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Copolymeric (1*R-trans*)-*N,N′*-1,2-cyclohexylene-bisbenzamide oligodimethylsiloxane chiral stationary phase for gas chromatography

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Abstract

A copolymeric stationary phase, consisting of a chiral selective part, i.e. (1*R-trans*)-*N,N′*-1,2-cyclohexylenebisbenzamide, and an efficient siloxane oligomeric part, was successfully applied to open tubular column GC analysis. The efficiency and the chiral selectivity of this stationary phase were studied in detail, and high capacity and efficiency at elevated GC temperatures were especially noted. Several drugs and other enantiomeric pairs were separated. The shown examples demonstrate a broad application range for this type of chiral stationary phase in GC analysis.

Keywords: Chiral stationary phases, GC; Enantiomer separation; Copolymeric structure; *trans*-Cyclohexylenebisbenzamide

1. Introduction

The increasing need for enantiomeric pure drugs [1] makes it necessary to further develop enantioselective analysis methods, which also makes it important to investigate new structures for chiral stationary phases (CSPs).

The derivatives of *trans*-cyclohexylenebisbenzamide chiral entities have been successfully applied for chiral LC [3] and supercritical fluid chromatography (SFC) [4,5], but the use in GC has only rarely been mentioned [6]. The chiral centers of these stationary phases have twisted, rigid units, which offer good chiral recognition features toward enantiomers having similar structures (e.g., vicinal diols, oxazolidine rings, epoxides, etc.) close to their respective chiral centers [7]. The benzamide substituent groups of these CSPs make hydrogen bonding (acceptors and donors) and π–π chiral recognition interactions possible for the analytes of interest [5,8].

Several (1*R-trans*)-*N,N′*-1,2,-cyclohexylenebisbenzamide units and methylsiloxane oligomers con-

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taining block copolymeric CSPs have been synthesized and tested in open tubular SFC [5,8].

In this study, one optimized member (named ChDA) of this type of CSPs was selected and thoroughly studied under GC conditions.

2. Experimental

A detailed procedure for the preparation of these CSPs, and effects of substitution characteristics on the elution pattern in SFC have already been published [5,8].

In this study fused-silica capillaries 20 m×0.2 mm I.D. (Polymicro Technologies, Phoenix, AZ, USA) were first hydrothermally treated and thereafter deactivated with polar hydrosilane agents [9]. The capillaries were thereafter statically coated (d_f ~ 0.15 μm) with the ChDA stationary phase.

Test compounds were purchased from Fluka (Buchs, Switzerland) or donated by Novartis (Basle, Switzerland). Standard achiral derivatization procedures were conventionally used to obtain higher volatility, less polarity or more selective forms of tested enantiomers [10].

Carlo Erba 5160 Mega and Hewlett-Packard 5890 II gas chromatographs equipped with flame ionization detection (FID) system were used with hydrogen as carrier gas in the split mode (1:100). The peak widths at half height were measured for calculation of resolution values. The maximum allowable operation temperature (MAOT) was defined to be exceeded, where the column lost 5% of its retention power or selectivity after purging overnight with carrier gas [9].

3. Results

Several (1*R-trans*)-*N,N′*-1,2,-cyclohexylenebisbenzamide containing CSPs were successfully used in SFC [5,8]; however, the *para*-substituted version with three silicone atoms in each siloxane achiral block (ChDA) proved to be the best alternative for GC applications (Fig. 1). For example, this optimized version of ChDA for GC gave an R_s value of 1.5 ($n=35\,500$, $\alpha=1.050$) for the enantiomers of trifluoroacetamide derivatives (*N*-TFA) of 1-phenylethanamine at 150 °C. Similar CSPs [5,8] with achiral units with two silicone atoms and meta substitution resulted in a decreased efficiency and resolution, although some selectivity improvement was achieved ($R_s=1.2$, $n=18\,000$, and $\alpha=1.057$) for the same test compounds and temperature. On the other hand, longer, five-silicone atom achiral units resulted in a significantly decreased chiral selectivity and resolution ($R_s=1.0$, $n=58\,000$, and $\alpha=1.025$) under identical analysis conditions. The optimized composition of ChDA gives no overloaded peaks for 100 ng enantiomers, which is better than the capacity of chemically bonded cyclodextrin CSPs [11].

The ChDA-coated column provided 97 600 plates (4880 plates/m) for enantiomers of *N*-TFA derivatives of 1-(2-naphthyl)ethanamine at high temperature (210 °C), which, however, diminishes at lower analysis temperature.

The efficiency loss and the selectivity improvement with decreasing temperature may produce a maximum of the resolution values as a function of temperature as demonstrated in Fig. 2. The efficiency loss was found to become serious below 110 °C in the GC mode. The slow mass transfer in the stationary phase, arising from increased rigidity of the CSP,

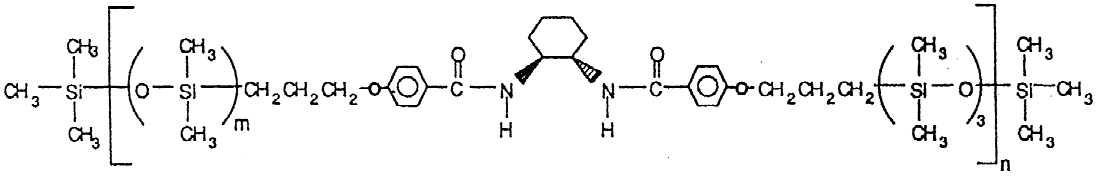


Fig. 1. Structure of copolymeric (1*R-trans*)-*N,N′*-1,2-cyclohexylenebisbenzamide oligodimethylsiloxane chiral stationary phase (ChDA) used for GC analysis.

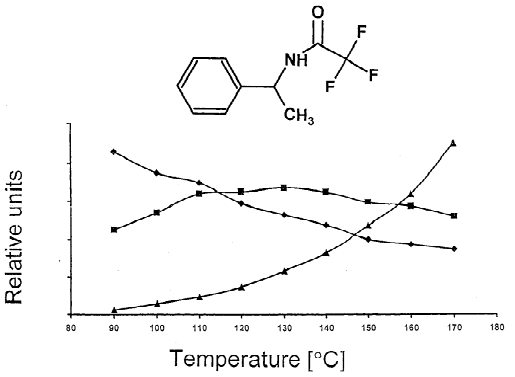


Fig. 2. Chromatographic characteristics of TFA derivatives of 1-phenylethanamine as a function of analysis temperature. Conditions: 20 m×0.20 mm I.D. fused-silica open tubular column coated with ChDA (0.15 μm); carrier, H_2. Symbol identification: (■) resolution values (1.1–1.7); (▲) number of theoretical plates (1900–67700); (♦) chiral selectivity (1.043–1.107).

causes a decrease in efficiency at low temperatures. The ChDA does not show a character of solid-phase, because the selectivity values give in continuous, unbroken increase with the decreasing temperature over the whole measured range. In SFC, however, even 50 °C analysis temperature could be used without efficiency loss, because the swelling effect of the supercritical mobile phase keeps the ChDA soft, preserving its fast mass transfer, high permeability character even at low separation temperature [5,12].

At the high-temperature end, the MAOT value of ChDA was found to be 260 °C. It was possible to reach this high MAOT value due to insertion of phenyl groups [9] between the rigid chiral centers and the flexible siloxane blocks. This high MAOT value is important when purging out remaining low volatile matrix components. The ChDA does, however, preserve surprisingly good chiral selectivity at relatively high analysis temperatures. Baseline resolution was thus observed for several compounds [e.g., *N*-(1-phenylethyl)benzamide, 1-(2-naphthyl)-ethanamine, metoprolol] even at 210 °C.

The retention of different classes of compounds was investigated. The five Rohrschneider–McReynolds constants of the ChDA thus gave the following values at 100 °C: benzene 118.8, *n*-butanol 310.8, 2-pentanone 202.5, nitropropane 275.0 and pyridine 275.2. These values show ability for both π–π and H-bond interactions.

A large number of enantiomeric pairs, having various structures and functional groups, have been separated using ChDA in the GC mode. Characteristic parameters of achieved selectivity and resolution values are summarized in Table 1. In some occasions higher separation values were achieved than referred to in Table 1, but those separations resulted in decreased resolution values or impractically long retention times.

High and favorable selectivity values were measured for several enantiomers having combined aromatic groups and hydrogen bonding abilities in the α positions from the asymmetric center (e.g., Table 1, Nos. 16–23, 29–30, 32, 34). The aromatic ring at the α position of the asymmetric center was necessary for the reasonable chiral recognition of these amines, because the amphetamine and metamphetamine with β position substitution did not show any resolutions. General for all enantiomeric amine was that the *N*-Ac derivatives gave higher selectivity values than the *N*-TFA derivatives at the same analysis temperature. On the other hand, the *N*-TFA derivatives had approximately five times less analysis times and only 1 unit in the third digit less selectivity values than *N*-Ac derivatives, which made the *N*-TFA derivatives also practical to be applied in several occasions.

Expanding the aromatic ring system from phenyl to naphthyl resulted in selectivity increase (Table 1, No. 16 versus 22, 23). It should be noted that the elution order of these derivatives of aryl-alkyl amines was (*S*) before (*R*), which becomes obvious from Fig. 3. This elution order is just the opposite from that observed on a Pirkle type stationary phase of a naphthylethyl amide containing siloxane polymeric CSP [13]. This complementary feature of these two CSPs offers good ability for exact determination of an extreme enantiomeric ratio of aryl amine derivatives, achieving primary first elution of the minor isomer [2].

ChDA is an excellent CSP for the chiral separation of β-blockers in their oxazolidine ring derivative forms (Table 1, Nos. 26–28, Fig. 4), similar to LC applications [3]. The rigid oxazolidine ring improves

Table 1
Chromatographic data of separated enantiomers on ChDA with GC

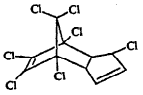
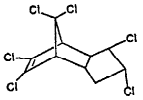
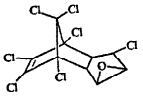
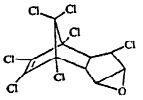
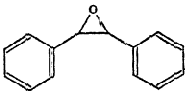
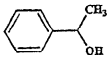
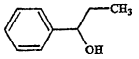
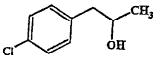
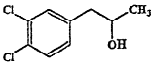
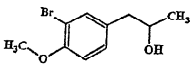
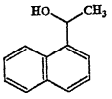
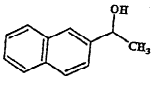
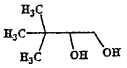
No.	Structure	Name	Functional group (analyzed form)	Analysis temp. (C°)	Retention time[a] (min)	Resolution (R_s)	Chiral selectivity (α)
1		Heptachlor	Chloro	160	68.024	1.1	1.017
2		*trans*-Chlordane	Chloro	150	86.167	0.9	1.009
3		*exo*-Heptachlor epoxide	Epoxide	150	102.962	1.0	1.016
4		*endo*-Heptachlor epoxide	Epoxide	140	177.551	1.0	1.019
5		*trans*-Stilbene-oxide	Epoxide	160	8.447	1.5	1.024
6		1-Phenylethanol	Alcohol	100	4.207	0.6	1.015
7		1-Phenylpropan-1-ol	Alcohol	100	7.341	0.9	1.023
8		1-(4-Chlorophenyl) propan-2-ol	Alcohol (benzoyl)	120	226.020	0.9	1.019
9		1-(3,4-Dichlorophenyl) propan-2-ol	Alcohol (Ac)	140	8.875	0.5	1.009
10		1-(3-Bromo-4-methoxyphenyl)propan-2-ol	Alcohol (Ac)	140	20.140	0.6	1.013
11		1-(1-Naphthyl)ethanol	Alcohol (Ac)	140	12.296	1.1	1.021
12		1-(2-Naphthyl)ethanol	Alcohol (Ac)	140	13.447	1.3	1.025
13		3,3-Dimethylbutane-1,2-diol	Diol	110	6.493	0.7	1.043

Table 1. Continued

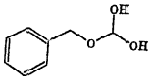
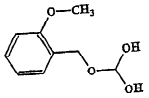
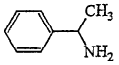
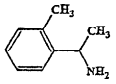
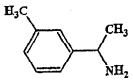
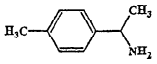
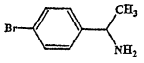
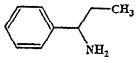
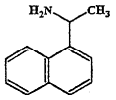
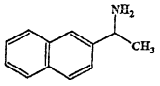
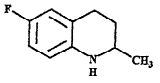
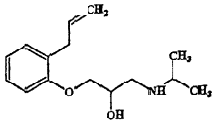
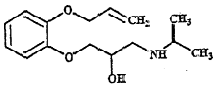
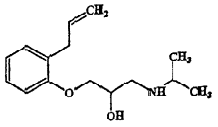
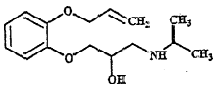
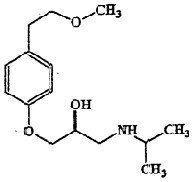
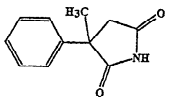
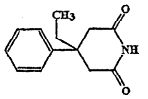
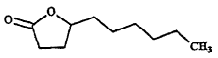
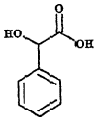
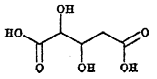
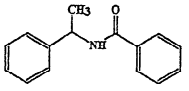
No.	Structure	Name	Functional group (analyzed form)	Analysis temp. (C°)	Retention time[a] (min)	Resolution (R_s)	Chiral selectivity (α)
14		3-(Benzyloxy) methanediol	Diol (cyclic carbonate)	170	33.195	0.8	1.012
15		Guafenesin	Diol (cyclic carbonate)	160	45.668	1.5	1.010
16		1-Phenylethanamine	Amine (*N*-Ac)	160	6.649	2.2	1.052
17		1-(2-Methylphenyl) ethanamine	Amine (*N*-Ac)	160	7.046	2.2	1.051
18		1-(3-Methylphenyl) ethanamine	Amine (*N*-Ac)	160	9.585	2.5	1.053
19		1-(4-Methylphenyl) ethanamine	Amine (*N*-Ac)	160	9.992	3.1	1.057
20		1-(4-Bromophenyl) ethanamine	Amine (*N*-Ac)	160	37.419	5.2	1.073
21		1-Phenylpropopan-1-amine	Amine (*N*-Ac)	160	8.825	3.3	1.063
22		1-(1-Naphthyl) ethanamine	Amine (*N*-Ac)	180	32.796	4.1	1.077
23		1-(2-Naphthyl) ethanamine	Amine (*N*-Ac)	180	43.781	3.9	1.059
24		6-Fluoro-2-methyl-1,2,3,4-tetrahydro-quinoline	Amine (*N*-Ac)	140	11.081	1.5	1.034
25		*trans*-2-Aminocyclohexylamine	Diamine (*N*-Ac)	170	24.439	3.4	1.064
26		Alprenolol	β-Aminoalcohol (oxazolidine)	180	53.271	1.4	1.026

Table 1. Continued

No.	Structure	Name	Functional group (analyzed form)	Analysis temp. (C°)	Retention time[a] (min)	Resolution (R_s)	Chiral selectivity (α)
27		Oxprenolol	β-Aminoalcohol (oxazolidine)	190	43.958	1.0	1.013
26		Alprenolol	β-Aminoalcohol (oxazolidine)	180	53.271	1.4	1.026
27		Oxprenolol	β-Aminoalcohol (oxazolidine)	190	43.958	1.0	1.013
28		Metoprolol	β-Aminoalcohol (oxazolidine)	200	85.469	1.5	1.023
29		3-Methyl-3-phenylpyrrolidine-2,5-dion	Imide	150	58.24	0.9	1.012
30		Glutethimide	Imide	150	73.11	1.5	1.025
31		5-Hexyldihydrofuran-2(3H)-one	Lastone	120	17.214	0.7	1.022
32		Mandelic acid	Hydroxy acid (methyl ester)	120	37.125	1.1	1.025
33		Tartaric acid	Hydroxy acid (diethyl ester)	130	41.871	1.7	1.051
34		*N*-(1-Phenylethyl) benzamide	amide	170	35.274	2.7	1.051

[a] Retention time of firstly eluting peaks.

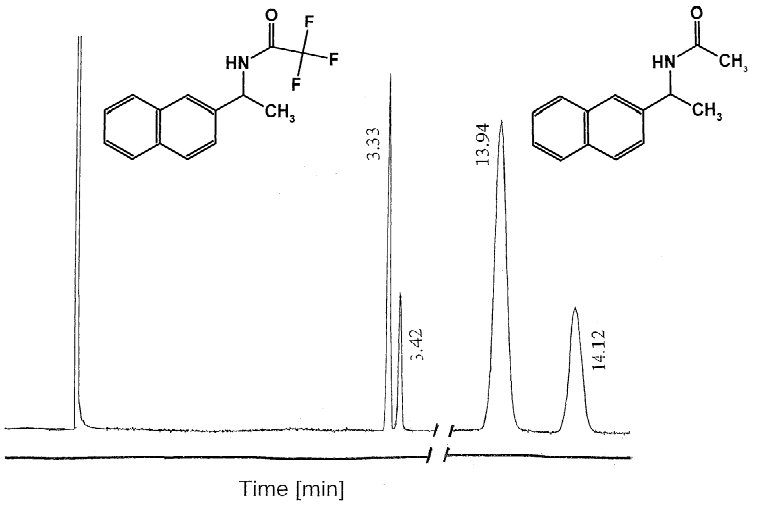


Fig. 3. Enantiomeric separation of 1-(2-naphthyl)ethanamine as trifluoroacetamide (*N*-TFA) and acetamide (*N*-Ac) derivatives with *S* isomer excess. Conditions: 20 m×0.20 mm I.D. fused-silica open tubular column coated with ChDA (0.15 μm); 210 °C; carrier, H_2.

the chiral recognition on the ChDA, because none of the measured β-blockers could be enantiomerically separated on ChDA using derivatization with acetic acid anhydride or trifluoroacetic acid anhydride.

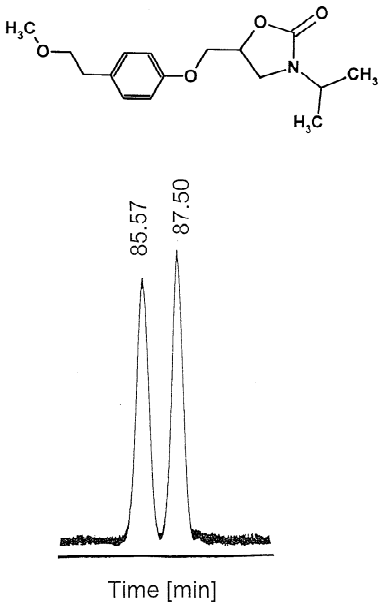


Fig. 4. Enantiomeric separation of rac-metoprolol (lopressor) β-blocker as oxazolidine derivatives. Conditions: 20 m×0.20 mm I.D. fused-silica open tubular column coated with ChDA (0.15 μm); 200 °C; carrier, H_2.

The enantiomers of aryl-alkyl alcohols having hydroxyl groups in their α position (Table 1, Nos. 6, 7, 11, 12) show also chiral recognition features on ChDA. By increasing the aromatic group size from phenyl to naphthyl, the selectivity also has improved. In some cases, optical isomers of aryl-alkyl alcohols with hydroxyl groups in the β position (Table 1, Nos. 8, 9) could also be separated.

Enantiomers of the frequently used chiral test compounds, *trans*-stilbene oxide (Table 1, No. 5) have also been separated on ChDA. The temperature dependence on chiral selectivity for *trans*-stilbene oxide shows an abnormal behavior. The change of α values of *trans*-stilbene oxide covers a very narrow band (1.024–1.021) comparing the normal behavior of α values (1.087–1.042) for *N*-TFA derivatives of 1-phenylethanamine in the observed temperature range (i.e., 110–190 °C). This behavior of *trans*-stilbene oxide has not been reported earlier for any other CSP.

Not only the aromatic, but also other ring systems show chiral selectivity on ChDA (Table 1, Nos. 1–4, 25, 31). The lack of the aromatic ring, however, results in moderate selectivity, but serving evidence for the hydrogen bonding ability and appropriate rigid structure of enantiomers can be enough for a good chiral recognition of ChDA.

Aliphatic enantiomers having twisted vicinal diol structures, however, are separated with high selec-

tivity on ChDA (Table 1, Nos. 13, 33). The chiral vicinal diols with higher molecular mass can be well separated in rigid cyclic derivatives, like cyclic carbonate (see Table 1, Nos. 11, 12).

The enantioselectivity is higher under GC than under SFC conditions for the same enantiomers and at the same analysis temperature on ChDA. For example, the *N*-TFA derivatives of 1-phenyl-ethanamine has an α value of 1.094 in GC and only 1.041 in SFC at 100 °C. The lower selectivity experienced in SFC are caused by the solvated sphere of mobile phase around the potential interaction sites [12].

The ChDA provides high-resolution separation of a broad spectrum of enantiomers in GC. This chiral stationary phase shows a working temperature range of 110–260 °C, but its efficiency is high only above 150 °C. A ring (e.g., aromatic, oxazoidine) in the α position to the chiral center, a twisted structure (diols, amino alcohols, hydroxyl acids, etc.) and hydrogen bonding interaction abilities plays a role in its chiral recognition character. It is expected that the ChDA stationary phase can solve further applications in GC and SFC, serving as a valuable alternative to the cyclodextrin and Chirasil-Val CSPs in the analysis of diols, amino alcohols and other enantiomers.

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References

[1] Department of Health and Human Service of USA Food and Drug Administration's Policy Statement for the Development of New Stereoisomeric Drugs, Fed. Reg. 57, 2 May 1992, 102.
[2] V. Schurig, J. Chromatogr. A 906 (2001) 275.
[3] F. Gasparrini, D. Misiti, C. Villani, F. La Torre, J. Chromatogr 539 (1991) 25.
[4] F. Gasparrini, D. Misiti, C. Villani, J. High Resolut. Chromatogr. 13 (1990) 182.
[5] P. Petersson, K.E. Markides, D.F. Johnson, B.E. Rossiter, J.S. Bradshaw, M.L. Lee, J. Microcol. Sep. 4 (1992) 155.
[6] H.J. Chaves das Neves, J.P. Noronha, H. Rufino, J. High Resolut. Chromatogr. 19 (1996) 161.
[7] F. Gasparrini, D. Misiti, M. Pierini, C. Villani, J. Chromatogr. A 724 (1991) 79.
[8] B.E. Rossiter, P. Petersson, D.F. Johnson, M. Euguchi, J.S. Bradshaw, K.E. Markides, M.L. Lee, Tetrahedron Lett. 32 (1991) 3609.
[9] Z. Juvancz, M.A. Pulsipher, M.M. Schirmer, R.S. Johnson, K.E. Markides, J.S. Bradshaw, M.L. Lee, J. Microcol. Sep. 1 (1989) 309.
[10] K. Blau, J.M. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, New York, 1993.
[11] Z. Juvancz, K. Grolimund, V. Schurig, J. High Resolut. Chromatogr. 16 (1993) 202.
[12] Z. Juvancz, K.E. Markides, LC·GC Int. 5 (4) (1992) 44.
[13] Z. Juvancz, K.E. Markides, C.A. Rouse, K. Jones, B.J. Tarbet, J.S. Bradshaw, M.L. Lee, Enantiomer 3 (1998) 89.