A Modified Analytical Method for Microgram Amounts of Metaldehyde in Plant Material

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A study of the Giang and Smith method was initiated because of high coloration in blanks. The objectionable color was found to originate from the chloroform used, and could be eliminated by evaporation of the chloroform to dryness. Emulsification problems were resolved by the use of Florex column filtration.

METALDEHYDE is used in agriculture to control slugs and snails on a variety of plants. A sensitive analytical procedure is necessary since the current federal tolerance for this pesticide on food crops is zero. Analytical methods for determining metaldehyde generally consist of hydrolysis to acetaldehyde, separation by distillation, and estimation of the aldehyde by one of several methods (*3*, *5*, *6*, *8*). Giang and Smith (*3*) used a very sensitive modification (*7*) of the *p*-phenylphenol color reaction for lactic acid (*1*). Bowman *et al.* (*2*) have recently extended this procedure to include acetals of acetaldehyde, vinyl ethers, and other compounds containing the combined acetaldehyde group.

Solvent interference in the Giang and Smith procedure, unimportant in the presence of a high concentration of metaldehyde, becomes a limiting factor in the analysis of low concentration samples, since it increases with the aliquot taken and varies from batch to batch of chloroform, regardless of grade. Chloroform purification by simple distillation as recommended, phenylhydrazine, or charcoal treatment followed by distillation does not reduce interference greatly. Low recovery of metaldehyde in the presence of chloroform-soluble plant material and emulsions resulting during bisulfite extraction indicated a need for a cleanup procedure. The following modifications are proposed to minimize solvent interference and emulsions, and to allow the use of technical chloroform in the extraction procedure.

Reagents and Apparatus

Florex, 60–100 mesh (Floridin Co., Tallahassee, Fla).

Hengar granules, uncoated, or other suitable boiling stones.

Other reagents are listed by Giang and Smith (*3*).

Column. A capillary tubing 80 × 0.5 mm., i.d., is sealed to a 300 × 20 mm., o.d., borosilicate glass tubing.

Evaporation apparatus. A 200 × 8 mm., o.d., glass tubing is held loosely inside of an ST 24/40 full length, inner member, ground joint (Pyrex No. 6560) using a collar made from a small sheet of polyethylene, through which a hole has been made to hold the 8-mm. tubing. When the joint is connected to the 100-ml., ST 24/40, round-bottomed boiling flask (Pyrex No. 4320), the 8-mm. tubing is adjusted by sliding the polyethylene collar to extend about 5 mm. or less into the sphere of the boiling flask. Compressed air line is connected to the upper end of the 8-mm. glass tubing.

Distillation unit. A Leibig condenser jacket (with screw-capped ends) was cut and resealed so that the distance between water inlet and outlet was about 11 cm. The inner tubing was cut so that 1.5 cm. extended from the ends of the capped condenser jacket. To one end was sealed an 18/9 socket joint and to the other a 140-mm. length of 6-mm., o.d., tubing. The 100-ml., round-bottomed, boiling flask, tilted 60° from the vertical, was connected to the condenser in the vertical position by means of a short adapter made from an ST 24/40 inner joint and a 18/9 ball joint.

Method

Extraction. Frozen samples of plant material are passed through a food chopper and mixed. A 100- to 200-gram laboratory sample is mixed in a fruit jar with sufficient sodium sulfate to remove most of the water within 1 to 2 hours. The sample is broken into small particles and chloroform added in a two to one volume per sample weight ratio. The mixture is shaken by an International shaker at 400 vertical 1-inch strokes per minute for 30 minutes. The liquid is separated by gravity filtration through a Whatman No. 1 paper or glass wool.

Cleanup and Evaporation. A 50-ml. aliquot is passed by gravity through a 90-mm. column of dry Florex. (About 13 grams of Florex is required for one determination.) The flow is regulated to about 5 to 10 ml. per minute by selection of a capillary of suitable length. The bottom of the tube is lined with a disk of glass-fiber filter paper. When the chloroform in the column reaches the level of the adsorbent, the sides are washed down, and more chloroform is added as necessary for washing. No metaldehyde is found in the first 20 ml. of the effluent; this portion may be used for rinsing. The next 60 to 70 ml. containing metaldehyde is collected in a graduated, 4-ounce medicine bottle for convenient storage. The Florex is discarded after one use. The effluent from the column is shaken in a separatory funnel with 3 ml. of fresh 2% sodium bisulfite solution (2 grams of sodium metabisulfite per 100 ml. of distilled water) and washed three times with 10-ml. portions of water. The chloroform phase is then transferred to a 100-ml. boiling flask and evaporated to about 10 ml. in a beaker of water at an initial temperature of 15° to 20° C. and just to dryness with agitation using filtered compressed air

Distillation. When the chloroform in the pesticide extract has just disappeared, 15 ml. of cold 0.14*N* sulfuric acid and a Hengar granule, or other boiling chip, are added. The flask is immediately attached to the distillation apparatus, which is prepared to collect the distillate at ice-bath temperature in a 25-ml. volumetric flask containing 2 to 3 ml. of aqueous 2% sodium bisulfite. The liquid is distilled by means of a microburner to 3 or 4 ml. of liquid. Agitation of the sample in the flask by means of the ball and socket joint to bring the acid in contact with the residue is important, especially during the initial part of the distillation. After the condenser is rinsed from the socket joint down, the receiver flask is filled to volume with water.

Color Development. The color is developed as recommended (*3*) using 1 ml. of the distillate or the diluted distillate containing an equivalent of not more than 5 μg. of metaldehyde.

The developed color is measured photometrically in a 1-cm., borosilicate-glass cuvette at the absorption peak of 570 mμ. A standard is run simultane-

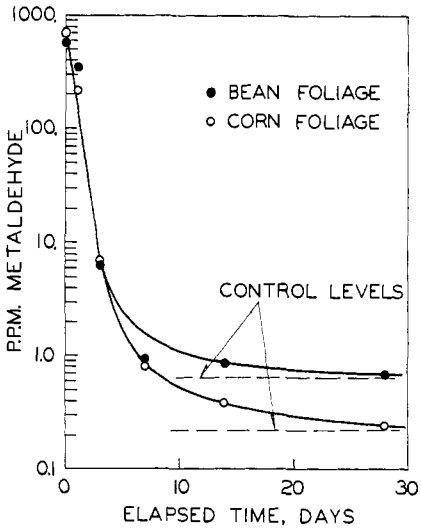

Figure 1. Relationship of metaldehyde concentration to time elapsed

ously beginning with the column filtration or the evaporation of a chloroform solution of standard metaldehyde.

Results and Discussion

During routine analyses by the Giang and Smith procedure, metaldehyde in chloroform was concentrated without appreciable loss by simple distillation in the absence of mineral acid. In so doing, solvent interference was also concentrated. However, in samples where chloroform was taken to dryness at low temperatures, in the absence of acid, metaldehyde remained but solvent interference was removed. This interference could not be removed by bisulfite extraction. Use, in the Giang and Smith procedure, of chloroform distilled from a strong base, increases the interference, indicating that it is caused by a product of chloroform hydrolysis.

With bean and corn foliage samples, a direct extraction of the ground foliage with chloroform was difficult because of emulsification. With the use of ethanol or isopropanol, the emulsion resolved rapidly and completely; however, these solvents interfered in the colorimetric step. Sodium sulfate, although requiring extra handling, did not interfere.

The use of Florex column was necessary because of emulsification of extracts during the bisulfite extraction. While the column does not remove all the undesirable impurities in the chloroform extract, it reduces the impurities to a point where emulsification is not a problem.

Table I. Recovery of Metaldehyde from Corn Foliage, Bean Foliage, and Strawberry Extracts

Extract	Added P.P.M.	Total Recovered, Av. P.P.M.	Control, Av. P.P.M.	Added P.P.M. Recovered, Av.	Replications
Corn foliage	2.00	2.11	0.31	1.80	4
	1.00	1.25	0.34	0.90	3
	0.40	0.71	0.28	0.42	4
	0.20	0.36	0.20	0.16	4
Bean foliage	2.00	2.19	0.76	1.43	5
	1.00	1.24	0.58	0.66	5
	0.40	1.09	0.64	0.45	5
	0.20	0.86	0.65	0.16	4
Strawberry, ripe fruit	1.00	1.08	0.19	0.89	7
	0.40	0.50	0.07	0.43	7
	0.20	0.29	0.14	0.15	7

Figure 1 shows metaldehyde recovery from corn and bean foliage after an initial spray application of 4 pounds per acre. The samples, immediately after harvest, were sealed in plastic bags and stored at −18° C. for 3 to 4 months preceding analysis. The control level expressed as metaldehyde was 0.22 p.p.m. for corn foliage. Control level for bean foliage was 0.65 p.p.m. These levels were approached, in each case, after 28 days. Figure 1 also shows one of the two basic types of organic pesticide behavior discussed by Gunther and Blinn (*4*). This characteristic degradation-persistance curve is described as a general phenomenon with organic pesticides applied to very oily or waxy surfaces.

The high control value for beans, even after solvent evaporation and bisulfite extraction, may be due to the presence of low molecular weight α-keto or α-hydroxy acids that had decomposed to the aldehyde on heating during the aldehyde distillation. Attempts to separate α-keto acids from the distillate by the use of basic resin column in the bicarbonate form on the assumption that some of the acids may have been distilled over, indicated that they were not present as such in the distillate. If, prior to color development, the solution is kept cool throughout the mixing with sulfuric acid, lactic acid does not react to any appreciable extent to form acetaldehyde. Thus, if a solution containing both pyruvic or lactic acid and acetaldehyde is given another treatment with *p*-phenylphenol reagent after the first steam bath treatment to destroy the excess *p*-phenylphenol and allowed to stand another 30 minutes at 30° C., the aldehyde formed by the keto or hydroxy acid can be determined.

The recovery of added standard from corn foliage extract (Table I) was comparable to that from strawberries. Recovery from bean foliage extracts was lower at levels of 1 to 2 p.p.m., but comparable to the others in the lower ranges. In all cases, 100 ml. of the extract were fortified. Fifty milliliters of this were passed through the column. Recovery above the level of 2 p.p.m. was not investigated since the primary area of interest was below 1 p.p.m.

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