Separation of a Mixture of Hydrophilic and Hydrophobic Herbicides by Ion-Interaction Reversed-Phase HPLC

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Abstract

An ion-interaction reversed-phase chromatographic method is presented for the separation of 2,4-dichlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propionic acid, 2,4,5-trichlorophenoxyacetic acid, 4-(2,4-dichlorophenoxy)butyric acid, 4-chloro-*o*-tolyloxyacetic acid, 4,6-dinitro-*o*-cresol, and 5-bromo-3-*sec*-butyl-6-methyluracil. These herbicides, which do not belong to the same functional classes, are characterized by different chemical properties, particularly in terms of hydrophilicity. The effect of mobile phase pH, organic modifier, ion-interaction reagent, and stationary phase packing material on analyte retention is investigated and discussed. The method permits detection at concentration levels less than 12 µg/L without preconcentration or pretreatment steps and is applied to the analysis of river water samples.

Introduction

To develop an analytical method capable of separating pesticides, we considered a mixture (see Figure 1) that contained 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 4-chloro-*o*-tolyloxyacetic acid (MCPA), 4,6-dinitro-*o*-cresol (DNOC), and 5-bromo-3-*sec*-butyl-6-methyluracil (bromacil). Phenoxy acids and DNOC are more hydrophilic, whereas bromacil is more hydrophobic.

Phenoxy acids, at concentration levels of approximately 1% (w/w), cause physiological, morphological, and histological modifications

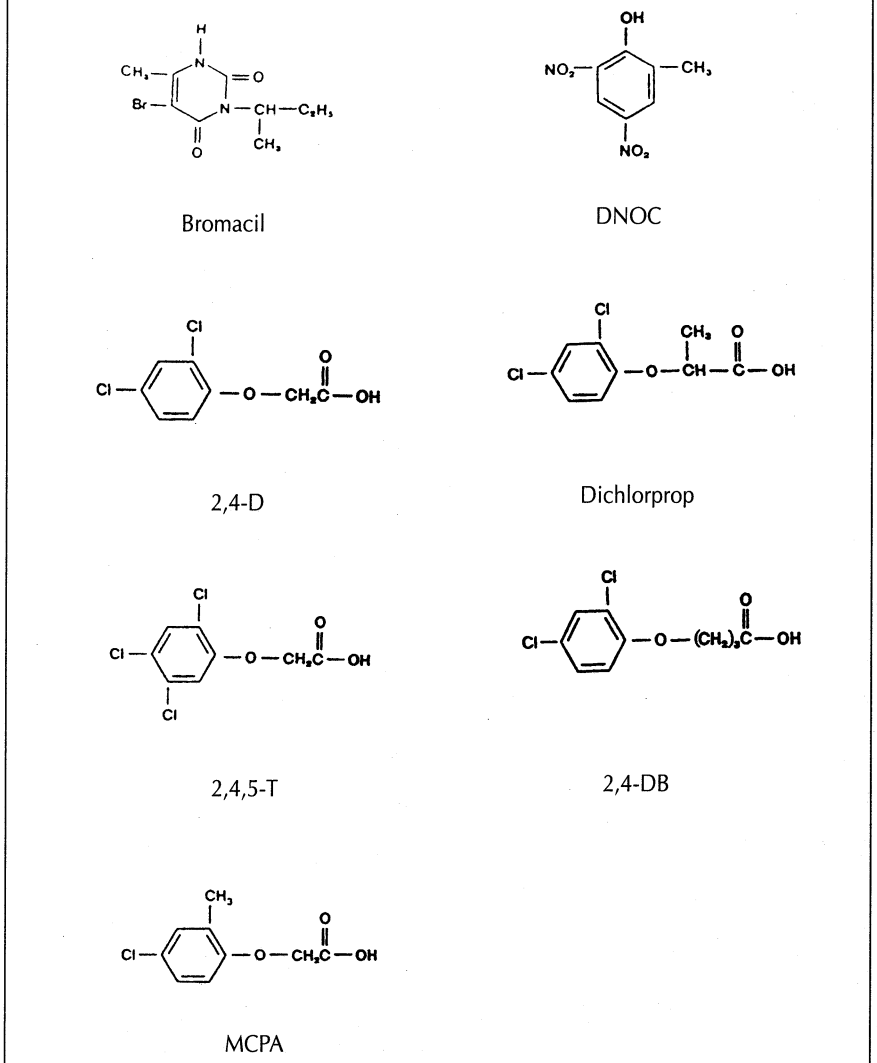

Figure 1. Chemical structures of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), DNOC (4,6-dinitro-*o*-cresol), 2,4-D (2,4-dichlorophenoxyacetic acid), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid), and MCPA (4-chloro-*o*-tolyloxyacetic acid).

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in the green parts of plants that lead to plant death. DNOC is a weed killer also used in winter seasonal protection of fruit trees. It affects the green parts of plants via a caustic contact action against cuticular integuments and the cell walls of cryptogams; its oral LD_{50} (dose that is lethal to 50% of test subjects) for rats is 25–40 µg/kg. Bromacil is a very strong herbicide used for total weed killing on untilled lands and weed killing on citrus plantations. It acts by adsorption from roots and leaves and by inhibition of photosynthetic processes in the Hill reaction; its oral LD_{50} for rats is 5200 mg/kg (1,2).

The European laws limit the amount of total pesticides in drinkable waters to 0.5 µg/L (0.1 µg/L for each pesticide) (3). No limit is explicitly declared for surface water, but amounts not more than 30 µg/L are generally accepted (4), whereas for fruits and crops (5) even higher quantities are allowed.

Table I. Detection Limits for the Analytes Investigated at 228 nm and Molar Absorptivity Values at This Wavelength

Analyte*	Detection limit (µg/L)	ε (L/mol · cm)
Bromacil	11.7	(5.29 ± 0.06) 10^3
DNOC	10.6	(8.16 ± 0.09) 10^3
2,4-D	9.4	(7.25 ± 0.09) 10^3
2,4-DB	12.2	(3.78 ± 0.05) 10^3
Dichlorprop	8.8	(8.53 ± 0.08) 10^3
2,4,5-T	6.0	(7.58 ± 0.07) 10^3
MCPA	4.5	(9.16 ± 0.08) 10^3

* Abbreviations: bromacil, 5-bromo-3-*sec*-butyl-6-methyluracil; DNOC, 4, 6-dinitro-*o*-cresol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DB, 4-(2,4-dichlorophenoxy)butyric acid; dichlorprop, 2(2,4-dichlorophenoxy)propionic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCPA, 4-chloro-*o*-tolyloxyacetic acid.

Many chromatographic methods have been published for herbicide separation and determination. Gas chromatography is applicable when associated with derivatization reactions that increase volatility and sensitivity. Preconcentration treatments are generally necessary for gas chromatography and high-performance liquid chromatographic (HPLC) analysis (6–9). For HPLC analysis, solid-phase extraction methods with C_8 or C_{18} phases (10,11) and graphitized carbon black (12), liquid membrane extraction (13), supercritical fluid extraction (14), liquid–liquid extraction (15), and liquid–solid extraction (4) are reported, and the methods are often associated with derivatization reactions (16). UV and diode-array detection are commonly used (9,10,12,15,17,18), as well as fluorescence (14,16) or electrochemical detection (15,19). Mass spectrometric methods including liquid–liquid extraction (20) and those using thermospray (21) or particle beam (4) interfaces are also reported.

We report a sensitive ion-interaction chromatographic method that permits the separation of bromacil, DNOC, 2,4-D, dichlorprop, 2,4,5-T, 2,4-DB, and MCPA. The effect of pH, organic modifier, and ion-interaction reagent concentration on analyte retention is comparatively studied in the optimization of the experimental conditions.

The method permits the achievement of detection limits constantly less than 12 µg/L without preconcentration processes and can therefore be advantageously applied to native water samples (i.e., surface water in which a concentration of 30 µg/L of each herbicide is the allowed limit) (4).

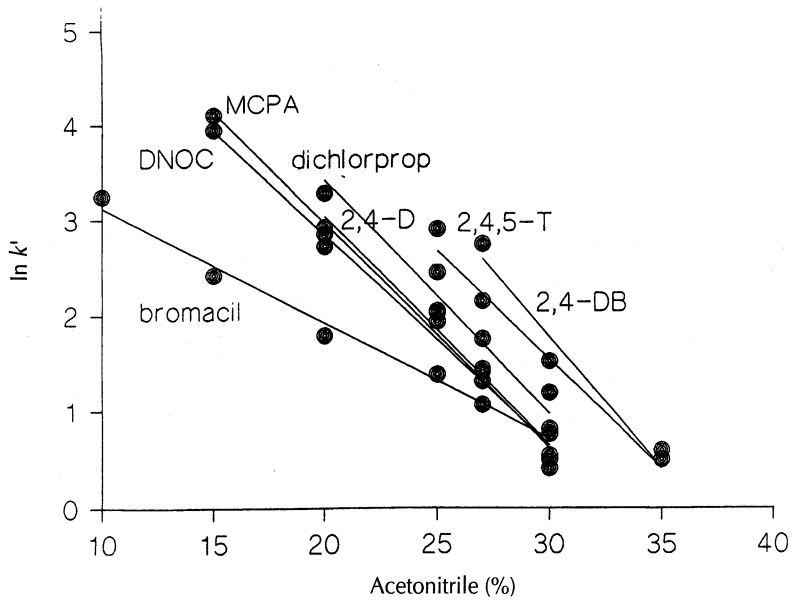

Figure 2. Plot of ln k' versus the acetonitrile percentage in the mobile phase, which contains, in all cases, 5.0mM of octylamine and is brought to pH 6.4 with *o*-phosphoric acid. Abbreviations: bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), DNOC (4,6-dinitro-*o*-cresol), MCPA (4-chloro-*o*-tolyloxyacetic acid), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid).

Experimental

Chemicals and reagents

Ultrapure water from a Millipore MilliQ (Milford, MA) system was used for the preparation of solutions. Sodium nitrate was of analytical grade from Merck (Darmstadt, Germany). 2,4-D, 2,4-DB, bromacil, dichlorprop, DNOC, 2,4,5-T, and MCPA were all of analytical grade from LabService Analytica (Anzola dell'Emilia; Bologna, Italy). Octylamine, *o*-phosphoric acid, and HPLC-grade acetonitrile were obtained from Fluka Chemicals (Buchs, Switzerland).

Apparatus

The chromatographic analyses were performed with a Merck-Hitachi (Tokyo, Japan) Lichrograph Model L-6200 chromatograph equipped with a two-channel D-2500 chromato-integrator interfaced with a UV–vis detector (Model L-4200) and a conductivity detector (Model L-3720) with temperature control, all of which were from Merck-Hitachi.

Absorbance measurements were performed with a Hitachi Model 150-20 spectrophotometer.

For pH measurement, a Metrohm 654 pH meter (Herisau, Switzerland) with a combined glass–calomel electrode was used.

Chromatographic conditions

A Phase Separations (Desidee; Clwyd, U.K.) Spherisorb S5 ODS-2 (250 × 4.6-mm i.d., 5-μm particle size) column with a carbon load of 12% (0.5 mmol/g) and Spherisorb S5 ODS-2 (250 × 4.6-mm i.d., 5-μm particle size) cartridges (unknown carbon load) were used together with a Lichrospher RP-18 guard precolumn (5 µm) (Merck).

Table II. Regression Functions of the Curves Reported in Figure 2

Analyte*	Regression functions	r^2
Bromacil	$y = 4.3324–0.1207x$	0.9885
DNOC	$y = 7.2373–0.2200x$	0.9912
2,4-D	$y = 7.8111–0.2389x$	0.9765
2,4-DB	$y = 8.4043–0.2179x$	0.9229
Dichlorprop	$y = 8.3199–0.2451x$	0.9674
2,4,5-T	$y = 8.3182–0.2260x$	0.9313
MCPA	$y = 7.6607–0.2349x$	0.9840

* Abbreviations: bromacil, 5-bromo-3-*sec*-butyl-6-methyluracil; DNOC, 4, 6-dinitro-*o*-cresol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DB, 4-(2,4-dichlorophenoxy)butyric acid; dichlorprop, 2(2,4-dichlorophenoxy)propionic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCPA, 4-chloro-*o*-tolyloxyacetic acid.

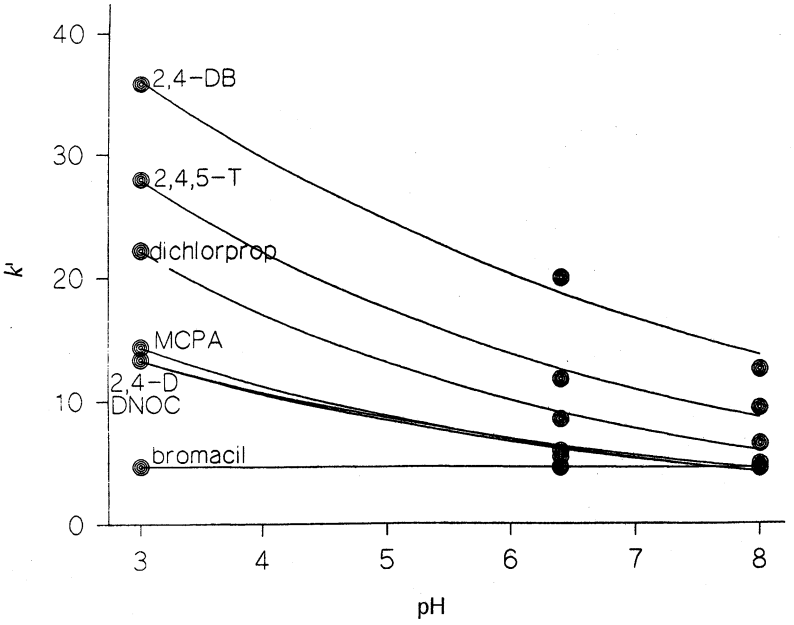

Figure 3. Plot of capacity factor k' versus pH of the eluent. Mobile phase consists of 5.0mM octylamine in 27% acetonitrile–73% water and is adjusted to the proper pH with *o*-phosphoric acid. Abbreviations: 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), MCPA (4-chloro-*o*-tolyloxyacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), DNOC (4,6-dinitro-*o*-cresol), and bromacil (5-bromo-3-*sec*-butyl-6-methyluracil).

The mobile phases were hydroorganic (water–acetonitrile) solutions of octylamine. *o*-Phosphoric acid was used to modify the pH. (The pH value obtained in the hydroorganic solution is reported as the "operational" pH [22].)

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained; a minimum of 1 h was necessary at a flow rate of 1.0 mL/min. After use, the column was washed with a water–acetonitrile mixture (50:50, v/v) (1.0 mL/min for 1 h) followed by a wash with acetonitrile (0.7 mL/min for 20 min).

Dead times were evaluated for all of the experimental conditions by injecting $NaNO_3$ (15.0 mg/L) solutions and using conductometric detection of the unretained sodium ion.

Repeatability of analyte-measured retention times was always within 3% for the same eluent preparation, and the interday reproducibility for different preparations was always within 6%.

Results and Discussion

Optimization of conditions

Different conditions were considered in order to optimize the separation of bromacil, DNOC, 2,4-D, 2,4-DB, 2,4,5-T, dichlorprop, and MCPA.

Detection wavelength

Spectra recorded for solutions containing 10.0 mg/L of the analytes showed an absorbance maximum at 228 nm for 2,4-D, 2,4-DB, 2,4,5-T, MCPA, and dichlorprop, whereas bromacil had an absorbance maximum at 278 nm. However, its absorbance at 228 nm was high enough that a wavelength of 228 nm was chosen for the analysis. The molar absorptivities obtained at 228 nm for all of the analytes are reported in Table I.

Concentration of the organic solvent in the mobile phase.

A series of experiments was performed with mobile phases containing both 5.0mM octylammonium phosphate and 5–35% acetonitrile; Figure 2 reports the values of ln k' versus acetonitrile concentration. The plots can be fitted by straight lines, and the regression functions and correlation coefficients of the lines are reported in Table II. The slopes obtained for the hydrophilic species (phenoxy acids and the substituted cresol) are similar (range, 0.2200–0.2451) and substantially different from the slope (0.1207) of the bromacil plot. This behavior is likely due to the different retention mechanisms that the analytes underwent as a function of their lipophilicity. It has been suggested (23) that two kinds of active sites are present in the stationary phase, namely, original reversed-phase sites and sites mod-

ified by the ion-interaction reagent. Each of them can participate in retention, and the predominance of one type of retention over the other depends on the chromatographic conditions, particularly the concentrations of the ion-interaction reagent and the organic modifier. It can be proposed that, under the chromatographic conditions used here, the more hydrophilic species undergo ion-interaction mechanisms, whereas bromacil, because of its predominantly lipophilic properties, is retained through conventional reversed-phase mechanisms. These different interaction equilibria can explain the different slopes observed.

Furthermore, the similar retention dependence on the organic modifier concentration obtained for the more hydrophilic analytes (phenoxy acids and DNOC) suggests that the organic solvent exerts its effect predominantly on the ion-interaction moiety adsorbed onto the surface of the stationary phase, which affects all the analytes similarly regardless of structure. The same behavior has already been observed (23) for hydrophilic analytes possessing different chemical structures (acids, amines, aminophenols).

The lower slope of the plot ln k' versus acetonitrile concentration obtained for bromacil can be explained as follows. First, bromacil retention is influenced only by the eluotropic force of the solvent. Second, there is another effect to be considered: As the organic modifier concentration increases and the ion-interaction reagent adsorbed onto the surface is progressively removed, a greater number of active sites is made available at the stationary phase surface for reversed-phase interactions. Thus, for bromacil, the retention decrease is lower than those observed for the other analytes.

From a practical point of view, the optimal acetonitrile concentration that produced the best resolution in a reasonable amount of analysis time was 27%.

Table III. Regression Curves of k' versus pH of the Mobile Phase

Analyte*	Curve	r^2
Bromacil	4.7587 exp (–0.0059x)	0.9708
DNOC	26.4119 exp (–0.2298x)	0.9823
2,4-D	25.5785 exp (–0.2183x)	0.9922
2,4-DB	64.4215 exp (–0.1935x)	0.9895
Dichlorprop	48.6950 exp (–0.2633x)	0.9959
2,4,5-T	56.2061 exp (–0.2345x)	0.9940
MCPA	30.0369 exp (–0.2467x)	0.9970

* Abbreviations: bromacil, 5-bromo-3-*sec*-butyl-6-methyluracil; DNOC, 4, 6-dinitro-*o*-cresol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DB, 4-(2,4-dichlorophenoxy)butyric acid; dichlorprop, 2(2,4-dichlorophenoxy)propionic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MCPA, 4-chloro-*o*-tolyloxyacetic acid.

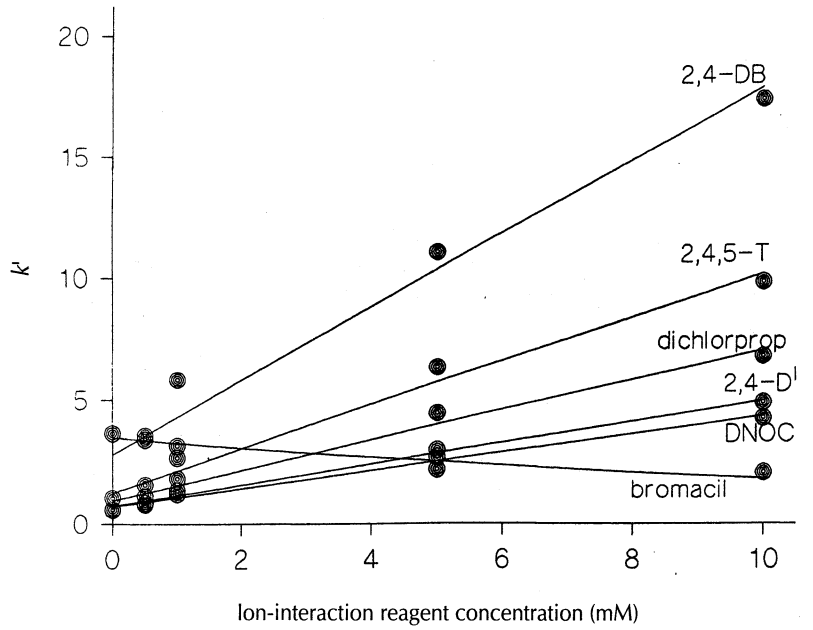

Figure 4. Plot of ln k' versus ion-interaction reagent (octylamine *o*-phosphate) concentration in the eluent. The mobile phase consists of the ion-interaction reagent (octylamine) and a hydroorganic (73% water–27% acetonitrile) solution adjusted to pH 6.4 with *o*-phosphoric acid. Abbreviations: 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), DNOC (4,6-dinitro-*o*-cresol), and bromacil (5-bromo-3-*sec*-butyl-6-methyluracil).

pH of the mobile phase

Figure 3 reports the capacity factors (k') for phenoxy acids, DNOC, and bromacil as a function of the pH of the mobile phase in the pH range allowed by the silica-based stationary phase. As expected for hydrophilic phenoxy acids and DNOC (24,25), retention decreases similarly as pH increases, whereas it is practically unaffected for hydrophobic bromacil (Table III).

As expected, less favorable values were obtained for sensitivity (expressed as the peak area given by the integrator for 1.00 µg/L) at the lowest pH values of the mobile phase, whereas comparable sensitivities were obtained for pH 6.4 and 8.0.

Resolution is comparable at pH 6.4 and 8.0, but analysis time is lower at pH 8.0.

Concentration of the ion-interaction reagent

Experiments were performed to optimize the ion-interaction reagent concentration. Retention was measured for mobile phases brought to pH 6.4 and 8.0; these mobile phases contained the optimized amount of acetonitrile (27%), and concentrations of the ion-interaction reagent ranged from 0.50 to 10.00mM. Figure 4 reports the k' values obtained as a function of the ion-interaction reagent concentration.

Once again, although the retention of hydrophilic analytes increased with the ion-interaction reagent concentration, bromacil retention did not increase but, in fact, decreased somewhat. This result might occur because, as the concentration of octylammonium phosphate in the mobile phase increased, a greater number of the original active sites on the column was modified (23),

and consequently, a lower number of reversed-phase sites was available for bromacil retention.

The optimal separation conditions included a mobile phase containing 5.0mM octylammonium *o*-phosphate in a water–acetonitrile (73:27, v/v) solution at pH 6.4 with a flow rate of 1.0 mL/min.

Reversed-phase elution

To confirm the hypothesis that two different retention mechanisms can take place under the same chromatographic conditions as a function of analyte lipophilicity, we performed a chromatographic run by using a mobile phase containing water–acetonitrile (73:27, v/v) in the absence of the ion-interaction reagent.

As expected under these conventional reversed-phase conditions, only bromacil, because of its lipophilic properties, was retained, and its retention time practically coincided with that observed in ion-interaction mode.

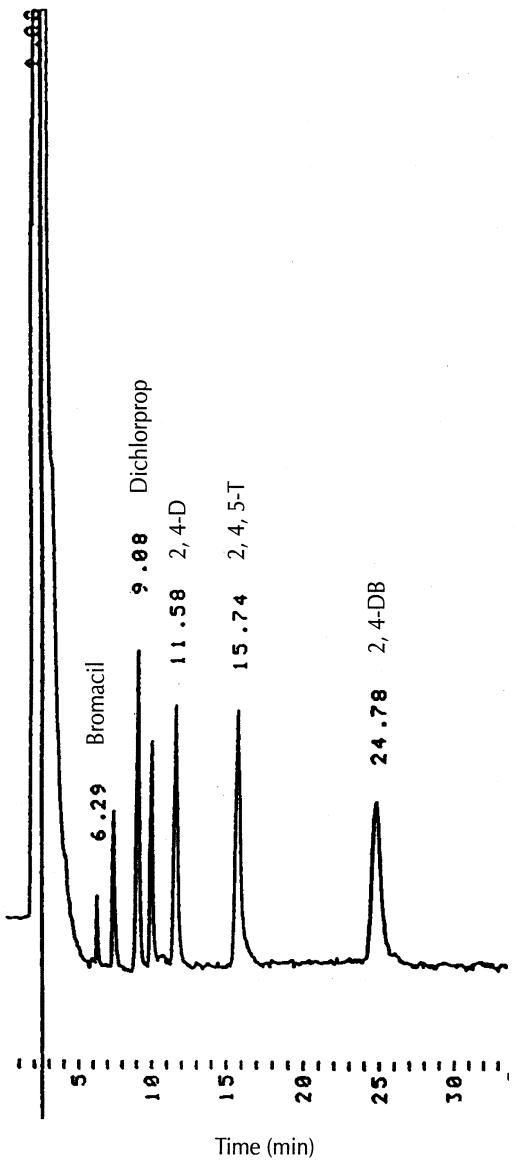

Figure 5. Chromatogram of a standard mixture of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid) (30 µg/L of each pesticide). Experimental conditions: column, Phase Separation Spherisorb 5S ODS-2 (cartridge type) (250 × 4.6-mm i.d., 5-µm particle size); mobile phase, 5.0mM octylamine in water–acetonitrile (73:27, v/v) adjusted to pH 6.4 with *o*-phosphoric acid; flow rate, 1.2 mL/min. Spectrophotometric detection at 228 nm.

Packing material

Previous work (26,27) showed that aspects of the stationary phase (not only the particle size but also the carbon percent load) can play relevant roles in retention. To confirm these results using the optimized conditions, we examined a new cartridge column that was produced by the same firm (Phase Separations) but whose carbon load content was not explicitly declared.

The cartridge produced a separation of the mixture that was comparable in resolution and total analysis time but different in retention times.

A different sequence elution order was observed between 2,4-D and dichlorprop; we are not able to explain this result. The sensitivity obtained with the cartridge was much higher.

As an example, Figure 5 shows the separation of bromacil, dichlorprop, 2,4-D, 2,4,5-T, and 2,4-DB (at concentration of 30 µg/L each) obtained using the cartridge-type column under the optimized conditions.

For all of the analytes, the calibration curves that show peak areas versus micrograms per liter yielded good linearities over the concentration range of 20–100 µg/L. Detection limits were evaluated by proportionally comparing the sensitivity (expressed as peak area for 1.00 µg/L) obtained in the calibration plot with a peak area in the chromatogram corresponding to a signal-to-noise ratio equal to 3. Detection limits are reported in Table I and range between 5 µg/L (MCPA) and 12 µg/L (2,4-DB).

Application of the method to the analysis of surface water samples

The method was applied to the analysis of samples of surface water. As an example, Figure 6 reports two chromatograms of a water sample of the Dese river, which flows into the Venice lagoon.

The chromatogram of Figure 6A recorded using the native sample seems to indicate the absence of the herbicides studied in this report, at least at the method detection limit (Table I).

Recovery yield studies were performed to take into account the matrix effect. Samples of water were spiked with a mixture of bromacil, dichlorprop, 2,4-D, 2,4-DB, and 2,4,5-T at final concentrations of 30 µg/L each (Figure 6B), and recovery yields evaluated by the standard addition method were always greater than 90%.

Conclusion

The analytical method presented can be advantageously used for the determination of bromacil, DNOC, and phenoxy acid pesticides in surface waters in instances in which the maximum

permitted concentration is 30 µg/L (4). No preconcentration step, derivatization procedure, or pH adjustment is required.

Acknowledgment

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References

1. G. Rapparini. *I Diserbanti.* L' Informatore Agrario, Verona, Italy, 1986.
2. M. Muccinelli. *Prontuario dei Fitofarmaci,* VI ed. Edizioni Agricole, Bologna, Italy, 1990.
3. *Drinking Water Directive.* Commission of the European Communities. July 15, 1980. Directive 80/778/CEE.
4. A. Cappiello, G. Famiglini, and F. Bruner. Determination of acidic and basic/neutral pesticides in water with a new microliter flow rate LC/MS particle beam interface. *Anal. Chem.* **66:** 1416–23 (1994).
5. Commission of the European Communities. August 23, 1993. Directive 93/57/CEE.
6. W.K. Wang and S.D. Huang. Rapid determination of seven herbicides in water or isooctane using C18 and Florisil Sep-Pak cartridge and gas chromatography with electron-capture detection. *J. Chromatogr.* **483:** 121–29 (1989).
7. C. Sánchez-Brunete, S. Pérez, and J.L. Tadeo. Determination of phenoxy ester herbicides by gas and high-performance liquid chromatography. *J. Chromatogr.* **552:** 235–40 (1991).
8. H. Steinwandter. Contributions to the analysis of chlorophenoxy acids. *Fresenius Z. Anal. Chem.* **334:** 133–35 (1989).
9. C. Schlett. Multi-residue-analysis of pesticides by HPLC after solid-phase extraction. *Fresenius Z. Anal. Chem.* **339:** 344–47 (1991).
10. S.H. Hoke, E.E. Brueggemann, L.J. Baxter, and T. Trybus. Determination of phenoxy acid herbicides using solid-phase extraction and high-performance liquid chromatography. *J. Chromatogr.* **357:** 429–32 (1986).
11. M. Åkerblom. Simple precolumn sample enrichment in high-performance liquid chromatography for determination of phenoxy acid herbicides in water samples from exposure studies. *J. Chromtogr.* **319:** 427–31 (1985).
12. A. Di Corcia and M. Marchetti. Multiresidue method for pesticides in drinking water using a graphitized carbon black cartridge extraction and liquid chromatographic analysis. *Anal. Chem.* **63:** 580–85 (1991).
13. G. Nilvé, G. Audunsson, and J.Å. Jönsson. Sample preparation by means of a supported liquid membrane for the determination of chlorophenoxyalkanoic acids. *J. Chromatogr.* **471:** 151–60 (1989).
14. E. Sauvage, J.L. Rocca, and G. Toussaint. The use of nitrous oxide for supercritical fluid extraction of pharmaceutical compounds from animal feed. *J. High Resolut. Chromatogr.* **16:** 234–38 (1993).
15. W. Schüssler. Automatic measurement of bentazone and phenoxy acid herbicides by HPLC with three different detectors. *Chromatographia* **29:** 24–30 (1990).
16. T. Suzuki and S. Watanabe. Screening method for phenoxy acid herbicides in ground water by high-performance liquid chromatography of 9-anthryldiazomethane derivatives and fluorescence detection. *J. Chromatogr.* **541:** 359–64 (1991).
17. H. Klöppel, J. Haider, C. Hoffmann, and B. Lüttecke. Simultaneous determination of the herbicides isoproturon, dichlorprop-p, and bifenox in soils using RP-HPLC. *Fresenius Z. Anal. Chem.* **344:** 42–46 (1992).
18. E.A. Hogendoorn, E. Dijkman, S.M. Gort, R. Hoogerbrugge, P. van Zoonen, and U.A.T. Brinkman. General strategy for multiresidue analysis of polar pesticides in ground water using coupled-column reversed-phase LC and step-gradient elution. *J. Chromatogr. Sci.* **31:** 433–39 (1993).

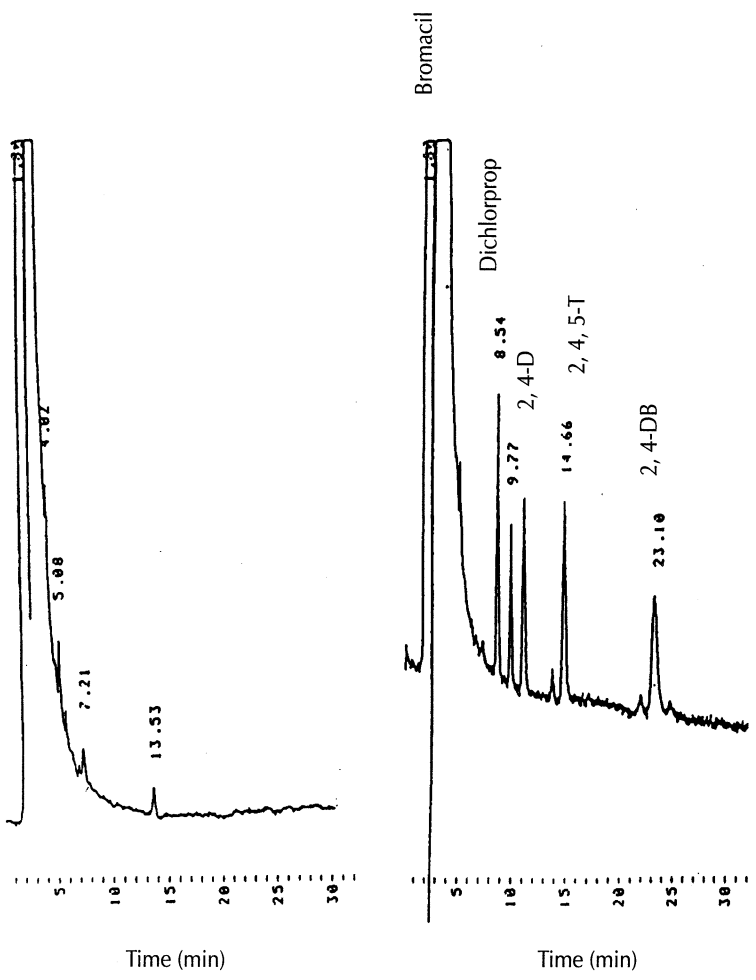

Figure 6. Chromatogram of a native (A) and spiked (B) (30 µg/L of each pesticide) sample of Dese river water collected near the outlet into the Venice lagoon. Experimental conditions: column, Phase Separation Spherisorb 5S ODS-2 (cartridge type) (250 x 4.6-mm i.d., 5-µm particle size); mobile phase, 5.0mM octylamine in water–acetonitrile (73:27, v/v) adjusted to pH 6.4 with *o*-phosphoric acid; flow rate, 1.2 mL/min. Spectrophotometric detection at 228 nm. Abbreviations: bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), dichlorprop (2-(2,4-dichlorophenoxy)propionic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and 2,4-DB (4-(2,4-dichlorophenoxy)butyric acid).

19. S. Yao, A. Meyer, and G. Henze. Comparison of amperometric and UV-spectrophotometric monitoring in the HPLC analysis of pesticides. *Fresenius Z. Anal. Chem.* **339:** 207–11 (1991).
20. J.A. Apffel, U.A.T. Brinkman, and R.W. Frei. Micro post-column extraction system for interfacing reversed-phase micro liquid chromatography and mass spectrometry. *J. Chromatogr.* **312:** 153–64 (1984).
21. D. Barceló, G. Durand, R.J. Vreeken, G.J. De Jong, H. Lingeman, and U.A.T. Brinkman. Evaluation of eluents in thermospray liquid chromatography–mass spectrometry for identification and determination of pesticides in environmental samples. *J. Chromatogr.* **553:** 311–28 (1991).
22. R.M. Lopes-Marques and P.J. Schoenmakers. Modelling retention in reversed-phase liquid chromatography as a function of pH and solvent composition. *J. Chromatogr.* **592:** 157–82 (1992).
23. M.C. Gennaro, C. Abrigo, E. Pobozy, and E. Marengo. Retention dependence on organic modifier and interaction reagent concentration in reversed-phase ion-interaction HPLC. *J. Liq. Chromatogr.* **18:** 311–30 (1995).
24. M.C. Gennaro, C. Abrigo, and E. Marengo. Retention of anions as a function of mobile phase pH in ion-interaction RP-HPLC *J. Liq. Chromatogr.* **17:** 1231–44 (1994).
25. M.C. Gennaro, D. Giacosa, and C. Abrigo. The role of pH of the mobile phase in ion-interaction RP-HPLC. *J. Liq. Chromatogr.* **17:** 4365–80 (1994).
26. M.C. Gennaro, P.L. Bertolo, and A. Cordero. Determination of nitrite and nitrate in Venice lagoon water by ion interaction reversed-phase liquid chromatography. *Anal. Chim. Acta* **239:** 203–209 (1990).
27. M.C. Gennaro and C. Abrigo. Caffeine and theobromine in coffee, tea and cola beverages. *Fresenius Z. Anal. Chem.* **343:** 523–25 (1992).

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