SHORT PAPERS

Determination of Methoprene in Poultry Manure by High-performance Liquid Chromatography

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Residues of methoprene [isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] were determined in poultry manure at levels of 0.05, 1, 5 and 10 mg kg^{-1}. Samples were extracted with acetonitrile and the extracts cleaned up by liquid - liquid partition and elution through a Florisil column. The mean recovery from spiked samples was 96.5% (range 91–104%). The lower limit of determination was 0.05 mg kg^{-1}.

Keywords: *High-performance liquid chromatography; methoprene; insect growth regulator; poultry manure*

Methoprene [isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] is a synthetic insect growth regulator that mimics the physiological actions of insect juvenile hormone.[1] Current studies include the use of methoprene as a feed-through larvicide to prevent flies developing in poultry manure. A number of analytical methods are available for the determination of insect growth regulators.[2] Measurements of residues of methoprene are difficult because selective detectors available for use in combination with gas - liquid chromatography (GLC) are not appropriate. For low-level residue determination, extensive clean-up is required prior to GLC analysis using flame-ionisation detection (FID). Miller *et al.*[3] reported a GLC - FID method developed specifically for methoprene residues in soil, plants, animal tissue and faeces. High-performance liquid chromatography (HPLC) has been employed to confirm methoprene metabolite structures from insects,[4] and for the clean-up and quantification of methoprene in bovine fat.[5]

It was considered that HPLC could probably be applied to the determination of methoprene residues in poultry manure, and this consideration forms the basis of this work.

Experimental

Reagents

Hexane. Glass-distilled, from Rathburn Chemicals Ltd.

Acetonitrile. HPLC grade, from Rathburn Chemicals Ltd.

Methanol. HPLC grade from Rathburn Chemicals Ltd.

Diethyl ether. Analytical-reagent grade, from BDH Chemicals.

Florisil (60–100 *mesh*). From BDH Chemicals. De-activated with distilled water (95 + 5, *m/m*) and allowed to equilibrate for 3 h before use.

Sodium chloride. Analytical-reagent grade from BDH Chemicals.

Anhydrous sodium sulphate. Analytical-reagent grade from BDH Chemicals.

Mobile phase for HPLC, methanol - water (90 + 10, *V/V*). De-gassed before use by purging with helium.

Elution solvent for clean-up column, hexane - diethyl ether (95 + 5, *V/V*).

Apparatus

The following apparatus was used: a Silverson laboratory blender; a Buchi rotary evaporator; an MSE GF6 centrifuge; and a Pye Unicam SP8-100 spectrophotometer. For HPLC a Waters Associates system was used consisting of the following: Model 6000 and M45 pumps, Model 660 solvent programmer, U6K septumless injector and a Model 450 variable-wavelength detector. A 30 cm × 3.9 mm i.d. μBondapak C_{18} reversed-phase column was used under the following operating conditions: mobile phase flow-rate, 2 ml min^{-1}; detector range, 0.02 absorbance unit full-scale deflection (a.u.f.s.); wavelength, 267 nm; and injection volume, 25 μl.

UV Spectrophotometric Analysis

The UV spectrum (220–400 nm) of methoprene was determined in methanol. From the spectral characteristics the absorption maximum ($\lambda_{max.}$) and molar absorptivity (ε) at the $\lambda_{max.}$ were obtained.

Sample Spiking

Fresh samples of poultry manure were collected and stored in glass jars at −10 °C until required. Spiked samples were prepared by adding an appropriate amount of a hexane solution of methoprene to 30 g of thawed manure to give levels of 0.05, 1, 5 or 10 mg kg^{-1}. The solvent was removed under a stream of nitrogen for 5 min.

Procedure

Extraction

Transfer 30 g of manure into a 250-ml centrifuge bottle. Add 100 ml of acetonitrile and homogenise the mixture at high speed for 2 min, using a laboratory blender. Centrifuge the homogenate at 2500 rev min^{-1} for 10 min and then decant the supernatant liquid into a 2000-ml separating funnel. Add a further 100 ml of acetonitrile to the centrifuge bottle and homogenise for 2 min. Centrifuge for 10 min and decant the supernatant liquid into the separating funnel.

Liquid - liquid partition

Add 300 ml of hexane to the combined acetonitrile extract in the separating funnel and shake vigorously for 1 min. Add 50 g of sodium chloride followed by 1500 ml of distilled water. Shake the mixture carefully for 2 min and then allow the layers to separate. Run off the aqueous layer and discard. Run off the hexane layer and dry over 10 g of anhydrous sodium sulphate for 10 min. Filter the hexane extract into a 500-ml round-bottomed flask and rinse the sodium sulphate with three 10-ml portions of hexane. Reduce the combined extract to approximately 25 ml using a rotary evaporator, the water-bath temperature being maintained at 45 °C.

Table 1. Recoveries of methoprene from poultry manure. Four determinations were carried out at each level

Methoprene added/mg kg^{-1}	Methoprene found/mg kg^{-1}	Recovery, %
0.05	0.052	104
1	0.91	91
5	4.9	98
10	9.3	93
		Mean: 96.5

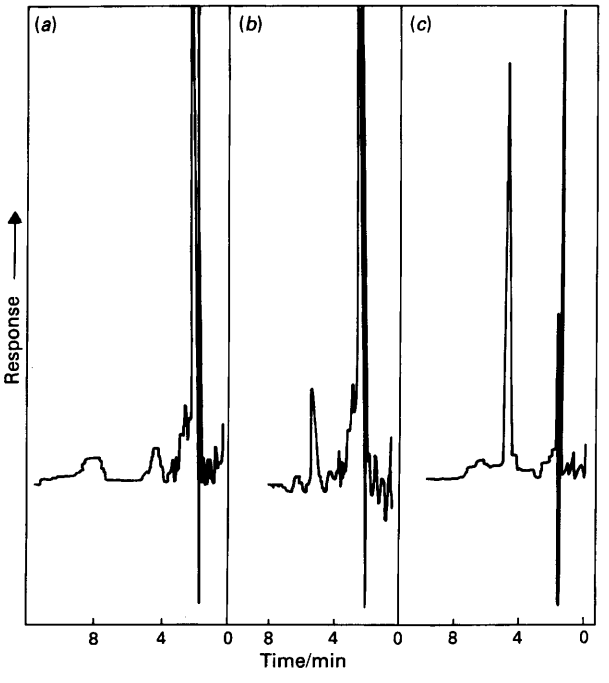

Fig. 1. Chromatograms for (*a*) control manure; (*b*) manure spiked at 0.05 mg kg^{-1}; and (*c*) standard methoprene, 1.0 μg ml^{-1}. (*a*) and (*b*) are in a final volume of 5 ml of methanol

Column chromatographic clean-up

Prepare a clean-up column by adding, in order, 5 g of sodium sulphate then 11 g of deactivated Florisil and finally 5 g of sodium sulphate to a glass chromatography column (300 × 25 mm i.d. fitted with a sintered-glass disc and PTFE tap) containing 50 ml of hexane. Allow the hexane to pass through the column until the liquid reaches the sodium sulphate layer.

Transfer the concentrated hexane extract on to the clean-up column. Allow the solution to pass through the column until the liquid reaches the top of the sodium sulphate layer. Rinse the round-bottomed flask with four 1-ml portions of hexane and transfer the washings to the column. Allow the washings to pass on to the column. Elute the column with 200 ml of the elution solvent mixture and discard the first 50 ml. Collect the remainder of the eluate and evaporate to a low volume (*ca.* 1 ml) in a rotary evaporator. Remove the final trace amounts of solvent under a stream of nitrogen at room temperature and dissolve the residue in an appropriate volume of methanol for subsequent HPLC analysis.

Results and Discussion

The UV spectrum of a methanol solution of methoprene (4.516×10^{-5} mol dm^{-3}) in a 1-cm path length silica cell showed a single absorption maximum at 267 nm. The molar absorptivity was calculated as 23×10^3 dm^3 mol^{-1} cm^{-1}.

Recoveries of methoprene from manure spiked at 0.05, 1, 5 or 10 mg kg^{-1} are given in Table 1. The mean recovery for all levels was 96.5% (range 91–104%). Extracts of control manure did not give rise to interfering peaks as all UV absorbing coextractives from the manure eluted before methoprene. Chromatograms obtained from control and spiked manure samples along with standard solutions are shown in Fig. 1. Under the chromatographic conditions described a 25-μl injection of a solution containing 0.5 μg ml^{-1} gave a response of 15% full-scale deflection.

The HPLC method described involves a less extensive clean-up programme than that required for methoprene residue determination by GLC - FID. The limit of determination of the procedure is 0.05 mg kg^{-1} (see Fig. 1 for conditions); however, Miller *et al.*[3] reported that the lower limit of determination for the GLC - FID method was 0.01 mg kg^{-1}.

Where very low residue determinations are not required, HPLC provides a simple, less time-consuming alternative to GLC because of the less extensive clean-up required.

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