Ion-pairing mechanism in the solid-phase extraction and reversed-phase high-performance liquid chromatographic determination of acidic herbicides in water

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Abstract

An alternative approach to the preconcentration of acidic herbicides from water and their HPLC determination utilizing an ion-pairing mechanism was developed. Triethylamine was applied as an ion-pairing reagent for increasing the retention of dicamba, bentazone, benazolin, 2,4-D and MCPA on C_{18}-bonded silica at neutral pH. Optimum conditions were found for solid-phase extraction in order to increase the recovery of analytes. Appropriate adjustments of the ion-pairing reagent and organic modifier in the mobile phase were made to optimize the HPLC retention and separation. An accurate and sufficiently sensitive analytical procedure was developed for the determination of the acidic herbicides in drinking and groundwater samples.

Keywords: Environmental analysis; Water analysis; Solid-phase extraction; Sample preparation; Ion-pairing mechanism; Pesticides; Triethylamine

1. Introduction

Acidic herbicides, such as dicamba, bentazone, benazolin, 2,4-D, MCPA and others, are in current use in agriculture. They are considered potential groundwater pollutants owing to their high soil mobility, long half-lives or large-scale application. Hence there is steadily increasing interest in methods for their determination in water.

Classical analytical procedures based on liquid–liquid extraction [1,2] are time consuming and require large volumes of environmentally hazardous solvents. Solid-phase extraction (SPE) using bonded silica sorbents has gained popularity for the extraction of pesticides from water [3]. It offers the advantages of short analysis time, low cost and the consumption of small volumes of organic solvents. C_{18}-bonded silica cartridges [4–6], styrene–divinylbenzene Empore extraction disks [7] and Carbopack cartridges [8,9] have been used for the extraction of acidic herbicides from water samples. Automated column-switching systems [10,11] and on-line SPE coupled to liquid chromatography [12–15] have been reported for the determination of acidic herbicides in drinking and surface water.

The determination of these substances by gas

chromatography (GC) with electron-capture detector (ECD) is only possible after derivatization, but the latter is time consuming and can be a source of additional errors. To avoid the derivatization step, reversed-phase (RP) high-performance liquid chromatography (HPLC) with UV or diode-array detection (DAD) has been frequently used for the determination of acidic herbicides. Thermospray mass spectrometry detector (TSP-MS) has been recommended as a powerful technique for their identification [4,7,16]. Multi-residue methods for the determination of acidic herbicides in aquatic media, based on RP chromatography, apply the approach of adjusting the pH value to be lower than the pK_a value of the analytes in order to increase the retention capacity on the sorbents in both SPE and HPLC [5–7,10–12,14,15,17]. The necessity to work under strongly acidic conditions gives rise to problems due to the instability of the bonded silica sorbents [7]. Ionization suppression could be achieved at milder conditions by applying an ion-pairing mechanism. Tertiary and quaternary amines are generally recommended as counter ions for the RP separation of anionic solutes [18]. This approach has been applied only in limited cases for the HPLC determination of acidic herbicides.

Arjmand et al. [19] used tetrabutylammonium phosphate as an ion-pairing reagent to improve the retention and separation of dicamba and its metabolite on a C_{18} HPLC column. Application of ion-pairing reagents in RP-SPE of acidic compounds from water does not appear to have been reported.

The aim of this work was to study the possibilities of utilizing the ion-pairing mechanism to increase the retention of strongly acidic herbicides at neutral pH on C_{18}-bonded silica for their preconcentration from water samples and for analytical HPLC separation, to optimize the SPE and HPLC conditions and to develop a procedure for the accurate and sensitive multi-residue determination in water of acidic herbicides including compounds of low retention such as dicamba and of compounds that are incompletely separated such as 2,4-D and MCPA.

2. Experimental

2.1. Chemicals and reagents

Doubly distilled water and methanol distilled in glass were used for HPLC. All reagents and solvents were of analytical-reagent grade. Triethylamine was obtained from Merck (Darmstadt, Germany) and 500-mg Sep-Pak C_{18} cartridges from Waters (Millipore, Milford, MA, USA).

Individual stock standard solutions were prepared in methanol by dissolving 100 mg of analytical-purity standards in 100-ml volumetric flasks; the solutions were stored at 4°C in the dark. Composite working standard solutions were prepared in the mobile phase by diluting the stock standard solutions as required.

2.2. Equipment

A Pye Unicam (Cambridge, UK) liquid chromatograph was equipped with a PU 4010 pump, a PU 4020 variable-wavelength UV detector and a Rheodyne Model 7125 injector with a 20-μl loop. A LiChrosorb RP-18 5-μm column (250 mm × 4.6 mm I.D.) (Merck) was used for HPLC separation.

2.3. Sample preparation

Samples of 1 l of drinking or groundwater were prepared by addition of triethylamine (TEA) to a concentration of 0.03 M and adjusting the pH to 6.9 with 1 M phosphoric acid. Samples were spiked with the herbicides studied, giving final concentrations in the range 0.05–1 μg/l. The Sep-Pak cartridges were conditioned with 10 ml of methanol and equilibrated with 10 ml of distilled water. The samples were passed through the cartridges under vacuum at a rate of ca. 10 ml/min. After washing with 5 ml of distilled water, the sorbent bed was dried under vacuum for 30 min. The analytes were eluted with 2 ml of methanol, the solvent was evaporated under a gentle stream of nitrogen and the residue was dissolved in the HPLC mobile phase to a final volume of 1 ml.

2.4. HPLC analysis

The mobile phase for isocratic elution was 0.01 M aqueous solution of TEA at pH 6.9 (adjusted with 1 M phosphoric acid)–methanol (80:20) at a flow rate of 1 ml/min. The compounds were detected at 230 nm. For programmed elution, the conditions were as follows: 100% A [0.01 M TEA (pH 6.9)–methanol (80:20)] for 4 min, then to 100% B [0.01 M TEA (pH 6.9)–methanol (70:30)] in 1 min at 100% B for 16 min; then back to A in 12 min.

Quantification of the herbicides was effected by external calibration and peak height measurement.

3. Results and discussion

3.1. Preconcentration studies

Acidic herbicides are water-soluble ionic compounds which are not retained by RP stationary phases in the ionized state. An increase in the retention capacity of bonded stationary phases used in SPE has usually been achieved by adjusting the pH of aqueous samples below 2. Since a C_{18}-bonded silica sorbent is not stable under these conditions, major interferences occur.

The retention of ionic compounds in a reversed-phase system can be increased by addition of ion-pairing reagents. The neutral ion pairs formed in situ adsorb firmly on the surface of a reversed-phase sorbent. The pH of the samples should be adjusted to maximize the concentration of the ionic form of the solutes. TEA was found to be a suitable counter ion in the preconcentration of acidic herbicides from water as it provided an effective retention of the analytes by C_{18} bonded silica. It is less expensive than other common ion-pairing reagents, e.g., tetrabutylammonium salts. This study was focused on the optimization of the retention step to increase the recovery of analytes.

The most useful method for controlling retention is to adjust the concentration of the ion-pairing reagent in the samples. The effect of different TEA concentrations on the sample
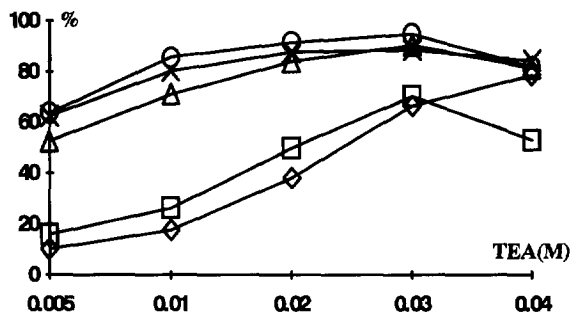

Fig. 1. Recovery of selected herbicides as a function of TEA concentration in 1-l water samples (pH 6.9) with 500-mg C_{18} cartridges. (◇) Dicamba; (□) bentazone; (△) benazolin; (×) 2,4-D; (○) MCPA.

recovery is shown in Fig. 1. The retention increases with increasing amount of TEA at low concentrations, levels off at about 0.03 M and then decreases at higher concentrations.

At the established optimum TEA concentration, 0.03 M, the effect of sample volume was studied using 125, 250, 500 and 1000 ml of water, fortified with constant amounts of herbicides (1 μg of dicamba, benazolin, 2,4-D and MCPA and 0.5 μg of bentazone). The recoveries obtained after passing different volumes of sample are shown in Fig. 2. The most polar early-eluting compounds dicamba and bentazone (Fig. 3) exhibited lower recoveries and the critical volume was 500 ml, while the other herbicides studied gave good recoveries at sample volumes of 1 l. At lower TEA concentrations (0.005–0.01 M) the recoveries of dicamba and bentazone
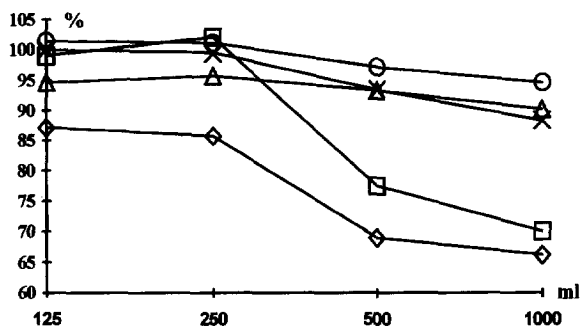

Fig. 2. Recovery of selected herbicides as a function of sample volume at a TEA concentration of 0.03 M with 500-mg C_{18} cartridges. (◇) Dicamba; (□) bentazone; (△) benazolin; (×) 2,4-D; (○) MCPA.
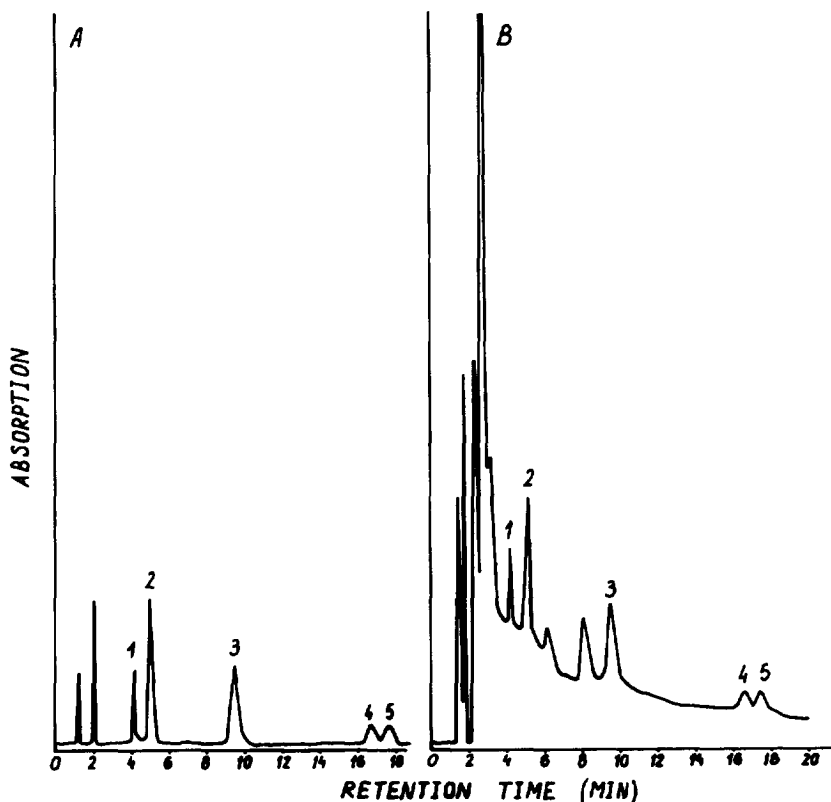
Fig. 3. HPLC of herbicides under optimum conditions (see Experimental). (A) Mixed working standard solution. Peaks: 1 = dicamba (4 ng); 2 = bentazone (2 ng); 3 = benazolin (4 ng); 4 = 2,4-D (4 ng); 5 = MCPA (4 ng). (B) 1-l drinking water sample fortified with dicamba, benazolin, 2,4-D and MCPA (0.2 μg/l each) and bentazone (0.1 μg/l), after preconcentration on C_{18} cartridges.

were lower even with a 250-ml volume, whereas the recoveries of benazolin, 2,4-D and MCPA were not influenced (data now shown).

3.2. Analytical LC separation

The pH of an acidic mobile phase used in the RP-LC of the acidic herbicides is a compromise between the separation efficiency and the decrease in the column lifetime. Ionization suppression of the anionic compounds could be achieved under milder conditions by applying an ion-pairing mechanism.

As dicamba is weakly retained on C_{18} sorbents, initially a counter ion with a longer alkyl chain, tetrabutylammonium hydrogensulphate, was selected in order to obtain an acceptable value of the capacity factor (k') for this solute. This resulted in an excessive increase in the elution times of 2,4-D and MCPA but not in an increase in the selectivity of their separation. TEA used with an appropriate composition of the mobile phase proved to be an adequate counter ion for the separation of the compounds studied. To achieve a better separation of 2,4-D and MCPA, application of a high-resolution LC column of length 25 cm and a particle size of 5 μm becomes necessary.

To optimize the analytical separation via changes in the mobile phase composition, we

concentrated on two parameters which cause a large change in retention and selectivity and are easy to vary: the concentration of the ion-pairing reagent and the concentration of the alcohol modifier.

The variation of the capacity factor (k') of herbicides as a function of TEA concentrations in the mobile phase is shown in Fig. 4. The hold-up time (t_0) of the system was determined by multiple injections of water and was found to be 0.85. This t_0 value was used for all k' calculations.

The results show that k' increases faster with increasing TEA concentration in the low concentration range up to 0.01 M. The increase in k' was slower at higher TEA concentrations. Concentrations higher than 0.02 M were not included in the study because they would have resulted in an unacceptable increase in the analysis time. The separation of the critical pair of compounds 2,4-D and MCPA was not improved by increasing the TEA concentration above 0.01 M. The latter value was accepted as optimum in the mobile phase composition.

As a result of the interaction between the counter ions and the ionic analytes, ion pairs with strong retention on the stationary phase are formed. An organic modifier in the mobile phase is needed to control the elution rate. The in-
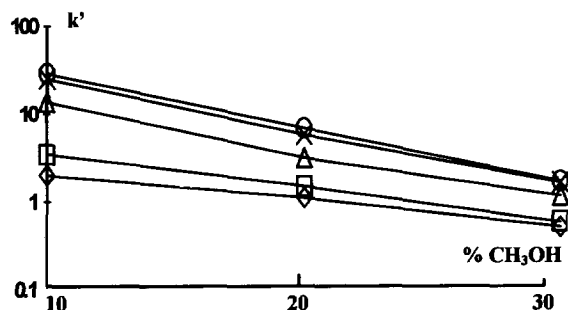

Fig. 5. Effect of methanol concentration in the mobile phase on capacity factor (k'). Mobile phase: 0.02 M TEA in water (pH 6.9) with different concentrations of methanol. Chromatographic conditions as described under Experimental. ($\Diamond$) Dicamba; ($\Box$) bentazone; ($\triangle$) benazolin; ($\times$) 2,4-D; ($\bigcirc$) MCPA.

fluence of methanol concentration on k' is shown in Fig. 5. The k' value decreased rapidly as the methanol content increased, but the resolution became worse. Optimum chromatographic retention and separation was achieved with 20% of methanol in the eluent.

The appropriate adjustment of the mobile phase composition gave the possibility of optimizing the chromatographic behaviour of the compounds studied. As is shown in the chromatograms in Fig. 3, excellent retention and separation of dicamba, bentazone and benazolin was achieved. 2,4-D and MCPA were not completely separated and the retention times were long. When gradient elution (as described under Experimental) was applied to shorten their retention times, the separation becomes worse.

The accuracy and precision of the method developed, evaluated at two fortification levels with 1-l samples of drinking and groundwater, are presented in Table 1. Limits of determination, defined as three times the baseline noise, are also given.

External calibration was used for the quantification of herbicides in water samples. Good linearity with correlation coefficients in the range 0.998-0.999 was found between the peak heights and amounts injected within the range of operation for all the compounds.
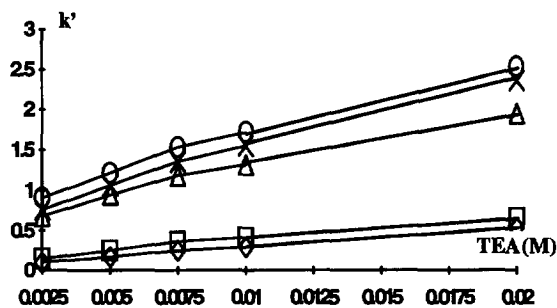

Fig. 4. Effect of TEA concentration in the mobile phase on capacity factor (k'). Mobile phase: methanol-water (20:80) containing different concentrations of TEA (pH 6.9). Chromatographic conditions as described under Experimental. ($\Diamond$) Dicamba; ($\Box$) bentazone; ($\triangle$) benazolin; ($\times$) 2,4-D; ($\bigcirc$) MCPA.

Table 1
Average recoveries and standard deviations (S.D.) of selected herbicides calculated for four replicates and limits of determination (LOD)

Compound	Recovery ±S.D. (%)				LOD (μg/l)
	Drinking water		Groundwater		
	0.2 μg/l	1 μg/l	0.2 μg/l	1 μg/l	
Dicamba	105.8 ± 5.4	82.3 ± 9.4	90.3 ± 16.4	63.2 ± 3.5	0.05
Bentazone	80.9 ± 9.1	84.1 ± 5.9	77.8 ± 6.8	72.2 ± 3.7	0.02
Benazolin	74.3 ± 12.1	82.0 ± 12.9	75.6 ± 5.2	95.4 ± 5.9	0.05
2,4-D	73.3 ± 8.8	70.8 ± 6.0	72.9 ± 4.8	94.6 ± 4.9	0.1
MCPA	76.7 ± 8.2	87.3 ± 11.8	73.0 ± 4.2	97.4 ± 3.1	0.1

4. Conclusions

The ion-pairing mechanism using TEA as a counter ion is an alternative approach to C_{18} SPE from water and to analytical RP-HPLC of acidic herbicides. It is particularly useful for increasing the retention capacity of weakly retained compounds such as dicamba.

The method based on optimized chromatographic conditions is reproducible and sufficiently sensitive. The milder chromatographic conditions are favourable for protecting the column lifetime and also when compounds susceptible to acid hydrolysis are present in samples.

Acknowledgement

References

[1] G. Durand, V. Bouvot and D. Barceló, J. Chromatogr., 607 (1992) 319.
[2] W. Schüssler, Chromatographia, 29 (1990) 24.
[3] E. Benfenati, P. Tremolada, L. Chiappetta, R. Frassanito, G. Bassi, N. Di Toro, R. Fanelli and G. Stella, Chemosphere, 21 (1990) 1411.
[4] D. Volmer and K. Levsen, J. Chromatogr. A, 660 (1994) 231.
[5] Th. Heberer, S. Butz and H.J. Stan, Abstracts of the 4th Workshop on Chemistry and Fate of Modern Pesticides, Prague, September 8–10, 1993, p. 42.
[6] A. Balinova, J. Chromatogr., 643 (1993) 203.
[7] S. Chiron, E. Martinez and D. Barcelo, J. Chromatogr. A, 665 (1994) 283.
[8] A. Di Corcia and M. Marchetti, Anal. Chem., 63 (1991) 580.
[9] A. Di Corcia, M. Marchetti and R. Samperi, Anal. Chem., 61 (1989) 935.
[10] R.B. Geerdink, C.A.A. van Balkom and H. Brower, J. Chromatogr., 481 (1989) 275.
[11] R. Hamman, M. Meier and A. Kettrup, Fresenius' J. Anal. Chem., 334 (1989) 231.
[12] J.V. Sancho-Llopis, F. Hernandez-Hernandez, E.A. Hogendoorn and P. van Zoonen, Anal. Chim. Acta, 283 (1993) 287.
[13] S. Chiron, A. Fernandez-Alba and D. Barcelo, Environ. Sci. Technol., 27 (1993) 2352.
[14] V. Coquart and M.C. Hennion, Sci. Total Environ., 132 (1993) 349.
[15] P. van Zoonen, E.A. Hogendoorn, G.R. van den Hoff and R.A. Baumann, Trends Anal. Chem., 11 (1992) 11.
[16] D. Barcelo, Org. Mass Spectrom., 24 (1989) 898.
[17] In Suk Kim, F.I. Sasinos, R.D. Stephens, J. Wang and M.A. Brown, Anal. Chem., 63 (1991) 819.
[18] E. Poole and S.A. Schuette, Contemporary Practice of Chromatography, Elsevier, Amsterdam, 1984.
[19] M. Arjmand, T.D. Spittler and R.O. Mumma, J. Agric. Food Chem., 36 (1988) 492.