COMMUNICATIONS

Determination of Difolatan Residues in Fruits by Electron-Capture Gas Chromatography

A procedure for the extraction and determination of Difolatan residues on fruits is presented. The residues are extracted with benzene and analyzed by electron-capture gas chromatography. Cleanup of the extracts is necessary only when a high degree of sensitivity is required. The technique is applicable to a variety of stone fruits, and as low as 0.01 p.p.m. of residue can be detected. Recovery values obtained from fortified control samples averaged 93%.

The *N* - (1,2,2 - tetrachloroethylsulfenyl) - *cis* - Δ^4-cyclohexene-1,2-dicarboximide) (Difolatan) is a promising new fungicide useful for the control of many of the major economic fungus diseases of fruits and vegetables (Chevron Chemical Co., 1965). However, no published information is yet available concerning the extraction and quantitative determination of Difolatan residues on food and feedstuffs.

In the present paper an analytical technique for the extraction and quantitative determination of Difolatan residues on cherries, apricots, peaches, prunes, and nectarines is described. The fruits are extracted with benzene and analyzed without additional cleanup by electron-capture gas chromatography. As low as 0.1 p.p.m. Difolatan can be detected with considerable reliability. If an even lower limit of detection (0.01 p.p.m.) is desired, the extracts are concentrated and subjected to a microcolumn chromatographic procedure for cleanup.

MATERIALS AND EQUIPMENT

Gas Chromatograph and Recorder. An Aerograph HY-FI gas chromatograph Model 600B (Varian Aerograph) equipped with an electron-capture detector was used for all analyses. The detector, which is partially exposed to the outside of the instrument, was covered with a small widemouth Thermos bottle to minimize temperature fluctuations. The detector signal was supplied to a 1-mv. Honeywell Electronik 16 recorder having a 1-second pen response. The recorder was operated at a chart speed of 20 inches per hour. Both chromatograph and recorder were operated in conjunction with a 500-watt standard sinusoidal voltage regulator to minimize line fluctuations.

Column and Operating Conditions. The chromatograph was fitted with a 2-foot × $^1/_8$-inch spiral borosilicate glass column containing 5% purified Dow-11 (w./w.) silicone oil on acid-washed 70- to 80-mesh DMCS-treated Chromosorb G. The column was conditioned in the chromatograph for 3 days at 225° C.

During the analyses the column temperature was maintained at 197° C., and the glass-lined injection port at 212° C. The nitrogen carrier gas was passed through a small molecular-sieve filter (Varian Aerograph) and was regulated to provide a flow rate of 75 cc. per minute through the column. The column was further conditioned by injecting nanogram quantities of Difolatan onto the column until a constant response was obtained.

Planimeter. A compensating polar planimeter (Keuffel and Esser Co.) was used to convert chart data to digital data.

Reagents. Double-distilled reagent-grade acetone, hexane, and benzene. Granular anhydrous sodium sulfate. Florisil, 60- to 100-mesh (Floridin Co., Tallahassee, Fla.). Difolatan standard (Chevron Chemical Co., Ortho Division, Richmond, Calif.).

PROCEDURE

Preparation of Standard Curve. A calibration curve was prepared by diluting aliquots of a stock solution (1 mg. of Difolatan per ml. of acetone) with hexane to contain from 0.10 to 1.00 μg. of Difolatan per ml. Aliquots of these solutions, ranging from 1 to 10 μl. were injected into the gas chromatograph.

An adequate time interval, 18 to 20 minutes, was allowed for the recorder to respond to the detector signal and to recover to the previous baseline before a subsequent injection was made. Then the area under the curve was determined and the average area of three consecutive injections for each concentration was plotted against the amount of Difolatan injected.

Extraction Method. A representative sample of the fruit was macerated in a Hobart food chopper. Three hundred grams of the macerated material was placed in a 1-gallon tin can, equipped with a metal baffle for mixing, and 600 ml. of benzene was added. The can was sealed tightly with a lid and rolled on a mechanical roller for 30 minutes at 35 r.p.m. The solvent layer was decanted into a beaker containing 200 grams of anhydrous sodium

sulfate and mixed thoroughly. The mixture was filtered through fluted filter paper (Whatman No. 1) and the eluate stored in a tightly sealed bottle until analyzed. In this study all sample extracts were analyzed within 48 hours after processing.

For recovery studies, the control samples were fortified with a known amount of Difolatan after placing the macerated material in the tin can, but before the addition of the benzene.

Removal of Interfering Substances. For the detection of very low levels of Difolatan (10 to 100 p.p.b.), the charcoal-Attaclay cleanup procedure developed by Thornburg (1963) was adapted for use by employing a microcolumn.

MODIFIED TECHNIQUE. One hundred milliliters of extract representing 50 grams of fruits was concentrated to 10 ml. The concentrate was saturated with distilled water and the wet solvent passed through a 9-inch disposable capillary pipet (Standard Scientific Supply Corp., New York, N.Y.; Catalog No. 832450), containing a small glass wool plug, granular anhydrous sodium sulfate (2 cm. high in column) and topped with 2 cm. of charcoal-Attaclay mixture (Varian Aerograph). Slight positive pressure may be required to force the extract through the microcolumn. The eluate was evaporated to near dryness and the volume adjusted to 1 ml. with nanograde benzene.

Gas Chromatographic Analyses. Undiluted 5-μl. aliquots of the extracts from the control were chromatographed to determine if any interfering substances were present. Identical aliquots of fortified samples were also chromatographed to establish actual recovery values.

Depending upon the amount of Difolatan residues in the samples, the volume of extract which can be placed on the column will vary considerably. Preliminary traces should be made for each treated sample before attempting to make an accurate measurement. If high levels of Difolatan residues are present, the extracts from the treated samples

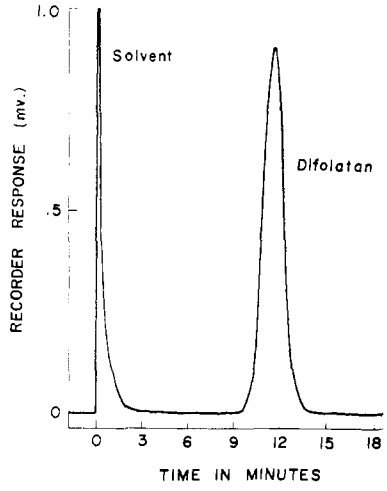

Figure 1. Gas chromatogram of Difolatan

Curve represents 1 nanogram of Difolatan

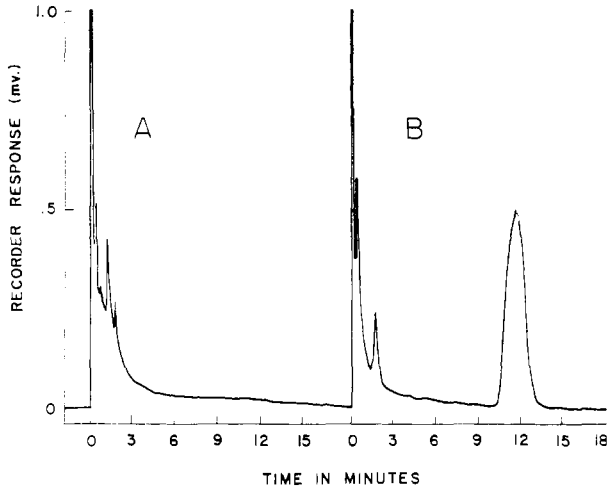

Figure 2. Gas chromatogram of Difolatan in cherry extract

A. Untreated control sample *B*. Untreated control sample fortified with 0.01 p.p.m. Difolatan

Table I. Recovery of Difolatan from Various Fruits

Fruit	Added, P.P.M.	Found, P.P.M.	Recovery, %	Av. Rec., %
Apricots	1.00	0.96	96	
	1.00	0.95	95	96
	1.00	0.96	96	
Cherries	0.50	0.42	84	
	0.50	0.45	90	87
	0.50	0.44	88	
Nectarines	1.00	0.97	97	
	1.00	0.94	94	96
	1.00	0.96	96	
Peaches	0.50	0.45	90	
	0.50	0.42	84	87
	0.50	0.43	86	
Prunes	2.00	1.92	96	
	2.00	2.00	100	99
	2.00	2.00	100	
Over-all av.				93

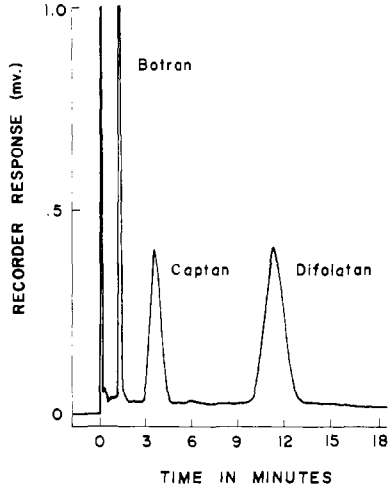

Figure 3. Chromatogram of field-treated cherries

Sample was treated with Botran, captan, and Difolatan. A 2-μl. aliquot of the benzene extract, equivalent to 1 mg. of fruit, was chromatographed. Response curve of Difolatan represents 500 picograms or 0.5 p.p.m.

may require dilution with benzene. The amount of residue in the sample is determined by comparing the averaged peak areas of a replicate series of three chromatograms with the calibration curve.

RESULTS AND DISCUSSION

The peak height, band width, and column retention time for a 1-nanogram sample of Difolatan are shown in Figure 1. The response of the electron-capture detection system to Difolatan was measured over a range of 0.2 to 10 nanograms. With a sensitivity setting of 1× and an attenuator setting of 2 on the instrument, the linearity of response was from 2 to 10 nanograms. At a more sensitive setting (10×) the response also was linear and ranged from 0.2 to 1 nanogram.

At levels of 0.5 to 2.0 p.p.m. recoveries of Difolatan from fortified control samples ranged from 84 to 100% (Table I). Because at these levels only negligible amounts of interfering substances were found in the untreated control samples, cleanup of the extracts was not required, the extracts simply being injected directly into the gas chromatograph. However, when an increased sensitivity was desired, it was necessary to purify and concentrate the extracts prior to analysis. Preliminary studies revealed that the recoveries of Difolatan using standard cleanup techniques (charcoal, Florisil, silicic acid, and alumina column chromatography; acetonitrile partitioning, etc.) were very poor. However, by using a modified charcoal-Attaclay procedure (Thornburg, 1963) as low as 0.01 p.p.m. Difolatan on cherries was detected with an average recovery of 83% (std. dev. ±1.0) (Figure 2). This level could possibly have been extended even lower had a greater sensitivity been desired.

To demonstrate the applicability of the procedure, actual field-treated samples were analyzed. The response curve of a cherry extract injected directly into the gas chromatograph is shown in Figure 3. This particular sample had also been treated with 2,6-dichloro-4-nitroaniline (Botran) and captan, but neither of these fungicides interfered with the determination of Difolatan.

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