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Liquid chromatographic separation of chiral triazolophthalazines

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Abstract

Investigations of the synthesis of N,N-linked heterobicyclic compounds, synthesized with cyclic hydrazine derivatives, have been the focus of the authors' research for some time. Using carbonyl compounds, the synthesis of chiral triazolophthalazines and thiadiazolophthalazines was recently achieved. The chromatographic investigation of these newly synthesized triazolophthalazines was of great interest. Different results were obtained with different chiral stationary phases. The influence of temperature and mobile phase composition was investigated.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Triazolophthalazines

1. Introduction

Numerous groups have been engaged in the search for potential pharmaceutical drugs, the development of new guiding structures being of special interest. In previous papers, we discussed the synthesis of N,N-linked heterobicyclic compounds [1,2]. We have now developed chiral triazolophthalazines with carbonyl compounds [3].

Especially for chiral substances, it is not advisable to disconnect synthesis and analysis, and history has shown how dangerous this can be [4]. For this reason, the synthesis and analysis of the substances under study here were carried out simultaneously (Fig. 1).

Chromatographic investigations were performed on four different chiral stationary phases (CSPs). First we chose Chiralcel OD-H, in which tris-3,5-dimethylphenyl carbamate is bound to silica. This CSP is suitable for analytes with carbonyl, hydroxyl and nitro groups, and also for substances with aromatic groups. The mobile phases were water-free (*n*-hexane–2-propanol–ethanol) compositions.

Since good results were obtained on these CSPs, we chose Chiralcel OD-R as a second stationary phase. Here the same cellulose derivative is bound to silica gel just as in Chiralcel OD-H, but the mobile phases are buffer mixtures. For cellulose phases, the chiral discrimination between enantiomeric solutes due to differences in their steric fits in the chiral cavity has been frequently discussed. [5,6]. The same has been discussed for cyclodextrin phases [7,8]. Because the relation to retention (k') and stereoselectivity (α) of cyclodextrin stationary

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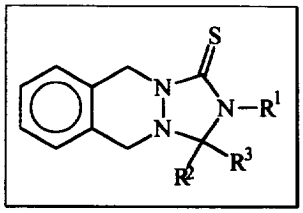

Sample-No.	R^1	R^2	R^3
1	CH_3	H	CH_3
2	CH_3	H	4'-NO_2-C_6H_4
3	CH_3	H	2'-NO_2-C_6H_4
4	C_6H_4	H	CH_3
5	C_6H_4	H	4'-NO_2-C_6H_4
6	C_6H_4	H	4'-Cl-C_6H_4
7	C_6H_4	H	4'-Br-C_6H_4
8	C_6H_4	CH_3	C_3H_7
9	4-NO_2-C_6H_4	H	CH_3
10	4-Cl-C_6H_4	H	CH_3
11	4-Br-C_6H_4	H	CH_3
12	4-Br-C_6H_4	H	4'-NO_2-C_6H_4
13	4-Br-C_6H_4	CH_3	C_3H_7

Fig. 1. Structures of analytes.

phase is of focal interest, we chose Cyclobond I 2000 Ac, an acetyl-β-cyclodextrin phase.

As mobile phases, water-free or water (buffer)-containing mixtures are possible. In the past, N-heterocyclic analytes investigated on Pirkle phases proved successful [9]. Pirkle et al. [10] developed an acceptor phase, in which D-dinitrobenzoylphenylglycine is bound to silica (Chiral D-DPG Si-100).

2. Experimental

2.1. Chemicals

HPLC-grade 2-propanol and *n*-hexane were obtained from J.T. Baker (Deventer, Netherlands), acetonitrile from Merck (Darmstadt, Germany) and ethanol, methanol, sodium perchlorate, phosphoric acid and sodium hydroxide from Apolda (Germany). The water used was doubly distilled.

2.2. Instrumentation

Liquid chromatography (LC) was performed with (i) a LiChroGraph (Merck–Hitachi, Darmstadt, Germany) equipped with a Model L-6000 pump, a Model L-4200 UV–Vis detector, a Model T-6300 column thermostat, a Rheodyne injector with a 20 μl loop and a Model D-2500 chromato-integrator, and (ii) an HP 1090 II HPLC system (Hewlett-Packard, Waldbronn, Germany) with a diode-array detector. A Chiralyser polarimetric detector (IBZ Messtechnik, Hannover, Germany) was used for the determination of the optical rotations of enantiomers. For pH measurements an MV instrument (Präcitronic, Dresden, Germany) was employed. A Model HR 30/3 ultrasonic bath (zft-Microelectronic, Berlin, Germany) was used for mobile phase degassing.

2.3. Sample preparation

The solutes (1 mg) were dissolved in 5 ml of 2-propanol.

2.4. Chromatographic conditions

Chiral separations were performed with several CSPs: (A) stainless-steel columns (250 × 4.6 mm I.D.) packed with tris(3,5-dimethylphenylcarbamate) cellulose adsorbed on microporous silica (A^1, Chiracel OD-H; A^2, Chiralcel OD-R column; Daicel Chemical Industries, obtained from J.T. Baker); (B) a similar column (250 × 4.6 mm I.D.) packed with acetyl-β-cyclodextrin adsorbed on microporous silica (Cyclobond I 2000 Ac; Astec, obtained from ICT, Frankfurt, Germany); and (C) a column (250 × 4.6 mm I.D.) packed with Pirkle phase (D-3,5-dinitrobenzoylphenylglycine, Chiral D-DPG Si-100; Serva, Heidelberg, Germany).

Mobile phases were mixed as described, filtered and degassed in an ultrasonic bath.

Table 1
Influence of 2-propanol content on retention and separation of the enantiomers of triazolophthalazines

Analyte	Content of 2-propanol (%, v/v) in *n*-hexane on Chiralcel OD-H																			
	10		20		30		40		50		60		70		80		90		100	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	1.18	1.35	1.04	1.28	0.79	1.33	0.89	1.18	0.79	1.18	0.67	1.21	0.63	1.20	0.59	1.19	0.60	1.19	0.52	1.22
2	4.08	0	3.51	1.17	2.37	1.20	2.27	1.24	1.95	1.28	1.65	1.32	1.33	1.38	1.23	1.43	1.22	1.52	0.91	1.63
3	1.37	1.63	1.25	1.60	1.07	1.59	1.18	1.39	1.10	1.39	0.99	1.38	0.98	1.39	0.84	1.36	0.87	1.30	0.69	1.33
4	1.61	1.65	1.46	1.56	1.11	1.55	1.24	1.39	1.12	1.38	0.95	1.37	0.83	1.37	0.82	1.36	0.84	1.38	0.77	1.46
5	4.07	1.65	4.14	1.91	2.77	1.86	2.74	1.84	2.39	1.84	2.03	1.86	1.77	1.89	1.74	1.91	1.80	1.97	1.79	2.09
6	1.02	1.52	1.09	1.54	0.85	1.55	1.06	1.41	0.99	1.40	0.93	1.44	0.88	1.46	0.89	1.47	0.94	1.49	0.92	1.57
7	1.13	1.53	1.19	1.53	0.95	1.57	1.15	1.44	1.09	1.43	1.02	1.47	0.97	1.49	0.99	1.50	1.05	1.53	1.04	1.62
8	1.01	2.09	0.87	1.96	0.70	1.93	0.76	1.69	0.72	1.65	0.65	1.6	0.58	1.59	0.61	1.56	0.63	1.56	0.61	1.67
9	4.19	2.06	3.05	1.81	2.48	1.79	2.31	1.68	1.95	1.66	1.59	1.64	1.56	1.62	1.38	1.64	1.43	1.68	1.41	1.80
10	1.66	2.24	1.59	2.05	1.14	2.01	1.29	1.78	1.09	1.72	0.96	1.70	0.87	1.71	0.85	1.68	0.89	1.72	0.83	1.84
11	1.85	2.18	1.59	1.82	1.18	2.08	1.42	1.72	1.21	1.66	1.87	1.66	0.97	1.65	0.98	1.63	0.99	1.66	0.95	1.76
12	5.06	1.59	4.84	1.76	3.18	1.73	3.23	1.67	2.79	1.65	2.39	1.66	2.15	1.66	2.33	1.63	2.16	1.69	2.20	1.73
13	1.37	3.46	1.15	3.14	0.81	3.06	0.96	2.61	0.91	2.55	0.81	2.48	0.72	2.43	0.76	2.36	0.82	2.37	0.82	2.53

Apparatus (i); flow-rate 1 ml/min for 10–80% 2-propanol, 0.8 ml/min for 90% 2-propanol and 0.5 ml/min for 100% 2-propanol; temperature, 25°C.

3. Results and discussion

3.1. Influence of stationary phase and mobile phase composition

As the first screening on Chiralcel OD-H was very successful, we investigated the retention behaviour and stereoselectivity of analytes **1–13** using different mixtures of mobile phases and increasing temperatures. Good separations were achieved for all analytes. Baseline separation was generally achieved; only a few compounds exhibited peak-valley factors (P) under 1 [11].

The effects of an increase in the proportion of 2-propanol in n-hexane on the retention and stereoselectivity of the analytes are shown in Table 1. It can be seen that the k' values of **6** and **7** are independent of 2-propanol concentration, confirming our expectations. However, the dependence of stereoselectivity on 2-propanol concentration varies to a large extent (Fig. 2).

Only **3**, **8**, **10** and **13** show a decrease in the α-values (Fig. 2a) with an increase in the polar component of the mobile phase (2-propanol). A minimal reduction can be seen for **4**, **9** and **11**, but the influence is so weak that they can be partly included in the group of substances without any dependence (cf., **1**, **7**, **12**; Fig. 2b). An exception in their behaviour is shown by **2** and **5**. The α-values increase with increasing 2-propanol

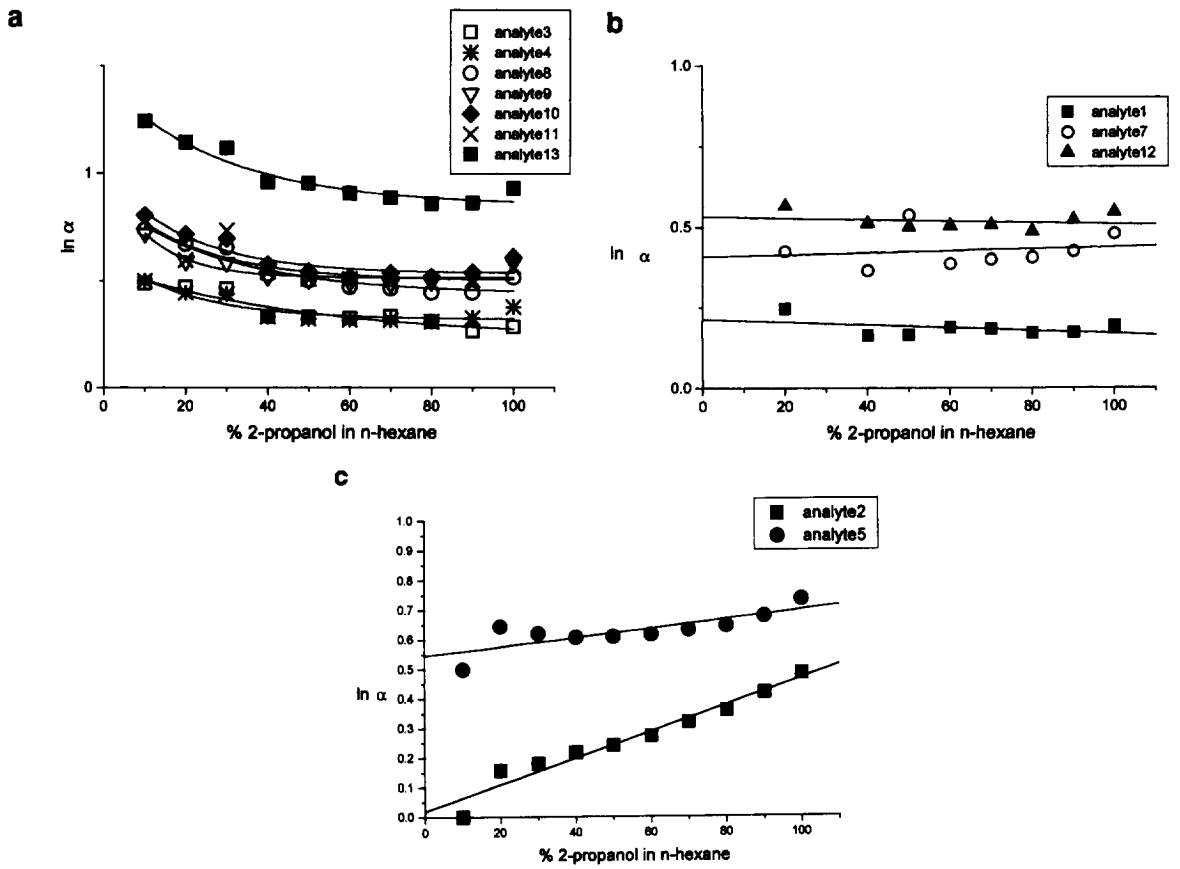

Fig. 2. Relationship between ln α and content of 2-propanol in n-hexane. For conditions, see also Table 1. (a) Analytes with decreasing α-values. Exponential decay: $y_0 + A \exp[-(x - x_0/t)]$. (b) Relationship between ln α and 2-propanol concentration in n-hexane for analytes showing no influence. Linear regression: $y = A + Bx$. (c) Relationship between ln α and content of 2-propanol in n-hexane for analytes with increasing α-values. Linear fit: $y = A + Bx$.

content in the mobile phase (Fig. 2c). Only for analyte **6** is an exact statement impossible.

All compounds with alkyl residues in position R 2 or 3 (Fig. 1) have short retention times, and no increase in α-values is observed. Substances with negative inductive and positive mesomeric effects are stabilized. The interaction with the stationary phase is strong, but there is no difference between the two enantiomers. There is also no influence of the 2-propanol concentration on stereoselectivity. The position of the electron-withdrawing substituent is also unimportant.

If there are substituents with electron-donating groups on the chiral centre of the molecule (position R 3) and a methyl group or a phenyl ring (apolar groups) in position R 2, then the α-values increase with increasing concentration of 2-propanol. To explain this phenomenon, we suggest the formation of a solvation shell around the analyte molecule. The proportion of the 2-propanol molecules in this solvation shell increases with increase in concentration. There are interactions between the N-heterocyclic structure of the analyte, the small polar group and the large apolar group of the solvent molecule and the stationary phase. This could be a reason for the different orientation of the substituent in position 3 on the stationary phase.

Direct interaction between CSP and the chiral carbon atom of the analyte is important for the stereoselectivity, in addition to the type of substituent on the chiral centre [5,12]. In general, substances with *p*-nitrophenyl residues are eluted later than other analytes, whereas those with alkyl residues have the shortest retention times.

Contrary to our expectations, in most cases elution on Chiralcel OD-R was not possible (1 *M* $NaClO_4$–acetonitrile with acetonitrile concentrations from 40 to 70% and temperatures up to 45°C). This supports our assumption that there must be an intensive interaction between the analyte and the stationary phase. It must also be borne in mind that the water molecule is very small and polar. Water cannot form a solvation shell around analytes such as 2-propanol. Hence dissolution of the analyte molecule from the stationary phase is not possible. The point of attack must be a polar structure in the triazolophthalazine molecule, but the spacious apolar groups shield it from the polar water molecules.

Similar results were obtained with Cyclobond I 2000 Ac. Starting with a mobile phase of 0.5% sodium acetate solution (pH 5.5) in methanol (4:6, v/v), continuing with acetonitrile–ethanol–0.5% sodium acetate solution (95:5:5, v/v/v) and ending with acetonitrile–methanol–water (95:5:5, v/v/v), we found that no elution was possible in any case. Only by taking 2-propanol as the mobile phase could the compounds of the stationary phase be dissolved; enantiomer separation, however, was not possible. It was evident that even the smallest amount of water in the mobile phase impaired or even prevented elution. This supports our theory of the formation of a solvation shell with 2-propanol around the analyte molecule, because there are polar and apolar structures. Variation of the mobile phase using *n*-hexane and ethanol had no effect on stereoselectivity.

This type of stationary phase is not suitable for enantiomer separation of the analytes, the substituents on the cyclodextrin (acetyl residues)

Table 2
Influence of mobile phase on separation of Chiral D-DPG Si-100

Sample	A	B	C	D	E	F
1		1.08	1.09	1.11	1.17	1.21
2	1		1.15		1.24	1.07
3	1	1	1.07		1.11	1.50
4	1.14	1.11	1.13	1.13	1.12	1.28
5		1.10	1.11	1.12	1	1.28
6	1	1.29	1.33	1.31	1	1.73
7	1	1.30	1.28	1.30	1.32	1.48
8	1.19	1.16	1.66	1.17	1.21	1.38
9		1.06	1.08	1.09	1.14	1.20
10		1.08	1.09	1.11	1.16	1.36
11		1.11	1.13	1.15	1.18	1.35
12		1.09	1.12	1.12	1.15	1.35
13	1.24	1.20	1.21	1.22	1.27	1.49

Apparatus (i); A, B = *n*-hexane–2-propanol (8:2, v/v); flow-rate, 0.5 ml/min; 25°C. A, 0% CH_3CN; B, 1% CH_3CN. C, D, E = *n*-hexane–2-propanol (9:1, v/v); flow-rate, 0.5 ml/min; 25°C. C, 0% CH_3CN; D, 1% CH_3CN; E, 2% CH_3CN; F, 5% CH_3CN.

being too small for steric adjustment of the triazolophthalazine molecules.

On Chiral D-DPG Si-100, different *n*-hexane–2-propanol mixtures with increasing acetonitrile concentrations were used. In most cases, a small separation was possible (Table 2); baseline separation was never possible (*P* always less than 0.5).

The delay of the retention of **6** and **7** (Fig. 3) was noteworthy. The expected decrease was only clearly seen for **1** and **9** with increase in acetonitrile concentration. The other substances are independent of the mobile phase composition, which was obviously caused by different substitutions.

Analytes **6** and **7** have a phenyl residue on position 2 and a positive mesomer group on the substituent on position 3. These groups push electrons into the molecule. The Pirkle stationary phase is a π-acceptor phase, hence intensive interactions with the analyte molecule are possible. Therefore, these analytes have the highest α-values.

All substances without electron-donating groups on the chiral carbon atom have k' values independent of the mobile phase; the α-values increase because acetonitrile supports the interaction.

3.2. Influence of temperature

The influence of temperature was investigated on Chiralcel OD-H for some of the analytes (Table 3, Fig. 4). As expected, there was a decrease in k' values. The quality of separation was also impaired, especially for some analytes with small separation factors. Baseline separation at high temperatures was not possible.

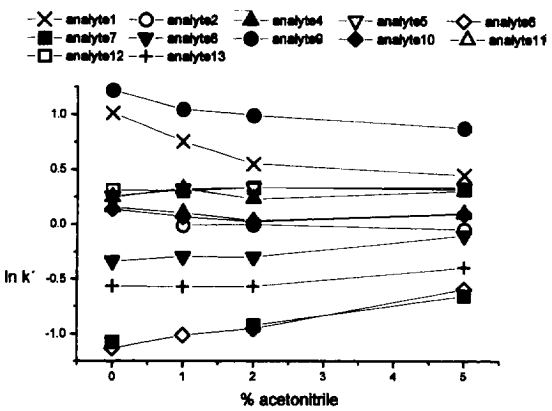

Fig. 3. Influence of acetonitrile concentration in the mobile phase on retention. Apparatus (i), column C. Mobile phase: *n*-hexane-2-propanol (9:1, v/v); flow-rate, 0.5 ml/min; 25°C.

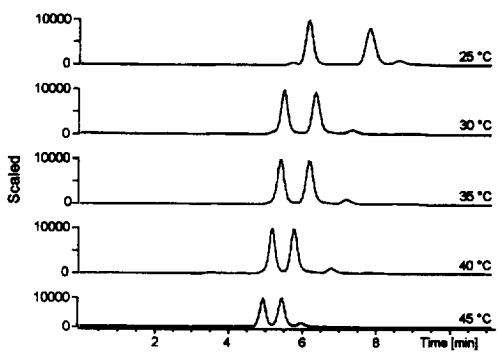

Fig. 4. Influence of temperature for analyte 3. For conditions, see also Table 3.

Table 3
Influence of temperature on retention and separation of some triazolophthalazines

Sample	25°C		30°C		35°C		40°C		45°C	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
1	1.18	1.35	1.13	1.34	1.05	1.31	0.98	1.28	0.92	1.26
3	1.37	1.63	1.30	1.57	1.20	1.47	1.11	1.38	1.02	1.99
8	1.01	2.09	0.70	2.87	0.92	1.99	0.81	2.00	0.73	1.99
9	4.19	2.06	4.00	2.05	3.65	2.03	3.33	2.00	3.04	1.98

Apparatus (ii); column A[1]; flow-rate, 1 ml/min; mobile phase, *n*-hexane–2-propanol (9:1, v/v).

4. Conclusion

Chromatographic separations on four chiral stationary phases have been investigated. In contrast to the separation results on Chiralcel OD-H, which were very good, retention on Chiralcel OD-R was not possible. One explanation could be the composition of the analytes and the resulting interactions with the stationary phase.

Chiral stationary phases based on cyclodextrin could be suitable for the enantiomeric separation of these substances. Other derivatives of cyclodextrin would be better. Pirkle phases are also suitable; the retention and separation with other mobile phases must be investigated.

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