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Simultaneous determination of insecticides, acaricides and fungicides by thin-layer chromatography

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ABSTRACT

A method is described for the determination of residual amounts of some insecticides, acaricides and fungicides in fresh and processed fruits. Optimum conditions were established for the extraction of eighteen pesticides, purification of the extracts by column chromatography and subsequent two-dimensional thin-layer chromatography on silica gel GF_{254} with cyclohexane–acetone (10:1) and light petroleum–benzene–ethanol (65:30:5) as solvents and with detection under UV light at 254 and 366 nm followed by a 0.1% solution of bromophenol blue.

INTRODUCTION

The wide application of pesticides requires methods for the detection and determination of pesticide residues in agricultural products and foodstuffs. Thin-layer chromatography (TLC) has proved advantageous for the determination of modern perticides belonging to different chemical classes[1–7].

The aim of this work was to develop a analytical procedure based on TLC for rapid determining various insecticides, acaricides and fungicides in fresh and processed fruits.

EXPERIMENTAL

Reagents

Standard acetone solutions (1000 $\mu g/cm^3$) of the following pesticides were prepared: dimethoate ($C_5H_{12}NO_3PS_2$), tetrachlorvinphos ($C_{10}H_9Cl_4O_4P$), pyrazophos ($C_{14}H_{20}N_3O_5PS$), diazinon ($C_{12}H_{21}N_2O_3PS$), phozalone ($C_{12}H_{15}ClNO_4PS_2$), pyrimiphosmethyl ($C_{11}H_{20}N_3O_3PS$), fenitrothion ($C_9H_{12}NO_5PS$), chlorpyriphos ($C_9H_{11}Cl_3NO_3PS$), deltametrin ($C_{22}H_{19}Br_2NO_3$), silhaletrin ($C_{23}H_{19}F_3ClNO_3$), triflumisol ($C_{15}H_{15}F_3ClN_3O$), fenarimol ($C_{17}H_{12}Cl_2N_2O$), iprodion ($C_{13}H_{13}Cl_2N_3O_3$), vinclosolin ($C_{12}H_9Cl_2NO_3$), hexathiazox ($C_{17}H_{21}ClN_2O_2S$),

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TABLE I

R_F VALUES OF INSECTICIDES, FUNGICIDES AND ACARICIDES WITH DIFFERENT SOLVENT SYSTEMS ON SILICA GEL G

Pesticide	*R_F value*				
	(1) Cyclohexane–acetone (10:1)	*(2) Light petroleum–benzene–ethanol (65:30:5)*	*(3) Benzene–acetone (95:5)*	*(4) Hexane–tetrachloromethane–ethyl acetate (65:30:5)*	*(5) Cyclohexane–benzene–acetone (5:2:1)*
Insecticides					
Dimethoate	0.05	0.07	0.03	0.03	0.07
Tetrachlorvinphos	0.19	0.22	0.12	0.05	0.27
Pyrazophos	0.29	0.27	0.43	0.07	0.44
Diazinon	0.45	0.29	0.53	0.13	0.53
Phozalone	0.31	0.33	0.55	0.17	0.50
Pyrimiphos-methyl	0.52	0.41	0.79	0.25	0.62
Fenitrothion	0.33	0.43	0.87	0.20	0.53
Chlorpyriphos	0.73	0.69	0.90	0.52	0.75
Deltametrin	0.39	0.56	0.78	0.45	0.70
Sihaletrin	0.30	0.59	0.83	0.48	0.66
Fungicides					
Trifiumisol	0.12	0.19	0.14	0.04	0.20
Fenarimol	0.15	0.17	0.16	0.07	0.25
Iprodion	0.17	0.24	0.40	0.13	0.35
Vinclosolin	0.45	0.44	0.85	0.56	0.66
Guazatin	0.53	0.39	0.82	0.50	0.78
Acaricides					
Hexathiazox	0.44	0.31	0.76	0.29	0.70
Chlofentisin	0.39	0.42	0.86	0.47	0.70
Flubenzimin	0.36	0.51	0.90	0.60	0.74

chlofentisin ($C_{12}H_{11}Cl_2N_4$), flubenzin ($C_{17}H_{10}F_6N$) and guazatin ($C_{15}H_{33}N_4O_6$). The TLC supports were silica gel G and silica gel GF_{254} (Merck). The solvent systems used are given in Table I. Detection was effected with (1) 0.1% bromophenol blue (BPB) solution prepared by dissolving 0.1 g of BPB and in 100 cm^3 of 1% $AgNO_3$ solution in 0.5 *M* ammonia, (2) UV light at 254 and 366 nm, (3) Dragendorff reagent[8] and (4) iodine vapour.

Procedure

A solutions containing 25 μg of a pesticide was mixed with 50 g of apple homogenate free from pesticides or with apple juice. All pesticides were extracted with 130 cm^3 of methanol for 50 min.

Each extract was filtered through a silica gel layer and the filtrate was mixed with 150 cm^3 of 10% sodium chloride solution. The pesticides were extracted twice from the solution with 80 cm^3 of chloroform and the extracts were evaporated to 4–5 cm^3 and passed down a chromatographic column packed with 5 g of adsorbent consisting of sodium sulphate, Florisil, Celite and charcoal (1:1:0.5:0.1). The pesticides in the column were eluted using 90 cm^3 of chloroform–diethyl ether (9:1). The eluate was evaporated to dryness at 40°C and the dry residue was dissolved in 0.5 cm^3 of acetone. A 250-μl aliquot of the acetone solution was spread on a chromatographic plate precoated with silica gel GF_{254}. A standard mixture of the pesticides was spread on another plate.

The pesticides were separated using two-dimensial TLC with the solvent systems cyclohexane–acetone (10:1) and light petroleum–benzene–ethanol (65:30:5). To identify the separated spots, the plates were first irradiated with UV light at 254 nm, which developed all pesticides except vinclosolin and dimethoate; the vinclosolin spot was then revealed under UV light at 366 nm and that of dimethoate by spraying with 0.1% BPB solution. The areas of the spots developed on the test plates and the plate with the standard mixture were compared in order to determine the pesticide contents in the samples.

RESULTS AND DISCUSSION

TLC has a number of advantages for determining a wide range of pesticides with different structures, owing to the rapidity and efficiency of separation and identification.

To establish the optimum conditions for the TLC analysis of the pesticides, a number of tests were performed on common chromatographic supports such as silica gel G, alumina, cellulose, Florisil and polyamide[1–8]. Silica gel G proved to be the most suitable and was adopted in subsequent work. The mobile phase for separating the pesticides under consideration was chosen after testing about 50 combinations of solvents, some of which are used in pesticide analysis and others in chromatographic analyses of similar compounds[1–11]. The most suitable are given in Table I. The results indicate that the most suitable systems for insecticides are 1 and 2, for acaricides 2 and 4 and for fungicides 5 and 2.

As none of the solvent systems could be used for the simultaneous determination of the whole combination of eighteen pesticides, two-dimensional TLC was tested with systems 1 and 2 successively. The latter was suitable for all three groups of

TABLE II

SENSITIVITY (μg) TO IDENTIFICATION OF INSECTICIDES, FUNGICIDES AND ACARICIDES WITH DIFFERENT DETECTION METHODS

Two-dimensional TLC on silica gel G with cyclohexane–acetone (10:1) and light petroleum–benzolene–ethanol (65:30:5)

Pesticide	*Detection*							
	0.1% BPB		*UV light (254 nm)*[a]		*Dagendorff reagent*		*Iodine vapour*	
	Sensitivity	*Colour of spot on pale lilac background*	*Sensitivity*	*Colour of spot on yellow-green background*	*Sensitivity*	*Colour of spot on pale yellow background*	*Sensitivity*	*Colour of spot on white background*
Insecticides								
Dimethoate	2	Dark blue	–	–	–	–	10	Brown
Tetrachlorvinphos	1	White	2	White	2	Orange	5	Brown
Pyrazophos	0.5	Blue-green	0.5	Light blue	2	Red-brown	2	Brown
Diazinon	1	White	5	Violet	0.5	Red-brown	10	Brown
Phozalone	2	Dark blue	5	Blue	5	Orange	5	Brown
Pyrimiphos-methyl	2	Dark lilac	1	Light blue	0.5	Red-brown	5	Brown
Fenitrothion	1	Dark lilac	1	Violet	5	Orange	10	Brown
Chlorpyriphos	5	Dark lilac	2	Blue	10	Orange	10	Brown
Deltametrin	1	Dark blue	5	Light blue	5	Orange	10	Brown
Sihaletrin	1	Dark blue	5	Light blue	10	Orange	10	Brown
Fungicides								
Triflumisol	2	Dark blue	2	Lilac	2	Red-brown	5	Brown
Fenarimol	0.5	Blue-green	2	Lilac	1	Red-brown	5	Brown
Iprodion	2	White	5	Blue	10	Orange	10	Brown
Vinclosolin	0.5	Dark lilac	2	Light blue (366 nm)	5	White	10	Brown
Guazatin	2	White	5	Lilac	10	Orange	20	Brown
Acaricides								
Hexathiazox	1	White	5	White	10	Orange	20	Brown
Chlofentisin	5	Dark lilac	0.5	Dark red	2	Red-brown	5	Brown
Flubenzimin	2	Yellow-green	2	Dark lilac	5	White	10	Brown

[a] Chromatographic support silica gel GF_{254}.

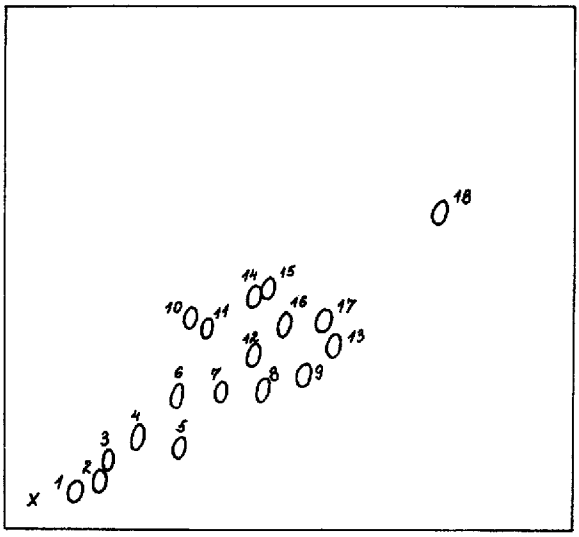

Fig. 1. Two-dimensional chromatogram of a mixture of 18 pesticides (10 μg each) on silica gel GF_{254}. Solvent systems: cyclohexane–acetone (10:1) and light petroleum–benzene–ethanol (65:30:5). Detection UV light (254 and 366 nm) and 0.1% BPB. 1 = Dimethoate; 2 = triflumisol; 3 = fenarimol; 4 = tetrachlorvinphos; 5 = iprodion; 6 = pyrazophos; 7 = phozalone; 8 = fenitrothion; 9 = flubenzimin; 10 = diazinon; 11 = hexathiazox; 12 = chlofentisin; 13 = sihaletrin; 14 = pyrimiphos methyl; 15 = guazatin; 16 = vinclosolin; 17 = deltametrin; 18 = chlorpyriphos.

TABLE III

ANALYTICAL RECOVERY AND DETECTION LIMIT IN TLC OF PESTICIDES IN APPLES

Pesticide	*Standard addition (mg kg^{-1})*	*Analytical recovery for apples (n = 12) (%)*	*Detection limit (mg kg^{-1})*
Diazinon	0.5	72	0.20
Dimethoate	0.5	80	0.08
Pyrimiphosmethyl	0.5	88	0.04
Chlorpyriphos	0.5	84	0.08
Tetrachlorvinphos	0.5	88	0.08
Phozalone	0.5	72	0.20
Fenitrothion	0.5	80	0.04
Pyrazophos	0.5	96	0.02
Deltametrin	0.5	80	0.20
Sihaletrin	0.5	72	0.20
Fenarimol	0.5	84	0.08
Vinclosolin	0.5	88	0.08
Triflumisol	0.5	80	0.08
Iprodion	0.5	72	0.20
Guazatin	0.5	80	0.20
Flubenzimin	0.5	80	0.08
Hexathiazox	0.5	76	0.20
Clofentisin	0.5	96	0.02

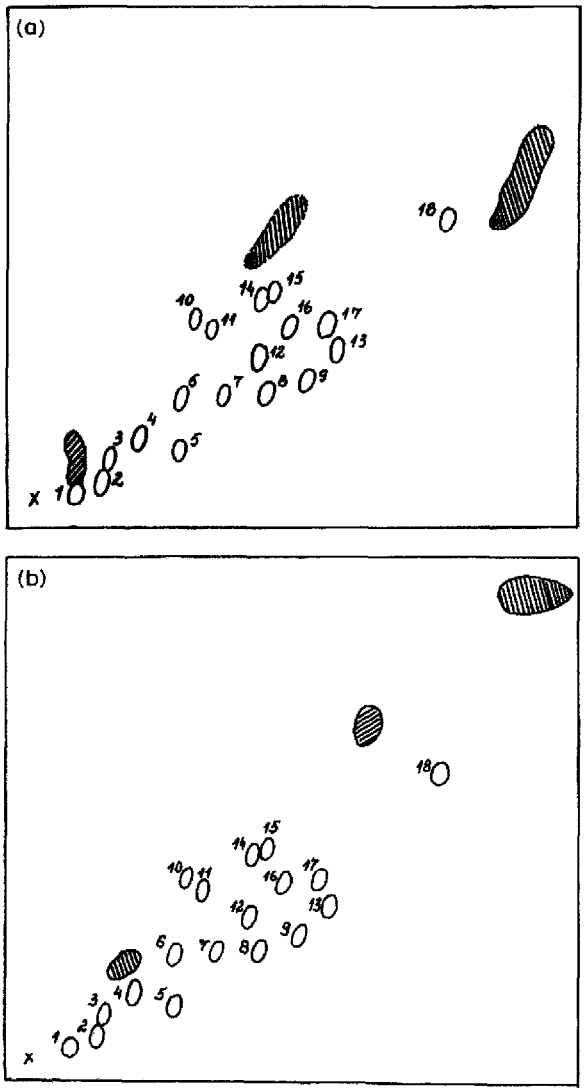

Fig. 2. Chromatograms of purified extract of (a) apples and (b) apple juice containing 18 pesticides (0.5 mg kg^{-1}) on silica gel GF_{254}.

pesticides, whereas system 1 ensured the best separation of the insecticides. Fig. 1 shows the results obtained from two-dimensional TLC on silica gel GF_{254}.

The detection reagents found to be suitable for the spots of the pesticides under consideration are listed in Table II. It can be seen that the highest sensitivity was obtained with identification using 0.1% BPB solution and UVirradiation at 254 nm. They produced intact spots of regular shape and their areas and intensities were proportional to the amounts of pesticides.

The results in Tables I and II indicate that the optimum conditions for the simultaneous TLC determination of the eighteen pesticides are the following: silica gel GF_{254} as the chromatographic support; two-dimensional chromatography for about 100 min with cyclohexane–acetone (10:1) and light petroleum–benzene–ethanol (65:30:5) and detection by successive UV irradiation at 254 and 366 nm for vinclosolin followed by spraying with 0.1% BPB solution for dimethoate.

Fig. 2 presents chromatograms of purified extracts of apples and apple juice (50 g) containing 0.5 ppm of each of the studies pesticides. Table III gives the results for pesticides determined by the standard additions method in apples and apple juice.

REFERENCES

1 W. Ebing, *J. Chromatogr.*, 65 (1972) 533.
2 H. Steinwandter and H. Schiuter, *Fresenius Z. Anal Chem.*, 286 (1977) 90.
3 V. Trdlicka, *J. Chromatogr.*, 130 (1977) 437.
4 E. Pozimek, E. Crabtree and J. Mullin, *Anal. Lett.*, A14 (1981) 825.
5 R. Sundarajan and R. B. Chawla, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1009.
6 C. Marution, C. Sarbu, M. Vlassa, C. Liteanu and P. Bogoda, *Analusis*, 14 (1986) 95.
7 A. Ambrus and H. P. Thier, *Pure Appl. Chem.*, 58 (1986) 1035.
8 E. Stahl, *Dünnschicht-Chromatographie, ein Laboratoriumshandbuch*, Springer, Berlin, 1962.
9 A. Necheva, E. Kovacheva and G. Marudov, *J. Chromatogr.*, 437 (1988) 249.
10 W. B. Wheeler, R. L. Edelstein and N. P. Thomson, *Pestic. Chem.: Hum. Welfare Environ. Proc. Int. Congr. Pestic. Chem. 5th. 1982*, 4 (1983) 49.
11 H. N. Niyg, L. G. Alberigo, H. E. Nordby and J. H. Stamper, *J. Agric. Food Chem.*, 29 (1981) 750.