Clean-Up of a Pesticide–Lanolin Mixture by Gel Permeation Chromatography

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Abstract

In this study, the efficiency of a clean-up method by gel permeation chromatography (GPC) for the separation of pesticides from lanolin is analyzed. The pesticides analyzed belong to two different families, organophosphorous and synthetic pyrethroids. Lanolin, a standard mixture of the pesticides, and a lanolin–pesticides mixture are injected in a GPC column. The recoveries and elution times from the GPC column of lanolin (by a gravimetric method) and pesticides (by gas chromatography–electron capture detector) are determined. From this column, a good separation of the lanolin–pesticides mixture is observed.

Introduction

The antiparasitic plague control for pasture and wool storage involves the use of pesticides remaining on wool fibers that (together with the grease secreted by the sebaceous glands of sheep and other impurities) must be eliminated. Otherwise, they cause an allergic reaction when wool is used by people. Therefore, it is necessary to ensure a thorough washing of wool, which means the use of surfactants and abundant water. This process generates a highly contaminant liquid with biochemical oxygen demand values between 20,000 and 40,000 mg/L and chemical oxygen demand up to 100,000 mg/L (1). This high-contaminant organic charge (20–60 times higher than effluents from the dyeing and finishing industry) causes serious problems in the depuration of these effluents, thus pesticide determination becomes necessary for their reuse.

Some wool scouring industries remove the grease from the liquid phase, because the product resulting from purifying the grease is known as lanolin, which is widely used as a moisturizer in cosmetics (2) and media for some pharmaceutical preparations (3,4) because of its high compatibility with human skin oils. Unfortunately, pesticides have been found in samples of lanolin, as revealed by other studies (2,5).

Pesticides that are allowed to be used for sheep are synthetic pyrethroid and organophosphorous (6). The presence of some organochlorine pesticides can be because of the ingestion of pastures treated with them, contaminated soil, and illegal use.

When a sample with a high fatty matter content is analyzed, a three-step procedure is carried out: an extraction stage allowing the separation of analytes from the sample bulk; a clean-up stage eliminating the interfering components; and an instrumental analysis for the separation, identification, and quantitation of analytes (7). The first two stages are considered to be the most critical of the analysis (8), because the achievement of the appropriate fractions needed in the further analysis depends on them.

Lipids from animals or vegetables consist of a primary mixture complex of long chains of acid and ester alcohols in which pesticides remain strongly retained because of their lipophilic character. Characteristics of these lipids include polar groups (H bonds), high content in hydrocarbon, high molecular weight (between 600 and 1500), and low volatility—in other words, characteristics that can be used for their separation from pesticides.

Experimental

Reagents and material

Lanolin solutions

Pesticide-free lanolin from Westbrook Lanolin (Verviers, Belgium) was weighed directly and dissolved in dichloromethane to form a stock solution of 25% (w/v).

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Table I. Chromatographic Method Parameters

Parameters	Mode	Initial temperature (°C)	Pressure (psi)	Flow rate (mL/min)	Average velocity (cm/s)
Injector	pulsed splitless	250	initial 6.25, pulsed 30	purge 25 (0.8 min)	–
Column	constant flow	100	nominal initial 6.25	initial 0.9	20
Detector	constant flow in column and make-up	340	–	purge anode 6.0, make-up 40.0	–

In order to prepare the spiked lanolin, pure lanolin was weighed and spiked with a concentrated mixture of the nine pesticides (calculated to have 5 ppm of each pesticide). Dichloromethane was added and placed into a rotatory system on a waterbath at 40°C. When the mixture was homogeneous, an amount of dichloromethane was added until a 2% final solution (w/v) was obtained.

Pesticides

Nine of the pesticides that are used for sheep or wool treatment were selected for this study—five of them belonging to the synthetic pyrethroids (cyhalothrin, cypermethrin, deltamethrin, fenvalerate, and tetramethrin) and four organophosphorous (carbophenothion, chlorpyriphos-methyl, diazinon, and propetamphos). A stock solution of 1000 ppm of each pesticide was prepared in ethyl acetate or cyclohexane. A mixture of 50 ppm of each pesticide was prepared from the stock solutions and used as a stock solution for the calibration standards. Purities were higher than 99%.

All solvents used were of Pestiscan-grade from Lab-Scan Analytical Sciences (Madrid, Spain).

Gas chromatograph

A Hewlett-Packard (Geneva, Switzerland) HP6890 gas chromatograph (GC) fitted with an automatic injector (Series Injector) was equipped with an HP-5 column (30 m × 320 µm × 0.25 µm) using helium as a carrier gas. Detection was made by a Model HP6890 ECD. Data were collected and statistically treated by Chemstation HP Software.

Gel permeation chromatography

Equipment fitted with a high-performance liquid chromatograph Varian (Middelburg, The Netherlands) Vista 5500 liquid chromatograph pump, an ultraviolet (UV)–visible (vis) Varian 634 detector, and Varian Star chromatography software integrator was used. The injector used was a Model 7000 stream switching valve with a 2-mL sample loop. Two chromatographic columns were used (Figure 1) that were made of transparent glass (450- × 15-mm i.d.) and slurry packed with BIO-BEADS S-X3 200–400 mesh (Bio-Rad, Eke, Belgium, ref. 152-2750). All connections were made in a Teflon pipe with a length of $^1/_{16}$ inch (0.8-mm i.d.) and $^1/_8$ inch (1.5-mm i.d.).

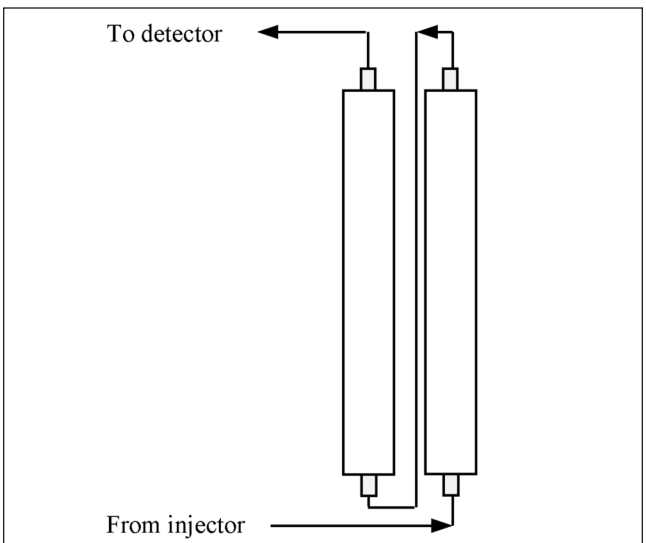

Figure 1. Configuration of the GPC columns used.

Procedure

All injections were always made at least by duplicate.

Clean-up method

Before initiating the clean-up study, the filling state of the gas

Table II. Statistical Analysis of the Capacity Factor for the Nine Pesticides

Pesticide	k' average	Standard deviation	Relative standard deviation	95% Confidence intervals
Propetamphos	1.87281	0.00043	0.02278	0.00031
Diazinon	1.92759	0.00048	0.02464	0.00034
Chlorpyrifos-met	2.37789	0.00043	0.0179	0.0003
Carbophenothion	5.0705	0.00085	0.01677	0.00061
Tetramethrin	6.0507	0.00142	0.02342	0.00101
Cyhalothrin	6.88809	0.00106	0.01546	0.00076
Cypermethrin	8.49533	0.00101	0.01185	0.00072
Fenvalerate	9.20656	0.00097	0.01055	0.00069
Deltamethrin	9.89721	0.00113	0.01147	0.00081

Table III. Statistical Analysis of the Selectivity for the Nine Pesticides

Pesticide	S average	Standard deviation	Relative standard deviation	95% Confidence intervals
Propetamphos	1.06084	0.05247	4.94645	0.03754
Diazinon	1.02925	0.00011	0.0107	0.00008
Chlorpyrifos-met	1.05919	0.00014	0.01293	0.0001
Carbophenothion	1.01385	0.00009	0.00929	0.00007
Tetramethrin	1.02371	0.00006	0.00576	0.00004
Cyhalothrin	1.02372	0.00003	0.00325	0.00002
Cypermethrin	1.00484	0.00004	0.00367	0.00003
Fenvalerate	1.04422	0.00005	0.00465	0.00003
Deltamethrin	1.01817	0.00004	0.00431	0.00003

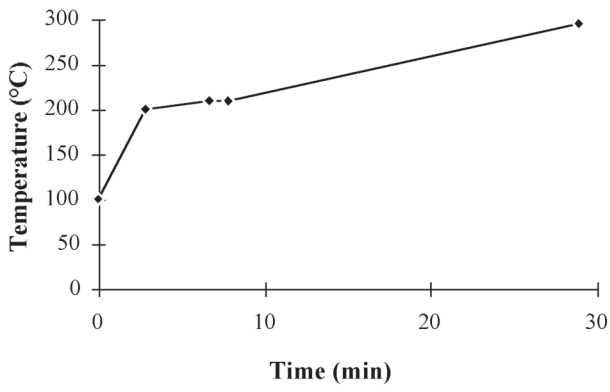

Figure 2. Oven temperature program.

permeation chromatograph (GPC) columns was verified by injecting a phthalate sample. The retention time and peak area were compared with the standard injected when the columns were just packed.

Two milliliters of a sample was injected for successive analyses. The solvent used for the elution of the grease and pesticides was dichloromethane at a flow rate of 4 mL/min. Detection was set at 254 nm.

Detection method

The optimized parameters for the detection method by GC–ECD are shown in Table I. The temperature gradient used for the separation of the nine pesticides is summarized in Figure 2.

Results and Discussion

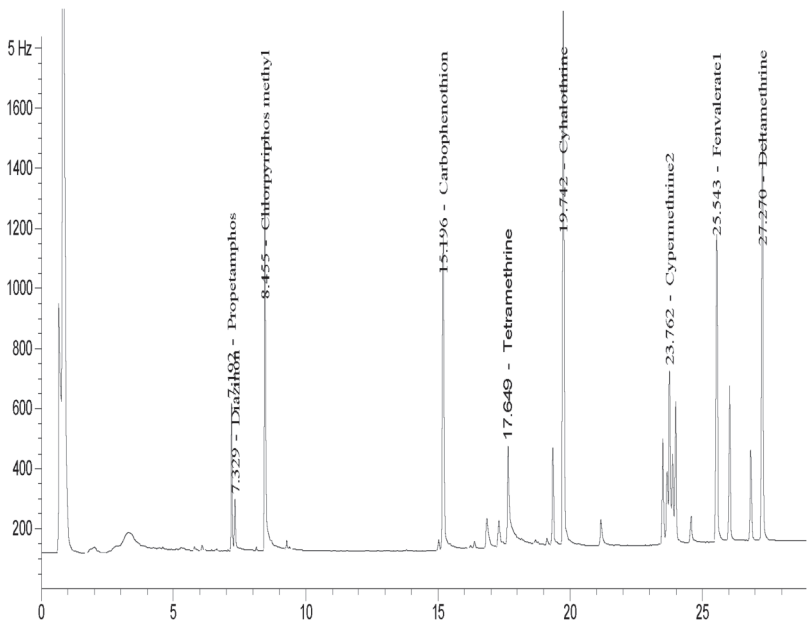

Figure 3. Chromatogram of the injection of a nine-pesticides standard sample containing 5 ppm of each.

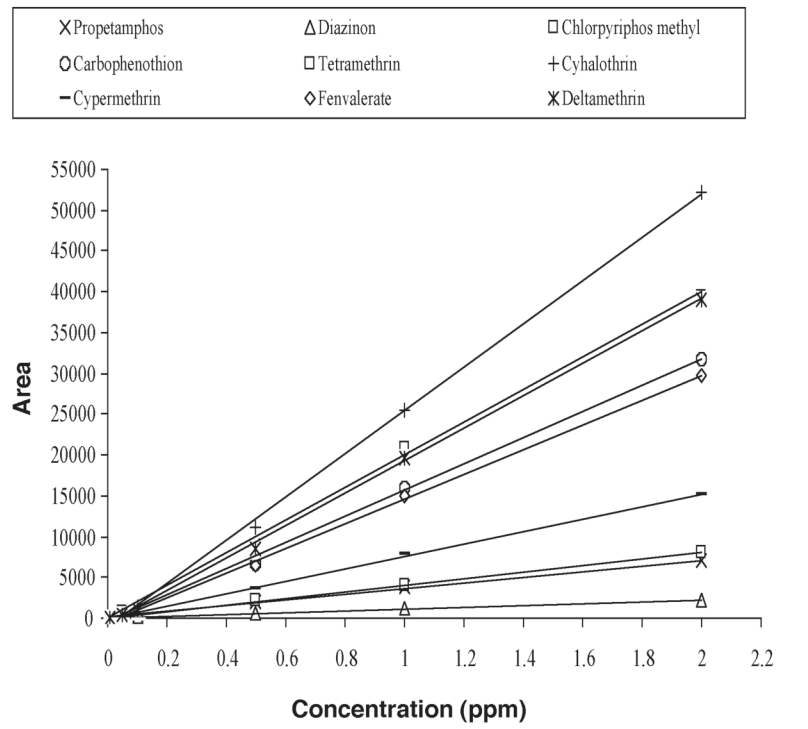

Figure 4. Calibration curves for the nine pesticides.

Detection method

Figure 3 shows the chromatogram obtained when a mixture of the nine pesticides was analyzed in the method described previously. The pesticides were eluted in a total time of 28 min.

Verification of the method

Verifications were necessary in order to establish if the parameters of the process developed were adaptable to further applications. The parameters studied in this work were: the capacity factor (k'), selectivity, linearity, and repeatability.

In order to carry out this analysis, a 0.5-ppm standard mixture was injected 10 times, and the chromatograms were analyzed in terms of the parameter studied.

Capacity factor

The k' value was defined as:

$$k' = \frac{t_r - t_o}{t_o} \qquad \text{Eq. 1}$$

where t_r is the retention time for a compound and t_o is the dead time (both in minutes). Results for the nine pesticides and the deviation of the average of the 10 injections are shown in Table II.

Selectivity

Selectivity (S) is the capacity of analyzing a compound in the presence of certain interferences. It is measured by the equation:

$$S = \frac{k'_{(b)}}{k'_{(a)}} \qquad \text{Eq. 2}$$

given that $t_r(a) < t_r(b)$. Table III shows the selectivity values obtained for each pesticide.

Linearity

As it is known, the response of an analytical method is proportional to a given value. This is a linear proportionality determined from a series of injections of standard mixtures at different concentrations (in this case 0.01, 0.05, 0.1, 0.5, 1, 2, and 5 ppm). They were injected by triplicate in the GC–ECD. The average of the pesticide areas were plotted, and the calibration curve was obtained for each component.

For each pesticide, the response was linear until 2 ppm—except for diazinon, which slightly deviated at this concentration (Figure 4).

Repeatability

The repeatability of the system was measured in order to determine the reliability of the method. In order to do this, a series of the same standard sample was injected and the results were statistically analyzed. Repeatability was obtained when the analysis was made in a short time by the same equipment, operator, and laboratory. Results are indicated in Table IV.

Table IV. Statistical Analysis of the Retention Time Repeatability for the Nine Pesticides

Pesticide	Average	Standard deviation	Relative standard deviation	95% Confidence intervals
Propetamphos	7.1864	0.0011	0.0148	0.00076
Diazinon	7.3235	0.0012	0.0162	0.00085
Chlorpyrifos-met	8.4499	0.0011	0.0126	0.00076
Carbophenothion	15.1856	0.0021	0.0140	0.00152
Tetramethrin	17.6375	0.0035	0.0201	0.00254
Cyhalothrin	19.7323	0.0027	0.0135	0.00191
Cypermethrin	23.7529	0.0025	0.0106	0.0018
Fenvalerate	25.5320	0.0024	0.0095	0.00174
Deltamethrin	27.2597	0.0028	0.0104	0.00203

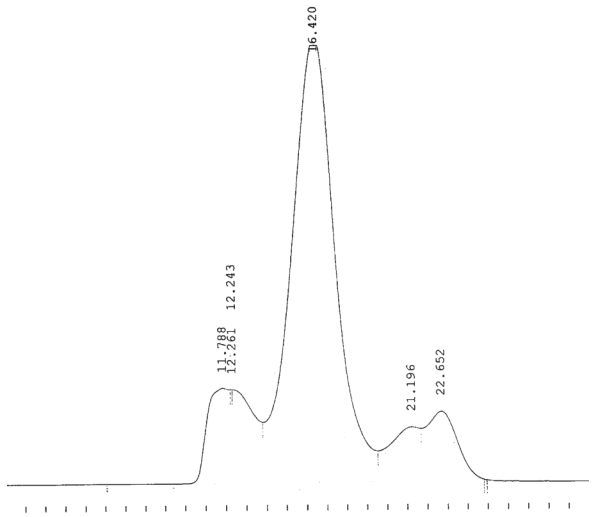

Figure 5. Elution spectrum of lanolin at 254 nm.

Table V. Recovery Percentages of Lanolin in Each Fraction Collected from the Discharge of the GPC Column

Elution time	%Lanolin recovered
10~12	1.34
12~14	12.04
14~16	17.16
16~18	24.88
18~20	32.14
20~22	5.6

Clean-up method

Lanolin elution

A 25% lanolin solution in dichloromethane was injected. This solution showed saturation in the UV–vis detector, thus a 2% solution was prepared and injected (Figure 5).

Table VI. Recovery Percentages of the Nine Pesticides Analyzed

Pesticide	%Recovery
Propetamphos	97.6
Diazinon	98.8
Chlorpyrifos-met	92.5
Carbophenothion	86.3
Tetramethrin	107.5
Cyhalothrin	122.5
Cypermethrin	97.6
Fenvalerate	101.2
Deltamethrin	97.1

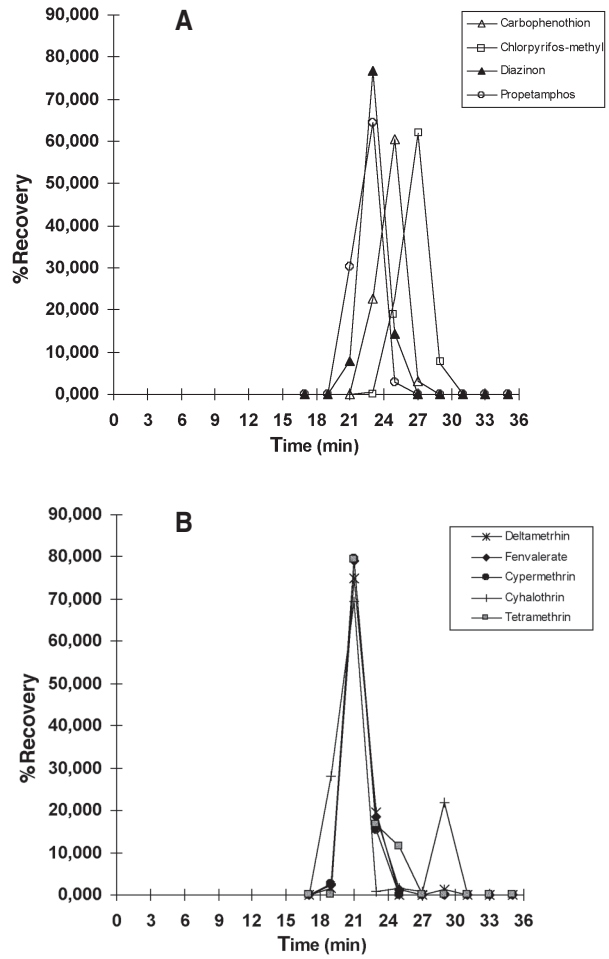

Figure 6. Recovery percentages of the different pesticides in each of the different fractions collected at the output of the GPC column: (A) organophosphorous pesticides and (B) synthetic pyrethroid pesticides.

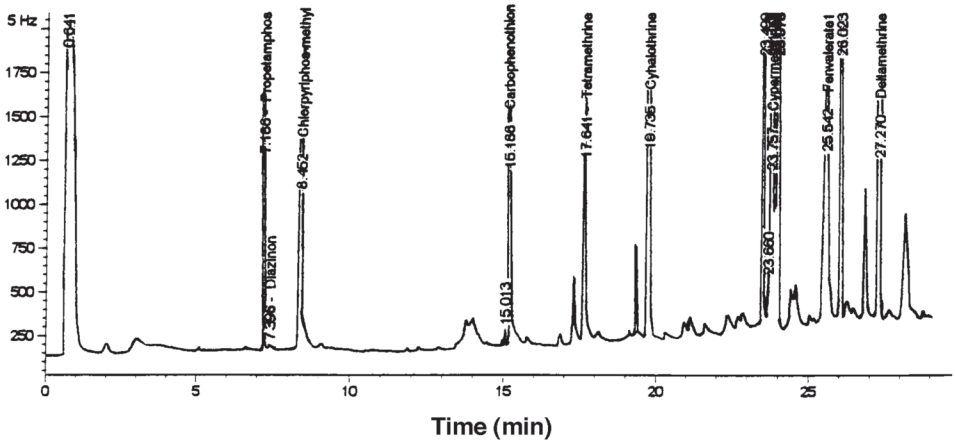

Figure 7. Chromatogram of an eluted sample from the GPC column after injection of a lanolin–pesticides mixture showing the interference by the remaining lanolin in the base line.

In order to determine exactly when lanolin is eluted from the GPC column, 2 mL of a 25% solution was injected, and the fractions eluted were collected in calibrated vials every 2 min from 10 to 26 min. The solvent was evaporated to dryness under a nitrogen atmosphere, and by difference, percentage recoveries were calculated for each eluted fraction (Table V). The maximum percentage of lanolin eluted was found between 18 and 20 min.

Elution of the pesticides mixture

A 5-ppm standard mixture was injected in the GPC with the same conditions as the lanolin injection, and a single fraction was collected between 16 and 38 min. The eluted fraction was evaporated to dryness and reconstituted in 5 mL of cyclohexane and injected in the GC system. After analysis, recovery percentages higher than 85% for each pesticide were obtained (Table VI).

In order to determine when the pesticides were exactly eluted from the GPC column, eluted samples after the injection were collected from 16 to 38 min every 2 min. The recovery percentage of each fraction versus the elution time was represented (Figure 6). Two elution groups can be differentiated: synthetic pyrethroid pesticides were eluted between 19 and 22 min, and organophosphorous was eluted between 22 and 28 min.

Elution of a lanolin–pesticide sample

Two milliliters of a 2% lanolin dissolution spiked with a 5-ppm mixture of the pesticides was injected. Eluted fractions were collected every 5 min. A GC analysis showed a chromatogram (Figure 7) with a non-flat baseline because of the interference of lanolin. Nevertheless, all peaks were able to be integrated. Recovery percentages higher than 85% (with the exception of diazinon) were found.

Conclusion

The GC–ECD method for the detection of the nine pesticides developed is valid and surpasses the 95% reliability test.

By means of the clean-up system, the separation of the pesticides into two groups constituted by each of the two families (organophosphorous and synthetic pyrethroids) is possible, which makes further analysis easier. The separation of the bulk of the lanolin from the pesticides is also possible. The presence of small residues of lanolin in the fraction of the pesticides eluted does not make analysis by GC–ECD difficult when obtaining recovery percentages greater than 85%.

Acknowledgments

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