Automatic Spotting of Pesticide Extracts on Thin-Layer Plates

by MORTON BEROZA and MELVIN E. GETZ
Entomology Research Division, Agr. Res. Serv., USDA
Beltsville, Maryland
and
C. W. COLLIER
Plant Pest Control Division, Agr. Res. Serv., USDA
Otis Air Force Base, Massachusetts

The demonstrated translocation of pesticides from soil to crop [e.g. (1,2)] has made it abundantly clear that certain crops should not be planted in soils contaminated with excessive amounts of pesticides. A rapid, simple means of analyzing soil for pesticide content was therefore needed, preferably one that could be conducted with inexpensive equipment by nonchemists with a minimum of training.

Thin-layer chromatography appeared to be suitable. The spot developed by an unknown could be compared with those of standards to determine whether the pesticide content of the soil exceeded the specified level for a particular crop. In pursuing this approach, an automatic spotting apparatus was devised for applying the pesticide extract to the thin-layer plate very slowly, thereby allowing the solvent to be evaporated by an unheated stream of air without excessive spread of the material being spotted. Although about 30 minutes was required for the spotting, the apparatus saved much time because no attention was required of the operator during the spotting period, and 9 extracts or standards could be applied and evaporated simultaneously.

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The apparatus has been used to estimate the amount of chlorinated insecticides in a variety of soils. The use of a simple extraction procedure followed by automatic spotting and chromogenic treatment of the developed plates enabled rapid semi-quantitative analyses in the 0.2- to 1-ppm range with detection down to the 0.05-ppm level.

Experimental

Apparatus. The spotting device is shown in Figure 1 and a cross-section of it in Figure 2. It consists of an air blower (Arthur H. Thomas Cat. No. 4739-X10, speed regulated by a transformer; alternatively dry compressed air may be used) connected successively via a taped-on No. 4 filter-adapter neoprene collar (Will Scientific Cat. No. 13011) to a one-holed rubber stopper and a 0.5-inch-o.d. copper tube in which nine 1/8-inch-o.d., 1-inch-long copper tubes are soldered. The 0.5-inch-o.d. tube is held in a rack made from 5/64-inch flat aluminum stock, and each 1/8-inch-o.d. tube is connected with a short length of rubber tubing to an 1/8-inch-o.d. copper tube that directs the air at the point of spotting. The rack holds a 4- X 8-inch thin-layer sheet or plate in its base and supports vertically at 2-cm intervals 9 glass tubes with male Luer tips. Each Luer holds a 1-inch-long, 30-gauge hypodermic needle (with a 45° tip or grind the sharp edge off). Needles are reinforced with a 7/8-inch length of 22-gauge needlestock held on by epoxy cement. The glass tubes are either barrels of 1-ml tuberculin syringes (1YTS/B Becton-Dickinson, Rutherford, N. J.) or 4-inch lengths of 7-mm o.d. glass tubing

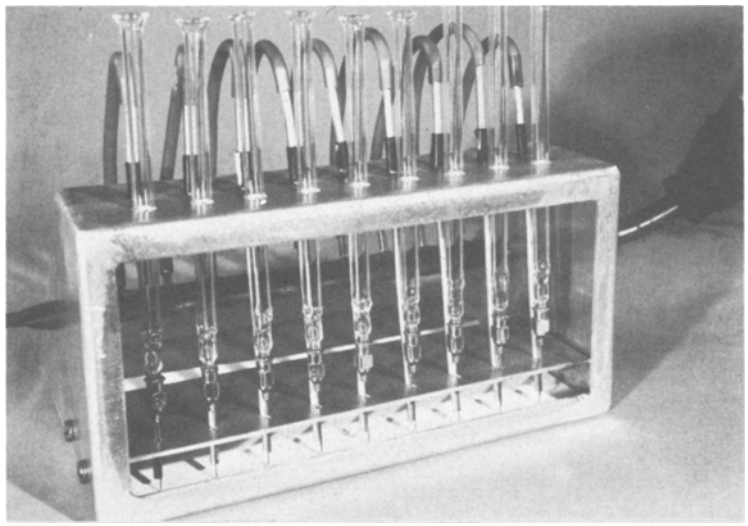

Figure 1. TLC Spotter

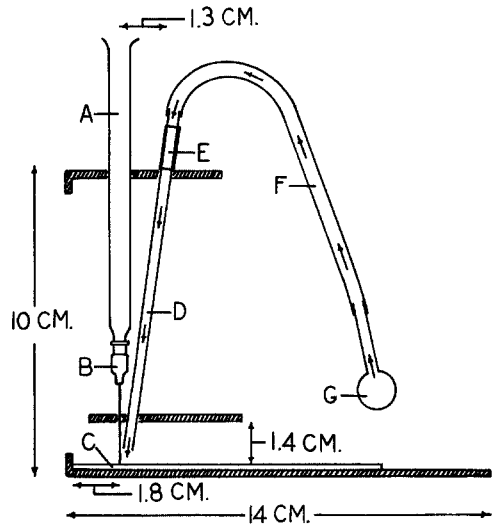

Figure 2. Cross-sectional View of TLC Spotter

A, glass tube that holds solution to be spotted; B, 30-gauge needle; C, TLC plastic plate; D, 1/8-inch-o.d. tube; E, tape holds D at desired height; F, rubber tubing; G, ½-inch-o.d. air inlet tube from air blower. Arrows indicate direction of air flow. Diagram drawn to scale. Dimensions not critical.

sealed to a glass Luer fitting (from adapter 420ST, Becton-Dickinson); the latter tubes have a 2-ml capacity. The point of spotting is fixed by the rack at 1.8 cm from the edge of the thin-layer sheet.

Laboratory shaker. A mechanical shaker such as the Burrell Wrist-Action Shaker (Burrell Corp., Pittsburgh, Pa.) is used.

Thin-layer Sheets and Equipment. Eastman 8- X 8-inch Chromagram® sheets K301R2 (silica gel) were cut in half (4 X 8 inch) and used with the Eastman chamber plate set. (Any thin-layer plates, sheets, and apparatus may be used.) The chromogenic reagent was applied by dipping the plates in a commercially available tank (Arthur H. Thomas 3106-H50) or by spraying.

Ultraviolet lamp. An intense source of ultraviolet light such as the Clean Ray ultraviolet sterilizer manufactured by the Infra Corp., Clarkston, Michigan is used. The lamp has two 15-watt Westinghouse sterilamps G15T8.

Pesticides. Reagent grade chemicals were made up in ethyl acetate to contain 0.05 to 1 μg/ml, as appropriate.

Reagents and Solvents. Ethyl acetate, 2,2,4-trimethylpentane, methylene chloride, and acetone were Burdick and Jackson (Muskegon, Mich.) distilled-from-glass solvents. The Norit SG-extra charcoal is available from R. W. Greef & Co., New York, N. Y.

The chromogenic reagent was that of Kóvacs (3). Add a solution of 0.5 gram of silver nitrate (C.P.) in 25 ml of distilled water to 50 ml of 2-phenoxyethanol (Eastman) and dilute to 1 liter with acetone.

Extraction of Soil. Weigh 25 grams of moist soil into a 125-ml glass-stoppered Erlenmeyer flask. (If soil is dry, add 5 ml of distilled water.) Add 25 ml of ethyl acetate, stopper, swirl to distribute the soil in the solvent, and allow the mixture to stand at room temperature for one hour but preferably overnight. Add 0.2 g of the Norit SG-extra charcoal and shake mechanically for 5 minutes. Filter by gravity through a few grams of anhydrous sodium sulfate on a moderately retentive filter paper (such as 589 S & S paper) into a bottle which is capped tightly after filtration.

Prewash of Thin-layer Sheet and Spotting. Scribe a line $8\frac{1}{2}$ cm from the uncut 8-inch end of the thin-layer sheet and prewash the sheet by allowing methanol to ascend the plate with the scribed line up. Allow the methanol to evaporate at room temperature before use.

Place the thin-layer sheet in the rack with the 8-inch uncut edge flush against the front edge of the rack and position the glass tubes and needles in the rack as shown in Figure 1 with the open side of the needles (marked with a dot) facing 180° away from the air stream. Start the blower, and add 1 or 2 ml of the standards and samples to each of the glass tubes. About 20-30 minutes per ml are required for the tubes to drain. Conduct evaporations in a hood. Push wire through needles after each run to be certain they are fully open.

Development and Visualization of Chromatograms. Develop the chromatograms to the scribed line with a solution 9:1 by volume

of 2,2,4-trimethylpentane:methylene chloride or other appropriate solvent. The scribed line serves as the marker for the solvent front. Remove the sheet and allow it to air dry. Dip the sheet in the chromogenic reagent with the scribed line up and again allow it to air dry. Expose the sheet to high intensity ultraviolet light until the spots are visible. Usually 10 to 15 min are sufficient, but for greater sensitivity expose for 20 to 30 min. Compare spots from soil samples with those of standards to estimate pesticide content.

Discussion

With simple modifications the apparatus will accommodate a wide variety of analyses. The 1-inch-long, 30-gauge needle restricts the flow of ethyl acetate sufficiently so that solvent evaporation limits spot size to about 5 to 6 mm in diameter, which for our purpose was considered satisfactory. Methylene chloride, acetone, and hexane solutions were also spotted with similar satisfactory results. Since spot size may be regulated by changing the air flow, a transformer was interposed to regulate blower speed. The temperature of the blower heating element (not used in this study) may be similarly regulated with a transformer to control spot size. Spot size may also be regulated by selecting the appropriate gauge of needle; such a change may be necessary when switching from one extraction solvent to another. The size of the spot is readily determined by observing the area wetted as the solvent flows from the glass tubes. (The open ends of the needles

are faced away from the air stream to prevent the solute from evaporating on the needle and clogging the opening.)

Plastic electrical tape on the airstream tubes was used to adjust the height of the tubes above the thin-layer plate (about 3/16 inch). The height of the tubes had to be changed if the glass plates were used in place of the thinner plastic thin-layer sheets. Recently, we have improved quantitation by numbering the needles so that they are always replaced in the same position and then adjusting the height of each airstream tube individually to give more uniform and reproducible spot sizes.

The efficiency of the simple procedure of extracting chlorinated insecticides from soils was checked with a variety of soils by gas chromatographic analysis; typical data are given in Table 1.

Table 1. Recoveries of insecticides from ethyl acetate extracts of soil by gas chromatographic analysis

Insecticide	% Recovery at 0.4-ppm fortification level		% Recovery at 0.1-ppm fortification level	
	Solvent contact		Solvent contact	
	1 Hr	Overnight	1 Hr	Overnight
Dieldrin	80	96	105	98
p,p'-TDE	74	100	91	90
o,p'-DDE	75	98	83	101
p,p'-DDT	90	95	90	96
Endrin	92	94	90	98
Heptachlor epoxide	84	99	90	92
Heptachlor	105	101	80	96

The data show that overnight contact with ethyl acetate resulted in good recoveries. (Recoveries from dry soils were low if water was not added.) Overnight contact caused no loss of the analyst's time since samples were set up on the afternoon before analysis. By comparing standards with unknown samples on a thin-layer plate, the amount of pesticide could be estimated to within 25% at the 0.4- to 1.0-ppm level. Thus, ten soil samples were analyzed for dieldrin by TLC, and 3 individuals judged the amounts present vs. standards. In no case was there a disagreement between the amount found by them and by GLC analysis.

Summary

An apparatus has been devised to apply on a thin-layer sheet 9 samples of 1 or 2 ml of a solvent extract at a rate of 1 ml/20 to 30 min, thereby allowing the solvent to be evaporated by an airstream. Spots were 5 to 6 mm in diameter, and no attention of the analyst was required during spotting. The apparatus has been used to analyze for chlorinated hydrocarbons in soils.

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