# THIN LAYER CHROMATOGRAPHY IDENTIFICATION OF PESTICIDES USING DIP-COATED PRESENSITIZED MICROSCOPE SLIDES

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(Received March 22nd, 1966)

## INTRODUCTION

Thin layer chromatography is widely used for qualitative identification of pesticide residues on agricultural products. KOVACS[4] describes a very sensitive method which will detect 5 ng of many pesticides. His procedure requires washing the adsorbent layer after the plates are coated, then spraying the plates with a chromogenic agent after development. DAMASKA[1] eliminated both of these manipulations by prewashing the adsorbent and incorporating the silver nitrate in the slurry before preparing the plates; however, the sensitivity of the plates was greatly diminished (50 ng). Many of the previous methods require relatively expensive and specialized equipment for the preparation of thin layer plates. The object of this paper is to describe a sensitive method for the qualitative identification of pesticides using inexpensive equipment commonly found in any analytical laboratory. The dipping technique has been described by PEIFER[8]; the use of microscope slides for thin layer plates was reported by STANLEY[9].

## EXPERIMENTAL

### *Reagents*

All reagents used were A.C.S. reagent grade unless otherwise specified. Camag D-5 aluminum oxide for thin-layer chromatography with $CaSO_4$ binder, without fluorescent indicator, was obtained from Arthur H. Thomas Co., Philadelphia, Pa.** The aluminium oxide was prewashed by shaking 30 g of adsorbent with 100 ml of 0.2 % $HNO_3$ in a 250 ml centrifuge bottle. The slurry was centrifuged at 1200 r.p.m. for 5 min, the supernatant liquid was decanted, and the residue was washed in a similar manner with three 100 ml portions of water followed by three 100 ml portions of 95% ethanol. The adsorbent may be stored with the final ethanol wash in the centrifuge bottle stoppered with an aluminum foil-covered rubber stopper.

A 1 % $AgNO_3$ solution was prepared by dissolving 1 g $AgNO_3$ in 1 ml of $H_2O$ and diluting to 100 ml with 95 % ethanol. This solution was stored in a stoppered brown glass bottle in the dark.

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** Manufactured by and available from Camag Co., Muttenz, Switzerland. Also distributed in the U.S.A. by Microchemical Specialties Co., 1825 Eastshore Highway, Berkeley 10, Calif.

The hydroquinone solution was prepared by dissolving 100 mg of hydroquinone (Fisher purified grade Cat. No. H-329) in 100 ml of 95 % ethanol and was stored in a stoppered brown glass bottle in the dark.

The fluorescent dye solution contained 100 mg of 2',7'-dichlorofluorescein in 100 ml of 70 % ethanol.

Two developing solvents were used, 2 % v/v acetone in *n*-heptane and *n*-heptane.

*Apparatus*

The exposure cabinet was constructed with four 15-W 18-in. GE germicidal U.V. lamps mounted in a metal case so that the chromatoslides rested 2 in. below the lamps. The chromatoslides were 3 × 1 in. microscope slides. The 1 $\mu$l spotting pipets were obtained from Kontes Glass Co., Vineland, N.J. The viewer was a Chromato-Vue, Model C-3F, obtained from Black Light Eastman Corp., Bayside, N.Y. and was equipped with both long- and short-wave U.V. lamps. The marking template for the slides consisted of two plastic rulers and one glass slide butt-plate glued to plate glass in the arrangement shown in Fig. 1.

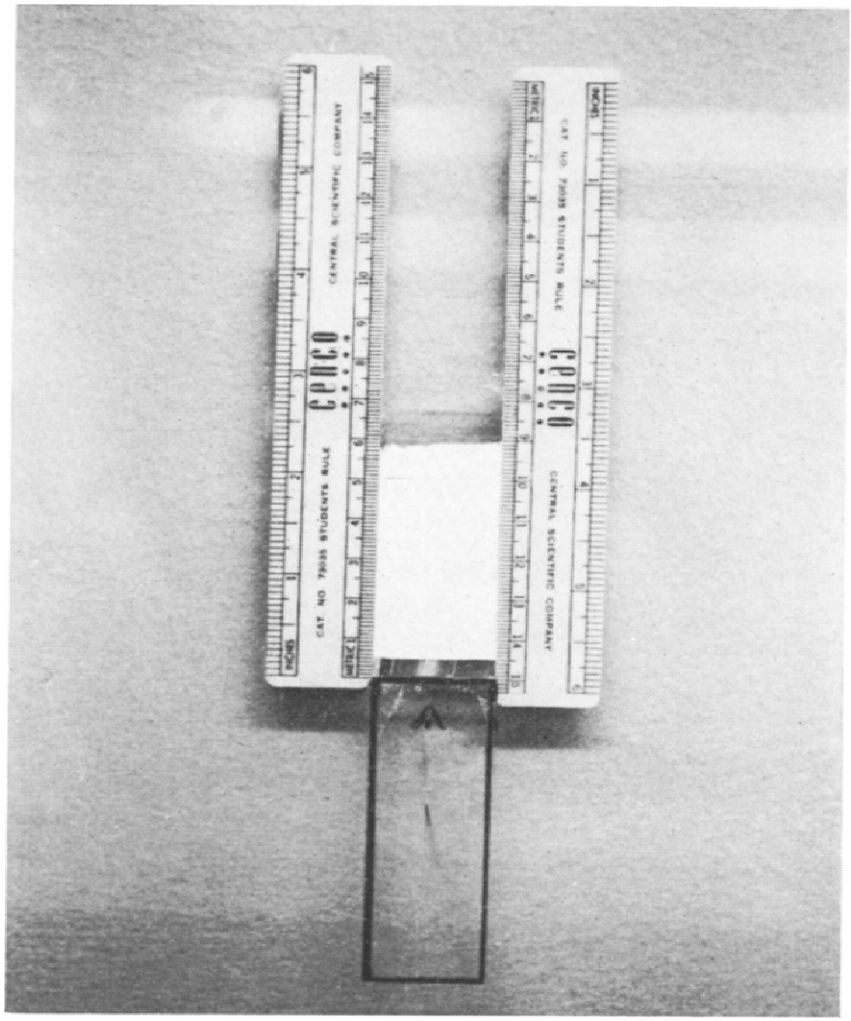

Fig. 1. Template for preparation of chromatoslides.

*Pesticide standards*

All standards were weighed on a Cahn microbalance. A stock solution was prepared by dissolving 5 mg of each standard in petroleum ether (b.p. range, 30–60°) and diluting to 100 ml. Further dilutions were made to prepare single and mixed standards containing 5 ng/$\mu$l in petroleum ether.

*Preparation of chromatoslides*

New slides were used directly from the box; however, slides that had been used were washed with detergent, rinsed with distilled water and ethanol, and dried in a metal microscope slide rack in a 100° oven. The slides were then inspected individually to insure cleanliness. All glassware was rinsed with distilled water and ethanol and allowed to drain dry.

The solution used in the preparation of the slurry was prepared by adding 6 ml of 1 % $AgNO_3$, 6 ml of 0.1 % dichlorofluorescein, and 50 ml of $CHCl_3$, in that order, to a 100 ml glass-stoppered graduate, diluting to about 95 ml with 95 % ethanol, and then adding 3 ml of 0.1 % hydroquinone. The solution was diluted to 100 ml with 95 % ethanol and mixed. This solution decomposes very rapidly and must be used immediately.

Of the above solution 85–90 ml was added to the centrifuge bottle containing 30 g of prewashed aluminum oxide (moist from final ethanol wash) and the mixture was shaken vigorously to form an homogeneous slurry. This slurry was immediately poured into a 100 ml tall-form beaker and the slides were dipped into the slurry to coat. Two slides were held together, dipped, and slid apart so that the adsorbent was applied to only one side. The slides were placed on glass plates and dried not less than 15 min in a 100° oven. The chromatoslides were stored in a desiccator cabinet by laying them on regular 8 in. × 8 in. thin-layer chromatographic plates. The slurry must also be used immediately after preparation or it will decompose. Approximately 175 slides can be prepared from one batch of slurry.

After drying, the chromatoslides were prepared for use by placing them in the template as illustrated in Fig. 1. The bottom 5 mm of the adsorbent was scraped off with a single-edged razor blade, small marks were placed at both edges of each slide 10 mm from the base to indicate the spotting line, and a line was made across each slide 50 mm from the base by pressing the edge of the blade firmly against the slide.

*Procedure*

The chromatoslides were spotted at the 10 mm mark (3 spots to a slide) and developed in 250 ml beakers lined with blotting paper, presaturated with the developing solution, and covered with watch glasses. Each beaker required about 20 ml of the solution. The developing solution was allowed to ascend to the 50 mm line (about 10 min). The slides were removed from the beakers, allowed to air-dry, and placed in the exposure cabinet. Full visualization of the spots required approximately 15 min of irradiation, with the slides placed 2 in. below the U.V. lamps. As the lamps aged, irradiation time was increased.

## RESULTS AND DISCUSSION

*Effect of 2',7'-dichlorofluorescein*

After 5 min of irradiation 5 ng spots of kelthane, methoxychlor, tedion, dieldrin,

endrin, lindane, heptachlor, heptachlor epoxide, γ-chlordane, α-chlordane, *o,p'*-DDT, *p,p'*-DDT, DDD, DDE, aldrin, and telodrin standards could be easily detected by using the Chromato-Vue with short-wave length U.V. An excellent example of the effect of dichlorofluorescein is the ability to detect 5 ng of methoxychlor with the aid of the Chromato-Vue before irradiation in the exposure cabinet. Ten nanograms of chlorbenside, chlorobenzilate, perthane, TCNB, PCNB, thiodan, BHC, and technical chlordane, and 20 ng of strobane, toxaphene, and iso-octyl ester of 2,4-D are required for detection with the Chromato-Vue after 5 min irradiation. The pesticides appear as dark purple spots (streaks in the case of strobane and toxaphene) on a background of the familiar yellow fluorescence of 2',7'-dichlorofluorescein. Prolonged exposure to the strong U.V. lamps destroys the fluorescent dye. This destruction takes place slowly so that even after the pesticide spots are visible to the unaided eye, some fluorescence still remains.

Many of the plant extracts which remain after the clean-up procedures[3,5,6] do not interfere with the fluorescent dye. Of the products which have been analyzed, onions have been the most troublesome. The pesticides chromatograph at the proper rate but usually appear as two small spots on the edges of the plant extract which appears as a white streak. The products analyzed were onions, celery, lettuce, carrots, hay, butter, whole milk, wheat, oats, corn and cabbage.

*Effect of hydroquinone*

The chromogenic reagent used by MITCHELL[7] and MILLS[5] utilized 2-phenoxyethanol and hydrogen peroxide to sensitize the silver nitrate and to retard darkening of the background. These compounds were found to be impractical for use on slides

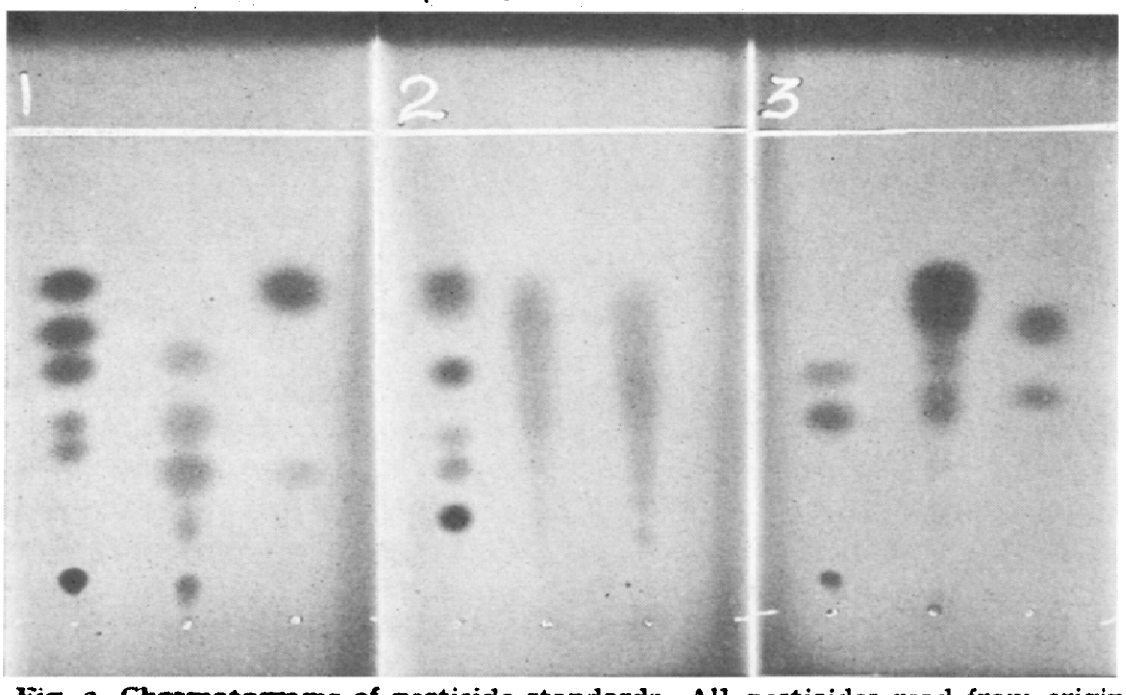


**Fig. 2. Chromatograms of pesticide standards. All pesticides read from origin in increasing $R_F$ values. Solvent system: 2% acetone in *n*-heptane. *Slide 1*, Spot 1 = kelthane, DDD, endrin, *p,p'*-DDT, *o,p'*-DDT, DDE; Spot 2 = BHC; Spot 3 = lindane, PCNB. *Slide 2*, Spot 1 = methoxychlor, dieldrin, heptachlor epoxide, perthane, heptachlor, aldrin; Spot 2 = toxaphene; Spot 3 = strobane. *Slide 3*. Spot 1 = thiodan, γ-chlordane, thiodan; Spot 2 = technical chlordane; Spot 3 = α-chlordane, TCNB.**

that would not be used immediately after preparation. Hydroquinone was substituted, and it was found that the sensitivity of the method increased 10-fold over plates prepared without it as described by DAMASKA[1]. Care must be exercised when preparing the solution for making the slurry. The silver nitrate and the hydroquinone solutions must be kept separated until the final mixing or the silver nitrate will be reduced to silver. The slurry begins to darken after about 20 min and therefore has to be used immediately after preparation. The adsorbent remains fairly stable after the slides are coated and dried. The sensitivity drops slightly with age. Plates that were seven weeks old still responded to 5 ng of the first group of pesticides listed above; however, the irradiation time necessary for full visualization of the spots increased to 45 min. The plates were somewhat light sensitive and were stored in the dark. Exposure to moisture over prolonged periods of time also darkened the layer.

*Effect of coating thickness*

The thickness of the adsorbent layer has a definite effect on sensitivity. Sensitivity drops as the thickness increases. When the slurry was prepared as described the adsorbent layer was approximately 75 $\mu$ thick. Attempts to produce a layer much thinner than 75 $\mu$ proved unsatisfactory. When the amount of solution used in the preparation of the slurry was increased, the adsorbent began to settle out and left an uneven layer on the slide. When the layer was more than 150 $\mu$ thick, it cracked after drying. A thick layer also slows the movement of the developing solution and lengthens the irradiation time.

The adsorbent layer applied in the manner described was very uniform, without the ridges which are often present when the layer is applied with a commercial applicator. The thickness of the layer varied by less than 5 $\mu$ between the 10 mm spotting line and the 50 mm stopping line. There was a slightly thicker layer on the very bottom portion of the slide but this was eliminated during the preparation of the chromatoslides by scraping off the bottom 5 mm of adsorbent.

*Qualitative analysis*

Samples were concentrated so that not more than five 1-$\mu$l applications were necessary to obtain the required sensitivity. When more than five applications were made, the spots became enlarged and the resolution was unsatisfactory.

When more than two standards were to be spotted on one spot, a mixed solution of the standards was prepared from the stock solutions. It was found that when three or more standards were overlaid on one spot the standards of higher $R_F$ values appeared as rings on the developed and irradiated chromatoslides.

Since the $R_F$ values of the pesticides varied slightly with the temperature, all the pesticides were compared with the $R_F$ for DDD. The $R_{DDD}$ values of 27 pesticides with 2 % acetone in *n*-heptane and *n*-heptane developing solvents are given in Table I. Most of the pesticides chromatographed in the same order with the two systems but for a few there was a marked difference. The two systems will identify tedion, lindane, heptachlor epoxide, endrin, chlorbenside, thiodan and the iso-octyl ester of 2,4-D and aid in the identification of the others.

The concentration of acetone in *n*-heptane is critical. It was noted that after this solution remained in the chromatographic tank for about 1 ½ hours the $R_F$ values approached those obtained with plain *n*-heptane. This indicates the acetone is lost

TABLE I

COMPARISON OF $R_{DDD}$ VALUES FOR 27 PESTICIDES IN 2% ACETONE–*n*-HEPTANE AND *n*-HEPTANE DEVELOPING SOLUTIONS

| *Pesticide* | $R_{DDD}$ (*2% acetone–heptane*) | $R_{DDD}$ (*n-heptane*) |
|---|---|---|
| Chlorobenzilate | 0.00 | 0.00 |
| Kelthane | 0.10 | 0.00 |
| Methoxychlor | 0.65 | 0.10 |
| Tedion | 0.85 | 0.00 |
| Dieldrin | 0.90 | 0.50 |
| Lindane | 0.98 | 1.22 |
| DDD | 1.00 | 1.00 |
| Heptachlor epoxide | 1.10 | 0.60 |
| $\gamma$-Chlordane | 1.17 | 1.20 |
| Endrin | 1.23 | 0.60 |
| Chlorbenside | 1.31 | 0.67 |
| $\alpha$-Chlordane | 1.45 | 1.80 |
| *p,p'*-DDT | 1.50 | 2.40 |
| Perthane | 1.66 | 1.20 |
| *o,p'*-DDT | 1.80 | 3.40 |
| TCNB | 1.90 | 3.35 |
| Heptachlor | 2.00 | 3.80 |
| PCNB | 2.15 | 3.55 |
| Aldrin | 2.15 | 4.40 |
| DDE | 2.20 | 3.80 |
| Telodrin | 2.20 | 2.80 |
| Thiodan | 0.12 | 0.00 |
| | 1.60* | 0.80 |
| Iso-octyl ester 2,4-D | 0.72* | 0.10 |
| | 1.12 | |
| BHC | 0.10 | 0.10 |
| | 0.30 | 0.50 |
| | 0.60 | 1.20* |
| | 1.05* | 1.60* |
| | 1.40* | 2.40 |
| | 1.67 | |
| Technical chlordane | 0.17 | Streak |
| | 0.56 | 0.10–2.00 |
| | 1.10* | |
| | 1.44* | |
| | 1.78 | |
| | 2.78* | |
| | 3.22* | |
| Toxaphene | Streak from 0.00–2.35 with spot at 1.25 | 0.00–2.80 |
| Strobane | Streak from 0.00–2.25 | 0.00–1.80 |

* Indicates major spots when more than one spot is present.

by selective evaporation. During the compilation of the data reported in Table I the 2% acetone–*n*-heptane solution was changed after every second set of slides was developed. Because of this change and the effect of temperature on the $R_F$ values, DDD was spotted on each slide until the unknown pesticide in the sample was narrowed down to a possible few. Then standards of the suspected pesticides were spotted with the sample.

The method as described is a rapid qualitative determination for pesticide residues. The most time-consuming portion of the method is the prewashing of the adsorbent but enough of this can be prepared at one time to prepare approximately 700 slides and can be stored for five months or longer. Once the slides were prepared, eight to ten slides with samples and standards were spotted, developed and irradiated to full exposure in less than 1 h.

SUMMARY

The use of thin layer chromatography for the qualitative analysis of pesticides using microscope slides as plates is described. The incorporation of hydroquinone, 2′,7′-dichlorofluorescein, and silver nitrate in the adsorbent layer and the effect on the sensitivity to pesticide residues are discussed. The preparation of microscope slide thin layer plates by dipping the slides into an ethanol–chloroform slurry of aluminum oxide adsorbent is described. A table of $R_F$ values computed against DDD is given for two solvent systems.

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