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Note

Detection limits for some carbamate and phenylurea pesticides by high-performance thin-layer chromatography

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The literature contains a number of papers dealing with the thin-layer chromatography (TLC) of one or more of the pesticides considered here (carbaryl, propoxur, aldicarb and diuron)[1–14,17,19,20] but in none of these is the use of high-performance (HP) plates reported. Of the pesticides listed there is very little information on the TLC detection of aldicarb other than by the enzyme inhibition technique. The object of this contribution is to report the lowering of detection limits by improving on current detection techniques in conjunction with the use of HPTLC silica gel plates.

EXPERIMENTAL

HPTLC plates were obtained from E. Merck (Darmstadt, G.F.R.) and cleaned in acetone and activated at 105° for 20 min before use. Plates with and without a fluorescent indicator were used.

Pure reagents were used in the experiments, details of which are to be found under the respective detection method.

Details of the pesticides studied are as follows:

Name (purity)	*Chemical formula*	*Supplier*
Carbaryl (99.9%)	1-Naphthyl methylcarbamate	Union Carbide (New York, N.Y., U.S.A.)
Propoxur (98.2%)	Isopropoxyphenyl methylcarbamate	Bayer (Leverkusen, G.F.R.)
Aldicarb (99%)	2-Methyl-2-(methylthio) propionaldehyde O-methylcarbamoyloxime	Union Carbide
Diuron (98%)	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	Bayer

Alternative names for the pesticides include Sevin (for carbaryl), Baygon and Bayer 39007 (for propoxur), Temik (for aldicarb) and Karmex (for diuron).

Working solutions of the pesticides with concentrations of 1000, 250 and 20 ng/μl were prepared in acetone (Analar; BDH, Poole, Great Britain) and were

spotted on the thin-layer plates using Pt–Ir tipped micropipettes, with volumes of 100 and 200 nl, obtainable from Camag (Muttenz, Switzerland).

A dual wavelength UV lamp (254 and 350 nm) available from Camag was used.

Chromatography

The spotted HPTLC plates were developed in chloroform (Merck, pro analysi) in saturated (paper-lined) beakers with ground glass tops. Approximate R_F values for the pesticides were as follows: carbaryl 0.28, propoxur 0.18, aldicarb 0.19, diuron 0.10.

The developed plates were thoroughly air-dried before detection of the pesticides.

Detection methods and results

Six different detection methods were carried out on all four pesticides involving the use of UV quenching, NaOH, $AgNO_3$, *p*-nitrobenzenediazonium tetrafluoborate, fisetin and enzyme inhibition, details for which are given below.

(1) UV quenching[1]. The pesticides were spotted on plates containing a fluorescent indicator (F254) and viewed under 254-nm UV light. All the pesticides appeared as dark spots. Detection limits (Table I) are given for the unaided eye and when using a Vitatron TLD 100 densitometer (Vitatron, Dieren, The Netherlands) in the fluorescent mode with excitation and emission wavelengths of 280 and 546 nm, respectively and a signal-to-noise ratio of 2.5:1.

TABLE I

DETECTION LIMITS FOR CARBARYL, PROPOXUR, ALDICARB AND DIURON ON HPTLC SILICA GEL PLATES (RESULTS IN ng)

Detection at maximum spotting of 1 μg. 1 = UV quenching; 2 = NaOH treatment; 3 = $AgNO_3$ treatment; 4 = *p*-nitrobenzenediazonium tetrafluoborate treatment; 5 = fisetin treatment; 6 = enzyme inhibition experiment. 1A = unaided eye; 1B = Vitatron densitometer; 4A = ethylene glycol; 4B = diethylene glycol; 5A = with bromine treatment; 5B = without bromine treatment. — = not detected.

Pesticide	*Method*								
	1A	*1B*	*2*	*3*	*4A*	*4B*	*5A*	*5B*	*6*
Carbaryl	100	30	6	12	25	12	25	200	0.5
Propoxur	400	100	—	—	200	12	25	200	3
Aldicarb	200	800	—	25	—	—	25	200	0.3
Diuron	8	75	—	15	—	—	10	150	—

The intensity of the quenched spots does not fade significantly over a period of several days.

(2) NaOH treatment. Only carbaryl was detected using this method (at the maximum spotting of 1 μg per pesticide used).

Reagents. NaOH (Merck, pro analysi) dissolved in double-distilled water to yield a 1 *N* solution.

Detection method[2] *and result.* The thin-layer plate was sprayed with 1 *N* NaOH, immediately covered with a glass slide and then observed under 350-nm UV light. The larger spots showed up almost immediately and remained visible for more than a day, but the smaller ones took 5–10 min before attaining maximum intensity and faded completely after 2 h. Examination under UV light took place with the cover glass on the plate as the act of its removal before complete conversion of carbaryl to the highly fluorescent 1-naphthol produced spot spreading.

The method detected 6 ng of carbaryl (as 1-naphthol) but, although sensitive, was somewhat erratic.

(3) $AgNO_3$ treatment. A variation of the method of Finocchiaro and Benson[3] was used with all the pesticides except propoxur being detected.

Reagents. $AgNO_3$ (Merck, pro analysi); H_2O_2 (30%, Cica, Kanto Chemical, Tokyo, Japan); H_2O (double-distilled); acetone (for analysis and chromatography, Riedel-de Haen, Seelze-Hannover, G.F.R.); HPTLC plates without fluorescent indicator.

Chromogenic agent. A 0.10-g amount of $AgNO_3$ dissolved in 1 ml H_2O, 10 ml 2-phenoxyethanol added and diluted to 200 ml with acetone. One drop H_2O_2 added. Stored in an amber bottle and prepared fresh every few days.

Detection method and results. The usual method is to spray the plate with the chromogenic agent, dry and then expose to 254-nm UV light until the pesticides appear as dark spots. Using this approach, however, often required 1–2 h before the spots reached maximum intensity, the time taken not being reproducible from one experiment to the next. A marked improvement in this situation was obtained by heating the plates at 108° for 10 min after spraying, and then viewing under 254-nm UV light. In this way the pesticides showed maximum intensity within 10 min of being heated.

The resulting spots maintained their intensity for at least a week when stored in the dark.

(4) p-Nitrobenzenediazonium tetrafluoborate treatment. Variations on the methods of Finocchiaro and Benson[3], Nagasawa *et al.*[1] and Henkel[4] were employed with carbaryl and propoxur only being detected.

Reagents. p-Nitrobenzenediazonium tetrafluoborate (Merck); ethylene glycol (Merck); diethylene glycol (Merck); ethanol (pro analysi, Merck); KOH (84%, Merck); HPTLC plates without fluorescent indicator.

Chromogenic agent. A saturated solution was prepared as follows: 25 mg of *p*-nitrobenzenediazonium tetrafluoborate was added to 100 ml of 10% diethylene glycol (or ethylene glycol) in ethanol, stirred for a few minutes and filtered. The solution was stored in a refrigerator and kept cold during use.

Detection methods and results. Four different experiments were performed as follows: (1) Chromogenic agent using ethylene glycol[1]. The thin-layer plate was sprayed with a 1 *N* KOH solution (in 95% ethanol), followed immediately by spraying with the chromogenic agent. The plate was then heated at 105° for 20 min and exposed to 254-nm UV light for 1–2 h. All the pesticides except aldicarb appeared as faint yellow spots which could be seen more clearly by holding the plate up to the light. The spots maintained their intensity for at least several days. The detection limits are recorded in Table I. (2) Experiments 2–4 were carried out using diethylene glycol to prepare the chromogenic agent. The thin-layer plate was dipped in the chromogenic

agent (rapid action) for 5 sec, dried for 15 min in a dark hood, then dipped in a 95% ethanolic 1 *N* KOH solution. No spots appeared on the plate at the 1-μg level even on subsequent heating at 105°. After several hours carbaryl appeared at the 1-μg level as a faint yellow spot which lasted for at least a few days. (3) The same procedure was followed under 2 above except that the plate was finally dipped in an aqueous 1 *N* NaOH solution. Carbaryl and propoxur showed up immediately as blue and rose pink spots which faded after 1 h. (4) The order of dipping was the reverse of that in 3 above. Carbaryl and propoxur showed immediately as intense blue and rose coloured spots respectively which faded only slightly with time. Detection limits are recorded in Table I.

(5) Fisetin treatment. A variation on the procedure of Frei *et al.*[5] was followed, with all four pesticides being detected.

Reagents. Fisetin (3,3′, 4′, 7-tetrahydroxy-flavone; Fluka, Buchs, Switzerland); carbon tetrachloride (pro analysi, Merck); bromine (Br_2) (Merck); ethanol (pro analysi, Merck); HPTLC plates without fluorescent indicator.

Chromogenic agent. A 0.05% solution of fisetin in ethanol.

Detection method and results. The thin-layer plate was heated for 5 min at 105°, then placed (while hot) in a tank containing bromine vapour for 10–20 sec (Frei *et al.*[5] use a 10% bromine in carbon tetrachloride solution but experimentation showed bromine alone to be more effective). The plate was dipped in the fisetin solution for 10 sec, followed by heating in an oven at 105° for 15 min, then exposure to 254-nm UV light.

Somewhat variable results were found in experimentation, the spots appearing immediately or taking up to one hour to appear. Diuron showed as a dark spot under UV light and was visible in daylight as a violet colour in contrast to the other pesticides which were not visible in daylight. The other pesticides appeared as white spots on a yellow-green background under UV light. All pesticides were somewhat more visible under 350-nm UV light due to a slightly lighter background. The spots faded only slightly over a period of several days.

Detection limits are recorded in Table I, both with and without the use of bromine.

(6) Enzyme inhibition experiment. The procedure of Gardner[6] worked very well for the carbamate pesticides but, as expected, diuron was not detected. This was the most sensitive of the six methods tried.

Reagents. Horse serum cholinesterase (Sigma, St. Louis, Mo., U.S.A.); tris-(hydroxymethyl)aminomethane (Merck, LAB); indoxyl acetate (BDH); acetone (Riedel-de Haen).

Preparation of chromogenic agents. Cholinesterase solution: 50 mg horse serum dissolved in 100 ml 0.5 *M* Tris buffer solution (made up in double-distilled water). Solutions kept refrigerated when not in use. Enzyme substrate: 0.3% indoxyl acetate in acetone prepared fresh immediately before use.

Detection method and results. The thin-layer plate was dipped in the cholinesterase solution for 10 sec (the dipping action must be rapid), then immediately placed flat in an empty, but closed, development chamber (laid on its side) for 30 min. Subsequent dipping in the enzyme substrate solution for 10 sec (rapid action) was followed by observation under 350-nm UV light. Initially the carbamate pesticides were visible as dark spots on a dark purple background (they were not visible in

daylight at this stage). As the background colour faded to a lighter purple the pesticides appeared as white spots on a light blue background in daylight (this took 5–10 min). The detection limit for the initial spots was lower than when they later appeared in daylight.

If kept in the dark the white spots last for at least a week without significant fading.

DISCUSSION

The modifications described here, used in conjunction with HPTLC plates, have lowered the detection limits on published data for several of the methods employed (Table II). The various methods used to detect carbamate pesticides in general have been shown to be equally applicable in most cases to aldicarb.

TABLE II

COMPARISON OF DETECTION LIMITS RECORDED HERE (A) WITH THOSE FROM OTHER PUBLICATIONS (B) (RESULTS IN ng)

1 = UV quenching; 2 = NaOH treatment; 3 = $AgNO_3$ treatment; 4 = *p*-nitrobenzenediazonium tetrafluoborate; 5 = fisetin treatment; 6 = enzyme inhibition experiment. — = not detected at the evel considered; × = not determined; 6B = use of bee-head esterase as enzyme source.

Pesticide	*Method*											
	1A	*1B*[1]	*2A*	*2B*[2]	*3A*	*3B*[1]	*4A*	*4B*[1]	*5A*	*5B*[5,7]	*6A*	*6B*[8]
Carbaryl	100	100	4	6	12	10	12	20	12	10	0.5	0.1
Propoxur	400	5	—	×	—	—	12	50	×	×	3	0.5
Aldicarb	200	×	—	×	25	×	—	×	25	×	0.3	5
Diuron	8	10	—	×	15	50	—	—	10	×	—	×

Of the methods discussed those involving the use of enzyme inhibition, *p*-nitrobenzenediazonium tetrafluoborate, $AgNO_3$ and UV quenching gave the most reproducible results for the carbamates. The last of these methods is not widely used because it lacks the sensitivity necessary for normal residue work. The reverse applies to the first two methods: enzyme inhibition has been used to detect carbamates at the nanogram to picogram level in vegetables and fruits[8], plant extracts[9], on sprayed surfaces[10] and in vegetables[11]; and *p*-nitrobenzenediazonium tetrafluoborate at similar levels in apples and lettuce[12] (carbaryl 0.10 ppm), in fruit and vegetables[13] (carbaryl 0.05–0.2 ppm) and in apples and vegetables[14] (carbaryl 0.02–0.03 ppm). Detection limits employing TLC compare favourably with methods using high-performance liquid chromatography[15,16], and the use of HPTLC should improve the situation further. The $AgNO_3$ method matches *p*-nitrobenzenediazonium tetrafluoborate as regards sensitivity but is not as selective being able to detect most classes of pesticides.

The enzyme inhibition experiment was carried out using horse serum cholinesterase but latest work[8] indicates bee-head esterase to be more sensitive in general. With the greater commercial availability of bee-head esterase, detection limits for the carbamates should be even lower than reported here, *cf.* the figure of 3 ng reported here for propoxur with 0.5 ng using bee-head esterase. Many authors[6,8]

report the use of bromine, and less seldom UV light, to oxidize the pesticides prior to detection with a chromogenic agent, but in general their use has been shown[17] to reduce the ability of the pesticides to inhibit the enzymes used. Carbaryl is one exception where an appreciable increase in sensitivity is obtained[17].

The only phenylurea pesticide to be considered here, diuron, is usually determined at the residue level by gas chromatography[18] (0.5–0.05 ppm). TLC has, however, been used to detect herbicide residues in soils and waters[19] (1 and 0.1 ppm, respectively) and grapes[20] (0.1 ppm) using chromogenic agents which appear to be less sensitive than those used here.

TLC is often used for identification or preparative work but seldom as a quantitative technique in pesticide residue work. It would appear that quantitation is the most uncertain factor in applying TLC but the more general availability of sensitive densitometers should help to change the situation.

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