

Separation and determination of acidic herbicides on a PRP-1 polymeric column

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ABSTRACT

The chromatographic behaviour of some acidic herbicides (phenoxyacetic acids, bentazon and dicamba) was investigated on a column laboratory-packed with PRP-1, using acetonitrile–water as mobile phase. Detection was performed with a UV detector set at 220 nm. This wavelength was selected as the optimum for the simultaneous determination of the herbicides. The elution of acidic compounds is affected by the pH of the mobile phase. The greatest retention and the best peak symmetry were observed at pH 2.3. The relationships between $\log k'$ and acetonitrile volume fraction (φ) in the mobile phase are parabolic. The detection limits of the tested substances were about 0.2 ng in the volume injected. The capacity factors in pure water (k'_w), extrapolated by a quadratic expression, showed that PRP-1 could be effective in extracting the examined compounds from water. Various experiments, carried out on spiked water samples using commercial PRP-1 cartridges, showed that the recovery of examined herbicides is nearly 100%. Further, such traps could make it possible to concentrate aqueous samples containing acidic herbicides at very low levels (ppt).

INTRODUCTION

Phenoxy acid herbicides are used extensively in agriculture for weed control and ultimately find their way into lakes, streams and groundwaters. Sometimes they may be found in tap water at very low concentrations (ppt). The common procedure for determining these pollutants in water involves extraction and derivatization prior to analysis by gas chromatography^{1–5}. The main disadvantages are the time-consuming sample preparation and the hazards associated with handling organic solvents.

High-performance liquid chromatography (HPLC) appears to be an attractive alternative as phenoxy acids absorb strongly in the UV region. Both reversed-phase^{6–9} and normal-phase^{6,9,10} applications have been reported. Ion-pair chromatography on octadecylsilica columns has also been employed¹¹. Chiral stationary phases were used

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to separate enantiomers of MCPP [2-(4-chloro-2-methylphenoxy)propionic acid] and of 2,4-DP [2-(2,4-dichlorophenoxy)propionic acid] in formulations^{12,13}.

In previous work, many substituted benzoic acids were studied on styrene-divinylbenzene columns and a polymeric packing, such as PRP-1, showed good properties for the elution of acidic compounds¹⁴; in fact, polymeric columns have better stability than silica-bonded phases at lower pH. It should be pointed out that phenoxy acids are usually separated on a C₁₈ reversed phase at a mobile phase pH of 3⁶⁻⁹. However, at this pH, some herbicides (such as 2,4-D and 2,4,5-TP) having $pK_a < 3$ are partially ionized during chromatographic runs¹⁵.

The aim of this work was to investigate the elution behaviour of some phenoxy acid herbicides on PRP-1 polymeric laboratory-prepared columns. The influence of the mobile phase pH on the retention data and the relationship between the capacity factor (k') and the mobile phase composition were investigated using water-acetonitrile eluents. Experiments were also carried out to find the optimum wavelength for the simultaneous determination of the herbicides; some workers have set the detector at 280 nm^{8,9} and others at lower wavelengths^{16,17}. The use of styrene copolymers as solid-phase extraction materials for the determination of phenoxy acids in water is discussed.

EXPERIMENTAL

The retention parameter measurements were carried out on a Waters Assoc. Model 501 solvent-delivery system (Millipore, Milford, MA, U.S.A.), equipped with a Waters, Assoc. Model 484 tunable absorbance detector. Chromatograms were recorded on a Spectra-Physics (San Jose, CA, U.S.A.) SP 4270 integrator. Samples were introduced with a Rheodyne Model 7125 injector (supplied by Millipore), equipped with a 10- μ l sampling loop. HPLC-quality acetonitrile (Rudi Pont, Eurobase, Milan, Italy) and deionized, distilled water were filtered through a 0.45- μ m Millipore filter and degassed with a helium purge. The pH was controlled with 0.05 *M* phosphoric acid and 0.05 *M* potassium dihydrogenphosphate or sodium monohydrogenphosphate. The reported pH values are those of the solution before the addition of acetonitrile. Solvent mixtures are expressed as percent by volume. The solutes, listed in Table I (Chem. Service, West Chester, PA, U.S.A.), were dissolved in acetonitrile. Bentazon and dicamba also were considered as they are the most effective herbicides under our environmental conditions. The solute concentrations were about 1 mg/l and allowed suitable detector responses without overloading the column. A PRP-1 (Hamilton, Bonaduz, Switzerland) column (150 \times 4.6 mm I.D.) was prepared as described previously¹⁸. Potassium nitrate was used as the unretained solute. All experiments were run in triplicate at a flow-rate of 1 ml/min. The repeatability of retention times was better than $\pm 1\%$.

PRP-1 commercial cartridges, supplied by Alltech (Milan, Italy), were employed in enrichment experiments. Before use the traps were treated as described previously¹⁹.

RESULTS AND DISCUSSION

Preliminary runs were performed to choose the optimum wavelength for the maximum absorbance. These experiments were carried out with a Waters Assoc.

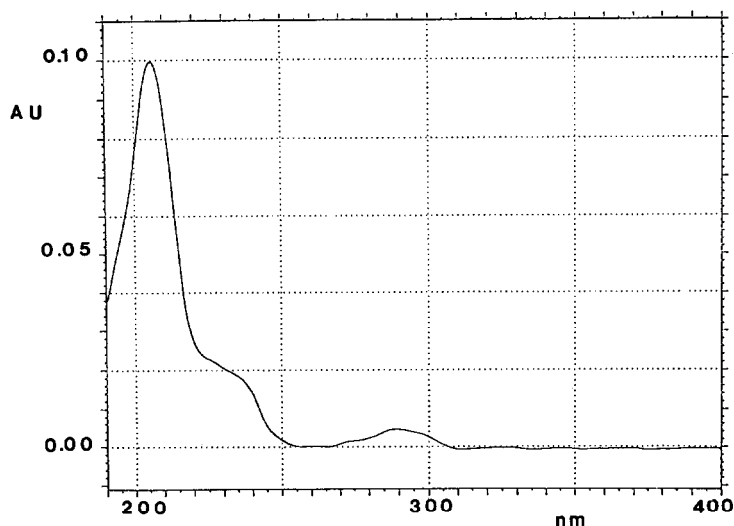


Fig. 1. UV spectrum of 2,4,5-TP. Mobile phase: acetonitrile–water (50:50) at pH 2.3.

Model 990 photodiode-array detector using acetonitrile–water (50:50) at pH 2.3. This pH (corresponding to a 0.05 *M* phosphoric acid solution) allows the phenoxy acids to be completely non-ionized.

The solutes showed absorbance bands at various wavelengths; some had a band with a maximum at a wavelength below the cut-off of acetonitrile (190 nm) and a shoulder between about 220 and 230 nm; others had a band with a maximum slightly above 190 nm and a similar shoulder. All the compounds examined showed bands between 250 and 320 nm.

The UV spectrum of 2,4,5-TP is reported in Fig. 1 as an example. In Table I the wavelengths of the maxima and of the shoulders are reported. For analytical purposes, 220 nm is a good compromise wavelength for all the solutes examined. Moreover, the molar absorptivities at 220 and 280 nm (Table I) show that a greater sensitivity may be obtained at the lower wavelength.

The retention mechanism of ionizable compounds in reversed-phase chromatography is highly dependent on the pH of the mobile phase²⁰. Measurements of *k'* in the pH range 2.3–5 were carried out with acetonitrile–water (50:50) as eluent. The maximum *k'* values were obtained at pH 2.3 and decreased as the pH was increased. At pH > 5 many solutes were eluted with the solvent. Further, the peak asymmetry factors were about 1 at pH 2.3 and became worse as the pH was increased. The data in Table II show that solute retention increases with increase in the number of methylene groups in the aliphatic chain and with an increase in the number of methyl and/or chloro substituents on the benzene ring. In fact, 2,4,5-TP is more retained than MCPP and 2,4,5-T more than MCPA. However, some pairs of phenoxy acid herbicides, such as 2,4-DB–MCPB and 2,4-D–MCPA, cannot be separated.

The behaviour of phenoxy acid herbicides on PRP-1 columns may be explained with the solvophobic theory²⁰ and is similar to the behaviour observed by other

TABLE I

UV SPECTRA OBTAINED WITH ACETONITRILE-WATER (50:50) OF pH 2.3 AS MOBILE PHASE

Compound	Systematic name	Wavelength of maximum absorbance (nm)			Molar absorptivity, $\epsilon \times 10^{-3}$ ($l \text{ mol}^{-1} \text{ cm}^{-1}$)	
					220 nm	280 nm
2,4-DB	4-(2,4-Dichlorophenoxy) butanoic acid	<190	220	280	9.97	1.78
2,4-D	2,4-Dichlorophenoxy acetic acid	<190	220	280	9.20	1.68
2,4,5-TP	(+)-2-(2,4,5-Trichloro phenoxy)propionic acid	205	220-230	290	9.05	0.98
2,4,5-T	2,4,5-Trichlorophenoxy acetic acid	200	220-230	290	9.12	1.01
MCPB	4-(4-Chloro-2-methyl phenoxy)butanoic acid	198	230	290	9.88	1.70
MCPD	(+)-2-(4-Chloro-2-methyl phenoxy)propanoic acid	198	230	290	10.10	1.55
MCPA	(4-Chloro-2-methyl phenoxy)acetic acid	198	230	280	10.90	1.62
Dicamba	3,6-Dichloro-2-methoxy benzoic acid	202	220-230	280	10.90	0.65
Bentazon	3-Isopropyl-(1 <i>H</i>)-2,1,3- benzothiadiazin-4(3 <i>H</i>)-one 2,2 dioxide	215	220-230	310	14.90	1.30

workers⁹ using octadecylsilica columns. The relationships between $\log k'$ and the acetonitrile volume fraction (ϕ) were investigated and the plots for some phenoxy acids are shown in Fig. 2. Regression analysis between $\log k'$ and ϕ showed that all data points may be fitted to a quadratic expression²¹:

$$\log k' = A\phi^2 + B\phi + C$$

TABLE II

 k' VALUES OF HERBICIDES OBTAINED WITH ACETONITRILE-WATER (50:50) AS THE MOBILE PHASE AT VARIOUS pH

Compound	pH					
	2.3	2.5	3.0	3.5	5.0	5.3
2,4-DB	5.15	5.07	4.94	4.63	2.89	2.15
2,4-D	2.65	2.49	1.96	0.99	0.15	—
2,4,5-TP	6.02	5.71	4.61	2.49	0.31	0.22
2,4,5-T	4.02	3.82	2.92	1.42	0.21	—
MCPB	5.33	5.18	5.15	4.87	3.12	2.82
MCPD	3.87	3.70	3.31	2.20	0.27	0.20
MCPA	2.71	2.56	2.19	1.27	0.17	—
Dicamba	2.09	1.74	0.86	0.29	—	—
Bentazon	3.28	2.96	1.89	0.80	0.52	—

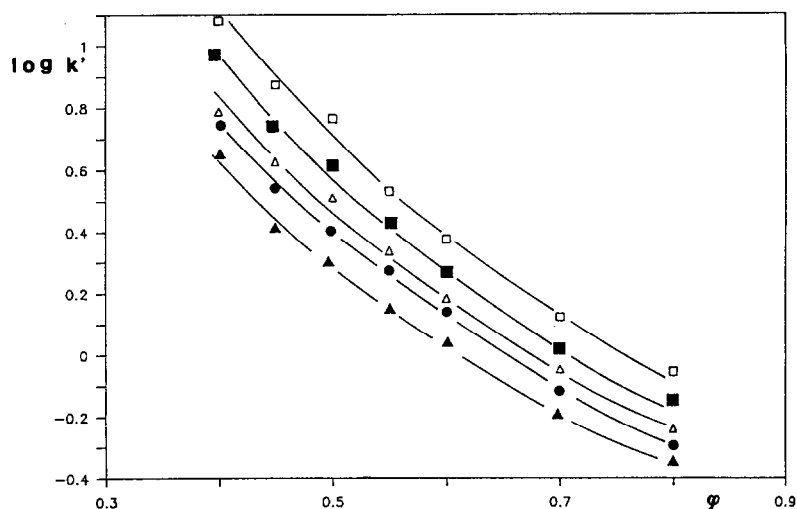


Fig. 2. Plot of $\log k'$ versus ϕ on PRP-1 column. Mobile phase: acetonitrile-water (pH 2.3). \square = MCPB; \blacksquare = 2,4,5-T; \triangle = bentazon; \bullet = 2,4-D; \blacktriangle = dicamba.

The regression parameters and correlation coefficients are reported in Table III. A similar behaviour was found for triazines on polymeric columns with water-acetonitrile eluents¹⁹.

Extrapolation to 0% organic modifier yields the logarithm of the capacity factor in pure water (k'_w). The high k'_w values suggest that PRP-1 could be effective in extracting phenoxy acids from water. In fact, herbicides are usually present at concentrations below the detection limits, so that a preconcentration step is necessary.

The suitability of PRP-1 for enrichment from aqueous solutions was evaluated by sampling distilled water containing herbicides at concentrations below the

TABLE III

REGRESSION PARAMETERS OF $\text{LOG } k' \text{ VS. } \phi$

$$\text{Log } k' = A\phi^2 + B\phi + C.$$

Compound	A	B	C	Correlation coefficient
2,4-DB	3.1	-6.6	3.2	0.999
2,4-D	2.4	-5.4	2.5	0.998
2,4,5-TP	3.4	-6.8	3.2	0.999
2,4-T	3.0	-6.2	2.7	0.999
MCPB	3.0	-6.5	3.2	0.996
MCPD	2.8	-6.2	2.9	0.999
MCPA	2.7	-5.9	2.6	0.998
Dicamba	1.9	-4.5	2.1	0.998
Bentazon	1.8	-4.8	2.4	0.995

TABLE IV

RECOVERIES OF HERBICIDES AT VARIOUS CONCENTRATION LEVELS IN DISTILLED WATER

Concentrations: column 1, 0.05 ppm; column 2, 0.5 ppm; column 3, 1.0 ppm; column 4, 0.5 ppm. pH of the aqueous sample = 2.3 with phosphoric acid (columns 1, 2 and 3) and 2 with hydrochloric acid (column 4).

Compound	Recovery (%)			
	1	2	3	4
2,4-DB	101	99	99	98
2,4-D	99	98	101	100
2,4,5-TP	99	103	101	101
2,4-T	97	99	100	99
MCPB	101	100	103	99
MCPD	100	98	98	100
MCPA	96	99	99	98
Dicamba	98	93	99	98
Bentazon	102	100	100	102

detection limits. These detection limits (signal-to-noise ratio = 3) were about 0.2 ng of the tested substance in the injected volume, *i.e.*, 0.01–0.05 p.p.m.

Commercial cartridges containing about 100 mg of PRP-1 were employed. Before use, the traps were washed with 2 ml of acetonitrile and 2 ml of water at pH 2.3. A vacuum was applied by a water pump and, after 250 ml of aqueous sample had



Fig. 3. Chromatogram obtained by concentrating 1000 ml of tap water spiked with 0.1 $\mu\text{g/l}$ of dicamba (peak 1), 0.1 $\mu\text{g/l}$ of 2,4-D (peak 2) and 0.2 $\mu\text{g/l}$ of bentazon (peak 3) using a PRP-1 cartridge. Mobile phase: acetonitrile–water (40:60), pH 2.

passed through, the herbicides were desorbed by passing 1 ml of acetonitrile and collected in a glass vial.

The recoveries of the herbicides were evaluated by comparing the peak height obtained with the sample and with a standard solution. The results obtained in three runs are reported in Table IV (first three columns of recoveries). These data do not vary with increase in the volume of the water samples (up to 1000 ml) or with increase in herbicide concentration (up to 1 mg/l). The repeatability of the percentage recovery data is $\pm 2\%$.

The re-usability of the cartridges was investigated by making repeated pre-concentration runs and by restoring the adsorbents with 10 ml of acetonitrile. After five extractions the recovery of herbicides had not decreased significantly.

Fig. 3 shows a typical chromatogram obtained by this procedure for a tap-water sample (1000 ml) acidified with hydrochloric acid to pH 2 and spiked with dicamba, bentazon and 2,4-D. The use of phosphoric acid in natural and in tap-water samples should be avoided in order to prevent the precipitation of insoluble phosphates. On the other hand, hydrochloric acid does not affect the recovery efficiency reported in Table IV (last column).

The results obtained showed that polymeric columns could be usefully employed in the determination of phenoxy acid herbicides. Further, such material could also make it possible to concentrate aqueous samples of these herbicides at very low concentration levels.

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