.8	97.1	3.0	1.10
Coumaphos	102.1	9.5	67.2	0.0	0.66
Cypermethrin	79.8	3.2	104.1	0.6	1.30
Deltamethrin	96.4	1.3	96.9	6.3	1.01

#### 4. CONCLUSIONS

This preliminary study shows that the extraction of pesticides from wool wax, with no further clean-up, may be successfully performed by the use of a non-inert support packed into the extraction cartridge and placed into the supercritical extraction equipment.

The best conditions for the extraction of pesticides were as follows: sample support of activated Florisil 4 g, 3 ml of ethyl acetate as co-solvent, chamber pressure of 150 atm, chamber and restrictor temperatures of 40 °C and 35 °C, respectively, and 10 ml in volume of CO<sub>2</sub>.

High pressures have been found to obtain higher pesticides recoveries, but also the amount of the unwanted wax co-extracted increases.

The supercritical fluid extraction may be used, therefore, as an extraction technique for the extraction of pesticides from wool wax with minimum extraction of wax components.

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