Journal of Chromatography, 208 (1981) 409–413
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,579

Note

Determination of triadimefon in grape juice and wine using capillary gas chromatography

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(Received December 11th, 1980)

Triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanone (Bayleton)] is being applied to an increasing number of crops, including grapes, for the control of fungal diseases[1–3]. After spraying, the chemical is metabolized to triadimenol [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-ol]. The usual method of residue determination is that due to Specht[4], in which the fungicide is extracted from the matrix with methylene chloride and determined, after Florisil column clean-up, by gas–chromatography (GC) with nitrogen-selective detection.

In this paper we report a procedure in which wine or juice is passed through a column of XAD-2 resin and the fungicide is recovered by elution with methylene chloride. The eluate is concentrated and subjected to capillary GC with flame-ionization detection.

EXPERIMENTAL

Reagents and standards

The solvents were obtained from BDH (Poole, Great Britain) and redistilled in an all-glass system prior to use. Analytical-reagent grade resin (XAD-2, particle size 100–200 μm) supplied by Serva (Heidelberg, G.F.R.) was purified by successive extraction with methanol, acetonitrile and diethyl ether[5]. Glass-wool was refluxed with 33% hydrochloric acid–65% nitric acid (3:1) for 3 h, dried at 120°C at 0.5 torr for 12 h, silanized with dimethyldichlorosilane and washed with methanol.

Triadimefon was extracted with chloroform for 12 h in a Soxhlet extraction system from a 25% (w/w) wettable powder (Bayleton) as supplied by Bayer UK (Agrochem Division, Bury, Great Britain). The resultant material was re-

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0021-9673/81/0000–0000/$02.50

crystallized twice from *n*-heptane. Triadimenol was obtained from triadimefon by reduction with lithium aluminium hydride in diethyl ether. Both chemicals were characterized by GC and nuclear magnetic resonance spectroscopy.

OV-17 and Carbowax 20M were supplied by Phase Separations (Queensferry, Great Britain). All glassware was cleaned with chromic acid and rinsed with distilled water prior to use.

Samples

The grape juice and white wine samples were obtained from vines (*Madelene angevine*) forming part of a fungicide spray trial for the control of powdery mildew (*Uncinula necator*) conducted at Long Ashton Agricultural Research Station in 1979. The vines were sprayed to run-off six times with 0.0025% a.i. (active ingredient) triadimefon and twice with 0.05% a.i. vinclozolin. The spraying dates (1979) for vinclozolin were 22/6 and 14/7 and for triadimefon 3/7, 16/7, 1/8, 20/8, 6/9 and 8/10. Samples of fruit were harvested on 16/10 and 29/10. An English white wine (1979 vintage) also prepared at Long Ashton from unsprayed vines was used as a blank when determining recovery values.

Capillary columns

Sodium silicate glass tubing of I.D. 2 mm and O.D. 7 mm (Schott Ruhrglas, Mainz, G.F.R.) wass drawn into capillaries of 0.22 mm I.D. on a horizontally operated capillary drawing machine (Hupe and Busch, Karlsruhe, G.F.R.). A 10-m length of capillary was etched with hydrogen chloride gas at 380°C for 12 h. The resultant column was then deactivated with Carbowax 20M and coated statically with OV-17[6,7]. The separation number was 16 for methyl decanoate and methyl undecanoate The thickness of the film coating was 0.3 μm, and using the Grob polarity mixture[8] dicyclohexylamine and *n*-octanol were eluted with 100% peak areas. No deterioration in column performance was noted after 100 temperature-programmed runs.

Sample preparation

A glass column (10 × 1.0 cm I.D.) fitted with a stop-cock at the lower end and a B14 female joint at the other end in order to take a liquid reservoir was plugged with silanized glass-wool at the stop-cock end. The column was then packed with a slurry of purified XAD-2 resin in methanol to a height of 60 mm and capped with silanized glass-wool. The methanol was replaced by three 15-ml washes of doubly distilled water. Grape juice or wine (100 ml) was passed through the column at a rate of 3 ml min^{-1}, and the column was washed three times with 15 ml of doubly distilled water and allowed to drain. The triadimenol/triadimefon retained on the column was eluted with methylene chloride (three 12-ml volumes). The combined eluates were concentrated to 0.5 ml at 35°C and 20 mmHg using a rotary evaporator. Dry ethanol (10 ml) was added and the sample again concentrated to 0.5 ml. Evaporation and addition of ethanol were repeated twice more. Finally, the extract was made up to 1 ml with dry ethanol.

Gas chromatography

Extracts were examined with a Carlo Erba Fractovap 2350 gas chromatograph equipped with a flame-ionization detector (FID) and a glass capillary column (10 m

× 0.22 mm I.D., coated with OV-17). The carrier gas was hydrogen at a flow-rate of 0.8 ml min^{-1}. The column was programmed from 100 to 200°C at 5°C min^{-1} with an isothermal hold for 20 min at 200°C. The injection port and the detector were maintained at 250°C. All injections (2.0 μl) were carried out with the split system closed for 60 sec. The retention times were 25.4 min for triadimefon and 29.5 and 30.4 min for the two triadimenol diastereomers.

RESULTS AND DISCUSSION

Initially the Specht procedure[4] was applied to the wine and juice samples, but the extraction procedure using methylene chloride proved troublesome because of the formation of emulsions. The adsorption of the fungicides on XAD-2 and their subsequent elution with methylene chloride has the advantage of providing extracts that are suitable for capillary GC without further purification. Fig. 1 demonstrates this point and is a typical chromatogram obtained from a grape juice sample. The juice was prepared from grapes collected 8 days after the final spray application of Bayleton [0.0025% (w/v) of active ingredient].

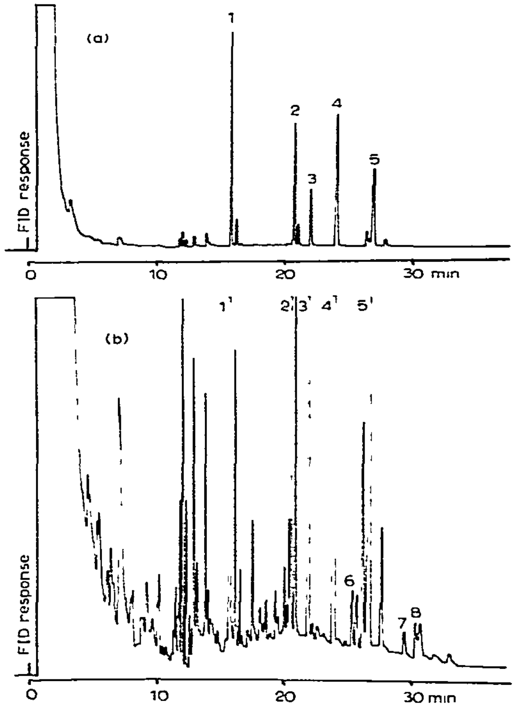

Fig. 1. Gas chromatograms of extracts of juice prepared from grapes 8 days after spraying vines with triadimefon. Column, OV-17, WCOT, 10 m × 0.22 mm I.D.; carrier gas, hydrogen at 0.9 ml min^{-1}, temperature, programmed at 5°C/min from 100 to 200°C, final hold 20 min; detector, FID; injection port and detector temperatures, 250°C (a) Full-scale response 5.2 · 10^{-11} A; (b) as (a) with a 10-fold increase in sensitivity. Peaks: 1 = diethyl phthalate; 2 = ethyl hexadecanoate; 3 = vinclozolin; 4 = di-*n*-butyl phthalate; 5 = ethyl linolate; 6 = triadimefon; 7 = triadimenol A; 8 = triadimenol B

TABLE I

TRIADIMEFON AND TRIADIMENOL RESIDUES IN GRAPE JUICE OR WINE PREPARED FROM GRAPES 8 (A) AND 21 (B) DAYS AFTER THE FINAL APPLICATION OF BAYLETON

Sample	*Triadimefon ($ng\ ml^{-1}$)*	*Triadimenol ($ng\ ml^{-1}$)*
Juice A	24	77
Juice B	5	60
Wine A	<5	50
Wine B	<5	30

TABLE II

RECOVERY OF TRIADIMEFON AND TRIADIMENOL FROM WINE

White wine (100 ml) prepared from untreated grapes was spiked with 1 ml of a solution of triadimefon and triadimenol (ratio of diastereomers 1:1) in acetone (10, 20 and 50 $\mu g\ ml^{-1}$)

Concentration ($ng\ ml^{-1}$)	*Triadimefon recovery (%)*	*Triadimenol recovery (%)*
100	94	98
200	89	96
500	97	104

Table I gives the concentration of triadimefon present in juice and wine. The ratio of the triadimenol diastereomers was 1:1, except for the 8-day wine sample, for which the later eluting isomer was in excess.

The recoveries given in Table II were determined by spiking wine samples with 0.1, 0.2 and 0.5 $\mu g\ ml^{-1}$ of triadimefon and triadimenol. Quantitative results were obtained by comparison of peak heights with external standards. Linear graphs of peak height *versus* amount were obtained over the range 1–50 ng for triadimefon and 1–25 ng for triadimenol, as shown in Fig. 2.

The precision of the determination may be improved by use of an internal standard, should this prove necessary. *n*-Butyl stearate is suitable, but if used acetone must be used instead of ethanol during sample preparation to avoid possible transesterification.

The detection limit was 0.5 ng for triadimefon and for each of the triadimenol

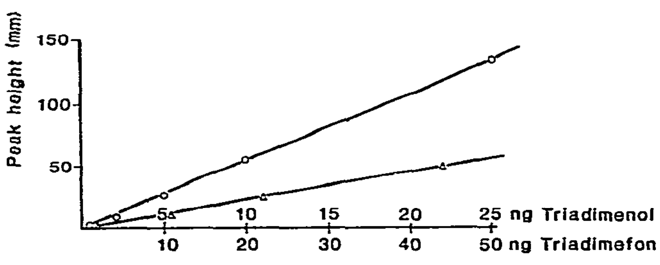

Fig. 2 Relationship between peak heights for triadimefon (○) and triadimenol A (△) and amount injected. GC–FID; 250 mm peak height equals $8 \cdot 10^{-12}$ A response, based on *n*-eicosane as internal standard; other conditions as in Fig. 1

diastereomers. These amounts produced peak heights that were a factor of five above the noise level. These limits correspond to 5 ng ml^{-1} of triadimefon and 10 ng ml^{-1} of triadimenol (A:B = 1:1) present in the original sample using a sample volume of 100 ml.

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] was also identified in the juice and wine extracts (Fig. 2). The residues were approximately 10–50 times greater than those for triadimefon and resulted from the use of Ronilan earlier in the season for the control of *Botrytis cinerea* on the grapes. Obviously, vinclozolin may be determined by a similar procedure to that used for triadimefon.

The major co-extractants were identified by GC–mass spectrometry. The phthalates contaminating the juice and wine may have arisen during processing and storage.

The method described here offers a distinct advantage over more complicated procedures that include solvent extraction and clean-up methods for triadimefon. At present the method is being extended to a wide range of fungicides that are to be found in grape juice and wine.

ACKNOWLEDGEMENT

One of us (T.S.) is grateful to the German Academic Exchange Service for providing a grant.

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