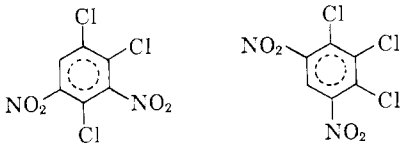
Electron Capture Gas Chromatographic Determination of Residues of Chemagro 2635 in Crops and Soil

J. S. THORNTON and C. A. ANDERSON
Chemagro Corp., Kansas City, Mo.

A gas chromatographic method for the determination of residues of Chemagro 2635 is described. Crop samples are initially extracted with acetone and filtered and the fungicide is partitioned into hexane with the aid of aqueous sodium sulfate solution. An aliquot of the hexane layer is injected into the gas chromatograph and the Chemagro 2635 detected by electron capture. For cottonseed samples, an acetonitrile partition is necessary to eliminate the oil after an initial Soxhlet extraction with Skellysolve B. Recoveries are generally better than 85% at the 0.1-p.p.m. level.

CHEMAGRO 2635, a fungicide, is an isomeric mixture of dinitrotrichlorobenzenes. The commercial product is composed of about 80% 1,2,4-trichloro-3,5-dinitrobenzene (I) and 20% 1,2,3-trichloro-4,6-dinitrobenzene (II). The structural formulas are:



Chemagro 2635 has been tested extensively for the control of soil-borne *Rhizoctonia* spp. and similar pathogenic fungi causing damping off and root and stem rotting.

The compound is a yellow crystalline solid with a melting point of 81° to 86° C. It is insoluble in water, slightly soluble in ethanol, and soluble in acetone, dioxane, and aromatic solvents. The compound is moderately volatile and is stable under normal use conditions.

Inasmuch as the compound contains three chlorine atoms and two nitro groups, it was suspected that it should exhibit a high electron affinity when introduced into a gas chromatographic electron capture detector. Such proved to be the case. The two isomers could be easily separated by gas chromatography and detected with good precision.

For a cleanup procedure, good recovery of Chemagro 2635 from a number of crops and soil was achieved by employing a modification of the procedure of Goodwin, Goulden, and Reynolds (*1*). In this case, the fungicide was extracted by blending with acetone and then partitioned into hexane solution. It was then possible to chromatograph the hexane solution without further cleanup.

The separation of Chemagro 2635 from cottonseed presents special problems because of the large amounts of oil and pigment present. Jones and Riddick (*3*) suggested extraction of a hexane solution with acetonitrile to separate pesticides and related molecules from glycerides. Using this system for Chemagro 2635, adequate recoveries for both isomers were obtained by using four extractions with acetonitrile. Very little interference from pigments was encountered in the gas chromatography by this procedure.

Experimental

Apparatus. Servall Omni - Mixer blender.

Hobart chopper.

Gas chromatograph, F & M Model 700 equipped with pulsed type electron capture detector.

Hamilton 701 N microliter syringe.

Soxhlet extraction assembly, extra-large, 1-liter capacity.

Wiley mill, standard model No. 3, A. H. Thomas Co.

Reagents. Acetonitrile, technical grade.

Acetone, reagent, ACS.

Chemagro 2635, analytical standard (available from Chemagro Corp., Kansas City, Mo.).

Petroleum ether, Skellysolve B, boiling range 60–65° C.

n-Hexane, analytical reagent.

Sodium sulfate solution, 2% in distilled water.

All solvents other than analytical reagents were redistilled in an all-glass apparatus.

Sample Preparation. VEGETABLES AND SOIL. Grind all vegetable samples in a Hobart food chopper with an equal weight of dry ice and mix thoroughly. Place the sample in frozen storage overnight to allow the dry ice to dissipate by sublimation (*2*). This step may be omitted with soils. Weigh a 50-gram sample into a 1-quart blender jar. Blend with 125 ml. of acetone at high speed for 4 minutes. Filter the macerate, under suction, through a pad of glass wool in a No. 2 Büchner funnel, pressing the pad with the bottom of a small beaker to squeeze out any remaining acetone. Rinse the blender jar and filter cake with fresh portions of acetone. Transfer the filtrate to a ground glass–stoppered graduated cylinder and make to 200-ml. total volume and mix. Transfer a 30-ml. aliquot of the acetone filtrate into a 200-ml. volumetric flask containing 10 ml. of *n*-hexane. Make to volume with 2% aqueous sodium sulfate and shake to mix. Analyze as instructed under Chromatography.

COTTONSEED. Grind the entire sample in a Wiley mill and mix thoroughly. Weigh a 50-gram portion of the sample into a large Soxhlet extraction thimble and cover with a plug of glass wool. Extract the sample for 20 hours in a Soxhlet extractor (1-liter capacity) using 800 ml. of Skellysolve B as the solvent. Transfer the extract to a 1000-ml. separatory funnel, add 150 ml. of acetonitrile, and shake vigorously for 1 minute to extract. Allow the phases to separate, draw off the lower (acetonitrile) layer into a 500-ml. separatory funnel containing 300 ml. of fresh Skellysolve B, and shake vigorously for 1 minute to extract. Allow the phases to separate and then draw off the acetonitrile layer into a 400-ml. beaker. Repeat the above acetonitrile extraction steps three times more with 75-, 75-, and 50-ml. portions of acetonitrile. Evaporate the combined acetonitrile extracts just to dryness on a steam bath under an air jet. Transfer the residue from the beaker to a 25-ml. volumetric flask with small portions of *n*-hexane and make to volume. Analyze as instructed under Chromatography.

COTTONSEED OIL. Weigh a 50-gram portion of the oil and dissolve it in 500 ml. of Skellysolve B. Transfer to a 1000-ml. separatory funnel and proceed with the acetonitrile extraction steps described for cottonseed. Analyze as instructed under Chromatography.

Chromatography. Using a microliter syringe inject 5 µl. of the hexane solution into the gas chromatograph. Identify each isomer by its retention time and measure the area produced on

the strip chart with a polar planimeter.

The conditions of gas chromatography are:

F & M Model 700 gas chromatograph equipped with a pulsed type electron capture detector.

Column, 4-foot, 3-mm. i.d. Borosilicate glass column of 5% w./w. QF-1 (Wilkens Instrument and Research Co., Walnut Creek, Calif.) coated on 60–80-mesh acid-washed Chromosorb W.

Carrier gas, 5% methane in argon, 70 ml. per minute.

Purge gas, 5% methane in argon, 30 ml. per minute.

Temperatures. Column, 160° C. Injection port, 210° C. Detector cell, 200° C.

Electrometer range setting, 100. Attenuation, 1.

Pulse interval, 15 microseconds.

Recorder chart speed, $^1/_2$ inch per minute.

Under these conditions, the retention time for the 1,2,4-trichloro-3,5-dinitro isomer is 4.0 minutes and the 1,2,3-trichloro-4,6-dinitro isomer is 5.8 minutes.

Calculations. The parts per million of Chemagro 2635 are calculated by comparing the response obtained for an unknown sample directly to the response for a standard solution of similar concentration. Under the above conditions for vegetables and soil, the response for an amount of standard solution containing 0.75 nanogram of each isomer injected corresponds to 0.1 p.p.m. For the cottonseed procedure, 1 nanogram injected corresponds to 0.1 p.p.m. In both cases, if aliquots and sample size are taken according to the above procedure, the calculations reduce to the simple equation:

$$\text{p.p.m.} = \frac{\dfrac{(\text{sample})}{\text{area}}}{\dfrac{(\text{standard})}{\text{area}}} \cdot \frac{\dfrac{(\text{sample})}{\text{attenuation}}}{\dfrac{(\text{standard})}{\text{attenuation}}} \cdot 0.1$$

Standards are injected periodically along with the samples as a check on the sensitivity of the instrument. The response usually varies less than 2% from day to day under normal conditions.

Results and Discussion

The isomers of Chemagro 2635 are easily separated in a single chromatogram. By preparing one standard containing each of the isomers in identical concentration, both isomers may be determined simultaneously.

The standard curve (Figure 1) of log area *vs.* log concentration (nanograms) of the isomers of Chemagro 2635 is prepared by making suitable dilutions of identical amounts of the isomers in hexane solution and chromatographing 4-μl. aliquots of the dilutions. If at least 4 μl. are injected, errors in volume measurement are negligible. Also, by injecting constant volumes, better reproducibility is obtained. Response of the electron affinity detector is practically identical in both cases and linear over at least a hundredfold range. On the basis of this standard curve, crop and soil samples having residues in excess of about 1.0 p.p.m. should be diluted and re-injected in order to have the response fall along the linear portion of the curve. Cottonseed and cottonseed oil samples may run as high as 3.0 p.p.m. before dilution is necessary.

Analyses were conducted on a number of crops to which known amounts of Chemagro 2635 isomers had been added in the blender. In the case of cottonseed, the samples were spiked before extraction on the Soxhlet apparatus. A 20-hour Soxhlet extraction was found to be necessary to obtain good recovery of the isomers. The data obtained in these experiments (Table I) indicate that the recovery of Chemagro 2635 isomers generally exceeded 85% for all crops examined. A chromatogram of a Lima bean sample spiked with 0.1 p.p.m. of each isomer is shown in Figure 2, with a chromatogram for the corresponding control.

Table I. Recovery of Chemagro 2635 from Crops and Soil

Crop	Added, P.P.M. Each Isomer	Average Recovery, %[a] Isomer I[b]	Isomer II	No. of Detns.
Cucumbers	0.1	89 ± 3.5	83 ± 2.0	4
Lima beans	0.1	91 ± 2.5	84 ± 7.5	4
	0.5	90	92	1
Potatoes	0.1	91 ± 2.5	90 ± 3.5	4
Spinach	0.1	91 ± 1.0	85 ± 5.3	3
Sugar beets	0.1	95 ± 2.6	87 ± 10.0	5
	0.5	102 ± 2.0	106 ± 0.0	2
Sugar beet tops	0.1	90 ± 7.0	87 ± 7.7	4
	0.5	97 ± 1.0	99 ± 1.0	2
Table beets	0.1	92 ± 2.4	92 ± 2.4	5
	0.5	100	100	1
Soil (clay)	0.1	94 ± 1.2	94 ± 5.0	4
	0.5	102	102	
Soil (muck)	0.1	95 ± 2.0	82 ± 6.8	4
	0.5	102 ± 4.0	97 ± 1.0	2
Cottonseed	0.1	91 ± 3.7	97 ± 3.0	3
Cottonseed oil	0.1	85 ± 5.0	93 ± 4.5	2

[a] Average recovery values followed by average deviation from mean.
[b] Isomer I. 1,2,4-Trichloro-3,5-dinitrobenzene.
Isomer II. 1,2,3-Trichloro-4,6-dinitrobenzene.

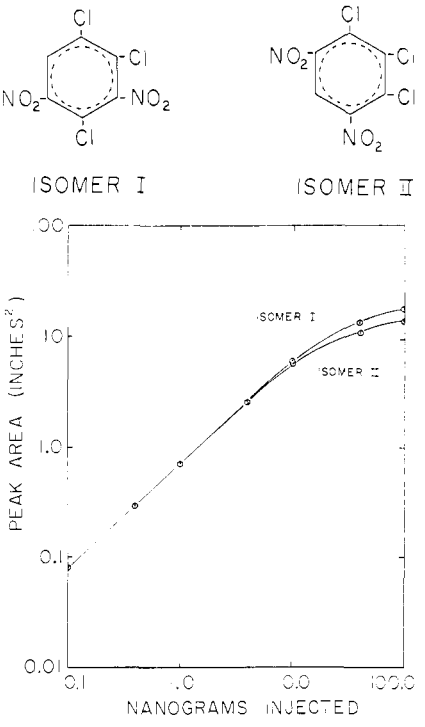


Figure 1. Standard curve of Chemagro 2635 isomers by electron affinity gas chromatography

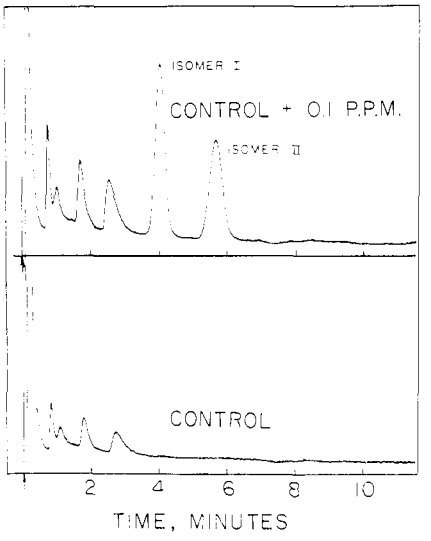


Figure 2. Chromatograms for recovery of Chemagro 2635 isomers from Lima beans

The sensitivity of the method is established at 0.1 p.p.m. Although it is possible to inject larger amounts of sample material to double or even triple the sensitivity, this tends to foul the system with extraneous crop extractives. In the case of cottonseed, larger samples cause peak broadening. Using the above procedure 0.1 p.p.m. produces an area on the recorder strip chart of about 0.5 square inch. This area can be measured with a polar planimeter with good accuracy.

In only one case was there any interference from peaks for crop extractives and in that case the value was less than half of that corresponding to 0.1 p.p.m. of Chemagro 2635. In no case did a control peak equal the response for 0.1 p.p.m. of fungicide.

Literature Cited

(1) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(2) Havens, R., Adams, J. M., Anderson, C. A., J. AGR. FOOD CHEM. **12,** 247–8 (1964).
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PESTICIDE ANALYSIS

Simultaneous and Selective Detection of Phosphorus, Sulfur, and Halogen in Pesticides by Microcoulometric Gas Chromatography

H. P. BURCHFIELD,[1] J. W. RHOADES and R. J. WHEELER

Southwest Research Institute, San Antonio, Tex.

Pesticides containing phosphorus, sulfur, or chlorine are separated from one another by gas chromatography and reduced to PH_3, H_2S, or HCl, respectively, with molecular hydrogen at 950° C. All three gases can be measured simultaneously with a microcoulometric titration cell equipped with silver electrodes. Alternatively, PH_3 and H_2S can be measured selectively by inserting a subtraction unit or GSC column between the outlet of the reduction tube and the inlet of the titration cell.

MICROCOULOMETRIC gas chromatography is widely used for the analysis of pesticides and drugs containing chlorine and sulfur. The compounds are separated from one another on a chromatographic column and then burned to yield CO_2, H_2O, HCl, and SO_2. HCl is measured specifically with a titration cell equipped with silver electrodes, while SO_2 is measured with an I_2/I^- cell. Details of this method are described by Coulson *et al.* (*3*).

This procedure has many advantages compared to other methods of analysis. It is highly sensitive, many compounds can be separated in a single operation, background from hydrocarbon and oxygenated hydrocarbon impurities in the sample is virtually eliminated, and interference from column bleed and tailing of the solvent peak is minimized. However, this method is not applicable to the detection of organic phosphates. The phosphate moiety is probably converted to P_4O_{10} in the combustion tube, but fails to emerge from it—probably because of its low vapor pressure and high reactivity. Consequently, a method was developed in which the column effluent is reduced with molecular hydrogen at 950° C. Phosphates are converted to PH_3, organically bound sulfur to H_2S, and organically bound chlorine to HCl. All three of these gases precipitate silver ion, and thus can be measured with a microcoulometric titration cell equipped with silver electrodes. This method is particularly suitable for the analysis of phosphorus-containing compounds, since phosphine is a chemically stable gas boiling at −87.8° C. By contrast, P_4O_{10}, which is formed on oxidation, sublimes at 360° C.

If the effluent from the reduction tube is passed directly into the titration cell, PH_3, H_2S, and HCl are measured simultaneously with a relative sensitivity of 2:2:1 if these gases are present. However, if a short subtraction tube containing Al_2O_3 is inserted between the exit line of the reduction tube and the inlet of the titration cell, H_2S and HCl are subtracted quantitatively. PH_3 passes through this packing unchanged, and thus can be measured with absolute specificity. PH_3 and H_2S can be measured in the presence of one another by inserting a short GSC column containing silica gel between the reduction tube and titration cell. Thus ronnel, which contains phosphorus, sulfur, and chlorine atoms, yields two peaks, the first of which represents PH_3 and the second H_2S. HCl is bound irreversibly by this column. Thus the method can be used to detect phosphorus alone, measure phosphorus and sulfur independently when they occur in the same compound, or measure the sum of phosphorus, sulfur, and chlorine. Moreover, sulfur bonded directly to phosphorus can be measured directly without interference from PH_3 by operating the reduction tube at a lower temperature (700° C.).

Reagents

Electrolyte. The electrolyte used in the Ag/Ag^+ titration cell was a 70 to 75% solution of acetic acid in water containing 0.2% Triton X-35, obtained from Rohm & Haas.

Aluminum oxide, Alcoa alumina, activated, grade F-20, 80- to 200-mesh,

[1] Present address, Pesticides Research Laboratory, U. S. Public Health Service, Perrine, Fla.