Journal of Chromatography, 78 (1973) 29–40

CHROM. 6463

THIN-LAYER CHROMATOGRAPHY AND ENZYME INHIBITION TECHNIQUES

INTRODUCTION

C. E. MENDOZA
Health Protection Branch, Department of National Health and Welfare, Ottawa, K1A 0L2 (Canada)

SUMMARY

Thin-layer chromatography continues to be an integral part of analytical chemistry because it is versatile, sensitive, simple and rapid. The application of enzymatic methods to thin-layer chromatography is becoming more important. This chromatographic–enzymatic technique has been developed for studies of metabolic pathways of pesticides, residue analyses and forensic chemistry. The discussions deal with the development of chromatographic techniques using enzymatic reactions to detect pesticides and other biologically active compounds, principles of the technique, comparison of detection limits by various methods and enzymes, and practical applications of the technique and methods of quantitation.

INTRODUCTION

A history of the development of thin-layer chromatography (TLC) has been discussed by STAHL[1]. The principle of the method was described as early as 35 years ago but the method has been considered to be indispensable only since the later 1950s. Its use had first been to separate and analyze biochemicals and other biological samples as well as inorganic compounds and elements. The application of enzymes to this method for pesticide determinations was not introduced until the middle 1960s (see MENDOZA[2]). Since 1968, numerous papers on the enzymatic determination of pesticides and enzyme inhibitors have been published.

PRINCIPLES OF THE TECHNIQUES

The principles of the technique can be illustrated as follows:

$$E + S \rightleftharpoons ES \rightarrow E + P \quad (1)$$

$$E + I \rightleftharpoons EI + S \not\rightarrow \text{no } P \quad (2)$$

where E = enzyme, S = substrate, I = inhibitor and P = substrate hydrolytic product. If the active sites on an enzyme molecule are blocked by an inhibitor, substrate reaction and hydrolysis on these sites are prevented and, as a result, no chromogenic product will be obtained. On a TLC plate, the areas where the pesticides are located will appear as colorless spots against an intensely colored background.

If a pH indicator is used, pesticide locations appear as colored spots against a colorless or lightly colored background.

Examples of enzyme substrates and their hydrolytic products are given below.

$$\text{Acetylcholine} + \text{water} \xrightarrow{E} \text{choline} + \text{acetic acid} \quad (3)$$

In the presence of Methylene Blue indicator, the spots that correspond to pesticide locations appear blue. The background, where the indicator was decolorized by the acetic acid, appears pale yellow.

With the following three substrates, the spots appear colorless on a mauve or blue background:

$$\text{1-Naphthyl acetate} + \text{water} \xrightarrow{E} \text{1-naphthol} + \text{acetic acid} \quad (4)$$

$$\text{1-Naphthol} + \text{diazonium salt} \rightarrow \text{azo compound (mauve)}$$

$$\text{Indophenyl acetate} + \text{water} \xrightarrow{E} \text{indophenol (blue)} + \text{acetic acid} \quad (5)$$

$$\text{Indoxyl acetate} + \text{water} \xrightarrow{E} \text{indoxol (fluorescent)} + \text{acetic acid} \quad (6)$$

$$2(\text{Indoxol}) + \text{oxygen} \rightarrow \text{indigo dye (blue)} \quad (6a)$$

$$\text{Indoxol} + \text{diazonium salt} \rightarrow \text{azo compound (mauve)} \quad (6b)$$

Nitrophenyl or naphthyl phosphate was used with phosphatases for pesticide determination (GEIKE[3]). In addition, N^{α}-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride was used with trypsin to detect organochlorine pesticides (GEIKE[4]).

THIN-LAYER CHROMATOGRAPHIC–ENZYME INHIBITION PROCEDURE

A flow diagram for the thin-layer chromatographic–enzyme inhibition (TLC–EI) procedure is shown in Fig. 1. The extracted pesticide residues are resolved on a TLC plate, derivatized or sprayed directly with an enzyme solution, and then sprayed

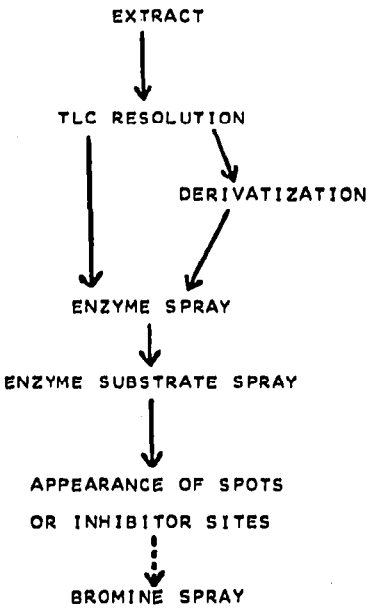


Fig. 1. Schematic diagram of TLC–EI procedure.

with a substrate solution. Sufficient time is allowed for enzyme–pesticide interaction before the substrate is applied. The substrate sprayed on a plate will not be hydrolyzed where the enzyme and the pesticide interacted. The development of the background color and spots takes place in only a few minutes. Bromine can be sprayed on to the plate as soon as maximum definition of spots is obtained so as to stop the reaction and to preserve the quality of the chromatogram. This step is applicable to indoxyl acetate and its derivative. The indophenol background is unstable to bromine.

SOME FACTORS THAT AFFECT THIN-LAYER CHROMATOGRAPHIC–ENZYME INHIBITION DETECTION

Table I shows the effects of gel types on the detection limits of nine carbamates (MENDOZA AND SHIELDS[5]). Aluminum Oxide DS-5 was more sensitive than Silica Gel G-HR or G. However, the quality of detection was not satisfactory because the spots corresponding to pesticide locations streaked. Silica Gel G gave slightly more sensitive detection than Silica Gel G-HR.

TABLE I

DETECTION LIMITS[a] IN NANOGRAMS OF CARBAMATE PESTICIDES WITH PIG LIVER EXTRACT AND 5-BIA (MENDOZA AND SHIELDS[5])

Compound	*Silica Gel*				*DS-5 Al_2O_3*	
	G-HR		*G*			
Aldicarb	50	(±)	30	(±)	10	(±)
Banol	0.5	(+)	0.5	(++)	0.5	(++)
Carbaryl	0.1	(+)	0.1	(+)	0.1	(±)
Carbofuran	1	(+)	1	(+)	1	(±)
Carbofuran 3-OH	10	(±)	10	(+)	5	(+)
Matacil®	10	(±)	5	(++)	1	(±)
Mesurol®	0.1	(±)	0.1	(±)	0.1	(±)
Tranid®	100	(±)	50	(+)	5	(++)
Zectran®	50	(+++)	0.5	(±)	5	(+++)

[a] (±) = spot lasted 1–2 min; (+) = 5 min; (++) = longer than 5 min but less than 30 min; (+++) = 30 min or longer.

Fig. 2 shows the effects of the thickness of the gel layer on the quality of detection when using pig liver esterases and 5-bromoindoxyl acetate or 5-bromo-4-chloroindoxyl acetate (see also MENDOZA *et al.*[6]). The upper region of the plate was about 500 μm thick and the lower region 250 μm thick. The thick layer gave a good contrast between spots and the background, probably owing to a better supporting medium for enzyme and substrate interaction.

The detection limit for each pesticide or group of pesticides varies with the types or sources of enzymes. This effect was reported by WINTERLIN *et al.*[7] and MENDOZA and co-workers[5,8–10].

The effects of enzyme dilution on the sensitivity of detection for some carbamates and organophosphorus pesticides are shown in Table II. Diluted enzyme sprays were generally more sensitive than the concentrated sprays, the improve-

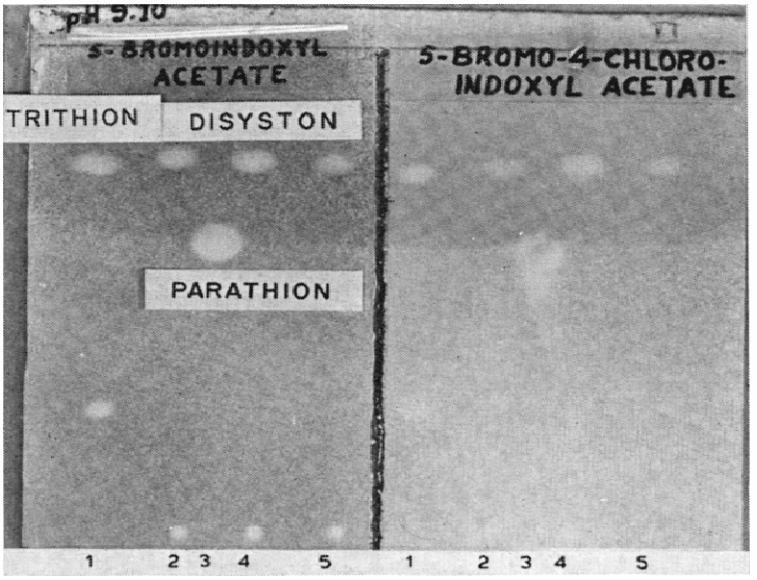


Fig. 2. Effect of thickness of the gel layer on the quality of detection of three organophosphorus pesticides. The top, darker area of the plate has a gel layer of about 0.5 mm. The bottom area has a gel layer of about 0.25 mm. Amount applied: 1 = 0.05 μg, 2 = 0.20 μg, 3 = 0.02 μg, 4 = 0.40 μg, 5 = 0.20 μg.

TABLE II

EFFECTS OF ENZYME DILUTION ON DETECTION LIMITS IN NANOGRAMS OF CHEMICAL STANDARDS ON SILICA GEL H[a] (MENDOZA AND SHIELDS[10])

Compound	*8 Pig*[b]	*100 Pig*	*8 Beef*	*25 Beef*
No bromine				
Aldicarb	50	25	250	250
Baygon®	10	5	500	500
Carbaryl	1	1	1	1
Carbofuran	5	2.5	250	100
With bromine				
Diazinon	10	5	1	0.5
Ethion	5	2.5	0.5	0.1
Malathion	50	50	1	1
Parathion	5	1	0.1	0.05

[a] IPA was used as a substrate.
[b] The numbers before the type of enzyme indicate the dilutions made from 20% liver extracts.

ment in detection varied by as much as 5-fold. The concentrated enzyme solution gave less sensitive detection because the number of active sites available was greater than that which the inhibitor could block. As a result, some substrate molecules could be enzymatically hydrolyzed, which caused partial or total masking of the spot.

The effect of the pH of the spray solutions on the detection of ten organophosphorus pesticides and carbaryl was reported by MENDOZA *et al.*[9]. The eleven compounds tested were not detected at pH 5.3 using beef liver esterases and 5-bromoindoxyl acetate. The plate appeared very pale yellow with a blue tinge, which indicated

TABLE III

DETECTION LIMITS[a] IN MICROGRAMS OF ORGANOCHLORINE INSECTICIDES OBTAINED BY THE TLC–EI TECHNIQUE USING PHOSPHATASES AND NITROPHENYL PHOSPHATE (NPP) OR NAPHTHYL PHOSPHATE (NP) (GEIKE[3])

Compound	*Acid phosphatase*		*Alkaline phosphatase*	
	NPP	*NP*	*NPP*	*NP*
DDT	70 *60*	30 *20*	50 *10*	8 *NC*
TDE	30 *NC*	10 *NC*	60 *30*	6 *2*
DDE	20 (*30*)	10 *NC*	50 *10*	6 *2*
Dicofol	30 *NC*	7 *NC*	20 (*30*)	2 *NC*
Methoxychlor	20 *NC*	7 *6*	30 *10*	6 *2*
Perthane	30 *NC*	2 (*4*)	20 *5*	2 *NC*
Benzenehexachloride	100 *NC*	100 *NC*	150 *NC*	—
Lindane	40 *NC*	30 *20*	150 *130*	70 (*100*)
Isodrin	40 *NC*	30 *NC*	70 *30*	70 *40*
Endrin	30 *NC*	20 *NC*	80 *40*	40 *10*
Aldrin	20 *10*	20 *7*	60 *40*	30 *6*
Dieldrin	40 *30*	20 *10*	80 *50*	30 *6*
Heptachlor	50 *NC*	10 (*30*)	50 *40*	10 *8*
Heptachlor epoxide	30 *20*	10 *NC*	70 *40*	70 *50*
Chlordane	50 *NC*	7 *6*	60 *20*	8 *6*
Isobenzan	10 (*50*)	10 (*30*)	80 *70*	70 *60*
Endosulfan	40 *30*	10 (*30*)	50 *20*	8 *NC*
Toxaphene	100 *NC*	80 *70*	50 *NC*	20 *10*

[a] Figures in italics are for detection limits after UV exposure. Figures in parentheses indicate a decrease in detection sensitivity. *NC* = no change; — = no data.

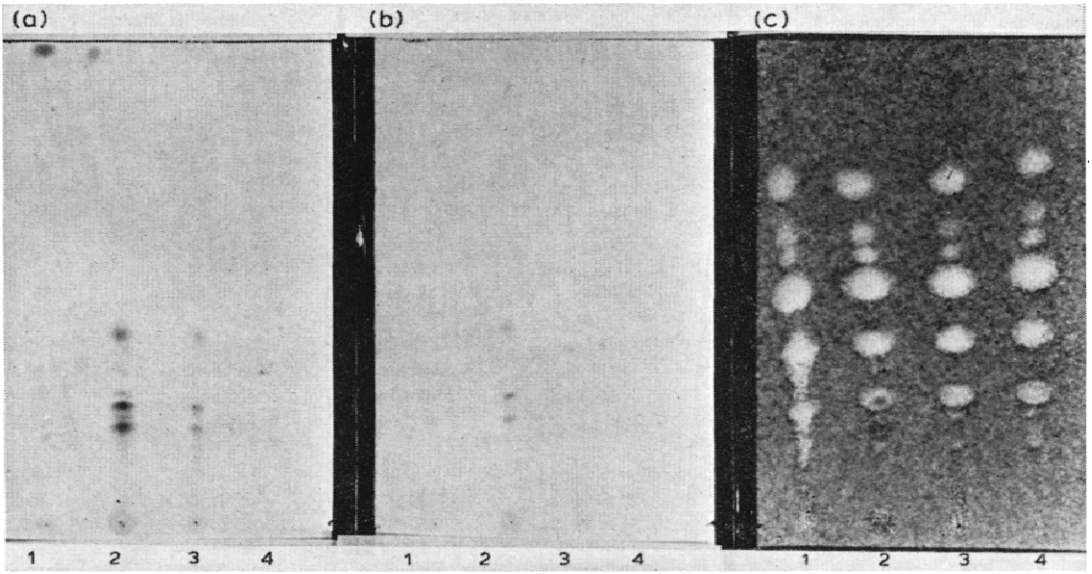


Fig. 3. TLC-EI procedure to detect pesticides in plant extracts. 1 = Carrot, 2 = pea, 3 = lettuce, and 4 = organophosphorus pesticide standard mixture. The sample equivalent weight is 150 mg. (a) A chromatogram obtained after development in 20% of acetone in hexane; (b) a chromatogram showing the effect of bromine on plant pigment; (c) a chromatogram showing TLC–EI detection of seven organophosphorus pesticides using liver homogenate and 5-bromoindoxyl acetate solution. White spots correspond to pesticide locations.

that the indoxols were not oxidized or that the indoxyl acetate molecules were not hydrolyzed. The detection at pH 7.19 was adequate although not as satisfactory as that at pH 8.32 or 9.10.

Table III shows the detection limits for organochlorine insecticides using two substrates (GEIKE[3]). The detection by phosphatase using naphthyl phosphate substrate was generally more sensitive than that using nitrophenyl phosphate. UV light has variable effects on the detection limits, varying from no change to a decrease or increase in detection sensitivity.

Fig. 3 illustrates the procedure for detecting some organophosphorus pesticides in plant extracts without elaborate clean-up. Fig. 3a is a chromatogram of carrot, pea and lettuce extracts developed in a system containing 20% of acetone in hexane. The dark spots are plant pigments. (The samples were extracted with acetonitrile and partitioned into hexane, and the hexane fractions were concentrated for TLC analysis.) Fig. 3b shows that the pigments were destroyed by bromine. Treatment of the plate with bromine was necessary in order to convert organophosphorus pesticides from P(S) to P(O) analogues or other potent enzyme inhibitors. Fig. 3c was obtained after spraying the plate with beef liver homogenate and then with 5-bromoindoxyl acetate solution.

APPLICATION OF THE THIN-LAYER CHROMATOGRAPHIC–ENZYME INHIBITION TECHNIQUE

Application of the TLC–EI technique to pesticide residue analyses has been reviewed recently (MENDOZA[2]). A continuation of this review is in progress so as to keep the information current and comprehensive.

It is also interesting to note that the detection limits obtained by our TLC–EI technique shown in Table IV (C. E. MENDOZA, unpublished work) concur with the toxicity index, LD_{50}, and pI_{50} obtained by FELTON[11]. The data indicate that the technique should be useful in toxicological evaluations of pesticides.

The application of the technique to forensic cases has been reported by HEYNDRICKX *et al.*[12], GELDMACHER-V. MALLINCKRODT AND ONG[13] and BOGUSZ AND BORKOWSKI[14].

HEYNDRICKX *et al.*[12] reviewed 40 cases of parathion poisoning in man. They also determined distributions of parathion in different organs by using fresh horse

TABLE IV

COMPARISON BETWEEN METHOMYL AND ITS ISOMER BASED ON TOXICITY INDEX, pI_{50}, AND TLC–EI DETECTION LIMITS

Test species	*Methomyl (syn)*	*Methomyl isomer (anti)*
Musca[a]	10	1
Musca (head)[b]	6.58	4.53
Pig (liver, TLC–EI)[c]	10	200–300

[a] Toxicity index = LD_{50} for parathion divided by LD_{50} for the test compound multiplied by 100 (FELTON[11]).

[b] pI_{50} values, where $pI_{50} = -\log_{10}(I_{50})$ (FELTON[11]).

[c] Detection limits (ng) using pig liver esterase and 5-bromoindoxyl acetate or indophenyl acetate substrate.

TABLE V

DETECTION LIMITS FOR SOME PESTICIDES INVOLVED IN FORENSIC CASES (GELDMACHER-V. MALLINCKRODT AND ONG[13])

Compound	*Detection limit (μg)*	*No. of cases analyzed*
Parathion	0.02	5
Oxydemetonmethyl	2	1
Demeton	2	1
Fenthion	2	1
Baygon®		

plasma and 1-naphthyl acetate on TLC plates. The detection limits were 0.1 μg for parathion, 20 μg for malathion, 3 μg for mevinphos, 1 μg for demeton, 25 μg for fenthion and 0.05 μg for paraoxon.

Table V shows the detection limits for five pesticides and a number of forensic cases analyzed by a TLC–EI method using 2-naphthyl acetate and beef liver esterases (GELDMACHER-V. MALLINCKRODT AND ONG[13]). The limits were only at the 2–4 μg level, except for parathion (0.02 μg).

Table VI shows five suicidal cases and a water sample analyzed by BOGUSZ AND BORKOWSKI[14] and the results show that the technique was very sensitive for thiometon, oxydemetonmethyl, dimethoate and malathion. The sensitivity of the

TABLE VI

CASES IN WHICH THE TLC–EI METHOD[a] WAS USED TO DETECT PESTICIDES IN LIVER OR STOMACH CONTENTS[b] (BOGUSZ AND BORKOWSKI[14])

No.	*Case*	*Results*	*TLC–EI detection*	*Other methods*[c]		
				A	*B*	*C*
1	Man, age 46, accidental intake of unknown insecticide	o	o	U	—	≪
2	Man, age 32, suicidal drinking of unknown insecticide	Thiometon	+++	+	—	o
3	Woman, age 19, suicidal drinking, empty container of Metasystox-R found	Metasystox-R	+++	+	+++	o
4	Man, age 58, suicidal drinking, empty container of dimethoate found	Dimethoate	+++	+	+++	±
5[d]	Woman, age 33, suicidal drinking, glass containing the remaining solution found	Malathion	+++	(+)[d]	+++	o
6	Study of water sample from a well	Thiometon	+++	+	+++	—

[a] The method used was that of MENDOZA *et al.*[6, 15].

[b] Symbols: o = none detected; U = unsuccessful; — = not tested or not applicable; ≪ = greatly decreased; ± = barely detectable; + = detectable; +++ = not explained by BOGUSZ AND BORKOWSKI[14] but interpreted as very definite enzyme inhibition or toxicity to houseflies.

[c] A = chemical TLC; B = bioassay with 5-day-old houseflies; C = acetylcholinesterase activity. Infrared spectrophotometric analysis was carried out on Case 2 but negative results were obtained.

[d] (+) = obtained from the solution in the glass.

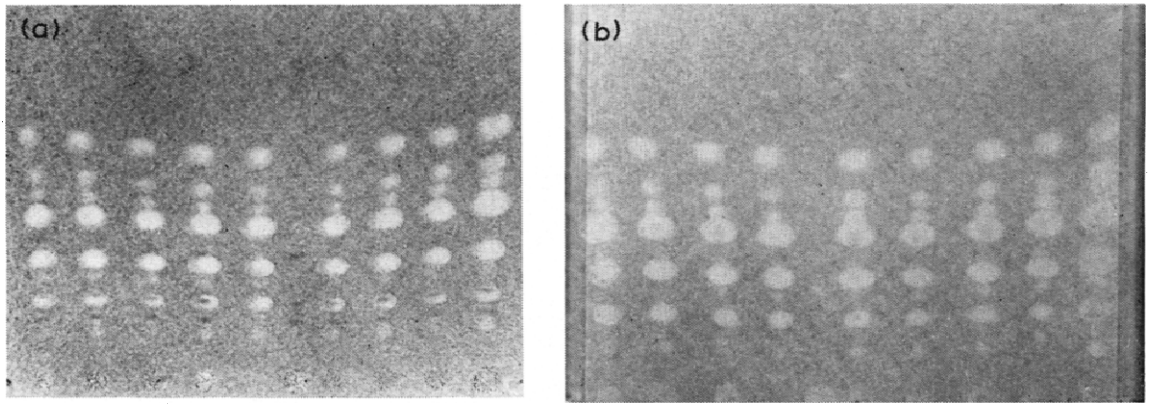


Fig. 4. (a) A chromatogram of fortified pea extracts developed and photographed in 1967. (b) A chromatogram of the same extracts also developed in 1967 and photographed in 1972.

method for eleven pesticide standards ranged from 10 to 500 ng; trichlorfon was detectable only at the $\geqslant 5$ μg level. BOGUSZ AND BORKOWSKI[14] also found, as we did, that the technique was stable and gave well defined spots.

Fig. 4 shows that the developed plates are indeed stable. The photograph on the left (a) was taken in March, 1967, shortly after the development, and the photograph on the right (b) was taken in May, 1972. Both plates were sprayed on March 14, 1967, with beef liver homogenate and 5-bromoindoxyl acetate. It should be noted that the quality of detection remained almost unchanged even after 6 years when the plates were protected with glass plates. Because of stability, 5-bromoindoxyl or the other derivatives of indoxyl acetate should be useful in forensic cases involving pesticides.

QUANTITATION

The mean recovery of methomyl (a carbamate insecticide) that was scraped off the plate after enzyme inhibition and analyzed by GLC was approximately 92 % (range 82–103 %). The recovery of methomyl that was added to the sprayed gel extracts was approximately 107 % (range 101–113 %). The GLC detection was based on dinitrophenylmethylamine, which is a reaction product of dinitrofluorobenzene and methylamine. Typical chromatograms of dinitrophenylmethylamine are shown in Fig. 5. The details of the derivatization technique will be published elsewhere. The low recoveries from spots that were scraped off the plate may be due to the residue adhering on the glass plate or to a loss during filtration. In spite of these shortcomings, the recoveries were well within the acceptable levels of efficiency ($\geqslant 80$ %) and precision (± 2 % standard error).

SEIFERT AND DAVÍDEK[16,17] reported the use of the TLC–EI technique in combination with polarography in the determination of some organophosphorus pesticides. The pesticides were detected with beef liver extract and 2-naphthyl acetate. The spots corresponding to pesticide locations were scraped off and eluted through chromatographic columns and the eluate was collected in a gelatin solution, deoxygenated and measured polarographically. The detection sensitivity was about 1 μg/ml

with a standard deviation of ±6%. The presence of the substrate, Fast Blue RR, and their coupled products did not interfere with the polarographic detection.

Several other methods have been proposed for the quantitative determination of compounds separated by TLC, and examples have been listed in an earlier review

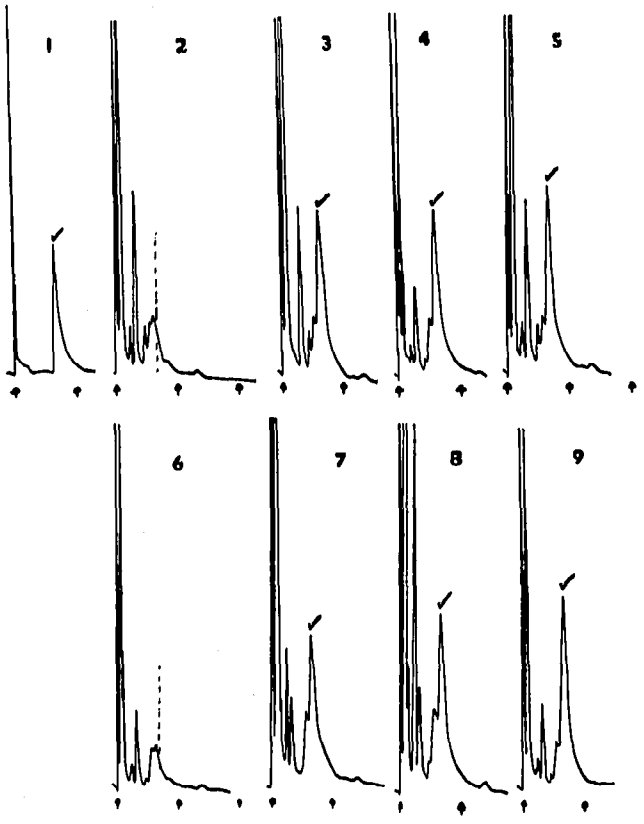


Fig. 5. Typical GLC chromatograms: (1) 1.3 ng of dinitrophenylmethylamine (DNPMA) standard. (2) Blank plate sprayed with indophenyl acetate (IPA). The vertical broken line indicates where the DNPMA peak is expected. (3) Recovery of 5 μg of methomyl from a plate sprayed with IPA. (4) Extract of gel sprayed with IPA and fortified with 5 μg of methomyl. (5) Extract of gel sprayed with IPA and fortified with 6.1 μg of DNPMA. (6) Blank plate sprayed with 5-bromoindoxyl acetate (5-BIA). (7) Recovery of 5 μg of methomyl from a plate sprayed with 5-BIA. (8) Extract of gel sprayed with 5-BIA and fortified with 5 μg of methomyl. (9) Extract of gel sprayed with 5-BIA and fortified with 6.1 μg of DNPMA.

(MENDOZA[2]). TOUCHSTONE *et al.*[18] surveyed the operating parameters for optimal quantitative spectrodensitometry of TLC plates, and found that transmittance was more sensitive than reflectance for absorbance, fluorescence quenching and fluorometric methods of quantitation. The use of double-beam instrumentation overcame some of the faults due to non-uniformity of the layer thickness, background scatter and sample application.

A flying-spot densitometer has been developed to overcome errors due to irregular patterns of the spots on the plate by dividing the measured areas into a large number of very small segments. The flying-spot densitometer measures the amount

of a substance in each segment separately and the amount in the whole spot is obtained by integrating all the segment amounts. (See also GOODALL[19].)

More recently, automation for TLC has been introduced[20,21]. A polyester tape conveys 5-inch chromatograms for automatic spotting, development, visualization and optical readout (Fig. 6). The developed chromatograms are measured by visible reflectance densitometry and the results are recorded on a strip chart or also stored on a computer tape. As many as 60 TLC determinations per hour can be performed.

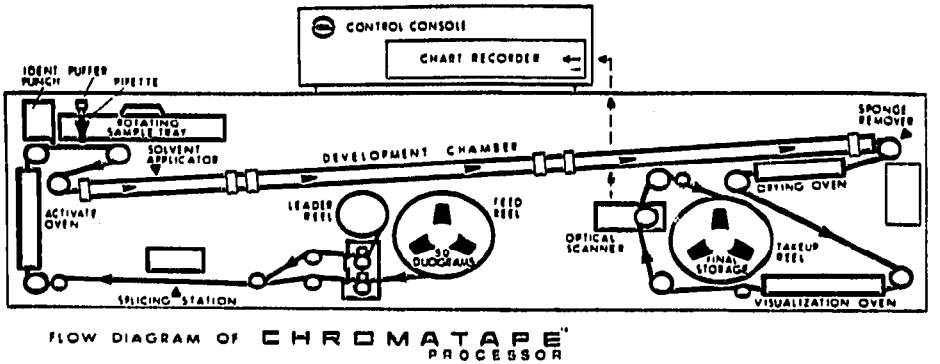


Fig. 6. Diagrammatic illustration of an automated apparatus for TLC development and quantitation. (Courtesy of the J. T. Baker Chemical Co., U.S.A.)

CONCLUSION

It can be summarized that TLC methods in combination with enzymatic techniques are versatile and sensitive for detecting pesticides. The combined technique is useful in the analysis of pesticide residues in foods and the environment, in metabolic and forensic investigations, and in the analysis of samples with interference that are too great for gas–liquid chromatography. Different types or sources of enzyme can be used to improve the detection sensitivity and selectivity. Sensitivity limits range from nanogram to picogram levels.

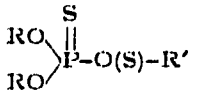
REFERENCES

1 E. STAHL (Editor), *Thin-Layer Chromatography*, Academic Press, New York, 1965, Section A, pp. 1–4.
2 C. E. MENDOZA, *Residue Rev.*, 43 (1972) 105.
3 F. GEIKE, *J. Chromatogr.*, 61 (1971) 279.
4 F. GEIKE, *J. Chromatogr.*, 52 (1970) 447.
5 C. E. MENDOZA AND J. B. SHIELDS, *J. Chromatogr.*, 50 (1970) 92.
6 C. E. MENDOZA, P. J. WALES, H. A. MCLEOD AND W. P. MCKINLEY, *Analyst (London)*, 93 (1968) 34.
7 W. WINTERLIN, G. WALKER AND H. FRANK, *J. Agr. Food Chem.*, 16 (1968) 808.
8 C. E. MENDOZA, D. L. GRANT, B. BRACELAND AND K. A. MCCULLY, *Analyst (London)*, 94 (1969) 805.
9 C. E. MENDOZA, P. J. WALES, D. L. GRANT AND K. A. MCCULLY, *J. Agr. Food Chem.*, 17 (1969) 1196.
10 C. E. MENDOZA AND J. B. SHIELDS, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 507.
11 J. C. FELTON, *J. Sci. Food Agr. (Suppl.)*, (1968) 32.

12 A. HEYNDRICKX, A. VERCRUYSSE AND M. NOË, *J. Pharm. Belg.*, (1967) 127.
13 M. GELDMACHER-V. MALLINCKRODT AND G.-L. ÖNG, *Arch. Kriminol. (Leipzig)*, 146 (1970) 154.
14 M. BOGUSZ AND T. BORKOWSKI, *Z. Rechtsmed.*, 68 (1971) 267.
15 C. E. MENDOZA, P. J. WALES, H. A. MCLEOD AND W. P. MCKINLEY, *Analyst (London)*, 93 (1968) 173.
16 J. SEIFERT AND J. DAVÍDEK, *J. Chromatogr.*, 59 (1971) 446.
17 J. SEIFERT AND J. DAVÍDEK *Z. Lebensm.-Unters. -Forsch.*, 146 (1971) 17.
18 J. C. TOUCHSTONE, S. S. LEVIN AND T. MUSAWEC, *Anal. Chem.*, 43 (1971) 858.
19 R. R. GOODALL, *J. Chromatogr.*, 78 (1973) 153.
20 T. A. GUERIN (J. T. Baker Chemical Co.), personal communication.
21 *Chem. Eng. News*, 50, No. 11 (1972).

DISCUSSION

H. ACKERMANN: Es wird hervorgehoben, dass die Kombination der Dünnschichtchromatographie mit der Enzymhemmtechnik zu einem äusserst empfindlichen Nachweis für insektizide Wirkstoffe vom Typ der phosphororganischen Verbindungen geführt hat. Da die meisten der im Pflanzenschutz verwendeten insektiziden Phosphororganika zunächst in der biologisch inaktiven Form als Thionophosphorsäureester mit der Grundstruktur



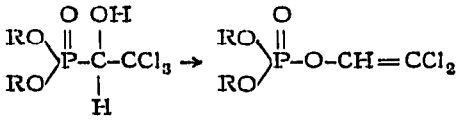
vorliegen, ist für den enzymatischen Nachweis eine Überführung in die biologisch aktive P=O-Form erforderlich. Nach der primär zur Anwendung gekommenen Aktivierung mit Bromdämpfen war der Nachweis der Thionophosphorsäureester im oberen Nanogramm- bzw. im unteren Microgrammbereich möglich. Damit waren allerdings die Möglichkeiten des enzymatischen Detektionsverfahrens für Thionoverbindungen im Vergleich zu den Hemmintensitäten der entsprechenden biologisch aktiven P=O-Verbindungen noch nicht ausgeschöpft.

Durch den Übergang zur Aktivierung der P=S-Verbindungen durch direktes Aufsprühen von wässrigen Bromlösungen auf die DC-Platte konnte eine bedeutende Empfindlichkeitssteigerung des enzymatischen Nachweises erreicht werden, die den Nachweis der meisten Thiono-Verbindungen im Picogrammbereich möglich machte.

Diese mit der Gaschromatographie vergleichbare Empfindlichkeit der DC-Enzymhemmtechnik ist eine wichtige Voraussetzung für den Einsatz dieser Technik zur Bestimmung von Rückstandsmengen in Lebensmitteln, besonders für die Erfassung von Rückständen im Bereich der "praktischen O-Toleranz" (p.p.b.-Bereich), in der Erfassung von Umweltverschmutzungen sowie für Untersuchungen im Bereich der Toxikologie und der Erforschung des Metabolismus der insektiziden Phosphororganika.

Es wurde weiterhin darauf verwiesen, dass der wichtige Dithiophosphorsäureester "Dimethoat" ("Rogor") mit der Bromoxidation nur mit ungenügender Empfindlichkeit erfassbar ist. Eine Empfindlichkeitssteigerung bis zur Hemmintensität der P=O-Verbindung konnte für das Dimethoat durch die Aktivierung mit UV-Licht (Bestrahlung der feuchtigkeitsgesättigten DC-Platte mit einer UV-Lampe über 15 Min) erzielt werden. Untersuchungen mit verschiedenen Strahlern zeigten allerdings, dass die Strahlenintensität und wahrscheinlich auch der Wellenlängenbereich des Strahlers die optimale Aktivierung beeinflussen.

Grosse Bedeutung besitzt die dem enzymatischen Nachweis vorausgehende Aktivierung auch für die Gruppe der primär ebenfalls biologisch inaktiven Phosphonsäureester von Typ des Trichlorphons ("Dipterex"). Durch Behandlung der DC-Platte mit einer verdünnten wässrigen Ammoniaklösung erfolgt die Umlagerung des Phosphonsäureesters in den esterasehemmenden Phosphorsäureester "DDVP" ("Dichlorphos") wodurch auch der Nachweis dieser Verbindungsklasse im Pico- bzw. unteren Nanogrammbereich möglich wurde.



Trichlorphon DDVP

Zum Schluss wurde nochmals die grosse Bedeutung der Enzymhemmtechnik als Detektionsverfahren für die DC herausgestellt.