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HPLC SEPARATION OF LINEZOLID ENANTIOMERS USING POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES

A. Bielejewska[1,2,*], *K. Duszczyk*[1], and *J. Żukowski*[3]

[1]Institute of Physical Chemistry PAS, Kasprzaka 44/52, 01-224 Warsaw, Poland
[2]Pharmaceutical Research Institute, Rydygiera 8, 02-187 Warsaw, Poland
[3]GlaxoSmithKline, Analyt Chem, Harlow CM19 5AD, Essex, England

SUMMARY

Separation of the enantiomers of linezolid has been compared on derivatized cellulose and amylose chiral stationary phases (Chiracel OD and Chiralpak AD) with mixtures of hexane with 1-propanol, 2-propanol, or ethanol as mobile phases. It was found that use of a small amount of water as mobile phase additive can improve or prevent enantioseparation, depending on the column. The order of elution of the linezolid enantiomers was different on the Chiracel OD and Chiralpak AD columns, and baseline resolution was achieved on the Chiralpak AD column only. Reversal of the order of elution of the enantiomers on changing from the propanols to ethanol was observed on both columns. The effect of temperature (in the narrow range 15–35°C) on retention and enantioselectivity was studied for both columns and a variety of mobile phases. The standard enthalpy ($\Delta H°$) and entropy ($\Delta S°$) changes for solute transfer between the mobile and stationary phases were also estimated.

INTRODUCTION

Linezolid, the structural formula of which is shown in Fig. 1, is a member of a new class of synthetic antibacterial drugs called oxazolidinone derivatives that are chemically unrelated to currently used antibiotics. The FDA has licensed linezolid since April 1, 2000. It is active against gram-positive bacteria by inhibiting the formation of ribosomal initiation complex [1]. Methods for monitoring linezolid in serum and urine by HPLC have already been reported [2].

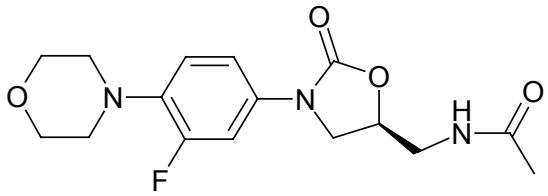

Fig. 1

The chemical structure of linezolid

Linezolid is a chiral compound and only its *S* enantiomer is administered as a therapeutic. In the development of a chiral HPLC method, it is usually desirable to use a chiral stationary phase (CSP) for direct separation of enantiomers, because of the simplicity of operation. Various types of CSP are available; among these cellulose and amylose-based CSP have been proved to be quite versatile [3,4]. The order of elution of enantiomers is of crucial importance in monitoring enantiomeric purity [5] – the minor enantiomer should be eluted before the major isomer, to avoid possible interference caused by tailing of the major enantiomer. This is especially true when the separation is relatively poor. Recent work [6,7] has shown that Chiracel OD and Chiralpak AD columns afford elution in the opposite order for some pairs of enantiomers. Reversal of the order of elution of some enantiomeric pairs on changing the mobile phase modifier from 2-propanol to ethanol was also observed [8].

The presence of a small amount of water in the mobile phase for some normal-phase separations on Chiracel OD columns has been found to be critical and indispensable for obtaining chiral separation of some compounds [9,10].

The two enantiomers of linezolid are well resolved on a Chiralpak AD column [11]. In this work we have compared separations obtained on Chiralpak AD and Chiracel OD columns. On both columns the effect of the alcohol used as mobile phase modifier and of addition of a small amount of water to the mobile phase has been investigated. To optimise the analytical procedure and to gain insight into the mechanisms of separation, the effect of temperature (in the narrow range 15–35°C) on retention and enantioselectivity was also studied for both columns using a variety of mobile phases.

EXPERIMENTAL

Materials

The *S* and *R* enantiomers of linezolid were synthesized and supplied by Adamed Company (Czosnów, Poland); solutions of the enantiomers of appropriate concentration were prepared in ethanol. The *n*-hexane was HPLC grade and the alcohols were of analytical reagent grade. All other reagents were of analytical grade, at least, and were used as received.

Apparatus and Procedures

Chromatography was performed with a Waters (Vienna, Austria) model 590 pump and model 490 UV–visible detector, a Rheodyne injector with 1-μL loop and 0.46 cm i.d. × 25 cm Chiralpak AD or Chiracel OD columns. The mobile phases used for enantioseparations were mixtures of hexane with 1-propanol (NPA), 2-propanol (IPA), or ethanol (EtOH); 0.2% water was added to some mobile phases. The flow rate was 1.2 and 0.8 mL min^{-1} for Chiralpak AD and Chiracel OD, respectively. Linezolid was detected at its absorption maximum at 260 nm. All chromatographic measurements, except for thermodynamic studies, were performed at ambient

Table I

Chromatographic data for the enantiomers of linezolid on Chiralpak AD and Chiracel OD columns

Mobile phase	Chiralpak AD column				Chiracel OD column			
	k_1	k_2	α	First enantiomer	k_1	k_2	α	First enantiomer
30%IPA	1.6	2.4	1.44	S	–	–	–	–
30%IPA + H_2O	1.7	2.2	1.27	S	–	–	–	–
20%IPA	3.8	5.0	1.30	S	9.0	9.3	1.03	R
20%IPA + H_2O	5.8	5.8	1.00	S*	8.1	8.5	1.06	R
20%NPA	3.4	4.1	1.21	S	7.5	7.5	1.00	–
20%NPA + H_2O	3.6	4.0	1.09	S	6.1	6.1	1.00	–
15%EtOH	12.7	14.6	1.16	R	–	–	–	–
15%EtOH + H_2O	11.8	12.4	1.05	R	–	–	–	–
10%EtOH	–	–	–	–	12.8	13.3	1.04	S
10%EtOH + H_2O	–	–	–	–	13.0	13.9	1.06	S

Chromatographic conditions: temperature 20°C, flow rate 0.8 mL min^{-1} on Chiracel OD and 1.2 mL min^{-1} on Chiralpak AD

*Separation of the enantiomers was achieved at a temperature >25°C

temperature in an air-conditioned room (20°C). For thermodynamic studies the temperature was controlled using a Waters model TCM oven.

The order of elution of the linezolid enantiomers was determined by injection of single enantiomers.

RESULTS AND DISCUSSION

Retention and Selectivity

Retention and enantioselectivity data for the enantiomers of linezolid on the Chiralpak AD and Chiracel OD columns with a variety of mobile phases are listed in Table I.

Enantioselectivity is much better and separation times shorter on the Chiralpak AD column than on the Chiracel OD column. On changing the alcohol modifier from propanols to ethanol, reversal of the order of elution of the enantiomers was observed on the Chiralpak AD column [11] (Fig. 2). For this column addition of 0.2% of water to the mobile phase made the separation worse, irrespective of the alcohol used in the mobile phase.

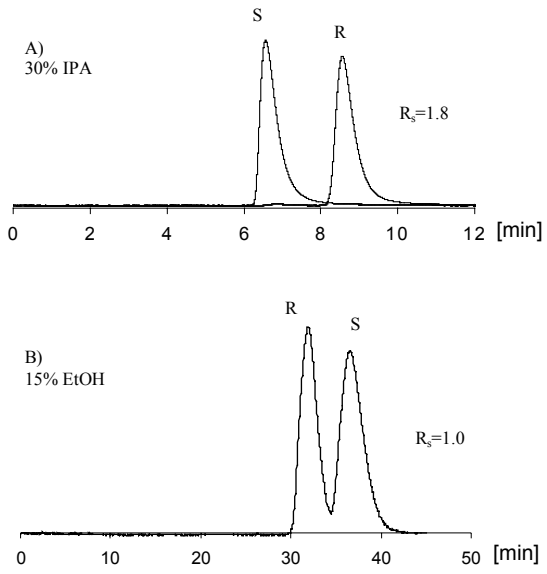

Fig. 2

Chromatograms of linezolid enantiomers on Chiralpak AD column with (A) IPA–hexane, 30:70 (*v*/*v*) and (B) EtOH–hexane 15:85 (*v*/*v*) as mobile phase

Although similar groups used for derivatization of both packings (Chiralpak AD is an amylose tris(3,5-dimethylphenylcarbamate) derivative and Chiracel OD is a cellulose tris(3,5-dimethylphenylcarbamate) derivative) opposite orders of elution are obtained from the columns, which suggests that the structure of the chiral support plays the main role in the recognition process.

On Chiralpak AD the *R* enantiomer was eluted after the *S* enantiomer with IPA–hexane mobile phases whereas on Chiracel OD the *R* enantiomer eluted first. Unfortunately the separation obtained on the Chiracel OD column was not satisfactory, and retention was longer than on the Chiralpak AD column. On changing from IPA to EtOH the order of elution was reversed but separation still remained poor. Addition of small amounts of water to mobile phases containing IPA or EtOH improved the separation of enantiomers slightly, but not enough to achieve baseline separation.

It has recently been reported [6,7] that Chiracel OD and Chiralpak AD columns had opposite chiral recognition properties. The authors attributed this to the different conformation of the chiral stationary phase. Another interesting phenomenon is the reversal of the order of elution of the enantiomers on changing the alcohol modifier in the mobile phase. The alcohols compete with the solute for hydrogen-bonding sites on the CSP, and this competition affects both retention and selectivity. Koller et al. [12] concluded that the polarity of the mobile phases may not be the key to their elution power. This is confirmed by results for the Chiralpak AD column – EtOH is more polar than IPA but at the same molar concentration (2.6 M) retention is shorter with IPA than with EtOH. The enantioselectivity and retention changes suggest that the alcohols affect the steric environment of the chiral cavities or channels of the stationary phase.

Temperature Study

The temperature can affect both enantioselectivity and retention. Study of the effect of temperature can also provide insight into separation mechanisms and enable the design of optimum conditions for analysis. In a chromatographic system with a CSP the retention factor of the solute depends on the partitioning process between the mobile and stationary phases. The van't Hoff expression for such a chromatographic system is [13]:

$$\ln k = \frac{-\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \varphi \qquad (1)$$

where k is the retention factor, $\Delta H°$ and $\Delta S°$ represent the standard enthalpy and entropy changes for transfer of the solute between the mobile and stationary phases, R is the gas constant, T is the absolute temperature, and φ is the volume phase ratio of the stationary to the mobile phase.

Furthermore:

$$\ln \alpha = -\frac{\Delta\Delta H^o}{RT} + \frac{\Delta\Delta S^o}{R} \tag{2}$$

If $\Delta H°$, $\Delta S°$, and φ are independent of temperature, plots of ln k and ln α against $1/T$ should be linear. The $\Delta\Delta H$ and $\Delta\Delta S$ values obtained from the plot can be used to obtain $\Delta\Delta G$ according to the equation:

$$\Delta\Delta G = \Delta\Delta H - T\Delta\Delta S \tag{3}$$

The effect of temperature in the narrow range 15–35°C on the retention of both enantiomers of linezolid was studied using the Chiralpak AD and Chiracel OD columns with mobile phases of different composition. The retention time for all the mobile phases investigated decreased with increasing column temperature, and the van't Hoff plots were indicative of linear behaviour in this temperature range (correlation coefficients were between 0.90 and 0.99). Enantioselectivity decreases with increasing temperature for the systems studied, except for Chiralpak AD with IPA when water was added, for which enantioselectivity increased with increasing temperature (Fig. 3). Figure 3 shows the van't Hoff plots for the Chiralpak AD column with IPA and IPA containing 0.2% water as mobile phases. It is apparent from the figure that addition of water affects transfer of the S enantiomer from the mobile phase to the stationary phase much more than it affects the R enantiomer.

According to eq. (2) the slope of the plot of ln k against $1/T$ provides information about enthalpy differences, whereas the intercept is related to $\Delta S/R$ + ln φ. The results for the R and S enantiomers of linezolid in all systems studied are presented in Table II.

As is apparent from Table II, changing the mobile phase affects both enthalpy and entropy changes for transfer of the enantiomers between the mobile and stationary phases. The enthalpy change for transfer from the mobile phase to the stationary phase is usually lower for the second eluted enantiomer, i.e. interaction between the stationary phase and the second enantiomer is more enthalpy-favourable. Only for Chiralpak AD with IPA and 0.2% water is ΔH lower for the first eluted enantiomer (S) than the second. For this isomer the unfavourable effect of the entropy of interaction

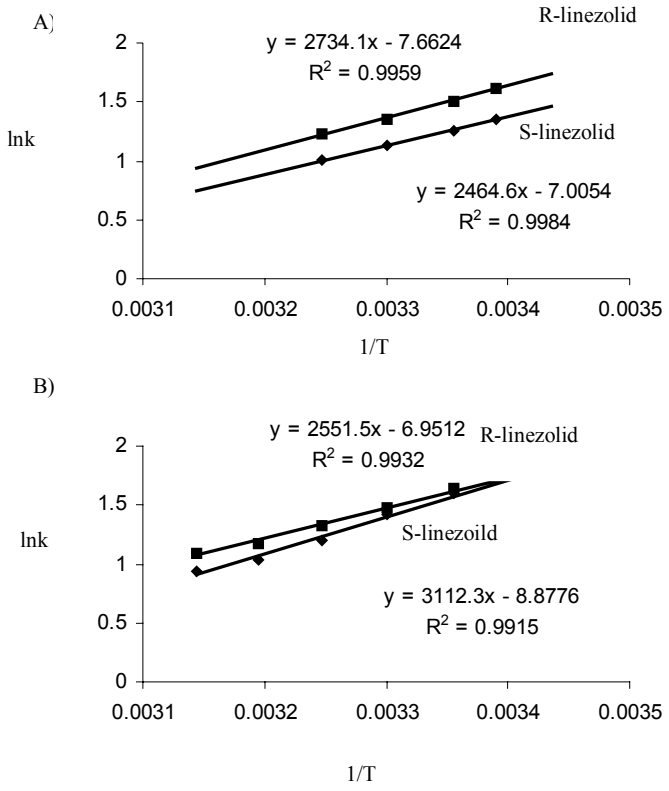

Fig. 3

Effect of addition of a small amount of water on the van't Hoff plots of linezolid enantiomers for the Chiralpak AD column: (A) mobile phase IPA–hexane, 30:70 (*v*/*v*); (b) mobile phase IPA–hexane, 30:70 (*v*/*v*) containing 0.2% water

between the stationary phase and compounds reduces the retention time. Unfortunately, it is not possible to estimate the entropy changes without knowing the phase ratio.

Comparison of the retention times of the enantiomers and the values of the thermodynamic functions reveals that the enthalpy changes for transfer between the mobile and stationary phase for IPA are more favourable but the retention is much shorter than for EtOH. This means that entropy changes connected with disorder in the stationary phase are more favourable for ethanol and this results in longer retention. These results confirm that changes occurring in the conformation of the stationary phase are because of changes of mobile phase composition and in this manner achiral organic solvents may contribute substantially to chiral recognition.

Table II

Thermodynamic data for linezolid enantiomers determined with different mobile phases on the Chiralpak AD and Chiracel OD columns

Mobile phase	Enantiomer	First eluted	ΔH (kJ mol^{-1})	$\Delta S/R + \ln \varphi$	$\Delta\Delta H$ (kJ mol^{-1})	$T\Delta\Delta S$ (kJ mol^{-1})	$\Delta\Delta G$ (kJ mol^{-1})
Chiralpak AD							
20% IPA	*R* *S*	*S*	−22.7 ± 1.0 −20.5 ± 0.6	−7.7 ± 0.4 −7.0 ± 0.2	−2.2 ± 0.4	−1.6 ± 0.4	−0.6
20% IPA + H_2O	*R* *S*	*S*	−21.2 ± 0.9 −25.2 ± 1.2	−7.0 ± 0.4 −8.9 ± 0.5	4.4 ± 0.8	4.4 ± 0.8	0
15% EtOH	*R* *S*	*R*	−13.2 ± 0.8 −15.5 ± 0.5	−2.7 ± 0.3 −3.5 ± 0.2	−2.3 ± 0.3	−1.9 ± 0.3	−0.4
15% EtOH + H_2O	*R* *S*	*R*	−15.4 ± 3.5 −19.5 ± 4.3	−3.7 ± 1.4 −5.3 ± 1.7	−4.1 ± 0.8	−3.9 ± 0.8	−0.2
Chiracel OD							
20% IPA	*R* *S*	*R*	−15.0 ± 2.0 −18.4 ± 0.4	−3.8 ± 0.8 −5.2 ± 0.2	−3.4 ± 2.4	−3.2 ± 2.3	−0.2
20% IPA + H_2O	*R* *S*	*R*	−14.4 ± 0.8 −17.1 ± 0.5	−3.7 ± 0.3 −4.8 ± 0.2	−2.8 ± 0.9	−2.6 ± 0.9	−0.2
10% EtOH	*R* *S*	*S*	−12.6 ± 1.0 −12.7 ± 0.8	−2.6 ± 0.3 −2.6 ± 0.4	0 ± 0.2	0.1 ± 0.2	≈0
10% EtOH + H_2O	*R* *S*	*S*	−17.3 ± 1.0 −16.8 ± 1.0	−4.3 ± 0.4 −4.1 ± 0.4	−0.5 ± 0.1	−0.4 ± 0.1	−0.1

T = 293 K

ΔH and ΔS are the enthalpy and entropy changes for transfer of the solute between the mobile and stationary phase

$\Delta\Delta H$, $\Delta\Delta S$, $\Delta\Delta G$ are the enthalpy ,entropy, and free energy differences between enantiomers

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