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Note

Separation of insecticides by reversed-phase high-performance liquid chromatography

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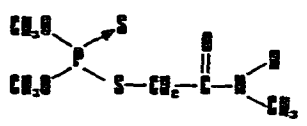
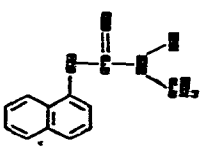
Dimethoate (I) [phosphorodithioic acid S-(2-methylamino-2-oxoethyl) O,O-dimethyl ester], carbaryl (II) (1-naphthalenol methylcarbamate) and tetrachlorvinphos (III) [phosphoric acid 2-chloro-1-(2,4,5-trichlorophenyl)ethenyl dimethyl ester (*Z*)] are pesticides currently employed against *Polychrosis botrana*, and many other insects and acari[1]. Etrimphos (IV) [phosphorothioic acid O-(6-ethoxy-2-ethyl-4-pyrimidinyl) O,O-dimethyl ester] is under experimental trial in our country against *Polychrosis botrana*.



(I)



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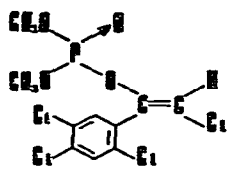

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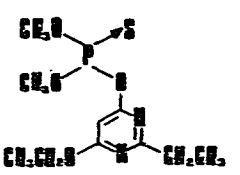

(IV)

Several analytical techniques are available for the separation and quantitative determination of these pesticides[3–6]. High-performance liquid chromatography (HPLC) has been recently introduced as a means of separating pesticide mixtures[7],

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and determinations of carbaryl[7–10], dimethoate[11] and tetrachlorvinphos[11,12] have been reported. However, few HPLC methods are available for the quantitative determination of these pesticides and have been provided only for carbaryl[8,10]. Moreover, to our knowledge no simultaneous determination of compounds I–III has been performed, and no chromatographic procedure has been applied to etrimphos.

Recently, we have successfully applied HPLC to the simultaneous quantitative determination of a mixture of chlorinated, carbamate and organophosphorus pesticides[12]. Here we describe the conditions which allow simultaneous quantitative determination of I–IV by reversed-phase high-performance liquid chromatography (RPLC).

EXPERIMENTAL

Apparatus

We used a Perkin-Elmer Model 601 liquid chromatograph equipped with a syringe-loading sample injector (Rheodyne 7105) and a variable-wavelength UV detector, Coleman Model LC 55. The column was a Perkin-Elmer reversed-phase, ODS-HC-Sil-X-1 (25 × 0.26 cm I.D.); analyses were performed at room temperature.

Chromatography

Elution was performed with a mixture (50:50) of distilled water and acetonitrile (Merck Analytical Grade, twice distilled) plus 10% of a buffer (0.067 *M* KH_2PO_4–1 *M* NaOH, pH 7.00; Carlo Erba, Milan, Italy). Mixtures of different proportions of these solvents, but without the buffer, at flows varying between 0.40 and 0.75 ml/min were also employed. The best wavelength for simultaneous determination was found to be at 221.0 nm. A standard curve was constructed for each pesticide. Plots of the peak area ratios (A_{prd}/A_{std}) *vs.* concentration showed good linearity in the range 0–100 ppm.

Chemicals

Standard solutions of pesticides were prepared by dissolving analytical-grade samples (purity $\geqslant$ 99.5%; BDH, Milan, Italy) of I–III in acetonitrile containing 30.0 ppm of benzene (purity $\geqslant$ 99.8% RP ACS, Carlo Erba) as internal standard. Etrimphos (IV) (purity $\geqslant$ 97.7%) was kindly donated by Sandoz (Milan, Italy) and prepared by the same procedure.

RESULTS AND DISCUSSION

Table I shows the results obtained with various water–acetonitrile mixtures as mobile phase. It is evident that the best separation was obtained at a 50:50 ratio plus 10% of the buffer, pH 7.00. A representative example of such a separation is shown in Fig. 1.

At a 63:37 ratio of water to acetonitrile the compounds can still be separated, but tetrachlorvinphos and etrimphos have too high retention times and the peaks were less sharp. Initially, we applied this method to the quantitative determination of pesticides I–IV in concentrated (200-fold) extracts of field-sprayed grapes prepared by the Office International du Vin technique[13]. Extracts were analyzed without any

TABLE I

RETENTION TIMES OF PESTICIDES AT DIFFERENT COMPOSITIONS OF MOBILE PHASE

Water–acetonitrile (%)	*Flow (ml/min)*	t_R*				
		I	*II*	*III*	*IV*	*STD*
63:37	0.75	0'42"	2'39"	17'54"	—	—
45:55	0.50	0'27"	1'00"	3'07"	5'10"	3'10"
35:65	0.50	0'20"	0'20"	1'50"	2'52"	—
50:50	0.40	0'40"	1'40"	5'50"	9'38"	2'40"
50:50 + 10% buffer	0.60	0'45"	1'27"	6'45"	12'50"	2'25"

* $t_R = t_{Rprd} - t_{RCH3CN}$.

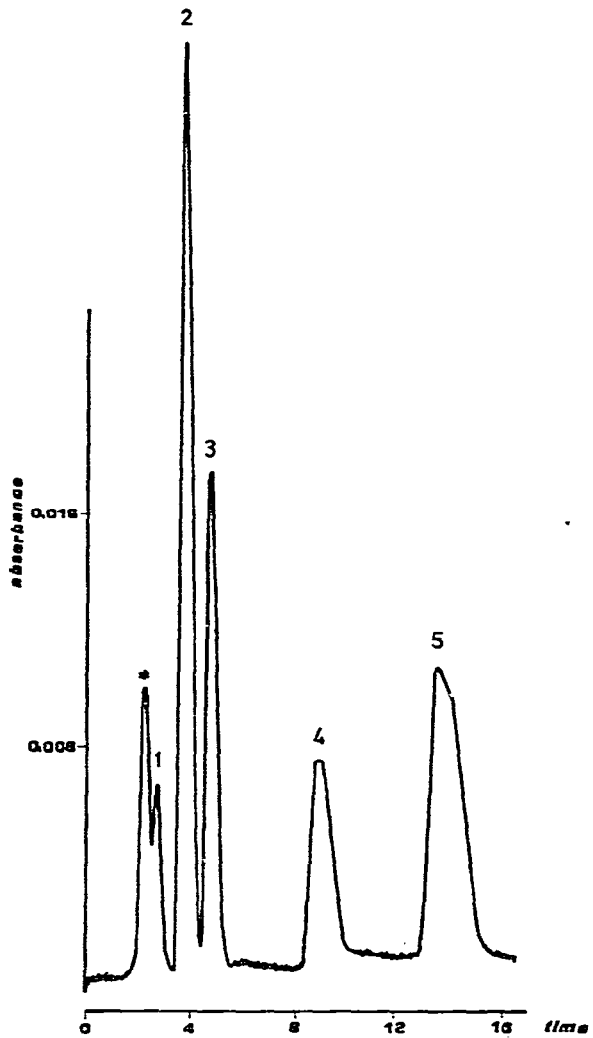

Fig. 1. Chromatogram of 3.0 μl of a mixture of pesticides. Peaks: * = acetonitrile; 1 = dimethoate (60 ppm); 2 = carbaryl (14 ppm); 3 = internal standard; 4 = tetrachlorvinphos (50 ppm); 5 = etrimphos (100 ppm).

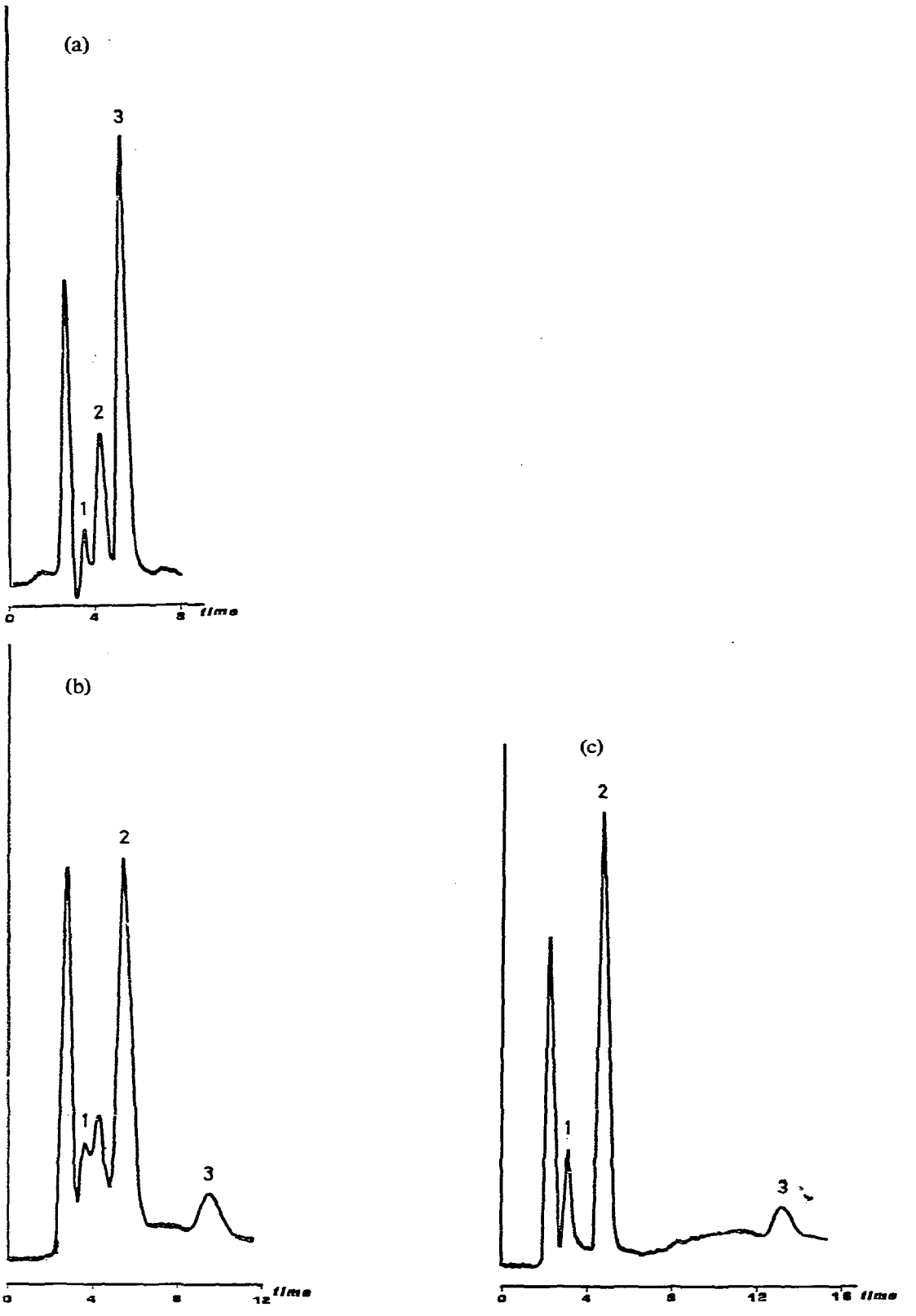

Fig. 2. Chromatograms of concentrated extracts from wine grapes sprayed with: (a) 1 = dimethoate, 2 = carbaryl, 3 = standard; (b) 1 = dimethoate, 2 = standard, 3 = tetrachlorovinphos; (c) 1 = carbaryl, 2 = standard, 3 = etrimphos.

further purification. Fig. 2 shows typical chromatograms of extracts from grapes sprayed with insecticides.

Table II reports the lowest concentration of these compounds that can be determined with reliable accuracy. The minimal acceptable values are considered as those giving a peak area ratio (A_{prd}/A_{std} for a 4.0-μl injection of concentrated extract) of *ca.* 0.04. This value is about three times the standard error of the instrument. The sensitivity of the determination of carbaryl compares favourably with that of other methods reported in the literature[8,10].

TABLE II

LOWEST CONCENTRATION OF PESTICIDES THAT COULD BE DETERMINED

Compound	*Concentration determined at 221.0 nm (ppm)*
I	~0.3
II	~0.002
III	~0.2
IV	~0.2

Although the sensitivity for dimethoate, tetrachlorvinphos and etrimphos is *ca.* 100 times lower than for carbaryl, the procedure enables the determination of these pesticides at concentrations below those permitted in Italy in foodstuffs (1.5, 1.5 and 0.5 ppm, respectively[14,15]).

In conclusion our technique provides a sensitive and rapid means for the simultaneous quantitative determination of chemically different pesticides in foodstuffs.

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