# Simultaneous Determination of 2,4-Dichlorophenoxyacetic Acid, 2,4,5-Trichlorophenoxyacetic Acid, and 2-Methoxy-3,6-dichlorobenzoic Acid in Soil and Water by Gas Chromatography with Electron Capture Detector

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Factors such as extraction, esterification, cleanup, stationary phases, and $^{63}Ni$ detector response were examined and a sensitive method using diethyl ether extraction, *n*-butylation, and Florisil cleanup was developed for the simultaneous detection and determination by gas chromatography of low levels of plant-growth regulating herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-methoxy-3,6-dichlorobenzoic acid (dicamba) in soil and water. The method is satisfactory with respect to background interference, resolution, and sensitivity using an OV-17/QF-1 or Carbowax 20M column. The soil background varied according to the type of soil, extraction method, esterification, cleanup, and gc conditions used. 2,4-D and 2,4,5-T exhibited good recovery from fortified soil samples using both methylation and *n*-butylation, whereas dicamba gave variable recovery with methylation but good recovery with *n*-butylation. Data on retention times and responses of the herbicide esters have been presented. The sensitivities (50% full scale values) of the compounds were in the subnanogram to low nanogram range. The sensitivity of the method is generally 0.03–0.05 ppm in soil. Water samples can be analyzed without cleanup.

Formulations containing more than one herbicide are in use in agriculture, in the management of land, water, and forest, and as defoliants in military operations. In Canada, in 1971 over 70 registered formulations contained either 2,4-D (2,4-dichlorophenoxyacetic acid) and dicamba (2-methoxy-3,6-dichlorobenzoic acid) or 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) for controlling weeds and brush, respectively. In order to get information on the environmental persistence of these compounds sensitive methods for their simultaneous determination are indispensable. While residues of 2,4-D (Woodham *et al.*, 1971) and of 2,4,5-T (McKone and Hance, 1972) in soil have been analyzed by using gas chromatography (gc) with an electron capture detector (ECD), no method has been reported for the simultaneous determination of 2,4-D, 2,4,5-T, and dicamba. The objective of this work was to develop such a method for these compounds at the level of 0.05 ppm or lower in soil and water.

## EXPERIMENTAL SECTION

**Gas Chromatography.** Gas chromatography was carried out using a Pye Series 104 chromatograph Model 74 fitted with a $^{63}Ni$ (10 mCi) electron capture detector. Two liquid phases were used for the gc separation: 11% (OV-17/QF-1) and 3% Carbowax 20M. These were coated on Gas-Chrom Q or Chromosorb W, 80–100 mesh, and packed in glass columns (5 ft × 0.25 in.). The detector was operated in the pulse mode at 15-$\mu$sec pulse space at 283°. The carrier gas flow was 60 ml of nitrogen/min.

**Extraction and Cleanup.** Two extraction techniques have been studied in detail: an acid–ether method which involved extraction at low pH (~2) by diethyl ether and an alkali–chloroform method where the herbicides were kept in aqueous solution at high pH (~12). In the latter, the impurities were removed by washing with chloroform and the salts of the herbicide acids were then converted to the acids by acidifying to low pH and extracted by chloroform. The two procedures used are outlined in Figures 1 and 2.

The cleanup was effected by acid–base partitioning of the free acids followed by column chromatography of their methyl esters on a Florisil column. Methylation was done by diazomethane. The Florisil used had a moisture content of 0.2–0.8% $H_2O$, and the column eluted with 5% diethyl ether in hexane and 20% diethyl ether in hexane.

## ANALYTICAL PROCEDURES

**Acid–Ether Extraction.** To the soil sample (20 g) in a 250-ml round-bottomed flask were added 5 ml of water, 5 ml of 18 *N* $H_2SO_4$, and 5 ml of 95% EtOH with hand stirring. The soil (pH <1.8) was evaporated just to dryness in a flash evaporator at 40°. Alternatively, to the soil sample, 18 *N* $H_2SO_4$ was added to make it acidic (pH 1.8). The amount of acid to be used for the soil type was determined previously by taking a separate sample, suspending it in deionized water, and adding the acid to it. The soil sample was dried by grinding in a mortar with anhydrous sodium sulfate. With the dried sample, sludge formation during extraction was prevented and the subsequent filtration was easier.

The soil was then shaken with 100 ml of diethyl ether in a 250-ml conical flask in a mechanical shaker for about 3 hr. The mixture was filtered through a fluted filter paper and washed with ether. The extract was transferred to a separatory funnel and shaken vigorously with 50 ml of 0.05 *N* NaOH and then with 4% $NaHCO_3$ (50 ml × 3). The ether phase was separated and put aside for the analysis of herbicide residues.

The combined aqueous solution was acidified to pH 1.6 with 18 *N* $H_2SO_4$ and extracted with diethyl ether (100 ml × 3). The ether layer was washed with 60 ml of saturated NaCl solution and dried with anhydrous $Na_2SO_4$ for 1 hr. After filtration the solvent was reduced to about 10 ml in a flash evaporator and finally to dryness in a centrifuge tube with an air stream. The residue was dissolved in 1 ml of diethyl ether and a few drops of methanol. Diazomethane (prepared from Diazald, Aldrich Chemical Co.) was passed through the solution for 10 min at room temperature. After standing for another 10 min, the solution was evaporated to dryness. The residue was taken up in 2 ml of 10% diethyl ether in hexane and transferred to a Florisil column (20 g, 99.8% dry matter) previously washed with 100 ml of hexane. The column was eluted first with 100 ml of 5% diethyl ether in hexane which was discarded, and then with 100 ml of 20% diethyl ether in hexane. The second eluate was concentrated to about 1 ml

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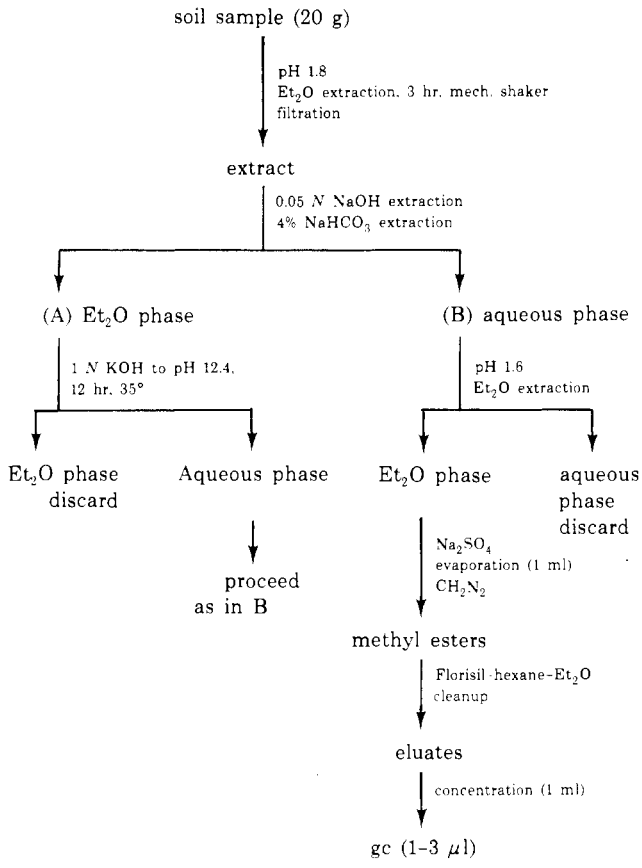


**Figure 1.** Schematic diagram of acid–diethyl ether extraction of soil.

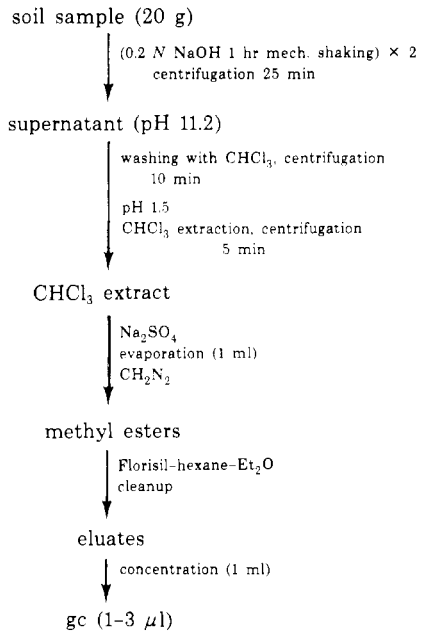


**Figure 2.** Schematic diagram of alkali–chloroform extraction of soil.

and an aliquot of this solution (1-3 μl) was used for gc analysis.

The ether phase containing esters of the herbicide acids was concentrated to about 20 ml and hydrolyzed overnight at 35° with 125 ml of 0.2 *N* ethanolic KOH. The aqueous solution was washed twice with 70 ml of diethyl ether acidified to pH 1.6 and extracted three times with 70 ml of diethyl ether. The extract was shaken twice with 50 ml of 0.05 *N* NaOH and then with 4% $NaHCO_3$ solution. All the aqueous phases were combined. This solution was again acidified to pH 1.6 and the diethyl ether extraction was repeated. The extract was dried, evaporated to a small volume, and methylated as described before. After the Florisil cleanup the sample was ready for analysis.

**Alkali–Chloroform Extraction.** (A) *Soil.* Twenty grams of soil was extracted for 1 hr in a mechanical shaker with 125 ml of 0.2 *N* NaOH. The mixture was centrifuged for 25 min at 5000 rpm. The soil residue was again extracted with 0.2 *N* NaOH and centrifuged off. The two supernatant liquids were combined in a separatory funnel (pH 11.2) and washed twice with 50 ml of chloroform. The aqueous phase was acidified to pH 1.5 with 18 *N* $H_2SO_4$ and extracted three times with 70 ml of chloroform. The chloroform extract was dried with anhydrous $Na_2SO_4$. After filtration the solvent was reduced to small volume in a flash evaporator and then to dryness in a centrifuge tube with an air stream. The residue methylated with diazomethane, chromatographed on Florisil, and analyzed as described above.

(B) *Water.* Samples (200 ml) were acidified to pH 1.8 with 18 *N* $H_2SO_4$ and extracted three times with 70 ml of diethyl ether. The aqueous phase was discarded and the ether phase was shaken with 50 ml of 0.05 *N* NaOH and then twice with 75 ml of 4% $NaHCO_3$. The organic phase at this step could be analyzed if esters of the herbicides were present. The aqueous solutions were combined and adjusted to pH 1.8. It was reextracted twice with 100 ml of ether and the ether phase was dried with anhydrous sodium sulfate, filtered, evaporated to about 1 ml, and methylated with diazomethane. The samples were analyzed as described before. Tap water samples fortified at 0.05 and 0.2 ppm were analyzed successfully without Florisil cleanup.

***n*-Butylation.** For the conversion of the herbicides to *n*-butyl esters diazobutane was used. It was prepared from its precursor *N*-*n*-butyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.). The reaction was carried out at room temperature for approximately 10 min, similar to methylation. This procedure was quicker than the existing procedure of heating at 70° for 1 hr (Woolson *et al.*, 1971; Woolson and Harris, 1967) with 14% $BCl_3$ in *n*-butyl alco-

**Table I. Retention Times and Response Characteristics of the Methyl and *n*-Butyl Esters of 2,4-D, 2,4,5-T, and Dicamba**[a]

| | 11% OV-17/QF-1 | | | | 3% Carbowax 20M | | | |
|---|---|---|---|---|---|---|---|---|
| | Methyl ester | | *n*-Butyl ester | | Methyl ester | | *n*-Butyl ester | |
| Herbicide | $T_r$, min | 50% FSD, ng | $T_r$, min | 50% FSD, ng | $T_r$, min | 50% FSD, ng | $T_r$, min | 50% FSD, ng |
| Dicamba | 1.65 | 0.48 | 3.62 | 0.42 | 1.66 | 0.56 | 2.92 | 0.38 |
| 2,4-D | 2.52 | 3.80 | 5.20 | 4.05 | 3.32 | 5.20 | 5.27 | 3.50 |
| 2,4,5-T | 4.10 | 1.01 | 8.20 | 1.32 | 5.53 | 3.50 | 8.10 | 2.20 |

[a] Operating conditions: pulse space 15 μsec; carrier gas flow, 60 ml/min; attenuation, $2 \times 10^{-10}$ A; detector temperature, 283°; column temperatures, 219° (OV-17/QF-1) and 231° (3% Carbowax 20M).

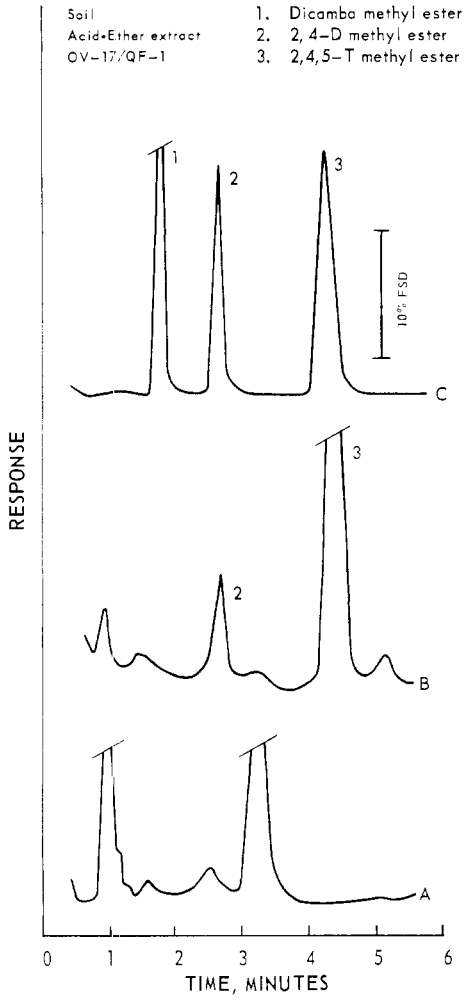


**Figure 3.** Gas chromatograms of acid-ether extracts of soil and soil fortified with 2,4-D (0.15 ppm) and 2,4,5-T (0.15 ppm), after methylation and cleanup on Florisil column, as separated on an OV-17/QF-1 column at 216°: (A) blank soil; (B) fortified soil; (C) methylated standard herbicides: dicamba, 0.4 ng; 2,4-D, 2.0 ng; 2,4,5-T, 1 2 ng.

hol or of heating with *n*-butyl alcohol and concentrated sulfuric acid for 30 min at 100° (McKone and Hance, 1972).

## RESULTS AND DISCUSSION

The conversion of herbicide acids to their esters enables them to be readily chromatographed, and by judicious choice of the esters, difficulties due to interfering substances can be avoided. Methyl esters are most commonly used for the phenoxyacetic acids. Accordingly, the initial development of the method described was based on this ester. However, on working with soil samples, difficulties were sometimes encountered in analyzing dicamba, in the presence of 2,4-D, and 2,4,5-T, due to its early elution and interference from soil extractives. To avoid similar difficulties in the case of 2,4-D in soil, Woodham *et al.* (1971) converted it to a 2-chloroethyl derivative and separated it on an OV-17/QF-1 column.

This procedure worked with 2,4-D, but with an OV-17/QF-1 column at 216° appreciable background interference occurred with the dicamba and 2,4,5-T peaks. These interferences were, however, negligible when a Carbowax 20M column was used.

The gc properties of the *n*-butyl and methyl esters of the three herbicides on two columns are given in Table I. The *n*-butyl esters could be cleaned up on a Florisil column. Thus, 91% of *n*-butyl ester of dicamba was recovered with the 5% diethyl ether–hexane eluate and 7% more in 20% ether–hexane eluate. All of the 2,4-D (92%) was recovered in the second eluate. The recovery of 2,4,5-T was 4 and 84% in the first and the second eluate, respectively.

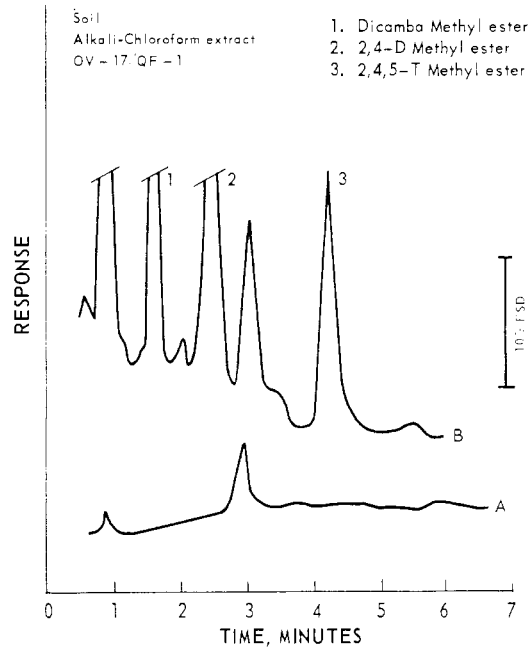


**Figure 4.** Gas chromatograms of alkali-chloroform extracts of soil and soil fortified with dicamba (0.17 ppm), 2,4-D (0.42 ppm), and 2,4,5-T (0.17 ppm), after methylation and cleanup on Florisil column, as separated on an OV-17/QF-1 column at 216°: (A) blank soil; (B) fortified soil.

**Interferences.** The results obtained with the acid-ether extraction and alkali-chloroform extraction of soil samples were satisfactory with respect to reproducibility and recovery. Methylated soil extracts were invariably analyzed on OV-17/QF-1 and Carbowax 20M columns, both of which gave good resolution of the individual herbicides. Chromatogram B in Figure 3 was obtained on an OV-17/QF-1 column at 216° with an acid-ether extract of soil fortified with 2,4-D and 2,4,5-T at 0.15 ppm. Analysis of a similar extract of blank soil on the same column showed small interferences with dicamba and 2,4-D, and none for 2,4,5-T (chromatogram A). The methyl esters of the standard herbicides were run under the same gc conditions as shown in chromatogram C. The alkali-chloroform extracts after methylation and cleanup gave similar results on an OV-17/QF-1 column (Figure 4). However, chromatograms obtained with such extracts on a Carbowax 20M column at 231° showed a large interference with dicamba and small interferences with 2,4-D and 2,4,5-T.

Some of the chromatograms obtained for the *n*-butyl esters are shown in Figures 5 and 6 which include the standard herbicides and acid-ether extracts of soil separated on both the gc columns. The fortification level in these soil samples was 0.4 ppm and the *n*-butylation was followed by the usual Florisil cleanup. The soil background obtained on OV-17/QF-1 and Carbowax 20M columns was satisfactory.

Sandy loam soils (organic matter: 3.0–5.6%) were examined in more detail for methyl ester interferences in electron capture detection. The results are reported in Table II for acid-ether and alkali-chloroform extractions. The alkali-chloroform extracts of the two soils showed that dicamba and 2,4,5-T regions were almost free from interferences (<5 ppb) on the gc columns. However, the acid-ether extract of soil no. 110 showed a higher interference (31 ppb) for dicamba on a Carbowax 20M column. Furthermore, appreciable interferences with 2,4-D methyl ester analysis on the gc columns were evident in acid-ether as well as alkali-chloroform extracts.

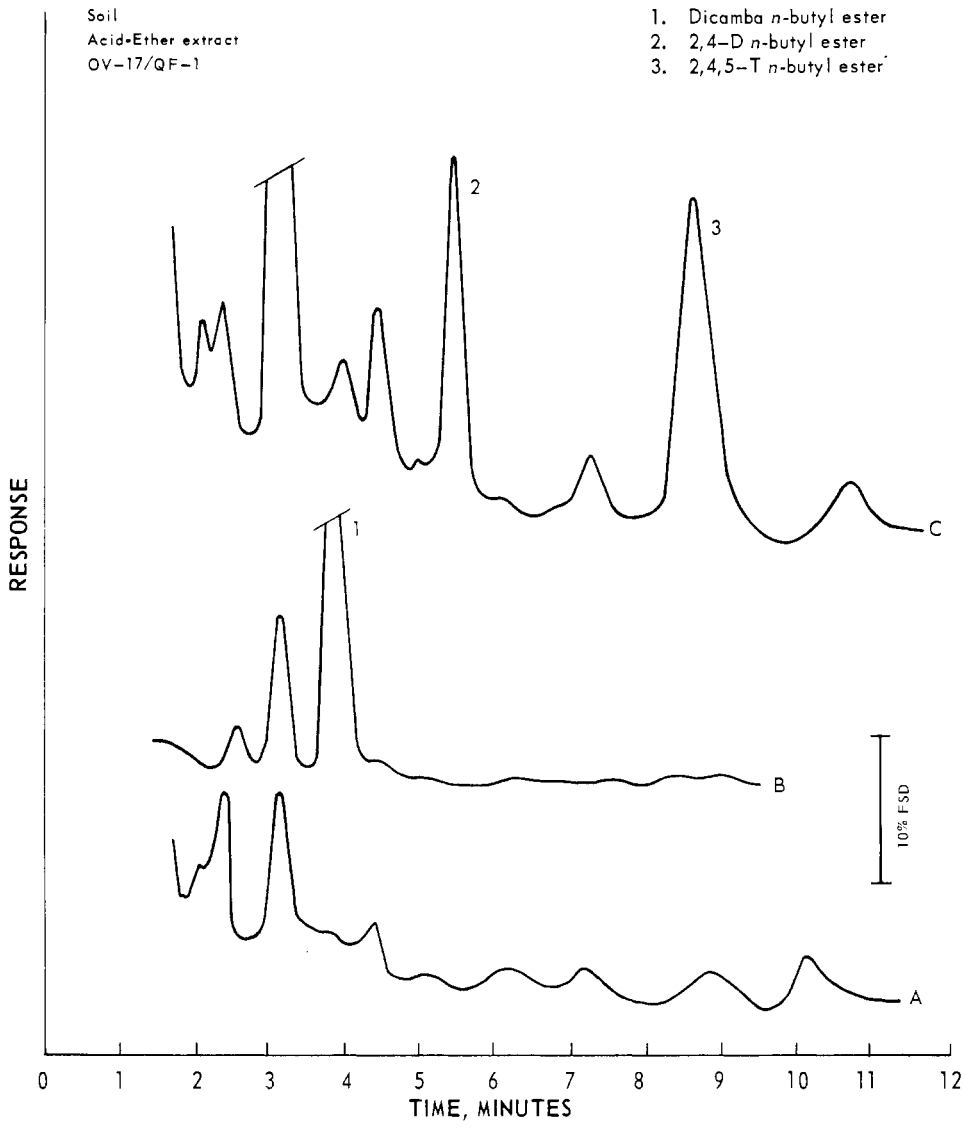


**Figure 5.** Gas chromatograms of acid–ether extracts of soil and soil fortified with dicamba (0.4 ppm), 2,4-D (0.4 ppm), and 2,4,5-T (0.4 ppm), after *n*-butylation and cleanup on Florisil column, as separated on an OV-17/QF-1 column at 216°: (A) blank soil (20% ether–hexane eluate from cleanup column); (B) fortified soil (5% ether–hexane eluate from cleanup column) representing a recovery of 88% of dicamba; (C) fortified soil (20% ether–hexane eluate from cleanup column) representing a recovery of 66% of 2,4-D and 58% of 2,4,5-T.

**Table II. Interference Levels in Soil Extracts Detected by Gc–EC of the Two Gc Columns**

| | | Interference level, ppm | | | | | |
|---|---|---|---|---|---|---|---|
| | | Soil no. 5 | | | Soil no. 110 | | |
| Gc column | Temp, °C | Dicama region | 2,4-D region | 2,4,5-T region | Dicamba region | 2,4-D region | 2,4,5-T region |
| | | Acid–Diethyl Ether Extract | | | | | |
| Carboxax[a] 20M | 227 | 0.000 | 0.034 | 0.007 | 0.031 | 0.079 | 0.007 |
| OV-17/QF-1[b] | 216 | 0.001 | 0.000 | 0.006 | 0.001 | 0.000 | 0.005 |
| | | Alkali–Chloroform Extract | | | | | |
| Carbowax[a] 20M | 227 | 0.001 | 0.005 | 0.002 | 0.000 | 0.083 | 0.000 |
| OV-17/QF-1[a] | 216 | 0.000 | 0.011 | 0.000 | 0.000 | 0.013 | 0.000 |

[a] Methyl ester. [b] Butyl ester.

**Recoveries from Fortified Soil.** The recovery of the herbicides using acid–ether and alkali–chloroform extractions from two soils at a low level of fortification (0.05–0.4 ppm) is reported in Table III. Moderate to satisfactory recoveries of 2,4,5-T (70–85%) were obtained after methylation or butylation of the extracts and cleanup on the Florisil column. The recovery of dicamba after butylation was relatively good on the OV-17/QF-1 column. However, poor recoveries of dicamba were obtained when the analyses were performed after methylation. Hence, quantitative analysis of dicamba residues in soil samples *via* methylation and Florisil cleanup cannot be strongly recommend-

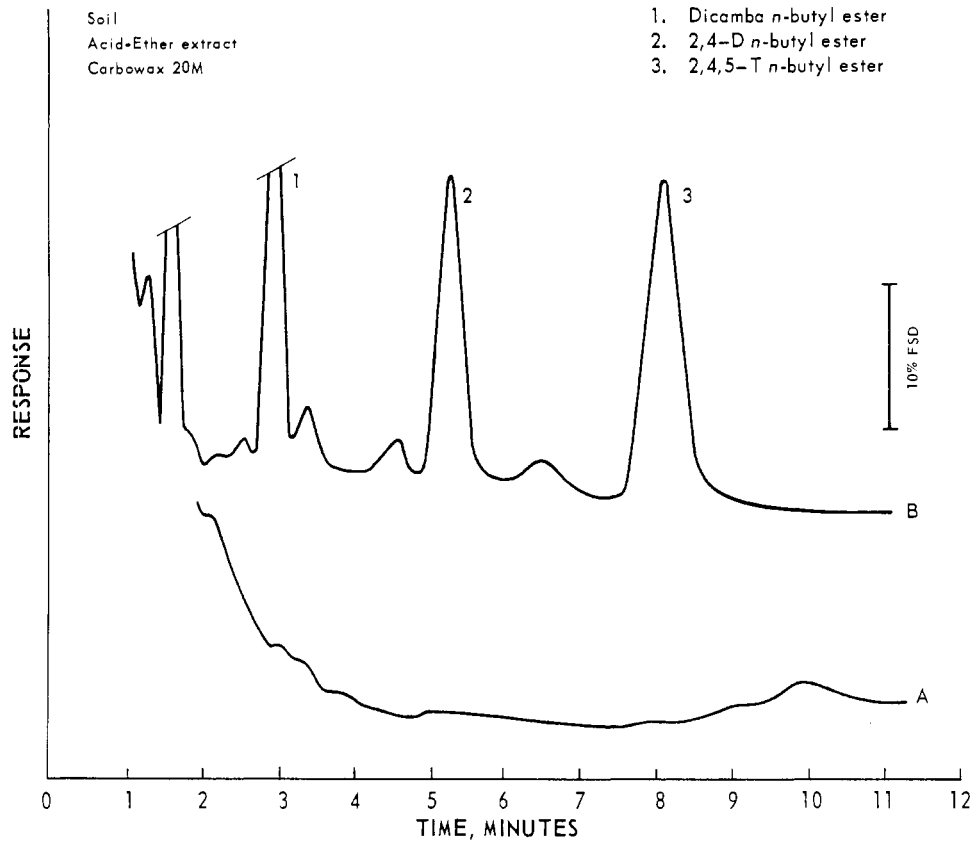


**Figure 6.** Gas chromatograms of acid-ether extracts of soil and soil fortified with dicamba (0.4 ppm), 2,4-D (0.4 ppm), and 2,4,5-T (0.4 ppm), after *n*-butylation and cleanup on a Florisil column; 20% ether-hexane eluate, as separated on Carbowax 20M column at 231°: (A) blank soil; (B) fortified soil representing a recovery of 58% of dicamba, 108% of 2,4-D, and 83% of 2,4,5-T.

**Table III. Recoveries of Dicamba, 2,4-D, and 2,4,5-T from Fortified Soils**

| Gc column | Soil no. | % recovery Dicamba | 2,4-D | 2,4,5-T |
|---|---|---|---|---|
| Acid–Diethyl Ether Extraction | | | | |
| Carbowax 20M[a] | 5 | 50 | 86 | 70 |
| | 110 | 52 | 110 | 70 |
| OV-17/QF-1[b] | 110 | 90 | 84 | 85 |
| Alkali–Chloroform Extraction | | | | |
| Carbowax 20M[a] | 5 | 21 | 86 | 79 |
| | 110 | 52 | 92 | 80 |
| OV-17/QF-1[a] | 5 | 67 | 83 | 72 |
| | 110 | 63 | 76 | 70 |

[a] Methyl ester. [b] Butyl ester.

ed, but it is adequate for the detection and quantitative estimation. The recoveries of 2,4-D using acid-ether and alkali-chloroform extractions from both soils appeared to be good (76–110%).

The recovery of herbicides from fortified soil as the *n*-butyl esters seemed to be quite good (84% and over). The chromatograms of such extracts are shown in Figure 5B for OV-17/QF-1 and Figure 6 for Carbowax 20M.

**Validity Test.** A validity test was made for the acid-ether extraction procedure for the simultaneous analysis of these herbicides in soil using the Carbowax 20M column. The extraction was followed by methylation and Florisil cleanup. The results are summarized in Table IV. The analyst was supplied with some blank soil samples to determine the interference levels, but the other samples (both blank and fortified) were unknowns. Since most of the results were satisfactory it could be concluded that the method is acceptable for low level detection of these herbicides in soil.

**Table IV. Validity Test for the Detection of 2,4-D, 2,4,5-T, and Dicamba in Soil Using Acid–Diethyl Ether Extraction[a]**

| Sample no. | Dicamba Fortification ppm | Dicamba Reported ppm | 2,4-D Fortification ppm | 2,4-D Reported ppm | 2,4,5-T Fortification ppm | 2,4,5-T Reported ppm |
|---|---|---|---|---|---|---|
| 1 | 0.000 | 0.020 | 0.000 | 0.034 | 0.100 | 0.072 |
| 2 | 0.000 | 0.016 | 0.000 | 0.003 | 0.000 | 0.004 |
| 3 | 0.000 | 0.014 | 0.000 | 0.016 | 0.000 | 0.002 |
| 4 | 0.000 | 0.001 | 0.150 | 0.096 | 0.120 | 0.081 |
| 5 | 0.000 | 0.028 | 0.000 | 0.012 | 0.000 | 0.000 |
| 6 | 0.160 | 0.072 | 0.170 | 0.100 | 0.000 | 0.000 |
| 7 | 0.320 | 0.190 | 0.000 | 0.010 | 0.100 | 0.065 |

[a] Carbowax 20M column.

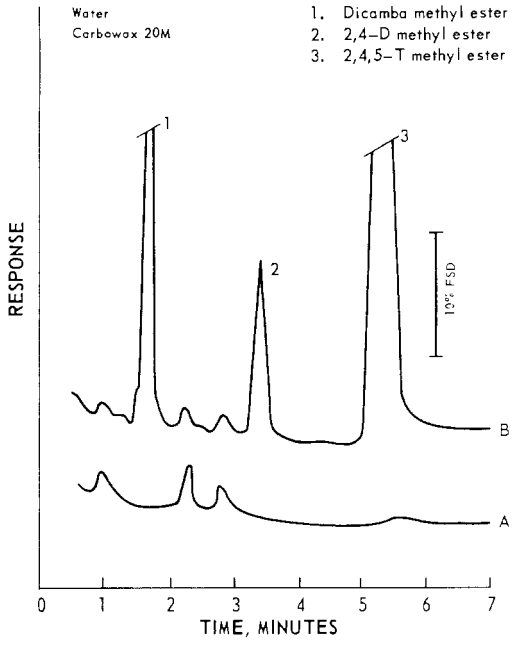


**Figure 7.** Gas chromatograms of acid–ether extracts of water and water fortified with dicamba, 2,4-D, and 2,4,5-T, after methylation without cleanup, as separated on Carbowax 20M at 231°: (A) blank water; (B) fortified water representing a recovery of 56% of dicamba at 0.05 ppm, 94% of 2,4-D at 0.05 ppm, and 103% of 2,4,5-T at 0.05 ppm.

**Water Samples.** The analysis of herbicides as methyl esters in tap water was performed on acid–ether extracts without cleanup. Water fortified at 0.05 ppm with the herbicides showed a recovery of 56% for dicamba, 94% for 2,4-D, and 103% for 2,4,5-T with a Carbowax 20M column at 231° (Figure 7B). No interferences were observed with the control water sample (Figure 7A). Similar results were obtained with an OV-17/QF-1 column. The results on the recovery of these herbicides as obtained with two columns (OV-17/QF-1 and Carbowax 20M) are summarized in Table V, from which it could be surmised that the water samples could be analyzed satisfactorily with the described method. No interferences were experienced with the Carbowax 20M column for all three herbicides. However, on the OV-17/QF-1 column, 2,4-D showed a maximum interference of 20 ppb while 2,4,5-T and dicamba were free from interference.

**Table V. Per Cent Recovery of Herbicides from Fortified Tap Water**

| | Dicamba | | 2,4-D | | 2,4,5-T | |
|---|---|---|---|---|---|---|
| Gc column | 0.05 ppm | 0.20 ppm | 0.05 ppm | 0.20 ppm | 0.05 ppm | 0.20 ppm |
| OV-17/QF-1[a] | 66 | 69 | 48 | 75 | 76 | 75 |
| Carbowax 20M[b] | 56 | 78 | 94 | 82 | 103 | 102 |

[a] 216°. [b] 231°.

In conclusion, the acid–ether method involving diethyl ether extraction at low pH followed by partition, *n*-butylation or methylation, Florisil cleanup, and $^{63}Ni$ gc with an OV-17/QF-1 or Carbowax 20M column has been found to give a sensitivity of 0.05 ppm or better for the simultaneous analysis of 2,4-D, 2,4,5-T, and dicamba residues in soil. The *n*-butylation provided better recovery of dicamba than that obtained by methylation. The procedure developed is also applicable without cleanup to water samples containing similar levels of these herbicides.

ACKNOWLEDGMENT

The author thanks W. R. McDowell for technical assistance.

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Received for review February 28, 1973. Accepted February 20, 1974. Presented at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 10–14, 1972. Contribution No. 748 from the Chemistry and Biology Research Institute, Canada Department of Agriculture.

# Chromatographic Parameters of the Bisquaternary Herbicides, Paraquat and Diquat

Charles W. Sharp* and Emile M. Lores, Jr.

Analysis of bisquaternary amines by thin layer chromatography represents a special problem. The diacetate, dichloride, and (mixed) acetate-chloride salts are resolved following development with a solvent composed of *n*-butyl alcohol, acetic acid, and water. Formation of the acetate salts could be suppressed by the addition of chloride ions to the system. However, from among several systems examined, solvents containing benzene–*n*-pentyl alcohol–methanol–1 *N* HCl, either 1:1:2:1 or 1.3:4:8:8, in combination with 300 MN cellulose thin layers are particularly suitable for the chromatography of paraquat, diquat, and perhaps other bisquaternary amines. This permitted the resolution of paraquat from diquat, as well as from impurities present in a technical grade of paraquat.

Deaths resulting from accidental ingestion of paraquat (1,1′-dimethyl-4,4′-bipyridinium) and the prevalent use of these bisquaternary amine herbicides, including diquat (1,1′-ethylene-2,2′-dipyridinium) and morfamquat [1,1′-bis(3,5-dimethylmorpholinocarbonylmethyl)-4,4′-bipyridinium], have stimulated an interest as to their possible environmental impact (Calderbank, 1968; Sharp *et al.*, 1972; "Touching up the Paraquat Picture," 1972; Kimbrough, 1973). Efforts to evaluate the effects of residues and any potential metabolite of these compounds require,

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