# Determination of enantiomer separation factors by nuclear magnetic resonance spectroscopy and by chiral liquid chromatography

Urban Skogsberg, Stig Allenmark*

*Department of Chemistry, Göteborg University, SE-41296, Göteborg, Sweden*

## Abstract

The equilibrium constants $K_+$ and $K_-$ for formation of the diastereomeric complexes of the two enantiomers of O,O'-dibenzoyltartaric acid (DBTA) with the chiral selector N,N'-diallyltartardiamide bis-(4-*tert*.-butylbenzoate) (TBB) have been determined by ^{1}H-NMR. The experiments were performed at different temperatures in CDCl$_3$ or in cyclohexane-$d_{12}$/2-propanol-$d_8$ mixtures. The equilibrium constants from the ^{1}H-NMR results have been compared with the retention factors ($k'$) obtained from the chromatographic resolution of *rac*. DBTA on a Kromasil CHI–TBB column with the same solvents as mobile phases. A satisfactory correlation between the ^{1}H-NMR data and the chromatographic data was found. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Chiral recognition; Nuclear magnetic resonance spectroscopy; Diastereomeric complexes

## 1. Introduction

In the development of new chiral sorbents for liquid chromatography (LC) a comparison of the selectivity displayed by the selector in free solution and immobilized on silica, respectively, is important. By chiral LC the retention factors $k'_1$ and $k'_2$, and thereby $\alpha$, are easily determined from the peaks' positions, including the solvent front [1]. Due to the many difficulties involved in the preparation of a column with the chiral sorbent containing the immobilized selector and with the elucidation of the contribution of non-selective interactions from the support, a fast method to evaluate the selectors is desirable. There are not so many options to study

chiral interactions free from the support, except for one frequently used method to study water soluble chiral selectors like cyclodextrins, viz, capillary electrophoresis (CE) [2]. However, many neutral selectors, such as the N,N'-diallyl-L-tartardiamide-(DATD) based selector TBB (Scheme 1), are not soluble in water and cannot be studied by CE [3,4]. On the other hand, ^{1}H-NMR can be a useful tool in the search for chiral recognition properties of the selector [5–12]. The possibility to correlate solution
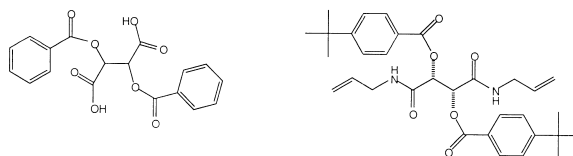


Scheme 1. Structures of DBTA (O,O'-dibenzoyltartaric acid; left) and TBB (N,N'-diallyl-tartardiamide bis-(4-*tert*.-butylbenzoate); right).

*Corresponding author. Tel.: +46-31-772-3841; fax: +46-31-772-3840.

*E-mail address:* stig.allenmark@oc.chalmers.se (S. Allenmark).

^{1}H-NMR data to chiral stationary phase properties is therefore an important matter. An obvious advantage of studying chiral selectors in solution is that there are no interfering achiral interactions present. It is therefore possible to obtain the "true" separation factor $(\alpha_t)$ of the selector from the equilibrium constants $K_+$ and $K_-$ found from NMR data for the respective enantiomer of the analyte.

In both chiral chromatography and chiral NMR the formation of diastereomeric complexes is essential for the observed enantiodiscrimination. Several attempts to use NMR to determine the equilibrium constants for the formation of diastereomeric complexes between a chiral selector and the enantiomers of a given solute have been reported [13–15]. The equilibria between the TBB selector and DBTA have been studied by ^{1}H-NMR in CDCl$_3$ at $-10°$C [16]; however, for the sake of comparison it would be more interesting to determine $\alpha_t$-values by NMR under conditions mimicking those normally used in chromatography. In this paper the results from such a comparison between data obtained from NMR and LC, respectively, are presented.

## 2. Experimental

Analytical chiral liquid chromatography was performed with the use of an equipment composed of a Varian mod. 9012Q solvent delivery pump and mod. 9050 variable wavelength UV detector. Samples were introduced via a Rheodyne injector equipped with a 20 µl loop onto a 4.6×250 mm Kromasil CHI–TBB column (EKA Chemicals AB, Bohus, Sweden). Mixtures of cyclohexane and 2-propanol (2.5–7.5%) as well as neat chloroform were used as mobile phases. All solvents were of analytical grade purity.

All NMR spectra were recorded at 500 MHz on a Varian Unity 500 NMR-spectrometer at room temperature or at 0°C. Thirty-two scans were run in each ^{1}H-NMR experiment and the cyclohexane solvent peak at 1.38 ppm was used as a reference. The solvents used for the NMR experiments were cyclohexane-d$_{12}$ (99.7%) and 2-propanol-d$_8$ (99%) both from Cambridge Isotope Laboratories. DBTA (purity ≥99%; Fluka) was used as a probe (P). The singlet at ca. 6.0 ppm from the methine protons of P

was used throughout for the studies of the selector-induced shifts in the NMR.

A solution of (+)-P (0.25 m*M*) was prepared by dissolving (+)-P (0.18 mg) in a solvent (2 ml) consisting of cyclohexane-d$_{12}$ and 2-propanol-d$_8$ (ratio 95/5, 97.5/2.5 or 92.5/7.5). A spectrum of free P was first run in order to determine the chemical shift position of the NMR signal of interest. To an NMR tube containing 700 µl of this solution, exact aliquots of a TBB solution were added prior to spectral recording. The observed chemical shift displacement of signal from the methine protons was determined with a precision of 0.1 Hz. The final volume was 1 ml. In order to keep the (+)-P concentration constant during the whole experiment, the TBB solution was made from TBB (1.30 mg) dissolved in the (+)-P solution. The experiment was repeated using a solution of (−)-P (0.31 m*M*) and a final volume of 1 ml.

The computational work involving the refinement of the equilibrium constant calculation via minimization with respect to the two unknown parameters was carried out using the MATLAB program (v. 5.3.1, The MathWorks, Inc.).

The curve fittings were done with the use of Igor Pro, ver. 2.04.

## 3. Results and discussion

As pointed out previously [16], a difference in the chemical shifts observed for two enantiomers in a chiral environment may be present even if their association constants are identical; that is, the effect is then caused entirely by different magnetic shielding of the diastereotopic nuclei observed. By studying the enantiomers in separate experiments at varying concentrations of added chiral selector it is possible, however, to calculate both equilibrium and maximum chemical shift displacement data, separately.

From NMR data it is possible to determine $\alpha_t$ by Eq. (1), where $K_A$ and $K_B$ are the equilibrium constants for the formation of the diastereomeric complexes. The interactions originate from the free selector in solution without any interfering contributions. These equilibria must therefore represent the "true $\alpha$" $(\alpha_t)$, since all other interactions will cause

a decrease of the $\alpha$-value. Consequently, the $\alpha_t$-value obtained by an NMR experiment should be larger than the $\alpha$-value obtained by LC under similar conditions due to the absence of nonselective interactions.

$$\alpha_t = \frac{K_B}{K_A} \qquad (1)$$

It is possible, in principle, to determine $K$ from concentration-dependent chemical shift measurements in the NMR. If a series of $^1$H-NMR experiments is performed in which the selector concentration is gradually increased while the probe concentration is held constant, then the chemical shift displacements obtained in each run can be measured. The precision within each run is of the order 0.1 Hz. Because the exchange of free and bound DBTA is fast on the NMR time scale it is not possible to see the signal corresponding to the diastereomeric complex; instead the observed NMR chemical shift corresponds to the equilibrium situation described by Eq. (2), where $X_p$ and $X_{ps}$ denote the mole fractions of free and complexed DBTA, respectively.

$$\delta^{obs} = X_p\delta_p + X_{ps}\delta_{ps} \qquad (2)$$

The chemical shift displacement is caused by an increase in the relative concentration of the diastereomeric complex. By a minimization of the function given in Eq. (3), $K$ can be determined with accuracy if the number of $^1$H-NMR experiments is large enough. This is done by means of a computer and a suitable mathematical program.

$$F(K) = \sum_i (\delta_i^{obs} - X_{pi}\delta_{pi} - X_{psi}\delta_{psi})^2 = \sum_i F_i^2 \qquad (3)$$

In order to use Eq. (3) the molar ratio [selector]/[probe], here denoted $m$, should be large enough to produce non-linearity in the $(\delta^{obs} - \delta_p)$ vs. $m$ plots.

If $m \gg 1$ it is possible to determine $K$ from a double reciprocal plot according to Eq. (4). This is a more simple but less accurate method which eliminates some of the drawbacks that may be associated with the minimization method.

$$
\begin{aligned}
1/\Delta &= 1/(K\Delta_0 B) + 1/\Delta_0 \quad \text{or} \\
1/\Delta &= 1/(KA\Delta_0 m) + 1/\Delta_0
\end{aligned} \qquad (4)
$$

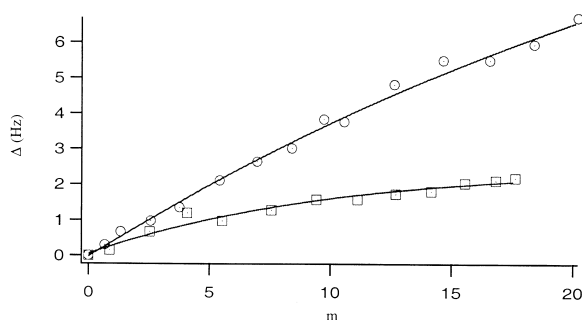Here $\Delta = |\delta^{obs} - \delta_p|$ and $\Delta_0 = |\delta_{ps} - \delta_p|$, whereas A



Fig. 1. TBB as selector and DBTA as probe (P) in $C_6D_{12}$/2.5% 2-propanol-$d_8$ at ambient temperature. Upper curve (circles): (+)-P; lower curve (squares): (−)-P.

and B denote the concentrations of the probe (P; here DBTA) and the selector (S; here TBB), respectively.

From experiments using three different solvents and two different temperatures, the chemical shift displacement $(\delta^{obs} - \delta_p)$ of the methine protons in (+)-P and (−)-P has been plotted against [TBB]/[DBTA], here denoted $m$ (Figs. 1–3). Fig. 1 corresponds to 2-propanol-$d_8$ (5%) in $C_6D_{12}$ and Fig. 2 to 2-propanol-$d_8$ (2.5%) in $C_6D_{12}$, both at ambient temperature. Fig. 3 corresponds to 2-propanol-$d_8$ (7.5%) in $C_6D_{12}$ at 0°C.

$K_-$ was determined to 105 $M^{-1}$ from the NMR data given in Fig. 1 by use of the function in Eq. (3), and to 100 $M^{-1}$ from a double reciprocal plot (Eq. (4)). $K_+$ was determined to 81 $M^{-1}$ from a double reciprocal plot (Eq. (4)) using $m$-values >10. An $\alpha_t$ of 1.30 can be calculated from Eq. (1). This separation is free from the achiral interactions present in
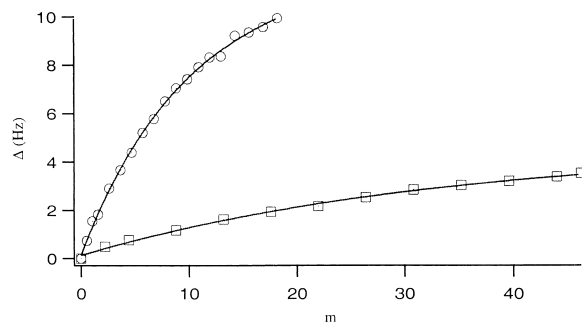


Fig. 2. TBB as selector and DBTA as probe (P) in $C_6D_{12}$/2.5% 2-propanol-$d_8$ at ambient temperature. Upper curve (circles): (+)-P; lower curve (squares): (−)-P.
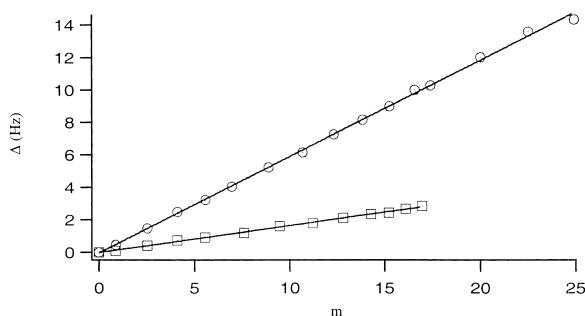
Fig. 3. TBB as selector and DBTA as probe (P) with 7.5% 2-propanol-$d_8$ in $C_6D_{12}$ at 0°C. Upper curve (circles): (+)-P; lower curve (squares): (−)-P.

the chromatographic situation and should therefore represent the highest possible $\alpha$ during these experimental conditions.

$K_-$ and $K_+$ for (−)-P and (+)-P in cyclohexane/ 2.5% 2-propanol were determined by double reciprocal plots to 214 $M^{-1}$ and 121 $M^{-1}$, respectively, which gives an $\alpha$ of 1.77. The maximum chemical shift displacement ($\Delta_0$) was determined from the double reciprocal plot in Fig. 4 to 6.4 Hz for (−)-P and 16.4 Hz for (+)-P. From the $^1$H-NMR data for (−)-P in cyclohexane/5% 2-propanol, $\Delta_0$ was determined from a double reciprocal plot to be 6.1 Hz; a satisfactory agreement between two different experiments.

From the plots given in Fig. 1, it is obvious that (−)-P reaches its maximum chemical shift displacement at a lower $m$-value than (+)-P. It is evident from Figs. 1–3 that a higher concentration of 2-propanol must be compensated by a higher selector/
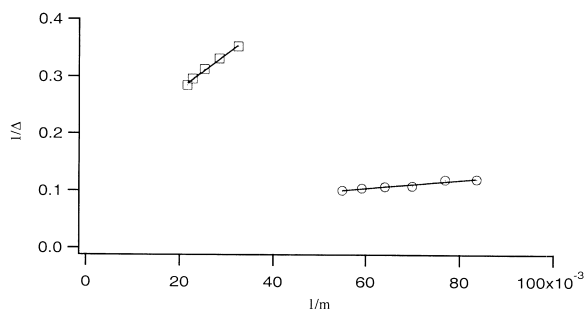


Fig. 4. Double reciprocal plot of (+)-P and (−)-P in 2-propanol-$d_8$ (2.5%) in $C_6D_{12}$ with TBB as selector at ambient temperature.

probe ratio in order to obtain the same curvature. The experimental data in Fig. 1 are located in the region where (+)-P is more sensitive than (−)-P for small errors in the measurement of the chemical shift. Therefore it was more advantageous to use the double reciprocal plot for the estimation of $K_+$.

A low solubility of the selector or probe is a limiting factor to get a sufficiently high $m$-value and therefore reach the plateau region in the concentration plots. Low $m$-values make it difficult to use both the minimization function and the double reciprocal plot when $K$ is to be determined. However, as can be derived from the general analytical expression [16], for very small $m$-values one obtains:

$$\Delta = m\Delta_0(KA/(KA + 1)) \qquad (5)$$

Therefore, since only $K$ is unknown and the other parameters are constants it should be possible to use the linear region where $m$ is small to determine $K$. If $\Delta_0$ is determined, this value can then be used to obtain $K$-values in similar solvent systems at the same temperature. This method could also be useful when small $K$-values are to be determined.

From a third $^1$H-NMR experiment using (+)-P and (−)-P in presence of TBB with 2-propanol-$d_8$ (7.5%) in $C_6D_{12}$ at 0°C, $K_+$ and $K_-$ were determined by a double reciprocal plot (Eq. (4)) to be 16 $M^{-1}$ and 14 $M^{-1}$, respectively. It is obvious from the curve fitting in Fig. 3 that both (+)-P and (−)-P are very far from reaching their plateau regions. Consequently, the magnitude of $K_+$ and $K_-$ is significantly lower than in the previous case. The $^1$H-NMR results are reasonably consistent with the chromatographic data obtained (Table 1). The $\Delta_0$ values of (−)-P and (+)-P are significantly higher in this experiment than the earlier two experiments, which is believed to be an effect of the decreased temperature [17].

In the chromatographic situation $\alpha$ is a function of both the chiral and achiral interactions (Eq. (6)), where $k'$ is the retention factor [18,19].

$$\alpha = \frac{k'_{ns} + k'_{2s}}{k'_{ns} + k'_{1s}} \qquad (6)$$

From chromatographic data $\alpha = 1.24$ was obtained when (−)-P and (+)-P are separated on the CHI–TBB column with 0.1% acetic acid in $CHCl_3$

Table 1
Equilibrium parameters and selectivity factors of (+)-P and (−)-P in cyclohexane/2-propanol obtained by $^1$H-NMR and by LC (with 0.05% of acetic acid added to the mobile phase) at room temperature

| Technique/parameter | Chloroform | 2-Propanol (2.5%) | 2-Propanol (5%) | 2-Propanol (7.5%) |
|---|---|---|---|---|
| NMR $K_+$ ($M^{-1}$) | 420[a] | 121 | 81 | 16[d] |
| NMR $K_-$ ($M^{-1}$) | 720[a] | 214 | 105 | 14[d] |
| NMR $\alpha_t$ | 1.71 | 1.77 | 1.30 | 0.9 |
| LC $k_1'$ | 16.9[b] | 19.1 | 6.93 | 4.71 |
| LC $k_2'$ | –[c] | 24.14 | 8.14 | 4.71 |
| LC $\alpha$ | –[c] | 1.26 | 1.17 | 1.0 |

[a] At −10°C.
[b] At −9.4°C and with 0.0005% acetic acid added.
[c] Could not be observed because of insufficient resolution.
[d] At 0°C.

as a solvent at room temperature. If the temperature was decreased, $k_1'$ also decreased (Table 2). This type of unusual effects in chromatography has earlier been reported [20].

$K_+$ and $K_-$ have earlier been determined by NMR in CDCl$_3$ at −10°C to 420 $M^{-1}$ and 720 $M^{-1}$, respectively. For the sake of comparison (±)-P was resolved on CHI–TBB with a minimum amount of acetic acid in CHCl$_3$ and $k_1'$ was determined to 16.9. The achiral contribution can be estimated by Eq. (6) from a comparison of the $\alpha_t$-values obtained from the $^1$H-NMR data and from the chromatographic results. From the separations of (+)-P and (−)-P in

cyclohexane/5% 2-propanol and cyclohexane/2.5% 2-propanol with TBB as stationary phase giving $\alpha = 1.17$ and 1.26, it is possible, by the use of Eq. (6), to estimate the the achiral contribution from a comparison of the $^1$H-NMR data and the chromatographic data. Since both the chromatographic experiments and the $^1$H-NMR experiments are performed in the same solvent mixtures and at the same temperature, a direct comparison is possible. The results from the $^1$H-NMR experiments and the chromatographic experiments are presented in Table 1.

Under the assumption that a low concentration (≤0.25%) of acetic acid would not change the equilibrium constant ratios obtained by $^1$H-NMR it is possible to calculate the selective and the non-selective interactions ($k_s'$ and $k_{ns}'$) by Eq. (6) from the data given in Table 1. The comparison indicates that both the chiral and the achiral interactions are higher when P is separated on CHI–TBB with 2.5% 2-propanol in C$_6$H$_{12}$ than when 5% 2-propanol is used; see Table 3.

Table 2
Temperature dependence of the separation of (±)-P on CHI–TBB with CHCl$_3$ and 0.1% acetic acid as mobile phase

| Temp. (°C) | $k_1'$ | $\alpha$ |
|---|---|---|
| RT | 5.9 | 1.24 |
| 10 | 5.4 | 1.25 |
| 0 | 5.1 | 1.27 |
| −9.4 | 4.8 | 1.30 |

Table 3
Calculated selective and non-selective interactions with different concentrations of 2-propanol and acetic acid

| $k'$ | 2-Propanol (5%) | | 2-Propanol (2.5%) | |
|---|---|---|---|---|
| | Acetic acid 0.05% | Acetic acid 0.25% | Acetic acid 0.05% | Acetic acid 0.25% |
| $k_{1s}'$ | 4.03 | 3.1 | 6.49 | 5.19 |
| $k_{2s}'$ | 5.24 | 4.03 | 11.49 | 9.19 |
| $k_{ns}'$ | 2.9 | 1.08 | 12.65 | 9.21 |

Table 4
Separation of (−)-P and (+)-P on CHI–TBB with cyclohexane/
2-propanol and 0.25% acetic acid at room temperature

| 2-Propanol concentration (%) | 2.5 | 5 | 7.5 |
|---|---|---|---|
| $k'_1$ | 14.4 | 4.18 | 2.51 |
| $k'_2$ | 18.4 | 5.11 | 2.99 |
| $\alpha$ | 1.28 | 1.22 | 1.19 |

As expected, a reduction of the non-selective interactions is observed both when the 2-propanol concentration and the acetic acid concentration is increased.

Since free silanol groups on the support are the main cause to non-selective interactions, an increase in solvent strength should reduce the silanol/analyte interaction. The reduction of the non-selective interactions is also believed to be caused by the blocking of free silanol groups on the sorbent with acetic acid (competition between the analyte and acetic acid).

From a comparison of Tables 1 and 4 it is found that an increase in the acetic acid concentration from 0.05% (Table 1) to 0.25% (Table 4), under otherwise identical chromatographic conditions, causes a considerable reduction of $k'$ accompanied by an improvement of the $\alpha$-value. This indicates that the major influence from the acetic acid is to reduce the non-selective interactions with the support and not the interactions with the selector.

The phase ratio ($\beta$) can be estimated by Eq. (7) from the equilibrium data given in Tables 1 and 3. The $K$ and $k'_s$-values obtained in the solvent systems containing 2.5% and 5% of 2-propanol (with 0.05% of acetic acid added in the chromatographic situation) give a $\beta$ of $0.052 \pm 0.002$.

$$k' = \beta \times K \tag{7}$$

The magnitude of this estimated phase ratio is in a region where it should be expected [21].

## 4. Conclusion

Attempts to correlate chromatographic data with ^{1}H-NMR data have shown that a reasonable prediction of chromatographic retention behavior and selectivity can be made as long as the equilibrium constants (and $k'$-values) are not too small. This is demonstrated by the satisfactory correlation between the experiments run in cyclohexane/2.5% 2-propanol, cyclohexane/5% 2-propanol and $CHCl_3$. The poor correlation between the experiments run in cyclohexane/7.5% 2-propanol is believed to be the result of the difficulties in determining small equilibrium constants by NMR.

In our investigated system, a substantial contribution to $k'$ from non-selective interactions with the chiral sorbent is present in solvents of low polarity, as expected in view of the protolytic nature of the analyte.

A mathematical approach to circumvent the problem appearing in the determination of small $K$-values has been suggested. The results also show that it is possible to get a good estimation of the phase ratio.

## References

[1] S. Allenmark, Chromatographic Enantioseparation, 2nd ed, Ellis Horwood, Chichester, 1991.
[2] B. Chankvetadze, G. Endresz, G. Schulte, D. Bergenthal, G. Blaschke, J. Chomatogr. A 732 (1996) 143.
[3] S. Allenmark, S. Andersson, P. Möller, D. Sanchez, Chirality 7 (1995) 248.
[4] S. Andersson, S. Allenmark, P. Möller, B. Persson, D. Sanchez, J. Chromatogr. A 741 (1996) 23.
[5] H.A. Benesi, J.H. Hildebrand, J. Am. Chem. Soc. 71 (1949) 2703.
[6] J.A.A. de Boer, D.N. Reinhoudt, S. Harkema, G.J. van Hummel, F. de Jong, J. Am. Chem. Soc. 104 (1982) 4073.
[7] R. Foster, C.A. Fyfe, Trans. Faraday. Soc. 61 (1965) 1626.
[8] M.W. Hanna, A.L. Ashbaugh, J. Phys. Chem. 68 (1964) 811.
[9] L. Fielding, Tetrahedron 56 (2000) 6151.
[10] U. Holzgrabe, H. Mallwitz, S.K. Branch, T.M. Jefferies, M. Wiese, Chirality 9 (1997) 211.
[11] E. Yashima, C. Yamamoto, Y. Okamoto, J. Am. Chem. Soc. 118 (1996) 4036.
[12] E. Yashima, M. Yamada, C. Yamamoto, M. Nakashima, Y. Okamoto, Enantiomer 2 (1996) 225.
[13] K.A. Connors, Binding Constants, John Wiley & Sons, New York, 1987.
[14] D. Parker, Chem. Rev. 91 (1991) 1441.
[15] H. Grosenick, M. Juza, J. Klein, V. Schurig, Enantiomer 1 (1996) 337.
[16] U. Skogsberg, L. Thunberg, S. Allenmark, Chirality 13 (2001) 272.

*U. Skogsberg, S. Allenmark / J. Chromatogr. A 921 (2001) 161–167*

167

[17] S. Allenmark, U. Skogsberg, Enantiomer 5 (2000) 451.

[18] S. Allenmark, Chirality 5 (1993) 295.

[19] G. Götmar, T. Fornstedt, G. Guiochon, Chirality 12 (2000) 558.

[20] F. Gasparrini, F. Marini, D. Misiti, M. Pierini, C. Villani, Enantiomer 2 (1999) 325.

[21] P.D. Ferguson, D.M. Goodall, S.S. Loran, Anal. Chem. 70 (1998) 4054.