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Applications of the Chiralpak AD and Chiralcel OD chiral columns in the enantiomeric separation of several dioxolane compounds by supercritical fluid chromatography☆

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Abstract

Two chiral columns based on polysaccharide derivatives (Chiralpak AD and Chiralcel OD) have been tested for the chiral separation of several dioxolane compounds, using supercritical fluid chromatography. The compounds studied included ketoconazole and some of its precursors. The effect of the different modifiers and the pressure, on the chromatographic parameters was also evaluated. In general, the alcohol modifiers provided better results than acetonitrile, and all the compounds could be separated with these two columns, but the selection of the column depends on the kind of compound.

Keywords: Chiral stationary phases, SFC; Supercritical fluid chromatography; Enantiomer separation; Pharmaceutical analysis; Dioxolanes; Ketoconazole

1. Introduction

Chiral separation is one of the fastest growing fields in chromatography and an important task in pharmaceutical research, due to the fact that the development of most today's pharmaceuticals, requires the determination of the enantiopurity of the drug, its precursors and metabolites [1]. This necessity is related to the different biological activity of each enantiomer.

Chromatography on chiral stationary phases is the most widely applied method [2–5], and in the last few years, supercritical fluid chromatography (SFC) has been demonstrated to be a good choice for chiral separations [6–13]. SFC provides higher efficiency, faster analyses, better resolutions and quicker method development.

A wide range of chiral stationary phases (CSPs) have been used in SFC including Pirkle type [14–17], cyclodextrins [18,19] and derivatized polysaccharides [11,20–22]. The latter, and mainly the tris(3,5-dimethylphenylcarbamate) of amylose (Chiralpak AD) and cellulose (Chiralcel OD), have

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proved to be two of the most successful and widely applied CSPs due to the high number of compounds resolved [9,10,23–27].

Ketoconazole is a dioxolane derivative used as an antimycotic drug. It is manufactured as the *cis* racemate, and its determination is usually accomplished by using achiral high-performance liquid chromatography (HPLC) [28] or spectrophotometric [29] methods. Nevertheless, differences in the biological activity among the ketoconazole isomers have been reported [30], and for this kind of investigation, enantioselective methods of analysis are necessary.

In previous works [31,32] we studied the application of the Chiralpak AD column in the SFC chiral separation of ketoconazole and some intermediates of its synthesis. We obtained very good results in the case of ketoconazole, but not all the intermediates studied could be resolved on this column. The aim of the present work, was to study the possibilities of the Chiralcel OD column in the enantiomeric separation of these compounds including some more precursors, and to compare the results with those ones obtained with the Chiralpak AD column. For this purpose, the effect of the modifier and the mobile phase composition on the separation were examined, in order to obtain the best enantioresolution.

2. Experimental

2.1. Reagents

The organic solvents: methanol, ethanol (98%), 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).

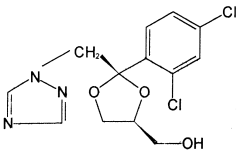
The compounds studied (Figs. 1 and 2) were synthesised in our laboratory according to the reaction scheme proposed by Heeres et al. [33], except ketoconazole that was obtained from Sigma–Aldrich. The standard solutions (500 mg/l) were prepared in methanol.

Carbon dioxide was SFC grade and purchased from Carburos Metálicos (Barcelona, Spain).

2.2. Instrumentation

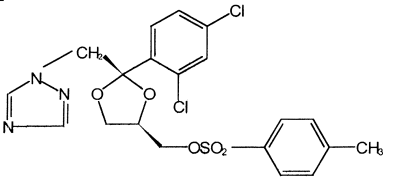
The supercritical fluid chromatograph used was a G1205A Model from Hewlett-Packard (Wilmington, DE, USA) equipped with a diode array detection (DAD) system and a 7410 Rheodyne (Cotati, CA, USA) valve (5 μl loop volume). The instrument was operated in the downstream mode.

Compound 1



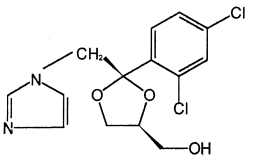
Cis- 2- (2,4- dichlorophenyl)- 2-(1H- 1,2,4- triazol- 1-ylmethyl) - 1,3- dioxolan- 4- methanol

Compound 2



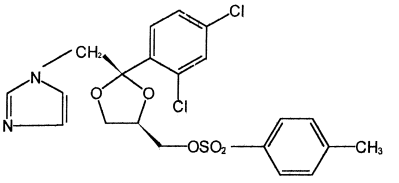
Cis- [2- (2,4- dichlorophenyl)- 2-(1H- 1,2,4- triazol- 1-ylmethyl)- 1,3- dioxolan- 4- yl] methyl p-toluensulfonate

Compound 3



Cis- 2- (2,4- dichlorophenyl)- 2-(1H- imidazol- 1-ylmethyl) 1,3- dioxolan- 4- methanol

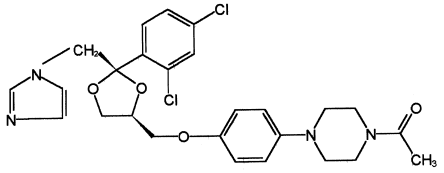
Compound 4



Cis- [2- (2,4- dichlorophenyl)- 2-(1H- imidazol- 1-ylmethyl)- 1,3- dioxolan- 4- yl] - methyl p-toluensulfonate

Fig. 1. Structures of the compounds studied.

Ketoconazole:



2- bromomethyl- 2(2,4- dichlorophenyl)-1,3 dioxolan-4 methyl benzoate

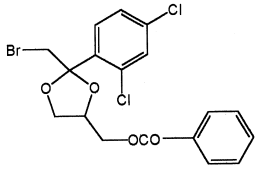


Fig. 2. Structures of the compounds studied.

The chiral columns employed were Chiralpak AD and Chiralcel OD they both were 250×4.6 mm and packed with the tris(3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose, respectively, coated on 10 μm silica-gel support. They were obtained from Daicel (Deventer, The Netherlands).

3. Results and discussion

The effect of the pressure on the separation using the Chiralcel OD column, was similar to the one obtained with the Chiralpak AD [31,32], for all the compounds studied. Increasing the pressure, retention decreased as a consequence of the increase in the density and solvating power of the mobile phase, but changes in the resolution were very small.

Table 1
Modifier properties [34]

Modifier	α	β	P
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

α, Hydrogen bond donating ability. β, Hydrogen bond accepting ability. P, Polarity coefficient.

According with the results obtained, a pressure of 200 bar was selected.

The column temperature and the flow-rate were fixed at 35°C and 2 ml/min, respectively.

3.1. Separation of compounds 1, 2, 3 and 4

The introduction of an organic modifier in the mobile phase was necessary in order to decrease the retention time of these solutes. Four organic modifiers with different characteristics (Table 1) were used: methanol, ethanol, 2-propanol and acetonitrile. The results obtained using each modifier, are shown in Tables 2 and 3. As can be seen, the variation of the capacity factors were similar in both columns, they increased from methanol to 2-propanol as the polarity of the modifier decreased, but in the case of acetonitrile, although it has a higher polarity than the alcohol-type solvents studied, a higher percentage was needed in order to elute the compounds. When the percentage of a given modifier was increased, retention decreased but the changes in the resolution were very small. In the case of the Chiralcel OD column, the polarity of the mobile phase affects more the achiral interactions (non stereoselective interactions), as we observed with the Chiralpak AD column [31,32], while other properties of the modifier such as hydrogen bond donating or accepting ability have a strong effect on the chiral interactions and thus, the highest variations on the enantioresolution were obtained by changing the nature of the organic modifier.

Resolution increased from methanol to 2-propanol except in the case of compounds 3 and 4 where the opposite effect was observed. When acetonitrile was used the results were worse.

It should be noted that whatever the organic modifier used, the compounds were more retained on the Chiralpak AD column than on the Chiralcel OD. As can be seen in Figs. 3 and 4, the best enantioresolutions were obtained using the Chiralcel OD column. Compound 4 could only be resolved using this column.

3.2. Separation of ketoconazole

Compared with the previous compounds,

Table 2
Effect of the modifiers on the separation, using the Chiralpak AD column

Modifier	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s
Compound 1 (5% of modifier)						
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
20% Acetonitrile[a]	19.19	22.10	12.23	14.24	1.16	0.53
Compound 2 (10% of modifier)						
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
20% Acetonitrile	13.84	14.87	8.17	8.85	1.08	0.56
Compound 3 (10% of modifier)						
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
25% Acetonitrile	Enantiomers did not elute in 60 min					
Compound 4 (10% of modifier)						
Methanol	19.74	19.74	11.82	11.82	1.00	0.00
Ethanol	26.90	26.90	15.81	15.811	1.00	0.00
2-Propanol	61.83	61.83	39.95	39.95	1.00	0.00
20% Acetonitrile	29.11	29.11	18.28	18.28	1.00	0.00

See text for chromatoghraphic conditions.
[a] Including 0.1% TEA and 0.1% TFA.

Table 3
Effect of the modifiers (10%) on the separation, using the Chiralcel OD column

Modifier	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s
Compound 1						
Methanol	6.16	6.77	3.19	3.61	1.13	2.57
Ethanol	8.11	9.13	4.41	5.09	1.15	2.64
2-Propanol	11.37	14.10	6.84	8.72	1.28	3.40
20% Acetonitrile	14.19	15.56	8.79	9.73	1.11	0.57
Compound 2						
Methanol	12.06	12.68	7.26	7.68	1.06	1.24
Ethanol	15.81	16.79	9.40	10.05	1.07	1.47
2-Propanol	23.91	24.43	15.27	15.62	1.02	1.31
20% Acetonitrile	10.38	10.38	6.16	6.16	1.00	0.00
Compound 3						
Methanol	8.47	8.88	4.80	5.08	1.06	1.16
Ethanol	15.08	15.96	8.92	9.50	1.06	0.44
2-Propanol	22.47	25.33	13.69	15.56	1.14	0.66
30% Acetonitrile	19.82	19.82	11.62	11.62	1.00	0.00
Compound 4						
Methanol	15.94	16.47	9.77	10.13	1.04	0.69
Ethanol	21.26	22.42	12.81	13.56	1.06	0.93
2-Propanol	45.93	45.93	29.22	29.22	1.00	0.00
30% Acetonitrile	10.46	10.46	5.66	5.66	1.00	0.00

See text for the other chromatographic conditions.

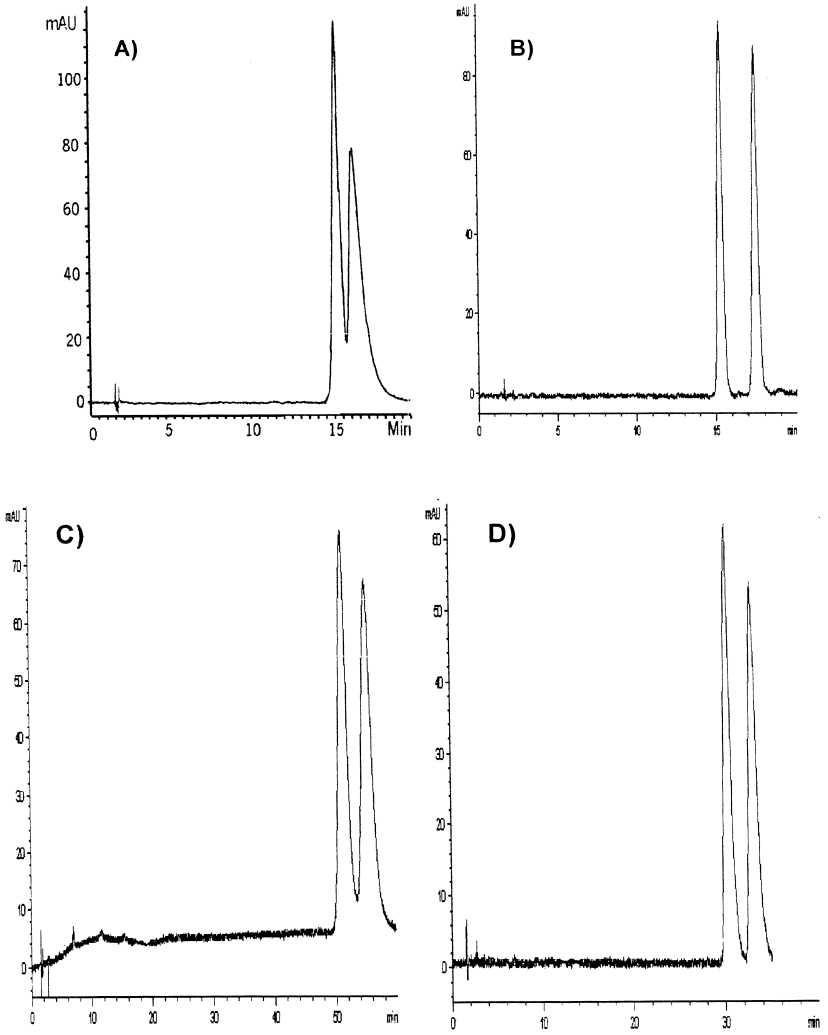


Fig. 3. Chromatograms obtained for compounds 1 and 2. (A) Compound 1, Chiralpak AD column, 10% 2-propanol, 200 bar, 2 ml/min, 35°C. (B) Compound 1, Chiralcel OD column, 5% methanol, 200 bar, 2 ml/min, 35°C. (C) Compound 2, Chiralpak AD column, 8% 2-propanol, 200 bar, 2 ml/min, 35°C. (D) Compound 2, Chiralcel OD, 8% 2-propanol, 200 bar, 2 ml/min, 35°C.

Table 4
Effect of the modifiers (30%) on the separation of ketoconazole

Modifier	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s
Chiralpak AD column						
Methanol	20.27	22.56	11.28	12.67	1.12	1.50
Ethanol	15.20	23.88	9.34	15.24	1.63	6.58
2-Propanol	14.72	18.85	9.08	11.91	1.31	2.94
Acetonitrile	Enantiomers did not elute in 60 min					
Chiralcel OD column						
Methanol	13.44	14.64	8.60	9.46	1.10	1.59
Ethanol	14.96	16.73	9.84	11.12	1.13	1.87
2-Propanol	27.19	32.56	19.29	23.30	1.21	2.72
Acetonitrile	Enantiomers did not elute in 60 min					

See text for the other chromatographic conditions.

ketoconazole needed a higher percentage of organic modifier to be eluted, which could be due to the bulkiness of the substituent at the second chiral center.

Taking into account the results obtained with the different modifiers (Table 4), it could be said that the columns behaved in an opposite way as far as retention was concerned. When the Chiralpak AD

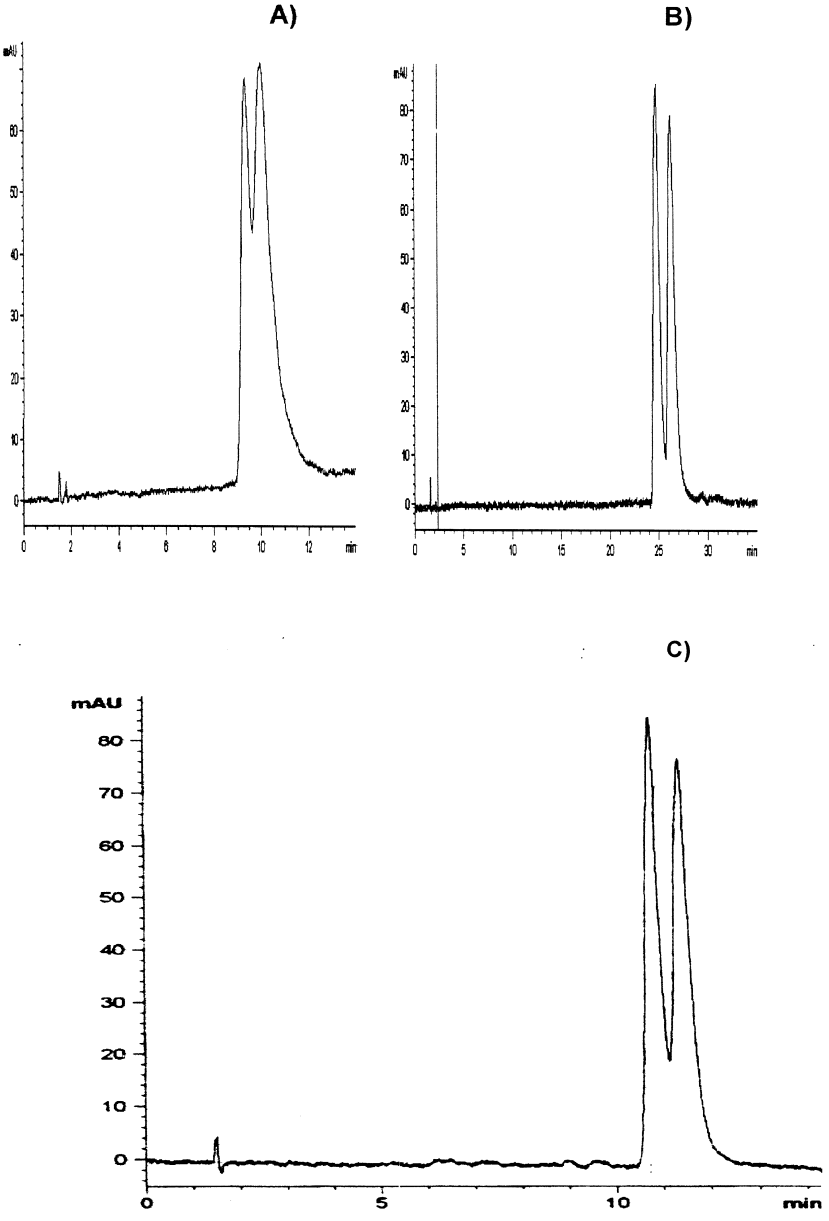


Fig. 4. Chromatograms obtained for compounds 3 and 4. (A) Compound 3, Chiralpak AD column, 10% methanol, 200 bar, 2 ml/min, 35°C. (B) Compound 3, Chiralcel OD column, 5% methanol, 200 bar, 2 ml/min, 35°C. (C) Compound 4 Chiralpak OD column, 15% ethanol, 200 bar, 2 ml/min, 35°C.

column was used, the decrease of the modifier polarity resulted in a decrease of the retention instead of an increase. Moreover, changes in the nature of the modifier produced higher variations on the resolution than using the Chiralcel OD column.

Although 2-propanol provided a good resolution using the Chiralcel OD column, the best separation in terms of analysis time (24 min) and resolution (6.58) was obtained using the Chiralpak AD column and ethanol as organic modifier (Fig. 5). The high resolution obtained under these conditions enables the use of higher flow-rates and additives in order to decrease the retention time [32], which allows to obtain the separation of ketoconazole enantiomers in 7 min (Fig. 6).

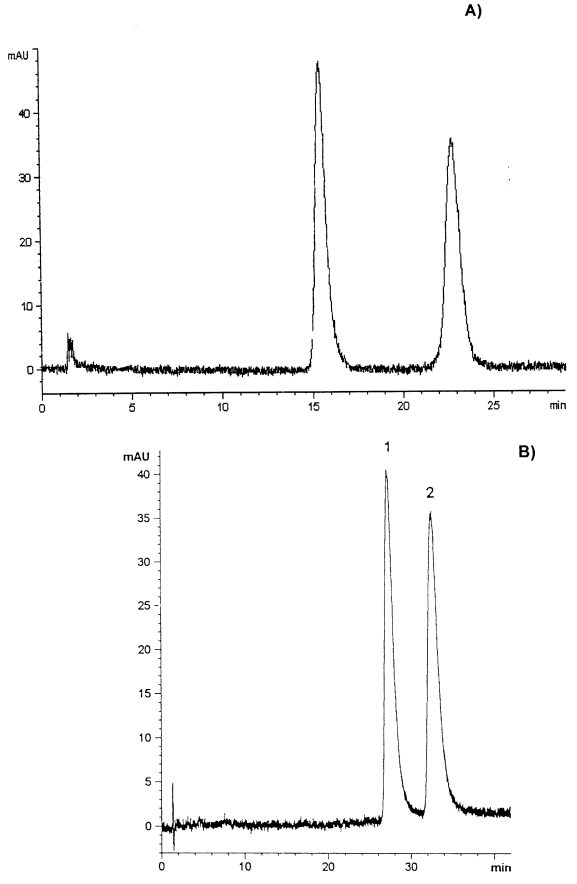


Fig. 5. Chromatograms obtained for ketoconazole. (A) Chiralpak AD column, 30% ethanol, 200 bar, 2 ml/min and 35°C. (B) Chiralcel OD column, 30% 2-propanol, 200 bar, 2 ml/min and 35°C.

3.3. Separation of 2-bromomethyl-2(2,4-dichlorophenyl)-1,3-dioxolan-4-methyl benzoate

This compound has two chiral centers, that means four optical isomers, and can be eluted with a lower percentage of organic modifier (it ranged between 4 and 10%).

As in the other cases, the retention decreased as the polarity or the percentage of a given modifier increased. The highest retention was obtained with acetonitrile.

The most noticeable variation in the enantioresolution was obtained by changing the nature of the modifier (Fig. 7).

Using the Chiralpak AD column the separation between diastereoisomers was possible with any of the modifiers assayed, but the enantiomeric separation is more dependent on the nature of the modifier employed. In this way, the *trans* enantiomers were baseline resolved with methanol while the

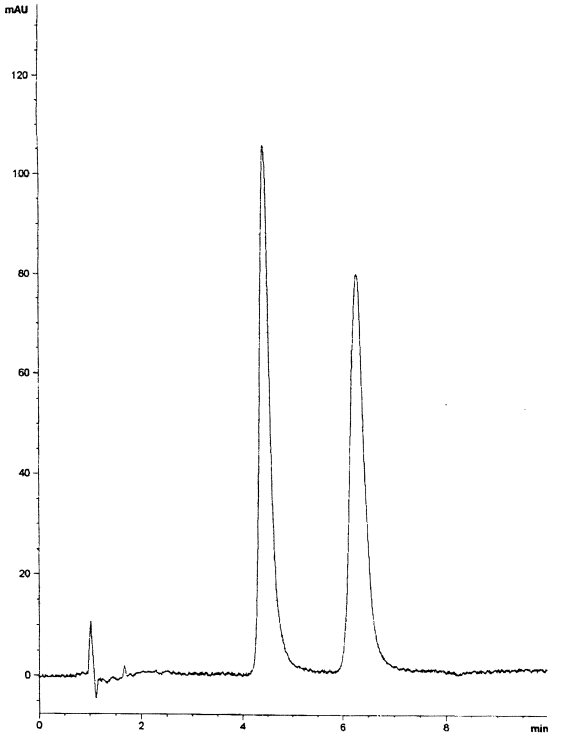


Fig. 6. Chromatogram of ketoconazole using the Chiralpak AD column, 30% ethanol containing 0.1% TFA and 0.1% TEA, 300 bar, 3 ml/min and 35°C.

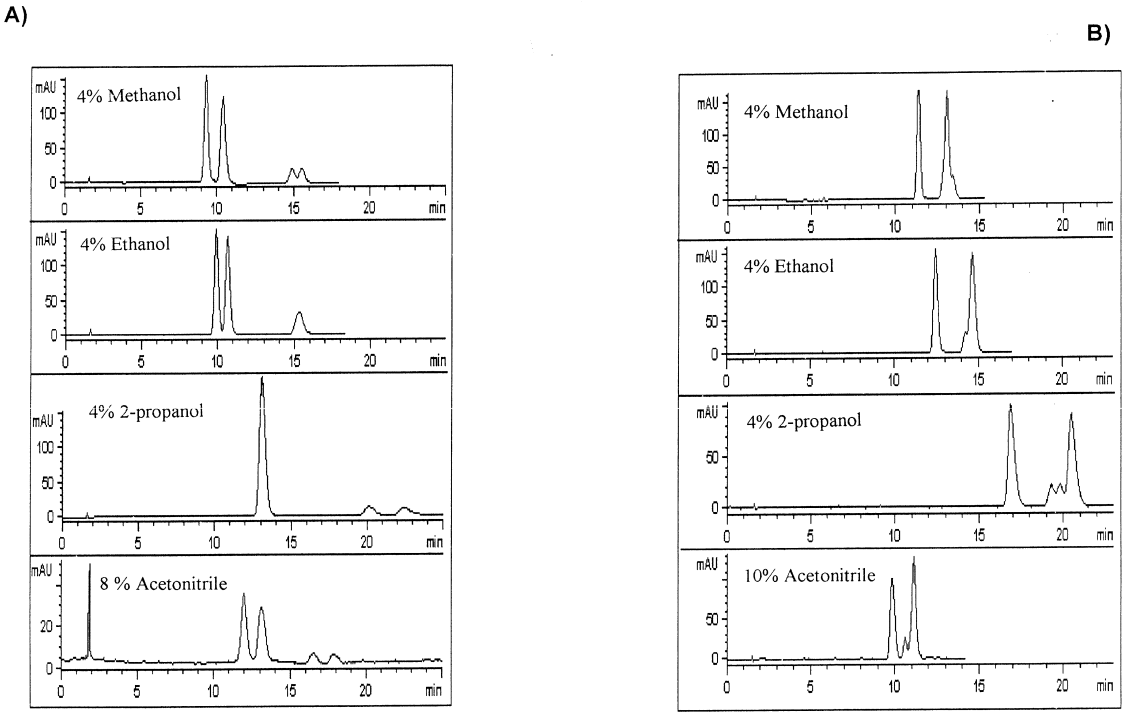


Fig. 7. Chromatograms obtained for the 2-bromomethyl-2(2,4-dichlorophenyl)-1,3-dioxolan-4-methyl benzoate at 200 bar, 2 ml/min and 35°C. (A) Chiralpak AD column. (B) Chiralcel OD column.

cis ones were partially separated. Ethanol provided a good resolution for the *trans* enantiomers but not for the *cis* ones, and the opposite effect was observed using 2-propanol. The four isomers were baseline resolved using acetonitrile.

Using the OD column the *trans* enantiomers were separated with any of the modifiers studied, but the *cis* enantiomers were not resolved and in some cases they coeluted with one of the *trans* enantiomers.

4. Conclusions

All the compounds studied can be enantiomerically resolved using supercritical fluid chromatography and chiral columns based on the tris(3,5-dimethylphenylcarbamate) of cellulose (Chiralcel OD) or amylose (Chiralpak AD). Obviously, the selection of the column depends on the kind of compound to be separated. This way, the first group of compounds are better resolved on the Chiralcel OD column than on the Chiralpak AD, but in the case of ketoconazole and 2-bromemethyl-2(2,4-dichlorophenyl)-1,3-dioxolan-4-methylbenzoate the best results are obtained with the Chiralpak AD column.

In all cases, the alcohol-type modifiers give better results than acetonitrile, except in the separation of the 2-bromemethyl-2(2,4-dichlorophenyl)-1,3-dioxolan-4-methylbenzoate on the Chiralpak AD column, where the baseline resolution of the four enantiomers is achieved using acetonitrile.

Changes in the modifier polarity have a bigger effect on the retention than on the enantioresolution, the latter being the most affected by changes in the nature of the modifier.

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