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THIN-LAYER CHROMATOGRAPHY IN THE DETECTION OF POISONING BY PESTICIDES

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SUMMARY

The relative efficiencies of various chromogenic reagents used in thin-layer chromatography (TLC) for the detection of commonly used pesticides in India have been evaluated. Combined diagnostic techniques using two sets of chromogenic reagents on a single TLC plate are suggested for the detection of certain organochlorine and organophosphorus pesticides.

INTRODUCTION

About 50–80% of the poisons detected in toxicology cases in India (homicides, suicides and accidents as referred to by the police) are organophosphorus and organochlorine pesticides. The main chemicals involved are parathion, malathion, endrin, diazinon, etc[1]. The detection of common pesticides, therefore, is a problem frequently faced by forensic toxicologists in India. There is an extensive literature on both the qualitative and quantitative analysis of pesticides and their metabolites[2–8]. Of the methods described a variety of thin-layer chromatographic (TLC) techniques have been found to be both sensitive and economical for the detection of individual members of different groups of pesticides[3–6,9–20]. Attempts have also been made with some success to achieve the multiple detection of pesticides[3,7,9,19,21,22]. The TLC procedures available for organophosphorus and organochlorine pesticides have been re-evaluated critically for the detection of any of the common pesticides on a single plate using one or more developing reagents. The resultant method could then be routinely adopted in toxicological analysis.

EXPERIMENTAL

Chromatographic procedure

Glass plates (20 × 20 cm) were spread with a 0.25-mm layer of a slurry of silica gel in water (type I) or of silica gel–alumina (7:3) in water (type II). The plates were activated at 120° and spotted with about 2 μl of a 2% solution of aldrin, dieldrin, DDT, chlordane, endrin, lindane, malathion, parathion, diazinon, dimethoate and phosphamidon, as well as a control extract of viscera in diethyl ether. The plates were then developed to a height of 11 cm with various solvent mixtures (Tables I, II and III) and dried, and the following chromogenic reagents were used as sprays.

A (i) 0.5% silver nitrate in ethanol[23].
(ii) Expose to ultraviolet (UV) light for 1 h.

B (i) 0.2% brilliant green in acetone[24].
(ii) Expose to bromine vapour.

C (i) 0.5% rhodamine B solution followed by 10% sodium carbonate solution[3].
(ii) Expose to UV light for 15 min.

D (i) Malachite green–1% benzoperpurine–isopropanol–1 *N* sodium hydroxide (4:1:7:5)[25].
(ii) Expose to UV light for 1 h (or to sunlight until the plate becomes green).

E (i) To 0.1 g of silver nitrate dissolved in 1 ml of water, add 20 ml of 2-phenoxyethanol, dilute to 200 ml with acetone and add a drop of 30% hydrogen peroxide[13].
(ii) Expose to UV light for 1 h after heating the plate for 20 min at 110 .

F Freshly prepared 2:1 mixture of 10% zinc chloride solution in acetone and 20% diphenylamine solution in acetone[11,26].

G (i) 0.625% *o*-dianisidine in ethanol[12].
(ii) Heat the plate at 90 for 10 min.
(iii) Expose to UV light for 30 min.

H (i) 0.5% chloramine T followed by 0.5% congo red solution in 1% ethanol[9].
(ii) Heat to 110 for 10 min.

J 0.5% palladium chloride solution in 18% hydrochloric acid[27].

K 0.1% mercuric nitrate followed by 0.5% diphenylcarbazone solution in ethanol[9].

L 0.05% g of bromophenol blue in 10 ml of acetone diluted to 100 ml with 1% silver nitrate solution in 3:1 water–acetone[14].

M (i) 0.5% congo red in 1% ethanol[28].
(ii) Expose plate to bromine vapour.

N (i) 20% dimethylformamide in diethyl ether.
(ii) Heat to 100 for 5 min.
(iii) 1% tetrabromophenolphthalein ethyl ester in acetone[10].
(iv) 0.5% silver nitrate in ethanol followed by 5% citric acid solution.

O (i) 0.5% 2,6-dichloroquinone-4-chlorimide in cyclohexane[15,16].
(ii) Heat to 100 for 10 min.

P (i) Mixture of 1 ml of 0.25% fluorescein solution in dimethylformamide and 49 ml of ethanol[13].
(ii) Expose to UV light for 7 min.

Q (i) 2% *p*-nitrobenzylpyridine in acetone[17,18].
(ii) Heat at 110 for 10 min.
(iii) Spray with 10% tetraethylenepentamine in acetone.

Extraction procedure

The extraction procedure for all of the pesticides involved direct extraction with diethyl ether of a neutral homogenate of the biological material. The residue obtained on evaporation was usually suitable for direct spotting on TLC plates and did not require an intermediate clean-up procedure. Alternatively, steam distillation of the acidified viscera sample gave volatile pesticides in the aqueous distillate, from

which they were extracted with *n*-hexane. The residue after distillation was filtered and extracted with acetone to give non-volatile organochlorine pesticides.

RESULTS AND DISCUSSION

Typical results obtained with the different chromogenic reagents used for the detection of six common organochlorine pesticides (Table I) indicated that the best separation was achieved on silica gel plates with *n*-hexane–acetone (4:1) as the mobile phase. However, when silver nitrate is used as chromogenic reagent[13], a silica gel–alumina adsorbent mixture (II) gave better spots for the chlorinated pesticides. The chromogenic reagents A, B, C and D gave false positive results with the control viscera extract, in addition to their failure to detect some of the pesticides in this group. With several of the chromogenic reagents studied, UV irradiation was necessary. Although silver nitrate[13] in some form was the common chromogenic reagent with a reported sensitivity of 0.05 μg, *o*-dianisidine[12] also served to identify all members of this class. With zinc chloride–diphenylamine reagent[26], the spots were rendered visible more easily by the characteristic colour difference for each member of this group. Aldrin and lindane, however, did not respond to this reagent, but they are only infrequently encountered in toxicological cases.

The results presented in Table II show the response of five commonly used organophosphorus pesticides to twelve chromogenic reagents. Not all the reagents gave similar responses to all members of this group. Chromogenic reagent N did not respond to phosphamidon although the other members could be detected. The dark background with reagents B, L, O and P–A made it difficult to identify the spots with certainty, although all five pesticides could be detected with these reagents. *p*-Nitrobenzylpyridine (reagent Q) and *o*-dianisidine (reagent G) were, however, found to be the most suitable for diagnostic work on organophosphorus pesticides. The spots obtained were distinct and persistent on a light background and the test was claimed to be sensitive to about 0.5 μg amounts of organophosphorus pesticides[17,18]. Silica gel plates were found to be preferable in this case, while *n*-hexane–acetone (4:1) was generally suitable as the mobile solvent phase.

Several procedures and chromogenic reagents listed in Tables I and II were combined for rapid screening and multiple detection of common organochlorine and phosphorus pesticides. It was observed that organochlorine compounds were more difficult to detect; several combinations of chromogenic reagents listed in Tables I and II were tried and the significant results obtained are given in Table III. In other instances, satisfactory results could not be obtained. Silver nitrate–2-phenoxyethanol reagent (E) was sensitive for all of the pesticides, except for aldrin, on a silica gel–alumina plate (type II). The identification was difficult, however, as the background turned black when an adequate exposure to UV light was made. This drawback made this reagent less suitable for general screening work. With reagents G and G–Q, although both classes of pesticides responded fairly well, the organophosphorus spots were not distinct with G and the dark background precluded the use of G–Q. By use of zinc chloride–diphenylamine reagent followed by *p*-nitrobenzylpyridine reagent (F–Q in Table III), organochlorine pesticides appeared first with the ircharacteristic colours while the organophosphorus compounds became visible later with a purple-blue colour. A lack of response to aldrin and lindane with this reagent combination,

TABLE I

DETECTION OF ORGANOCHLORINE PESTICIDES BY TLC

Rf values are given in parentheses where relevant.

No.	*Chromogenic reagent*		*Adsorbent type*[1]	*Solvent system*	*Aldrin*	*Dieldrin*	*DDT*	*Chlordane*	*Endrin*	*Lindane*	*Control (viscera extract)*	*Colour of spots observed*
	Code	*Components*										
1	A	Silver nitrate in ethanol	I	*n*-Hexane–liquid paraffin–dioxane (7:2:1)	–	–	–	+	–	–	+	Black spots on grey background
2	B	Brilliant green	I	*n*-Hexane–acetone (4:1)	–	–	+	+	+	–	+	Light yellow spots on green background
3	C	Rhodamine B	I	*n*-Hexane–acetone (4:1)	+	–	+	+	+	+	+	Violet spots on pink background
4	D	Malachite green	I	*n*-Hexane	+	–	+	+	+	+	+	Dark green on green background
5	E	Silver nitrate–2-phenoxy ethanol	II	Acetone–*n*-heptane (2:98)	–	+ (0.76)	+ (0.75)	+ (0.75)	+ (0.38)	+ (0.38)	–	Black spots on dark brown background
6	F	Zinc chloride–diphenylamine	II	*n*-Hexane–acetone (4:1)	–	+ (0.64)	+ (0.69; 0.36)	+ (0.72)	+ (0.6)	–	–	Different colours (purple, orange, green, etc.) on light blue background
7	G	*o*-Dianisidine	I	*n*-Hexane	+ (0.81)	+ (0.81)	+ (0.89; 0.77)	+ (0.86)	+ (0.16)	+ (0.29)	–	Brown spots on dirty white background (dark spots in UV light)

[1] I = Silica gel; II = silica gel alumina (7:3).

TABLE II

DETECTION OF ORGANOPHOSPHORUS PESTICIDES BY TLC

R_F values are given in parentheses where relevant.

No.	Chromogenic reagent		Adsorbent type*	Solvent system	Malathion	Parathion	Diazinon	Dimethoate	Phosphamidon	Control (viscera extract)	Colour of spots observed
	Code	Components									
1	H	Chloramine T	I	*n*-Hexane–acetone (4:1)	–	–	–	–	–	–	–
2	C	Rhodamine B	I	*n*-Hexane acetone (4:1)	–	+	–	–	–	–	Violet spots on pink background in UV light
3	J	Palladium (II) chloride	I	*n*-Hexane–acetone (4:1)	+	–	+	–	–	–	Yellow spots on dirty white background
4	K	Mercury(II) nitrate–diphenylcarbazone	I	*n*-Hexane–acetone (4:1)	+	+	+	–	–	–	White spots on violet background
5	M	Congo red	I	*n*-Hexane–acetone (4:1)	+	+	+	–	–	–	Brown spots on light brown background
6	B	Brilliant green	I	*n*-Hexane–acetone (4:1)	+	+	+	+	+	–	Yellow spots on grey background
7	N	Tetrabromophenolphthalein ethyl ester–silver nitrate	II	Methylcyclohexane	+	+	+	+	–	–	Blue spots on yellow background
8	L	Bromophenol blue	I	*n*-Hexane–acetone (4:1)	+	+	+	+	+	–	Yellow spots that turn violet with time on dark background
9	O	2,6-Dichloroquinone-4-chloroimide	I	*n*-Hexane–acetone (4:1)	+	+	+	+	+	–	Purple on dark background
10	P–A	Fluorescein and silver nitrate	II	*n*-Hexane–acetone (4:1)	+	+	+	+	+	–	Pinkish spots in UV light White spots on brown background
11	Q	*p*-Nitrobenzylpyridine–tetraethylenepentamine	I	*n*-Hexane–acetone (4:1)	+ (0.43)	+ (0.41)	+ (0.65)	+ (0.0)	+ (0.08)	–	Purple-blue spots on light brown background
12	G	*o*-Dianisidine	I	*n*-Hexane–acetone (4:1)	+	+	+	+	+	–	White spots on light yellow background

* I = Silica gel; II = silica gel–alumina (7:3).

TABLE III

SIMULTANEOUS DETECTION OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES BY TLC

R_F values are given in parentheses where relevant.

No.	Chromogenic reagents		Adsorbent type*	Solvent system	Aldrin	Dieldrin	DDT	Chlordane
	Code	Components						
1	E	Silver nitrate–2-phenoxyethanol	I	*n*-Hexane–acetone (4:1)	–	–	–	+
2	E–P	Silver nitrate–2-phenoxyethanol and fluorescein	II	Acetone–*n*-heptane (2:98)	–	+ (0.84)	+ (0.82)	+ (0.84)
3	F–L	Zinc chloride–diphenylamine and bromophenol blue	I	*n*-Hexane–acetone (4:1)	–	+	+	+
4	F–Q	Zinc chloride–diphenylamine and *p*-nitrobenzylpyridine	II	*n*-Hexane–acetone (4:1)	–	+ (0.68)	+ (0.73)	+ (0.76)
5	G	*o*-Dianisidine	I	*n*-Hexane–acetone (4:1)	+	+	+	+
6	G–O	*o*-Dianisidine and 2,6-dichloroquinone-4-chloroimide	I or II	*n*-Hexane–acetone (4:1)	+	+	+	+
7	G–Q	*o*-Dianisidine and *p*-nitrobenzylpyridine–tetraethylenepentamine	I	*n*-Hexane–acetone (4:1)	+ (0.96; 0.32)	+ (0.96; 0.76)	+ (0.85)	+ (0.88)
8	G–Q	As for 7	II	*n*-Hexane–acetone (4:1)	+ (0.92)	+ (0.78)	+ (0.82)	+ (0.90)

* I=silica gel; II=silica gel–alumina (7:3).

however, limited its general applicability, although the technique was otherwise acceptable.

o-Dianisidine followed by *p*-nitrobenzylpyridine (G–Q in Table III) as a double spray technique on silica gel plates using *n*-hexane–acetone (4:1) as the mobile phase was found to be more useful. It revealed all of the organochlorine pesticides as brown-coloured spots on exposure to UV light and a purple-blue colour was finally obtained with organophosphorus compounds. This double spray procedure could be used as the basis for general screening work. A silica gel–alumina plate (type II), however, was not found to be satisfactory with this technique as the organochlorine pesticides sometimes tended to move with the solvent front. The thin-layer chromatogram obtained by method No. 7 (reagent G–Q in Table III) is shown in Fig. 1.

The results obtained for a given pesticide should be further confirmed by comparison with known standards using one or more of the specific chromogenic reagents already discussed.

Endrin	*Lindane*	*Malathion*	*Parathion*	*Diazinon*	*Dimethoate*	*Phosphamidon*	*Colour of spots observed*
−	−	+	+	+	+	+	Dark spots on UV exposure
+ (0.75)	+ (0.62)	+ (0.2)	+ (0.41)	+ (0.49)	+ (0.0)	+ (0.1)	E: black spots on UV exposure P: white spots on brown background (violet in UV light)
+	+	+	+	+	+	+	F: different colours L: yellow spots that turn purple on dark grey background
+ (0.61)	−	+ (0.51)	+ (0.66)	+ (0.74)	+ (0.09)	+ (0.15)	F: different colours Q: purple-blue on light brown background
+	+	+	+	+	+	+	G: light brown (violet in UV light): white on light brown background for phosphorus compounds
+	+	+	+	+	+	+	G: brown spots on dark background O: purple spots on dark background
+ (0.76)	+ (0.62)	+ (0.3)	+ (0.32)	+ (0.56)	+ (0.0)	+ (0.0)	G: brown spots on light brown background Q: purple-blue spots on light brown background
+ (0.79)	+ (0.67)	+ (0.37)	+ (0.58)	+ (0.65)	+ (0.0)	+ (0.0)	As for 7

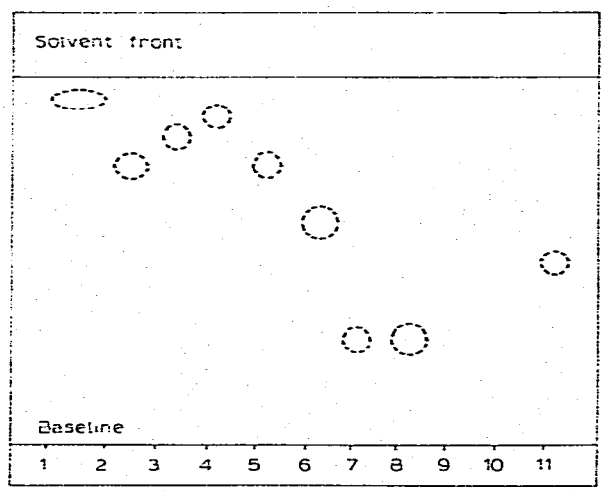

Fig. 1. Thin-layer chromatogram of six organochlorine and five organophosphorus pesticides using the double reagent G–Q (see No. 7 in Table III). 1 = Aldrin; 2 = dieldrin; 3 = DDT; 4 = chlordane; 5 = endrin; 6 = lindane; 7 = malathion; 8 = parathion; 9 = phosphamidon; 10 = dimethoate; 11 = diazinon.

CONCLUSION

Analytical techniques based on advanced gas–liquid chromatographic (GLC) equipment are increasingly becoming the methods of choice for the quantitative determination of pesticides at even nanogram levels[4,8]. The application of infrared spectrophotometry[4] as a specific characterisation technique requires a larger and purer sample, even though this can be coupled with GLC. TLC is an alternative inexpensive but sensitive technique for the rapid screening and multiple detection of pesticide residues at even 0.5 *µg* levels[6-29]. An appraisal of the existing techniques and a combination of the spray reagents has resulted in a rapid diagnostic survey of pesticides commonly involved in human poisoning in India. Considering that a large volume of work has to be carried out by the toxicologist in this field, a simpler and more economical technique is of great use for preliminary screening, which can be further supported by other qualitative and quantitative techniques.

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