COMMUNICATIONS

Thin-Layer Chromatography of Bromacil Residues in Soils

A thin-layer chromatographic method for the identification of bromacil residues in agricultural soils is described. Cleanup of a solvent extract of the soil is performed on a Florisil column using acetone as solvent. Thin-layer analysis is carried out on silica gel G and aluminium oxide G with acetone-benzene (1 to 9, v./v.) as developing solvent in both cases. Exposure to ultraviolet irradiation, then spraying with a silver nitrate chromogenic reagent followed by a further period of irradiation is the method of detection. Limit of detection is 0.2 to 0.3 μg. of bromacil.

Pease (1966) and Jolliffe *et al.* (1967) have analyzed for bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) residues in soils and plant materials. The final analysis in each case was carried out using gas-liquid chromatography coupled with a microcoulometric detector (Pease, 1966) or an electron-capture detector (Jolliffe *et al.*, 1967).

It is important in residue analysis to confirm the identity of a compound. A single chromatographic property is usually considered incomplete evidence for identification. The thin-layer chromatographic method described in this paper complements the gas chromatographic method of Jolliffe *et al.* (1967) in the identification of bromacil.

EXPERIMENTAL

Apparatus and Reagents. THIN-LAYER CHROMATOGRAPHIC PLATES. Glass plates (20 × 20 cm.) were spread with silica gel G to a thickness of 0.25 mm. The plates were allowed to air-dry and were then washed using aqueous acetone (50% v./v.). After air-drying, the plates were stored in an atmosphere of 40% relative humidity at 25° C. Aluminum oxide G plates were prepared and treated in a similar manner.

CHROMATOGRAPHIC COLUMNS, 1.6-cm. I.D. incorporating Teflon stopcocks.

BROMACIL, recrystallized bromacil was obtained from E. I. du Pont de Nemours & Co., Wilmington, Del.

FLORISIL, 60- to 100-mesh, 660° C., factory treated (Floridin Co., Tallahassee, Fla.).

CHROMOGENIC REAGENT, silver nitrate reagent prepared according to the method of Mitchell (1958).

Method. THIN-LAYER CHROMATOGRAPHY OF BROMACIL. The sample, dissolved in acetone (distilled), is applied 2 cm. from the base of a plate using a capillary pipet (1, 2, or 5 μl.). A portion of adsorbent 12 cm. from the sample origin is scraped off, and the developing solvent is run to this line using the overrunning technique of Dallas (1965).

The developing solvent is acetone-benzene (1 to 9 v./v.), and the atmosphere of the chamber is kept saturated by lining the walls with filter paper. Development time is approximately 30 minutes.

After development, the plate is allowed to dry in an air draft. It is then exposed to the unfiltered ultraviolet light from a medium pressure mercury arc for 30 minutes. After an even spraying with chromogenic reagent, the plate is further exposed to the ultraviolet radiation (2 minutes for silica gel G, 5 minutes for aluminum oxide G). The spots appear black on a light fawn background.

EXTRACTION FROM SOIL. The method of Jolliffe *et al.* (1967) was followed to the point where the evaporated residue was dissolved in acetone. This solution, when concentrated, was unsuitable for thin-layer chromatography. The plate was easily overloaded if too much of the solution was applied. An additional cleanup step remedied this.

Pack Florisil (10 grams) in a 1.6-cm. diameter chromatographic column. Place a 1-cm. layer of anhydrous sodium sulfate on top of the Florisil. Pour 50 ml. of acetone through the column, not allowing it to go dry.

Transfer the dissolved residue quantitatively to the top of the column with a small volume of acetone. Begin collection of the eluate immediately, discarding the first 5 ml. and retaining the next 50 ml. The eluting solvent is acetone. Evaporate the 50 ml. of acetone to 0.1 ml. in a graduated centrifuge tube, using a stream of dry nitrogen on a steam bath.

ANALYSIS. Apply 5, 10, and 15 μl. from the 0.1-ml. solution to each of the thin-layer plates. Carry out the thin-layer chromatography as described previously.

DISCUSSION

To obtain a sensitivity in the thin-layer method approaching that of the gas chromatographic method, it is necessary to apply a considerable fraction of the final extract to the thin-layer plate. The additional Florisil cleanup allows one to do this without overloading the plate with extractives from the soil. The limit of sensitivity of the method is about 0.03 p.p.m. in soil.

Exposure of the plate to ultraviolet radiation prior to spraying gives a higher sensitivity of detection and lower background color than the normal procedure for chlorinated compounds, which involves spraying with a silver nitrate chromogenic reagent followed by exposure to ultraviolet light. The limit of detectability under the conditions used in the present method is approximately 0.2 μg. for silica gel G and 0.3 μg. for aluminum oxide G.

No attempt was made to obtain reproducible R_f values. Standard quantities of bromacil were applied to each plate.

This gave an R_f comparison and also provided a semiquantitative measurement of the amount of bromacil present in a sample. Approximate R_f values observed were 0.20 on silica gel G and 0.33 on aluminum oxide G. Saturation of the chamber with solvent vapor helped to prevent edge effect, permitting easy comparison of R_f values.

Four kinds of soil, a light brown loamy sand, a gray-brown loam, a yellowish brown sandy clay loam, and a light gray-brown clay were fortified with bromacil at the 0.1- and 1.0-p.p.m. level. The cleanup method proved adequate for the thin-layer chromatography step for each type of soil.

LITERATURE CITED

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The Chromatographic Determination of 4-Trifluoromethyl-2,4′-dinitrodiphenyl Ether Residues in Soybeans

Soybeans are ground to pass a 20-mesh screen and blended with a 1 to 1 mixture of acetone-hexane. The herbicide is partitioned into the hexane portion by the addition of distilled water. The hexane portion is placed on a silica gel column and soybean oil and other interfering compounds are eluted with hexane. The herbicide is eluted with acetone and the concentration determined using electron capture gas chromatography. The method is sensitive to 0.05 p.p.m. with recovery of better than 85%. No residues were found in soybeans treated pre-emergence at herbicidal rates of 2 to 6 pounds per acre.

Four-trifluoromethyl-2,4′-dinitrodiphenyl ether is a herbicide recently introduced by the CIBA Corp. (C-6989). It shows considerable promise for use in agronomic crops, especially soybeans. However, no method has been published for residue determinations. The presence of a CF_3 and two NO_2 groups on the herbicide molecule indicated that it could be readily detected using electron capture gas chromatography.

MATERIALS AND METHODS

Soybean samples were harvested from plots in Alabama, South Carolina, Wisconsin, Kentucky, and Missouri. These plots had been treated pre-emergence with C-6989 at rates of 0, 2, 3, 4, or 6 pounds per acre.

Samples were ground in a Wiley mill to pass a 20-mesh screen. Alcohol, acetone, hexane, benzene, and various combinations of these chemicals were used as extracting solvents. A 1 to 1 mixture of hexane and acetone extracted the herbicide most efficiently. Twenty grams of soybean powder were blended with 50 ml. of the mixture for 5 minutes in a Waring Blendor. After suction filtration, the soybeans were again blended using 50 ml. of the mixture. The blender should be tightly stoppered, as overflow vapors present a possible fire hazard. The filtrates were combined and the herbicide was partitioned into the hexane portion by addition of 50 ml. of water. The water-acetone portion was extracted with a second 50 ml. of hexane. The two hexane extractions were combined and washed with 50 ml. of water.

A silica gel column was prepared by pouring 100- to 200-mesh silica gel into a 1.2-cm. inside diameter buret plugged with glass wool. The column was approximately 30 cm. high and was preconditioned by washing with both hexane and acetone.

The entire hexane extract was poured onto the silica gel column and the column washed with an additional 10 ml. of hexane. The herbicide was retained on the column, but most of the interfering compounds were eluted with the hexane. The herbicide was eluted from the column with acetone and its concentration determined using electron capture gas chromatography. The herbicide was concentrated in the first 10 ml. of acetone eluted.

Samples were analyzed with a Barber-Colman Model 5360 gas chromatograph equipped with a radium-226 electron capture detector. A 6-foot spiral glass column which had an inside diameter of 5 mm. was packed with 10% DC-200 on 100- to 200-mesh Gas-Chrom Q. The temperatures of the detector, column, and injector were 240°, 210°, and 265° C., respectively. The carrier gas was prepurified nitrogen at a flow rate of approximately 90 ml. per minute.

Recovery percentages and herbicide concentrations in field samples were determined by comparing peak heights produced by the samples to peak heights produced by known quantities of herbicide.

RESULTS AND DISCUSSION

The recovery of C-6989 from soybeans is shown in Table I. There was a linear relation between peak height and herbicide content over a range of concentrations from 0 to 0.5 μg. per ml. This corresponds to concentrations

Table I. Recovery of 4-Trifluoromethyl-2,4′-dinitrophenyl Ether from Soybean Seed

Herbicide Added, P.P.M.	Herbicide Found, P.P.M.	% Recovery
0.25	0.220	88.0
0.20	0.172	86.0
0.15	0.127	84.7
0.10	0.092	92.0
0.05	0.048	96.0
0.00	0.000	00.0