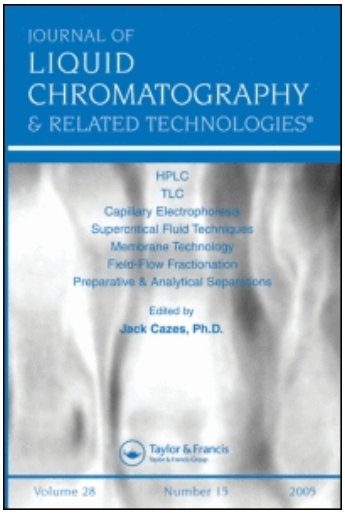

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Interconversion of Oxazepam Enantiomers During HPLC Separation. Determination of Thermodynamic Parameters

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Abstract: Oxazepam undergoes reversible enantiomerization at ambient temperature. Standard solutions of this compound and extracts from tablets (both with c = 0.1 mg/mL) were injected on a chiral HPLC column (Chirobiotic T) at various temperatures (273 K-313 K, increment 5 K). The mobile phase with the composition MeOH/TEA/Hac (100/0, 1/0, 1) and flow rate 1.0 mL/min were used.[1] Both the separation and the interconverson process occurred simultaneously. The various profiles obtained by a UV detector included two peaks (unreacted zone) and a plateau sandwiched between two peaks (reacted zone). These profiles could be explained by on-column enantiomerization. The unreacted molecule zone on the profile was fused with the reacted molecule zone. The computer assisted peak deconvolution procedure (Origin 7.0 section peak fitting) was used for the determination of peak areas in peak clusters. The deconvolution of experimentally obtained chromatograms enabled the resolved and determined areas of each zones. As follows, the peak areas were used

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for calculation of thermodynamic parameters of enantiomerization – apparent rate constants (k_1^{app} and k_{-1}^{app}), Gibbs free energy (ΔG_1^{app}, ΔG_{-1}^{app}), enthalpy (ΔH_1^{app}, ΔH_{-1}^{app}), and entropy (ΔS_1^{app}, ΔS_{-1}^{app}).

Keywords: Oxazepam, Energy barriers, Enantiomerization, Deconvolution

INTRODUCTION

There are many enantiomeric drugs which have a different biological activity for each enantiomer.[2] Benzodiazepines (BDZs) are widely employed in therapy for their anxiolitic, sedative, hypnotic, and myorelaxing properties. From a toxicological point of view, BDZs have been considered quite safe among the drugs acting on the central nervous system. BDZs, in fact, have only few side effects and a low incidence of dependence and tolerance. Pure 3-hydroxy-1,4-benzodiazepines enantiomers are difficult to isolate, they are quickly racemized in aqueous medium and are clinically used in racemic forms.[3]

Enantiomers of some 3-hydroxy-1,4-benzodiazepines, however, undergo inversion of their respective configuration at elevated temperatures.[4–6] This interconversion is an undesired attribute of some chiral drugs. The extent of interconversion of enantiomers depends on the energy barriers to enatiomerization and can be determined by several methods. Interconversion of 3-hydroxy-1,4-benzodiazepines occurs due to the keto-enol tautomerization. This reaction strongly depends on temperature, as well as stationary and mobile phase composition.[7]

It is also known that, when the time scales of interconversion and separation are comparable, typical peak interconversion profiles are obtained in which the signal between the elution of the two enantiomers forms a plateau, rather than returning to the baseline.[8] This is caused by the fact, that during the analysis, a fraction of the enantiomers species is converted into its mirror image. They will, therefore, migrate during the first part of the analysis with the characteristic of one enantiomer, and during the analysis, the remaining time with characteristic of the other.[3]

EXPERIMENTAL

Instrumentation

HPLC separation was performed by using a Merck Hitachi pump (L-6000A), a Rheodyne 7725i injector equipped with 20 μL sample loop, LCT 5100 thermostat (Czech Republic), and UV detector Waters 484. The HPLC column Chirobiotic T, 250 mm × 4 mm I. D., 5 μm is a commercially available product of Astec, USA.

Chromatographic Conditions

Standard solution of oxazepam and extract from tablets (both with c = 0.1 mg/mL) were eluted at various temperatures (273 K-313 K). The mobile phase consisted of MeOH/TEA/Hac (100/0.1/0.1), flow rate 1.0 mL/min. The detection at 230 nm was used. We needed distinct zones and deconvoluated envelopes of peak clusters. We have used the program Origin 7.0 section Peak fitting. For details of this method of deconvolution see the theoretical section.

Preparation of Solution

A standard of oxazepam (Zentiva, Hlohovec) was dissolved in methanol (Merck Germany) (0.1 mg/mL) and it was isolated from the tablet by extraction from the same solvent and the suspension was then filtrated.

THEORY

Enantiomerization constitutes a reversible first order reaction.[9]

$$\mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{B}$$

This arises from the interconversion of a stereogenic element in a particular molecule.[10]

The apparent rate constants for this irreversible approach may be determined using the following equations:

$$k_1^{app} = \frac{1}{t_{R,A}} \ln \frac{c_{A0}}{c_A} = \frac{1}{t_{R,A}} \ln \frac{A_{A0}}{A_A} \tag{1}$$

$$k_{-1}^{app} = \frac{1}{t_{R,B}} \ln \frac{c_{B0}}{c_B} = \frac{1}{t_{R,B}} \ln \frac{A_{B0}}{A_B} \tag{2}$$

where t_R is the retention time, A is the peak area of enantiomer A and B at the time prior to separation (at time $t = 0$, the peak areas are A_{A0} or A_{B0}). After the separation (at time t, the peak areas are A_A or A_B), respectively.[11]

The apparent energy barriers to $A \rightarrow B$ and $B \rightarrow A$ enantiomerization can be than found from the apparent rate constants using the Eyring equation:[10]

$$-\Delta G_{A\rightarrow B}^{app} = RT \cdot \ln\left(\frac{h \cdot k_1^{app}}{\kappa \cdot k_b \cdot T}\right) \tag{3}$$

$$-\Delta G_{B\rightarrow A}^{app} = RT \cdot \ln\left(\frac{h \cdot k_{-1}^{app}}{\kappa \cdot k_b \cdot T}\right) \tag{4}$$

where R is the universal gas constant, T is the temperature in K, κ is transmission coefficient, k_B is the Boltzmann constant, and h is Planck's constant.

The dependence of the apparent enantiomerization barrier ($\Delta G^{app}_{A\rightarrow B}$, $\Delta G^{app}_{B\rightarrow A}$) on temperature can be used for the calculation of the apparent activation enthalpy ($\Delta H^{app}_{A\rightarrow B}$, $\Delta H^{app}_{B\rightarrow A}$) and entropy ($\Delta S^{app}_{A\rightarrow B}$, $\Delta S^{app}_{B\rightarrow A}$) using the Gibbs-Helmholz equation:

$$\Delta G^{app}_{A\rightarrow B} = \Delta H^{app}_{A\rightarrow B} - T\Delta S^{app}_{A\rightarrow B} \quad (5)$$

$$\Delta G^{app}_{B\rightarrow A} = \Delta H^{app}_{B\rightarrow A} - T\Delta S^{app}_{B\rightarrow A} \quad (6)$$

From the above written equations, it follows that the enantioselectivity of a chiral selector might be responsible for any differences in the apparent thermodynamic data for both enantiomers.[12]

Deconvolution Procedure

The basic requirement for deconvolution of chromatograms of peak envelopes is to discern parameters, which are characteristic for individual peaks in cluster (retention times, areas, height, and width of peaks). At first, it is necessary to find a mathematic function, which describes the shape of peaks on the studied part of the chromatogram, and as follows, to estimate the probable number of peaks in the cluster. The shape of asymmetric peaks is often described by the Exponential Modified Gauss function, for symmetric peaks Gauss function. It is possible to estimate the number of peaks in a cluster by using information about the sample (e. g., number of components). It is not possible to strictly determine the general number of peaks, but only to estimate them, because selectivity and separation efficiency is not sufficient to adequately recognize all compounds in the sample. Deconvolution procedures require the best estimate of initial parameters. This is a problem when the number of peaks in a chromatogram is not known. To find the minimum of number of compounds, we can use the number of maxima on chromatograms. Peaks are often co-eluted and, in this case, practically impossible to determine the number of peaks in an envelope from one chromatogram. The result of a deconvolution procedure is the number of notable outputs and their parameters (areas, retention times, width for peaks, which we have found in a peaks cluster of experimental chromatogram). The use of this method doesn't require information about the mechanism of separation.[13]

RESULTS AND DISCUSSION

As pointed out in the introduction, the typical interconversion profile with the plateau formation was observed. As can be seen in Figure 1, the elution profile was dependent on the temperature. By increasing the temperature, the height of plateau increased because the interconversion was faster. The co-elution of

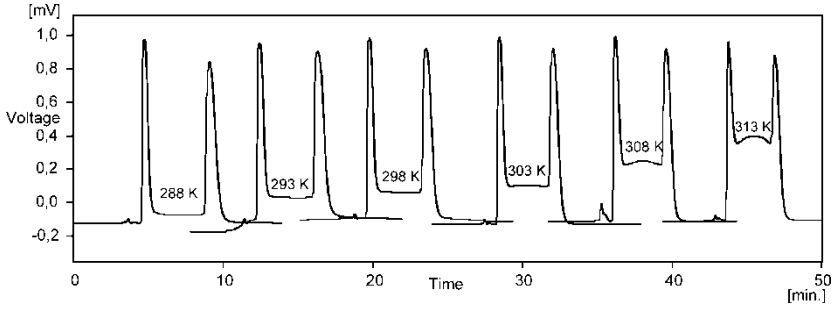

Figure 1. Chromatogram of HPLC enantiomeric separation of oxazepam at different temperatures. Chromatographic conditions: temperature 293 K, column Chirobiotic T, mobile phase MeOH/TEA/Hac (100/0.1/0.1 v/v/v), flow rate 1 mL/min, other details in experimental section.

the individual enantiomers was not observed in the studied temperature range. The high stereoselectivity of Chirobiotic T was confirmed — it was capable to separate the individual enantiomers of oxazepam in spite of its interconversion.

Deconvolution Procedure

It is known from the literature,[7,11] that two different approaches (the three or four peaks concept) can be used in the deconvolution procedure. The existence of three peaks in a peak envelope was assumed according to the interconversion process, which occurred during the oxazepam HPLC enantiomeric separation. Thus, the three-peak concept was used in our study. The commercial available software, Origin Microsoft with section Peak Fitting™ was employed for evaluation of experimentally obtained chromatograms. Individual chromatograms were exported from ChromWin station to the Origin Microsoft. As follows, the deconvolution procedure was applied, and the individual peaks areas were determined (Table 1). The chromatogram after the deconvolution procedure is shown in Figure 2.

Calculation of Interconversion Thermodynamic Parameters

The apparent rate constants were calculated from peaks areas (determined computer deconvolution) according to Eqs. (1) and (2). The values of the apparent rate constants for forward and backward interconversion are summarized in Table 2. The results showed that the rate of forward and backward interconversion was not the same. The apparent rate constants for

Table 1. The peak areas obtained after deconvolution procedure

Temperature (K):	273	278	283	288	293	298	303	308	313
A_A(mAU^2)	1098.9	1101.3	1151.3	1111.1	1095.2	1111.1	1174.6	1169.6	1151.5
A_R(mAU^2)	14.4	24.6	30.8	37.1	131.3	158.0	233.8	389.4	494.0
A_B (mAU^2)	996.7	1022.8	1038.6	1028.8	1014.1	970. 2	927.7	903.4	889.1
Correlation coeficient	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
A_0, B_0 (mAU^2)	1055.1	1074.1	1110.4	1088.5	1120.3	1119.7	1168.0	1231.2	1267.3

A_A – the peak area of the first eluted enantiomer after interconversion.
A_R – the unresolved zone of racemate after interconversion.
A_B – the peak area of the second eluted enantiomer after interconversion.
A_0, B_0 – the peak area of the individual enantiomers before interconvesion.
$A_A \pm 0.1$ (mAU^2), $A_B \pm 0.2$ (mAU^2), $A_R \pm 0.1$ (mAU^2), $A_0 \cdot B_0 \pm 0.2$ (mAU^2).

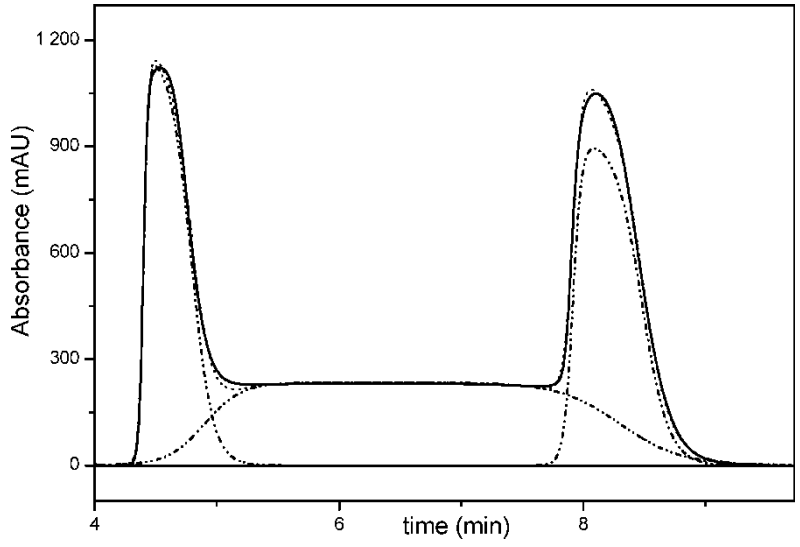

Figure 2. The chromatographic deconvolution using Peak Fitting™ (Origin Microsoft). The full line is experimentally obtained chromatogram; broken line is simulated peak envelope with resolved internal peaks areas calculated by computer. Chromatographic conditions: temperature 293 K, column Chirobiotic T, mobile phase MeOH/TEA/Hac (100/0.1/0.1), flow rate 1 mL/min, other details in experimental section.

forward interconversion (A → B) were higher than the apparent rate constants for backward interconversion ($k_1^{app} > k_{-1}^{app}$) in the entire studied temperature range. Consequently, the interconversion A → B occurred faster than B → A. Probably, the reason of this phenomenon was the different stability of the diastereoisomeric complexes between individual enantiomers and chiral stationary phase (CSP). The first eluted enantiomer (A) was less retained on CSP than the second one. The second eluted enantiomer (B) created more stable transient complexes with CSP and, due to this, underwent the interconversion slower than the first one. Thus, the interconversion A → B was faster than the interconversion B → A.

Thus, the dependence of the apparent rate constants on the temperature was studied. The dependence of k_{-1}^{app} on the temperature was monotonous — the increase of the temperature, the increase of the apparent rate constants.

Next, the apparent rate constants were used for calculation of the apparent activation (Gibbs-Free) energy according to the Eyring equation Eqs. (3) and (4). The results were summarized in Table 3. As can be seen in Table 3, the ΔG_1^{app} and ΔG_{-1}^{app} values were similar. The absolute value of the apparent activation energy increased with the increase of the temperature. The dependences $\Delta G_{1(-1)}^{app} = f(T)$ were used for the determination of the apparent enthalpy and entropy of interconversion (Figures 3 and 4).

Table 2. The apparent rate constants for forward and backward interconversion of oxazepam enantiomers

T (K):	273	278	283	288	293	298	303	313
k_1^{app} (s^{-1})	1.28×10^{-3}	1.30×10^{-3}	1.27×10^{-3}	1.33×10^{-3}	1.38×10^{-3}	1.37×10^{-3}	1.33×10^{-3}	1.41×10^{-3}
k_{-1}^{app} (s^{-1})	7.04×10^{-4}	7.10×10^{-4}	7.18×10^{-4}	7.50×10^{-4}	8.15×10^{-4}	8.64×10^{-4}	9.40×10^{-4}	1.07×10^{-3}

k_1^{app} – the apparent rate constant of interconversion A $\rightarrow$ B.
k_{-1}^{app} – the apparent rate constant of interconversion B $\rightarrow$ A.
$k_1^{app} \pm 0{,}08 \cdot 10^{-3}\ s^{-1}$, $k_{-1}^{app} \pm 0 \cdot 04 \cdot 10^{-4}\ s^{-1}$.

Table 3. The apparent activation (Gibbs free) energy for forward and backward interconversion of oxazepam enantiomers

T (K):	273	278	283	288	293	298	303	313
ΔG_1^{app} (kJ/mol)	80.20	81.68	83.25	84.65	86.07	87.61	89.20	92.07
ΔG_{-1}^{app} (kJ/mol)	80.92	82.63	84.12	85.87	87.52	89.08	90.55	93.52

ΔG_1^{app} – the apparent activation (Gibbs free) energy of interconversion A → B.
ΔG_{-1}^{app} – the apparent activation (Gibbs free) energy of interconversion B → A.
$\Delta G_1^{app} \pm 0.02$ kJ/mol, $\Delta G_{-1}^{app} \pm 0.03$ kJ/mol.

The apparent enthalpy ($\Delta H_{1(-1)}^{app}$) was determined from the slope of linear dependence $\Delta G_{1(-1)}^{app} = f(T)$, the apparent entropy ($\Delta S_{1(-1)}^{app}$) was determined from intercept of linear dependence $\Delta G_{1(-1)}^{app} = f(T)$ according to the Gibbs-Helmholz equation (Eqs. (5) and (6)).

The following equations were determined from linear regression:

$$\Delta G_1^{app} = -0.88 + 0.30T \quad r = 0.999$$
$$\Delta G_{-1}^{app} = -5.34 + 0.32T \quad r = 0.999$$

The obtained correlation co-eficients (r = 0.999) referred to high linearity of the dependence of $\Delta G_{1(-1)}^{app} = f(T)$. A comparison of slopes and intercept with the absolute terms in Eqs. (5) and (6) allowed to calculate the enthalpy and entropy terms with the results, which are summed up in Tables 4 and 5.

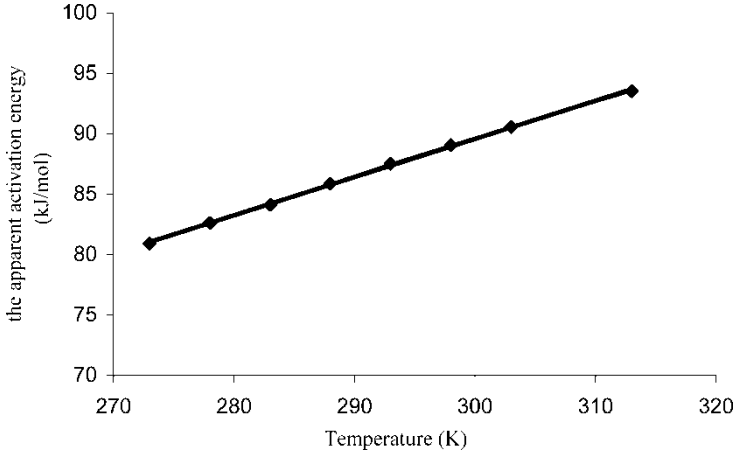

Figure 3. The dependence of apparent activation (Gibbs-Free) energy on temperature for backward interconversion (B → A).

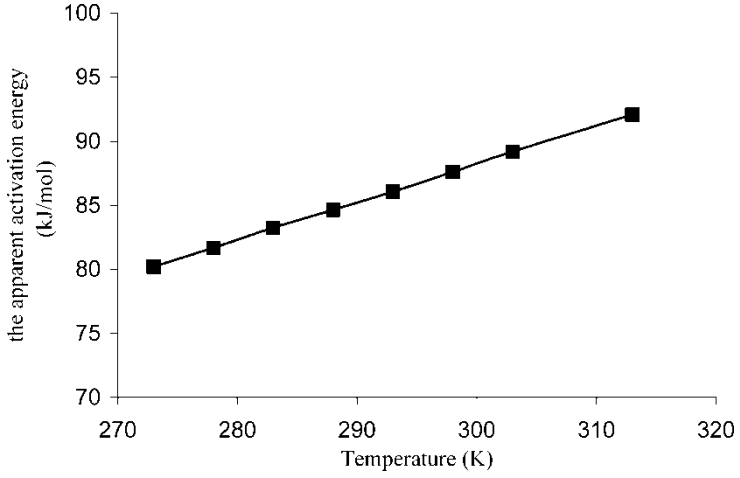

Figure 4. The dependence of apparent activation (Gibbs-Free) energy on temperature for forward interconversion (A → B).

The aim of the study was to determine the dominant contribution (enthalpic or entropic) to the apparent activation energy. As can be seen in Tables 4 and 5, the entropic contribution played an important role. Comparing the enthalpic contributions to the apparent activation energy (Tables 4 and 5), it can be concluded that the apparent enthalpy for interconversion B → A is higher than that the apparent enthalpy for interconversion A → B. It confirmed our previous assumption that the interactions of individual enantiomers with chiral stationary phase played an important role in the interconversion process.

CONCLUSION

The aim of this study was to study the calculation of the apparent rate constants and the apparent activation (Gibbs free) energy for on – column

Table 4. The contribution of the apparent enthalpy and entropy to the apparent activation energy for forward interconversion A → B

T (K):	273	278	283	288	293	298	303	313
$-\Delta H_i^{app}$ (kJ/mol)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
$-T \cdot \Delta S_i^{app}$ (kJ/mol)	81.90	83.40	84.90	86.40	87.90	89.40	90.90	93.90

$\Delta H_i^{app} \pm 0.02$ (kJ/mol), $-T \cdot \Delta S_i^{app} \pm 0.26$ (kJ/mol).

Table 5. The contribution of the apparent enthalpy and entropy to the apparent activation energy for backward interconversion B → A

T (K):	273	278	283	288	293	298	303	313
$-\Delta H_{-1}^{app}$ (kJ/mol)	5.34	5.34	5.34	5.34	5.34	5.34	5.34	5.34
$-T \cdot \Delta S_{-1}^{app}$ (kJ/mol)	87.40	89.00	90.60	92.20	93.80	95.40	97.00	100.20é

$\Delta H_{-1}^{app} \pm 0.06$ (kJ/mol), $-T \cdot \Delta S_{-1}^{app} \pm 0.33$ (kJ/mol).

interconversion of oxazepam enantiomers. The interconversion of oxazepam enantiomers was observed in the whole studied temperature range. The typical chromatographic profile depended on temperature – by increasing of the temperature, the height of plateau between two peaks increased. Coelution and collapse of peak cluster was not observed in the studied temperature range. For the evaluation of experimental chromatograms, computer assisted deconvolution was used. The determination of the peak areas included in the peak envelope was necessary for calculation of the thermodynamics parameters. According to the obtained results it can be concluded that:

The interconversion A → B occurred faster than interconversion B → A.
The rate of interconversion B → A increased with the increase of temperature.
According to Gibbs-Helmholz equation, the main contribution to the activation energy represented the entropy term ($-T \cdot \Delta S$).

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