COMMUNICATIONS

Gas Chromatographic Determination of Vinclozolin and Endosulfan in Strawberries

A gas chromatographic method is described for the determination of vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] and endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) residues in strawberries. After extraction by acetone homogenization and cleanup by Florisil column chromatography, vinclozolin and endosulfan isomers I and II were identified by using an electron-capture detector. Recovery of vinclozolin averaged 93 ± 16% from the various samples fortified at levels ranging from 2.1 to 26.4 ppb. Quantitative recoveries of endosulfan I (91 ± 14%) and endosulfan II (87 ± 24%) at different levels of fortification were also obtained. The detection limit was 0.05 ng for vinclozolin and 0.04 and 0.2 ng for endosulfan I and II.

Vinclozolin is a new fungicide being developed for use on grapes, strawberries, and many other cultivations. It is a fungicide effective against *Botrytis* sp., *Sclerotina* sp., and *Monilia* sp. (Di Giusto, 1978). Endosulfan is one of the most important insecticides in use as a contact poison on eating and sucking arthropods. Molinari and Del Re (1978) investigated the quantitative evaluation of vinclozolin on field-treated samples of grapes, must, and wine; they suggested an extraction with benzene, without following cleanup: the dried extracts were analyzed by gas chromatography with electron-capture detection. A number of papers have been written referring to the gas chromatographic analysis of endosulfan: Maier-Bode (1968) and Zweig and Sherma (1972) extensively reviewed them. To obtain necessary residue data we describe in this report a gas chromatographic method to determine vinclozolin in strawberries in the presence of endosulfan.

MATERIALS

Apparatus. A. C. Erba 2350 gas chromatograph, equipped with a ^{63}Ni electron-capture detector and a Spectra Physics System I integrator, was employed for the analysis. The gas chromatographic column was a 1 m × 4 mm i.d. glass column packed with 3% SE 52 on 100–120 mesh Chromosorb W.

Reagents. All solvents were reagent grade, but additional purification by distillation was necessary. Sodium sulfate was reagent grade (anhydrous). Florisil (Baker grade), 60–100 mesh, was activated 6 h at 200 °C and cooled in a desiccator before use. Vinclozolin reference standard (99%) was supplied by Dr. S. n. I. Ehrenstorfer, D-8900 Augsburg; endosulfan I (99%) and II (99%) were Pestanal by Hoechst.

ANALYTICAL PROCEDURE

Sample Preparation and Extraction. Strawberries were chopped up in a Braun mixer and 10–50-g samples of fresh whipped matter were dried in a Krist freeze-drier to remove water and to conserve products without losing the constituents and the pesticide residues. The very hygroscopic dried samples had already been put into protected closing containers immediately before removal from the plant and were stored at −20 °C.

Table I. Recovery from Strawberries Fortified before Extraction

fortification, ng/g	fortified sample, ng/g	nonfortified sample, ng/g	recov., %
	Vinclozolin		
2.1	9.2	7.7	71
2.1	9.1	7.2	90
4.3	6.3	2.8	81
4.3	23.2	19.2	93
4.3	15.1	10.1	116
9.2	18.8	8.8	109
9.6	138.0	128.6	98
9.6	31.0	24.2	71
10.8	41.6	32.6	84
10.8	48.4	36.4	112
10.8	55.1	43.0	112
12.3	30.7	19.0	95
20.8	67.8	48.0	95
21.2	75.8	52.2	111
24.2	171.6	153.6	74
26.4	54.8	35.4	74
		av 93 ± 16%	
		SE 4%	
	Endosulfan I		
6.3	10.5	5.2	84
6.3	12.3	6.7	89
18.8	16.5	0.0	88
19.4	17.6	0.0	91
19.6	24.9	0.0	127
20.1	18.2	1.9	81
22.0	28.0	9.2	85
25.0	30.0	6.2	95
53.9	68.6	26.1	79
		av 91 ± 14%	
		SE 5%	
	Endosulfan II		
9.8	14.2	2.6	118
9.8	24.4	13.7	109
10.3	8.9	0.0	86
10.6	8.3	0.0	78
10.7	11.5	0.0	107
10.9	12.6	3.7	82
12.0	33.2	28.4	40
13.6	28.9	19.2	72
29.4	43.9	17.4	90
		av 87 ± 24%	
		SE 8%	

A dried strawberry sample was extracted with 200 mL of acetone for 3 min in an Ultra-Turrax blender; 2 g of

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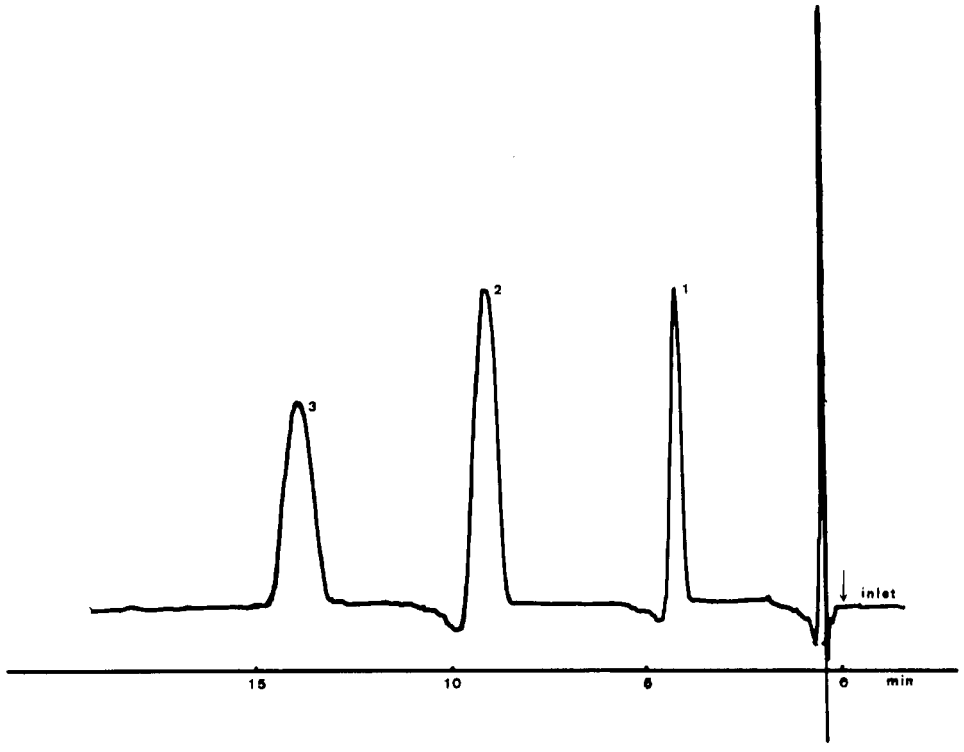

Figure 1. Chromatographic separation of a standard mixture of vinclozolin (1), endosulfan I (2), and endosulfan II (3).

Table II. Elution Data from Activated Florisil for Vinclozolin and Endosulfan I and II: Elution at Different Percentages of Ethyl Ether in Hexane (v/v; Eluant Amount, 150 mL)

ethyl ether, %	replications	% recoveries		
		vinclozolin	endosulfan I	endosulfan II
10	6	98 ± 4	97 ± 11	8 ± 25
20	4	112 ± 6	110 ± 13	10 ± 17
30	4	111 ± 7	115 ± 17	104 ± 19
40	4	116 ± 7	102 ± 9	108 ± 10
50	4	93 ± 4	101 ± 9	106 ± 11

anhydrous sodium sulfate was added to reduce moisture in the sample. The homogenate was filtered and the extract was quantitatively transferred to a 300-mL, round-bottomed flask. The acetone was removed by a rotary vacuum evaporator at 25 °C and substituted by 100 mL of hexane. The extract was then processed through the Florisil clean-up step.

Florisil Column Cleanup. A 10 × 200 mm chromatographic column was prepared by adding a plug of glass wool and 5 g of activated Florisil topped with 2 g of sodium sulfate. The column was rinsed with 50 mL of hexane and the wash was discarded. Three 5-mL rinses of hexane were added to extract. When the last of the rinses reached the top, the column was eluted at 5 mL/min with 150 mL of 30% ethyl ether in hexane (v/v). The eluate was completely evaporated and the residue was taken up in an appropriate amount of hexane for analysis.

Gas Chromatographic Analysis. The following conditions were used for gas chromatographic analysis. Temperature: inlet, 225 °C; oven, 185 °C; detector, 250 °C; gases: nitrogen (carrier), 40 mL/min; nitrogen (scavenger), 20 mL/min. With these chromatographic conditions vinclozolin eluted in approximately 4.5 min, endosulfan I in 9.7 min, and endosulfan II in 14.8 min.

A 2-μL aliquot from a total volume of 1.0 mL was injected into a gas chromatograph equipped with a ^{63}Ni electron-capture detector. Peak areas determined by a computing integrator were compared to standard curves of vinclozolin and endosulfan I and II in order to calculate the amount of residues present.

RESULTS AND DISCUSSION

Gas chromatography has been used for the analysis of pesticide residues in a countless number of diverse investigations widely reviewed by Zweig and Sherma (1972). Optimum response for vinclozolin and endosulfan was given by the above-mentioned conditions. This method was applied to field-treated samples and not very high residues were found in any of the samples analyzed. Standards were added as a dilute hexane solution to the sample before freeze-drying to test the loss of pesticides

Table III. Elution Data from Activated Florisil for Vinclozolin and Endosulfan I and II: Elution at Different Volumes of Eluant (Ethyl Ether in Hexane)

ethyl ether, %	replications	pesticide	% recoveries in increasing volume of eluant				
			30 mL	+30 mL	+30 mL	+60 mL	total recov., %
10	3	vinclozolin	85	6	0	0	91
	3	endosulfan I	10	0	0	0	10
	3	endosulfan II	0	0	0	0	0
20	6	vinclozolin	95	2	0	0	97
	3	endosulfan I	97	0	0	0	97
	3	endosulfan II	0	8	24	26	58
30	6	vinclozolin	98	0	0	0	98
	3	endosulfan I	91	0	0	0	91
	3	endosulfan II	8	63	10	3	84

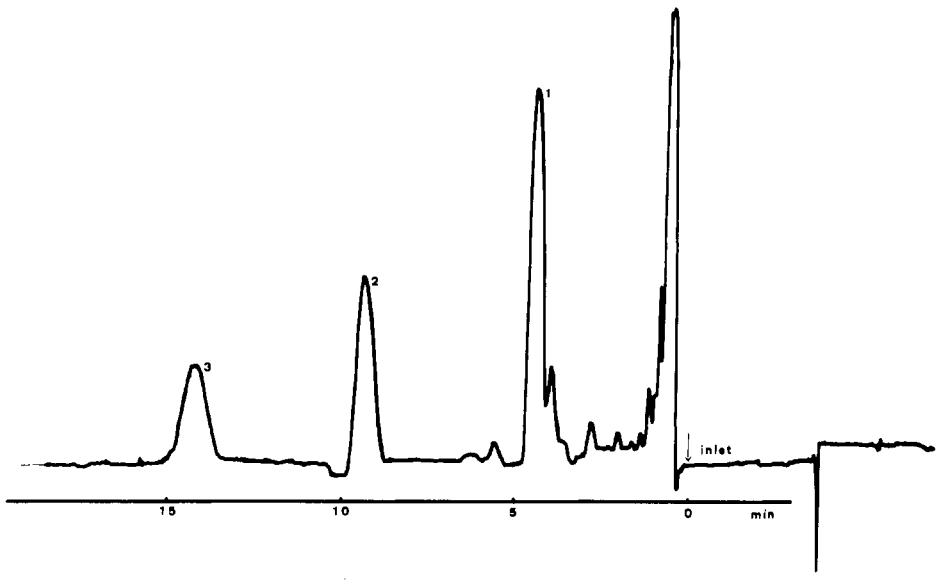

Figure 2. Chromatogram of strawberries, sample no. 6: vinclozolin (1), endosulfan I (2), and endosulfan II (3).

Table IV. Residues of Vinclozolin and Endosulfan in Treated Strawberries (μg/kg Fresh Weight)[a]

		sample collected at beginning of ripening			sample collected at end of ripening (after 3 weeks)		
sample no.	cv.	vinclozolin	endosulfan I	endosulfan II	vinclozolin	endosulfan I	endosulfan II
1	Gorella	54.0	0.0	0.0	10.8	0.0	0.0
2	Gorella	106.1	1.1	0.5	20.2	0.0	0.0
3	Red Gaulett	145.7	1.3	0.5	21.4	0.0	0.0
4	Umigento	141.9	0.0	0.0	30.8	0.0	0.0
5	Gorella	63.2	30.6	30.2	18.6	16.8	25.8
6	Gorella	133.2	31.3	22.4	52.9	28.7	34.0
7	Gorella	46.2	11.4	16.0	27.4	68.2	30.8
8	Gorella	64.2	1.4	0.0	25.6	1.5	1.8

[a] Samples 1-4 have not been treated with endosulfan; sample 7 has not been treated with vinclozolin, but it could be contaminated by drift.

during lyophilization: recoveries averaged in eight replications 90 ± 17% for vinclozolin, 86 ± 3% for endosulfan I, and 115 ± 8% for endosulfan II.

Table I shows recovery data from strawberry samples fortified after freeze-drying and before the addition of solvent and extraction. Fortification levels ranged from 2.1 to 26.4 ppb of vinclozolin, from 6.3 to 53.9 ppb of endosulfan I, and from 9.8 to 29.4 ppb of endosulfan II. The recoveries of vinclozolin averaged 93 ± 16% from added dried samples. Endosulfan I recoveries averaged 91 ± 14% and endosulfan II recoveries averaged 87 ± 24%. These results agree with those reported in the literature: Molinari and Del Re (1978) reported vinclozolin recoveries ranging from 76 to 103%, Bonelli (1965) recovered 90 ± 10% of endosulfan on fruits, and Graham et al. (1964) showed recoveries on strawberries ranging from 86 to 115%.

Chromatograms of standard mixture and treated samples are presented in Figures 1 and 2.

To ensure complete recoveries the Florisil column should be checked for each group of samples. An elution with 60 mL of 10% v/v ethyl ether in hexane could be enough to isolate vinclozolin in the column cleanup. Tables II and III present elution data of vinclozolin and endosulfan from activated Florisil at different percentages of ethyl ether in hexane and at different volumes of elution.

Table IV presents some analytical results of treated samples collected in the spring of 1978 at the beginning and at the end of ripening. No statistical difference was found between replicated measures. The detection limit for strawberries was 0.05 ng of vinclozolin, 0.04 ng of endosulfan I, and 0.2 ng of endosulfan II.

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