Determination of Residues of 4-Amino-3,5,6-trichloropicolinic Acid in Cereal Grains by Gas Chromatography

E. L. BJERKE, A. H. KUTSCHINSKI, AND J. C. RAMSEY

A gas chromatographic method for determining residues of 4-amino-3,5,6-trichloropicolinic acid in cereal grains is described. Samples are extracted with aqueous KOH, and cleanup is accomplished with an alumina column followed by $KMnO_4$ oxidation. The acid is esterified with diazomethane, and residues as low as 0.05 p.p.m. are determined by gas chromatography as the methyl ester using a LAC-446/H_3PO_4 column and electron capture detection. Recoveries were 74% from straw and 86% from grain. Samples of wheat and barley treated with the acid at rates from 0.2 to 1 ounce per acre were analyzed. Seventy-five per cent of the samples contained residues less than 0.05 p.p.m. However, residues as high as 0.22 p.p.m. in wheat grain, 0.44 p.p.m. in wheat straw, and 0.64 p.p.m. in barley grain were found.

Tordon acid (Dow Chemical Co.), 4-amino-3,5,6-trichloropicolinic acid, is a herbicide which has been proposed for weed control in cereal grains (*3*, *4*); it has thus been necessary to develop a method for residue determination of the acid. Since the acid is highly active (*5*), very low application rates are effective. Therefore, any residue determination method used should be sensitive to submicrogram levels of 4-amino-3,5,6-trichloropicolinic acid.

Gas chromatography was selected as the best method for analysis because the electron-capture detector is highly sensitive to the methyl ester of the acid, methyl 4-amino-3,5,6-trichloropicolinate. The major considerations in the development of a specific gas chromatographic method were: an extraction procedure which would remove the acid completely from grain and straw, a procedure to give sufficient cleanup of the sample extract for gas chromatography, and a column packing which would give good resolution of the ester.

In the procedure described, the samples are extracted with aqueous potassium hydroxide. Coextracted chromatographic interferences are removed in two steps involving an alumina column and oxidation with potassium permanganate. Following esterification with diazomethane, residues of 4-amino-3,5,6-trichloropicolinic acid are determined as the methyl ester.

Recently, gas chromatographic methods for determining residues of 4-amino-3,5,6-trichloropicolinic acid in soil and plant materials have been developed (*6–8*). In these methods also, the acid is determined as the methyl ester. A method bovine urine analysis includes direct gas chromatography of the acid (*2*).

Apparatus

Gas chromatograph, Barber-Colman Model 5000 or Model 10, equipped with a Sr^{90} ionization detector. A 45-volt B battery is the voltage source. The desired voltage is produced by means of a 300,000-ohm, 10-turn potentiometer. A 0.008-μf. capacitor is connected between the anode of the detector cell and chassis ground to reduce base line noise at high sensitivity.

Gas chromatographic column, U-shaped borosilicate glass, 74 inches × 3 mm. i.d.

Bioproducts Department The Dow Chemical Co., Midland, Mich.

Lourdes Model MM-1 Multi-mixer equipped with 50-ml. stainless steel centrifuge tubes (Lourdes No. 50-STF).

Centrifuge tubes, 35-ml. screw-cap (Corning No. 8422) equipped with caps lined with either foil or polyseal.

Hamilton 701 N, 10-μl. syringe.

Reagents

4-Amino-3,5,6-trichloropicolinic acid. Prepare aqueous standards containing 1.0 and 10.0 μg. of acid per ml.

Methyl 4-Amino-3,5,6-trichloropicolinate. Prepare a standard solution of the ester in benzene by dissolving 106 mg. in 100 ml., giving a concentration equivalent to 1 mg. of the acid per ml. Make volumetric dilutions of this solution with benzene to obtain standard solutions equivalent to the range from 0.01 to 0.20 μg. of acid per ml. Both of the above reagents are obtainable from Bioproducts Department, The Dow Chemical Co., Midland, Mich.

Ethereal diazomethane reagent (*1*).

Woelm basic alumina, activity grade 1.

Redistilled reagent grade or nanograde benzene.

All other solvents used were reagent grade.

Procedure

Gas Chromatographic Column Preparation. Dissolve 150 mg. of LAC-2R-446 (Cambridge Industries Co., Inc., 101 Potter St., Cambridge, Mass.) and 0.05 ml. of 85% phosphoric acid in 80 ml. of acetone in a round-bottomed flask. Add 15 grams of 80- to 100-mesh Gas-Chrom Z (Applied Science Laboratories, Inc., 140 N. Barnard St., State College, Pa.), and boil off most of the acetone on a steam bath. Dry the column packing on a rotary evaporator under vacuum over a steam bath. Sieve the dried material on a 100-mesh screen and discard the fines. The liquid loading on the support is 1% LAC-2R-466 and 0.5% phosphoric acid by weight. Fill the column with packing and tamp it on a solid surface until no further settling occurs. The packing in the inlet arm should be 3 inches below the injection septum. Insert a small glass wool plug over the packing only at the effluent end of the column. Condition the column at 210° C. overnight with a nitrogen flow of about 100 ml. per minute.

Gas Chromatograph Operating Conditions. Typical operating conditions of the gas chromatograph are:

Column temperature, 196° C.

Injector block temperature, 220° C.

Detector temperature, 215° C.

Detector operating voltage, 13 volts

Carrier gas, prepurified nitrogen at a flow rate of about 95 ml. per minute

Recorder, 0–5 mv.

Chart speed, 20 inches per hour

Electrometer sensitivity, 3×10^{-10} ampere full scale

Sample Preparation and Storage. Grind grain and straw samples in a Wiley mill fitted with a 2-mm. screen. Store them in polyethylene bags at room temperature.

Grain Extraction. Weigh 5.0 grams of grain into a stainless steel centrifuge tube. Add 15 ml. of 0.1*N* potassium hydroxide to the tube, mix, and let stand 5 minutes. Attach the tube to the Multi-mixer and lower it into an ice bath. After 5 minutes, blend the contents at full speed for 5 minutes, keeping the tube immersed in the ice bath. Centrifuge the sample for about 5 minutes and decant the supernatant liquid into a 50-ml. graduated cylinder having a glass stopper.

Add 15 ml. of 0.1*N* potassium hydroxide to the residue in the tube and mix with a spatula. Place a rubber stopper in the tube and shake it in a mechanical shaker for 15 minutes. Remove the stopper, centrifuge the mixture, and decant the supernatant liquid into the graduated cylinder. Dilute the combined supernatant liquid to 50 ml. with water and mix.

Straw Extraction. Weigh 5.0 grams of straw into a 4-ounce, wide-mouthed bottle. Add 50 ml. of a solution of 0.05*N* potassium hydroxide in 10% potassium chloride (*6*). Cap the bottle tightly with a foil-lined cap and shake it for 15 minutes in a mechanical shaker. Filter the mixture through a Büchner funnel having a coarse sintered glass disk covered with about 1 cm. of packed diatomaceous earth.

Sample Cleanup. Prepare a column of 1 gram of alumina, in acetone, in a 1-cm. chromatograph tube. Wash the column with about 20 ml. of acetone and then about 20 ml. of ether.

Pipet 15 ml. of grain or straw extract into a 35-ml. glass centrifuge tube. Acidify the extract with 1.0 ml. of 85% phosphoric acid. Add about 4 grams of sodium chloride and 15.0 ml. of ether. Cap the tube and shake it in a mechanical shaker for 5 minutes. Centrifuge the mixture for 5 minutes to separate the phases.

Pipet 10 ml. of the ether phase onto the alumina column and allow the solution to run through. Wash the column with 20 ml. of ether and then 20 ml. of acetone. Discard the eluate. Elute with 20 ml. of 0.25*M* sodium bicarbonate, collecting the eluate.

Acidify the eluate by carefully adding 1.5 ml. of 6*N* sulfuric acid. For wheat grain and straw, add 0.5 ml. of saturated potassium permanganate solution, stir, and let stand for 5 minutes; for barley grain, treat with 0.1 ml. of permanganate for 2 minutes. Add 5*M* sodium bisulfite solution dropwise with stirring until the solution is clear and colorless.

Dissolve about 4 grams of sodium chloride in the solution, and carefully extract it with 20 ml. and then 10 ml. of ether, using a separatory funnel. Combine the ether extracts, adding a small amount of anhydrous granular sodium sulfate to absorb any water present, and concentrate them to about 2 ml. on a steam bath.

Grain Esterification. Use about 2.5 ml. of ether to transfer the ether extract to a 5-ml. volumetric flask. Add a glass bead to the flask, and concentrate the solution to about 1 ml. on a steam bath. Add about 1 ml. of benzene and 0.5 ml. of diazomethane reagent, and heat the solution on the steam bath until the yellow color disappears and the ether is nearly all evaporated (10 to 15 minutes). Dilute to volume with benzene.

Straw Esterification. Add 1 ml. of diazomethane reagent to the ether extract of straw, cover the beaker, and heat the solution on a steam bath until it is colorless. Do not allow the solution to evaporate completely.

Prepare a second alumina column according to the procedure given for sample cleanup. Pour the esterified straw extract onto the column and elute with 20 ml. of ether, collecting all of the eluate. Concentrate it to about 2 ml. on a steam bath, and use benzene to transfer it to a 5-ml. volumetric flask. Dilute to volume with benzene.

Injection Technique. Fill the needle of a 10-μl. syringe with benzene, carefully excluding air. Draw 1 or 2 μl. of sample extract or standard methyl 4-amino-

Table I. Summary of 4-Amino-3,5,6-trichloropicolinic Acid Recovery Data

Acid Added, P.P.M.	Number of Determinations	Recovery, % Average	Recovery, % Range
	WHEAT GRAIN		
0.05	4	85	80–90
0.10	6	84	75–90
0.20	2	92	92–93
0.30	4	92	90–97
0.50	4	85	83–87
0.69	2	81	81–82
1.00	6	86	81–89
		Av. 86	
	WHEAT STRAW		
0.05	3	75	72–78
0.10	3	72	70–75
0.20	2	71	70–72
0.50	2	72	68–76
0.70	2	77	75–79
1.00	2	77	75–79
		Av. 74	
	BARLEY GRAIN		
0.05	2	84	82–86
0.10	2	84	82–87
0.20	2	85	85
0.50	5	85	83–89
0.80	2	81	81–82
		Av. 84	

3,5,6-trichloropicolinate solution into the syringe and inject it into the gas chromatograph.

Standard Curve. Inject standard benzene solutions of methyl 4-amino-3,5,6-trichloropicolinate covering the concentration range equivalent to 0.01 to 0.20 μg. of Tordon acid per ml. Establish a standard curve by plotting peak heights as per cent full scale deflection *vs.* corresponding acid concentrations in μg. per ml. By multiplying these numerical concentrations by five, a second scale is obtained which gives parts-per-million residue directly.

Calculations. The final 5 ml. of sample extract represents 1 gram of plant material. The concentration of Tordon acid in this final extract, expressed as micrograms per milliliter, is obtained from the peak height by referring to the standard curve. Therefore, five times this concentration corresponds to micrograms of Tordon acid per 5 ml. of sample extract—i.e., per gram of crop—this is equivalent to parts-per-million residue in the sample.

Recovery Determination. Fortify 5-gram aliquots of untreated control samples at concentration levels from 0.05 to 1.00 p.p.m. by adding appropriate amounts of aqueous solutions of Tordon acid before extraction. Then analyze these fortified samples by the method used for treated samples and determine recovery efficiency.

Results

Results of the recovery studies are summarized in Table I, and typical chromatograms are given in Figure 1. These results have been corrected for the corresponding blank of the control sample.

Samples from many locations of several varieties of spring and winter wheat and barley were analyzed. These samples were obtained from plots of grain treated with postemergence herbicide applications at growth stages ranging from three leaf to completely tillered. Three formulations were used: Tordon 202 Mixture, Tordon 303 Mixture, and Tordon 22K Weed Killer. Tordon 202 Mixture is a formulation containing 1.4% of 4-amino-3,5,6-trichloropicolinic acid and 22.1% of 2,4-dichlorophenoxyacetic acid as the triisopropanolamine salts. Tordon 303 Mixture contains 1.35% of 4-amino-3,5,6-trichloropicolinic acid and 21.50% of 2-methyl-4-chlorophenoxyacetic acid as the potassium salts. Tordon 22K Weed Killer contains 2 pounds per gallon of 4-amino-3,5,6-trichloropicolinic acid as the potassium salt.

The grain was harvested, dried, and threshed. Samples were placed in cloth bags for shipment and storage. After being ground, the samples were stored in polyethylene bags. Experiments with each type of sample indicated that 4-amino-3,5,6-trichloropicolinic

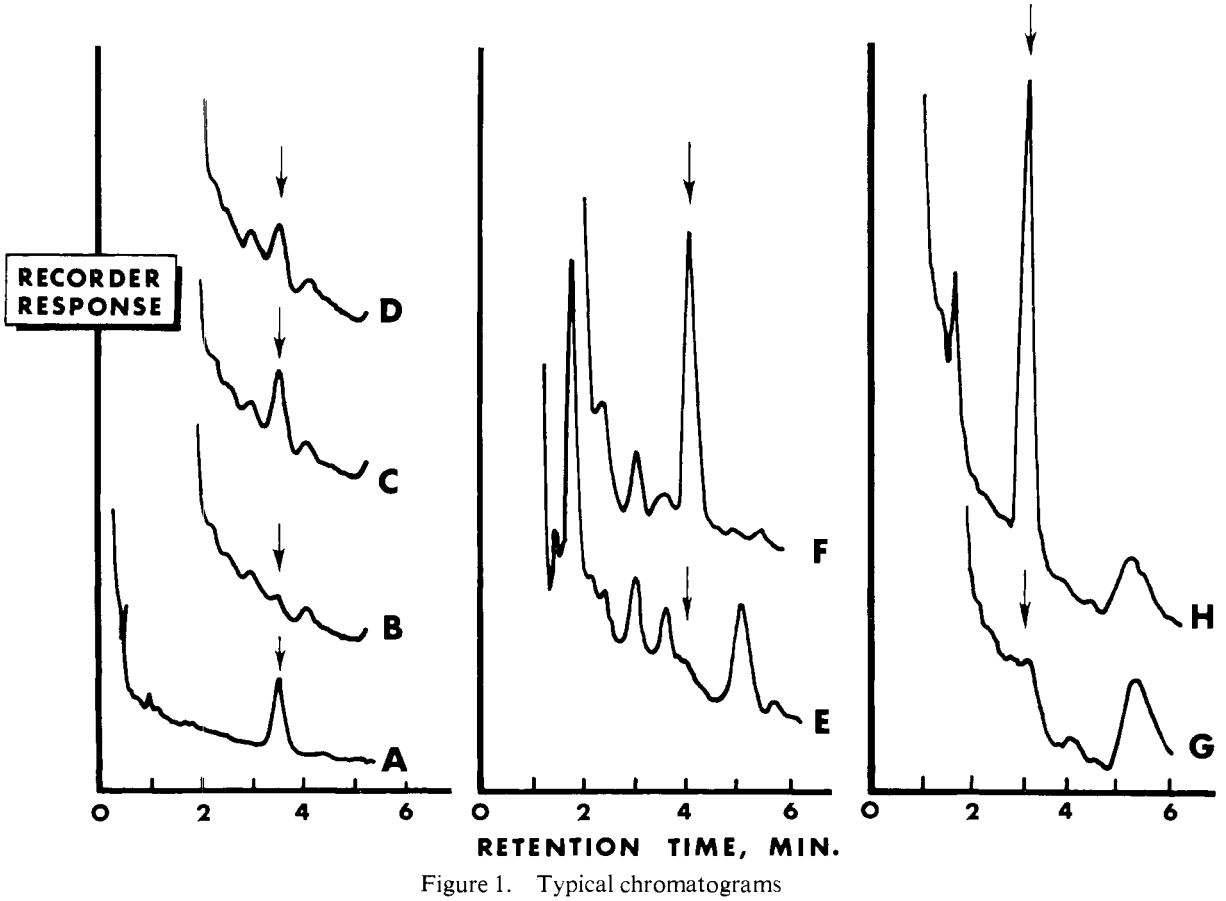

Figure 1. Typical chromatograms

Arrows indicate retention time of methyl 4-amino-3,5,6-trichloropicolinate. Curves were obtained from chromatography of: *A*, 0.02 ng. of ester. *B*, control wheat grain. *C*, recovery at 0.05 p.p.m. level. *D*, treated wheat grain. *E*, control wheat straw. *F*, treated wheat straw. *G*, control barley grain. *H*, treated barley grain

acid was stable in the samples under these storage conditions.

Results from treated samples, summarized in Tables II and III, are reported as parts per million of Tordon acid. They have been corrected for any control sample blank and also for the average recovery obtained for each type of sample. Application rates are reported as ounces of acid per acre.

Sixty-eight of 91 samples analyzed contained less than 0.05 p.p.m. of residue. Greater amounts of residue were found in some samples treated at the higher application rates—as high as 0.22 p.p.m. in wheat grain, 0.44 p.p.m. in wheat straw, and 0.64 p.p.m. in barley grain.

Discussion

The most complete extraction of 4-amino-3,5,6-trichloropicolinic acid from grain and straw was obtained with an aqueous alkaline solvent. A straw sample containing in-grown Tordon acid was found to

Table II. Residues of Tordon Acid Found in Wheat Grain and Straw Grown in the United States, Canada, and Australia

				Grain		Straw	
Location	Variety	Growth Stage at Application	Application Rate, Oz./A.	No. of samples	Apparent residue range, p.p.m.	No. of samples	Apparent residue range, p.p.m.
Michigan	Selkirk	5–7 leaf	3/8	2	<0.05	2	<0.05
		3 leaf	1/2	2	<0.05		
Montana	Cheyenne[a]	Fully tillered	3/8	6	<0.05–0.05		
S. Dakota	Omaha[a]	About to joint	3/8	2	<0.05	1	<0.05
N. Dakota		5–7 leaf	3/8	2	<0.05		
S. Dakota	Pembina	1–1 1/2 leaf	3/8	2	<0.05		
S. Dakota	Justin	5 leaf	3/8	2	0.05–0.06		
Montana	Winnalta[a]	Tillering	3/8	2	0.15–0.22	2	0.25–0.44
Minnesota	Justin	5–6 leaf	3/8	2	<0.05	2	<0.05
		5–6 leaf	3/4	2	<0.05–0.05	2	0.06–0.10
N. Dakota	Justin	6–8 leaf	3/8	2	<0.05	2	<0.05
		6–8 leaf	3/4	2	<0.05–0.05	2	<0.05
California	Ramona	3–5 leaf	1/2	4	<0.05	2	0.14–0.27
Manitoba	Pembina	3–3 1/2 leaf	1/2	4	<0.05		
		3–3 1/2 leaf	1	2	<0.05		
	Selkirk	3–3 1/2 leaf	1/2	8	<0.05		
		3–3 1/2 leaf	1	2	<0.05		
Saskatchewan	Selkirk	4–5 leaf	1/2	7	<0.05		
Saskatchewan	Selkirk	4–5 leaf	1	2	<0.05		
Alberta	Thatcher	3–4 leaf	1/2	4	<0.05–0.16		
		3–4 leaf	1	4	0.05–0.09		
New S. Wales	Olympic	Tillered	1/5	2	<0.05–0.05		
		Tillered	2/5	2	<0.05–0.06		
		Tillered	3/5	2	<0.05–0.13		
W. Australia		...	1/3	1	<0.05		
		...	3/5	1	<0.05		
		...	4/5	1	<0.05		
S. Australia	Insignia	Preplant	1	1	<0.05		
			2	1	<0.05		

[a] Winter wheat.

Table III. Residues of 4-Amino-3,5,6-trichloropicolinic Acid in Barley Grain

Location	Variety	Growth Stage at Application	Application Rate, Oz./A.	No. of Samples	Apparent Residue Range, P.P.M.
Montana	Compana	4–5 leaf	3/8	6	0.08–0.19
Minnesota	Larker	5–6 leaf	3/8	4	0.16–0.23
		5–6 leaf	3/4	4	0.28–0.43
N. Dakota	Larker	5 1/2 leaf	3/8	4	<0.05–0.08
S. Dakota	Trophy	3–4 leaf	3/8	2	<0.05
Minnesota	Larker	4 leaf	1/2	6	0.15–0.19
Montana	Compana	Tillering	1/2	1	0.12
		Tillering	1	2	0.45–0.64

contain 7.70 p.p.m. acid by exhaustive extraction with aqueous KOH–KCl solvent. Ninety-four per cent of the Tordon acid in this sample was extracted with KOH–KCl solvent by the procedure described for straw extraction. Only 13% was extracted when a mixture of 2% concentrated H_2SO_4, 10% H_2O, and 88% acetone by volume was used. In contrast, this acidic solvent extracted 70 to 80% of Tordon acid which, instead of being in-grown, was added to finely ground straw or grain.

The conditions used for cleanup of plant extract containing Tordon acid by potassium permanganate oxidation must be carefully controlled. Oxidation at greater than room temperature destroys the acid to a prohibitive degree. Oxidation for longer periods of time than those described generates new gas chromatographic interferences. Oxidation in an acidic solution gives a much better chromatogram than is obtained by oxidation in a neutral or basic solution. However, varying the amount of permanganate used does not seem to be as important; this might be due to the large excess of potassium permanganate relative to Tordon acid that is necessary for satisfactory cleanup. Using the conditions described in this method, oxidation causes a loss of about 10% of the Tordon acid added to samples for recovery determination (Table I).

The use of a trace of mineral acid to promote esterification of pesticide-related acids by diazomethane has been reported (*9*). Complete esterification of a solution of 4-amino-3,5,6-trichloropicolinic acid with diazomethane can be obtained consistently only when a trace of mineral acid is present. However, Tordon acid in crop extracts prepared by the procedure described is esterified completely by diazomethane; it is probable that a trace of mineral acid enters the ether phase and catalyzes the reaction.

About 55% full scale deflection occurred when 0.1 ng. of methyl 4-amino-3,5,6-trichloropicolinate was chromatographed. The detector response was linear up to this point, but relative response decreased for larger quantities of the ester; thus, all of the points used to establish the standard curve should be checked frequently. Sample extracts did not affect the detector response adversely.

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