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Effect of temperature on the separation of chlorophenoxy acids and carbamates by capillary high-performance liquid chromatography and UV (or diode array) detection

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Abstract

In this work, the effect of temperature in isothermal and programmed modes on several chromatographic parameters such as retention factor, selectivity, resolution and plate number has been discussed. A critical comparison of isocratic/isothermal, gradient/isothermal and isocratic/program temperature modes has been made. Two representative families of pesticides have been selected for this study. One includes ionisable chlorophenoxy acids and two of their esters, some of which show similar polarities. The other one contains several weakly polar carbamates. Analysis was carried out using a reversed-phase capillary high-performance liquid chromatography (HPLC) system and focusing technique with UV or diode array detection (DAD).

Keywords: Temperature effects; Programmed temperature; Chlorophenoxy acids; Carbamates; Pesticides

1. Introduction

In the last few decades, application of pesticides on agricultural crops for pest control has progressively increased, and their residues are present in quite different matrices. Thus, analysis of pesticide residues at the levels established by legal directives continues to be important in the field of method development. Usual procedures for separation and quantitation of pesticides involve chromatographic techniques. For carbamates [1,2] and chlorophenoxy acid residues [3], the use of GC is limited because of their thermolability, polar nature and/or low volatility, while liquid chromatography (LC) techniques can be used without derivatisation and a wide variety of reversed-phase LC methods have been proposed.

The use of LC packed capillary columns with inner diameters between 100 and 350 μm and sorbent packing particles with diameter ranging from 3 to 10 μm has received special attention during recent years to improve chromatographic separations. Packed capillary columns exhibit properties of both packed and open tubular columns [4]. Compared to conventional LC [5,6], packed capillary LC increases the sensitivity because it reduces chromatographic dilution, requires lower sample volumes and reduces both the amount of stationary phase and the consumption of mobile phase. Main disadvantages of capillary columns include the loss of sensitivity due to the small volumes or masses required [7] and difficulties to control gradient elution at the required flow rates. These problems could be overcome by the use of the so-called on-column focusing modes by injections of larger sample volumes, and by elution with temperature programs. In fact, the small inner diameter of capillary columns allows the use of temperature programs without previous heating of the solvents, giving rapid response to temperature changes due to favourable geometry, minimised radial temperature gradients and relatively low heat capacity [3,4,8,9]. In conventional high-performance liquid chromatography (HPLC), column temperature has been adjusted to optimise resolution with many samples [10]; temperature controls both retention (k) and resolution (R_s), and also it affects intrinsically many parameters of the chromatographic systems, such as

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pK_a values of both sample and buffer [11]. Temperature increases the analyte diffusion and decreases the viscosity of mobile phase; consequently, the lower backpressure allows higher flow rates, the use of stationary phases with smaller particles sizes for increased efficiency [12] or longer columns containing higher plate numbers [13]. The increased mass transfer rate between mobile and stationary phases, usually resulting in narrow peaks and improved column efficiency.

In the last few years, the development of more stable stationary phases has allowed the use of higher temperature in LC, and actually, temperature programs combined with packed capillary columns seems to be a feasible alternative to commonly used gradient elution [14,15]. Moreover, from an instrumental point of view, thermal gradients are easier to engineer than gradient elution for small diameter columns, and require less maintenance [16]. In addition, the effects observed by changes in temperature or mobile phase can be complementary and more or less orthogonal to each other [10], and often it is possible combine changes on temperature and solvent strength. Temperature programs offer several advantages with some detectors such as MS and NMR [13]. Separations based on temperature programs have been used with capillary columns to analyse phenols [9], chlorobenzenes [11], β-lactoglobulins [17] and high-molecular weight hindered amine light stabilisers [18]. Using on column focusing with a low eluting strength and temperature program, several compounds such as chlorophenoxy acids [3], retinoids [4], triazines [8], antidepressants [14] and isocyanates derivates [19] have been successfully separated.

Although temperature program with capillary columns in LC has been found to be a promising alternative to gradient elution, it has been scarcely investigated by research groups; hence, it continues to be essential for the development of separation methods, especially in combination with on-column focusing modes. Consequently, the aim of the present study is to explore the influence of temperature on reversed-phase separations when capillary columns are employed, by comparison on the retention of ionised and non-ionised solutes (Fig. 1). We use the on-column focusing technique that we have successfully employed for the determination of acid herbicides [3] and carbamate pesticides [20].

2. Experimental

2.1. Materials and reagents

Acetonitrile and methanol of HPLC quality were supplied by Scharlab (Barcelona, Spain). Analytical grade sodium acetate was obtained from Probus (Badalona, Spain), phosphoric acid (85% pure) and hydrochloric acid (35% pure) were purchased from Panreac (Barcelona, Spain). Deionised water from a Milli-Q system was used in all procedures (Millipore, Bedford, MA, USA).

Pesticides included in the study were of the following purity: 2,4-D (99%), 2,4,5-TP (97%) and MCPA (95%) provided by Aldrich; 2,4-DP (95%), 2,4-D-1-methyl ester (97%) and 2,4-DB (97%) from Sigma; MCPP (99%), MCPB (99%), 2,4-D-1-butyl ester (98.3%) and carbaryl (99.7%) from Riedel-de Häen. Aminocarb (98%), propoxur (99%), carbofuran (99%) and methiocarb (98.5%) purchased from Chem Service.

Names, structural formulas, pK_a values and log $K_{o/w}$ [21] of chlorophenoxy acid herbicides and carbamate pesticides are shown in Fig. 1.

2.2. Preparation of standard solutions

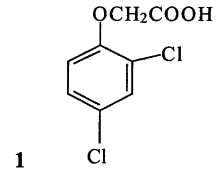
Stock solutions of chlorophenoxy acid herbicides were prepared by dissolving 20 mg of each herbicide in 100 mL of methanol, while those of carbamates were prepared by dissolving 10 mg of each one in 10 mL of acetonitrile and then diluting to 50 mL with purified water. These one stock solutions were stored in the dark at 4 °C for 2 months maximum. Standard mixtures of pesticides for focusing purposes were prepared by diluting the stock solutions in an aqueous solution containing 10% of methanol. In order to prevent the influence of the possible analyte degradation on the results, the standard solutions were prepared daily.

2.3. Packed capillary LC instrumentation

The capillary LC system used for the separation of chlorophenoxy acid herbicides by isocratic elution at constant or programmed temperature consisted of a Beckman 125 S Solvent Module System Gold (Beckman, Fullerton, CA, USA) pump, and a Beckman UV programmable variable wavelength 166 Detector System Gold with a microcell (35 nL, 8 mm pathlength). The components were interfaced to a Dell computer equipped with a MMX Pentium processor and a Gold Nouveau Chromatography Workstation Software (version 1.6) for Windows (Beckman). Mobile phase was continuously degassed with helium.

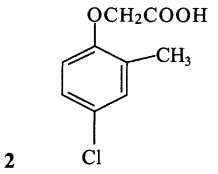
The micro (μ) HPLC instrument used for the separation of chlorophenoxy acids by gradient elution under isothermal conditions, and carbamates by isocratic elution with constant or programmed temperature was an Agilent Model 1100 Series (Agilent Technologies, Spain), which was equipped with a solvent delivery system composed of a binary capillary pump G1376 A, a degasser G1379 A and a diode array detector G1315B with a 500-nL flow cell (10 mm pathlength), coupled with a HP computer containing a Pentium 4 processor, an Agilent Chemstation Software for LC systems G2170AA and a spectral evaluation module G2180AA for Microsoft Windows XP Professional operating system.

In all cases, injections were performed with a Rheodyne injection valve (Beckman, Fullerton, CA, USA) with a stainless steel external loop of 20 μL, and temperature programs were achieved with a MISTRAL programmable oven (Spark-Holland, Emmem, The Netherlands). Separations of pesticides were carried out on a 15 cm × 300 μm i.d. LC Packings



1

2,4-dichlorophenoxy-acetic acid (2,4-D)
pK_a 2.7 log $K_{o/w}$ 2.6



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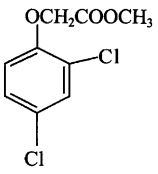
4-chloro-2-methylphenoxy-acetic acid (MCPA)
pK_a 3.1 log $K_{o/w}$ 2.8



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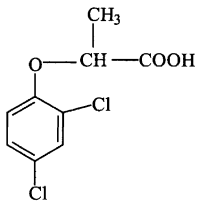
2,4-D-1-methyl ester
log $K_{o/w}$ 2.9



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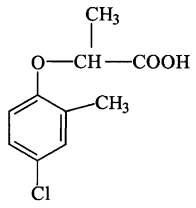
2-(2,4-dichlorophenoxy)-propanoic acid (2,4-DP)
pK_a 3.0 log $K_{o/w}$ 3.4



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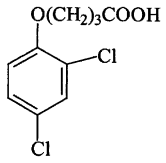
2-(4-chloro-2-methyl)-phenoxy-propanoic acid (MCPP)
pK_a 3.8 log $K_{o/w}$ 3.1



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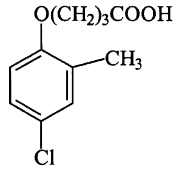
4-(2,4-dichlorophenoxy)-butanoic acid (2,4-DB)
pK_a 4.8 log $K_{o/w}$ 3.4



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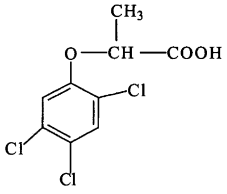
4-(4-chloro-2-methylphenoxy)-butanoic acid (MCPB)
(a) **pK_a 3.1 log $K_{o/w}$ 3.5**



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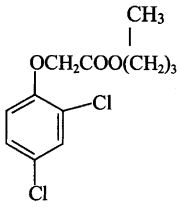
2-(2,4,5-trichlorophenoxy)-propanoic acid (2,4,5-TP)
pK_a 3.6 log $K_{o/w}$ 3.8



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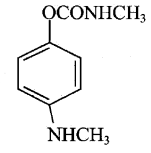
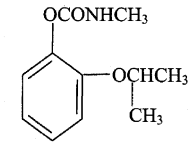
2,4-D-1-butyl ester
log $K_{o/w}$ 4.4



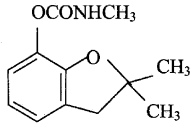
1

Aminocarb
log $K_{o/w}$ 1.7



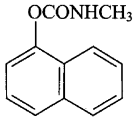
2

Propoxur
log $K_{o/w}$ 1.6



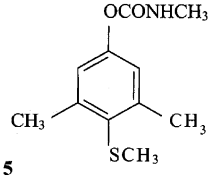
3

Carbofuran
(b) **log $K_{o/w}$ 1.6**



4

Carbaryl
log $K_{o/w}$ 2.3



5

Methiocarb
log $K_{o/w}$ 2.9

Fig. 1. Names, structural formulas, pK_a values and partition coefficient between *n*-octanol and water ($K_{o/w}$, as the log value) of cholorophenoxy acid (a) and carbamate (b) pesticides.

Analytical Column packed with 3 μm Hypersil C_{18} BDS (LC Packings, Amsterdam, The Netherlands).

2.4. Chromatographic conditions

Different chromatographic conditions were used for carbamate and chlorophenoxy acids analysis. The mobile phase flow rate through the column was 8 μL min^{-1} for chlorophenoxy acids separation and 8 or 10 μL min^{-1} for carbamate mixtures. Injections of pesticides were performed at an initial temperature column of 20 °C in a low eluting strength solution (10% methanol) for focusing purposes.

Isocratic elution of chlorophenoxy acid herbicides was carried out with a mixture of methanol–0.8% aqueous phosphoric acid solution (45:55) as mobile phase. For separations under isothermal conditions, temperature column was maintained at 20, 40, 50 and 65 °C. Gradient elution was made by a mobile phase composition methanol–0.8% aqueous phosphoric acid (40:60) solution for 25 min, then a linear increase to 70% methanol over 15 min and final isocratic step till the end of the chromatogram. The optimum conditions found for the separation with programmed temperature were: an initial step at 20 °C during 25 min, then a linear increase to 30 °C in 10 min, another linear increase to 65 °C in 7 min, and finally, an isothermal step at 65 °C till the end of the chromatogram.

Regarding to carbamate pesticides, separation was made in isocratic elution mode using acetonitrile—acetic acid/sodium acetate 20 mM pH 5.5 buffer (30:70) as mobile phase. Isothermal separations were carried out at 20, 30, 40 and 50 °C. For separation by temperature program, an isothermal step at the initial temperature for 7 min was made, following by a linear increase to 50 °C for 5 min, then another isothermal step at 50 °C for 5 min, and finally, return to the initial temperature in a time of 5 min.

Wavelength for UV detection or diode array detection (DAD) was fixed at 232 nm for chlorophenoxy acids and at 220 nm for carbamates.

3. Results and discussion

Advantages and disadvantages of employing temperature programs are discussed by comparing separations achieved by the isothermal mode with those achieved by the gradient elution mode. Experimental parameters such as peak width ($w_{1/2}$), resolution, retention factor and column efficiency (N) have been measured in all tested conditions. To decide about optimum separation, a compromise based on the reasonable analysis time, narrow peaks and acceptable resolution was taken.

First, isocratic elution conditions were established. Several mixtures of acetonitrile or methanol–water were tested. For chlorophenoxy acid herbicides separation, only mixtures of methanol–water with methanol ratios between 45 and 50% allowed good resolution. The best conditions found are shown in Section 2. On the contrary, mixtures of acetonitrile–water provided better results for carbamates separation. Ratios of acetonitrile were studied in the range of 27–30%. Selected conditions are described in Section 2.4.

3.1. Temperature behaviour

The influence of temperature on the retention is given by the well known Van't Hoff equation:

$$\ln k = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \beta$$

where ΔH is the standard enthalpy change due to the transfer of the solute between stationary and mobile phase, ΔS is the entropy change, R is the thermodynamic molar gas constant and T is the absolute temperature. The phase ratio of the column, β, was not considered for discussion because the same column was used in all the experiments. In reversed-phase HPLC, a column temperature increase usually leads to a decrease in retention times and higher column efficiencies.

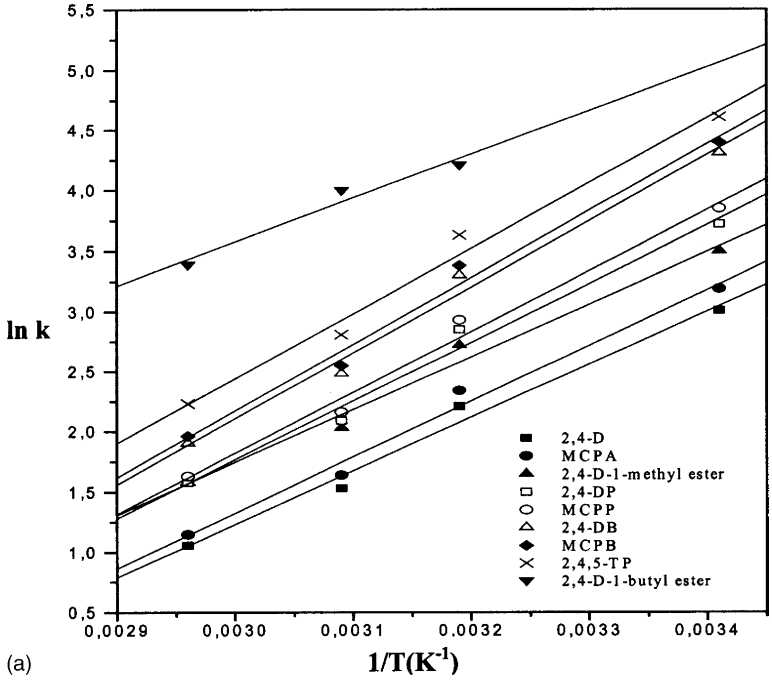
In the working temperature ranges, not degradation symptoms were observed for both chlorophenoxy acid and carbamate pesticides.

Graphs of the logarithm of the retention factor versus the inverse of temperature are shown in Fig. 2. As can be seen in Fig. 2a, the Van't Hoff plots for all chlorophenoxy acid herbicides fit into a linear curve (R^2 0.960–0.988), indicating that the retention mechanism does not change throughout the whole temperature interval. The slope is positive but different for each herbicide. This fact suggests a negative change in enthalpy and, therefore, an enthalpically favourable transfer from mobile to stationary phase. For the same temperature change, solutes with higher ΔH will be more strongly affected. Solutes with small ΔH changes will not suffer significant variation in the retention factor when temperature is modified. Retention factors less affected by temperature are those of the more strongly retained compounds. This is the case of 2,4-D-1-butyl ester.

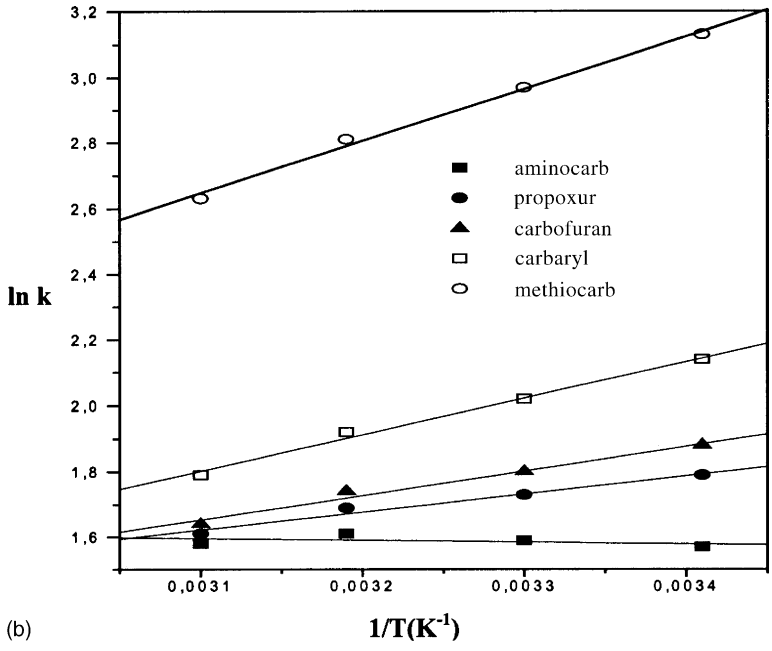
Van't Hoff plots for carbamate pesticides can be observed in Fig. 2b. A linear behaviour (R^2 0.970–0.994) and a positive slope were also obtained for all of them with the exception of aminocarb, again indicating a negative enthalpy change and thus an exothermic transfer to the stationary phase. As can be observed in this graph, the slope of the critical pair propoxur–carbofuran is similar. For aminocarb, the retention factor remains virtually constant during the studied temperature interval, thus it was not affected by temperature changes up to 50 °C, while methiocarb was the most significantly affected by temperature.

For all the studied pesticides, differences in slopes allows exploring the use of temperature changes to control resolution and to estimate the ranges that might be useful for temperature programming. The convenience of isothermic steps, especially at higher temperatures, is also apparent from these Van't Hoff graphs.

Tables 1 and 2 show the values of several chromatographic parameters at different temperatures under isocratic elution



(a)



(b)

Fig. 2. Van't Hoff plots of the chlorophenoxy acid herbicides (a) and carbamate pesticides (b), illustrating effect of temperature on retention factor (*k*).

conditions for chlorophenoxy acids and carbamates, respectively. For chlorophenoxy acid herbicides, acceptable resolution was obtained working isothermically at temperatures of 20, 40 and 50 °C but the analysis time was unacceptable long and the later eluted peaks were broadened at 40 and specially at 20 °C. As can be observed in Table 1, for the most retained and non polar compound, the 2,4-D-1-butyl ester, a strongly increase in the plate number was observed at 65 °C, according with its lower peak width. Higher efficiencies, lower retention times and lower peak widths were observed in all cases when temperature increased. But in the case of 2,4-D, which is the most acidic compound, the increase of efficiency at 65 °C could not be only explained by temperature changes, and the acid-basic equilibrium must be involved.

For carbamates, Table 2 shows that a temperature increase also reduces the retention time (negative temperature dependence) and peak widths, yielding narrow and very sharp peaks. Efficiency for aminocarb and carbofuran was strongly increased from 20 to 50 °C, although high-column plate numbers were observed in general for all of them. A

Table 1
Influence of temperature on the chromatographic parameters for chlorophenoxy acid herbicides capillary LC separation

Herbicide	Temperature (°C)																							
	20					40					50					65					Programmed temperature[a]			
	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	R_s
2,4-D	34.4	20.3	1.5	3076		16.3	9.1	0.6	3829		9.0	4.6	0.4	3663		6.3	2.9	0.2	4986		16.3	2.4	1.0	
MCPA	40.8	24.4	1.7	3229	2.4	18.4	10.4	0.7	4056	1.9	9.9	5.1	0.4	3394	1.3	6.7	3.2	0.3	3679	1.1	18.8	2.9	1.0	2.5
2,4-D-1-methyl ester	54.7	33.0	2.2	3520	4.2	26.2	15.3	1.0	3960	5.5	13.4	7.6	0.5	3541	5.1	9.4	4.8	–	–	2.8	24.3	4.1	1.3	4.7
2,4-DP	68.1	41.3	2.7	3659	3.3	29.3	17.2	01.0	4952	1.9	14.7	8.1	0.5	4262	0.9	9.4	4.8	–	–	0	28.6	5.0	1.5	3.1
MCPP	77.0	46.8	2.9	4004	1.9	31.8	18.8	1.1	4715	1.4	15.6	8.7	0.6	3292	0.9	9.8	5.1	0.4	4343	0.7	31.3	5.5	1.5	1.8
2,4-DB	121.4	74.4	4.8	3604	6.9	45.5	27.2	1.6	4594	6.0	20.9	12.0	0.8	3781	4.4	12.4	6.7	0.4	4607	3.9	40.5	7.4	0.6	9.1
MCPB	133.1	81.7	5.0	4022	1.4	49.0	29.4	1.8	3972	1.2	22.3	12.8	0.9	3401	1.0	13.1	7.1	0.5	3385	0.8	41.5	7.6	0.7	1.6
2,4,5-TP	162.7	100.0	5.9	4199	3.2	62.5	37.8	2.1	4815	4.0	28.5	16.7	1.1	3719	3.7	16.6	9.3	0.6	4103	3.6	44.3	8.2	0.6	4.3
2,4-D-1-butyl ester	–	–	–	–	–	110.2	67.4	8.9	860	5.1	89.3	54.5	3.4	3937	15.8	49.2	29.5	1.6	5238	17.3	69.0	13.4	0.3	5.6

Mobile phase composition: methanol–0.8% aqueous phosphoric acid solution (45:55). Flow rate 8 μL min^{-1}. Concentration of pesticide 25 μg L^{-1}. Injection volume 20 μL. Focusing solution: methanol–water (10:90).
[a] Conditions explained in Section 2.4.

Table 2
Influence of temperature on the chromatographic parameters for carbamate pesticides capillary LC separation

Pesticide	Temperature (°C)																							
	20					30					40					50					Programmed temperature[a]			
	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	N	R_s	t_R (min)	k	$w_{1/2}$ (min)	R_s
Aminocarb	8.7	4.4	0.3	4363		7.9	4.4	0.3	5115		7.6	4.4	0.3	5120		7.0	4.3	0.2	7520		6.8	2.0	0.4	
Propoxur	10.3	5.4	0.3	5084	2.9	8.9	5.0	0.3	5597	2.1	8.2	4.8	0.3	5511	1.3	7.3	4.4	0.2	6100	0.7	8.0	2.5	0.4	3.0
Carbofuran	10.9	5.9	0.4	4114	1.1	9.3	5.3	0.3	4680	0.9	8.5	5.0	0.3	5491	0.7	7.5	4.6	0.2	7066	0.6	8.5	2.7	0.4	1.3
Carbaryl	13.7	7.6	0.5	3998	3.5	11.2	6.6	0.4	4134	3.0	9.8	5.9	0.3	4603	2.5	8.4	5.2	0.3	4986	2.1	10.4	3.5	0.4	4.5
Methiocarb	34.0	20.4	1.3	3971	13.5	27.0	17.3	01.0	4205	13.3	22.2	14.7	0.7	5124	13.6	17.9	12.4	0.6	5099	12.9	19.5	7.5	0.8	14.4

Mobile phase: acetonitrile-acetic acid/sodium acetate 20 mM pH 5.5 buffer (30:70). Flow rate 8 μL min^{-1}. Concentration of pesticide 0.2 mg L^{-1} for carbaryl and 0.5 mg L^{-1} for the rest. Injection volume 20 μL. Focusing solution: methanol–water (10:90).
[a] Conditions explained in Section 2.4.

loss in resolution was observed for the critical pair formed by propoxur and carbofuran at temperatures higher than 20 °C. To increase resolution, the separation was made at 10 or 15 °C, but, due to overpressure problems even at a flow rate of 8 μL min^{-1}, temperatures lower than 20 °C were not suitable. Consequently, both flow and composition of mobile phase were readjusted to optimise the separation and resolution of the critical pair. At this temperature and a flow of 10 μL min^{-1}, changing the organic ratio solvent to 28% of acetonitrile led to a greater resolution for the critical pair (R_s 1.5); however, as can be observed in Fig. 3, analysis time is unfortunately not practical.

3.2. Gradient elution for chlorophenoxy acid herbicides

Separation at isothermal temperature was optimised using gradient elution. Several gradient steepness were tested at 25 °C using a mixture of methanol–0.8% aqueous phosphoric acid solution as the elution solvent. Initial and final ratios of methanol were 40 and 70%, respectively, and the best conditions found for the mobile phase composition are described in Section 2.4. As can be seen in Fig. 4a, analysis time was 55 min and the resolution obtained was reasonable for all the herbicides studied, including the critical pair of 2,4-DB and MCPB (R_s 1.4). Besides the decrease in peak dispersion of the analytes with the elution time, narrow and sharp peaks were observed for the last eluted herbicides, specifically for 2,4-DP, MCPP, 2,4-DB, MCPB and 2,4,5-TP. However, for the first eluted peaks, 2,4-D, MCPA and 2,4-D-1-methyl ester, relatively high values of the retention factors were in the range between 4.3 and 7.0 were obtained, thus, to decrease the retention factor of these analytes while main-

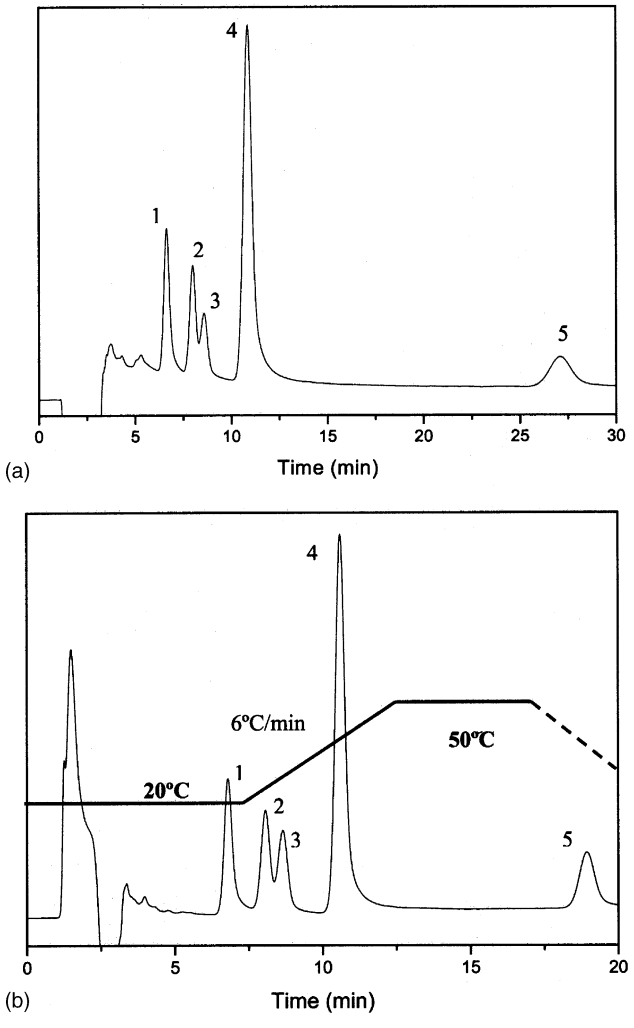


Fig. 3. Capillary HPLC chromatograms of carbamates standard working solution. (a) Isocratic elution with the mobile phase acetonitrile-acetic acid/sodium acetate 20 mM pH 5.5 (28:72) at 20 °C. (b) Temperature program from 20 to 50 °C. Peaks: (1) aminocarb; (2) propoxur; (3) carbofuran; (4) carbaryl; (5) methiocarb. Amount injected 200 μg L^{-1} for carbaryl and 500 μg L^{-1} for all the other pesticides. Flow rate 10 μL min^{-1}.

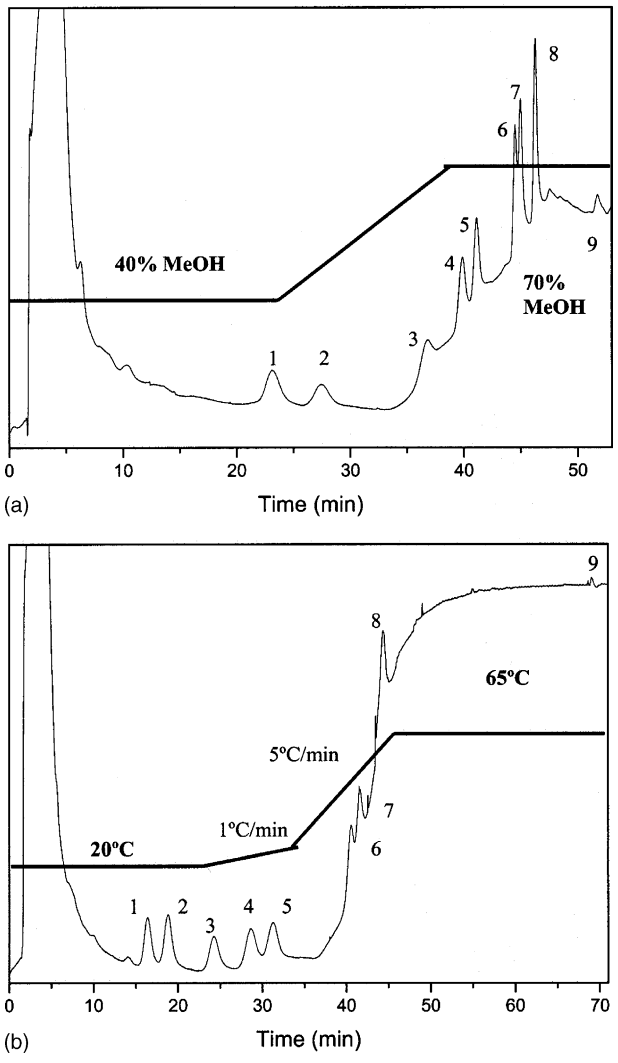


Fig. 4. Capillary HPLC chromatograms of chlorophenoxy acids standard solution. (a) Gradient elution at 25 °C. (b) Isocratic elution with the mobile phase methanol–0.8% aqueous phosphoric acid solution (45:55) and temperature program from 20 to 65 °C. Peaks: (1) 2,4-D; (2) MCPA; (3) 2,4,-D-1-methyl ester; (4) 2,4,-DP; (5) MCPP; (6) 2,4-DB; (7) MCPB; (8) 2,4,5-TP; (9) 2,4-D-1-butyl ester. Amount injected 25 μg L^{-1}. Flow rate 8 μL min^{-1}.

taining the resolution, the effect of temperature program was studied.

3.3. Temperature program for elution purposes

Working in the isocratic conditions described above (Section 2.4), different temperature programs were explored from 20 to 65 °C to improve separation of chlorophenoxy acid herbicides. Fig. 4b shows the chromatogram obtained for the best temperature program. As can be observed in Table 1, the temperature program decreased the analysis time with respect to that of the isothermal separation at 40 °C; in addition, acceptable resolution including the critical pair formed by 2,4-DB and MCPB was obtained. Although retention factors (see Table 1) for the last eluted peaks were similar to those obtained by gradient elution, the first eluted peaks showed lower *k* values. In spite of this, the retention time for 2,4-D-1-butyl ester was unacceptable long because it is the least affected by temperature changes.

In the case of carbamate compounds, although several elution gradients at 20 °C in isothermal mode were tested to decrease the analysis time, results were not significantly improved; therefore, to reduce this time while maintaining the quality of the separation, temperature programs were tried. For all the cases, initial column temperature was 20 °C ending at 50 °C with a gradient rate of 6 °C min^{-1}, using a mixture of acetonitrile—acetic acid/sodium acetate 20 mM pH 5.5 buffer (28:72) as mobile phase. The optimum conditions for the program are described in Section 2.4. As can be seen in the chromatogram shown in Fig. 3b, analysis time was reduced to 20 min and a quite stable baseline was achieved. Separation of the critical pair propoxur–carbofuran was possible with slightly improved resolution, as can be observed in Table 2. Moreover, high-sensitivity and less peak widths with respect to the isothermal separation were obtained for all the carbamates studied, especially for methiocarb, the last eluted compound.

4. Conclusions

The usefulness of temperature programs, which makes the effect of increasing elution strength on separation of chlorophenoxy acid and carbamate pesticides by capillary LC, has been established. Temperature effects on chromatographic parameters such as retention factor, peak width, resolution and plate number has been taken as a basis to select temperature programs.

Temperature programming allows finer changes on elution strength than their equivalent gradients in mobile phase composition do. This leads to wider *k* ranges, starting from lower values, and it involves improvements in the resulting chromatographic parameters.

Combining isocratic optimisation with temperature programs, chromatographers have another tool to control resolution and efficiency in capillary LC separations, as has been clearly proved for the separation of the carbamates studied group.

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