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Note

The liquid chromatographic assay of permethrin

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Permethrin (FMC 33297 = NRDC 143), 3-phenoxybenzyl (±)-*cis*, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, is a synthetic pyrethroid developed by Elliott *et al.*[1]. This compound has exhibited high light stability and has been shown to be an effective insecticide against several major insect species[2–7]. Elliott *et al.* have also published an extensive study of the metabolism of permethrin in rats[8] which indicated that the parent compound and its metabolites are not extensively retained in the organs of the test animals. These features make this compound a potentially safe, general purpose insecticide.

Analysis of this compound has been usually carried out by gas chromatography (GC)[4,7,9–11]. GC is quite suitable for the analysis of this compound if certain procedural precautions are taken. These precautions generally center upon the conditioning of the instrument to be used for analysis. The most ideal situation when performing the GC analysis of this compound is to have a dedicated instrument. We have found that conditioning for constant response may take 10–100 injections. If this is not done, the drift in analytical error can be rather slow and subtle, causing highly erroneous results which may not be quickly recognized by the analyst who only occasionally works with this compound. Having a dedicated instrument does eliminate this problem, but this is often impractical. Recently Lam and Grushka have described the analysis of permethrin by high-performance liquid chromatography (HPLC) using a silver-loaded aluminosilicate column[12]. This rapid elution method holds great promise for the rapid analysis of technical permethrin though we speculate interference will probably be encountered with formulated or residue samples due to early eluting sample components.

For the routine analysis of this compound we have developed two HPLC methods which can be quickly initiated to give reliable precise and accurate results. These methods are viable alternatives to GC and are reported in this paper.

EXPERIMENTAL

Two different LC systems were employed in this study. Reversed-phase studies were carried out on an instrument comprised of components obtained from Waters

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Assoc. (Milford, Mass., U.S.A.). The pumping system consisted of two 6000A pumps controlled by a Model 660 solvent programmer. Injections onto a Partisil-ODS column (Whatman), 25 cm × 4.6 mm I.D., were made via a model U6K sample injection valve. The column was jacketed and temperature was controlled to 40° using a system described elsewhere[13]. A Model 440 absorbance detector capable of monitoring 254 nm and 280 nm simultaneously was employed. The mobile phase consisted of 65% methanol (Burdick & Jackson, Muskegon, Mich., U.S.A.) and 35% distilled deionized water. Data were collected on an Omniscribe dual-pen recorder (10 mV full scale, chart speed 0.5 cm/min).

Normal-phase studies were carried out on a constant-pressure liquid chromatograph. The pumping system consisted of a 160-ft. coil of stainless-steel tubing filled with solvent (*ca.* 1.2 l) driven by constant nitrogen pressure. Injections were made via a Rheodyne (Berkeley, Calif., U.S.A.) Model 7120 sample injection valve onto a Brownlee Labs. Si-5A silica gel column 25 cm × 4.6 mm I.D. A Gilson Holochrome variable-wavelength detector set to 280 nm or 220 nm and equipped with a 32-μl flow-through cell was used. The mobile phase consisted of 99.9% *n*-heptane and 0.1% acetonitrile (both from Burdick & Jackson). In this case analysis is carried out under ambient conditions. Data were collected on a Heath Schlumberger Model 204 recorder set for 10 mV full scale using a chart speed of 1 in./min or an LDC Model 3402 dual-pen recorder set for 10 mV full scale using a chart speed of 30 mm/min.

GC was carried out on a Hewlett-Packard Model 7620 gas chromatograph equipped with a thermal conductivity detector operating at 200 mA. The column used was 24 × 1/8 in. O.D. packed with 20% SE-30 on Anakrom ABS (110–120 mesh).

RESULTS AND DISCUSSION

Using the reversed-phase system, the *trans* isomer eluted before the *cis* isomer, as shown by a typical chromatogram (Fig. 1). A complete analysis required 24 min using the specified conditions. By proper calibration with a standard, the *cis–trans*

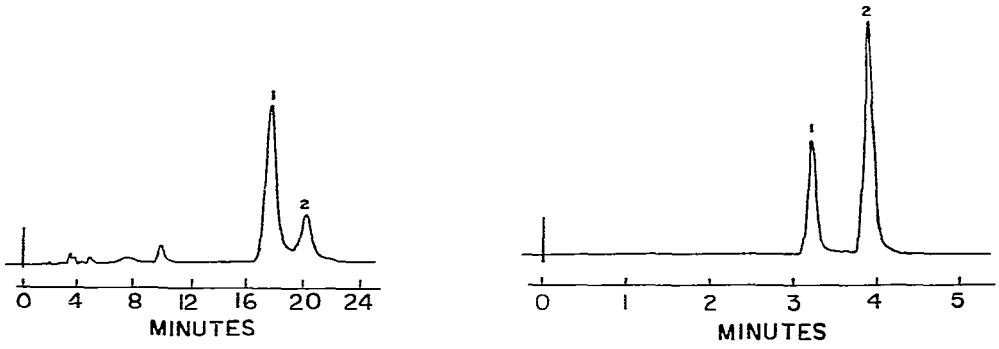

Fig. 1. Sample, 1.5 μg permethrin in 10 μl of methanol; column, Partisil-ODS, 25 cm × 4.6 mm I.D.; mobile phase, methanol–water (65:35); temperature, 40°; pressure, 600 p.s.i.; flow-rate, 1 ml/min; detector, 254 nm at 0.05 a.u.f.s.; chart speed, 0.5 cm/min. 1 = *trans*; 2 = *cis*.

Fig. 2. Sample, 21.9 μg permethrin in 19.5 μl of *n*-heptane; column, Brownlee Si-5A, 25 cm × 4.6 mm I.D.; mobile phase, 99.9% *n*-heptane + 0.1% acetonitrile, temperature, 28°; pressure, 1,120 p.s.i.; flow-rate, 1.6 ml/min; detector, 280 nm at 0.5 a.u.f.s.; chart speed, 30 mm/min. 1 = *Cis*; 2 = *trans*.

ratio of each sample could be obtained easily. As expected, polar impurities tended to elute early.

Normal-phase chromatography produced contrary effects; the *cis* isomer eluted before the *trans* isomer and the analysis required only 5 min under specified conditions (Fig. 2). The most critical factor in this system was the heptane–acetonitrile ratio. If too much acetonitrile was used, poor resolution of the isomers resulted. On the other hand, if too much heptane was present, analysis time was extended unnecessarily[14]. Again, using this system, the *cis–trans* isomer ratio could be calculated.

Both systems have been successfully used to analyze technical and formulated materials. By applying proper clean-up procedures[9,11], their usefulness can be extended to include extremely low-level residue studies. Normally we use either 254 nm or 280 nm to monitor assays of technical material or formulated goods. For trace analysis we recommend that 220 nm be used since this increases sensitivity by more than an order of magnitude.

Currently a study is in progress to determine the deposition pattern and the persistence of various permethrin formulations on cotton. Using the normal-phase system with the detector operating at 220 nm, we have been able to detect as little

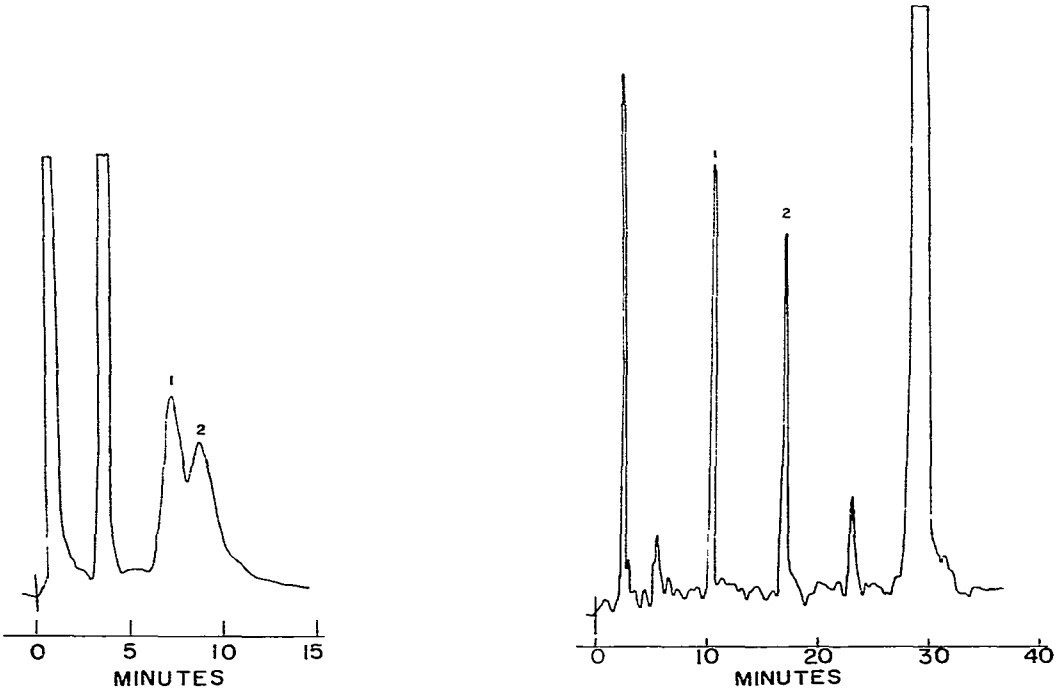

Fig. 3. GC scan of permethrin oil-based formulation. 1 = Oil interference; 2 = permethrin (2 μg). Instrument, HP 7620; column, 20% SE-30 on Anakrom ABS, 24 × 1/8 in. O.D.; carrier gas, helium; inlet pressure, 80 p.s.i.; flow-rate, 20 ml/min; oven temperature, 220°; inlet temperature, 300°; detector (thermal conductivity bridge at 200 mA) temperature, 300°.

Fig. 4. HPLC scan of permethrin oil-based formulation extracted from a cotton leaf 2 weeks after application. 1 = *Cis*; 2 = *trans*. Total permethrin, 0.07 μg corresponding to 2.8 ppm (w/w) on the leaf; column, Brownlee Si-5A, 25 cm × 4.6 mm I.D.; mobile phase, 99.9% *n*-heptane + 0.1% acetonitrile; temperature, 25°; pressure, 1,120 p.s.i.; flow-rate, 1.5 ml/min; Detector, Gilson Holochrome at 220 nm 0.05 a.u.f.s. 200 μl of a 2-ml heptane extract were injected via a Rheodyne model 7120 injection valve equipped with a 200-μl loop.

as 40 ppb (w/w) deposition on cotton leaves before any significant interference from plant extracts is encountered. This result was obtained without any prior clean-up of the heptane extract from a cotton leaf. Since sensitivity is limited by interference from the leaf, we estimate that by employing known sample clean-up procedures[9,11] we could extend the limit of detection by another order of magnitude. It should be emphasized that at the present time detection limits in the ppb range using GC are not attainable unless an extensive sample clean-up is performed.

The following two figures emphasize the difference between GC and HPLC methods. Fig. 3 shows a typical GC analysis of an oil-based permethrin formulation. Sample clean-up or system reoptimization would be required for acceptable results. Fig. 4 shows the liquid phase chromatogram of a cotton leaf extract directly injected into the liquid chromatograph. Even at the 2.8-ppm level, well resolved peaks are obtained for permethrin. The leaf was treated with the oil-based formulation prior to extraction. Fig. 4 then combines the chromatogram of the oil base, permethrin and the cotton leaf extract materials. In general, the oil base gives rise to the early eluting impurities pictured. The elution of such early impurities may cause problems with the silver-loaded aluminosilicate column[12] discussed before. The low-level baseline noise and the late eluting peaks are attributable to the cotton leaf. The baseline noise of course determines the limits of detection prior to any clean-up steps. It should be noted that the retention time increase in Fig. 4 for permethrin, as compared to Fig. 2, is due to a minor decrease in acetonitrile content in the system of Fig. 4, although both nominally contain 0.1% acetonitrile.

CONCLUSION

We have found HPLC to be operationally easier than GC methods previously employed for the analysis of permethrin. Assays can be performed quickly without any significant interference encountered with technical, formulated and residue level samples. The short and long term precision and accuracy of these HPLC methods distinguishes them from GC methods previously used and makes the use of HPLC particularly attractive.

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