ELSEVIER

Journal of Chromatography A, 663 (1994) 79–85

JOURNAL OF CHROMATOGRAPHY A

Gas chromatographic separation of the enantiomers of volatile fluoroether anesthetics by derivatized cyclodextrins II. Preparative-scale separations for isoflurane

Daniel U. Staerk, Aroonsiri Shitangkoon, Gyula Vigh *

Department of Chemistry, Texas A & M University, College Station, TX 77843-3255, USA

(First received October 19th, 1993; revised manuscript received November 30th, 1993)

Abstract

Preparative-scale gas chromatographic separation of the enantiomers of isoflurane, a volatile anesthetic, has been achieved with 2 m × 10 mm I.D. columns, packed with 80–100 mesh Chromosorb W AW, coated with up to 23% (w/w) trifluoroacetyl γ-cyclodextrin as stationary phase. The type and particle size of the support, the concentration of the stationary phase and the separation conditions were varied to improve product purity and the production rate under overloaded elution mode conditions. Using a sampling interface located between the exit of the preparative column and the fraction collector, effluent samples were directed onto a short, high-efficiency analytical capillary column every 30 s to achieve on-line enantiomeric analysis of the eluting bands, which allowed the calculation of purity, recovery and production rate for the separations.

1. Introduction

In the early 1970s, there were some exciting developments in preparative-scale gas chromatography (GC) which culminated in 1–4 in. (1 in. = 2.54 cm) I.D. packed columns with plate heights on the order of 1 mm, cycle times less than 1 h and production rates of 50 mg/day to 100 g/day [1–3]. Though preparative GC never replaced distillation as an industrial production method, due to advances in modelling and engineering in the 1980s, Bonmati *et al.* [4] were able to design and realize systems with production rates as high as 100 g/day to 100 kg/day.

Though there is a strong, documented need for pure enantiomers in the life sciences [5], very little has been published about the efforts aimed at their production by preparative GC. Schurig and Leyrer [6,7] were able to isolate, in 2- to 20-h long runs, up to 10-mg quantities of the pure enantiomers of pheromones using 3.5 m × 4 mm I.D. columns packed with 60–80 mesh (approximately 175–250 μm) Chromosorb W, coated with 0.7% (w/w) of nickel(II) bis[(6-heptafluorobutanoyl)-(5 *S*)-carvonate] in squalane as stationary phase. The limited success is due, primarily, to the small chiral selectivity factors ($\alpha \leq 1.5$), and the accompanying large k' values that are commonly observed with the current, mostly cyclodextrin-based, chiral GC stationary phases [8–11].

Following the first report [12] on the successful capillary GC analysis of fluoroethers, in Part I of this series [13] we studied the factors that led to maximized separation selectivities for the enantiomers of one of the volatile fluoroether

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1280-D

anesthetics, isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane]. Of the commercially available cyclodextrin-based chiral stationary phases, trifluoroacetyl γ-cyclodextrin, operated at 40°C, showed both sufficiently high α and acceptably low k' values, suggesting that preparative-scale enantiomer separations using a packed GC column could be attempted. Trifluoroacetyl γ-cyclodextrin is, as most derivatized cyclodextrins used as neat stationary phases, a mixture of isomers and homologues [8], a viscous, honey-like liquid that can be coated onto the walls of capillary columns, or onto the surface of diatomite-based GC supports. In this paper, we will describe some of the early results of these preparative-scale separation efforts including column preparation, the development of an on-line fraction analysis scheme for the determination of the enantiomeric purity of the eluting bands, as well as load, recovery and production rate studies.

2. Experimental

Preparative-scale GC separations were completed on a PSGC 10-40 preparative gas chromatograph (Varex, Burtonsville, MD, USA), equipped with a septumless injector, a large-capacity oven and a thermal conductivity detection (TCD) system. A Chrom-1 AT data acquisition board (Keithley-Metrabyte, Tauton, MA, USA) installed in a 386SX-20 NEC personal computer, and our ChromPlot1 software [14] was used for data acquisition and analysis. An HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a cryostate, a septumless split/splitless injector, a flame ionization detector, and a ChemStation data collection/analysis system was used for the semi-preparative and analytical-scale separations.

An effluent sampling interface, based on a Series 7000 switching valve (Rheodyne, Cotati, CA, USA), which was connected between the TCD outlet of the PSGC 10-40, the cooled fraction collector unit and the inlet of an analytical capillary column, allowed for the on-line enantiomeric purity analysis of the effluent of the preparative column. A 15 m × 0.25 mm I.D. fused-silica capillary, coated with a 0.25-μm thick film of trifluoroacetyl 2,6-O-dipentyl γ-cyclodextrin (ASTEC, Whippany, NJ, USA) was used as the analytical column for the on-line analysis. A 0.5–3-s wide effluent sample could be injected into the capillary column (and the enantiomeric analysis could be completed) every 30 s. Semi-preparative and preparative columns of 2.1 mm I.D., 5.3 mm I.D. (Supelco, Bellefonte, PA, USA) and 10.0 mm I.D. (Varex) were constructed from copper and stainless-steel tubing. Chromosorb W AW and W HP, 80–100, 100–120 and 120–140 mesh (Supelco) were used as support materials (approximately 150–175 μm, 125–150 μm and 105–125 μm fractions, respectively).

Hydrogen and helium were used as carrier gases at various average linear velocities with methane as the unretained compound. All separations were performed isothermally in the −10 to 70°C range. Isoflurane was obtained from Anaquest (Murray Hill, NJ, USA), a division of BOC Health Care.

Semi-preparative (2.1 mm I.D.) and preparative (> 2.5 mm I.D.) columns were fabricated in essentially the same fashion. The calculated amount of trifluoroacetyl γ-cyclodextrin was dissolved in enough dichloromethane to yield a thin slurry when mixed with the desired amount of dry diatomite support. Dichloromethane was then removed in a Rotavap (Fisher Scientific, Pittsburgh, PA, USA), under slight vacuum, and the packing was transferred into a fluidization apparatus where it was conditioned with dry nitrogen at 105°C for 1–1.5 h. Columns were packed with a combination of tapping and turning, while drawing dry nitrogen through the column. Plate heights were determined by injecting dichloromethane, which is as retained on trifluoroacetyl γ-cyclodextrin as isoflurane.

3. Results and discussion

3.1. Column packing studies

Chromosorb W AW and W HP supports (80–100, 100–120 and 120–140 mesh) were coated

with 4.7% (w/w) trifluoroacetyl γ-cyclodextrin and packed into straight, 1 m × 1/4 in. O.D. columns using different packing methods. Invariably, the denser 80–100 mesh Chromosorb W AW material yielded more efficient columns (Fig. 1), with varying degrees of lumping for both the W HP material and the smaller particle size fractions. Next, the concentration of the trifluoroacetyl γ-cyclodextrin stationary phase was increased through 7.5, 10, 15 and 20% (w/w) to 23.4% (w/w). Up to this point, the plate heights did not increase with increasing loading of the stationary phase (Fig. 1). Finally, when the 23.4% (w/w) trifluoroacetyl γ-cyclodextrin coated 80–100 mesh Chromosorb W AW material was packed into 10 mm I.D. columns (the largest used in this study), the column efficiency increased slightly indicating that the efficiencies were limited by the homogeneity of the bed structure, rather than by liquid phase mass transfer problems. [Incidentally, about twice as many plates were obtained when 25% (w/w) PS255-coated 80–100 mesh Chromosorb W AW was packed into the same 10 mm I.D. columns.]

Therefore, in the rest of the studies, 2-m long sections of 10 mm I.D. columns packed with 23.4% (w/w) trifluoroacetyl γ-cyclodextrin on 80–100 mesh Chromosorb W AW were used.

3.2. Loading studies

The loading characteristics of the 2 m × 10 mm I.D. columns were tested next. The injected sample size was increased from 6 mg to 1200 mg and the elution profiles were determined as shown in Fig. 2. The separation cycle can be completed in about 30 min.

In order to determine the enantiomeric purity *vs.* sample load characteristics, as well as the positions of the cut-points for fraction collection, the enantiomeric composition in the effluent of the preparative column has to be known. Since no chiroptical GC detector was available for this study, a capillary column, coated with the same trifluoroacetyl γ-cyclodextrin as the preparative column, but capable of completing the enantiomer analysis in less than 30 s was made using the static coating procedure [15]. The width of the effluent plug injected into the capillary column could be varied from 0.5 s upwards; 3-s aliquots

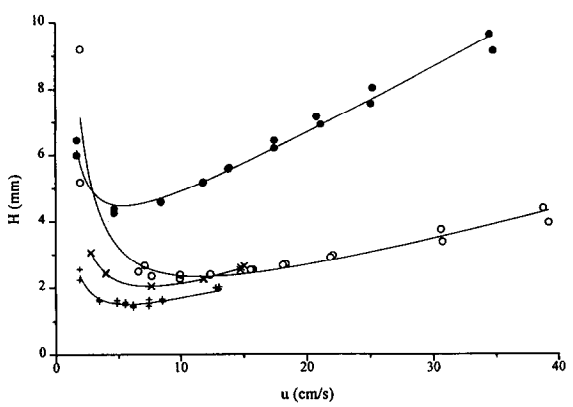

Fig. 1. Van Deemter plots (H = plate height, u = linear velocity) for the packed trifluoroacetyl γ-cyclodextrin columns. Column length: 1 m; temperature: 40°C, isothermal; carrier gas: hydrogen. Packing: 4.7% (w/w) trifluoroacetyl γ-cyclodextrin on Chromosorb W HP 80–100 mesh (●), 4.7% (w/w) trifluoroacetyl γ-cyclodextrin on Chromosorb W AW 80–100 mesh (○) in 2.1 mm I.D. semi-preparative columns; 23.4% (w/w) trifluoroacetyl γ-cyclodextrin on Chromosorb W AW 80–100 mesh in 5.3 mm I.D. column (x); 23.4% (w/w) trifluoroacetyl γ-cyclodextrin on Chromosorb W AW 80–100 mesh in 10 mm I.D. column (+).

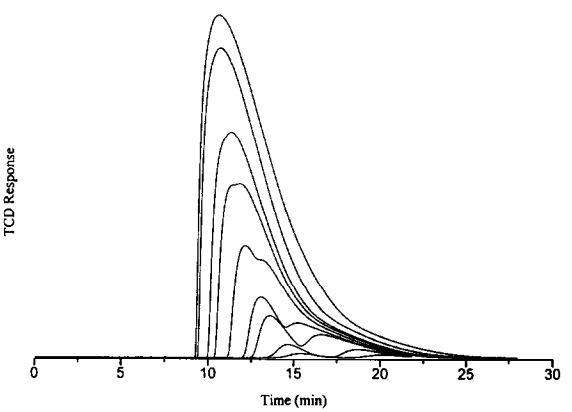

Fig. 2. Sample load study on the 2 m × 10 mm I.D. preparative column packed with 23.4% (w/w) trifluoroacetyl γ-cyclodextrin on 60–80 mesh Chromosorb W AW. Column temperature: 40°C, isothermal; carrier gas: helium at 2.2 cm/s. Racemic isoflurane sample loads: 12, 36, 120, 195, 335, 525, 675, 900 and 1200 mg, increasing as peak height.

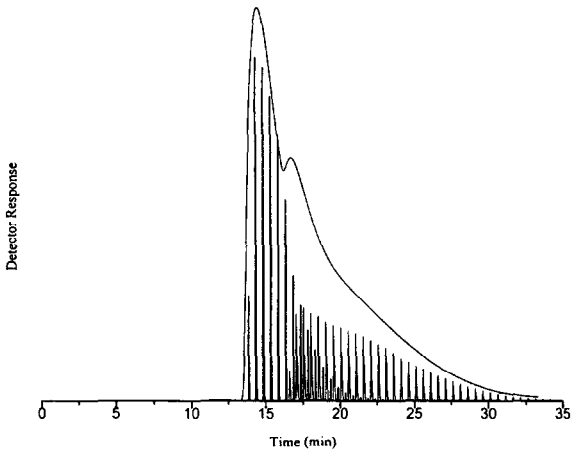

Fig. 3. Example of an on-line enantiomeric fraction analysis. Sample: 195 mg racemic isoflurane. 2 m × 10 mm I.D. preparative column packed with 23.4% (w/w) trifluoroacetyl γ-cyclodextrin on 80–100 mesh Chromosorb W AW, operated at 40°C with 2.2 cm/s helium as carrier gas. Analytical column: 15 m × 0.25 mm I.D. fused-silica capillary coated with a 0.25-μm thick film of trifluoroacetyl γ-cyclodextrin. Analysis temperature: 40°C, isothermal; carrier gas hydrogen at 50 cm/s. Sampling time: 3 s, sampling interval: 30 s. Dark peaks: (+)-isoflurane; light peaks: (−)-isoflurane.

allowed for the analysis of less than 0.01% of the minor enantiomer, so that value, coupled with a 30-s analysis cycle time, was used in the rest of the studies.

The TCD trace of the effluent of the preparative column, together with the corresponding on-line enantiomer analysis chromatograms, is shown in Fig. 3 for a 195-mg injection of racemic isoflurane sample. The peaks in the fraction analysis corresponding to the less retained enantiomer are in black, and those for the more retained enantiomer in white. The peak areas and the respective calibration curves allow for the determination of the quantities of the individual enantiomers in each fraction, and the calculation of production, production rate and enantiomeric purity of the pooled fractions.

The enantiomeric purity *vs.* production (lower horizontal axis) curves and recovery (upper horizontal axis) curves are shown in Figs. 4–7, with the actual reconstructed chromatogram appearing as an inset, for sample loads of 75, 195, 375 and 675 mg, respectively. The normalized

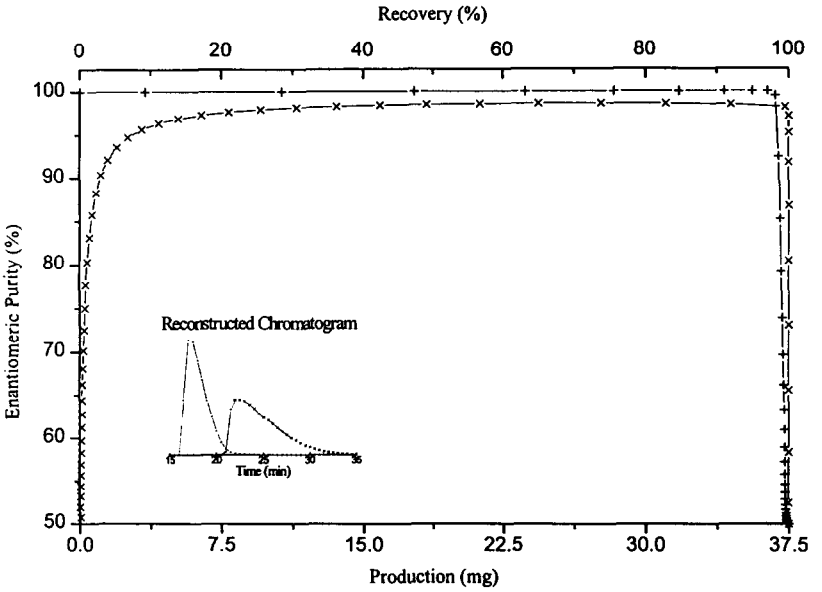

Fig. 4. Cumulative enantiomeric purity of the effluent as a function of enantiomer production (mg) on the lower horizontal axis, recovery (%) on the upper horizontal axis, with the reconstructed chromatogram shown in the inset. Sample load: 75 mg racemic mixture. Symbols: + = (+)-isoflurane; x = (−)-isoflurane.

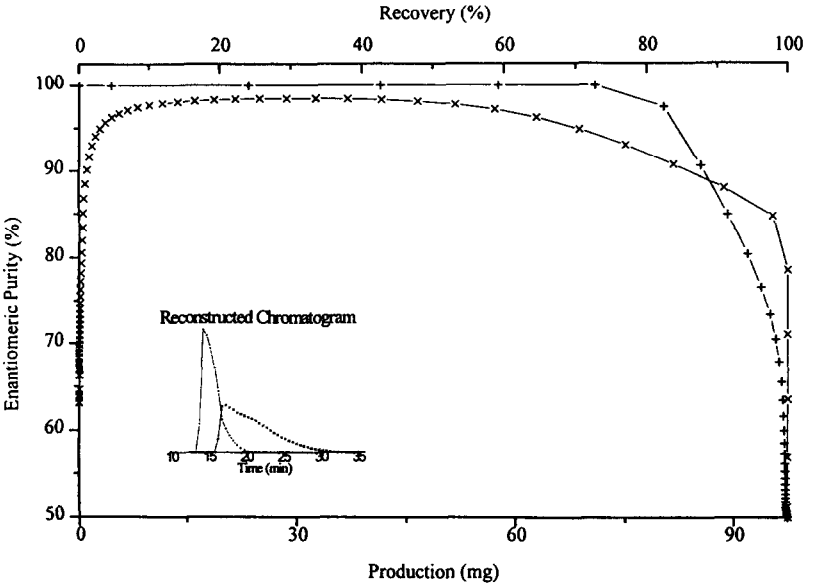

Fig. 5. Cumulative enantiomeric purity of the effluent as a function of enantiomer production (mg) on the lower horizontal axis, recovery (%) on the upper horizontal axis, with the reconstructed chromatogram shown in the inset. Sample load: 195 mg racemic mixture. Symbols as in Fig. 4.

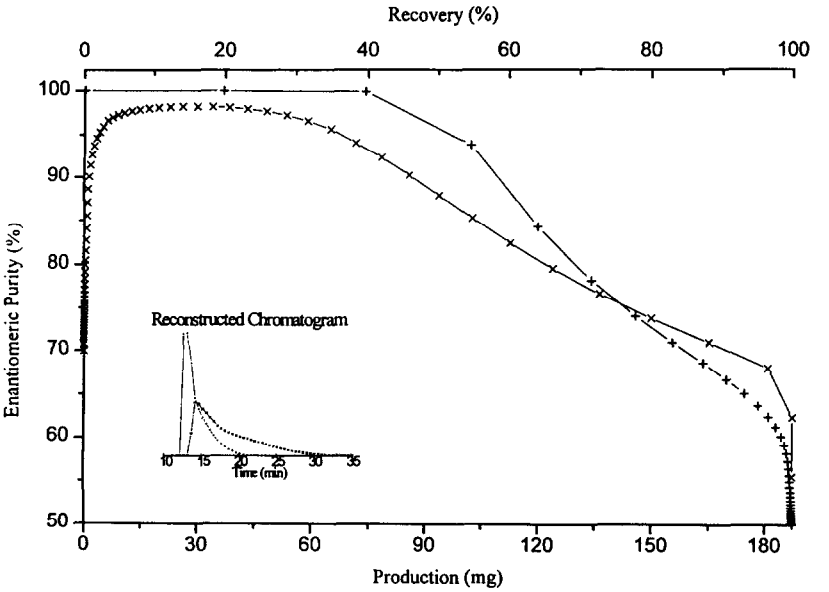

Fig. 6. Cumulative enantiomeric purity of the effluent as a function of enantiomer production (mg) on the lower horizontal axis, recovery (%) on the upper horizontal axis, with the reconstructed chromatogram shown in the inset. Sample load: 325 mg racemic mixture. Symbols as in Fig. 4.

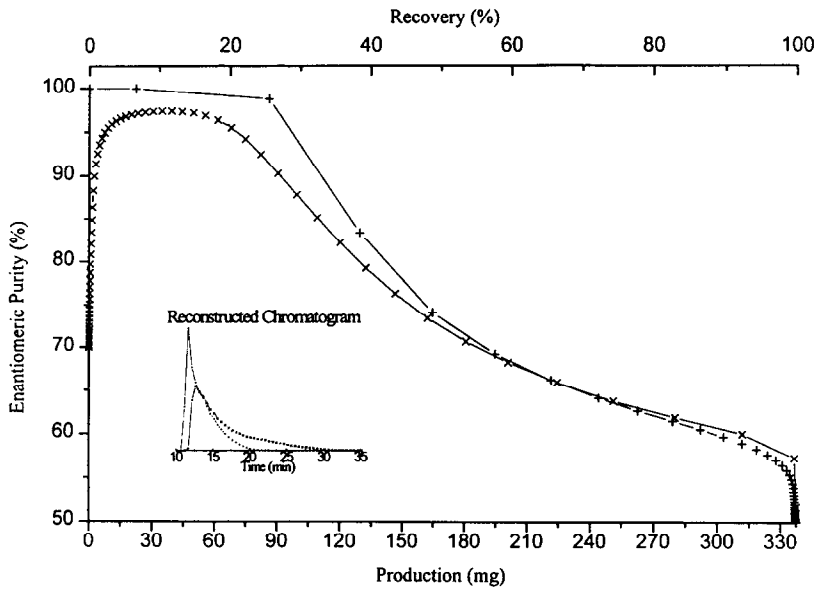

Fig. 7. Cumulative enantiomeric purity of the effluent as a function of enantiomer production (mg) on the lower horizontal axis, recovery (%) on the upper horizontal axis, with the reconstructed chromatogram shown in the inset. Sample load: 675 mg racemic mixture. Symbols as in Fig. 4.

production rate *vs.* sample load curves for both enantiomers at 99, 97 and 95% purity levels are shown in Fig. 8. It can be concluded that, with the help of the on-line fraction analysis method, the production rate can be steadily increased by increasing the size of the injected sample up to 395 mg. Above this point, increased load does not result in increased production rates.

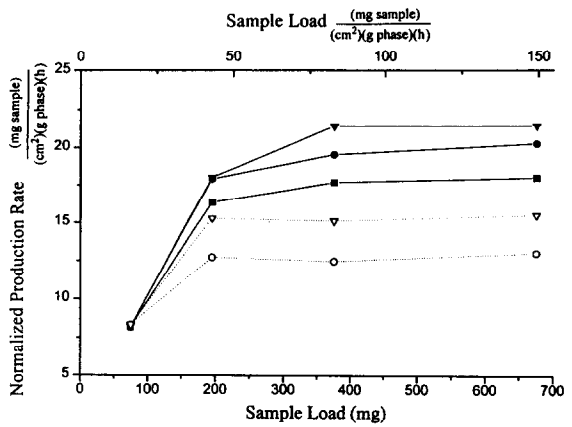

Fig. 8. Normalized production rate as a function of the sample load for various levels of product purity. Production rate has been normalized for column cross-section, stationary phase load and cycle time. Symbols: ▼ = (+)-isoflurane at 95 + % purity; ● = (+)-isoflurane at 97 + % purity; ■ = (+)-isoflurane at 99 + % purity; ▽ = (−)-isoflurane at 95 + % purity; ○ = (-)-isoflurane at 97 + % purity.

4. Conclusions

By maximizing the value of the separation selectivity factor through the selection of the appropriate cyclodextrin derivative to be used as the chiral stationary phase, and the column temperature as a compromise between high selectivity and strong retention, small-scale preparative GC separations of the enantiomers of isoflurane, a volatile anesthetic, have been successfully realized using 2 m × 10 mm I.D. columns. The sampling and switching interface allowed for the on-line analysis, in an efficient capillary column connected to the TCD exit of the preparative GC system, of the enantiomeric purity of the effluent stream and the aggressive, yet safe selection of the cut-points for fraction collections. Further work is under way in our laboratory to extend the method to the production of other valuable enantiomers.

5. Acknowledgements

Partial financial support for this project by the National Science Foundation (CH-8919151), the US Department of Education (415004), Anaquest Inc. and the Dow Chemical Company is gratefully acknowledged. ASTEC is acknowledged for providing the stationary phase used in this study.

6. References

[1] E. Bayer, K.P. Hupe and H. Mack, *Anal. Chem.*, 35 (1963) 492.
[2] M. Verzele, in J. Krugers (Editor), *Instrumentation in Gas Chromatography*, Centrex, Eindhoven, 1968, p. 159.
[3] A. Zlatkis and V. Pretorius, *Preparative Gas Chromatography*, Wiley-Interscience, New York, 1971, p. 73.
[4] R. Bonmati, G. Chapelet-Letourneux and G. Guiochon, *Sep. Sci. Technol.*, 19 (1984) 113.
[5] S.C. Stinson, *Chem. Eng. News*, 71 (1993) 38.
[6] V. Schurig, *Naturwissenschaften*, 74 (1987) 190.
[7] V. Schurig and U. Leyrer, *Tetrahedron: Asymmetry*, 1 (1990) 865.
[8] W.A. König, *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*, Hüthig, Heidelberg, 1992.
[9] V. Schurig, M. Jung, D. Schmalzing, M. Schleimer, C. Duvekot, J.C. Buyten, J.A. Peene and P. Mussche, *J. High Resolut. Chromatogr.*, 13 (1990) 470.
[10] D.W. Armstrong, W. Li, A.M. Stalcup, H.V. Secor, R.R. Izac and J.I. Seeman, *Anal. Chim. Acta.*, 234 (1990) 365.
[11] A. Shitangkoon and Gy. Vigh, *J. High Resolut. Chromatogr.*, 16 (1993) 504.
[12] J. Meinwald, W.R. Thompson, D.L. Pearson, W.A. König, T. Runge and W. Francke, *Science*, 251 (1991) 560.
[13] A. Shitangkoon, D.U. Staerk and Gy. Vigh, *J. Chromatogr. A*, 657 (1993) 387.
[14] Gy. Vigh, G. Quintero and Gy. Farkas, in Cs. Horváth and J. Nikely (Editors), *Analytical Biotechnology*, American Chemical Society, Washington, DC, 1990, p. 181.
[15] J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.