

compounds without degradation. The SFC/FTIR spectra were able to distinguish between the structurally similar pyrethrins continuing to maintain the structural information provided by GC/IR analysis.

Future Developments

The future of SFC in pesticide and environmental analysis is not predictable. Currently more work is being reported in supercritical fluid extraction than in chromatography. However, research continues in a few locations with a few select individuals. The future of SFC seems to lie with chiral separations and pharmaceutical analysis and not with the environmentally oriented work presented in this review. This is not to suggest that the work presented here is without value. Advances into enhanced fluidity chromatography were most likely initiated from the work in SFC. As with other analytical techniques, success in one area leads to attempts in others.

Low waste generation in SFC is not enough to carry the technique into the arena where it can compete equally with LC and GC, the investment in training and equipment is too large a barrier to be overcome with what has fallen into the category of being a 'niche' technique. Liquid and gas chromatography are well cemented into environmental analysis. SFC of pesticides will continue to go beyond academic exercises to solve real problems, but most like

ly only when LC and GC do not yield a satisfactory result.

Further Reading

- Bright FV and McNally MEP (1992) *Supercritical Fluid Technology: Theoretical and Applied Approaches to Analytical Chemistry*. Washington: American Chemical Society.
- Brown PR and Grushka E (1994) *Environmental Applications of Supercritical Fluid Chromatography*. Advances in Chromatography, vol. 34, ch. 5. New York: Marcel Dekker, Inc.
- Charpentier BA and Sevenants MR (1988) *Supercritical Fluid Extraction and Chromatography: Techniques and Applications*. Washington: American Chemical Society.
- Johnston KP and Penninger (1989) *Supercritical Fluid Science and Technology*. Washington: American Chemical Society.
- Lee ML and Markides KE (1990) *Analytical Supercritical Fluid Chromatography and Extraction*. Provo: Chromatography Conferences.
- Smith RM (1988) *Supercritical fluid chromatography*. London: The Royal Society of Chemistry.
- Smith RM and Hawthorne SB (1997) *Supercritical fluids in chromatography and extraction*. Reprinted from *Journal of Chromatography A*, vol. 785. Amsterdam: Elsevier.
- Westwood SA (1993) *Supercritical Fluid Extraction and Its Use in Chromatographic Sample Preparation*. Boca Raton: CRC Press, Inc.
- White CM (1988) *Modern Supercritical Fluid Chromatography*. Heidelberg, Huthig Verlag.

Thin-Layer (Planar) Chromatography

J. Błażek and A. Rostkowski, Military University of Technology, Warsaw, Poland

Copyright © 2000 Academic Press

Introduction

Pesticides are a group of chemicals designed for killing weeds, pest control and plant growth regulation. They are poisons by design. Some of them also demonstrate carcinogenic potential and/or teratogenic activity. Pesticides (or products of their transformation in the environment) can penetrate soil, water, air and also food and fodder. As a result, pesticides are currently present in all parts of the environment. Many of them undergo degradation, but others are persistent and may accumulate in the food chain.

Development of residue analysis for pesticides is driven by toxicological purposes or by the need to

identify residues. In the first case, the compounds are identified as being potentially hazardous to human health or to the environment. In the second case, the determination of residues is mainly aimed at inspecting and monitoring of food or environmental samples. Thin layer chromatography (TLC), including its modern developments (high-performance adsorbents, application of new, automatic techniques of spotting and development of chromatograms, spray-on technique of sample application), is still used for such analyses, especially in combination with selective biochemical detection methods or multidimensional methods although gas chromatography and high-performance liquid chromatography with selective detectors (ECD, NPD, AED or MS) are more important.

There is now a considerable literature describing pesticide analysis by TLC, with environmental and food monitoring, generally being the main aim in such

research (see Fodor-Csorba in Further Reading). There are also numerous works of cognitive character, concerning investigation of the most advantageous chromatographic systems, separation techniques and methods of visualization and quantification. Assessment of physical and chemical properties of pesticides, e.g. their mobility, bioaccumulation and biotransformation, is also an important area of TLC study.

General Principles of Pesticide Analyses

The high selectivity, high detectability and reliability of analysis under fairly simple conditions contribute to the effective use of TLC for pesticide applications. Unfortunately, in the great majority of cases, pesticides need to be determined in complex matrices at extremely low concentrations over a wide polarity range. Therefore the analysis of samples without some preliminary preparation is almost impossible. Separation of pesticides in such samples to determine chemical identity and achieve detection and quantification should be followed by obtaining representative samples, sample clean-up and analytes enrichment.

Sample Preparation

Sample preparation techniques for analysis of pesticide residues by TLC are similar to those applied in the analysis of other pollutants. In the case of liquid matrices (water, milk, oil), liquid-liquid extraction (LLE) and, more recently, solid phase extraction (SPE) are most often used. For instance, C_{18} cartridges are useful for the extraction and purification of phenylurea herbicides, N-methylcarbamate and organophosphorous insecticides from water. For solid matrices (soil, meat, fruit and vegetable) liquid extraction is the most effective, although SPE has also been used after liquid extraction. Nowadays there is an increase in the application of supercritical fluid extraction, especially for the isolation of pesticides from solid matrices. This technique is useful for the trace analysis of pesticides because of much reduced amount of co-extracted interfering material.

It should be emphasized that TLC has no limitations in the scope of solvents that can be used for sample preparation, since solvent is removed and does not take part in the chromatographic process. The choice of TLC solvent is limited only by the physico-chemical properties of particular groups of pesticides. For example, carbamates are relatively labile chemicals so that during their extraction, strong bases are to be avoided to prevent losses due to chemical reaction. Extraction is usually carried out with acetonitrile or methanol (solubility in petroleum

solvents is limited). A small amount of water often allows actively absorbed pesticides to be released into the solvent. Strongly retained pesticides may be removed by elution with a mixture of water and methanol (1 : 4, v/v) and then partitioned into methylene chloride or eluted directly with ethyl acetate if the extract is clean. Extraction of organophosphorous or organochlorine pesticides is similar. These substances are much less labile than the carbamate pesticides and may, therefore, be retained on more active sorbents.

The solvent chosen to extract residues of pesticides depends not only on the solubility of the chemical, but also on the nature of the information required. This feature has special meaning in the analysis residues of pesticides from plant tissue. For example, the determination of residues for the purpose of establishing of safe re-entry times of workers after crops have been sprayed, requires a surface extraction of residue. On the other hand, determination of pesticide residues in fruits or vegetables to ensure the safety of food for consumers usually requires homogenization of the whole sample to extract the total impurities.

The goal of clean-up is to remove as much interfering, co-extracted substances and to lose as little of the pesticides as possible. Clean-up of samples depends on the type of matrix, detection limits required and the visualization technique employed. Usually about 80% of water samples had not been cleaned, but almost all analyses of pesticides in soil and food samples requires at least some clean-up. Selective methods of pesticide visualization such as fluorescence or enzymatic methods may minimize the need of clean-up.

Development Techniques

The majority of pesticide separations are performed on un-modified sorbents such as silica gel, cellulose aluminium oxide and polyamide. Modified sorbents (amino- NH_2 octyl-RP- C_8 , and octadecyl-RP- C_{18} or impregnated silica gel) are also used (Table 1).

The composition of the mobile phase is the second parameter, defining the conditions for chromatographic separation. During pesticide analysis in normal phase systems, mixtures of organic solvents are often applied. In reversed-phase systems mixtures of polar solvents (e.g. methanol, acetonitrile) with water, organic acids (e.g. acetic or formic acid) or ammonia are used. In some cases, organic salts or ion exchangers are dissolved in the mobile phase to improve the selectivity of the system.

All works concerning research into the best chromatographic systems are commonly named 'behaviour'. An investigation of the relation between pesticide structure and retention provides informa-

Table 1 Examples of chromatographic systems and techniques of development used in pesticide analyses

Analytes	Chromatographic systems		Technique of development
	Stationary phase	Mobile phase	
Pesticides of different classes	HPTLC silica	Gradient based on <i>tert</i> -butylmethyl ether + 5% acetonitrile, hexane, formic acid and ammonia	AMD
Pesticides of different classes	HPTLC silica	Gradient based on acetonitrile, dichloromethane and hexane	AMD
Pesticides of different classes	Silica gel impregnated by paraffin oil	Aqueous sodium chloride solution modified by β -cyclodextrin polymer	Classical TLC
Triazine herbicides	HPLC silica	Chloroform : ethyl acetate (1 : 3)	OPLC
Carbamates	RPC-18	Acetonitrile : water (17 : 3) or chloroform : acetonitrile : acetone (4 : 1 : 1)	Classical TLC
Cyanophenyl herbicides	Silica bonded β -cyclodextrin	Water : methanol (7 : 3) or glycine : methanol	Sandwich DS chamber

tion about the character of interactions in the chromatographic system and the possibility of prediction of their separation. Such works concern the behaviour of different groups of pesticides on silica gel, impregnated silica gel, reversed-phases, water insoluble β -cyclodextrin polymer, etc. Perišić-Janjić and co-workers have carried out research on the chromatographic behaviour of four groups of *s*-triazine derivatives on aminoplast (a carbamide-formaldehyde polymer) and cellulose. Chromatograms were developed with three aqueous mobile phases. The basic aim of the investigation was the evaluation of aminoplast (cellulose was used as comparative adsorbent) for the separation of triazines. Because the *s*-triazines are weak bases, the influence of mobile phase pH on the chromatographic retention was also examined. The authors demonstrated that retention behaviour of *s*-triazine derivatives on aminoplast and cellulose is similar. The greatest changes in R_F values occur in the pH region of protonation and dissociation of the triazine derivatives. It was also shown, that changes in retention factor, k , with pH allows the determination of ionization constants of the analytes.

One-dimensional ascending or horizontal techniques have usually been applied for the separation of pesticides in a closed chamber; multiple and two-dimensional development techniques have been rarely used. The new instrumental techniques such as forced flow planar chromatography (FFPC), automated multiple development (AMD) and gradient

development techniques are being used more frequently. The work of Mazurek and Witkiewicz is an example of the investigation of good separation techniques for pesticides. The direct aim of the work was the analysis of organophosphorous warfare agents, but they were analysed in the presence of 22 pesticides. The main features of the work are the application of the Prisma model for the mobile phase optimization, two-dimensional development, a biochemical method of visualization and separation by overpressure TLC (OPLC). The authors demonstrated faster separations in pressure chambers compared with classical TLC. It leads to a decreased spot diffusion and an increase in the number of theoretical plates. It was also shown that complete separation of all components of the mixture is possible only by two-dimensional OPLC (Figure 1). Examples of pesticides separation using automated multiple development (AMD) and gradient development technique are presented below.

Detection, Identification and Quantification

Pesticides are visualized using chemical or biochemical, physicochemical and physical methods. Chemical methods are based on wetting the adsorbent by solvents or aerosols of different agents, which react with the pesticides, resulting in coloured products. Fluorescence and enzymatic methods are particularly useful in pesticide investigations. They are dis-

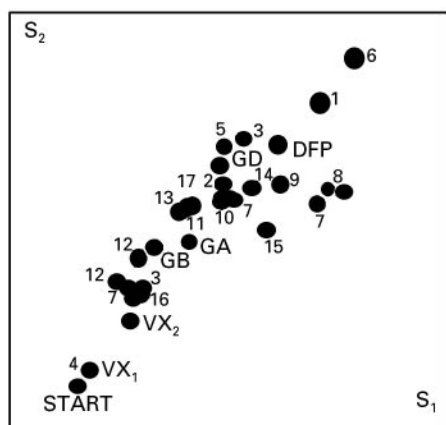


Figure 1 Two-dimensional OPLC separation of pesticides in the presence of organophosphorous warfare agents. Stationary phase – silica gel (TLC 10 × 10 cm); mobile phase: first direction (S_1) diisopropyl ether–benzene–tetrahydrofuran–n-hexane (10 + 7 + 5 + 11, v/v); second direction (S_2) tetrahydrofuran–n-hexane (2 + 3, v/v). Development distance – 6 cm; total development time – 30 min. Visualizing reagent – enzymatic. 1 = Co-Ral; 2 = DDVP; 3 = diazinon; 4 = disyston; 5 = ethion; 6 = fenclor-phos; 7 = gution; 8 = malathion; 9 = monitor; 10 = naled; 11 = thimet; 12 = trichlorphon; 13 = zolone; 14 = carbaryl; 15 = thiram; 16 = fenuron; 17 = linuron. (Reproduced with permission from Mazurek, 1991.)

tinguished by high sensitivity and specificity and allow the analysis of pesticides in the presence of background impurities that do not interfere. In enzymatic detection chromatograms are first sprayed with

an enzyme solution, then, after appropriate incubation, components altered by enzyme are detected by reaction with a suitable reagent. This method is characterized by very low detection limits and sometimes it allows analysis of pesticides without resorting to enrichment of the sample. Radiometric visualization methods are used for detection of radiolabelled pesticides. Radiometric methods are mainly applied to studies of pesticide metabolism in plants and animals, the uptake of pesticides by plants from soil and the fate of pesticides in the environment. The principal methods for the detection and quantification of radiolabelled pesticides separated on TLC plates are autoradiography, scraping followed by scintillation counting, and direct measurement using radiation detectors. The common pesticide visualization methods are presented in Table 2.

Lawrence, Frei, Mallet, and their co-workers focused attention on the visualization and quantification of pesticides by fluorescence methods. A comprehensive account of their works was presented by Hurtubise (see Further Reading). Most fluorescence analyses of pesticides require pre-treatment of the compounds to convert them to a fluorescent species. Certain organophosphorous pesticides fluoresce after just heating others (e.g. carbaryl or benzomyl) form fluorescent anions as a result of hydrolysis, and others (e.g. organothiophosphorous compounds) can be quantified in the presence of metal chelating compounds. Sulfur-containing pesti-

Table 2 Reagents for visualization of pesticides

Class of pesticide	Reagent
Organochlorine	Sodium hydroxide-cobalt (II)-acetate- <i>o</i> -tolidine <i>N,N</i> -Dimethyl-1,4-phenylenediamine
Organophosphorous	4-Nitrobenzenediazonium tetrafluoroborate Mercury (II) salt-diphenylcarbazone 4-(4-Nitrobenzyl)pirydyne Enzymatic methods
Carbamate	Bratton–Marshall reagent Fast Blue salt 4-(Dimethyloamino)-benzaldehyde-sulfuric acid <i>N,N,N',N'</i> -Tetramethyl-1,4-phenylenediamine 4-Nitrobenzenediazonium tetrafluoroborate
Phenoxy acid	2,6-Dichloroquinone-4-chloroimide
s-Triazine derivatives	<i>N,N,N',N'</i> -Tetramethyl-1,4-phenylenediamine Chlorine-4,4'-tetramethyldiaminodiphenylmethane Chlorine- <i>o</i> -tolidine-potassium iodide Tetrabromophenolphthalein ethyl ester-silver nitrate-citric acid
Urea derivatives	<i>N,N,N',N'</i> -Tetramethyl-1,4-phenylenediamine Bratton–Marshall reagent

cides are determined quantitatively by pH-sensitive fluorescent reagents. The best fluorogenic reagents are dansyl chloride and fluorescamine. Fluorescence allows detection of certain pesticides at the level of 10 ng and linearity in the range 10 ng to 10 µg.

In the review articles written by Sherma, several new spray reagents, recently introduced for the selective detection of pesticides, are presented. For instance 20% sodium hydroxide, 5% cupric acetate, 1% phosphomolybdic acid followed by 0.1% *o*-toluidyne in acetic acid was proposed for the determination pyrethroid insecticides containing a nitrile group. These form blue spots with a detection limits of 1 µg without interference from organophosphorous, organochlorine and carbamate insecticides. Synthetic pyrethroids (fenvalerate, cypermethrin, fenprothrin) can be visualized as pink-coloured spots at 1 µg by their alkaline hydrolysis to liberate HCN, which reduces 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-tetrazonium chloride to formazan in the presence of phenazonium methosulfate. Organophosphorous, organochlorine and carbamate insecticides do not interfere. Another example is the application of 2-trichloromethylbenzimidazole to determination pesticides containing either an azine or anazole ring. Excellent detection limits (20 ng to 10 µg) and very high selectivity makes this agent as very useful chromogenic agent for identification as well as for detection of these compounds.

Quantitative analyses of pesticides are performed mainly by UV-VIS densitometric measurement or by fluorescence. Quantitative analyses of pesticides can also be performed by combining TLC with other analytical techniques, e.g. TLC/HPLC, TLC/GC, TLC/MS, etc. Theoretical considerations, the correct approach to multidimensional methods development, instrumental requirements and contemporary applications of these approaches have been reviewed by Poole *et al.*

Applications

The agricultural use of pesticides gives rise to most analysis. Instrumental TLC has been applied for sensitive and fully quantitative analyses of environmental samples and control of food. Applications in forensic toxicology are less common. A feature of these analyses are investigations of pesticide residues in very completed matrices. Samples of vegetables, meat, food or biological material (for forensic toxicology) are analysed identically (Table 3).

Monitoring of the Environment

Butz and Stan have demonstrated the application of the AMD technique for the monitoring of environmental samples and the strategy of the whole proced-

ure, which has become a German Standard (DIN 38407 part II). Pesticides isolated from water are spotted on the layer with standards, and separated using gradient elution. Gradient elution allows the separation of pesticides belonging to different classes of compounds such as phenylureas, carbamates, triazines, phenoxy-carboxylic acids, and others. In total, 283 pesticides were analysed and only eighteen of them give detection limits of more than 100 ng and can therefore not be analysed from one litre of drinking water without further treatment. Two examples of drinking water spiked at the 100 ng L⁻¹ level are presented to demonstrate the merits of the method. Further research confirmed that the AMD technique can be easily applied to screen for pesticide residues in drinking water and ideally supplements other analytical techniques (Figure 2).

In the AMD technique in the first ten isocratic development steps, the starting zones are focused into sharpened bands. The elution gradient starts with an alkaline solvent mixture and ends with an acidic solvent. In this way it is possible to move or fix particular compound classes, such as acids or amines. The pH change provides better peak shapes and improves separation performance. Figure 3 shows a densitogram of a pesticide mixture separated using a gradient (UV absorption measurement with multiple wavelength scan). The limit of detection of most pesticides was 10 ng. However, it should be emphasized that in real samples the resulting spectra of the peak may be altered by overlapping matrix compounds, so that a pesticide, although present may not be recognizable. In such cases the authors recommend another gradient to confirm positive results.

TLC/AMD and SPE have been used for the analyses of pesticide residues in strongly contaminated samples of soil (Figure 4). Chromatograms were developed in a normal-phase system by AMD gradient elution. Limitations of detectability were compensated for by the application of relatively large volumes (by use of a spray-on technique) of analysed solutions. Quantitative assessment (linear relationship $A = f(c)$, where A is densitometric peak area, c mass of pesticide in band formulated in ng) was achieved by UV absorption measurement scanning of the chromatograms by a 'zig-zag' technique (Table 4).

Recovery and error of the method was estimated; the recovery level was 80% and the R.S.D. was less than 9%. The result presented confirm the advantages of modern TLC, which result principally from equipment development. It was demonstrated that the greatest benefits in the trace analysis of pesticides are achieved by the use of the 'spray-on' technique of sample application.

Table 3 Examples of TLC application to pesticide analysis in different matrices

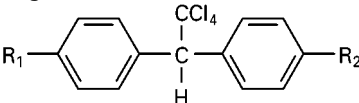
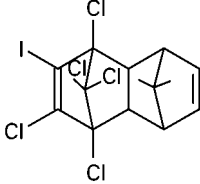
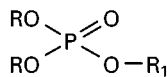
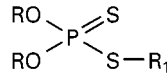
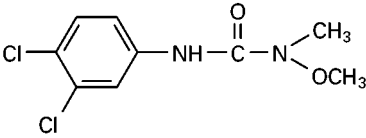
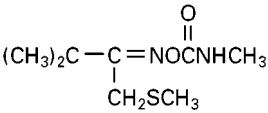
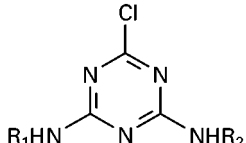
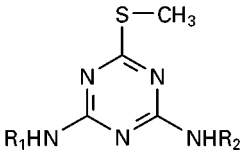
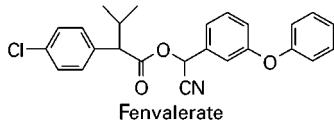
Class of pesticide and examples of its structure	Analyte	Matrix	Chromatographic system	
			Stationary phase	Mobile phase
Organochlorine				
 <p>Metoxychlor: $R_1 = R_2 = \text{OCH}_3$ DDT: $R_1 = R_2 = \text{Cl}$</p>	Metoxychlor in presence of DDT, isomers, γ -HCH, toxaphene, dieldrin, aldrin, etc. DDT and DDE DCP	Water Plants Poultry	Silica gel Silica gel Silufol	Hexane-acetone (9 : 1) Hexane-ethyl ether (17 : 3) Hexane-benzene-ethyl acetate (6 : 4 : 1)
 <p>Aldrin</p>	DDP, DDT, parathion, metoxychlor Aldrin, dieldrin	Water Water	HPTLC Silica gel	Gradient based on dichloromethane and hexane Heptane
Organophosphorous				
 <p>Dichlorfos: $R = \text{CH}_3$, $R = \text{CHCl}_2$</p>	Metaphos Chlorpyrifos metabolites	Vegetables Banana pulp	Silica gel + gypsum + Zn Silica gel	Hexane-acetone (4 : 1) Hexane-chloroform (4 : 1)
 <p>Malation: $R = \text{C}_2\text{H}_5$, $R_1 = \text{CH}(\text{CH}_2\text{COOC}_2\text{H}_5)\text{COOC}_7\text{H}_9$</p>	Bromofos Methidation	Peanut crops Clinical samples	RPC-18 W RPC-18	Acetic acid-chloroform-isooctane (1 : 4 : 15) Acetonitrile-water (3 : 1)
Carbamates				
 <p>Linuron</p>	Diflubenzuron Diuron, isopropuron, linuron, metoxuron, monolinuron, nrburon	Water Plant	HPLC HPTLC	Ethyl acetate-toluene (1 : 3) Gradient based on acetonitrile, dichloromethane, acetic acid, toluene and hexane
 <p>Thiofanox</p>	Metoxuron and its breakdown product Aldicarb and thiofanox	Potato Sugar beet	RPC-18 RPC-18	Acetonitrile-water (17 : 3) Chloroform-acetonitrile-acetone (4 : 1 : 1)
Triazines				
 <p>Simazine: $R_1 = R_2 = \text{C}_2\text{H}_5$</p>	Metribuzin	Soil	Silufol	Chloroform-ethyl ether (2 : 1)

Table 3 Continued

Class of pesticide and examples of its structure	Analyte	Matrix	Chromatographic system	
			Stationary phase	Mobile phase
 Desmetryne: R ₁ = CH ₃ , R ₂ = CH(NH ₃) ₂	Simazine, atrazine, promazine, prometon, desmetryne, ametryne, terbutryne	Water	Zn carbonate	Benzene–acetone (19 : 1)
Pyrethroids  Fenvalerate	Halogenated pyrethroid insecticide and its <i>trans</i> -isomers of permethrin and cypermethrin from valerate	Fruits and plants	Silufol	Hexane–chloroform (3 : 2)

Investigations of Pesticides in the Environment

In environmental research not only the level of pesticide residues is controlled but also their behaviour in different matrices. In such research TLC is used for the measurement of the mobility of pesticides, their bioaccumulation and biodegradation.

Residues of pesticides introduced into the environment are adsorbed onto soil particles and may end up as sediments at the bottom of lakes and rivers. Therefore, knowledge of the mobility of pesticides in soil is an essential element of environmental investigations. Measurements of pesticide mobility are usually performed using STLC (soil thin-layer chromatography). In this technique a stationary phase is prepared from a soil sample, in which the mobility of pesticides is to be determined. Solutions of persistent pesticides (sometimes radiolabelled pesticides) are spotted onto

the prepared layer and then developed with water as the mobile phase. STLC is also used for the evaluation of the influence of exogenous organic matter on the mobilities of pesticides in the soil. For example, examination of the mobility of diazinon and linuron demonstrated that simultaneous addition of organic compounds and other pesticides to the soil in agricultural practice may alter the mobility of sparingly soluble pesticides.

In another example researchers exposed Bluegill Sunfish to [¹⁴C] metolachlor at a concentration of 1 mg L⁻¹ for 34 days in a flow-through system. After that time all the fish were removed from the tank and dissected into tissues. After extraction and clean-up, eluates were spotted (together with unlabelled standards) onto layers, and then separated by two-dimensional development. Radioactive zones were detected using X-ray film. The non-radiolabelled

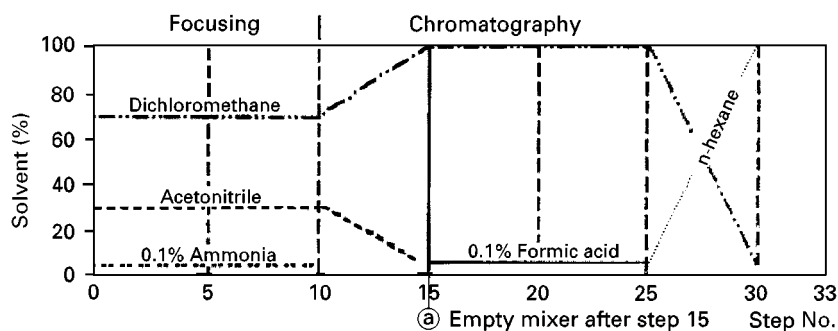


Figure 2 Gradient example for pesticide screening in DIN 38407 part 11. (Reproduced from Morlock, 1996 with permission from Elsevier Science.)

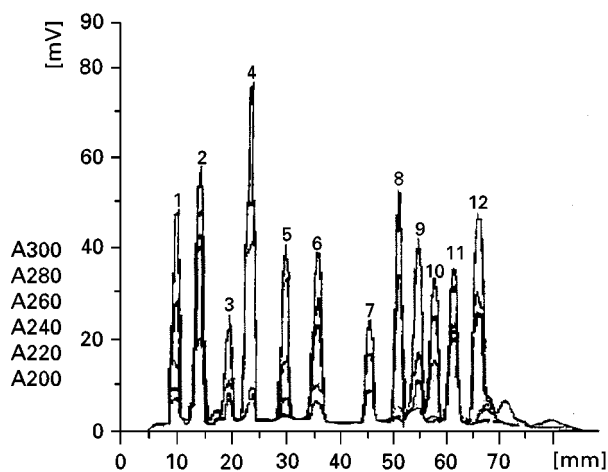


Figure 3 Multi-wavelength of a pesticide mixture separated according to the gradient in Figure 2. 1 = hydroxyatrazin, 2 = formetant, 3 = triadimenol, 4 = matalaxyl, 5 = isoproturon, 6 = diuron, 7 = dimethylaminosulfanilide, 8 = methidathion, 9 = 2,4-*p*-isobutyl ester, 10 = endrin, 11 = 2,2-*bis*-(4-chlorophenyl)-1,1-dichloroethane. (Reproduced from Morlock, 1996 with permission from Elsevier Science.)

standards were visualized with UV light. Detection of known metabolites was performed by the removal of radioactive zones and scintillation counting (in this case TLC was used as a clean-up technique). Unknown metabolites were identified using FAB/MS and NMR. The result showed that the pathways of the transformation of metolachlor by Bluegill Sunfish are very similar to those observed in animals, soils, and plants. In a similar way, examinations of bioaccumulation of pesticides may be performed.

TLC as a Clean-up Technique

An application of TLC as a clean-up technique is based on the separation of components of analysed mixture on TLC followed by layers introduction of analytes directly to the detector of other analytical instrument (on-line technique) or the removal of the analytes together with adsorbent and its elution with appropriate solvent (off-line technique). Identification of pesticides or their metabolites is performed

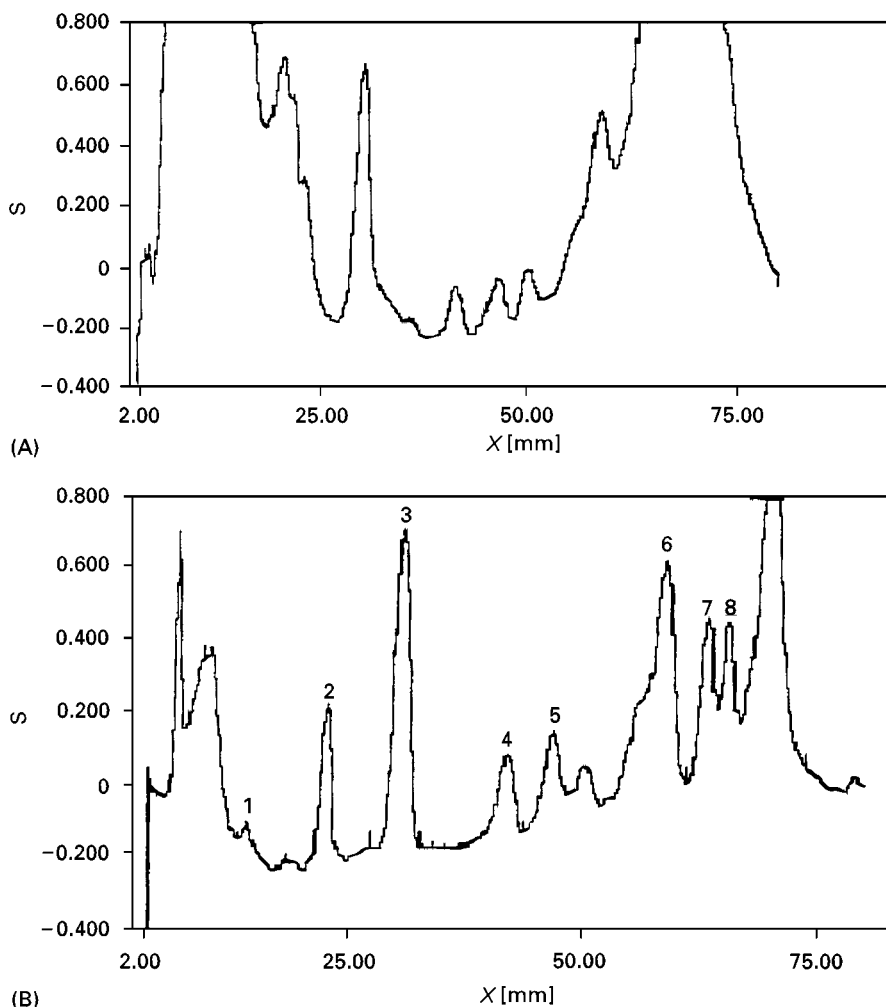


Figure 4 Chromatograms of soil sample: (A) before purification, (B) after purification by SPE. S = absorbance, x = distance of bands. Peaks: 1 = oxamyl, 2 = pirimicarb, 3 = carbaryl, 4 = phosalone, 5 = malathion, 6 = fenitrothion, 7 = tetradifon, 8 = metoxychlor. (Reproduced with permission from Bładek, 1996.)

Table 4 Parameters of quantification

Type of pesticide	λ_{max} (nm)	Calibration curves			Detection limits		
		$A = f(c)$	Correlation coefficient	Max. range of linearity (ng/band)	In band (ng)	RSD (%)	In soil ($\mu\text{g kg}^{-1}$)
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
Fenitroton	280	$A = 84782c + 1821$	0.9981	500	50	9.0	2.6

(Reproduced from Błażek, 1996 with permission from Elsevier Science.)

mainly by GC/MS, FTIR or NMR. The work in Bluegill Sunfish mentioned above is an example of such an application of TLC in pesticide analyses.

Conclusions

In the history of applications of TLC for pesticide analysis two periods (pre- and post- 1980s) may be distinguished. Within the first period, analyses were performed on home-made layers using equipment allowing for detection of substances at the μg level. During that period most chromatographic systems and methods of visualization of pesticides were developed. The second period is mainly connected with development of instrumental TLC which has enabled application of the technique for analysis of pesticides at the desired level, appropriate to the needs of food and environmental analysis.

It has not been possible to cover all applications of TLC in pesticide analysis extensively and therefore, only the demonstration of the method's abilities are presented here. TLC can be used for the clean-up of samples, for performing simple semi-quantitative screening analyses and, in the case of instrumental TLC, for the full quantitative analyses of pesticides.

Further Reading

Błażek J, Rostkowski A and Miszczak M (1996) Application of instrumental thin-layer chromatography and solid phase extraction to the analyses of pesticide residues in grossly contaminated samples of soil. *Journal of Chromatography A* 754: 273–278.

Butz S and Stan HJ (1995) Screening of 265 pesticides in drinking water by thin-layer chromatography with auto-

mated multiple development. *Analytical Chemistry* 67: 620–630.

Cruz SM, Scott MN and Merritt AK (1993) Metabolism of ^{14}C metolachlor in bluegill sunfish. *Journal of Agriculture and Food Chemistry* 41: 662.

Fodor-Csorba K (1996) Pesticides. *Handbook of Thin Layer Chromatography* (eds Sherma J and Fried B), pp. 753–817. New York: Marcel Dekker.

German standard methods for the examination of water waste and sludge. Berlin: German Standard Institute (Deutsches Institut Für Normung, DIN) DIN 38407, Part II.

Hurtubise RJ (1981) Pesticide analysis. In: *Solid Surface Luminescence Analysis* (ed.: Guilbault), pp. 151–175. New York: Marcel Dekker.

Mazurek M and Witkiewicz Z (1991) The analysis of organophosphorus warfare agents in the presence of pesticides by overpressured thin layer chromatography. *Journal of Planar Chromatography* 4: 379–384.

Morlock GE (1996) Analysis of pesticide residues in drinking water by planar chromatography. *Journal of Chromatography A* 754: 423–430.

Perišić-Janjić NU, Jevrić LR, Bončić-Carić GA, Jovanović BŽ and Ilić SR (1995) The chromatographic behaviour of some s-triazine derivatives on various supports. *Journal of Chromatography A* 703: 573–612.

Poole CF and Poole SK (1995) Multidimensionality in planar chromatography. *Journal of Chromatography A* 703: 573.

Sánchez-Camazano M, Sánchez-Martín MJ, Poveda E and Iglesias-Jiménez E (1996) Study of the effect of oxogenous organic matter on the mobility of pesticides in soil using soil thin-layer chromatography. *Journal of Chromatography A* 754: 279–284.

Sherma J (1997) Determination of pesticides by thin-layer chromatography. *Journal of Planar Chromatography* 10: 80–89.