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Research Article

Chiral separation of aryloxyphenoxy-propionate herbicides in a permethyl-β-cyclodextrin based column. Influence of temperature and mobile phase composition on enantioselectivity

We used a permethyl-β-cyclodextrin chiral stationary phase under reversed-phase conditions for the chiral separation of four aryloxyphenoxy-propionate herbicides (fenoxaprop-*p*-ethyl, quizalofop-*p*-ethyl and tefuryl, and haloxyfop-*p*-methyl) with mixtures of methanol, ethanol, 2-propanol, *n*-propanol, *tert*-butanol, or acetonitrile and water as mobile phases and investigated the influence of mobile phase composition and column temperature (from 0 to 50°C) on the separation. The retention factors (k) and selectivity factors (α) of all the herbicides investigated decreased with increasing temperature. The $\ln\alpha$ versus 1/T and $\ln k$ versus 1/T plots for the enantiomers of the chiral pesticides were linear within the range of 0–50°C with all alcohol/water mixtures constituting the mobile phase, but the $\ln k$ versus 1/T plots were nonlinear for all the enantiomers chromatographed in acetonitrile/water mixtures. The thermodynamic parameters based on linear van't Hoff plots were calculated. The influence of temperature and mobile phase composition on the enantioseparation of the solutes has rarely been considered simultaneously. The temperature and the solvents used in the mobile phase, however, were found to have a profound effect on the enantioseparation of these herbicides.

Keywords:
Aryloxyphenoxy-propionate herbicides / Mobile phase composition effects / Permethyl-β-cyclodextrin / Reversed-phase liquid chromatography / Temperature effects
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1 Introduction

The aryloxyphenoxy-propionate herbicides (FOPs) control graminaceous weeds in broad-leaved crops such as soybean or sugar beet and constitute a class of selective postemergence herbicides [1]. FOPs can be used alone or in combination with other herbicides to enhance production rates also in the cultivation of cereals [2]. Those compounds are administered as esters, which derivatives are rapidly hydrolyzed in soil and plants to yield the corresponding acids having the herbicidal activity [3,4]. All FOPs have an asymmetrically substituted carbon atom in the α-position with respect to the propionic acid or ester moiety. These compounds were developed and introduced in the 1970s and 1980s as racemates. The enantiomers of chiral herbicides, however, usually exhibit different degrees of bioactivity and toxicity, with the *R*-isomers of the FOPs being more biologically active than the *S*-isomers. In fact, the *S*-isomers fail to eliminate agricultural crop pests while exhibiting toxic side effects [5–7]. For example, the enantiomeric activities of haloxyfop-*p*-methyl against annual grass weeds are mainly performed by the *R*-form, which enantiomer has a herbicidal activity 1000-times higher than the *S*-form [8].

Several analytical methods have been proposed for the analysis of chiral herbicides. Owing to the selectivity, sensitivity, and accuracy, chromatographic technology has been widely and successfully implemented for the separation of enantiomers or stereoisomers [9]. Some of the relevant technology includes gas chromatography [10, 11], supercritical fluid chromatography [8], capillary electrophoresis [12, 13], and HPLC [14, 15]. Using normal phase HPLC, Zhou et al. separated quizalofop-*p*-ethyl with R_s values of 1.21, with a coated amylose *tris*(3,5-dimethylphenylcarbamate) as a stationary phase and 100% *n*-hexane as a mobile phase [16]. Employing the same chiral column, Wang et al. separated fenoxaprop-*p*-ethyl with a R_s value of 1.75, with 99.5:0.5 *v/v* *n*-hexane:2-propanol, whereas quizalofop-*p*-ethyl had the largest R_s of 1.71 when chromatographed at a (98:2) *v/v* mobile phase composition with the same stationary phase. For fenoxaprop-*p*-ethyl, an even further decrease in the percentage of 2-propanol resulted in a higher resolution, though with

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Abbreviations: CSP, chiral stationary phase; **FOP**, aryloxyphenoxy-propionate herbicide

also a longer retention. Finally, the retention of quizalofop-*p*-ethyl increased while the resolution factor decreased with a reduction in the content of 2-propanol, reaching a maximum resolution with 2% *v/v* 2-propanol, after which point the resolution became affected by peak tailing [15].

Reversed-phase HPLC (RPLC), has many advantages over normal-phase HPLC, namely: (i) a satisfactory solubility of polar compounds in the mobile phase, which is especially useful for enantiomeric analysis of chiral pesticides [17]; (ii) reduced chromatographic costs by replacing normal-phase HPLC solvents by usually cheaper ones or, alternatively, by using stronger solvent conditions to reduce analysis times; and (iii) an easier method development along with compatibility with mass spectrometric detection. Tian et al. [18] employed an amylose *tris*(3,5-dimethylphenylcarbamate) stationary phase and achieved a baseline separation of fenoxaprop-*p*-ethyl (R_s between 1.75 and 2.76) at room temperature using acetonitrile/water mobile phase compositions of 100:0, 80:20, and 70:30, and $R_s < 1.5$ for fenoxaprop-*p*-ethyl and quizalofop-*p*-ethyl using methanol/water mobile phase compositions of 100:0, 95:5, and 85:15, though the authors obtained no resolution for quizalofop-*p*-tefuryl with acetonitrile/water or metanol/water at different mobile phase compositions.

Recently, Yan et al. used a permethyl-β-cyclodextrin (PM-β-CD) stationary phase in RPLC with methanol/water mixtures to separate fenoxaprop-ethyl along with other chemically different herbicides [19]. Those authors obtained R_s values between 1.32 and 3.78 at 20°C when the mobile phase compositions were 90:10 and 65:35 methanol/water, respectively. No other organic solvents, however, were systematically tested [19].

Column temperature affects retention, selectivity, system pressure, and column stability. The adjustment and control of temperature are therefore useful for optimizing chiral separations, for protecting the chiral column, and for designing more effective chiral stationary phases (CSPs). Moreover, by changing column temperature, one can gather useful information for gaining insight into the mechanisms of chiral recognition.

In the present work, we studied the separation of enantiomers of four chiral herbicides—namely, haloxyfop-*p*-methyl, fenoxaprop-*p*-ethyl, quizalofop-*p*-ethyl, and quizalofop-*p*-tefuryl—using a PM-β-CD column and several aqueous mobile phase mixtures and conducted a systematic investigation on the influence of the organic modifiers methanol, ethanol, *n*-propanol, 2-propanol, *tert*-butanol, and acetonitrile and of the column temperature from 0 to 50°C.

2 Materials and methods

2.1 Apparatus

Chromatographic studies were performed on an Agilent liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with vacuum degasser (model 1100), binary pump (model 1100), autosampler (model 1260), thermostated column device (model 1100), and photodiode array detector (DAD, model 1290). The detector was set at 240 nm for all analytes.

The chiral column was a Nucleodex-β-PM (200 × 4 mm id) purchased from Macherey-Nagel (Düren, Germany). The flow rates were set between 0.18 and 0.70 mL/min depending on the fluid viscosity, that is, on mobile phase composition and temperature. The injection volume was 10–30 μL. The influence of column temperature was studied within the range of 0–50°C.

2.2 Chemicals and materials

Reagents were of analytical grade or better. The chiral pesticides haloxyfop-*p*-methyl, quizalofop-*p*-tefuryl, quizalofop-*p*-ethyl, and fenoxaprop-*p*-ethyl were kindly provided by Agrofina S.A. (Buenos Aires, Argentina). Uracil, used as dead-volume marker, was purchased from Aldrich (Aldrich, Milwaukee, WI, USA).

Stock solutions of these herbicides were prepared in methanol and stored at 4°C. Working standard solutions (200 μg/mL) were prepared by dilutions of the stock solutions in methanol and filtered through a 0.22 μm cellulose–nitrate membrane before injection.

The solvents used for the mobile phases were HPLC grade methanol and acetonitrile (J. T. Baker, Ciudad de México, México), ethanol (Cicarelli, Buenos Aires, Argentina), *n*-propanol (Merck, Darmstadt, Germany), 2-propanol (Sintorgan, Buenos Aires, Argentina), *tert*-butanol (Mallinckrodt, New York, NY, USA).

The organic solvents were filtered through 0.22-μm Nylon membranes (Osmonics-Magna, Westborough, MA, USA). Water was purified with a Milli-Q system (Millipore, Damstadt, Germany), and filtered through 0.45-μm cellulose-nitrate filters (Micron Separations, Westborough, MA, USA) before use.

3 Results and discussion

Figure 1 depicts the chemical structure of the four chiral aryloxyphenoxypropionate herbicides studied.

Fenoxaprop-*p*-ethyl and quizalofop-*p*-ethyl had been separated in amylose and cellulose-based chiral columns under RPLC conditions at room temperature [18, 20]. In this study, we tested the chiral recognition ability of a PM-β-CD–based column using different organic modifiers, such as mixtures of a primary, secondary, or tertiary alcohol and acetonitrile with water. Table 1 summarizes the retention factors (k_i), enantioselectivity factors (α), and resolution (R_s) for the four herbicides measured by using those organic modifiers (i.e. acetonitrile and alcohols) at different compositions and at 20°C. This CD-based chiral column displayed excellent enantiomeric recognition for fenoxaprop-*p*-ethyl. Those enantiomers were easily separated with practically all the mobile phases tested,

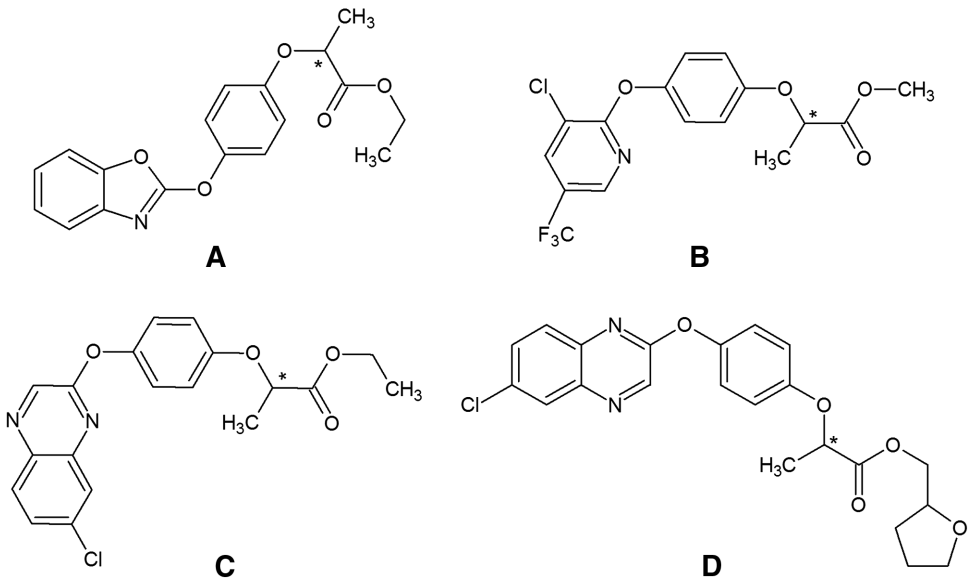

Figure 1. Chemical structure of (A) fenoxaprop-*p*-ethyl (B) haloxyfop-*p*-methyl (C) quizal ofop-*p*-ethyl (D) quizalofop-*p*-tefuryl.

Table 1. Chromatographic results for the four herbicides at 20°C

Mobile phase		Fenoxaprop-*p*-ethyl	Haloxyfop-*p*-ethyl	Quizalofop-*p*-ethyl	Quizalofop-*p*-tefuryl
30% ACN	k_1	26.09	17.50	19.66	14.96
	α	1.26	1.17	1.18	1.10
	R_s	1.65	1.30	1.31	0.66
40% ACN	k_1	6.92	3.53	4.71	3.69
	α	1.17	1.12	1.14	1.07
	R_s	1.40	1.00	1.13	0.60
50% ACN	k_1	2.23	1.42	1.93	—
	α	1.16	1.11	1.13	—
	R_s	1.21	0.76	0.94	—
60% MeOH	k_1	10.85	6.88	8.69	7.48
	α	1.48	1.24	1.30	1.18
	R_s	3.13	1.32	1.56	0.86
70% MeOH	k_1	3.32	2.05	2.58	11.24[a]
	α	1.39	1.22	1.25	1.18[a]
	R_s	2.45	1.15	1.39	0.92[a]
45% EtOH	k_1	2.05[b]	11.38	11.17	8.67
	α	1.34[b]	1.21	1.27	1.17
	R_s	1.88[b]	1.20	1.27	0.83
50% EtOH	k_1	7.67	5.67	5.37	4.38
	α	1.38	1.20	1.15	1.16
	R_s	2.10	1.09	1.01	0.73
30% NPA	k_1	9.15	17.75[c]	7.23	13.35[a]
	α	1.17	1.10[c]	1.14	1.08[a]
	R_s	1.24	0.69[c]	0.96	0.49[a]
45% IPA	k_1	3.76	2.72	3.02	2.30
	α	1.24	1.15	1.19	1.13
	R_s	1.51	0.86	1.11	0.64
50% IPA	k_1	2.12	1.54	1.70	1.31
	α	1.24	1.15	1.19	1.13
	R_s	1.17	0.69	0.85	0.59
30% TBA	k_1	18.60	13.00	16.18	13.33
	α	—	1.11	1.14	1.09
	R_s	—	0.75	1.43	0.70

ACN, acetonitrile; MeOH, methanol; EtOH, ethanol; NPA, n-propanol; IPA, 2-propanol; TBA, *tertiary*-butanol.
a) 55% MeOH.
b) 60% EtOH.
c) 25% NPA.

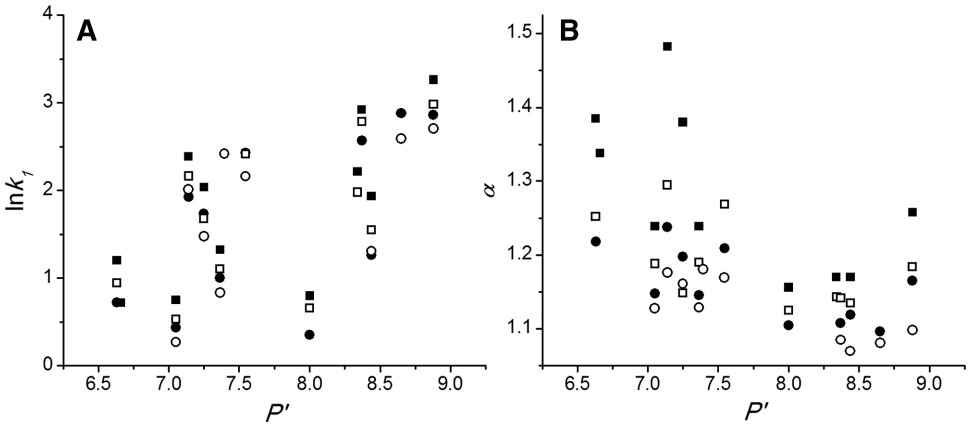

Figure 2. Retention factors (A) or selectivity factors (B) for the four herbicides as a function of the polarity (*P′*) of the mobile phase (■ fenoxaprop-*p*-ethyl, • haloxyfop-*p*-methyl, □ quizalofop-*p*-ethyl, ○ quizalofop-*p*-tefuryl).

for instance, an R_s value of 3.13 was achieved with 60% *v/v* aqueous methanol. Likewise, baseline enantioseparations of quizalofop-*p*-ethyl and of haloxyprop-*p*-methyl were readily obtained in this PM-β-CD-column with methanol/water as mobile phase. Only for quizalofop-*p*-tefuryl relatively poor resolutions were observed: the highest R_s value achieved at 20°C was 0.92, using 55% *v/v* methanol, though better resolution was obtained when the temperature was decreased.

3.1 Influence of mobile phase composition

CD-based chiral columns—and specifically PM-β-CD based ones—have been used in numerous enantioseparations [19, 21], mainly because CDs offer the possibility of coordinating molecules of different sizes and recognizing different guest functional groups, including diastereomers as well as enantiomers [22].

Under reversed-phase conditions, polar analyte–CD interactions may be attenuated in a number of ways by adjusting the mobile phase pH, the buffer type and concentration, and the column temperature [23]. Organic modifiers, such as acetonitrile, methanol, ethanol, 1- and 2-propanol, *n*-butanol, tetrahydrofuran, triethylamine, and dimethylformamide have been used for the optimization of chiral resolution on these CD-based CSPs [24]. Since the effect of the type and concentration of these organic modifiers varies from one analyte to another, anticipating the results with different organic modifiers is very difficult. Once the modifier has been chosen, a higher concentration of the polar component (water) in the mobile phase generally has resulted in better resolutions and longer retention times. Table 1 presents a comparison between retention factors and selectivity factors for the four compounds at a given composition of different modifiers. The use of methanol induced significantly larger retention factors than the other less polar alcohols. A similar behavior has been described previously for the enantioseparation of zopiclone, fluoxetine, and norfluoxetine from a β-CD column [25]. The authors postulated that retention and chiral discrimination were controlled by a competition between solutes and the organic modifier for the hydrophobic CD cavity. The hydrophobicity of the organic modifier increased the latter's affinity for the CD cavity, where methanol had a weak displacing ability that led to longer retention times.

The retention of chiral solutes in a stationary phase is given by two contributions: specific interactions between each enantiomer with the CSP (k_R and k_S), and other contributions, equal for both enantiomers, that are nonenantioselective (k_{ne}). Thus, the experimentally measured enantioselectivity factor, α_{exp}, will be $\alpha_{exp} = k_{R,exp}/k_{S,exp} = (k_R + k_{ne})/(k_S + k_{ne})$, where k_R and k_S were arbitrarily chosen, and $k_{i,exp}$ represents the experimentally measured retention factor. In chiral separations, only enantioselective interactions with the chiral selector lead to enantioresolution. In an attempt to evaluate the influence of elution strength and polarity over retention and enantioseparation, we plotted the retention factors (Fig. 2A) and selectivity factors (Fig. 2B) as a function of the mobile phase polarity, *P'* [26]. Clearly, no correlation occurs between *k* (nor ln*k*) and *P'* for any of the aryloxyphenoxypropionate herbicides. Nonetheless, from the observation of the overall data points (for the four herbicides) we can be envisage a rough trend of an increment in retention as the polarity increases. Similarly, in plot (b), the entire group of points reveals that the enantioselectivity factors were relatively larger in mobile phases of lower *P'*-values. These two plots indicate that the increase in retention owing to the mobile phase polarity could have a nonenantioselective (hydrophobic) origin since the experimental enantioselective factors decrease relative to what is observed in less polar mobile phases. This observation demonstrates that the contribution of the enantioselective interactions to the total retention times can fail to dominate under certain mobile phase conditions.

From a practical point of view, because nonenantioselective retention will compromise the authentic enantioselectivity, mechanisms to decrease achiral retenion (e.g. the substitution of the modifiers, or the use of different additives) without altering chiral retention would improve the experimental selectivity factors. Figure 2 would indicate that the choice of less polar alcohols than methanol as a modifier will lead to a better compromise between optimal separation factors and analysis times.

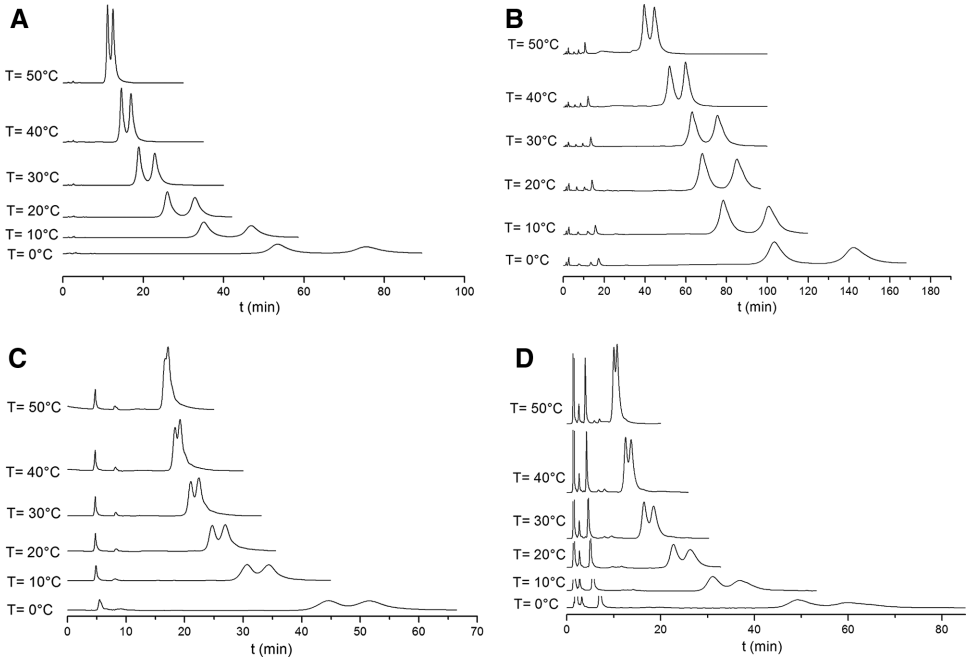

Figure 3. Chromatograms for the four herbicides at different temperatures: (A) quizalofop-*p*-ethyl with a mobile phase of (60:40) MeOH/H_2O and F = 0.700 mL/min; (B) fenoxaprop-*p*-ethyl with a mobile phase of (30:70) ACN/H_2O and F = 0.700 mL/min; (C) haloxyfop-*p*-methyl with a mobile phase of (50:50) 2-propanol/H_2O and F = 0.180 mL/min; (D) quizalofop-*p*-tefuryl with a mobile phase of (60:40) MeOH/H_2O and F = 0.700 mL/min Injection volume = 10 μL, detection was set at 240 nm for all runs.

3.2 Influence of temperature

Temperature plays a fundamental role in retention and selectivity in HPLC using CDs bonded to a silica surface [19, 27]. Figure 3 (A–D) shows chromatograms of quizalofop-*p*-ethyl, fenaxoprop-*p*-ethyl, haloxyfop-*p*-methyl, and quizalofop-*p*-tefuryl obtained within the range between 0 and 50°C, respectively.

Retention factors usually decrease as the temperature is raised and follow a simple van't Hoff relationship—i.e. a linear behavior of $\ln k_i$ versus (1/T) with a slope representing the enthalpy changes for the transfer of the enantiomer from the mobile to the stationary phase ($\Delta H°_i$):

$$\Delta H_i^\circ = -R\partial(lnk_i)/\partial(1/T). \quad (1)$$

The van't Hoff plots of these herbicides obtained with different mobile phases revealed two different patterns. The upper panels A–C of Fig. 4, present the plots of $\ln k_i$ versus 1/T in mobile phases consisting of 30, 40, and 50% *v/v* aqueous acetonitrile. These plots are not linear within the temperature range. Nonlinear van't Hoff plots are usually attributed to a change in the retention mechanism as a result of either the existence of more than one binding site, a change in the solvation state of the analyte and/or the stationary phase, or the presence of a retention mechanism that is not independent of temperature within the range investigated [28, 29]. The behavior for the four herbicides when acetonitrile was used as modifier clearly prevented the achievement of meaningful thermodynamic quantities. Although the dependence of retention on temperature was very unusual, the enantioselectivity factors decreased regularly as the temperature was increased over the interval investigated (*cf.* also Fig. 4, bottom panels).

In contrast, the $\ln k_i$ as a function of 1/T for these four compounds in alcohol/water mobile phases was linear (not shown). Table 2 lists the apparent enthalpy changes for retention as obtained from the regression slopes of Eq. (1). The estimated values for apparent $\Delta H°i$, for all the herbicides were negative. This enthalpy change is related to the interaction energy between the enantiomer and the mobile and stationary phases. The entropic term (also negative for all conditions) suggested an increase in the system order as a consequence of the interactions.

The (apparent) thermodynamic properties for the enantioseparation process can be estimated from the following expressions:

$$\Delta(\Delta\Delta G^\circ) = -RTln\alpha \quad (2)$$

$$\Delta(\Delta H^\circ) = -R\partial(ln\alpha)/\partial(1/T) \quad (3)$$

$$-T\Delta(\Delta S^\circ) = \Delta(\Delta G^\circ) - \Delta(\Delta H^\circ), \quad (4)$$

where $\Delta(\Delta G°)$, $\Delta(\Delta H°)$, and $\Delta(\Delta S°)$ are the differences in the Gibbs-free energy, enthalpy, and entropy of transfer between the two enantiomers from the eluent to the CSP, respectively. Table 2 also includes the estimated values for those properties in different mobile phases.

All four compounds have comparable differential enthalpies. The $\Delta(\Delta H°)$ values are from about −2 to −4 kJ/mol that are relatively high if we only consider the chiral discrimination on the basis of steric hindrance (i.e. inclusion into the CD cavity). Indeed, those values would indicate the contribution of a second type of interaction, e.g. dipole–dipole or weak hydrogen bonding, added to the steric hindrance [30]. The entropic contributions, however, are also negative for all the discrimination systems and, as a result, their chiral selectivities are comparable in magnitude.

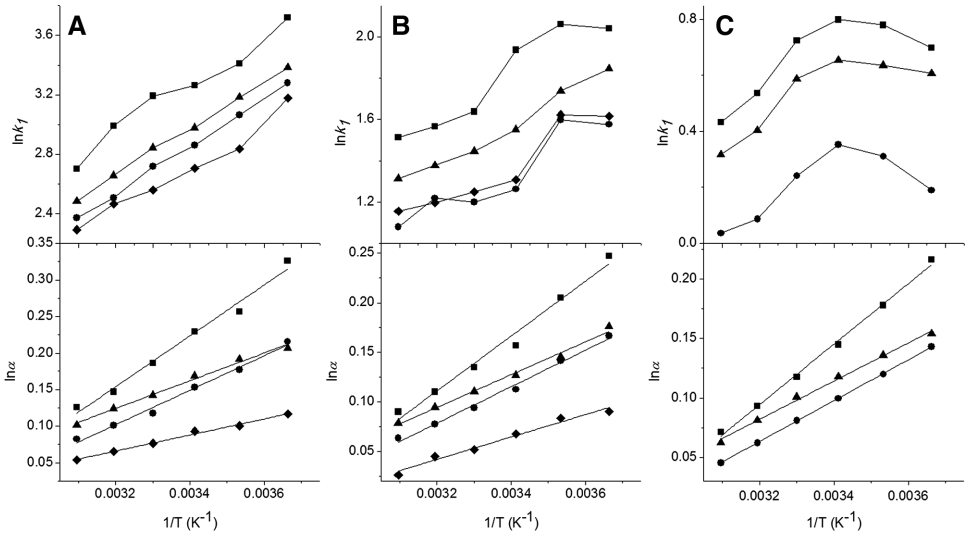

Figure 4. Plots of $\ln k_1$ and $\ln\alpha$ versus 1/T) in (A) 30%, (B) 40%, and (C) 50% *v/v* ACN for the four herbicides. Symbols are the same as those in Fig. 1.

Table 2. Apparent thermodynamic functions of transfer of chiral herbicides to permethyl-β-cyclodextrin

	ACN			MeOH		EtOH		NPA	IPA		TBA
	30%	40%	50%	60%	70%	45%	50%	30%	45%	50%	30%
Fenoxaprop-p-ethyl											
ΔH°1 (kJ/mol)	—	—	—	−23.2	−17.7	−22.1[a)]	−28	−16.1	−19.1	−18.2	−29
r^2	—	—	—	0.997	0.998	0.996[a)]	0.996	0.997	0.997	0.996	0.990
Δ(ΔH°) (kJ/mol)	−2.9	−2.3	−2.11	−6	−5.3	−5[a)]	−4.9	−2.31	−2.9	−3.14	−1.67
−TΔ(ΔS°) (kJ/mol) (20°C)	2.3	1.9	1.75	5	4.4	4.2[a)]	4.1	1.92	2.38	2.61	1.34
r^2	0.982	0.978	0.996	0.993	0.986	0.980[a)]	0.992	0.993	0.996	0.997	0.997
Haloxyfop-p-ethyl											
ΔH°1 (kJ/mol)	—	—	—	−21.7	−16.5	−26.2	−24	−24[b)]	−18.8	−18.3	−37
r^2	—	—	—	0.995	0.997	0.999	0.989	0.979[b)]	0.996	0.996	0.987
Δ(ΔH°) (kJ/mol)	−2	−1.53	−1.429	−2.7	−2.66	−2.32	−2.6	−0.96[b)]	−1.56	−1.85	−1.467
−TΔ(ΔS°) (kJ/mol) (20°C)	1.6	1.25	1.186	2.2	2.18	1.86	2.1	0.73[b)]	1.23	1.52	1.218
r^2	0.988	0.990	0.999	0.994	0.998	0.998	0.977	0.984[b)]	0.989	0.998	0.999
Quizalofop-p-ethyl											
ΔH°1 (kJ/mol)	—	—	—	−22.3	−17.8	−26	−25	−15.6	−19.4	−18.2	−33
r^2	—	—	—	0.994	0.999	0.999	0.988	0.995	0.996	0.993	0.989
Δ(ΔH°) (kJ/mol)	−1.58	−1.38	−1.34	−3.22	−2.91	−2.86	−2.75	−1.7	−2	−2.19	−1.73
−TΔ(ΔS°) (kJ/mol) (20°C)	1.18	1.06	1.05	2.58	2.35	2.28	2.2	1.38	1.6	1.77	1.41
r^2	0.988	0.989	0.995	0.997	0.998	0.999	0.996	0.995	0.985	0.999	0.989
Quizalofop-p-tefuryl											
ΔH°1 (kJ/mol)	—	—	—	−23.2	−24[c)]	−26.2	−24.7	−23[b)]	−18.7	−19.2	−38
r^2	—	—	—	0.998	0.988[c)]	0.997	0.997	0.991[b)]	0.995	0.996	0.983
Δ(ΔH°) (kJ/mol)	−0.91	−0.94	—	−1.86	−1.8[c)]	−1.81	−1.74	−0.96[b)]	−1.57	−1.5	−1.38
−TΔ(ΔS°) (kJ/mol) (20°C)	0.69	0.78	—	1.47	1.4[c)]	1.43	1.39	0.77[b)]	1.28	1.2	1.19
r^2	0.991	0.972	—	0.999	0.997[c)]	0.995	0.988	0.997[b)]	0.990	0.972	0.995

RSDs values ranged between 0.55 and 11.81%. The significant figures correspond to the precision of the regression parameters.
a) 60% EtOH.
b) 25% NPA.
c) 55% MeOH.

The existence of a compensation between $\Delta H°_i$ and $\Delta S°_i$ for a process involving a group of related compounds with moderate differences in their structures determines that the data points fall approximately on a straight line, where the slope is referred to as the compensation temperature. This phenomenon of enthalpy–entropy compensation has been verified for a considerable number of chiral separation systems in both gas and liquid chromatography. We examined the measured retention data from this perspective by plotting the $\ln k_i$ as a function of the $\Delta H°_i$ for the different alcohols (Fig. 5). The different symbols correspond to thermodynamic data for the first enantiomer of the four compounds in a given

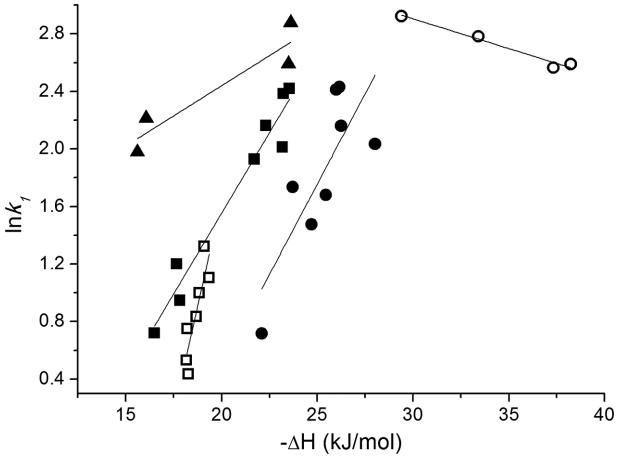

Figure 5. Plots of lnk_1 versus ($-\Delta H_1$) for the four herbicides in different modifiers. The solid lines correspond to the regression lines. Symbols: ■: MeOH ($r^2 = 0.957$), •: EtOH ($r^2 = 0.638$), ▲: 1-propanol ($r^2 = 0.879$), □: 2-propanol ($r^2 = 0.772$), and ○: *tert*-butanol ($r^2 = 0.971$).

alcohol/water mobile phase. The data yield reasonable linear dependencies (solid lines) for methanol, for *tert*-butanol, and for *n*-propanol and *2*-propanol, though there with lower correlation coefficients ($r^2 = 0.88$ and 0.77, respectively). These relationships suggest the existence of this compensation for those systems, whereas no such correlation was observed for ethanol. The slopes of the regression lines for the different alcohols are, however, quite different, and even slightly negative for *tert*-butanol. These plots suggest that the four compounds are retained by a similar mechanism, and one depending on the nature of the alcohol. Because both enthalpy and entropy are influenced by changes in the solvation that accompanies analyte transfer, we could conceive that the nature of the eluent could influence the magnitude (and even the sign) of the $\Delta(\Delta G°)$. The results of this study emphasize the profound influence of the mobile phase components on the mechanism of separation. One must keep in mind, however, that, as the nonenantioselective interactions contribute to retention and are not discounted from experimental *k*-values, the thermodynamic functions reported in Table 2 are only estimations of the absolute thermodynamic parameters. Consequently, the discussion presented here on the basis of these quantities stems from the observations of the trends exhibited by these solutes as a whole.

4 Concluding remarks

The main conclusions obtained from this study are the following:

(1) Enantioseparation of four aryloxyphenoxy-propionate herbicides in a permethyl-β-cyclodextrin column under reversed-phase conditions was feasible with different alcohols or acetonitrile as modifiers.

(2) A comparison between the types and compositions of different solvents revealed that the eluent polarity increased the solute retention, but not the enantioselectivity factors. These findings suggest that a high retention of the compounds does not a guarantee a baseline separation. Enantioseparation is caused by discrimination by the CSP, whereas a high retention factor is caused by strong interactions of different nature with the surface. Moreover, a high *k* may well be attributable to a strong nonenantioselective component in the solute-CSP interaction.

(3) The four herbicides run in alcohol/water mixtures exhibited satisfactory linear van't Hoff plots. These solutes had the expected behavior, i.e. the enantioselectivity decreased with increased temperature, indicating that the differential enthalpy of interaction of the enantiomers with the CSP dominated enantiorecognition. A study of enthalpy–entropy compensation with the solutes solvated by different alcohols gave satisfactorily linear plots within these narrow solvent compositions for methanol, 2-propanol and *tert*-butanol, but a poor correlation ($r^2 = 0.63$) was observed for ethanol. We must consider, however, that since the nonenantioselective interactions also contributed to retention and were not under consideration in the study, the thermodynamic quantities reported here should be regarded as rough estimations of the true thermodynamic parameters. Nonetheless, because the conclusions made from these quantities are based on the observations of a large number of systems, even though the individual values are not necessarily absolute, the overall data nevertheless point to clear trends.

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