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Il Farmaco 55 (2000) 637–640

IL FARMACO

# Extraction and clean-up procedure for analysis of organochlorine pesticide residues in ethoxylated lanolin

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Received 6 September 1999; accepted 5 September 2000

This paper is dedicated to the memory of Professor Waldemar Saffioti.

**Abstract**

In the present study an evaluation was made of a method for the determination of organochlorine pesticide residues in ethoxylated lanolin. Samples were homogenized with Celite, transferred to chromatographic columns, prepacked with silica gel deactivated to 10%. The pesticide elution was processed with *n*-hexane–dichloromethane and the concentrated eluate was analyzed using gas–liquid chromatography (GC) with electron capture detection (ECD). The composition of the elution solvent was a significant factor for the recovery of the pesticides. Mean recoveries obtained for fortified samples ranged from 87 to 94% for *p,p′*-DDE, dieldrin, endrin, *p,p′*-DDD and *p,p′*-DDT. Optimization of the experimental conditions resulted in a small-scale method that combines extraction and cleanup in a single step. 

*Keywords:* Organochlorine pesticides; Ethoxylated lanolin; Gas chromatography

## 1. Introduction

Lanolin, produced from sheep's wool wax and lanolin derivatives are widely used as raw materials for cosmetic production. Since a variety of pesticides are used to control sheep ectoparasites [1] studies were carried out to evaluate the contamination by pesticides in wool wax [1,2], lanolin [1,3–5] and cosmetics [5–9].

Although the influence of these complex matrices on the analytical method performance is recognised [1,2,5], the development of efficient procedures has been published in the literature [1,2,4,5,9]. William Jones [1] studied the effects of modifications in the procedure previously described [10,11] for the determination of organochlorine and organophosphorus pesticides. The experimental conditions were also optimized for the analysis of pyrethroid pesticides. The selected compounds in lanolin and wool wax were analysed using gel permeation and GC. Recovery data from fortified samples ranged from 71 to 108%. In a further study [2] supercritical fluid extraction with $CO_2$ was employed as a clean-up procedure for the analysis of pesticide residues in wool wax. Recovery data varied from 83 to 108% for samples fortified with eight pesticides. Miyahara et al. [4] described a method for the determination of organochlorine and organophosphorus pesticides in lanolin. Pesticides were extracted with *n*-hexane, partitioned into acetonitrile, purified by Florisil column chromatography and analyzed by GC. Recovery data ranged from 75 to 110%. In the procedure proposed by Diserens [5] for the determination of organochlorine and organophosphorus pesticides, the clean-up of lanolin samples was improved using solid phase extraction on Extrelut and $C_{18}$ cartridges. An additional clean-up step using a Florisil column was necessary before GC analysis of organochlorine compounds could be carried out. The recoveries of 13 pesticides ranged from 80 to 90%. Although the use of organochlorine pesticides has been restricted or forbidden by legislation, these compounds are still under investigation and they are included in the method adopted by the USP — United States Pharmacopeial Convention [13].

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0014-827X/00/$ - see front matter 
PII: S0014-827X(00)00089-6

This paper describes an analytical methodology for the determination of organochlorine pesticide residues in ethoxylated lanolin, one of the most important raw materials used in cosmetic products such as shampoos, hair conditioners, and lotions. The extraction and clean-up procedures are carried out in a single step by transferring the samples, previously homogenized with Celite, to glass columns packed with silica gel. The pesticide compounds are quantitatively eluted with *n*-hexane–dichloromethane and analised by GC–ECD.

## 2. Experimental

### 2.1. Apparatus

A Varian 3300 gas–liquid chromatograph equipped with a 200 cm × 2 mm i.d. glass column packed with 1.5% OV-17/1.95% QF-1 on 100-120 mesh Chromosorb WHP, a constant current $^{63}$Ni electron capture detector and a Varian 4290 integrator were used. The operating conditions were as follows: injector temperature 230°C, oven temperature 200°C, detector temperature 300°C and nitrogen flow at 30 ml/min.

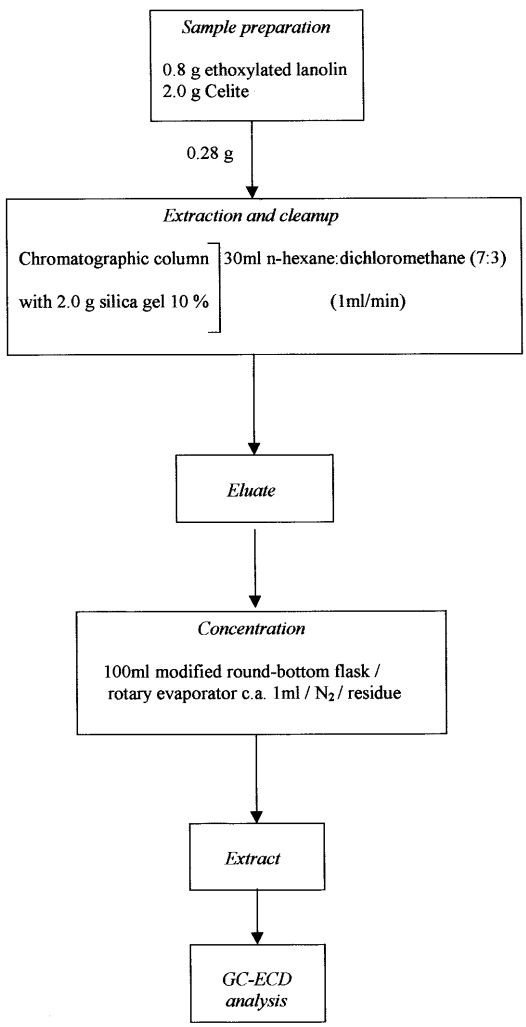


Fig. 1. Scheme of analytical procedure.

### 2.2. Reagents

*n*-Hexane and isooctane were of pesticide grade (Mallinckrodt). Dichloromethane (analytical grade, Merck) was heated under reflux and distilled as described previously [12]. Celite (Reagen) was of analytical grade. Silica gel 60 (70–230 mesh ASTM, Merck) was heated at 130°C for 24 h, deactivated with 10% (w/v) deionized water and stored in a closed glass flask for 24 h before use. Reference standards of all pesticides were obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC). Standard pesticide solutions were made up in isooctane and stored at −18°C.

### 2.3. Sample fortification

Fortified ethoxylated lanolin samples were prepared by adding 2.0 ml of the standard solution to 0.8 g of sample.

### 2.4. Analytical procedure

Ethoxylated lanolin (0.8 g) was mixed with 2.0 g of Celite using a glass stirring rod and 0.28 g of the homogeneous mixture was transferred to the top of a glass chromatographic column (35 cm × 10 mm i.d.) prepacked with 2.0 g of 10% deactivated silica gel. The elution was processed with 30 ml of 7:3 *n*-hexane–dichloromethane (v/v) at 1 ml/min. The eluate was collected in a 100-ml modified round-bottom flask and concentrated to 1 ml using a rotary evaporator. The solvent was removed under a gentle stream of nitrogen and the residue dissolved to an adequate volume (5 ml) with isooctane. The analytical procedure is presented in Fig. 1.

Procedure blanks, consisting of all reagents and glassware used during the analysis were checked for contamination.

### 2.5. Gas chromatographic analysis

Suitable aliquots of sample extracts and standard solutions were injected into a gas chromatograph. The percentage recoveries were calculated by comparing the average chromatographic peak areas or heights of the standard, fortified and unfortified samples.

Table 1
Recoveries of organochlorine pesticides from fortified ethoxylated lanolin by using different solvent systems

| Pesticides | Fortification level (μg/g) | Recovery range (%) | | | |
|---|---|---|---|---|---|
| | | Solvent system [a] | | | |
| | | A [b] | B [c] | C [c] | D [c] |
| HCB | 0.6 | 16–32 | 35–44 | 36–43 | 38–41 |
| α-HCH | 0.6 | 37–47 | 46–53 | 52–54 | 50–54 |
| γ-HCH | 0.6 | 50–56 | 53–57 | 62–63 | 60–65 |
| Heptachlor | 0.8 | 38–48 | 45–54 | 58–61 | 58–63 |
| Aldrin | 0.8 | 42–50 | 49–58 | 59–64 | 59–61 |
| *p,p′*-DDE | 1.0 | 62–65 | 65–68 | 75–77 | 69–74 |
| Dieldrin | 1.0 | 39–45 | 63–64 | 76–78 | 68–72 |
| Endrin | 2.0 | 46–51 | 65 | 79–81 | 72–76 |
| *p,p′*-DDD | 2.4 | 64–69 | 69–72 | 80 | 70–72 |
| *p,p′*-DDT | 2.4 | 61–68 | 61–63 | 78–80 | 74–76 |

[a] 30 ml *n*-hexane–dichloromethane: **A**, 9:1; **B**, 8:2; **C**, 7:3; **D**, 6:4 (v/v).
[b] Six analyses.
[c] Two analyses.

Table 2
Recovery and precision of the proposed method

| Pesticides | Retention time (min) | Fortification level (μg/g) | Mean recovery ± RSD [a] (range) (%) |
|---|---|---|---|
| *p,p′*-DDE | 6.06 | 1.0 | 87 ± 9.9 (77–96) |
| Dieldrin | 6.46 | 1.0 | 91 ± 13.2 (76–105) |
| Endrin | 7.83 | 2.0 | 90 ± 9.1 (79–99) |
| *p,p′*-DDD | 9.21 | 2.4 | 91 ± 9.2 (80–99) |
| *p,p′*-DDT | 11.06 | 2.4 | 94 ± 12.4 (78–104) |

[a] Six analyses; solvent system: 30 ml 7:3 *n*-hexane–dichloromethane (v/v).

## 3. Results and discussion

Experimental conditions were based on a small-scale method previously developed in our laboratory [3] for the determination of eight organochlorine pesticides in lanolin samples. In this method, a lanolin solution (0.04 g/ml) was made up in *n*-hexane and 1.0 ml was transferred to a chromatographic column prepacked with 2.0 g of 10% deactivated silica gel. The pesticide elution was processed with 30 ml of *n*-hexane.

In the present study, the ethoxylated lanolin samples were mixed with Celite and transferred to columns prepared as described above.

Preliminary analyses were made by using *n*-hexane for pesticide elution from the column. Recovery values ranged from 73 to 119% for heptachlor, aldrin, *p,p′*-DDE; *p,p′*-DDD and *p,p′*-DDT. No reproductive recovery data (52 to 111%) were obtained for HCB, α-HCH, and γ-HCH and the pesticides dieldrin and endrin were not eluted from the column.

Four mixtures of *n*-hexane–dichloromethane (9:1; 8:2; 7:3 and 6:4 v/v) were then tested for the elution of the selected pesticides. As shown in Table 1 these solvent systems promoted the elution of dieldrin and endrin. However, the recovery percentages were low (16–65%) for HCB, α-HCH, γ-HCH, heptachlor and aldrin in all cases, demonstrating that this method was not adequate for the determination of these compounds. The highest percentage recoveries for *p,p′*-DDE, dieldrin, endrin, *p,p′*-DDD and *p,p′*-DDT were achieved by using 7:3 *n*-hexane–dichloromethane (v/v). In this study, the composition of the elution system appears to be one of the main factors for the quantitative elution of pesticides from the column.

In order to evaluate the efficiency of the method additional recovery analyses were carried out using 7:3 *n*-hexane–dichloromethane (v/v). Table 2 shows the recovery and precision expressed as relative standard deviation (RSD). Mean recoveries from samples fortified with *p,p′*-DDE, dieldrin, endrin, *p,p′*-DDD and *p,p′*-DDT at levels from 1.0 to 2.4 μg/g ranged from 87 to 94% with RSD values between 9.1 and 13.2%. These data have demonstrated the efficiency of the proposed method.

Gas chromatograms concerning an ethoxylated lanolin sample, a fortified sample and a standard solution are shown in Fig. 2. The unfortified ethoxylated lanolin sample used in the recovery analyses was free of pesti-

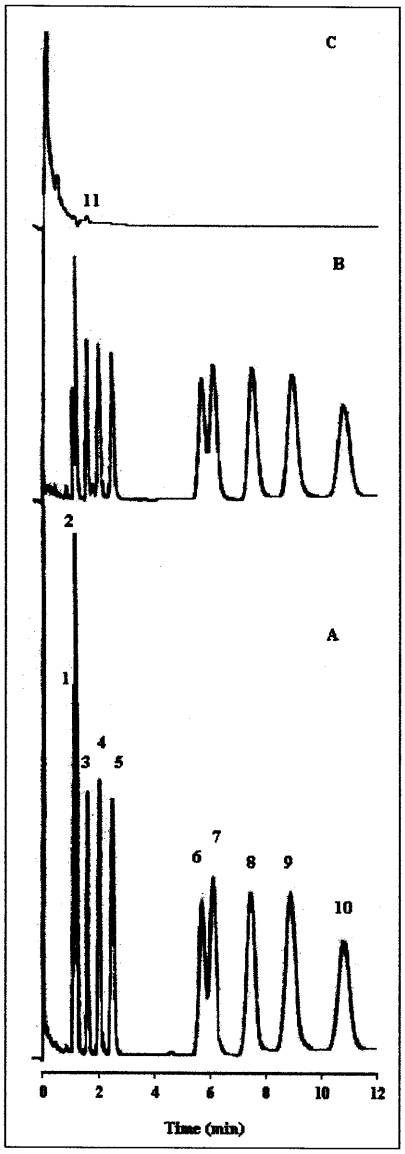


Fig. 2. (**A**) Gas chromatograms of standard solution: (1) HCB (38 pg); (2) α-HCH (38 pg); (3) γ-HCH (38 pg); (4) heptachlor (51 pg); (5) aldrin (51 pg); (6) *p,p′*-DDE (61 pg); (7) dieldrin (64 pg); endrin (128 pg); *p,p′*-DDD (154 pg); *p,p′*-DDT (154 pg). (**B**) Fortified ethoxylated lanolin sample (solvent system: 7:3 *n*-hexane–dichloromethane (v/v)). (**C**) Unfortified ethoxylated lanolin sample. (11) Solvent impurity peak.

cide residues and interfering compounds. The total running time of the GC–ECD analysis was, under the proposed conditions, approximately 12 min.

The analytical methodology was applied to three commercial ethoxylated lanolin samples. The analyses were performed in duplicate, and no detectable amounts of the pesticides were found in any of the samples under the conditions described herein.

Further investigations are necessary in order to evaluate the analytical method for the determination of other classes of pesticides [13] such as pyrethroids [1] used to control sheep ectoparasites.

## Acknowledgements

The authors would like to thank Doris Barnes for the English revision.

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