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Note

High-performance liquid chromatographic determination of phenoxyalkanoic acid herbicides using iron(II) 1,10-phenanthroline as a mobile phase additive

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The increasing agricultural consumption of chlorinated phenoxy acid herbicides has led to greater possibilities of contamination of soil, water and food by their residues and their phenolic metabolites. This class of herbicides is considered to be moderately toxic, whereas their chlorinated phenolic metabolites are highly toxic to man and to aquatic organisms[1]. Therefore, the development of methods for the determination of this class of compounds is essential. Among other methods, chlorinated phenoxy acid herbicides can be determined spectrophotometrically[2–4], either directly or after derivatization of the phenols with 4-aminoantipyrine, which results in cleavage of the ether linkage. Differential pulse polarography has been used for the determination of phenoxy acids after nitration[5]. Chromatographic methods[6–8], including paper, thin-layer and gas–liquid modes, are also used for determination of this class of compounds. A great disadvantage of these methods is that derivatization is required before measurement. Liquid chromatography[9–15] using normal- and reversed-phase columns can also be used for the separation of phenoxy acids, with UV detection at 254 or 280 nm. At these wavelengths, however, many compounds may absorb, making detection non-selective.

In this work, the separation and selective detection of phenoxy acids was achieved by an indirect photometric high-performance liquid chromatographic (HPLC) method using $Fe(phen)_3^{2+}$ as a mobile phase additive. The ion-pairing reagent $Fe(phen)_3^{2+}$ has previously been used for the separation and indirect photometric detection of inorganic anions such as Cl^-, Br^- and I^-[16] and organic acid anions such as acetate, succinate and citrate[16]. In these studies, the stationary phase was modified by adding $Fe(phen)_3^{2+}$ to the mobile phase. As it is hydrophobic, $Fe(phen)_3^{2+}$ is adsorbed on the reversed stationary phase to form a double layer composed of $Fe(phen)_3^{2+}$ and its counter anion[17]. The type of analyte interaction mechanism was classified as an ion-interaction mechanism[18], where the analyte anion competes with the counter ion provided by the buffer or electrolyte anions. The separated analyte anions can be detected indirectly at the absorption maximum of $Fe(phen)_3^{2+}$ (510 nm), because absorbance within the analyte band differs from the background absorbance provided by the $Fe(phen)_3^{2+}$ salt-containing mobile phase.

Several factors cause the $Fe(phen)_3^{2+}$ concentration and thus absorbance to

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change within the analyte band: (1) the type of stationary phase, which affects the amount of $Fe(phen)_3^{2+}$ retained; (2) the concentration of $Fe(phen)_3^{2+}$; (3) the percentage of organic solvent in the mobile phase, which affects the separation; (4) the pH of the mobile phase, especially when the analyte anions are derived from weak acids; and (5) the concentration of salts and the ionic strength of the mobile phase, which affect both the amount of $Fe(phen)_3^{2+}$ retained and the direction of the peaks.

In this work, these parameters were optimized for the separation and indirect detection of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenoxybutytric acid (2,4-DB) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

EXPERIMENTAL

Reagents

$Fe(phen)_3SO_4$ solution (ferroin) was obtained from Fluka. The inorganic salts used were of analytical grade. 2,4-D, 2,4-DB and 2,4,5-T were analytical standards obtained from Supelco. Organic solvents were of LC quality. LC-quality water was obtained by passing doubly distilled water through a LiChroprep RP-C_8 (40–63 μm) column (Merck). LiChrosorb RP-C_{18} and RP-C_2 (7 μm) prepacked columns (250 × 4.6 mm I.D. were obtained from Knauer.

Instrumentation

The HPLC instrumentation was obtained from Beckman and consisted of a Model 114M solvent-delivery system, Model 340 injection organizer with 20-μl sample loop, Model 165 multiscanning UV–VIS detector, Model 420A controller and an SP 4270 integrator (Spectra-Physics).

Procedure

Stock solutions (1000 ppm) of 2,4-D, 2,4-DB and 2,4,5-T were prepared by dissolving weighed amounts of the acids in acetonitrile. Solutions containing 800, 600, 400, 200, 100, 50, 10, 5 and 2.5 ppm of the acids were prepared by appropriate dilution of aliquots of the stock solutions with acetonitrile. Sample aliquots of 20 μl were introduced by syringe. Mixed mobile phase solvents are expressed as percent by volume. Mobile phases containing 68.0, 34.0, 25.5, 17.0 and 8.5 μM $Fe(phen)_3^{2+}$ were prepared by adding 1.36, 0.68, 0.51, 0.34 and 0.17 ml of a 0.025 M solution of $Fe(phen)_3SO_4$ to 500 ml of methanol–water (40:60). Mobile phases containing salts were prepared by dissolving the appropriate amounts of sodium sulphate or sodium chloride in 500 ml of 40% (v/v) methanol–water mobile phase which was 34 μM in $Fe(phen)_3^{2+}$. The chromatographic runs were performed at two pH values: at pH 7, which was the pH obtained on mixing the mobile phase constituents, and at pH 3, which was obtained by dropwise addition of 0.1 M sulphuric acid to the mobile phase.

Column breakthrough volumes were determined by passing a $Fe(phen)_3^{2+}$-containing mobile phase of known concentration through the column at a constant flow-rate until the colour of $Fe(phen)_3^{2+}$ appeared in the effluent, as indicated by a sharp increase in the detector response, which was monitored at 510 nm where $Fe(phen)_3^{2+}$ has maximum absorbance. The amount of $Fe(phen)_3^{2+}$ retained was calculated from the breakthrough volume and the concentration of $Fe(phen)_3^{2+}$ in the mobile phase.

Columns were conditioned prior to use with the desired mobile phase by passing at least 150 ml (flow-rate 1 ml/min) of mobile phase more than the volume required to reach breakthrough. The flow-rate was 1 ml/min and the inlet pressure between 1000 and 1500 p.s.i., depending on the mobile phase. Detection was performed at 510 nm. Capacity factors (k') were calculated from $k' = t_R - t_R^0/t_R^0$, where t_R is the retention time of the analyte peak (min) and t_R^0 is the retention time of the solvent peak (min).

RESULTS AND DISCUSSION

The parameters that affect the separation and indirect detection of 2,4-D, 2,4-DB and 2,4,5-T using $Fe(phen)_3^{2+}$ as a mobile phase additive were optimized.

Stationary phase and column conditioning

Two types of reversed stationary phases were tried, RP-C_2 and RP-C_{18}. The amount of $Fe(phen)_3^{2+}$ retained on the stationary phase can be calculated from the volumes of $Fe(phen)_3^{2+}$ solution required to saturate the column. For this purpose, the $Fe(phen)_3^{2+}$ solution was pumped through the column until the column effluent showed the presence of $Fe(phen)_3^{2+}$, as noted by the appearance of an orange colour and an increase in the detector absorbance. A 7-μmol amount of $Fe(phen)_3^{2+}$ was needed to equilibrate the RP-C_{18} column, whereas 17 μmol were required with the RP-C_2 column.

Although RP-C_2 and RP-C_{18} are both of low polarity, RP-C_2 has the higher polarity owing to its shorter carbon chain. This difference in polarity might be responsible for differences in the retention of $Fe(phen)_3^{2+}$, which although hydrophobic, carries two positive charges, leading to greater interaction with the stationary phase of higher polarity. As $Fe(phen)_3^{2+}$ carries two positive charges, the number of

TABLE I

VARIATION OF PEAK AREAS WITH $Fe(phen)_3^{2+}$ CONCENTRATION IN THE MOBILE PHASE

Compound	*Concentration of $Fe(phen)_3^{2+}$ (μM)*	*Peak area (arbitrary units)*
2,4-D	8.5	2.00
	17.0	2.34
	26.0	2.52
	34.0	3.12
	68.0	3.10
2,4-DB	8.5	1.40
	17.0	1.50
	26.0	1.82
	34.0	2.30
	68.0	2.29
2,4,5-T	8.5	1.77
	17.0	1.81
	26.0	1.96
	34.0	2.44
	68.0	2.44

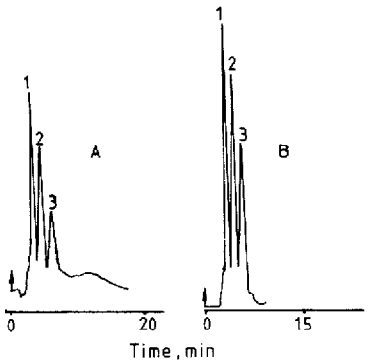

Fig. 1. Chromatograms of 10 μg each of (1) 2,4-D, (2) 2,4,5-T and (3) 2,4-DB. Column, RP-C_2; detection, 510 nm. Mobile phase: (A) 17 μM $Fe(phen)_3^{2+}$ and (B) 34 μM $Fe(phen)_3^{2+}$ in methanol–water (40:60, v/v).

available anion-exchange sites is $7 \times 2 = 14$ μequiv. with RP-C_{18} and $17 \times 2 = 34$ μequiv. with RP-C_2. Hence RP-C_2 has a greater capacity than RP-C_{18} to exchange analyte anions. Further, less solvent and a shorter time were required to clean the RP-C_2 column to remove retained $Fe(phen)_3^{2+}$. Also, under the above experimental conditions no analyte anion exchange could be detected when using the RP-C_{18} column. For these reasons, the RP-C_2 column was used in subsequent work.

Concentration of $Fe(phen)_3^{2+}$

Increasing the concentration of $Fe(phen)_3^{2+}$ in the mobile phase results in an increase in the peak areas of the three acids (Table I). Fig. 1 shows chromatograms of a mixture of the three acids in the presence of 17 and 34 μM of $Fe(phen)_3^{2+}$. The optimum $Fe(phen)_3^{2+}$ concentration when using methanol–water (40% v/v) as the mobile phase was found to be 34 μM. Lower concentrations resulted in a decrease in the peak areas of the three phenoxy acids. Although higher concentrations result in an increase in anion-exchange sites, eventually a level is reached where additional $Fe(phen)_3^{2+}$ will repel the retained $Fe(phen)_3^{2+}$. This occurs because $Fe(phen)_3^{2+}$ is a divalent cation and builds up surface charge. Also, if higher concentrations of $Fe(phen)_3^{2+}$ are used, it will become more difficult to offset the detector electronically.

Percentage of organic modifier in the mobile phase

Methanol and acetonitrile were used as organic modifiers. Increasing the percentage of methanol or acetonitrile in the aqueous mobile phase decreased the retention time of the phenoxy acids (Table II). The effect of adding an organic modifier to the mobile phase (water) is to decrease the polarity compared with pure water, which leads to a decrease in the retention time on a reversed-phase column. In this work, acetonitrile was used rather than methanol as the organic modifier because the retention times are shorter and lower pressures are obtained for acetonitrile at the same flow-rates.

pH of the mobile phase

Theoretically, a pH range of 2.0–9.0 over which $Fe(phen)_3^{2+}$ is stable could be

TABLE II

VARIATION OF CAPACITY FACTORS (k') USING METHANOL–WATER AND ACETONITRILE–WATER IN DIFFERENT PROPORTIONS ON THE RP-C_2 COLUMN

Compound	*Methanol (%, v/v)*				*Acetonitrile (%, v/v)*		
	25	*30*	*40*	*45*	*20*	*27*	*30*
2,4-D	11.89	5.38	3.49	2.69	3.91	2.03	1.53
2,4-DB	34.00	16.01	5.67	3.99	7.05	2.45	1.98
2,4,5-T	23.90	10.82	4.41	3.39	13.77	4.63	2.68

used[19]. However, at low pH (*ca.* 3) the analyte anions could not be detected as they are derived from weak acids, which are slightly dissociated at such pH values. On the other hand, pH values higher than 7.5 could not be applied as the RP-C_2 column is silica based and cannot tolerate pH values higher than 7.5, which cause hydrolysis of the chemically bonded groups[20]. The pH of the mobile phase was therefore adjusted to 7, at which the stationary phase and the $Fe(phen)_3^{2+}$ solution are stable and the analyte anions could be detected.

Effect of ionic strength and counter anion selectivity

If the ionic strength of the mobile phase is increased, the amount of $Fe(phen)_3^{2+}$ retained increases, as indicated by the disappearance of the colour of the column effluent[18]. This was observed when mobile phases containing 100 and 200 μM sodium sulphate or 300 μM sodium chloride were used. When a mobile phase containing 150 μM sodium chloride was used, negative peaks were observed for the three acids (Fig. 2) at longer retention times. On increasing the ionic strength of the mobile phase, the amount of $Fe(phen)_3^{2+}$ retained on the column and hence the number of anion-exchange sites increase, leading to longer retention times of the analyte anions.

Fig. 2. Chromatogram of 100 μg each of (1) 2,4-D, (2) 2,4-DB and (3) 2,4,5-T. Column, RP-C_2; detection, 510 nm. Mobile phase: 34 μM $Fe(phen)_3^{2+}$ and 150 μM NaCl in methanol-water (40:60, v/v).

The negative peaks can be explained by assuming that chloride ions (counter anion) has a lower anion-exchange selectivity by mass action than the phenoxy acid anions, so the equilibrium amount of $Fe(phen)_3^{2+}$ retained increases and the $Fe(phen)_3^{2+}$ concentration decreases in the analyte band by an equivalent amount, leading to negative peaks when the analyte band passes through the detector[18].

Calibration and detection limits

For all three acids the linear working range was between 50 and 10 000 ng under the optimum chromatographic conditions, *i.e.*, RP-C_2 column and a mobile phase containing 34 μM $Fe(phen)_3^{2+}$ in 18:82, 20:80 and 28:72 (v/v) acetonitrile–water for 2,4-D, 2,4,5-T and 2,4-DB, respectively. The pH of the mobile phase is about 7 and no electrolytes are added to the mobile phase.

The detection limits were found to approach 50 ng for the three acids. The detection limit could be lowered by raising the pH of the mobile phase to increase the dissociation of the acids. This is possible only if another kind of column, such as poly(styrene–divinylbenzene), which can tolerate high pH values is used instead of the RP-C_2 column.

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