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Note

Reversed-phase high-performance liquid chromatography of pesticides

VII*. Analysis of Vinclozolin, Iprodione, Procymidone, Dichlozolinatate and their degradation product 3,5-dichloroaniline on white must and wine extracts

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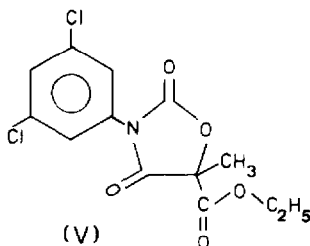
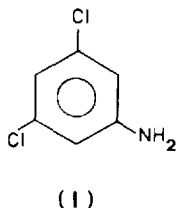
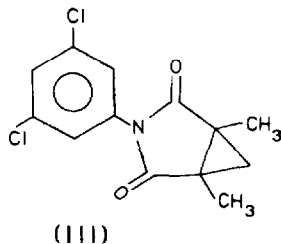
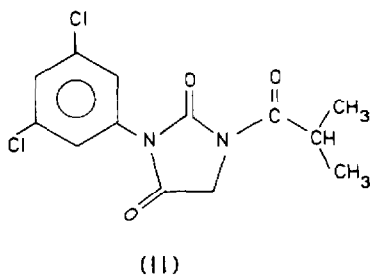
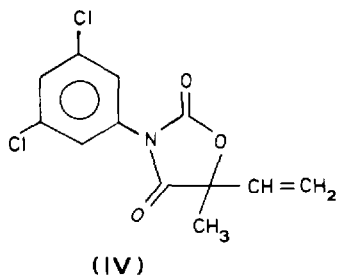
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Dicarboximidic fungicides have been widely used to control *Botrytis cinerea* in vineyards^{1,2}. Among these fungicides Vinclozolin (IV) and Iprodione (II) are currently employed in the field in Italy, while Procymidone (III) and Dichlozolinatate (V) are waiting for licences to be issued by the Italian Health Department³.



* For Part VI, see ref. 13.

Although these fungicides show reduced toxicity⁴⁻⁶, the probable common final product of their degradation or metabolic pathway, 3,5-dichloroaniline (I), seems to be as hazardous as other aromatic amines⁷ even if, so far, few data are available in the literature^{8,9}.

In order to determine the residue levels of II-V, which could be applied simultaneously to grapes, and their metabolite (I) on grape derivatives we developed a reversed-phase high-performance liquid chromatographic method that allows the separation of I-V and their determination in white must and wine extracts.

EXPERIMENTAL

Apparatus

A Varian 5020 liquid chromatograph equipped with a UV-50 UV-visible variable-wavelength detector, a heater block, a Valco AH-20 injector (50 μ l loop) and a Hewlett-Packard 3390 A reporting integrator were used.

Chromatography

Merck Hibar (250 \times 4.0 mm I.D., 10 μ m) columns were employed. Routinely, the mobile phase was water-acetonitrile (45:55) at a flow-rate of 1 ml/min. Based on their UV spectra (see Table I), 210 nm was chosen for the simultaneous determination of compounds I-V. Very good linearity was achieved in the range 0-5 ppm.

Materials and chemicals

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-ethenyl-2,4-oxazolidinedione], Iprodione [3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinocarboxamide], Dichlozolate [ethyl-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-5-oxazolidinocarboxylate] were AnalGrade standards furnished by BASF Agritalia, Rhône-Poulenc and Farmoplant (Milan, Italy), respectively. Procymidone [3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.9]hexane-2,4-dione] and 3,5-dichloroaniline were analytical standards kindly furnished by Dr. M. Barbina-Taccheo. Acetonitrile was of HPLC grade (J. T. Baker, Deventer, The Netherlands); water was distilled twice and filtered through a Millipore apparatus before use. Diethyl ether was of reagent grade (Merck, Darmstadt, G.F.R.) distilled over calcium chloride and benzene and light petroleum (b.p. 40-60°C) were of HPLC and RS for pesticide analysis grades, respectively (Carlo Erba, Milan, Italy).

We employed must and wine from Nuragus grapes with the following com-

TABLE I
UV SPECTRA OF FUNGICIDES AND 3,5-DICHLOROANILINE IN ACETONITRILE SOLUTION

Compound	$\epsilon_{254} \cdot 10^3$	$\epsilon_{220} \cdot 10^3$	$\epsilon_{210} \cdot 10^3$	λ_{max}	$\epsilon_{max} \cdot 10^3$
3,5-Dichloroaniline	6.35	9.6	19.1	212	19.7
Iprodione	3.80	27.6	49.4	210	49.4
Procymidone	2.30	15.30	36.7	204	43.4
Vinclozolin	1.05	14.4	30.6	200	52.0
Dichlozolate	4.18	15.1	26.5	200	43.1

TABLE II
RECOVERY OF COMPOUNDS I-V WITH LIGHT PETROLEUM EXTRACTION FROM MUST AND WINE

Results are means of three duplicate experiments.

Compound	Fortification level (ppm)	Recovery \pm RSD (%)	
		Must	Wine
3,5-Dichloroaniline	1.00	54.5 \pm 5.7	74.8 \pm 4.7
Iprodione	1.00	85.5 \pm 2.0	96.8 \pm 4.3
Procymidone	1.07	106.5 \pm 3.5	106.8 \pm 4.9
Vinclozolin	1.50	103.9 \pm 6.5	100.4 \pm 5.2
Dichlozolate	1.00	96.9 \pm 0.2	107.9 \pm 4.8

positions. Must: reducing sugars 188.6 g/l; pH 3.03; dry extract 214.1 g/l; density 1.0822; total acidity 7.50. Wine: reducing sugars 1.54 g/l; pH 3.56; dry extract 21.4 g/l; total acidity 5.6 g/l; alcoholic content 11.54%. These data were determined by using standard methods.

Extraction procedure

A 5.0-g amount of must (or wine) was placed in a screw-capped tube (160 \times 16 mm), 5.0 ml of organic solvent were added and the tube was stirred for 10 min on a rotary apparatus at 30 rpm. The two phases were allowed to separate, 1.0 ml of the organic layer was evaporated to dryness under reduced pressure and the residue was recovered with 1.0 ml of eluting mixture.

Percentage recoveries from blank musts and wines were checked by the simultaneous addition of known amounts of pure pesticides; the data obtained are given in Table II.

RESULTS AND DISCUSSION

The separation of pesticides II-V together with 3,5-dichloroaniline (I) was studied using different experimental conditions on an RP-8 column (see Fig. 1). The capacity factors (k') of I-V increased with increasing water content of the mobile phase. The separation selectivity is good and allows simultaneous quantitative determinations up to levels of 0.05 ppm for each pesticide. These limits were calculated for the injection of 50 μ l of an extract sample giving a signal three times higher than that of the blank at the pesticide retention times.

Light petroleum was found to be the best solvent for pesticide extractions and the extracts can be injected directly without any further clean-up. Figs. 2-5 show the chromatograms of a blank extract of must (Fig. 2), wine (Fig. 3) and of fortified must (Fig. 4) and wine (Fig. 5). Extractions performed with benzene or diethyl ether and with benzene-diethyl ether mixtures (1:2 and 1:1) gave lower recoveries for 3,5-DCBA and higher relative standard deviations for pesticide recoveries; further, the extracts contained more interfering materials.

The relatively reduced contents of interfering materials (see Figs. 2-5) when

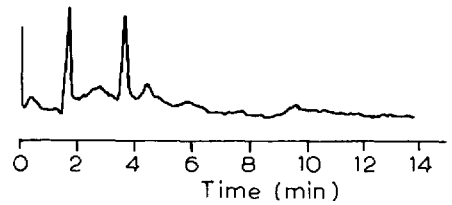
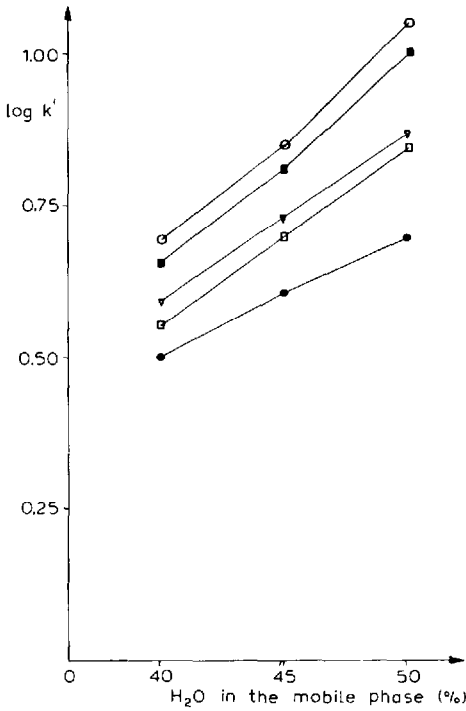


Fig. 1. Log k' of studied compounds *versus* water content of the mobile phase. ● = 3,5-DCBA; □ = Iprodione; ▽ = Procymidone; ■ = Vinclozolin; ○ = Dichlozolate.

Fig. 2. Chromatogram of a blank extract of must extracted with light petroleum. Column, RP-8; flow-rate, 1.0 ml/min; $\lambda = 210$ nm; 0.016 a.u.f.s.; eluent, water-acetonitrile (45:55).

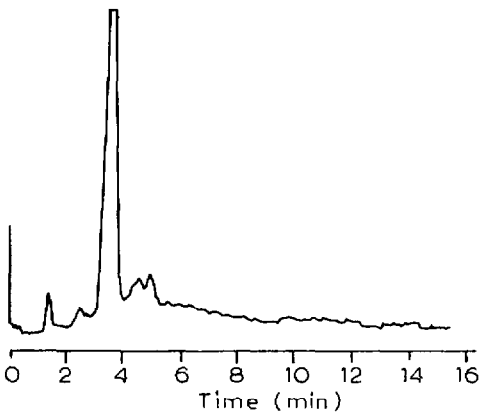


Fig. 3. Chromatogram of a blank extract of wine extracted with light petroleum. Conditions as in Fig. 2.

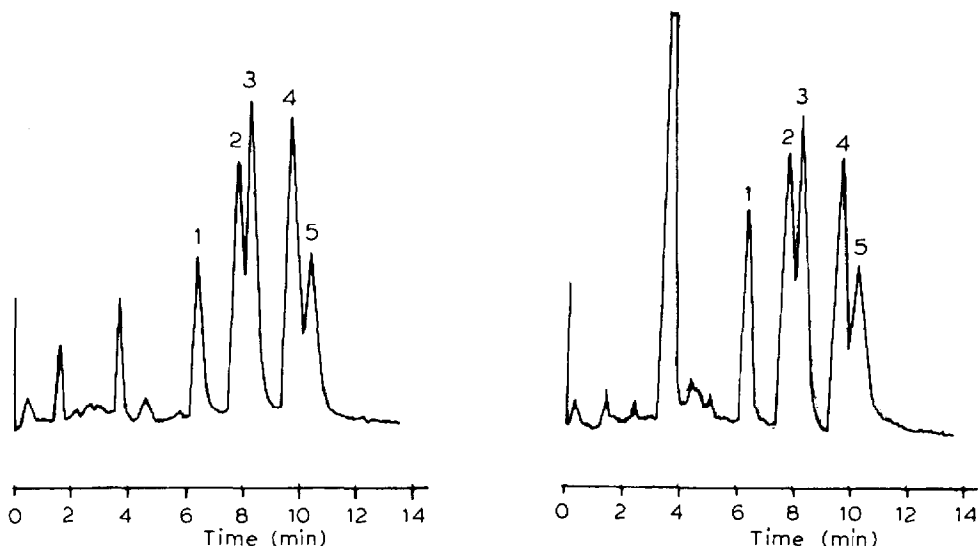


Fig. 4. Chromatogram of a light petroleum extract from a must sample fortified with (1) 3,5-dichloroaniline (1.0 ppm), (2) Iprodione (1.0 ppm), (3) Procymidone (1.0 ppm), (4) Vinclozolin (1.5 ppm) and (5) Dichlozolate (1.0 ppm). Conditions as in Fig. 2.

Fig. 5. Chromatogram of a light petroleum extract from a wine sample fortified with (1) 3,5-dichloroaniline (1.0 ppm), (2) Iprodione (1.0 ppm), (3) Procymidone (1.0 ppm), (4) Vinclozolin (1.5 ppm) and (5) Dichlozolate (1.0 ppm). Conditions as in Fig. 2.

using light petroleum in the extraction procedure could be related to its low polarity, as observed also on similar extracts of grapes^{10,11} and tomatoes¹².

For routine analysis we used water-acetonitrile (45:55) as the eluent and the detector was set at 210 nm. However, different conditions could be used depending on the particular problem. In this way, the determination of a single pesticide could be performed at its maximum wavelength and, moreover, different mixtures could be used to reduce the analysis time [e.g., with water-acetonitrile (35:65) at 212 nm 3,5-dichloroaniline was detected at levels of *ca.* 0.03 ppm in 5 min].

In conclusion, the reported procedure allows quantitative determinations of compounds I-V with good reproducibility, and at levels lower than the legal limits set in many European countries. Finally, we should emphasize that in this procedure the clean-up of the samples is not complicated and can also be applied to foodstuffs such as tomatoes and lettuces.

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