Supercritical Fluid Extraction as a Cleanup Technique for Gas Chromatographic Analysis of Pesticides in Wool Wax

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Supercritical fluid extraction with CO_2 was investigated as a sample cleanup procedure for the gas chromatographic analysis of pesticide residues in wool wax. Spiked samples were used to optimize the extraction parameters. Recoveries of between 85% and 108% with relative standard deviations of 2−8% were obtained for a range of pesticides that represented those most likely to be found in raw wool wax as a result of treating sheep with legal pesticides to control ectoparasites.

Keywords: *Analysis; carbon dioxide; gas chromatography; lanolin; pesticides; supercritical fluid extraction; wool wax*

INTRODUCTION

Wool wax is the lipid secreted by the sebaceous glands of sheep and is recovered during the scouring of raw wool. Due to its lipophilic nature, the raw wool wax is generally contaminated with trace levels of the pesticides used to control ectoparasites that afflict sheep. The type and amount of these residues vary with the parasite to be controlled and the time of treatment relative to the harvesting of the raw wool. These residues are difficult to remove from wool wax, and although their levels are substantially reduced during conventional refining, more specialized processes are required to comply with the maximum pesticide residue specifications in the U.S. Pharmacopeia Modified Lanolin monograph (U.S.P., 1993).

Wool wax is a complex matrix comprised principally of a mixture of esters in which over 138 different aliphatic acids (Motiuk, 1979a) are combined with about 75 different alcohols (Motiuk, 1979b). Natural weathering of the wax on the sheep adds to this complexity by the conversion of a portion of these esters to "free" wool wax acids, alcohols, and sterols as well as some high molecular weight "oxidized" material. As a result of this complexity, it has proved difficult to apply conventional cleanup procedures used for the analysis of pesticides in lipids. Diserens (1989) has described a solvent partitioning process, based on a solid-phase diatomaceous earth support using hexane and acetonitrile with a subsequent cleanup on a C_{18} column, which was effective but rather labor intensive. The U.S.P. (1993) specifies in its lanolin monographs a gel permeation chromatography (GPC) procedure that uses a 1:1 mixture of dichloromethane and hexane as the eluant. The use of this procedure to determine pesticide levels in a range of lanolin-containing samples has been reported by Heikes and Craun (1992), and modifications to the protocol to optimize the recovery of synthetic pyrethroids have been described by Jones (1996).

The above methods all require the use of considerable amounts of toxic solvents. Accordingly, an alternative procedure utilizing supercritical carbon dioxide has obvious advantages. Many studies have demonstrated the feasibility of using supercritical fluids to extract pesticides from solid matrices such as soil (Lopez-Avila et al., 1989; Reindi and Höfler, 1994) and grains (Skopec et al., 1993; King et al., 1993), but the reported isolation of pesticides from matrices containing high lipid levels has generally involved the subsequent use of conventional cleanup procedures involving solvents prior to gas chromatographic (GC) analysis (King, 1989; Hopper and King, 1991).

Recent work (Cygnarowicz-Provost et al., 1994; Jones et al., 1997) on the solubility of wool wax in supercritical CO_2 has shown that the bulk of the wool wax is much less soluble than the triglycerides of vegetable oils and animal fats and, accordingly, high pressures and temperatures were required to solubilize a significant amount of the wool wax esters. However, at pressures and temperatures used to extract pesticides from other substrates (King, 1989) the extracts of wool wax contained principally free wool wax acids and alcohols with only traces of the more volatile wool wax esters. In the present paper the use of these extraction conditions to isolate pesticide residues and their subsequent analysis by GC are described.

EXPERIMENTAL PROCEDURES

Wool Waxes. Pesticide-free wool wax or pesticide-free grade of lanolin (Westbrook, U.K.) were dissolved in hexane/diethyl ether (6:4) to give a 10% w/v solution. To determine the degree of pesticide recovery, these solutions were spiked with a range of pesticides commonly used to treat wool ectoparasites at the levels listed in Table 1.

Supercritical Fluid Extraction. Unless otherwise specified, supercritical fluid extractions of wool wax were carried out in a fume hood using SFE/SFC grade CO_2 (BOC Gases, Melbourne, Australia) pressurized to 250 atm and heated to 80 °C. Liquid CO_2 at 20 °C was pressurized by an ISCO 100DM syringe pump (Lincoln, NE) and delivered to an ISCO SFX 2-10 extractor fitted with 10 mL extraction cells. Depressurization was achieved using a stainless steel capillary (120 mm × 0.25 mm id) which was crimped at the outlet end to produce pump flow rates in the range of 2−3 mL of liquid CO_2. The depressurization capillary was thermostated at 10 °C above the extraction temperature used.

Supercritical Fluid Extraction Procedure. Typically, the spiked wool wax solution (2 mL) was added to 2 g of Chromosorb W-HP, 60−80 mesh (Maneville, Denver, CO) in the extraction cell. Concurrently, a gentle stream of dry air was drawn through the cell to spread the wool wax as a thin layer over the Chromosorb. The removal of the solvent was completed at 80 °C. The wool wax was extracted with the delivery of 25 mL of liquid CO_2 to the extractor. The extract was trapped in toluene (6 mL) containing chlorpyrifos ethyl

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Table 1. Recovery of Pesticides from Spiked Raw Wool Wax

	spike level			
	high		low	
pesticide	recovery[a] (%)	RSD (%)	recovery[a] (%)	RSD (%)
propetamphos[b]	92	3.3	83	4.3
diazinon[c]	90	5.2	85	5.2
chlorfenvinphos[b]	104	4.5	95	6.3
carbophenothion[b]	104	3.9	96	3.0
cyhalothrin[b]	105	5.4	101	4.5
coumaphos[b]	105	5.3	89	4.8
cypermethrin[b]	104	3.2	87	4.5
deltamethrin[b]	108	5.3	103	7.8

[a] Average of 6 replicates. [b] High-level spike = 20 μg/g of wool wax; low-level spike = 2 μg/g of wool wax. [c] High-level spike = 40 μg/g of wool wax; low-level spike = 4 μg/g of wool wax.

(0.4 mg/L) as an internal standard, applied to a silica solid phase extraction column, and eluted with a mixture of ethyl acetate/hexane (5 mL, 2:3 v/v). The combined eluant was reduced in volume under N_2 as required, and the pesticides were analyzed by GC.

GC. A Varian 3400 GC (Varian, Harbor City, CA) fitted with a 1093 septum-equipped programmable injector and a DB-5 capillary column (15 m × 0.25 mm i.d., 0.25 μm film, J&W Scientific 123-5032, Folsom CA) was used. The capillary column outlet was split and coupled to an electron capture detector and a thermionic specific detector via a 1:1 outlet splitter (VSOS, Scientific Glass Engineering, Melbourne, Australia) using 2 × 30 cm lengths of deactivated 0.22 mm i.d. fused silica tubing. The usual operating conditions were as follows. The injector temperature was ramped from 65 to 250 °C at 100 °C/min and held at 250 °C for 23 min. The column oven temperature was initially held at 80 °C for 2 min, ramped to 340 °C at 15 °C/min, and held at 340 °C for 6 min. The detector temperature was 350 °C. A helium carrier gas flow rate of 23 cm/s and an injection volume of 0.5 μL were used.

RESULTS AND DISCUSSION

The pesticides chosen for this study not only represent the ones most likely to be found in wool wax as a result of legal usage but also span the range of molecular size and polarity of typical lipophilic pesticides which would be retained by the wool wax. Initial experiments showed that the ease of extraction of these pesticide residues generally correlated to their GC retention order on a nonpolar column, suggesting that molecular weight rather than the small differences in polarity was the most important factor. Deltamethrin, which was the largest compound, was observed to be the least soluble of the pesticides in the supercritical CO_2 and therefore the most difficult to extract. Accordingly, this compound was used to optimize the extraction conditions to achieve quantitative recovery of the pesticides. For GC determination of the pesticides it is desirable that the amount of wool wax present is minimized and the wool wax components be readily eluted from the GC column. As shown in Figure 1, the amount of wool wax coextracted at 80 °C increased with increasing pressure. This together with the observation (Jones et al., 1997) that the molecular size of the wool wax components extracted also increases with increasing pressure makes it desirable to use the lowest extraction pressure possible. Above 300 atm the rate of extraction of deltamethrin was relatively independent of the pressure and diffusion controlled, but below 300 atm the amount of CO_2 required to completely extract deltamethrin increased

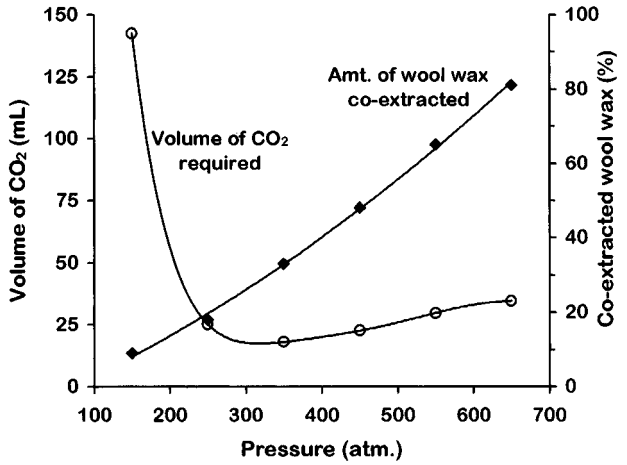

Figure 1. Effect of pressure on the amount of supercritical CO_2 required for the extraction of deltamethrin at 80 °C and amount of wool wax coextracted.

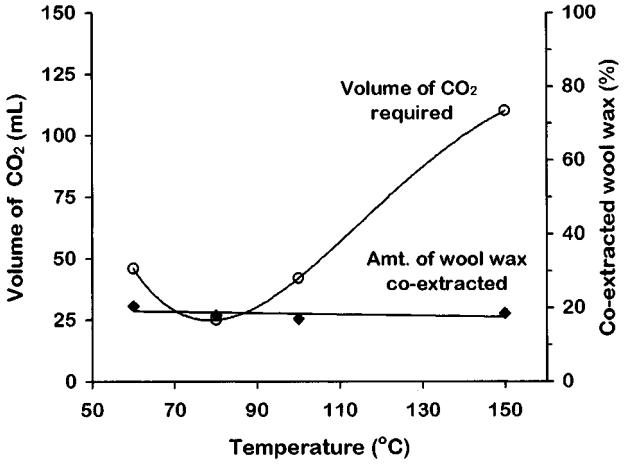

Figure 2. Effect of temperature on the amount of supercritical CO_2 required for the extraction of deltamethrin at 250 atm and amount of wool wax coextracted.

substantially as the solubility of deltamethrin in supercritical CO_2 became the dominant factor. As a compromise between the minium amount of coextracted wool wax and an acceptable amount of CO_2 usage the pressure of 250 atm was used throughout the rest of this study. The effect of temperature on the rate of extraction of deltamethrin at 250 atm is quite complex (Figure 2). The initial fall in the amount of CO_2 required as the temperature is raised to 80 °C probably reflects the increasing solubility of deltamethrin with increasing temperature, whereas the increasing amount of CO_2 required above 80 °C is probably associated with the observed retrograde solubility characteristics of the wool wax below 300 atm (Jones et al., 1997). At higher pressures the temperature had very little effect on the amount of CO_2 required.

Under the extraction conditions of 250 atm and 80 °C about 18% of the raw wool wax was coextracted with the pesticides. This material consisted primarily of the free wool wax acids and alcohols and appeared to readily elute from the GC column. However, after about 30−40 analyses the resolution and peak shape of the organophosphorus compounds began to deteriorate. The column performance could be corrected by cleaning the injector insert and removing the first 15 cm of the GC column, which suggested some of the wool wax components were not eluting from the column. A prolonged bake-out at or near the upper temperature limit of the

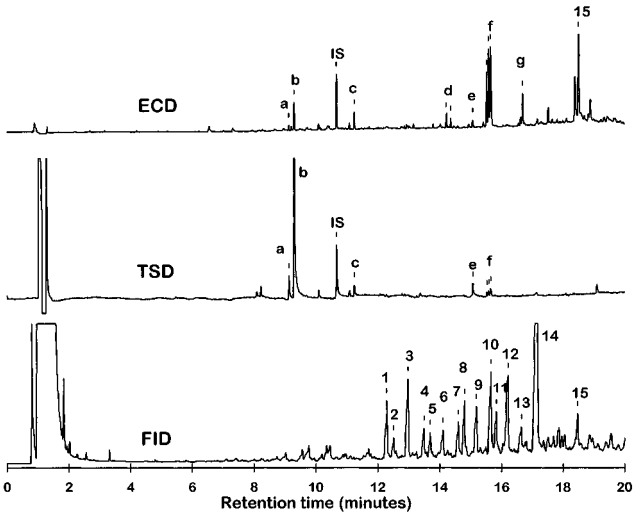

Figure 3. GC traces of a raw wool wax using different detectors: [long-chain alcohols] (1) iso-C_{20}, (2) *n*-C_{20}, (3) anteiso-C_{21}, (4) iso-C_{22}, (5) *n*-C_{22}, (6) anteiso-C_{23}, (7) iso-C_{24}, (8) *n*-C_{24}, (9) anteiso-C_{25}, (10) iso-C_{26}, (11) *n*-C_{26}, (12) anteiso-C_{27}, (13) iso-C_{28}; [sterols] (14) cholesterol, (15) 7-oxocholesterol; [pesticides] (a) propetamphos, (b) diazinon, (c) chlorfenvinphos *E* and *Z* isomers, (d) cyhalothrin isomers, (e) coumaphos, (f) cypermethrin isomers, (g) deltamethrin; (IS) chlorpyriphos ethyl as internal standard.

stationary phase was tried, and while this removed most of the wool wax esters injected, the accompanying decomposition gave rise to substantial ghost peaks in subsequent chromatograms and the severe conditions were found to unacceptably shorten the life of the GC column. Without the extended bake-out the GC columns used still only had a limited life. Similar degradation of the GC column has been observed with pesticide analysis of wool wax using GPC cleanup previously (Jones, 1996). In this case the degradation of the GC column performance was ascribed to the presence of certain unidentified wool wax compounds which were thought to be sterol peroxides, and a secondary cleanup on a short proprietary alumina column was used. In the present study the secondary cleanup was successfully carried out using either basic alumina or silica proprietary columns. These columns removed some of the more polar components of the extracted wool wax, reducing the amount of coextracted wool wax to about 10% for raw wool waxes and to about 5% for refined wool waxes. The analyte solution still contained small amounts of residual wool wax esters, but these did not affect the analysis and were found to elute very slowly as a slight increase in background noise in subsequent injections. Well in excess of 100 analyses were performed without any indication of significant retention time drift or peak tailing occurring.

Figure 3 shows the GC traces obtained from a raw wool wax sample using electron capture, thermionic specific, and flame ionization detection. The major peaks due to the wool wax observed with the flame ionization detector were the "free" wool wax alcohols and sterols. These compounds did not appear to contribute to the background of the traces obtained from the electron capture or thermionic detectors.

To test the efficacy of using supercritical fluids to extract pesticides from wool wax for GC analysis, wool wax was spiked at the two levels shown in Table 1. Excellent, highly reproducible recoveries of all the pesticides were obtained. Quantification of an analysis method is considered satisfactory if the recovery is over

Table 2. GC Detection Limits in Raw Wool Wax and Refined Lanolin

	detection limits[a] (μg/g)			
	raw wool wax		refined lanolin	
pesticide	ECD	TSD	ECD	TSD
propetamphos	5.0	0.1	1.2	0.1
diazinon	0.4	0.01	1.0	1.0
chlorfenvinphos	0.1	0.05	0.1	0.1
carbophenothion	0.4	0.2	0.1	0.1
cyhalothrin	0.1		0.1	
coumaphos	0.6	0.1	0.3	0.1
cypermethrin	0.05		0.1	
deltamethrin	0.8		0.5	

[a] Detection limits were defined as 4 times the level of background noise in the retention window of the pesticide.

Table 3. Pesticide Levels in a Sample of Raw Wool Wax As Determined Using Four Different Cleanup Procedures

	pesticide levels[a] (μg/g) by different cleanup procedures			
pesticide	SFE	GPC	SPE	SCD
propetamphos	2.2 (4.4)	2.3 (9.1)	1.7 (10.3)	1.7 (10.1)
diazinon	28 (4.9)	29 (7.2)	17 (6.7)	23 (7.1)
chlorfenvinphos	2.5 (5.7)	2.4 (8.3)	2.2 (7.9)	1.8 (8.2)
cyhalothrin	1.5 (5.9)	1.3 (12.1)	1.5 (7.5)	1.2 (15.3)
coumaphos	3.9 (6.3)	4.0 (6.8)	3.9 (8.7)	3.4 (7.5)
cypermethrin	34 (3.2)	33 (4.7)	32 (4.3)	15 (11.3)
deltamethrin	4.1 (7.2)	4.0 (7.5)	3.7 (8.2)	2.2 (12.1)

[a] Figures in parentheses represent the % RSD of six replicate analysis. SFE, supercritical fluid extraction; GPC, gel permeation chromatography (Jones, 1996); SPE, liquid−liquid extraction on a solid-phase support (Diserens, 1989); SCD, sweep codistillation (Pharmacopial Forum, 1989).

70−80% with a relative standard deviation of 10% for repeatability (Ambrus and Thier, 1986). In this study the average recovery levels for all pesticides were >85%, with most >90%. Relative standard deviations ranged from 2.5% to 8%. The lowest recoveries were associated with the most volatile compounds, propetamphos and diazinon.

Analyte solutions prepared from the pesticide-free wool waxes were used to estimate the detection limits of the pesticides used in this study (Table 2). The traces from the electron capture detector were the most noisy, but the background peaks lacked the sharpness of the pesticide peaks and could be avoided by manual integration or by the careful selection of integration parameters, such as the peak threshold limit or peak slope function. Excellent detectability was observed for most compounds using the most appropriate detector. Care must be exercised in the identification of deltamethrin at trace levels, particularly in raw wool waxes, due to the presence of some relatively sharp peaks of wool wax origin close to its retention time.

The supercritical CO_2 cleanup procedure described in this paper was compared with previously published cleanup procedures in the analysis of the raw wool wax sample shown in Figure 3 (Table 3). The SFE results were generally slightly higher with a lower relative standard deviation than the other procedures, which reflects the excellent recoveries of the pesticide residues by this procedure.

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