Determination of *N,N*-Dimethyl-2,2-diphenylacetamide (Diphenamid) in Plant Tissues Using Gas-Liquid Chromatography

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A method for determining the herbicide diphenamid in various crop samples, based on vapor phase chromatography and partition and column chromatography, is described. The sensitivity limit is 0.05 p.p.m. No detectable residue was found in field-treated tomato, pepper, white potato, sweet potato, and peanut.

THE selective herbicide *N,N*-dimethyl - 2,2 - diphenylacetamide, commonly known as diphenamid, has been found useful for control of weeds in a number of agricultural crops. Therefore, it was important to develop methods for the determination of diphenamid residues in the various produce.

Gas chromatography has been used increasingly in recent years for the determination of pesticide residues. The development of instruments with various types of detectors has also been rapid. The flame ionization detector and the thermal conductivity detector respond to diphenamid, but the latter was selected because of its high sensitivity.

The application of hydrogen flame detection to analysis of pesticide residue, formulation, and food analyses has been reported by several workers. The following have been described: determination of ethylene dichloride in aqueous solutions (*5*), determination of the antioxidants BHA and BHT in dried potato (*3*), analysis of herbicide residues using the flame ionization and electron capture detector (*9*), analysis of pesticide formulations (*1*), analysis of pesticides in surface and ground waters using flame ionization and electron capture detectors (*4*), determination of benzaldehyde in flavorings (*2*), analysis of spices in solid form (*10*), analysis of food products for aroma quality control (*11*), determination of monuron and diuron (*6*) and analysis of Trithion and other pesticides (*7*).

The flame ionization detector is not selective, since it responds to all carbon-containing compounds. Among its advantages are sensitivity and linearity of response over a relatively wide range of concentrations. The inherent lack of specificity of this detector is offset by adequate cleanup, which is required for best column and detector behavior regardless of the type of detector used. The method for diphenamid described here includes careful but not laborious cleanup. The method has been adaptable without major alterations to fruit, nuts, foliage, and tubers.

Equipment and Materials

Gas Chromatography Apparatus. F & M Model 500 gas chromatograph with F & M Model 1609 flame ionization detector attachment and Honeywell-Brown 1-mv. recorder with 1-second response.

F & M Model 810 gas chromatograph with flame ionization detectors and Honeywell-Brown 1-mv. recorder with 1-second response.

Gas Chromatography Columns. Stainless steel, $^1/_4$-inch o.d., 2 feet long, $^1/_8$-inch i.d., packed with 0.1 or 0.2% Carbowax 20M on 60- to 80-mesh glass beads (for F & M Model 500).

Stainless steel, $^1/_4$-inch o.d., 3 feet long, $^1/_8$-inch i.d., packed with 0.2% Carbowax 20M on 60- to 80-mesh glass beads (for F & M Model 810).

Syringe, Hamilton, 10-μl.

Sample tubes, Shevky-Stafford albumin tube, 6.5 ml., graduated in 0.01 to 0.40 ml.

Extractor, Waring Blendor with stainless steel or glass containers

Evaporator, Rinco rotary vacuum evaporators with water bath

Centrifuge, International Model U with head carrying 250-ml. bottles

Chromatographic columns, glass, 19-mm. i.d., 2 feet long, Fischer-Porter Co.

Scintillation counter, Model 314 EX-2 Tri-Carb liquid scintillation spectrometer system, Packard Instrument Co., Inc.

Benzene, redistilled in glass, b.p. 80–81° C.

Hexane fraction, redistilled in glass, b.p. 81–83° C.

Methylene chloride, redistilled in glass, b.p. 39.5–41.0° C.

Chloroform, reagent grade, Mallinckrodt

Alumina, Woelm neutral, Activity Grade I

Florisil, Floridin Co., 60- to 100-mesh, activated at 1225° to 1250° F., held at 130° C. until used

Standard diphenamid, *N,N*-dimethyl-2,2-diphenylacetamide found to be 99.7 ± 0.4% pure by phase solubility analysis.

Solutions of suitable concentration in methanol were used for fortifying crop samples. Solutions of 25, 50, and 100 μg. per ml. in benzene were used to check instrument response and to plot dosage-response curves.

Procedure

Extraction. Homogenize the plant sample in a Waring Blendor or a Hobart food chopper and take a 50- to 100-gram aliquot. Add water or sodium sulfate if needed to avoid emulsions or aid extraction. Blend the aliquot with 200 to 400 ml. of benzene in the Waring Blendor. A suitable procedure for most samples is three 30-second cycles at high speed with 30-second intervals between. Longer slow-speed cycles help prevent emulsion formation in samples such as peanuts. Centrifuge the resulting slurry at 1800 to 2000 r.p.m. for 10 minutes. Take a $^2/_3$ or $^3/_4$ aliquot of the benzene phase, using a glass syringe. Evaporate the benzene to dryness using a Rinco evaporator.

For crops like cottonseed and for green foliage, extract 50 grams of the ground plant material in a 1-liter flask with 400 ml. of benzene on a wrist-action shaker for 30 minutes. Filter through a Büchner funnel. Evaporate an aliquot of the filtrate using the Rinco equipment.

Partitioning. Dissolve the dried benzene residue in 50 ml. of acetonitrile saturated with hexane. Extract the solution with two 50-ml. portions of hexane saturated with acetonitrile. Discard the hexane layers. For oily crops such as peanuts and soybeans, use 100-ml. portions of hexane and acetonitrile. Evaporate the acetonitrile solution to dryness on the Rinco equipment.

Column Chromatography. Partially inactivate 50 grams of alumina by shaking with 100 ml. of benzene and 3 ml. of water for 30 minutes. Pour the slurry into the chromatography column and allow to settle. Place a plug of borosilicate glass wool on top of the alumina. When the benzene has drained down to the top of the alumina, transfer the acetonitrile residue to the column using five 3-ml. portions of benzene. Develop with benzene. Discard approximately

the first 300 ml.; collect the next 400 ml. and evaporate it to dryness. The volumes discarded and collected vary somewhat with different crops and batches of alumina. Run preliminary chromatographic profiles at intervals to determine the optimum collection volumes.

For cottonseed and green forage crops a Florisil column cleanup gives better results. Partially deactivate the Florisil to be used during one day by adding 8% by weight of water and tumbling for one hour. Place 50 grams of the Florisil in a column. Transfer the acetonitrile residue to the column using five 3-ml. portions of a mixed solvent (95 to 5, methylene chloride–chloroform, v./v.). Develop with the same solvent. Discard approximately the first 200 ml. Collect approximately 650 ml. and evaporate to dryness. Run chromatographic profiles periodically to determine optimum volumes and occasionally increase chloroform content of the developing solvent to 10% for some Florisil batches.

Vapor Phase Chromatography. Transfer the residue from column chromatography to a Shevky-Stafford albumin tube, using a total of 5 ml. of benzene in several portions. Place the tube in a water bath at 40° C. Evaporate to approximately 0.1 ml., using a stream of nitrogen. Rinse the sides of the tube with benzene during the evaporation. Adjust the volume to exactly 0.20 ml. with benzene and mix by twirling the tube. Inject 5 μl. onto the vapor phase chromatography column.

Either isothermal or temperature-programmed chromatography may be used. Representative isothermal conditions for two instruments are:

F & M Model 500, 2 Foot Carbowax Column	
Injection port temperature, ° C.	300
Detector temperature, ° C.	300
Range	1
Attenuation	32
Column temperature, ° C.	155–65
Air flow, ml./min.	350
Hydrogen flow, ml./min.	50
Carrier gas flow (N_2), ml./min.	25
F & M Model 810, 3-Foot Carbowax Column	
Injection port temperature, ° C.	300
Detector temperature, ° C.	300
Column temperature, ° C.	165–175
Range	1
Attenuation	32
Air flow, ml./min.	300 (18 p.s.i.)
Hydrogen flow, ml./min.	60 (20 p.s.i.)
Carrier gas flow (He), ml./min.	100 (40 p.s.i.)

For programmed temperature operation the conditions are similar, except that the temperature is programmed from 125° to 200° C. at 4° C. per minute, and attenuation is 64. Start programming 4 minutes after the injection.

Under isothermal operation the retention time for diphenamid is 8 to 11 minutes. When programmed, the retention time is 14 to 17 minutes. Measure the peak heights.

Calibration and Fortification

Establish a standard curve and check daily by injecting benzene solutions of standard diphenamid containing 25, 50, and 100 μg. per ml. Fortify untreated crop samples at the level expected in the unknown sample by adding a methanol solution (5 μg. per ml.) of standard diphenamid to the macerated sample. Macerate for 3 minutes before extraction.

Calculations

$$\text{P.p.m.} = \frac{\text{peak height (mm.)} \times \text{standard curve factor } (\mu\text{g./mm.}) \times 100}{\text{aliquot factor} \times \text{partition factor} \times \text{recovery} \times \text{sample weight (g.)}}$$

The aliquot factor arises from loss of diphenamid when only $^2/_3$ or $^3/_4$ of extracting benzene is taken.

The partition factor arises from loss of diphenamid during partition, based on a distribution of 20 to 1 between acetonitrile and hexane.

Table I. Efficiency of Extraction

	Operation	Recovery, %
A.	Extraction of tomato	93.6
B.	Partition	
	Hexane	4.7
	Acetonitrile	95.3
C.	Column chromatography	
	Fractions 1, 2, and 3	Nil
	Fraction 4	11.8
	Fraction 5	70.3
	Fraction 6	15.1
	Fraction 7	3.0
	Fraction 8	Nil

Efficiency of Extraction and Cleanup Procedure

The procedure was followed through all the steps using tomatoes fortified with diphenamid-C^{14}, labeled in the 1-position (specific activity 0.80 mc. per mmole). Samples taken from each step were counted with a Tri-Carb liquid scintillation spectrometer, Model 314 EX-2. The counting efficiency was determined by adding a known quantity (5000 d.p.m.) of toluene-C^{14}, followed by recounting. To follow the alumina column chromatography, eight 100-ml. fractions of the eluate were taken. Results are shown in Table I.

Discussion of Method

Programmed temperature vapor phase chromatography gives better resolution of the diphenamid peak and the column stays cleaner because of periodic operation at high temperature. Isothermal operation gives a broader peak but is more convenient. Linearity and reproducibility of peak height are approximately the same for both methods.

Since the flame ionization detector is sensitive to all carbon-containing compounds, the purity of solvents used is critical. The suitability of the benzene used for extraction may be determined by evaporating 500 ml. to 0.20 ml. and in-

Table II. Recovery of Diphenamid from Fortified Crop Samples

Crop	Diphenamid Added, P.P.M.	Sample Size, G.	Procedure	% Recovery
Tomato	0.20	100	Programmed	86, 81, 76
	0.10	100	Programmed	76, 89, 69, 79, 82, 82, 62, 66
	0.05	100	Programmed	74, 76
	0.025	100	Programmed	60, 70
	0.20	100	Isothermal	69, 81, 78, 95
	0.05	100	Isothermal	97
	0.20	50	Isothermal	88
	0.10	50	Isothermal	72, 88, 68, 75
	0.05	50	Isothermal	78, 64, 94
Pepper	0.10	100	Programmed	87, 83, 93, 93, 80, 70
	0.20	50	Isothermal	85, 82, 95, 87
	0.10	50	Isothermal	67, 78
	0.05	50	Isothermal	92, 78
Potato (Irish)	1.00	100	Programmed	87, 77
	0.20	100	Programmed	86
	0.10	50	Programmed	74, 88
	0.05	50	Programmed	98, 98
Potato (sweet)	0.20	100	Programmed	75, 86, 111, 82, 80, 61
	0.10	50	Programmed	74, 74, 74, 82, 108, 108
	0.20	50	Isothermal	88
	0.20	100	Isothermal	73, 71
	0.10	50	Isothermal	94, 85
	0.05	100	Isothermal	68, 74
Peanut	0.10	50	Programmed	94, 82, 80, 94
	0.10	100	Programmed	74, 81, 63, 69, 85, 80
	0.20	50	Isothermal	76, 81
	0.05	50	Isothermal	60, 76, 84, 70

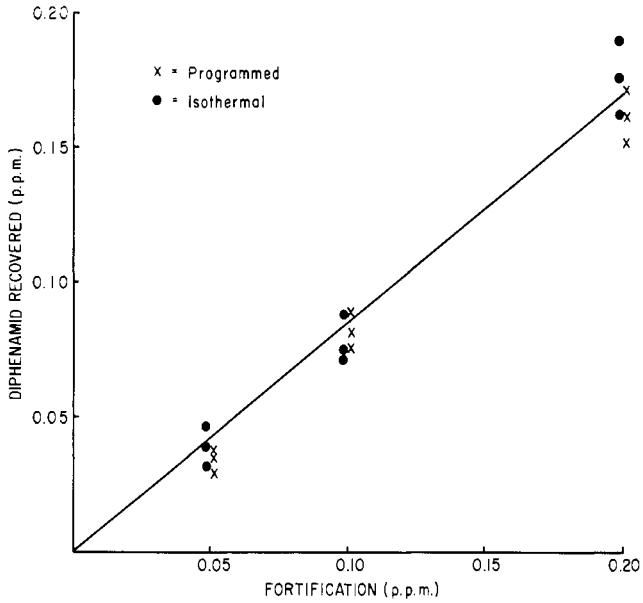

Figure 1. Recovery of diphenamid from fortified tomato

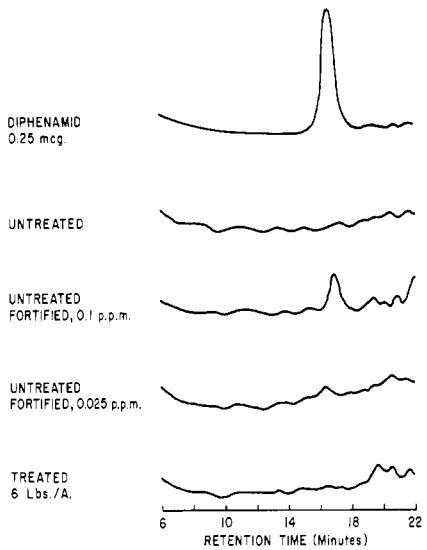

Figure 2. Determination of diphenamid residue in tomato

Programmed vapor phase chromatography, 100-gram samples

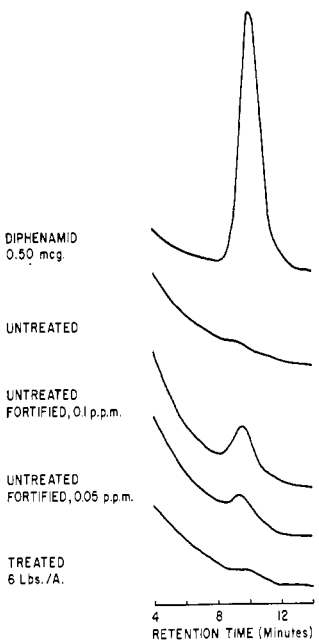

Figure 3. Determination of diphenamid residue in tomato

Isothermal vapor phase chromatography, 50-gram samples

jecting 5 μl. No peaks greater than 2 mm. should be seen for 30 minutes after injection. Solvents purchased from Burdick and Jackson, Inc., Muskegon, Mich., were found uniformly satisfactory.

The sensitivity of the method is considered to be 0.05 p.p.m. Fortification at this level consistently gives a small peak. Fortification at 0.10 or 0.20 p.p.m. was carried out in parallel with each set of samples, and at least daily, to determine recovery. Figure 2 shows a small peak from 0.025-p.p.m. fortification, but such recovery was not consistent.

A study of these peaks and fortification recovery data presented in Table II using a modification of the statistical method of Redman *et al.* (*8*) led to the conclusion that, if a true difference of 0.04 p.p.m. exists between treated and untreated samples, this method will classify the sample as having no residue approximately nine out of ten times.

Untreated samples occasionally and randomly show peaks corresponding to up to 0.03 p.p.m. of diphenamid.

Results

Tomato, pepper, Irish potato, sweet potato, and peanut kernels treated under recommended conditions were analyzed for diphenamid residue. None was found in harvest samples.

Figure 1 presents recoveries of diphenamid from tomato samples fortified at 0.05-, 0.10-, and 0.20-p.p.m. levels, using both programmed and isothermal gas chromatography. Figures 2 and 3 present gas chromatography curves obtained from tomato analyses using temperature-programmed and isothermal gas chromatography.

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