Determination of Some Acidic Herbicides by Thin-Layer Chromatography

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A thin-layer chromatographic (tlc) procedure for the identification and quantitation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methoxy-3,6-dichlorobenzoic acid (Dicamba), 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP), 4-(2,4-dichlorophenoxy)butyric acid 4-(2,4-DB), 4,6-dinitro-*o*-cresol (DNOC), and 4,6-dinitro-*o*-*sec*-butylphenol (DNBP) has been developed. After extraction and cleanup, the herbicide residues were converted to the nitro derivatives. These derivatives were then subjected to tlc and visualized by reducing the nitro to the amine followed by diazotization and coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride. In this manner, 0.5 μg of herbicide was easily detected. This afforded a sensitivity of ≤0.05 ppm in actual crop residue determination.

The reported herbicides are representative of several structurally similar compounds used for selective weed control. Residues can be found in various combinations (U.S. Dept. of Agriculture, 1969) in leguminous plants, small grain cereals, forages, and turfs. The free acids can become conjugated with sugars or other metabolites from the plant tissues. These conjugates are difficult to detect by normal procedures and must be hydrolyzed. Utilizing gas–liquid chromatographic (glc) methods, the analyst is often confronted with dubious identification and quantitation because of poorly resolved or overlapping peaks and confirmation of results becomes necessary. A procedure utilizing the Bratton–Marshall reagent (Bratton and Marshall, 1939; Onley and Yip, 1969) has been developed for the tlc detection and quantitation of the above mentioned herbicides after formation of their nitro derivatives (Bache *et al.*, 1964).

Solvents and Reagents. The hexane and acetone were nanograde quality, the chloroform was spectrograde, and the methyl alcohol was either spectrograde or nanograde. All the above were obtained from the Mallinckrodt Chemical Works, Saint Louis, Mo. The ethyl alcohol was pesticide quality (Matheson Coleman and Bell, East Rutherford, N.J.). The water used was deionized. All other materials were reagent grade.

Herbicides. MCPP, 2,4-D, and 4-(2,4-DB) were obtained from Rhodia Inc., Chipman Division; Dicamba was obtained from Velsicol Chemical Corp.; DNOC came from Eastman Organic Chemicals; DNBP was from Dow Chemical Corp.; Benefin (Balan) and Trifluralin (Treflan) were obtained from Eli Lilly Co.; Vernolate (Vernam) came from Stauffer Chemical Co.; and Diphenamide (Enide) was obtained from Upjohn Co.

Reducing Agent. A 15% w/w stannous chloride stock solution in 0.5 *N* HCl was prepared, shaken well until a milky solution was obtained, and then stored in a refrigerator. Six milliliters of this solution was mixed with 10 ml of concentrated HCl and then made up to 100 ml by addition of a 50:50 mixture of water and methyl alcohol. This solution was prepared fresh daily.

Diazotizing Agent. One gram of sodium nitrite was dissolved in 2 ml of distilled water and diluted with methyl alcohol to 100 ml. This solution was also prepared daily.

Chromogenic Agent (Bratton–Marshall Reagent). One gram of *N*-(1-naphthyl)ethylenediamine dihydrochloride (Matheson Coleman and Bell) was dissolved in 2 ml of water by warming the flask in a stream of tap water. This solution was then diluted to 100 ml with methyl alcohol. The solution was prepared fresh daily.

Methanolic HCl. Twenty milliliters of concentrated HCl was placed in a 100-ml volumetric flask and made up to volume with methyl alcohol.

Extraction. Fifty grams of a representative sample (peanuts, soybeans, alfalfa, stalks, leaves, or fruit) were placed into a Waring blender. Fifty grams each of Dry Ice and anhydrous sodium sulfate were added and the mixture was ground to a powdery consistency. Acetone (200 ml) was added and the resultant mixture was homogenized for 1–2 min at high speed.

The homogenate was filtered under vacuum through a Buchner funnel containing a Whatman No. 1 filter paper moistened with water, covered with a glass wool pad and a layer of Hyflo Super-Cel (Johns-Manville Corp., Manville, N.J.). The residue from the blender was washed three times with 50 ml of acetone and the washings were poured through the Buchner funnel. The filter cake was then washed with an additional 200 ml of acetone. The combined filtrates were then transferred to a 1-l. round-bottomed flask and evaporated to semidryness under vacuum by means of a rotary flash evaporator at a water bath temperature not exceeding 40–45° C.

Cleanup by Alkaline Hydrolysis and Liquid–Liquid Partition. To the above residue was added 5 ml of 5 *N* NaOH to afford a minimum pH of 10. The mixture was heated under reflux for 30 min, cooled to room temperature, and the hydrolyzed residue transferred to a 250-ml separatory funnel. Herbicides which may have been present in the crop as the free acid, amine salt, isooctyl ester, or "conjugated" with a carbohydrate were now present as the sodium salts. The hydrolysis flask was washed with small portions of 50 ml of $CHCl_3$ and then with a few milliliters of water. The rinsings were combined in a separatory funnel and shaken, and the $CHCl_3$ layer was discarded. This operation was repeated three more times with 25-ml portions of $HCCl_3$ and the organic fractions were discarded. Herbicides which were not readily soluble in water, such as Benefin, Trifluralin, Vernolate and Diphenamid, were retained in the $CHCl_3$ layer. The aqueous layers were acidified with 20 ml of 2 *N* H_2SO_4 to afford a pH of 2 or less and then 50 ml of $CHCl_3$ were added. The mixture was well shaken, the layers were allowed to separate, and the organic layer was filtered through anhydrous sodium sulfate into a 250-ml round-bottomed flask. This operation was repeated five times with 25-ml portions of $CHCl_3$. The organic extract was evaporated to near dryness under vacuum using a rotary evaporator with a water bath temperature not exceeding 40° C.

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Table I. R_f Values and Colors of Nitrated Acidic Herbicides Chromatographed on Silica Gel F-254 Plates Reduced and Visualized with the Bratton–Marshall Reagent

Herbicide	R_f	Spot color
Dicamba	0.14	Pink
2,4-D	0.25	Purple
MCPP	0.36	Purple
4-(2,4-DB)	0.54	Pink garnet
DNOC	0.69	Blue
Dinoseb	0.74	Blue

Conversion of the Herbicide Free Acids to Nitro Derivatives. A 2% solution of sodium nitrate in concentrated *ortho*-phosphoric acid was prepared by heating the mixture to 85–90° C. This nitrating mixture must be freshly prepared before use. Five milliliters of the hot nitrating solution were added to the acidified residue and the mixture was heated to 80–90° C for 30 min with occasional swirling. The cooled mixture was transferred to a 250-ml separatory funnel by rinsing with 100 ml of a 10% aqueous sodium sulfate solution and then with 25 ml of ethyl ether in small portions. The mixture was well shaken, the layers were allowed to separate, and the organic layer was filtered as described above. The contents of the separatory funnel were extracted four more times with 25-ml portions of ethyl ether and then filtered. The combined filtrates were taken to near dryness on the rotary evaporator at reduced pressure with a water bath temperature of not more than 30° C. The residue was quantitatively transferred to a 10-ml evaporative concentrator (Kontes Glass Co., Vineland, N.J.) with small amounts of ethyl ether and evaporated in a current of nitrogen to a volume of 0.5 ml so that a minimum of 0.05 ppm of the desired herbicide was detectable by tlc when spotting, typically, 100 μl.

Preparation of Reference Standards. The following stock solutions were prepared to afford concentrations of 1 mg/ml: 2,4-D, MCPP and 4-(2,4-DB) in acetone; Dicamba and DNOC in methyl alcohol; and DNBP in ethyl alcohol. Suitable aliquots of these stock solutions were nitrated as described above, either singularly or in the desired combinations.

Thin-Layer Chromatography. Tlc was done on precoated 20 × 20 cm Merck (Brinkman Instruments, Inc., Westbury, N.Y.) silica gel F-254 glass plates. The plates were activated for 30 min in an oven at 130° C and cooled to room temperature in a desiccating cabinet prior to use. Using a 100-μl pipette, the derivatized crop residue was spotted 2.5 cm from the plate bottom adjacent to 0.5, 1.0, 3.0, and 5.0 μg (acid equivalents) of the derivatized reference standards which were spotted using a 10-μl pipette. Concurrently, solvent vapors of benzene–glacial acetic acid, 85:15, were permitted to saturate the developing tank which was lined with Whatman No. 1 filter paper. The plate was then rapidly inserted into the tank and permitted to develop to a height of 14 cm above the spotting point. After air drying, the plate was examined under shortwave uv light to determine if the chromatography was successful (a clear separation of spots); if not, the chromatography was repeated.

Visualization. The plate was sprayed heavily and uniformly with the reducing agent. After the coating had dried for about 5 min, the plate was sprayed with the methanolic HCl solution and permitted to air dry for 5 min, then sprayed heavily with the diazotizing agent. After 15–20 min the plate was sprayed with the Bratton–Marshall reagent, after which colored spots appeared. The maximum color intensity occurred in about 15–30 min and did not fade for several days, provided the plate was protected from light. The concentration of the crop residue derivatives was then visually determined by comparison with the reference standards. The R_f values and spot colors are given in Table I. Examination of Dinoseb under uv light afforded better visualization than with the spray reagent.

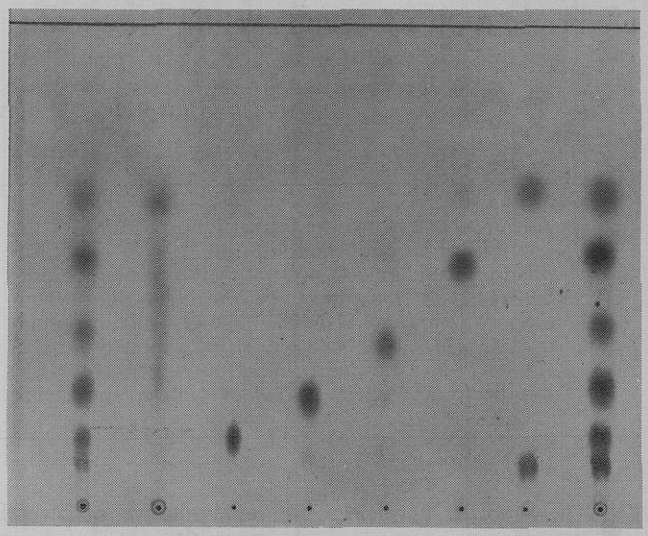

Figure 1. Chromatogram of some acidic herbicides after formation of nitro derivatives, reduction to amino groups, and visualization with the Bratton–Marshall reagent. Spots, left to right: 1. Recovery test with soybean crop substrate spiked at 0.4 ppm (20 μg of each herbicide per 50 g sample). 100 μl of a 500-μl sample of cleaned-up extract, theoretically containing 4 μg equivalent of each herbicide per 100 μl. Recoveries approximate 80% or better. 2. Soybean control, 100 μl of 50 g crop substrate sample in 500 μl. Limit of detection of the herbicides established at 0.5 μg each, equivalent to <0.05 ppm. 3–7. 5-μg equivalents of Dicamba, 2,4-D, MCPP, 4-(2,4-DB), and Dinoseb, respectively. 8. 10-μg equivalents of 3, 4, 5, 6, and 7, respectively

RESULTS AND DISCUSSION

It has been experimentally determined in this laboratory (Guardigli, 1970) that one of the electron-capture gas chromatographic peaks of the methyl ether derivative of the dinitro preemergence herbicides DNBP (technical grade) eluted at the same retention time as the 2,4-D methyl ester. Attempts to separate these compounds with several typically polar and nonpolar columns failed to satisfactorily resolve the peaks, making quantitation difficult.

In view of the importance of these several structurally similar compounds, a tlc method was developed in which mixtures of these acidic herbicides could be detected, as shown in Figure 1.

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