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Short communication

Chiral separation of optically active 2,3-benzodiazepine derivatives by high performance liquid chromatography[1]

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1. Introduction

Some 3,4-dihydro-2,3-benzodiazepines show different pharmacological effects. Since these compounds are optically active, the determination of the enantiomer ratio is an important part of their analysis. Therefore a chiral high performance liquid chromatography (HPLC) method has been elaborated for the separation of enantiomer forms. Methods for separation of enantiomeric 1,4-benzodiazepines on different chiral stationary phases can be found in the literature [1–3]. Cellulose derivatives as stationary phases proved to be useful in some cases for separation. Therefore in this work cellulose-type chiral phases were used for the separation of 2,3-benzodiazepine enantiomers. Two different types of chemical structure were chosen. One of them contained a cellulose tribenzoate derivative as the chiral phase (Chiralcel OJ); the other column contained a cellulose triphenylcarbamate derivative (Chiralcel OF) capable of hydrogen bonding and dipole–dipole interactions with the solute. A detailed description and characterization of these cellulose derivatives are given in the literature [4,5]. The influence of structural units of the benzodiazepines on the separation has been studied using these stationary phases of different chemical structure.

2. Experimental

The structures of the racemic compounds tested are illustrated by the following formula:

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

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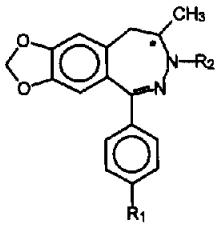
Table for the Structure 1.



Compounds	R_1	R_2
I	NH_2	H
II	NO_2	H
III	NH_2	$CO-CH_3$
IV	$NH-CO-CH_3$	H
V	$NH-CO-CH_3$	$CO-CH_3$
VI	NH_2	$CO-NH-CH_3$

All of the compounds were synthesized at the Institute for Drug Research, Budapest. Pure enantiomers of compounds **II**, **III** and **VI** were available, compounds **I**, **IV** and **V** were studied as racemates.

2.1. Chromatographic conditions

The following equipment was used: an LKB HPLC 2248 pump; an LKB HPLC UV detector operating at 240 nm; and an LKB column oven. Data processing was performed with an IBM PC/AT computer using Nelson 5.0 software. The columns used were Chiralcel OF and OJ (0.46 cm × 25 cm; 10 μm; Daicel). A Rheodyne 7125 injector with a 20 μl sample loop was used. The mobile phases used were: (A) hexane:2-propanol,

Table 1
Separation characteristics of compounds **I–VI** on Chiralcel OF column using different mobile phases and column temperatures

Compound	Parameter	Mobile phase (B) (22°C)	Mobile phase (B) (40°C)	Mobile phase (A) (40°C)
I	k_1	13.17	7.00	7.77
	k_2	16.17	8.20	9.27
	N_1	740	1821	1839
	N_2	663	1345	1417
	R_s	1.26	1.40	1.60
II	k_1	5.41	3.84	3.54
	k_2	6.33	4.28	4.00
	N_1	1482	3241	3266
	N_2	1239	2788	3022
	R_s	1.22	1.20	1.33
III	k_1	8.33	5.10	5.41
	k_2	12.00	7.00	7.77
	N_1	694	2229	2625
	N_2	623	2304	1839
	R_s	2.09	3.00	3.59
IV	No separation on Chiralcel OF			
V	k_1	No separation	6.40	5.36
	k_2		7.00	5.91
	N_1		No separation	1600
	N_2			1444
	R_s			0.8
VI	k_1	11.67	8.2	7.77
	k_2	15.83	10.64	9.27
	N_1	699	1748	1839
	N_2	564	1456	1417
	R_s	1.75	2.30	1.83

Table 2
Separation characteristics of compounds I–VI on Chiralcel OF and OJ columns using mobile phases (A) and (C) at 40°C

Compound	Parameter	Chiralcel OF (Mobile phase (A))	Chiralcel OJ (Mobile phase (A))	Chiralcel OJ (Mobile phase (C))
I	k_1	7.77		9.45
	k_2	9.27		10.49
	N_1	1839		
	N_2	1417		
	R_s	1.60		
II	k_1	3.54	2.77	2.91
	k_2	4.00	3.41	3.68
	N_1	3266	3061	3364
	N_2	3022	2352	3273
	R_s	1.33	2.00	2.58
III	k_1	5.41	1.64	1.09
	k_2	7.77	3.00	1.50
	N_1	2625	2152	2344
	N_2	1839	632	1936
	R_s	3.59	3.16	2.04
IV	k_1	No separation	3.18	2.41
	k_2		3.54	2.90
	N_1			2500
	N_2			2414
	R_s			1.69
V	k_1	5.36	0.45	0.32
	k_2	5.91	0.64	0.55
	N_1	1600	2843	3136
	N_2	1444	1296	2736
	R_s	0.8	1.25	2.18
VI	k_1	7.77	1.77	1.32
	k_2	9.27		6.55
	N_1	1839		2601
	N_2	1417		
	R_s	1.83		

50:50 (v/v); (B) hexane:2-propanol:diethylamine, 50:50:0.1 (v/v/v); (C) hexane:absolute ethanol, 50:50 (v/v). Solvents were of HPLC quality (Reanal, Hungary). Mobile phases (A), (B) and (C) were used for the Chiralcel OF column; mobile phases (A) and (C) were used for the Chiralcel OJ column (an ethanol-containing mobile phase must not be used on the Chiralcel OF column at 40°C). The flow rate used was 0.7 ml min^{-1}. The column temperatures used were 22 and 40°C. The sample concentration was about 100 μg ml^{-1} in mobile phase (A).

3. Results and discussion

For studying the effect of diethylamine added to the mobile phase and that of increased column temperature chromatography was carried out at ambient temperature (22°C) and at 40°C using solvent systems (A) and (B) on the Chiralcel OF column. Separations were characterized by calculating $k=(t_r-t_0/t_0)$, $N=16(t_r/W)^2$ and $R_s=2(t_r-t_0)/(W_1+W_2)$, where W is the peak width measured by extrapolating the relevant straight sides to the baseline. k_1 and k_2, N_1 and N_2 refer to

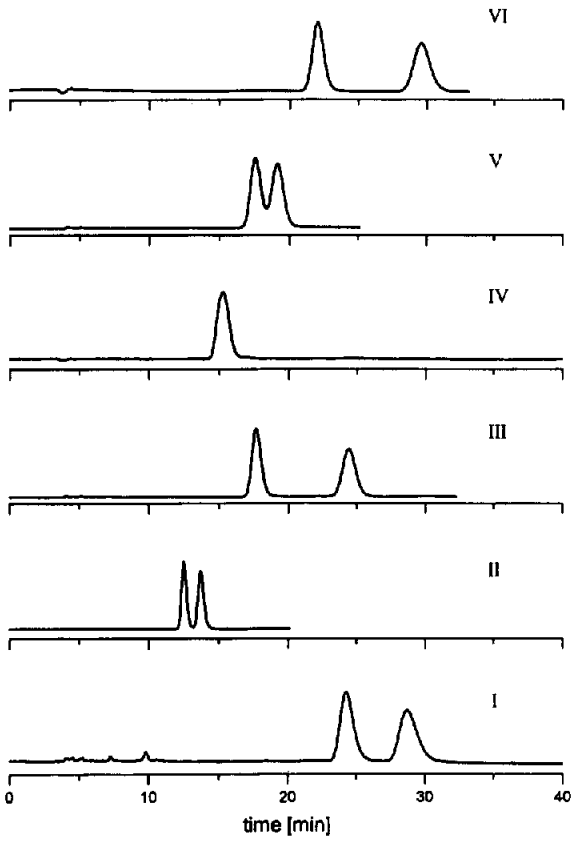


Fig. 1. Chromatograms of the racemates of compounds I–VI. Column: Chiralcel OF; mobile phase: (A); flow rate: 0.7 ml min^{-1}; temperature: 40°C; detection: UV detector at 240 nm.

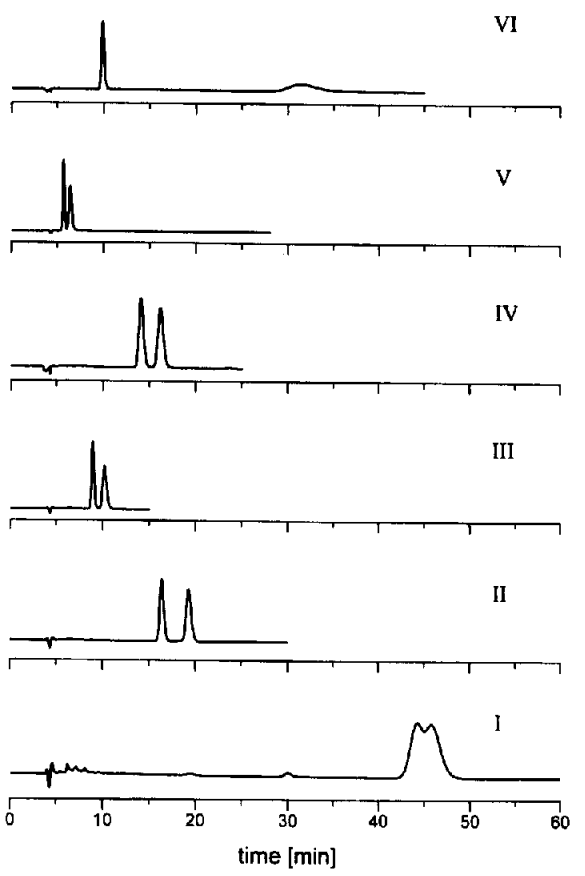


Fig. 2. Chromatograms of the racemates of compounds I–VI. Column: Chiralcel OJ; mobile phase: (C); flow rate: 0.7 ml min^{-1}; temperature: 40°C; detection: UV detector at 240 nm.

the enantiomers having lower and higher retention times respectively. Table 1 shows the values obtained. As can be seen from these data, elevated temperature improved the column efficiency significantly when comparing corresponding k and N values. (N_1, N_2 and R_s values for compound **V** in mobile phase (B) at 40°C could not be evaluated because of the poor separation.) However, there was no significant advantage using diethylamine (see corresponding values using mobile phases (B) and (A) at 40°C). Similar experiences were found with the Chiralcel OJ column. On this basis chromatograms obtained with mobile phases without diethylamine at 40°C on both of the columns were compared. Separation data are given in Table 2. Missing data could not be determined either because of poor separation (compound **IV**) or because of very strong retention (compounds **I** and **VI**).

Chromatographic separation of the enantiomers of compounds **I–VI** is illustrated in Figs. 1 and 2. As can be seen in the Figures and Tables, compounds **II**, **III** and **VI** can be separated on both of the columns and on Chiralcel OJ with both mobile phases. The (+)-enantiomer of compound **VI**, however, shows a very strong retention on Chiralcel OJ, therefore its detection and quantification is very poor. The same phenomenon was observed when chromatographing the racemate of **I** on Chiralcel OJ. The retention time with mobile phase (A) was over 80 min; separation with mobile phase (B) was also very poor. Enantiomers of compounds **IV** and **V** were separated on the Chiralcel OJ column using mobile phase (B). Enantiomers of the most polar compounds (**I** and **VI**) were resolved on the Chiralcel OF column with mobile phase (A).

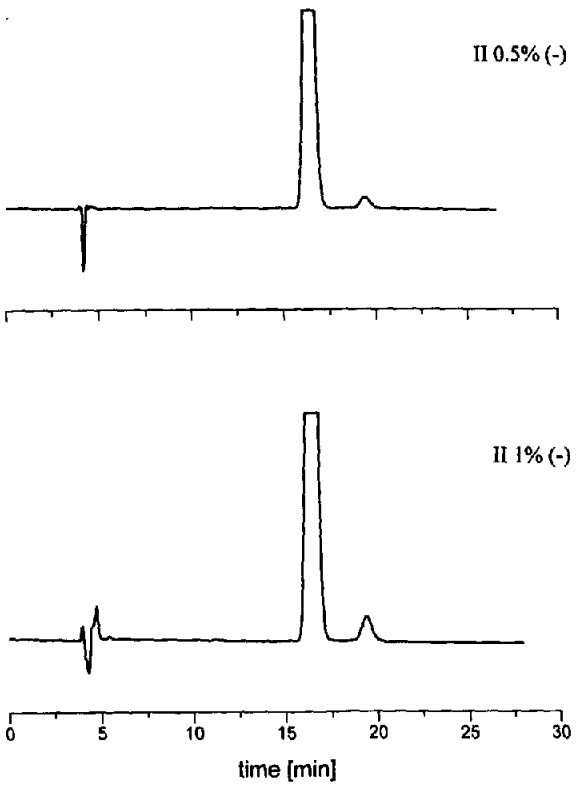


Fig. 3. Chromatograms of mixtures containing 1% and 0.5% of the (−)-enantiomer of compound II added to the solution of the (+)-enantiomer of compound II. Column: Chiralcel OJ; mobile phase: (B); flow rate: 0.7 ml min^{-1}; temperature: 40°C; detection: UV detector at 240 nm.

When comparing the separation on both columns — considering the structural characteristics of these compounds — it can be stated that on the Chiralcel OF column the presence of a free amino or nitro group on the aromatic ring is necessary for enantiomeric resolution (compounds **I**, **II**, **III** and **VI**). The presence of an acetylated aromatic amino group caused poor separation (compound **V**) or no separation at all (compound **IV**). The Chiralcel OJ column was capable of separating enantiomers **IV** and **V**. Retention in the two mobile phases decreased in the case of compound **V**, containing two acetyl groups, when compared with the retention of compounds **I**, **III** and **IV**.

The limit of detection is illustrated for the (−)-enantiomer of compound **II** in Fig. 3. Similar quantities of enantiomers of compounds **III** and **VI** were detected using a suitable column and mobile phase, see Tables 1 and 2 and Figs. 1 and 2. Concerning the elution order of the pure enantiomers available it can be said that, in the case of compounds **III** and **VI** on the OF column the (+)-enantiomer elutes with the shorter retention time, and on the OJ column the (−)-enantiomer elutes with the shorter retention time. On the OJ column there was no difference in elution order using mobile phases (B) or (C). In the case of compound **II** the (+)-enantiomer showed shorter retention times on both columns.

Considering the sample concentrations, the peaks on the chromatograms corresponding to 0.5% enantiomer contain 10 ng of substance. This quantity was easily detected even in the case of long retention times (compounds **III** and **VI**), when a suitable column and mobile phase were chosen as given in the Figures.

4. Conclusion

Determination of the optical purity of 4,5-dihydro-2,3-benzodiazepine derivatives by HPLC is an important part of their analysis. Cellulose derivatives as chiral stationary phases were used for separation (Chiralcel OF and OJ columns) and the effect of the substituting groups of the benzodiazepines on their chiral recognition was studied. On cellulose triphenyl carbamate (Chiralcel OF column) a higher degree of resolution was obtained if the molecule contained an aromatic NO_2 or NH_2 group. Acetyl substitution of the aromatic amino group reduced resolution; acetylation of the 3-NH group in the benzodiazepine ring, however, did not influence chiral recognition. Cellulose tribenzoate stationary phase (Chiralcel OJ) resolved derivatives containing an acetylated aromatic amino group, but the resolution power decreased if the 3-NH group in the benzodiazepine ring was acetylated.

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