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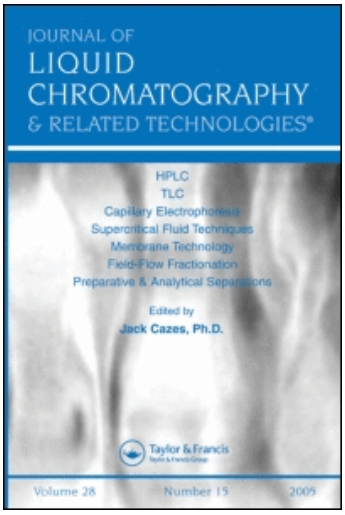


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# Thin Layer Chromatography of Pesticides-A Review of Recent Techniques and Applications

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# THIN LAYER CHROMATOGRAPHY OF PESTICIDES - A REVIEW OF RECENT TECHNIQUES AND APPLICATIONS

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## ABSTRACT

Recent advances in the techniques and applications of TLC for the separation, detection, and quantitative determination of pesticides are reviewed. Analyses of a variety of samples for residues of pesticides of various classes are considered.

## INTRODUCTION

Thin layer chromatography (TLC) is used in pesticide residue analysis for the separation and qualitative detection of residues in samples; for comparison with standards for confirmation of residues tentatively identified by gas chromatography; and for quantitative determination of residues, usually by densitometric scanning. Previous reviews (1-3) have covered the techniques, equipment, and applications of TLC for these purposes. This paper reports the advances and publications that have occurred in this field since the preparation of the last review (3).



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## MATERIALS AND TECHNIQUES

### Sample Preparation

More attention has been paid recently to the very important topic of sample preparation prior to chromatographic determination A book chapter (4) on sample preparation for quantitative TLC described techniques for direct sample application, application of sample solutions or extracts, cleanup of extracts by partition, cleanup by column chromatography, disposable cleanup systems, evaporation of solutions, and dissolving of evaporating residues. Many examples given were for pesticide analyses.

A recent paper (5) discussed solvent extraction and preconcentration, solvent concentration, solvent purity problems, and preconcentration with sorbent traps in relation to analyses of trace organic constituents in environmental samples. The critical problem of organic residue loss upon evaporation of solutions, especially to volumes below 1 ml, is emphasized and illustrated with data in this reference. A paper in a previous issue of this Journal (6) described cleanup of organophosphorus and carbamate pesticide extracts on a cellulose column and other applications of carbon columns and sweep co-distillation. Microcolumn cleanup, continuous flow cleanup, and macroporous silica gels have been used in TLC analyses of organophosphorus and carbamate pesticides (7).

It is important to use the best available solvents for extraction and cleanup procedures, as well as for the preparation of TLC mobile phases. Distilled-in-glass solvents have the highest purity available commercially, but even these solvents may contain unacceptably high impurity levels for trace analyses where high concentration factors are required. Initially pure solvents can become contaminated during storage or by migration of impurities from glue holding foil liners to the bottle caps. Impurities in the TLC mobile phases can cause altered $R_F$ values and diffuse zones.

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In addition to its use as a primary determinative method, TLC is widely applied as a cleanup procedure prior to GC or HPLC determination. For example, the acaracide benzomate was extracted from fruit with methanol, the extract was partitioned with methylene chloride, the extract was further purified by silica gel TLC, and the pesticide was determined by reversed phase column HPLC (8).

## Sample Application

Application of samples is normally made using Drummond Microcap micropipets, a Drummond Dialamatic microdispenser, or an automatic spotting apparatus. Special techniques are required for application of volumes below 1 µl for high performance (HP) TLC unless preadsorbent HP plates (Whatman) are used (9).

Touchstone and Levin (10) have verified that application of samples as thin horizontal streaks rather than round spots can lead to improved resolution in TLC. Preadsorbent layers facilitate sample application since thin streaks are automatically produced. In addition, relatively large volumes of dilute solutions can be rapidly applied, and all initial zones formed at the silica gel-preadsorbent interface are the same size regardless of the volume applied, thus allowing accurate and precise in situ quantitation. One or two predevelopments up to the interface with chloroform-methanol (1:1) may be necessary to form tight initial zones when impure samples are applied to the preadsorbent. Automatic sample applicators were found to be advantageous if more than 15 µl is to be applied to non-preadsorbent layers.

## Thin Layer Plates

Commercial, precoated, organic-bound, "hard" layers are recommended over laboratory-made silica gel G because of uniformity and stability. Silica gel is by far the most widely used sorbent for pesticide analyses, but an increasing trend to high performance silica gel and chemically bonded $C_{18}$ reversed phase layers (11) is evident. The Certificate of Analysis Program

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instituted by Whatman (12) is a notable advance that assures the quality of their commercial TLC plates and guarantees reproducibility and suitability for qualitative and quantitative TLC.

Silica gel, high performance silica gel, and reversed phase plates with preadsorbent spotting areas are highly recommended for pesticide analysis by TLC because of the ease in uniform spotting of variable volumes of samples and standards (10,13). The separation and quantitation of ioxynil, MCPA, and flurenol (flurecol) were studied on high performance silica gel 60 with and without a preadsorbent zone and on high performance cellulose (14). The mobile phase for silica gel was hexane-ethyl acetate-formic acid (40:60:0.5), and the migration distance was 5 cm. The resolution was superior, detection limits lower, and calibration curves steeper on the preadsorbent layer because of the more highly concentrated initial zones produced automatically at the interface of the layers. The mobile phase was butanol-water-25% $NH_3$ (80:20:1) for cellulose, on which sorbent the development time was much longer and resolution poorer but which gave much better sensitivity of detection (fluorescence quenching) and steeper calibration curves for reflectance scanning.

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## Mobile Phase Selection and Development

Mobile phases are generally composed of appropriate pure organic or mixed aqueous-organic solvent systems. Polarity (and selectivity) is varied by using different solvents or by altering the ratio of solvents in a mixture.

A very recent innovation in TLC is to prepare highly selective mobile phases using dilute aqueous solutions of micelles (e.g., sodium dodecylsulfate and hexadecyltrimethyl-ammonium bromide) and cyclodextrins (cetyltrimethylammonium bromide) (15). These totally aqueous mobile phases have been used successfully with polyamide and $C_{18}$ layers, but not with silica gel. Separations of chlorinated insecticides and PCBs have been demonstrated (16,17), and many more applications to pesticides are to be anticipated.

Development of the plate with the mobile phase is generally carried out in traditional rectangular glass chambers. The use of miniature tanks for HPTLC plates (which are usually 10 x 10 cm rather than the 20 x 20 cm TLC layers) is convenient but does not change results to any significant extent (18). Circular high performance chromatograms are developed with one of several commercial devices, most often the Camag U-chamber.

*Detection of Zones*

Pesticides are detected on thin layers by their natural color or fluorescence under ultraviolet (UV) light, by quenching of fluorescence on a phosphor-containing layer, or as colored or fluorescent zones after reaction with a chromogenic or fluorogenic reagent. It may be possible to apply reagents more uniformly by dipping rather than spraying for improved results by in situ quantitation. A gaseous electrical discharge technique has been devised for production of fluorescence in some organophosphate insecticide zones on TLC plates (19).

The carbamate and phenylurea pesticides carbaryl, propoxur, aldicarb, and diuron were studied on silica gel HPTLC plates prewashed in acetone. After predevelopment with chloroform, six detection reagents were evaluated. Enzyme inhibition, fluoborate reagent, silver nitrate reagent, and UV quenching were most reproducible, but the last was not sufficiently sensitive for residue analysis. The selectivity of fluoborate reagent was superior to silver nitrate, while the enzyme detection was most sensitive. Detection limits in general compared favorably to column HPLC (20).

Photosynthesis inhibiting herbicides such as triazines, phenylureas, phenylcarbamates, uracils, and acyl anilides were detected at levels of 100-500 pg/spot by spraying the plate with a mixture of spinach or bean leaf chloroplasts and the redox indicator 2,6-dichloroindophenol. In samples such as potatoes, carrots, and corn, detection limits with no extract cleanup were 1 ppb or less for linuron and 5-10 ppb for atrazine (21).

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The detectability of 188 pesticidal compounds using o-tolidine + KI, p-nitrobenzenediazonium fluoborate, bioassay with fungi and enzyme sources, $AgNO_3$ + UV radiation, and p-dimethylaminobenzaldehyde was studied. $R_F$ values in several mobile phases and minimum detectable amounts were listed (22).

## Identification of Zones

A confirmatory procedure on TLC plates for organophosphorus insecticides containing a nitro group has been described (23). Pesticides were spotted on a plate, the spots were covered with 2-3 drops of a derivatizing (reducing) reagent, and the plate was heated at 100°C for 10 minutes. After cooling, parent compound was separately spotted on the same plate. After development with hexane-acetone-ethanol (6:2:2) and drying, the chromatogram was sprayed with mercuric nitrate followed by diphenylcarbazone. Spots of the reduction product were eluted with ethanol, and the extract was subjected to a conventional test of diazotization for confirmation of an amine.

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## Quantitation by Densitometry

Pesticides have been quantitated by in situ measurement of color, fluorescence, or fluorescence quenching. Fluorescence is often most advantageous in terms of sensitivity and selectivity. Those pesticides that possess native fluorescence can be measured directly and immediately after separation. Many other nonfluorescent compounds can be rendered fluorescent by treatment with heat, acid, base, or inorganic salts; a derivative can be prepared in solution by fluorogenic labeling prior to TLC; or a reagent that becomes fluorescent on contact with the pesticide may be used after chromatographic development. All of these alternatives have been described and their applications reviewed (3,24).

Dansyl chloride (25) and fluorescamine (26) are examples of two reagents that have been widely applied for production of pes-

ticide fluorescence prior to or post-TLC. A large variety of chromogenic reagents have also been used for determinations of pesticides; in most cases, the reagents have been applied by spraying or dipping after mobile phase development and just prior to scanning (13).

## RECENT APPLICATIONS OF TLC

### Chlorinated Pesticides

Quantitative TLC has been used in forensic chemistry to estimate the distribution of OCl, OP, and carbamate pesticides in human cadavers following poisoning episodes (27,28). Laboratory-made silica gel G plates, chromogenic spray reagents, and transmission densitometry were used to analyze tissue extracts, which required little cleanup (partition, batch adsorbents) because of the relatively high residue levels present.

The separation of nine chlorinated insecticides was studied on chemically bonded $C_{18}$ reversed phase layers with seven mobile phases such as acetontrile-water (75:25). Detection was by spraying with o-tolidine reagent and exposure to UV light. The densitometric calibration curve for α-BHC was linear from 300-1100 ng, and 100 ppb of this pesticide was determined in natural water with an average recovery of 85.1% by direct spotting of hexane extract (29).

Five BHC isomers were separated on silica gel 60 HPTLC plates and also detected with o-tolidine. Reflectance scanning produced a straight calibration curve for δ-BHC from 50-500 ng, the slope of which was about twice as great as that obtained on a conventional TLC plate. The baseline noise was also less on the HP plate (14). Six BHC isomers have been separated on silica gel by means of cyclohexane-chloroform (80:20) and detected with 0.1% diphenyl solution in acetone (30).

MCPA (2-methyl-4-chlorophenoxyacetic acid), its soil metabolites (4-chloro-o-cresol and 3-methyl-5-chlorocatechol), and

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their pentafluorobenzyl derivatives were studied on silica gel layers with 19 mobile phases. The best separation of the individual compounds occurred with toluene-benzene-acetic acid (2:2:1), while chloroform-diethyl ether-toluene (1:1:1) was suitable for the group separation of pentafluorobenzyl derivatives (31).

MCPA residues were quantitated in apples by extraction with hexane-diethyl ether and reextraction with trichloromethane. TLC on silica gel was carried out with diethyl ether-methanol-water (94:4.5:1.5) as the mobile phase, and zones were scanned at 278 nm. The recovery rate was 103-114% for MCPA in the concentration range of 0.1-0.5 ppm (32).

Five different spray reagents were compared for detection of lindane (33); DDT and its metabolites were detected and identified in the presence of PCBs on alumina plates (34); spraying with $SnCl_2$ in HCl followed by 0.01% fuschin basic and heating at 110°C for 5-10 minutes was found to be a specific detection reagent for endrin (35); n-hexane-xylene-benzene-toluene-cyclohexane-methyl cyclohexane (1:1:1:1:1:1) was found to be a successful mobile phase for separation of chlorinated and organophosphate insecticides and pentachlorophenol on silica gel (36); adsorption and reversed phase TLC were used to determine residual amounts of chloroalkanecarboxylic and chlorophenoxyalkanecarboxylic acids in environmental samples (37); and pentachlorophenol was determined in water in the presence of OCl and OP pesticides (38).

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## Organophosphate (OP) Pesticides

The TLC separation of 13 OP pesticides with 17 mobile phases has been studied (39), and ITLC silicic acid impregnated glass fiber sheets have been used for OP pesticide separations (40). A popular technique for detection of OP and carbamate pesticides is by enzyme inhibition (41). Relative $R_F$ values and limits of detection have been reported for 65 pesticides with drosophila, rat-liver, and bee-head esterases and naphthyl acetate as substrate (42).

p-Phenyl organothiophosphate insecticides were differentiated from other OP insecticides by use of mercurous nitrate reagent.

The phenyl compounds gave black zones that turned to pink, while the other compounds gave only a black color (43). An improved ammonium molybdate method was described for detecting OP residues on TLC plates. After development, the plates were heated at 110°C for 2 hours. The resulting inorganic phosphate was reacted with ammonium molybdate to form phosphomolybdate, which was then reduced to a blue complex by ascorbic acid. Quantities of ca. 0.1-0.2 µg were detected (44).

Organophosphate and carbamate pesticide residues were determined in was by densitometry on Whatman preadsorbent plates (13). Total extracts and standards were applied to the channeled plates with a Drummond Dialamatic dispenser in volumes of 40-50 µl. Phorate sulfoxide and carbaryl were determined in fortified river water at 8 ppb levels without cleanup. Phorate oxygen analog and carbofuran were determined at the same level in tap water after cleanup of the extract on a macroporous silica gel column. The OP compounds were developed with hexane-acetone-chloroform (60:35:5) and detected as orange zones with $MgCl_2$-TCQ reagent. The carbamates were developed with cyclohexane-ethyl acetate (40:30); carbaryl was detected with p-nitrobenzenediazonium fluoborate reagent and carbofuran with KOH-TCQ, both as blue zones. The Kontes scanner was used in the single beam, transmission mode. Recoveries for fortified samples ranged from 85-95%.

Fluorescence densitometry has been applied for purposes other than direct analytical residue determinations. For example, coumaphos, coroxon, and chlorferone have been determined in water after extraction, concentration, spotting, development with carbon tetrachloride-methanol (100:7), and heating to produce fluorescence. The purpose of the study was to follow the degradation of coumaphos in water as a function of pH (45). In addition, TLC with densitometry was used in the evaluation of Amberlite XAD-4 resin for recovery of the insecticide fenitrothion from water (46,47).

Organophosphorus pesticides were extracted from paprika, spinach cream, and other pigment-rich foods with acetonitrile,

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followed by cleanup on an activated carbon-silica gel (1:3 w/w) column (acetonitrile elution) and TLC on silica gel using chloroform-ethanol (24:1) mobile phase. Spot visualization was carried out in UV light following bromination and treatment with an enzyme preparation (human plasma), or treatment with the chromogenic reagent 4-(4-nitrobenzyl)pyridine. The minimum detectable level ranged from 2 ppb for chlorfenvinphos to 1 ppm for dimethoate (48).

Cyanophos (Cyanox) insecticide was determined in plant samples by extraction with $CCl_4$ and silica gel TLC with hexane-acetone (2:1) mobile phase. Detection as a light brown spot was made with 500 ng sensitivity (49).

## Carbamate, Urea, and Related Pesticides

Asulam (Asulox) herbicide was determined in river water at 0.2 ppm by direct spotting of a concentrated ethyl acetate extract. Asulam in spinach at 0.2 ppm was extracted with acetone containing 0.1% acetic acid, concentrated, converted to the salt form by the addition of ammonia, and cleaned up by elution with 0.2% ammoniacal methanol from a two-layer alumina-Florisil column (24:10 w/w). The pH was adjusted to pH 2.5 before spotting. Chromatography was carried out on a 10 x 10 cm high performance preadsorbent-containing silica gel layer that was divided into 6 mm channels. Development with acetone-conc. $NH_3$ (95:5) gave an $R_F$ value of 0.40. The amino group of asulam was detected by spraying with 1% sodium nitrite in 1 N HCl, followed by N-(1-naphthyl)ethylenediamine dihydrochloride in 2 N HCl. Scanning of red zones with the transmission mode of the Kontes fiber optics densitometer gave a linear calibration curve for 10-90 ng amounts. Recoveries from spiked river water at 0.02 ppm averaged 83.9% and from spiked spinach at 0.2 ppm averaged 80.3% (50). Asulam residues in soil have been determined by solution colorimetry after extraction, TLC, scraping of zones, and elution (51).

Urea herbicides, e.g., linuron, diuron, metoxuron, and chlorbromuron, were catalytically hydrolyzed to the corresponding anilines on a silica gel plate making use of a reaction with

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acidic silanol groups. The anilines were then further reacted in situ with dansyl chloride, and the fluorescent derivatives separated on the same plate. The sensitivity and selectivity of the technique permitted analysis of urea herbicide residues in soil and water samples with good reproducibility and a minimum of sample cleanup (52). Urea and aniline herbicides have also been determined in plants by extraction, partition and column cleanup, and TLC with an azo reaction for detection (53).

A TLC method using the Hill-reaction inhibition detection technique was tested for determination of 11 urea and triazine herbicides (e.g., ametryne, atrazine, monolinuron, monuron) in concentrated water extracts. Four mobile phases were described for separation of all of the compounds by silica gel TLC, and visual comparison was used for quantitation after detection by the Hill-reaction inhibition technique. Recoveries of 95% or better were achieved for all of the compounds (54).

The herbicidal thiocarbamate EPTC (Eptam) and two of its metabolites were separated on silica gel using either cyclohexane-acetone-acetonitrile (16:3:1) or light petroleum ether-methyl ethyl ketone (9:1) as the mobile phase. The detection limits with 2,6-dibromo-*N*-chlorobenzoquinoneimine reagent were below 0.1 μg for all three compounds (55). EPTC and Sutan (butylate) were determined in vegetables by extraction with acetonitrile, cleanup on a silica gel column (benzene elution), and TLC on silica gel using hexane-ethyl acetate (9:1) mobile phase and the detection reagent above. The sensitivity was 50 ng/spot (56).

TLC methods for identification of propoxur, aldicarb, carbaryl, carbofuran, methomyl, mancozeb (Dithane M-45), thiram, ziram, and zineb from autopsy tissues have been described (57). Macerated tissue was heated with acetonitrile plus sodium sulfate, the mixture was filtered, and extraction was performed with chloroform. The concentrated extract was chromatographed on silica gel with benzene-methyl ethyl ketone (9:1), and carbamates were detected with an ethanolic solution of Fast Blue B and sodium

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hydroxide. Detection limits were ca. 0.5 μg, and recovery was 90-95%.

Aminocarb and its four major metabolites were separated by TLC on silica gel using hexane-acetone (1:1) and ethyl ether-hexane-ethanol (77:20:3). Spot visualization was achieved with ninhydrin or cholinesterase inhibition (58).

Phenylurea and N-phenylcarbamate residues (0.1 ppm) were determined in carrots, potatoes, and wine by sample extraction, spotting, hydrolysis on the origin with 7% methanolic KOH, development, and detection of the resulting anilines with an acetone fluorescamine solution. Extracts of some samples of carrots and potatoes required cleanup by solvent partitioning (59).

Kerb herbicide (pronamide or propyzamide) was extracted from water and plants with chloroform and from soil with acetone, purified on an alumina column eluted with HCl, and determined by TLC on silica gel with chloroform or benzene-ethanol (15:1) mobile phase. Recoveries ranged from 67-87%, and sensitivity of determination was 0.02-0.1 ppm (60).

Metolachlor (Dual) herbicide was determined in plants and soil by extraction with chloroform or benzene, evaporation of the solvent, dissolution of the dry residue in acetone, and silica gel TLC. Sensitivity was 2-3 μg, and recovery was 87-96% (61).

Fluometuron (Cotoran) was determined in air and water using $CCl_4$-diethyl ether (3:2) and methanol-toluene (1:4) as mobile phases. Detection with a sensitivity of 5 μg was made without hydrolysis and after hydrolysis to trifluoromethylaniline (62).

## Triazine Herbicides

Atrazine and simazine were extracted from natural water with chloroform, and the extracts were concentrated and spotted along with standards onto a silica gel G layer preimpregnated with silver nitrate. After development with chloroform-acetone (9:1), the plate was exposed to UV light to produce black spots that were scanned with the Kontes fiber optics densitometer. Alumina column

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cleanup of extracts was required for tap water samples. Recovery of samples fortified at 10 ppb was greater than 80%, and the CV was better than 7.5%. Calibration curves were linear from 0.1 to 1.2 µg per spot (63).

Residues of metribuzin (Sencor) were extracted from tomatoes with acetonitrile and water, the solution was filtered, the acetonitrile evaporated, and the solution refrigerated overnight. The solution was then filtered and extracted with chloroform. The extract was chromatographed on silica gel G plates with benzene-ethyl acetate-chloroform (2:1:1), and the plates were dried with cold air, exposed to chlorine gas, and sprayed with o-tolidine-KI solution to give blue spots of metribuzin. The limit of detection was 50-100 ng, and mean recovery was 75.5% for 150-600 ng of the herbicide (64).

The thin layer and paper chromatography of potential degradation products of s-triazine herbicides were studied (65).

## Miscellaneous Pesticides

The fungicide benomyl and its breakdown product MBC (carbendazim) were extracted from cucumber samples into ethyl acetate, then re-extracted with 0.1 M HCl. The acid layer was washed with benzene, and its pH was then adjusted to 6.5-7.0 with 6.5 M NaOH (converting benomyl into MBC). Total MBC was extracted into $CHCl_3$, the extract was evaporated to dryness, and a solution of the residue was analyzed by silica gel G TLC with ethyl acetate as the mobile phase. The fluorescent zone ($R_F$ ca. 0.35) containing MBC was scanned at 315 nm, with excitation at 285 nm. The sensitivity was 8 ppb of benomyl in a 100 g sample, and recovery of 0.2 ppm was 114% (66). Carbendazim was also detected using o-tolidine-KI reagent after silica gel TLC with chloroform-acetone (4:6) mobile phase. A linear relationship was found between square root of spot area and concentration (67).

Gibberellins $A_4A_7$ and 6-benzyladenine act as growth promoters for Delicious Apples. The gibberellins were extracted from apple

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pulp with pH 7 buffered aqueous acetone. After concentrating the extract to the aqueous phase, the solution was acidified, and $A_4A_7$ were extracted into ethyl acetate followed by back extraction into pH 7 aqueous buffer. The buffer was acidified and $A_4A_7$ reextracted into ethyl acetate. The dried residue was subjected to preparative TLC on a 10 x 20 cm silica gel G layer developed with $CHCl_3$-acetic acid (8:2). The $A_4A_7$ ($R_F$ ca. 0.7) was scraped and eluted with methanol. The solvent was evaporated and the residue taken up in 200 µl of methanol. Samples and standards were spotted from a 10 µl microsyringe on a silica gel G plate. The plate was equilibrated 16 hours in a chamber containing the lower, aqueous phase of benzene-acetic acid-water (8:3:5), and it was then developed in the upper benzene phase. The plate was then air dried, sprayed lightly with 95% $H_2SO_4$ in ethanol, and heated at 100°C for 10 minutes to develop fluorescence. $A_4$ gave a blue-purple spot at $R_F$ ca. 0.8 and $A_7$ a light yellow spot at $R_F$ ca. 0.7. The zones were scanned with a densitometer having 380 nm excitation and 460 nm emission filters. The method quantitated $A_4$ and $A_7$ at levels as low as 1 ppb (40 g samples) with 1-sigma recoveries of 50-76% and 67-93%, respectively. 6-Benzyladenine was determined by cleanup on a cation-exchange column, preparative TLC, derivatization with pentafluorobenzyl bromide, and electron capture gas chromatography (68). Gebberellins and their precursors have also been separated as their p-nitrobenzyl esters by argentation TLC (69).

A one-dimensional TLC method was used to determine 100 ng of the herbicide glyphosphate and 50 ng of its major metabolite aminomethylphosphonic acid in water samples. The mobile phases consisted of methanol-water containing sodium chloride, and ninhydrin was used for detection (70).

Residues of the pyrethroid decamethrin (Decis) were determined in soil, water, or plant samples by n-hexane extraction and TLC of the dried, concentrated extract on silica gel with chloroform-hexane (3:1) and acetone-hexane (2:5) solvent systems. Detection was by spraying with a saturated solution of diphenylamine in acetone or a 0.5% solution of ammoniacal silver nitrate, followed by

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UV irradiation. Sensitivity was 3-5 µg, and quantitation was made by comparison of samples with reference spots (71).

The fungicide ethirimol (Milgo) was determined by solvent extraction, cleanup, and silica gel TLC. Sensitivity was 1-2 µg, and recovery 85-95% (72).

The urea rodenticides Dirax and Vacor (pyriminil) were determined in biological samples by acetone extraction and TLC with methanol-chloroform (3:97) or acetone-chloroform (8:2), respectively. Grote reagent and p-dimethylaminobenzaldehyde were sprayed for zone detection; Vacor was reduced with $SnCl_2$ prior to visualization. The detection limit was 250 ng of pesticide (73).

Additional TLC determinations that have been reported include the herbicide lenacil in grain (5 µg sensitivity) (74); the herbicide ethofumesate (Nortron) in water, soil, and sugar beet (3-5 µg sensitivity) (75); the fungicide metalaxyl (Ridomil) in plants (76); the plant growth regulator chlormequat in cereals, straw, pears, grapes, raisins, and milk at 0.05 ppm (77) and in environmental samples (74); and OCl, OP, and carbamate insecticides in the gastric content and urine of humans by silica gel and reversed phase TLC (79).

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## RECENT BOOKS ON PESTICIDE TLC

Two books on pesticide analysis including chapters on TLC have been recently published. Chapter 1 of the book edited by Das (80) is titled "Thin Layer Chromatography" and was written by C.E. Mendoza. This chapter is not an up-to-date survey of the field (the latest reference is from 1976, most are from the 1960's and the early 1970's). Nothing is included on HPTLC or chemically bonded reversed phase layers, and almost nothing on precoated TLC plates or determinations by scanning densitometry. Chapter 10 of this same book is on confirmatory tests, written by J. F. Lawrence. Section III of this chapter is on TLC for confirmation of qualitative results obtained by gas chromatography or other analytical methods, and includes discussions of in situ spray reactions for various pesticide classes and derivatization reactions before

TLC. Again, virtually all references are from the 1960's and early 1970's. The chapter by MacNeil and Frei in the book edited by Moye (81) is a very good review of the quantitative in situ analysis of pesticides on thin layer chromatograms published through 1977. In situ analyses of colored, quenched, and fluorescent zones are covered, including discussions of the different procedures for producing fluorescence in various pesticides.

## CONCLUSION

The increased use of column HPLC for pesticide determinations has caused a reduced frequency of new reports on pesticide TLC. However, as reviewed above, significant new research is still being published, and TLC remains widely used for the analysis of a variety of samples for residues of pesticides of all types.

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