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Note

Thin-layer chromatographic technique for the separation and identification of carbamate pesticides in post mortem material

S. N. TEWARI and RANJEET SINGH
Toxicology Division, Chemical Examiner's Laboratory, Agra (India)
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Carbamic acid esters are highly toxic to mammals[1–3]. Carbamate pesticides, being cholinesterase inhibitors, differ from organophosphorus insecticides in that they induce inhibition more rapidly and produce the symptoms of poisoning more quickly. The extensive use of carbamate pesticides in agriculture has resulted in accidental, suicidal and homicidal deaths in recent years.

Different techniques for the isolation[4–6], identification and detection by thin-layer chromatography (TLC)[7–10] and determination by various techniques such as gas–liquid chromatography[11–13] in tissues and biological materials have been reported. The identification of carbamate pesticides by TLC has also been described[14]. However, no systematic work has yet been reported for the analysis of carbamate pesticides from autopsy materials. We have therefore developed a technique for the isolation and clean-up of carbamate pesticides and for their detection and identification using TLC.

The following nine common carbamate pesticides were studied:

(1) Aldicarb (Temik; 116-06-3), 2-methyl-2-(methylthio)propionaldehyde; (2) Baygon (Bayer 39007), 2-isopropoxyphenyl N-methylcarbamate; (3) Carbaryl (Sevin; experimental insecticide 7744), 1-naphthyl N-methylcarbamate; (4) Carbofuran =Furadan, Rallis India Ltd.), 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; (5) Lannate, N-[(methylcarbamoyl)oxy]thioacetimidic acid; (6) Mancozeb, manganese ethylenebis(dithiocarbamate); (7) Thiram (Thiured; Arasan; TMTD), tetramethylthiuram disulphide; (8) Ziram (Milban; Fuklasin; zerlate), zind bis-(dimethyldithiocarbamate); (9) Zineb (Dithane Z-78; Parzate), Zinc ethylenebis-dithiocarbamate.

In this paper we describe the isolation and detection of these carbamate pesticides in autopsy tissues by TLC.

EXPERIMENTAL

Materials

Analytical-reagent grade reagents and chemicals were obtained from BDH (Poole, Great Britain). As controls, recrystallized technical-grade carbamate pesticides were used.

Extraction

Twenty grams of tissue (*e.g.*, stomach, liver, kidney, spleen) were macerated into a fine slurry and transferred into a conical flash, 30 g of anhydrous sodium sulphate and 50 ml of acetonitrile were added and the flask fitted with a condenser, was heated on a water-bath. After 30 min the contents of the flask were cooled and filtered. The process was repeated twice with 25-ml portions of acetonitrile. The filtered acetonitrile portions were combined and transferred into a separating funnel, 100 ml of distilled water and 30 ml of a saturated solution of sodium sulphate were added and the solution was extracted for 5 min with three 10-ml volumes of chloroform (25 shakes per min). The extracted chloroform layers were separated, combined and filtered through a layer of anhydrous sodium sulphate. This solution was then concentrated to 2 ml on a water-bath at 80° and then dried with a current of dry air. The residue was dissolved in 1 ml of acetone.

Method

Glass plates (20 × 20 cm) coated with a 0.25-mm layer of silica gel G and activated at 110° for 1 h were used.

An aliquot (10 μl) of the extracted residue in acetone was spotted on a TLC plate together with control carbamate pesticides and the plate was developed in a TLC chamber containing the solvent system benzene–ethyl methyl ketone (9:1) (1 h saturation). When the solvent front had moved 10 cm, the plate was removed, dried at room temperature and then viewed under UV light (254 nm). The plate was sprayed with a 1% ethanolic solution of Fast Blue B, dried for 30 min and then sprayed with 20% sodium hydroxide solution.

RESULTS AND DISCUSSION

The R_F values and the colours of the spots are given in Table I, and indicate that the solvent system used provides a clear separation of all nine carbamate pesticides. Several other solvent systems were also tried, but none gave a satisfactory resolution of all nine carbamate pesticides.

TABLE I

R_F VALUES AND COLOURS OF THE SPOTS OF CARBAMATE PESTICIDES OBTAINED USING THE SOLVENT SYSTEM BENZENE–ETHYL METHYL KETONE (9:1)

No.	*Pesticide*	$R_F \times 100$	*Colour of spots*		
			In UV light (254 nm)	*With Fast Blue B*	*With 2,6-dibromoquinone chlorimide*
1	Aldicarb	63	Dark	Brown	Yellow
2	Baygon	33	Dark	Red	Grey
3	Carbaryl	42	Dark	Purple	Steel grey
4	Carbofuran	47	Dark	Reddish orange	Brownish yellow
5	Lannate	54	Dark	Brown	Steel grey
6	Mancozeb	57	—	Reddish brown	Green
7	Thiram	23	Dark	Orange	Yellow
8	Ziram	69	Dark	Orange-red	Bluish yellow
9	Zineb	70	—	Brown	Yellow

In addition to Fast Blue B, ten other chromogenic reagents were tried as spray reagents. Of these, 0.5% 2,6-dibromoquinone chlorimide in dimethylformamide (followed by heating the TLC plate for 1 h in a hot chamber at 80°) was found to be a sensitive spray reagent and hence the colours the spots with this reagent are also incorporated in Table I. However as reported earlier for chlorinated pesticides[15], in the present study some false spots were obtained for tissue extracts.

The limit of detection was *ca.* 0.5 μg of the carbamate pesticides, and control experiments showed that the recovery of the pesticides by the proposed method was 90–95%.

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REFERENCES

1 R. Gasser, *Ber. Schweiz. Bot. Ges.*, 62 (1952) 66.
2 M. Vandekar; R. Plestina and K. Wilhelm, *Bull. W.H.O.*, 44 (1971) 241.
3 S. N. Tewari, *Pesticides in Suicides and Homocides, Proceedings of the Chemistry Section of 58th Session of the Indian Science Congress, Bangalore, Jan. 1971*, Indian Science Congress Association, Calcutta, 1971, p. 5.
4 T. I. Golubev and V. A. Volkova, *Vopr. Pitan.*, 26 (6) (1967) 64.
5 G. C. Morrison and W. F. Durham, *J. Amer. Med. Ass.*, 216 (1971) 298.
6 M. A. Klisenko, *Zh. Anal. Khim.*, 20 (1965) 634.
7 B. Mikolajczak, *Zesz. Nauk. Akad. Roln. Wroclawiu, Weter.*, 31 (1974) 153.
8 O. M. Malinin, *Kormov, Vnesh. Sredy, 2nd*, (1971) 172; *Ref. Zh., Khim.*, Abstr. No. 11 N 410 (1972).
9 A. Mikan, *Nippon Hoigsku Zasshi*,. 27 (1973) 316.
10 O. A. Malinin, *Veterinariya (Moscow)*, (1974) 104.
11 N. C. Jam, C. R. Fontan and P. L. Kirk, *J. Pharm. Pharmacol.*, 17 (1962) 362.
12 F. J. Biros and A. C. Walker, *J. Agr. Food Chem.*, 18 (1970) 425.
13 J. Wozniak and A. Tokarz, *Farm. Pol.*, 31 (1975) 589.
14 S. N. Tewari and R. Singh, *Z. Anal. Chem.*, (1978) in press.
15 S. N. Tewari and I. C. Sharma, *J. Chromatogr.*, 131 (1977) 275.