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Preparative gas chromatographic separation of the enantiomers of methyl 2-chloropropionate using a cyclodextrin-based stationary phase

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Abstract

The enantiomers of methyl 2-chloropropionate, a volatile synthetic precursor, have been separated by preparative-scale gas chromatography using a 1 m × 22.5 mm I.D. column, packed with 20% (w/w) trichloroacetyl β-cyclodextrin-coated 60–80-mesh Chromosorb A. The preparative column was installed in a custom-designed preparative gas chromatograph and operated in the isothermal mode. An effluent-sampling interface valve, located between the exit of the preparative column and the fraction collector took 10- μl samples of the effluent gas every 20 s and sent the samples onto a short, efficient analytical capillary column to provide on-line enantiomeric analysis of the eluting bands. This near-real-time knowledge of the enantiomer composition of the effluent gas leads to aggressive, yet safe, fraction pooling schemes and permits the precise calculation of product purity, recovery and production rate. Results for the preparative separation of racemic as well as enantiomerically enriched (95% enantiomeric excess) feedstock are presented in this paper.

1. Introduction

In three previous papers [1–3] we have described the design, construction and use of a first-generation custom-designed preparative gas chromatograph which combines the preparative-scale separation of enantiomers with integrated on-line analysis of the enantiomeric composition of the effluent from the preparative column. We have demonstrated that the system could provide sub-g/day-scale separation of the enantiomers of volatile anesthetics, enflurane and isoflurane, using a 1 m × 10 mm I.D. column packed with Chromosorb A, coated with 20% (w/w) trifluoroacetyl γ-cyclodextrin as chiral stationary phase. The objective of the present paper is to demonstrate that with a slight modification of the injector system and the injection sequence, the separation efficacy of the integrated preparative/analytical system can be preserved even when the diameter of the preparative column is doubled to 22.5 mm, offering production rates as high as 5 to 10 g/day using a chiral stationary phase, trichloroacetyl β-cyclodextrin.

The methyl ester of α-chloropropionic acid (MCP) was used as test compound in these studies. α-Chloropropionic acid esters are chiral synthons which can be used, among others, in the synthesis of enantiomerically pure herbicides, such as the (*R*)-α-phenoxypropionic acid esters [4]. While MCP is commercially available in enantiomerically enriched form (95.7% enantiomeric excess), higher-purity material is not readily available. MCP is quite suitable as a test

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compound for scale-up studies in chiral preparative gas chromatography, because a selectivity factor of 1.5 can be achieved for its enantiomers with an accompanying k_2' of 150 using the relatively inexpensive chiral stationary phase, trichloroacetyl β-cyclodextrin [5].

2. Experimental

The custom-designed preparative gas chromatographic system has been described in detail in Ref. [3]. Briefly, it was built from a modified Model 439 gas chromatograph (Chrompack, Middelburg, Netherlands), equipped with a septumless split/splitless injector with a modified, heated Series 7000 (Rheodyne, Cotati, CA, USA) switching valve-based vaporization loop, a flame ionization detection (FID) system, and a Model 4270 integrator (Varian, Walnut Creek, CA, USA) for data acquisition, a modified Model FE thermostatted circulating oil-bath system (Science Electronics, Dayton, OH, USA), modified HP 5982 MS interface heated transfer lines (Hewlett-Packard, Avondale, PA, USA), a modified, heated Series 7000 (Rheodyne) switching valve-based effluent sampling interface, a modified variable-split injector (Tracor, Houston, TX, USA) and a custom-made analytical capillary column installed in an HP 5890 Series II gas chromatograph (Hewlett-Packard), a septumless split/splitless injector, a FID system, and a ChemStation data collection/analysis system.

The preparative-scale GC separations were completed on 1.0 m × 22.5 mm I.D. stainless-steel preparative columns, which were packed as described in Ref. [3] with 60–80 mesh (approximately 175–250 μm) Chromosorb A (Alltech, Deerfield, IL, USA), coated with 20% (w/w) trichloroacetyl β-cyclodextrin (AMP-5). AMP-5 was synthesized by refluxing partially pentylated β-cyclodextrin in a mixture of 1,4-dioxane and trichloroacetyl anhydride, as described and characterized in Ref. [5]. The preparative column, which contained 44 g of AMP-5, was operated isothermally at 60°C and yielded a selectivity factor of 1.5 and an accompanying k_2' of 150 for MCP.

The effluent of the preparative column was analyzed on-line for enantiomeric purity using a 5.5 m × 0.15 mm I.D. fused-silica capillary column, statically coated [6] with a 0.15-μm thick film of AMP-5. Hydrogen was used as carrier gas at a linear velocity of 50 cm/s (methane was used as unretained compound). The capillary column was operated isothermally at 60°C.

MCP was obtained in racemic form as well as enantiomerically enriched form (lot No. 11401LZ, factory analyzed 95.7% ee) from Aldrich (Milwaukee, WI, USA), and used without further purification.

3. Results and discussion

3.1. Equipment modification

In order to accommodate the larger sample loads expected with the use of the 22.5 mm I.D. preparative column, the injector [2] has been modified by extending the original injector of the Chrompack Model 439 gas chromatograph with an expansion vessel constructed from a 1 m × 5.3 mm I.D. stainless-steel tube. The enlarged injector is connected between ports 2 and 3 of the Rheodyne Series 7000 heated valve. Ports 5 and 6 of the valve are connected by a short section of a 0.75 mm I.D. stainless-steel capillary. The carrier gas source is connected to port 1, the preparative column to port 4. In the load cycle, the valve routes the carrier gas through ports 1, 6, 5 and 4 to the preparative column, while the liquid sample, up to 1.5 ml in volume, is injected into the closed loop formed by ports 2 and 3, and the extended injector. After a brief evaporation time, the valve is turned routing the carrier gas through ports 1 and 2, the extended injector and ports 3 and 4 to the preparative column. The carrier gas is allowed to flush the sample onto the column only for a predetermined period of time: as soon as the vapor phase concentration begins to decrease at the exit of the expansion vessel, the valve is switched back to the load position providing a square-wave like injection plug devoid of a dilute tail. Though this solution ensures sharp boundaries for the injected sample

plugs, it results in incomplete sample transfer. Therefore, the analytical part of the system has to be calibrated through the analytical injector, the appropriate response factors must be determined and used to calculate the amount of material actually introduced into the preparative column.

The 1 m × 22.5 mm I.D. preparative column was packed with 60–80 mesh Chromosorb A, coated with 20% (w/w) AMP-5, as described in Refs. [2] and [3]. The Van Deemter curves of the column were determined at 60°C with hydrogen as carrier gas and 0.5 μl samples of racemic MCP as probe. At the optimum linear velocity of 4 cm/s, the column could provide 825 theoretical plates, at 8 cm/s linear velocity (with an inlet pressure of 70 p.s.i.; 1 p.s.i. = 6894.76 Pa), this decreased to 600 theoretical plates.

3.2. Column load studies and on-line effluent analysis

The preparative column was loaded with several samples of racemic MCP and the preparative detector traces (envelope chromatograms) were recorded as shown in Fig. 1. The touching-band condition develops at 37 mg load; a clear shoulder for the second enantiomer is observable up to the 206-mg load, the envelope chromatograms show only a single, distorted peak between the 425- and 1070-mg loads. In all cases, the envelope chromatograms indicate that the sample leaves the preparative column in 35 min.

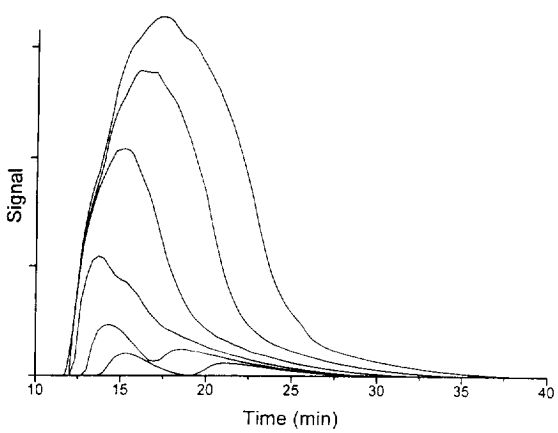

Fig. 1. Preparative FID traces (envelope chromatograms) for racemic MCP samples of increasing size. Column: 1 m × 22.5 mm I.D.; temperature: 60° C, isothermal; carrier gas: hydrogen at 8 cm/s linear velocity. Sample loads: 37, 87, 206, 425, 728 and 1070 mg, respectively, increasing as peak height.

In order to realize on-line enantiomeric analysis of the effluent gas of the preparative column, the cycle time of the analytical system must first be determined. As the worst case, a new, 10-μl effluent gas fraction can be injected by the interface into the analytical column as soon as the analytical FID signal returns to the baseline following the elution of the more retained enantiomer. However, as described in Ref. [3], the sampling frequency can be increased if the duration of the analytical chromatogram is an integer number multiple of the cycle time. The cycle time is made up from the duration of the pressure pulse that is caused by the turning of the interface valve to the inject position and back to the refill position, the duration of the flat baseline section before the peak of the less retained enantiomer, the duration of the first peak, the duration of the second peak (both determined at the highest effluent concentration expected during the preparative separation), and the duration of the flat baseline section after the peak of the more retained enantiomer necessary for reliable peak area determination. In this study the actual separation time on the analytical capillary column is 40 s, while the cycle time is 20 s. The detector trace is very clean, and can be easily integrated to yield the enantiomeric composition of the effluent gas of the preparative column. This results in 70 to 80 enantiomeric composition data points during the course of an average preparative separation. As an example, the analytical FID trace is shown in Fig. 2 as a function of time during the preparative-scale separation of a 206-mg MCP sample. The corresponding envelope chromatogram is shown in Fig. 1: it is the last one where the shoulder caused by the second enantiomer is still discernable.

The reproducibility of the entire integrated preparative/analytical system is indicated by the analytical FID trace of a 2-h segment of a production campaign shown in Fig. 3: three

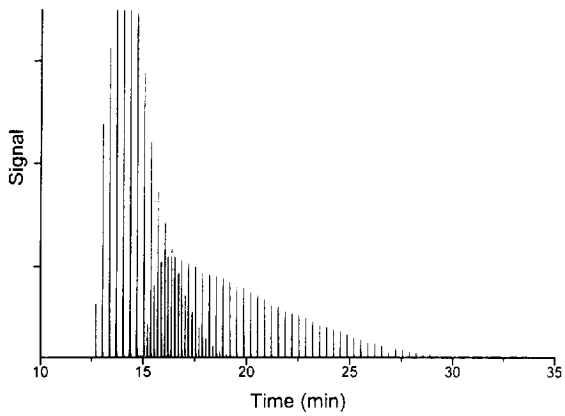

Fig. 2. Example of an on-line enantiomeric fraction analysis. Sample: 206 mg racemic MCP. Preparative column and conditions as in Fig. 1. Sampling volume: 10 μl, sampling cycling time: 20 s. Light peaks: (*R*)-(+)-MCP, dark peaks: (*S*)-(−)-MCP.

preparative cycles can be completed in slightly over 2 h. Since the MCP feedstock contains three slightly retained contaminants, amounting to about 5% of the sample, the preparative cycles cannot be nested as with the isoflurane and enflurane samples [2,3]. This lowers the daily production rates and indicates the importance of the chemical purity of the feedstock.

3.3. Preparative results

Using the on-line enantiomer analysis data of the effluent (an example of which is shown in Fig. 2 for the 206-mg load) and the appropriate response factors of the analytical system established by direct calibration, the enantiomeric purity vs. production and enantiomeric purity vs. recovery curves can be calculated, as shown, for example, in Fig. 4 for the 206-mg injection. The left-hand inset in Fig. 4 shows the envelope chromatogram, the right-hand inset shows the reconstructed chromatogram of the enantiomers. It can be seen that for a 206-mg injection of the racemic mixture, about 70 mg of the less re-

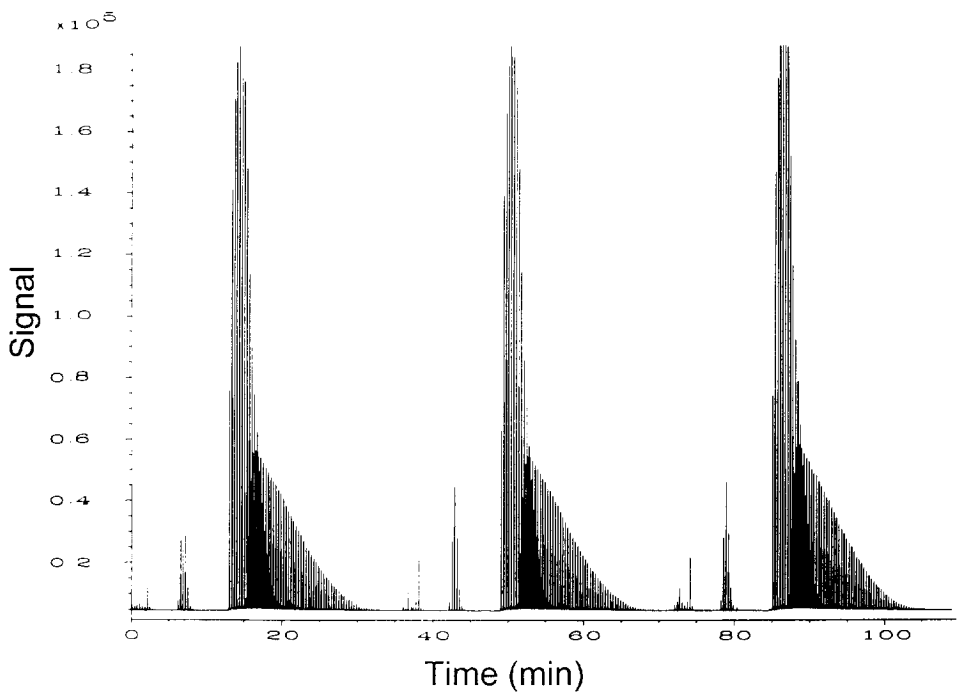

Fig. 3. Stability and reproducibility of the preparative GC system: a 2-h segment of the analytical FID trace during a single day's production campaign (206 mg racemic MCP injections, 35-min preparative cycle time, 20-s interface valve cycling time). Other conditions as in Fig. 2.

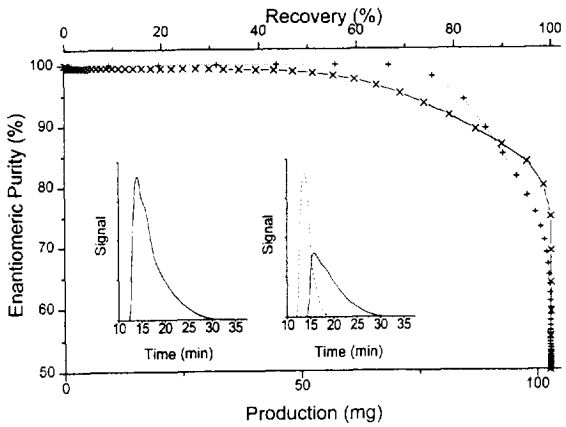

Fig. 4. Enantiomeric purity as function of production and % recovery for a 206-mg injection calculated from the on-line enantiomeric fraction analysis. Insets: preparative envelope chromatogram at left, reconstructed chromatogram of the enantiomers at right. Conditions as in Fig. 2. Symbols: + (dotted line) = less retained enantiomer; × (solid line) = more retained enantiomer.

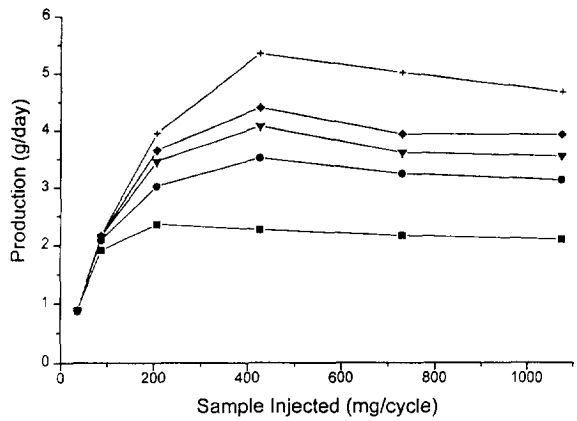

Fig. 5. Production for the less retained MCP enantiomer as a function of the injected sample amount at various levels of product purity. Feedstock: racemic MCP. Conditions as in Fig. 2. Symbols: + = 80%; ◆ = 90%; ▼ = 95%; ● = 99%; ■ = 100% purity.

tained enantiomer leaves the column at a purity higher than 99.99%. However, only about 2 mg of the more retained enantiomer are available at such a high enantiomeric purity. A small relaxation of the enantiomeric purity requirement leads to negligible increase in production for the less retained enantiomer, but significant increase for the more retained enantiomer.

Knowing that each run takes 35 min, one can calculate the daily production rates (g/day) for various enantiomeric purities as a function of the injected load in each cycle. The results are shown in Fig. 5 for the less retained enantiomer and Fig. 6 for the more retained enantiomer. If the less retained enantiomer is to be produced at greater than 99.99% purity, the maximum injection size must be about 206 mg. This leads to about 2.2 g/day of pure product. Larger injections slightly decrease the daily production at this purity level. If the purity requirement is relaxed to 99%, the daily production rate increases by about 50% (to 3.3 g/day) as the injection size is increased to 425 mg. Larger injections again result in slightly lower production rates. The amount of 425 mg remains the desirable injection size even when the product purity is relaxed successively to 95, 90 and eventually, 80%. At the 95% purity level (which is of interest, because it is close to that of the commercially available, enantiomerically "pure" material), the production rate is about 4 g/day. Even a drastic reduction of the target purity to 80% does not increase the production rate above 5.5 g/day.

The situation is different for the more retained enantiomer (Fig. 6). At greater than 99.99%

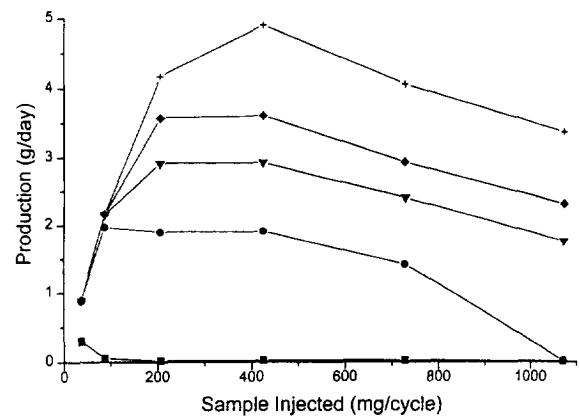

Fig. 6. Production for the more retained MCP enantiomer as a function of the injected sample amount at various levels of product purity. Feedstock: racemic MCP. Conditions as in Fig. 2. Symbols: + = 80%; ◆ = 90%; ▼ = 95%; ● = 99%; ■ = 100% purity.

purity, only 0.3 g/day can be produced, and only when the injection size is just below the "touching-band" load. When the purity requirement is relaxed to 99%, there is a dramatic increase in the production rate to 2 g/day. This is only a third less than the maximum production rate for the less retained enantiomer, and can be realized by injections as small as 80 mg. Quite fortunately, production rate remains flat (decreases only slightly) at this product purity as the injection size is increased to 425 mg. This means that one can achieve maximum production rate both for the less retained enantiomer and the more retained enantiomer at the same injection size (425 mg), without sacrificing either product purity or production rate.

It was instructive to see what happens when the racemic feedstock is replaced with an enantiomerically enriched feedstock (95.7% ee for the less retained enantiomer). The analytical FID trace at 420 mg load obtained with this feedstock is shown in Fig. 7, and the daily production rate vs. injected amount curves for greater than 99.99% purity and 99% purity in Fig. 8. There is no significant accumulation of the more retained enantiomer at the tail-end of the less retained enantiomer (Fig. 7); instead the

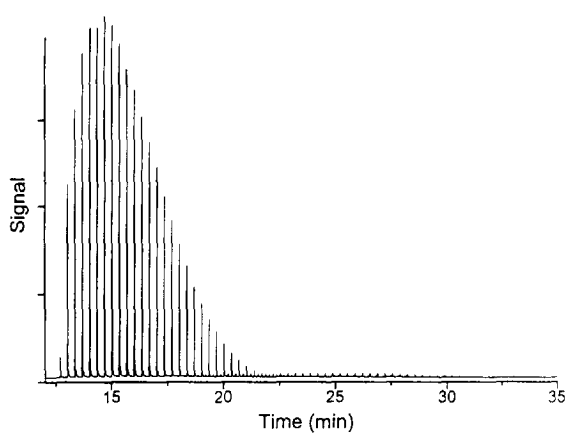

Fig. 7. Example of an on-line enantiomeric fraction analysis. Sample: 420 mg enantiomerically enriched (*R*)-(+) (95.7% ee) MCP. Preparative column and conditions as in Fig. 1; analytical column and conditions as in Fig. 2. Sampling volume: 10 μl, sampling cycling time: 20 s. Light peaks: (*R*)-(+)-MCP, dark peaks: (*S*)-(−)-MCP.

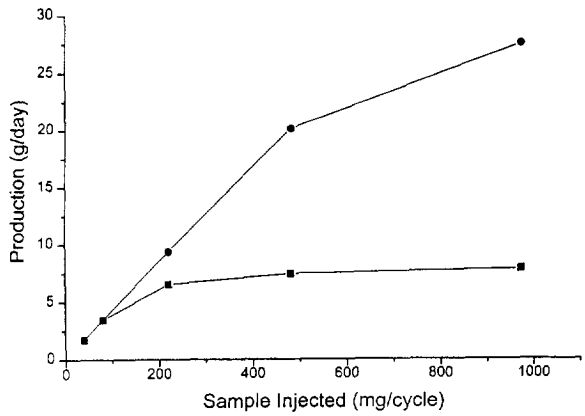

Fig. 8. Production for the less retained MCP enantiomer as a function of the injected sample amount at various levels of product purity. Feedstock: enantiomerically enriched (*R*)-(+)-MCP (95.7% ee). Conditions as in Fig. 7. Symbols: ● = 99% purity; ■ = 100% purity.

concentration of the second enantiomer remains rather constant during the last third of the peak of the first enantiomer. It can be seen in Fig. 8, that about 7 g/day of the less retained enantiomer can be produced at greater than 99.99% purity, a three-fold increase compared to what could be obtained with the racemic feedstock. At this level of enantiomeric purity, the production rate remains flat as the injection size is increased. However, when the purity requirement is relaxed to 99%, the production rate becomes as high as about 30 g/day, and the production rate maximum is not achieved even at 970 mg injection, the highest tested in this work.

4. Conclusions

The previously described integrated preparative/analytical GC system has been modified to accommodate columns as large as 1 m × 22.5 mm I.D. A liquid chiral stationary phase, AMP-5 could be successfully coated onto the 60–80 mesh Chromosorb A support and packed into 1 m × 22.5 mm I.D. columns yielding 825 theoretical plates at the optimum point of the Van Deemter curve. Operated isothermally at 60°C, the column resulted in $\alpha = 1.5$ and $k_2' = 150$ values for the enantiomers of the methyl ester of

α-chloropropionic acid. A 5.5-m long capillary column, coated with a 0.15-μm thick film of the same stationary phase permitted the on-line enantiomeric analysis of the effluent of the preparative column with a cycle time of 20 s, resulting in accurate effluent purity vs. production rate curves and production rate vs. injected sample size curves. It also leads to aggressive, yet safe, production regimes yielding 1 to 5 g/day production rates, depending on the level of required purity, with a racemic feedstock, and 7 to 30 g/day production rates (for 100 and 99% purity, respectively) with an enantiomerically enriched feedstock. Further work is underway in our laboratory to extend the system for the production of other enantiomers of value or interest.

Acknowledgements

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