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Chiral separation of four 1,3-dioxolane derivatives by supercritical fluid chromatography on an amylose-based column

J.L. Bernal, L. Toribio*, M.J. del Nozal, E.M. Nieto, J.J. Jiménez

Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Prado de la Magdalena s/n, E-47005 Valladolid, Spain

Abstract

The chiral separation of four 1,3-dioxolane derivatives by supercritical fluid chromatography on an amylose-based column is described. The effects of mobile phase composition, temperature and pressure have been investigated. The nature of the modifier is the parameter which has the highest impact on the chiral resolution and it is more important than the polarity of the mobile phase. The organic modifier used for the best enantiomeric separation was different for each compound, because it depends strongly on the molecular structure of the compound.

Keywords: Enantiomer separation; Mobile phase composition; Thermodynamic parameters; Dioxolanes

1. Introduction

It is well known that chirality plays an important role in areas such as pharmaceuticals or agrochemicals due to the different activity and toxicological profiles of each enantiomer [1–6]. Because of that, analytical methods for the enantiomeric determination of the final drug and of some synthesis intermediates are needed.

Chromatographic methods using chiral stationary phases (CSPs) have been the most widely applied in the last few years [7], with high-performance liquid chromatography (HPLC) being the technique which predominates [8–17] due to its extended use and to the number of CSPs that can be employed. Nevertheless numerous papers focus on the possibilities that supercritical fluid chromatography (SFC) has for chiral separations [18–24]. The advantages of SFC over HPLC are the higher diffusivities of the analytes and faster analysis times, which provide higher resolution in shorter times.

The CSPs that can be employed in SFC are the same as in HPLC. A large number of chiral selectors have been reported in the literature [18,24–27], and the chiral mechanism responsible for the separation has been studied for some of them; but it is difficult to extrapolate rules and to predict which CSP will resolve a particular compound. However, one of the most popular CSP types in terms of the wide number of compounds resolved are those based on the polysaccharide derivatives, mainly the 3,5-dimethylphenyl carbamates (Chiralpak AD and Chiralcel OD) [28–30].

The 1,3-dioxolane derivatives studied in this work, are intermediates in the synthesis of several antimycotic drugs with medical application in the treatment of fungal diseases [31,32]. Their enantiomeric separations have not been reported yet, so the aim of this work was to study the enantiomeric separation by SFC on a Chiralpak AD column. For

*Corresponding author.

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this purpose the effects of the different parameters such as pressure, composition of the mobile phase and temperature, were investigated to obtain the best chromatographic conditions for the enantioresolution.

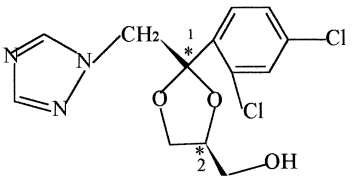
2. Experimental

2.1. Reagents

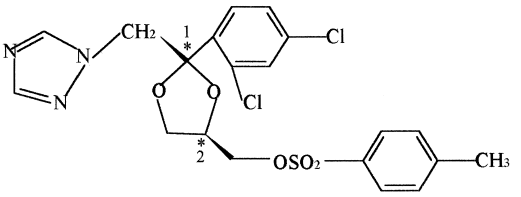
Methanol, ethanol, acetonitrile and 2-propanol were all HPLC-grade and purchased from Lab-Scan (Dublin, Ireland). Triethylamine (TEA) and trifluoroacetic acid (TFA) were obtained from Sigma–Aldrich (Madrid, Spain). The compounds studied (Fig. 1) were: *cis*-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-methanol (compound 1), *cis*-[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl *p*-toluensulfonate (compound 2), *cis*-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-methanol (compound 3) and *cis*-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl *p*-toluensulfonate (compound 4). They were synthesized in our laboratory according to the reaction scheme proposed by Heeres et al. [31,32]. The stock solutions of the compounds, were prepared in methanol at the 100 mg/l level. Carbon dioxide was SFC-grade and purchased from Carburos Metálicos (Barcelona, Spain).

2.2. Instrumentation

An HP 1205A Model SFC system from Hewlett-Packard (Palo Alto, CA, USA) furnished with a diode-array detection (DAD) system and a 7410 Rheodyne (Cotati, CA, USA) valve (5-μl loop volume) was used. The instrument was operated in the downstream mode. The chiral column employed, a Chiralpak AD, 250×4.6 mm, packed with the 3,5-dimethylphenyl carbamate derivative of amylose, coated on a 10-μm silica-gel support, was obtained from Daicel (Deventer, The Netherlands).



Compound 1



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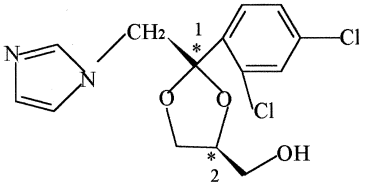
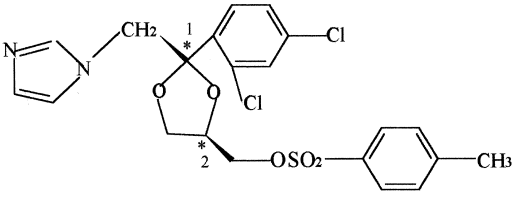


Compound 3



Compound 4

Fig. 1. Structures of the compounds studied.

Table 1
Effect of the different modifiers on the separation

Modifier	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s
Compound 1						
Chromatographic conditions: 5% of modifier, 200 bar, 35°C, 2 ml/min						
Methanol	23.08	24.08	13.70	14.34	1.05	0.25
Ethanol	30.88	32.72	19.72	20.96	1.06	0.99
2-Propanol	57.73	61.64	28.76	30.77	1.07	0.89
Acetonitrile (20%)[a]	19.19	22.10	12.23	14.24	1.16	0.53
Compound 2						
Chromatographic conditions: 10% of modifier, 200 bar, 35°C, 2 ml/min						
Methanol	17.06	17.06	10.45	10.45	1.00	0.00
Ethanol	20.52	20.52	12.50	12.50	1.00	0.00
2-Propanol	33.00	35.18	17.75	18.99	1.07	1.23
Acetonitrile	55.48	60.16	33.68	36.60	1.09	0.53
Compound 3						
Chromatographic conditions: 10% of modifier, 200 bar, 35°C, 2 ml/min						
Methanol	9.39	10.04	5.26	5.69	1.08	0.76
Ethanol	13.95	14.85	8.05	8.63	1.07	0.59
2-Propanol	38.04	38.04	24.70	24.70	1.00	0.00
Acetonitrile (enantiomers did not elute in 60 min even using 30%)						
Compound 4						
Chromatographic conditions: 10% of modifier, 200 bar, 35°C, 2 ml/min						
Methanol	19.74	19.74	11.82	11.82	1.00	0.00
Ethanol	26.90	26.90	15.81	15.81	1.00	0.00
2-Propanol	61.83	61.83	39.95	39.95	1.00	0.00
Acetonitrile (20%)	29.11	29.11	18.28	18.28	1.00	0.00

[a] Including 0.1% TEA and 0.1% TFA.

3. Results and discussion

3.1. Effect of pressure on the separation

The effect of the pressure was checked using 10% of organic modifier in the case of compounds 2–4 and 5% in the case of compound 1, the other chromatographic conditions were a temperature of 35°C, and a flow-rate of 2 ml/min. When the pressure was varied between 100 and 300 bar, little effect on the resolution was observed. The higher impact was appreciated on the retention, which decreased when the pressure increased. Taking into account these results a pressure of 200 bar was selected to continue the study because it provided an acceptable time of analysis.

3.2. Effect of modifier

As it can be seen in Fig. 1 all the compounds studied have several functional groups which can interact with the stationary phase through hydrogen bonding. As a consequence, when pure CO_2 was used as mobile phase, they were highly retained. The introduction of an organic modifier in the mobile phase, was then necessary in order to reduce the retention by acting in two ways: blocking the active sites of the stationary phase and changing the

Table 2
Modifier properties [37,38]

Modifier	α[a]	β[b]	P[c]
Methanol	0.93	0.62	5.1
Ethanol	0.83	0.77	4.3
2-Propanol	0.76	0.95	3.9
Acetonitrile	0.19	0.31	5.8

[a] α: Hydrogen bond donating ability.
[b] β: Hydrogen bond accepting ability.
[c] P: Polarity coefficient.

solvating power and the selectivity of the mobile phase.

Four organic modifiers, belonging to two different families, were tested in this work: alcohol type modifiers (methanol, ethanol and 2-propanol) and a non-alcohol type modifier (acetonitrile). The influence of the modifier nature on the retention, was studied at a temperature of 35°C, a flow-rate of 2 ml/min, a pressure of 200 bar and at a constant percentage of the modifier in the mobile phase. The results obtained are presented in Table 1, as it can be seen the capacity factors of both enantiomers, increased from methanol to 2-propanol for each of the compounds studied. The selectivity and the resolu-

Table 3
Effect of modifier concentration on the separation of compounds 1 and 2 (chromatographic conditions: 200 bar, 35°C, 2 ml/min)

	Modifier	t_1	t_2	k_1	k_2	α	R_s
Compound 1	Methanol (%)						
	5	23.08	24.08	13.70	14.34	1.05	0.25
	15	4.95	4.95	2.30	2.30	1.00	0.00
	20	3.78	3.78	1.54	1.54	1.00	0.00
	Ethanol (%)						
	5	30.88	32.72	19.72	20.96	1.06	0.99
	6	22.12	24.87	13.17	14.94	1.13	1.10
	8	15.22	15.56	8.88	9.10	1.02	0.75
	10	10.64	10.64	6.00	6.00	1.00	0.00
	20	4.10	4.10	1.73	1.73	1.00	0.00
	2-Propanol (%)						
	5	57.73	61.64	28.76	30.77	1.07	0.89
	8	23.55	24.96	14.81	15.75	1.06	0.82
	10	15.22	16.30	8.76	9.45	1.08	0.87
	12	10.77	11.31	6.18	6.54	1.06	0.78
	15	7.37	7.75	3.91	4.17	1.07	0.82
	Acetonitrile (%)[a]						
	20	19.19	22.10	12.23	14.24	1.16	0.53
	25	11.42	13.37	6.05	7.25	1.20	0.54
	30	8.49	9.86	4.18	5.01	1.20	0.56
Compound 2	Methanol (%)						
	5	50.45	50.45	25.55	25.55	1.00	0.00
	10	17.06	17.06	10.45	10.45	1.00	0.00
	20	6.60	6.60	3.20	3.20	1.00	0.00
	Ethanol (%)						
	5	73.37	73.37	52.95	52.95	1.00	0.00
	10	20.52	20.52	12.50	12.50	1.00	0.00
	15	11.65	11.65	6.66	6.66	1.00	0.00
	2-Propanol (%)						
	8	40.90	42.80	25.55	26.79	1.05	1.37
	10	33.00	35.18	17.75	18.99	1.07	1.23
	12	22.91	24.22	14.80	15.70	1.06	1.11
	15	14.93	15.67	8.95	9.45	1.06	0.91
	Acetonitrile (%)						
	10	55.48	60.16	33.68	36.60	1.09	0.53
	15	22.47	24.24	13.69	14.84	1.08	0.56
	20	13.84	14.87	8.17	8.85	1.08	0.57
	25	8.04	8.56	4.03	4.35	1.08	0.56

[a] Including 0.1% TEA and 0.1% TFA.

tion have also a tendency to increase from methanol to 2-propanol, except for compound 3. Although acetonitrile has a polarity coefficient higher than the alcohols assayed (Table 2) the compounds needed a higher percentage of modifier to be eluted. Probably this is because hydrogen bonding interaction, between the enantiomers and the stationary phase, plays a role in the non specific retention mechanism. Acetonitrile cannot compete with the analytes in this interaction, which could explain the higher retention and the failure to obtain a higher enantiomeric resolution.

It should be noted that using acetonitrile the peaks obtained for compound 1 were severely tailed, so the addition of 0.1% of TEA and 0.1% of TFA [28], to the mobile phase, was necessary in order to improve the peak shapes. The effect of these additives was also investigated on the others modifiers and compounds, but as the results did not improve, so the additives were not added anymore.

The effect of modifier concentration on the retention was also studied. The data (Tables 3 and 4) were obtained at a pressure of 200 bar, a temperature of 35°C and a flow-rate of 2 ml/min. As can be seen, the capacity factor decreased markedly when the percentage of modifier was increased (polarity of the mobile phase increased), which caused a slight change on the resolution. This could indicate that the achiral interaction (non specific interactions) depends more on the polarity of the mobile phase than the chiral interactions. So the polarity of the mobile phase affected the overall interaction of the two enantiomers with the stationary phase. Increasing or decreasing the percentage of modifier affected in the

Table 4
Effect of modifier concentration on the separation of compounds 3 and 4 (chromatographic conditions: 200 bar, 35°C, 2 ml/min)

	Modifier	t_1	t_2	k_1	k_2	α	R_s
Compound 3	Methanol (%)						
	5	33.93	37.05	20.47	22.45	1.10	0.74
	8	15.24	16.21	9.09	9.74	1.07	0.72
	10	9.83	10.70	5.51	6.09	1.11	0.76
	15	5.24	5.57	2.54	2.76	1.09	0.60
	Ethanol (%)						
	5	57.83	61.68	35.14	37.55	1.07	0.49
	8	23.01	24.01	13.90	14.55	1.05	0.53
	10	14.14	15.19	8.55	9.26	1.08	0.59
	20	4.36	4.56	1.95	2.08	1.07	0.55
	30	2.89	2.89	0.93	0.93	1.00	0.00
	2-Propanol (%)						
	5	63.45	63.45	39.41	39.41	1.00	0.00
	10	38.04	38.04	24.70	24.70	1.00	0.00
	15	14.43	14.43	8.82	8.82	1.00	0.00
	20	7.77	7.77	4.36	4.36	1.00	0.00
Compound 4	Methanol (%)						
	5	69.97	69.70	43.85	43.85	1.00	0.00
	10	18.71	18.71	11.15	11.15	1.00	0.00
	Ethanol (%)						
	5	53.40	53.40	33.01	33.01	1.00	0.00
	10	26.90	26.90	15.81	15.81	1.00	0.00
	20	7.59	7.59	4.20	4.20	1.00	0.00
	2-Propanol (%)						
	10	61.83	61.83	39.95	39.95	1.00	0.00
	20	12.88	12.88	8.07	8.07	1.00	0.00
	Acetonitrile (%)						
	20	29.11	29.11	18.28	18.28	1.00	0.00

same way the two enantiomers and the chiral resolution was slightly changed. Greater variations of the chiral resolution, which were caused by changes of the chiral interaction, were obtained by changing the nature of modifier and not by varying the percentage of a given modifier.

It should be noted that compound 4 was not resolved with any of the organic modifiers tested.

The effect of the organic modifier on the separation, is different according to the molecular structure of the compounds. Changing the hydroxyl group at the second chiral center (compound 1) by the sulfonate group (compound 2), the retention increased using the alcohol type modifiers. Resolution for compound 2 was only obtained with 2-propanol. When acetonitrile was used, the retention decreased and a small resolution was obtained for both compounds. It seems that when the steric hindrance at the second chiral center increases, a bulkier modifier (isopropanol) or a modifier with small H-bond character (acetonitrile, see Table 2) gives better results. This can be explained by saying that the competition between the modifier and the analyte for the interaction with the stationary phase is favorable for the analyte.

When the imidazole group (compound 3) was changed by the triazole group (compound 1), bulkier modifiers (isopropanol) and modifiers with small H-bond characteristics (acetonitrile) provided better results than the other modifiers.

3.3. Effect of the temperature

Temperature is an important factor in chiral separations [33,34]. Chromatographic selectivity and capacity factors are related to the temperature according to the van 't Hoffs equation:

$$\ln k = -\Delta H^0/RT + \Delta S^0/R + \ln \phi$$

$$\ln \alpha = \ln (k_2/k_1) = -\Delta(\Delta H^0)/RT + \Delta(\Delta S^0)/R$$

$$\ln \alpha = -\Delta(\Delta G^0)/RT$$

where R is the ideal gas constant, T the absolute temperature, ϕ the phase ratio, and ΔH^0 and ΔS^0

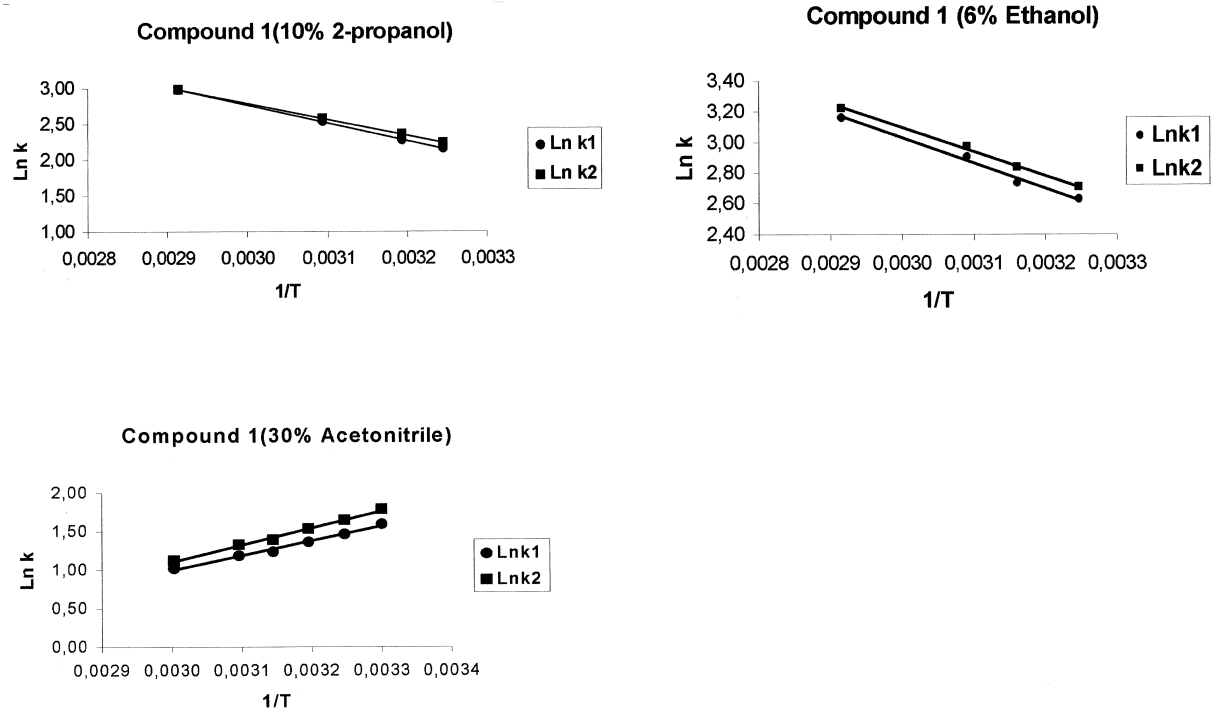


Fig. 2. Variation of ln k versus $1/T$ for compound 1. Chromatographic conditions: 200 bar, 2 ml/min.

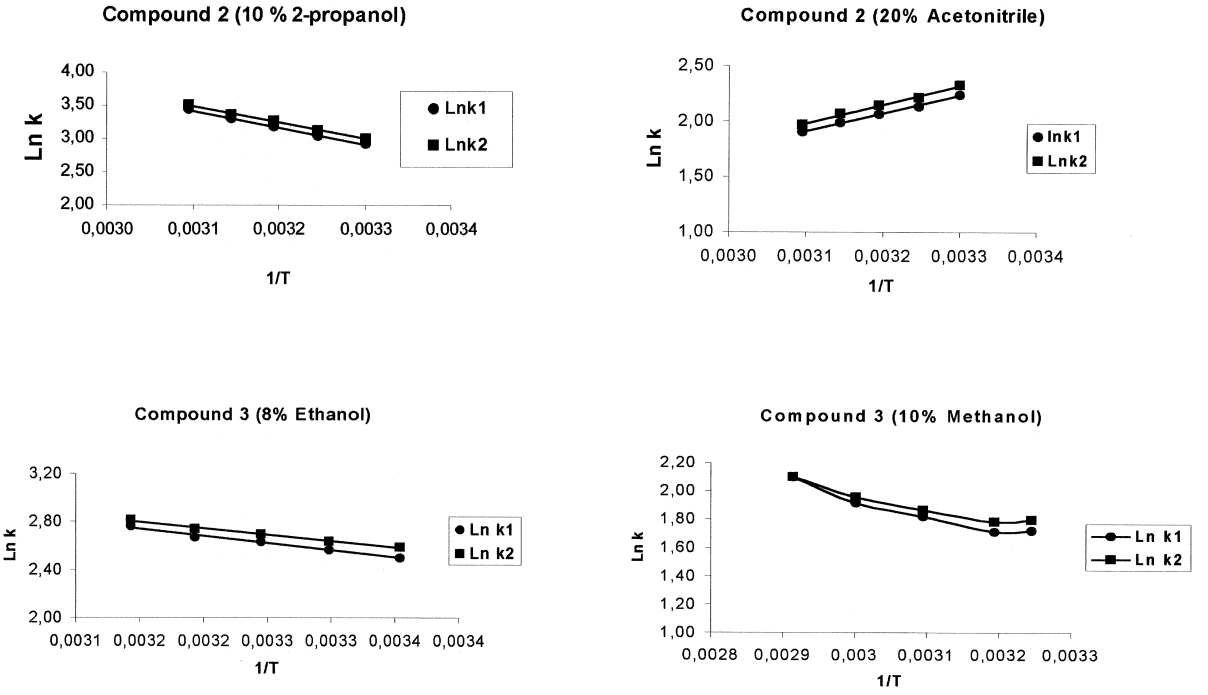


Fig. 3. Variation of ln k versus $1/T$ for compounds 2 and 3. Chromatographic conditions: 200 bar, 2 ml/min.

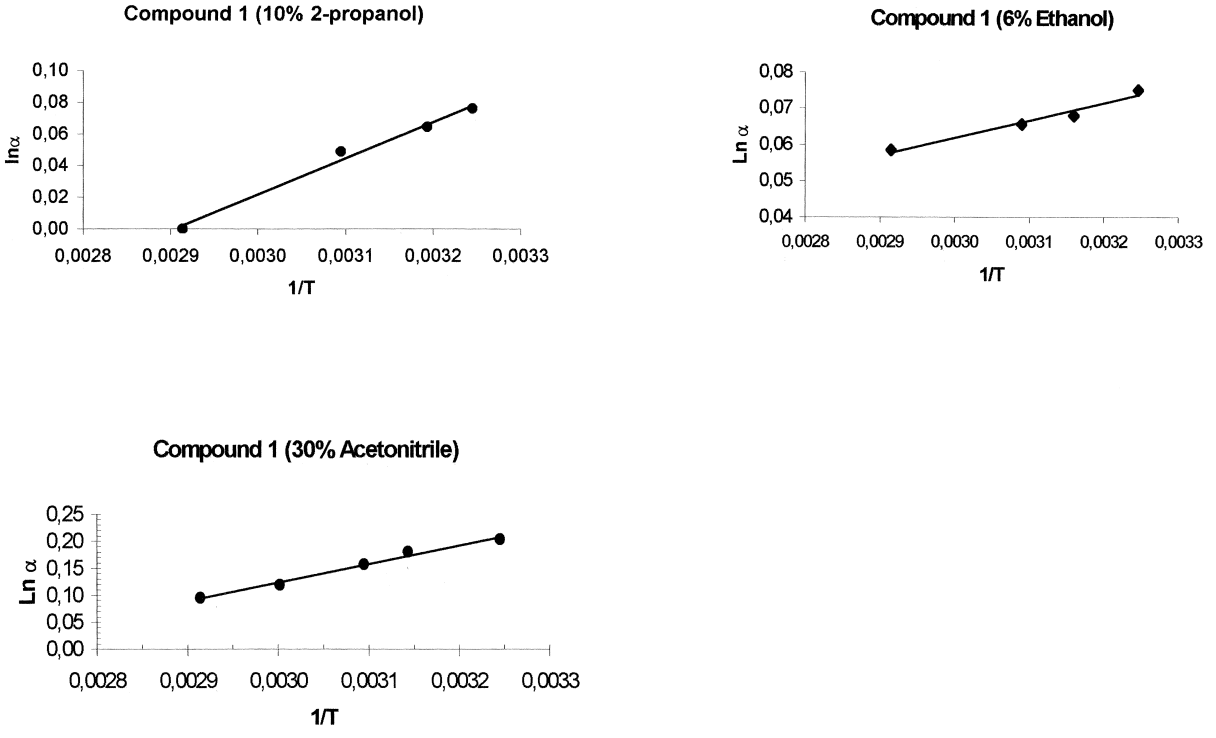


Fig. 4. Variation of ln α versus $1/T$ for compound 1. Chromatographic conditions: 200 bar, 2 ml/min.

Table 5
Enthalpy and isoelution temperature values obtained for the compounds studied (1 cal = 4.184 J)

Compound	Modifier	ΔH_1^0 (cal/mol)	ΔH_2^0 (cal/mol)	T_{iso} (°C)
1	Ethanol	3276.31	3182.45	314
	2-Propanol	4827.80	4375.20	70
	Acetonitrile	−3814.04	−4387.29	108
2	2-Propanol	5046.82	4898.2	163
	Acetonitrile	−3174.62	−3379.09	138
3	Ethanol	2377.58	2111.87	94
3	Methanol	–	–	70

represent the enthalpic and the entropic differences of the enantiomers interaction with the stationary phase.

From a thermodynamic point of view, retention and selectivity are controlled by an enthalpic contribution which decreases with the temperature and an entropic contribution which is independent of the temperature. The effect of temperature on the enantiomeric separation was investigated for the modifiers which provided some kind of resolution. As can be seen in Figs. 2 and 3 the plots of ln k versus $1/T$ are straight lines for all the compounds, except for compound 3 when using methanol; this non-linear behavior has been observed by other authors and for other compounds and the causes are not well understood [35]. Using the alcohol type modifiers the retention increased when the temperature increased, but in the case of compounds 1 and 2, using

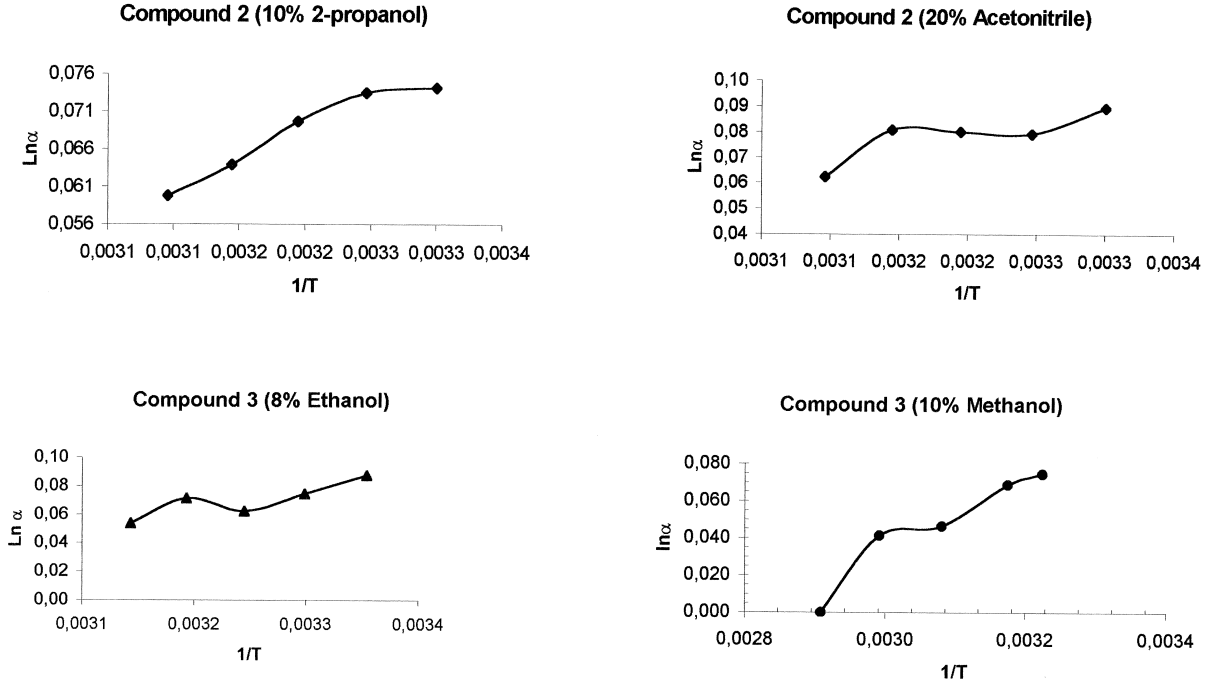


Fig. 5. Variation of ln α versus $1/T$ for compounds 2 and 3. Chromatographic conditions: 200 bar, 2 ml/min.

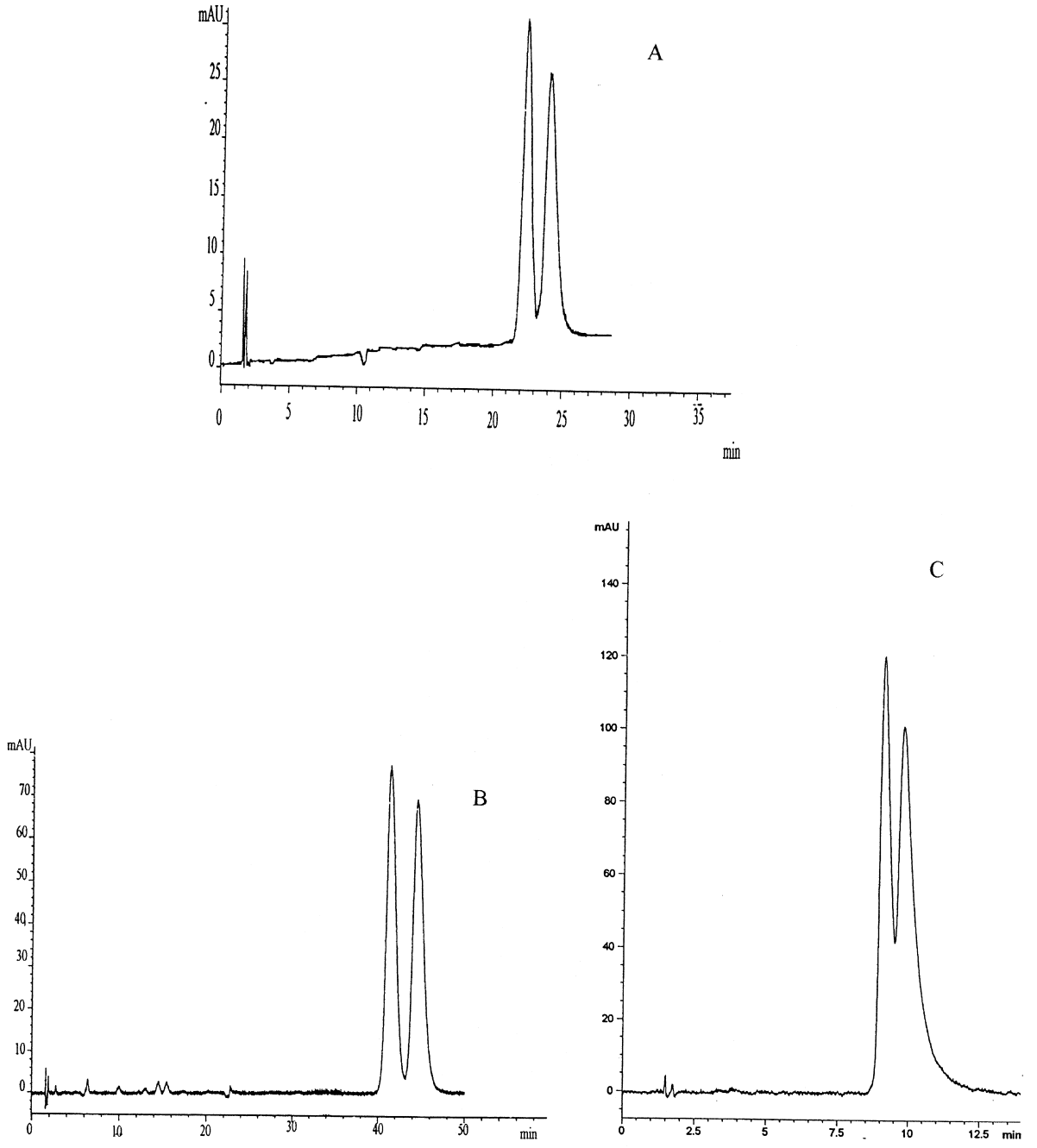


Fig. 6. Chromatograms of the best separation obtained for each compound. (A) Compound 1 (50 mg/l), 6% of ethanol, 35°C, 200 bar, 2 ml/min. (B) Compound 2 (100 mg/l), 8% of 2-propanol, 35°C, 200 bar, 2 ml/min. (C) Compound 3 (100 mg/l) 10% of methanol, 35°C, 200 bar, 2 ml/min.

acetonitrile as modifier, the opposite effect was observed. This could be caused by the high percentage of acetonitrile used, which makes the separation to be more similar to HPLC than to SFC. This agrees with the decrease on the retention, described in chiral HPLC, when the temperature increases [33].

The ΔH^0 values calculated from the linear correlation are given in Table 5. As it can be seen, for compound 1 2-propanol provided the highest values followed by acetonitrile, this fact was also observed for compound 2. In the case of compound 3 these values were calculated only for ethanol, because it was not resolved either using 2-propanol or acetonitrile, and had a non-linear behavior when methanol was used. The temperature of isoelution (T_{iso}) was calculated from the intersection of the lines obtained for the two enantiomers. As can be seen (Table 5) this temperature varies between the compounds, and for the same compound it depends on the modifier nature. The isoelution could only be observed in the case of compound 1 using 2-propanol (70°C) and compound 3 using methanol (70°C). According to the studies of Stringham et al. [35,36], above this temperature the elution order of the enantiomers will be reversed, and the chiral separation is said to be entropically driven. In our case, the temperature was not increased further than 70°C to avoid damaging the column, and so these effects could not be confirmed.

Plots of ln α versus $1/T$ (Figs. 4 and 5) gave straight lines for compound 1. For compounds 2 and 3 the relationship was not linear, which could indicate a variation, due to the temperature, in the mechanism of interaction between the enantiomers and the stationary phase.

This variation could be attributed to changes in the amount of the modifier adsorbed on the stationary phase.

Taking into account all the data obtained, the best enantiomeric separation obtained for each compound is shown in Fig. 6.

4. Conclusion

The enantiomeric resolution of several 1,3-dioxolane derivatives can be achieved by SFC chromatography on an amylose-based column. The compounds are highly retained on this CSP making the use of organic modifiers necessary. Using the alcohol type modifiers the retention increases from methanol to 2-propanol. Acetonitrile provides the highest retention and, for compound 1, the addition of 0.1% TEA and 0.1% TFA is necessary in order to decrease the peak tailing.

Polarity of the mobile phase seems to affect in the same way both enantiomers producing a decrease on the overall retention but very small changes in the resolution. Marked changes on the resolution are obtained when the nature of the modifier is changed.

When the steric impediment on the second chiral center increases, it seems that a bulkier modifier (isopropanol) or a modifier with small H-bond character (acetonitrile) gives better results. The same effect is observed when an imidazole group is changed by a triazole group, in the first chiral center.

The van ‘t Hoff plots were linear for all the compounds studied except for compound 3, and the isoelution temperature were observed for compound 1 (70°C) and 3 (70°C).

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