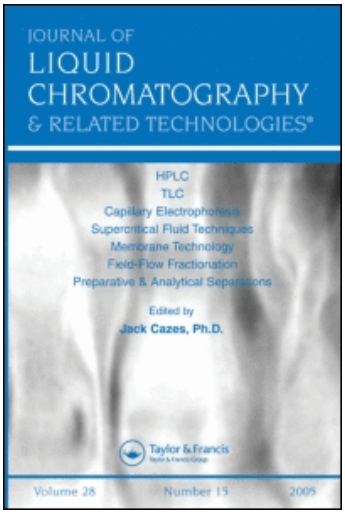


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# Determination of Deltamethrin Levels in Wool by Reversed-Phase High Performance Liquid Chromatography

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# DETERMINATION OF DELTAMETHRIN LEVELS IN WOOL BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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## ABSTRACT

A method is described for the extraction and analysis of deltamethrin in wool after pour-on or plunge dipping application. Acetonitrile was used for extraction followed by simple clean-up on Florisil or C18 cartridges. Deltamethrin levels were determined by liquid scintillation spectrometry of [$^{14}$C]-deltamethrin and high performance liquid chromatography at 217-222 nm. Results showed good recovery and correlation between the two methods. This extraction procedure is relatively simple and rapid, it eliminates the need for prolonged liquid-liquid extraction and solid-phase steps thus making it more suitable for pharmacokinetic studies.

## INTRODUCTION

Deltamethrin ((s)-α-cyano-3-phenoxybenzyl (IR,3R)-cis-3-(2,2-dibromo-vinyl)-2,2-dimethylcyclopropanecarboxylate; DM) is a particularly photostable synthetic pyrethroid (SP) and has been used extensively against many insect pests (1,2), its high potency proving very valuable in the treatment of the sheep body louse, Damalinia ovis. The lipophilic nature of DM has been utilized in the

development of formulations which were originally solvent, but latterly aqueous based, which facilitated SP diffusion in wool grease after topical application on the sheep's backline. These treatments have been widely used, albeit with little knowledge of their diffusion kinetics, for over a decade (3,4). During the course of a study to determine the diffusion kinetics of backline application of SP drugs, a simple and accurate assay procedure for quantitation of SP in wool was required.

Only limited information is available on extraction and analytical procedures necessary for the determination of SP content in wool or animal hair. These procedures generally involve the extraction of SP with non-polar solvents such as hexane or petroleum spirit followed by prolonged clean-up using liquid-liquid and solid-phase extractions (4-6). These multi-step procedures are time consuming and reduce SP recovery (unpublished observations). Acetonitrile (ACN) has been reported to be an effective extraction solvent for cypermethrin residues in vegetable and animal tissues (6); the relatively low fat solubility of ACN lowers the quantity of contaminating lipid extracted although the extracts can be frozen to further separate residual lipids (6). Gas liquid chromatography (6-9), normal and reversed-phase high performance liquid chromatography (HPLC) is then used to determine SP concentration of commercial formulations and vegetable or animal tissues (4, 7, 10).



This paper describes a method which uses ACN to remove DM from wool without prolonged extractions, followed by a simple clean-up procedure. This method has been utilized effectively in subsequent studies to define the pharmacokinetics of DM in wool following backline or plunge dipping application to sheep after shearing and of alphacypermethrin after long wool application.

## MATERIAL AND METHODS

### Instrumentation

The HPLC system consisted of a Millipore Waters (Milford, MA., USA) Model 510 pump, Model 710B automatic injector, with the elution profile

monitored by a Model 481 UV detector and peak analysis determined using a Model 730 data module. A 4 μ Nova-Pak C18 radial-pak cartridge contained in an 8 x 10 mm Radial Compression Module, preceded with a C18 Guard-Pak pre-column, was used to separate SP compounds. Assay of [$^{14}$C]-DM concentrations utilized a Packard 2000CA Tri-Carb scintillation spectrometer (Canberra Packard, Melbourne, Australia). Sample sonication was carried out in a Bransonic 220 bath (Branson, Shelton, CN., USA).

Reagents

Standard DM was provided by Roussel Uclaf (Paris, France). [$^{14}$C]-DM formulation was provided by Pitman-Moore (Sydney, Australia). The acetonitrile used for extraction and HPLC elution was of HPLC-grade (Mallinkcrodt, Melbourne, Australia), and petroleum spirit of Univar grade (AJAX, Sydney, Australia). Florisil Sep-Pak Cartridges were obtained from Millipore Waters, C18 Bond Elut from Analytichem International (Harbour, CA., USA) and C18 Maxi-Clean and Nylon 66 filters from Alltech Associates (Deer Field, IL., USA). The scintillant OCS was obtained from Amersham (Arlington Heights, IL., USA). All water used for dilution and elution was of HPLC-grade.

HPLC Conditions

The isocratic system utilized 83-88% ACN in water as the elution solvent, pumped at a flow rate of 1-2 ml/min with the eluent analysed for absorbance at a wavelength 217-222 nm. Injection volumes ranged between 10 and 200 μl and the total run time was 10 min.

Wool Samples

Shorn sheep were treated by pour-on or plunge dipping methods. Only the pour-on formulations contained [$^{14}$C]-DM, these having a specific activity of 1.25 MBq/ml and a final concentration of 10 mg DM/ml. The dose rate for the pour-on treatment was 1 ml per 4 kg bodyweight and the dip concentration was 15 μg

DM/ml. Wool samples were collected over 98 days from various sites around the fleece.

Extraction Procedure

Wool samples (1-5 g) were weighed into 25 or 50 ml glass stoppered Erlenmeyer flasks and ACN added at 10 ml/g wool. The mixture was sonicated for 5 min, the flasks covered with aluminium foil to prevent exposure to light and stirred intermittently overnight. The samples were again sonicated for 3 min, and then stored at 4°C.

Quantitation

Standard curves were prepared by spiking an ACN extract of untreated wool with 0.5-100 μg DM/ml. DM content in unknown wool samples was calculated by comparing peak area with that of the standard curve.

Sample clean-up

ACN extracts of wool were subjected to one of the following three alternative clean-up procedures:

1. Florisil clean-up: A Florisil Sep-Pak was conditioned with 25 ml ACN, then 1 ml ACN extract containing DM was passed through the Sep-Pak into an HPLC vial, and followed by elution with a further 3 ml ACN (final volume 4 ml).
2. Bond Elut: A C18 Bond Elut column was conditioned by successive washes with 5 ml ACN, 3 ml water then 3 ml 50% ACN/water. One ml of ACN extract containing DM was diluted with 1 ml water and applied to the column which was then washed with 3 ml water followed by 3 ml 50% ACN/water. These fractions were discarded and DM was then eluted with 4 ml ACN into an HPLC vial.
3. Maxi-Clean cartridges: Since these cartridges require more pressure than Sep-Pak or Bond-Elut columns for solvent elution a vacuum manifold was

used. A cartridge was conditioned with 10 ml ACN, 5 ml water and then 5 ml 50% ACN/water. One ml ACN extract containing DM was diluted with 1.0 ml water and applied to the cartridge, followed by a 5 ml water and 5 ml 50% ACN/water wash. These fractions were discarded and DM was then eluted with 4 ml ACN into an HPLC vial.

The collected ACN eluents were then analysed by HPLC for DM concentration.

The efficiency of ACN extraction of DM

The efficiency was determined using wool samples containing 1-1000 μg DM/g which were obtained from sheep previously treated with [$^{14}$C]-DM. After extraction with ACN, the wool was removed from the flask, the remaining solvent removed under vacuum and the wool blotted to dryness and placed in a scintillation vial. Petroleum spirit (10 ml) was added and the vial sonicated for 5 min and then allowed to stand in the dark overnight. Ten ml OCS scintillant was added and residual [$^{14}$C] content determined.

Recovery of DM from solid-phase extraction

For each of the alternative solid-phase clean-up procedures the recovery of [$^{14}$C]-DM in the ACN eluent was determined for a range of wool samples containing 1-1000 μg/g. After the final 4 ml elution the cartridges or columns were washed with 3 x 5 ml volumes of ACN and each washing was examined for [$^{14}$C] content. The solid-phase adsorbent material was also removed and assayed for [$^{14}$C] content.

Verification of HPLC Results

ACN extracts of wool obtained after pour-on treatment of sheep were analysed by both HPLC and liquid scintillation spectrometry. Aliquots (2-5 ml) of the of ACN extract of wool were added to scintillation vials followed by 10 ml OCS and allowed to stand in the dark for at least 2 hours to eliminate

TABLE 1

Typical Extraction Recoveries of DM from Wool Treated with [$^{14}$C]-Pour-on Formulation

| Wool Sample | % Recovery |
|---|---|
| 1 | 99.0 |
| 2 | 98.3 |
| 3 | 98.7 |
| 4 | 99.3 |
| 5 | 98.9 |
| 6 | 99.6 |
| 7 | 98.5 |
| 8 | 98.0 |
| 9 | 99.7 |
| 10 | 98.6 |
| mean ± SD = | 98.9 ± 0.5 |



luminescence before determination of [$^{14}$C] content. The results obtained were compared with those determined by HPLC using analysis of variance.

## RESULTS AND DISCUSSION

Based on total [$^{14}$C] in wool samples, the recovery of DM from either clean or dirty wool samples using ACN was > 98% (n=100) (Table 1) although total recovery was probably closer to 100% since it was extremely difficult to remove all the extractant from the wool fibre. Recovery of [$^{14}$C]-DM in the fractions used for HPLC analysis was 100% from Florisil Sep-Pak while that from Bond Elut and Maxi-Clean was 96.4 and 96.0 respectively (Table 2). Complete recovery of DM from Florisil was probably due to its relatively high lipophilicity which facilitated

TABLE 2

Recovery of DM from Solid-Phase Extraction of Wool Treated with [$^{14}$C]-Pour-on Formulation

| ACN Elution Volume (ml) | 3 | 4 | 9 |
|---|---|---|---|
| | % recovery | | |
| Florisil Sep-pak | > 95 | 100 | 100 |
| Bond Elut | > 80 | 96.4 | 96.4 - 96.6 |
| Maxi-Clean | > 80 | 96.0 | 96.0 - 96.2 |



partioning into ACN, hence no [$^{14}$C]-DM remained on the solid-phase after elution.

HPLC separation of the ACN extract obtained from the Florisil eluent contained less contaminating peaks than that obtained from samples injected directly without clean up or those filtered through Nylon 66 filter (Fig. 1). With clean wool repeated use (up to 8 times) of a Florisil Sep-Pak was possible as no interfering peaks were observed. Florisil clean-up was suitable for most samples except those from particularly soiled wool or those containing less than 2 μg DM/g wool where large injection volumes were required. In these cases the relatively slower fractionation by Bond Elut columns was used. This provided separations with fewer interfering peaks and cleaner chromatograms than those obtained with Florisil (Fig. 2).

When wool samples from a related plunge dipping experiment were analysed by HPLC following clean-up with Florisil or Bond Elut, contaminating peaks co-eluted with the DM peak. When the dip formulation containing no DM was

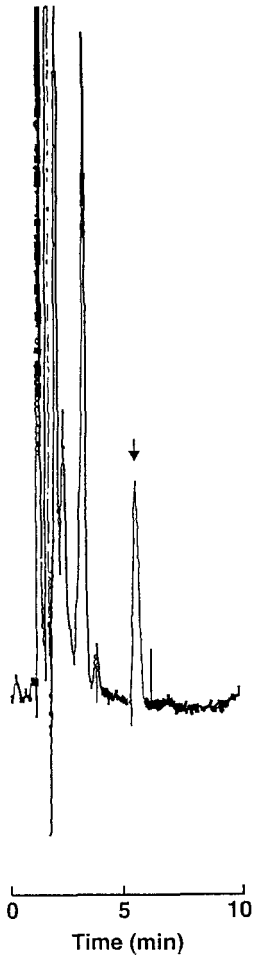


FIGURE 1. Typical elution of deltamethrin from wool extracts after pour-on treatment and Florisil clean-up. Mobile phase 86% acetonitrile in water, flow rate 2 ml/min, detection 217 nm. Injection volume 100 $\mu$l containing 9 ng deltamethrin (indicated by arrow).

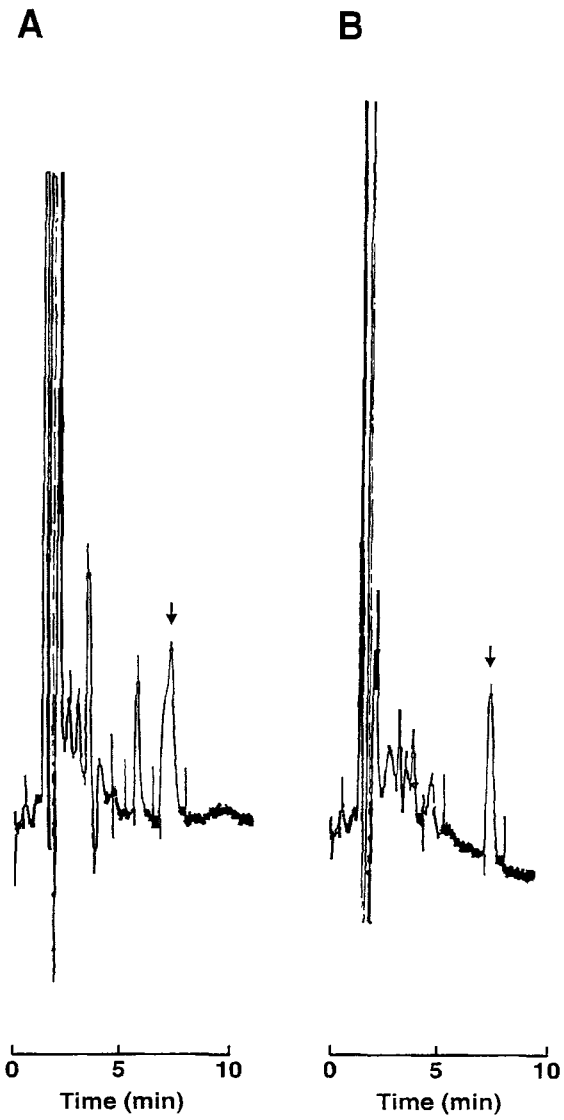


FIGURE 2. Elution of deltamethrin from soiled wool extracts after pour-on treatment and clean-up with Florisil (A) and Bond Elut (B). Mobile phase 87% acetonitrile in water, flow rate 1.5 ml/min, detection 217 nm. Injection volume 100 $\mu$l containing 9.5 ng deltamethrin.

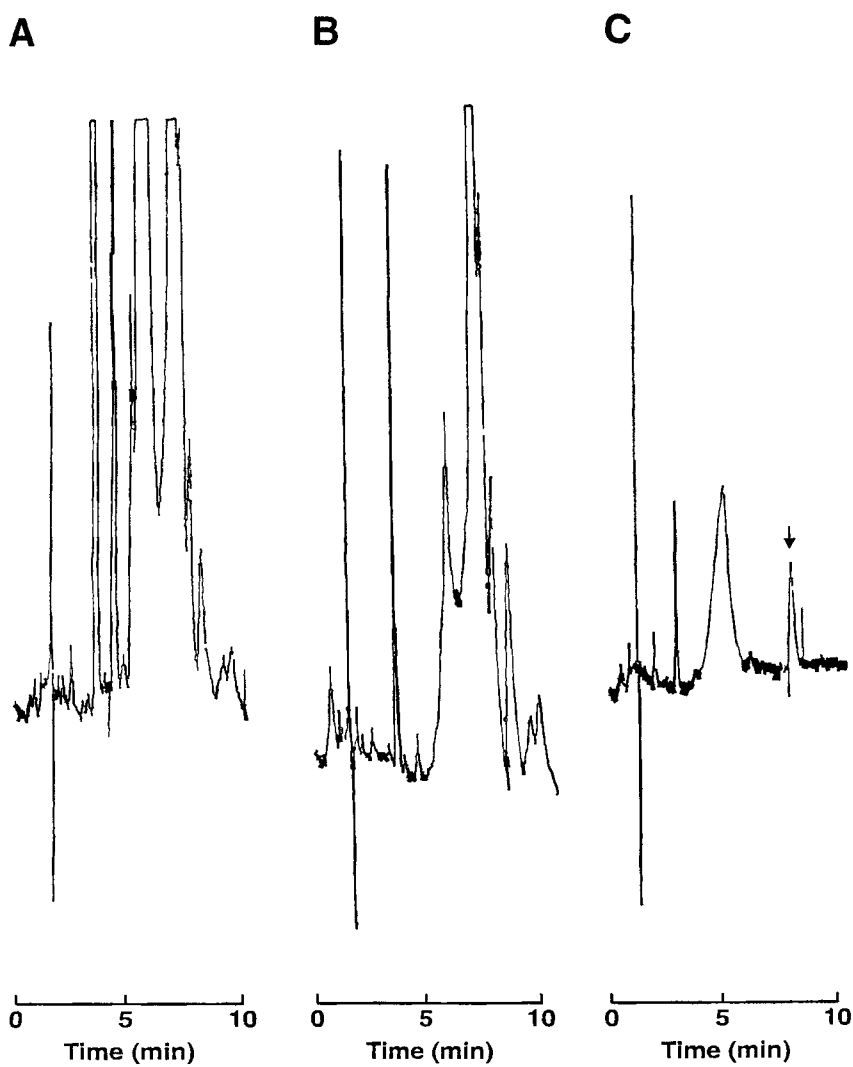


FIGURE 3. Elution of deltamethrin from wool extracts after plunge dipping and clean-up with Florisil (A), Bond Elut (B) and Maxi-Clean (C) chromatographic conditions as for Figure 2. Injection volume 10 $\mu$l containing 5.3 ng deltamethrin.

TABLE 3

Quantitation of Spiked Wool Extract by HPLC after Florisil or Bond Elut Clean-up

| Spike Level µg/ml | Recovered µg/ml | % Error |
|---|---|---|
| 98 | 103 | +5.1 |
| 69 | 70 | +1.4 |
| 37 | 36 | -2.7 |
| 28.2 | 28.8 | +2.1 |
| 14.2 | 14.3 | +0.7 |
| 7.1 | 7.2 | +1.4 |
| 3.4 | 3.5 | +2.9 |
| 3.0 | 3.0 | 0 |
| 1.36 | 1.35 | -0.7 |
| 0.70 | 0.71 | +1.4 |
| | | Mean: +1.2 |



similarly examined the same interfering peaks were present. In this case Maxi-Clean cartridges minimised interference and provided clear chromatograms (Fig. 3). Regardless of the clean-up method used, occasional ACN washing of the HPLC column (after every 20-40 injections) ensured little increase in back pressure and minimal column deterioration. Standardisation used in this study for DM quantitation gave linear and reproducible curves and the correlation co-efficient exceeded 0.99. The amount of DM added to wool samples before extraction and those calculated from standard curves over a wide range were in good agreement (Table 3). For wool containing DM outside the range of the standard curve the ACN extract was diluted accordingly. Final calculations of DM content in wool were made by incorporating the dilution factors and the weight of wool samples extracted. The detection limit was 0.2 µg DM/g wool.

TABLE 4

Comparison of [$^{14}$C] and HPLC Quantition of DM from Wool Treated with [$^{14}$C] Pour-on Formulation

| Wool Sample | [$^{14}$C] | HPLC after Florisil or Bond Elut Clean-up | % Error |
|---|---|---|---|
| | μg DM/g wool | | |
| 1 | 395 | 410 | +3.8 |
| 2 | 224 | 221 | -1.3 |
| 3 | 136 | 139 | +2.2 |
| 4 | 64 | 63 | -1.6 |
| 5 | 27 | 28 | +3.7 |
| 6 | 17.9 | 17.8 | -0.6 |
| 7 | 7.8 | 7.5 | -3.8 |
| 8 | 1.9 | 2.0 | +5.2 |
| 9 | 1.6 | 1.6 | 0.0 |
| 10 | 0.8 | 0.7 | -12.5 |
| | | | Mean: -0.5 |



Following pour-on application of [$^{14}$C]-DM, the concentrations of DM determined by [$^{14}$C] count and HPLC after Florisil or Bond Elut clean-up were in close agreement (Table 4). Analysis of variance of these results demonstrated no significant differences between the two methods for over 600 samples examined. The good correlation and reproducibility of the standard curves and the close agreement of the results obtained with [$^{14}$C] and HPLC quantitation negated the need for internal standards.

In this study ACN extraction provided significant time savings over the use of more polar solvents such as hexane since the latter often require subsequent

liquid-liquid partioning and solid-phase clean-up especially when reversed-phase HPLC is used (4, 6). The present method provides simple and rapid procedures for the analysis of DM from clean and contaminated wool. The use of this assay in describing the distribution kinetics of topically applied DM and other related SP compounds will be detailed in subsequent reports.

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