Determination of 2-Chloro-4-isopropylamino-6-hydroxymethylamino-*s*-triazine (ACD15M) in Corn Plants

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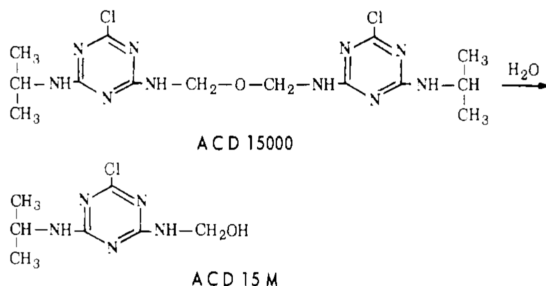
A method for determining residues of the herbicide ACD15M 2-chloro-4-isopropylamino-6-hydroxymethylamino-*s*-triazine in sweet corn ears or plants (leaves and stalks) is presented. After extraction with methanol, the extractives were transferred to methylene chloride, passed through a column of Florisil, and the residue transferred to acetone for analysis by gas chromatography using a nitrogen detector. The procedure will easily measure 0.1 ppm in corn ears and 0.2 ppm in corn plants, using a 50-g sample. The average recovery was 80% in corn ears and 82% in plants.

The experimental compound ACD15M (2-chloro-4-propylamino-6-hydroxymethylamino-*s*-triazine) (Allied Chemical Corporation) is a promising herbicide for pre- or post-emergence control of broadleaf and grassy weeds in corn and other crops. An analytical method for determining residues of this pesticide in corn ears or plants (leaves and stalks) was developed, utilizing a gas chromatograph equipped with a nitrogen detector. Residues of 0.1 ppm in corn ears and 0.02 ppm in corn plants were readily determined, using 50-g samples.

The colorimetric procedures proposed for determining Dyrene [2,4-dichloro-6-(*o*-chloro-anilino) *s*-triazine] by Burchfield and Storrs (1956) and modified for simazine [2-dichloro-4,6-bis(ethylamino)-*s*-triazine] as reported by Zweig (1964) is applicable to the analysis of several pesticides containing active halogen atoms. Burchfield and Schuldt (1958) found a qualitative response to a number of such compounds. The reaction of ACD 15000 {*N,N'*-[2-chloro-4-isopropylamino(1,3,5-triazinyl)] diamino methyl ether}, the dimer of ACD15M, after hydrolysis to form ACD15M, with the alkali-pyridine reagent, was investigated by Westlake (1969) in detail and conditions for color formation optimized.



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The instability of the color, due to dependence on exact reaction conditions involving control of several difficultly controllable factors, made it impossible consistently to duplicate results with this particular compound and led to a search for a more dependable and, hopefully, simpler procedure. The availability of a gas chromatographic procedure for determining nitrogen, already proven for determining other triazines, offered a logical solution.

PROCEDURE

Sweet corn ears (grain and cob) were ground in a Hobart food cutter, thoroughly mixed, and a 50-g sub-sample was blended in an Omni-mixer for 10 min at a speed setting of 7 with 100 ml of reagent-grade methanol. Chopped leaf and stalk samples were blended similarly with 200 ml of methanol for a 50-g sample. The Omni-mixer jar was cooled in an ice bath during blending to prevent pressure buildup due to heating.

The macerate was filtered with suction through Whatman No. 2 filter paper in a Buchner funnel, then cake and filter were washed twice with 25-ml portions of methanol, adding the washings to the filtrate.

Corn Ears. The filtrate from each corn ear sample was transferred to a 500-ml round-bottomed flask, 25 ml of 10% sodium chloride solution was added, and the solution was reduced to an aqueous residue on a rotary vacuum evaporator at a water bath temperature of 65–70° C. (The volume should be 60–65 ml. If the volume is reduced to the point where the yellow-colored material begins to coagulate, this material, and the pesticide, will remain on the column in the succeeding cleanup step. The yellow material must elute from the column.)

The aqueous residue was transferred to a 250-ml separatory funnel with 50 ml of methylene chloride, and shaken for 30 sec. After separation of the phases, the methylene chloride

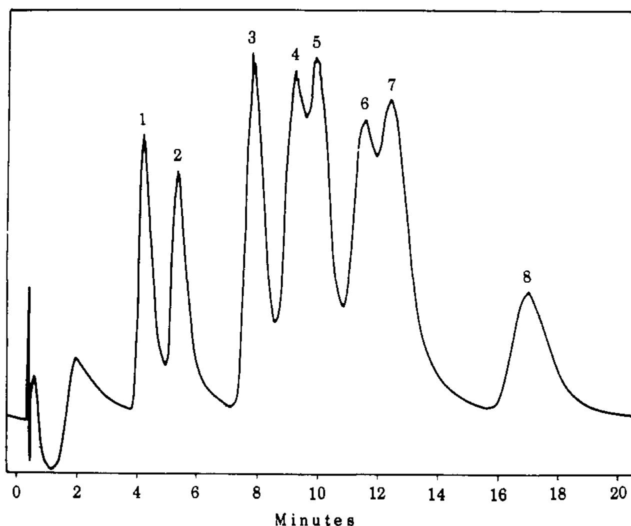

Figure 1. Chromatogram of ACD15M and other triazines

1 = GS-28304; 2 = Prometone; 3 = simetone; 4 = atrazine; 5 = Prometryne; 6 = simazine; 7 = ametryne; 8 = ACD15M. Amount injected = 3.6 μl of a solution containing 5 μg/ml of 1 and 2, and 10 μg/ml of 3–8 (18 ng of 1 and 2; 36 ng of 3–8). Operating conditions were as given in text in section on gas chromatography, for a 5.5-ft Reoplex 400 column

was drawn off into a 250-ml Erlenmeyer flask and the procedure was repeated with a second 50-ml portion of methylene chloride, combining the two extracts. A few grams of anhydrous sodium sulfate were added with swirling.

A 15 mm o.d. chromatographic column was prepared, using about 8 cm of Florisil (60/100 mesh, PR grade, heated at 130° C for 1 hr), topped with 1 cm of anhydrous sodium sulfate. The column was wet with methylene chloride and the sample added without concentration, eluting at 2 to 3 drops per sec. After the sample had entered the column, an additional 50 ml of methylene chloride was added and the elution continued to completion.

The methylene chloride eluate was evaporated to a few ml in a Kuderna-Danish evaporative concentrator, then taken to dryness with a gentle jet of air at about 30° C. The residue was then dissolved in 5 ml of acetone for gas chromatography.

Corn Plants. The methanolic extract from each corn plant sample was transferred to a 500-ml separatory funnel, 25 ml of 10% sodium chloride solution was added, and the solution was shaken with three successive 50-ml portions of hexane, discarding the hexane washes. Following this, the procedure was identical to that used for corn ears, beginning with "and reduced to an aqueous residue . . ."

Gas Chromatography. A gas chromatograph equipped with a Coulson electrical conductivity detection system (available from Tracor, Inc.) and a 3.5-ft × 6 mm o.d. glass column packed with 2% Reoplex 400 on Gas Chrom Q, 80/100 mesh, was used for the determinative step. This column will give good separation of ACD15M from other triazine herbicides, but if resolution of all such herbicides is desired, a 5 to 6 ft column may be necessary. Figure 1 illustrates the type of chromatogram that results. If separation from other triazines is not required, a 2 ft column packed with 2% Epon resin 1001 on Gas Chrom Q, 80/100 mesh, may be used for routine analyses, one advantage being a retention time of only 3.8 min for ACD15M. At temperatures of 195° C for the column, 224° C for the inlet, and 820° C for the furnace, and gas flow rates of 39 ml per min for the carrier (helium) and 200 ml per min of hydrogen, retention times were 5.6 min and 17 min for 3.5 ft and 5.5 ft Reoplex 400 columns, respectively. The plant samples contained an interfering substance not present in the corn ear samples that gave rise to a large peak at a retention time about three times that of ACD15M (see Figure 2). Attempts to eliminate this interference by different cleanup procedures were unsuccessful. Two injections could be made in succession (see Figure 3) but it was necessary then to wait for the interfering peaks to elute before injecting again. If the short Epon 1001 column is used, six samples per hr may be injected; a reasonable rate for routine work.

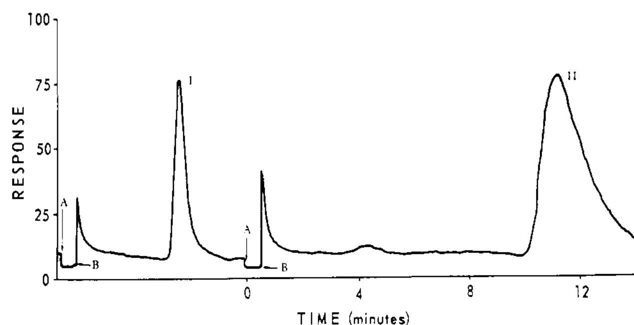

Figure 2. Chromatogram of 50 ng of ACD15M standard solution (I) and extract equivalent to 59 mg of untreated corn plant (II)

A = point of injection and B = point vent was closed

The corn ear samples gave chromatograms similar to those shown for the corn plants, but the large contaminant peak was not present and injections could be made as soon as the ACD15M peak had emerged.

No effect on column performance or sensitivity was evident after several hundred injections.

DISCUSSION

The availability of gas chromatographic detectors that are relatively specific for nitrogen makes this the method of choice for triazine herbicides. While the electrical conductivity detector was used in this study, the microcoulometric detector cell developed for the Dohrmann instrument should be equally satisfactory. Other columns and operating conditions for the gas chromatograph could probably be found that would give equally good results. Those reported, however, were very satisfactory for our instrument. Other liquid phases tested included SF-96 and Carbowax 20M, but these were not as satisfactory as Reoplex 400.

ACD 15000 and ACD15M, when injected into the gas

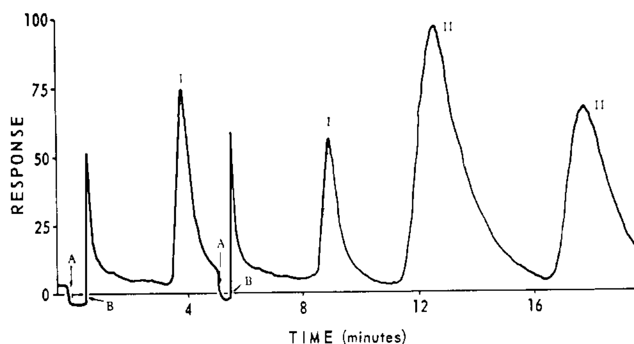

Figure 3. Chromatogram of two injections of corn plant extract fortified with 1.5 ppm of ACD15M

I = ACD15M peaks, II = contaminant peaks, A = point of injection, and B = point vent was closed

Table I. Recovery of ACD15M from Fortified Control Samples of Corn Bars and Plants (Leaves plus Stalks)

50 g subsamples		Recovery (%)	
μg added	ppm	Ears	Plants
100	2.0	81, 79	71
75	1.5	77	69
50	1.0	83, 74	69
40	0.8	76, 79	80
20	0.4	72, 91	94
10	0.2	79, 85	112
5	0.1	75, 91	...[b]
0	0	ND[a]	ND[a]

[a] None detectable. [b] Too much interference.

chromatograph, gave identical retention times on the columns used, leading to the conclusion that the dimer was converted to the monomer by cleavage at the ether linkage either in the gas chromatograph or in the solution prior to injection. Attempts to distinguish the two compounds from one another by thin-layer chromatography were likewise unsuccessful; R_f values for the two compounds were identical. An earlier report by Ulmer (1969) gave a tentative R_f value for ACD 15000 that was different from that for ACD15M, but this could not be verified in the present study. It has also been reported by Ulmer (1968) that ACD15M appears to be the initial product formed by hydrolysis of ACD 15000. The present study indicates that this reaction may occur in the solvent systems used for processing prior to gas or thin-layer chromatography, and/or in the gas chromatograph and on the thin-layer plates.

The recovery of ACD15M from fortified control samples of corn ears (without husks) and plants (leaves plus stalks) is shown in Table I. They are reasonably uniform, for ears, over the entire range (0.1 to 2.0 ppm), but there is an indication of an increased apparent recovery in the plant samples at levels below 0.4 ppm. The linear range of the standard curve was from about 20 to 50 ng and the response was about 1.5% of full scale on a 1 mv recorder for one ng of this herbicide. The minimum detectable quantity (5× noise level) was in the order of five ng, but accurate measurement of amounts less than 20 ng was difficult because of nonlinear response in this region.

The method was not tested on crops other than corn, as this was the only crop on which use of ACD15M was being considered at the time. There is no reason, however, to suspect that the method would not be adaptable to other crops.

ACKNOWLEDGMENT

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