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Study of the enantiomeric separation of an acetamide intermediate by using supercritical fluid chromatography and several polysaccharide based chiral stationary phases☆

L. Toribio*, M.J. del Nozal, J.L. Bernal, J. Bernal, M.T. Martín

I.U. CINQUIMA, Analytical Chemistry Group, University of Valladolid, Vallodolid, Spain

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ABSTRACT

Four chiral stationary phases, based on the phenylcarbamate derivatives of amylose or cellulose: Chiralcel OD-H, Chiralpak AD, Lux Cellulose-2 and Lux Amylose-2, were evaluated for the enantiomeric separation of an acetamide chiral intermediate, the (4S-trans)-4-(ethylamino)-4-(N-acetamide)-5,6-dihydro-(6S)-methyl-4H-thieno-[2,3-b]thiopyran-7,7-dioxide, using SFC. The effect of the different modifiers and temperatures, on the separation, was also studied. The chiral separation could not be achieved using the Chiralpak AD column, nevertheless the other columns provided excellent results with analysis times close to 6 min and resolutions higher than 2. The highest enantioresolutions and retentions were obtained with the Lux Cellulose-2 column and 2-propanol as organic modifier. The isoelution temperatures were estimated from the van't Hoff plots, and in all the cases they were above the temperature range studied which means that the enantiomeric separation was enthalpy driven.

1. Introduction

Since it is well known that a pair of enantiomers can display quite different activities and toxicological profiles, the pharmacological evaluation of each enantiomer and the enantiomeric purity of a drug, are important tasks in drug development [1–5]. As a consequence, chiral separation methods are subjects of growing interest in the pharmaceutical industry [6]. Regulations focus on the enantiomeric purity analysis of not only the active ingredient or finished form, but also the chiral intermediates, in order to ensure the robustness of the synthetic process.

HPLC is one of the most frequently used separation techniques for chiral drugs [7], nevertheless the combined advantages of speed, efficient separations and environmental friendliness have made supercritical fluid chromatography (SFC) the preferred instrumental approach, for enantiomeric analysis, in many laboratories [8–17].

Among the numerous chiral stationary phases (CSPs) commercially available, those based on the phenylcarbamate derivatives of polysaccharides have been the most widely employed ones. They have shown a broad applicability not only in HPLC but also in SFC, providing excellent enantiomeric separations for a high number of chiral compounds [7,10,16,18–22]. The 3,5-dimethylphenylcarbamates of amylose and cellulose have been extensively studied and are recognized as the most efficient ones [23–27], nevertheless the introduction of a chloro group on the phenyl moieties has shown to produce a positive effect on the chiral recognition [28–31]. This has encouraged the development of new CSPs which have been recently marketed, being necessary its evaluation in chiral SFC.

The (4S-trans)-4-(ethylamino)-4-(N-acetamide)-5,6-dihydro-(6S)-methyl-4H-thieno-[2,3-b]thiopyran-7,7-dioxide is a chiral intermediate in the synthesis of an ophthalmic drug used in the treatment of glaucoma and ocular hypertension. The determination of its enantiomeric purity is an important task to control the enantiomeric purity of the final drug, and as a consequence the development of chiral methods of analysis for this compound, is necessary. To the best of our knowledge, the enantiomeric separation of this compound using SFC has not been reported yet. So, taking into account all the points stated above, the aim of this work was to evaluate four different chiral stationary phases using SFC. The CSPs studied were: Chiralpak AD (tris-3,5-dimethylphenylcarbamate of amylose), Chiralcel OD-H

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* Corresponding author at: Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Dr. Mergelina s/n, 48011 Valladolid, Spain. Tel.: +34 983 423262; fax: +34 983 423013.
E-mail address: ltoribio@qa.uva.es (L. Toribio).

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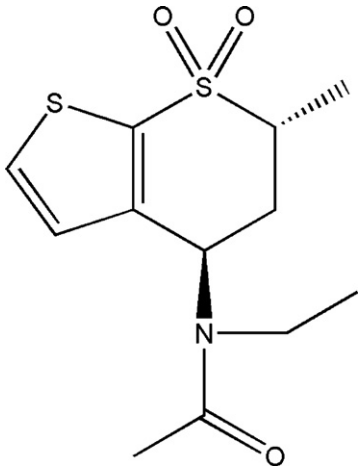

Fig. 1. Structure of the compound studied.

(tris-3,5-dimethylphenylcarbamate of cellulose), Lux Amylose-2 (tris-5-chloro-2-methylphenylcarbamate of amylose) and Lux Cellulose-2 (tris-3-chloro-4-methylphenylcarbamate of cellulose).

2. Experimental

2.1. Reagents

All the organic solvents used were of HPLC grade and obtained from Lab-Scan (Dublin, Ireland). Carbon dioxide was of SFC-grade and purchased from Carburos Metálicos (Barcelona, Spain).

The enantiomers of the compound studied (Fig. 1) were kindly supplied by Gadea Pharmaceutical Group (Boecillo, Valladolid, Spain). The stock solutions of the individual enantiomers were prepared in methanol at the 100 mg/l level and the volume injected was 20 μl.

2.2. Instrumentation

The supercritical-fluid chromatograph used was manufactured by Jasco (Tokyo, Japan). It was equipped with two PU-2080 pumps for supplying the carbon dioxide and the modifier, and an AS-2059-SF autosampler with a variable injection volume. The column was thermostated in a CO-2065 oven and the detector employed was a MD-2015 diode-array model. The pressure was controlled by a BP-2080 pressure regulator.

The chiral columns employed (Fig. 2) were: Chiralpak AD, 250 mm × 4.6 mm, packed with the 3,5-dimethylphenylcarbamate derivative of amylose and coated on a 10-μm silica-gel support, Chiralcel OD-H, 250 mm × 4.6 mm, packed with the 3,5-dimethylphenylcarbamate derivative of cellulose and coated on a 5-μm silica-gel support, both of them obtained from Daicel Chemical Industries, LTD (Deventer, Holland). Lux Amylose-2, 250 mm × 4.6 mm, packed with the 5-chloro-2-methylphenylcarbamate derivative of amylose and Lux Cellulose-2, 250 mm × 4.6 mm, packed with the 3-chloro-4-methylphenylcarbamate derivative of cellulose, both of them coated on a 5-μm silica-gel support and obtained from Phenomenex (Torrance, CA, USA).

3. Results and discussion

3.1. Effect of the organic modifier

The enantiomeric resolution of the compound was studied using organic modifier in order to decrease the retention and to obtain the separation within an acceptable analysis time.

Table 1
Effect of the organic modifier on the retention time (t), selectivity factor (α), resolution (R_s) and column efficiency (N). Chromatographic conditions: 200 bar, 35 °C, 2 ml/min.

Organic modifier (15%)	$t_{(+)}$ (min)	$t_{(-)}$ (min)	α	R_s	N[a]
Lux Cellulose-2					
Methanol	11.03	13.83	1.29	5.30	8388
Ethanol	11.55	15.24	1.37	6.26	7830
2-Propanol	16.52	22.84	1.42	6.47	6004
Chiralcel OD-H					
Methanol	4.45	4.80	1.13	1.36	5703
Ethanol	4.67	5.15	1.17	1.63	4822
2-Propanol	6.64	7.87	1.24	2.73	4021
Lux Amylose-2					
Methanol	6.13	6.65	1.12	1.61	6004
Ethanol	6.96	7.85	1.17	2.65	7910
2-Propanol	8.92	9.93	1.26	1.82	4291
Chiralpak AD					
Methanol	9.82	9.82	1	0	3965
Ethanol	10.51	10.51	1	0	3031
2-Propanol	14.32	14.32	1	0	2897

[a] Measured on the last eluting enantiomer.

The type of organic modifier is one of the factors that most influence the chiral separation. It increases the polarity and the density of the mobile phase and, as a consequence of its adsorption, it changes the three dimensional structure of the stationary phase.

Based on our previous experience [32–34], the effect of three organic modifiers: methanol, ethanol and 2-propanol, was investigated at 200 bar, 35 °C and 2 ml/min. As it can be seen (Table 1), using the Chiralpak AD column, the compound could not be enantiomerically resolved with any of the modifiers assayed, on the contrary the chiral separation was successfully achieved on the other columns using any type of the modifier. In all the columns, retention increased from methanol to 2-propanol as a consequence of the decrease of the modifier polarity.

Considering any column, the selectivity factors (α) were different for each modifier and it was improved when methanol was changed by ethanol and this last by 2-propanol.

Column efficiency (N) was calculated for the last eluting peak using the width at half height. As it can be seen from Table 1, using the Lux Cellulose-2 and Chiralcel OD-H columns, N decreases in the order methanol > ethanol > 2-propanol, as the viscosity of the organic modifier increased. The increase of the modifier viscosity slows the solute diffusion in the mobile phase and contributes to the band broadening which causes a decrease in column efficiency. Nevertheless using the Lux Amylose-2 column the highest values of N were obtained with ethanol, not with methanol. This fact has also been described by other authors [14].

As far as enantioresolution is concerned, it increased in the order methanol < ethanol < 2-propanol, except in the case of using the Lux Amylose-2 column where the highest enantioresolution was obtained with ethanol. This could be explained by the higher column efficiencies obtained with this organic modifier.

It should be noted that the elution order was affected neither by the type of modifier nor by the column used, and always the first eluted peak was the (+)-enantiomer.

In all the cases, with the increase of the percentage of organic modifier in the mobile phase (Table 2) retention and resolution decreased, being this effect more pronounced on the Lux Cellulose-2 column, which presented the highest retention for this compound. As a general rule, using this column, is necessary to increase twice the percentage of organic modifier to obtain a simi-

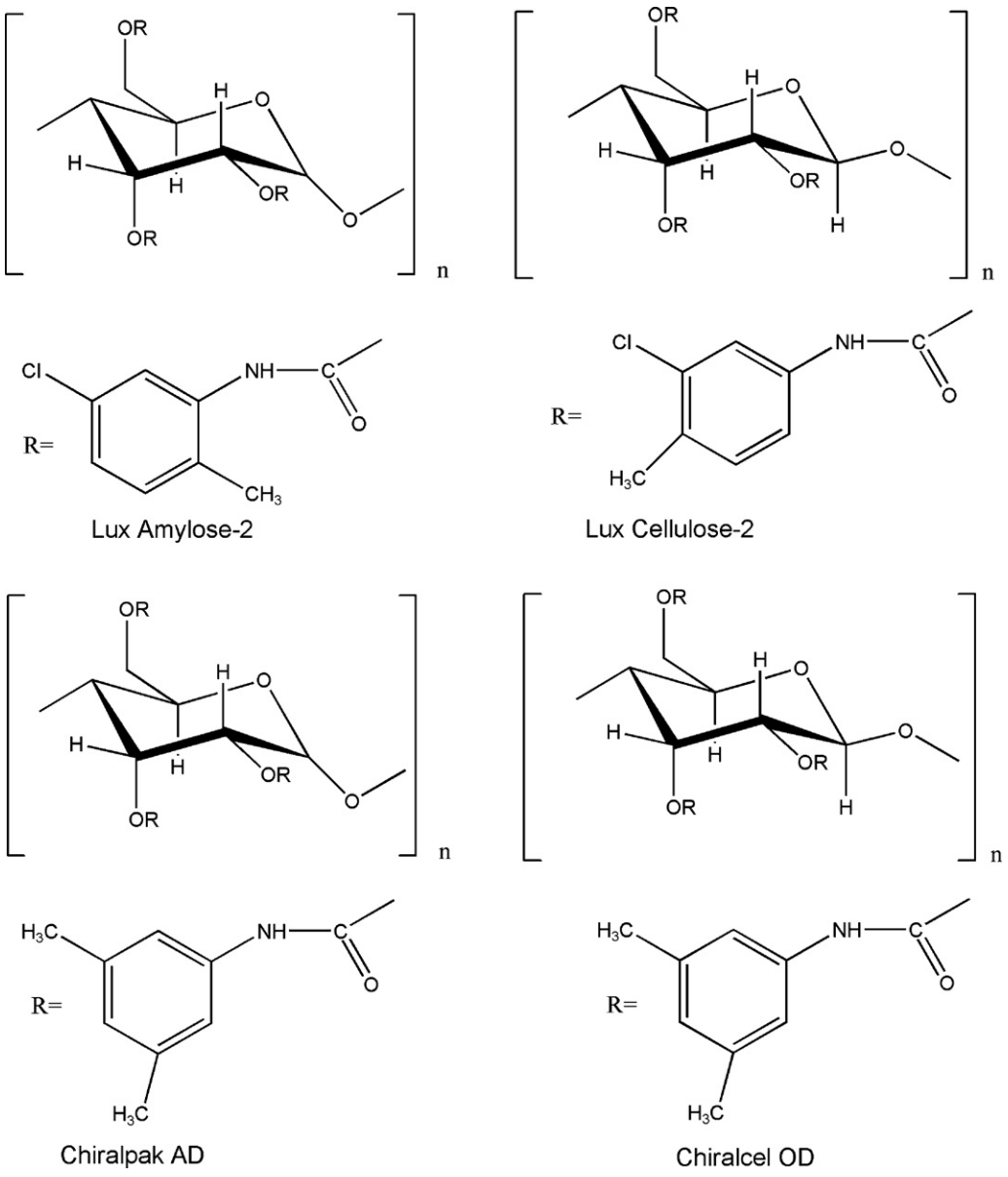

Fig. 2. Chiral stationary phases employed.

Table 2
Effect of the organic modifier percentage on the retention and resolution. Chromatographic conditions: 200 bar, 35 °C, 2 ml/min.

Organic modifier	Percentage (%)	Lux Cellulose-2			Lux Amylose-2			Chiralcel OD-H		
		$t_{(+)}$ (min)	$t_{(-)}$ (min)	R_s	$t_{(+)}$ (min)	$t_{(-)}$ (min)	R_s	$t_{(+)}$ (min)	$t_{(-)}$ (min)	R_s
Methanol	10	19.37	25.2	6.40	9.40	10.33	1.88	6.37	7.00	1.60
	15	11.01	13.83	5.30	6.13	6.65	1.61	4.45	4.80	1.36
	20	7.69	9.51	4.90	4.83	5.21	1.49	3.44	3.65	0.97
	30	5.00	5.98	4.21	3.56	3.79	1.20	2.89	3.15	0.45
Ethanol	10	21.08	28.77	7.44	10.57	12.07	2.69	8.13	9.15	2.21
	15	11.55	15.24	6.26	6.96	7.85	2.65	4.67	5.15	1.63
	20	7.72	9.92	5.86	5.11	5.67	2.06	3.93	4.28	1.46
	30	4.83	5.97	4.91	3.52	3.87	1.74	2.48	3.85	1.26
2-Propanol	10	30.60	44.11	7.79	14.71	16.43	1.95	10.41	12.64	3.04
	15	16.52	22.84	6.47	8.92	9.93	1.82	6.64	7.87	2.73
	20	10.37	13.5	6.33	6.32	6.94	1.70	4.81	5.57	2.46
	30	5.00	5.98	4.18	4.00	4.32	1.20	3.45	3.80	1.41

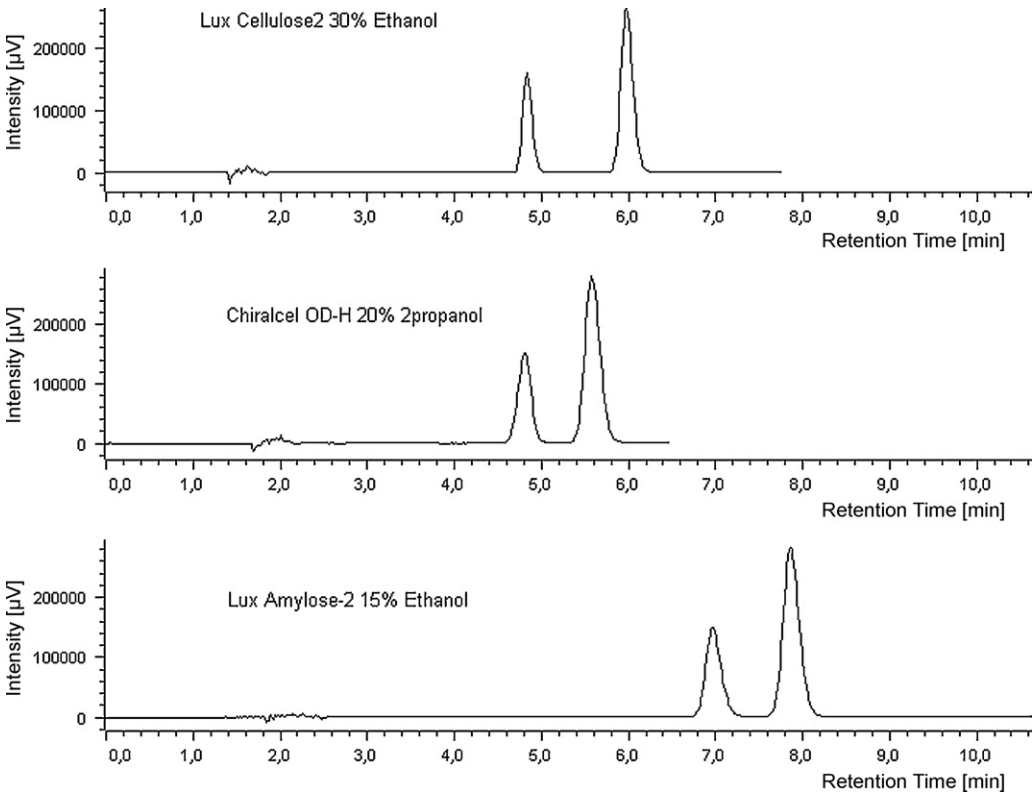

Fig. 3. Chromatograms obtained on the best conditions, using the different chiral columns. Chromatographic conditions: 200 bar, 35 °C, 2 ml/min.

lar retention to the other columns. It should be also noted that using the Lux Cellulose-2 column and 30% of organic modifier the enantioresolution decreased paradoxically, when ethanol was changed by 2-propanol.

The best separations obtained on the three columns studied, in terms of low analysis time and high enantioresolution, are shown in Fig. 3.

3.2. Effect of temperature

Temperature is an important factor in chiral separations. Its study could contribute to a better understanding of the mechanism that controls the chromatographic process, and to improve the enantiomeric separation.

The relationship between the temperature and the selectivity factor can be expressed in terms of the van't Hoff equation:

$$\ln k = -\left(\frac{\Delta H^\circ}{RT}\right) + \left(\frac{\Delta S^\circ}{R}\right) + \ln \Phi,$$

$$\ln \alpha = -\left(\frac{\Delta \Delta H^\circ}{RT}\right) + \left(\frac{\Delta \Delta S^\circ}{R}\right)$$

where T is the absolute temperature, R is the ideal gas constant, Φ is the phase ratio and ΔH° and ΔS° are the enthalpic and entropic changes of the enantiomer–chromatographic system interaction, respectively. From a thermodynamic point of view, retention and selectivity are controlled by an enthalpic contribution which decreases with temperature and an entropic contribution which is independent of the temperature. Although using SFC this independence does not occur in many cases.

If $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ are independent of the temperature, $\ln \alpha$ vs. $1/T$ should be linear, and the isoelution temperature, T_{iso} (where the enthalpic and entropic contributions to the selectivity are balanced and coelution of enantiomers occurs) can be calculated as the ratio between the molar differential enthalpy ($\Delta\Delta H^\circ$) and entropy ($\Delta\Delta S^\circ$) of the enantioselective interaction. The isoelution temperature may, or may not, be in the practical operating range of the chromatographic system, but it is clear that temperature determines the column selectivity for closely eluting peaks. Above the T_{iso} the elution order will be reversed and the selectivity will increase as the temperature increases.

In this work the effect of temperature was studied between 25 and 40 °C, at 200 bar, 2 ml/min and using the percentage of modifier that provided the best separation on each column, because these will be the percentages used to carry out the enantiomeric separation.

In all the cases, retention and selectivity decreased as temperature increased. Linear van't Hoff plots for selectivity factors were obtained using the Lux Cellulose-2 column with methanol or ethanol, and the Lux Amylose-2 column with any of the modifiers assayed (Fig. 4). On the contrary, employing the Chiralcel OD-H column, the plots were nonlinear which could be attributed to a change in the separation mechanism due to temperature (Fig. 5). This variation could be caused by a modification in the conformation of the stationary phase as a consequence of the modifier adsorption [35].

The thermodynamic parameters, estimated from the van't Hoff plots, as well as the correlation coefficients, are shown in Table 3. As it can be seen the values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ were always negative. This means that the separation is enthalpy driven, nevertheless the values of $\Delta\Delta H^\circ$ are small which could indicate that the interaction difference between the two enantiomers is small and so the temperature has slight influence on the separation. In the case of the Lux Cellulose-2 column the values of $\Delta\Delta H^\circ$ were similar for both modifiers, nevertheless using the Lux Amylose-2 column, the highest values were obtained with ethanol. Considering the isoelution temperature (T_{iso}), it can be observed that in all the cases it was above the range of temperatures assayed, and taking into

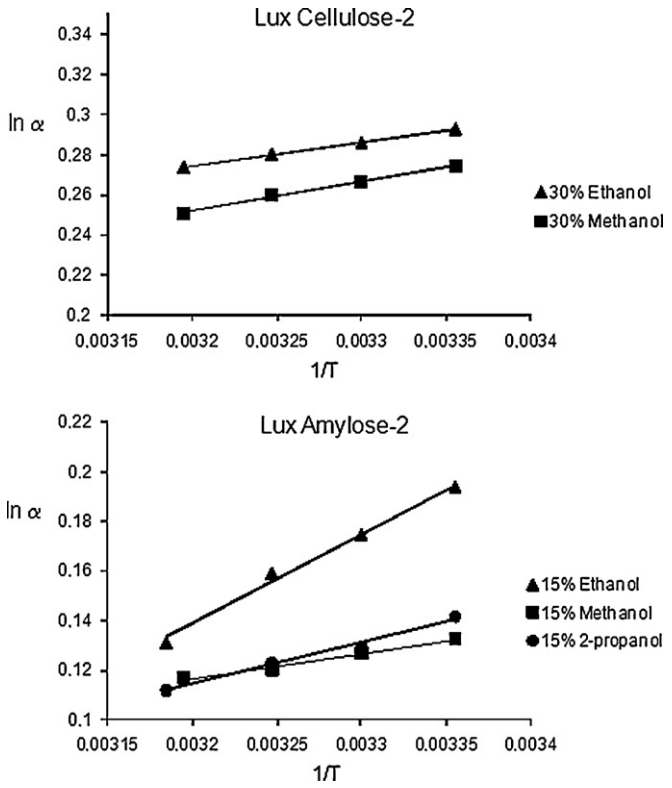

Fig. 4. van't Hoff plots. Chromatographic conditions: 200 bar, 2 ml/min.

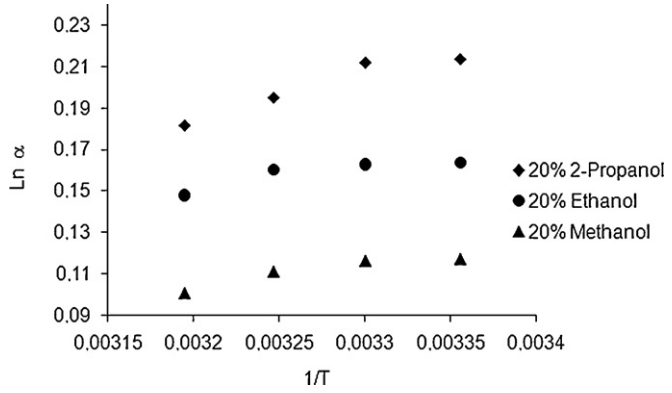

Fig. 5. van't Hoff plots obtained for the Chiralcel OD-H column. Chromatographic conditions: 200 bar, 2 ml/min.

account that the maximum temperature recommended for these columns is 50 °C, the isoelution temperature cannot be reached for these compounds. On the other hand, it should be noted that all the values presented here are estimations obtained from the linear correlations, and they cannot be considered as exact values.

Table 3
Thermodynamic parameters. Chromatographic conditions: 200 bar, 35 °C, 2 ml/min.

Modifier	R^2	$\Delta\Delta H^\circ$ (cal mol^{-1})	$\Delta\Delta S^\circ$ (cal mol^{-1} °K^{-1})	T_{iso} (°C)
Lux Cellulose-2				
30% methanol	0.993	−292.1	−0.43	399.9
30% ethanol	0.999	−235.5	−0.21	856.7
Lux Amylose-2				
15% methanol	0.998	−201.5	−0.41	214.5
15% ethanol	0.992	−715.4	−2.00	82.3
15% 2-propanol	0.996	−331.2	−0.83	124.8

3.3. Comparison of the columns

The carbamate group is considered to be the most important adsorbing site for chiral recognition on the phenylcarbamate derivatives of polysaccharides. It can interact with the analyte via hydrogen bonding on the NH or C=O groups and dipole–dipole interactions on the C=O [36]. Nevertheless the chiral recognition abilities of the different polysaccharide derivatives are greatly influenced by the position and kind of substituents on the phenylcarbamate group [28,29].

As it can be seen from Tables 1 and 2 the Lux Cellulose-2 column provided the highest enantioresolutions and retentions, followed by the Lux Amylose-2, while the lowest values were obtained using the Chiralcel OD-H column. It should be noted that both, Lux Cellulose-2 and Lux Amylose-2 columns, possess an electron withdrawing substituent (the chlorine atom) on the phenylcarbamate group which increases the acidic strength of the NH group. This enhances the resolving power for the analytes that could interact with the NH groups of the carbamate moieties. On the contrary the substituents in the Chiralcel OD-H column are methyl groups with electron-donating ability, which decreases the acidic strength of the NH group. In this case, the proportion of free NH groups is lower due to interactions with the carbamate moiety of the neighboring glucose units, and the resolving power for analytes interacting with the NH groups will be diminished [29].

The analyte studied (Fig. 1), possesses sulphone and acetamide groups which can interact via hydrogen bonding with the NH groups of the stationary phase and contribute to the chiral recognition. According to the points stated above, the compound may interact more strongly with the Lux Cellulose-2 and Lux Amylose-2 columns than with the Chiralcel OD-H. This could explain the lower retention and resolution obtained with the last column. On the other hand, the Lux Amylose-2 column showed lower chiral recognition ability, for this compound, than the Lux Cellulose-2, which could be due to the higher steric hindrance of the CH_3 group in the Lux Amylose-2 column, that could prevent or decrease the interaction with the NH group. Other reason could be the differences in the higher order structure between the cellulose and amylose derivatives.

4. Conclusions

The enantiomeric separation of the (4S-trans)-4-(ethylamino)-4-(N-acetamide)-5,6-dihydro-(6S)-methyl-4H-thieno-[2,3-b]thiopyran-7,7-dioxide, was successfully achieved using supercritical fluid chromatography and chiral stationary phases based on the phenylcarbamate derivatives of amylose or cellulose. The best results were obtained with the CSP that contained a chloro substituent on the phenyl group, Lux Amylose-2 and Lux Cellulose-2. This could be explained by the stronger interaction of the analyte with the NH group of the carbamate moiety, due to the presence of the chlorine atom. On the contrary, any sign of separation was achieved on the Chiralpak AD column.

As far as the effect of the organic modifier is concerned, 2-propanol provided the highest retentions and enantioresolutions, except in the case of using the Lux Amylose-2 column, where the best resolutions were obtained with ethanol. In all the cases retention and selectivity decreased as temperature increased and the van't Hoff plots were linear only with the Lux Amylose-2 and Lux Cellulose-2 columns. The values of T_{iso} and $\Delta\Delta H^\circ$ estimated, revealed that the separation was enthalpy driven.

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