Chromatographic identification of some polar organophosphorus insecticides and their residues using formamide-impregnated paper and thin-layer systems

In his general procedure for analysis of organophosphorus pesticide residues BATES[1] described a system for the identification of some of the polar organophosphorus pesticides using a formamide-impregnated paper run in benzene–chloroform (9:1). We have extended this work to cover other compounds and report separations of P=O dimethoate from vamidothion sulphoxide and menazon and of vamidothion from demeton-S-methyl sulphone using two-way chromatography on formamide-impregnated paper in benzene–chloroform (6:4) and then in a 9:1 mixture of these solvents.

We also report separation of P=S dimethoate, P=O dimethoate, menazon, vamidothion, vamidothion sulphoxide, demeton-*S*-methyl sulphoxide and demeton-*S*-methyl sulphone on formamide-impregnated Silica Gel HF plates run in 1,2-dichloroethane–benzene (2:1) and then in *cis*-1,2-dichloroethylene.

The application of these separations to apple, pear, blackcurrant, cabbage, orange, pea and cauliflower extracts is described.

Experimental

Chromatography papers. 10-in. corner-hole Whatman No. 20 papers were used in Shandon frames and tanks. Papers were spotted 1 in. from a corner using a micropipette or capillary. After spotting and drying, the papers were dipped rapidly in 20 % formamide in acetone contained in a Shandon trough. The papers were again air-dried before running in the tank.

Chromatography plates. Formamide-impregnated 0.5 mm thick layers (on 20 × 20 cm plates) were prepared by slurrying 30 g Silica Gel HF_{254} and 100 ml 20 % formamide in ethanol and spreading on four plates with a Desaga spreader. The spread plates were air-dried for 5–10 min and then oven-dried at 50° for 10 min before being stored in a desiccator. Different batches of Silica Gel HF_{254} were found to require different amounts of ethanolic solution to achieve a slurry of suitable consistency.

Some latitude in the temperature (± 5°) and time of oven drying of the plates (5–15 min) after spreading is allowable but much change in conditions will lead to different R_F values between batches of plates.

Solvents. Paper chromatography: (A) benzene–chloroform (6:4) and (B) benzene–chloroform (9:1).

Thin-layer chromatography: (a) 1,2-dichloroethane–benzene (2:1) and (b) *cis*-1,2-dichloroethylene (obtainable from the Aldrich Chemical Co. Inc.).

Visualization reagent. The 4-(*p*-nitrobenzyl)-pyridine reagent of WATTS[2] was used. The papers were heated for 5 min and the thin-layer plates for 10 min at 110°.

Pesticide solutions. 1 mg/ml standard solutions of research grade pesticides in chloroform were used.

Crop extracts. Crops were extracted and first cleaned-up by FREHSE's[3] method. This method comprises extraction with acetone, evaporation of the acetone, filtration, extraction of the aqueous extract with chloroform and purification of the chloroform

extract by chromatography on Brockman Grade V neutral aluminium oxide using chloroform–carbon tetrachloride (1:1) as eluent. Aliquots of eluates from the columns corresponding to 25 g vegetable or fruit were then shaken with 1 g Nuchar C 190 N for 5 min and filtered through Whatman 2V (No. 12) papers. The colourless filtrates were evaporated to 1–2 ml on a warm water-bath in a stream of air, transferred to a tapered tube and finally evaporated to 20–25 μl for chromatography. In the cases of orange, pea and cauliflower extract it was necessary to purify the column eluate after evaporation by partition between 25 ml acetonitrile and four times 10 ml hexane, the insecticide being retained in the acetonitrile. The latter was shaken with Nuchar, filtered, concentrated and spotted. If this further clean up is not used, depressed R_F values were observed due to excessive amounts of oils. Marker spots of pure pesticides were run near the edges of chromatograms on which crop extracts were spotted.

Where quantitative assays of the percentage efficiency of the extraction and clean-up were required the column eluate was evaporated and the residue wet-ashed with nitric and perchloric acids before formation of a phosphomolybdate complex which was reduced with stannous chloride/hydrazine to give a blue colour indicating the amount of phosphorus present.

Results

Paper chromatographic system. R_F values for the polar organophosphorus pesticides and metabolites in the absence of crop extract are given in Table I and a typical chromatogram is shown in Fig. 1.

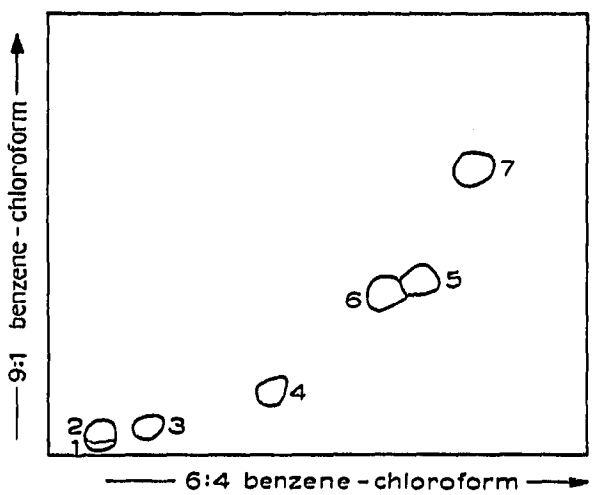
When chromatograms are run, the position of spots may vary slightly from that given in Table I because of the difficulty of impregnating the paper to the same extent each time and also traces of crop extract affect the running of the pesticides. For example, the R_F values of P=S dimethoate in solvent systems A and B respectively varied between 0.69–0.83, and 0.58–0.71, and those of P=O dimethoate between 0.13–0.23, and 0.04–0.06, with apple, pear, blackcurrant, cabbage, orange, pea and cauliflower extracts. The sensitivity was better than 0.1 p.p.m. (2–3 μg) insecticide. Untreated crops gave clean chromatograms except for some slight coloration at the junction of the two solvent fronts: none of the polar organophosphorus pesticides investigated appeared near this area.

TABLE I

R_F VALUES FOR SOME POLAR ORGANOPHOSPHORUS PESTICIDES AND METABOLITES ON FORMAMIDE-IMPREGNATED PAPER

Solvent systems: A = benzene–chloroform (6:4); B = benzene–chloroform (9:1).

Organophosphorus compounds	*A*	*B*
P=S dimethoate	0.78	0.65
P=O dimethoate	0.18	0.06
Menazon	0.09	0.04
Vamidothion	0.70	0.40
Vamidothion sulphoxide	0.09	0.04
Demeton-S-methyl sulphoxide	0.41	0.14
Demeton-S-methyl sulphone	0.61	0.36



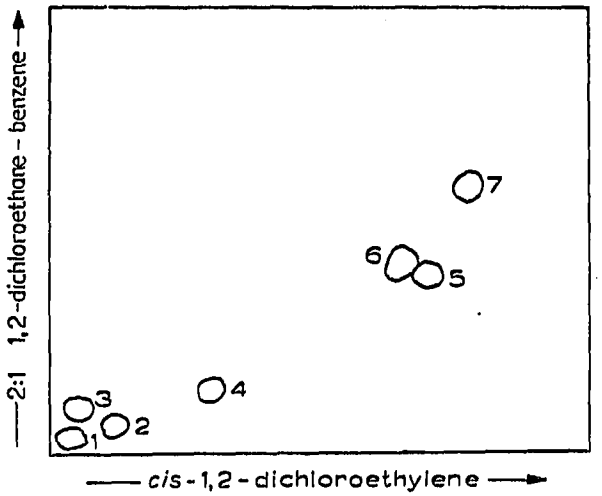


Fig. 1. Chromatogram of some organophosphorus pesticides and metabolites on formamide-impregnated paper. 1 = vamidothion sulphoxide; 2 = menazon; 3 = P=O dimethoate; 4 = demeton-S-methyl sulphoxide; 5 = vamidothion; 6 = demeton-S-methyl sulphone; 7 = P=S dimethoate.

Fig. 2. Chromatogram of some organophosphorus pesticides and metabolites on formamide-impregnated silica gel thin-layer plates. 1 = vamidothion sulphoxide; 2 = P=O dimethoate; 3 = menazon; 4 = demeton-S-methyl sulphoxide; 5 = vamidothion; 6 = demeton-S-methyl sulphone; 7 = P=S dimethoate.

The following less polar organophosphorus pesticides ran to the solvent fronts: azinphos-methyl, mevinphos, parathion, diazinon, disulfoton, phosphamidon, phorate, ethion, "Imidan", formothion, mecarbam, "Aphidan", fenchlorfos, azinphos-ethyl, phenkapton, fenthion, malathion, morphothion, fenitrothion, carbophenothion and malaoxon.

Thin-layer chromatographic system. R_F values for the more polar organophosphorus pesticides and metabolites with and without crop extract are given in Table II and a typical chromatogram is shown in Fig. 2.

Again, marker spots of pure pesticides run near the edges of plates on which

TABLE II

R_F VALUES FOR SOME POLAR ORGANOPHOSPHORUS PESTICIDES AND METABOLITES ON FORMAMIDE-IMPREGNATED SILICA GEL THIN-LAYER PLATES

Solvent systems: a = 1,2-dichloroethane–benzene (2:1); b = *cis*-1,2-dichloroethylene.

Organophosphorus compound	*Without crop material*		*Range with different crop extracts**	
	a	*b*	*a*	*b*
P=S dimethoate	0.60	0.79	0.50–0.58	0.63–0.75
P=O dimethoate	0.07	0.12	0.05–0.07	0.10–0.16
Menazon	0.10	0.07	0.08–0.14	0.06–0.07
Vamidothion	0.40	0.71	0.35–0.46	0.65–0.74
Vamidothion sulphoxide	0.03	0.05	0.02–0.03	0.05–0.08
Demeton-S-methyl sulphoxide	0.13	0.30	0.11–0.17	0.31–0.41
Demeton-S-methyl sulphone	0.43	0.65	0.37–0.49	0.60–0.70

* Crops were apple, pear, blackcurrant, cabbage, orange, pea and cauliflower.

crop extracts were spotted, after being cleaned-up as described above, showed that crop material had little effect on the R_F values of the pesticides and their metabolites. The sensitivity was better than 0.1 p.p.m. insecticide (< 2 μg). Untreated crops again gave clean chromatograms except for some slight coloration at the junction of the two solvent fronts; none of the polar organophosphorus pesticides under investigation appear near this area.

Malathion, malaoxon, diazinon, disulfoton sulphoxide, mevinphos and dichlorvos ran to the solvent fronts in this system and it is reasonable to suppose that other less polar insecticides that run to the front in the paper system would do so on this thin-layer system.

Recovery of added pesticide through extraction and clean-up. Recovery of the insecticides and metabolites through the extraction and clean-up stages dealt with in this paper are shown in Table III.

TABLE III

RECOVERY OF ADDED PESTICIDE OR METABOLITE BY FREHSE'S METHOD

Organophosphorus compound	*Recovery* (%)
P=S dimethoate	90
P=O dimethoate	65
Menazon	70
Vamidothion	100
Vamidothion sulphoxide	45*
Demeton-S-methyl sulphoxide	80
Demeton-S-methyl sulphone	70

* The sample was only 75% chloroform-extractable.

Discussion

Both with the paper and thin-layer systems a number of solvent systems was tried so as to obtain the best resolution and the combination of systems reported above are those found to give the best overall separation. The separation of demeton-S-methyl sulphone and vamidothion is not absolutely complete in either of the suggested techniques; if both are run together a dumb-bell shaped spot is obtained as shown in the figures. Complete separation of these two can, however, be obtained by thin-layer chromatography using chloroform–benzene (1:1) or chloroform–hexane (2:1) but other separations are impaired.

It is necessary to add the formamide to the slurry before spreading the plates; dipping spread silica gel plates in solutions of formamide did not give satisfactory results.

The variations in R_F values of the compounds recovered from different crops and of the pure organophosphorus compounds, which were less in the thin-layer than the paper technique, can be explained as being due to the difficulty in obtaining reproducible impregnation of formamide between batches of plates, the drying time of plates, the effect of traces of crop extracts and temperature variations.

Both sets of systems described in this note have been found to be of considerable use in identifying residues of the polar organophosphorus pesticides and their meta-

bolites at low levels in a wide range of fruits and vegetables. FREHSE's extraction and clean-up technique is at present being studied collaboratively in the United Kingdom as a standard method for quantitative assay of dimethoate residues.

Preliminary investigations using cabbage and pea have indicated that LAWS AND WEBLEY's clean-up method[4] may be used with slight modification in conjunction with the chromatographic systems described here.

Ministry of Agriculture, Fisheries and Food,
Plant Pathology Laboratory, Hatching Green,
Harpenden, Herts. (Great Britain)

N. A. SMART
A. R. C. HILL

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The identification of colistin and polymyxin B by thin-layer chromatography

Colistin and polymyxin B are members of the same family of polypeptide antibiotics and have been well characterized[1]. However, a simple test to identify and differentiate them is lacking. A paper chromatographic method[2] exists, but it requires a long development time. We have developed a simple method to differentiate these two antibiotics by thin-layer chromatography.

A 5 mg sample of polymyxin B sulfate, colistin sulfate or sodium colistimethate is dissolved in 1 ml of 5 *N* hydrochloric acid and sealed in a glass tube in air. The sample is hydrolyzed by heating 6 h at 120°. The tube is then broken and the contents are evaporated to dryness in a watch glass on a steam bath. One ml of water is added and the evaporation is repeated. This is done twice more to remove any traces of hydrochloric acid. The final residue is dissolved in 0.5 ml of water.

Thin-layer plates, 20 × 20 cm, are coated with MN-Silica Gel G-HR (Macherey, Nagel, Düren, Germany, or available from Brinkmann Instruments, Westbury, N.Y.) to a thickness of 0.25 mm and are air-dried overnight. Approximately 0.7 μl of each sample is applied from the tip of a 145-mm disposable capillary pipette (Arthur H. Thomas Company, Philadelphia, Pa.) at 1 cm intervals along a starting line 2 cm from the bottom of the plate, to form spots of about 1.5 mm diameter. Solutions of L-2,4-diaminobutyric acid·HCl (K and K Laboratories, Inc., Plainview, N.Y.), D-leucine, L-leucine, D-phenylalanine, D-serine and L-threonine (Mann Research Laboratories, Inc., New York, N.Y.) are prepared in water at 5 mg/ml. About 0.7 μl of each of these standards is also spotted at the starting line, as well as approximately 1.4 μl of a 30 mg/ml solution of the intact antibiotic in water. A finish line is scratched across the plate 14 cm from the starting line to stop solvent migration.

A MITCHELL tank[3] is lined with Whatman No. 3 MM paper, using no staples or