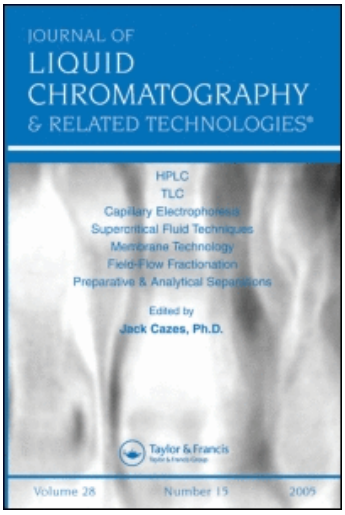

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G. M. F. Pinto[a]; I. C. S. F. Jardim[a]
[a] Department of Analytical Chemistry, Chemistry Institute, State University of Campinas, Campinas, SP, Brazil

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MOBILE PHASE OPTIMIZATION FOR THE SEPARATION OF SOME HERBICIDE SAMPLES USING HPLC

G. M. F. Pinto,* I. C. S. F. Jardim

Department of Analytical Chemistry
Chemistry Institute
State University of Campinas
Cx. Postal 6154
13083-970 Campinas, SP, Brazil

ABSTRACT

To separate and determine a mixture of herbicides containing bentazon, 2,4-D, cyanazine, simazine, atrazine, fluazifop acid, diuron, linuron, and ametryn, an intense study was made to optimize the chromatographic conditions, emphasizing the composition of the mobile phase. After consideration of both analysis time and resolution, the optimum mobile phase to carry out the separation of mixtures of these herbicides was found to be methanol :water 60:40, v/v, pH = 4.6 (adjusted with phosphoric acid). These conditions may be used to analyze mixtures of the cited herbicides present in water samples.

INTRODUCTION

The use of herbicides in agriculture has increased in recent years although people have become more conscious of the risks arising from intense use of herbicides on large areas.[1-7] Thus, European countries and the US EPA (Environmental Protection Agency) have established legal limits of 0.1 μg/L for individual herbicides and 0.5 μg/L for the sum of herbicides present in water.[1,4,5,7-13]

HPLC and GC, after appropriate sample enrichment procedures, are widely used to monitor the concentrations of herbicides in water.[1,4-9,13,14] HPLC has many advantages over GC methods, because it permits the simultaneous analysis of acidic, basic and neutral compounds, ionic compounds, non volatile, and thermally unstable compounds, without a derivation step.[5,6,8-10,15-17]

An important factor in HPLC analysis is the mobile phase, because it interacts with solute species of the sample and has a significant influence on the separation. In the case of ionizable compounds, the mobile phase is even more important, because controlling the pH of the mobile phase may determine if the compound will be dissociated or not. In this study potentially ionized compounds were analyzed utilizing a C-18 column, which requires that these compounds not be dissociated to have useful retention on the column. The method of ionic suppression was used to achieve a mobile phase that permits the separation of acidic, basic, and neutral herbicides present in an aqueous sample.

2,4D is a pre or post emergence herbicide used mainly on cane and coffee crops.[18-21] Because of its acidic and polar character, 2,4D can not be analysed by GC without derivatization such as methylation or acetylation.[22]

Bentazon is a post emergency herbicide used mainly on corn and bean crops.[19-22] It also has acidic and polar character and can only be analysed by GC after diazomethane derivatization.[23-24]

Simazine, cyanazine, atrazine, and ametrin are triazine groups. They have weak basic character. The triazines are a very important class of herbicides with atrazine and simazine being the most widely used and the most persistent in the environment.[25-28]

Diuron and linuron are substituted urea compounds. They have nearly neutral character but present difficulty in analysis by GC because they are thermally degradable. The substituted ureas are persistent chemically and can survive in the soil for months after application. Beyond this, they are soluble in water, so they can migrate in the soil and can thereby enter the food chain, where they are degraded and metabolized by mammals.[25,29,30]

Fluazifop acid is a post emergence herbicide used mainly on cane, coffee, and corn crops.[18-21] It has acidic and polar character so it can not be analysed by GC without derivatization.

These herbicides can present significant environmental hazards and, thus, it is important to develop an analytical method to analyse for them in the aqueous samples.

EXPERIMENTAL

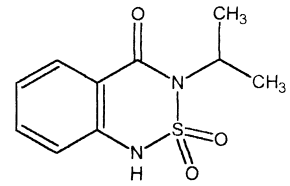
Reagents

Table 1 presents information about the supplier, purity grade, chemical family, and agricultural use of the herbicides determined in this work. Figure 1 illustrates the structures of these herbicides. The structures of some compounds, such as 2,4D and fluazifop acid, clearly show ionic character. Stock solutions were prepared in methanol at 0.1 g/L, except for 2,4-D which was pre-

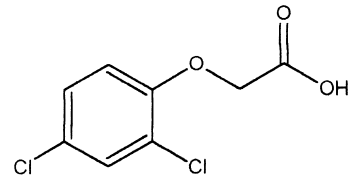
Table 1

Supplier, Purity Grade, Chemical Family and Agricultural Use of Some Herbicides

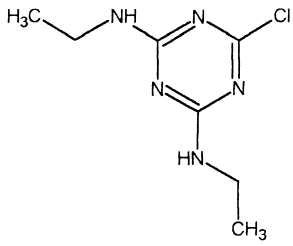
Herbicide	Supplier	Purity Grade (%)	Family[19,20]	Agricultural Use[19,20]
Bentazon	Basf	99.9	Tiodiazine	soybeans, corn, wheat, peanut
Fluazifop acid	Zeneca	97.4	aryl-oxy-phenoxy-acid	lettuce, cotton
2,4-D	Dow	> 99	Phenoxyacetic acid	coffee, cane
Cyanazine	Cyanamid	98.0	Triazine	cotton, soy-bean, cane
Simazine	Novartis	98.3	Triazine	corn, strawberry, pineapple
Atrazine	Novartis	97.7	Triazine	pineapple, cocoa, cane, coffee
Diuron	DuPont	99.27	Substituted urea	cotton, soy-bean, banana
Linuron	Hoescht	99.5	Substituted urea	garlic, manioc, wheat
Ametryn	Novartis	96.8	Triazine	pineapple, coffee, corn



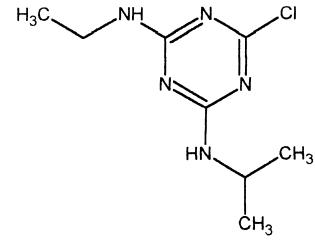
bentazon



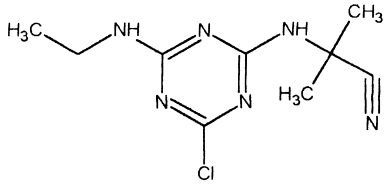
2,4-D



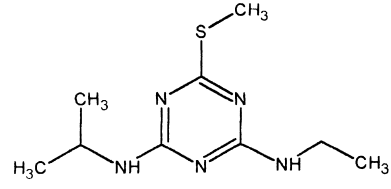
simazine



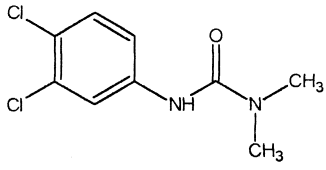
atrazine



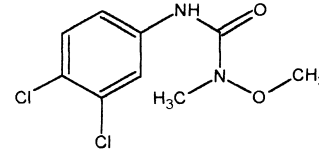
cyanazine



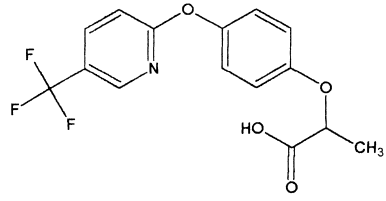
ametryn



diuron



linuron



fluazifop acid

Figure 1. Structures of the herbicides used in this study.[18-20]

pared at 1 g/L. The mixture of herbicides for developing the separation was prepared in mobile phase and stored in the refrigerator (T = 4°C). The concentrations of each herbicide in the analytical mixture were: 1330.0; 3150.0; 1110.0; 1050.0; 360000.0; 5000.0; 1120.0; 4880.0, and 1880.0 µg/L, for bentazon; 2,4-D; cyanazine; simazine; fluazifop acid; diuron; atrazine; linuron, and ametryn, respectively.

The solvents methanol (Omnisolv- Merck) and acetic acid (Mallinckrodt) were chromatographic grade. Sodium acetate (Mallinckrodt) and phosphoric acid (Synth) were analytical reagent grade. Water was purified with a Millipore Milli-Q Plus System.

Instrumentation and Methods

The chromatography was performed with a modular HPLC system equipped with a Rheodyne 7725i injector with a 10 µL loop, a Waters 510 pump, a UV/Vis absorbance detector (Waters Model 486) coupled to a Chrom Perfect for Windows, version 3.03, computer program, for acquisition and treatment of data. The mobile phase flow rate was set at 0.8 mL/min and detection was at 230 nm. The column used was a Waters Nova-Pak C-18 (150 x 3.9 mm i.d.) and the guard column was also Nova-Pak C-18 (20 x 3.9 mm i.d.). All measurements were carried out at ambient temperature.

The mobile phase was prepared volumetrically from individually measured aliquots of methanol and water. The pH of the mobile phase was adjusted with use of a Digimed, model DM21, pHmeter, with glass and thermal compensation electrodes.

The column hold-up time, t_M, was determined using methanol as an unretained compound. The chromatographic factors measured were retention time (t_R), plate number per meter (N/L), asymmetry factor at 10% of peak height (As) and resolution (Rs).

RESULTS AND DISCUSSION

Figure 2 illustrates that it is not possible to determine quantitatively all components of the mixture of herbicides without pH adjustment of the mobile phase. This is because the compounds 2,4-D and bentazon are not retained by the C-18 column at neutral pH, appearing with retention times less than the apparent hold-up time. This result occurs for the mobile phases methanol:water 50:50, 60:40, and 70:30, v/v. Besides, for the 50:50 v/v composition of the mobile phase the total analysis time is relatively long, approximately 21 minutes, and ametryn is difficult to determine. With the 70:30 v/v mobile phase the resolution (Rs) between atrazine and diuron and between bentazon and

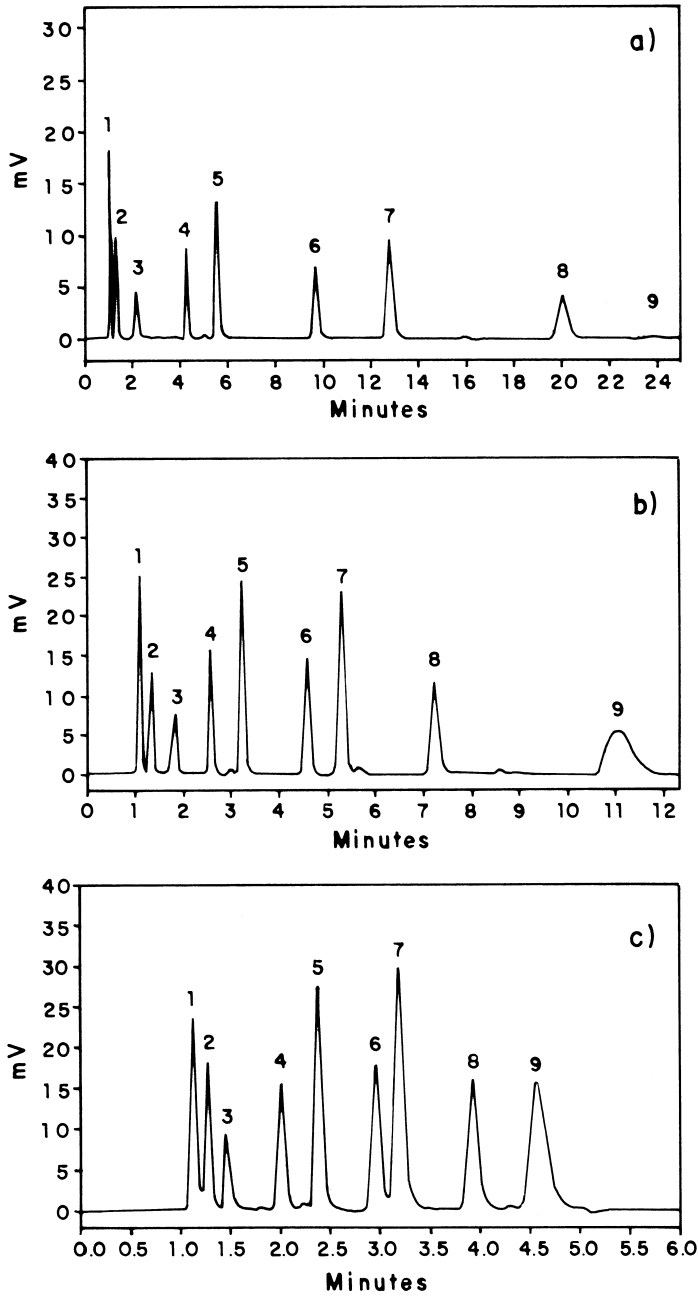


Figure 2. Chromatographic separation of herbicides using the mobile phase methanol : water without pH adjustment, a) 50:50, v/v; b) 60:40, v/v; c) 70:30, v/v. Flow rate = 0.8 mL/min, λ = 230 nm, injection volume = 10 µL. 1= bentazon; 2= 2,4D; 3= fluazifop acid; 4= cyanazine; 5= simazine; 6= atrazine; 7= diuron; 8= linuron; 9= ametryn.

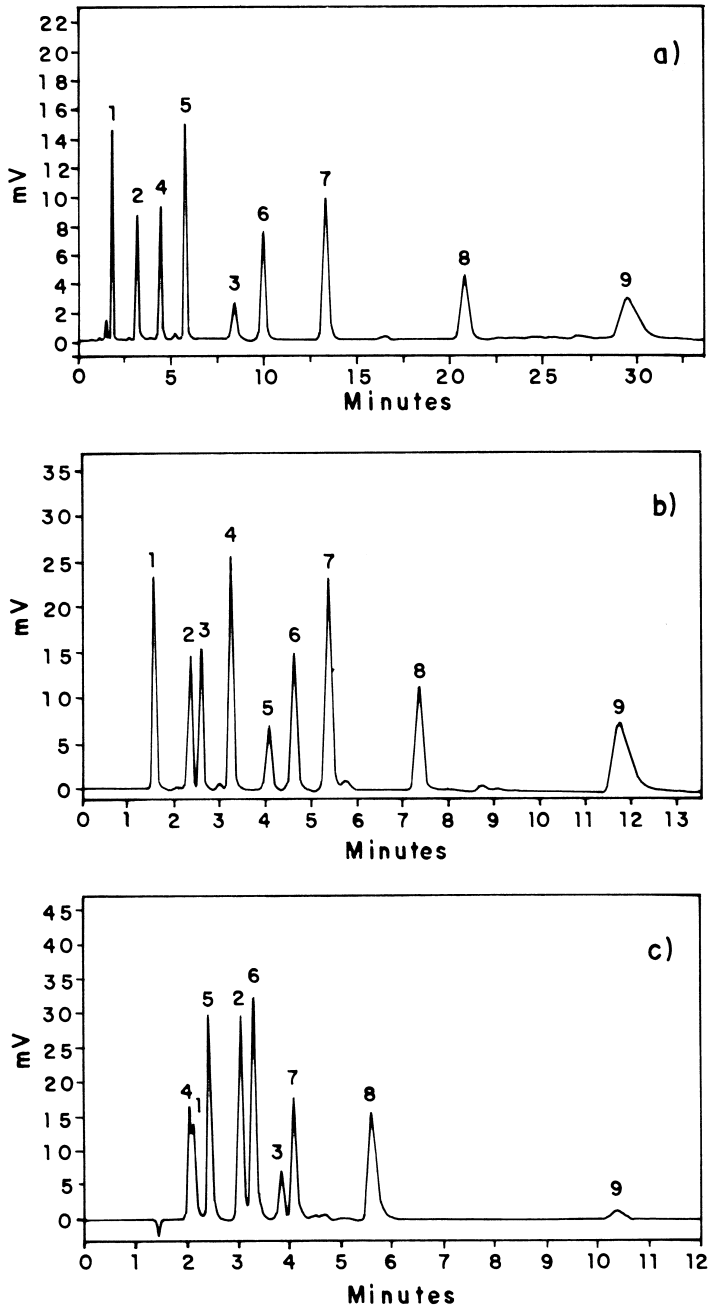


Figure 3. Chromatographic separation of herbicides using the mobile phase methanol : water with pH adjustment with phosphoric acid. a) 50:50, v/v pH=4.6; b) 60:40, v/v pH=4.6; c) 70:30, v/v pH=3.6. Peak identification as in Figure 2.

2,4-D is low (Rs= 1.46). However, with the 60:40 v/v mobile phase, the analysis time and Rs are satisfactory (analysis time = 12 minutes and Rs ≥ 1.5 for all the peaks).

To determine the optimum pH for separation of the herbicides, chromatographic analyses were carried out over the pH range: 3-7. Figure 3 presents some chromatograms from separations of the herbicides, utilizing acidified mobile phases. In this study, phosphoric acid was used instead of acetic acid to lower the pH because it has lower absorption in the UV and is a stronger acid. Similar quantities of a 0.1% solution of phosphoric acid give the same pH as does 1% acetic acid. Besides, phosphoric acid does not attack the chromatographic connections. In Figures 3a and 3b, the pH of each mobile phase was adjusted to 4.6. This value was found to be the optimum pH for the separation of complete herbicide mixtures. In Figure 3c the pH of the mobile phase is adjusted to 3.6, to illustrate that a small alteration in pH causes a significant modification in herbicide separation.

With pH adjustment, the compounds 2,4-D, bentazon and fluazifop acid appear with retention times longer than the hold-up time, indicating that these compounds are present in the non-dissociated form. This procedure is called ionic suppression, which consists of reducing the ionization of solutes by alteration of the pH of the mobile phase. Compounds with weak acidic character may be described by the equilibrium: $HA = H^+ + A^-$. In this case the pH of the

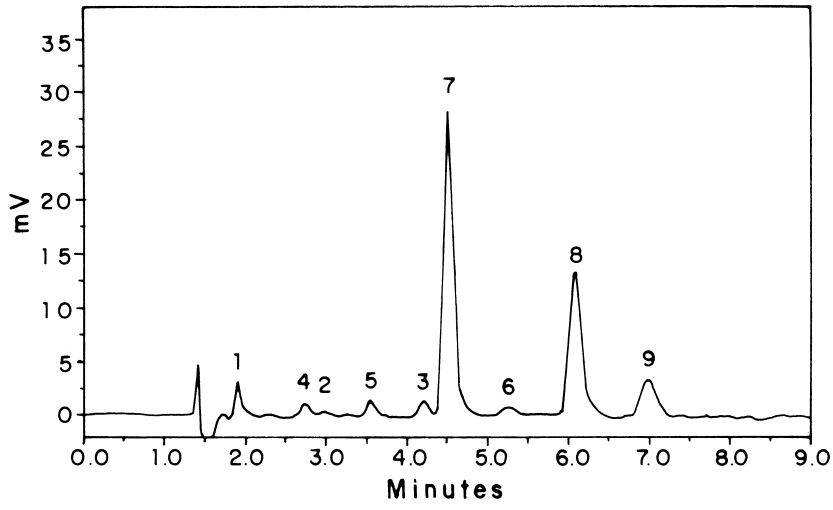


Figure 4. Chromatographic separation of herbicides using an acetate buffer mobile phase: methanol:NaAc/HAc pH=3.8 buffer (50:50), v/v. Peak identification as in Figure 2.

mobile phase must be decreased to shift the equilibrium to the non-dissociated form to have useful retention on the C-18 column. Compounds which are commonly added to the mobile phase to decrease the pH are acetic acid, phosphoric acid, acetate buffer and phosphate buffer. Figure 4 shows the poor results obtained with acetate buffer in the mobile phase. The intensity of the peaks is drastically reduced, decreasing the chromatographic sensitivity, except for diuron, linuron, and ametryn. Besides this, the well known disadvantages of the use of buffers include: the frequent need to exhaustively wash the chromatographic system to avoid the problems of salt encrustation and an increase in baseline noise.

CONCLUSION

A mobile phase consisting of methanol:water 60:40, v/v, at pH=4.6, adjusted with phosphoric acid, permits the chromatographic separation of the herbicide mixture: bentazon, 2,4D, fluazifop acid, cyanazine, simazine, atrazine, diuron, linuron, and ametryn (which includes acidic, basic, and neutral compounds, with some having ionic character), with good resolution (≥ 1.5) and a convenient analysis time (12 minutes), without the problems of buffer type mobile phases. This mobile phase is economical, easy to prepare, and to use in the separation of mixtures of the several herbicides, which can not be conveniently separated using GC.

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