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Application of instrumental thin-layer chromatography and solid-phase extraction to the analyses of pesticide residues in grossly contaminated samples of soil

J. Błądek[a,*], A. Rostkowski[a], M. Miszczak[b]

[a]*Institute of Chemistry, Military University of Technology, Kaliskiego 2, St., 01-489 Warsaw, Poland*

[b]*Military Institute of Armament Technology, Wyszyńskiego 7, St., 05-220 Zielonka, Poland*

Abstract

Modern TLC equipment was used for the quantitative determination of pesticide residues in soil that was considerably contaminated by petroleum derivatives. Chromatograms were developed in a normal-phase system by automated multiple development (AMD) gradient elution. Limitations of detectability by TLC were compensated for by application of relatively large volumes (by Linomat IV-Y) of analysed solutions on start lines. Quantitative assessment was achieved by UV absorption measurement scanning of the chromatograms by a "zig-zag" technique. An excess of petroleum derivatives was removed by solid-phase extraction; it was shown that this technique can be used not only in analyses of pollution contaminating liquid matrix but in research with solid matrix, as well. Recovery and error of the method were estimated (the recovery level was 80% and the R.S.D. was less than 9%).

Keywords: Soil; Environmental analysis; Pesticides

1. Introduction

The use of TLC for separation, identification and quantitation of environmental pollutants has a long history and there are innumerable references in the literature. Windhorst and De Kleijn [1] proved that results of quantitations obtained by use of TLC are comparable with those obtained by HPLC and GC. The range of TLC application in pesticides analysis is also wide. Rathore and Begum [2] refer to ca. 300 papers in their recapitulation of TLC methods for use in pesticide residue analysis. This list is systematically supplemented by review articles written by Sherma [3,4].

The collection of tasks in TLC applications for pesticide investigation could be classified based on the criteria of goals of experiments. The environmental monitoring is mostly the main aim in such research — information about the type and quantity of pesticide in a sample should be given [5,6]. On the other hand, assessment of the physical and chemical properties of pesticides, their mobility [7], bioacumulation [8], biotransformation [9] etc., is also an important area of TLC interest, in such kinds of experiments TLC is used as a research tool. Separate groups of tasks are investigations in which TLC is used only as clean-up technique.

In this work, experimental results concerning the use of HPTLC with automated multiple development (AMD) gradient elution for the monitoring of pesticide residues in soil are presented. A specific problem of this research was the contamination of analysed samples by considerable amounts of interfering admixtures, mainly petroleum derivatives (the

*Corresponding author.

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samples originated from military ranges in Poland recently abandoned by ex-USSR army). Separation conditions and a method of sample preparation as well as the limit of detectability and reproducibility of the method were determined, too. It was confirmed that TLC can be used in environmental research.

2. Experimental

2.1. Apparatus and materials

The apparatus used included an applicator Linomat IV-Y (Camag); AMD system (Camag); densitometer CS-9000 (Shimadzu); solid-phase extraction (SPE) set (J.T. Baker); TLC plates with silicagel 60 F_{254} HPTLC (Merck, cat. No. 5548). Solvents: acetone, *n*-hexane, dichloromethane and water were obtained from J.T. Baker. Pesticide standards: oxamyl, pirimicarb, carbaryl, malathion, phosalone, fenitrothion, tetradifon and methoxychlor were obtained from IPO (Warsaw).

2.2. Selection of the chromatographic system and quantitative relations assessment

Experiments to select the chromatographic systems were conducted with pesticide standard solutions. Eight insecticides from three different groups of compounds were selected for this purpose: organophosphorous, organochlorine and carbamate pesticides. Pesticide solutions were prepared in dichlomethane (10^{-3}%), and then the solutions were applied by "spray on" techniques onto TLC plates (10 or 50 μl), prewashed with methanol and activated at 100°C. The chromatograms were developed by the AMD technique, after selection of an effective elution gradient, number and duration of development steps. In this stage of research the correctness of the separation was estimated by the observation of fluorescence quenching. Application volumes of malathion, oxamyl and phosalone (these compounds quench fluorescence weakly) were five times greater than the rest of the pesticides. The most efficient separation (Fig. 1) was obtained by applying the elution gradient whose profile is shown in Fig. 2. After each step of development, the TLC plate was dried (3 min) and then activated by vapours of a binary mixture of solvents (dichloromethane–*n*-hexane, 2:1). Particular information about the composition of solvents in bottles and the number and period of each development step are listed in Table 1.

Amounts of each pesticide in the bands were determined by densitometric scanning of chromatograms with the use of "zig-zag" technique (UV absorption in reflection mode of densitometer). For this purpose first UV absorption spectrum of each pesticide was measured in order to find out the wavelength corresponding absorbance maximum (λ_{max}). Next, by scanning of chromatograms with characteristic for each pesticide value of λ_{max} (Table 2) position, the amounts of all pesticides were

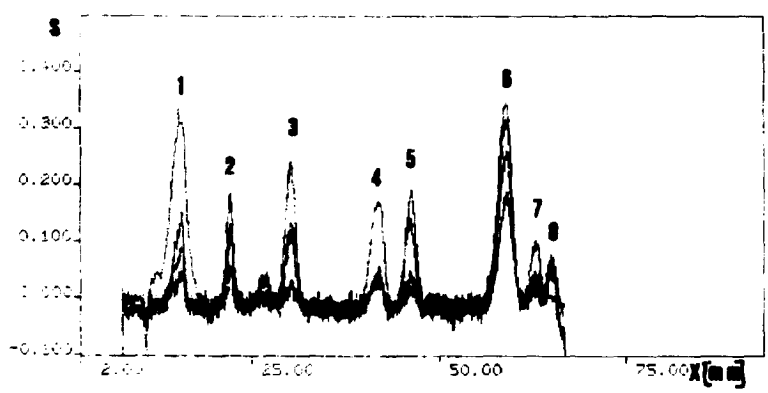

Fig. 1. A chromatogram of pesticides residues: three scannings at wavelengths 220, 265 and 300 nm. S=absorbance, x=distance of bands. Peaks: 1=oxamyl, 2=pirimicarb, 3=carbaryl, 4=phosalone, 5=malathion, 6=fenitrothion, 7=tetradifon, 8=methoxychlor.

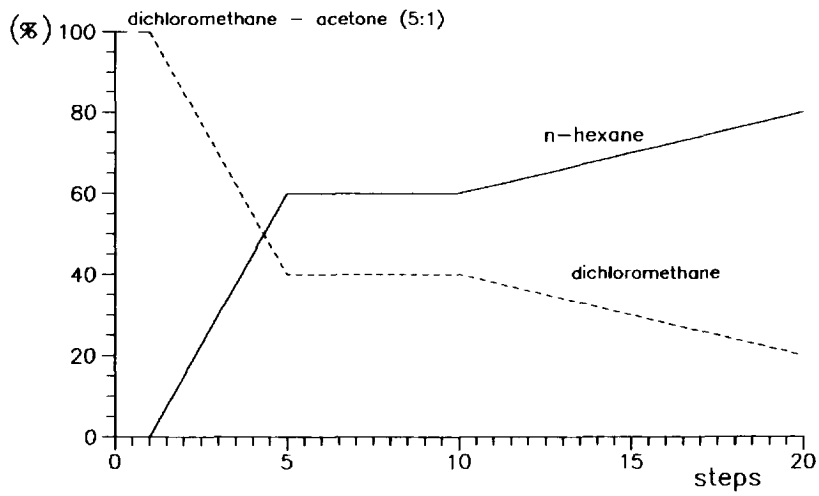

Fig. 2. Elution gradient profile.

determined. As a measure of detectability limits such amount of a pesticide in the band was taken, which densitometric peak area (expressed in relative absorbance units for the densitometer) was 10 times greater than the largest peak of noise.

In order to estimate the quantitative relation $A = f \times c$, where A=densitometric peak area, c=amount of the pesticide in the band, various volumes of solvents mentioned above were applied to the start line. After development of the chromatograms, they were scanned (saving λ_{max} condition) in a direction which was parallel to the head of the mobile phase, at heights relative to the location of particular pesticides. Results of measurements are specified in

Table 1
Filling of bottles and time duration of each step of development

Step No.	Time of step duration (min)	Bottle No.	Composition of solvents (v/v)		
			Acetone	Dichloromethane	Hexane
1	0.4	1	1	5	
2	0.7				
3	1.1	2		4	6
4	1.4				
5	1.7				
6	2.1				
7	2.6				
8	3.1	3		4	6
9	3.7				
10	4.2				
11	5.0				
12	5.7				
13	6.3	4		3	7
14	7.1				
15	7.9				
16	8.9				
17	10.0				
18	11.5	5		2	8
19	13.0				
20	15.0				

Table 2
Parameters of quantitations

Type of pesticide	λ_{max} (nm)	Calibration curves			Detection limits		
		$A = f \times c$	Correlation coefficient	Max. range of linearity (ng/band)	In band (ng)	R.S.D. (%)	In soil (μg/kg)
Oxamyl	220	$A=3939c+183$	0.9932	1200	150	6.1	7.7
Carbaryl	265	$A=21793c+1561$	0.9961	800	70	5.6	3.3
Pirimicarb	220	$A=92115c+1010$	0.9993	200	25	9.0	1.2
Malathion	200	$A=5556c+9970$	0.9958	4000	400	3.1	20.0
Phosalone	210	$A=11695c+1420$	0.9964	2000	200	4.4	10.0
Methoxychlor	220	$A=63653c+1955$	0.9991	500	50	8.7	2.4
Tetradifon	220	$A=75175c+580$	0.9994	500	50	8.0	2.5
Fenitrothion	280	$A=84782c+1821$	0.9981	500	50	9.0	2.6

Table 2. For all pesticides linear functions $A=f\times c$ were obtained; these functions differed in slope and range of linearity.

2.3. Preparation of soil samples to the analyses

Five representative samples (25 g each) were selected and then, they were spiked by the pesticides under investigation (Table 3). Each sample was extracted in a Soxhlet apparatus with 150 ml of dichloromethane for 8 h. The extracts were transferred into a separator to separate the water fractions. Organic fractions were dried in anhydrous sodium sulphate (20 g) during 24 h, and then (after filtration) they were concentrated by evaporation to ca. a 10-ml volume. The solid components were separated from the suspensions obtained (5 min, rotation at 3000 rpm) and then the clear solvents were concentrated in a nitrogen stream to a 2-ml volume. Aliquots (20 μl) of the concentrated solutions were applied to the chromatographic plate and the chromatograms were developed using the procedure described above. Results are shown in Fig. 3a. It was obvious that considerable amounts of pollution interfered with determined pesticides; some of the pollutants were identified by a GC–MS technique as humic compounds and petroleum derivatives.

Attempts at direct purification of the extracts in cartridges filled with silica gel with chemically bonded phases (C_8, C_{18} and C_{18} Polar Plus) did not give the results expected; in each case the recovery of pesticides was not more than 10%. One may suppose that in these experiments methylene chloride played a dual role as a matrix and also as an eluent and that it disturbed the retention. The best results (in terms of recovery and degree of sample purification) were obtained using the following procedure. A 1-ml volume of the extract was dissolved in methanol and then it was mixed with 800 ml of double-distilled water. The obtained emulsion was extracted on a solid-phase cartridge (C_{18} Polar Plus) according to the SPE method used in liquid matrix researches. Substances retained in the column were dried in a nitrogen atmosphere for 20 min and then the column

Table 3
Characterisation of the sample purification technique

Type of pesticide	Mass of pesticides spiked into 25 g of soil (μg)	Recovery; mean value from five measurements (%)	SD (%)
Oxamyl	50	72.0	7.5
Carbaryl	20	81.6	8.4
Pirimicarb	10	81.3	7.6
Malathion	100	75.6	6.6
Phosalone	60	76.5	9.1
Methoxychlor	20	80.0	9.9
Tetradifon	20	76.6	9.6
Fenitrothion	20	71.6	8.3

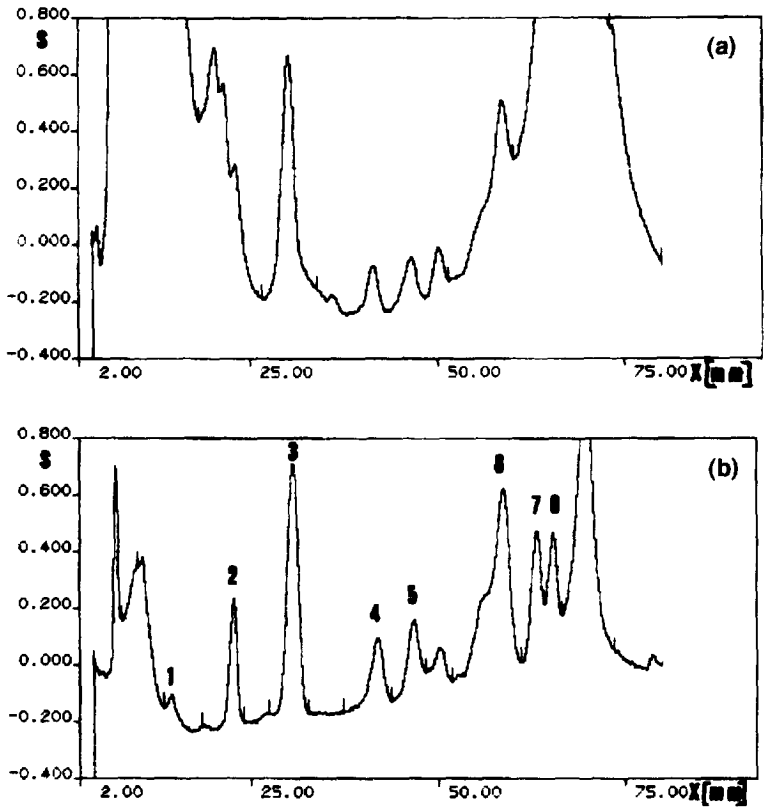

Fig. 3. Chromatograms of soil sample: (a) before purification, (b) after purification by SPE. Notation as in Fig. 1.

was washed by 6 ml of *n*-hexane divided into three portions — 2 ml each. Pesticides remaining in the column were eluted by two portions of methanol — 2 ml each. An eluent which had been concentrated in nitrogen stream to a volume of 1 ml was analysed according to the HPTLC–AMD technique used for pesticide standard analysis described above. Results of measurements (Fig. 3b) demonstrated not only the efficiency of the purification technique but also the possibility of quantitative estimation of the pesticide content in particular bands. This feature was used for the assessment of recovery (Table 3).

3. Discussion

The results presented confirm the advantages of modern TLC which originate principally from its equipment. It seems that the greatest benefits of trace analysis are achieved by the use of the "spray on" technique of sample application (Linomat IV-Y). This technique makes it possible to apply large volumes (100–500 μl) of analysed solvent while preserving the advantageous distribution of concentration at the start line. The importance of the influence of starting concentration distribution on the final result of the analysis was discussed earlier [10]. It is easy to demonstrate that the "spray on" technique makes (at level of detection limit) analysis of mixtures possible at concentrations nearly 1000 times smaller than was possible with classic application devices. Another decrease in the concentration level of the analysed mixture (at least one order of magnitude) enables the development of chromatograms by the AMD technique. At each step of development a band is concentrated and shaped (after scanning) to a peak with an area at least 10 times greater than the area obtained during development in classic chambers. For that reason, the investigations were limited to measurements of UV

absorption as it was possible to region employment of colour reaction which in classical method usually increases the detectability and specificity of a determination.

The possibility to use gradient elution is also an advantage of the AMD technique. It enables the separation of multicomponent mixtures in one properly programmed run. Using the AMD method in the first step of development, the most polar compound was isolated (oxamyl); steep elution gradient was used in the following four steps that made it possible to separate carbamates and isolate them from organophosphorous pesticides. Phosalone and malathion were separated in an isocratic elution process performed at steps 6–10. A relatively gentle elution gradient in the following ten steps (11–12) made it possible to separate organochlorine pesticides. In this way, the separation of eight pesticide mixtures was performed in 20 steps at a thedistance of 65 mm of mobile phase head during last step.

A method which use densitometric measurements for quantitative analyses (in this case, of pesticides) is described elsewhere and does not require comment. Linear characteristics of changes of measured signals vs. concentration of pesticides confirm possibility of such determinations.

The method of sample preparation these investigations is very interesting (usually, sample preparation is the most difficult stage of an investigation). Application of SPE for this purpose in analyses of solids has been used only recently. The recovery of pesticides and their detection limit related to their amounts in soil (Table 3) confirm the usefulness of this purification method to environmental monitoring.

HPTLC–AMD appeared to be useful for the detection and quantitation of pesticides with various chemical structures. Pesticides were separated in one analytical run only. It is worth adding that the level of the detection limit for malathion, phosalone or oxamyl is relatively poor and also that an increase in the specificity of determinations of all pesticides can be achieved by the use of chemical or biochemical methods of visualisation. A review on the importance of these methods is described in Jork et al. [11].

References

[1] G. Wihdhorst and J.P. De Kleijn, J. Planar Chromatogr., 5 (1992) 229.
[2] H.S. Rathore and T. Begum, J. Chromatogr., 643 (1993) 271.
[3] J. Sherma, J. Planar Chromatogr., 7 (1994) 265.
[4] J. Sherma, Anal. Chem., 67 (1995) 1R.
[5] J. Bladek, J. Planar Chromatogr., 6 (1993) 495.
[6] R.M. Johnson and A.B. Pepperman, J. Liq. Chromatogr., 18 (1995) 739.
[7] G.E. Schneiders, M.K. Koeppe, M.V. Naidu, P. Horne, A.M. Brownn and C.F. Mucha, J. Agric. Food Chem., 41 (1993) 2404.
[8] P.W. Lee, A.D. Forbis and L. Franklin, J. Agric. Food Chem., 38 (1990) 323.
[9] M. Chessels, W.D. Hawker and D.W. Connel, Chemosphere, 22 (1991) 1175.
[10] J. Bladek, A. Rostkowski, J. Planar Chromatogr., 8 (1995) 430.
[11] H. Jork, W. Funk, W. Fischer and H. Wimmer, Thin Layer Chromatography (Vol. 1, 2). Physical and Chemical Detection Methods. VCH, Weinheim, 1990.