Rapid Determination of Diphenylamine in Apples by Direct Bromination and Gas Chromatography

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A rapid method is described for the determination of the scald-control chemical, diphenylamine, in apples. The method consists of direct bromination of an acetone-hexane extract of apple to yield presumably the ortho-, para-hexabromo derivative of diphenylamine. This electrophilic compound is chromatographed and determined by electron affinity spectroscopy. The method is sensitive to about 0.02 p.p.m. of diphenylamine. Complete analysis requires about 1 hour per sample. The recovery of diphenylamine from the fruit is good. Residue data from the analysis of treated apples are presented.

Smock (*4*) showed that diphenylamine effectively controls apple scald. A tolerance of 10 p.p.m. of diphenylamine in apples has recently been granted. Spectrophotometric methods (*1*, *3*) have been used for determining residues of this chemical. The present method involves direct bromination of an acetone-hexane extract of treated fruit to yield presumably the ortho-, para-hexabromo derivative. This electron-capturing compound is chromatographed and determined by electron affinity spectroscopy. Diphenylamine itself does not give sufficient response for this type of detection.

Equipment

A Barber-Colman Model 10 gas chromatograph was used in this study. It was equipped with a battery operated (*2*) Barber-Colman Model No. A-4071, 6-cc. detector containing 56 μc. of radium-226. The detector was operated at 22 volts. The bromo derivative of diphenylamine was found to give the largest response at this voltage setting. The electrometer was modified to give a gain of 3000, and this setting was used exclusively. The recorder was a Wheelco, 0 to 50 mv. equipped with 10-inch chart paper, running 10 inches per hour.

The column was borosilicate glass, U-shaped, 9-mm. o.d. and 2 feet long. The packing was 5% ethyl acetate-fractionated, Dow Corning high-vacuum silicone grease on 80- to 100-mesh, acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing, glass elbows, and silicone rubber through-septums. The operating temperatures for the column, flash heater, and detector were 235°, 275°, and 265° C., respectively, and nitrogen (60 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 245° C. before use.

Procedure

Place 10 to 15 apples in a Hobart Food Cutter and thoroughly chop the sample. Mix the chopped sample manually and place a 25-gram subsample in a semi-micro Waring Blendor jar. Add 70 ml. of distilled acetone and blend the mixture for 2 minutes. Transfer the contents to a sintered glass funnel (40 mm., coarse porosity disk) upon which a thin layer of glass wool has been placed. Filter by suction into a 125-ml. flask marked at approximately 100 ml. Rinse the sample with two 20-ml. portions of acetone each time compressing the sample with the bottom of a 50-ml. beaker to squeeze out the remaining acetone. Pour the filtrate into a graduated cylinder and adjust the volume to 100 ml. either by evaporation with an air stream or by the addition of acetone.

Place 1 to 5 ml. of the acetone solution in a 100-ml. volumetric flask. Add 5 to 15 ml. of distilled hexane to the flask and make to volume with distilled water. The water must not come in contact with rubber tubing which usually contains diphenylamine as an antioxidant. The volumes of acetone and hexane used will depend on the level of diphenylamine in the fruit. For instance, 1 ml. of acetone and 15 ml. of hexane were the volumes required for 3 p.p.m. of diphenylamine in the fruit. Shake the flask vigorously for 1 minute. Pipet 1 ml. of the hexane (upper) layer into a 10-ml. test tube. Add 0.1 ml. of a carbon tetrachloride solution saturated with iodine crystals and containing 5% (by volume) of liquid bromine. Shake the tube for 10 minutes in a water bath maintained at 40° to 45° C. Evaporate off the solvent and halogens with a gentle air stream. The air is passed through a glass tube containing cotton and through Tygon tubing. Again add 0.1 ml. of the bromine-iodine reagent and shake the test tube for 10 minutes in the water bath. After shaking, allow the tube to stand in the water bath for an additional 10 minutes. Evaporate the contents to dryness with air. Add 1 ml. of distilled hexane to the tube, dissolve the contents by swirling, and inject 5 to 10 μl. of the solution into the column. The retention time for the brominated product is about 15 minutes.

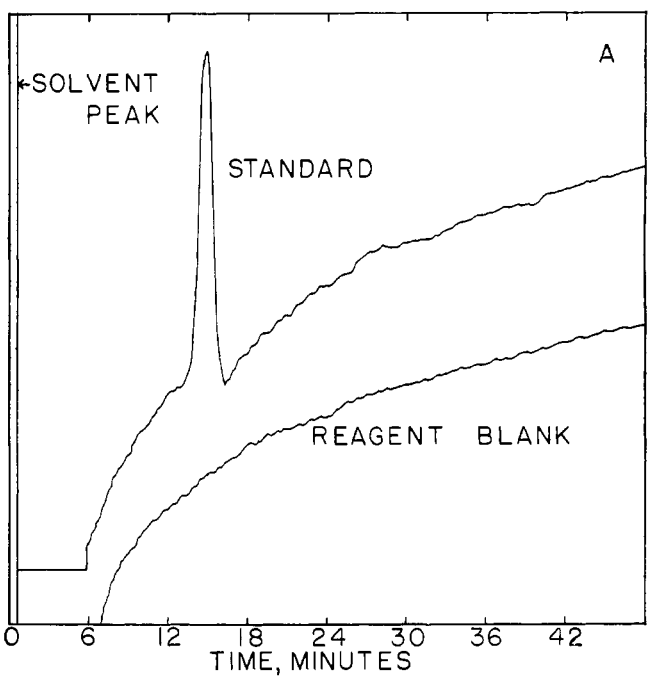
The standard curve for diphenylamine was developed as follows. Pipet 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml. of a 0.1 μg. per ml. solution of diphenylamine in distilled hexane into a series of 10-ml.

Table I. Recovery of Diphenylamine Added to Apples

Apple Variety	Amount Added, P.P.M.	Recovery, %
Wagener	0.1	120
	0.3	97
	0.6	101
	1.0	118
	2.0	110
	5.0	105
Cortland	1.0	108, 110
McIntosh	1.0	118

Table II. Residues of Diphenylamine in Apples from Three Methods of Application

Apple Variety	Treatment	Residue, P.P.M.
McIntosh	Dip, 1000 p.p.m.	2.36
	Dip, 2000 p.p.m.	3.31
	Flood, 1000 p.p.m.	1.76
	Flood, 2000 p.p.m.	3.07
Check	...	0.10
Cortland	Tree spray, 2000 p.p.m.	1.70, 2.33
Check	...	0.04, 0.02



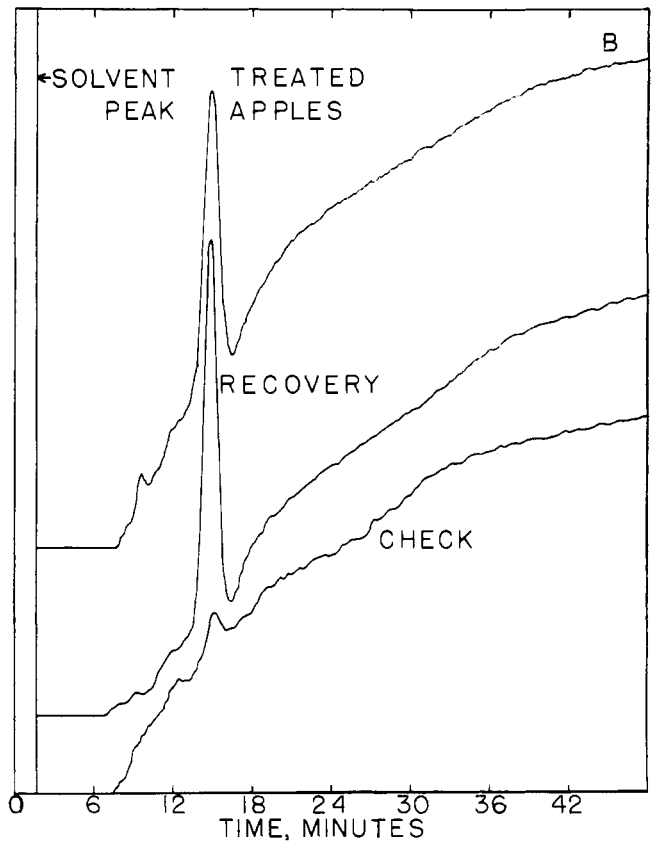


Figure 1. Chromatograms of (A) brominated standard solution of diphenylamine (0.0006 μg. injected) and reagent blank, and (B) Wagener apples untreated, fortified with 0.30 p.p.m. diphenylamine, and the 1000-p.p.m. dip-treated McIntosh apples

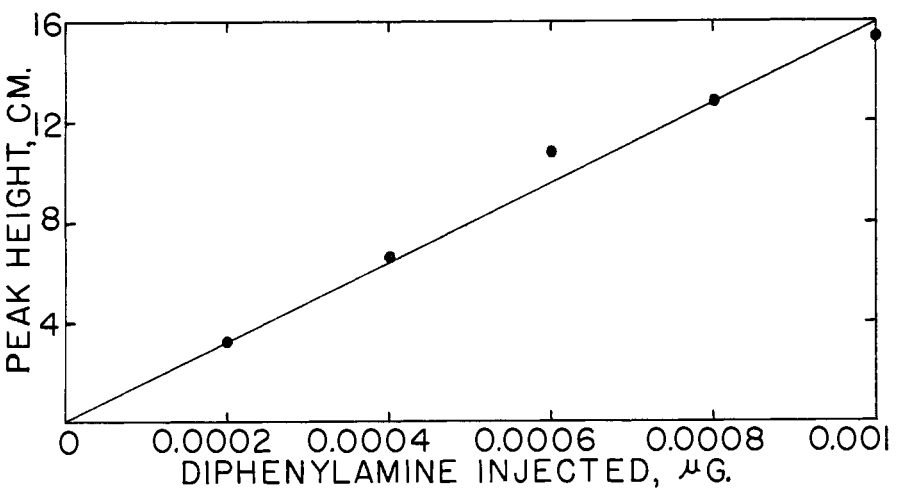


Figure 2. Diphenylamine standard curve

test tubes. Make each to a total volume of 1 ml. with hexane. Add 0.1 ml. of the bromine-iodine reagent and proceed as in the analysis of apples. Inject 10 μl. of each standard into the column. Plot peak height in centimeters against micrograms of diphenylamine injected.

Results and Discussion

Figure 1*A* shows the chromatograms of a brominated diphenylamine standard and the reagent blank. The flat portion in the chromatograms immediately following the solvent peak represents the time required for the detector to recover from injection of the solvent. This effect is pronounced in larger electron affinity detectors (6 cc.) (*2*). The chromatograms of untreated apples, treated apples, and recovered diphenylamine are shown in Figure 1*B*.

The recoveries of diphenylamine added to untreated apples are listed in Table I. The check value for the Wagener apples was 0.02 p.p.m. The check values for the other varieties are listed in Table II. The method is sensitive to about 0.02 p.p.m. of diphenylamine. This concentration would yield a peak height equal to about a 5% full-scale deflection when injecting 10 μl. of the sample. Figure 2 shows the standard curve for diphenylamine.

In September 1962, diphenylamine was applied to apples for scald control by the Cornell University Pomology Department. Solutions containing 1000 and 2000 p.p.m. of the actual chemical were prepared from Amchem's 83% dry wettable powder. One method of application involved dipping the fruit in the solutions. A second method of application involved a flood treatment in which the solution was allowed to flood through whole bushel crates of fruit. In both methods, the time of contact of the solution and fruit was from 2 to 4 seconds. A third method of application involved spraying the apples on the trees just before harvest with the 2000 p.p.m. solution. About 250 gallons of spray were used on 16 trees. The yield of apples per tree was about 15 bushels. Samples taken shortly after these treatments were analyzed by the method. Table II lists the residues found.

The residues of 1.70 and 2.33 p.p.m. represent apples from two separate crates which were harvested after the tree spray. Diphenylamine has been used by the Pomology Department here for apple scald control for about 5 years. Traces of diphenylamine were found in the untreated fruit. These values probably represent small amounts of the chemical in the fruit by contamination

from the surrounding atmosphere (diphenylamine has an appreciable vapor pressure) in which treated apples were stored. Contamination by contact with used wooden crates and other equipment was also possible.

An amino group on an aromatic ring activates the ortho and para carbons for bromine substitution. Iodine may be used as a catalyst to incorporate one atom of the bromine molecule into an anion, which can act as acceptor for the hydrogen atom to be displaced. The other atom is left as a positively charged ion—i.e., $Br^+(IBr_4)^-$. This may explain the course of bromination of diphenylamine when iodine is the catalyst. The bromination step must be conducted as described. Shorter reaction time leads to a mixture of partially brominated derivatives which will appear earlier in the chromatogram.

Solvents must be distilled before use to avoid an appreciable blank value. Diphenylamine is volatilized by an air stream but the brominated product is not. The bromination is therefore conducted in hexane to obviate evaporation.

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INSECTICIDE RESIDUES

Gas Chromatographic Determination of Organophosphorus Insecticides Using the Zeisel Alkoxyl Reaction

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A rapid gas chromatographic method is described for determining organophosphorus insecticides by electron affinity detection. A solution of the insecticide is evaporated in a small vial. Hydriodic acid is introduced, and the vial is sealed and heated. Methyl or ethyl iodides are evolved upon cleavage of the alkoxyl groups. The alkyl iodides are then syringed from the headspace and determined by electron affinity spectroscopy. Ethion and malathion have been determined by the method.

With a few notable exceptions, the organophosphorus insecticides are not exquisitely sensitive to the electron affinity detector as are many of the chlorinated compounds. Iodine imparts much greater sensitivity when introduced into an organic compound than any of the other halogens (*3*). The organophosphorus insecticides usually contain methoxyl or ethoxyl groups and yield the corresponding alkyl iodides upon reaction with hydriodic acid [Zeisel alkoxyl reaction (*4*)]. In this paper, this reaction is the basis for the gas chromatographic determination of ethion, $[(C_2H_5O)_2PS_2]CH_2$, and malathion, $(CH_3O)_2PS_2CH(COOC_2H_5)$-$(CH_2COOC_2H_5)$.

Equipment

A Barber-Colman Model 10 gas chromatograph was used with a battery-operated (*1*, *2*), No. A-4071, 6-cc. detector containing 56 μc. of radium-226. An optimum voltage of 11 volts was applied to the detector, and an electrometer gain of 3000 was used. The recorder was a Wheelco, 0 to 50 mv. equipped with 10-inch chart paper, running 10 inches per hour.

The column was U-shaped made of borosilicate glass, 9-mm. o.d. and 6 feet long. The column packing was 5% ethyl acetate-fractionated Dow Corning high vacuum silicone grease on 80- to 100-mesh, acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing, glass elbows, and silicone rubber through-septums. The operating temperatures for the column, flash heater, and detector were 35°, 40°, and 90° C., respectively, and nitrogen (10 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

Prepare a series of standard solutions (5 to 20 μg. per ml.) of the insecticide in a suitable solvent. Pipet 1 ml. of the solution into a No. 2 screw-cap vial. Evaporate the solution just to dryness with a gentle air stream. Add 1 ml.

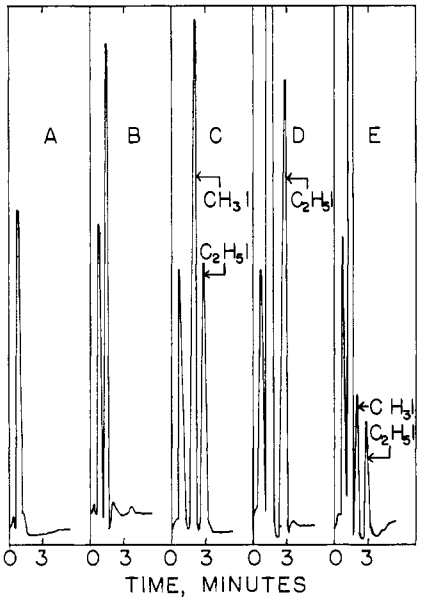

Figure 1. Chromatograms of (*A*) air, (*B*) reagent blank, (*C*) methyl and ethyl iodides in air, (*D*) ethion (13.6 μg.), and (*E*) malathion (9.8 μg.)