

Thin-Layer Chromatographic Separation and Colorimetric Detection of Malathion and Some of Its Metabolites from Stored Grains

A thin-layer chromatographic (tlc) procedure for separation and determination of some of the metabolic and hydrolytic products of malathion has been developed. The method reported herein advocates the use of one of the three solvent systems: benzene:hexane:acetic acid (40:40:20), benzene:acetic acid (80:20), or hexane:acetic acid:ethyl ether

(75:15:10). The metabolites which have very close R_f values were resolved by two-dimensional tlc using the two solvent systems: benzene:hexane:acetic acid (40:40:20) and hexane:acetic acid:ethyl ether (75:15:10). This method is suitable for separation and determination of malathion and its metabolites in stored grain.

Many papers have been published on thin-layer chromatographic analysis (tlc) of organophosphorus pesticides (Abbott *et al.*, 1967; Bunyan, 1964; El-Refai and Hopkins, 1965; Walker and Beroza, 1963). Many workers have reported malathion but only Rowlands (1964) has mentioned malathion-half ester and malathion dicarboxylic acid. In his work Rowlands indicated that the chromogenic reagent used is not suitable for detecting P = 0 compounds; therefore, he has used the cholinesterase inhibition technique for the detection of malaoxon. Potassium salt of O-demethyl malathion has not been mentioned by any of the above workers. Because previous work using gas liquid chromatography has failed to determine either potassium salt of O-demethyl malathion or malaoxon (Kadoum, 1969), a thin-layer chromatographic method has been developed for detecting malathion and its metabolites. The important metabolic products and the parent compound are listed in Table I.

EXPERIMENTAL

Apparatus and Reagents. THIN-LAYER CHROMATOGRAPHIC PLATES. Silica gel G and cellulose thin-layer plates were purchased from Brinkmann Instruments, Westbury, N.Y.

STANDARD SOLUTIONS. Standards of malathion, malathion-half ester, malathion dicarboxylic acid, potassium salt of O-demethyl malathion, malaoxon, sodium dimethyl phosphate, potassium dimethyl phosphorothioate, and potassium dimethyl phosphorodithioate were supplied by American Cyanamid Corp., Princeton, N.J., and were used without further purification. Standard solutions of 1 μg per μl in redistilled acetone were prepared by weighing an exact amount of each compound and diluting to an exact volume with redistilled acetone.

CHROMOGENIC REAGENTS. Mitchell's reagent (Mitchell, 1960) and the reagent of Menn *et al.* (1957), DCQ (2,6-dibromo-N-chloro-*p*-quinoneimine), Fisher Scientific Co., St. Louis, Mo., are the two sets of chromogenic reagents em-

Table I. Thin-Layer Chromatographic Systems for Separating Malathion and its Indicated Metabolic Products using Silica-Gel Plastic Plates

Compound	R_f values		
	Solvent System 1 benzene:hexane: acetic acid 40:40:20	Solvent System 2 benzene:acetic acid 80:20	Solvent System 3 hexane:acetic acid ether 75:15:10
Malathion	0.79 \pm 0.01 ^a	0.75 \pm 0.00	0.82 \pm 0.03
Malaoxon	0.55 \pm 0.01	0.64 \pm 0.00	0.23 \pm 0.02
Malathion-half ester	0.54 \pm 0.01	0.61 \pm 0.01	0.61 \pm 0.02
Malathion dicarboxylic acid	0.26 \pm 0.00	0.37 \pm 0.01	0.23 \pm 0.02
Potassium salt of O-demethyl malathion	0.07 \pm 0.01	0.12 \pm 0.00	0.06 \pm 0.01
Potassium dimethyl phosphorodithioate	0.10 \pm 0.00	0.17 \pm 0.00	0.09 \pm 0.00
Potassium dimethyl phosphorothioate	0.20 \pm 0.01	0.30 \pm 0.01	0.17 \pm 0.00
Sodium dimethyl phosphate	0.14 \pm 0.01	0.22 \pm 0.01	0.12 \pm 0.01

^a Mean deviation of six replicates.

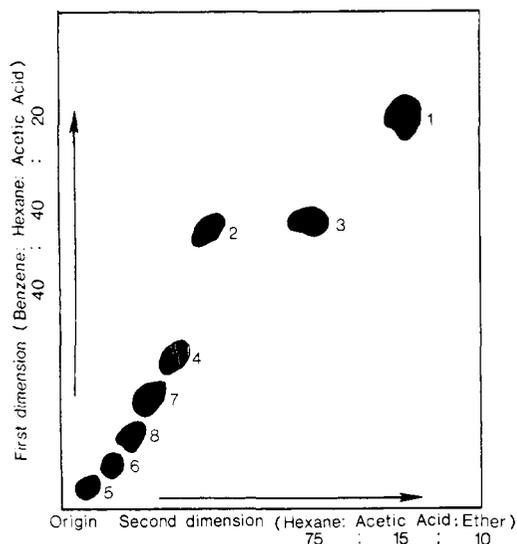


Figure 1. Two-dimensional thin-layer chromatography for malathion and its metabolites from treated stored grain residue

The spots identified using DCQ spray are: 1-malathion, 2-malaoxon, 3-malathion-half ester, 4-malathion dicarboxylic acid, 5-potassium salt of O-demethyl malathion, 6-potassium dimethyl phosphorodithioate, 7-potassium dimethyl phosphorothioate, and 8-sodium dimethyl phosphate

ployed to detect the compounds studied. Mitchell's reagent and 1% (v/v) hydrogen peroxide were prepared as described previously (Kadoum, 1968). Fresh 0.5% solution of DCQ must be prepared in redistilled acetone daily.

SOLVENTS. Reagent grade solvents were redistilled before use. The solvent systems consisted of benzene, hexane, ethyl ether, acetic acid, 2,2,4-trimethylpentane, and N,N-dimethylformamide.

APPARATUS. The chromatographic chambers ($8\frac{1}{2} \times 4 \times 8\frac{1}{2}$ ") purchased from Brinkmann Instruments, Inc., Great Neck, N.Y., were used.

Procedure. The procedure used to extract and clean malathion and its metabolites from stored grain has been described by Kadoum (1969). The modified thin-layer chromatographic technique published previously (Kadoum, 1968) was used to detect these compounds with the following minor variations: thin-layer chromatography was carried out in a chromatographic chamber ($8\frac{1}{2} \times 4 \times 8\frac{1}{2}$ ") according to Walker and Beroza (1963). The plates were developed in one of the following solvent systems: 2,2,4-trimethylpentane (mobile phase) and 35% (v/v) N,N-dimethylformamide in ethyl ether (immobile phase), benzene:hexane:acetic acid (40:40:20), and benzene:acetic acid (80:20), benzene:acetic acid:ethyl ether (75:15:10). However, for two-dimensional chromatography, the plates were allowed to develop for 2 hr in hexane:acetic acid:ether (75:15:10), and for $\frac{1}{2}$ hr in

benzene:hexane:acetic acid (40:40:20) system. The chromatograms were air dried, sprayed with a 0.5% solution of DCQ in redistilled acetone, and the plate heated at 110° C for 20 min.

RESULTS AND DISCUSSION

The modified thin-layer chromatographic technique (Kadoum, 1968), which advocated the use of the solvent system 2,2,4-trimethylpentane and N,N-dimethylformamide, failed to separate the metabo-hydrolysis products of malathion. However, thin-layer chromatographic separation and determination of malathion and certain of its metabolic products were achieved by using the solvent systems consisting of benzene, hexane, and acetic acid as shown in Table I. For the three solvent systems, DCQ spray produced pink-reddish spots of compounds with R_f values recorded in Table I. The dark yellow color was observed first for malaoxon, which was not detected by Rowlands (1964). This suggested that redistilled acetone is a better solvent for 0.5% DCQ spray than the cyclohexane, which Rowlands (1964) used.

The metabolites which have very close R_f values were resolved by two dimensional tlc using two separate solvent systems, illustrated in Figure 1. Tlc provides a procedure for determination of malaoxon, potassium salt of O-demethyl malathion, as well as the aforementioned metabolites, which were not detected in the gas-chromatographic-analysis procedure previously described by Kadoum (1969). Sensitivity obtained from tlc was in the microgram level and could be used for confirming glc analysis of malathion and its metabolites in stored grain.

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