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Thin-layer chromatographic technique for the identification of bithionol*

Bithionol, 2,2'-thiobis(4,6-dichlorophenol) (Bitin®; Tanabe Seiyaku Co., Ltd., Japan), is an anthelmintic which has shown promising results in the treatment of paragonimiasis and tapeworm infections in recent years[1-3]. Thus methods for detecting bithionol and the related compounds dichlorophene, hexachlorophene, and hexylresorcinol are becoming important. BRAVO AND HERNANDEZ[4] and PORCARS[5] have used paper and thin-layer or gas–liquid chromatographic methods for resolving mixtures of dichlorophene and hexachlorophene. However, the thin-layer chromatographic method did not effectively separate bithionol from dichlorophene, hexachlorophene and hexylresorcinol in a single system[4]. Therefore we developed a two-dimensional thin-layer chromatographic method to separate these compounds.

The following chemicals were used: bithionol (Actamer®; Hilton-Davis Co.), 2,4-dichlorophenol (Eastman Organic Chem.), hexachlorophene (Givaudan Co.), hexylresorcinol (Sigma Chem. Co.), Silica Gel G (E. Merck), potassium ferricyanide (Eastman Kodak Co.), and ferric chloride, *n*-heptane, formic acid, and toluene (Fisher Scientific Co.). All solid chemicals were recrystallized and the solvents distilled before use. Individual solutions and a mixture of bithionol, dichlorophene, hexachlorophene and hexylresorcinol were prepared in concentrations of 50 μg/ml each in a 1:1 mixture of chloroform and methyl alcohol.

A suspension of Silica Gel G was made by shaking 30 g of powder in 60 ml of distilled water for a few minutes. This produced enough suspension to prepare five 20 × 20 cm glass plates with a 0.25 mm thick layer of Silica Gel G with a Brinkmann Spreader. The plates were allowed to dry at room temperature for 2 h, activated in an oven at 110° for 1 h, and then stored in a desiccator until use.

The thin-layer plates were prepared and developed according to the schematic diagram shown in Fig. 1. A sample volume of 0.4–0.6 μl was applied with a glass capillary pipette at positions A, B, C, D and M. The plate was then developed in the first direction to a height of 15 cm at room temperature with heptane saturated with

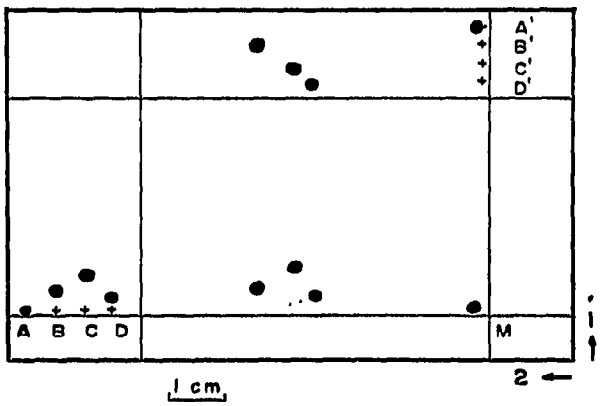

Fig. 1. Two-dimensional thin-layer chromatogram on Silica Gel G. A = hexylresorcinol; B = hexachlorophene; C = dichlorophene; D = bithionol; and M = mixture. Run 1 was performed with *n*-heptane, and run 2 with toluene, both saturated with formic acid.

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formic acid, requiring about 3 h. The plate was then allowed to dry at room temperature for 30 min. Samples were then applied at points A′, B′, C′, and D′ and the plate was developed in the second direction to a height of 15 cm at room temperature with toluene saturated with formic acid, requiring about 1 h. Then the plate was air-dried and sprayed with a freshly-prepared mixture of equal portions of 1 % ferric chloride and 1 % potassium ferricyanide in water[3]. After drying at room temperature, dichlorophene, hexachlorophene and hexylresorcinol appeared as blue spots, and bithionol as a brown spot on a white background.

TABLE I

R_F VALUES OF BITHIONOL AND RELATED COMPOUNDS

Compound	*Solvent system*	
	n-Heptane[a]	*Toluene*[a]
Hexylresorcinol	0.01	0.03
Bithionol	0.07	0.52
Hexachlorophene	0.10	0.70
Dichlorophene	0.21	0.59

[a] Saturated with formic acid.

Fig. 1 is a tracing of an actual separation. Table I presents typical R_F values for these compounds. The addition of formic acid to both heptane and toluene decreased the mobility of the compounds but still permitted adequate separation. Bithionol and hexachlorophene could not be separated with the first solvent system, but they were with the second solvent system. On the other hand, bithionol and dichlorophene could not be separated completely with the second solvent system, but they were with the first solvent system. Using both systems, all four compounds could be separated.

The methods of BRAVO AND HERNANDEZ[4] and GÄNSHIRT[6] do not permit separation of bithionol and hexachlorophene. Our two-dimensional thin-layer chromatographic method separates bithionol, dichlorophene, hexachlorophene and hexylresorcinol. This method was reproducible and easy to perform.

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