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Note

Thin-layer chromatographic detection of some systemic fungicides and their metabolites

ANNA BALINOVA
Plant Protection Institute, Kostinbrod (Bulgaria)
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The simultaneous determination of multicomponent pesticide residues in the environment and in foods is an important analytical problem because of the increasing number and scale of application of pesticides with different chemical structures. This problem is particularly important with systemic fungicides because, in order to prevent the possibility of resistance to individual groups of compounds arising, the successive application of fungicides with different chemical structures became indispensable. As many of these substances cannot be determined by the commonly used gas-chromatographic methods at present, thin-layer chromatography (TLC) remains the best means of separating and identifying different systemic fungicides in mixtures.

The application of the two-dimensional TLC technique for separating fungicidal compounds of the benzimidazole group was described by Von Stryk[1], but a chromogenic spray reagent with a sufficiently good sensitivity was found only for methyl 2-benzimidazolylcarbamate (MBC).

Baker *et al.*[2] proposed a method for the separation and detection of eight fungicidal compounds with different chemical structures, but a suitable solvent system was not found and the results of separations obtained with four different solvent systems had to be collated in order to identify the compounds.

There was also some discrepancy regarding the development of MBC in these two studies[1,2]. Kvalvåg[3] explained this discrepancy in terms of the different properties of the adsorbents used, and he considered that the prior conversion of MBC into 2-aminobenzimidazole (2-AB) by hydrolysis on a TLC plate is necessary for the development of MBC. On the contrary, Von Stryk[1] finds that under the same conditions not 2-AB but MBC only can be developed.

In the work described in this paper, a spray reagent with good sensitivity was discovered for the detection of benomyl and of MBC and 2-AB produced in plants through the hydrolysis of benomyl (derivatives of benzimidazole), and also of ethirimol and dimethirimol (derivatives of pyrimidine), all of which are typical systemic fungicides.

Three solvent systems were established, each of which permits good separations of the above five compounds to be obtained.

EXPERIMENTAL

Materials

Reagents. Analytical standard samples of ethirimol (5-*n*-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine) and dimethirimol (5-*n*-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine) were obtained from Plant Protection Ltd. (Yalding, Great Britain) and of benomyl [methyl 3-(butylcarbamoyl)-2-benzimidazolyl carbamate], MBC (methyl 2-benzimidazolyl carbamate) and 2-AB (2-aminobenzimidazole) from DuPont (Wilmington, Del., U.S.A.).

TLC plates. TLC plates coated with silica gel G nach Stahl (Merck, Darmstadt, G.F.R.) with a layer thickness of about 250 μm were used.

Development systems. Chloroform–ethyl acetate–methanol (3:2:1), chloroform–acetone–acetic acid (5:2:0.5) and benzene–acetic acid (5:1) were used. All solvents were of analytical-reagent grade and all mixtures were freshly prepared.

Detection reagents. (a) A mixture for chlorination consisting of a 3% aqueous solution of potassium permanganate and an equal volume of dilute hydrochloric acid (concentrated hydrochloric acid–water, 1:2); (b) a chromogenic spray reagent consisting of a mixture of a 1% aqueous solution of potassium iodide, a 3% aqueous solution of soluble starch and ethanol (analytical-reagent grade) in the proportions 1:1:0.4. Both reagents were prepared freshly immediately before use.

Procedure

The TLC plates were activated for 30 min at 110°. Substances for investigations in amounts of 0.05, 0.1, 0.3, 0.5, 1.0 and 2.0 μg, dissolved in suitable solvents, were applied by means of a micropipette or a syringe on to the TLC plates. The plates were developed in each of the solvent systems for a distance of 10 cm at room temperature in a saturated chamber. Following the development, the plates were air-dried until the solvents were completely removed. The plates were then placed in a chlorine saturated chamber for 5 min, removed and kept in the open air for about 5–10 min. After the disappearance of the chlorine smell, the plates were sprayed with chromogenic spray reagent.

RESULTS AND DISCUSSION

Fungicides were detected as intense blue spots on a white background. The chromatograms remained unchanged for 2–3 h, then began to turn dark, but they

TABLE I

R_F VALUES OF FUNGICIDES IN DIFFERENT SOLVENT SYSTEMS

Solvent system	*Fungicide*				
	Dimethirimol	*Ethirimol*	*Benomyl*	*MBC*	*2-AB*
Chloroform–ethyl acetate–methanol (3:2:1)	0.62	0.62	0.90	0.72	0.24
Chloroform–acetone–acetic acid (5:2:0.5)	0.44	0.52	0.89	0.72	0.08
Benzene–acetic acid (5:1)	0.07	0.34	0.61	0.21	0.02

TABLE II

MINIMUM DETECTABLE AMOUNTS OF THE FUNGICIDES TESTED

Fungicide	*Minimum detectable amount (μg)*
Dimethirimol	1.0
Ethirimol	0.5
Benomyl	0.3
MBC	0.3
2-AB	0.05

could be interpreted satisfactorily after 1–2 days. The use of the above three solvent systems in the one-dimensional TLC technique enables good separations of the five fungicidal compounds to be achieved. The R_F values obtained for each solvent system are given in Table I and the minimum detectable amounts of each compound are given in Table II. Starch–iodide reagent produces a sensitive colour reaction with the two groups of fungicidal compounds of different chemical structures, namely derivatives of benzimidazole and of pyrimidine. The same spray reagent has previously been used for detecting herbicides as the *sym*-triazine derivatives[4]. The colour reaction with starch–iodide reagent after treatment with chlorine shown by all of these compounds is due to the presence of a heterocyclic nitrogen atom in the molecules. In comparison with methods of detection based on chlorination[1,3], use of the starch–iodide reagent after treatment with chlorine leads to increased sensitivity and durability of the chromatograms and the results are not affected by the type of adsorbent applied.

As a result of these features, combined with the excellent separation by one-dimensional TLC, the method described overcomes the disadvantages of previously used methods for the determination of systemic fungicides in mixtures.

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