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Semi-preparative gas chromatographic separation of *all-trans*-perhydrotriphenylene enantiomers on a chiral cyclodextrin stationary phase

Stefan Schürch[a,*], Andreas Saxer[a], Saturnin Claude[b], Raffaele Tabacchi[b], Bernhard Trusch[a], Jürg Hulliger[a]

[a]*Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, CH-3012 Berne, Switzerland*
[b]*Institut de Chimie, Université de Neuchâtel, Avenue de Bellevaux 51, CH-2000 Neuchâtel, Switzerland*

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Abstract

Enantiomers of *all-trans*-perhydrotriphenylene (PHTP) were separated by gas chromatography using heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (TBDMS-β-CD) as the chiral selector. Conditions for semi-preparative separations were established using a 2 m×2 mm I.D. packed column and subsequently extended to a 1.8 m×4 mm I.D. column which enabled separations on a mg scale. The column packing was TBDMS-β-CD dissolved in SE-54 coated on Chromosorb P AW-DMCS 80–100 mesh. Optimization of the chromatographic conditions (oven temperature, carrier gas flow, and column load) with respect to better efficiency and peak retention resulted in a system capable of separating up to 10 mg of the racemate per day. Purities of separated enantiomers were determined by capillary gas chromatography. Yields and purities of the fractions obtained by single- and double-step separations are compared. Highly enriched enantiomers with purities of up to 99.6% (99.2% ee) were obtained by a single separation step.

Keywords: Enantiomer seperation; Chiral stationary phases, GC; Perhydrotriphenylene

1. Introduction

Cyclodextrins are playing a key role as chiral selectors in gas chromatography and capillary electrophoresis. In 1983, Koscielski et al. demonstrated the first successful gas chromatographic separation of chiral analytes into their enantiomers [1]. Subsequently, great effort has been put into the design of novel stationary phases based on cyclodextrins for enantioselective gas chromatography. Native cyclodextrins are insoluble in polysiloxanes and hinder efficient gas diffusion, thus, severely hampering the separation efficiency. Therefore, many studies focussed on the synthesis of derivatized cyclodextrins to be used as stationary phases in either pure form or diluted in polysiloxanes [2]. One of the main driving forces behind that development was the need for a rapid and accurate technique for the analysis of chiral flavour constituents in essential oils, since the determination of enantiomeric ratios provides a reliable indicator for a sample's authenticity. This

*Corresponding author. Fax: +41-316-313-421.
E-mail address: schuerch@ioc.unibe.ch (S. Schürch).

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development has been reviewed by several authors, who also emphasize the versatility of cyclodextrin stationary phases for a great variety of analytical applications and demonstrate that enantioselective gas chromatography has rapidly emerged into many fields, such as asymmetric organic synthesis, pharmacology and environmental analysis [3,4].

Chiral recognition is based on interaction of the cyclodextrin host molecules with the analyte guest molecules, which, in chromatographic systems, allows for separation of enantiomers. The enantioselectivity of a chiral stationary phase is primarily determined by the stability of the intermediate diastereomeric host–guest complexes. The interaction between many combinations of chiral guest molecules and cyclodextrins has been investigated using various experimental and theoretical approaches. However, a generally accepted comprehensive theory of the chiral recognition mechanism has not yet been presented. Nevertheless, the formation of intermediate diastereomeric complexes and the interaction of the guest molecules with the peripheral region of the cyclodextrin macrocycles are considered to be two fundamental aspects thereof [5–9]. Additionally, it must be taken into consideration that complexation takes place at the gas/liquid interface and that the cyclodextrin is dissolved in a polysiloxane matrix, which also affects the mass transport in the chromatographic system [10,11]. The lack of a well understood recognition mechanism impedes the controlled development of novel stationary phases with predictable enantioselectivity. This fact is reflected by the large number of published work describing protocols for optimized separation of specific chiral compounds.

Only a relatively small number of publications addresses the issue of (semi)-preparative separations based on cyclodextrin stationary phases [12–18]. The lack of generally applicable working protocols for large-scale enantiomer separation is primarily due to the difficulties associated with the inherently low separation factor (α) and the low sample capacity of cyclodextrin stationary phases in gas chromatographic systems. Consequently, time-consuming adjustment of the system parameters is needed for each specific application.

Here we demonstrate the gas chromatographic separation of *all-trans*-perhydrotriphenylene (PHTP) [19] into its enantiomers on analytical and preparative scales. PHTP is one of todays most versatile inclusion forming host molecules, because of its capability of including a large number of guest molecules [20]. Due to its D_3 symmetry, PHTP exists as enantiomers. In 1970, Farina et al. succeeded in separating the enantiomers via functionalization [21]. At the time, 100% purity was assumed, however, only based on results using isotopic dilution. An $[\alpha]_D$ of $-93°$ and a melting point 142°C were measured for (−)-PHTP, further purified by liquid chromatography and sublimation (for a review see Ref [22].). Configuration R was assigned to the (−)-enantiomer. PHTP is now being separated and characterized for the purpose of a precise analysis of its inclusion capacity and structural difference when compared to inclusion compounds using racemic PHTP.

2. Experimental

2.1. Chiral selector and analyte

Heptakis(6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (TBDMS-β-CD) was synthesized following the protocol of Takeo et al. [23]. Synthesis of racemic PHTP was performed by hydrogenation of dodecahydrotriphenylene, as described previously [24].

2.2. Preparation of the capillary column

A fused-silica capillary column (25 m×0.30 mm I.D.) was deactivated with Superox-4 (Socochim, Lausanne, Switzerland) following the procedure of Arrendale et al. [25–27]. The column was statically coated with 25% (w/w) of TBDMS-β-CD dissolved in OV-1701 (7% phenyl-, 7% cyanopropyl-, methylpolysiloxane, Ohio Valley, Marietta, OH) [28]. In short, the column was filled with the cyclodextrin/polysiloxane mixture dissolved in pentane/methylene chloride (1:1) and the solvent was subsequently removed by applying a controlled vacuum in the range of 50–60 mbar, regulated by a solenoid valve. With this procedure, a uniform coating with a film thickness of 0.25 μm was achieved.

2.3. Preparation of packed columns

The stationary phase was prepared by coating a mixture of 89% (w/w) TBDMS-β-CD and 11% SE-54 (5% phenyl-, 1% vinyl-, methylpolysiloxane) on Chromosorb P AW-DMCS 80–100 mesh (Supelco, Bellefonte, PA). 2.76 g of TBDMS-β-CD were dissolved in 20 ml of freshly distilled chloroform and 0.31 g of SE-54 were added. This mixture was poured onto 46 g of Chromosorb placed on a glass-frit, with slight vacuum applied to the bottom side of the frit. The solvent was removed in vacuum (50 mbar) for 2 h at room temperature. After packing, the column was baked at 180°C for 4 h under nitrogen flow to remove residual solvent. The coating of the cyclodextrin/polymer mixture on the solid support was 6%, the total packing of the 2 m×2 mm I.D. column after baking was 3.2 g. Packing of the 1.8 m×4 mm I.D. column was performed in exactly the same manner, with the exception of using Chromosorb P AW-DMCS 100–120 mesh instead. The total column packing was 18.1 g.

2.4. Instrumentation

Analytical separations were performed on a HP 5890 (Hewlett-Packard, Palo Alto, CA) gas chromatograph. Operating conditions were as follows: carrier gas: helium (95 kPa); split injection (1:20) of 0.2 μl of a 1 mg/ml solution of PHTP in pentane; injector temperature: 180°C; oven temperature: 180°C; flame ionization detector.

Separations on the 2 m×2 mm I.D. packed glass column were performed on a HP 5890 gas chromatograph; carrier gas: nitrogen (24–30 ml/min); injection volume: 1 μl of a 50 mg/ml PHTP solution in pentane; injector temperature 220°C; oven: isothermal operation at temperatures in the range of 160–190°C; thermal conductivity detector with a reference gas flow (nitrogen) of 13 ml/min.

Semi-preparative gas chromatographic separations on the 1.8 m×4 mm I.D. packed stainless steel column were performed on a Perkin-Elmer F21 gas chromatograph, equipped with a fraction collector. The injector temperature was at 190°C and the column was operated isothermally at temperatures in the range between 140 and 190°C. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min (100 kPa). The column effluent was split at a ratio of 1:40 and about 2.5% of the total flow were fed to a flame ionization detector for monitoring. Injections of 2 mg PHTP in 40 μl pentane were performed repetitively with a cycle time of 60 min. Sample fractions were collected in glass traps held at −15°C, the transfer lines to the traps were held at 220°C. The column effluent was directed to the desired trap by switching solenoid valves in the transfer lines.

2.5. Characterization

The melting point of 99.0% pure enantiomer was measured with a Mettler Toledo (Dübendorf, Switzerland) DSC 25 differential scanning calorimeter. A melting point of 142.0°C was obtained for the first eluting (−)-enantiomer. Optical rotation was measured on a Perkin-Elmer (Rotkreuz, Switzerland) model 241 polarimeter using a paraldehyde solution (4 mg of enantiomer per 2 ml of paraldehyde). Paraldehyde was used for the measurements because with this solvent, no inclusion formation takes place. An $[\alpha]_D$ of −92.4° was obtained for the first eluting enantiomer at 20°C and λ=589 nm. These results are in good agreement with the data obtained from previous measurements of enantiomerically pure PHTP, showing a melting point of 144°C [21,22].

3. Results and discussion

Separations of PHTP were performed on heptakis(6-*O*-*tert.*-butyldimethylsilyl-2,3-*O*-dimethyl)-β-cyclodextrin (TBDMS-β-CD) dissolved in polysiloxane as the stationary phase [29–35]. Separations of the racemic mixture were first performed on the analytical column (25 m×0.30 mm I.D., coated with 25% of TBDMS-β-CD dissolved in OV-1701), which later on also served for determination of the enantiomeric purities of the fractions collected from semi-preparative separations. The chromatographic data obtained for PHTP demonstrates the ability of TBDMS-β-CD to fully separate the two enantiomers and a resolution R of 2.48 was obtained (Fig. 1). The low separation factor α of 1.04 is typical for enantioselective separations on cyclodextrin stationary phases and is comparable to

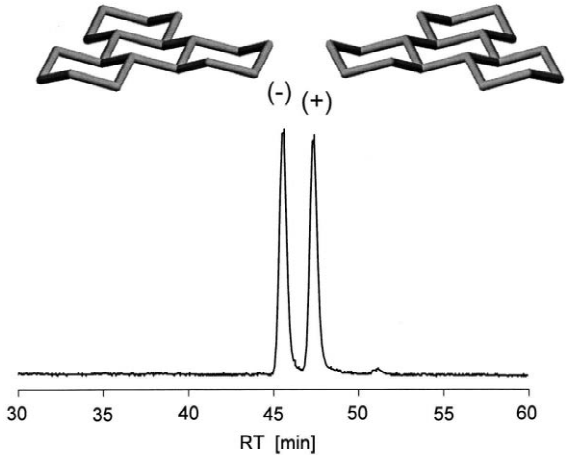

Fig. 1. Analytical separation of a racemic mixture of *all-trans*-perhydrotriphenylene showing a separation factor (α) of 1.04 and a resolution (R) of 2.48 (column: 25 m×0.30 mm i.d; TBDMS-β-CD in OV-1701; film thickness 0.25 μm; 160°C isothermal; Helium 95 kPa).

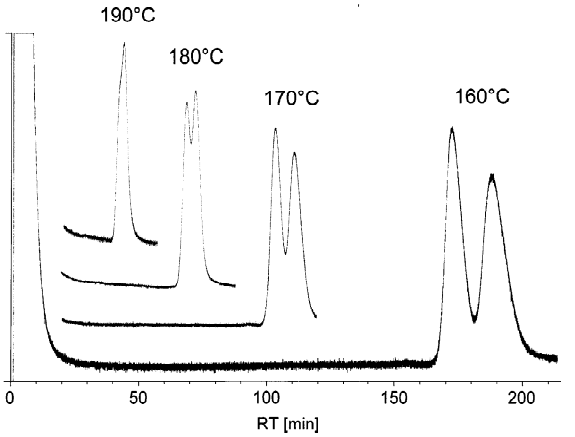

Fig. 2. Separations of PHTP performed on a 2 m×2 mm I.D. packed column, demonstrating the influence of increased oven temperature on the separation and retention.

the results obtained for a variety of chiral compounds [36].

Transfer to a 2 m×2 mm I.D. packed column was successful in terms of enantiomer separation. The column packing was 89% of TBDMS-β-CD dissolved in SE-54 and coated on Chromosorb P AW-DMCS 80-100 mesh. The operating conditions were adjusted with respect to the greater internal diameter and the column packing. The carrier gas flow was set to 24 ml/min (160 kPa) and good separation (R= 1.40) of the enantiomers was achieved at a column temperature of 160°C with the less retained enantiomer eluting after 165 min. Higher oven temperatures resulted in significantly decreased retention of 100 min at 170°C, 62 min at 180°C, and 38 min at 190°C, but a severe loss of resolution was observed for higher temperatures, as depicted in the chromatograms in Fig. 2. A reduction of the retention time without notably affecting the separation efficiency was achieved by increasing the carrier gas flow to 30 ml/min (270 kPa), while keeping the oven temperature at 160°C. Under these conditions elution of the two enantiomers was observed within 110 min and 120 min, respectively.

The limited sample capacity on the order of 100 μg per injection prompted us to transfer the methodology to a packed column of larger dimensions to perform separations on a milligram scale. A 1.8 m ×4 mm I.D. stainless steel column was packed with the same stationary phase as used in the previous experiment, but coated on Chromosorb P AW-DCMS 100–120 mesh as the solid support. The column effluent was split at a ratio of 1:40 and the smaller portion of the flow was fed to a flame ionization detector for monitoring the elution of the enantiomers. The performance of the system was tested for different oven temperatures, column loads, and carrier gas flow rates, demonstrating the effect of the larger column dimensions on resolution and efficiency. The effect of various oven temperatures is illustrated by the FID-traces of the eluting enantiomers in Fig. 3. Heights and widths of the peaks are reproduced at the same scale. Baseline separation of the enantiomers was achieved at an oven temperature of 140°C (chromatogram not shown). However, retention times of 780 min and 870 min for the two enantiomers and an overall peak width of 220 min were observed, which were detrimental to the desired high production rate and were not acceptable for further experiments. Partial separation of the enantiomers at reasonable retention times was achieved by slightly increasing the oven temperature. At temperatures above 180°C the two enantiomers eluted as a single peak. An additional reduction of the peak retention was achieved by increasing the carrier gas flow rate. The column load study was performed at 160°C with injections of 5, 10, 20, 40 and 80 μl

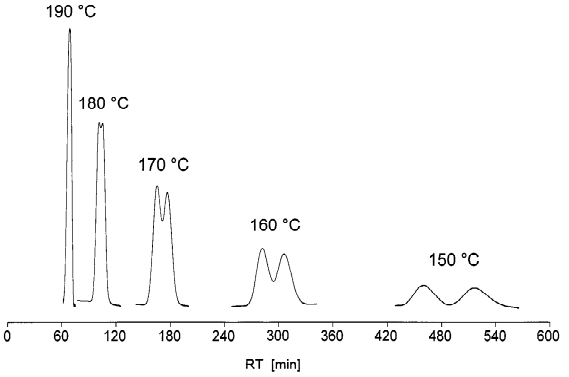

Fig. 3. Influence of the oven temperature on resolution and sample retention on a 1.8 m×4 mm I.D. packed column. FID-traces of separations at oven temperatures in the range from 150°C to 190°C are shown. Separations were performed with 0.25 mg of racemic PHTP at a carrier gas flow of 40 ml/min.

volumes of PHTP dissolved in pentane, corresponding to 0.25, 0.5, 1, 2, and 4 mg of racemic PHTP. As illustrated by the corresponding FID-traces (Fig. 4), a rapid loss of resolution was observed for higher column loads and the retention of the enantiomers decreased slightly, thus, indicating overloading effects due to saturation of the cyclodextrin stationary phase. The results obtained for different working conditions are summarized in Table 1.

Based on these results, a compromise between low retention and maximum resolution was chosen to achieve a good production rate. All further separations were performed with the oven operated in the isothermal mode at 160°C and a carrier gas flow rate of 50 ml/min. Repetitive injections of 2.0 mg of racemic PHTP dissolved in 40 μl pentane were performed every 60 min. Such timing allowed for 4 injections prior to elution of the first PHTP peak pair. Additional injections were performed between the elution of adjacent PHTP peak pairs which were spaced sufficiently to avoid overlapping with the readily eluting solvent peak. The FID-trace shows the two enantiomers partially separated, with the less retained enantiomer eluting after 220 min and a total baseline peak width of 48 min (Fig. 5). Since only partial separation of the enantiomers was obtained, samples had to be collected in four fractions. Material eluting between consecutive PHTP peaks pairs was collected in a fifth trap. The time scheme for fractionation was defined in a test run by monitoring

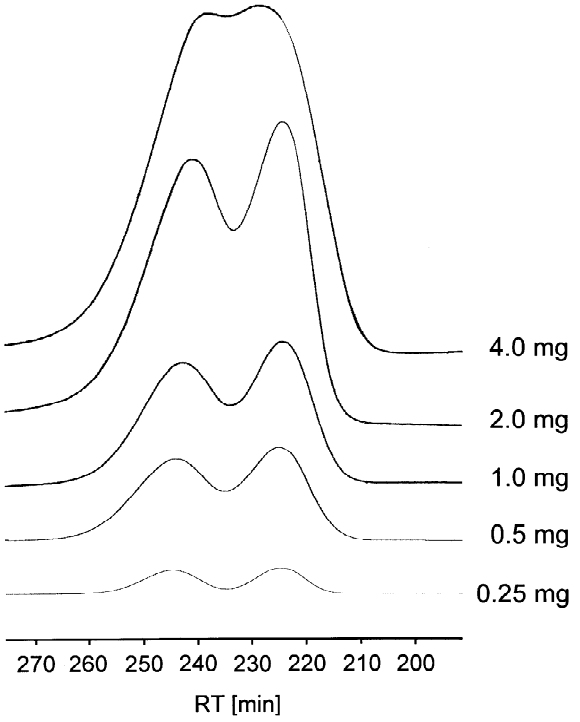

Fig. 4. Column load study on the 1.8 m×4 mm I.D. packed column with injections of 0.25, 0.5, 1, 2, and 4 mg of racemic PHTP. A loss of separation power was observed with increased quantities of analyte injected.

the FID-signal, and the same timing was applied to all subsequent separations. The results of separations performed with different cut-point positions are summarized in Table 2. This procedure allowed to perform 5 repetitive injections of totally 10 mg of the racemate per day.

Purities and yields of the separated enantiomers were adjusted by setting the cut-point positions for fractionation and by repeated separation (fraction recycling). Fraction collection with the cut-point positions at the peak maxima resulted in 47% of the total material recovered as separated enantiomers with purities between 90 and 95%. Differences of a few percent in yield and purity of the enantiomers were observed within a series of separations, caused by slight variations of the timing for trap valve switching. Thus, automated fraction collection controlled by the detector signal of the eluting compounds is recommended for maximum reproducability. Enantiomers of higher purity were obtained by

Table 1
Resolution and retention times obtained for the separation of PHTP enantiomers on the 1.8 m×4 mm I.D. packed column under various operating conditions

Temperature [°C]	Load [mg]	Flow [ml/min]	Resolution	t_{R1} [min]	t_{R2} [min]
140	0.25	40	1.20	780	872
150	0.25	40	1.23	460	514
160	0.25	40	0.82	280	302
170	0.25	40	0.47	165	174
180	0.25	40	0.20	102	106
190	0.25	40	0	71	71
150	0.25	40	1.23	460	514
150	0.25	50	0.94	353	390
150	0.25	60	0.88	329	363
150	0.25	70	0.66	307	332
160	0.25	50	0.75	223	240
160	0.5	50	0.67	223	240
160	1.0	50	0.65	222	239
160	**2.0**	**50**	**0.55**	**220**	**235**
160	4.0	50	0	220	220

subjecting the already enriched material from fractions 1 and 4 to an additional separation step. After two separation steps 24% of the initial material was collected as separated enantiomers with purities of up to 99.4%. The total recovery of material, including mixed fractions, was on the order of 95% for each step of separation. However, the production rate was strongly decreased due to the need for a second separation step. An overall increase of the production rate and high enantiomeric purities were achieved simultaneously by performing a single separation step with the cut-point positions for fraction collection shifted into the peak flanks. This method yielded 36% of the injected material as separated enantiomers with purities of 99.6% and 98.8%. Consequently, the single-step separation proved to be advantageous over the double-step separation, resulting in higher yields of enantiomers of comparable high purities. As a result of the measurements of optical rotation, it is the (−)-enantiomer (R configuration) showing the lower retention time.

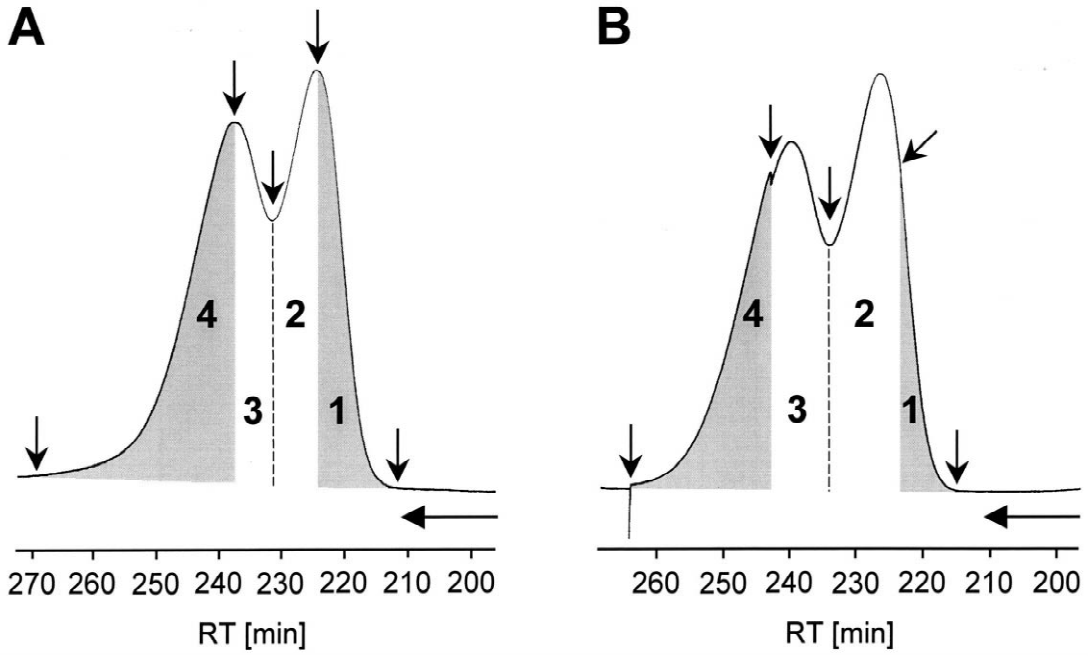

Fig. 5. Separation of PHTP enantiomers on the 1.8 m×4 mm I.D. packed column. The peak of partially separated PHTP enantiomers eluted after 220 min. Arrows indicate the cut-point positions for fraction collection. (A) Trap valve switching at the peak maxima. (B) Trap valve switching in the peak flanks. The small peak in the flank of the second enantiomer is an electronic artifact generated by trap valve switching.

Table 2
Yields and enantiomeric excess of separated PHTP enantiomers[a]

Method	Trap	Yield [%]	ee (−)-PHTP [%]	ee (+)-PHTP [%]
A	1	24	79.2	
	2	29	49.8	
	3	15		44.6
	4	23		89.2
	5	5		
	Σ	96		
B	1	22	97.0	
	2	29	49.8	
	3	15		44.6
	4	23		89.2
	5	6		
	Σ	93		
C	1	16	99.2	
	2	25	78.6	
	3	28		68.8
	4	20		97.6
	5	4		
	Σ	93		

[a] (A) Single separation step, cut-point position at peak maxima. (B) Repetitive separation of fractions 1 and 4 from previous separations, cut-point positions at peak maxima. (C) Single separation step with cut-point positions in peak-flanks.

4. Conclusions

Enantioselective gas chromatography on cyclodextrin stationary phases has proven to be a reliable alternative to enantioselective synthesis for the preparation of pure optically active compounds. Semi-preparative separations on a milligram scale, yielding enantiomers of high purity, can be performed within a short period of time by repetitive injections. Since low separation factors are typically obtained with cyclodextrin stationary phases, the working conditions of the gas chromatographic system have to be evaluated carefully in order to obtain optimum separation efficiency at reasonable sample retention. The experiments demonstrate that baseline separation can be obtained for the PHTP enantiomers at low oven temperatures. However, time considerations often prohibit operation at maximum resolution. Therefore, separations at higher oven temperatures, resulting in faster elution, may be preferable and the drawback of partial separation may be overcome by effluent fractionation. Enantiomers of high purity are obtained by adequately setting the cut-point positions for fraction collection, and mixed fractions are purified further by additional recycling steps. Regardless of the absolute peak retention, the production rate may be maximized by repetitive injections with the minimal cycle time defined by the overall peak width of the eluting enantiomers. The availability of pure enantiomers of PHTP opens up new work on structural chemistry when investigating their inclusion properties with achiral or chiral guest molecules.

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