

THIN-LAYER CHROMATOGRAPHY OF ORGANOPHOSPHORUS
PESTICIDES AND ACIDS ON MICROCHROMATOPLATES*

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INTRODUCTION

While thin-layer chromatography (TLC) has been applied to a wide variety of separations in the past few years, it has been applied relatively little to organophosphorus pesticides or to organophosphorus acids. WALKER AND BEROZA¹ have summarized the literature on pesticides and have presented data on 62 chlorinated or organophosphorus pesticides in three solvents (chloroform, benzene, and hexane) and in mixed solvent systems consisting of each of these solvents with ethyl ether, ethyl acetate, acetone, methanol, and acetic acid. KOVACS² extended WALKER AND BEROZA's studies and obtained better resolution of chlorinated pesticides and BLINN³ has applied TLC to the determination of the pesticide phorate residue. All of these studies have been conducted with standard glass plates, 20 cm in length.

The substitution of microscope slides for the standard plates provides an economical, rapid TLC method. These microchromatoplates may be prepared by dipping the plates into a suspension of the solid phase⁴, by spraying^{5,6}, by spreading with a commercially available applicator⁷, or by spreading with an easily constructed applicator⁸. The microchromatoplates require less preparation time than standard plates since no preliminary cleaning of the plate is required and the slides may be discarded after use. These microchromatoplates require less solvent and a shorter time for development than the standard plates. Therefore, these plates are ideal for the screening of solvents to establish the requisite conditions for any desired separation. The R_F values obtained on these microchromatoplates differ somewhat from those obtained on the standard plates but resolution of two components can be demonstrated.

EXPERIMENTAL

Apparatus

Standard TLC plates were prepared with the Desaga/Brinkmann Model S-II Adjustable Applicator**. Desaga developing tanks** were used for these standard plates.

The microchromatoplates were prepared with essentially the same apparatus as that described by WASICKY⁸ and shown in Fig. 1. The applicator is made of brass, with dimensions as given by WASICKY. The base plate, made of brass bar stock, is 92 cm long and 3.5 cm wide. Along the back edge and the left end, there is a 0.9 cm

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** Brinkmann Instruments, Inc., Westbury, N.Y.



Fig. 1. Applicator and developing jars for microchromatoplates.

wide retaining ledge, raised 1 mm above the base. As this ledge is thinner than the 1.2 mm microscope slides, the applicator rides on the surface of the slides during the slurry spreading. Standard microscope slides*, 25.4 mm \times 76.2 mm, were used without preliminary cleaning. Coplin staining jars** were used as developing tanks (see Fig. 1).

Chemicals

The solid phases, Silica Gel G, Silica Gel H, Aluminum Oxide G, and Kieselguhr G (Brinkmann Instruments, Inc.) were used as solid phases. The developing solvents were of reagent grade and were used without further purification. The pesticides were obtained as analytical standards through the courtesy of various producers and were used as received. Organophosphorus acids were either purchased from chemical supply houses or were furnished by Victor Chemical Division of Stauffer Chemical Company, Chicago, Ill. A stock solution of each compound was prepared at a concentration of 1 mg/ml by dissolving a weighed quantity in a solvent. Benzene was used as the solvent for all compounds except for some acids, for which a mixed benzene-acetone solvent (4:1) was used.

Preparation of plates

The standard plates were prepared with a nominal thickness of 0.25 mm according to instructions supplied with the Desaga Applicator.

In the preparation of the microchromatoplates, 12 microscope slides were placed end to end against the retaining ledge on the applicator base plate. For Silica Gel G,

* Catalog No. M6125, Protecta-Slide, S/P. Scientific Products, Division of American Hospital Supply Corporation, Evanston, Ill.

** Catalog No. S7660, Scientific Products.

Kieselguhr G, and Aluminum Oxide G, 6 g of powder were weighed into a 125 ml, glass-stoppered, Erlenmeyer flask and 12 ml of distilled water were added. The flask was shaken vigorously for 30 sec, and the slurry was poured into the applicator which was resting at the right-hand end of the row of slides. As soon as the slurry began to run out of the slot at the back of the applicator, the applicator was pushed as smoothly as possible along the row of slides to spread the layer of solid phase onto the slides. After the plates had set for a few minutes, the slides were removed from the base plate and were dried at 110° in an oven. Silica Gel H was spread in the same fashion except that 6 g of powder were mixed with 14.5 ml of water. The plates coated with Silica Gel H were allowed to air dry for at least 4 h after which they were dried in the oven. The ratio of solid phase to water was fairly critical with all the solid phases which were used and particularly with Silica Gel H. The usual precautions with the "G" phases, which contain a calcium sulfate binder, were followed. Since the water content of the solid phase might influence R_F values with nonaqueous solvents, plates were used as soon as possible after being removed from the drying oven. With aqueous developing solvents the water content of the solid phase was not critical.

Development of chromatograms

Two samples were spotted with either a 10 μ l or a 50 μ l Microliter Syringe* on a microchromatoplate at 1 cm from one end of the plate. The pesticides were usually 10 μ l in volume; the acids were 10, 20, or 50 μ l in volume. A mark was placed 5 cm above the point of sample application to note the end of solvent travel. The end of the microchromatoplate on which the sample had been spotted was immersed to a depth of 0.5 cm in 3 ml of solvent in a Coplin jar, and the chromatogram was permitted to develop at room temperature (about 24°). The time required for development varied with the volatility of the solvent; for example, benzene required 9 min for a solvent travel of 5 cm.

Chromogenic reagents

The chromogenic reagents which were sprayed onto the plates for sample detection were:

Iodine. Dissolve 0.5 g of iodine in 100 ml of ethanol⁹. This chromogenic reagent gives spots with many organophosphorus compounds. It is also always used before Fluorescein or 4-MUB.

FCSSA. (a) Dissolve 0.1 g of ferric chloride hexahydrate in 1 ml of 1 N hydrochloric acid and dilute the solution to 100 ml with 80 % ethanol; this solution is stable for about 2 weeks. (b) Dissolve 1 g of sulfosalicylic acid in 100 ml of 80 % ethanol¹⁰. Spray first with (a), then with (b). This chromogenic reagent or the Hanes reagent was used for detection of alkylphosphoric acids.

IOP. Dissolve 1 g of platinum chloride in 10 ml of water and mix this solution with 10 g potassium iodide dissolved in 250 ml of water. To prepare the spray solution, dilute one volume of this stock solution with six volumes of water¹⁰.

AgNO₃. Dissolve 1.7 g of silver nitrate in 5 ml of water, add 10 ml of 2-phenoxyethanol, and dilute the solution to 200 ml with acetone¹. Spray the plate with the

* Hamilton Company, Whittier, Calif.

AgNO₃ spray reagent and then expose the plate to 3660 Å ultraviolet light for about 10 min.

Fluorescein. Prepare a 0.01 *M* solution in 0.01 *N* sodium hydroxide. Dilute this stock solution 3 to 100 with ethanol for spraying¹¹. The plate is sprayed first with Iodine spray reagent, any visible spots are recorded, and then the plate is resprayed with Fluorescein. Some compounds will produce spots visible under room light; others will produce spots visible under either 2547 Å or 3660 Å ultraviolet light.

4-MUB. Dissolve 0.075 g of 4-methylumbelliferone in 100 ml of ethanol-water (1:1) and add 10 ml of 0.1 *N* ammonium hydroxide¹². The plate is first sprayed with Iodine spray reagent, any visible spots are recorded, and then the plate is resprayed with 4-MUB. The plate is observed under either 2547 Å or 3660 Å ultraviolet light.

Hanes reagent. Dissolve 0.5 g of ammonium molybdate tetrahydrate in 5 ml of water and add 1.5 ml of hydrochloric acid and 2.5 ml of perchloric acid. Cool the solution and dilute it to 50 ml with acetone¹³. This solution gives better results if aged for a day and is stable for about three weeks. After the reagent is sprayed on the plate, expose the plate first to an infrared heat lamp at a distance of 30 cm for 2 min, then to a 3660 Å ultraviolet light for 7 min.

These spray reagents were adapted from the many sprays used in paper chromatography. Their lower limit of detection was not determined; most will detect at least 1 μg of the compound spotted on a TLC plate. About 0.5 ml of each solution will cover a single plate when a 10 ml tube sprayer* is used with compressed air. Some sprays were applied consecutively; for example, a plate might be sprayed with Iodine, then with either Fluorescein or 4-MUB, and finally with AgNO₃.

When volatile organic solvents were used as developers, a suitable chromogenic spray could readily be found. However, when acidic or basic aqueous solvents were used as developers, some reagents would give a good color test when the sample was spotted directly on a TLC plate; after the sample had been developed with the solvent, there was no color produced upon spraying. The alkylphosphoric acids gave the most trouble in this respect.

DISCUSSION AND RESULTS

Pesticides

Thirty-one organophosphorus pesticides (given with chemical names in Table I) were studied on Silica Gel G in an eluotropic series of six solvents. The results are summarized in Table II. If streaking occurred, the *R_F* values are the leading and trailing edges of the spot. When a single *R_F* value is given, the width of the spot is about ± 0.03. Many of these pesticides contain isomers, manufacturing impurities, or decomposition products; these pesticides give multiple spots with one or more developing solvents. The nonpolar solvent, cyclohexane, moved appreciably only Merphos, which is a phosphite. Although no solvent separated all pesticides, benzene produced the best general separation.

Four pesticides and two acids were studied on 20 cm plates, with a solvent travel of 15 cm. The *R_F* values obtained on microchromatoplates for a solvent travel of 5 cm and on standard plates for a solvent travel of 15 cm are given in Table III. The results show that *R_F* values are lower for the longer solvent travel. *R_F* values

* Catalog No. 2 C-50, Microchemical Specialties Co., Berkeley, Calif.

TABLE I
CHEMICAL NAMES OF ORGANOPHOSPHORUS PESTICIDES

<i>Pesticide</i>	<i>Name</i>
Betasan ^a	N-[2-(O,O-Diisopropylthiophosphoryl)ethyl]benzenesulfonamide
Ciodrin ^b	O,O-Dimethyl O-[1-methyl-2-(1-phenylcarbethoxy)vinyl] phosphate
DDVP ^c	O,O-Dimethyl O-2,2-dichlorovinyl phosphate
DEF ^c	S,S,S-Tributyl phosphorotrithioate
Delnav ^d	2,3- <i>p</i> -Dioxane S,S-bis(O,O-diethyl phosphorodithioate)
Demeton ^e	O,O-Diethyl O(or S)-2-(ethylthio)ethyl phosphorothioate
Diazinon ^e	O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate
Dicaphon ^f	O,O-Dimethyl O-(2-chloro-4-nitrophenyl) phosphorothioate
Dimethoate ^f	O,O-Dimethyl S-(N-methylcarbamoyl)methyl phosphorodithioate
Di-Syston ^c	O,O-Diethyl S-2-(ethylthio)ethyl phosphorodithioate
EPN ^g	O-Ethyl O- <i>p</i> -nitrophenyl phenylphosphonothioate
Malathion ^{f,h}	O,O-Dimethyl S-[1,2-bis(ethoxycarbonyl)ethyl] phosphorodithioate
Merphos ⁱ	S,S,S-Tributyl phosphorotrithioate
Methyl Demeton ^e	O,O-Dimethyl O(or S)-(2-ethylthio)ethyl phosphorothioate
Methyl Parathion ^{f,h}	O,O-Dimethyl O- <i>p</i> -nitrophenyl phosphorothioate
Methyl Trithion ^a	O,O-Dimethyl S-(<i>p</i> -chlorophenylthiomethyl) phosphorodithioate
Naled ^j	O,O-Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate
OMPA ^k	Octamethylpyrophosphoramidate; Bis-N,N,N',N'-tetramethyl-phosphorodiamidic anhydride
Para-Oxon ^f	O,O-Diethyl O- <i>p</i> -nitrophenyl phosphate
Parathion ^l	O,O-Diethyl O- <i>p</i> -nitrophenyl phosphorothioate
Phorate ^f	O,O-Diethyl S-ethylthiomethyl phosphorodithioate
Phosdrin ^b	O,O-Dimethyl O-(2-carbomethoxy-1-methylvinyl) phosphate
Phosphamidon ^l	O,O-Dimethyl O-[2-chloro-2-(N,N-diethylcarbamoyl)-1-methylvinyl] phosphate
Phosfon ^l	Tributyl 2,4-dichlorobenzylphosphonium chloride
Ronnel ^m	O,O-Dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate
Ruelene ^m	O-(4- <i>tert.</i> -Butyl-2-chlorophenyl) O-methyl N-methyl phosphoramidate
TEPP ^l	Tetraethyl pyrophosphate; Bis-O,O-diethyl phosphoric anhydride
Trichlorfon ^e	O,O-Dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate
Trithion ^a	O,O-Diethyl S-(<i>p</i> -chlorophenylthiomethyl) phosphorodithioate
VC-13 ^l	O,O-Diethyl O-(2,4-dichlorophenyl) phosphorothioate
Zinphos ^f	O,O-Diethyl O-(2-pyrazinyl) phosphorothioate

Obtained from:

- ^a Stauffer Chemical Co., Richmond, Calif.
- ^b Shell Chemical Co., New York, N.Y.
- ^c Chemagro Corp., Kansas City, Mo.
- ^d Hercules Powder Co., Wilmington, Del.
- ^e Geigy Chemical Corp., Yonkers, N.Y.
- ^f American Cyanamid Co., Princeton, N.J.
- ^g E. I. du Pont de Nemours & Co., Wilmington, Del.
- ^h Thompson-Hayward Chemical Co., Kansas City, Mo.
- ⁱ Virginia-Carolina Chemical Co., Richmond, Va.
- ^j California Chemical Co., Richmond, Calif.
- ^k Pennsalt Chemicals Corp., Tacoma, Wash.
- ^l American Potash & Chemical Corp., Los Angeles, Calif.
- ^m Dow Chemical Co., Midland, Mich.

reported by WALKER AND BEROZA¹ for the four pesticides with a solvent travel of about 10 cm are shown for comparison. Their R_F values are intermediate between the 5 cm and the 15 cm solvent travel for most of the pesticides. PEIFER⁴ stated that the polarity of the developing solvent necessary to obtain the same R_F value is partially dependent upon the distance traveled by the solvent front but he presented no comparative data.

TABLE II
R_F VALUES FOR PESTICIDES ON MICROCHROMATOPLATES
 Solvent travel: 5 cm. Solid phase: Silica Gel G.

Pesticide	Solvent						Chromogenic reagent
	Cyclohexane	Benzene	Acetone	Ethyl acetate	Isopropanol	Methanol	
Betasan ^a (Disan ^a)	o	0.03	1.0	1.0	0.95	1.0	IOP
Ciodrin ^a	o	o	0.96	0.66	0.77	0.94	Iodine
DDVP (Dichlorovos)	o	0.05	o	0.75	o	o	AgNO ₃
DEF ^a	o	o	0.93	0.73	0.75	0.82	IOP
	0-0.12 ^b	0.04	1.0	1.0	0.93	1.0	
Delnav ^a	o	o	o	o	o	0.96	Iodine
Demeton (Systox)	o	0.33	1.0	1.0	0.88	o	Iodine
		o	o	o	0.85	0.87	
		0.54	0.95	0.77	0.90		
			1.0	0.98			
Diazinon ^a	o	0.08	1.0	1.0	0.83	0.92	AgNO ₃
Dicapthon	o	0.64	1.0	1.0	0.89	0.95	AgNO ₃
Demethoate (Cygon ^a)	o	o	0.88	0.39	0.77	0.92	Iodine
Di-Syston ^a	o	0.68	1.0	1.0	0.92	1.0	Iodine
EPN	o	0.64-0.74 ^b	1.0	1.0	0.87	0.86-1.0 ^b	AgNO ₃
Malathion	o	o	o	o	o	0.86	Iodine
		0.07	1.0	0.94	0.88	0.94	
Merphos ^a	o	o	1.0	1.0	0.92	1.0	IOP
	0.06-0.16 ^b	0.09					
	0.22-0.34 ^b	1.0					
Methyl Demeton (Meta-Systox ^a)	o	o	o	o	0.04	0.91	Iodine
			0.93	0.66	0.74-0.86 ^b		
Methyl Parathion	o	0.55	1.0	1.0	0.89	0.96	Iodine
Methyl Trithion ^a	o	0.68-0.78 ^b	1.0	1.0	0.84-0.94 ^b	1.0	IOP
Naled (Dibrom ^a)	o	o	o	o	o	o	AgNO ₃
		0.08	0.96	0.85	0.80	0.90	
OMPA (Schradan)	o	o	0.04	0.01	0.19-0.36 ^b	0.76	Iodine
Para-Oxon	o	o	0.94	0.77	0.79	0.96	Iodine
Parathion	o	0.60	0.98	0.98	0.87	0.89	Iodine
Phorate (Thimet ^a)	o	o	o	o	0.89	1.0	Iodine
		0.68	1.0	1.0			
Phosdrin ^a	o	o	0.88	0.45	0.68	0.85	AgNO ₃
				0.59			
				0.82			
Phosphamidon	o	o	o	o	0.02	0.09	Iodine
			0.82	0.26	0.60	0.85	
Phosfon ^a	o	o	o	o	0-0.38 ^b	0.18-0.84 ^b	Iodine
						0.87	
Ronnel	o	0.86	1.0	0.98	0.83	0.96	Hanes reagent
Ruelene ^a	o	o	0.86	0.43	0.79	0.93	Iodine
TEPP, 40 % (Tetron-100 ^a)	o	o	o	o	0.07	0.92	Hanes reagent
			0.81	0.35	0.84		
Trichlorfon (Dipterex ^a)	o	o	0.88	1.0	0.82	0.88	AgNO ₃
			0.98		0.97	0.98	
Trithion ^a	o	0.76-0.86 ^b	1.0	1.0	0.92	1.0	IOP
VC-13 ^a	o	0.86	1.0	1.0	0.90	1.0	Hanes reagent
Zinophos	o	0-0.14	0.96	0.90	0.71-0.83 ^b	0.80-0.96 ^b	IOP

^a Trade mark.^b Streaking.

TABLE III

 R_F VALUE AS A FUNCTION OF LENGTH OF SOLVENT TRAVEL

Solid phase: Silica Gel G.

Solvent system: a = Benzene

b = *n*-Propanol-*n*-butanol-2 *N* ammonium hydroxide (50:20:30, v/v).c = *n*-Butanol-acetic acid-water (60:20:20, v/v).

Compound	Solvent travel			Solvent system
	5 cm	15 cm	10 cm ¹	
Demeton	0	0	0	a
Malathion	0.54	0.39	0.43	a
	0	0	0.08	
Methyl Parathion	0.07	0.05	0.41	a
	0.55	0.37		
Parathion	0.60	0.52	0.47	a
Chloromethylphosphonic acid	0.06-0.15	0.12		b
Chloromethylphosphonic acid	0.30-0.42	0.29		c
Phenylphosphonic acid	0	0		b
Phenylphosphonic acid	0.44-0.58	0.39		c

The repeatability of R_F values for a solvent travel of 5 cm is shown in Table IV. Excellent agreement was obtained between two runs for both demeton and para-oxon. The samples in Run 2 had been sprayed with the Iodine spray reagent before the chromatogram was developed. This may account for the absence of the component at $R_F = 0$ for demeton. The component at this R_F in Run 1 gave a much fainter spot than the other two components and is probably the sulfoxide.

TABLE IV

REPEATABILITY OF R_F VALUES

Solvent travel: 5 cm. Solid phase: Silica Gel G.

Pesticide	Solvent	R_F values	
		Run 1	Run 2
Demeton	Acetone	0	—
		0.95	0.93
		1.0	1.0
Para-Oxon	Ethyl acetate	0.77	0.75

Organophosphorus acids

The organophosphorus acids require more polar solvents than the neutral esters such as the pesticides. The solvent systems selected for trial were based on those systems used in paper chromatography of phospholipids¹³, amino acids¹⁴, and organophosphorus acids^{15, 16}. Preliminary results on the TLC for some alkylphosphoric and organophosphonic acids are summarized in Table V. These solvent systems are not satisfactory since considerable streaking was evident.

Table III shows that the R_F values for standard plates tend to be somewhat lower than R_F values with the microchromatoplates for the acids as well as for the neutral esters.

TABLE V

 R_F VALUES FOR ORGANOPHOSPHORUS ACIDS

Solvent travel: 5 cm. Solid phase: Silica Gel G, except alkylphosphoric acids in solvent 4 are on Silica Gel H.

Solvent systems: 1 = 2-Propanol-water-conc. ammonium hydroxide (75:24:1, v/v),
 2 = *n*-Propanol-*n*-butanol-2 *N* ammonium hydroxide (50:20:30, v/v),
 3 = 2-Propanol-conc. ammonium hydroxide (75:25, v/v),
 4 = *n*-Butanol-formic acid-water (60:20:20, v/v),
 5 = *n*-Butanol-acetic acid-water (60:20:20, v/v),
 6 = *n*-Butanol-acetic acid-water (80:20:100, v/v); use the top phase.

Acid	Solvent system					
	1	2	3	4	5	6
Monomethylphosphoric	0	0.04	0-0.12 ^a	0		
Dimethylphosphoric	0.45	0.39	0.37	0.68-0.78 ^a		
Monoethylphosphoric	0	0.09	0.03	0	0.28-0.46 ^a	
Diethylphosphoric	0.60	0.50	0.50	0.74-0.90 ^a	0.28-0.46 ^a	
Monoisopropylphosphoric	0.08	0-0.18 ^a	0	0		
Diisopropylphosphoric	0.74	0.64	0.57	0.74-0.92 ^a		
Monobutylphosphoric	0-0.14 ^a	0-0.26 ^a	0.03	0		
Dibutylphosphoric	0.73	0.61	0.59	0.74-0.94 ^a		
Chloromethylphosphonic	0-0.16 ^a	0.06-0.15 ^a	0	0.54	0.30-0.42 ^a	0.10-0.24 ^a
Phenylphosphonic	0-0.20 ^a	0	0	0.24-0.61 ^a	0.44-0.58 ^a	0.24-0.46 ^a

^a Streaking

Table VI shows that the solid phase had little effect on the R_F value for the organophosphorus acids when Silica Gel H and Aluminum Oxide G were substituted for Silica Gel G. Preliminary work with Kieselguhr G had indicated little advantage for this solid phase over other solid phases.

Suitable chromogenic agents for organophosphorus acids have been difficult to find. Several agents gave good tests for samples spotted on TLC plates; however, after the plates had been developed, the agents produced poor spots or none. The reasons for this behavior have not been elucidated; the residual solvent may interfere or the free acid may have been converted to a salt. This behavior is still being studied.

TABLE VI

EFFECT OF SOLID PHASE ON ACID R_F VALUES

Solvent system: Isopropanol-conc. ammonium hydroxide (75:25, v/v).

Acid	Solid phase		
	Silica Gel G	Silica Gel H	Aluminum Oxide G
Monoisopropylphosphoric	0	0.03	0
Diisopropylphosphoric	0.57	0.59	0.57
Monobutylphosphoric	0.03	0.07	0
Dibutylphosphoric	0.64	0.65	0.51

SUMMARY

R_F values are given for 31 organophosphorus pesticides in six solvents on microchromatoplates. The use of microscope slides as supports for thin-layer chromatog-

raphy adsorbents is described and results obtained for four pesticides and four acids on the microchromatoplates are compared with those obtained with standard thin-layer chromatography plates. Preliminary results on the thin-layer chromatography of alkylphosphoric and organophosphonic acids are presented.

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