Residue Determination of GS 13005, a New Insecticide

D. O. EBERLE, R. G. DELLEY, G. G. SZÉKELY, AND K. H. STAMMBACH

Colorimetry, thin layer chromatography, and electron-capture gas chromatography have been applied for residue determination of GS 13005, a new organophosphorus insecticide, in various crops. Of the methods evaluated, a combination of thin layer chromatography and electron-capture gas chromatography was most versatile and specific. Its sensitivity limit is 0.01 p.p.m. Excellent recoveries were obtained from known amounts of insecticide added to the crop material before extraction.

GS 13005 is the active ingredient of a new organophosphorus insecticide of J. R. Geigy S.A., Basle, Switzerland, registered under the trademark of Supracide or Ultracide, with the structure *O,O*-dimethyl - *S* - [2 - methoxy - 1,3,4 - thiadiazol - 5 - (4*H*)-onyl-(4)-methyl]-dithiophosphate (*4*). Its potent insecticidal activity, toxicological behavior (*3*), and metabolic pathway (*1*, *2*) in animals and plants have been described.

The mode and rate of dissipation of GS 13005 residues depend on the type of crop, temperature, concentration, and other factors. A sensitive and specific method of analysis is required for registration of the compound for use on various crops.

Different analytical procedures have been developed in these laboratories. Because of its extreme sensitivity and high selectivity, a combination of thin layer chromatography and electron-capture gas chromatography was best.

Apparatus

Spectrophotometer. Beckman DU, tungsten lamp, slit width 0.01 to 0.04 mm., 1-cm. glass cuvettes.

Branson Sonifier. Type S 110, 20 kc. per second, 110 watts.

Gas Chromatograph. Aerograph Hi-Fi with a tritium electron-capture detector and a Honeywell-Brown electronic 1-mv. recorder. The column was a 60-cm., 2-mm. glass tube packed with 5% Silicon Oil Dow 11 on Chromosorb W, 60 to 80 mesh. Purified nitrogen was used as the carrier gas at a flow rate of 60 cc. per minute. The column was operated at 160° C. and the injection port at 200° C. The amplifier was set at 1×10^{-9} ampere sensitivity and the detector voltage at 90 volts. Under these conditions, GS 13005 has a retention time of 6 minutes.

Analytical Department, J. R. Geigy S.A., Basle' Switzerland.

Reagents

Petroleum ether, b.p. 50–60° C., reagent grade.

Acetonitrile, reagent grade.

Methylene chloride, reagent grade.

48% hydrobromic acid, reagent grade; add 5 ml. of water to 95 ml. of acid before using.

Zinc acetate reagent: Mix 130 ml. of 1% aqueous zinc acetate with 5 ml. of 12% sodium hydroxide.

Ferric chloride reagent: Dissolve 0.62 gram of $FeCl_3 \cdot 6\ H_2O$ in 9.6 ml. of concentrated hydrochloric acid and make up to 100 ml. with distilled water.

p-Aminodimethylaniline reagent: 0.1 gram of *p*-aminodimethylaniline hydrochloride dissolved in 100 ml. of 1:1 hydrochloric acid.

Sodium hydroxide: 12% aqueous solution.

Silica gel: Silica gel G fluorescent UV 254 mμ (Macherey, Nagel & Co.).

Extraction and Cleanup of Crop Samples

The crops are cut into small pieces and 200 grams are macerated in a mixer with 100 ml. of water. The macerate is rinsed with an additional 100 ml. of water into a 1-liter flask. Four-hundred milliliters of petroleum ether are added and the flask is shaken mechanically for ½ hour. The mixture is stored overnight. The next day, 200 ml. of the petroleum ether extract (equivalent to 100 grams of sample) are decanted through a fluted filter into a 500-ml. separatory funnel. The petroleum ether phase is extracted twice with 20 ml. of acetonitrile and the acetonitrile is transferred to a second 1000-ml. separatory funnel which contains 400 ml. of water. The insecticide in this aqueous solution is extracted with two 20-ml. portions of methylene chloride and the solvent evaporated to dryness on a flash evaporator.

Colorimetric Sulfide Procedure

Procedure and hydrolysis apparatus are similar to those described for Diazinon (*5*).

Hydrolysis. For the colorimetric procedure the residue is transferred quantitatively to a 50-ml. hydrolysis flask with a few milliliters of ethyl ether and the solvent removed by a gentle stream of air. Fifteen milliliters of hydrobromic acid are added to the flask and 7 ml. of zinc acetate reagent are placed in the receiver tube. Nitrogen, washed by bubbling through pyrogallol, is passed through the apparatus for 10 minutes to displace all air in the system, the flow being adjusted to a rate of 40 to 50 bubbles per minute. The acid solution is heated electrically just to boiling and maintained at a reflux rate of 3 to 4 drops per minute from the condenser tip for $1\frac{1}{2}$ to 2 hours. Nitrogen is bubbled through the system for the entire heating period (see Figure 1).

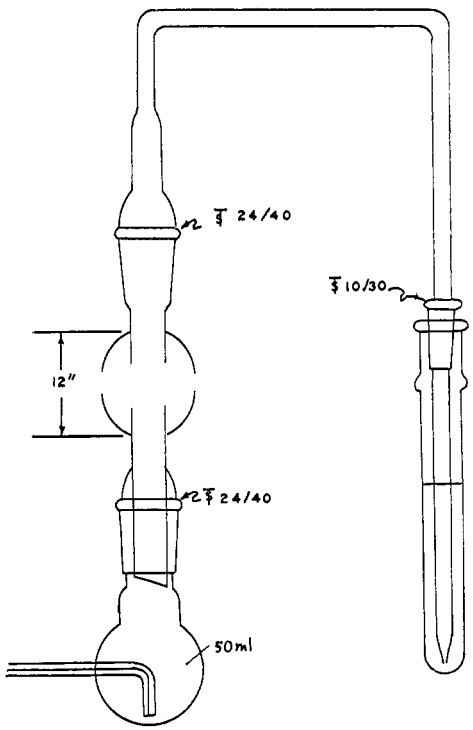

Figure 1. Hydrolysis apparatus

Color Development. To the zinc acetate reagent in the receiver tube are added 1.4 ml. of *p*-aminodimethylaniline and 0.3 ml. of ferric chloride reagent. The solution is stirred well, allowed to stand for 15 minutes, and the volume made to 14 ml. with distilled water. The color is read on a spectrophotometer at 665 mμ in 1-cm. cells against a reagent blank.

The amount of GS 13005 is determined from a standard curve prepared by following all the above steps with fortified crop extracts containing 5 to 500 μg. of GS 13005 per 200 ml. of petroleum ether. Five micrograms of GS 13005 (equivalent to 0.05 p.p.m.) give an absorptivity value of 0.12.

Thin Layer Chromatographic and Gas Chromatographic Procedure

For thin layer chromatographic identification of GS 13005, the residue of the methylene chloride extract is dissolved in 100 μl. of acetone. Five microliters (corresponding to 5 grams of crop sample) are spotted on a 200 × 200 mm. plate together with a 0.05-, 0.1-, 0.2-, 0.5-, 1.5-, and 10-μg. standard of the pure compound and the plate is developed with methylene chloride (unsaturated chamber). After drying, the plate may be sprayed with a 0.1*N* aqueous silver nitrate solution and irradiated for 15 minutes with unfiltered short-wave ultraviolet light. GS 13005 yields a brownish spot at R_f 0.65. The limit of detection is 0.1 μg. (equivalent to 0.02 p.p.m.). The amount of insecticide in a crop sample is determined semiquantitatively by visual comparison with standards. For the gas chromatographic identification the R_f area corresponding to GS 13005 is marked with a pencil and scraped off. The spots are extracted in a small test tube with 0.5 ml. of hot benzene by agitating the mixture by ultrasound (20 kc. per second and 110 watts) for 1 minute. A 10-μl. aliquot of the benzene solution (100-mg. crop) is injected directly into the gas chromatograph.

Determination of Standard Curve and Per Cent Recovery

The standard curve is determined by scraping and extracting the standard spots (0.05 to 10 μg. of pure GS 13005) from the thin layer chromatographic plate as described above and injecting a 10-μl. aliquot of the benzene solution. The area of the insecticide peak, calculated from peak height times half width, is plotted on double logarithmic paper *vs.* nanograms of GS 13005 injected. The standard curve is almost linear from 1 to 200 nanograms. It should be checked occasionally during the day since changes in column conditions, oven temperature, or flow rate will affect the calibration. The comparison with standards of directly gas chromatographed GS 13005 (without prior thin layer chromatography cleanup) shows that the extraction with benzene from silica gel gives a recovery of 45 to 55% only, but if the silica gel–benzene slurry is exposed to ultrasound the recovery can be increased to 85 to 95%.

Results and Discussion

For determination of GS 13005 residues in apples, pears, potatoes, potato vines, cherries, peaches, grapes, prunes, Brussels sprouts, and cotton foliage, the above procedure can be applied as described.

Crops with higher oil content like nuts, olives, and olive oil demand extraction with methanol instead of petroleum ether, followed by an additional cleanup step. The methanol solution has then to be diluted with 300 ml. of water and shaken with 100 ml. of methylene chloride and the extract passed through a Florisil column 1.5 cm. in diameter and 10 cm. in height. The eluate is evaporated to dryness, redissolved in petroleum ether, and partitioned into acetonitrile as described above. Methanol also may be used for extraction of all the above crops if emulsions occur. Only 50% of GS 13005 is extracted from olive oil with methanol.

Of the analytical methods described, the sulfide procedure provides low selectivity and only medium sensi-

Table I. Recovery of Added GS 13005

Crop	Equivalent Sample Weight Injected, Mg.	GS 13005 Added P.p.m.	GS 13005 Added Nanograms	GS 13005 Recovered P.p.m.	GS 13005 Recovered Nanograms	GS 13005 Recovered %
Apples	50	1.0	50	0.84	42	84
	50	0.16	8	0.15	7.4	95
	100	0.08	8	0.06	6	75
	100	0.02	2	0.015	1.5	75
	100	0.0	0	0.0	0	...
Cherries	50	1.0	50	0.92	46	92
	50	0.16	8	0.15	7.6	95
	100	0.08	8	0.088	8.8	110
	100	0.02	2	0.012	1.2	60
	100	0.01	1	0.0065	0.65	65
	100	0.0	0	0.0	0	...
Grapes	50	1.0	50	0.80	40	80
	100	0.10	10	0.065	6.5	65
	100	0.02	2	0.015	1.5	75
	100	0.01	1	0.0055	0.55	55
	100	0.0	0	0.0	0	...
Cotton foliage	25	1.0	25	0.84	21	84
	25	0.5	12.5	0.40	10	80
	25	0.1	2.5	0.08	2	80
	50	0.05	2.5	0.036	1.8	72
	50	0.0	0	0.0	0	...

tivity, but for routine analysis it is more suitable because of its simple instrumental requirements. The sensitivity of the thin layer chromatographic and gas chromatographic method depends appreciably on the kind of crop sample being analyzed and how well extraneous material can be removed. Table I shows recoveries of GS 13005 added to macerated crop samples before extraction.

Figure 2 is a typical thin-layer chromatogram of treated grapes. Two 10-μg. standards, a check sample, and field samples zero, 1, 4, 7, 14, 21, and 28 days after treatment are shown. The strong spot below GS 13005 in this figure cannot be the oxygen analog because untreated check samples show the same spot. With methylene chloride, used for the development of the TLC plate, the oxygen analog stays at the origin of the chromatogram.

For most fruits, 1 nanogram of GS 13005 can be measured quantitatively by gas chromatography in the presence of the equivalent of 100 mg. of crop extract (0.01 p.p.m.). The gas chromatogram of an untreated crop sample, fortified at the 0.01-p.p.m. level, positively shows the peak of GS 13005 in comparison to a check sample (Figure 3).

Cotton foliage extracts contain green interfering material even after the solvent-partition step, so that the amount of sample spotted on the thin layer chromatographic plate must be reduced considerably; the detection limit by visual determination of the spot intensity is 0.5 p.p.m. After the R_f area of GS 13005 has been scraped and extracted, 0.05 p.p.m. is detectable by electron-capture gas chromatography.

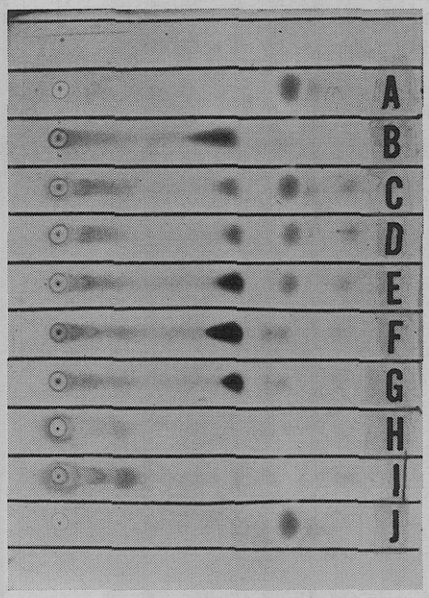

Figure 2. Thin layer chromatogram of grapes treated with GS 13005

A. 10 μg. of GS 13005
B. Blank
C. 0-day sample
D. 1-day sample
E. 4-day sample
F. 7-day sample
G. 14-day sample
H. 21-day sample
I. 28-day sample
J. 10 μg. of GS 13005

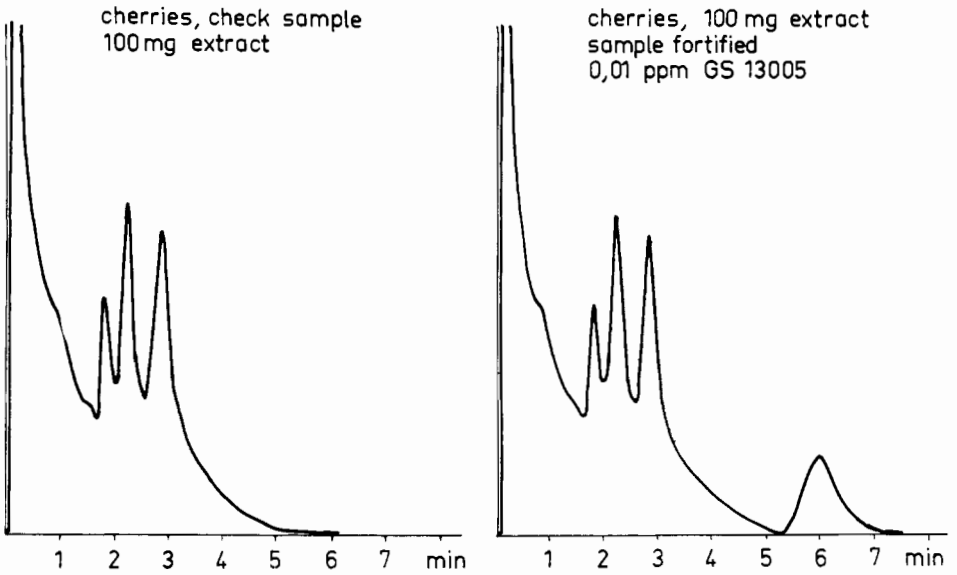

Figure 3. Gas chromatogram of cherry extracts

This example shows the superiority of this combination of two modern analytical methods. Thin layer chromatography provides medium sensitivity and good specificity and it can serve for cleanup, identification, and measurement of the parent compound with most crops being analyzed routinely. The combination with gas chromatography as a second sensitive and selective instrumental method yields a significant increase in versatility, specificity, and sensitivity.

Literature Cited

(1) Bull, D. L., "Metabolism of GS 13005 in Plants and Animals," paper presented at 1965 Entomological Society of America annual meeting, New Orleans.
(2) Esser, H. O., Mueller, P. W., *Experientia*, in press.
(3) Gasser, Rudolf, Grob, Hans, Ruzette, M. E., "Further Investigations with GS 13005 for Use in Orchards and Vineyards," Proc. 3rd Brit. Insecticide and Fungicide Conf., 1965, Brighton, England, pp. 451–77.
(4) Ruefenacht, Kurt (to J. R. Geigy S.A.), Swiss Patents **392,521** (Oct. 15, 1965) and **395,632** (Dec. 31, 1965).
(5) Zweig, G., "Pesticides, Plant Growth Regulators, and Food Additives," Alfred Margot and K. H. Stammbach, Eds., Vol. **II**, pp. 109–24, Academic Press, New York and London, 1964.

Received for review May 19, 1966. Accepted December 7, 1966.