

THIN-LAYER CHROMATOGRAPHY OF PESTICIDES – A REVIEW OF APPLICATIONS FOR 2002–2004

J. Sherma

Department of Chemistry, Lafayette College, Easton, PA 18042-1782, USA

SUMMARY

Applications of thin-layer chromatography and high-performance thin-layer chromatography for the separation, detection, and qualitative and quantitative determination of pesticides, other agrochemicals, and related compounds are reviewed for the period mid-2000 to mid-2004. Analyses are covered for a variety of samples, such as food, crops, biological, environmental, pharmaceuticals, and formulations, and for residues of pesticides of various types, including insecticides, herbicides, and fungicides, belonging to different chemical classes. In addition to references on residue analysis, studies of pesticide–structure relationships, metabolism, degradation, adsorption, uptake, dissipation, mobility, and lipophilicity are covered, many of which make use of thin-layer radiochromatography.

INTRODUCTION

The purpose of this paper is to selectively review the literature on the thin-layer chromatography (TLC) and high-performance thin chromatography (HPTLC) of pesticides, metabolites, breakdown products, and some related agrochemicals published and/or abstracted in the period from mid-2002 through mid-2004. It updates the previous review on this topic [1] in a series dating back to 1982. Pesticide analysis remains one of the leading applications of TLC and HPTLC for qualitative and quantitative analysis of foods and crops [2]; environmental samples such as soil, drinking water [3], and sand [4]; forensic and medical samples [5]; biological samples; and commercial formulations. In addition, TLC is very widely used in a variety of pesticide studies, such as determination of quantitative structure–activity relations (QSAR) that describe how the molecular structure, in terms of descriptors (lipophilic, electronic, steric), affects the biological activity of a compound.

TLC and HPTLC complement gas chromatography (GC) and high-performance column liquid chromatography (HPLC) for pesticide separation, detection, identification, and quantification because of their following unique advantages over column chromatography: single use of the layer simplifies sample preparation procedures; simplicity of development by dipping the plate into a mobile phase in a chamber; high sample throughput with low operating cost because multiple samples can be run simultaneously with standards on a single plate using a very low volume of solvent; high resolution through multiple development or two-dimensional (2D) development on a plate with a single adsorbent or dual adsorbents; selective and sensitive postchromatographic detection and identification with a very wide variety of chromogenic, fluorogenic, and biological reagents and coupled spectrometric techniques; high resolution and accurate and precise quantification achieved on HPTLC plates, especially with automated sample application, development, and densitometric scanning methods; visual observation and direct recording of the entire chromatogram including all sample components, the origin, and the mobile phase front; and the ability to repeat detection and quantification steps under different conditions. Thin layer radiochromatography (TLRC) is used routinely for metabolism, degradation, and other studies of pesticides in plants, animals, and the environment, and these applications will be covered as well as studies of lipophilicity and pesticide migrations through soils.

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 yearly print or electronic editions of the Pesticide Dictionary of the Crop Protection Handbook (Meister Media Worldwide, Willoughby, OH USA; <http://www.meistermedia.com>).

MATERIALS AND TECHNIQUES

Sample Preparation

Although traditional sample preparation methods such as liquid-liquid and Soxhlet extraction together with large column chromatographic cleanup on adsorbents such as Florisil are still widely used prior to TLC analysis [6], modern extraction and cleanup methods are being used to an increasing degree. For example, pyrethrins and piperonyl butoxide in soil extracts were purified and concentrated using Isolute C₁₈ solid phase

extraction (SPE) cartridges eluted with methanol prior to reversed phase (RP)-TLC analysis [7].

Matrix solid phase dispersion (MSPD) was used in the determination of the organophosphorus (OP) pesticides diazinon and ethion in pistachio nuts. Ground nut samples were blended with C₁₈ adsorbent and packed into a column on top of lanthanum silicate ion exchanger as a co-column. After washing the column with hexane, the purified residues were extracted with dichloromethane–ethyl acetate (1 + 1) before TLC analysis [8].

Additional sample preparation methods are described in some of the applications cited in the sections below.

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

Most pesticide determinations are performed on silica gel TLC or HPTLC commercially precoated plates. The layers almost always contain a gypsum binder (designated as G layers) [2] or an organic binder. Silica gel H layers [9] are especially hard and rugged and permit the use of high concentrations of water in mobile phases without loss of adherence. Layers often contain a fluorescent indicator or phosphor to facilitate detection of compounds that absorb 254 nm UV light as dark zones on a fluorescent background. These are termed F layers or F₂₅₄ layers by the manufacturers. Cellulose, alumina [10], and kieselguhr layers are also used occasionally for pesticide TLC.

Chemically bonded silica gel phases are being used with increasing frequency for TLC analysis of pesticides, most notably cyano, amino, and diol normal phase (NP) layers and C₁₈ (octadecyl) and C₁₈W (water wettable octadecyl) reversed phase (RP) layers. A novel, non-chromatographic use of C₂ and C₁₈ bonded layer plates was as extractants for pesticides in a passive sampling environmental screening method: a 50 mm × 51 mm plate was placed between a plastic mount and a wire guard cage, the sampler was placed for a number of days in the water source (e.g., river or creek) to be sampled, the layer was removed, and the pesticides eluted for analysis by HPLC [11].

Impregnated layers are also sometimes applied for pesticide analysis, e.g., silica gel impregnated with mineral oil for the RP separation of pyrethrins and piperonyl butoxide [7].

The separation of a pesticide mixture containing metoxuron, deisopropylatrazine, cyanazin, and trifluralin was obtained in 260 s on an ultra-thin (10 μm) silica gel layer with a monolithic structure having a defined meso- and macropore structure. A volume of 20 nL of sample (0.1% so-

lution in acetonitrile) was applied with an ATS 4 sample applicator, the layer was developed over a distance of 2 cm with petroleum ether–acetone (7 + 3), and the chromatogram was evaluated with a diode array densitometer. These layers have advantages of short migration distances, short development times, low solvent consumption, and high sensitivity [12].

Lanthanum silicate or lanthanum tungstate ion exchange layers were used for the separation and detection of diazinon and ethion residues recovered from pistachio nuts by MSPD. Mobile phases were methanol–10% ammonia (95 + 5) and methanol–dichloromethane–10% ammonia (65 + 31 + 4), respectively, and visualization was obtained with palladium chloride reagent. Diazinon and ethion R_F values were 0.79 and 0.69, respectively, on lanthanum silicate and 0.98 and 0.90 on lanthanum tungstate [8].

Mobile phases for the NP TLC of pesticides are less polar than the layer and are composed of aqueous–organic solvent mixtures or fully organic mixtures, such as heptane plus a polar modifier [ethyl acetate, tetrahydrofuran (THF), dioxane, or diisopropyl ether] for silica gel. Acid or base may be added to the mobile phase in a small amount to reduce zone tailing [2]. Mobile phases for RP TLC are more polar than the layer, e.g., acetonitrile–water, methanol–water, or THF–water with C_{18} bonded silica gel. Mobile phases are chosen by trial and error guided by literature searches, or by use of by a systematic, computer-assisted optimization method such as window diagrams [13].

One-dimensional ascending, capillary flow development with the mobile phase in a covered glass chamber or tank having a relatively large volume is the technique most often used for pesticide TLC. Less often, a small-volume sandwich chamber has been used for ascending or horizontal development. Two-dimensional development involves the use of two mobile phases with complementary selectivity at right angles in order to better resolve a mixture zone, spotted in a corner of the plate, over the entire layer rather than in a single track as in one-dimensional development. For example, a mixture of urea herbicides and fungicides was separated by 2D TLC on chemically bonded cyanopropyl silica gel stationary phase using a nonaqueous mobile phase in the first direction and an aqueous reversed phase mobile phase in the second direction [14]. Figure 1 shows the separation obtained in a horizontal Teflon DS chamber as recorded by UV scanning at 254 nm.

A variation of 2D TLC is to use a plate with two different layers having diverse mechanisms and selectivity. Figure 2 shows the complete

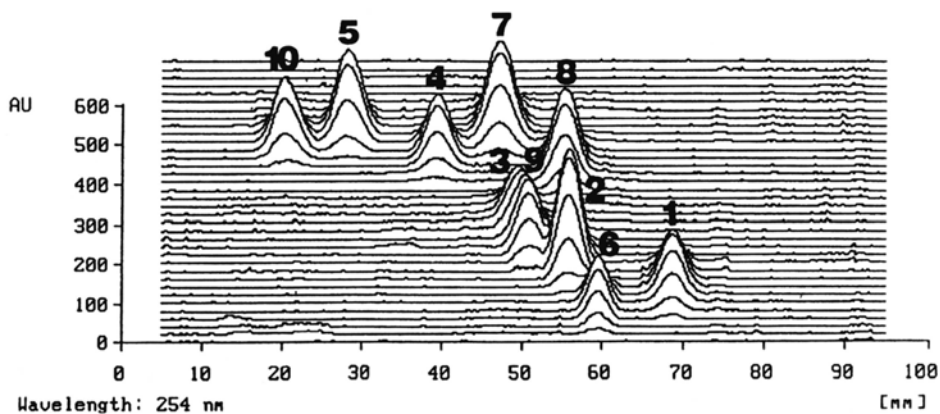


Fig. 1

Densitogram (tracks 1 to 36) showing the 2D separation of a ten-component pesticide mixture on a chemically bonded cyanopropyl layer after development with ethyl acetate-*n*-heptane (2 + 8) (NP) followed by dioxane-water (4 + 6) (RP). The pesticide peaks are: 1, fenuron; 2, monuron; 3, fluometuron; 4, buturon; 5, neburon; 6, benzthiazuron; 7, monolinuron; 8, methabenzthiazuron; 9, chlorotoluron; 10, penycycuron [14]

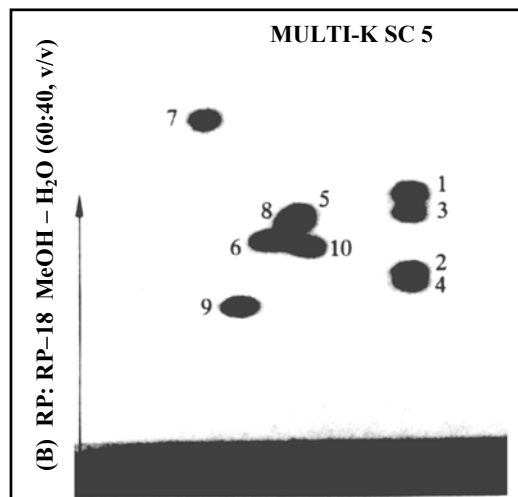


Fig. 2

Videoscan of the chromatogram showing the separation of a ten-component pesticide mixture by 2D development with THF-*n*-hexane (4 + 6) on silica gel (step A) and with methanol-water (6 + 4) on C₁₈ silica gel (B). The pesticide zones are: 1, monolinuron; 2, linuron; 3, metobromuron; 4, chlorbromuron; 5, chlortoluron; 6, diuron; 7, metoxuron; 8, isoproturon; 9, chloroxuron; 10, methabenzthiazuron [15]

separation of ten urea herbicides on a Multi-K SC5 plate having a 2 cm × 3 cm strip of K5 F plain silica gel (NP) adjacent to the main 20 cm × 17 cm C₁₈ F bonded silica gel layer (RP). The mobile phases given in the figure legend were chosen from correlation plots of R_F data obtained in RP and NP systems. Development was in a horizontal Teflon DS chamber, and zones were detected by viewing under 254 nm UV light [15].

Forced-flow overpressured-layer chromatography (OPLC; now termed optimum-performance laminar chromatography by some) under RP conditions was used to assess the hydrophobicity properties of a newly synthesized group of antimycotic compounds [16]. RP-8 F₂₅₄ HPTLC plates were spotted with 1 μL samples of the compounds (0.5 mg mL⁻¹ in methanol) using an AS30 applicator and developed over a distance of 7 cm in an automatic OPLC BS-50 chamber with mobile phases composed of water–methanol in the concentration range of 0.5–1.0 v/v methanol in 0.5 intervals. Spots were detected with a dual wavelength densitometer at 325 nm. Development in a horizontal sandwich chamber was carried out for comparison. The concentration range in which the correlation between log k (capacity factor) and methanol content is linear was established and used to determine the hydrophobicity parameters of log k_w (the retention factor of a solute with pure water as the mobile phase) by linear extrapolation. Also, the effect of substituents on retention constants was quantified by using the group contribution parameters (t_w). OPLC proved to be a rapid method for determination of physicochemical properties of a large number of organic compounds.

Specific layers, mobile phases, and development techniques are stipulated in applications described in other sections in this review.

Detection, Identification, and Documentation of Zones

Pesticide zones can be detected on layers in daylight as colored zones or under 366 nm UV light as fluorescent zones in their natural state, or after postchromatographic derivatization with a chromogenic or fluorogenic reagent applied by spraying or dipping. In the case of chlorinated pesticides, silver nitrate detection reagent can be impregnated into a silica gel layer prior to development, followed by exposure to UV light for about 10 min to allow observation of dark zones on a light brown background; this method was used to detect the pyrethroid insecticide fenvalerate after development with hexane–acetone (3 + 1) during toxicity and residue studies with the freshwater fish *Channa punctatus* (Bloch) [17].

Many pesticides absorb shortwave UV light and can be detected on F layers as dark zones on a bright fluorescent background (green fluorescence with most commercial layers) by inspection in a viewing box under illumination from a 254 nm lamp; this detection method is based on quenching of the layer fluorescence by the pesticides.

Various biological detection procedures are also used to make pesticide bands visible on TLC plates. As an example, the detection of bactericide, fungicide, algicide, phytotoxic, and genotoxic bands was studied using bacteria (*Bacillus subtilis*, *Vibrio fischeri*, *Salmonella typhimutium*), fungi (*Aspergillus niger*), algae (*Pseudokirchneriella subcapitata*), and pollen (*Impatiens walleriana*) as test organisms [18].

In another study [19], zones of leaf and stem extracts from *Cleome viscosa* L (Capparaceae) were tested for anti-bacterial activity by autobiography on silica gel 60 F₂₅₄ aluminum-backed sheets by spraying separately with *Bacillus subtilis* and *Pseudomonas fluorescens* spore suspensions in nutrient broth and then incubated in the dark for 24 h at 37°C; compounds having inhibitory action on the growth of the bacteria appeared as white zones against a purple–pink background after spraying with iodotetrazolium salt solution. Anti-fungal activity was measured by spraying a silica gel layer with a spore suspension of the plant pathogenic fungus *Cladosporium cucumerinum* in nutrient medium and then incubating for 72 h; fungicides appeared as white zones on a dark grey background. Fungicides were also detected postchromatographically by bioassay with *Penicillium* and insecticides with *Cholinesterase* [20].

Pesticide identification is initially based on comparing R_F values between sample and standard zones and colors with selective detection reagents. Confirmation of identity is carried out by spectrometric analysis [e.g. UV–visible, fluorescence, Fourier transform infrared, and mass spectrometry (MS)] in situ or after recovery of localized zones from the plate by scraping and elution. For example, off-line TLC/electron impact ionization (100 eV) MS was used to study the determination of 151 pesticides of many different classes in biological samples of importance in toxicology and forensic medicine [21]. Samples were prepared by liquid–liquid extraction or SPE, and extracts were separated on silica gel PF₂₄₅ plates with the following mobile phases: methanol–25% aqueous ammonia (100 + 1.5), cyclohexane–toluene–diethylamine (75 + 15 + 10), chloroform–methanol (9 + 1), *n*-hexane–acetone (8 + 2), toluene–acetone (95 + 5), and chloroform–acetone (1 + 1). The reagents used for color detection were Dragendorff, Ludy Tenger, potassium iodoplatinate, palladium chloride, ferric chloride–

sulfuric acid, and mercury nitrate–mercury sulfate. EI mass spectra were obtained by an MAT212 spectrometer equipped with a spectroscopy system SS 300; compounds were transferred from the layer into quartz crucibles and then evaporated into the direct inlet system of the instrument by controlled heating at 200°C. The eight-peak mass spectra and R_F values in the six mobile phases are tabulated for the pesticides.

Documentation and evaluation of results is becoming increasingly important in all analytical methods, including TLC. TLC documentation is most often achieved today by use of an instrument containing a visible and UV lighting module plus a video camera or a digital camera, or with a flatbed office computer scanner. An integrated software concept was described for qualitative and quantitative HPTLC that allows evaluation, electronic documentation of all instrumental parameters, and forgery-proof picture documentation in visible and long- and shortwave UV ranges of chromatograms with zones that are naturally detectable or require post-chromatographic derivatization [22].

Additional specific detection and identification methods are stated in applications described in earlier sections and below.

Quantitative Determination

Quantitative TLC of pesticides is usually performed by measuring the visible absorption, UV absorption, or fluorescence of standard and sample analyte zones in situ on a high-performance layer using a slit-scanning densitometer in the reflection mode. As an example, an HPTLC–densitometry method was developed for the quality control and stability analysis of commercial emulsifiable concentrate (EC) formulations of the synthetic pyrethroids cypermethrin, α -cypermethrin, and λ -cyhalothrin. Reference standards and the EC formulation, applied with a Linomat IV band applicator, were chromatographed on an aluminum backed silica gel 60 F₂₅₄ layer in a vapor-presaturated twin-trough chamber with hexane–toluene (1 + 1) mobile phase. Quantification was carried out by single wavelength reflectance scanning at 220 nm using a TLC Scanner II (Fig. 3). Calibration plots were linear in the range 8–24 μ g, and the linearity correlation coefficients ranged between 0.97 and 0.99. Recoveries from laboratory-prepared test samples of the EC formulations were in the range 95–99% [23]. Similar methods were described for analysis of formulations of the pyrethroids fenvalerate and deltamethrin [24]. Calibration plots for these pesticides were linear in the range 3–23 μ g, and recoveries from laboratory prepared EC formulations were 96–100%. In both cases, HPTLC results

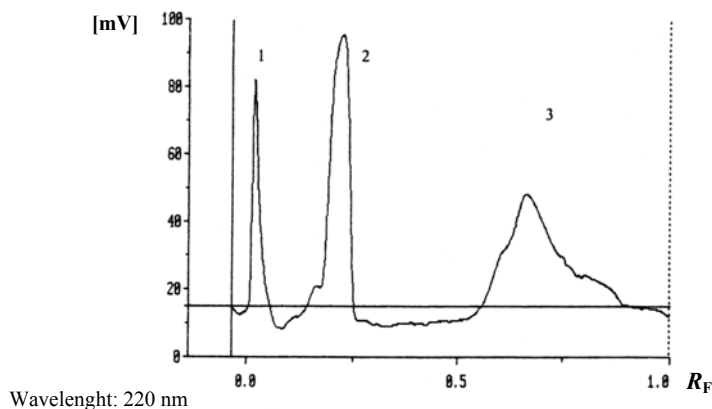


Fig. 3

Densitogram obtained from α -cypermethrin formulation. Peaks: 1, application position; 2, α -cypermethrin (15 $\mu\text{g}/6$ mm band); 3, mobile phase front [23]

were comparable to those obtained using a more complex, slower, and more costly GC–flame ionization detection method.

A more recent approach is to perform imaging documentation [20] and quantification using a videodensitometer consisting of a video documentation system incorporating quantification software. As an example of quantification [13], atrazine, alachlor, and α -cypermethrin were determined in water samples using preconcentration on Empore 47 mm C₁₈ disks eluted with hexane followed by dichloromethane; TLC separation on RP-18 F_{254S} HPTLC plates developed with 2-propanol–water (6 + 4; R_F values 0.64, 0.51, and 0.28, respectively); and quantification by videodensitometry at 254 nm. Recoveries ranged from 94.7–104.2% for 250–1000 mL spiked water samples. Densitograms for a standard, sample, and blank are shown in Fig. 4.

Additional applications of quantitative TLC are given throughout this review.

Preparative-Layer Chromatography (PLC)

Classical preparative layer chromatography (PLC) allows the separation and isolation of compounds in amounts up to about 1 g by applying larger samples in long bands onto plates with increased layer thickness. Rotation planar chromatography (RPC) and forced flow development can provide higher resolution, shorter separation times, and on-line band detection. Zone detection in PLC is by fluorescence quenching on layers con-

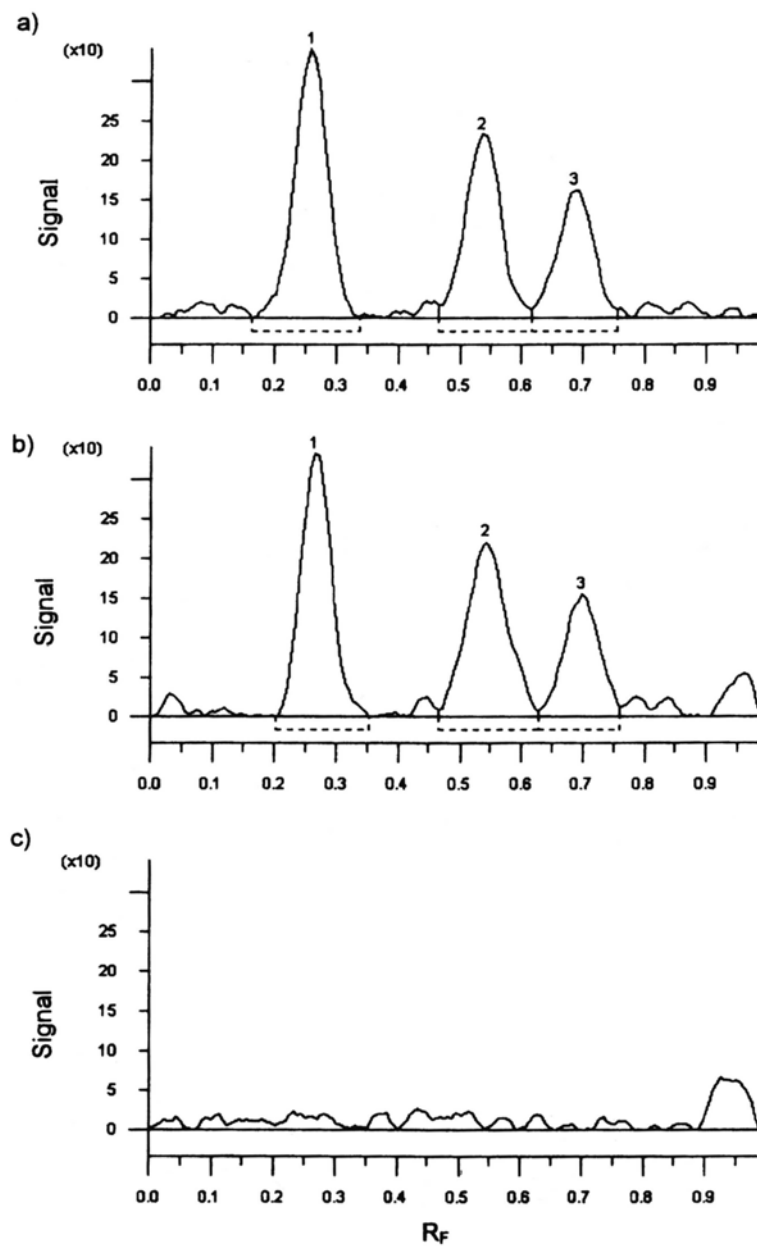


Fig. 4

Videoscans of chromatograms obtained from a standard pesticide mixture (a), from a pesticide mixture extracted from a spiked water sample (b), and from a blank extract (c). 1, α -cypermethrin; 2, alachlor; 3, atrazine [13]

taining a fluorescent indicator or use of physical, chemical, or biological detection techniques that are either reversible (e.g., iodine vapor) or carried out on the side edge of the plate to guide subsequent recovery by scraping and elution. As an example of biological detection, the silica gel PLC fractionation of a hexane extract obtained from the medicinal plant *Myroxylon balsamum* (red oil) in order to obtain larvicidal compounds was guided by use of a bioassay on third instar *Aedes aegypti* larvae, NPPN colony [25].

Relationships between R_F values and mobile phase composition were determined in NP systems of the type silica gel–nonpolar or weakly polar diluent (heptane, toluene, diisopropyl ether) + polar modifier (ethyl acetate, THF, dioxane, methyl ethyl ketone, 2-propanol) and used as a retention database for choosing optimum mobile phases for preliminary fractionation of a multicomponent pesticide mixture by zonal micropreparative TLC. The mixture was applied from the edge of the layer in the ‘frontal + elution’ mode, which increased the separation efficiency because of displacement effects, and the separated simpler fractions were applied to a silica gel plate and rechromatographed [26].

The multifunctional ExtraChrom instrument was used for the rotation planar extraction (RPE) of antimicrobial and scavenging components from oak (*Quercus robur*) bark [27]. Milled and sieved bark were extracted on the instrument using a six-step gradient ranging from ethyl acetate–hexane (3 + 1) to methanol–water (3 + 1) in the preparative separation. The planar column was filled with 40 g bark using a rotation speed of 1700 rpm. The volume of material to be extracted stays constant along the separation distance due to the special design of the chamber [28]. The bark was wetted with 135 mL extraction solvent at 1400 rpm and a solvent flow of 6 mL min⁻¹. The extracts were collected after a 60 min equilibrium time using a rotation speed of 1700 rpm and analyzed for bactericidal and fungicidal components by TLC on silica gel 60 plates developed with ethyl acetate–formic acid–water (85 + 10 + 15). Detection was by natural fluorescence at 366 nm and documentation of chromatograms by videodensitometry.

An approach for the choice of mobile phases for PLC is discussed in the section on ‘Chemometric Retention Studies’ below.

Thin-Layer Radiochromatography (TLRC)

Direct detection and quantification of radiolabeled compounds on thin layers using classical autoradiography, liquid scintillation counting (LSC) of scraped zones, or a linear analyzer or phosphor imaging plate

analyzer system for in situ measurement is a very important procedure for many types of pesticide studies, such as metabolism, adsorption–desorption, and mobility (soil-TLC) [29]. The following are examples of the use of TLRC, and additional applications are given in other sections below.

The fate of ^{14}C -diazinon in compost, compost-amended soil, and uptake by earthworms was determined by analysis of extracts using TLC and autoradiography with Kodak XRP-1 X-ray film. Spots visualized by autoradiography or by UV light were removed from the plates and quantitatively analyzed by LSC. Identification was carried out on silica gel 60 F₂₅₄ HPTLC plates, but quantification was performed on silica gel GF plates because larger samples could be separated on these thicker layers. The mobile phases used were chloroform–acetone (8 + 1) and ethyl acetate–benzene–chloroform–propanol (2 + 2 + 1 + 1); the respective R_F values of diazinon and its degradation and hydrolysis products pyrimidine thiol, diazoxon, and 2-isopropyl-4-methyl-6-hydroxypyrimidine were 0.88, 0.58, 0.38, and 0.08 in the first mobile phase and 0.86, 0.81, 0.65, and 0.25 in the second [30].

Samples obtained from crop or animal metabolism studies after treatment with radiolabeled pesticides (paddy water, grain, tomato, hen excreta, and goat urine extracts) were analyzed by 2D TLRC on silica gel 60F₂₅₄ layers in a saturated chamber. Radioactive zones on the plates were localized with a bioimaging analyzer by using typical counting times of ca. 16 h. Quantification of spots was performed by computer integration of the photostimulated luminescence values, with background correction done by subtraction of an integrated blank zone. The purpose of the study using unnamed, confidential pesticides was to compare TLRC with a new combined narrow-bore HPLC and microplate scintillation counting (TopCount) method, and the results showed that the performance of TLRC was essentially equivalent to the new method in terms of resolution and sensitivity [31].

The fate of seven currently used ^{14}C -labeled soybean and corn pesticides in two tropical soils of Brazil was studied in a laboratory incubation experiment. Soils were extracted by shaking with acetone–ethyl acetate–water (2 + 2 + 1) and extracts analyzed by silica gel TLC with mobile phases consisting of *n*-hexane–ethyl acetate (1 + 1) for alachlor, *n*-hexane–toluene (7 + 3) for chlorpyrifos, toluene–cyclohexane (7 + 3) for deltamethrin, *n*-hexane–acetone (9 + 1) for endosulfan and endosulfan-sulfate, acetonitrile–water (3 + 2) for monocrotophos, *n*-hexane–acetone (1 + 1) for simazine, and *n*-hexane–acetone (3 + 1) for triflurolin. The spatial distribution of ra-

radioactivity on the TLC plates was measured and integrated by electronic autoradiography with an Instant Imager Analyzer. Dissipation half-lives of pesticides ranged between 2 days (monocrotophos) and 90 days (endosulfan) [32].

The nonextractable residues of cyprodinil in heterotrophic cell suspension cultures of wheat were studied by application of (2-pyrimidyl-¹⁴C) or (2-pyrimidyl-¹³C)cyprodonil. After incubation for 12 days, 20% of the applied ¹⁴C was detected as nonextractable residues. The cell debris was treated with 0.1 M HCl (reflux), 1.0 M HCl (reflux), buffer, or 2 M NaOH (50°C); Bjoerkman lignin and acidolysis lignin fractions were also prepared from the debris. Radioactivity liberated and solubilized by these procedures was examined by TLC on silica gel SIL G-25 UV₂₅₄ plates using chloroform–methanol–formic acid–water (90 + 10 + 4 + 1) mobile phase. Separated ¹⁴C peaks were located and quantified with a Tracemaster 40 radiochromatogram scanner or BAS 1000 bio-imaging analyzer and BAS-MS imaging plates, and nonlabeled cyprodinil and metabolites were detected using 254 nm UV light. The results of TLC, as well as those obtained by HPLC and solid state and liquid ¹³C nuclear magnetic resonance (NMR) spectrometry, showed that cyprodinil and primary metabolites contributed to the fungicide's bound residues [33].

TLC as a Pilot Method for Column LC

Experimental data for 50 pesticides, presented as log *k* (HPLC) vs *R_M* (TLC) correlations, showed that RP-18W F₂₅₄ layers can be applied successfully as a pilot technique to choose mobile phases for C₁₈ HPLC. Pesticides were spotted as 0.5% solutions and the HPTLC plates developed for 9 cm in the horizontal mode in a Teflon DS chamber. Zones were detected under 254 nm UV light. Mobile phases used to determine correlation coefficients were acetonitrile–water (70 + 30 and 60 + 40), methanol–water (80 + 20 and 70 + 30), and THF–water (50 + 50 and 40 + 60) [34].

The transfer of TLC retention data to HPLC is also discussed in the section on 'Chemometric Retention Studies' below.

APPLICATIONS OF TLC

Insecticides

The herbal drug *Pyrethri flos* (from the flowers of *Pyrethrum cinerariaefolium*, Asteraceae, a Dalmatian plant) has insecticidal properties related to the esters of pyrethrins and cinerins that are its main chemical

constituents. Petroleum ether, chloroform, and ethanol extracts of the herbal drug were analyzed on silica gel 100 F₂₅₄ plates with *n*-heptane–acetone (4 + 1) mobile phase. Visualization of analytes was first done under 254 nm UV light and then by spraying with a solution of anisaldehyde in sulfuric acid. The herbal drug was also analyzed by the TAS technique for thermo-microseparation and application of volatile samples to the TLC plate [35].

A mixed microbial culture capable of degrading endosulfate, the toxic metabolite of the organochlorine insecticide, endosulfan. The products of degradation were characterized by TLC on neutral alumina F₂₅₄ plates using petroleum ether–acetone (85 + 15) or chloroform–ethyl acetate (3 + 1) as the mobile phase and detection by spraying with silver nitrate-saturated 5% aqueous methanol and exposure to UV light. Zones were scraped and eluted for further analysis by GC/MS [36].

OP Pesticides

Seven OP pesticides (listed in Fig. 5) were determined in water at 0.1 µg L⁻¹ by SPE enrichment on SBD-1 (RP-polymer phase) and C₁₈

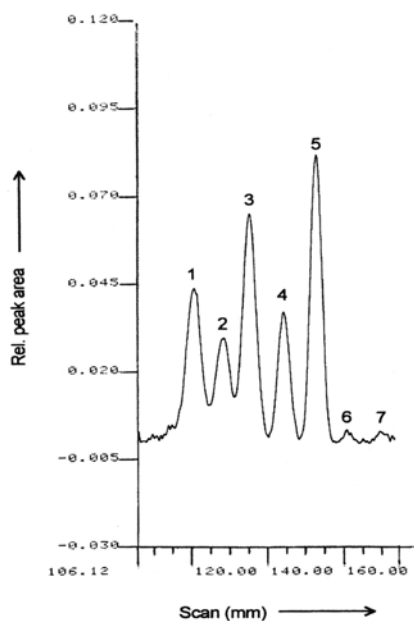


Fig. 5

Separation of seven OP pesticides on silica gel 60 F₂₅₄ with *n*-hexane–acetone (75 + 30) as mobile phase. 1, chlorpyrifos; 2, diazinon; 3, fenthion; 4, methidathion; 5, azinphos methyl; 6, omethoate; 7, methamidophos; each 2 µg spot⁻¹ [37]

cartridges followed by separation on silica gel 60 F₂₅₄ plates by development with *n*-hexane–acetone (75 + 30) for 10 cm in a saturated chamber [37]. Zones were quantified by scanning fluorescence quenched zones at 220 nm with a dual wavelength, flying-spot densitometer in the reflectance mode; a densitogram is shown in Fig. 5. Calibration plots were linear between 100 and 2000 ng for all pesticides, and correlation coefficients, *r*, were >0.999. Recovery rates were between 94 and 102% for both cartridge types except for chlorpyrifos on C₁₈; relative standard deviation (RSD) values were 0.7–4.9%. In addition, an enzymatic inhibition screening method was described using 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (maleimide-CPM) as fluorogenic reagent. It reacts with thiocholine released after hydrolysis of acetylcholine with acetylcholinesterase at pH 8 to form a strongly blue fluorescent background against which the OP pesticides can be detected as dark zones with a limit of detection (LOD) of 1–10 ng spot⁻¹ (Fig. 6).

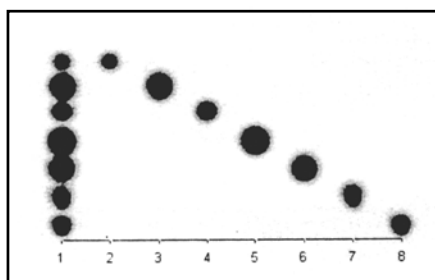


Fig. 6

Chromatogram obtained from the seven OP pesticides after spraying with acetyl cholinesterase, acetylthiocholine, and maleimide CPM. 1, mixture of seven pesticides; 2, chlorpyrifos; 3, diazinon; 4, fenthion; 5, methidathion; 6, azinphos methyl; 7, omethoate; 8, methamidophos [37]

A fluorescence polarization immunoassay based on a monoclonal antibody for the detection of parathion-methyl (PM) was described, and the influences of immunogen and tracer structures on the assay characteristics were investigated [38]. During method development, fluorescein-labeled PM derivatives (tracers) with different structures were synthesized and purified by TLC of 20–50 μ L of reaction mixture on silica gel 60 plates developed with chloroform and then, after drying, with chloroform–methanol (4 + 1). The major separated yellow bands were collected, eluted with 0.5 mL methanol, and stored at -20°C in the dark.

Herbicides

A new TLC method for determination of 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); and 2-naphthoxyacetic acid (NAA) was reported based on TLC separation and spectrometric quantification. The compounds were separated on silica gel G plates with the mobile phase dioxane–acetic acid (4 + 1; R_F values 0.63, 0.68, and 0.52, respectively) and detected as blue, blue, and green zones, respectively, by hydrolysis with aqueous NaOH followed by spraying with *p*-anisidine (0.2% in methanol), *N*-chlorosuccinimide (0.1% in water), and sodium nitroprusside– MnO_2 (10 mg mL⁻¹, 1 + 1). Zones of standards and samples were scraped from the plate, eluted with 2 M NaOH, and measured by visible-mode spectrometry for analysis of the pesticides in water, wheat, rice, blood, and urine samples [39].

A new method for analyzing drinking water for the phenylurea herbicides shown in Fig. 7 used TLC on diol-modified silica gel with water–

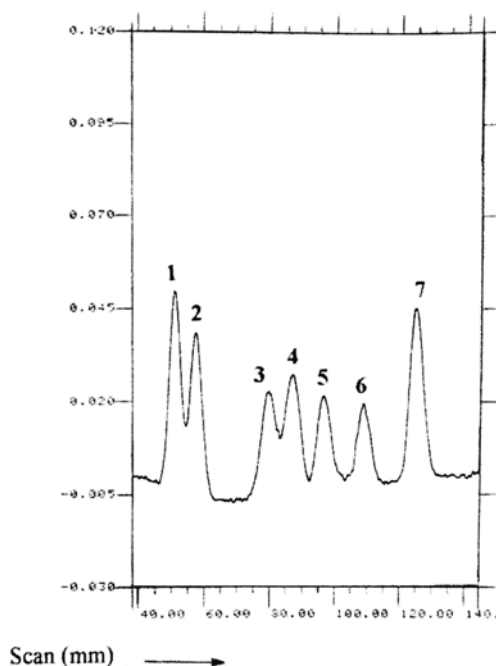


Fig. 7

Densitogram obtained from separation of seven phenylureas on silica gel 60 F₂₅₄ after 15-cm development with benzene–triethylamine–acetone (15 + 3 + 2). 1, linuron; 2, neburon; 3, isoproturon; 4, methabenzthiazuron; 5, chlortoluron; 6, fluometuron; 7, diuron [40]

acetone–methanol (6 + 1 + 3) mobile phase, on amino-modified silica gel with chloroform–toluene (4 + 1), and on silica gel with benzene–triethylamine–acetone (15 + 3 + 2). Detection was by fluorescence quenching at 254 nm, and quantification at ng levels by reflectance-mode dual-wavelength flying-spot densitometry at 245 and 265 nm. Calibration plots were linear between 50 and 2500 ng for all of the herbicides, with an *r*-value between 0.996 and 0.999. The herbicides were extracted from water by C₁₈ SPE, with recoveries of 87–102%. A rapid fluorodensitometric screening method, involving thermal hydrolysis and subsequent derivatization with fluorescamine, was also developed and improved the limit of detection twentyfive-fold [40].

The six triazine herbicides terbutryn, ametryn, atrazine, propazine, terbuthylazine, and simazine were determined in drinking water after C₁₈ SPE; separation on silica gel 60 F₂₅₄ (*n*-heptane–ethyl acetate (1 + 1) mobile phase), C₁₈ F₂₅₄ (acetonitrile–water, 7 + 1), or diol F₂₅₄ (acetone–water–tetrahydrofuran, 4 + 6 + 1) layers; and reflectance dual-wavelength densitometry at 222 nm. Recoveries between 88% and 99% were achieved, and the LOD was 100 ng L⁻¹. A fluorescence densitometry screening method was also described involving SPE recovery, derivatization with dansyl chloride, and scanning with an excitation wavelength of 280 nm; the linearity range was 20–1200 pg spot⁻¹ and detection limit 2 pg spot⁻¹ [41].

The sulfonylurea herbicides metasulfuron-methyl, chlorsulfuron, bensulfuron-methyl, tribenuron, and chlorimuron-methyl were separated on aluminum-backed silica gel F₂₅₄ sheets developed with chloroform–acetone–acetic acid (90 + 10 + 0.75) (mobile phase A) in a twin-trough chamber. The optimum humidity range was 18–42%, as determined using a Vario-TLC chamber and various water–sulfuric acid ratios for vapor phase conditioning. Quantification of bensulfuron-methyl added to tapwater at levels of 5–20 µg kg⁻¹ was carried out using extraction by C₁₈ SPE, separation on alumina with double development by mobile phase A and then toluene–ethyl acetate (1 + 1), and densitometric scanning at 201 nm. Detection limits ranged from 2–8 ng zone⁻¹ for the herbicides [42].

Fungicides

TLC–densitometry was successfully applied as a stability indicating method to separate and quantify clotrimazole (CZ), alone or in presence of byproducts, impurities, and/or its acid degradation products, in pharmaceutical formulations [43]. TLC was performed on silica gel 60 F₂₅₄ plates by development with a mobile phase consisting of chloroform–acetone–

25% ammonia (7 + 1 + 0.1). CZ (R_F 0.75) was well separated from its related substances and quantified by scanning at 260 nm with a dual wavelength flying spot densitometer. Validation studies with the standard addition technique gave a mean CZ recovery of $100.72 \pm 0.46\%$ for three preparations, LOD was $2.5 \mu\text{g zone}^{-1}$, and linear calibration range $5\text{--}25 \mu\text{g zone}^{-1}$.

An antifungal agent produced by *Streptomyces aureofaciens* was extracted from soil by *n*-butanol, separated by silica gel TLC with chloroform–methanol (24 + 1) mobile phase, and characterized by bioautography techniques and other tests. The collected data was suggestive of 20-macrolactone structure [44].

Benomyl, captan, furanocoumarins, and quinoline and quinolone alkaloid natural fungicides from *Ruta graveolans* L leaves were separated on silica gel with various proportions of hexane–ethyl acetate and methanol–ethyl acetate as mobile phases. Detection was by exposure to iodine vapor, under 254 and 366 nm UV light, and spraying with Dragendorff and anisaldehyde reagents. PLC was done on thicker silica gel layers, and bioautography by spraying with a spore suspension of *Colletotrichum* spp. and incubation in a moisture chamber for 3 days at 26°C [45].

TLC was used for isolation and purification of antifungal metabolites produced by *Bacillus cereus* [46]. Biologically active antibiotics were extracted with methanol from solid and liquid culture medium, and concentrated crude extracts were purified by C_{18} SPE. The X16_sI fraction eluted with 100% water was developed on silica gel 60 F₂₅₄ plates with butanol–water–pyridine–toluene (10 + 6 + 6 + 1) followed by spraying with ninhydrin detection reagent and heating. Detected bands were recovered from the layer by scraping and extraction and then bioassayed on seeded *Cladosporium* plates. It was shown that *B. cereus* X16 produces more than one antifungal antibiotic.

Multiclass and Miscellaneous Pesticide Determinations

A multiclass, multiresidue screening method was described for the determination of atrazine, carbaryl, carbofuran, chloroxuron, diuron, dimethoate, imazalil, oxamyl, and methamidophos in tomatoes. The samples were extracted with ethyl acetate, and the extracts were cleaned up by gel permeation chromatography with a semiautomatic KL-SX-3 instrument and additionally on a silica gel column, if necessary. Evaporated column extracts were applied to a silica gel F₂₅₄ layer that was developed with ethyl acetate or dichloromethane. Two reagents, *o*-toluidine plus potassium

iodide (o-TKI) and *p*-nitrobenzene diazonium tetrafluoroborate (NBDTFB), detected all of the pesticides as colored zones. The LOD values ranged from 12–125 ng for o-TKI and 60–70 ng for NBDTFB; on a concentration basis in spiked tomato samples, minimum concentration values were 1.1–32.3 ng μL^{-1} for o-TKI and 3.5–4.3 ng μL^{-1} for NBFB. Sizes of standard zones were measured with a ruler, and calibration plots of average diameter vs weight spotted were constructed for semiquantitative analysis [47].

Chemometric Retention Studies

Plots of R_F against modifier concentration (C_{mod}) were reported for 31 moderately polar pesticides in systems of the type silica gel 60 F₂₅₄–heptane + modifier (ethyl acetate, THF, dioxane). Pesticides were spotted as 0.5% solutions, mobile phase development was for a distance of 9 cm in a horizontal Teflon DS chamber, and zones were detected under 254 nm UV light. Typical plots obtained were in accordance with the Snyder–Soczewinski competitive adsorption model, with heptane–ethyl acetate and heptane–THF showing greater selectivity than heptane–dioxane. The relationships found constitute a retention database that can be used to choose a suitable, selective mobile phase for the analysis of a given set of pesticides; to plan 2D separations of complex pesticide mixtures in combination with videoscanning; to choose optimum conditions for preparative column separation into weakly polar, moderately polar, and polar fractions; and to plan preconcentration by SPE [48].

A later, similar study [49] was carried out with 20 additional pesticides using the same mobile phases and silica gel and other adsorbent layers. The greatest selectivity based on R_F –mobile phase composition plots was obtained by use of nonaqueous mobile phases on silica gel and methanol–water (6 + 4) on RP-18W F₂₅₄. The most selective NP and RP systems were chosen for the separation of nine pesticides by 2D TLC on a plate comprising adjacent layers of plain silica gel and C₁₈ bonded silica gel.

Metabolism, Degradation, Adsorption, Uptake, Dissipation, Interaction, Mobility, and Lipophilicity Studies

The metabolism of the cyano-oxime fungicide cymoxanil and analogs was studied on several strains of *Botrytis cinera* owing to their difference in sensitivity towards cymoxanil. It was found that HPLC was suitable for analysis of cymoxanil but not its ionizable metabolites, so an ion-pairing HPTLC method with C₁₈W F₂₅₄ plates was devised to monitor these metabolites in unextracted culture media for substrates that were

demonstrated to decompose most rapidly. Prior to chromatography, plates were impregnated by dipping into 50 or 70 mM methanolic TBAB (tetrabutylammonium bromide), initial zones were applied with an Automatic TLC Sampler, plates were developed with phosphate buffer (0.01 M, pH 6)–methanol mobile phase in a horizontal chamber, and quantification done by UV reflectance densitometry at 243 nm [50].

Photodegradation products of the herbicide EPTC (*S*-ethyl-*N,N*-dipropylthiocarbamate) and the safener dichlormid upon irradiation with 254 nm UV light in methanol and water solutions were identified by TLC, GC, and MS. For TLC, silica gel plates were developed with water–methanol (1 + 1 and 9 + 1), butanol–acetic acid–water (6 + 1 + 1), hexane–diethyl ether (7 + 3), and cyclohexane–acetone–acetonitrile (80 + 15 + 5), and zone detection was by viewing in daylight or under UV light. Localized zones were scraped off, eluted with methanol, and identified by MS. For detection of the sulfoxide and sulfone zones, plates were sprayed with a 0.5% solution of 2,6-dichloro-4-(chloroimino)-2,5-cyclohexadiene-1-one in acetic acid (Gibb's reagent) and heated for 10 min at 110°C [51].

In a study of the effects of moisture, temperature, and biological activity on the degradation of the herbicide isoxaflutole in soil, ethyl acetate extracts containing isoxaflutole, ¹⁴C-isoxaflutole, and diketonitrile-isoxaflutole hydrolysis product were separated on silica gel with ethyl acetate–methanol–acetic acid (92 + 5 + 3) [52].

Adsorption of pyrethrins and piperonyl butoxide to soil organic matter was studied using C₁₈ SPE and RP-TLC on silica gel F₂₅₄ layers impregnated with mineral oil; development with methanol–water (8 + 2) completely separated pyrethrins I and II and piperonyl butoxide, zones of which were detected under 254 nm UV light and by iodine vapor [7].

Uptake of ¹⁴C-DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl ethylene)] by marine clams was studied by TLC analysis of acetone Soxhlet extracts partitioned with hexane. Silica gel F plates were developed with hexane–acetone–methanol–diethylamine (100 + 1 + 1 + 1) to yield *R_F* values of 0.37, 0.53, and 0.66 for DDD, DDT, and DDE, respectively. The plates were then subject to phosphor imaging, DDE zones were scraped, and the radiation in the scrapings was measured in a liquid scintillation counter [53].

Dissipation of ¹⁴C-carbaryl and quinalphos in soil in a semi-arid Indian groundnut field was investigated under field use conditions [54]. Residues were recovered by methanol extraction and partitioning with dichloromethane followed by cleanup on Florisil or silica gel columns. TLC

for qualitative analysis of ^{14}C -carbaryl was done with silica gel Baker-Flex sheets or Linear K-TLC plates that were developed with chloroform–methanol (49 + 1); R_F values were ca. 0.56 for carbaryl and 0.30 for 1-naphthol degradation product. The visualized zones were extracted with methanol and analyzed by HPLC. One cm increments scraped-off from the developed layer were radioassayed by LSC.

The interaction of 18 diverse pesticides (three herbicides, 14 fungicides, and one plant growth regulator) with a water soluble β -cyclodextrin polymer (BCDP) was studied by RP-TLC. Pesticides dissolved in methanol were chromatographed on RP-18W F₂₅₄ layers in sandwich chambers with mobile phases consisting of methanol–water mixtures with methanol concentration varying from 30–60% in 5% steps; pesticide zones were detected by their UV absorbance or by iodine vapors. Principle components analysis (PCA) was employed to elucidate the relationship between the relative strength of interaction and calculated surface parameters of the guest molecules. Except for one compound (penconazole), the lipophilicity of the pesticides decreased in the presence of BCDP. PCA indicated that apolar surface parameters are highly positively correlated with strength of interaction, and the data showed that agrochemical characteristics (adsorption, leakage, decomposition, etc.) of complexed pesticides are different from uncomplexed ones, resulting in modified efficacy [55].

The mobilities of the acid herbicides MCPA [(4-chloro-2-methoxyphenoxy)acetic acid] and MCPP [4-chloro-2-methylphenoxy)propionic acid] in soils of northwest Croatia were studied by soil-TLC (STLC). Glass plates were coated with layers of 0.5 mm thickness composed of sand or kaolin plus microcrystalline cellulose and of two soils without cellulose addition. Mobile phases were water and 1% aqueous solutions of KCl, NaCl, Na₂SO₄, CaCl₂, and MgCl₂ for the sand and kaolin layers, and 1–5% solutions of KCl, (NH₄)₂HPO₄, and NH₄NO₃ and 5% solutions of two fertilizers (calcium ammonium nitrate and a multnutrient complex fertilizer) for the soil layers. Results showed that the mobility of MCPA and MCPP was influenced by the change in the concentration of soluble salts and the effect of the content of kaolin and sand in the soil thin layer. Mobility increased with application of fertilizers, especially on soil with low clay and low organic matter content [56].

RP-TLC was used to study the hydrophobic properties of 16 new herbicides [2-(chlorophenoxy)acyl derivatives and *N*-aryltrichloroacetamides]. Log k_w factors were determined by linear extrapolation and by a new method based on Ošćik's equation. RP-18 F₂₅₄ plates were developed with

aqueous Na_2HPO_4 –citric acid buffer (pH 5.3, 7.3, and 8.3)–methanol mixtures as mobile phases in saturated sandwich chambers. The herbicides were dissolved in ethylene chloride (0.3 mg mL^{-1}), and $1 \mu\text{L}$ samples of each were applied to plates by means of a microsyringe. A Reprostar 3 video camera and Videostore software were used for visualization and evaluation of chromatograms. It was concluded that retention factors in water determined by the new method can be used as descriptors of the hydrophobic properties of the herbicides and so were compared with calculated $\log P$ values (partition coefficients) in *n*-octanol–water systems [57].

The chromatographic behavior of four groups of *s*-triazine derivatives (14 compounds) was studied by TLC on silica gel 60 GF₂₅₄ plates impregnated with 2.5% paraffin oil without chamber saturation and in the dark. Zones were detected by fluorescence quenching at 254 nm. Mobile phases were water mixed with acetone, acetonitrile, or dioxane in different ratios. Retention constants, R_M° , were determined by extrapolation, and good correlation was obtained between R_M° and the slope, m , of equations expressing the dependence of retention on mobile phase composition. There was also satisfactory correlation between these retention constants and the $\log P$ of the investigated triazines. Better correlation was obtained between lipophilicity, C_0 , and $\log P$, indicating C_0 can be used as a measure of the lipophilicity of the compounds investigated [58].

Lipophilicity was also studied for eight pesticides (simazine, atrazine, propazine, prometryn, alachlor, sethoxydim, haloxyfop, trifluralin) by TLC and PCA in which classical R_M° values were compared with the factor scores based on retention data [59]. Samples ($3 \mu\text{L}$) in methanol (1 mg mL^{-1}) were applied to C_{18} bonded phase plates, which were developed with water containing 50–70% methanol in steps of 5%. Compound zones were detected under 254 nm UV light.

Pesticide-Related Studies

TLC has been used to study the effects of pesticides on the content of other compound types in certain samples. For example, alteration of the lipid metabolism in the plant pathogen *Phytophthora infestans* by a mandelamide pesticide [60,61] was determined by extraction of lipids from cultures exposed to the pesticides followed by TLC separation on silica gel 60G plates. Mobile phases for polar lipids were chloroform–methanol–acetic acid–water (170 + 30 + 20 + 7), or chloroform–methanol–ammonia (60 + 25 + 4). For neutral lipids, single development was carried out with petroleum ether (60–80°C)–diethyl ether–acetic acid (80 + 20 + 1), or doub-

le development with diethyl ether–toluene–ethanol–acetic acid (40 + 50 + 2 + 0.2) run three-quarters up the plate followed, after drying under nitrogen, by diethyl ether–hexane (6 + 94) to the top of the plate. Separated lipid bands were routinely detected by spraying the plates with 8-anilino-1-naphthalenesulfonic acid (ANSA) in anhydrous methanol (0.2%).

The insecticidal effect for an extract of *Burkholderia* (*Pseudomonas cepacia*) was evaluated in neonate larvae of *Spodoptera frugiperda* by bioassay. TLC of the extract using Dragendorff reagent showed the presence of alkaloids [62].

The mobility of amino acids was measured through soil amended with commonly used pesticides (dicofol, lindane, metasystox-R, thiram) using soil-TLC. Layers of natural soil or soil plus fertilizer containing 0–100 mg 100 g⁻¹ of each pesticide were spread on glass plates, aqueous amino acid solutions were applied, the layers were developed with distilled water, and zones detected with ninhydrin reagent. Mobility was found to increase or decrease uniquely for each amino acid–pesticide combination, as well as the presence or absence of fertilizer [63].

Earlier Publications Reviewing Information on Pesticide TLC

A handbook chapter described sample preparation, chromatographic systems, detection and identification of zones, quantitative determination and validation, and applications to the TLC of all pesticide classes [64]. A book chapter on environmental analysis covered the TLC of pesticides and other pollutants such as polycyclic aromatic hydrocarbons (PAHs), metals, and explosives [65].

An updated article in the second edition of the Encyclopedia of Chromatography [66] covered the materials and techniques of pesticide analysis by TLC and provided examples of applications. Reviews on the chromatographic analysis of environmental samples for chlorophenols, which are used mostly as insecticides, fungicides, herbicides, bactericides, anti-septics, disinfectants, and wood preservatives [67], and on methods of analysis of biological samples to detect poisoning by the phosphorus-containing amino acid herbicide glufosinate [68] included references on TLC.

REFERENCES

- [1] J. Sherma, *J. AOAC Int.*, **86**, 602 (2003)
- [2] S. Jayaraman, M. Naika, and H. Das, *J. Food Sci. Technol.-Mysore*, **40**, 319 (2003)
- [3] M. Lekic, M. Mijanovic, and Z. Pujic, *Pharmacia*, **13**, 39 (2002)
- [4] K. Al-Mutlaq, A.I. Rushdi, and B.R. Simoneit, *Arab Gulf J. Sci. Res.*, **20**, 141 (2003)
- [5] H. Mori, *Yakugaku Zasshi*, **122**, 625 (2002)
- [6] B. Das, Y. Sharif, A. Khan, P. Das, and S.M. Shaheen, *Environ. Pollut.*, **120**, 255 (2002)
- [7] G.A. Antonious, G.A. Patel, J.C. Snyder, and M.S. Coyne, *J. Environ. Sci. Health B*, **39**, 19 (2004)
- [8] S.W. Husain, V. Kiarostami, M. Morrovati, and M.R. Tagebakhsh, *Acta Chromatogr.*, **13**, 208 (2003)
- [9] A. Liu and Z. Yu, *Huaxue Tongbao*, **66**, w056/1 (2003)
- [10] T.D. Sutherland, I. Horne, R.J. Russell, and J.G. Oakeshott, *Appl. Environ. Microbiol.*, **68**, 6237 (2002)
- [11] C.J. Leblanc, W.M. Stallard, P.G. Green, and E.D. Schroeder, *Environ. Sci. Toxicol.*, **37**, 3966 (2003)
- [12] H.E. Hauck and M. Schulz, *J. Chromatogr. Sci.*, **40**, 550 (2002)
- [13] S. Babic, D. Mutavdzic, and M. Kastelan-Macan, *J. Planar Chromatogr.*, **16**, 160 (2003)
- [14] T. Tuzimski and A. Bartosiewicz, *Chromatographia*, **58**, 781 (2003)
- [15] T. Tuzimski and E. Soczewiński, *J. Planar Chromatogr.*, **16**, 263 (2003)
- [16] J.K. Różyło, A. Żabińska, J. Matysiak, and A. Niewiadomy, *J. Chromatogr. Sci.*, **40**, 1 (2002)
- [17] K.S. Tilak, K. Veeraiah, and K.S. Vardhan, *Bull. Environ. Contam. Toxicol.*, **71**, 1207 (2003)
- [18] U. Baumann, C. Brunner, E. Pletscher, and N. Tobler, *Umweltwissenschaften und Schadstoff-Forschung*, **15**, 163 (2003)
- [19] L.A.D. Williams, E. Vasques, W. Reid, and R. Porter, *Naturwissenschaften*, **90**, 468 (2003)
- [20] C. Weins and P. Collet, *Lebensmittelchemie*, **56**, 128 (2002)
- [21] H. Brzezinka and N. Bertram, *J. Chromatogr. Sci.*, **40**, 609 (2002)
- [22] M. Werther, *GIT Labor-Fach.*, **47**, 32 (2003)
- [23] K.K. Sharma, *J. AOAC Int.*, **85**, 1420 (2002)
- [24] K.K. Sharma, *J. Planar Chromatogr.*, **15**, 67 (2002)
- [25] N.K. Simas, E. Lima, S. Conceicao, R. Kuster, A. Martins de Oliveira Filho, and C. Lage, *Quim. Nova*, **27**, 46 (2004)

- [26] T. Tuzimski and E. Soczewiński, *Chromatographia*, **59**, 121 (2004)
- [27] S. Andrensek, B. Simonovska, I. Vovk, P. Fyhrquist, H. Vuorela, and P. Vuorela, *Int. J. Food Microbiol.*, **92**, 181 (2004)
- [28] Sz. Nyiredy, *J. Planar Chromatogr.*, **14**, 393 (2001)
- [29] A. Satrallah, A. Dahchour, M. Badraoui, E. Essassi, and M.J. Sanchez-Martin, *J. Environ. Prot. Ecol.*, **3**, 390 (2002)
- [30] J.E. Leland, D.E. Mullins, and D.F. Berry, *J. Environ. Sci. Health B*, **38**, 697 (2003)
- [31] M. Kiffe, A. Jehle, and R. Ruembeli, *Anal. Chem.*, **75**, 723 (2003)
- [32] V. Laabs, W. Amelung, G. Fent, W. Zech, and R. Kubiak, *J. Agric. Food Chem.*, **50**, 4619 (2002)
- [33] M. Sapp, T. Ertunc, I. Bringmann, A. Schaeffer, and B. Schmidt, *Pestic. Manag. Sci.*, **60**, 65 (2003)
- [34] T. Tuzimski, *Chromatographia*, **56**, 379 (2002)
- [35] M. Lekic, E.E. Kovac, F. Korac, and F. Pajalic, *Pharmacia*, **13**, 24 (2002)
- [36] T.D. Sutherland, K.M. Weir, M.J. Lacey, I. Horne, R.J. Russell, and J.G. Oakeshott, *J. Appl. Microbiol.*, **92**, 541 (2002)
- [37] M. Hamada and R. Wintersteiger, *J. Planar Chromatogr.*, **16**, 4 (2003)
- [38] A.Y. Kolosova, J.-H. Park, S.A. Eremin, S.-J. Kang, and D.-H. Chung, *J. Agric. Food Chem.*, **51**, 1107 (2003)
- [39] A. Asthana, G. Sunita, and V.K. Gupta, *Chem. Environ. Res.*, **11**, 31 (2002)
- [40] M. Hamada and R. Wintersteiger, *J. Planar Chromatogr.*, **15**, 11 (2002)
- [41] M. Hamada and R. Wintersteiger, *J. Biochem. Biophys. Methods*, **53**, 229 (2002)
- [42] R. Zhang, Y. Yue, R. Hua, F. Tang, H. Cao, and S. Ge, *J. Planar Chromatogr.*, **16**, 127 (2003)
- [43] E.M. Abdel-Moety, F.I. Khattab, K.M. Kelani, and A.M. AbouAl-Alamein, *Il Farmaco*, **57**, 931 (2002)
- [44] H.M. Atta, A.T. Abul-Hamd, and M.E. Zain, *Afr. J. Mycol. Biotech.*, **11**, 51 (2003)
- [45] A. Oliva, K.M. Meepagala, D.E. Wedge, D. Harris, A.L. Hale, G. Aliotta, and S.O. Duke, *J. Agric. Food Chem.*, **51**, 890 (2003)
- [46] N. Sadfi, M. Cherif, M.R. Hajlaoui, A. Boudabbous, and R. Belanger, *Ann. Microbiol.*, **52**, 323 (2002)
- [47] S.L. Moraes, M.O.O. Rezende, L.E. Nakagawa, and L.C. Luchini, *J. Environ. Sci. Health B*, **38**, 605 (2003)

- [48] T. Tuzimski, *J. Planar Chromatogr.*, **15**, 124 (2002)
- [49] T. Tuzimski and S. Soczewiński, *J. Planar Chromatogr.*, **15**, 164 (2002)
- [50] F. Tellier, R. Fritz, P. Leroux, A. Carlin-Sinclair, and J.-C. Cherton, *J. Chromatogr. B*, **769**, 35 (2002)
- [51] A.W. Abu-Qare and H.J. Duncan, *Chemosphere*, **46**, 1183 (2002)
- [52] S. Taylor-Lovell, G.K. Sims, and L.M. Wax, *J. Agric. Food Chem.*, **50**, 5626 (2002)
- [53] S.T. Mehetre, S.P. Kale, P.D. Sherkhane, and N.B.K. Murthy, *J. Nuclear Agric. Biol.*, **31**, 105 (2002)
- [54] P. Menon and M. Gopal, *Chemosphere*, **53**, 1023 (2003)
- [55] T. Cserhati, E. Forgacs, Y. Daewish, G. Oros, and Z. Illes, *J. Incl. Phenom. Macrocycl. Chem.*, **42**, 235 (2002)
- [56] A.J.M. Horvat, M. Kastelan-Macan, M. Petrovic, and Z. Barbaric, *J. Environ. Sci. Health B*, **38**, 305 (2003)
- [57] M. Janicka, B. Ościk-Mendyk, and B. Tarasiuk, *J. Planar Chromatogr.*, **17**, 186 (2004)
- [58] N.U. Perisic-Janjic and B.Z. Jovanovic, *J. Planar Chromatogr.*, **16**, 71 (2003)
- [59] C. Sarbu, *Rev. Roum. Chim.*, **47**, 1113 (2002)
- [60] R.G. Griffiths, J. Dancer, E. O'Neill, and J.L. Harwood, *New Phytologist*, **158**, 337 (2003)
- [61] R.G. Griffiths, J. Dancer, E. O'Neill, and J.L. Harwood, *New Phytologist*, **158**, 345 (2003)
- [62] R. Figueroa Brito, J. Martinez Herrera, A.N. Hernandez Lauzardo, and M. Velazquez Del Valle, *Rev. Latinoam. Quim.*, **31**, 28 (2003)
- [63] V.S. Shrivastava and S.N. Vaishnav, *Pollut. Res.*, **22**, 427 (2003)
- [64] M. Kastelan-Macan and S. Babic, *Pesticides*. In: J. Sherma and B. Fried (eds) *Handbook of Thin Layer Chromatography*, 3rd edn, Marcel Dekker, New York, 2003, pp. 767–805
- [65] J. Sherma, *Thin Layer Chromatography in Environmental Analysis*. In: Sz. Nyiredy (ed.) *Planar Chromatography—A Retrospective View for the Third Millennium*, Springer, Budapest, Hungary, 2001, pp. 569–584
- [66] J. Sherma, *Pesticide Analysis by Thin Layer Chromatography*. In: J. Cazes (ed.) *Encyclopedia of Chromatography*, 2nd edn, Marcel Dekker, New York, 2005, pp. 1230-1238
- [67] I.-U. Haque, *Sci. Int. (Lahore)*, **14**, 227 (2002)
- [68] Y. Hori, *Jap. J. Forensic Toxicol.*, **21**, 1 (2003)

