ELSEVIER

Journal of Chromatography A, 699 (1995) 215–221

JOURNAL OF CHROMATOGRAPHY A

Backbone modification of Chirasil-Val I. Effect of loading on the separation of enantiomers by gas chromatography

Bernhard Koppenhoefer*, Ulrich Mühleck, Konrad Lohmiller

Institut für Organische Chemie der Universität, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

First received 29 November 1994; revised manuscript received 10 February 1995; accepted 16 February 1995

Abstract

For the gas chromatographic phase Chirasil-Val, the effect of loading of the polysiloxane backbone with L-valine *tert.*-butylamide units on the chromatographic properties is described. For three enantiomeric pairs of N-trifluoroacetyl amino acid *n*-propyl esters, a maximum separation factor (α) was observed when the ratio x:y of x dimethylsiloxane units per y chirally modified dialkyl siloxane units was adjusted to the range of x:y = 5.6–5.9; whereas a ratio x:y = 7 has proven more suitable for the complete separation of all 39 components present in a mixture of the 20 proteinogenic amino acids.

1. Introduction

Enantiomer separation via gas chromatography (GC) [1] is an ever increasing branch of analytical chemistry; a release (3/94) of the molecular database Chirbase/GC [2] documents 10700 separations of 4648 enantiomeric pairs on 324 chiral stationary phases (CSPs). This methodology allows the determination of the enantiomeric purity down to 0.01% [11,14]. One of the most prominent CSPs, commercially available under the tradename Chirasil-Val (Chrompack, Middelburg, Netherlands) [3–6] is applicable for a large number of compounds, such as dipeptides [7,8], sulfur compounds [9,10], diols [11,12], hydroxy- and carbonyl compounds [13], and others. However, this well-documented phase is still good for a surprize: resolution factors higher than 2.0 were recently observed for imidazolyl compounds [15], thus pointing to an unexploited potential for separating chiral drugs by GC.

One of the key issues in fine-tuning the properties of a CSP is the proper adjustment of the polarity to the given separation problem. The simultaneous determination of all 20 proteinogenic amino acids, important for medical analysis as well as peptide synthesis (automatic amino acid analysis, ASA) [16], on Chirasil-Val columns requires that no peak overlap occurs. Variation of the temperature program alone is not sufficient to accomplish this cumbersome task, unless the polarity of the stationary phase is adapted with great care. To avoid peak interference is particularly difficult for the eight enantiomeric

* Corresponding author.

0021-9673/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(95)00215-4

pairs and one achiral analyte typically eluting in the temperature range of 90–120°C, i.e., the derivatives of Val, Gly, Thr, α-Ile, Ile, Ser, Pro and Leu. The successful optimisation of the polarity during chemical synthesis requires complete control of the two main steps, (i) synthesis of a copolymer with well-defined structure, and (ii) coupling of valine *tert.*-butylamide to the copolymer carboxylic function with a high and reproducible yield.

Following preliminary studies [17], we describe here the synthesis of Chirasil-Val type CSPs from copolymers with different x:y ratios of x dimethylsiloxane units and y monomethyl(2-carboxypropyl)siloxane units that were coupled with L-valine *tert.*-butylamide. Our final goal was to accomplish a complete peak resolution of all proteinogenic amino acids by systematically changing the x:y ratio.

2. Experimental

2.1. Materials

1,1′-Carbonyldiimidazole (CDI), 1,3-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS), hexamethyldisiloxane and octamethylcyclotetrasiloxane (D_4) were purchased from Fluka (Buchs, Switzerland); O-benzotriazolyl-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), oxalyl chloride, perfluoropropanoic anhydride and trifluoroacetic anhydride were supplied commercially by Merck (Darmstadt, Germany).

2.2. Copolymerisation

β-(Dichloromethylsilyl)-α-methylpropanoic acid [18], γ-(dichloromethylsilyl)-*n*-butyronitrile [18], poly-(β-methylsiloxy-α-methylpropanoic acidmethylester) [18], poly-(γ-methylsiloxy-*n*-butyronitril) [18], poly-(γ-methylsiloxy-*n*-butanoic acid) [18], poly-(γ-methylsiloxy-α-methylpropanoic acid [18], dimethyl-methyl(2-carboxypropyl)siloxane copolymer 5:1 (variation 1 with dichlorodimethylsilane) [18], were prepared according to the literature. All compounds gave the ^{1}H NMR spectra expected.

2.3. Dimethyl-methyl(2-carboxypropyl)siloxane copolymer 5:1, variation 2 with octamethylcyclotetrasiloxane (D_4)

In a 100-ml three-necked flask, equipped with mechanical stirrer and reflux condenser, poly-(β-methylsiloxy-α-methylpropanoic acid (10.1 g, 69.1 mmol), D_4 (25.6 g, 86.4 mmol), and hexamethyldisiloxane (3.17 ml, 15.2 mmol) were mixed, and concentrated sulfuric acid (0.65 ml) was added. The mixture was stirred at 100°C for four days, and caoline (0.5 g) was added. After cooling, the copolymer was dissolved in dichloromethane and filtered. The organic layer was washed trice with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo with a rotary evaporator to give a viscous mass, yield 78.9%. Elemental analysis: found, C 34.50%, H 7.80%; calculated, C 34.85% H 7.79%.

2.4. Dimethyl-methyl(3-carboxypropyl)siloxane copolymer 5:1

In a 100-ml three-necked flask, equipped with mechanical stirrer and reflux condenser, poly-(γ-methylsiloxy-*n*-butanoic acid) (10.1 g, 69.1 mmol), D_4 (25.6 g, 86.4 mmol), and hexamethyldisiloxane (3.17 ml, 15.2 mmol) were mixed, and concentrated sulfuric acid (0.65 ml) was added. The mixture was stirred at 100°C for four days, and caoline (0.5 g) was added. After cooling, the copolymer was dissolved in dichloromethane and filtered. The organic layer was washed trice with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to give a viscous mass, yield 82.9%. Elemental analysis: found, C 34.48%, H 7.75%; calculated, C 34.85% H 7.79%.

2.5. Monomeric valine derivatives

tert.-Butyloxycarbonyl-L-valine [18,19], *tert.*-butyloxycarbonyl-L-valine *tert.*-butylamide [18] and L-valine *tert.*-butylamide × -HCl [18] were synthesised according to literature procedures.

2.6. L-Chirasil-Val

Dimethyl methyl(2-carboxypropyl)siloxane copolymer 5:1 was coupled to L-valine *tert*-butylamide with 1,1′-carbonyldiimidazole (CDI) as described [18].

Alternatively, O-benzotriazolyl-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) [20] and oxalyl chloride [21], respectively, served as the coupling reagent.

2.7. Derivatisation of the amino acids [22–24]

Prior to the esterification reaction, a mixture of acetyl chloride (20%, v/v) in *n*-propanol was reacted at 0°C to give a solution of hydrochloric acid in *n*-propanol [note: for methyl and ethyl esters, a mixture (10%, v/v) of acetyl chloride in the respective alcohol has proven sufficient]. A sample of the amino acid (3 mg) was placed in a Reacti-Vial (Macherey-Nagel), the reagent (0.3 ml) described above was added, and the mixture was heated for 30 min to 110°C [note: for isopropyl- and 3-pentyl esters, the reaction time had to be extended to 1 h]. After cooling the vial to ambient temperature, the volatiles were removed in a gentle stream of nitrogen.

Acylation was performed in the same Reacti-Vial by addition of trifluoroacetic acid anhydride (0.1 ml) (TFA anhydride, note: an analogous procedure may be applied for the preparation of perfluoropropanoates and heptafluorobutanoates, respectively). The mixture was treated for 10 min at 110°C [note: Arg derivatives were treated for 1 h at 140°C]. After cooling the vial, the solvent and excess reagents were removed in a gentle stream of nitrogen, and the residue was dissolved in dichloromethane (0.1 ml).

2.8. Analysis of the stationary phase composition: gas chromatography ASA and determination of the enantiomeric purity by enantiomer labelling [3,25–29]

The chirally modified polysiloxane (2 mmol) was placed in a Reacti-Vial. After addition of a solution (1 ml) of 6 *M* hydrochloric acid in *n*-propanol, the compound was hydrolysed for 24 h at 110°C. The solvent was removed in a stream of nitrogen, and 0.1 *M* hydrochloric acid (0.1 ml) was added. The solution was transferred into a graduated cylinder and filled up to 10 ml. Two portions (100 μl each) of this solution were transferred to two ampoules (supplied by CAT, Tübingen, Germany), and one of them was mixed with the D-amino acid standard. Both samples were evaporated to dryness in a stream of nitrogen, derivatised and analysed by gas chromatography on a Chirasil-Val column.

2.9. Glass capillaries

Glass capillaries (0.3 mm I.D.) were drawn from washed (50% HNO_3, 10% HF, 40% H_2O) Duran 50 glass tubes on a Hupe and Busch glass capillary drawing machine. After treatment with 0.3% colloidal SiO_2 in acetone [30,31], the capillaries were leached with 18% hydrochloric acid for 12 h at 150°C, washed with 0.1% hydrochloric acid, and dried (24 h at 270°C) [32], followed by deactivation with 1,3-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) (12 h at 250°C) and static coating with a 0.3% solution of the chiral polysiloxane in a mixture (9:1, v/v) of pentane and dichloromethane.

2.10. Instrumentation

Gas chromatography was performed with hydrogen as the carrier gas on a Carlo Erba Fractovap Model 2101, equipped with a flame ionisation detector (FID) and an Hewlett-Packard 3390 A electronic integrator.

Proton nuclear magnetic resonance (^{1}H NMR, ^{29}Si NMR) spectra were recorded on a Bruker AC 250 at 250 MHz, ^{13}C NMR spectra on a Bruker WP 80, and solid ^{29}Si NMR spectra on a Bruker MSL.

Elemental analyses were carried out on a Carlo Erba Elemental Analyser Model 1104.

3. Results and discussion

In previous investigations, the problem of batch-to-batch variation of the polarity of Chirasil-Val was addressed in different ways.

Thumm [33] described two methods to correct the polarity: (i) variation of the polysiloxane backbone by inserting diphenylsiloxy groups; (ii) blending the chiral phase with the achiral polar modifier poly(γ-methylsiloxybutanoic acid-*n*-butylamide), either chemically linked by insertion during the synthesis, or physically mixed after the reaction. Küsters [34] achieved a shift in polarity by mixing batches of Chirasil-Val prepared by coupling with N,N′-dicyclohexylcarbodiimide (DCCI) with those prepared by coupling with CDI. Walser [18] investigated binary mixtures of Chirasil-Val with either the Kovats phase or with the achiral cyanoallyl silicon phase poly(β-methylsiloxy-α-methylpropanoic acid methylester), better known as OV-275.

In contrast to these early attempts to improve the resolution of different amino acids by addition of a modifier to Chirasil-Val, we describe here an almost complete resolution of all proteinogenic amino acids merely by systematic variation of the copolymer composition itself. To this end, a series of copolymers with different ratios *x*:*y* were synthesised and coupled with L-valine *tert.*-butylamide under similar conditions.

All copolymers and chiral phases were characterised by ^{1}H NMR spectroscopy. The amino acid content of the chiral phases was determined by acidic hydrolysis of a defined amount of stationary phase in a Reacti-Vial in 6 *M* hydrochloric acid–propanoic acid at 110°C for 24 h [36], followed by two-step derivatisation, addition of a D-standard and gas chromatography on L-Chirasil-Val (see Experimental); in the following, this procedure is referred to as ASA. A comparison of the valine contents determined by ASA with those derived from ^{1}H NMR spectroscopy showed a good agreement of the two methods.

A definite *x*:*y* ratio could be obtained by equilibration of poly-(γ-methylsiloxy-*n*-butanoic acid) with D_4 at increased temperature. The same procedure was applied to the branched-chain analogon poly-(β-methylsiloxy-α-methylpropanoic acid). For both derivatives, it was even possible to alter the ratio *x*:*y* after close examination by ^{1}H NMR spectroscopy. The ^{29}Si NMR spectrum gives a clue to the proportion of different siloxane units present. High coupling yields of L-valine *tert.*-butylamide with the polysiloxane carboxylic acid functions were observed for both coupling reagents, CDI and HBTU.

A series of polysiloxanes linked to amides of the branched side-chain β-methylsiloxy-α-methylpropanoic acid are compiled in Tables 1 and 2. For each phase, two capillary columns were made. To this end, a Duran-glass capillary was wall-deactivated with chlorodiphenyl methylsilane (CDPMS) and coated statically with a 0.25% solution of the polysiloxane in a mixture of dichloromethane–pentane (1:9, v/v). The enantioselectivity was tested with a mixture of three O,N-TFA-amino acid propyl esters, prepared in a two-step procedure from the

Table 1
Valine content c_{Val} of L-Chirasil-Val for different loadings *x*:*y*

Ratio *x*:*y*[a]	Valine content c_{Val} (mmol/g)		Coupling yield[c] (%)
	calculated	found[b]	
2.6	2.02	2.10	103.9
3.5	1.78	1.60	89.7
5.6	1.40	1.43	102.2
5.9	1.36	1.36	100.3
7.1	1.21	1.26	103.9

[a] As determined by ^{1}H NMR spectroscopy.
[b] As determined by ASA.
[c] Calculated from the results of ^{1}H NMR spectroscopy and ASA.

Table 2
Resolution factors α of selected N-TFA-amino acid-O-*n*-propyl esters on L-Chirasil-Val for different loadings x:y

Ratio x:y	Resolution factor α[a]		
	Thr	Pro	Leu
2.6	1.15	1.035	1.25
3.5	1.17	1.037	1.28
5.6	1.24	1.041	1.40
5.9	1.24	1.040	1.40
7.1	1.17	1.035	1.32

[a] $\alpha = (t'_L - t_0)/(t'_D - t_0)$, where t'_L and t'_D are net retention times of L- and D-enantiomer, respectively, and t_0 is the dead time.
Conditions: isothermal at 100°C; $p_i = 0.35$ kg/cm^2.

amino acids proline, threonine, and leucine, respectively.

It should be noted that the resolution factors (α) do not necessarily increase for a higher loading of the polymer backbone with chiral groups. In fact, the stationary phases tested gave the highest α-values for a ratio x:y = 5.6. A smaller ratio x:y gives rise to stronger intramolecular hydrogen bonds that also result in a slight increase in viscosity. It turned out that these stronger interactions between the chiral groups of the polymer are accompanied by a decreased enantioselectivity of these phases. Unfortunately, the softening temperatures of the heavily loaded phases turned out rather high, reaching up to 120°C. Thus, the use of these phases is restricted to higher temperatures where the separation is diminished considerably by an unfavorable entropy contribution. Below the softening temperature, on the other hand, the separation performance in terms of the effective number of theoretical plates is rather poor. This corresponds with the observations of Thumm [33].

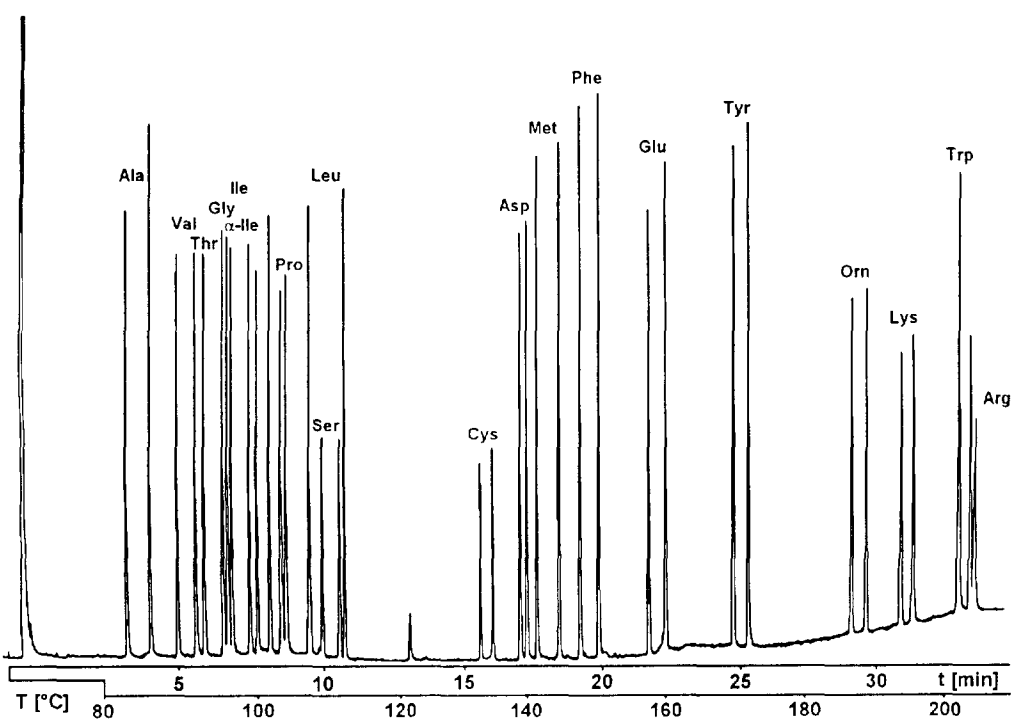

Fig. 1. Gas chromatogram of the separation of racemic amino acids on L-Chirasil-Val 5.6:1 (thermally immobilised); Conditions: TFA-amino acid propyl esters; Temperature program: 80°C, 3 min, 4°C/min to 200°C; $p_i = 0.35$ kg/cm^2, H_2 as carrier gas; 20 m × 0.3 mm glass capillary, deactivated with DPTMDS.

More recent investigations showed that the long-term stability of columns coated with Chirasil-Val may be improved by immobilisation of the stationary phase, which can be achieved either thermally or through free radicals [36]. Although both methods tend to reduce the enantioselectivity of the chiral stationary phase, a complete resolution of all components of the amino acid mixture could still be achieved. After coating a capillary column with L-Chirasil-Val 5.6:1, immobilisation of the phase took place by prolonged thermal conditioning. The separation of the amino acid test mixture by gas chromatography on this column is shown in Fig. 1.

4. Conclusions

We have described the tailoring of a chirally modified polysiloxane copolymer by equilibration of the corresponding homopolymer with the cyclotetrasiloxane D_4. This variation could be easily adapted to any given separation task. For the separation of amino acid enantiomers, it turned out that the optimum of the enantioselectivity, as determined by the resolution factors (α) of three trifluoroacetyl amino acid propyl esters, is reached at a ratio $x:y = 5.6$, while the polarity adjustment necessary for a complete separation of all 39 components of the proteinogenic amino acid text mixture favors a ratio $x:y = 7$. As displayed in Fig. 1, the latter ratio meets both criteria in a very fortunate way.

Acknowledgements

We are indebted to Prof. Dr. E. Bayer and Mr. G.J. Nicholson for helpful discussions. Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie are acknowledged for financial support.

References

[1] E. Gil-Av, J. Mol. Evol., 6 (1975) 131.
[2] B. Koppenhoefer, A. Nothdurft, J. Pierrot-Sanders, P. Piras, C. Popescu, C. Roussel, M. Stiebler, U. Trettin, Chirality, 5 (1993) 213.
[3] H. Frank, G.J. Nicholson, E. Bayer, J. Chromatogr. Sci., 15 (1977) 174.
[4] E. Bayer, Z. Naturforsch., 38b (1983) 1281.
[5] E. Bayer, H. Frank, J. Gerhardt, G.J. Nicholson, J. Assoc. Off. Anal. Chem., 70 (1987) 234.
[6] E. Bayer, G.J. Nicholson, H. Frank, in C.W. Gehrke, K.C.T. Kuo, R.W. Zumwalt (Editors), Amino Acid Analysis by Gas Chromatography, Vol. II, CRC Press, Boca Raton, FL, Ch. 2, 1987.
[7] B. Koppenhoefer, H. Allmendinger, P.C. Lu, B.C. Lin, J. Chromatogr., 441 (1988) 89.
[8] B.C. Lin, E. Bayer, V. Muschalek and B. Koppenhoefer, Science in China (Series B), 34 (1991) 769.
[9] E. Küsters, H. Allgaier, G. Jung, E. Bayer, Chromatographia, 18 (1984) 287.
[10] E. Bayer, E. Küsters, G.J. Nicholson, H. Frank, J. Chromatogr., 320 (1985) 393.
[11] B. Koppenhoefer, U. Trettin, R. Figura, B. Lin, Tetrahedron Lett., 30 (1989) 5109.
[12] B. Koppenhoefer, B. Lin, J. Chromatogr., 481 (1989) 17.
[13] B. Koppenhoefer, H. Allmendinger, G. Nicholson, Angew. Chem., 97 (1985) 46.
[14] B. Koppenhoefer, V. Muschalek, M. Hummel, E. Bayer, J. Chromatogr., 477 (1989) 139.
[15] J.M. Briody, D. Keenan, G.J. Nicholson, B. Koppenhoefer, Chirality, 5 (1993) 229.
[16] J. Gerhardt, Thesis, University of Tübingen, Tübingen, Germany, 1984.
[17] E. Bayer, H. Frank, ACS Symp. Ser., 121 (1980) 341.
[18] M. Walser, Thesis, University of Tübingen, Tübingen, Germany, 1987.
[19] L. Moroder, A. Hallett, E. Wünsch, O. Keller, G. Wersin, Hoppe-Seyler's Z. Physiol. Chem., 357 (1976) 1651.
[20] V. Dourtoglou, B. Gross, V. Lambropoulou, C. Zioudrou, Synthesis, (1984) 572.
[21] W.A. König, The Practice of Enantiomer Separation by Capillary Gas Chromatography, Hüthig Verlag, Heidelberg, Germany, 1987, p. 14.
[22] H. Frank, D. Bimboes, G.J. Nicholson, Chromatographia, 12 (1979) 168.
[23] H. Frank, G.J. Nicholson, E. Bayer, J. Chromatogr., 146 (1978) 197.
[24] H. Frank, G.J. Nicholson, E. Bayer, Angew. Chem., 90 (1978) 396.
[25] H. Frank, G.J. Nicholson, E. Bayer, J. Chromatogr., 167 (1978) 187.
[26] W. Woiwode, H. Frank, G.J. Nicholson, E. Bayer, Chem. Ber., 111 (1978) 3711.
[27] H. Frank, W. Woiwode, G. Nicholson, E. Bayer, Liebigs Ann. Chem., (1981) 354.
[28] G.J. Nicholson, H. Frank, E. Bayer, J. High Resolut. Chromatogr., Chromatogr. Commun., 2 (1979) 411.
[29] E. Wünsch, Synthese von Peptiden, in Methoden in der Organischen Chemie (Houben–Weyl–Müller, Ed.), Vol. XV/1, Georg Thieme Verlag, Stuttgart, Germany, 1974.
[30] E. Schulte, Chromatographia, 9 (1976) 315.
[31] G. Nicholson, personal communication.

[32] K. Grob, Making and Manipulating Capillary Columns for Gas Chromatography, Hüthig Verlag, Heidelberg, Germany, 1986, p. 105.
[33] D. Thumm, Thesis, University of Tübingen, Tübingen, Germany, 1980.
[34] E. Küsters, Thesis, University of Tübingen, Tübingen, Germany, 1983.
[35] Shui-Tein Chen, Shyh-Horno Chiou, Yen-Ho-Chu, Kung-Tsung Wang, Int. J. Pepide Protein Res., 30 (1987) 572.
[36] G. Lai, G. Nicholson, E. Bayer, Chromatographia, 26 (1988) 229.