Recent Advances in Thin-Layer Chromatography of Pesticides

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Advances in the applications of thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) for the separation, detection, and qualitative and quantitative determination of pesticides, other agrochemicals, and related compounds are reviewed for the period 1998–2000. Analyses are covered for a variety of samples, such as food, biological, and environmental, and for residues of pesticides of various types, including insecticides, herbicides, and fungicides, belonging to different chemical classes. References on formulation analysis, hydrophobicity studies, and the use of TLC and thin-layer radiochromatography (TLRC) for studies of pesticide metabolism, degradation, uptake, and related studies are also included.

The purpose of this paper is to selectively review the literature on thin-layer chromatography (TLC) analysis of pesticides, metabolites, breakdown products, and some related agrochemicals published and/or abstracted in the period from mid-1998 through mid-2000. It updates the review on the same topic published in this journal in 1999 (1).

TLC is widely used for the determination of residues of pesticides in a variety of samples, such as foods, drinking water, environmental matrices (soil, ground water, wastewater), biological samples, and commercial formulations. Analytical methods applied range from routine, fast qualitative or semiquantitative TLC screening of one or a few compounds with traditional sample preparation methods to sensitive quantitative multiresidue analyses performed by high-performance thin-layer chromatography (HPTLC) using modern sample preparation methods such as solid-phase extraction (SPE) combined with automated instrumental techniques for sample application, plate development, and densitometric scanning of zones. TLC and HPTLC complement the other primary methods used for pesticide determinations, i.e., gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography, spectrometry, immunoassay, biosensors, and capillary electrophoresis. High sample throughput, low operating costs, selective and sensitive detection and identification with a variety of color reagents and coupled spectrometric techniques, and the high resolution achieved on HPTLC plates, especially with instrumental development methods such as automated multiple development (AMD) or overpressured-layer chromatography (OPLC), are among the advantages of TLC for pesticide analysis. Thin-layer radiochromatography (TLRC) is used routinely for metabolism, breakdown, and other primarily nonanalytical studies of pesticides in plants, animals, and the environment. Roughly 30% of TLC papers each year are on the subject of food and environmental analysis, and pesticide determinations represent a significant part of these applications and will continue to be of high importance in the foreseeable future.

In most cases, generic (common) names of pesticides have been used, rather than trade names. Chemical names and formulae, trade names, properties, uses, and other information are contained in the Pesticide Dictionary of the Farm Chemicals Handbook (Meister Publishing Co., Willoughby, OH [1997]).

Materials and Techniques

Sample Preparation

The use of SPE in preference to conventional solvent extraction methods continued to increase in the review period. Advantages of SPE include faster analyses, lower cost, and less organic solvent usage. SPE was applied in the analyses of fruits and vegetables by HPTLC with AMD as described below. Ultrasonic extraction was reported for the recovery of pesticides from soil prior to TLC (2).

Thin-Layer Plates, Mobile Phase Selection, and Plate Development

Most pesticide determinations are performed on silica gel TLC and HPTLC plates, but other types of layers have been applied in some analyses, including C-18 and other nonpolar and polar bonded phases, plain and modified cellulose, polyamide, and ion exchangers. For example, some organochlorine (OC) pesticides were separated and identified on hydrated zirconium oxide layers with methyl acetate–formic acid (8 + 2), ethanol–hexane (1 + 1), and acetone–cyclohexane (6 + 4) mobile phases (4). LiChrospher Si 60 and LiChrospher RP-18 plates are relatively new products from Merck (EM Science, Gibbstown, NJ) that contain layers of spherical silica gel and C-18 bonded-silica gel, respectively, on glass or plastic backing (5). These thin layers of spherical sorbents provide rapid, high efficiency separations (Figure 1). Specific layers are stipulated in applications described in the sections below. Empirical and systematic optimization meth-
ods are applied for mobile phase selection and optimization of pesticide separations (6).

Pesticides in fruits and vegetables were determined by the official German Multimethod S19 using gradient elution–AMD and ultraviolet (UV) detection with the computer-controlled Camag AMD instrument (Wilmington, NC). Eluates of the mini silica gel column required SPE on an amino cartridge to remove matrix interferences prior to TLC. In some cases, detection limits were achieved that satisfied the German legal limits (7). AMD, which was described and a photograph of the apparatus shown in ref. 8, was the basis of the pesticide separation shown in Figure 1.

Detection and Identification of Zones

Crystal violet (methyl violet) was used as a selective chromogenic agent for visualizing OC insecticides in chromatograms of toxicological samples (viscera). Spectral studies of resulting complexes extracted from the layer with chloroform provided additional information for identifying the insecticides (9).

The insecticide and fumigant dichlorvos was selectively detected as a pink zone with a sensitivity of 3 µg/zone after silica gel TLC with hexane–acetone–methanol (16 + 6 + 1) by drying the layer at room temperature and spraying with 2% sodium hydroxide solution, then with 2% 2-thiobarbituric acid solution, and heating at 90°C for 10 min. Semiquantitative determination in minced visceral tissue was performed by visual comparison of sample zones against standard zones (10).

Organophosphorus (OP) pesticides were detected by a method in which the oxidation reaction of o-dianisidine with hydrogen peroxide is catalyzed by the pesticides on a silica gel layer surface. The detection limits for metaphos and fozolone were 0.6–0.7 µg, and the method was applied to the analysis of apples (11).

A sensitive and selective detection was described for 15 OP pesticides using petroleum ether–chloroform–ethyl acetate (65 + 30 + 5) and petroleum ether–tetrahydrofuran (THF; 9 + 1) mobile phases on silica gel R and petroleum ether–THF (19 + 1) on amino bonded silica gel R, and detection by spraying with 0.05% 9-methylacridine in ethanol followed by viewing under 366 nm UV light. The zones had different colors and minimum detection limits of 0.1–10 µg (12).

An enzymatic method was devised for low-level detection of phosphorus-containing pesticides and warfare agents (e.g., soman, sarin, tabun, diazinon, fenitrothion, phosalone) using 4-methylumbelliferone esters. Silica gel 60 plates were developed with diisopropyl ether–benzene–THF–hexane (10 + 7 + 5 + 11; warfare agents) or benzene–ethyl acetate (9 + 1; pesticides) and dried. A chromatographic paper sheet was impregnated with 0.3 mg/mL cholinesterase in potassium dihydrogen phosphate/disodium hydrogen phosphate pH 7.6 buffer (67mM; buffer A) and pressed against the TLC plate for 10 min at 0.35 MPa. A second paper sheet was impregnated with 5 mg/mL 7-acetyl-4-methylumbelliferone (I) in chloroform, the solvent was evaporated, and the paper impregnated with buffer A. This sheet was pressed for 60 min at 0.6 MPa against the first sheet that contained the zones in which cholinesterase activity was inhibited by the analytes. The OP compounds were visualized, with detection limits ranging from 0.01–1 ng, as black or grey zones of unhydrolyzed I against a fluorescent background of 4-methylumbelliferone resulting from enzymic hydrolysis of its ester (13).

A new spray reagent was found that detects synthetic pyrethroids containing a nitrile group with a sensitivity of...
0.5–1 μg but not OP, OC, or carbamate pesticides or pyrethroids without a nitrile group. λ-Cyhalothrin, deltamethrin, cyfluthrin, and fluvalinate upon alkaline hydrolysis yield a cyanohydrin derivative that degrades to give HCN and a corresponding aldehyde. The liberated HCN reduces 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium (INT) to formazin, a pink product in the presence of phenazonium methosulfate (PMS). The pink zones are stable for >24 h (14).

In situ, coupled TLC-surfaced enhanced Raman spectroscopy (SERS) was shown to be an effective method for determination of heterocyclic pesticides in organic materials (15). Three-orders of magnitude improved sensitivity (nanogram range) was obtained by use of an evaporated thin film of silver onto the layer rather than the usual application of silver colloids.

Cationic pesticides in the picogram range were analyzed by coupled TLC/matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) on silica gel, C-18, and cellulose 300 layers. The developed layer containing the separated zones is sprayed with an appropriate extraction solvent and sandwiched under pressure for 10–60 s against a MALDI cell to be transferred with the solvent to the matrix layer by the squeezing action, and the entire TLC plate/MALDI complex is analyzed by MS (16).

Quantitative Determination

The newest approach to quantitation of pesticides involves video densitometry rather than use of a traditional slit-scanning densitometer. Reversed-phase (RP) TLC with a methanol–water (8 + 2) mobile phase was used in conjunction with video densitometry for the determination of propanil, chlorpropham, atrazine, difluorobenzuron, tetramethrin, and α-cypermethrin. Quantitation was validated for linearity, precision, and detection limit, and the method was tested for determination of residues in spiked soil using ultrasonic extraction with various solvents (17). A comparative study was made of validation of TLC quantitation with a charge-coupled device (CCD) versus a slit-scanning densitometer for the analysis of the same pesticides on silica gel developed with methanol–water (4 + 1). It was found that slit scanning is more sensitive and precise, but that the RSD of 3.5–5.3% for video densitometry was acceptable. Linearity was very good and almost identical for the 2 methods. The advantages of video densitometry were speed of scanning (few seconds vs 20 min), excellent archiving facility, and that fact that image and chromatographic data can be stored together, edited, and used for many tasks (18).

A mixture of 10 pesticides (MCPA, atrazine, propanil, chlorpropam, ofurace, tridimefon, biteranol A, biteranol B, tetramethrin, and α-cypermethrin) was separated by 2-dimensional (2-D) TLC on a cyano-bonded HPTLC plate with methanol–water mobile phase (polar) in the first direction and hexane–diethyl ether (nonpolar) in the second. The use of 2 separation mechanisms improved resolution, and recording of chromatograms with a CCD camera and evaluation with Camag VideoScan software allowed quantitation. Video densitometry is advantageous for quantitating 2-D chromatograms because data collection is faster and reproducibility is not affected as greatly as with classical linear densitometry by greater diffusion of the double-developed zones and inability to chromatograph standards in both directions in parallel with the sample (standards can be run only in the second development; 19).

The herbicides atrazine, propazine, and simazine were used as models to study the use of 2 weighted regression functions for calibration in quantitative TLC. One method was based on relative error in y (YWLS) and the other on the basis of 1/x (XWLS) as the weighting factor for calculation of the calibration functions, and these were compared with conventional ordinary (OLS) and simple weighted least square method (WLS). Instead of the coefficient of correlation, 6 coefficients of quality were used to characterize the quality of the calibration functions. Silica gel developed with toluene–acetone (17 + 3) and reflectance densitometry at 225 nm were used (20).

Applications of TLC

OC and Pyrethroid Insecticides

Chlordimeform residues in honey were determined by TLC on silica gel with benzene–chloroform–acetone (5 + 5 + 1) mobile phase and detection by spraying with 5% N-(1-naphthyl)ethylenediamine dihydrochloride. The detection limit was 10 ng, and results agreed well with those obtained by an established GC method (21).

Cypermethrin, fenvalerate, fenpropathrin, cyhalothrin, flucythrinate, and allethrin residues were determined in forensic and other samples by TLC on silica gel G using hexane–benzene–acetone (8 + 2 + 1) mobile phase and 2-orthophosphoric–tannic acid reagent in acetone for chromogenic detection by spraying (22).

The TLC of numerous hydrolysis products of pyrethroids (e.g., 2-hydroxy- and 4-hydroxy-deltamethrin, chrysanthemic acid) was described on silica gel with the following mobile phases: hexane–ethyl acetate (1 + 1), ethyl acetate–petroleum ether (4 + 6 or 3 + 7), and ethyl acetate–petroleum ether–acetic acid (20 + 79.9 + 0.1 or 30 + 69 + 1). Detection was performed by viewing under UV light, exposure to iodine vapor, or spraying with 0.03% potassium permanganate in concentrated sulfuric acid or 1% silver nitrate solution (23).

OP Pesticides

The rapid separation of malathion, dimecron, chlorpyrifos, monocrotophos, dimethoate, and methyldemeton was performed on hydrated stannic oxide layers using various mobile phases. The Rf values were related to the polarity of the mobile phases and to interactions with the layer material (24).

Methods were described for the separation, detection, and quantitation of the thio (PS-containing) dialkyl phosphates DMTP, DMDTP, DETP, and DEDTP by TLC on C-18 bonded silica gel layers with methanol–0.5M NaCl (12 + 13) and THF–0.5M NaCl (2 + 3) mobile phases and detection with...
0.3% N,N,2,6-trichlorobenzoquinoneimine (TCQ) reagent as pink zones. Calibration curves were demonstrated for use in quantitation by visible reflectance scanning at 440 nm. The PO-containing compounds DMP and DEP were not detected and, therefore, did not interfere (25).

HPTLC on silica gel with hexane–acetone (4 + 1) and toluene and on C-18 bonded silica gel with methanol–water (7 + 3) with detection under 254 nm UV light, spraying with 2% 4-(4-nitrobenzyl)pyridine, and heating at 110°C for 5 min or with 5% PdCl₂ in 10% HCl was used for detection and qualitative identification of OP insecticides in human serum after acute poisoning (26).

Silica gel TLC with palladium chloride detection reagent was used together with GC/MS for qualitative analysis and GC with an electron capture detector for quantitative analysis in a chronological study of diazinon in putrefied viscera of rats. Interpretation of data was outlined regarding relative contents of organs (stomach, intestine) and the factors affecting persistence of diazinon in the viscera (27).

In a study of toxicological interactions of chlorpyrifos and methyl mercury in the amphipod Hyalella azteca, chemical–chemical interactions were evaluated by TLC analysis of a reaction mixture on a silica gel GF plate with hexane–acetone (7 + 3) mobile phase and zone detection with iodine vapor (28).

**Herbicides and Plant Growth Regulators**

TLC was used to study the synthesis of 24 new unsaturated chlorotriazine derivatives with herbicidal activity. Fourteen different mobile phases were used with silica gel 60F plates, visualization was under UV light, and quantitation by densitometry (29).

The separation of 19 photosensitive E-Z isomers of pyrazole, pyrimidine, and purine derivatives with potential cytokinin activity as plant growth regulators was studied on 7 different types of layers (adsorption, reversed phase, and impregnated) with a variety of aqueous and nonaqueous mobile phases. The best performance was observed with silica gel as stationary phase and hexane–ethyl acetate (1 + 9) mobile phase (30).

Bioactivity of herbicides and fungicides was assessed by use of TLC combined with enzyme-inhibition and antibiotic activity tests. The chromatography was performed according to German DIN 38407, part 11, which is a universal AMD method for the separation of pesticides with varying polarity. Identification at nanogram to picogram levels was obtained by multiwavelength in situ scanning at 7 UV wavelengths (31).

**Fungicides**

Analytical and preparative TLC on silica gel G layers were used to study the antagonism and structural identification of antifungal compounds from Chaetomium cochliodes against phytopathogenic fungi. The respective mobile phases were cyclohexane–ethyl acetate (1 + 1) and cyclohexane–methylene chloride–acetone (0.5 + 8 + 2). Identification of inhibitory compounds was performed by direct inhibition bioassay of antifungal activity on the analytical plates; a spore suspension of Botrytis cinerea in 1% potato dextrose agar was sprayed on the developed layer, and inhibition zones were observed after 4–5 days of incubation at 28°C in a humidified box (32).

Normal-phase HPTLC on silica gel and RP-TLC on rice starch and cellulose with detection under 254 nm UV light were studied for separation of some fungicidal 3-(2′-furroyl)-5-X-2,4-dioxotetrahydro-1,3-thiazole derivatives. The best system was found to be silica gel with hexane–ethyl acetate (4 + 1) mobile phase (33).

A new 2-D TLC method with direct bioautographic assay of separated zones using Colletotrichum fragariae as the indicator species was described for analysis of new natural products with activity against agriculturally important fungal pathogens. The mobile phases were methanol–dichloromethane (1 + 9) followed by ethyl acetate–hexanes (1 + 1; 34).

Residues of fungicidal ethylenebisdithiocarbamates and ethylthioxures in plants after oxidative inactivation with potassium permanganate were analyzed by TLC on silica gel using either chloroform–butanol–methanol–water (200 + 10 + 2 + 1) or chloroform–ethyl acetate–methanol (3 + 2 + 1) and detection under 254 nm UV light or spraying with 2% aqueous sodium nitroferricyanide reagent (35).

The relationship between the fungistatic activity of thiobenzanilides and their lipophilicity as determined by TLC was reported (36). Other studies involving TLC determination of lipophilicity are cited below.

**Multiclass and Miscellaneous Pesticide Determinations**

A quick and easy TLC method was reported for detection of pesticide residues in textiles for quality control application in textile mills. Sixteen pesticides of different classes were analyzed using a silver nitrate reagent for detection of halogenated compounds and enzymic tests for P- and S-based pesticides (37).

Quantitative determination of combinations of the pesticides atrazine, propanil, chlorpropham, difluubenzuron, α-cypermethrin, and tetramethrin from spiked soil was performed by ultrasonic extraction with acetone, RP-TLC with computer-assisted optimization of mobile phases, and slit-scanning densitometry. Recoveries ranged from 79–103% for the 6 pesticides (38).

Determination of some insect repellents in cosmetic products was achieved by silica gel HPTLC with benzene–diethyl ether–cyclohexane (5 + 3 + 2) mobile phase. N,N-diethyl-ethyl-m-toluamide (DEET) and dimethyl phthalate (DMP) were quantitated by densitometry at 230 nm (39).

A new method for analysis of liver and crop samples for the insecticide imidacloprid and its primary metabolite, 6-chloronicotinic acid, in suspected bird poisoning cases with a detection limit of 0.25–0.5 μg/g involved HPTLC on silica gel with chloroform–acetone–methanol (23 + 1 + 1; for imidacloprid) or acidified chloroform–acetone–methanol (13 + 1 + 10; for imidacloprid plus the metabolite) and densitometry at 275 nm (40).

The chemical reduction of zoalene to ANOT (3-amino-5-nitro-α-toluamide) and primary metabolites was studied by TLC for potential use in zoalene residue studies in
Silica gel plates were developed with chloroform–ethyl acetate–methanol (5 + 5 + 1). Detection was under 366 nm UV light, by exposure to nitrous acid vapors, and by spraying with 0.4% naphthylethenediamine dihydrochloride in methanol (Bratton-Marshall reagent; 41).

TLC is often used for the study of pesticide residual products and main active ingredients in commercial formulations. For example, the insecticides fenpropatrin and fluvinate were determined in their formulations by TLC on silica gel with hexane–acetone (9 + 1). Detection was by spraying with potassium permanganate–sulfuric acid and quantitation after scraping and elution from the layer by spectrophotometry (42).

Theoretical, Degradation, Metabolism, Uptake, Hydrophobicity, and Soil Mobility Studies

Rf values were chemometrically characterized for 30 pesticides on silica gel layers with mobile phases composed of heptane with a polar modifier (ethyl acetate, THF, or dioxane) and detection under 254 or 366 nm UV light or by exposure to iodine vapor. Plots of Rf against mobile phase composition were used for selection of suitable TLC systems for compound separations. Pesticide retention and resolution was described in terms of the selectivities of the TLC systems, and information was obtained that is useful for designing silica gel column SPE isolation and preconcentration procedures (43, 44).

A study was reported (45) on the relationship between the Rf values for a group of OP insecticides and a series of topological descriptors. By using multivariate regression, the corresponding connectivity functions were obtained and used to identify and predict different structural features that determine the Rf values of the pesticides.

A multilinear retention model for 10 carboxylic acid herbicides on a variety of stationary phases in 19 different mobile phases was studied. Relationships were proposed between compound structure and measured Rf values (46).

The TLC of radiolabeled pesticides and metabolites, which usually involves scraping and liquid scintillation counting or in situ measurement of radioactive zones by autoradiography or use of a radioscanner such as a linear analyzer or phosphor imager, is used extensively for studies such as pesticide metabolism in plants and animals, uptake of pesticides by plants from soil, and pesticide fate and degradation in the environment. The following are examples of such studies using TLRC and TLC of nonradioactive compounds. The metabolism of the postemergence herbicide 2H-1,4-benzoxazin-3(4H)-one (S-53482) in rats was studied by using multivariate regression, the corresponding connectivity functions were obtained and used to identify and predict different structural features that determine the Rf values of the pesticides. A study was reported (45) on the relationship between the Rf values for a group of OP insecticides and a series of topological descriptors. By using multivariate regression, the corresponding connectivity functions were obtained and used to identify and predict different structural features that determine the Rf values of the pesticides.

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Hydrophobicity (or lipophilicity) is the molecular parameter most frequently used in quantitative structure–activity relationship studies for determination of physicochemical parameters of solutes by TLC (53). The hydrophobicity and biological activity of some newly-synthesized 2-hydrazonehetiazolic derivatives (I; 54) and dihydroxythiobenzanilides (II; 55) with antifungal activity were evaluated by RP-TLC using C-8 and C-18 bonded silica gel layers with water–methanol and water–acetone mobile phases and detection under 254 nm UV light (for I) and densitometry at 325 nm (for II). RP-18W plates developed with water–acetone mobile phases were used to model the hydrophobicity of 18 antifungal dihydroxythiobenzanilides and make predictions on their biological activity (56). Structure–activity relationships for fungicidal substances were studied by RP-HPTLC on RP-18WF layers developed in a horizontal sandwich chamber with water containing 0.4–0.75% acetone and detection under 254 nm UV light; it was found that hydrophobic character and structure of substituents were important in determining the antimycotic activity of the examined compounds (57).

Charge transfer RP-TLC was used to study the strength of interaction of 18 commercial pesticides with a water-soluble β-cyclodextrin polymer (BCDP) and assess the relationship between the surface characteristics of pesticides and their complex-forming capacity. Formation of inclusion complexes with BCDP decreased pesticide lipophilicity, and significant quadratic relationships were found between the relative strength of interaction and polar surface area and polar surface energy of pesticides. The goal was to use the information about pesticide–BCDP complexing to develop new, more effective formulations with higher biological efficiencies and lower toxic side effects (58).

Soil TLC with water or water–methanol mobile phase allows observation and measurement of the mobility of isotope-labeled pesticides through soil microstructures. Eleven different sieved matrixes were studied for preparation of lay-
ers, including pure humine, pure clays, schists, and soils, and $R_f$ values were related to pesticide ionization and log P values. The rate of water movement (WR) changed widely from one matrix to another, and pesticide movement (M) in soil under the action of rain could be described by the equation $M = WR \times R_f$ (59). The mobility of the herbicides alachlor, metolachlor, simazine, and atrazine in soils was determined by TLC without the use of radio labeled compounds. After development with water, the soil layer was separated into various bands and the compounds were extracted with acetone–methanol (2 + 1) and analyzed by GC (60).

**Multidimensional Methods**

The advantages of coupling HPLC and TLC were reviewed and applications described for pesticide analysis in foods, wash additives in sewage plants, and pesticides in surface waters; the HPLC-TLC interface DuoChrom was also described (61).

**Earlier Reviews Containing Information on Pesticide TLC**

Two reviews of TLC methods for analysis of pesticide residues in environmental samples were published in 1998 (62, 63) covering the earlier literature. TLC in food and agricultural analysis was reviewed for the period 1995–mid-1999 (64).

**References**


