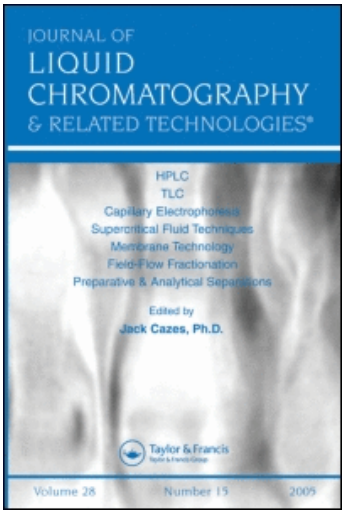

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Two-Stage Fractionation of a Mixture of 10 Pesticides by TLC and HPLC

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Two-Stage Fractionation of a Mixture of 10 Pesticides by TLC and HPLC

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Abstract: Relationships between R_F values and mobile-phase composition have been determined for 10 pesticides in normal-phase (NP) systems, which have enabled choosing optimum systems for preliminary fractionation of the multicomponent mixture of pesticides by zonal micropreparative thin-layer chromatography (TLC). The 10-component mixture was applied from the edge of the silica layer and developed with ethyl acetate–diisopropyl ether (10 : 90, v/v). The zones were detected in UV light at $\lambda = 254$ nm. The separated eight simpler fractions were applied to an octadecyl silica layer wettable with water (TLC, RP-18W) and rechromatographed. The separated eight simpler fractions were also applied to a cyanopropyl-bonded silica layer and developed with (NP) and reverse-phase (RP) systems. The plates were scanned and videoscanned, furnishing real pictures of the plates showing complete separation of the fractions on the RP-18W plate with the RP and NP systems on cyanopropyl silica layer. The simpler fractions were also separated on a cyanopropyl and octadecyl silica high performance liquid chromatography (HPLC) columns. Preparative separation of the complex mixture by TLC on silica (non-aqueous eluent, NP system), combined with TLC or/and HPLC (aqueous eluent, RP system) or HPLC (NP system), gives a good perspective of full separation of the simpler fractions in the second stage, by using the two methods with the possibility of full quantitative TLC and HPLC analysis.

Keywords: Thin-layer chromatography, NP/NP and NP/RP systems, fractionation of a mixture of pesticides, HPLC

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INTRODUCTION

Synthetic pesticides have been used since the early to mid-twentieth century. Organic pesticides, the most employed group, due to their widespread use in agriculture in the control, prevention, and elimination of plagues that attack plantations and herds, require effective analytical methods.

Pesticides occur frequently in the form of multicomponent mixtures (contaminations of rivers, dumping areas of toxic waste, stores), difficult to analyse in a single analytical process. The separation of volatile and thermostable pesticides can be enhanced by using comprehensive two-dimensional gas chromatography (GC × GC), where all separated peak clusters are transferred to a second column (with a different separation mechanism in comparison with a first column) and providing orthogonal resolution on a comprehensive $n_1 \times n_2$ basis (n_1 and n_2 are the number of the separated peaks in the first and second dimensions, respectively).[1,2]

Thin-layer chromatography (TLC) analysis of pesticides is especially suitable at sites where the concentration of pesticides might be high, e.g., sites of dumping grounds of toxic substances. The analysis of complex mixtures of pesticides can be simplified by preliminary fractionation of the mixture into simpler mixtures by micropreparative chromatography.[3] The analysed set of pesticides was chosen merely as an example of a complex mixture, the objective being the illustration of the analytical procedure.

The main problem in the separation of complex mixtures is finding systems of different selectivities for effective separation. For preparative separations, the normal-phase (NP) systems are preferable because of their wide range of differentiated selectivity of various mobile phases on polar adsorbents. The main purpose of preparative layer chromatography is isolation of pure compounds from a mixture with maximal yield. Sample application is one of the most important steps of a successful preparative separation. The zonal application of the sample from the edge of the layer is preferable.[4,5] Nyiredy[6,7] described sample application and other characteristics of classical preparative layer chromatography, overpressured layer chromatography, other special techniques, and trends in preparative layer chromatography. Waksmundzka-Hajnos and colleagues[8,9] described the strategy of preparative separation in TLC. Dzido et al.[10] also described the effect of temperature on the separation of test solutes in preparative TLC. Guiochon and colleagues[11–14] described theory, instruments, and practical issues of preparative chromatography. The separation of a certain target component from a multicomponent mixture using isocratic preparative elution chromatography was also studied theoretically.[15]

A good perspective of separation of compounds is obtained by use, in the second stage, systems of different selectivities compared with the first stage, e.g., NP system on silica in the first stage followed by a reversed-phase (RP) system on octadecyl silica adsorbent in the second stage [TLC or high

performance liquid chromatography (HPLC)]. Also possible is the use of NP or RP systems with different retention mechanisms on hydrophilic modified stationary phases, e.g., cyanopropyl, aminopropyl, and diol. These phases have many other advantages—an extended range of selectivity, and graduated surface polarity, and show less influence of the vapour phase on retention behaviour and, therefore, have better reproducibility.[16]

NP HPLC has also several advantages:[17] pressure drop across the column is lower in non-aqueous RP system than in aqueous RP system (because of lower viscosity of non-aqueous eluents); columns are usually more stable in non-aqueous solvents than in aqueous solvents; some samples are more soluble, or less prone, to decomposition in organic mobile phases.

However, RP chromatography generally offers better selectivity for the separation of molecules with different sizes of their hydrocarbon part.

EXPERIMENTAL

Standards of Pesticides

Standards of pesticides 1–10, listed in Table 1, were purchased from the Institute of Organic Industry (IPO, Warsaw, Poland). The standards were dissolved in methanol.

Solvents

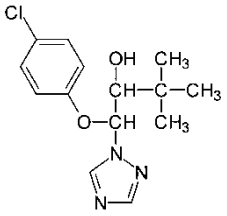
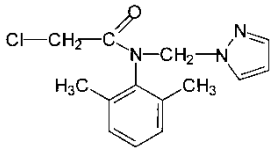
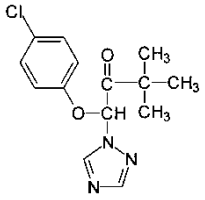
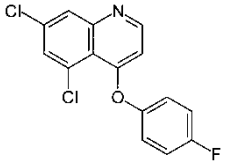
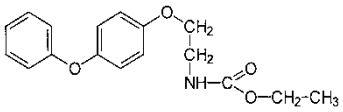
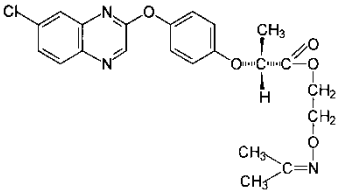
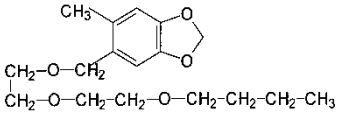
Dioxane, *n*-heptane, acetonitrile, methanol, and tetrahydrofuran were of chromatographic grade from Merck (E. Merck, Darmstadt, Germany); ethyl acetate and diisopropyl ether were of analysis grade from Polish Reagents (POCh, Gliwice, Poland).

Analytical TLC

TLC experiments were performed on $10 \times 20\,cm^2$ glass-backed silica gel TLC 60 F_{254} plates (E. Merck; #1.05729.0001), $10 \times 10\,cm^2$ glass-backed silica gel HPTLC RP-18W F_{254S} plates (#1.13124.0001), and $10 \times 10\,cm^2$ glass-backed cyanopropyl silica layer HPTLC CN F_{254S} plates (#1.16464.0001).

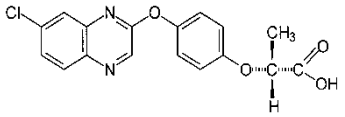
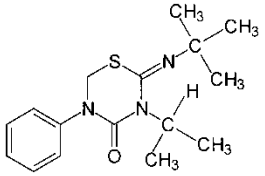
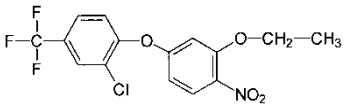
The pesticides were spotted as 0.5% solutions, and the plates were developed to a distance of 9 cm in horizontal, Teflon DS chambers (Chromdes, Lublin, Poland).[18,19] The spots were detected at $\lambda = 254$ and 366 nm.

Table 1. Pesticides investigated by TLC

No.	Compound	Activity	Structure
1	Triadimenol	Fungicide	
2	Metazachlor	Herbicide	
3	Triadimefon	Fungicide	
4	Quinoxyfen	Fungicide	
5	Fenoxycarb	Insecticide	
6	Propaquizafop	Herbicide	
7	Piperonyl butoxide	Synergist	

(*continued*)

Table 1. Continued.

No.	Compound	Activity	Structure
8	Quizalofop-P	Herbicide	
9	Buprofezin	Insecticide	
10	Oxyfluorfen	Herbicide	

Micropreparative TLC

Micropreparative zonal chromatography was performed using $20 \times 20\,cm^2$ plates coated with a 0.5 mm layer of silica gel 60 F_{254} (#5744). All micropreparative separations were performed using DS chambers. A solution of a mixture of pesticides (0.4–0.7 mL) (3% of no. 1; 2% of nos. 2, 3, 5, 7, and 1% of the remaining pesticides) was introduced to the edge of the layer through the glass distributor, by means of a glass syringe. The plate was developed by use of the NP non-aqueous eluent ethyl acetate–diisopropyl ether (10 : 90, v/v) chosen from the retention–eluent composition plots. The zones were detected in UV light at $\lambda = 254$ nm. The located zones were scraped from the plate into small funnels in which the narrow outlets were closed with glass wool. The adsorbed fractions were then isolated by elution with methanol.

Analytical HPLC

The chromatographic experiments were performed at $22^{\circ}C \pm 1^{\circ}C$ using a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with gradient pump, LC 10 AT, at a flow-rate of 1 mL/min, and UV–VIS detector SPD-10 AV (at $\lambda = 254$ and 223 nm). Solutions of the simpler fractions of pesticides (I–VIII) were injected in the eluent with the help of a Rheodyne

20 μL injector. The HPLC apparatus was equipped with SUPELCOSIL™ LC–CN $150 \times 4.6\,mm^2$ column, $d_p = 5\,\mu m$ (Supelco, Bellefonte, PA, USA) with the NP systems dioxane–*n*-heptane (2 : 98, 3 : 97, 5 : 95, v/v). The HPLC analysis was also obtained with SUPELCOSIL™ LC-18 $150 \times 4.6\,mm^2$ column, $d_p = 5\,\mu m$ (Supelco, Bellefonte, PA, USA) with the RP system, acetonitrile–water (70 : 30, v/v).

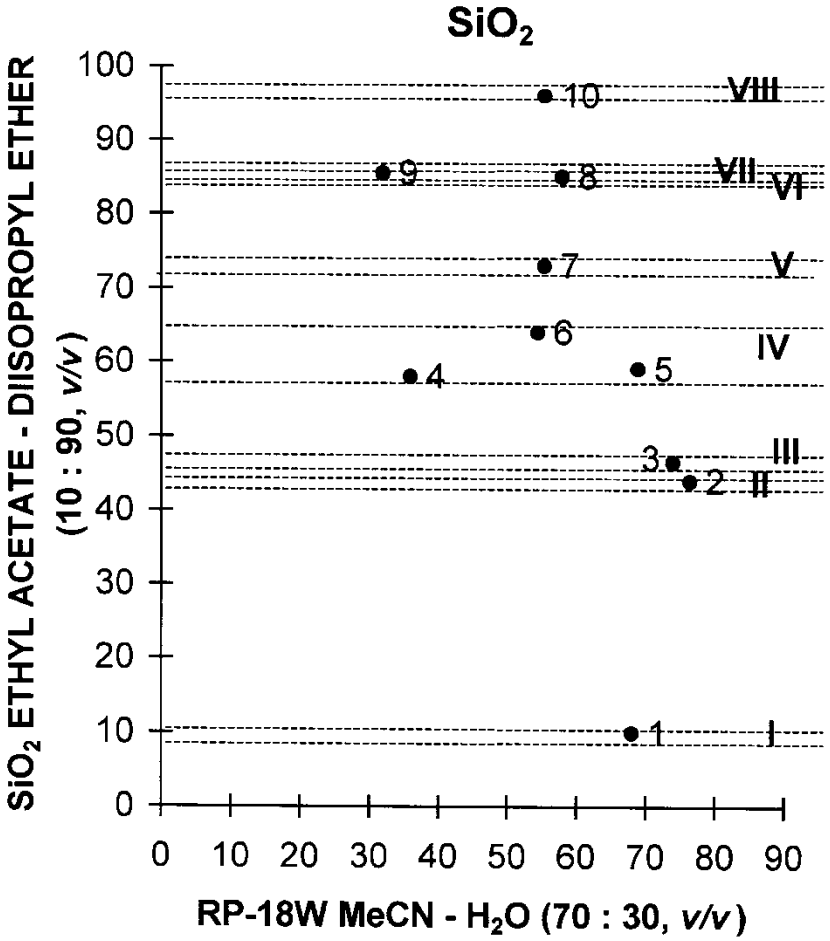


Figure 1. Correlation hR_F vs. hR_F system—RP: acetonitrile–water (70 : 30, v/v) on octadecyl silica wettable with water (RP-18W) and NP: ethyl acetate–diisopropyl ether (10 : 90, v/v) on silica gel. Illustrates possibilities for the separation of the 10-component mixture of pesticides (nos. 1–10) into eight fractions (nos. I–VIII) in the NP system on silica; the numbering of solutes in all figures is as given in Table 1.

Data Recording and Processing

The plates were videoscanned at $\lambda = 254$ nm by means of a Hitachi 3 CCD videoscanner controlled by Videostore 2 software. In addition, densitograms of plates were recorded (Camag TLC Scanner with the program CATS4). The scanning wavelength was 254 nm and the light source was a deuterium lamp.

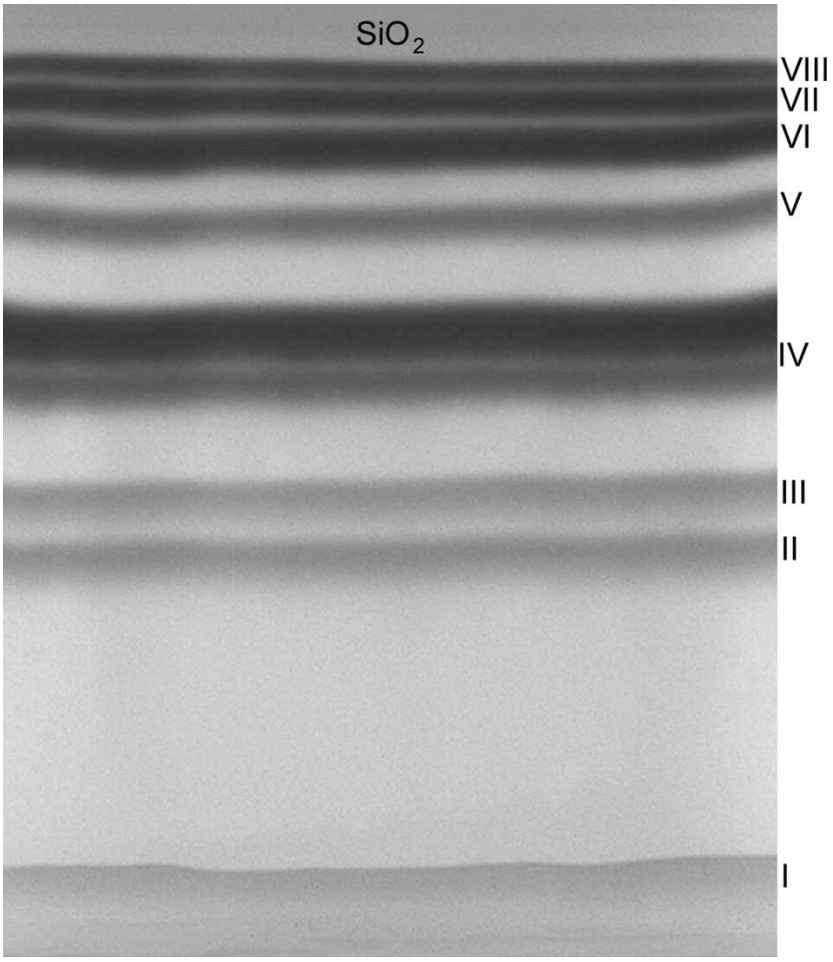


Figure 2. Chromatographic separation of pesticides on silica (0.5 mm layer) with ethyl acetate–diisopropyl ether (10 : 90, v/v) as mobile phase: zone diagrams of eight fractions of mixture of 10 pesticides applied as a solution (1–3%, 0.4 mL) to the plate from the edge of the silica layer through the glass distributor.

RESULTS AND DISCUSSION

Figure 1 illustrates possibilities for the separation of the 10-component mixture of pesticides (nos. 1–10) into fractions (nos. I–VIII) in the system, silica–ethyl acetate–diisopropyl ether (10 : 90, v/v).

A solution of the mixture of pesticides (1–3% of pesticides, 0.4 mL) was applied to the plate from the edge of the silica layer (0.5 mm) through the glass distributor. The plate was developed with ethyl acetate–diisopropyl ether (10 : 90, v/v). Bands were visualised in UV light at $\lambda = 254$ nm (Figure 2). Next, the zones were scraped from the plate, and the adsorbed fractions were isolated by elution with methanol. Each of the pesticide fractions (I–VIII) was applied on a silica gel 60 F_{254} HPTLC plate. In addition, standard substances were applied to the plates (1–10) and the plates developed to a distance of 9 cm with ethyl acetate–diisopropyl ether (10 : 90, v/v) as mobile phase. The real picture of the silica gel plate obtained by videoscanning (Figure 3) showed the separation of eight fractions of pesticides.

The fractions were applied 0.5 cm from the edge on a RP-18W plate and developed with a RP aqueous eluent, acetonitrile–water (60 : 40, v/v) [Figure 4(a) and (b)]. It can be seen that the fractions are separated into single components.

In the next series of experiments, the selectivity was investigated for cyanopropyl silica adsorbent. The cyanopropyl-bonded layer was developed with the RP system, acetonitrile–water (55 : 45, v/v). [Figure 5(a) and (b)] and with the NP system, dioxane–*n*-heptane (30 : 70, v/v) [Figure 6(a) and (b)].

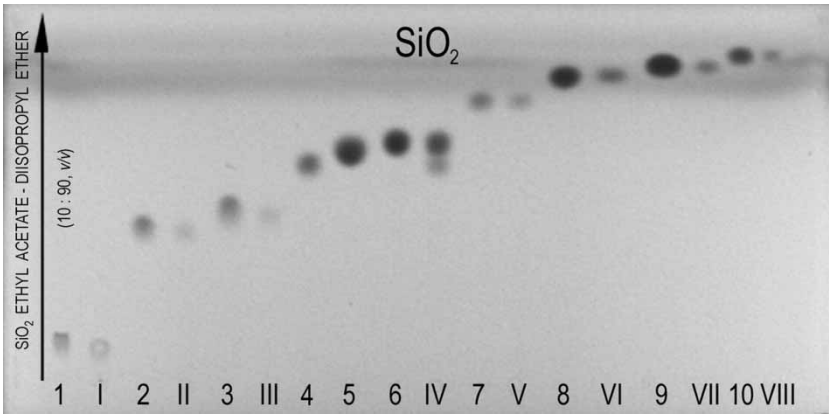


Figure 3. Videoscan showing a real picture of the silica plate and fractionation of the mixture of 10 pesticides (fractions I–VIII) by use of 10% ethyl acetate in diisopropyl ether as mobile phase. Standard substances (1–10) were also applied to the plate; the numbering is as given in Table 1.

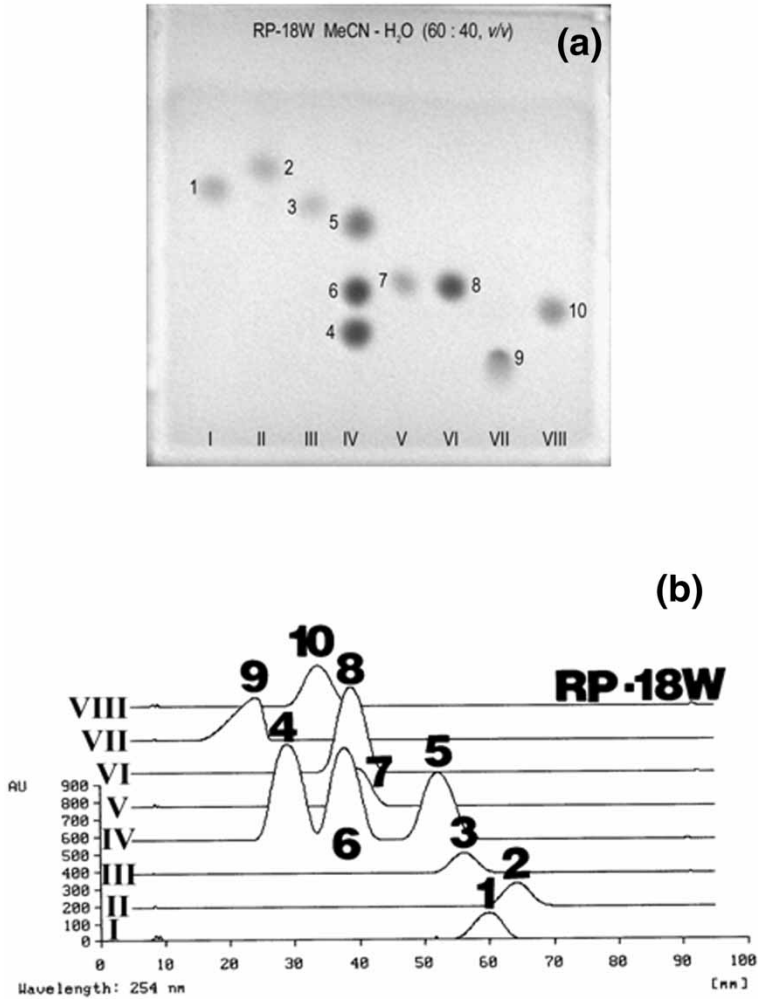


Figure 4. Videoscan (a) and densitogram (b) of the RP-18W plate, which shows separation of eight fractions of mixture of 10 pesticides (5 μL of each fraction) by RP system: acetonitrile–water (60 : 40, v/v).

It follows from Figure 6 that the selectivity of the CN/NP system is satisfactory for HPLC analysis on a CN column.

The fractions (I–VIII) were also injected on a cyanopropyl column (LC–CN) and developed by NP eluents composed of dioxane and *n*–heptane (2 : 98, 3 : 97, and 5 : 95, v/v). The fractions were also separated on a octadecyl silica column (LC-18) and analysed by the RP system with acetonitrile–water (70 : 30, v/v).

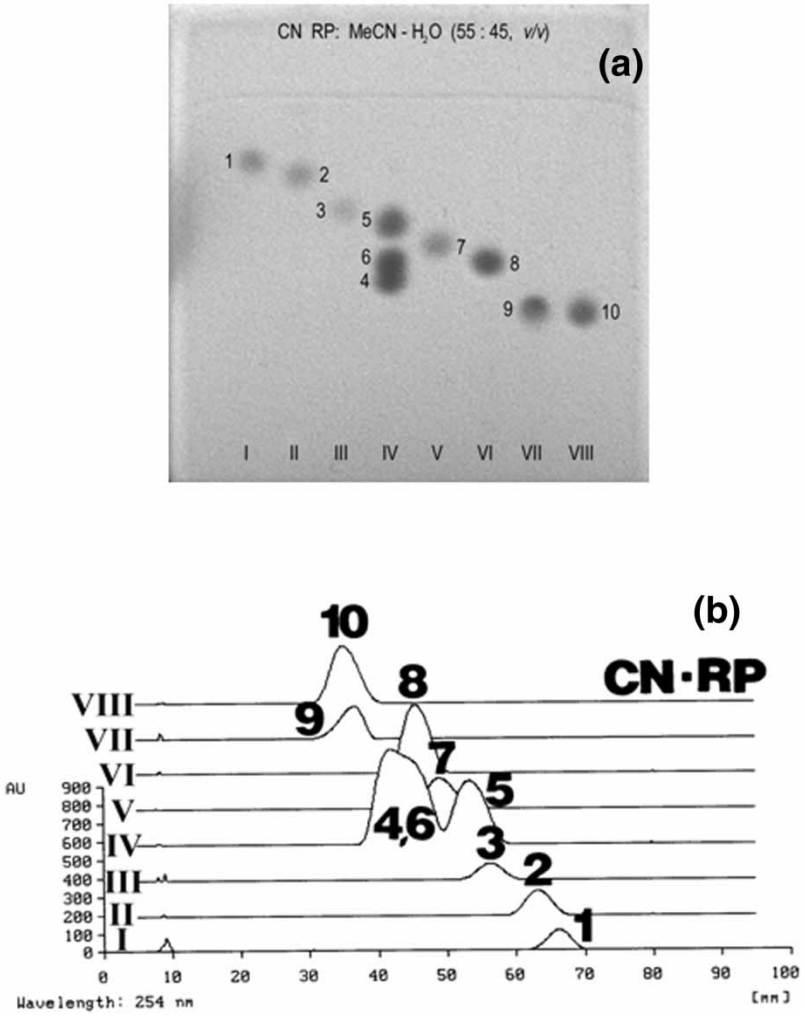


Figure 5. Videoscan (a) and densitogram (b) of the cyanopropyl plate, which shows separation of eight fractions of mixture of 10 pesticides (5 μL of IV–VII fractions; 10 μL of remaining fractions) by RP system: acetonitrile–water (55 : 45, v/v).

The retention times (t_r), number of theoretical plates on meter of peaks (N), and the values of resolution factor of peaks (R_S) separated in each fraction I–VIII containing more than one pesticide are presented in Table 2. The data of Table 2 showed larger R_S values for the majority of a set of pesticides of the I–VIII fractions in acetonitrile–water system on an LC-18 column, than nonaqueous system on an LC–CN column. The more

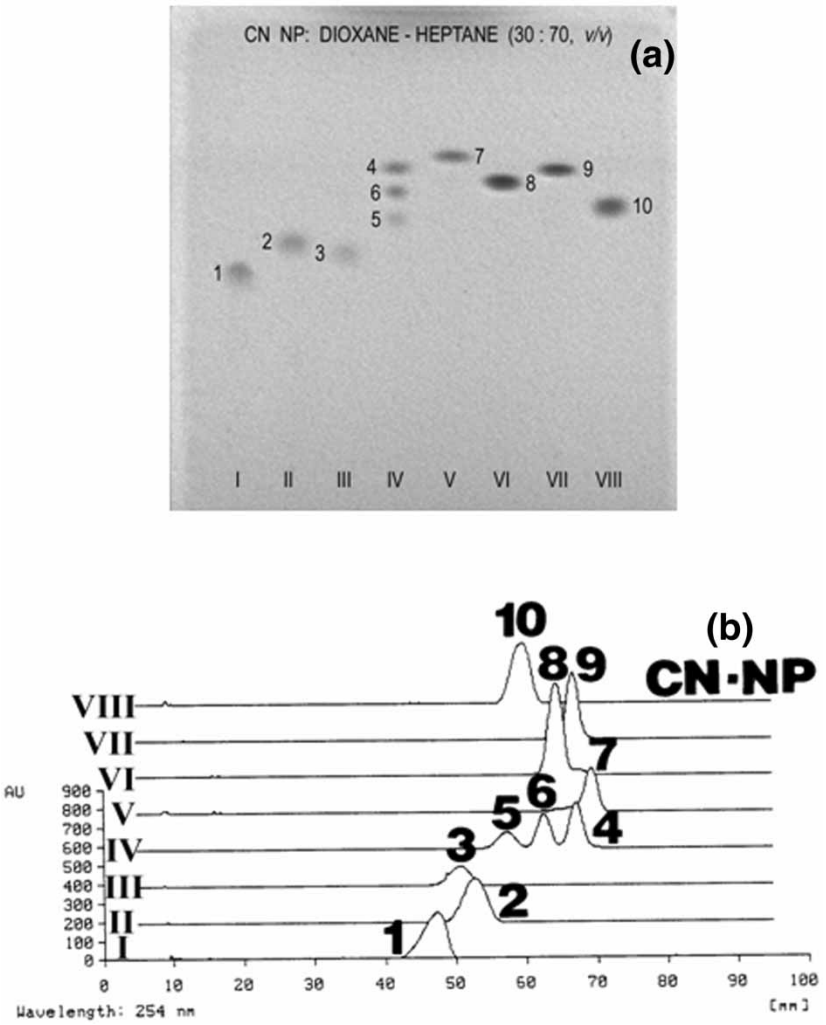


Figure 6. Videoscan (a) and densitogram (b) of the cyanopropyl plate, which shows separation of eight fractions of mixture of 10 pesticides (5 μL of IV, VI, VII fractions; 10 μL of remaining fractions) by NP system: dioxane–*n*-heptane (30 : 70, v/v).

sensitive HPLC analysis shows that some peaks are accompanied by small peaks of neighbouring compounds, e.g., in the fraction II: peak of metazachlor (no. 2) is accompanied by a small peak of neighbouring triadimefon (no. 3). The values of R_S of the pair were 0.78 and 2.78, respectively, on Supelcosil^{TM} LC–CN and Supelcosil^{TM} LC-18 columns. These values indicate successful resolution of the compounds in the acetonitrile–water system on the octadecyl

Table 2. Data of HPLC analysis of the I–VIII fractions of 10-component mixture of pesticides on cyanopropyl LC–CN column and on octadecyl silica LC-18 column

Fraction no.	Pesticide no.	Supelcosil™ LC–CN column dioxane–*n*-heptane (2 : 98, v/v)			Fraction no.	Pesticide no.	Supelcosil™ LC-18 column acetonitrile–water (70 : 30, v/v)		
		t_r	N/m	R_S			t_r	N/m	R_S
I	1	7.967	1,890	—	I	1	2.650	12,940	—
II	2	7.075	241,560	—	II	2	2.558	21,370	—
	3	7.317	25,980	0.78		3	3.083	26,980	2.78
III	2	7.092	180,860	—	III	3	3.067	24,070	—
	3	7.358	189,590	1.54		5	3.400	26,830	1.59
IV	4	3.245	22,880	—	IV	5	3.392	28,140	—
	6	5.408	31,830	7.25		6	6.433	44,860	11.64
	5	7.075	165,880	6.77		4	7.875	36,850	3.91
V	7	3.800	25,310	—	V	8	6.258	48,310	—
	8	4.183	26,940	1.50		7	7.200	47,540	2.97
VI	8	4.175	26,920	—	VI	8	6.250	46,480	—
VII	9	4.275	41,060	—	VII	9	10.100	53,510	—
VIII	10	6.308	46,080	—	VIII	10	7.442	54,310	—

silica column. For the majority of the compounds, larger values of N were for the RP system on the LC-18 column, besides compounds (nos. 2, 3, 5).

The solutions of some pesticides, during prolonged storage, development of chromatograms, elution and visualisation of bands in UV light, may decompose and can cause the appearance of additional peaks on their chromatograms. The correct identification and separation of the decomposition of the labile pesticides is possible.

The retention values of the compounds increase with addition of polar groups for NP chromatographic systems, e.g., the addition of polar substituents, –OH, whereas the same change in molecular structure decreases their retention value in RP systems, but less strongly.[20] The values of the hR_F of the pair of compounds, triadimefon (no. 3) and triadimenol (no. 1), were 46 and 10, respectively, in NP systems on silica gel (Figures 1–3); 46 and 51, respectively, in RP systems on octadecyl silica (Figure 4; Table 2).

CONCLUSIONS

A good perspective of separation of all components of fractions was obtained by using the systems of different selectivity in both stages, e.g., NP system on silica in first stage and RP system on octadecyl silica adsorbent in the second stage (TLC or HPLC). The procedure gives successful separation of the fractions by using the two methods with the possibility of full quantitative TLC and HPLC analysis.

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