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Note

Reversed-phase high-performance liquid chromatography of fungicides

Determination of Vinchlozolin in presence of Benomyl, BMC and Methylthiophanate

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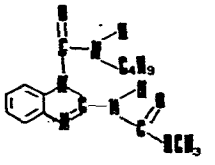
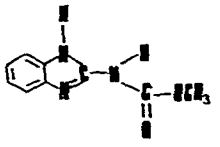
Many chemically different fungicides such as benzimidazolic and thiophthalimidic derivatives have been employed in control of *Botrytis cinerea*. Benomyl (I), BMC (II) and Methylthiophanate (III) are currently used although resistance of *Botrytis* to their action has been observed in European countries[1]. Recently, Vinchlozolin (IV) has been introduced to control *Botrytis* because of its high potency and absence of secondary effects[2,3] such as the inhibition of yeast fermentation which can occur with Captafol, Folpet, Captan and Diclofuanid[4,5].

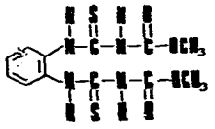
Although high-performance liquid chromatography (HPLC) determinations of compounds I–III have been reported in the literature[6–9], few simultaneous deter-



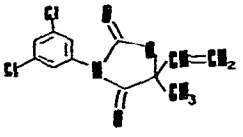
(I)



(II)



(III)



(IV)

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minations are avilable and/or have been performed on standard samples only. Moreover, to our knowledge, the determination of Vinchlozolin has not been performed by this analytical technique, Benomyl and BMC have not been separated by other authors[6] and for Benomyl and Methylthiophanate only a quantitative determination has been reported[8].

In this note we report the conditions which allow the separation of these four fungicides and their quantitative determination in samples extracted from field-sprayed grapes by reversed-phase HPLC.

EXPERIMENTAL

Apparatus

We used the apparatus previously described[10]. Column temperatures were from 20 to 50°. According to UV spectra of compounds I–IV, the optimum wavelength for a simultaneous determination was found to be 221.0 nm. UV spectra of fungicides were recorded in acetonitrile solution with a Beckman 25 spectrophotometer and are reported in Table I.

TABLE I

UV SPECTRA OF FUNGICIDES IN ACETONITRILE SOLUTION

Fungicide	λ_1 *(nm)*	$10^3 \cdot \varepsilon_1$	λ_2 *(nm)*	$10^3 \cdot \varepsilon_2$	$10^3 \cdot \varepsilon_{221}$
Benomyl	287	1.41	221.5	1.61	1.60
BMC	225	1.70	210.0	2.60	1.80
Methyl thiophanate	272.5	1.46	227.5	1.56	1.40
Vinchlozolin	280	0.26	230.0	1.56	1.31

Chemicals

Benomyl [1-(butylamino)carbonyl]1H-benzimidazole-2-carbamic acid methyl ester), Methylthiophanate [1,2-phenylenebis(iminothiocarbonyl carbamid acid) dimethyl ester] and Vinchlozolin [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-oxazolidine 2,4-dione] were analytical grade samples kindly provided by DuPont (Milan, Italy), SIPCAM (Milan, Italy) and BASF Agritalia (Milan, Italy). BMC (1H-benzimidazole-2-carbamic acid methyl ester) was extracted from a commercial product containing 50% of fungicide. The extract was purified on silica gel with benzene–methanol (9:1) and crystallized twice from benzene (colourless needles, m.p. 48°). Standard solutions were prepared by dissolving known amounts of fungicides in acetonitrile containing 30.0 ppm of benzene (Carlo Erba, Milan, Italy; purity $\geqslant$99.5%) as internal standard. A calibration curve was constructed for each fungicide by plotting peak area ratio ($A_{\text{compound}}/A_{\text{standard}}$) *vs.* concentration and good linearity was achieved in the range 0–100 ppm.

Chromatography

The mobile phase was a mixture of distilled water and acetonitrile (E. Merck, Darmstadt, G.F.R.; suitable for chromatography of pesticides) employed with buffers in different ratios and flow-rates as shown in Table II. Phosphate (0.067 *M*, pH 7.00, E. Merck) and acetic acid (0.2 *M*)–sodium acetate (0.2 *M*) pH 4.00, buffers were employed.

Extraction procedure from grapes[11]

Field-sprayed grapes (*ca.* 2.0 kg) were milled and 200 ml of the homogenate were shaken twice for 30 min with 200 ml of purified light petroleum ether (b.p. 40–60°). The organic layers were dried with anhydrous Na_2SO_4, evaporated to dryness under reduced pressure and the residue dissolved in 1 ml of acetonitrile containing the internal standard. The extract can be analyzed without clean-up.

RESULTS AND DISCUSSION

Table II shows the capacity factors, k', of four fungicides using various water–acetonitrile mixtures as mobile phase. A good separation was obtained under the conditions of experiment 3 (see Fig. 1). Other conditions (*i.e.*, experiments 1 and 2) give the same retention time for BMC and the solvent acetonitrile.

TABLE II

CAPACITY FACTORS, k', OF FUNGICIDES UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Experiment No.	*Water–acetonitrile (%)*	*T (°C)*	*Flow-rate (ml/min)*	*k'*				
				I	*II*	*III*	*IV*	*Stand.*
1	50:50	20	0.4	1.98	0.02*	0.35	2.56	0.75
2	50:50**	20	0.5	2.61	0.03*	0.23	3.70	0.84
3	55-45**	35	0.6	3.02	0.13	0.43	4.44	1.03
4	55-45**	40	0.6	2.66	0.08*	0.07*	4.09	1.05
5	55:45**	50	0.6	1.30*	0.04*	0.04*	3.70	1.28*
6	55:45***	30	0.6	3.49	0.25	0.44	5.24	1.09

* See text.
** Plus 10% of the buffer, pH 7.00
*** Plus 5% of buffer, pH 4.10.

It is evident that the separation depends on the water content of the mobile phase and on the temperature and pH. Increasing temperature gives lower retention times but also causes overlap of some peaks (*i.e.*, at 50°, experiment 5, Benomyl with standard and Methylthiophanate with BMC). The pH of buffer affects the separation: going from pH 7.00 to pH 4.00 we found an increase of retention times for four fungicides (*cf.*, experiments 3 and 6) and their peaks were less sharp.

The effect of pH may be tentatively explained as follows. In the reversed-phase liquid chromatography an increase of retention time depends on the solubility of compounds in the mixture water–acetonitrile; the solubility can be correlated to Beroza's[12] *p*values of compounds as shown by Seiber[13] so that larger p values give higher retention times. Going from neutral pH to acidic pH would increase the p value of each fungicide and, consequently, would lead to an increase of retention time. We are checking this hypothesis on compounds I–IV and on insecticides like Carbaryl, Tetrachlorvinphos, Dimethoate, etc. separated by reversed-phase liquid chromatography as described elsewhere[10].

We have applied this separation procedure to extracts from field-sprayed grapes under the conditions of experiment 3. The recovery of known amounts of

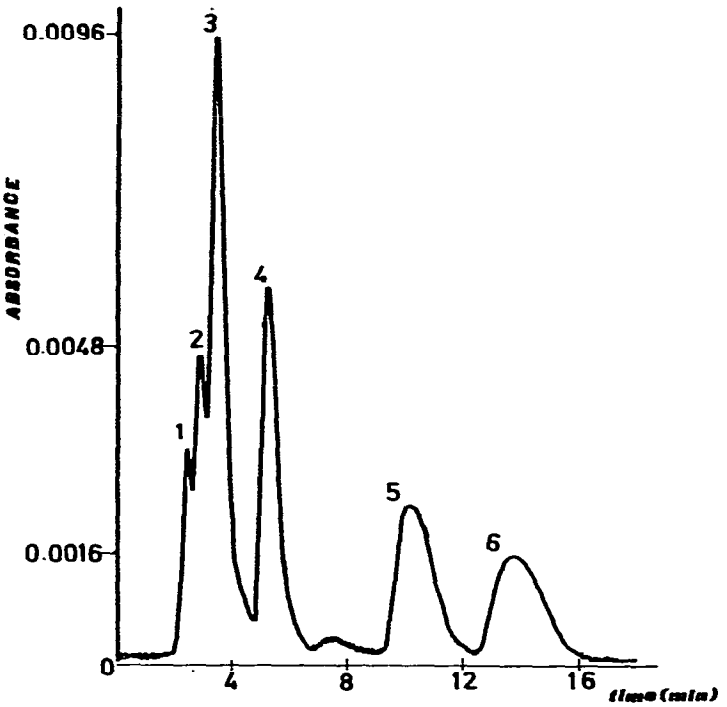

Fig. 1. Injection of 3.0 μl of mixture of fungicides. Peaks: 1 = acetonitrile; 2 = BMC (*ca.* 4.0 ppm); 3 = Methylthiophanate (*ca.* 32 ppm); 4 = internal standard; 5 = Benomyl (*ca.* 38 ppm); 6 = Vinchlozolin (*ca.* 34 ppm).

each fungicide added to a water–ethanol–glucose solution is reported in Table III. The "true" concentration $[\text{pesticide}]_{smp}$ of fungicides in samples of grapes was calculated according to

$$[\text{pesticide}]_{smp} = \frac{[\text{pesticide}]_{sc}}{f_c \cdot f_e} \quad (1)$$

TABLE III

PERCENT RECOVERY OF FUNGICIDES PERFORMED BY EXTRACTION PROCEDURE

Fungicide	*Concn. (ppm)*	*Recovery (%)*	
		after one extraction	*after two extractions*
Benomyl	50	63	88
	25	65	89
	10	65	88
BMC	50	75	90
	25	74	94
	10	75	91
Methylthiophanate	50	36	75
	25	38	75
	10	38	70
Vinchlozolin	50	20	55
	25	22	50
	10	25	52

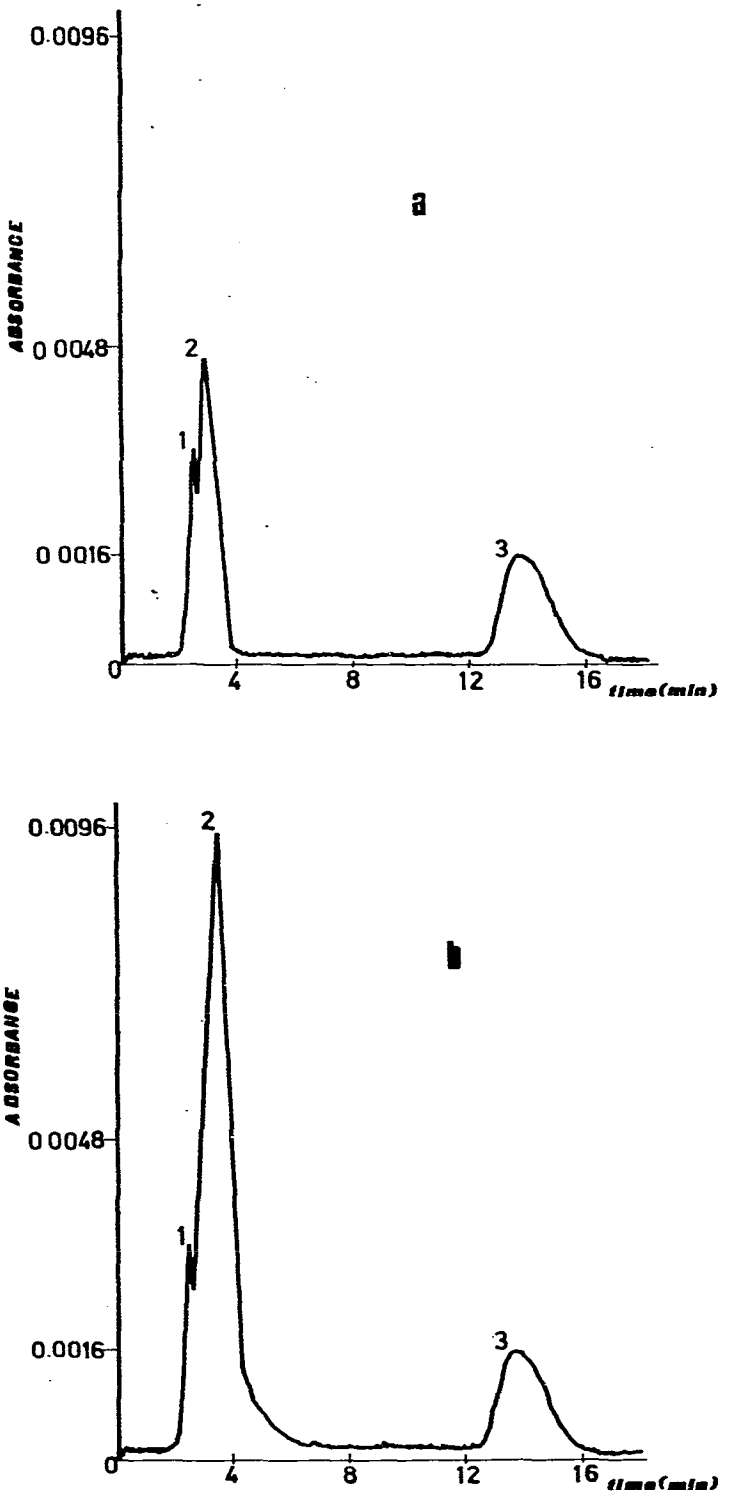
0.0096
ABSORBANCE
0 0048
0 0016
0
a
1
2
3
4
8
12
16
time(min)
0.0096
ABSORBANCE
0.0048
0.0016
0
b
1
2
3
4
8
12
16
time(min)

Fig. 2.

where $[\text{pesticide}]_{sc}$ represents the concentration of pesticide obtained from standard curves using the ratio $A_{compound}/A_{standard}$ found experimentally for each fungicide on chromatograms of extracts (average of at least three determinations), f_e is the percent recovery of each fungicide obtained by the extraction procedure and f_c represents the ratio between the starting and the final volume of the extract (*ca.* 200).

Our procedure enables a quantitative analysis with sensitivity levels[10] which are of the same order as those reported in refs. 6–8 for Benomyl and Methylthiophanate. As shown in Table IV, for Vinchlozolin these levels are 30 times lower than the limits permitted on foodstuffs in Italy[14] and 20 and 10 times lower for Benomyl and Methylthiophanate, respectively.

A very good sensitivity (*ca.* 100 times lower than the legal limit) was

TABLE IV

LOWEST CONCENTRATION OF FUNGICIDES THAT COULD BE DETERMINED

Fungicide	*Sensitivity limit (ppm)*	*Maximum residue level permitted in foodstuffs (ppm)*
Benomyl	0.05	1.00
BMC	0.01	1.00
Methylthiophanate	0.05	0.50
Vinchlozolin	0.05	1.50

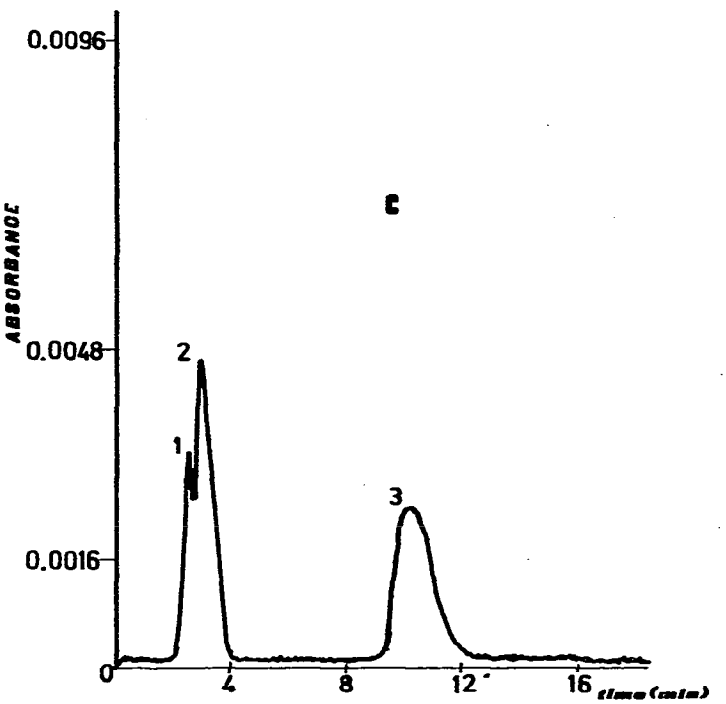

Fig. 2. Injection of 4.0 μl of concentrated extract from grapes sprayed with: a, BMC (2) and Vinchlozolin (3); b, Methylthiophanate (2) and Vinchlozolin (3); c, BMC (2) and Benomyl (3). Peak 1 is acetonitrile.

achieved for BMC. Since BMC is a degradation product (by a metabolic or photolytic pathway) of Benomyl and Methylthiophanate[15,16], our method permits the identification and the separation of the parent compounds from this potentially hazardous[17] metabolite.

Fig. 2 shows a typical chromatogram of concentrated extract from grapes sprayed with (a) Vinchlozolin and BMC, (b) Methylthiophanate and Vinchlozolin and (c) BMC and Benomyl.

These results indicate that our procedure could provide a rapid and sensitive tool for quantitative routine determination of these fungicides in foodstuffs.

ACKNOWLEDGMENTS

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REFERENCES

1 M. Bisiach, *Atti Giornate Fitopatologiche*, (1975) 763.
2 E. Kuehle, E. K. Pauke and F. Grewe, *Angew. Chem.*, 76 (1964) 807.
3 D. Mangold and E. H. Pommer, *Meded. Fac. Landbouwwet Rijksuniv. Gent*, 40 (1975) 713.
4 W. D. Thomas, P. H. Eastburg and M. D. Bankuty, *Phytopathology*, 52 (1962) 754.
5 E. Minarik and P. Ragala, *Mitteilungen*, 3 (1975) 187.
6 J. Kirkland, H. Holtr and H. Pease, *J. Agr. Food Chem.*, 21 (1973) 368.
7 J. F. Lawrence and D. Turton, *J. Chromatogr.*, 159 (1978) 207.
8 J. Katayama and T. Hotta, *Osaka-fu Norin Gijstu Senta Kenkyu Hokoku*, 12 (1975) 131; *C.A.*, 88 (1978) 16964v.
9 M. Meloni, F. M. Pirisi and P. Cabras, *Rend. Seminario Fac. Sci. Univ. Cagliari*, 48 (1978) 293.
10 P. Cabras, M. Meloni and F. M. Pirisi, *J. Chromatogr.*, 176 (1979) 473.
11 P. G. Garoglio, *Enciclopedia Vitivinicola Mondiale*, Edition. Scint. Unione Italiana del Vino, Milan, 1973, Vol. II, p. 186.
12 M. Beroza, M. N. Inscoe and M. C. Bowman, *Residue Rev.*, 30 (1969) 1.
13 J. N. Seiber, *J. Chromatogr.*, 94 (1974) 151.
14 *Italian Health Department Act Jan. 6 1979*, Gazzetta Ufficiale of Italian Republic, Feb. 8, 1979, p. 1282.
15 S. Foschi, I. Ponti, G. Svampa and P. Flori, *Ital. Agr.*, 112 (1975) 96.
16 D. A. M. Watkins, *Chemosphere*, 3 (1974) 239.
17 J. A. Styles and R. Garner, *Mutat. Res.*, 26 (1974) 177.