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Trace level determination of organochlorine, organophosphorus and pyrethroid pesticides in lanolin using gel permeation chromatography followed by dual gas chromatography and gas chromatography–negative chemical ionization mass spectrometric confirmation ☆

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Abstract

A methodology for multi-class pesticide determination at trace level in lanolin is presented. Gel permeation chromatography on a Bio-Beads SX-3 column followed by a dual GC chromatographic determination has been developed. The effluent of the analytical column (50% diphenyl–methyl- or 14% cyanopropyl–phenylpolysiloxane) was split into an electron-capture and a nitrogen–phosphorus detection system. The chromatographic system was optimised for 28 pesticides commonly used to control sheep pests and corresponding to organochlorine, organophosphorus and pyretroid classes. Identification has been carried out by gas chromatography coupled to negative chemical ionization mass spectrometry. Recoveries ranged from 72 to 94% and the detection limits from 20 to 97 ng/g depending on the pesticide class, the RSDs were below 10%. Finally, the developed analytical methodology has been successfully applied to the determination of pesticides in several lanolin samples.

Keywords: Waxes; Organochlorine compounds; Organophosphorus compounds; Pyrethroids; Pesticides; Lanolin

1. Introduction

To control the sheep pests, insecticides such as organochlorine and organophosphorus compounds and pyrethroids are applied. Since most of these pesticides are lipophilic, they tend to accumulate into lipids, so a main part of them can be found in lanolin, the wool wax of sheep. Also, indirect input of pesticides in lanolin appears to come from the ingested grass [1]. Despite the fact that organochlorine pesticides are commonly forbidden, they are still in use in several countries.

Lanolin is a complex mixture of fatty acids and alcohols, diols, hydroxy-acids, sterols, esters, sterol esters and diesters. This blend forming the wool wax has similar properties to the human skin wax; so

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lanolin and its by-products are widely used in many applications such as baby care, cosmetics and pharmaceuticals. Furthermore, it is suspected that lanolin can vehiculate pesticide transport through the human skin. Direct ingestion by infants is possible after treatment of the mother nipple [2]. For these reasons, pesticide occurrence in lanolin has to be closely controlled. Pesticide content, according to the 1990 European Pharmacopoeia, cannot exceed 50 ng/g for individual organochlorine pesticides, 500 ng/g for other individual pesticides and 1 μg/g as total.

Pesticides occurring in lanolin have already been analysed by gas chromatography (GC) following clean up by gel permeation chromatography (GPC) [3–6]. Several detection systems such as electron-capture detection (ECD) [3–8], flame photometric detection (FPD) [3,7,8], atomic emission detection (AED) [7], thermionic specific detection (TSD) [4] and mass spectrometry (MS) [3,7] have been used for this purpose. However, due to the wide range of pesticides occurring in lanolin, the use of several detectors with different selectivity to target different analytes appears to be the most promising approach.

In this work, we present a fast methodology based on a dual GC system with ECD and nitrogen–phosphorus detection (NPD). The use of this system has already been described for pesticide analyses [9,10] but it has not been used yet for trace level determination of pesticides in lanolin. The dual detection system offers advantages over mono-elemental detection systems, such as higher sensitivity than AED for nitrogen and higher selectivity than GC–MS in the election impact ionization (EI) mode. Moreover, GC–negative chemical ionization (NCI) MS has been selected for confirmation due to its high selectivity and sensitivity for most of the target pesticides [11]. Special attention has been paid to pyrethroids since they are increasingly replacing the organophosphorus pesticides. Furthermore, they show poor response in both NPD and ECD. Therefore, detection has been optimised for these specific compounds.

The main goal of this work is to show the suitability of a single-step clean up by GPC followed by a dual GC determination of pesticides occurring in lanolin. GPC has been demonstrated to be the best option as lanolin clean-up method, but the detection system needs an improvement to permit a faster analysis.

2. Experimental

2.1. Standards and reagents

Ethyl acetate was obtained from Merck (Darmstadt, Germany). HPLC-grade cyclohexane was purchased from Panreac (Barcelona, Spain). Analytical-grade allethrin, cypermethrin, chlorpyriphos-methyl, deltamethrin, bromophos-methyl, fenthion, ethion, coumaphos, chlorfenvinphos, permethrin, hexachlorbenzene, 4,4′-DDT, 4,4′-DDD and 4,4′-DDE were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Quantitative grade endosulfan, pirimiphos-ethyl, diazinon and malathion were from Riedel-de Häen (Seelze, Germany). Qualitative grade lindane, allethrin, heptachlor, aldrin, dieldrin and endrin were obtained from PolyScience (Niles, IL, USA). Lanolin 1 was a refined wool wax, Lanolin 4 a technical product, they were purchased from a local brand. Lanolins 2 and 3 are wool wax extracts obtained by supercritical fluid extraction (SFE) in our own laboratory.

2.2. Sample preparation

Standard stock solutions of 2000 μg/g have been prepared with ethyl acetate. All solutions were stored at 4 °C. Lanolin samples (1 g) were dissolved in a known volume of GPC mobile phase (5 ml). Hexachlorobenzene (HCB) (0.4 μg) dissolved in ethyl acetate, was used as surrogate and spiked into the solution that was filtered through a 0.45 μm nylon membrane (Lida, Konosha, WI, USA). Samples were injected into a high-performance liquid chromatographic system (HPLC) system equipped with a Rheodyne high-pressure injection valve (Rhonert Park, CA, USA) and a 100 μl loop. The HPLC system used was from Shimadzu (Kyoto, Japan). The system consisted of two LC-10AT pumps, an SPD-10AV UV detector, an SCL-10A controller and Class-VP software. A 450 mm×10 mm I.D. column packed with Bio Beads SX-3, 200–400 mesh (Bio-Rad, Hercules, CA, USA) was used. Ethyl acetate–cyclohexane (1:1) with a 2 ml/min flow-rate was used as mobile phase for GPC. To carry out the pesticide determination, each lanolin sample was injected four times, each of them corresponding to ca. 20 mg of lanolin on column.

Two different fractions, high-molecular-mass

compounds (t_R<8 min) and low-molecular-mass (t_R>8 min) were collected. The latter that may contain pesticides, was rotaevaporated to a volume of ca. 2 ml. Then the samples were placed into tared conic vials before evaporation under a gentle nitrogen current to ca. 100 μl, then the vials were weighed to know the exact lanolin concentration before injection into the dual GC system.

2.3. Instrumental analysis

2.3.1. Dual GC system

For the GC analysis, a chromatograph MFC 500 from Carlo Erba (Milan, Italy) coupled to NPD 800 and ECD 800 detectors from Fisons was used. Helium from Abello Linde (Barcelona, Spain) was used as carrier gas at 190 kPa, and nitrogen as ECD make-up gas at 80 kPa from Abello Linde. In NPD, the additional gas was hydrogen at 50 kPa and air at 30 kPa and as make-up gas, helium at 70 kPa. Several capillary columns were tested: 5% phenyl–methylpolysiloxane (META TX-5, Teknokroma, Sant Cugat, Spain; 30 m×0.25 mm I.D., 0.25 μm), 50% phenyl–methylpolysiloxane (DB17, J&W, Folsom, CA, USA; 30 m×0.25 mm I.D., 0.25 μm) and a 14% cyanopropyl–phenyl–methylpolysiloxane (DB1701, J&W; 30 m×0.25 mm I.D., 0.25 μm). The best results in terms of resolution were achieved with the latter two columns. Oven temperature was programmed from 70 °C (1 min), at 10 °C/min to 220 °C (0 min), and then at 5 °C/min to 280 °C (18 min). Injector and detector temperatures were held at 280 °C except the ECD 800 body that was maintained at 310 °C. A vitreous fused-silica outlet splitter purchased from SGE (Ringwood, Australia) is used to split the capillary column effluent to the two detectors. An AS 200 autosampler from CTC Analytics (Zwingen, Switzerland) was used.

2.3.2. GC–NCI-MS system

A GC system from Agilent Technologies 6890A (Geneva, Switzerland) was used, coupled to an MS detector 5973N. Ammonia was chosen as ionization gas and its pressure was optimised to $1.6 \cdot 10^{-4}$ Pa in the ion source that was held at 175 °C. Transfer line and the quadrupole temperatures were maintained at 280 and 150 °C, respectively. Helium at a constant flow-rate of 1 ml/min was used as carrier gas.

2.4. Calibration curve and quantification

The calibration curves did not have to be corrected by the internal standard because a strong linear correlation has been found without correction ($0.996<R^2<0.999$). In order to minimise detector variability, samples and calibration standards were injected in a randomised order.

3. Results and discussion

3.1. GPC fractionation

The cut-off time has been selected by injecting lanolin samples and pure pesticide solutions into the GPC system. Different pesticide classes have been tested, and lindane eluted first. Therefore, the choice of cut-off time depends on the trade off between lindane recovery and clean-up efficiency. In Fig. 1, two GPC chromatograms of a lanolin sample (Lanolin 1) and lindane are shown where the cut-off time of 8 min is indicated. To evaluate the efficiency of clean-up, the UV response of all lipidic compounds was considered to be equivalent. With the chosen cut-off time, a lindane recovery of 85% was obtained by injecting a lindane stock solution into the GPC system. The clean-up efficiency evaluated by injection of a lanolin sample into the GPC system was 79%.

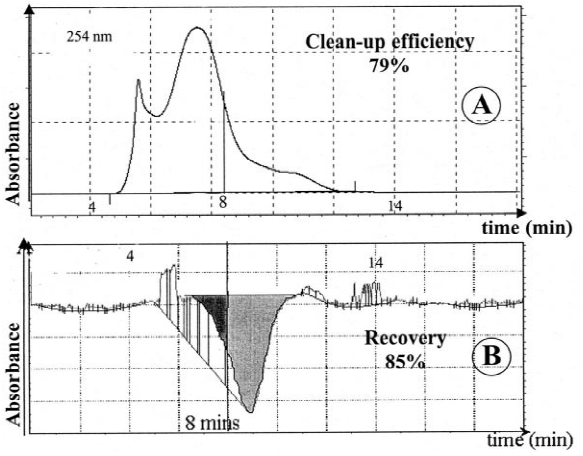

Fig. 1. GPC chromatograms of a (A) lanolin sample (20 mg) and (B) lindane (17 mg) injected on column.

3.2. Optimisation of GC–ECD–NPD

In order to optimise the chromatographic conditions, a mixture of 23 pesticides has been analysed by dual detection GC. The oven temperature program has been optimised to obtain a satisfactory separation for all target compounds. Three different pesticide classes are present in the mixture: nine organophosphorus pesticides (diazinon, chlorpyrifos-methyl, malathion, pirimiphos-ethyl, bromophos-methyl, fenthion, chlorfenviphos, ethion, and coumaphos); 10 organochlorine pesticides (heptachlor, lindane, aldrin, dieldrin, endrin, endrin ketone, endosulfan, 4,4′-DDT, 4,4′-DDD and 4,4′-DDE); and four pyrethroid pesticides (allethrin, permethrin, cypermethrin, and deltamethrin). A 14% cyanopropyl–phenyl–methylpolysiloxane (DB1701) also recommended for pesticide analysis has been evaluated but poor separation was observed between three organophosphorus pesticides (malathion, pirimiphos-ethyl and bromophos-methyl) with an α value of 1.004 between pirimiphos-ethyl and malathion, and 1.003 between malathion and bromophos-methyl. The ECD and NPD chromatograms of the mixture are shown in Fig. 2.

As confirmation method, GC–NCI-MS has been used. The mixture has been injected into the GC–NCI-MS system in full-scan mode in order to define the characteristic ions that will be used later to confirm the presence of pesticides in real samples by selected ion monitoring (SIM). A total ion current trace of the pesticide mixture by GC–NCI-MS is shown in Fig. 3 and Table 1 presents relevant data for pesticide identification.

3.3. Real lanolin and wool wax sample analysis

Recovery was calculated using hexachlorobenzene as surrogate. Recoveries from 72 to 94% were obtained ($n=4$) and are shown in Table 2. Lanolin represents a very complex mixture with a wide range of molecular masses and polarities. For this reason, the ECD chromatogram contains a lot of peaks that makes identification and integration difficult. How-

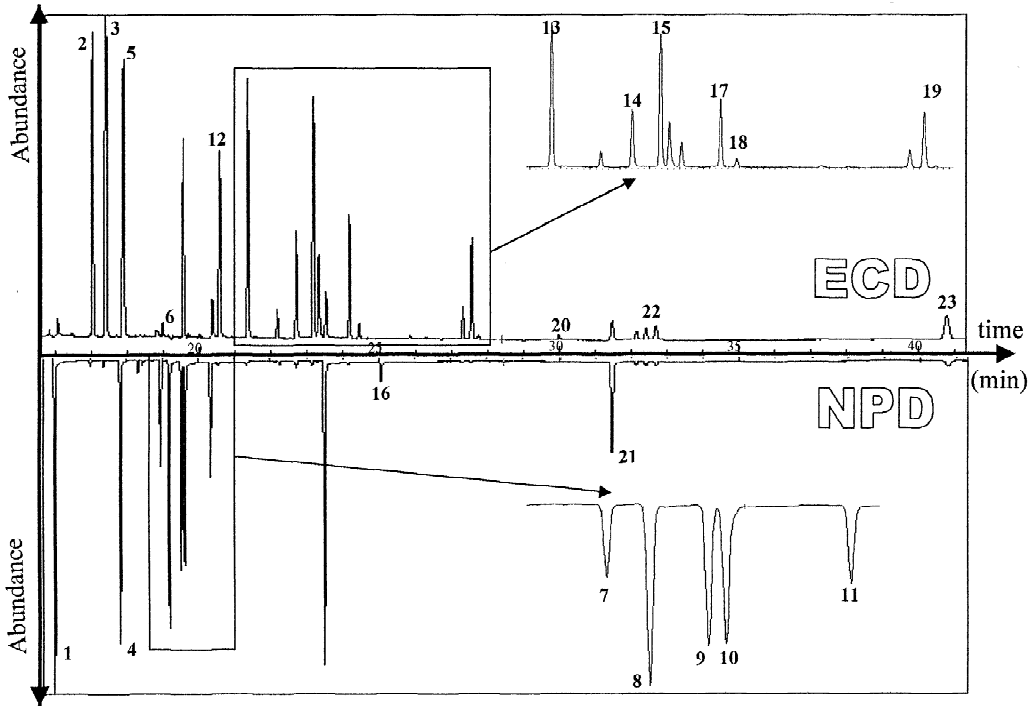

Fig. 2. Gas chromatogram of a 23-component pesticide mixture detected by ECD and NPD at concentrations ranging from 500 to 1500 μg/ml.

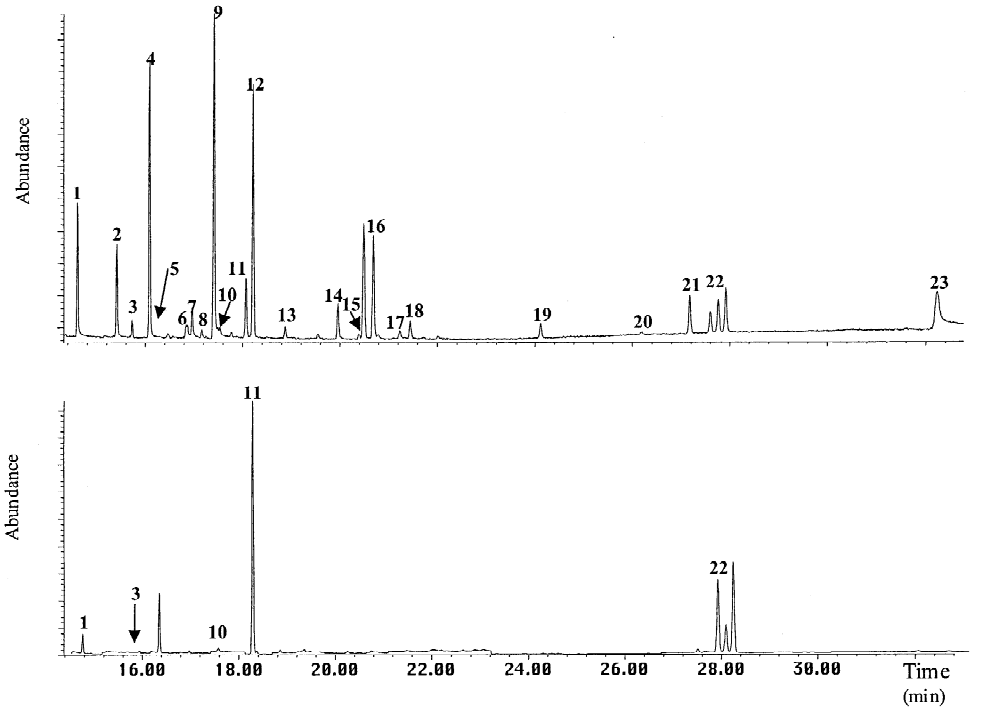

Fig. 3. Two total ion current traces obtained by NCI-MS using NH_3 at $1.2 \cdot 10^{-4}$ Pa. (A) Standard mixture of 23 pesticides with concentrations between 500 and 1500 μg/ml in the full scan mode and, (B) a real lanolin sample detected in the SIM mode.

ever, the NPD chromatogram is cleaner due to a higher detector selectivity (Fig. 4).

First of all, a qualitative study has been carried out looking for the presence of target compounds. Several of them have been detected (diazinon, chlorfenviphos, fenthion, bromophos-methyl, lindane, 4,4′-DDE and cypermethrin). Peak identification has been confirmed by GC–NCI-MS. The technique proved to be excellent, allowing high sensitivity and additional information for compound identification.

Pesticides present at higher concentrations (i.e., diazinon, chlorfenviphos, lindane and cypermethrin) have been quantified. Limits of quantitation (LOQs) are not limited by the detectors themselves but by the matrix background. Also, the splitter ratio can be modified to reach the configuration limit of a single detector, if needed. Even with the 1:1 splitter ratio used, limits of detection (LODs) of 76 ng/g were obtained for diazinon, 30 ng/g for chlorfenvinphos, 21 ng/g for lindane and 97 ng/g for cypermethrin. The LOQs of these compounds were suitable, with no important differences for any of the pesticide classes, so they can be considered representative if no coelution is observed. We cannot exclude possible coelutions because major compositional differences can be observed between samples depending on the origin of lanolin. Diazinon and chlorfenviphos have been determined using NPD, cypermethrin and lindane by ECD. Limits of detection were evaluated using three times the area of the background matrix response in the neighborhood of the retention time of the target peak and 10 times the area ratios to obtain limits of quantification (Table 3). RSDs for the different compounds are shown in Table 2. Levels above the allowed limits were observed for three samples out of the four analysed (Fig. 5). So, it is evident that this kind of methodology is suitable for routine analysis.

4. Conclusion

The developed methodology is suitable for the determination of pesticides in real lanolin samples.

Table 1
Summary of data for the identification of 29 pesticides obtained by GC–NCI-MS

Name	Number	Pesticide class	Confirmation ions	k_{DB17}	k_{DB1701}	Detection method
Dichlorvos		Organophosphorus	125, 205, 170	8.45	–	NPD
Diazinon	1	Organophosphorus	169, 303, 275	15.83	15.09	NPD
Heptachlor	2	Organochlorine	266, 300, 237	16.85	15.92	ECD
Lindane	3	Organochlorine	255, 145	17.27	17.92	ECD
Parathion-methyl		Organophosphorus	263, 154, 141	17.99	17.67	NPD
Pirimicarb		Carbamate	237, 193, 166	17.64	–	NPD
Chlorpyrifos-methyl	4	Organophosphorus	141, 212, 95	17.72	16.52	NPD
Aldrin	5	Organochlorine	237, 330, 214	17.75	16.54	ECD
Allethrin	6	Pyrethroid	167, 134, 301	18.49	18.67	ECD
Malathion	7	Organophosphorus	157, 172, 125	18.86	18.11	NPD
Pirimiphos-ethyl	8	Organophosphorus	169, 332, 304	19.13	18.03	NPD
Bromophos-methyl	9	Organophosphorus	257, 81, 141	19.47	18.16	NPD
Fenthion	10	Organophosphorus	277, 263, 247	19.57	23.88	NPD
Chlorfenvinphos	11	Organophosphorus	153, 288, 322	20.32	19.29	NPD
Endosulfan	12	Organochlorine	406, 242, 372	20.54	19.06	ECD
4,4′-DDE	13	Organochlorine	281, 246, 315	21.37	19.69	ECD
Dieldrin	14	Organochlorine	237, 346, 380	22.76	20.73	ECD
4,4′-DDD	15	Organochlorine	248, 283, 319	23.28	22.08	ECD
Ethion	16	Organophosphorus	185, 355, 153	23.63	22.26	NPD
4,4′-DDT	17	Organochlorine	281, 249, 260	24.34	22.61	ECD
Carbophenotion		Organophosphorus	185, 143, 153	24.47	–	NPD
Endrin	18	Organochlorine	272, 380, 308	24.61	23.59	ECD
Endrin ketone	19	Organochlorine	308, 272, 346	27.82	25.95	ECD
Permethrin	20	Pyrethroid	207, 354, 390	30.39	27.94	ECD
Azinphos-ethyl		Organophosphorus	185, 316, 133	31.79	–	NPD
Coumaphos	21	Organophosphorus	225, 362, 191	31.93	31.00	NPD
Cypermethrin	22	Pyrethroid	207, 171, 379	32.62	30.82	ECD
Deltamethrin	23	Pyrethroid	81, 297, 217	41.45	36.84	ECD

The dual chromatographic system supplies more information in less time and helps us to improve identification by comparing the response of a compound by two different detectors. Sample clean-up is the most important step of analysis for this kind of complex mixture. GPC eliminates 79% of the lipidic compounds but matrix peaks still appear in the ECD chromatogram. Therefore, an additional clean-up is necessary for most real samples if organochlorine pesticides are targeted. However, the developed

Table 2
HCB recoveries for the different samples and RSDs for the different compounds analysed

		Recovery (%)		RSD (%, $n=4$)
Lanolin 1	Replicate 1	83.30	HCB	6.50
Lanolin 1	Replicate 2	72.40	Diazinon	12.80
Lanolin 1	Replicate 3	87.10	Chlorfenvinphos	15.00
Lanolin 1	Replicate 4	93.50	Cypermethrin	8.50
Lanolin 2	SFE wool extract	83.4	Lindane	12.10
Lanolin 3	SFE wool extract	79.40		
Lanolin 4	Technical lanolin	82.50		

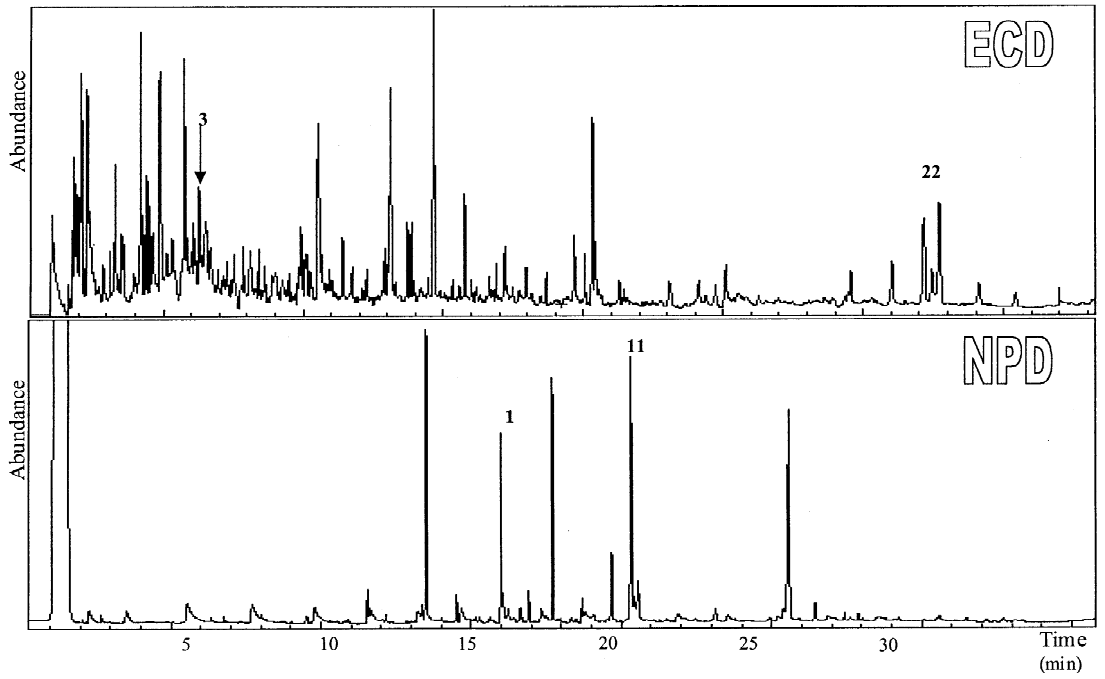

Fig. 4. Gas chromatogram of a real lanolin (1.1 mg on column) sample after GPC detected by ECD and NPD.

analytical methodology is successful for organophosphorus and pyrethroid pesticide classes that are used most nowadays.

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Table 3
LODs and LOQs for the target compounds evaluated in real lanolin samples (1.1 mg on column) by GC–ECD–NPD

	Test compound	LOD (ng/g)	LOQ (ng/g)
Organochlorine	Lindane	21	57
Organophosphorus	Diazinon	76	253
	Chlorfenvinphos	30	117
Pyrethroid	Cypermethrin	97	331

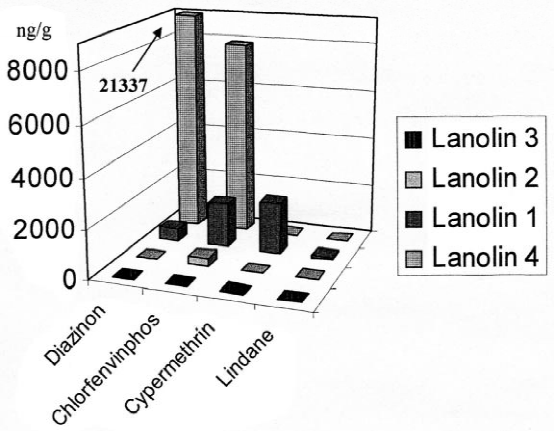

Fig. 5. Quantification of the main pesticides in four lanolin samples by GC–ECD–NPD.

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