Micromethod for Determining Insect Growth Regulator Methoprene in Bovine Fat

A micromethod to determine methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) in 2 g of bovine fat is presented. Samples were cleaned by elution through an acid alumina column and by high-performance liquid chromatography. Quantitation was by high-performance liquid chromatography, and confirmation was by gas–liquid chromatography. The percentage of recovery from fortified samples averaged 88% (range 84–96%). The lower limit of sensitivity is less than 0.008 ppm.

Methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) as a feed additive is effective in the control of larvae of the horn fly, *Haematobia irritans* (L.), and stable fly, *Stomoxys calcitrans* (L.), in the manure of cattle (Harris et al., 1973, 1974). A method to determine residues of methoprene in feces and tissues of cattle quantitated by gas–liquid chromatography (GLC) was presented by Miller et al. (1975). Because of the relatively large (20 g) sample size required by the Miller method, a method was needed in which smaller samples were used in order to determine, in duplicate, the residues of methoprene in fat samples taken from cattle by omentectomy. The study reported here was undertaken to develop an effective micromethod to determine methoprene residues in fat, one offering high sensitivity and requiring a relatively small sample size.

EXPERIMENTAL SECTION

All solvents used were uv grade and were obtained from Burdick and Jackson. Aluminum oxide Woelm acid was obtained from ICN Pharmaceuticals and Celite 545 from Supelco. The aluminum oxide used in the cleanup column was heated 8 h at 650 °C, and the sodium sulfate and Celite were heated 3 h at 600 °C. All three reagents were then stored in a 37 °C oven until further use. The glass cleanup column was 8 mm i.d. × 320 mm and was connected to a 250-ml separatory funnel. A Micro-Tek Model 220 equipped with a flame ionization detector and a 6.35 mm × 180 cm glass column packed with Chromosorb W H/P (100–120 mesh) coated with 3% OV-101 were used for confirmation. Carrier gas was prepurified nitrogen at 55 ml/min. The column was operated at 185 °C, the injector at 240 °C, and the detector at 230 °C.

Extraction of Methoprene from Fat. All samples were run in duplicate. A 2-g sample of fat was weighed into a 50-ml, round-bottomed centrifuge tube. A 20-ml aliquot of acetonitrile was added to the tube and, in order, 10 g of anhydrous sodium sulfate and 1 g of Celite. The mixture was blended at medium speed for 5 min with a Polytron homogenizer. After the residue settled, the supernatant liquid was decanted into a 50-ml centrifuge tube. Another 15-ml aliquot of acetonitrile was added to the residue, and it was blended at high speed for an additional 4 min. The entire contents of the tube were transferred and filtered through Whatman No. 1 filter paper into the 50-ml centrifuge tube. The generator of the homogenizer and the centrifuge tube were washed twice with small aliquots of acetonitrile, and the washings were poured over the residue on the filter paper. After filtration was complete, the extract was chilled to −10 °C and centrifuged at that temperature for 45 min. The extract was decanted into a 50-ml graduated tube, and the centrifuge tube containing the fat was washed twice with small aliquots of chilled acetonitrile. The acetonitrile extract was then evaporated in a 37 °C water bath under a stream of dry filtered nitrogen to a volume of 1 ml.

Cleanup and Quantitation. The concentrated sample was transferred to the cleanup column immediately with two washings of 0.25 ml of acetonitrile. The cleanup column was prepared by adding, in order, a glass wool plug, 3 g of anhydrous sodium sulfate, 8.4 g of acid alumina, and 3 g of sodium sulfate. The column was tapped gently after each addition until no more settling occurred. The column was not prewashed. The methoprene was eluted from the column with 50 ml of hexane at a rate of 1 ml/min for the first 5 min and 3 ml/min for the rest of the elution time. The column was allowed to free flow with the elution solvent for 155 mm before pressure was applied. The sample was evaporated to 0.5 ml in a 37 °C water bath with a jet-dry filtered nitrogen before further purification by high-resolution liquid chromatography (HRLC).

The HRLC preparatory column was a 0.9 × 120 cm Porasil A with a solvent system of hexane–tetrahydrofuran (20:1) and a flow rate of 7.0 ml/min. A Waters ALC 202/401 liquid chromatograph equipped with a UV_{254} detector was used. The 0.5-ml concentrated sample was injected on the preparatory column, and the appropriate part was collected as determined by injecting standard hexane solutions containing methoprene. The methoprene fraction was again evaporated in a water bath to a volume of 0.1 ml, and the entire sample was injected on a 6.35 mm × 30 cm μ Porasil column for quantitation by HRLC. The solvent system was composed of hexane–tetrahydrofuran (99:1), and a flow rate of 3 ml/min was used. Methoprene in the samples of fat was quantitated by comparison of peak heights with known amounts of methoprene. Methoprene fractions were collected from the μ Porasil column for confirmation by GLC.

RESULTS AND DISCUSSION

The efficiency of the method was tested by fortifying duplicate control samples of fat with 95% technical methoprene at levels of 0.008–7.000 ppm. The percentage of recovery averaged 88 (range 84–96%). Minimum background and no interfering peaks made possible a 21% full-scale deflection for 17.5 ng (Figure 1). Residues of methoprene of somewhat less than 0.008 ppm could be quantitated because the detector response is linear.

Samples of fat were taken by omentectomy from cattle offered methoprene in mineral blocks. Duplicate analyses of the methoprene in these samples showed residues ranging from 0.02 to 6.09 ppm. There were no significant differences when methoprene in duplicate samples was compared by the paired differences t test ($t = 0.453$, df = 17; $P = 70.5$).

Other methods for cleanup, partitioning, and solvent extractions were investigated. They were not reproducible at the levels described in this paper.

The procedure reported here offers high sensitivity and requires relatively small sample size, and it could be

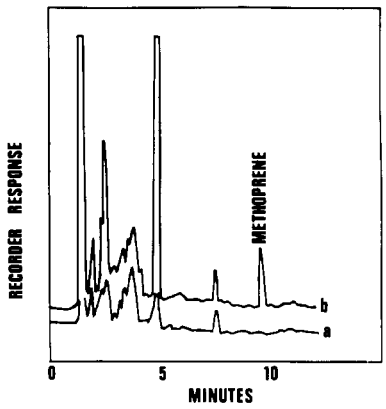

Figure 1. High-performance liquid chromatograms of (a) an extract of bovine fat and (b) an extract of bovine fat containing 0.008 ppm of methoprene.

adaptable to the determination of methoprene in a variety of animal tissues.

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Metabolism and Residues of ^{3}H- and ^{35}S-Labeled Ferbam in Sheep

^{35}S- and ^{3}H-labeled ferbam (Fermate) (ferric dimethyldithiocarbamate) was administered orally to 1 sheep, and only ^{35}S-labeled ferbam was administered to another sheep. By 76 h, 82% of the ^{3}H moiety and 23% of the ^{35}S moiety had been excreted in feces and urine. None of the radiolabeled metabolites co-chromatographed with available metabolic standards, dimethylamine and tetramethylthiuram disulfide. Tissues of slaughtered sheep contained radiolabeled materials.

The dialkyldithiocarbamates are an important class of fungicides. One of the representatives of this group, ferric dimethyldithiocarbamate (ferbam), is often applied to fruit crops, and because dried citrus pulp is used as a supplemental ration for livestock, ferbam residues may potentially be consumed by these animals. The maximum tolerance level for ferbam in citrus is 7.0 ppm. Because of this potential hazard, the authors investigated the excretion and deposition of residues of the sulfur and tritium moieties as well as that of the parent compound when the dithiocarbamate fungicide Fermate was administered orally to sheep.

EXPERIMENTAL SECTION

Chemicals. New England Nuclear Corportion, Boston, Mass., supplied the labeled ferbam and the metabolites, dimethylamine and TMTD (tetramethylthiuram disulfide). The ferric dimethyldithio-^{35}S-carbamate had a specific activity of 36 mCi/mmol, and that of ferric dimethyl-^{3}H-dithiocarbamate was 89.5 mCi/mmol.

Exposure and Sampling. Two Delaine-cross yearling ewes in good condition, each weighing 32 kg after being sheared, were conditioned in metabolism stalls for 7 days. During this pretreatment and the 76-h posttreatment period, the ewes were provided free choice a protein supplement, hegari, and water.

After preconditioning, sheep A was treated orally, via a gelatin capsule, with ferbam-^{35}S at 0.45 mg/kg of body weight, and also with ferbam-^{3}H equivalent to 0.74 mg/kg, or a total dose of 1.19 mg/kg of ferbam. The second ewe (sheep B) was treated orally with 14.5 mg of ferbam-^{35}S, equivalent to 0.45 mg/kg.

Retention catheters allowed separate collection of urine and feces. Blood and urine for each animal were collected at periodic intervals. The blood was heparinized, and the samples of blood and urine were quick-frozen for subsequent analysis. Samples of feces were also collected at intervals, freeze dried, ground in a CRC micro-mill, and frozen for later analysis. The ewes were electrocuted 76 h after treatment, and tissues were collected, ground in a Latapie grinder, and frozen for analysis. Bone was ground in a CRC micro-mill after the marrow had been removed.

TLC of Urine and Feces. Aliquots of whole urine, chloroform extracts of urine, urine after extracts, and chloroform and methanol extracts of feces were spotted on 250-μm silica gel GF thin-layer chromatographic plates and developed 15 cm in a solvent mixture containing benzene–methanol–acetic acid (12:2:1) and then in the second dimension in formamide–acetone (1:5). The plates were dried and placed under Ansco nonscreen x-ray film for 14 days. After the film was developed, appropriate gel regions, as indicated by the exposed film, were scraped and quantitated by liquid scintillation. R_f values of two of the potential ferbam metabolites, dimethylamine and TMTD (tetramethylthiuram disulfide), were determined nonradiometrically, but an analytical standard of the alkyldithiocarbamic acid, another metabolite, was not available for study.

Sample Preparations and Counting. Duplicate 100-mg samples of each tissue, other than blood, were digested for liquid scintillation counting (LSC) as described by Budd et al. (1968). Digestion time in the 130 °C sand bath varied from 3 to 5 h, depending on the tissue.