Journal of Chromatography A, 658 (1994) 437–443
Elsevier Science B.V., Amsterdam

CHROM. 25 584

Enantiomer fractionation by preparative gas chromatography

G. Fuchs and M. Perrut*

Separex B.P. 9, F-54250 Champigneulles (France)

ABSTRACT

The preparative gas chromatographic separation of a "mushroom odour" racemate, 1-octenyl 3-acetate, was performed on two columns connected in series (1 m × 2 cm I.D.) packed with modified β-cyclodextrin coated on Chromosorb support. The process feasability was evaluated, leading to a production rate of 15 g/day of enantiomers with an optical purity of 80%.

INTRODUCTION

Derivatized cyclodextrins (CDs) can be used as reliable chiral stationary phases for the capillary GC separation of volatile racemates of widely different structural characteristics. They were first introduced by Koscielski *et al.* [1] for use in packed columns, but only recently has the possibility of preparing capillary columns coated with different modified CDs been systematically investigated by various workers [2–14].

This paper aims to show the behaviour of modified CDs in the separation of the enantiomeric aroma compound 1-octenyl 3-acetate by preparative gas chromatography on packed columns. Several derivatized (peracetylated, perbenzoylated, permethylated) CD phases were examined in order to find the appropriate chiral phase for preparative separation.

EXPERIMENTAL

Stationary phases

Stationary phases were prepared by Fellous [15] according to a proprietary method; several materials were supplied, *viz.*, peracetylated, perbenzoylated and permethylated β-cyclodextrin and permethylated maltocyclodextrin.

Column preparation

Columns were prepared by coating 10% (w/w) of CD on Chromosorb P NAW (Johns-Manville, Denver, CO, USA) by the usual procedure: the chiral phase was diluted in dichloromethane (*e.g.*, 1 g in 25 ml of CH_2Cl_2), this mixture was added to the solid support and the solvent was evaporated under slight agitation at 35°C in order not to create fine particles; evaporation must be "smooth" in order to lead to a homogeneous layer of chiral phase on the support.

Equipment

Analyses were performed with a GC 180 gas chromatographic system (GIRA, Morlaas, France) with a katharometer as detector.

The preparative GC unit (THN 102, Chromatelf) (Fig. 1), equipped with a katharometer as detector, was fully controlled by a computer that ensured periodical injection of feed and recovery of the fractions in the different traps by condensation. Placed in the oven and connected in series, two columns (1 m × 2 cm I.D.) were packed according to the "vibrations and shocks" procedure, which is known to lead to high-efficiency packings [16].

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1025-U

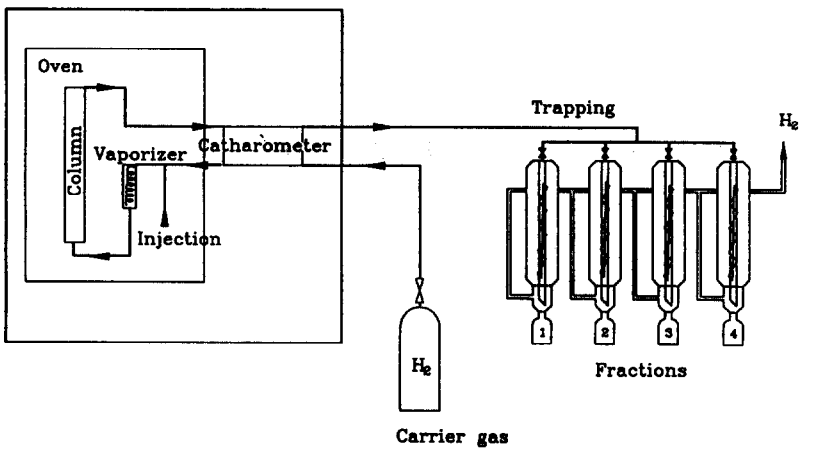

Fig. 1. Preparative GC unit.

RESULTS AND DISCUSSION

Analytical study

On an analytical scale, the main objective was to establish the operating conditions leading to good resolution between the two enantiomers. First, the various stationary phases were tested and the best results were obtained with permethylated β-CD (with peracetylated β-CD low retention and no selectivity, with perbenzoylated β-CD strong retention and no selectivity and with permethylated maltocyclodextrin containing a mixture of α-, β- and γ-CD low selectivity were obtained). Consequently, further work was carried out with permethylated β-CD.

With the analytical column (2 m × 2.2 mm I.D.), the optimum operating conditions were found to be as follows: temperatures, oven 120°C, detector 220°C and injection 220°C; and carrier gas, helium at 16.8 ml/min (20°C, 1 bar). Typical chromatograms are presented in Figs. 2 and 3.

As shown in Fig. 2a, the results were satisfactory, with good enantioselectivity and resolution for acceptable retention times (t_{r_1} = 27 min, t_{r_2} = 32 min), good peak symmetry and a relatively short delay between each injection (16 min). When the oven temperature was fixed at 140°C a loss of resolution occurred (Fig. 2b). In order to evaluate the feasibility of fractionation on a preparative scale, overloading tests were performed on the analytical column (Fig. 3). Extrapolation of these results led to the conditions for preparative-scale operation reported in Table I.

In order to improve the analytical procedure, a 4-m long analytical column was prepared and

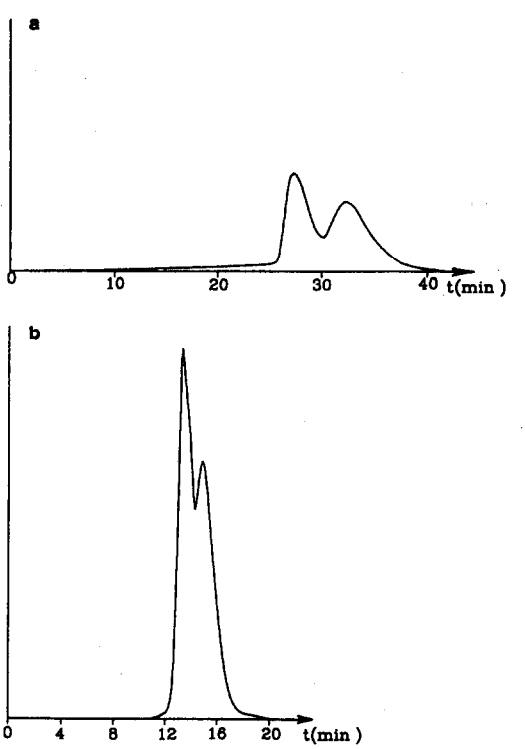

Fig. 2. Analytical chromatogram with an injection volume of 1 μl and an oven temperature of (a) 120 and (b) 140°C.

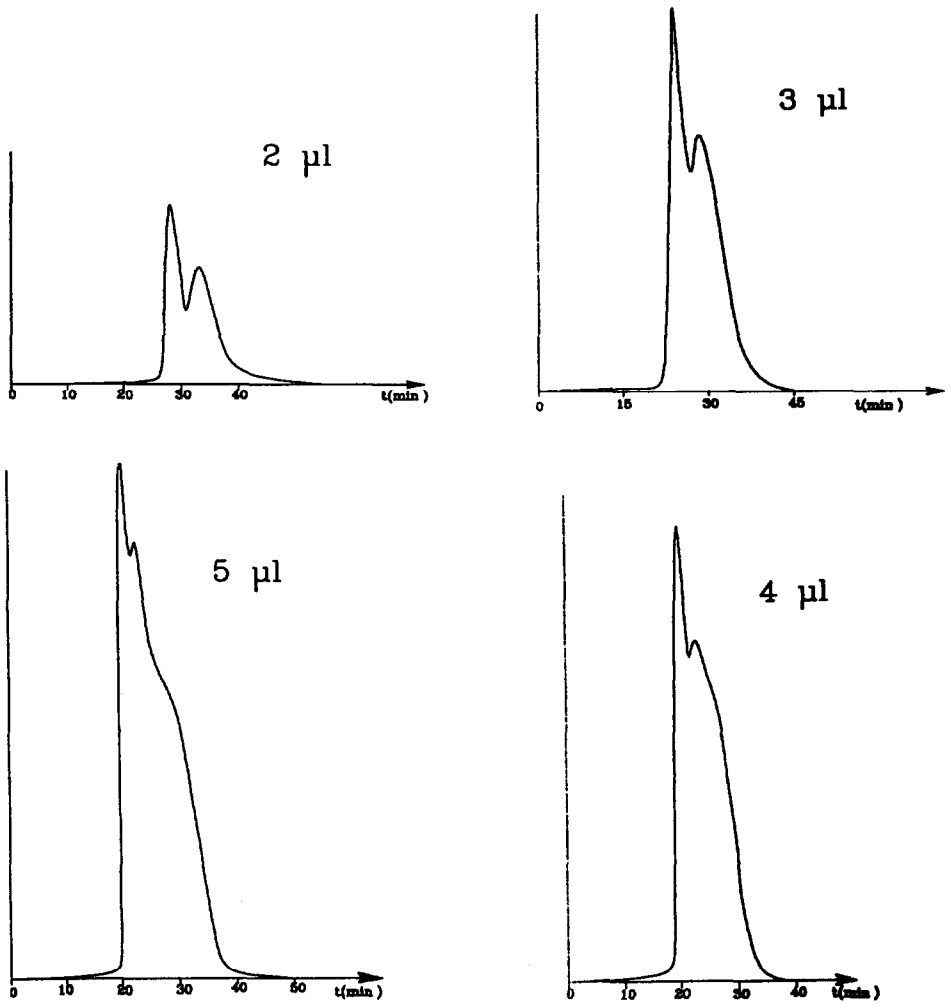

Fig. 3. Analytical chromatograms with different injection volumes.

TABLE I

EXTRAPOLATION OF INJECTION VOLUME FROM ANALYTICAL TO PREPARATIVE GAS CHROMATOGRAPHY

Injection volume (µl)		Cycle duration (min)
Column I.D. 2.2 mm	Column I.D. 20 mm	
1	100	16
2	200	20
3	300	23
4	400	23
5	500	25

several packings and operating parameters for analytical purpose were examined, resulting in a good quality of separation (Fig. 4).

Column conditioning was very important for obtaining satisfactory resolution and good reproducibility of the chromatograms. A sufficient temperature was necessary (in this work, the operating temperature was 160°C for 24 h at least) to remove traces of solvent and impurities from the stationary phase, and also to modify the crystalline structure of the β-CD. A tentative explanation proposed by Venema and Tolsma [14] was related to the structure of the modified CD (liquid crystal structure) that needs to be present in order to be selective, so that the column must be conditioned above the melting point of the CD.

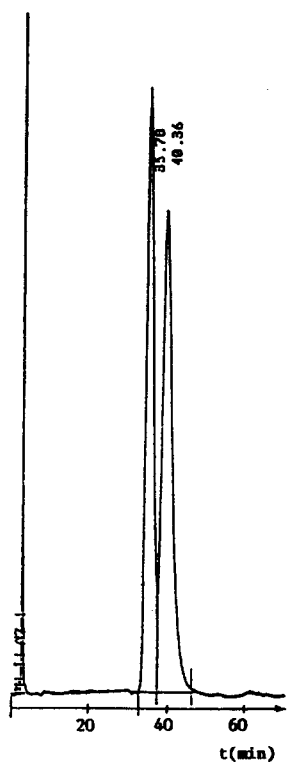

Fig. 4. Analytical chromatogram of racemic mixture. Column, 4 m × 2.2 mm I.D.; oven temperature, 130°C; flow-rate, 20 ml/min; injection volume, 0.3 μl.

Preparative study

Linear extrapolation of the carrier gas flow-rate from analytical- to preparative-scale GC led to a value of 1.4 l/min. However, as we know that the packing performances are excellent and the HETP varies slowly when the eluent flow-rate is increased, we preferred to increase the flow-rate in order to decrease the time required. For economic reasons, experiments were carried out using hydrogen as the carrier gas instead of helium, under the following conditions: temperatures, oven 120°C, detector 150°C and injection 120°C; and carrier gas, Hydrogen at 2.6 l/min (20°C, 1 bar).

From our experience, direct extrapolation must be considered cautiously, so we performed different mass injections of the racemic mixture in order to establish the phase behaviour *versus* amount injected (Fig. 5). It appears that, surprisingly, the peak shape does not change significantly with the amount injected; the volume injected was fixed at 500 μl (*e.g.*, 0.43 g) with a cycle duration of 16 min.

Many injections were repeated every 16 min, permitting the recovery of fractions with different cut-point positions (Fig. 6, Table II). Unfortunately, the recovery was low (about 75%), as the trap size was too large in comparison with the volumes injected. The recovery of the first-eluted enantiomer was 31.4% (expected recovery for total trapping = 42%) with a purity of *ca.* 65% and that of the second-eluted enantiomer was 23.7% (expected recovery for total trapping = 32%) with a purity of 72%.

The first three fractions were recycled in order to increase the purity. The cut-point positions were varied with the injected fraction composi-

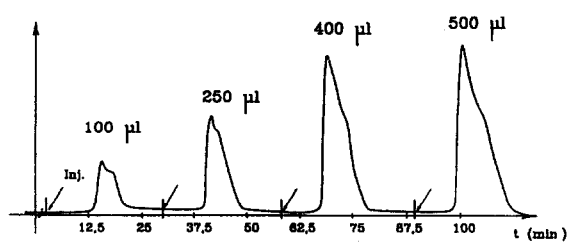

Fig. 5. Preparative chromatograms. For experimental details, see text.

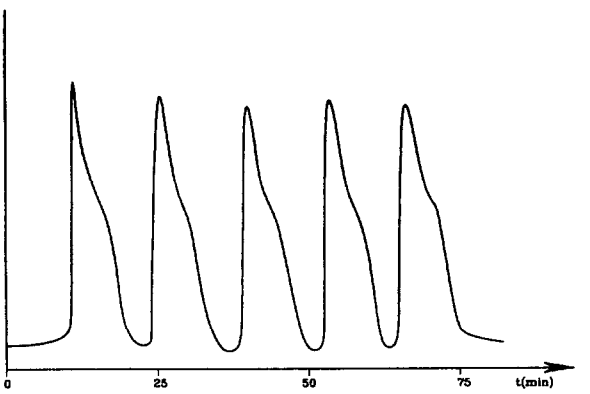

Fig. 6. Production step: injection volume, 500 μl; cycle duration, 16 min.

tion: the cycle duration for the first fraction was then 12 min whereas that for the second fraction was maintained at 16 min.

In this recycling step, a purity of *ca.* 80% for both enantiomers was obtained. According to these results, a fractionation process diagram is presented in Fig. 7, from which the production rates were determined, with the assumption of total recovery, and are reported in Table III.

Repeated chromatograms were reproducible; however, after 3 weeks of operation the retention times decreased from 12 to 5 min for which several explanations can be suggested: stationary phase leakage (bad coating on the support); irreversible adsorption of impurities on chiral sites; or deterioration of stationary phase.

TABLE II

RESULTS OBTAINED FOR ONE PASS

For details, see text.

No. of injections of 500 μl	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Total recovery (%)
	Mass recovered (g)	Recovery (%)	Mass recovered (g)	Recovery (%)	Mass recovered (g)	Recovery (%)	Mass recovered (g)	Recovery (%)	
21	2.95	32.2	1.94	21.5	1.43	15.8	0.31	3.4	73
20	3.06	35.6	1.66	19.3	1.24	14.4	0.59	6.8	76
14	2.12	35.2	1.08	17.9	1.12	18.6	0.34	5.6	77
21	2.64	29.2	1.94	21.5	2.21	24.5	0.67	7.4	83
21	1.11	21.5	0.89	17.2	1.04	20.1	0.02	0.39	59
Total: 88	11.88	31.4	7.51	19.8	7.04	18.6	1.93	5.1	75
Concentration of first-eluted enantiomer (%)	65		53		30		20		

TABLE III

PRODUCTION RATES OF EACH ENANTIOMER FOR A TOTAL RECOVERY

Parameter	First-eluted enantiomer	Second-eluted enantiomer
Productivity for one pass (g/day)	16.25	12.38
Purity (%)	65	72
Recovery (%)	42	32
Productivity according to process in Fig. 7 (g/day)	7	7.4
Purity (%)	82	80
Recovery (%)	48	52

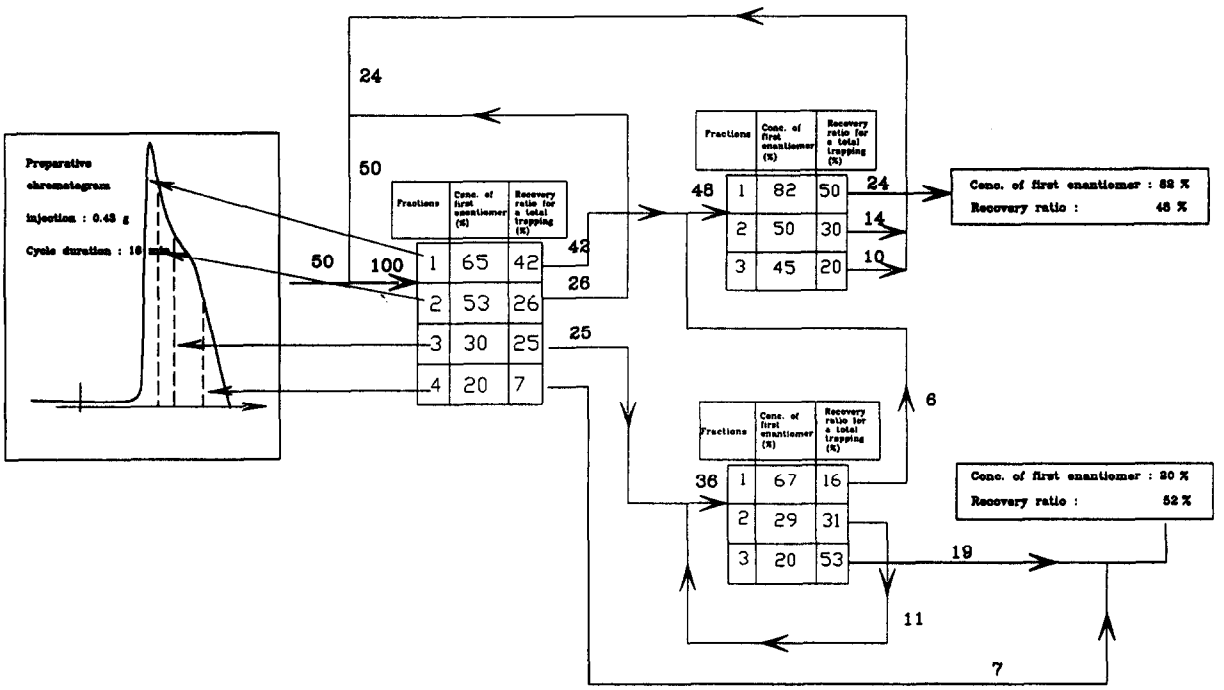

Fig. 7. Fractionation process diagram in GC mode.

CONCLUSIONS

Although experiments with other cut-point positions might lead to better optimization, the separation of this racemic mixture with a production rate of 15 g per day and enantiomer purities of 80% demonstrates the potential of preparative GC for this application.

Finally, although the knowledge regarding CD stability remains limited, the new modified CD phases might extend the possibilities of volatile racemate resolution without derivatization.

ACKNOWLEDGEMENTS

We are grateful to Professor R. Fellous (Université de Nice) for providing the racemate and stationary phases, and the Société Nationale Elf Aquitaine for financial support.

REFERENCES

1 T. Koscielski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 280 (1983) 131.
2 V. Schurig and H.P. Nowotny, *Angew. Chem.*, 29 (1990) 939.
3 W.A. Koenig, R. Krebber, P. Evers and G. Bruhn, *J. High Resolut. Chromatogr.*, 13 (1990) 328.
4 W.A. Koenig, D. Icheln, T. Runge, I. Pforr and A. Krebs, *J. High Resolut. Chromatogr.*, 13 (1990) 702.
5 G. Wenz, P. Mischinick, R. Krebber, M. Richters and W.A. Koenig, *J. High Resolut. Chromatogr.*, 13 (1990) 724.
6 V. Schurig, M. Jung, D. Schmalzing, M. Schleimer, J. Duvekot, J.C. Buyten, J.A. Peene and P. Mussche, *J. High Resolut. Chromatogr.*, 13 (1990) 470.
7 V. Schurig, D. Schmalzing, U. Muhleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot and J.C. Buyten, *J. High Resolut. Chromatogr.*, 13 (1990) 713.
8 D.W. Armstrong, C.D. Chang and W. Li, *J. Agric. Food Chem.*, 38 (1990) 1674.
9 D.W. Armstrong, W. Li, A.M. Stalcup, J.I. Seemen and H.V. Secor, *Anal. Chim. Acta*, 234 (1990) 365.

10 W. Blum and R. Alchholz, *J. High Resolut. Chromatogr.*, 13 (1990) 515.
11 U. Hener, P. Kreis and A. Mosandl, *Flavour Fragrance J.*, 6 (1991) 109.
12 C. Bicchi, G. Artuffo, A. d'Amato, G.M. Nano, A. Galli and M. Galli, *J. High Resolut. Chromatogr.*, 14 (1991) 301.
13 C. Bicchi, G. Artuffo, A. d'Amato, V. Manzin, A. Galli and M. Galli, *J. High Resolut. Chromatogr.*, 15 (1992) 710.
14 A. Venema and P.J.A. Tolsma, *J. High Resolut. Chromatogr.*, 12 (1989) 32.
15 R. Fellous, University of Nice, personal communication.
16 H. Colin and P. Hilaireau, *Actual. Chim.*, *April–May* (1988) 151.