Journal of Chromatography, 623 (1992) 329–336
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 390

Enantiomer fractionation of phosphine oxides by preparative subcritical fluid chromatography

G. Fuchs, L. Doguet and D. Barth

LPCI–ENSIC, 1 rue Grandville, B.P. 451, F-54001 Nancy Cédex (France)

M. Perrut

SEPAREX, 5 rue Jacques Monod, B.P. 9, F-54520 Champigneulles (France)

(First received November 8th, 1991; revised manuscript received May 26th, 1992)

ABSTRACT

The subcritical fluid chromatographic separation of three enantiomeric pairs of phosphine oxides (two phosphanorbornadienes and 1,2,5-triphenylphospholane-1-oxide) was performed on a pilot plant equiped with a 60-mm axial compression column packed with Pirkle-type phases, the mobile phase being carbon dioxide–ethanol mixtures. With selectivities ranging from 1.1 to 1.3, both enantiomers were obtained with an optical purity greater than 95%. The resolution of one phosphanorbornadiene pair was optimized with a production rate reaching 510 mg/h with a good yield (80%).

INTRODUCTION

Traditionally, chromatography has been used to separate compounds having distinctly different chemical and physical properties. Only relatively recently has attention been focused on chiral chromatography, where selective adsorption is used to separate enantiomeric mixtures into optically pure compounds. Most investigations have been directed towards the development of highly enantioselective and broadly applicable chiral stationary phases (CSPs) [1–3]. Among the numerous classes of chiral stationary phases, those developed from Pirkle's concept are probably the best known and most widely used, essentially on an analytical scale [3,4].

The principle of chromatographic separation is based on the formation of labile diastereoisomeric complexes with a chiral complexing agent (selector) bonded on a silica support. The different stabilities of these diastereoisomers lead to selectivity. In other words, the enantioselectivity factor, α, is related to the difference in free energies of binding ($\Delta\Delta G$) to the CSP for two enantiomers by the equation

$$\Delta\Delta G = \Delta\Delta H - T\Delta\Delta S = -RT \ln \alpha \quad (1)$$

where $\Delta\Delta H$ and $\Delta\Delta S$ are bonding enthalpy and entropy differences, respectively. Consequently, the chiral recognition ability of the CSP decreases with increasing column temperature [5,6]. We have to operate at the lowest temperature possible (20°C) on our pilot plant in order to maximize selectivity.

Several workers [7,8] have reported enantiomer resolutions on a wide variety of CSP using carbon dioxide enriched with different alcohols as a subcritical eluent for analytical purposes. However, no preparative resolution has been published so far.

In this work, preparative subcritical fluid chromatography (SubFC) was used for the resolution of three tertiary phosphine oxides which play a prominent role in asymmetric catalysis, *e.g.*, the pure enantiomer of 1,2,5-triphenylphospholane 1-oxide

Correspondence to: Dr. D. Barth, LPCI–ENSIC, 1 Rue Grandville, B.P. 451, F-54001 Nancy Cédex, France.

0021-9673/92/$05.00

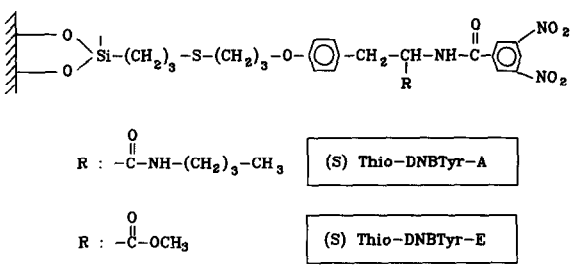

Fig. 1. Chiral stationary phases.

(compound **2**) is used as a ligand for transition metal complexes in enantioselective catalytic organic reactions [9]. Moreover, in addition, high-performance liquid chromatography (HPLC) and SubFC were compared.

Two chiral π-acceptor stationary phases derived from (*S*)-N-(3,5-dinitrobenzoyl)tyrosine (DNBTyr) covalently bonded to a γ-mercaptopropyl silica gel were used (Fig. 1). The mobile phase in SubFC was a classical mixture for a Pirkle-type phase, carbon dioxide–ethanol [8]. The three enantiomeric pairs of compounds considered were phosphine oxides (Fig. 2). Compound **1a** was fractionated on (*S*)-thio-DNBTyr A and compounds **1b** and **2** on (*S*)-thio-DNBTyr E.

EXPERIMENTAL

Analytical chromatography

HPLC. The analytical liquid chromatography consisted of a pump (Isochrom LC, Spectra-physics, Les Ulis, France) and a spectrophotometer (Model 481, Waters, Montigny-le-Bretonneux, France) connected with a computer–integrator (SP 4270, Spectra-physics).

SubFC. Liquid carbon dioxide was pumped through a cooled head metering pump (Minipump, Dosapro Milton Roy, Pont Saint-Pierre, France). Polar modifier was added through a pump (Model 510, Waters) and mixed with carbon dioxide prior to a heat exchanger. An HPLC valve (Rheodyne Model 7125, Touzart et Matignon, Vitry-sur-Seine, France) was used as an injector. The column temperature was controlled by a heat exchanger. A variable-wavelength UV detector (Spectra 100, Spectra-physics) was connected with an integrator (SP 4270, Spectra-Physics). The pressure was controlled by two manually adjustable back-pressure regulators (LTH 400, Alphagaz, Bois D'Arcy, France). Finally, the gaseous carbon dioxide was released to the atmosphere after flow-rate measurement using a rotameter (Teflinox, Wasselone, France).

Preparative subcritical fluid chromatography (PSubFC)

The PSubFC pilot plant, with total recycle of the eluent, has been under development in our laboratory since 1982 [10,11] (Fig. 3). It should be noted that eluent–product separation is performed through high-performance separators [12] and the axial compression technique is used to increase column efficiency. The column efficiencies are now at least similar to those obtained in analytical SubFC [13]. An original set-up called a saturator permits

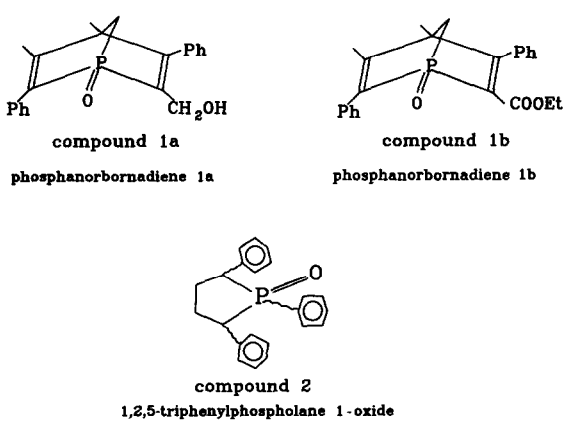

Fig. 2. Phosphine oxides.

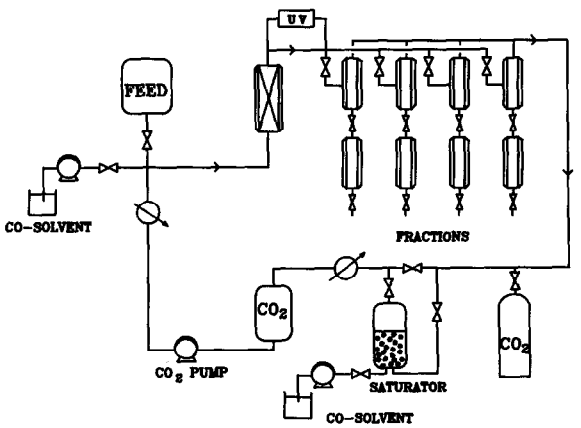

Fig. 3. PSubFC pilot plant.

TABLE I

RESOLUTION OF THREE RACEMIC PHOSPHINE OXIDES

Mobile phase: *n*-hexane–ethanol for HPLC; CO_2–ethanol, pressure 20 MPa, for SubFC. UV detection at 230 nm. Column temperature, 293 K.

Compound	Stationary phase	HPLC		Sub FC		Ethanol in mobile phase (% v/v)
		$\alpha_{1,2}$	k'_1	$\alpha_{1,2}$	k'_1	
1a	(*S*)-Thio-DNBTyr A	1.27	12	1.35	32	8
1b	(*S*)-Thio-DNBTyr E	1.19	17	1.19	17	12
2	(*S*)-Thio-DNBTyr E	1.11	22	1.15	41	8

both polar modifier addition to the mobile phase and recycled eluent cleaning [14]. The pilot plant is fully controlled by a computer which ensures periodical injection of the feed and the recovery of the different fractions.

Apparatus

Melting points (m.p.) were measured on a Kofler hot-stage apparatus (OSI, Paris, France) and are given without correction.

Optical purities were obtained using a Model 141 micropolarimeter with a 1-dm length quartz cell (Perkin-Elmer, St.Quentin en Yvelines, France).

Solvents

HPLC-grade hexane and absolute ethanol were purchased from Prolabo (Paris, France). Technical-grade carbon dioxide for preparative SubFC was supplied by Carboxyque Française (Puteaux, France) and high-purity carbon dioxide (>99.9995%) for analytical SubFC from Air Gaz (Mitry-Mory, France).

Stationary phases

The phases (*S*)thio-DNBTyr A bonded on LiChroprep Si-60 silica (10 μm, irregular) (Merck) and (*S*)-thio-DNBTyr E on LiChroprep Si-60 silica (7 μm, irregular) (Merck) have been developed recently by Tambute and Begos [15]. The stationary phase (*S*)-thio-DNBTyr A is now commercially available as ChyRoSyne A from Touzart et Matignon.

Racemic mixtures

Phosphanorbornadienes **1a** and **1b** were supplied by Le Goff (SNPE, Vert-le-Petit, France) and 1,2,5-triphenylphospholane 1-oxide by Fiaud (Institut de Chimie Moléculaire, Orsay, France). The products were dissolved in ethanol for the injection.

RESULTS AND DISCUSSION

Analytical study

The chromatographic results obtained for the direct resolution of racemic compounds under HPLC and SubFC conditions are reported in Tables I and II. For SubFC the temperature (T) was 293 K and the pressure (P) 20 MPa. The selectivity, α_{12}, is greater with the subcritical eluent than with the liquid. The capacity factor, k', is much higher in SubFC than HPLC, especially for **1a**, but the low viscosity of carbon dioxide ($\eta = 10.3 \cdot 10^{-5}$ Pa s,

TABLE II

OVERLOADING TESTS FOR THREE RACEMIC PHOSPHINE OXIDES IN SubFC

(A) Stationary phase, (*S*)-Thio-DNBTyr A; column, 250 × 4.6 mm I.D.; mobile phase, CO_2–ethanol (92:8, v/v); flow-rate, 4.42 ml/min; pressure, 20 MPa; temperature, 293 K; UV detection at 230 nm. (B) Stationary phase, (*S*)-Thio-DNBTyr E; column, 150 mm × 4.6 mm I.D.; mobile phase, CO_2–ethanol (88:12, v/v); flow-rate, 2.53 ml/min; other conditions as in (A). (C) Stationary phase, (*S*)-Thio-DNBTyr E; column, 150 mm × 4.6 mm I.D.; mobile phase, CO_2–ethanol (92:8, v/v); flow-rate, 3.88 ml/min; other conditions as in (A).

Compound	Conditions	Mass injected (mg)	R_S
1a	A	4.00	1.2
1b	B	0.32	0.9
2	C	0.15	1.0

TABLE III

PREPARATIVE SCALE COLUMN EFFICIENCIES

Column	Phase	TP/m[a]	*h* (reduced HETP)	Column length (mm)
1	(*S*)-Thio-DNBTyr A	45 000	2.2	76
2	(*S*)-Thio-DNBTyr E	56 000	2.5	100

[a] Theoretical plates per metre.

T = 293 K, P = 20 MPa) [16] permits much higher flow-rates to be used than those classicaly used in HPLC. Consequently, in SubFC the retention times are shorter (Fig. 4). It is noteworthy that the maximum amount injected in order to obtain a resolution of 1 is very low. In fact, only 19% of the initial mercaptopropyl groups have reacted with the chiral selector, which results in a small number of chiral sites and hence in a low stationary phase capacity [15].

Preparative study

The chiral stationary phases [146 g of (*S*)-thio-DNBTyr A and 184 g of (*S*)-thio-DNBTyr E] were packed successively into the column (60 mm I.D.) by axial compression (2 MPa) (Table III). The other

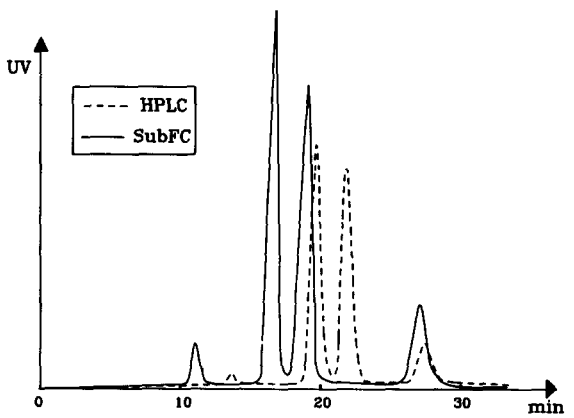

Fig. 4. Resolution of 1,2,5-triphenylphospholane 1-oxide under HPLC and SubFC conditions. HPLC conditions: stationary phase, (*S*)-thio-DNBTyr E; column, 150 mm × 4.6 mm I.D.; mobile phase, *n*-hexane–ethanol (92:8, v/v); flow-rate, 2 ml/min; column temperature, 293 K; UV detection at 230 nm. SubFC conditions: stationary phase, (*S*)-thio-DNBTyr E; column, 150 mm × 4.6 mm I.D.; pressure, 20 MPa; column temperature, 293 K; mobile phase, CO_2–ethanol (92:8, v/v); flow-rate, 3.88 ml/min; UV detection at 230 nm.

operating conditions were as follows: column pressure 20 MPa; separator pressure, 5 MPa; column temperature, 293 K; separator temperature, 338 K; eluent, carbon dioxide–ethanol (for **1a** 92:8, for **1b** 88:12 and for **2** 92:8 v/v); and flow-rate, 10–16 l/h.

The extrapolation rules are complex and several parameters have to be taken in to account: number of theoretical plates, injection shape and peak asymmetry [17]. As a first choice, the amounts injected were linearly extrapolated with the volume of stationary phase as shown in Table IV; we shall now present the results for the three fractionations in order of increasing difficulty (**1a**, α = 1.3, **1b**, α = 1.2; **2**, α = 1.1).

*Resolution of phosphanorbornadiene **1a** enantiomers*

For the first enantiomer fractionation, the conditions of preparative separation were not optimized and small amounts of solute (100 mg) were injected in order to obtain the first and second enantiomers with purities of 100% and 97%, respectively (by HPLC). The resolution was correct (R_s = 1.1). The optical purity of each enantiomer was also measured in order to confirm the purity attainable by preparative chromatography: (+)-phosphanorbornadiene (**1a**) m.p. = 117°C, $[\alpha]_D^{22}$ = +192.26° (c = 1, $CHCl_3$); (−)-phosphanorbornadiene (**1a**) m.p.

TABLE IV

EXTRAPOLATION OF AMOUNT INJECTED FROM ANALYTICAL TO PREPARATIVE SubFC

Compound	Mass injected (mg)	
	Analytical SubFC	Preparative SubFC
1a	4.00	207
1b	0.32	36
2	0.15	17

= 123°C, $[\alpha]_D^{22}$ = −173.53° (*c* = 1, $CHCl_3$). The absolute configurations are not given in the literature.

Resolution of phosphanorbornadiene ***1b*** *enantiomers*

For the second enantiomer fractionation, the preparative separation parameters (flow-rate, amount injected, particle size, column length and diameter, injection frequency, cut point position) can be optimized. In fact, some parameters are imposed (column geometry, particle size). This study was performed as follows. The recovery ratio of pure enantiomer at two different flow-rates 12 and 16 l/h) was reported *versus* the injected mass, and the production rate (Tables V and VI) was quantified by

$$Q_L \text{ (mg/s)} = \frac{\text{solute recovery at each injection (mg)}}{\text{retention times of first-eluted enantiomer (s)}}$$

From 70 injections, the optimum Q_L value was determined for each flow-rate. It could be related to the fact that it is difficult to choose good cut-point positions which change with amount injected owing to non-linear adsorption isotherms. As the amount injected is increased, the band of the profile moves toward shorter retentions (Table V) owing to the non-linear elution conditions.

The fractions are considered to be correct if they reach the following enantiomeric purities: first enantiomer (+), enantiomeric excess (e.e.) = 96%; second enantiomer (−), e.e. = 94%. The enantiomeric purity and solute recovery were determined by comparing individual fractions with a standard solution containing 0.5 mg/ml of solute on an analytical column by HPLC.

According to our analytical work, the optimum amount injected is 36 mg (see Table IV). In fact, we found that the amount injected ranged from 40 to 100 mg. Consequently, direct extrapolation must be considered cautiously. This could be related to the fact that the plate number *N* is higher in the preparative column than in the analytical column ($N_{anal.}$ ≈ 25 000 theoretical plates (TP)/m, *l* = 25 cm; $N_{prep.}$ ≈ 50 000 TP/m, *l* = 10 cm), owing to the excellent performance of the axial compression technique.

For a flow-rate of 12 l/h, the mass injected is optimum around 80 mg for a recovery ratio of 90%. However, for a flow-rate of 16 l/h, the recovery ratio is lower than 90% for the same optimum mass injected.

The curves of Q_L *versus* mass injected show that higher flow-rates may lead to higher production rates (Table VI). Indeed, for a flow-rate of 16 l/h, the delay between each injection can be decreased to 7 min under such conditions, and production rate can reach 510 mg/h higher than that obtained by HPLC (250 mg/h) with a ternary mixture as eluent [18].

Although experiments with other flow-rates might lead to a better optimization, it seems that the best results are obtained at higher flow-rates. The low viscosity of carbon dioxide permits high flow-rates to be used without too high a pressure drop, which is the main advantage in PSubFC.

TABLE V

Q_L *VERSUS* MASS INJECTED FOR RESOLUTION OF PHOSPHANORBORNADIENE **1b**

Flow-rate, 12 l/h; stationary phase, (*S*)-Thio-DNBTyr E; column, 100 mm × 60 mm I.D.; mobile phase, CO_2 –ethanol (88:12, v/v); pressure, 20 MPa; column temperature, 293 K; UV detection at 230 nm.

First-eluted enantiomer				First- + second-eluted enantiomers	Second-eluted enantiomer			
Mass injected (mg)	Mass recovered (mg)	Retention time of first-eluted enantiomer (s)	Q_L	Q_L	Mass injected (mg)	Mass recovered (mg)	Retention time of first-eluted enantiomer (s)	Q_L
40	16	950	0.017	0.035	40	17	950	0.018
40	19	950	0.020	0.039	40	19	950	0.019
60	25	900	0.028	0.056	60	25	900	0.028
80	33	850	0.039	0.076	80	32	850	0.037
100	38	750	0.050	0.098	100	36	750	0.048

TABLE VI

Q_L *VERSUS* MASS INJECTED FOR RESOLUTION OF PHOSPHANORBORNADIENE **1b**

Flow-rate, 16 l/h; other operating conditions as in Table V.

First-eluted enantiomer				First- + second-eluted enantiomers	Second-eluted enantiomer			
Mass injected (mg)	Mass recovered (mg)	Retention time of first-eluted enantiomer (s)	Q_L	Q_L	Mass injected (mg)	Mass recovered (mg)	Retention time of first-eluted enantiomer (s)	Q_L
60	18	520	0.036	0.065	60	15	520	0.029
70	28	600	0.046	0.090	70	26	600	0.044
80	32	590	0.054	0.102	80	28	590	0.048

The purified enantiomers were obtained as follows: (*R,R*)-(+)-phosphanorbornadiene (**1b**), m.p. = 120°C, $[\alpha]_D^{22}$ = +162.6° (*c* = 1, $CHCl_3$); (*S,S*)-(−)-phosphanorbornadiene (**1b**), m.p. = 118°C, $[\alpha]_D^{22}$ = −154.1° (*c* = 1, $CHCl_3$). Literature data [19] are as follows: (*R,R*)-(+) -**1b**, m.p. = 139–141°C, $[\alpha]_D^{22}$ = +178° (*c* = 1, $CHCl_3$); (*S,S*)-(−)-**1b**, m.p. = 138–140°C, $[\alpha]_D^{22}$ = −172.3° (*c* = 1, $CHCl_3$).

Resolution of 1,2,5-triphenylphospholane 1-oxide enantiomers

Regarding the resolution of *trans*-1,2,5-triphenylphospholane 1-oxide, the separation of enantiomers with good enantiomeric purity is a challenge as the selectivity factor is very low (α = 1.1). The feed contains two *cis*/*trans* isomers (ratio 14:83 and 3% of unknown impurities); the *trans* isomer is chiral and gives the absolute configurations *R,R*/*S,S*, which were separated on the (*S*)-thio-DNBTyr-E stationary phase. The mass injected was fixed at 38 mg with an eluent flow-rate of 15 l/h, leading to a resolution of 1.16.

The total amount of compound fractionated on a preparative scale was *ca.* 3 g and the recovery ratios were low (*ca.* 50%) (Table VII). This result can be explained by two factors: on the one hand, the size of the pilot unit is too large in comparison with the mass injected, and on the other, the initial feed contains 17% of impurities (14% *cis* isomer + 3% impurities). Although the separation factor is low (α = 1.1), each enantiomer is obtained with high opti-

TABLE VII

RECOVERED MASS AND OPTICAL PURITIES FOR EACH INJECTION SERIES OF 1,2,5-TRIPHENYLPHOSPHOLANE 1-OXIDE

Operating conditions as in Fig. 5.

Σ mass injected for each series (mg)	First fraction		Second fraction		Third fraction	
	Mass recovered (mg)	% of first enantiomer	Mass recovered (mg)	% of first enantiomer	Mass recovered (mg)	% of first
304	64	96	49	39	52	15
342	80	99	17	42	60	11
190	32	100	3	24	28	6
266	31	100	33	11	25	11
418	125	98	37	33	111	7
380	87	98	16	35	133	8
152	32	99	1	35	32	5
380	84	100	14	48	87	7
456	81	100	79	40	94	6

cal purity, as checked by HPLC (Fig. 5) (Table VII): first enantiomer (−), 100% e.e.; second enantiomer (+), 90% e.e. The enantiomeric purity of the second fraction is slightly reduced by tailing of the first band. Moreover, enantiomeric purity (>99% e.e.) could be obtained by a single recrystallization from ethanol–water (60:40) from a fraction showing 84% e.e. [9]. The (−)-enantiomer was obtained as with m.p. = 204°C and $[\alpha]_D^{22} = -150°$ (c = 1, CH_3OH).

The absolute configurations of the enantiomers are unknown. The racemate has m.p. = 170°C, which is lower than that measured for the (−)-enantiomer. Consequently, the enantiomer mixture gives a conglomerate; this is of particular interest because conglomerates are far easier to resolve than are racemic compounds by crystallization [20].

CONCLUSIONS

The separation of three enantiomer pairs with a production rate of a few hundred milligrams per hour demonstrates the great potential of PSubFC with short cycle times for difficult separations (α = 1.1) of molecules that can be eluted using subcritical carbon dioxide with a polar modifier. Moreover, preparative chromatography will become more attractive with the developement of new chiral stationary phases of greater capacity.

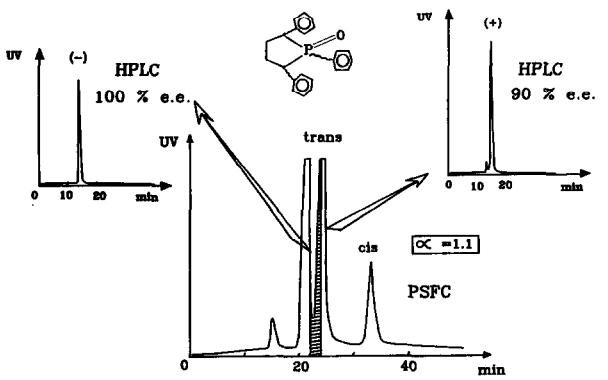

Fig. 5. Separation of *trans*-1,2,5-triphenylphospholane 1-oxide. Stationary phase, (*S*)-thio-DNBTyr E; column, 100 mm × 60 mm I.D.; mobile phase, CO_2–ethanol (92:8, v/v); flow-rate, 15 l/h; pressure, 20 MPa; column temperature, 293 K; UV detection at 230 nm; mass injected, 38 mg.

SYMBOLS

$\alpha_{ij} = k'_j/k'_i$	Selectivity
k'_i	Capacity factor
$\text{e.e.} = \frac{[R] - [S]}{[R] + [R]}$	Enantiomeric excess for R
$h = H/d_p$	Reduced height equivalent to a theoretical plate
d_p	Particle size
$H = L/N$	Height equivalent to a theoretical plate
L	Column length
N	Theoretical plates per meter

ACKNOWLEDGEMENTS

We are grateful to Dr. J. C. Fiaud, Dr. P. Le Goff and A. Tambute for providing racemic compounds and stationary phases and for helpful discussions. We thank Prochrom (Champigneulles, France) for financial support.

REFERENCES

1 W. Wairner, *Trends Anal. Chem.*, 6 (1987) 125.
2 R. Dappen, H. Arm and V. R. Meyer, *J. Chromatogr.*, 373 (1986) 1.
3 W. H. Pirkle, M. H. Hyon, A. Tsipouras, B. C. Hamper and B. Banks, *J. Pharm. Biomed. Anal.*, 2 (1984) 173.
4 W. H. Pirkle and J. Finn, *J. Org. Chem.*, 46 (1981) 2935.
5 W. H. Pirkle and T. C. Pochapsky, *Adv. Chromatogr.*, 27 (1987) 73.
6 F. Gasparini, D. Misiti and C. Villani, *J. High Resolut. Chromatogr.*, 3 (1990) 182.
7 S. Hara and A. Dobashi, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 2 (1979) 531.
8 P. Macaudière, M. Caude, R. Rosset and A. Tambute, *J. Chromatogr.*, 27 (1989) 383.
9 J. C. Fiaud and J. Y. Legros, *Tetrahedon Lett.*, 32 (1991) 5089.
10 M. Perrut, *Fr. Pat.*, 2 527 934 (1982); *Eur. Pat.*, 0 099 765 (1983); *US Pat.*, 4 478 702 (1983).
11 C. Berger and M. Perrut, *J. Chromatogr.*, 505 (1990) 37.
12 M. Perrut, *Fr. Pat.*, 2 584 618 (1985); *Eur. Pat.*, 0 212 999 (1986); *US Pat.*, 4 724 087 (1986).
13 L. Doguet and M. Perrut, presented at the *2nd Symposium on Supercritical Fluids, Boston, MA, May 1991.*
14 P. Jusforgues and M. Perrut, *Fr. Pat.*, 2 601 883 (1986); *Eur. Pat.*, 0 254 610 (1987).
15 A. Tambute and A. Begos, *New J. Chem.*, 13 (1989) 625.
16 A. L. Horvath, *Physical Properties of Inorganic Compounds*, Edward Arnold, London, 1975, p. 163.
17 P. Gareil, C. Durieux and R. Rosset, *Sep. Sci. Technol.*, 18 (1983) 441.

18 A. Tambute, M. Lienne, P. Macaudière, M. Caude and R. Rosset, presented at the *1st International Symposium on Separation of Chiral Molecules, Paris, May 31 and June 1–2, 1988.*

19 A. Breque, J. M. Alcaraz, L. Ricard and F. Mathey, *New J. Chem.*, 13 (1989) 369.

20 A. Collet, M. J. Brienne and J. Jaques, *Chem. Rev.*, 80 (1980) 215.