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Note

Thin-layer chromatography of phenoxyacetic acid herbicides

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In our laboratory, the thin-layer chromatography (TLC) of chlorinated catechols[1] and cresols[2] was examined previously on five different layer materials with several solvent systems in order to develop separation and clean-up methods for these potentially important residues from environmental samples. 4-Chloro-2-methylphenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are the most important herbicides in Finland[3]. The crude extracts treated with pentafluorobenzyl bromide are important for the gas chromatographic determination of MCPA[4,5], MCPA and its metabolites[6–13] and MCPA, 2,4-D and 2,4,5-T[14–16] in soil. The TLC method has been applied for the separation and identification of MCPA, 4-chloro-*o*-cresol and 5-chloro-3-methylcatechol and their pentafluorobenzyl (PFB) derivatives by using 19 solvent systems on a silica gel layer[17]. In the present study, TLC of compounds I–VI (see Fig. 1) on silica and alumina layers with 11 solvent systems has been extended for the

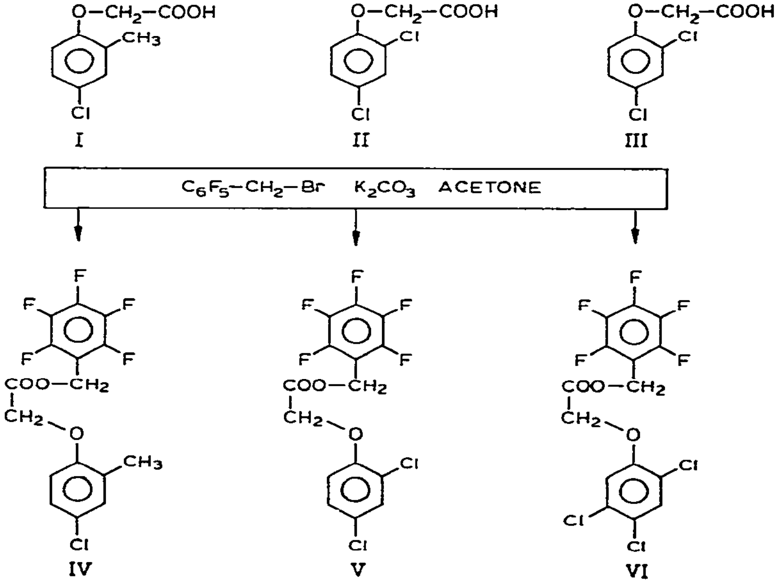

Fig. 1. Structures of MCPA (I), 2,4-D (II) and 2,4,5-T (III), and their pentafluorobenzyl derivatives (IV–VI).

separation, identification and determination of these important pesticides and their derivatives.

EXPERIMENTAL

Apparatus and methods

The standard chromatoplates (20 × 20 cm; Merck, Darmstadt, G.F.R.) were used. The plates were pre-coated with silica gel G 60 and aluminium oxide 60 F_{254} (Type E) with a layer thickness of 0.25 mm. The samples were spotted with 10–50 µl pipettes (H. E. Pedersen, Copenhagen, Denmark) in a line 1 cm from the bottom of the layer, the first and the last spots being 1 cm from the side of the plate and the other 4 spots were applied at 3-cm intervals. Ascending development was carried out in a closed glass chamber (Desaga, Heidelberg, G.F.R.). A Desaga scale plate was used to measure the R_F values of the spots. The colours of the spots of each sample were examined at 1-h, 1-day, 1-week and 1-month intervals.

Samples

MCPA (Pestanal; Riedel de Haen, Hannover, G.F.R.), 2,4-D (Merck), 2,4,5-T (Merck) and their PFB bromide derivatives were used in the experiment. Three fresh (I–III) and three crude derivative (IV–VI) samples were applied and they represent 1 mg/ml in diethyl ether. The six compounds used are as follows (see Fig. 1): (I) MCPA, fresh sample; (II) 2,4-D, fresh sample; (III) 2,4,5-T, fresh sample; (IV) MCPA crude derivative, sample from I; (V) 2,4-D crude derivative, sample from II; and (VI) 2,4,5-T crude derivative, sample from III.

Preparation of derivatives

A 50-mg amount of compound I, II or III was shaken vigorously for 5 min in a glass-stoppered flask with 1 ml of a 1% solution of PFB bromide in acetone, 500 µl of potassium carbonate (30% solution in water) and 4 ml acetone and was allowed to stand for 3 h at room temperature. The sample was mixed with 2 ml hexane and evaporated to dryness with a stream of nitrogen, and the residue was transferred with diethyl ether into a volumetric flask to make up 50 ml of a crude derivative stock solution which represents 1 mg/ml of I, II or III as its derivative (IV, V or VI, respectively) (Fig. 1).

Solvent systems

The preliminary screening of 45 different solvents or solvent systems was tested in order to select those giving good spots and reasonable R_F values. All solvent systems produced sharp spots with acetic acid. The compositions of the 11 most suitable solvent systems are listed below:

(1) Dichloromethane–acetone–acetic acid (8:1:1)
(2) Acetone–toluene–acetic acid (2:2:1)
(3) Dichloromethane–toluene–acetic acid (2:2:1)
(4) Toluene–benzene–acetic acid (2:2:1)
(5) Dichloromethane–heptane–acetic acid (2:2:1)
(6) Ethyl acetate–dichloromethane–acetic acid (8:1:1)
(7) Ethyl acetate–acetic acid (49:1)

(8) Acetone–acetic acid (97:3)
(9) Dichloromethane–acetone–acetic acid (5:4:1)
(10) Acetone–chloroform–heptane–acetic acid (3:3:3:1)
(11) Toluene–benzene–dichloromethane–acetic acid (3:3:3:1)

Development of chromatograms

The development with each solvent system was continued until the solvent front had ascended 15 cm. The plates were then dried in air and sprayed with chromogenic reagent.

Chromogenic reagent

A 2% solution of 3,5-dichloro-*p*-benzoquinonchlorimine (Merck) in toluene was used for spot detection.

RESULTS AND DISCUSSION

Colour reactions

3,5-Dichloro-*p*-benzoquinonchlorimine variously influenced the colour reactions of compounds I–VI during different periods of observation (Table I). Initially (after 1 h), a yellow colour was produced which changed to light yellow, then to white and finally disappeared almost entirely after 1 month except when a large concentration of compound I (2 µg/µl) was applied. The colours detected with the crude derivative samples (IV–VI) changed rapidly and disappeared finally even when a large concentration (2.5 µg/µl) was applied. Usually MCPA (I) spots persisted longer. The developing solvent systems (1–11) sharply affected the reactions of IV–VI. Both silica and alumina layers have shown similar colour reactions during the different periods of observation.

TABLE I

CHARACTERISTIC COLOUR REACTIONS OF MCPA (I), 2,4-D (II), 2,4,5-T (III) AND THEIR PENTAFLUOROBENZYL DERIVATIVES (IV–VI) IN DIFFERENT TIMES AFTER SPRAYING SILICA AND ALUMINA LAYER TLC PLATES WITH A 2% SOLUTION OF 3,5-DICHLORO-*p*-BENZOQUINONCHLORIMINE IN TOLUENE

Sample volume: 20 µg.

Compound	*Colour observation time*			
	1 h	*1 day*	*1 week*	*1 month*
I	Deep yellow	Yellow	Light yellow/white	Light yellow/ white/disappear
II	Deep yellow	Yellow/white	Light yellow/ white/disappear	White/disappear
III	Deep yellow	Yellow/white	Light yellow/ white/disappear	White/disappear
IV	Yellow	Light yellow/white	Light yellow/white	White/disappear
V	Light yellow	Light yellow/white	White/disappear	Disappear
VI	Light yellow	Light yellow/white	White/disappear	Disappear

TABLE II

R_F VALUES OF MCPA (I), 2,4-D (II), 2,4,5-T (III) AND THEIR PENTAFLUOROBENZYL DERIVATIVES (IV–VI) ON A SILICA GEL 60 LAYER WITH SOLVENT SYSTEMS 1–11 AND STANDARD DEVIATIONS (S.D.)

Solvent system	*Compound*						*S.D. I–III*	*Elution time (min)*
	I	*II*	*III*	*IV*	*V*	*VI*		
1	0.60	0.53	0.53	0.57	0.50	–	0.040	55
2	0.70	0.70	0.73	0.93	0.73	–	0.017	55
3	0.57	0.50	0.53	0.57	0.50	–	0.035	60
4	0.50	0.44	0.44	0.50	0.50	–	0.035	70
5	0.56	0.47	0.50	0.56	–	–	0.046	55
6	0.73	0.67	0.70	0.90	0.57	–	0.030	60
7	0.50	0.47	0.47	0.90	0.30	–	0.017	55
8	0.63	0.60	0.57	0.90	0.33	–	0.030	40
9	0.67	0.60	0.60	0.90	0.90	–	0.040	50
10	0.57	0.50	0.53	0.73	0.37	0.37	0.035	50
11	0.47	0.43	0.43	0.73	0.33	0.33	0.023	45

Silica gel layer

The R_F values of I–VI with silica gel 60 are reported in Table II. All compounds produced sharp spots except VI which was detected with only two solvent systems. All of the different solvent systems applied were adequate for the separation and identification of I–III. The standard deviations (S.D.) showed the differences of separations between the three compounds (I–III) when solvent systems 1–11 were applied. For the separation of I–III as a group, 2, 3 and 11 are good, and for separation of the individual compounds solvent systems 5, 3, 10, 6 and 8 are adequate (in that order). The overall solvent effects of separation on the R_F values of I (S.D., 0.085), II (S.D., 0.092) and III (S.D., 0.097) were 0.59, 0.54 and 0.55, respectively. Samples of IV–VI could be applied for individual observation. Elution times varied from 40–70 min.

TABLE III

R_F VALUES OF MCPA (I), 2,4-D (II), 2,4,5-T (III) AND THEIR PENTAFLUOROBENZYL DERIVATIVES (IV–VI) ON AN ALUMINIUM OXIDE 60 F_{254} LAYER WITH SOLVENT SYSTEMS 1–11 AND STANDARD DEVIATIONS (S.D.)

Solvent system	*Compound*						*S.D. I–III*	*Elution time (min)*
	I	*II*	*III*	*IV*	*V*	*VI*		
1	0.40	0.27	0.20	0.33	–	0.10	0.101	70
2	0.57	0.53	0.60	0.67	–	0.67	0.035	60
3	0.47	0.37	0.37	0.50	–	–	0.058	60
4	0.43	0.30	0.30	0.40	–	0.27	0.075	70
5	0.33	0.20	0.20	0.30	0.10	–	0.075	70
6	0.30	0.23	0.27	0.27	–	–	0.035	75
7	0.10	0.07	0.07	–	0.07	–	0.017	70
8	0.07	0.03	0.03	0.07	0.07	–	0.058	45
9	0.30	0.23	0.23	–	–	–	0.040	70
10	0.33	0.20	0.20	0.30	0.10	0.10	0.075	85
11	0.30	0.23	0.20	0.33	0.17	0.20	0.051	60

Alumina layer

The fresh samples of MCPA (I), 2,4-D (II) and 2,4,5-T (III) produced sharp spots, but the crude derivative samples of IV–VI were affected by the different solvent mixtures. The R_F values observed on the alumina layer have been reported in Table III. The solvent systems 9, 3 and 10 were good for the group separation, and 1 and 11 could be applied for individual identification of I–III. In case of small differences in R_F values, 2 and 6 could be tested with I–III. The R_F values observed were 0.33, 0.24 and 0.24 for I (S.D., 0.147), II (S.D., 0.135) and III (S.D., 0.152), respectively, regardless of the solvent mixtures used. The crude derivatives IV–VI were detected with 10 (S.D., 0.115) and 11 (S.D., 0.85), and they could be used for the separation and identification. The elution time observed was between 45 and 85 min.

The silica and alumina layers were detected differently with all 11 solvent systems applied, the overall R_F ratio being 2:1 for silica–alumina regardless of compounds and solvents used in the experiment.

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