Determination of Chloroacetaldehyde-2,4-dinitrophenyl Hydrazone in Apples, Peaches, and Cherries

METHRATTA P. THOMAS,[1] HENRY J. ACKERMANN, and EDWARD J. KUCHAR

Olin Mathieson Chemical Corp., New Haven, Conn.

A method to determine microgram quantities of chloroacetaldehyde-2,4-dinitrophenylhydrazone in apples, peaches, and cherries is described. A weighed amount of the fruit is extracted with methylene chloride. An aliquot of the extract is taken on a silicic acid column and eluted with methylene chloride. The eluate is evaporated to dryness. The residue is redissolved in petroleum ether and alcohol, and extracted with a 9.4 pH buffer. The buffer layer is filtered and its optical density measured at 360 mμ. The amount of the chemical in the sample is then calculated using a preconstructed standard curve.

CHLOROACETALDEHYDE - 2,4 - DINITROPHENYL HYDRAZONE, OM-1763, is a promising experimental fungicide. During its development, a method to determine microgram quantities of this chemical in apples, peaches, and cherries became necessary. The procedure described was developed for this purpose.

Experimental

Apparatus. Chromatographic tubes (19 × 450 mm.) with Teflon stopcocks (Fischer and Porter Co., Warminster, Pa.).

Filter tubes (10 × 150 mm.) with coarse-porosity fritted disk (Will Scientific Inc., Rochester 3, N. Y.).

Separatory funnels, globe-shaped, 500 ml. with Teflon stopcocks (Scientific Glass Apparatus Co., Bloomfield, N. J.).

Osterizer blender, 1-mm. thick Teflon gaskets for the cutting assembly, and 1/2-gallon jars. Fabricate the Teflon gaskets from the proper size Teflon tubes.

Ultrasonic generator and high-intensity transducerized tanks (Cole-Parmer, Chicago, Ill.).

Rotating vacuum evaporator (California Laboratory Equipment Co., Oakland, Calif.).

Beckman DU spectrophotometer and cells.

Reagents. SOLVENTS. Distilled methylene chloride and petroleum ether. Reagent grade acetone and ethyl alcohol (denatured formula 2B).

BUFFER. Dissolve 13.4 grams of ammonium chloride in deionized water. To the solution, 22 ml. of concentrated ammonium hydroxide are added and the solution is made up to 1 liter. The pH of this solution is usually 9.4 ± 0.1.

DRY SILICIC ACID. Mallinckrodt's chromatographic grade silicic acid is heated at 100° ± 5° C. overnight in a vacuum oven. The sample is stored in a desiccator.

20% MOIST SILICIC ACID. To 100 grams of dry silicic acid in a screw cap bottle add 20 ml. of deionized water. Shake vigorously to break up the lumps and equilibrate the contents by mechanically tumbling the bottle overnight.

NEUTRAL CELITE. Celite 545 is slurried with dilute (1/1) HCl, filtered, and washed with distilled water until free of acid. The material is dried at 100° ± 5° C. overnight and stored in screw cap bottles.

Chloroacetaldehyde - 2,4 - dinitrophenyl Hydrazone (CADNPH). About 2 grams of OM-1763 containing 95% CADNPH are dissolved in 100 ml. of methylene chloride. Ten-milliliter portions of this solution are chromatographed on 5-gram dry silicic acid columns. The fast moving band in each column is eluted with methylene chloride, combined, and evaporated almost to dryness at 43° ± 2° C. The pale greenish yellow crystals of CADNPH are dried further overnight in a vacuum oven at 35° C. and stored in a brown screw cap bottle.

Preparation of the Standard Curve

Weigh 0.1 gram of CADNPH accurately into a 100-ml. volumetric flask. Dissolve and make up the solution to volume with methylene chloride. Dilute aliquots of this to give standards containing 4, 10, 20, 40, 50, 80, and 100 μg. of CADNPH per milliliter.

One milliliter of a standard is pipetted into a 250-ml. Erlenmeyer flask. The methylene chloride is evaporated off carefully using a 45° C. bath. The residue is dissolved in 8.5 ml. of alcohol. To the solution, 10 ml. of petroleum ether are added, followed by 8.5 ml. of buffer. The flask is shaken well and emptied into a 60-ml. separatory funnel with Teflon stopcock.

The separatory funnel is allowed to stand undisturbed until the solution separates into two layers. The lower alcohol-buffer layer then is filtered through 0.5 gram of neutral Celite packed in a filter stick. The absorbance of the filtrate is measured at 360 mμ in 5-cm. quartz optical cells against water.

This procedure is repeated with 1 ml. of each of the remaining standards. The absorbances are plotted against the corresponding micrograms of CADNPH and the slope, M, of the best straight line is determined. The value of the slope is equal to the absorbance of the final solution contributed by 1 μg. of CADNPH. The data developed to obtain the slope, M, are given in Table I.

Extraction of CADNPH

The stalks are removed from the fruit. Cherries are not pitted, but stones are removed from the peaches. Both apples and peaches are cut into small pieces to prepare subsamples.

To each 200-gram aliquot in a 1/2-gallon jar, about 900 grams of anhydrous sodium sulfate and 500 ml. of methylene chloride containing 0.1% (v. v.) glacial acetic acid are added. The contents of the jar are blended for 5 minutes in an Osterizer blender and then equilibrated for 10 minutes in a sonic vibrator. The methylene chloride in the jar is poured into a 500-ml. separatory funnel containing a static bed of anhydrous sodium sulfate (300 grams) and a thin layer of neutral Celite (0.5 gram) and allowed to drain into a 250-ml. graduated cylinder.

Cleanup of the Extract

A 250-ml. aliquot of the extract is concentrated to about 3 ml. over a steam bath using a strong stream of nitrogen. The concentrate is transferred quantita-

Table I. Data for the Standard Curve[a]

CADNPH, μg.	Net Absorbance,[b] A
220	5.079
110	2.529
88	2.019
55	1.268
44	1.021
22	0.505
11	0.252
4.4	0.101

[a] Slope of the line = M = 0.023 absorbance per μg.

[b] Apparent absorbance = 0.021. (0.021 = average reagent absorbance). Absorbance values under 1 were measured in 5-cm. cells, and those above 1 taken in 1-cm. cells.

[1] Present address, Wallace & Tiernan Inc., Belleville, N. J.

Table II. Control Blanks and Recovery Values Obtained with Apples

Source and Variety	CADNPH Blanks, P.P.M.	CADNPH Recoveries Added, p.p.m.	CADNPH Recoveries Found, net %
Michigan, McIntosh	0.02	0.05	63.0
	0.02	0.10	74.0
	0.02	0.20	81.0
Wisconsin, McIntosh	0.02	0.05	73.0
	0.02	0.10	71.0
	0.02	0.10	85.0
North Carolina, Red Delicious	0.02	0.10	66.0
	0.01	0.20	81.0
	0.02	0.20	72.0
North Carolina, Golden Delicious	0.01	0.05	81.0
	0.01	0.20	79.0
	0.01	0.40	76.0
Delaware, Blaxstayman	0.02	0.05	72.0
		0.61	86.0
		1.23	89.0
Pennsylvania, Stayman	0.02	0.05	72.0
Oregon, Rome	0.02	0.10	71.0
		0.62	82.0

Table III. Control Blanks and Recovery Values Obtained with Peaches and Cherries

Replicate No.	CADNPH Blanks, P.P.M.	CADNPH Recoveries Added, p.p.m.	CADNPH Recoveries Found, net %
	Elberta Peaches		
1	0.01	0.06	101.0
2	0.01	0.12	101.0
3	0.01	0.23	92.0
4	0.01	0.45	90.0
5	0.01	0.94	92.0
	Bing Cherries		
1	0.01	0.09	84.0
2	0.02	0.11	94.0
3	0.02	0.23	93.0
4	0.01	0.51	89.0
5	0.02	0.80	90.0
6	0.01	1.14	91.0

tively with methylene chloride into a chromatographic column of 3 grams of dry silicic acid prepared using methylene chloride. About 2 grams of 20% moist silicic acid are added to the column and slurried with the concentrate using a long glass rod without disturbing the dry silicic acid bed. The column then is eluted with methylene chloride at about 5 ml. per minute. To speed up the elution, a positive pressure of about 10 inches of Hg is applied to the column. About 85 ml. of the eluate are collected and evaporated in a rotating vacuum evaporator using gentle heat until free of methylene chloride.

Determination of CADNPH

The residue from the rotating evaporator is dissolved in 8.5 ml. of alcohol and 10 ml. of petroleum ether using an ultrasonic vibrator. To the solution, 8.5 ml. of the buffer are added, processed as described in Preparation of Standard Curve, and the absorbance, A, of the alcohol–buffer layer is measured. The apparent parts per million of pesticide in the sample is calculated using the formula $M/100A$.

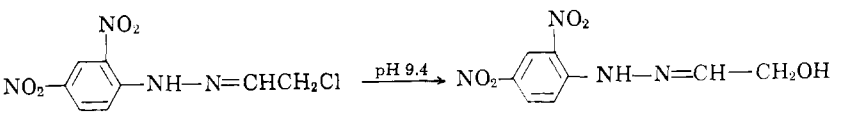
Blanks and recoveries for the method are established by analyzing aliquots of the control samples before and after fortifying with known amounts of CADNPH. The results are calculated from the following equations.

$$\text{Recovery, net \%} = \frac{(\text{apparent p.p.m. recovery} - \text{apparent p.p.m. control}) \times 100}{\text{p.p.m. CADNPH added}}$$

$$\text{Control, net p.p.m. CADNPH} = \frac{\text{apparent p.p.m. in control sample} \times 100}{\text{net \% recovery}}$$

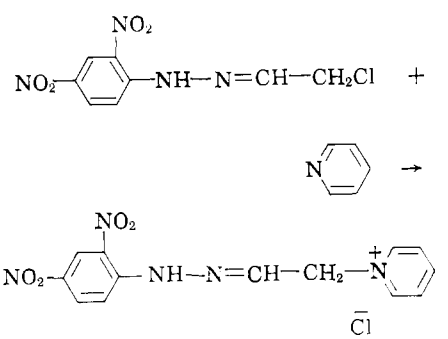
Results and Discussion

CADNPH is stable at room temperature and in neutral or acidic solutions (*3*). It contains a reactive chlorine, which is easily removed at a pH of about 9.4, to form glycolic aldehyde-2,4-dinitrophenylhydrazone (GADNPH).



In solutions of higher pH the nitro groups of 2,4-dinitrophenyl hydrazones polarize to produce a wine-red colored solution (*1*, *2*). Both CADNPH and GADNPH are yellow and stable in the buffer solution. They give a wine-red color with alcoholic potassium hydroxide. This color is very unstable. Therefore, the ammonia buffer does not polarize the nitro groups of CADNPH or GADNPH and the pH of the buffer is critical to produce a stable color. The authors have not investigated the pH limits of the buffer and have had no problem with the buffer solutions prepared as directed in the procedure.

In an attempt to substitute pyridine for the buffer, the authors found the pyridine reacted with CADNPH in a different way. The reaction product gave a deep blue color with alcoholic potassium hydroxide. The GADNPH did not produce the blue color under the same circumstances. This suggested that the presence of the active chlorine is necessary to form the pyridine–CADNPH compound and that most probably the compound is a quaternary salt.



Aqueous alcohol (50/50) quantitatively extracts GADNPH from petroleum ether in the presence of very persistent fruit pigments. Besides, the color of this extract is stable for at least an hour. These two properties together make the method highly sensitive and easily applicable.

Loss of the pesticide has been noticed when blended with apples and methylene chloride. The nature of this loss is not understood clearly. However, the use of methylene chloride containing 0.1% glacial acetic acid has prevented any significant loss of CADNPH during the analysis. The control blanks and recoveries obtained with different samples are listed in Tables II and III.

The 3-gram dry silicic acid column cleans the extracts effectively. However, some of the extracts form a dense sludge on the column and make further elution extremely slow. This situation is prevented by slurrying 2 grams of 20% moist silicic acid, which adsorbs the sludge-forming material and settles to form a favorably porous bed.

The color of the alcohol–buffer solution of CADNPH has an absorption peak at 360 mμ. This peak is seen distinctly in a test solution containing as little as 0.4 μg. of CADNPH per ml. The fruit pigments do not have this peak. Therefore, the peak at 360 mμ can be used as a confirmatory test for CADNPH when its concentration exceeds 0.4 μg. per ml. Thus, the method is selective as well as sensitive.

Literature Cited

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