Determination of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Grain and Seed

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The 2,4-D acid is extracted from a dry sample of meal with an acidified ether-chloroform solvent. By further treatment, including a chromatographic separation, interfering materials are removed. The color produced by the reaction of 2,4-D acid with chromotropic acid in concentrated sulfuric acid is measured on a suitable spectrophotometer. As little as 5 γ of 2,4-D acid in a 200-gram sample can be detected.

THE USE OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D acid) as a weed killer on a variety of crops has made it desirable to develop an analytical method capable of detecting minute quantities of 2,4-D acid which may be picked up by the plant. The possibility of translocation of the 2,4-D acid must also be determined.

The method of Marquardt and Luce (*4*) was not adaptable directly to this problem, and the procedure of Gordon and Beroza (*3*) did not offer a means of separation from the substrata or indicate the possibility of detecting only trace quantities.

A method of extraction and separation of the 2,4-D acid was developed and the colorimetric method of Freed (*2*) further refined to give the accuracy demanded for this problem.

Reagents

Chloroform, technical grade. Ether, U.S.P. grade. Acetic acid, glacial. Sodium hydroxide, approximately 5*N*. Hydrochloric acid, concentrated. Sodium hydroxide, approximately 0.5*N*.

Phosphotungstic acid. Dissolve 40 grams of phosphotungstic acid (approximately $P_2O_5.24WO_3.25H_2O$) in water and dilute the solution to 100 ml.

Hyflo Super-Cel, a Celite product, diatomaceous silica, made by Johns-Manville.

Absolute methanol in U.S.P. chloroform, 3.0 and 7.0% by volume (see section on chromatographic separation of 2,4-D acid).

Buffered extraction solution, pH 6.85. Dissolve 19.0 grams of dibasic sodium phosphate, $Na_2HPO_4.7H_2O$, and 10.0 grams of monobasic sodium phosphate, $NaH_2PO_4.H_2O$, in water and dilute to exactly 1.0 liter.

Chloroform, analytical reagent grade.

Chromotropic acid. Dissolve 0.10 gram of the sodium salt of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in 100 ml. of concentrated sulfuric acid (95.5%).

Sulfuric acid–stannous chloride solution, dilute. Mix 200 ml. of concentrated sulfuric acid with 800 ml. of water and cool the dilute acid to room temperature; then dissolve 50 grams of stannous chloride, $SnCl_2.2H_2O$, in the acid.

2,4-Dichlorophenoxyacetic acid, standard solution. Dissolve 0.500 gram of 2,4-D acid in chloroform and dilute to 500 ml. Dilute a 10.0-ml. aliquot to 100 ml. with chloroform. Dilute a 10.0-ml. aliquot of this second solution to 100 ml. with chloroform. The final solution contains 0.01 mg. of 2,4-D acid per ml.

Apparatus

The tube for the chromatographic column is a glass tube, 17 mm. in inside diameter and 50 cm. long, one end of which is constricted and attached to a smaller glass tube, 5 mm. in inside diameter and 10 cm. long.

A suitable apparatus for regulating air pressure and an interval timer are also required.

A Coleman spectrophotometer, Model 11, equipped with a PC-4 filter and 5-cm. cuvettes, was used in this investigation. Any photometer measuring light transmittance at 565 mμ should be suitable.

Procedure

Grind the grain or seed to a fine meal to pass through a No. 10 sieve. In this work a No. 1 Wiley mill was used.

Place a 200-gram sample of the meal in a 2-liter glass-stoppered bottle, and add 500 ml. of technical grade chloroform, 500 ml. of ether, and 10 ml. of acetic acid. Stopper the bottle, fastening the stopper with Okonite tape. Place the bottle and contents in a shaking machine for 1 hour.

Use a 2-liter filtering flask, a Büchner funnel 16 cm. in diameter, and Whatman's No. 1 or similar grade filter paper. Rinse the bottle by adding 50 ml. of technical grade chloroform and 50 ml. of ether and wash the insoluble material with the rinsing solution.

Discard the insoluble material. Using a 2-liter separatory funnel, extract the 2,4-D acid from the filtrate once with a solution of 200 ml. of water and 50 ml. of 5*N* sodium hydroxide and twice with a solution of 200 ml. of water and 10 ml. of 5*N* sodium hydroxide. Shake well each time to ensure complete extractions. Discard the organic layer and wash the combined aqueous extracts three times with 100-ml. portions of technical grade chloroform.

Acidify the aqueous solution with 50 ml. of concentrated hydrochloric acid. Extract the 2,4-D acid from the solution with 125 ml. of ether. Repeat the extraction with 100 ml. of ether and combine the extracts. Discard the aqueous solution.

Filter the ether solution, using Whatman's No. 1 or equivalent grade filter paper, into a 250-ml. separatory funnel. Extract the 2,4-D acid from the filtered solution once with 50 ml. of 0.5*N* sodium hydroxide and twice with 25-ml. portions of 0.5*N* sodium hydroxide. Combine the caustic extracts.

Pour the caustic solution into the 250-ml. separatory funnel and wash it three times with 10-ml. portions of chloroform, discarding the washings. Acidify the solution with 10 ml. of concentrated hydrochloric acid and add 5 ml. of phosphotungstic acid solution. Extract the 2,4-D acid with three 10-ml. portions of chloroform and combine the extracts.

Prepare the column for the chromatographic separation by placing a plug of glass wool in the bottom of the tube at the constriction. Add Hyflo Super-Cel and settle it in the tube by bouncing the tube gently on a large rubber stopper. Continue adding more Super-Cel and bouncing the tube gently until the Super-Cel is packed in the tube for a distance of 20 cm.

Filter the chloroform solution into the tube, using No. 1 Whatman's or equivalent grade filter paper. Apply air pressure at the top of the tube to push the solution into the Super-Cel.

Using proper air pressure, percolate 150 ml. of the 3.0% methanol in chloroform solution through the column at the rate of 150 ml. per hour, followed by 25

ml. of 7.0% methanol in chloroform. Discard the effluent.

Then percolate 150 ml. of 7.0% methanol in chloroform through the column at the same rate, collecting the effluent in a 250-ml. beaker.

Pour the effluent into a 250-ml. separatory funnel. Extract the 2,4-D acid from the solution with three 25-ml. portions of buffered extraction solution and combine the extracts.

Return the extract solution to the separatory funnel and wash it three times with 10-ml. portions of analytical reagent grade chloroform. Discard the washings.

Acidify the solution with 2 ml. of concentrated hydrochloric acid and extract the 2,4-D acid with three 10-ml. portions of analytical reagent grade chloroform. Combine the extracts in a 30-ml. beaker.

Decant the chloroform solution from the globules of water adhering to the beaker into another clean, dry 30-ml. beaker. Gently evaporate the chloroform by use of a steam bath (test tube clamps may be used to hold the beakers deep in the steam bath to facilitate the evaporation). Evaporate just to dryness and remove the beaker immediately. Cool.

Add 5.0 ml. of chromotropic acid reagent to the residue in the beaker and swirl to make a homogeneous solution. Place the beaker and contents in an oven set at 150° ± 2° C. for exactly 10.0 minutes (use interval timer). Cool the solution to room temperature (wine-purple colored if 2,4-D is present).

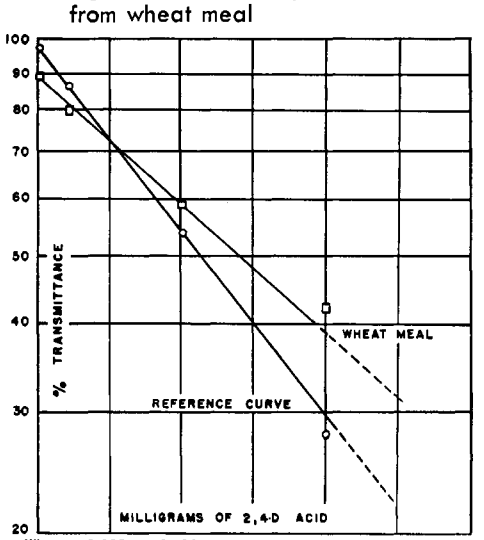
Pour the solution with stirring into about 30 ml. of sulfuric acid–stannous chloride solution, rinsing the beaker with a few milliliters of the solution. Start timing with the interval timer and cool the solution to approximately room temperature, using a cold water bath.

After about 5 minutes, pour the solution into a 50-ml. volumetric flask and make to volume with sulfuric acid–stannous chloride solution. Mix well and filter through a Whatman's No. 42 or equivalent grade of filter paper.

After 30 to 45 minutes, determine the per cent transmittancy of the filtrate at 565 mμ, using 5-cm. cuvettes with water as the reference liquid set at 100%.

Read the milligrams of 2,4-D acid contained by the sample from a graph made from the data obtained by analysis of untreated meal to which known quantities of 2,4-D acid had been added.

Figure 1. Recovery of 2,4-D acid from wheat meal



Calculation

Calculate the parts per million of 2,4-D acid present in the sample as follows:

$$\frac{\text{Mg. of 2,4-D acid} \times 1000}{\text{grams of sample}} = \text{p.p.m. of 2,4-D acid}$$

Preparation of Graph

Add 0.0, 1.0, 5.0, and 10.0 ml. of the standard solution containing 0.01 mg. of 2,4-D acid per ml. to 200-gram samples of untreated meal, and determine 2,4-D acid according to the procedure. Using the data obtained, construct a suitable graph.

Data obtained by the authors on wheat meal are shown in Table I.

Reference data on known amounts of 2,4-D acid were obtained by pipetting 0, 1.0, 5.0, and 10.0 ml. of the standard solution containing 0.01 mg. of 2,4-D acid per ml. into four 50-ml. beakers and gently evaporating the chloroform by use of a steam bath. Then 5.0 ml. of chromotropic acid reagent were added and the color was developed according to the procedure. Results are given in Table II.

The data from Tables I and II are shown graphically in Figure 1. Comparison of the two sets of data shows that the recovery of 2,4-D acid, although not complete, is adequate to detect and estimate trace quantities of the acid in wheat meal. The over-all loss is corrected in the preparation of the graph.

Graphs made for meals of barley, flax, dried peas, and oats were similar to the one prepared for wheat meal. For oats, it was found expedient to use 100-gram samples.

The analytical data found on the samples of grain and seed listed above correlate very well with the information available in each case.

Chromatographic Separation of 2,4-D Acid

The developing solution and the eluting solution used with the Hyflo Super-Cel for the chromatographic separation of the 2,4-D acid should be carefully standardized in order to make a proper separation.

The adsorptive strength of Hyflo Super-Cel was found to vary somewhat from batch to batch. Therefore, a uniform batch of Super-Cel, in adequate supply for standardization of the chromatographic separation and for use in many subsequent separations, should be set aside.

Table I. Transmittancy Values for 2,4-D Acid in Wheat Meal

2,4-D Acid, Mg.	% Transmittancy
Nil (blank)	89.0
0.01	79.8
0.05	58.8
0.10	42.0

Table II. Transmittancy Values for 2,4-D Acid

2,4-D Acid, Mg.	% Transmittancy
Nil (blank)	97.1
0.01	86.2
0.05	53.7
0.10	27.8

The presence of water was found to affect the elution strength of the solutions used in the chromatographic separation. Since the water content in technical methanol may vary from drum to drum, several gallons of methanol from a selected drum should be stored and used for standardizing the solutions and for the duplication of these solutions thereafter. In this investigation, methanol containing 0.03% water was used.

It is probably not necessary to store chloroform, as variation in quality from various lots appeared to have no significant effect on the elution strengths of the solutions.

Prepare the column for the chromatographic separation by packing the tube with Super-Cel for a distance of 20 cm. Pour about 30 ml. of chloroform containing 0.10 mg. of 2,4-D acid into the tube. Apply air pressure at the top of the tube to force the solution into the Super-Cel.

Percolate 150 ml. of 3.0% methanol in chloroform solution through the column at the rate of 150 ml. per hour, followed by 25 ml. of 7.0% chloroform in methanol. Discard the effluent. Then pass 50 ml. of 7.0% methanol in chloroform through the column at the same rate and save the liquid in a 100-ml. beaker. Repeat this three times with the similar portions of the eluting solution.

Test for 2,4-D acid in each beaker as follows: Evaporate the liquid by use of a steam bath, add 10 ml. of chromotropic acid reagent, and swirl to make a uniform solution with any residue. Place the beaker and contents in an oven set at 150° ± 2° C. for 10 minutes. Then examine the solutions for the wine-purple color which indicates the presence of 2,4-D acid.

For a good chromatographic separation of the 2,4-D acid, results should be:

1. At most, a faint wine-purple color in the first beaker, indicating a trace of 2,4-D acid.
2. A deep wine-purple color in the second beaker, indicating most of the original 0.10 mg. of 2,4-D acid.
3. A pale wine-purple color in the third beaker, indicating only a small portion of the original 0.10 mg. of 2,4-D acid.
4. No wine-purple color noticeable in the fourth beaker, indicating the absence of 2,4-D acid.

The fourth 50-ml. portion of 7.0% methanol in chloroform is not run through the tube during a regular analysis. It is done here for testing the chromatographic separation to make sure that all of the 2,4-D acid is eluted with the first 150 ml. of 7.0% methanol in chloroform.

If the results do not show a good separation of the 2,4-D acid, the amount of methanol in the two solutions of chloroform should be adjusted.

Discussion

Phosphotungstic acid is used to separate proteins when the 2,4-D acid is extracted with chloroform from the acidified aqueous solution.

The buffered extraction solution separates the 2,4-D acid from some of the small amount of acidic material remaining with the 2,4-D acid after the chromatographic separation.

After the final separation of the 2,4-D acid, a very small amount of acidic material from the grain sample may be present, which will give an amber color when heated with chromotropic acid reagent. The stannous chloride in the dilute sulfuric acid bleaches the amber color, but under the conditions of the experiment it does not bleach the wine-purple color. Though the solution from an untreated grain sample obtained by analysis is not water-white, the per cent transmittancy is high and constant.

The color reaction of 2,4-D acid with chromotropic acid is not quite specific. Formaldehyde will produce the same wine-purple color (*1*). According to Freed (*2*), phenoxyacetic acid and its derivatives will give the same color, perhaps because formaldehyde is a decomposition product.

The procedure can probably be used for phenoxyacetic acid and many of its derivatives besides 2,4-D acid, although the solutions of methanol in chloroform may have to be modified to make a proper chromatographic separation of the desired compound. The procedure as given has been used to determine 4-chloro-*o*-toloxyacetic acid (MCP acid).

Although this procedure was developed principally for the determination of 2,4-D acid in grain and seed, it can probably be used for other agricultural products. Fresh vegetables such as peas should be dried by suitable means and the analysis made on a meal of the dried sample.

It is possible to detect less than 0.05 p.p.m. of 2,4-D acid in samples by this analytical procedure. Interferences by other compounds were not experienced as, under the conditions of the procedure, the color test is specific for aryloxyacetic acids.

Literature Cited

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INSECTICIDE MIXTURE ANALYSIS

Chromatographic Separation of Dichlorodiphenyltrichloroethane and Dichlorodiphenyldichloroethane Mixtures

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Previous attempts to analyze mixtures of dichlorodiphenyltrichloroethane with dichlorodiphenyldichloroethane had proved unsatisfactory because of the similarity of chemical reactions. The physical differences of the two indicated they might be separable by chromatography. Dichlorodiphenyltrichloroethane is separated from dichlorodiphenyldichloroethane upon a silicic acid chromatographic column with *n*-hexane saturated with nitromethane, as the solvent. Dyes D and C red No. 18 and violet No. 2 can be used as a visual means of determining the point of separation. The compounds are recovered quantitatively upon evaporation of the solvent. This is a rapid, simple, and accurate means of analyzing mixtures of these compounds. It eliminates the uncertainty found in attempts to analyze the two simultaneously.

Because of the similarity of dichlorodiphenyltrichloroethane to dichlorodiphenyldichloroethane, analysis of mixtures of the two compounds based upon a chemical reaction is not completely satisfactory. Reactions which are used to determine dichlorodiphenyltrichloroethane quantitatively cannot be used in the presence of dichlorodiphenyldichloroethane. Wichmann *et al.* (*5*) have reported methods of determining dichlorodiphenyltrichloroethane or dichlorodiphenyldichloroethane quantitatively by analysis for total and mobile chlorine. Although the ratio of total to mobile chlorine in the two compounds is different, the procedures are not sufficiently accurate to enable an analysis of mixtures of the two compounds to be made.

The oxidation of the compounds to the ketones and subsequent spectrophotometric determination of the 2,4-dinitrophenylhydrazones of the ketones (*5*) cannot be used because both compounds yield the same ketone upon oxidation.

Colorimetric determination of nitration products is unsatisfactory because of the color's fading as well as the uncertainty of the products of nitration.

Infrared analysis of the mixtures has been found to be unsatisfactory (*4*).