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Influence of temperature on chiral high-performance liquid chromatographic separations of oxazepam and Prominal on chemically bonded β-cyclodextrin as stationary phase

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ABSTRACT

β-Cyclodextrin, chemically bonded to silica, was used as a chiral stationary phase for the HPLC separation of two chiral pharmaceuticals, oxazepam and Prominal. The influence of temperature on the separation of these two compounds was studied in detail. A decrease in temperature caused an increase in the retention for both compounds. Chiral separation of oxazepam was optimized by decreasing the temperature whereas that of Prominal was improved by increasing the temperature. Thermodynamic data reveal that the separation of Prominal is an interesting case of entropy-controlled separation whereas for oxazepam the expected enthalpy-controlled separation was observed. The enantiomerization of oxazepam that occurs during its separation can be suppressed by using low temperatures.

INTRODUCTION

The high specificity of cyclodextrins (CDs) for the discrimination of enantiomers was first observed by Cramer in 1952 [1]. Owing to this property and their availability in large amounts, CDs are now widely used as chiral selectors for the separation of enantiomers with various chromatographic techniques.

In gas chromatography (GC), for example, cyclodextrin derivatives have been introduced as versatile enantioselective stationary phases by different groups [2]. Schurig and Nowotny [3] described the use of peralkylated cyclodextrins dissolved in a liquid stationary phase (polysiloxane) whereas König *et al.* [4] and Armstrong *et al.* [5] applied peralkylated/acylated cyclodextrin derivatives as undiluted liquids. In high-performance liquid chromatography (HPLC), cyclodextrins have been mainly used in their underivatized form either as chiral additives to the mobile phase [6,7] or chemically bonded to the stationary phase. Armstrong and co-workers [8–10] and many others have demonstrated the broad enantioselectivity of chemically bonded cyclodextrin phases for numerous HPLC separations of enantiomers.

Whereas in GC temperature is the main parameter to be varied for optimizing separations, in HPLC little attention has been focused on this parameter. This is mainly due to the limitations imposed by the low boiling points of the solvents commonly used in normal-phase HPLC. Even with reversed-phase systems, which are operated with aqueous mobile phases, modification of the mobile phase composition has been preferred to temperature changes.

So far, only a few workers have studied the effect of temperature on the resolution of enantiomers by HPLC. The different chiral stationary phases used include protein phases [11–13], Pirkle phase [14] and some others [15–17]. It was

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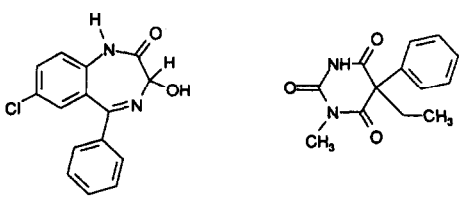
often found that a decrease in temperature causes an increase in enantioselectivity. This is consistent with thermodynamics because HPLC separations are usually enthalpy controlled. Recently, Pirkle and Murray [18] described an interesting study using a brush-type stationary phase where a progressive decrease in temperature initially lowered the enantioselectivity, then inverted the elution order of the enantiomers and finally caused the enantioselectivity to increase. In GC, the influence of temperature on the elution order has been well documented. A change in elution order was predicted by Koppenhöfer and Bayer [19] based on thermodynamic considerations and later observed experimentally by Schurig *et al.* [20] and Watabe *et al.* [21].

β-CD, which consists of seven D-glucose molecules forming a ring structure, is the most widely applied CD. The cyclic arrangement of β-CD gives rise to a chiral cavity in which molecules of different structures can be included [22]. Enantiomers, for instance, can form diastereomeric inclusion complexes with β-CD.

It is known from binding studies that an increase in temperature diminishes the extent of complexation between the guest molecule and β-CD [23]. Therefore, it is expected that separations on chemically bonded β-CD at different temperatures should give different results. In fact, chiral separations on such a stationary phase have been improved by decreasing the temperature [24,25].

This paper describes the influence of temperature on the chiral HPLC separation of two pharmaceuticals using chemically bonded β-CD (ChiraDex) as the stationary phase: oxazepam, a benzodiazepinone, and Prominal, a barbiturate (Fig. 1).



Oxazepam Prominal

Fig. 1. Structures of oxazepam and Prominal.

EXPERIMENTAL

All solvents used for the preparation of the mobile phases were of LiChrosolv grade from Merck (Darmstadt, Germany). The HPLC column was LiChroCART ChiraDex, 5 μm (β-CD chemically bonded to silica) (250 × 4 mm I.D.) from Merck. The instrumentation consisted of an HPLC system from Merck–Hitachi with an L-6200 intelligent pump, an L-4000 UV detector, a D-2500 integrator and a Rheodyne injection valve. Temperature experiments were performed with an Inlabo F 10 UC thermostat.

RESULTS AND DISCUSSION

The enantiomers of oxazepam and Prominal (Fig. 1) were chromatographed at different column temperatures from 5 to 40°C using ChiraDex, chemically bonded β-CD, as the stationary phase. The chromatograms are shown in Figs. 2 and 3. The capacity factors (k') and separation factors ($\alpha = k'_2/k'_1$) obtained are given in Table I. As expected, the retention times of both compounds increase with decrease in column temperature. Further, van't Hoff plots (plots of ln k' against the reciprocal of absolute temperature) show linear behaviour (Figs. 4 and 5). This usually occurs when there is no change in the retention mechanism as a function of temperature. Whereas the retention behaviour is very similar for both compounds, the chiral discrimination behaviour, described by ln α as a function of the reciprocal of absolute temperature ($1/T$), is very different for oxazepam and Prominal (Figs. 6 and 7). An increase in temperature results in smaller α values for oxazepam and larger α values for Prominal. Obviously the change in temperature influences differently the interaction of enantiomers with the stationary phase. This may be better understood if one considers the thermodynamics involved in the separation.

The different interaction of two enantiomers (R and S) leading to chiral discrimination can be expressed as the difference in the free energy

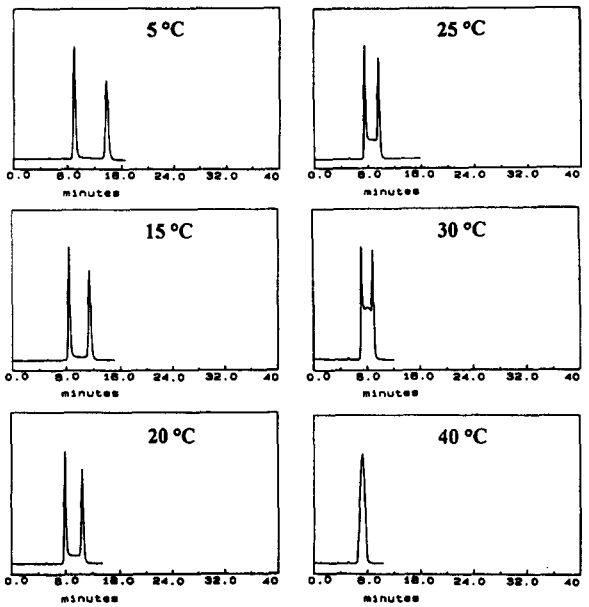


Fig. 2. Chromatograms of oxazepam separations at different temperatures. Column, ChiraDex; mobile phase, methanol–0.01 *M* NaH_2PO_4 (pH 2.8) (40:60, v/v); flow-rate, 0.5 ml/min; detection, UV at 220 nm.

$-\Delta_{R,S}\Delta G^0$ calculated from the separation factor α:

$$-\Delta_{R,S}\Delta G^0 = RT \ln \alpha,\ \alpha = k'_R/k'_S \text{ with } k'_R > k'_S \,. \quad (1)$$

The combination of eqn. 1 with the Gibbs–Helmholz relationship:

$$-\Delta_{R,S}\Delta G^0 = -\Delta_{R,S}\Delta H^0 + T\,\Delta_{R,S}\Delta S^0 \quad (2)$$

gives

$$\ln \alpha = -\Delta_{R,S}\Delta H^0/R \cdot 1/T + \Delta_{R,S}\Delta S^0/R \quad (3)$$

According to eqn. 3, the enthalpy and entropy differences for the interaction of the two enantiomers with the stationary phase can be obtained by plotting ln α *versus* $1/T$. The slope of these plots represents $-\Delta_{R,S}\Delta H^0/R$ and the intercept is related to $\Delta_{R,S}\Delta S^0/R$. Further, as follows from the Gibbs–Helmholz relationship (eqn. 2), there exists a certain temperature T_{iso} (isoenantioselective temperature) where $-\Delta_{R,S}\Delta G^0 = 0$ owing to enthalpy–entropy compensation:

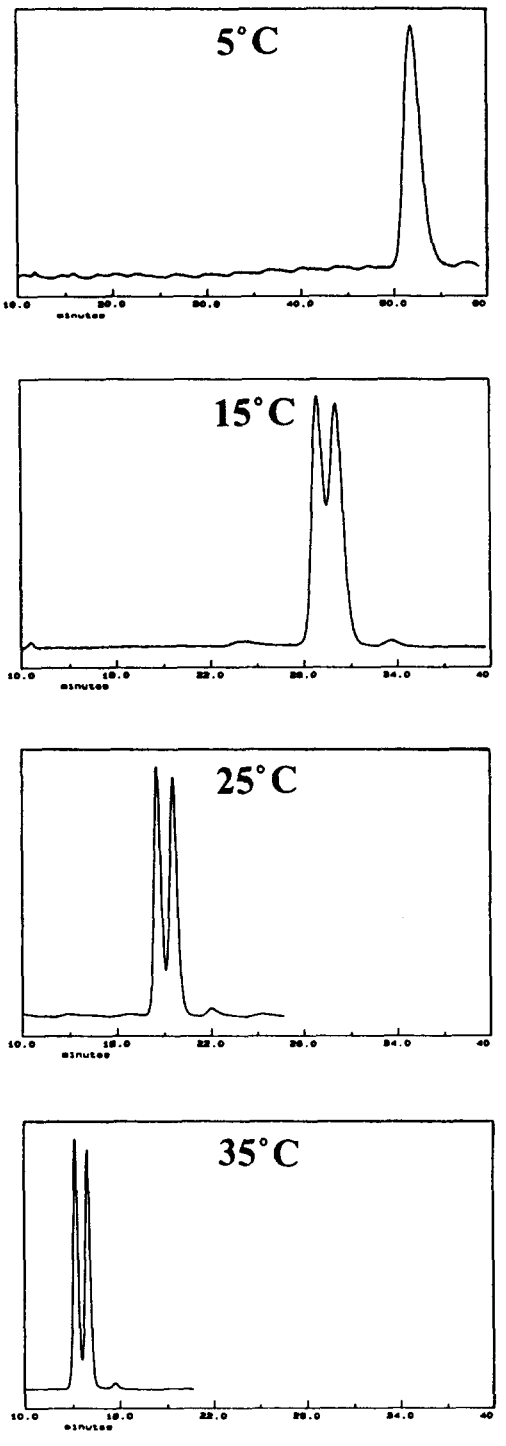


Fig. 3. Chromatograms of Prominal separations at different temperatures. Column, ChiraDex; mobile phase, methanol–water (50:50, v/v); flow-rate, 0.5 ml/min; detection, UV at 220 nm.

TABLE I

VALUES OF k' AND α FOR THE ENANTIOMERS OF OXAZEPAM AND PROMINAL

Chromatographic conditions: column, ChiraDex; flow-rate, 0.5 ml/min; detection, UV at 220 nm; mobile phase, for oxazepam methanol–0.01 *M* NaH_2PO_4 (pH 2.8) (40:60, v/v) and for Prominal methanol–water (50:50, v/v).

Temperature (°C)	Oxazepam			Prominal		
	k'_1	k'_2	α	k'_1	k'_2	α
5	0.85	1.69	1.994	4.54	4.54	1.000
10	0.71	1.41	1.979	3.55	3.55	1.000
15	0.60	1.18	1.962	2.74	2.87	1.048
20	0.51	0.99	1.945	2.15	2.30	1.067
25	0.43	0.83	1.922	1.68	1.82	1.082
30	0.37	0.70	1.896	1.31	1.43	1.091
35	–	–	–	1.04	1.14	1.100
40	0.38	0.38	1.000	0.83	0.92	1.106

$$T_{iso} = \Delta_{R,S}\Delta H^0/\Delta_{R,S}\Delta S^0 . \quad (4)$$

At T_{iso} no separation of enantiomers will occur. Koppenhöfer and Bayer [19] predicted that there will be an inversion of elution order because below T_{iso} the separation of enantiomers is enthalpy controlled whereas above T_{iso} the separation is entropy controlled.

Thermodynamic data for the chiral separations of oxazepam and Prominal are given in Table II. Interestingly, the $\Delta_{R,S}\Delta H^0$ values for the two pharmaceuticals have opposite signs, being negative for oxazepam but positive for Prominal,

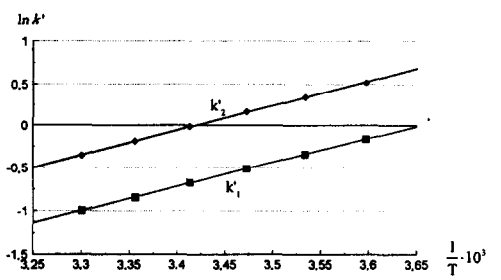


Fig. 4. Van't Hoff plots for the chiral separation of oxazepam. Column, ChiraDex; mobile phase, methanol–0.01 *M* NaH_2PO_4 (pH 2.8) (40:60, v/v); flow-rate, 0.5 ml/min; detection, UV at 220 nm.

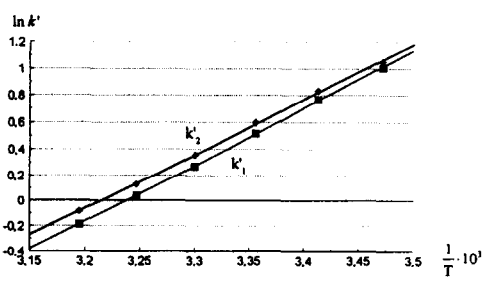


Fig. 5. Van't Hoff plots for the chiral separation of Prominal. Column, ChiraDex; mobile phase, methanol–water (50:50, v/v); flow-rate, 0.5 ml/min; detection, UV at 220 nm.

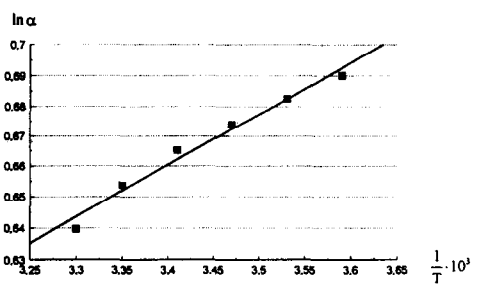


Fig. 6. Ln α *versus* $1/T$ for the separation of oxazepam.

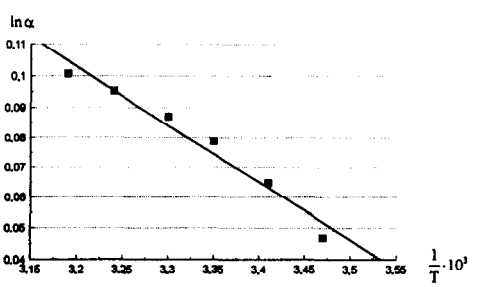


Fig. 7. Ln α *versus* $1/T$ for the separation of Prominal.

TABLE II

THERMODYNAMIC DATA OBTAINED FOR THE SEPARATION OF OXAZEPAM AND PROMINAL

Data calculated from the plots of ln α *versus* $1/T$ (Figs. 6 and 7).

Parameter	Oxazepam	Prominal
r^2	0.979	0.965
$-\Delta_{R,S}\,\Delta H^0/R$	166.85	−188
$\Delta_{R,S}\,\Delta S^0/R$	0.093	0.707
$-\Delta_{R,S}\,\Delta G^0$ (cal/mol)[a]	386.63	45.1
$\Delta_{R,S}\,\Delta H^0$ (cal/mol)	−331.56	373.59
$\Delta_{R,S}\,\Delta S^0$ (cal/mol)[a]	55.06	418.69
T_{iso} (K)		265.9

[a] At $T = 298$ K.

which is unexpected. Usually, as most HPLC separations are enthalpy controlled, one would expect negative values. Obviously, with Prominal a special case was found where the temperature dependence of the separation is entropy controlled. This is clearer by looking at the thermodynamic data in more detail. At $T = 298$ K, $\Delta_{R,S}\,\Delta S^0 = 418.69$ cal/mol (1 cal = 4.186 J) calculated, which is a much higher value than that for oxazepam (55.06 cal/mol) (see Table II). The difference in free energy, $-\Delta_{R,S}\,\Delta G^0 = 45.1$ cal/mol, leads to the chiral discrimination, which is mainly driven by the drastic increase in entropy with increase in temperature. Because of enthalpy–entropy compensation, T_{iso} was calculated and determined for Prominal to be −7.1°C. Below this temperature there should be a reversal of the elution order. Unfortunately, it was not possible to confirm this with practical experiments. The two enantiomers of Prominal are not separated at 5°C (Fig. 3). At this temperature long retention times are observed and strong peak broadening prevents chiral separations because lower separation efficiencies due to peak broadening compensate enantioselectivity.

As can be seen in Fig. 2, the chiral separation of oxazepam at 15°C and above is accompanied by a baseline shift between the elution of the two enantiomers, which indicates "enantiomerization". Consequently, under these conditions it is not possible to determine the chiral purity quantitatively. According to Bürkle *et al.* [26], "enantiomerization" is a process in which the individual antipodes of a racemic mixture of enantiomers undergo inversion of their respective configurations during chromatographic resolution. This effect has been described by several workers in GC [26,27] and HPLC [28]. Enantiomerization of oxazepam increases at higher column temperatures. It is known from other studies [29] that benzodiazepinones such as oxazepam tend to racemize as a result of their chemical structure. The chiral centre of oxazepam is located in a seven-ring structure (Fig. 1) which opens easily, thereby causing a change in configuration. The problem of enantiomerization can, however, be avoided if the chromatography is performed at column temperatures below 15°C.

In conclusion, a systematic variation of column temperature should be considered as one way to improve chiral separations in HPLC. From a practical point of view it is easier to vary column temperatures than mobile phase composition. Both the column and the eluent can be thermostated within a temperature range of, *e.g.*, 0–40°C without problems. The change in temperature may have unexpected effects, *e.g.*, as for Prominal, where an improvement in separation is obtained by increasing the temperature. With oxazepam it was possible to avoid "enantiomerization" at lower temperatures.

REFERENCES

1 F. Cramer, *Angew. Chem.*, 64 (1952) 136.
2 V. Schurig and H.P. Nowotny, *Angew. Chem.*, 102 (1990) 969.
3 V. Schurig and H.P. Nowotny, *J. Chromatogr.*, 441 (1988) 155.
4 W.A. König, S. Lutz, P. Mischnick-Lübbecke, G. Brassat and G. Wenz, *J. Chromatogr.*, 441 (1988) 471.
5 D.W. Armstrong, W.Y. Li and J. Pirka, *Anal. Chem.*, 62 (1990) 217.
6 J. Debowsky, D. Sybilska and J. Jurczak, *Chromatographia*, 16 (1982) 198.
7 Y. Zukowski, D. Sybilska and J. Bojarski, *J. Chromatogr.*, 364 (1986) 225.
8 T.Y. Ward and D.W. Armstrong, in M. Zief and L.Y. Crane (Editors), *Chromatogr. Sci. Ser.*, 40 (1988) 131.
9 D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner, Jr., and S.-Ch. Chang, *Anal. Chem.*, 62 (1990) 1610.

10 S.H. Lee, A. Berthod and D.W. Armstrong, *J. Chromatogr.*, 603 (1992) 83.
11 R.K. Gilpin, S.E. Ehtesham and R.B. Gregory, *Anal. Chem.*, 63 (1991) 2825.
12 S. Jönsson, A. Schön, R. Isaksson, C. Pettersson and G. Pettersson, *Chirality*, 4 (1992) 505.
13 R.C. Williams, J.F. Edwards and M.J. Potter, *J. Liq. Chromatogr.*, 16 (1993) 171.
14 W.H. Pirkle, *J. Chromatogr.*, 558 (1991) 1.
15 J.N. Akanya and D.R. Taylor, *Chromatographia*, 25 (1988) 639.
16 T. Takagi and T. Suzuki, *J. Chromatogr.*, 625 (1992) 163.
17 B. Galli, F. Gasparrini, D. Misiti, M. Pierini, C. Villani and M. Bronzetti, *Chirality*, 4 (1992) 384.
18 W.H. Pirkle and P.G. Murray, *J. High Resolut. Chromatogr.*, 16 (1993) 285.
19 B. Koppenhöfer and E. Bayer, *Chromatographia*, 19 (1984) 123.
20 V. Schurig, J. Ossig and R. Link, *Angew. Chem.*, 101 (1989) 197.
21 K. Watabe, R. Charles and E. Gil-Av, *Angew. Chem.*, 101 (1989) 195.
22 W. Saenger, *Angew. Chem.*, 92 (1980) 343.
23 W.L. Hinze, *Sep. Purif. Methods*, 10 (1981) 159.
24 W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
25 R. Furuta and H. Nakazawa, *J. Chromatogr.*, 625 (1992) 231.
26 W. Bürkle, H. Karfunkel and V. Schurig, *J. Chromatogr.*, 288 (1984) 1.
27 V. Schurig, *J. Chromatogr.*, 441 (1988) 135.
28 H. Fujima, H. Wada, T. Miwa and J. Haginaka, *J. Liq. Chromatogr.*, 16 (1993) 879.
29 Y. Aso, S. Yoshioka, T. Shibazaki and M. Uchiyama, *Chem. Pharm. Bull.*, 36 (1988) 1834.