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SEPARATION OF PHENOXYACID HERBICIDES USING LIQUID COLUMN CHROMATOGRAPHY ON CHEMICALLY BONDED PHASES

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SUMMARY

Possibilities for the liquid chromatographic (LC) separation of phenoxyacid herbicides were tested. Two complementary LC systems are suggested for the separation and identification of these compounds. In the first system, an octadecylsilica column is used together with aqueous-methanolic mobile phases containing an inorganic salt or a buffer, such as sodium sulphate or potassium dihydrogen phosphate. The second system makes use of a chemically bonded amino phase and of mobile phases containing acetic acid in water, methanol or other organic solvents and their mixtures. The influence of the mobile phase composition and of the structure of phenoxyacid herbicides on retention and selectivity of separation was investigated. The separation methods suggested can be used in the analysis of commercial herbicide formulations and in environmental analyses.

INTRODUCTION

Phenoxyacid herbicides have become widely used because of their relative cheapness and effectiveness in controlling the presence of unwanted broad-leaf weeds in crops. Their introduction required the development of an appropriate analytical method for the control of the content of the active herbicides in commercial formulations, fruits and vegetables and of the level of environmental pollution. Chromatographic methods are obviously promising for this purpose. However, gas chromatography requires derivatization to the volatile silyl esters of the free acids, which can lead to errors. Therefore, various high-performance liquid chromatographic methods have been developed to overcome this difficulty.

The earliest of these methods made use of pellicular ion exchangers for the separation of herbicide acids'. Esters of phenoxyacids and their mixtures with free acids were separated by chromatography on microparticulate silica using mobile phases formed by solutions of dichloromethane in trimethylpentane[2]. Separations of mixtures of free acids were achieved on silica using organic mobile phases containing acetic acid[2–4]. Mixtures of free phenoxyacids (and with their esters) were separated on columns packed with alkylsilica chemically bonded phases using mobile phases

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containing solutions of buffers in aqueous acetonitrile[2,5–8]. Ion-pair chromatography on an octadecylsilica column was employed for the separation of amino conjugates of 2,4-dichlorophenoxyacetic acid[9].

The aim of this work was to compare various possibilities for separations of phenoxyacid herbicides on an octadecylsilica column using mobile phases containing buffers or ion-pairing ions or a neutral salt-sodium sulphate (these mobile phases were successfully used in separations of sulphonic acids[10]), with chromatography on a bonded amino phase, which had proved useful for separations of other carboxylic acids[11].

EXPERIMENTAL

The equipment used included a Model 6000 pump, a U6K injector and an M440 UV detector (all from Waters Assoc., Milford, MA, U.S.A.). Some experiments were performed using simultaneous detection at two different wavelengths, 254 and 280 nm, others using detection at 254 or 280 nm only. Stainless-steel columns (300 × 4.2 mm I.D.) were packed in the laboratory, using a slurry-packing technique, with an octadecylsilica material (Silasorb C_{18}, 10 μm) and with an aminosilica material (Silasorb Amin, 10 μm), both obtained from Lachema (Brno, Czechoslovakia). The void volumes, V_0, of the packed columns were determined as the retention volumes of ^2H_2O measured with the aid of a differential refractometer (R-401, Waters Assoc.) and, as the values of V_0 for a given column did not change significantly with changing composition of the mobile phase, an average value of V_0 was used for calculations of the capacity ratios, $k' = (V_R - V_0)/V_0$ (where V_R = retention volume).

The mobile phases were prepared by dissolving the calculated amount of potassium dihydrogen phosphate, sodium sulphate, tetrabutylammonium hydrogen sulphate (TBAS) or acetic acid in a pure solvent or in a mixed solvent prepared previously by mixing the solvents in the required volume ratios. The methanol, *n*-propanol, n-hexane, acetic acid, sodium sulphate and potassium dihydrogen phosphate used for preparation of mobile phases were all analytical-reagent grade chemicals and the water used was doubly distilled in glass with addition of potassium permanganate. The pH of the solutions of phosphate buffers was measured and, if necessary, adjusted to the required value by addition of a few drops of phosphoric acid. TBAS was prepared using a procedure described elsewhere'[2].

The samples of phenoxyacid herbicides were obtained from East-Bohemian Chemical Works (Synthesia, Semtin, Czechoslovakia): (1) phenoxyacetic acid; (2) 2,4-dichlorophenoxyacetic acid (2,4-D); (3) 2-methyl-4-chlorophenoxyacetic acid (MCPA); (4) 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP); (5) 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP); (6) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); (7) 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB); (8) 4-(2-methyl-4-chlorophenoxy)butyric acid (MCPB); and (9) 2-methyl-4,6-dichlorophenoxyacetic acid.

The procedures used in the analysis of water samples containing 2,4-D are briefly described in the captions to Figs. 12 and 13.

RESULTS AND DISCUSSION

Chromatography on octadecylsilica

In chromatography on an octadecylsilica column, phenoxyacid herbicides are usually eluted near or even prior to the column void volume, often as strongly distorted or split peaks with irreproducible shapes, if mobile phases composed only of water and an organic solvent (methanol, acetonitrile, etc.) are employed. This is caused by ionic exclusion effects, as in the chromatography of other carboxylic and sulphonic acids under analogous conditions[10,13]. This difficulty can be overcome either by suppressing the ionization of the acids using buffered mobile phases of lower pH[5–8], by adding an ion-pairing reagent such as a quaternary ammonium salt to the mobile phase[9,13,14] or by using mobile phases containing an inorganic salt such as sodium sulphate, where salting-out of the organic solutes occurs from the mobile to the non-polar stationary phase[10,12,13,15]. We studied these three possibilities by using aqueous-methanolic mobile phases containing different concentrations of (a) sodium sulphate; (b) potassium dihydrogen phosphate (phosphate buffer) and (c) tetrabutylammonium hydrogen sulphate (TBAS). Table I gives the capacity ratios (k') and separation factors (α) found experimentally in the various mobile phases.

The order of elution of phenoxyacid herbicides does not depend on the mobile phase used, but the retention (k' values) can be varied by changing the composition of the mobile phase in order to improve the separation. From a set of preliminary experiments, we found that 0.04 *M* sodium sulphate allows the concentration of methanol in the mobile phase to be adjusted in the range 0–60% (v/v) and that this concentration is sufficient to achieve good retention of phenoxyacid herbicides, which are eluted as symmetrical peaks.

The retention of phenoxyacid herbicides decreases with increasing concentration of methanol (c) in the mobile phases containing sodium sulphate. The plots of log ***k'*** decrease linearly with increasing c (Fig. 1).

An increase in concentration of a salt in the mobile phase leads to an increase in retention of phenoxyacid herbicides, as can be seen from comparison of mobile phases V and VI in Table I (these mobile phases differ in the concentration of potassium dihydrogen phosphate). In mobile phases containing a phosphate buffer and those containing TBAS, the retention of the tested solutes decreases with increasing concentration of methanol, as in the mobile phases containing sodium sulphate (Table I). The optimum concentration of phosphate buffer in the mobile phase was found to be 0.03 ***M*** potassium dihydrogen phosphate (pH 3), which gives a comparable retention of phenoxyacid herbicides to 0.04 ***M*** sodium sulphate, and the same applies with 0.005 ***M*** tetrabutylammonium hydrogen sulphate in the ion-pair reversed-phase chromatography of these compounds (Table I).

The retention of the solutes studied increases with increasing number of methylene groups in the aliphatic chain and with increasing number of methyl and chloro substituents on the benzene ring. The contributions of one CH_2 group, one CH_3 group and one Cl atom to the retention of phenoxyacid herbicides are approximately equal. A substituent on the benzene ring in the o-position with respect to the $-O(CH_2)_nCOOH$ chain contributes to the retention less than a substituent in the m-position. This retention behaviour is in agreement with general structural effects in reversed-phase chromatography on chemically bonded alkylsilica phases, where

TABLE I

CAPACITY RATIOS (k') AND SEPARATION FACTORS (a = k'/k'_s) OF PHENOXYACID HERBICIDES ON A SILASORB C_{18} (10 pm) COLUMN (300 × 4.2 mm I.D., V_0 = 3.10 ml) IN DIFFERENT MOBILE PHASES

The separation factors are relative to 2.4-D as the standard. Mobile phases: I = 0.04 *M* Na_2SO_4 in methanol-water (40:60); II = 0.04 *M* Na_2SO_4 in methanol-water (45:55); III = 0.04 A4 Na_2SO_4 in methanol–water (50:50); IV = 0.04 *M* Na_2SO_4 in methanol&water (55:45); V = 0.1 *M* KH_2PO_4 in methanol-water (50:50), pH = 3; VI = 0.05 *M* KH_2PO_4 in methanol-water (50:50), pH = 3: VII = 0.03 *M* KH_2PO_4 in methanolwater (60:40), pH = 3; VIII = 0.03 *M* KH_2PO_4 in methanol water (70:30), pH = 3; IX = 0.005 *M* tetrabutylammonium hydrogen sulphate (TBAS) in methanol-water (50:50), pH = 6.7; X = 0.005 *M* TBAS in methanol-water (60:40), pH = 6.7; XI = 0.005 *M* TBAS in methanol- water (70:30), pH = 6.7. All methanol-water ratios in v/v units.

Compound	*Parameter*	*Mobile phase*										
		I	*II*	*III*	*IV*	*v*	*VI*	*VII*	*VIII*	*IX*	*x*	*XI*
Phenoxyacetic acid	*k'*	0.45	0.27	0.19	0.12	1.53	1.07	0.61	0.35	0.81	0.62	0.11
2.4-D		4.32	2.49	1.76	1.09	9.26	7.82	2.69	1.53	3.38	2.65	0.42
MCPA		4.22	2.49	1.76	1.08	10.70	9.11	2.91	1.65	4.12	2.91	0.47
MCPP		6.96	4.01	2.73	1.63	17.20	16.20	4.63	2.52	6.55	4.48	0.56
2,4-DP		7.59	4.27	2.89	1.74	16.50	15.03	4.45	2.28	6.10	4.32	0.53
2,4.5-T		10.49	5.75	3.85	2.23	–	–	5.16	–	–	5.04	–
2,4-DB		18.02	9.27	6.90	3.62	–	–	7.08	–	–	6.67	–
MCPB		20.55	9.48	7.54	3.64	35.7	31.1	7.40	3.74	12.10	6.88	1.59
2-Methyl-4,6-dichlorophenoxyacetic acid		7.11	4.01	2.77	1.66	18.34	17.00	4.76	2.58	7.10	4.68	0.71
Phenoxyacetic acid	α	0.104	0.109	0.108	0.111	0.165	0.137	0.226	0.229	0.240	0.233	0.262
2,4-D		1	1	1	1	1	1	1	1	1	1	1
MCPA		0.975	1.002	1.000	0.994	1.156	1.165	1.083	1.078	1.219	1.098	1.119
MCPP		1.609	1.609	1.550	1.504	1.857	2.072	1.723	1.647	1.938	1.688	1.333
2,4-DP		1.756	1.714	1.640	1.600	1.782	1.922	1.657	1.490	1.805	1.629	1.262
2,4,5-T		2.426	2.311	2.184	2.056	–	–	1.922	–	–	1.905	–
2,4-DB		4.169	3.726	3.920	3.329	–	–	2.635	–	–	2.514	–
MCPB		4.755	3.810	4.279	3.353	3.855	3.977	2.754	2.444	3.580	2.593	3.786
2-Methyl-4,6-dichlorophenoxyacetic acid		1.646	1.609	1.571	1.529	1.981	2.174	1.772	1.686	2.101	1.767	1.690

the compounds are eluted in order of increasing hydrophobicities. Consequently, the following pairs of phenoxyacid herbicides are difficult to separate on an octadecyl-silica column: 2,4-D and MCPA; 2,4-DP and MCPP; 2-methyl-4,6-dichlorophenoxyacetic acid and MCPP and 2,4-DB and MCPB. With these exceptions, all the solutes tested can be separated in mobile phases containing either TBAS, potassium dihydrogen phosphate or sodium sulphate. The separations of a mixture containing phenoxyacetic acid, 2,4-D, 2,4-DP, 2,4,5-T and MCPB in these three systems are compared in Figs. 24. For this particular mixture, the best selectivity (particularly for the separation of 2,4-DP and 2,4,5-T) and the shortest time of separation are achieved in the mobile phase containing sodium sulphate (Fig. 4). This separation demonstrates minor differences in the selectivities of separation between the three types of mobile phase tested, which can be used for "fine tuning" of particular separations. Owing to the high price of ion-pairing reagents, mobile phases containing

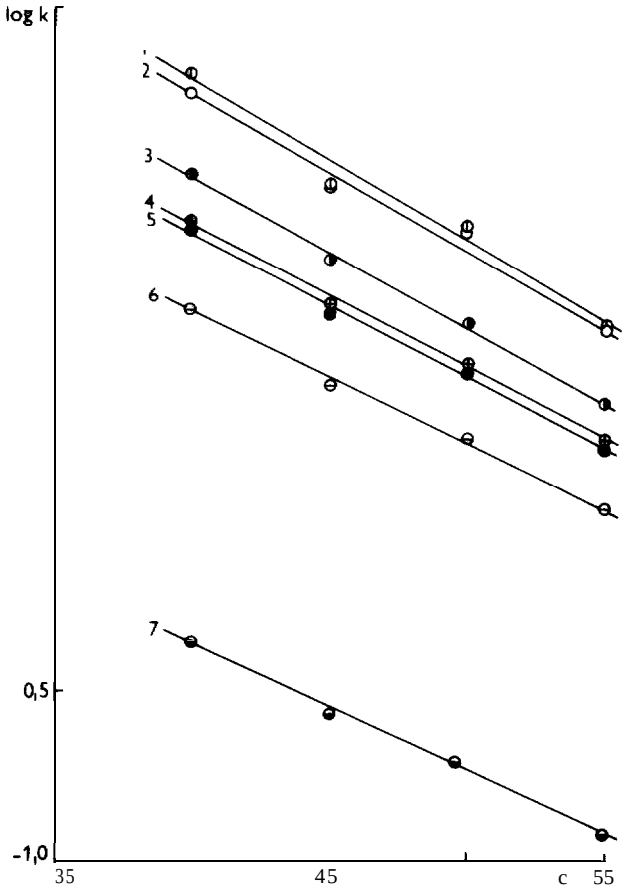


Fig. 1. Plots of the logarithms of capacity ratios (k') of phenoxyacid herbicides on a Silasorb C_{18} (10 μm) column as a function of concentration (c, % v/v) of methanol in a mobile phase containing 0.04 M Na_2SO_4 in methanol-water mixed solvents. Compounds: 1 = MCPB; 2 = 2,4-DB; 3 = 2,4,5-T; 4 = 2,4-DP; 5 = MCPP: 6 = 2.4-D; 7 = phenoxyacetic acid.

phosphate buffers and sodium sulphate are to be preferred. Figs. 5 and 6 show examples of separations of the components of a commercial herbicide formulation containing MCPA in the two types of mobile phases, where again certain differences in selectivities of separation are apparent. Other salts or buffers could probably be used as additives to the mobile phase and efficient separations would result with other slight differences in selectivities, but it does not seem realistic to expect from these mobile phases an improvement in the separation of the pairs of phenoxyacid herbicides that are difficult to separate.

Chromatography on Silasorb Amin

In an acidic medium, chemically bonded amino phases become protonated and may act as weak anion exchangers, and their ion-exchange interactions with weak organic acids are potentially useful for chromatographic separations of acidic com-

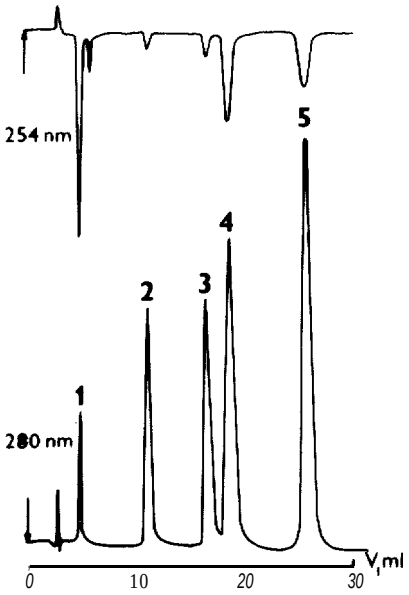
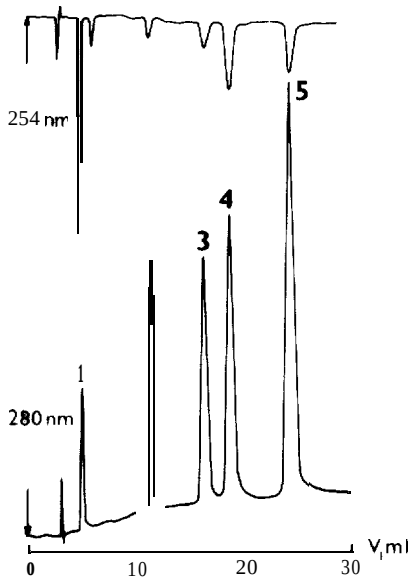


Fig. 2. Separation of a mixture of phenoxyacid herbicides on a Silasorb C_{18} (10 μm) column (300 × 4.2 mm I.D., V_0 = 3.10 ml) by ion-pair chromatography. Mobile phase: 0.005 M TBAS in methanol-water (60:40). Flow-rate: 0.97 ml/min. Detection: UV. 254 nm (upper trace) and 280 nm (lower trace), both at 0.05 a.u.f.s. Compounds: 1 = phenoxyacetic acid; 2 = 2,4-D; 3 = 2,4-DP; 4 = 2,4,5-T; 5 = MCPB. V = volume of the eluate.



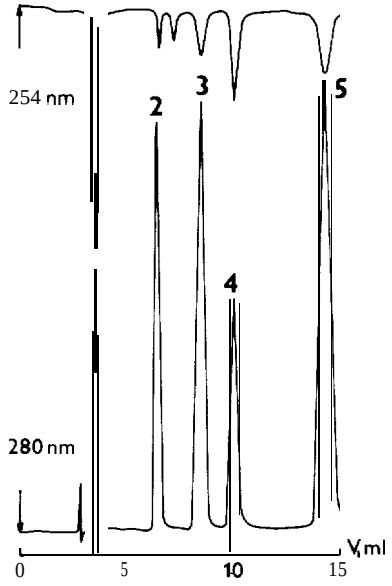
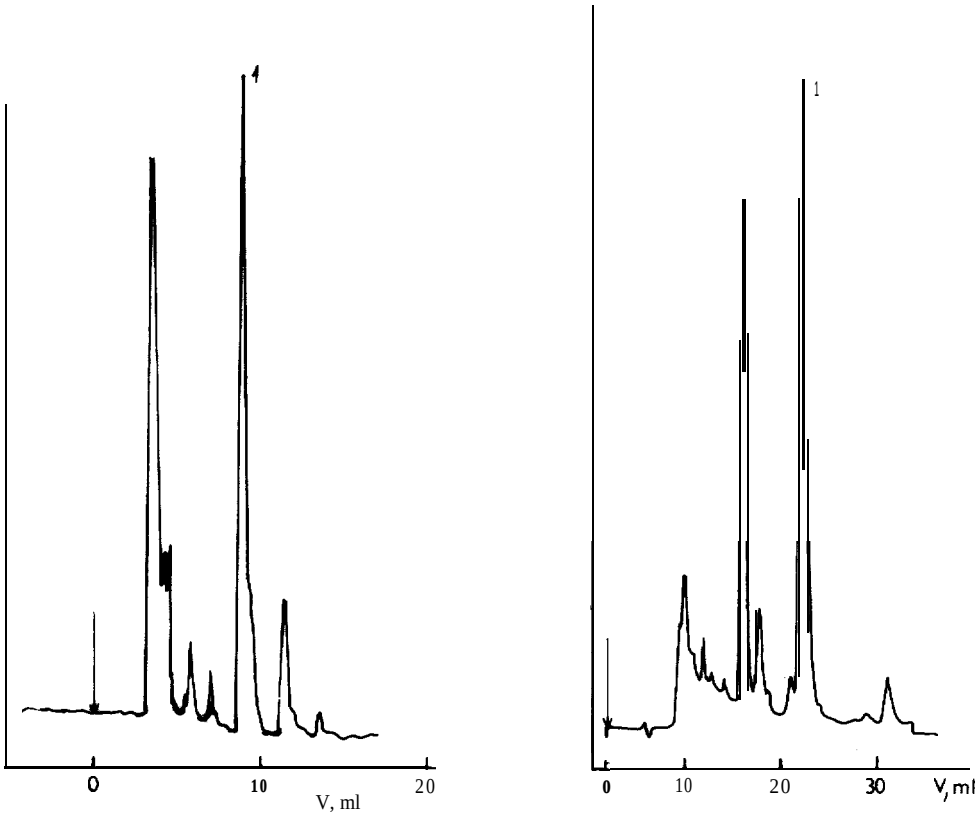


Fig. 3. Separation of a mixture of phenoxyacid herbicides on a Silasorb C_{18} column using a buffered mobile phase. Column, compounds and operating conditions as in Fig. 2, except for the mobile phase: 0.03 M KH_2PO_4 in methanol–water (60:40), pH = 3.0.

Fig. 4. Separation of a mixture of phenoxyacid herbicides on a Silasorb C_{18} column using salting-out chromatography. Column, compounds and operating conditions as in Fig. 2, except for the mobile phase: 0.04 M Na_2SO_4 in methanol&water (55:45).



Fig, 5. Chromatogram of the commercial herbicide formulation Dikotex (Chemical Works CHZJD, Bratislava, Czechoslovakia) on a Silasorb C_{18} column using salting-out chromatography. Column as in Fig. 2. Mobile phase: 0.04 *M* Na_2SO_4 in methanol-water (50:50). Flow-rate: 0.7 ml/min. Detection: UV, 280 nm. Peak 1 = MCPA.

Fig. 6. Chromatogram of Dikotex on a Silasorb C_{18} column using a buffered mobile phase. Column as in Fig. 2. Mobile phase: 0.03 *M* KH_2PO_4 in methanol-water (50:50), pH = 3.0. Flow-rate: 0.9 ml/min. Detection: UV. 280 nm. Peak 1 = MCPA.

pounds. Therefore, we studied the possibilities of separating phenoxyacid herbicides on a bonded amino phase, Silasorb Amin.

In water, methanol, mixtures of water with methanol and mixtures of various neutral organic solvents, the phenoxyacid herbicides are relatively strongly retained on the column of Silasorb Amin and their elution cannot be achieved in a reasonable time. To speed up the elution, it is necessary to add a reagent to the mobile phase that is able to compete with the acidic solutes for amino groups of the bonded phase. The speed of migration of the acids along the column is then determined by ion-exchange equilibrium constants as in chromatography on other ion exchangers. We tested solutions of acetic acid in aqueous-methanol solutions and in organic solvents as mobile phases (these mobile phases have frequently been used in the chromatography of phenoxyacid herbicides on silica[2–4]) and compared them with mobile phases containing a phosphate buffer in aqueous-methanol solutions.

TABLE II

CAPACITY RATIOS (k') AND SEPARATION FACTORS ($\alpha = k'/k'_s$) OF PHENOXYACID HERBICIDES ON A SILASORB AMIN (10 μm) COLUMN (300 × 4.2 mm I.D., V_0 = 3.22 ml) IN DIFFERENT MOBILE PHASES CONTAINING A PHOSPHATE BUFFER

The separation factors are relative to 2,4-D as the standard. Mobile phases: I = 0.005 M KH_2PO_4; II = 0.01 M KH_2PO_4; III = 0.05 M KH_2PO_4; all in methanol--water (50:50); pH = 3.4.

Compound	*Mobile phase*					
	I		*II*		*III*	
	k'	α	*k'*	α	*k'*	α
Phenoxyacetic acid	2.53	0.968	1.59	1.021	0.42	1.072
2,4-D	2.62	1	1.56	1	0.39	1
MCPA	1.96	0.750	1.21	0.777	–	–
MCPP	1.50	0.575	0.95	0.612	–	–
2,4-DP	2.09	0.797	1.27	0.814	–	–
2,4,5-T	2.66	1,015	1.68	1.082	0.34	0.879
2,4-DB	0.04	0.017	0.02	0.010	–	–
MCPB	0.04	0.017	0.00	0.000	–	–
2-Methyl-4,6-dichlorophenoxyacetic acid	2.21	0.844	1.30	0.835	–	–

Table II gives the capacity ratios (k') and separation factors (α) of phenoxyacid herbicides in mobile phases containing three different concentrations of potassium dihydrogen phosphate in aqueous methanol (pH = 3.4). The retention of the solutes tested decreases with increasing concentration of potassium dihydrogen phosphate in the mobile phase, which is in agreement with the proposed ion-exchange mechanism of separation. In 0.005 M potassium dihydrogen phosphate in aqueous methanol (50:50, v/v), the retention and selectivities are adequate for the chromatographic separation of some of the acids tested (Table II). However, the peaks of the compounds eluted under these conditions show tailing, as illustrated by the separation

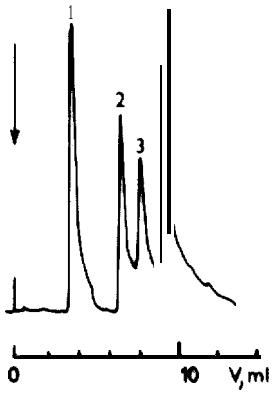


Fig. 7. Separation of a mixture of phenoxyacid herbicides on a Silasorb Amin (10 μm) column (300 × 4.2 mm I.D., V_0 = 3.22 ml) using a buffered mobile phase: 0 01 M KH_2PO_4 in methanol-water (50:50), pH = 3.0. Flow-rate: 0.96 mlimin. Detection: UV. 254 nm. Compounds: 1 = 2,4-DB; 2 = MCPP; 3 = 2,4-DP; 4 = 2.4,5-T.

example in Fig. 7. Therefore, the use of mixed aqueous–methanol mobile phases containing phosphate buffers does not seem advantageous for the separation of phenoxyacid herbicides.

The capacity ratios and separation factors of phenoxyacid herbicides in mobile phases containing various concentrations of acetic acid in methanol, aqueous methanol and water are given in Table III. The retention decreases with increasing concentration of acetic acid in the mobile phase (Fig. 8) and the tested solutes are strongly retained in mobile phases containing 0.1–0.5 *M* acetic acid. In solutions containing 1-2 *M* acetic acid, the retention and selectivities are adequate for successful chromatographic separation.

TABLE III

CAPACITY RATIOS (k') AND SEPARATION FACTORS ($\alpha = k'/k'_s$) OF PHENOXYACID HERBICIDES ON A SILASORB AMIN (10 μm) COLUMN (300 × 4.2 mm I.D.. V_0 = 3.56 ml) IN DIFFERENT MOBILE PHASES CONTAINING ACETIC ACID, WATER AND METHANOL

The separation factors are relative to 2.4-D as the standard. Mobile phases: I = 0.1 *M* acetic acid in methanol; II = 0.5 *M* acetic acid in methanol; III = 1 *M* acetic acid in methanol; IV = 1.5 *M* acetic acid in methanol; V = 2 *M* acetic acid in methanol: VI = 2 *M* acetic acid in methanol–water (50:50); VII = 2 *M* acetic acid in water.

Compound	*Parameter*	*Mobile phase*						
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*
Phenoxyacetic acid	k'	–	12.90	5.99	4.26	2.80	1.71	2.38
2,4-D		–	13.88	6.44	4.55	2.98	2.00	3.58
MCPA			6.62	3.05	2.23	1.47	1.25	2.44
MCPP		–	3.61	2.11	1.21	0.76	0.82	2.19
2,4-DP		–	6.70	3.05	2.16	1.39	1.35	3.37
2,4,5-T		–	15.66	7 11	5.23	3.35	2.39	4.36
2,4-DB		0.88	0.22	0.11	0.07	0.05	0.00	0.16
MCPB		0.70	0.17	0.09	0.05	0.02	0.00	0.13
2-Methyl-4,6-dichlorophenoxyacetic acid		–	13.65	6.13	4.60	2.90	1.53	2.88
Phenoxyacetic acid	α	–	0.929	0.931	0.935	0.940	0.857	0.664
2,4-D			1	1	1	1	1	1
MCPA		–	0.477	0.474	0.490	0.493	0.623	0.681
MCPP			0.260	0.327	0.265	0.256	0.409	0.612
2,4-DP			0.483	0.474	0.475	0.469	0.677	0.941
2,4,5-T		–	1 128	1 104	1.150	1.126	1.196	1.218
2,4-DB		–	0.016	0.018	0.015	0.015	0.001	0.045
MCPB			0.012	0.014	0.010	0.008	0.000	0.036
2-Methyl-4,6-dichlorophenoxyacetlc acid			0.984	0.952	1.01	0.974	0.764	0.804

Table III also shows the influence of the content of methanol in the mobile phase on retention at a constant concentration of acetic acid. Most of the phenoxyacid herbicides tested are retained more strongly in pure water as the solvent for acetic acid (mobile phase VII) than in solutions in pure methanol (mobile phase V), but the differences in capacity ratios measured in the two mobile phases are small. For most compounds, the retention in 2 *M* acetic acid in methanol-water (1:1, v/v)

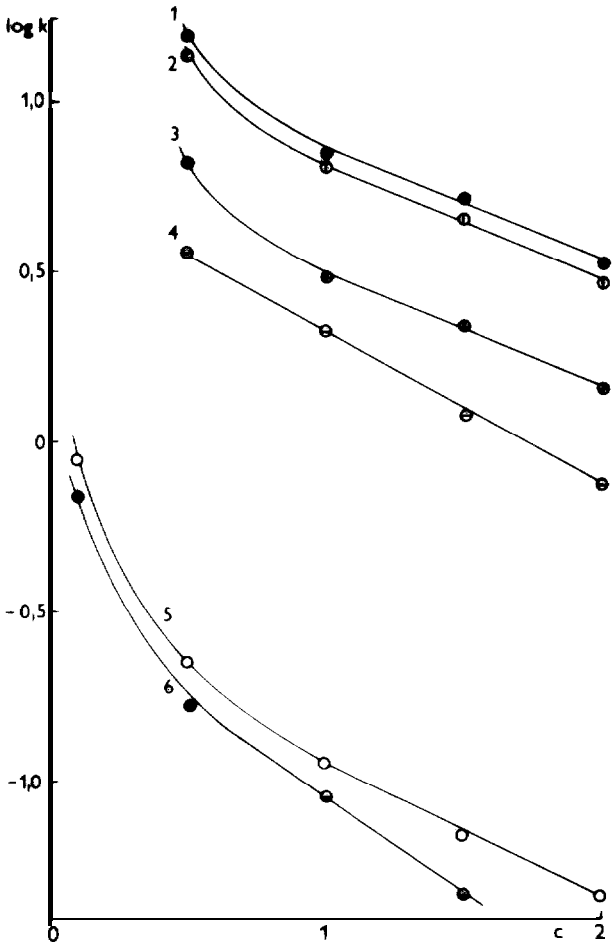


Fig. 8. Plots of the logarithms of capacity ratios (k') of phenoxyacid herbicides on a Silasorb Amin (10 μm) column (300 × 4.2 mm I.D., V_0 = 3.56 ml) as a function of concentration (c. mol/l) of acetic acid in methanol as the mobile phase. Compounds: 1 = 2.4.5-T; 2 = 2,4-D; 3 = MCPA; 4 = MCPP; 5 = 2,4-DB; 6 = MCPB.

(mobile phase VI) is lower than the retention in both mobile phases V and VII. This means that minimum retention occurs at a certain ratio of methanol to water in the mobile phase. The reason for this behaviour is not clear; it may possibly be attributed to the opposite effects of the solubilities of compounds in the mobile phase and of the dielectric constant of the mobile phase on retention, as in other ion-exchange systems[16] The order of elution is similar in all the mobile phases tested and does not depend on the concentration of acetic acid in the mobile phase, but certain changes in selectivities and even in the order of elution occur when the ratio of methanol to water in the mobile phase is changed. This is illustrated in Figs. 9 and 10, where the separation of five phenoxyacid herbicides in both methanol and methanollwater (1: 1, v/v), containing 2 *M* acetic acid, is shown. In this example, the

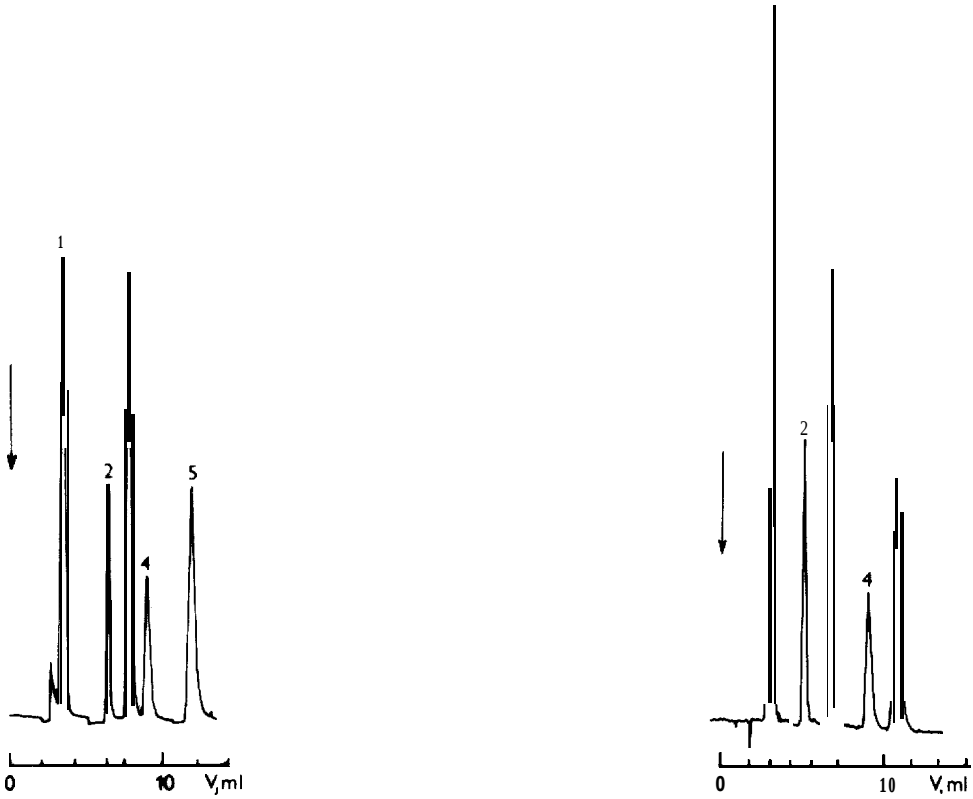


Fig. 9. Separation of a mixture of phenoxyacid herbicides on a Silasorb Amin (10 μm) column (300 × 4.2 mm I.D.. V_0 = 3.56 ml) using a mobile phase containing 2 A4 acetic acid in methanol-water (50:50) as the solvent, Flow-rate: 1.0 ml/min. Detection: UV. 254 nm. Compounds: 1 = 2,4-DB; 2 = MCPP; 3 = MCPA; 4 = 2-methyl-4,6-dichlorophenoxyacetic acid; 5 = 2,4,5-T.

Fig. 10. Separation of a mixture of phenoxyacid herbicides on Silasorb Amin using a mobile phase containing 2 *M* acetic acid in methanol as the solvent. Column, operation conditions and compounds as in Fig. 9.

selectivity of separation is better when pure methanol is used as the solvent for acetic acid.

Table IV shows the experimental capacity ratios and separation factors of phenoxyacid herbicides in mobile phases containing acetic acid in a less polar solvent than methanol, namely *n*-propanol–*n*-hexane (70:30, v/v). Here, the capacity ratios are lower than in mobile phases containing an equal concentration of acetic acid in methanol (Table III). The order of elution is similar to that in methanolic solutions of acetic acid (with one or two exceptions), but the selectivities of separation are different An example of the separation of six herbicides is shown in Fig. 11.

The retention of the phenoxyacid herbicides studied on Silasorb Amin in the mobile phases tested generally decreases with increasing number of CH_2 groups in the aliphatic chain, increases with increasing number of Cl atoms on the benzene ring and decreases with substitution of the benzene ring with a CH_3 group. Thus,

TABLE IV

CAPACITY RATIOS (k') AND SEPARATION FACTORS ($\alpha = k'/k'_s$) OF PHENOXYACID HERBICIDES ON A SILASORB AMIN (10 μm) COLUMN (300× 4.2 mm I.D., V_0 = 3.32 ml) IN ORGANIC MOBILE PHASES CONTAINING ACETIC ACID

The separation factors are relative to 2.4-D as the standard Mobile phases: I = 1 *M* acetic acid; II = 2 *M* acetic acid: both in n-propanol-n-hexane (70:30).

Compound	*Mobile phase*			
	I		*II*	
	k'	*a*	*k'*	α
Phenoxyacetic acid	3.20	0.861	1.45	–
2,4-D	3.72	1	–	
MCPA	1.71	0.459	–	–
MCPP	0.82	0.219	–	–
2,4-DP	1.39	0.373	–	–
2,4.5-T	3.65	0.982	1.58	
2,4-DB	0.24	0.066	–	–
MCPB	0.14	0.038	–	
2-Methyl-4,6-dichlorophenoxyacetic acid	2.40	0.646	–	–

the order of elution is in rough agreement with increasing acidities of the acids tested and is different (almost opposite) to the order of elution of phenoxyacid herbicides in reversed-phase chromatography on octadecylsilica. The pairs of compounds 2,4-D and MCPA, 2,4-DP and MCPP. and 2-methyl-4,6-dichlorophenoxyacetic acid and MCPP, which are difficult to separate on octadecylsilica. can be easily separated on Silasorb Amin, where, in contrast. the separations of 2,4-D from 2,4,5-T and of MCPA from 2,4-DP are more difficult to achieve than on an octadecylsilica column. MCPB and 2.4-DB are difficult to separate on both Silasorb C_{18} and Silasorb Amin, being only slightly retained in the mobile phases tested.

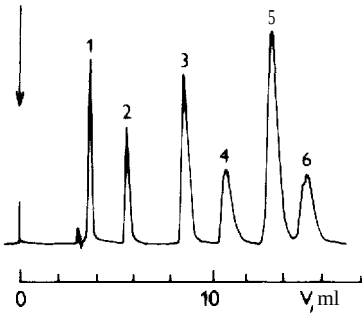


Fig. 11. Separatron of a mixture of phenoxyacid herbicides on a Silasorb Amin (IO μm) column (300 × 4.2 mm I.D., V_0 = 3 32 ml) using a mobile phase containing 1 *M* acetic acid in n-propanol-n-hexane (70:30) as the solvent Flow-rate: 0.88 ml min. Detection: UV, 254 nm. Compounds: 1 = 2,4-DB; 2 = MCPP: 3 = MCPA: 4 = 2-methyl-4,6-dichlorophenoxyacetic acid: 5 = phenoxyacetic acid; 6 = 2,4,5-T.

It is probable that the use of other organic solvents and modification of the acetic acid concentration in the mobile phase could lead to further improvements in selectivities useful for separations. but a detailed study of these possibilities is beyond the scope of the present work.

CONCLUSIONS

A number of phenoxyacid herbicides can be separated by liquid column chromatography in two different chromatographic systems. An octadecylsilica column and aqueoussmethanolic mobile phases containing 0.04 *M* sodium sulphate or 0.03 A4 potassium dihydrogen phosphate can be recommended as the first system, and a column packed with a bonded amino phase and solutions of acetic acid in water,

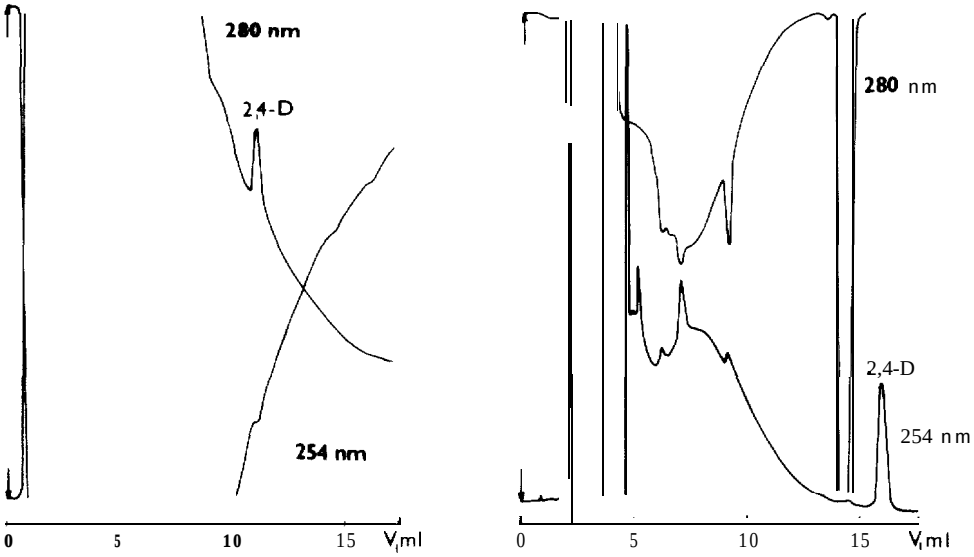


Fig. 12. Chromatographic analysis of a sample of potable water containing 2,4-D at a concentration of 10^{-8} mol l on a Silasorb C_{18} analytical column using direct on-line sample enrichment. Analytical column as in Fig. 2 Enrichment column: 40 × 2.3 mm I.D., packed with Spheron DEAE 1000 (25-40 μm) anion exchanger (Lachema). A IO-ml sample of water was passed through the enrichment column at a Bow-rate of 0.3 ml mm. then the columns were switched and the acid sorbed on the pre-column was desorbed and swept on to the analytical column with 2 ml of 5 *M* lithium nitrate solution at 0.3 ml/min. Analytical separation was performed on the Silasorb C_{18} column using 0.04 A4 sodium sulphate in methanol-water (45:55) as the mobile phase at a flow-rate of 0.93 ml mm Simultaneous UV detection was performed at 254 nm (lower trace) and at 280 nm (upper trace). both at 0.005 a.u.f.s. The peak of 2,4-D is detected only at 280 nm

Fig. 13. Chromatographic analysis of a sample of potable water containing 2,4-D at a concentration of 10^{-8} mol 1 on a Silasorb Amin analytical column usıng direct on-line enrichment. Analytical column as in Fig 9. Enrichment column: 40 × 2.3 mm I.D.. packed with Aquapak 440A (37 75 μm) (Waters Assoc.). A phosphate buffer was added to the sample of water at a concentration of 0.1 *M* (pH = 3); 10 ml of sample were then passed through the enrichment column at a flow-rate of 0.5 ml/min. The columns were then switched and the acid was desorbed from the pre-column and swept on to the analytical column with 0.2 ml of water and 1 ml of methanol at 0.5 ml 'min. Analytical separation was performed on the Silasorb Amin column usıng l *M* acetic acid in methanol as the mobile phase at a flow-rate of 0.91 ml/min. Detection was performed as in Fig. 12.

methanol or other organic solvents and their mixtures as the second system, complementary to the first one. Silasorb C_{18} and Silasorb Amin are suitable as column packings for this purpose. Individual phenoxyacid herbicides can be identified on the basis of their retentions in these two systems.

Simultaneous UV detection at two wavelengths, i.e., 254 and 280 nm, is helpful in the identification of phenoxyacid herbicides, because they absorb more strongly at 280 nm than at 254 nm, in contrast to most other herbicides and non-herbicide sample components (see Figs. 24).

In addition to the analytical control of commercial herbicide formulations, the methods proposed can be used in environmental analysis of herbicide pollution, as is demonstrated by the examples in Figs. 12 and 13. In the analytical problem shown, the method using a column packed with Silasorb Amin is far more sensitive than that with Silasorb C_{18}. Environmental applications are currently being studied in more detail and the results will be published elsewhere.

In some commercial herbicide formulations, phenoxyacid herbicides are present in the form of alkyl esters of phenoxyacids. In this instance, the free acids can be separated after alkali hydrolysis using the methods proposed, or the herbicides can be separated as the esters. The separation of esters is generally less demanding than the separation of free acids and a variety of gas and liquid chromatographic methods suitable for non-ionic compounds may be used for this purpose.

REFERENCES

1 N. E. Skelly. R Russell and D. Porter. *J* Ass. *Offic. Anal. Chem.*, 59 (1976) 748.
2 J. F. Lawrence and D. Turton, *J*. Chromatogr., 159 (1978) 207.
3 F. Eisenbeiss and H. Sieper, *J*. *Chromatogr.*, 83 (1973) 439.
4 J. C. van Damme and M. Galoux, *J*. Chromatogr., 190 (1980) 401.
5 A. D. Drinkwine. D W. Bristol and J. R. Fleeker. *J*. Chromatogr., 174 (1979) 264.
6 T. S. Stevens, N. E. Skelly and R. B. Grorud, *J. Ass. Offic Anal.* Chem., 61 (1978) 1163.
7 N. E. Skelly, T S Stevens and D. A. Mapes, *J Ass. Offic. Anal. Chem.*, 60 (1977) 868.
8 T. S. Stevens. *Advan*. Chromatogr., 19 (1981) 91.
9 M. Armjand, R H. Hamilton and R. 0. Mumma, *J. Agr. Food* Chem., 26 (1978) 971.
10 P. Jandera and J. Churáček, *J*. Chromatogr., 197 (1980) 181
11 H. J. Cortes. *J*. Chromatogr., 234 (1982) 517.
12 P. Jandera. J. Churáček and B. Taraba. *J. Chromatogr.*, 262 (1983) 121.
13 P. Jandera and H. Engelhardt. *Chromatographia*, 13 (1980) 18.
14 D. Wittmer. N. 0. Nuessle and W. G. Haney, Jr.. *Anal* Chem., 47 (1975) 1422.
15 P. Jandera, J. Churáček and J. Bartošová, *Chromatographia, 13 (1980) 485.*
16 D. J. Pietrzyk. *Talanta*, 13 (1966) 209 and 225.