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A Rapid, Inexpensive, Quantitative Procedure for the Extraction and Analyses of Penncap-M (Methyl Parathion) from Honeybees (*Apis mellifera* L.), Beeswax, and Pollen

A rapid, inexpensive, quantitative procedure for the acetone–*o*-xylene (19:1) extraction of Penncap-M (methyl parathion) from honeybees (*Apis mellifera* L.), beeswax, and pollen is reported. Mean recoveries for six concentrations of methyl parathion were 98.0, 97.8, and 94.2% from honeybees, beeswax, and pollen, respectively. Mean recovery of methyl parathion from honey seeded at five concentrations of Penncap-M was 87.3%.

Penncap-M insecticide is a water-based slurry of microcapsules (30–50-μm cross-linked nylon polymer) containing methyl parathion [*O,O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate)]. Because methyl parathion is sparingly soluble in water, the moist microcapsules provide slow, controlled release of methyl parathion by diffusion through capsule walls, maintaining pesticide levels on crops for much longer than nonencapsulated formulations (Ivy, 1972).

Because Penncap-M microcapsules mimic pollen grains in size, honeybees (*Apis mellifera* L.) are unable to discriminate the microcapsules and pollen and transport contaminated pollen back to the hive (Stoner et al., 1978). Thus, Penncap-M contaminates foraging honeybees in the field, but it also is introduced into the food chain of the colony (Stoner et al., 1979; Burgett and Fisher, 1977). Methyl parathion residues have been found in honeybee combs stored for 14.5 months; introduction of these combs into small colonies resulted in death of adult honeybees (Rhodes et al., 1979).

Currently, there is no quantitative method for analysis of microencapsulated methyl parathion in honeybees, beeswax, or pollen. Rhodes et al. (1979) utilized warm acetonitrile–hexane for extraction of Penncap-M from honey, pollen, and beeswax. Their method involved an extrapolation based on a 30% recovery of methyl parathion. Pennwalt Corp. (Carlson, 1980) utilized the Association of Official Analytical Chemists (1975) method of analysis for nonmicroencapsulated methyl parathion with modifications for analysis of honeybees, pollen, and beeswax. They reported an 80% recovery of methyl parathion. We found Pennwalt's modified AOAC method of analysis time consuming and expensive, and we recovered only 65% methyl parathion. Therefore, we developed a rapid, less expensive method for the quantitative analysis of microencapsulated methyl parathion in honeybees, beeswax, and pollen.

MATERIALS AND METHODS

Solvents and Reagents. Penncap-M, technical grade (907.2 g of methyl parathion/3.8 L), was supplied by Pennwalt Corp., AgChem Division, Fresno, CA. A methyl parathion standard of 99.0% purity was obtained from Chem Service, West Chester, PA. Acetone and hexane, spectrograde, were distilled in glass obtained from Burdick and Jackson, Muskegon, MI; *o*-xylene was purchased from Eastman Organic Chemicals.

Sample Preparation and Extraction. Triplicate samples (2.5 g) of honey bees, beeswax, and pollen were spiked individually with 0.1, 1.0, 5.0, 10.0, or 25.0 ppm of Penncap-M and placed in 250-mL Erlenmeyer flasks. One-hundred milliliters of acetone–*o*-xylene (19:1) was added, and samples were placed in a hot-water bath (100 °C) for 3 min. Fifty milliliters of hexane was added, and the sample was blended with a Polytron equipped with a PT 20ST probe generator (Brinkmann Instruments, Westbury, NY) for 30 s at high speed. The samples were then transferred with two 5-mL acetone washes to 250-mL round-bottom flasks and concentrated to 40 mL on a rotary evaporator at 40 °C. The samples were transferred to 50-mL centrifuge tubes with two 2.5-mL acetone washes, placed in an ice bath (0 °C), and shaken for 10 min (Grussendorf et al., 1970; McLeod and Wales, 1972). The samples were then centrifuged in an International Model NH tabletop centrifuge (International Equipment Co., Neeham Heights, MS) at 10000*g* for 15 min.

The supernatants were decanted into 250-mL round-bottom flasks, concentrated to less than 10 mL on a rotary evaporator at 40 °C, transferred to 10-mL volumetric flasks, and diluted to volume with *o*-xylene.

Gas–Liquid Chromatography. A Hewlett-Packard 5730A chromatograph equipped with a flame photometric detector in the phosphorus mode was used for analysis. Five microliters of sample extract was injected on a 91.44 cm long glass column (6.35-mm i.d.) packed with 10% (w/w) OV-1 on Chromosorb (80–100 mesh) WHP. Analyses were carried out at the following temperatures: column oven, 200 °C; injection port, 250 °C; detector, 200 °C. Flow rates of the gases were as follows: nitrogen carrier, 60 mL/min; hydrogen, 200 mL/min; air, 50 mL/min; oxygen, 20 mL/min. Samples were quantitated

Table I. Recovery of Methyl Parathion by Acetone-o-Xylene (19:1) Extraction from Samples of Honeybees, Beeswax, and Pollen Spiked with Penncap-M

	mean % recovery (N = 3)		
concn, ppm	honeybees	beeswax	pollen
0.1	97.3	95.4	91.7
0.5	95.4	102.1	94.3
1.0	98.8	98.7	93.5
5.0	95.6	98.9	98.6
10.0	99.3	96.7	95.1
25.0	101.3	95.1	91.9
mean	98.0	97.8	94.2

by comparison to an external standard on a Hewlett-Packard 3380A integrator.

RESULTS AND DISCUSSION

The mean percentage recoveries of methyl parathion from triplicate analyses of honeybees, beeswax, and pollen spiked with Penncap-M and measured by the acetone–o-xylene (19:1) extraction procedure are listed in Table I. Mean recoveries for the six concentrations of Penncap-M were 98.0, 97.8, and 94.2% from honeybees, beeswax, and pollen, respectively. Triplicate honey samples were seeded at 0.1, 0.5, 1.0, 5.0, and 10.0 ppm of Penncap-M, and mean methyl parathion recovery utilizing this method was 91.7, 85.6, 87.2, 86.7, and 85.2%, respectively. When this method was used for analyses of honeybees, beeswax, and pollen exposed to Penncap-M in the field, quantitation at 1.0 ppb was achieved (Ross and Harvey, 1981).

This improved method for analysis of methyl parathion residues can be used for quantitating Penncap-M in contaminated honeybees, beeswax, and pollen.

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A Comparison of High-Performance Liquid Chromatography and Proton Nuclear Magnetic Resonance in Determining the Phosphatidylcholine Content in Soy Lecithin

A study was conducted comparing HPLC and ^{1}H NMR as methods for determining the phosphatidylcholine content in soy lecithin. The HPLC method employs detection of the phosphatidylcholine at 210 nm while the ^{1}H NMR utilizes the resonance of the choline at δ 3.3. Both methods gave similar precision data with good correlation between methods.

Lecithin is a generic name for an emulsifier and surface active agent derived from many sources. The highest lecithin content is in egg yolk with 8–10% generic lecithin while soybean oil contains ~2.5% generic lecithin. Soy lecithin consists of three major phosphatides (Minifie, 1980): ~20% phosphatidylcholine, 20% phosphatidylethanolamine, and ~20% phosphatidylinositol.

The analysis of lecithin has usually been performed by the method of acetone insolubles (Horwitz, 1975).

This paper reports the comparative analysis of one of the phosphatides in soy lecithin using HPLC and ^{1}H NMR.

EXPERIMENTAL PROCEDURES

HPLC Analysis. The HPLC conditions were those specified in earlier studies (Hurst and Martin, 1980).

^{1}H NMR Analysis. The ^{1}H NMR consisted of a Varian T-60 NMR. Fifty milligrams of sample or standard was dissolved in 500 μL of acetic acid-d_4. Figures 1 and 2 show the ^{1}H NMR spectra of phosphatidylcholine standard and lecithin extract. The peak at δ 3.3 is the phosphatidylcholine peak, and the integration of the standard vs. in-

Table I. Percent Phosphatidylcholine in Different Lecithin Lots[a]

lot no.	HPLC	^{1}H NMR
1	27.88	25.84
2	24.70	23.03
3	23.91	22.47
4	27.31	24.72
5	26.43	24.16
6	28.67	25.84
$\bar{x}$	26.49	24.34
Cv	6.9	6.7

[a] That is, same type but differing lot numbers.

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