Determination of Phenoxy Acid Herbicides in Water By Electron-Capture and Microcoulometric Gas Chromatography

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By DONALD F. GOERLITZ *and* WILLIAM L. LAMAR

ORGANIC SUBSTANCES IN WATER

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Identification, measurement, and stability of phenoxy acid herbicides in water samples

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By DONALD F. GOERLITZ and WILLIAM L. LAMAR

ABSTRACT

A sensitive gas chromatographic method using microcoulometric titration and electron-capture detection for the analysis of 2,4-D, silvex, 2,4,5-T, and other phenoxy acid herbicides in water is described. The herbicides are extracted from unfiltered water samples (800–1,000 ml) by use of ethyl ether; then the herbicides are concentrated and esterified. To allow the analyst a choice, two esterification procedures—using either boron trifluoride-methanol or diazomethane—are evaluated.

Microcoulometric gas chromatography is specific for the detection of halogenated compounds such as the phenoxy acid herbicides whereas it does not respond to nonhalogenated components. Microcoulometric gas chromatography requires care and patience. It is not convenient for rapid screening of 1-liter samples that contain less than 1 microgram of the herbicide. Although electron-capture gas chromatography is less selective and more critically affected by interfering substances, it is, nevertheless, convenient and more sensitive than microcoulometric gas chromatography.

Two different liquid phases are used in the gas chromatographic columns—DC–200 silicone in one column and QF–1 silicone in the other. The performance of both columns is improved by the addition of Carbowax 20M. The Gas Chrom Q support is coated with the liquid phases by the "frontal-analysis" technique.

The practical lower limits for measurement of the phenoxy acid herbicides in water primarily depend upon the sample size, interferences present, and instrumentation used. With 1-liter samples of water, the practical lower limits of measurement are 10 ppt (parts per trillion) for 2,4-D and 2 ppt for silvex and 2,4,5–T when electron-capture detection is used, and approximately 20 ppt for each herbicide when analyzed by microcoulometric-titration gas chromatography.

Recoveries of the herbicides immediately after addition to unfiltered water samples averaged 92 percent for 2,4-D, 90 percent for silvex, and 98 percent for 2,4,5-T. Studies on the stability of herbicides added to water samples showed that 2,4-D may be rapidly degraded, especially if the samples are obtained from areas which have been repeatedly sprayed with 2,4-D. When degradation was observed, added 2,4-D rapidly decomposed within 10 days. At concentrations of about 200 ppt, however, the degradation rate was diminished. In 20 days the concentration of 2,4-D was reduced to 160–180 ppt.

INTRODUCTION

Phenoxy acid herbicides are used extensively for weed control. Esters and salts of 2,4-dichlorophenoxyacetic acid, 2-(2,4,5-trichlorophenoxy) propionic acid, and 2,4,5-trichlorophenoxacetic acid (commonly called 2,4-D, silvex, and 2,4,5-T, respectively) make up most of the organic herbicide production in the United States (Am. Chem. Soc., 1963). Silvex and 2,4-D in the form of water-soluble salts and water-soluble and insoluble esters are effective as aquatic herbicides. The general use of phenoxy acid herbicides and especially the increasing use of 2,4-D and silvex as aquatic herbicides in lakes, streams, and irrigation canals makes apparent the need for rapid and convenient methods for the identification and measurement of these substances in water.

Early methods of analysis for 2,4-D in agricultural products were colorimetric procedures using chromotropic acid (Freed, 1948; Marquardt and Luce, 1951). Yip (1962) reported a gas chromatographic method for the analysis of 2,4-D in spinach and wheat which involved extracting this herbicide by use of solvents, and then several treatments to isolate the acid. The relatively nonvolatile acid was converted to the methyl ester by use of diazomethane, and the herbicide was determined by microcoulometric gas chromatography. Since Yip's (1962) contribution, many workers have developed gas chromatographic methods for the analysis of herbicides in plant and food products using different gas chromatigraphic columns, detectors, and other esterification techniques.

Aly and Faust (1963) used chromotropic acid for the determination of 2,4-D in surface waters by colorimetric and ultraviolet absorption techniques. They stated that the major analytical interferences are the naturally occurring color materials in surface water and the impurity, 2,4-dichlorophenol, in the commercial formulation. Silicic acid column chromatography was used to remove these interfering substances. The colorimetric method is sensitive to 7 μg per l (micrograms per liter) whereas the ultraviolet procedure is sensitive to 30 μg (micrograms).

Abbott, Hammond, and Thomson (1964) published a thin-layer and paper-chromatographic procedure for the determination of six organochlorine herbicides in soil and water. These compounds included 2,4-D and 2,4,5-T. The phenoxy acid herbicides were quantitatively extracted from acidified water samples by ethyl ether and concentrated before chromatographic spotting. The sensitivity stated is about 0.5 μg on the paper chromatogram.

A brief procedure was developed by Gutenmann and Lisk (1964) for the electron-capture gas chromatographic analysis of silvex in water. The method is sensitive to 0.05 mg per l (milligram per liter) based on

a 25-g (gram) water sample, and the authors suggest using the technique to trace the disappearance of silvex in water.

Goerlitz and Lamar (1965) published a preliminary report on the application of microcoulometric gas chromatography for identification and measurement of 2,4-D, silvex, and 2,4,5-T in water. The practical limit of measurement for these herbicides in 4-liter water samples was reported to be 10 ppt (parts per trillion).

Storrs and Burchfield (1962) reported that 2,4,-D and related compounds were not quantitatively esterified by hydrochloric acid-methanol or boron trifluoride-methanol procedures commonly used to esterify fatty acids. Diazomethane was avoided because of its extreme toxicity. The use of a suitable internal standard was proposed to bypass the many difficulties in residue analysis.

Bevenue, Zweig, and Nash (1962) modified Yip's (1962) procedure for the analysis of 2,4-D in dry crops and walnuts. They reported quantitative esterification of 2,4-D using diazomethane even with traces of water present and also that gas chromatographic column efficiency (recovery) was 100 ± 5 percent. The method is sensitive to 0.05 ppm (part per million) of 2,4-D in 25–50 g of crop sample.

Information on the persistence of the phenoxy acid herbicides in water supplies is sparse. Newman and Thomas (1950) gave information on decomposition of 2,4-D in soil and liquid media exposed to soil that had previously been treated with the herbicide. Aly and Faust (1964) studied biological decomposition of 2,4-D and several of its esters in lake waters and bottom muds. The 2,4-D persisted for 120 days in aerated natural lake waters. Esters of 2,4-D were oxidized in 7–9 days using a domestic sewage seed, but the evidence indicated that only the alcohol part of the esters was involved and the 2,4-D acid remained. Aly and Faust (1964) found, by adapting the microorganisms in lake-bottom mud to increasing amounts of 2,4-D, that subsequent doses of 2,4-D were degraded within 24 hours.

The purpose of the research work described in this paper was to develop a convenient and sensitive method for the analysis in water of 2,4-D, silvex, 2,4,5-T, and related compounds. Both electron-capture gas chromatography and microcoulometric gas chromatography were investigated. Because Storrs and Burchfield (1962) had indicated that the boron trifluoride-methanol esterification is not quantitative and because some analysts are reluctant to use diazomethane for esterification owing to its extreme toxicity, the evaluation of both procedures is pertinent. Further, the results of this research study will provide the analyst with substantive grounds for a choice of procedures.

The boron trifluoride-methanol esterification reported by Metcalfe and Schmitz (1961) and the diazomethane esterification given by Schlenk and Gellerman (1960) were evaluated and modified.

This paper also serves to provide information on another significant problem, that is, the stability of the herbicides in surface-water samples. Several studies (Alexander, 1965; Aly and Faust, 1964; and Newman and Thomas, 1950) have indicated that 2,4-D is particularly susceptible to rapid degradation, whereas silvex and 2,4,5-T are resistant to biological attack. Thus, infomation concerning the stability of the herbicides is important for the reliable analysis of 2,4-D, silvex, 2,4,5-T, and related compounds in water.

APPARATUS

Microcoulometric-titrating gas chromatograph.—An Aerograph Hy-FI, model 550–B gas chromatographic oven specially modified for coupling the gas chromatographic column to the combustion unit. A quartz liner is used in the injection port, and the carrier gas is regulated with a differential-flow controller. The microcoulometric titrating system is a product of the Dohrmann Instrument Co. and consists of model S–100 sample inlet-combustion unit, model C–200 microcoulometer, and model T–300–S titration cell.

Electron-capture gas chromatograph.—An Aerograph Hy-FI, model 610–D, equipped with electron-capture detector, quartz-injection port-liner insert, isothermal temperature controller model 328, and carrier-gas-differential flow controller.

Concentrating apparatus.—A Kuderna-Danish concentrator, 250 ml capacity with a 1-ball Snyder column, is used for the initial concentration step. Final concentration is performed in the receiver using a 1-ball Snyder microcolumn. A 4.00-ml graduated receiver tube is used for the diazomethane esterification, and a 5.00-ml volumetric flask receiver is utilized for the boron trifluoride-methanol esterification.

Gas chromatographic columns.—The gas chromatographic columns are fabricated in a coiled configuration from 5-foot lengths of borosilicate glass tubing. Four analytical columns were prepared and used —two each for electron capture and for microcoulometric analysis. The two different columns for electron-capture gas chromatography are 3 mm (millimeters) OD (outside diameter) and 1.5 mm ID (inside diameter), packed with 60–80 mesh Gas Chrom Q support, previously coated as follows: (1) with 5 percent by weight DC–200 (viscosity 12,500 centistokes) and 0.5 percent by weight Carbowax 20M; (2) with 5 percent by weight QF–1 fluorinated silicone oil (also designated FS–1265) and 0.5 percent by weight Carbowax 20M. The two different columns for microcoulometry are 6 mm OD and 4 mm ID, packed with 60–80 mesh Gas Chrom Q, support previously coated as follows: (1) with 10 percent by weight DC–200 silicone oil and 0.5 percent by weight Carbowax 20M; (2) with 10 percent by weight QF–1 and 0.5 percent

by weight Carbowax 20M. The Gas Chrom Q support is coated in each instance by the "frontal-analysis" technique (Smith, 1960). Chloroform is used as the solvent for DC–200 silicone oil and Carbowax 20M mixture. A solution of 1:1 v/v (volume per volume) acetone-chloroform is used as solvent for the QF–1 silicone oil and Carbowax 20M.

Recorder.—Honeywell Brown Electronik, class 15, 0.9166 mv (millivolt) nominal span, 1-second pen speed, ½-inch-per-minute chart speed. The recorder has a Disc chart integrator.

Sandbath.—Tecam fluidized sandbath.

Saponification apparatus.—Erlenmeyer flasks, 250 ml capacity with 24/40 ground-glass joint to accept 1-ball Snyder column.

REAGENTS

All reagents should be checked for purity as reagent blanks by the gas chromatographic procedure. Much time and effort is saved by selecting high-quality reagents that do not require further preparation. However, some purification of reagents may be necessary as outlined below. If more rigorous treatment is indicated, the procurement of the reagent from an alternate source may eliminate this necessity.

Boron trifluoride-methanol.—Esterification reagent, 14 percent boron trifluoride by weight.

Benzene.—Suitable for pesticide-residue analysis, such as Nanograde (distilled in glass and meeting A.C.S. specifications).

2-(2-Ethoxyethoxy)ethanol.—High purity, N_D 26°C 1.4068.

Water.—Distilled water obtained from a high-purity tin-lined still. The feed water is passed through an activated carbon filter. The distillate is collected in a tin-silver-lined storage tank and the water is constantly irradiated with ultraviolet light during storage. A gravity delivery system is used, and no plastic material other than Teflon is allowed to contact the distilled water.

Ethyl ether.—Reagent grade, redistilled from an all-glass packed column still, after refluxing over granulated sodium-lead alloy for 8 hours. Purity is checked by gas chromatography after a part is evaporated to one-tenth of the original volume.

Florisil adsorbent.—A synthetic magnesium silicate 60–100 mesh, factory activated at 650°C. The Florisil is stored in an oven at 130°C and is checked from time to time for contamination. To determine the presence of electron-capturing components, about 1 g of the material is washed with 1 ml of benzene, and the washings are analyzed by gas chromatography.

Herbicides.—Chlorinated phenoxy acids, analytical grade; 2,4-D mp (melting point) 138–139°C, silvex, mp 181–182°C, and 2,4,5-T,

mp 154–155°C. To ensure selection of high-quality standards for this study, each phenoxy acid herbicide was obtained from at least two different sources and recrystallized from an acetone-water solution. The crystals were dried in a vacuum oven at 35°C for 8 hours. Melting points were determined. The chlorine was measured using microcoulometric gas chromatography, and the methyl esters for this purpose were prepared using diazomethane in 10 percent v/v methanol-ether solution. The recrystallized acids which exceeded 98 percent purity and which appeared as a single component on the chromatogram were selected as standards for this study. Methyl esters of the herbicides used to obtain gas-chromatographic calibration data were prepared from the recrystallized acids. About 5 g of methyl ester for each acid was prepared using boron trifluoride-methanol. After reaction, the esters were taken into ether solution, washed once with distilled water, once with 5 percent sodium carbonate, and then twice with distilled water. The ether solutions were dried over anhydrous sodium sulfate, filtered, and the ether removed on a rotary vacuum evaporator. The crystallized esters were finally dried at 30°C for 8 hours in a vacuum oven.

Methanol.—Reagent grade, redistilled from an all-glass packed column still after reacting with 5 g of magnesium turnings per liter of solvent.

N-*methyl*-N-*nitroso*-p-*toluenesulfonamide.*—Melting range 60–62°C.

Potassium hydroxide reagent.—A 37 percent aqueous solution, prepared from reagent-grade pellets and distilled water, is treated by refluxing for 8 hours to reduce interfering substances. An Ascarite-filled calcium chloride tube is used at the top of the condenser to exclude carbon dioxide.

Silicic acid.—Chromatographic grade, 100 mesh, stored at 130°C. This material is checked for contamination by washing 1 g with 1 ml of ether and by analyzing the washings by gas chromatography.

Sodium sulfate.— Reagent grade, anhydrous, granular; heat treated at 300°C for 24 hours. The heat-treated material is divided, and one part is labeled "neutral sodium sulfate" and stored at 130°C. The other part is slurried with enough ether to cover the crystals and acidified to pH 4 by adding a few drops of purified sulfuric acid. To determine the pH, a small quantity of the slurry is removed, the ether evaporated, water is added to cover the crystals, and the pH is measured on a pH meter. The ether is removed by vacuum from the acidified sodium sulfate. This fraction is labeled "acidified sodium sulfate" and stored at 130°C.

Sulfuric acid.—Reagent grade, purified by distilling off water until a constant boiling solution remains. The acid is refluxed for about 4 hours.

GAS-CHROMATOGRAPHIC PROCEDURE

All glassware, except volumetric flasks and pipettes, is heat treated at 300°C overnight to remove organic contamination. Glassware and glass wool which come in contact with the phenoxy acid herbicides are rinsed with dilute hydrochloric acid prior to the heat treatment. The volumetric glassware is cleaned in the conventional manner with sodium dichromate in concentrated sulfuric acid. This cleaning is followed by a rinsing in dilute hydrochloric acid and steam cleaning for 20 minutes. The steam is generated from an all-glass apparatus using a solution of basic potassium permanganate in "organic-free" distilled water.

EXTRACTION AND HYDROLYSIS

The extraction of the phenoxy acids from unfiltered water samples and the subsequent hydrolysis are conducted in the following manner:

1. Acidify (pH 2.0) the water sample (approximately 800 ml) with concentrated sulfuric acid.
2. Pour the sample into a 1-liter separatory funnel. Add 150 ml of ether to the sample bottle, rinse the sides, and pour the solvent into the separatory funnel. Shake the mixture vigorously for 1 minute.
3. Allow the contents to separate for at least 10 minutes. Occasionally, emulsions may prevent adequate separation. In this event, draw off the separated aqueous layer, invert the separatory funnel and shake rapidly. **Caution:** Vent the funnel frequently to prevent excessive pressure buildup. Addition of small measured volumes of distilled water often aids removal of sediment from the ether layer. Collect the extract in a 250 ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.
4. Extract the sample two more times, using 50 ml of ether each time, and combine the extracts in the 250 ml Erlenmeyer flask.
5. Add 15 ml of distilled water and a small boiling chip to the flask containing the ether extract and fit the flask with a 1-ball Snyder column. Remove the ether on a steam bath and continue heating for a total of 90 minutes.
6. Transfer the concentrate to a 60-ml separatory funnel. Extract the basic solution once with 20 ml and two more times each with 10 ml of ether and discard the ether layers. The herbicides remain in the aqueous phase.
7. Add 2 ml of cold 25 percent sulfuric acid to the contents of the separatory funnel to bring the pH below 2.0 and extract the herbicides once with 20 ml and two times each with 10 ml. of ether. Collect the extracts in a 125-ml Erlenmeyer flask contain-

ing about 0.5 g of acidified anhydrous sodium sulfate. Allow the extract to remain in contact with the sodium sulfate preferably in an explosion-proof refrigerator for at least 2 hours.

8. Transfer the ether solution into the Kuderna-Danish apparatus through a funnel plugged with glass wool; use liberal washings of ether. It is necessary to crush any hardened sodium sulfate to obtain a quantitative transfer.
9. Concentrate the extract to about 0.5 ml using a fluidized sandbath heated to 60°–70°C. Do not allow the extract to evaporate completely.

ESTERIFICATION WITH DIAZOMETHANE

1. A 4.00-ml graduated receiver tube is used with the Kuderna-Danish apparatus when the sample is to be esterified with diazomethane. Add a quantity of anhydrous methanol equal to 10 percent of the volume of the concentrated extract.
2. Connect two 20 by 150-mm test tubes in series with glass tubing through neoprene stoppers so that incoming nitrogen gas bubbles through liquid in the tubes. At the outlet, position a piece of glass tubing with a right-angle bend and a drawn-out tip so that the gas can be bubbled through the sample.
3. Add about 5 ml of ether to the first test tube. To the second test tube add 0.7 ml of ether, 0.7 ml of 2-(2-ethoxyethoxy)ethanol, 1.0 ml of 37-percent aqueous potassium hydroxide solution, and 0.1–0.2 g of N-methyl-N-nitroso-p-toluenesulfonamide.
4. Immediately position the second test tube and adjust the nitrogen flow through the apparatus to about 10 ml per minute. **Caution:** Diazomethane is a toxic and explosive gas. The use of a good fume hood is absolutely necessary.
5. Place the Kuderna-Danish receiver so that the gas bubbles through the sample. Allow the reaction to proceed for about 10 minutes, or less if the yellow color of diazomethane can be observed to persist in the sample tube.
6. Remove the receiver containing the sample; stopper the receiver and allow to stand in the hood for about 30 minutes. Carefully discard all waste from the reaction.
7. Add about 0.1–0.2 g of silicic acid to the sample solution to destroy excess diazomethane. After the evolution of nitrogen has subsided, pass the solution through a disposable pipette plugged with glass wool and packed with 1.5 cm (centimeters) of neutral anhydrous sodium sulfate over 1.5 cm of Florisil adsorbent. The eluate is collected in a graduated receiver tube. The transfer is completed by washing the receiver tube several times with small quantities of ether to a final volume of 2.00 ml.

8. The tube is stoppered and the contents thoroughly mixed and analyzed by gas chromatography.
9. To approach the practical limit of detectability, further concentration of the esterified extract is necessary. Add a minute boiling chip and fit the graduated receiver tube with a small 1-ball Snyder column. Concentrate the extract on the sandbath. Accelerate the solvent removal by gently aspirating the vapors from the column just above the ball. Do not allow the receiver to go dry. Remove the apparatus from the heat and allow the vapors to condense and wash down the sides of the tube. The final volume of the concentrate, usually 0.05–0.20 ml, depends on the volume of the apparatus. The volume may be further reduced by directing a gentle stream of nitrogen on to the surface of the liquid. Mix the contents by pumping with a syringe and analyze the solution by gas chromatography.

ESTERIFICATION WITH BORON TRIFLUORIDE-METHANOL

1. A 5.00-ml volumetric receiver flask is used with the Kuderna-Danish apparatus when the sample is to be esterified with boron trifluoride-methanol reagent. Prior to the concentration step, 0.5 ml of benzene is added to the extract in the Kuderna-Danish apparatus. The extract is concentrated to less than 1 ml, and the walls of the flask are washed down with a small amount of ether. The receiver is fitted with a small 1-ball Snyder column, and the extract is concentrated to 0.5 ml in the sandbath.
2. After the benzene solution in the receiver has cooled, 0.5 ml of boron trifluoride-methanol reagent is added. The small 1-ball Snyder column is used as an air-cooled condenser, and the contents of the receiver are held at 50°C for 30 minutes in a sandbath. Afterwards the reaction mixture is allowed to cool to room temperature.
3. About 4.5 ml of 5-percent aqueous sodium sulfate solution is added to the reaction mixture so that the benzene-water interface is observed in the neck of the Kuderna-Danish receiver flask. Close the flask with a ground-glass stopper and shake vigorously for about 1 minute. Allow to stand for about 3 minutes for phase separation. Twirling the flask between the palms of the hands from time to time aids separation.
4. The benzene layer is pipetted from the receiver and passed through a small column prepared by plugging a disposable pipette with glass wool and packing with 2.0 cm of neutral anhydrous sodium sulfate over 1.5 cm of Florisil adsorbent. The eluate is collected in a 2.50-ml graduated centrifuge tube. The transfer is completed by repeating the extraction step with small quantities of benzene until a final volume of 2.00 ml is attained.

5. Add a few crystals of neutral anhydrous sodium sulfate to the benzene solution and thoroughly mix for gas chromatographic analysis.

RESULTS AND DISCUSSION

The gas chromatographic columns used in this procedure are described in the apparatus section. Initially, Chromosorb P, Chromosorb W, and Chromosorb G were tried as solid supports. These were found to be unsatisfactory when coated with silicone oils alone. Excessive tailing and poor resolution of the herbicide methyl esters suggested interaction of these compounds with the solid supports. The elution characteristics are improved when silanized Chromosorb W is used. Carbowax treatment (Lamar and others, 1966), originally used to improve the elution characteristics of insecticides, is also effective for the herbicide methyl esters. The addition of 0.5 percent Carbowax 20M to the silanized Chromosorb W during the coating operation not only improves the elution characteristics of the esters from the column but also improves the quantitative aspects of the technique. The recovery of the herbicide methyl esters as determined by microcoulometric gas chromatography closely approaches 100 percent of the theoretical value when Carbowax 20M is used in the column. The columns as described require only about 24 hours of preconditioning before use for either electron-capture or microcoulometric gas chromatography.

The Gas Chrom Q support was later chosen because it appears to allow a more symmetrical elution of the herbicide methyl esters. Figures 1 and 2 are reproductions of chromatograms obtained by using the columns described and electron-capture detection. The uncorrected retention times relative to 2,4-D methyl ester on the DC–200 column are 1.00 for 2,4-D methyl ester, 1.42 for silvex methyl ester, and 1.91 for 2,4,5-T methyl ester. These values were obtained at a column temperature of 156°C, an injection-port temperature of 190°C, a detector temperature of 196°C, and a nitrogen carrier-gas flow rate of 45 ml per minute. The chromatogram of the herbicides separated on the DC–200 column is shown in figure 1.

The uncorrected relative retention times on the QF–1 column are 1.00 for 2,4-D methyl ester, 1.22 for silvex methyl ester, and 1.80 for 2,4,5-T methyl ester. These data were obtained at a column temperature of 145°C, an injector-port temperature of 190°C, a detector temperature of 177°C, and a nitrogen carrier-gas flow of 45 ml per minute. The separation of the herbicides as shown in figure 2 on the QF–1 column is not complete but it is adequate. The heavier loaded QF–1 column used in the microcoulometric gas chromatograph separates 2,4-D and silvex methyl esters completely. The difference in rel-

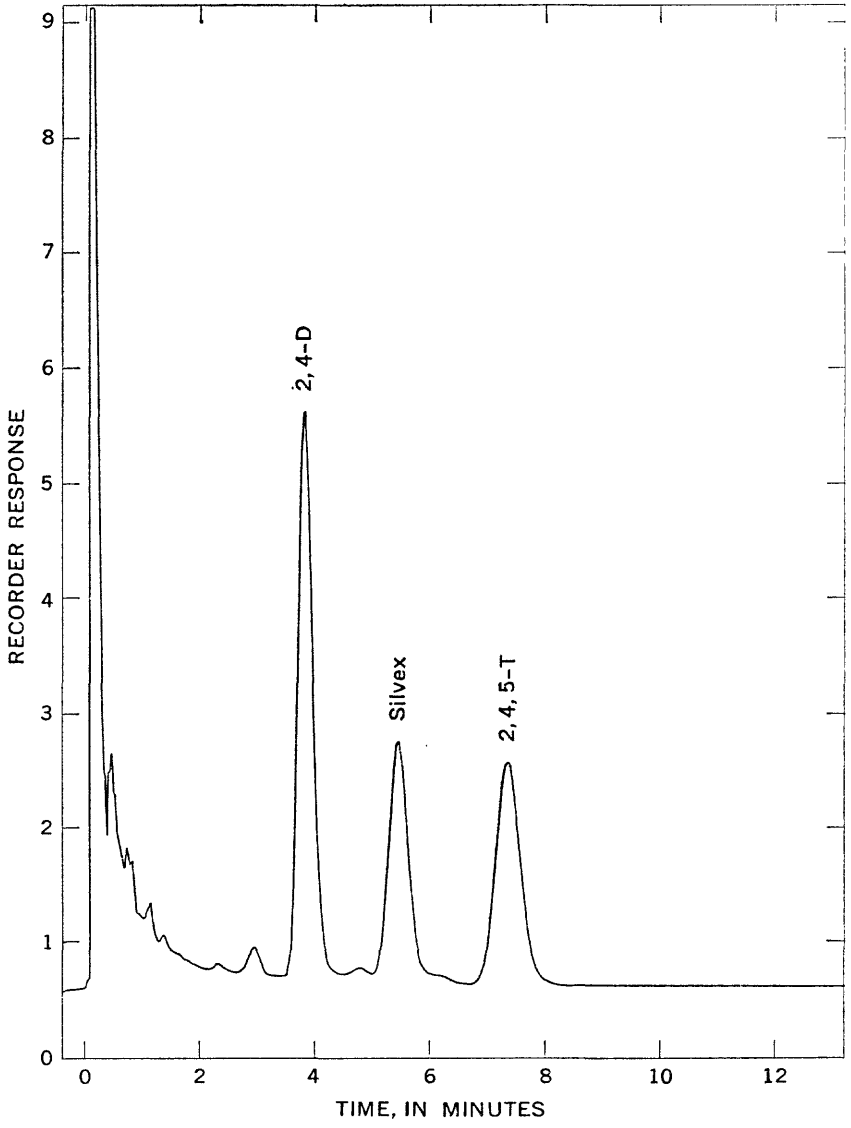

FIGURE 1.—Chromatogram of herbicide methyl esters on DC–200 column.

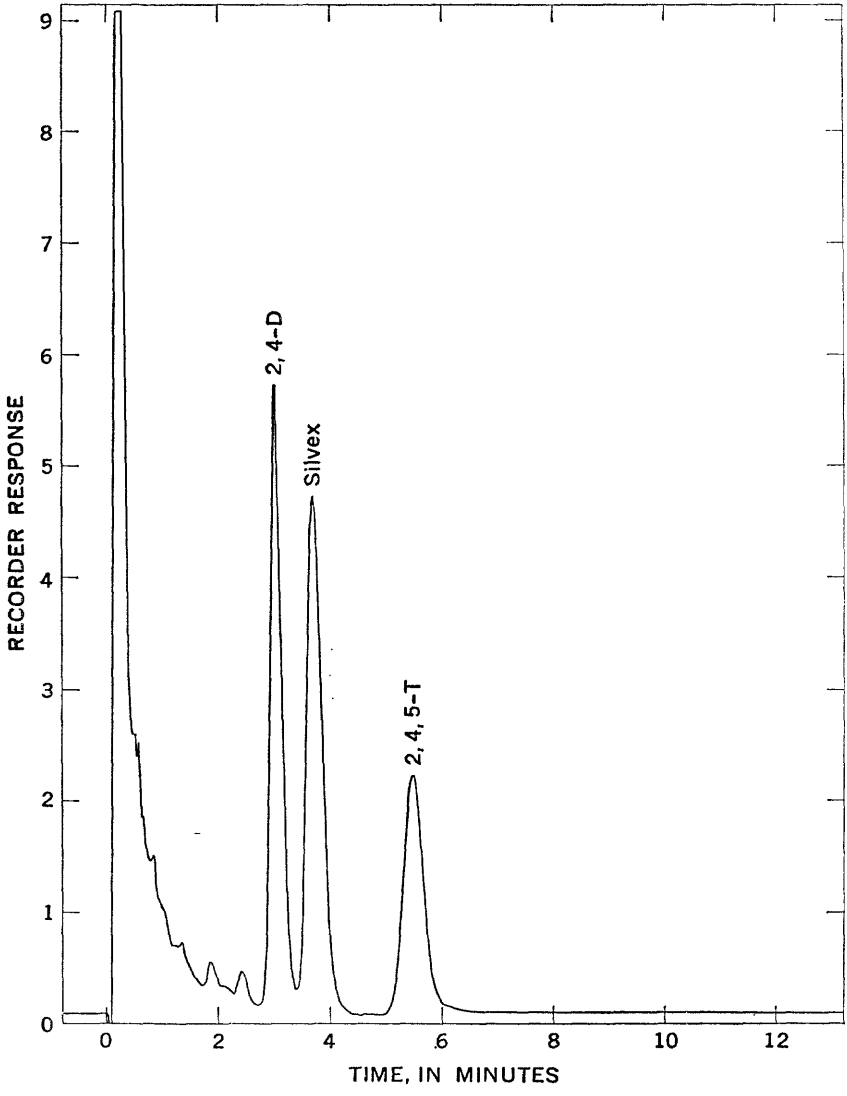

FIGURE 2.—Chromatogram of herbicide methyl esters on QF–1 column.

ative retention times aids identification when the two-column analysis is used.

Detector-response curves for both chromatographs were prepared from purified methyl esters of each acid. Additional standards were prepared from stock solutions so that the response curves could be reestablished to correct instrument-response fluctuations. During the recovery studies, standards were prepared daily, and their concentrations were adjusted so that the quantity injected was equivalent to that in the sample. The response was recorded in square inches as a function of the amount injected into the chromatograph.

Injections of 5μl (microliters) of standards and esterified sample extracts were used for electron-capture gas chromatography and as much as 40μl injections were used for microcoulometric gas chromatography. The practice of reading the syringe before and after injection was observed. Septums were replaced after about 15 penetrations.

When esterification procedures were initially investigated, poor recoveries of the herbicides resulted, especially at low concentrations. The reason for these low recoveries was found to be caused by the basicity of the glassware and of the anhydrous sodium sulfate. When this was corrected by acid treatment, the results were quantitative for both esterification procedures. Each step of the analysis was checked during the developmental stages. The data in table 1 are the results of electron-capture analysis of six prepared samples of herbicides in ether solution carried through the saponification and concentration steps of the procedure and treated in the following manner: Three of the samples were esterified with boron trifluoride-methanol; the other three were esterified with diazomethane. The average recovery of the three herbicides using boron trifluoride-methanol was 98 percent and the average recovery using diazomethane was 94 percent.

Initially, a 1 : 1 v/v benzene-ether solution was used to extract quantitatively the herbicide acids and esters from 4-liter water samples (Goerlitz and Lamar, 1965). The purpose of the benzene was to retard the ether from dissolving in the aqueous phase. It was found, however, that benzene was not effective for this purpose. Furthermore, many analysts seek to avoid the continued use of benzene because this solvent is a chronic systemic poison. Subsequently, 1-liter water samples were analyzed and ether alone was used as the extractant. It is necessary to use a large quantity of ether in the first extraction because 60–80 ml dissolve in the aqueous phase. The extraction set forth in the procedure is quantitative, provided the pH of the aqueous phase is 2 or below. The distribution coefficient for 2,4-D in an ether and water

TABLE 1.—*A comparison of two esterification procedures*

Test	Esterification procedure	Herbicide	Amount added (nanograms)[1]	Recovered	
				Nanograms	Percent
1	BF_3-CH_3OH	2,4-D	669	678	101
		Silvex	34	31	91
		2,4,5-T	64	67	105
2	BF_3-CH_3OH	2,4-D	669	693	104
		Silvex	34	32	94
		2,4,5-T	64	69	108
3	BF_3-CH_3OH	2,4-D	669	635	95
		Silvex	34	30	88
		2,4,5-T	64	63	98
4	CH_2N_2	2,4-D	669	622	93
		Silvex	34	33	97
		2,4,5-T	64	57	89
5	CH_2N_2	2,4-D	669	657	98
		Silvex	34	36	106
		2,4,5-T	64	65	102
6	CH_2N_2	2,4-D	669	564	84
		Silvex	34	31	91
		2,4,5-T	64	53	83

[1] A nanogram is 10^{-9} gram.

mixture was found to be greater than 95. This value was determined by electron-capture gas chromatographic analysis of a fraction of the ether layer after thorough mixing of equal volumes of ether and ether-saturated distilled water at pH 2.0. Three independent trials at three different concentrations of 2,4-D were made at a temperature of 25°C±1°C.

To calculate the quantity of herbicide acid removed with each extraction, assume a distribution coefficient of 95 for ether-ether saturated water at pH 2.0. Extracting 1 liter of water with 50 ml of ether removes 82.6 percent of the herbicide. Three such extractions recover 99.5 percent of the acids. In actual tests, the recovery of the three herbicides from distilled water after three independent trials averaged 96 percent. The extracts were concentrated and esterified by the diazomethane procedure.

Six esters of the herbicides were obtained for evaluating the saponification step of the procedure. The results of this experiment are shown in table 2. Except for the purified methyl ester of 2,4,5-T, the other esters were technical-grade compounds. The recovery values from this experiment, averaging 97 percent, indicate that the saponification is adequate for the analysis of herbicide esters in water.

TABLE 2.—*Saponification of selected herbicide esters*

Ester	Amount added (nanograms)	Recovered	
		Nanograms	Percent
2,4-D propylene glycol butylether ester	659	518	79
2,4-D isopropyl ester	420	425	101
Silvex butoxyethanol ester	219	201	92
Silvex 2-ethylhexyl ester	212	175	83
2,4,5-T methyl ester	190	205	108
2,4,5-T butyl ester	164	197	120

As a final procedure check, recovery studies were made on actual samples obtained from surface water in California and Florida. The samples were collected in 4-liter bottles and sealed by aluminum-foil-covered rubber stoppers. Each sample was composited in the laboratory in a 5-gallon carboy having a stopcock at the bottom. The water-sediment composite was mixed with a Lightnin stirrer and the sample was divided, during the constant stirring, into 12 subsamples of about 800 ml each.

Purified herbicide acids were weighed into volumetric flasks—100-ml flasks were used for 2,4-D and 500-ml flasks were used for silvex and 2,4,5-T. An excess of potassium hydroxide was added and the flasks were brought to volume with distilled water. Appropriate amounts of the herbicide solutions were added to six of the subsamples. The remaining six subsamples were retained as controls. Duplicate subsamples and controls were analyzed immediately, at 10 days, and again at 20 days after "spiking" (adding the herbicides). The data from these experiments obtained by electron-capture gas chromatography, using boron trifluoride-methanol esterification, are shown in table 3. The samples were stored at 72°–74°F in the dark for the duration of the experiment. The pH of the water was measured before spiking and at the beginning of each operation. No significant pH change was noted. The recovery data immediately after spiking show the reliability of the analytical method. These values range from 81 to 110 percent. The average recovery is 92 percent for 2,4-D, 90 percent for silvex, and 98 percent for 2,4,5,-T.

The recoveries for the samples analyzed immediately after adding the herbicides are acceptable for the levels used, although a few are somewhat low. Apparently, a considerable amount of extraneous material eluted with the herbicide methyl esters, because it took about 20 minutes for the recorder pen to return to the baseline. Initially, the elevated baseline was most apparent for the sample from Eagle Bay and the recovery values were lower, averaging less than 85 percent.

TABLE 3.—*Recovery and degradation studies of phenoxy acid herbicides in surface-water samples*

Designation	Herbicide	Amount added (nanograms)	Percent recovered after addition		
			Immediately	10 days later	20 days later
San Joaquin River near Tracy, Calif.	2,4-D	308	94	66	52
	Silvex	70	87	84	84
	2,4,5-T	92	86	68	79
American River at Sacramento, Calif.	2,4-D	308	82	98	110
	Silvex	70	99	97	123
	2,4,5-T	92	110	90	109
Sacramento River at Sacramento, Calif.	2,4-D	308	90	95	99
	Silvex	70	81	111	100
	2,4,5-T	92	104	104	108
Cosumnes River at Michigan Bar, Calif.	2,4-D	470	91	92	82
	Silvex	126	91	92	81
	2,4,5-T	140	86	85	87
Mokelumne River near Clements, Calif.	2,4-D	470	97	75	74
	Silvex	126	86	76	85
	2,4,5-T	140	96	73	81
Eagle Bay at Okeechobee, Fla.	2,4-D	515	99	3	0
	Silvex	222	98	100	101
	2,4,5-T	221	104	80	80

This water was highly colored (170 units on the platinum-cobalt scale) by "humic acids" (Lamar and Goerlitz, 1966). Whenever the baseline is noticeable raised on the electron-capture chromatogram, the response of the detector is somewhat reduced. A similar situation may be observed when column material from a newly prepared column elutes through the detector.

Recoveries averaging 100 percent were obtained for the Eagle Bay sample after a large amount of the extraneous material was removed by the Florisil treatment. The baseline was only slightly elevated, about the same as for a benzene solution of pure methyl esters. These observations indicate that the Florisil treatment effectively reduces the interferences from the natural organic matter.

Table 3 also shows the results of analyses following periods of sample storage after spiking. Disappearance of added 2,4-D in the sample from Eagle Bay clearly shows that degradation in the sample bottle does occur. This sample is the only one that contained 2,4-D (180 ppt) prior to spiking. It was obtained from an area which had been sprayed with 2,4-D amine about bimonthly for more than a year. Apparently, acclimated micro-organisms capable of decomposing 2,4-D were present in the sample. Losses of added 2,4-D were also observed in the

sample from the San Joaquin River, Calif. Because the San Joaquin River passes through an area of intensive agricultural activity, it is conceivable that acclimated micro-organisms were present. The other California rivers selected for study have less exposure to agricultural activity, and this fact may explain why no significant degradation was observed for these streams.

When degradation was observed, the added 2,4-D rapidly decomposed to about 200 ppt (parts per trillion) within 10 days. At this concentration, however, the rate of degradation was diminished. In 20 days the concentration of 2,4-D was reduced to the 160–180 ppt level. The reduction of 2,4-D to this level occurred (1) when 2,4-D was present in the sample as collected, (2) when 2,4-D was added to a sample which contained this herbicide, and (3) when 2,4-D was added to another sample which did not contain 2,4-D. It is significant that this degradation of 2,4-D occurred only in samples suspected of having acclimated micro-organisms from areas subject to repeated spraying with 2,4-D.

In an earlier work (Goerlitz and Lamar, 1965), surface-water samples from the Loxahatchee National Wildlife Refuge, Palm Beach County, Fla. were analyzed. Herbicide spraying, principally 2,4-D and silvex, had taken place in the area, and some of the samples were collected at the time of, and in the vicinity of, spraying operations. Very little 2,4,5-T (90 ppt or less) was detected because it was sprayed on few occasions. However, silvex was found at concentrations as high as 51,500 ppt. Although spraying records obtained by the authors show that most of the spraying was done with 2,4-D, the analytical results indicated that the 2,4-D in the samples at the time of analysis was not greater than 250 ppt. A subsequent field study designed to determine the distribution of applied 2,4-D amine in the canal cross section resulted in finding levels below the anticipated concentrations. Even though the water samples were analyzed promptly after receipt in the laboratory, it is apparent that 2,4-D degradation had already occurred. Decomposition of 2,4-D in the water was considered, but the rapidity of the degradation was not anticipated.

The indications that degradation of 2,4-D had occurred in the Loxahatchee studies prompted investigation of phenoxy acid herbicide stability in various surface-water samples. In retrospect, it is noted that whenever 2,4-D was found in the water, it was at concentrations less than 250 ppt. The observations from previous investigations and the stability studies described in this report support a conclusion that 2,4-D may be rapidly decomposed by acclimated micro-organisms but that at about 200 ppt the rate of degradation is significantly reduced.

SUMMARY

A sensitive gas chromatographic method using microcoulometric titration and electron-capture detection for the analysis of 2,4-D, silvex, 2,4,5-T, and other phenoxy acid herbicides in water is described. The herbicides are extracted from unfiltered water samples (800–1000 ml) by use of ethyl ether, and are then concentrated and esterified. Two esterification procedures—boron trifluoride-methanol and diazomethane—are evaluated. Two types of analytical columns are used—DC–200 and QF–1, which are improved by the addition of Carbowax 20M. Recovery studies and stability of the herbicides with time are presented. Degradation of 2,4-D by acclimated microorganisms is related to water from areas which have been repeatedly sprayed with this herbicide.

The analysis of a variety of water samples is difficult if different interfering substances are present. The microcoulometric gas chromatograph was selected because it provides specific detection of halogenated compounds. This detection system is least affected by interfering substances. Microcoulometric gas chromatography, however, requires care and patience by the analyst, and it is not suitable for rapid screening of 1-liter water samples that contain less than 1 μg of the herbicide.

For convenience, electron-capture gas chromatography was used in most of the developmental stages of the procedure. Although the electron-capture detector is less selective and requires more attention to cleanliness of apparatus and reagents, it has several distinct advantages over the microcoulometric-titrating detector. The electron-capture detector is more sensitive by several orders of magnitude. It is easier to operate and consequently a greater number of analyses may be performed per unit time.

Water-soluble amine salts and insoluble esters of 2,4-D, silvex, and 2,4,5–T are often used in and around water courses. It is possible for a given chlorinated phenoxy acid herbicide to exist in the water in various forms—salts, esters, and ester-hydrolysis products. Because of this possibility, it was decided to simplify the analysis by employing a saponification step to isolate the important acid part of the herbicide.

Many investigators prefer to avoid using diazomethane for esterification because of its dangerous nature. Boron trifluoride-methanol reagent was investigated as an alternative. Both boron trifluoride-methanol and diazomethane provided quantitative results. Boron trifluoride-methanol can also be used as an interesterification agent for preparing methyl esters from herbicide esters of the higher alcohols, and may be done without going through the saponification step. However, to perform interesterification or esterification at this point the concentrated extract must be relatively free from extraneous halogen-

ated or electron-capturing components—otherwise a meticulous "cleanup" procedure to remove interfering compounds is necessary.

The saponification step is in itself a "cleanup." Ether-soluble compounds are removed by extraction of the basic aqueous solution. Water samples with little organic matter usually need no more "cleanup" than the saponification. However, removal of extraneous matter is desirable, especially for analysis of highly colored water samples. The extractable organic material from the water reduces the separating ability of the gas-chromatographic columns. Even though this does not appear to affect the quantitative aspects of the microcoulometric-titrating detector, a sharper, more distinctive chromatogram is obtained if the esterification reaction mixture is passed through a small amount of Florisil adsorbent. If this procedure is used in the analysis of water samples having moderate amounts of agricultural or similar pollution, a gas-chromatographic column will function normally for more than a month of almost continuous use. This experience shows that it is more convenient to prepare gas-chromatographic columns than to process each sample through a laborious "cleanup" procedure. This finding also applies to electron-capture gas chromatography where, in addition to column degradation, detector fouling is a problem.

In regard to compounds that may cause interference with the herbicide analysis, boron trifluoride-methanol reagent is used to advantage for electron-capture gas chromatography whereas diazomethane reagent is the choice for microcoulometric gas chromatography. Boron trifluoride-methanol reagent reacts specifically with carboxylic acids to forms the methyl esters. Diazomethane reacts not only with carboxylic acids but it may also react with phenols and other compounds with relatively active hydrogens. The action of diazomethane may increase the volatility of the extraneous substances by allowing them to "bleed" through the chromatographic column and thus interfere with the electron-capture detector. This "bleeding" does not appear to hamper the operation of the microcoulometric detector, and it may extend the usefulness of the gas-chromatographic column over a longer period of time. Of the two esterification procedures, diazomethane is more convenient to use than boron trifluoride-methanol.

The practical lower limits for measurement of the phenoxy acid herbicides in water primarily depend upon the size of the sample and also the instrumentation used. If the extract from a 1-liter water sample is concentrated to 2.00 ml and a 5.0-μl part of the concentrate is injected into the electron-capture gas chromatograph, reliable measurement of 50 ppt of 2,4-D and 10 ppt of silvex and 2,4,5-T is feasible. Concentrating the extract to 0.50 ml permits detection of approximately 10 ppt of 2,4-D and 2 ppt of silvex and 2,4,5-T when 5.0 μl

aliquots are analyzed. The sensitivity of the electron-capture detector is often adversely affected by extraneous material in the sample or reagents. Concentrating the extract may progressively amplify this complication. Owing to this problem, the practical lower limits of measurement for the electron-capture gas chromatographic method are difficult to define.

The minimum reliable value or practical lower limit of measurement is approximately the same for each herbicide when analyzed by microcoulometric gas chromatography. Compared to the electron-capture detector, the relative insensitivity of the microcoulometric detector is somewhat offset by its mode of response. Because microcoulometric detection is specific, sensing only halogenated compounds, larger injections from more concentrated extracts are possible, and the lower limits of measurement are more easily defined. The injection of 40 μl from 2.00 ml of concentrate extract permits quantitative determination down to about 1,000 ppt for each of the herbicides. Concentrating the extract to 0.05 ml and injecting 40 μl (80 percent of the total sample) allows a minimum reliable measurement of approximately 20 ppt.

The practical limits of quantitative analysis were calculated from gas-chromatographic peaks having measureable areas and were not obtained from a statistical treatment. Because the absolute lower limit of detection (2 : 1 signal to noise ratio) is variable, depending on the sample and analytical conditions, no attempts were made to establish the specific minimum limits of detection for the compounds studied.

The surface-water samples obtained for this investigation were analyzed by use of both DC–200 and QF–1 chromatographic columns. The herbicides, detected by electron-capture gas chromatography, were also analyzed by microcoulometric gas chromatography to provide an additional parameter of identification.

Studies on the fate of the herbicides in water samples showed that 2,4-D may be rapidly degraded by biological action. This degradation was related to acclimated micro-organisms from areas subject to repeated spraying with 2,4-D, because samples from other areas did not show significant degradation in 20 days. The degradation was pronounced when the water supply had been repeatedly sprayed with 2,4-D.

When degradation was observed, 2,4-D added to samples rapidly decomposed to about 200 ppt within 10 days. At this concentration, however, the rate of degradation was diminished. The concentration of 2,4-D, found in the controls or found after the addition of this herbicide to the samples, was not reduced below the 160–180 ppt level in 20 days.

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