Journal of Chromatography, 365 (1986) 309–314
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 916

SELECTIVITY AND SENSITIVITY OF SOME THIN-LAYER CHROMATOGRAPHIC DETECTION SYSTEMS

K. FODOR-CSORBA* and F. DUTKA
Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest (Hungary)

SUMMARY

The selectivity and sensitivity of some thin-layer chromatographic detection systems widely used internationally and developed in our laboratory were studied. Halogenated organophosphorus pesticides were found to interfere with the detection of organochlorine pesticides when using silver nitrate–2-phenoxyethanol. The stability of colours formed by the 4-(4′-nitrobenzyl)pyridine–tetraethylenepentamine system was enhanced by spraying with acetic acid and allowed densitometric evaluation. The most sensitive detection method for thiocarbamates is the reaction with 2,6-dichlorobenzoquinone-N-chloroimine or its dibromo analogue (50 ng). Application of this method for sulphur-containing organophosphorus insecticides results in the same or better sensitivity. Quantitation of these compounds was carried out by densitometry.

INTRODUCTION

For the analysis of pesticide residues in foods[1] the application of at least two alternative methods is required. The preliminary estimation is usually made by the quick and simple thin-layer chromatography (TLC), and the official, final-action determination is carried out by gas or liquid chromatography. If the detection system is not sufficiently selective, some compounds may interfere with the determination of the other pesticides in the sample studied.

The method of the Association of Official Analytical Chemists (AOAC)[2] for the determination of the residues of organochlorine (OC) and organophosphorus (OP) insecticides is an internationally used multiresidue procedure. We have extended this AOAC method to the determination of residues of thiocarbamate herbicides in food of plant origin[3] and developed a new and sensitive TLC detection by 2,6-dichlorobenzoquinone-N-chloroimine[4] (DCBC).

The present paper deals with the selectivity and sensitivity of three detection systems, (1) silver nitrate–2-phenoxyethanol; (2) 4-(4′-nitrobenzyl)pyridine[5] (NBP); 2,6-dibromobenzoquinone-N-chloroimine (DBBC) and its analogue (DCBC), for the visualization of OC and OP insecticides and thiocarbamate herbicides.

0021-9673/86/$03.50

EXPERIMENTAL

Standards

Lindane(1α,2α,3β,4α,5α,6β-hexachlorocyclohexane), pp'DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], pp'DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] and Methylparathion [O,O'-dimethyl O''-(*p*-nitrophenyl) phosphorothioate] were from Supelco (Gland, Switzerland). Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethylphosphate), Dimethoate (O,O'-dimethyl S-methylcarbamoylmethyl phosphorodithioate) and Fenthion [O,O'-dimethyl Ö-(3-methyl-4-methylthiophenyl) phosphorothioate] were obtained from Farmitalia Carlo Erba (Milan, Italy). Chlorfenvinphos [2-chloro-1-(2',4'-dichlorophenyl) vinyl diethyl phosphate] and Trichlorphon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) were gifts from EPA Research (Triangle Park, NC, U.S.A.). These compounds were of "Chromatographic standard" quality and were used as received. EPTC (S-ethyl dipropylthiocarbamate), Butylate (S-ethyl diisobutylthiocarbamate), Molinate (S-ethyl N,N-hexamethylene thiocarbamate) and Cycloate (S-ethyl N-cyclohexyl-N-ethylthiocarbamates) were commercial products supplied by Nitrokemia (Fűzfögyártelep, Hungary) and purified by distillation.

TLC

Plates used in TLC studies were either glass (g), aluminium (a) or plastic (p) backed: Kieselgel 60 (g), silica gel 60/Kieselguhr F_{254} (g) (Merck, Darmstadt, F.R.G.); aluminium oxide G (g), Polygram SIL G/UV_{254} (p), Polygram Cel 300 (p) (Machery-Nagel, Düren, F.R.G.) and Silufol (a) (Kavalier, Votice, Czechoslovakia). All plates were washed with acetone and activated at 110°C for 10 min. Aluminium oxide plates for OC separation (Table I) were developed either in *n*-hexane or, after impregnation with 8% (v/v) paraffin oil in light petroleum, in acetonitrile–acetone–methanol–water (40:18:40:2, v/v/v/v). For OP separation the plates (Table II) were developed in acetone. Thiocarbamates and OP compounds (Table III) were chromatographed in *n*-hexane–diethyl ether–acetone (7.5:2:0.5, v/v/v) or in *n*-hexane–diethyl ether (8:2, v/v). One- or two-dimensional TLC analyses of the biological samples were carried out in these latter systems.

The colour reagents, DCBC, DBBC and tetraethylenepentamine (TEPA), were purchased from Merck, NBP from Fluka (Buchs, Switzerland) and 2-phenoxyethanol from Janssen Chimica (Beerse, Belgium).

The silver nitrate–2-phenoxyethanol reagent was prepared as described in the AOAC method[2]. NBP–TEPA modified detection: after spraying with a freshly prepared 4% (w/v) solution of NBP in acetone, the plates were sprayed with acetic acid, dried at room temperature, then heated at 110°C for 10 min. After spraying with a 10% (v/v) solution of TEPA in water (freshly prepared), the spots formed were scanned ($\lambda_{max.}$ 250 nm). DCBC and DBBC[3]: plates were sprayed for semiquantitative determination and dipped for densitometric measurements using a solution of DCBC or DBBC (1%, w/v) in acetic acid. After heating at 110°C for 2–10 min, yellow spots of the thiocarbamates and orange spots of Dimethoate and Fenthion appeared against a white background. The thiocarbamates (EPTC, Butylate, Cycloate, Molinate) were measured at 450 nm, Dimethoate at 415 nm and Methylparathion at 495 nm.

Densitometry

Densitometric measurements were carried out with a Shimadzu CS-920 scanner at the absorption maximum of the spots determined *in situ*. Linearizer settings were determined empirically. The area counts were linearly dependent on the concentration of the thiocarbamates in the range 0.1–2.5 μg ($r^2 = 0.99$, $n = 20$), the reproducibility of repeated scans of a single spot being 5%.

Materials and apparatus used for biological samples

Hyflosupercel, Florisil, phosphoric acid, ammonium chloride, anhydrous sodium sulphate and urea were from Reanal (Budapest, Hungary). The solvents were purchased from Reanal and distilled from glass before use. Kuderna-Danish apparatus was obtained from Kontes Glass Co. (Vineland, NJ, U.S.A.).

Food samples were prepared for TLC either by the AOAC method[3] described briefly as follows or by a modified method, see below.

Extraction. The food sample (100 g) was mixed with Hyflosupercel (10 g) and extracted with acetonitrile (100 ml) in a high speed blender for 2 min.

Liquid–liquid partition. After filtering with suction, petroleum ether (b.p. 40–70°C) (100 ml) was added to the filtrate and shaken for 2 min, then saturated sodium chloride solution (50 ml) and finally water (300 ml) were added and the mixture shaken again. The organic layer was washed with water (2 × 100 ml), dried over anhydrous sodium sulphate and evaporated to 5–10 ml in a Kuderna-Danish apparatus.

Column chromatography. A Florisil column (22 mm I.D.) was activated before use at 650°C for 5 h and deactivated by 20% (w/w) of water. The extract was eluted with 200 ml of light petroleum (b.p. 40–70°C) containing 6 and 15% diethyl ether. These fractions collected were evaporated in a Kuderna-Danish concentrator and the thiocarbamate content was determined by TLC and densitometry.

Modified AOAC 1975 method: coagulation. After extraction with acetonitrile, the filtrate was acidified by phosphoric acid to pH 2 and 50 ml of the coagulating solution (ammonium chloride, 0.5%, w/v) in water acidified to pH 2 by phosphoric acid then Hyflosupercel (10 g) were added. The precipitate was filtered off and washed with a mixture of 15 ml coagulating solution and 15 ml acetonitrile. 3 *M* Urea (60 ml) in aqueous solution and Hyflosupercel (10 g) were added to the filtrate and the mixture filtered again. The procedure was continued with liquid partition as described above.

RESULTS AND DISCUSSION

In accordance with literature data[6,7], we found that semiquantitative detection of OC pesticides on impregnated aluminium oxide G plates with silver nitrate–2-phenoxyethanol is satisfactory. However, we achieved a lower detection limit on Polygram Cell 300 plates (Table I). The limits of detection for halogen-substituted OC and OP compounds (Trichlorphon, Chlorfenvinphos) were nearly the same. Surprisingly, Dimethoate also gave a positive reaction and the detection limit was one order of magnitude lower than that of OC compounds. No interference from OP pesticides with the semiquantitative detection of OC compounds can occur because they appear in different fractions during the AOAC multiresidue procedure[2].

TABLE I

TLC RESULTS OBTAINED WITH THE DETECTION SYSTEM: SILVER NITRATE–2-PHENOXYETHANOL

Plate	*Detection limit (μg)*						
	Lindane	*pp'DDT*	*pp'DDE*	*Dimethoate*	*Mevinphos*	*Chlorfenvinphos*	*Trichlorphon*
Alumina G + paraffin oil	0.05	0.05	0.05	–	–	0.1	0.1
Alumina G	0.05	0.05	0.05	0.5	–	0.05	0.05
Polygram Cel 300	0.01	0.01	0.01	0.05	–	0.05	0.05

The importance of NBP–TEPA[8,9] has been reduced because the detection limit is higher than that of enzyme-inhibition procedures, and the spots formed are not sufficiently stable (Table II). Nevertheless, its selectivity is excellent, being specific for the OP insecticides. In addition, the colour stability of this very simple detection could be significantly enhanced by acetic acid spray, as we found experimentally. The spots formed on Polygram SIL G/UV_{254} plates were visible for several hours, allowing densitometric evaluation. The detection limit of this method was dependent on the sorbent media, Kieselgel 60 > Silufol ⩾ Polygram SIL G/UV_{254}, and the sensitivity for Chlorfenvinphos was 20 times better on the latter plate (Table II) than on Kieselgel or Silufol.

OP insecticides act by inhibition of cholinesterases[10]. Analytical exploitation of this phenomenon has led to a more sensitive and selective TLC determination of OP residues[11] in the ng range. Thiocarbamates do not interfere with the determination of OP compounds by the enzymatic method because the detection limits for the former are three orders of magnitude higher (1 μg) than for the OP compounds[12] (⩽ 1 ng).

In our experiments, DCBC and DBBC were used under acidic conditions for the detection of thiocarbamates[3,4]. Their residues were determined quantitatively by densitometry[13]. The detection limit for the thiocarbamates using this colour-forming procedure is definitely lower (Table III) than that with enzyme inhibition. OP pesticides containing sulphur atoms have the same or even lower detection limits than these for thiocarbamates (Table III). These compounds can also be determined by densitometry at their absorption maxima.

The biological materials, deep frozen beans, peas, potatoes[3] and maize prod-

TABLE II

TLC RESULTS OBTAINED WITH THE DETECTION SYSTEM 4(4'-NITROBENZYL)PYRIDINE

Plate	*Detection limit (μg)*				
	Mevinphos	*Chlorfenvinphos*	*Dimethoate*	*Methylparathion*	*Fenthion*
Silufol	0.05	2.0	0.05	0.05	0.05
Polygram SIL G	0.05	0.1	0.05	0.05	0.05

TABLE III

TLC RESULTS OBTAINED WITH THE DETECTION SYSTEM 2,6-DICHLORO- OR 2,6-DIBROMOBENZOQUINONE-N-CHLOROIMINE

Plate	*Detection limit (μg)*					
	Methylparathion	*Dimethoate*	*Fenthion*	*EPTC*	*Butylate*	*Cycloate*
Kieselgel 60	0.10	0.10	0.10	10.00	10.00	10.00
Silica gel 60/Kieselguhr	0.05	0.05	0.05	0.05	0.05	0.05
Silufol	0.02–0.05	0.005–0.01	0.02–0.05	0.05	0.05	0.05

ucts (shelled grain, flour, grits), were analysed for thiocarbamates. The sample preparation was according to the AOAC method[2] (procedure for the determination of OC residues in non-fatty foods). The thiocarbamate content was determined by the use of DCBC or DBBC and densitometry. The recoveries of the thiocarbamates were determined in the presence (Table IV) and absence of the biological samples. Without biological samples, 85–90% (±8%) of the standards added were recovered. EPTC and Butylate were completely eluted by 6% diethyl ether containing light petroleum, Molinate (and Cycloate) only partially. The elution of these compounds was accomplished with light petroleum containing 15% diethyl ether (Fig. 1). The recoveries given in Table IV are the sums of the values obtained with the 6 and 15% diethyl ether eluents. In the extracts of maize products, some coextracted material interfered with the quantitation of the thiocarbamate residues. The introduction of the coagulation step eliminated this interference and thiocarbamates were determined after two-dimensional TLC. OP pesticides studied do not disturb the determination of the

TABLE IV

DENSITOMETRIC DETERMINATION (AOAC 1975) OF THIOCARBAMATES IN FOOD SAMPLES

Food	*Recovery (%)*					
	With coagulation			*Without coagulation*		
	Molinate	*Butylate*	*EPTC*	*Molinate*	*Butylate*	*EPTC*
Deep frozen						
Pea	87	91	90	80*	78	100
Potato	81	95	84	82*	99	77
Bean	84	82	85	81*	55	58
Maize						
Shelled grain	80	73	78			
Flour	76	99	85	Cannot be		
Grits	87	90	85	evaluated		

* Interference from coextracted material; evaluation uncertain.

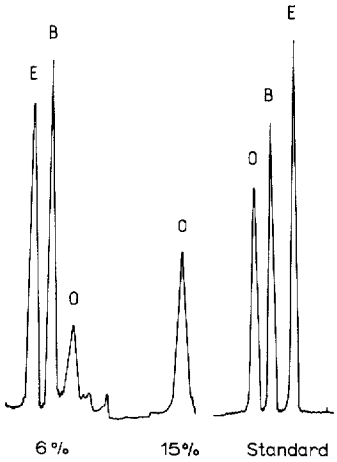

Fig. 1. Densitogram of maize grits prepared by the AOAC method with coagulation. Eluent: 6 and 15% diethyl ether containing light petroleum. Developing solvents: *n*-hexane–diethyl ether–acetone (7.5:2.0:0.5); *n*-hexane–diethyl ether (8:2); E = EPTC; B = Butylate; O = Molinate.

thiocarbamates, even when they are eluted from the column, because Dimethoate, Methylparathion and Fenthion giving a positive reaction with DCBC and DBBC can be separated by TLC. Moreover, Dimethoate was found to remain on the Florisil column.

ACKNOWLEDGEMENT

We are grateful to Dr. Miklos Vajda of our Institute for valuable discussions.

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