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Detection and quantitative determination of certain chlorinated pesticides by micro-thin layer chromatography

An appreciable amount of work[1-5] has been carried out to evaluate the quantity of a chemical on a thin-layer chromatogram by employing the relationship that exists between the weight of the compound in the located spot and the size, density of coloration (visual or photometric) or radioactivity of the spot. The measurement of spot area avoids the difficulties associated with the elution of the material from the adsorbent and the possibility of further pollution of the purified sample.

PURDY AND TRUTER[6,7] found a linear relationship between the square root of the area and the logarithm of the weight of the compound, while OSWALD AND FLUCCK[8] observed in many cases that the area–weight relationships were not linear. A limiting sensitivity method is employed for the quantitative estimation of aflatoxins in groundnut[9] and ascorbic acid in potato tubers[10].

The limits allowed for various pesticidal residues in foods under various food laws vary from 0.1 to 10 p.p.m. To estimate these low levels sophisticated and costly methods such as gas–liquid chromatography, infrared spectroscopy and mass spectroscopy are necessary. The present investigation envisages the possibility of utilizing a combination of spot diameter and visual extinction methods on micro thin-layer chromatography (micro-TLC) slides for the rapid detection and quantitative estimation of certain chlorinated insecticides, both in the pure state and in treated materials.

Experimental

Insecticides. All the insecticides screened were from E. Merck A.G., Darmstadt, G.F.R.

Chromatoplate. Microslides of 2.5 × 7.5 cm size were used instead of the conventional 10 × 20-cm TLC.

Preparation of micro-TLC plates. Silica Gel G (National Chemical Laboratory, Poona, India) (40 g) was made into slurry with 100 ml of chloroform and transferred into a glass container (diameter 3.5 cm; height, 10 cm). The microslide was dipped into the slurry and immediately taken out to dry in front of a fan. The silica gel coating on one side of the plate was removed and the plates were kept overnight over anhydrous calcium chloride in an air-tight desiccator. This technique of preparation of micro-TLC slides is simple, rapid and does not require activation, in comparison with the conventional method.

Spotting. Graded concentrations of each pesticide under test (10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05 and 0.02 μg) were spotted on the micro-TLC plate using a Lamda-pipette or standard nanogram microcapillaries.

Chromogenic reagent. 1% *o*-tolidine dissolved in acetone was sprayed over the spotted micro-TLC plates and the solvent was evaporated. Coloured spots of various intensities appeared when the plates were exposed to sunlight.

Limiting sensitivity technique for semi-quantitative estimation. For a rapid semi-quantitative determination of pesticide present in thin-layer spots, a visual extinction method was followed by running a series of spots of standard pesticide solution and

keeping a record of the least quantity made visible by the chromogenic reagent or by a 5-min exposure to iodine vapour in a closed chamber.

Diameter of the spot. The diameter of the spot was measured by placing a sheet of transparent paper over the chromatogram and tracing the outline. The mean value of the four measurements of the spot diameter was taken for the calibration curve.

Clean-up procedure. Wheat samples, treated with pesticides, were powdered in a grinder. Hexane was added and the mixture was shaken thoroughly and filtered. The hexane washing was repeated four times and the filtrate was concentrated to about 10 ml. To the hexane concentrate, 5 ml of acetonitrile was added, the mixture was shaken thoroughly and the acetonitrile layer was separated. This extraction was repeated three times and the acetonitrile fraction was treated with 0.5 g of activated animal charcoal to remove the colouring material. The solution was filtered and made up to a known volume for chromatographic analysis.

Results and discussion

The investigations conducted in earlier work have revealed[11] that micro-TLC can advantageously be utilized in detecting and estimating pesticides by visual extinction techniques, and is simple, economical and rapid compared with conventional TLC.

For a rapid, quantitative determination of pesticides present in thin-layer spots, a visual extinction method combined with the diameter of the spot and the spot intensity was used. KATZ[12] detected certain chlorinated pesticides on thin-layer chromatograms from the different colours produced when a mixture consisting of 0.5 g of diphenylamine and 0.5 g of zinc chloride in 100 ml of acetone was sprayed on, with subsequent heating of the plate to about 200°. Sensitivities claimed by the author were captan 3–4 μg, methoxychlor and chlor-DDT 1 μg, and DDT and toxaphene 2 μg. In order to avoid the use of a mixed spray reagent and subsequent heating to a temperature as high as 200°, the use of *o*-tolidine as a spray reagent was studied on micro-TLC plates spotted with various chlorinated pesticides. The different colours produced with various quantities of pesticides are shown in Table I. These different colours offer an easy method of detection of the common chlorinated pesticides in

TABLE I

QUANTITATIVE DETERMINATION OF CHLORINATED PESTICIDES BY VISUAL COMPARISON OF SPOTS

Pesticide	*Minimum quantity detectable (μg)*	*Colour of spot at the 0.02–0.2 μg level*	*Colour of spot at the 0.2–2.5 μg level*	*Colour of spot at the 2.5–10 μg level*
DDE	0.3	Yellow	Faint buff	Dark buff
DDT	0.02	Yellow	Greenish yellow	Leafy green
DDD	0.25	—	Very faint brown	Clear brown
Lindane	0.02	Faint blue	Clear blue	Dark blue
Aldrin	0.25	—	Very faint yellowish green	Pale yellowish green
Endrin	0.15	—	Very faint pink	Clear pink
BHC	0.02	Very faint blue	Clear blue	Dark blue
Captan	0.015	Very faint bottle green	Clear bottle green	Dark bottle green

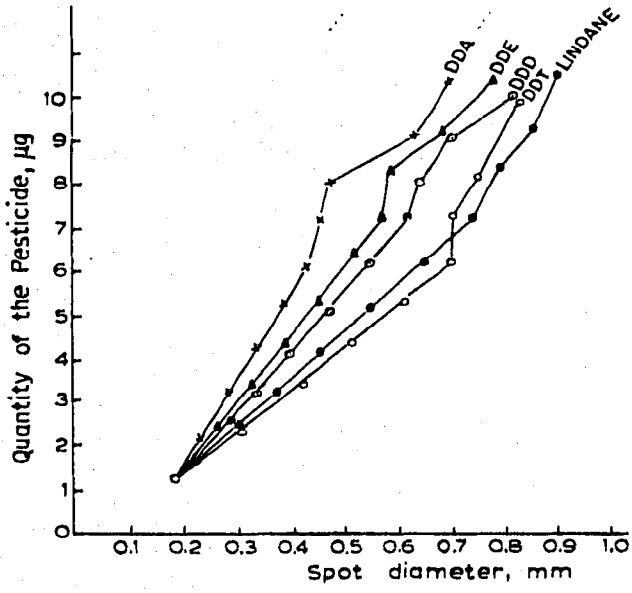
a one-step process. In order to further simplify the procedure and save time, the detection and estimation were investigated on micro-TLC plates. A series of spots of known concentrations was run on the micro-TLC plate and the minimum quantity of each pesticide detectable with the chromogenic reagent was recorded. In the case of DDT, lindane, captan and BHC, the minimum quantity detectable by visual extinction was in the range 0.015 to 0.02 μg (Table I). Aldrin, dieldrin, endrin, DDD, DDA and DDE could be detected at the 0.3 μg level and above. The higher sensitivity of DDT, BHC, lindane and captan is due to the more labile chlorine atoms in these molecules[13] compared with DDD, DDE, DDA, aldrin, dieldrin and endrin. The concentration range in which each pesticide is present can also be assessed by the different shades of colour produced on the micro-TLC plate. In the case of DDT, a yellow spot appeared between 0.02 to 0.2 μg, greenish yellow in the range 0.25 to 2.5 μg and leafy green between 2.5 and 10 μg. Similarly, lindane, captan, BHC, DDD, etc., can be detected by the various colours and estimated semi-quantitatively by the various shades of colour produced depending on the concentration (Table I). The blue colour produced by lindane and BHC can be further distinguished on the micro-TLC by the appearance of 4–5 blue spots in the case of BHC and a single blue spot with lindane, when developed in hexane and sprayed with *o*-tolidine solution.

TABLE II

DETERMINATION OF PESTICIDE IN KNOWN SAMPLES BY VISUAL EXTINCTION AND SPOT DIAMETER METHOD

Pesticide	*Quantity taken (mg)*	*Visual extinction method*		*Spot diameter method*	
		Quantity determined (mg)	*Error (%)*	*Quantity determined (mg)*	*Error (%)*
Lindane	(a) 25.0	23.7	5	23.9	4.6
	(b) 75.0	72.1	4	70.8	5.6
DDT	(a) 25.0	23.9	4	23.6	5.8
	(b) 75.0	71.5	5	71.8	4.2
BHC	(a) 50.0	47.9	4	47.3	5.3
	(b) 100.0	96.6	4	95.4	4.4
Captan	(a) 25.0	24.2	3	23.8	4.8
	(b) 75.0	72.2	4	71.2	5.1

The quantitative method of relating spot size to the quantity of compound present was studied next. Since the spot size varied with the thickness of the silica gel layer, particle size, activity of the absorbent, etc., the use of chloroform instead of distilled water to prepare the silica gel slurry was studied. By this technique, many of the factors which affect the spot size were largely eliminated. In order to increase the reproducibility and accuracy of the method, the technique of spotting the pure pesticide solution on a micro-TLC slide with a standard nanogram capillary (300 μm diameter) was followed. This procedure gave distinct and well defined circular spots. In order to obtain circular spots, care was taken that the entire volume of the spotting solution was delivered from the micro-pipette at one time, as a second spotting with fresh solution affected the size and diameter of the spot.



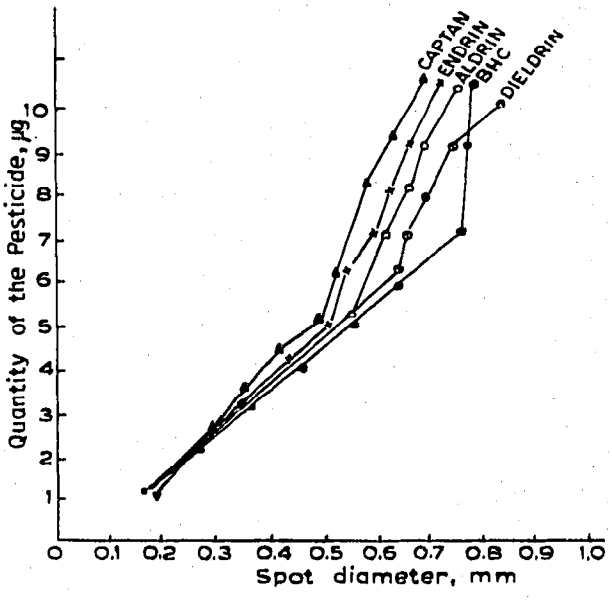


Fig. 1. Relation between spot diameter and quantity of pesticide present for DDA, DDE, DDD, DDT and lindane.

Fig. 2. Relation between spot diameter and quantity of pesticide present for captan, endrin, aldrin, BHC and dieldrin.

The quantity of the pesticide in relation to the area of the spot was studied using DDT. In the case of DDT, a straight-line relationship was observed from 1 to 6 μg, corresponding to a spot diameter from 0.22 to 0.7 cm (Fig. 1). The other analogues of DDT, such as DDD, DDE and DDA, exhibited the same straight-line relationship between 1 to 7 μg, corresponding to a diameter of 0.2 to 0.6 mm. Aldrin, dieldrin and endrin obeyed a straight-line relationship between 0.1 and 0.6 mm and captan between 0.2 and 0.5 mm (Fig. 2). In general, a linear relationship was observed in all cases from 1 to 4 μg and then a deviation slowly increased from the cyclodine group of pesticides to the lindane and DDT series. However, there was no correlation between the spot diameter and the molecular weight of the pesticide

TABLE III

ESTIMATION OF CERTAIN CHLORINATED PESTICIDES IN DUST FORMULATIONS

Pesticide	*Quantity added (mg)*	*1% dust estimated by*		*Quantity added (mg)*	*5% dust estimated by*		*Quantity added (mg)*	*10% dust estimated by*	
		Visual extinction method (%)	*Spot diameter method* (%)		*Visual extinction method* (%)	*Spot diameter method* (%)		*Visual extinction method* (%)	*Spot diameter method* (%)
DDT	10	95	94	50	96	95	100	95	95
Lindane	10	95	95	50	96	95	100	98	96
BHC	10	95	94	50	97	96	100	98	96
Aldrin	10	—	—	50	96	95	100	96	95
Endrin	10	95	94	50	96	94	100	98	95

screened. While examining the variation in the spot size of each of these pesticides, an optimum constantly linear region of sample size was found above which the spot size decreased greatly as the amount of substance present increased. On the other hand, below this level, the small size of the spot greatly increased the errors. These observations agree with the findings of OSWALD AND FLUCCK[14] who investigated the optimum region of sample size for the alkaloids (3–4 μg).

In order to check the reproducibility and accuracy of the visual extinction and spot diameter methods, determinations were carried out on known quantities of pure pesticide samples (Table III).

The error in the visual extinction method varied between 3 and 5 % compared with 4–5.7 % in the spot diameter method. When the method was applied to certain pesticide dust formulations, after the usual clean-up technique, the results revealed that the pesticides can be recovered and estimated within 6 % error (Table III).

Wheat samples treated with lindane, DDT and captan were estimated by the above methods with the necessary clean-up technique. The recovery of pesticides was in the range 90–92 % (Table IV).

TABLE IV

PESTICIDE RESIDUE ESTIMATIONS IN TREATED WHEAT SAMPLES

Pesticide	*Visual extinction method*		*Spot diameter method*	
	Weight of pesticide added (mg)	*Weight of pesticide recovered (mg)*	*Weight of pesticide added (mg)*	*Weight of pesticide recovered (mg)*
Lindane	50	48	50	46
DDT	50	45	50	44
Captan	50	48	50	47

The different colours produced by DDT, Lindane, BHC, and the cyclodine group of insecticides offer an easy method of distinguishing various insecticides on micro-TLC with the chromogenic agent. This technique will be of great advantage in field work and routine analysis.

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Central Food Technological Research Institute,
Mysore 2A (India)

K. VISWESWARIAH
M. JAYARAM

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