An Improved Gas Chromatographic Method for the Analysis of 2,4-D Free Acid in Soil

An improved method has been developed for the determination of 2,4-D residues in soil. The method involves a diethyl ether extraction of acidified soil, an alkali wash to remove interfering substances, and an improved esterification procedure using a 10% BCl_3 in 2-chloroethanol reagent. This method is subject to less interference and has equal or greater sensitivity than the methyl ester. Information is also given for the esterification of other acid-type herbicides.

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely used for a number of years in the control of many undesirable plants. It is applied both as a preemergence treatment in the soil and as a postemergence treatment of the growing plants. Although there is no evidence of buildup of 2,4-D residues in soil, such widespread use requires that a simple, reliable method of analysis be available for monitoring any possible accumulation.

A method of analysis was needed which would permit residue determination of 2,4-D in soil following multiple applications of the herbicide.

Colorimetric methods of analysis for 2,4-D (Warshowsky and Schantz, 1950) are both cumbersome and insensitive. Electron capture gas chromatography of the acid herbicide ester provides a decided improvement both in sensitivity and simplicity.

Diazomethane is most commonly used for esterification as the methyl ester (Yip, 1963); however, it is decidedly toxic and presents safety hazards. Other reagents for preparing methyl esters of herbicides include boron halide-methanol, 3-methyl-*p*-tolyltriazine, H_2SO_4-methanol, and others.

Other authors (Bottcher *et al.*, 1959; James, 1959) have described methods for the preparation of methyl esters of fatty acids prior to analysis by gas-liquid chromatography (glc). These methods normally require the use of anhydrous methanol containing an acidic or basic catalyst and involve a reflux period of 30 min to 2 hr.

Other methods describe the use of boron trifluoride-methanol reagent for the esterification of carboxylic acid (Mitchell and Smith, 1948; Metcalfe and Schmitz, 1961).

Landry and Collier (1969) reported a method using 3-methyl-*p*-tolyltriazine reagent to form the methyl ester.

The methyl ester has a very short retention time and is subject to interferences from soil extractives, esterifiable impurities in the ether, and from certain pesticides.

Gutenmann and Lisk (1964) reported a method describing the use of boron trifluoride : 2-chloroethanol reagent for the esterification of 2-methyl-4-chlorophenoxyacetic acid (MCP) analysis by glc.

Woodham *et al.* (1967) determined residues of 2,4-D in soil sediment and water using a boron trichloride : 2-chloroethanol reagent for esterification. Although this method had the desired sensitivity, produced less interference, and gave a longer retention time for the ester, it was still subject to interference from certain soil extractives. A cleanup step was added employing an alkaline wash method whereby ether soluble impurities are removed from an aqueous sodium salt solution of the herbicide. After this extraction, the herbicide salt is reacidified and reextracted into an ether phase.

MATERIALS AND METHODS

Esterification Reagent. Weigh 900 g of freshly redistilled reagent grade 2-chloroethanol (Distillation Products Co.) into a 2-l. flask and cool in an ice bath. With the flask still in the bath, bubble BCl_3 through a glass tube into the alcohol until 100 g are dissolved. Agitate the solution with a magnetic stirrer during the entire procedure. Place a trap in the gas line to prevent liquid from being drawn back into the gas cylinder valve. Prepare the reagent in a good fume hood and regulate the gas flow so that white fumes do not emerge from the flask.

Extraction. Weigh 75 g of soil into a 350-ml extraction bottle and add 30 ml of distilled H_2O and 150 ml of Nanograde diethyl ether. Add sulfuric acid (1 to 1, v/v) until a pH of 3 or below is obtained. Rotate the mixture on a concentric rotator for 4 hr and filter through a layer of sodium sulfate-Celite into a 350-ml sample bottle.

Aliquot 50.0 ml of the ether extract representing 25 g of soil into a 125-ml separatory funnel and add 50 ml of 0.05 *N* aqueous NaOH. After mixing, test the pH of the aqueous layer. If the pH at this point is not 10 or greater, add more alkali. Shake the funnel and, after phase separation, drain the aqueous layer into another 125-ml separatory funnel.

Adjust the pH of the aqueous layer in the second funnel to 3 or below with a 1 to 1 aqueous solution of H_2SO_4. Add 50 ml of Nanograde ether and shake the funnel vigorously. Allow the layers to separate and discard the bottom aqueous layer.

Drain the cleaned-up ether layer into a 125-ml Erlenmeyer flask. Add 1 ml of 0.01% Nujol in hexane and evaporate slowly to approximately 5 ml through a Snyder column on a warm water bath (*ca.* 50° C). Transfer the concentrated extract quantitatively to a 15-ml graduated centrifuge tube and evaporate just to dryness in a warm water bath using a gentle stream of air to speed the evaporation.

Esterification. Add 1.0 ml of the boron trichloride reagent down the sides of the centrifuge tube containing the 2,4-D residue. Immerse the tube in a 90° C water bath for 10 min. After cooling, add 5.00 ml of Nanograde hexane to the tube followed by 10 ml of a 7% aqueous solution of sodium sulfate. Shake the tube and allow the layers to separate.

Analysis. Inject 5.0 μl portions of the esterified sample in hexane into the gas chromatograph. Calculate residues by comparing with 2,4-D standards which are esterified in a

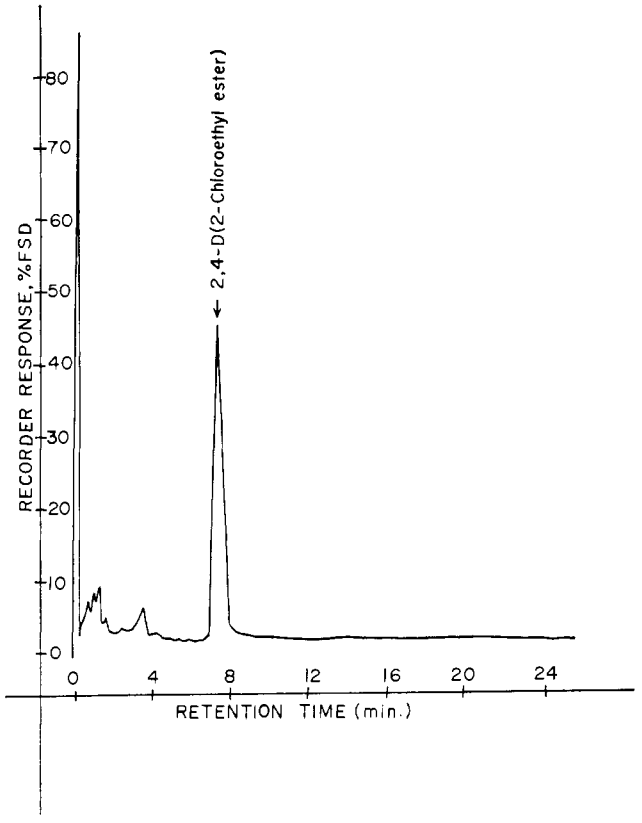

Figure 1. Chromatographic tracing of 2-chloroethyl ester of 2,4-D (2 ng). Column used was a 6-ft glass column packed with 11% OV-17:QF-1 mixture of 80–100 mesh Gas-Chrom-Q. Column temperature was 205° C, detector temperature 210° C, and injector temperature 250° C. Nitrogen gas flow was 80/min

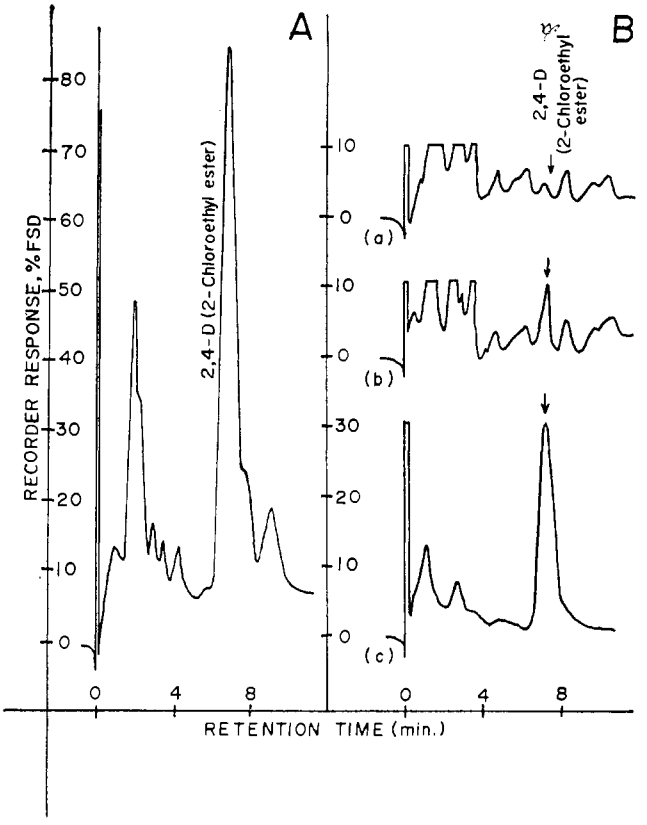

Figure 2 (A). Chromatographic tracing of a 2,4-D fortified soil sample (2-chloroethyl ester) without alkali treatment. Figure 2 (B) (a) shows a tracing of a blank soil sample; (b) a soil sample fortified with 0.013 ppm 2,4-D acid; and (c) a soil sample fortified with 0.667 ppm 2,4-D acid; the latter three samples received the alkali prewash

similar manner. Instrument operating conditions were as follows:

	11% OV-17: QF-1	10% DC-200
Instrument	Jarrell-Ash	Jarrell-Ash
Support	Gas-Chrom-Q (80–100 mesh)	Gas-Chrom-Q (100–120 mesh)
Column	6-ft glass	6-ft glass
Carrier gas	Nitrogen	Nitrogen
Flow rates		
Carrier gas	80 ml/min	80 ml/min
Tank pressure	60 p.s.i.g.	60 p.s.i.g.
Temperatures		
Column	205° C	190° C
Detector	210° C	200° C
Injection port	250° C	250° C
Chart speed: 15 in. per hr		15 in. per hr
Sensitivity: 10^{-9}		

RESULTS AND DISCUSSION

A series of 2,4-D fortified soil samples, as the free acid, was analyzed employing this method. Results are summarized in Table I. Recoveries ranged from 84.3% to 102.6%; these percentages are an average of two replicates.

The 2-chloroethanol ester has a retention time of approximately 3.3 that of the methyl ester on the 10% DC-200 column. A chromatographic tracing of this is shown in Figure 1.

Table I. Recoveries of 2,4-D from Fortified Soil[a]

Sample No.	ppm added	ppm recovered	% recovery	Average
1	0	negative	...	...
2	0	negative	...	...
3	0.013	0.011	84.6	...
4	0.013	0.011	84.6	84.6
5	0.053	0.035	66.0	...
6	0.053	0.060	113.2	89.6
7	0.133	0.132	99.2	...
8	0.133	0.141	106.0	102.6
9	0.667	0.616	92.3	...
10	0.667	0.679	101.8	97.1
11	1.333	1.022	76.7	...
12	1.333	1.226	92.0	84.3

[a] Values obtained using a 6-ft glass column packed with a 11% OV-17: QF-1 mixture on Gas-Chrom-Q (80–100 mesh): column temperature, 205° C with a nitrogen flow rate of 80 ml per min.

A comparison of a soil sample with and without the alkali cleanup is shown in Figure 2. As can be seen from Figure 2(A), numerous interferences are encountered which can interfere with a routine 2,4-D residue analysis. Figure 2(B) shows chromatographic tracings of a blank soil sample (curve a); the same soil fortified with 0.01 ppm 2,4-D (curve b); and this soil fortified with 0.67 ppm 2,4-D (curve c).

It was determined in experimental work that the length of varying reaction times of the herbicide with esterifying reagent up to 30 min were not critical, as shown in Table II. However, the amount of reagent added was critical. When a volume greater than 1 ml was added, the ester peak began to

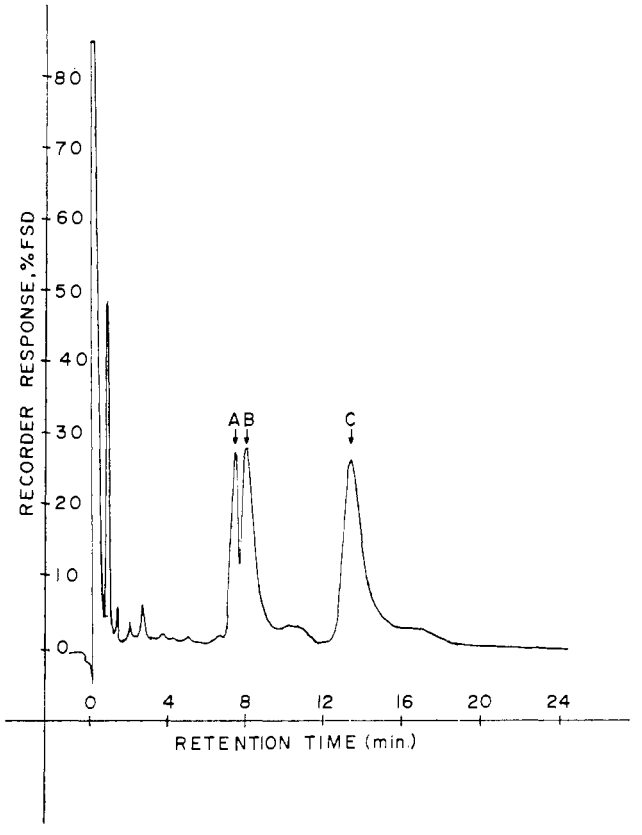

Figure 3. Chromatographic tracing of a mixture of 0.19 ng fenac (peak A), 3 ng 2,4-D (peak B), and 2.83 ng 2,4,5-T (peak C), on a 11% OV-17:QF-1 mixture on 100–120 mesh Gas-Chrom-Q column

Table II. Response of 2,4-D (2-Chloroethyl Ester) Using Various Esterification Times[a,b,c]

Sample No.	Reaction time-min	Peak height, mm
1	5	11.0
2	5	10.4
3	10	11.4
4	10	11.0
5	15	11.1
6	15	11.8
7	20	11.5
8	20	11.3
9	25	11.4
10	25	10.5
11	30	10.7
12	30	10.8

[a] Values obtained using a 6-ft glass column packed with 10% DC-200 on Gas-Chrom-Q (100–120 mesh) operated at 190° C with a nitrogen flow rate of 80 ml per min. [b] Ten micrograms 2,4-D acid added to each sample. [c] 1.0 ml reagent added.

decrease. The results of these tests are given in Table III.

The 2-chloroethanol method is also applicable to the analysis of certain other acid herbicides. Table IV gives data for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,3,6-trichlorophenylacetic acid (fenac), 3-amino-2,5-dichlorobenzoic acid (amiben), and 4-amino-3,5,6-trichloropicolinic acid (picloram). Sensitivities and retention times (relative to the 2,4-D ester) are given. Fenac and 2,4,5-T are very sensitive to this method, while amiben and picloram are somewhat lower in sensitivity.

The method described in this paper is rapid and simple. The alkali wash treatment described provides an adequate separation of the interfering components in soil and no further cleanup is necessary for routine soil samples. The lower limits of sensitivity for this method is approximately 0.01 ppm for 2,4-D.

Table III. Response of 2,4-D (2-Chloroethyl Ester) Using Various Volumes of Esterification Reagent[a,b,c]

Sample No.	Reagent vol., ml	Peak height, mm
1	0.5	10.5
2	0.5	11.3
3	1.0	10.8
4	1.0	11.2
5	2.0	10.4
6	2.0	9.2
7	3.0	8.8
8	3.0	9.2
9	4.0	6.3
10	4.0	6.2
11	5.0	4.6
12	5.0	4.5

[a] Values obtained using a 6-ft glass column packed with 10%, DC-200 on Gas-Chrom-Q (100–120 mesh) operated at 190° C with a nitrogen flow rate of 80 ml per min. [b] Ten micrograms 2,4-D acid added to each sample. [c] Ten minute reaction period.

Table IV. Retention Times and Sensitivities of Four Phenoxy Acid Herbicides (2-Chloroethyl Ester) Relative to the 2,4-D Ester[a]

Herbicide	Relative retention time	Relative sensitivity
2,4-D	1.00	1.00
2,4-5-T	1.76	1.00
Amiben[b]	1.65	0.25
Picloram[b]	2.95	0.07
Fenac	1.10	15.50

[a] Values obtained using a 6-ft glass column packed with 10% DC-200 on Gas-Chrom-Q (100–120 mesh) operated at 190° C with a nitrogen flow rate of 80 ml per min. [b] Esterified for 2 hr at 90° C before esterification was complete.

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