Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1034 (2004) 117–123
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison study of Chiralpak AD-H with AD columns in chromatographic enantioseparation of dihydropyrimidinone acid and its methyl ester

Fang Wang*, Thomas Dowling, Dean Ellison, Jean Wyvratt

Department of Analytical Research, Merck Research Laboratories, Merck & Co., Inc., P.O. Box 2000, RY818 B-208, Rahway, NJ 07065, USA

Received 13 November 2003; received in revised form 27 January 2004; accepted 6 February 2004

Abstract

This paper reports a comparison study of the difference between Chiralpak AD-H and AD columns in enantioseparation of dihydropyrimidinone (DHP) acid and its methyl ester under normal phase LC conditions. Unlike those of the AD phase, the van't Hoff plots of retention factors for DHP acid on the AD-H phase were linear. The cyclic van't Hoff plots of selectivity factors for DHP acid on the AD-H phase were non-linear and slightly non-superimposable. No conformational transition was observed on the AD-H phase in the whole temperature range. A single-step temperature program on the AD-H phase showed that the selectivity factors of DHP acid only increased approximately 1.7% in 24 h (versus approximately 50% on the AD phase). For DHP ester, the single-step temperature program showed that the selectivity factors on the AD-H phase remained the same in 24 h while those on the AD phase increased around 3.1%. The enantioselectivity of DHP acid on the AD-H phase was lower than that on the AD phase while the enantioselectivity of DHP ester on the AD-H phase was higher than that on the AD phase. The resolution of DHP acid on the AD-H phase was about the same as that on the AD phase while the resolution of DHP ester on the AD-H phase was much higher than that on the AD phase. The results of DHP acid are opposite of what the vendor suggested while the results of DHP ester are the same as the vendor's application notes. This indicates that the differences between Chiralpak AD-H and AD columns are not only in their particle size, but also in the solvated conformations.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Cyclic van't Hoff plots; Step-temperature programs; Chiralpak AD-H and AD; Dihydropyrimidone acid; Amylose tris-(3,5-dimethylphenylcarbamate)

1. Introduction

Enantioseparation by liquid chromatography plays an important role in pharmaceutical research and development. The selection of chiral stationary phases (CSPs) is the key to the success of chromatographic enantioseparation method development. To date, different types of CSPs, such as proteins, Pirkle-type phases, cyclodextrins, derivatized amylose and cellulose and antibiotics, etc., have been successfully applied for enantioseparation of different pharmaceutical compounds [1–7]. Among these CSPs, the carbamate-derivatized amylose (Chiralpak AD-H/AD) and cellulose (Chiralcel OD-H/OD) stationary phases are the most popular phases because of their selectivity and versatility [8–12]. Originally, the column vendor introduced these phases (Chiralpak AD and Chiralcel OD) with 10 μm particle size. Recently, the vendor recommended using the columns with 5 μm particle size (Chiralpak AD-H and Chiralcel OD-H) to start method development because of their higher selectivity and resolution as stated by the vendor [13]. For the existing methods using the AD/OD columns, the replacement of the old columns (10 μm particle size) with the new columns (5 μm particle size) could generate potential problems if there are some differences in the manufacturing processes of these new columns. Even though the vendor originally stated that the only difference among these phases was the particle size, the first glance at their mobile phase suggestions shows that the AD phase cannot be used with mobile phase containing 15 to 60% ethanol in alkanes, while the AD-H phase can be used with 0–100% ethanol [14]. There is no such difference in the OD and OD-H phases. To date, to the best of the authors' knowl-

* Corresponding author. Fax: +1-732-594-3887.
E-mail address: fang_wang@merck.com (F. Wang).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.02.035

edge, there has been no comparison study on these phases under different experimental conditions.

Previously, we reported some preliminary results on the unusual chromatographic behaviors of DHP acid and ester on the AD phase when a mixture of 15:85:0.1 EtOH:*n*-hexane:TFA was used as the mobile phase [15]. The cyclic van't Hoff plots showed the non-linear and non-superimposable nature in enantioseparation of DHP acid, indicating the change of the phase conformation during the temperature program. The step-temperature programs showed that the thermally-induced conformational transition was controlled by a kinetic process [16]. Both solid-state NMR and chromatographic data show that the differences in the solvation of the AD phase by the different organic modifiers in *n*-hexane are the key to generate the conformational transition. Based on these studies, we recognize that both DHP acid and ester can be used as probe compounds for the evaluation of the differences in the solvated phases because this kind of change in the AD phase conformation (caused by the column temperature in such a relatively narrow range) has not been observed in enantioseparation of other compounds in the past.

In this paper, we report the comparison of the solvated Chiralpak AD-H with Chiralpak AD column in enantiomeric separation of DHP acid and ester by the cyclic van't Hoff and step-temperature programs. The van't Hoff plots of the AD-H phase with 15% ethanol in *n*-hexane as the mobile phase were different from those on the AD phase. This indicates the solvated AD-H phase behaves differently from the solvated AD phase.

2. Experimental

2.1. Apparatus

All experiments were performed on an Agilent G1100 system with a column oven (which was used for the temperature control of the separation) and a photodiode array detector (Santa Clarita, CA, USA). The column temperature was measured by a calibrated Ertco High-Precision Thermometer (accuracy ±0.015 °C) (West Paterson, NJ, USA). The chromatographic data were acquired and analyzed by P.E. Nelson Turbochrom software (Cupertino, CA, USA). Chiralpak AD-H/AD (amylose tris-(3,5-dimethylphenylcarbamate) columns (4.6 mm × 250 mm, 5 and 10 μm) were purchased from Chiral Technologies (Exton, PA, USA). Their structures are shown in Fig. 1. The water content of the mobile phases was measured by a 756 Brinkmann Coulometric KF instrument (Westbury, NY, USA).

2.2. Chemicals

Dihydropyrimidinone (DHP) acid and methyl ester racemates and the pure enantiomers were prepared by the Process Research Department, Merck Research Laboratories (Rahway, NJ, USA). The structures of these compounds are shown in Fig. 1. 1,3,5-Tri-*tert*-butyl-benzene was used as the void volume (t_0) marker and was purchased from Aldrich (Milwaukee, WI, USA). Trifluoroacetic acid (TFA) was purchased from Fisher Scientific (Springfield, NJ, USA). *n*-Hexane (greater than 85% *n*-hexane, greater than 99.8% total C6 isomers), 2-propanol (2-PrOH), 1-propanol (1-PrOH), ethanol (EtOH), 1-butanol (1-BuOH), 2-butanol (2-BuOH), *tert*-butanol (*t*-BuOH) and methanol (MeOH) were HPLC grade and purchased from EM Sciences (Gibbstown, NJ, USA).

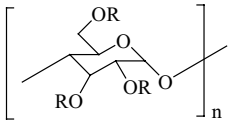
2.3. Chromatographic conditions

The mobile phases were prepared at ambient by mixing the alcohol modifiers and TFA in *n*-hexane. Samples and the t_0 marker were prepared together in the mobile phases at a concentration of 0.5 mg/ml. A 10 μl volume of each sample was injected. The detector wavelength was set up at 220 nm with a 4 nm bandwidth. The flow rate was 1.0 ml/min. The column temperature programs were the same as previously reported [16]. The elution orders were determined by spiking the pure enantiomeric standards with the racemic samples.

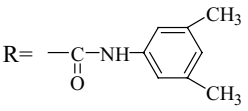
3. Results and discussion

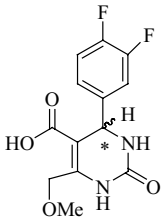
3.1. Cyclic van't Hoff plots

Previously, we studied the enantioseparation of DHP acid and ester (Fig. 1) on the AD and OD phases with both EtOH and 2-PrOH in *n*-hexane with 0.1% TFA as the mobile

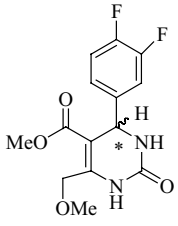


Chiralpak AD-H/AD





(+/-)-DHP acid



(+/-)-DHP ester

Fig. 1. Structures of Chiralpak AD-H and AD phases and DHP acid and methyl ester analytes.

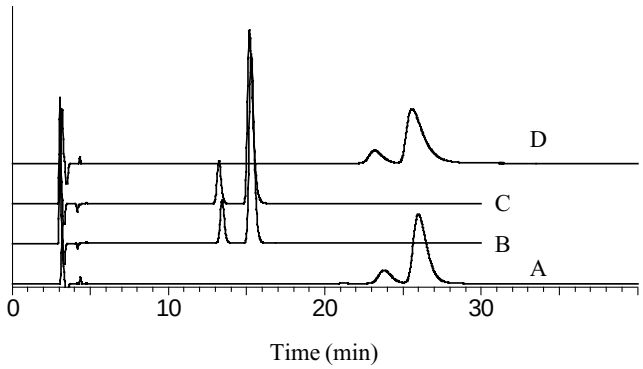
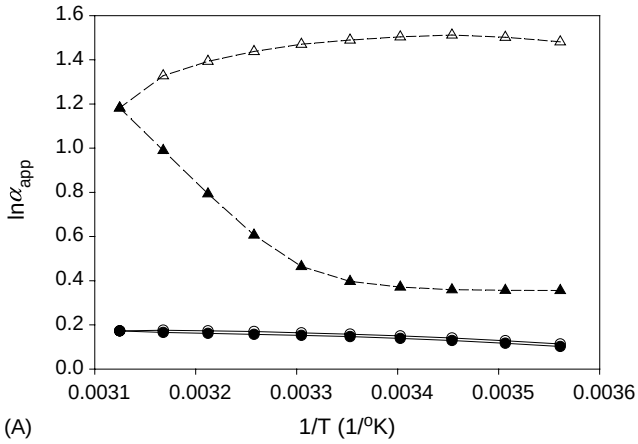


Fig. 2. Enantioseparation of DHP acid on Chiralpak AD-H column. Mobile phase: 15% EtOH in hexane with 0.1% TFA. Column temperature: (A) 5 °C, heating; (B) 45 °C, heating; (C) 45 C, cooling; (D) 5 °C, cooling.

phases. Since the thermally-induced, kinetically-controlled conformational transition of the AD phase was only evidenced by these two compounds so far [15,16], it is our interest to use the compounds as the molecular probes to explore the AD-H phase to determine if the AD-H phase behaves the same as the AD phase (besides the possibility of morphology difference in the CSPs caused by the difference in the pore size of the silica of the two phases). Fig. 2 shows the enantioseparation of DHP acid at 5 and 45 °C during a heating (first)/cooling (second) cycle. The elution order on the AD-H phase was the same as that on the AD phase. Unlike those on the AD phase, the retention times and peak shape of *S*-(+)-DHP acid did not change much on the AD-H phase at the same temperature no matter how the temperature was reached (i.e., by heating or cooling). A comparison of the van't Hoff plots of retention factors of both phases in a heating process is shown in Fig. 3. Note that the plots of the AD phase in Figs. 3–5 were previously reported [15]. They are used here for the comparison purpose only. For



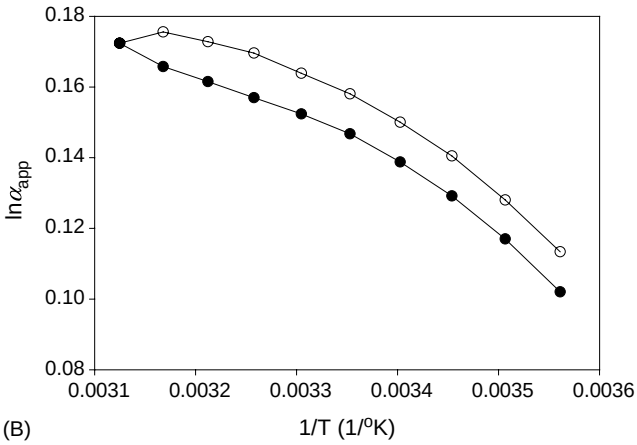


Fig. 4. Comparison of cyclic van't Hoff plots of α of DHP acid on Chiralpak AD-H and AD columns. Mobile phase: 15% EtOH in hexane with 0.1% TFA. (A) van't Hoff plots of α on both phases. (B) Enlarged cyclic van't Hoff plots of α on Chiralpak AD-H column. Processes: Chiralpak AD-H column, heating (filled circle), cooling (open circle); Chiralpak AD column, heating (filled triangle), cooling (open triangle).

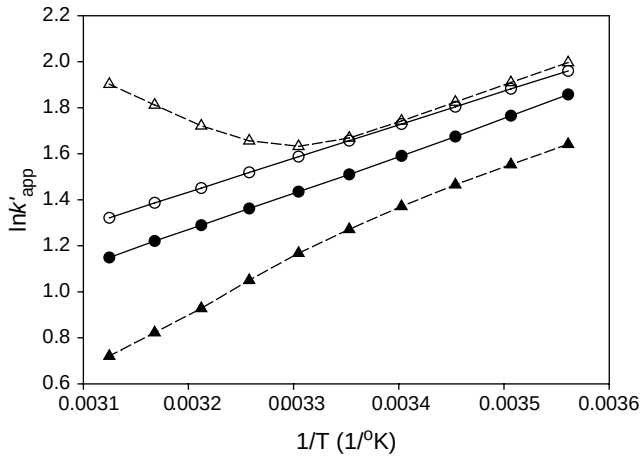


Fig. 3. Comparison of van't Hoff plots of k' of DHP acid on Chiralpak AD-H and AD columns during a heating process. Mobile phase: 15% EtOH in hexane with 0.1% TFA. *S*-(+)-DHP acid: Chiralpak AD-H (open circle), Chiralpak AD columns (open triangle). *R*-(−)-DHP acid: Chiralpak AD-H column (filled circle), Chiralpak AD column (filled triangle).

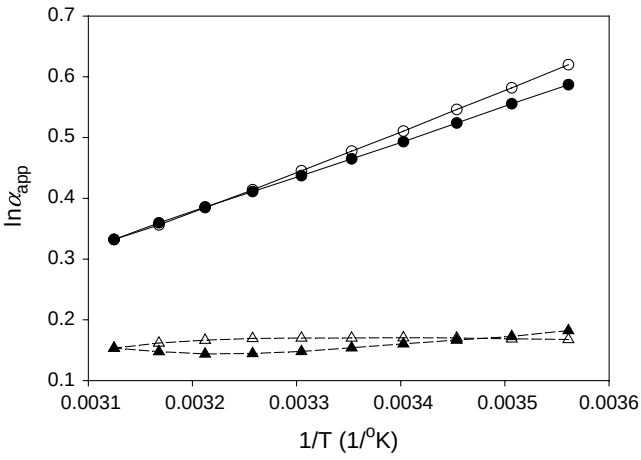


Fig. 5. Comparison of cyclic van't Hoff plots of α of DHP ester on Chiralpak AD-H and AD columns. Mobile phase: 15% EtOH in hexane with 0.1% TFA. Processes: Chiralpak AD-H column, heating (filled circle), cooling (open circle); Chiralpak AD column, heating (filled triangle), cooling (open triangle).

both DHP acid enantiomers, the van't Hoff plots of retention factors are linear on the AD-H phase during the heating process while those of the AD phase are not. The retention factors of DHP acid on the AD-H phase decreased with the increase in the column temperature in a classical pattern. There was no transition temperature observed in the plots for both enantiomers on the AD-H phase. Fig. 4 shows the van't Hoff plots of selectivity factors of the phases in a single cyclic van't Hoff temperature program. The most noticeable difference between the two phases was the selectivity on the AD-H phase was much smaller than that on the AD phase during the whole temperature range, which was opposite to what the vendor showed in their application notes. The van't Hoff plots of selectivity factors for the acid on the AD-H phase are non-linear during both heating and cooling processes. They have the same curve-shape and are nearly superimposable (Fig. 4B). Therefore, it is obvious that no conformation transition occurred on the AD-H phase during the cyclic van't Hoff temperature program.

For DHP ester, the van't Hoff plots of selectivity factors of the AD-H phase are linear during the heating and cooling processes (Fig. 5), although they are not exactly superimposable. The shape of the plots of the AD phase in the heating process is different from that in the cooling process. The selectivity of DHP ester on the AD-H phase was higher than that on the AD phase throughout the temperature range. From the ester, the same conclusion can be drawn that there was no conformational change in the AD-H during the cyclic van't Hoff temperature program.

3.2. Step-temperature programs

Previously, we used the step-temperature programs to study the kinetic behavior of the thermally-induced conformational transition of the AD phase by using DHP acid as a probe compound [16]. The apparent selectivity factors increased approximately 50% in 24 h when the column temperature stepped up from 20 to 50 °C. In a two-step temperature program, we found that the apparent selectivity factors increased with time when the temperature was stepped above the transition temperature. However, the apparent selectivity factors remained the same when the temperature was stepped below the transition temperature (∼30 °C). In our current study, Fig. 6 shows the change of selectivity factors of DHP acid with time on the AD-H phase when the column temperature stepped from 10 to 50 °C. The trend of the change was similar to what was observed on the AD phase. However, the magnitude of the change was only approximately 1.7% in 24 h, which was much smaller than that of the AD phase (∼50%). The two-step temperature program on the AD-H phase showed a similar curve to that of the AD phase, but with a much smaller magnitude (data not shown here).

For DHP ester, no change in selectivity factors was observed on the AD-H phase when the single-step temperature program was applied. The change of apparent selectivity factors was approximately 3.1% in 24 h when the single-step temperature program was applied on the AD phase (data not shown here). Compared with that of DHP acid, the change of apparent selectivity factors of DHP ester on the AD phase was much smaller. This indicates that DHP acid is a better probe compound to elucidate the thermally-induced conformational transition even though the molecular size difference between DHP acid and its methyl ester is very small. From the comparison data, we can conclude that the AD-H phase is more rigid than the AD phase using 15% EtOH in *n*-hexane with TFA as the mobile phase.

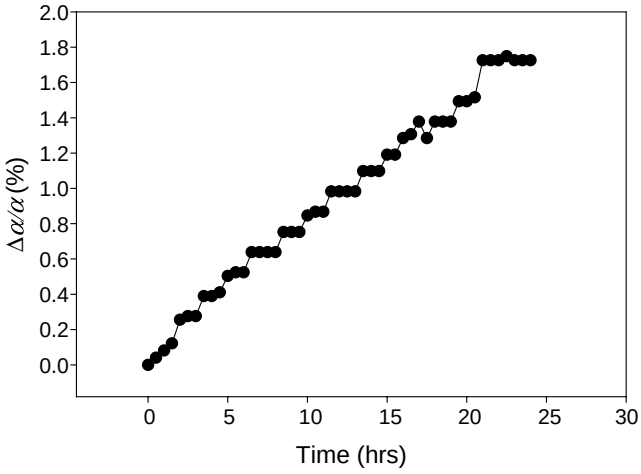


Fig. 6. Single step-temperature study of α change of DHP acid with time on Chiralpak AD-H column. Conditions are the same as Fig. 4.

3.3. Separation efficiency

Fig. 7 shows the effect of the column temperature on the separation efficiency for DHP acid on both AD-H and AD phases. Here, the efficiency is calculated by Foley–Dorsey equation [17]: $N = 41.7(t_r/w_{0.1})/(A/B+1.25)$, where t_r is the retention time, $w_{0.1}$ is the width at 10% of peak height and A/B is an empirical asymmetry ratio (determined by Turbochrom software). On both phases, the general trend was that the efficiency of both enantiomers was higher during the heating processes than that during the cooling processes. For the *R* enantiomer, the efficiency increased with the increase in the column temperature on both phases (Fig. 7). On the AD-H phase, the *S* enantiomer showed different curve shape in the heating/cooling cycles. They were not superimposable (Fig. 7A). The efficiency difference in the cyclic van't Hoff temperature program was more noticeable compared with that of the selectivity factors (Fig. 4B). Fig. 7B and C shows the effect of the column temperature on the efficiency on the AD phase. From 5 to 20 °C in the heating process, the efficiency of the *S* enantiomer increased with the increase in the column temperature (Fig. 7C). It reached a maximum at 20 °C. Between 20 and 40 °C, the efficiency decreased with the increase in the temperature, which in-

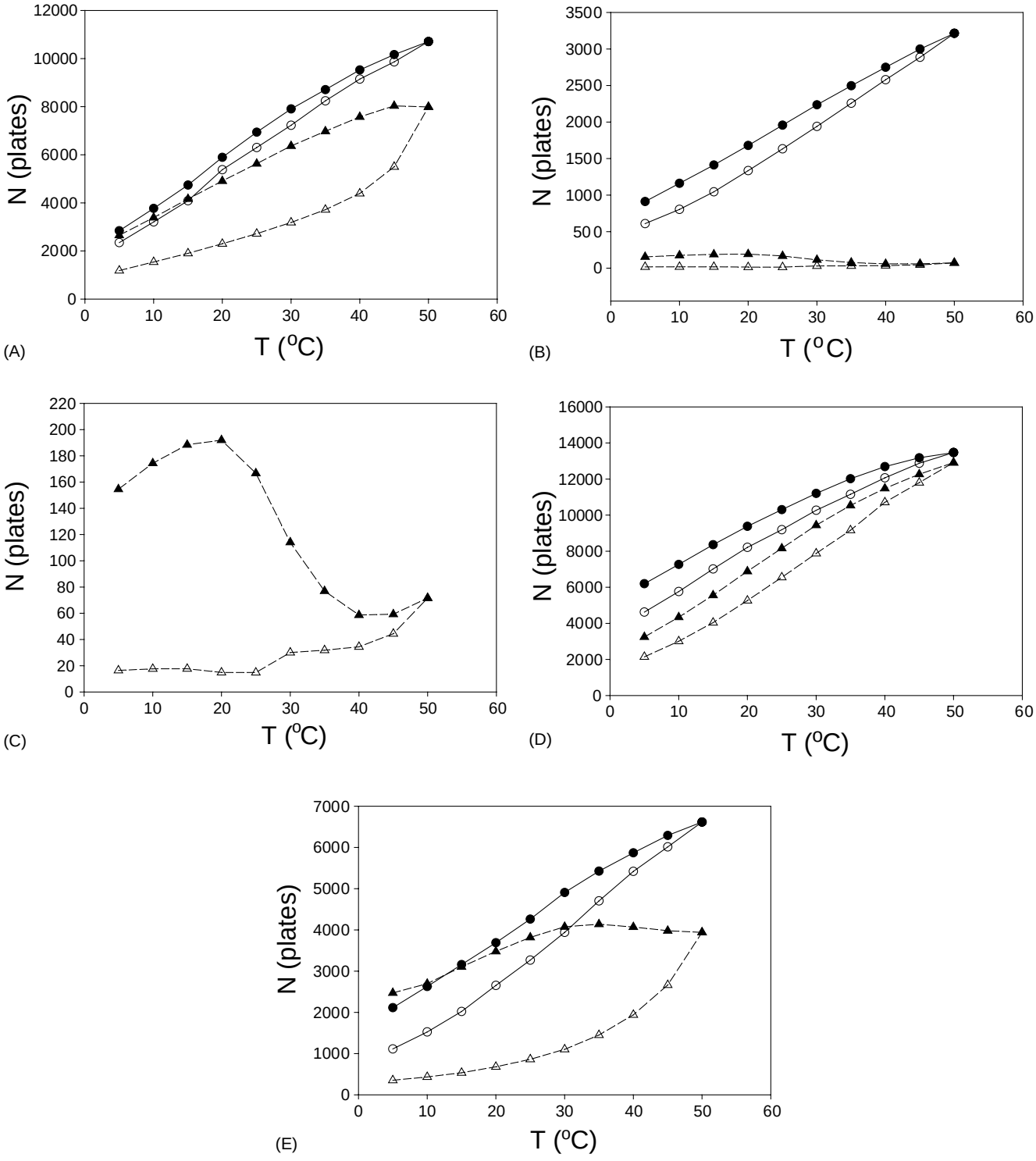


Fig. 7. Comparison of column efficiency of DHP acid and ester on both Chiralpak AD-H and AD columns. Mobile phase: 15% EtOH in hexane with 0.1% TFA. Processes: *R* enantiomers—heating (filled circle), cooling (open circle); *S* enantiomers—heating (filled triangle), cooling (open triangle). (A) DHP acid on Chiralpak AD-H column, (B) DHP acid on Chiralpak AD column, (C) enlarged (B) of *S*-(+)-DHP acid on Chiralpak AD column, (D) DHP ester on Chiralpak AD-H column, (E) DHP ester on Chiralpak AD column.

dicated the change in the conformation of the AD phase (Fig. 7C). It reached a minimum at 40 °C. A clear transition can be observed in Fig. 7C. The efficiency increased slightly when the temperature was over 40 °C. During the cooling process, the efficiency decreased with the decrease in the column temperature (Fig. 7C). There was no transition during the cooling process, which was consistent with the van't Hoff plots.

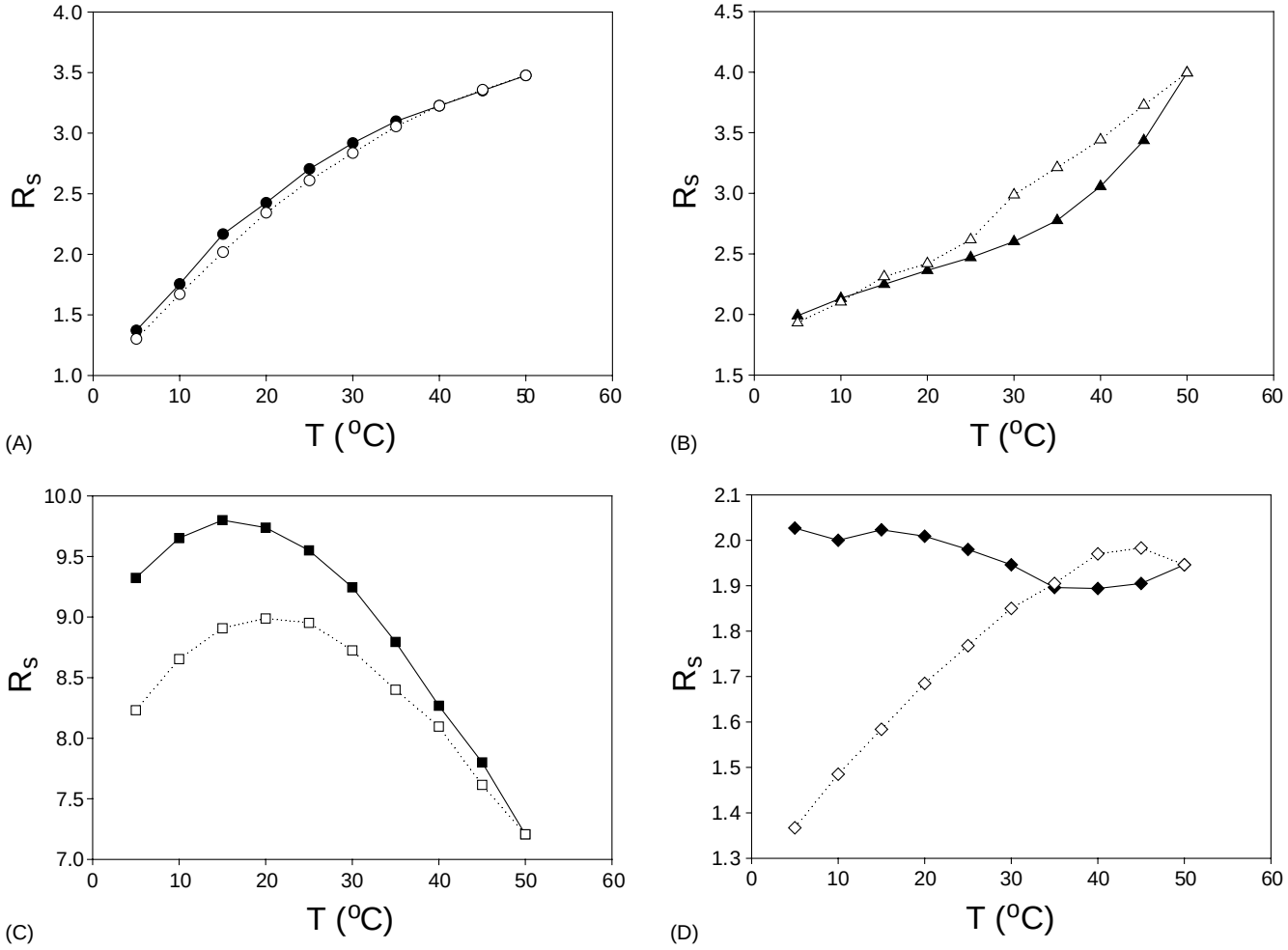


Fig. 8. Comparison of resolution of DHP acid and ester on Chiralpak AD-H and AD columns. Mobile phase: 15% EtOH in hexane with 0.1% TFA. (A) DHP acid on Chiralpak AD-H column, (B) DHP acid on Chiralpak AD column, (C) DHP ester on Chiralpak AD-H column, (D) DHP ester on Chiralpak AD column. Processes: heating (filled symbols), cooling (open symbols).

For DHP ester, the efficiency curves for both enantiomers on the AD-H phase showed the same curve-shape in a heating/cooling cycle (Fig. 7D). The efficiency curves for DHP ester on the AD phase (Fig. 7E) were similar to those of DHP acid on the AD-H phase (Fig. 7A). Again, different trends were observed on both phases by using DHP ester as a molecular probe.

3.4. Resolution

Fig. 8 shows the effect of the column temperature on the resolution of DHP acid and ester. On the AD-H phase, the increase in the column temperature increased the resolution of DHP acid (Fig. 8A) because both selectivity factors and efficiency increased with the increase in the temperature (Figs. 4B and 7A). On the AD phase, the increase in the column temperature increased the resolution of the acid (Fig. 8B). However, the curves of the AD phase were not superimposable because of the conformational transition. The overall magnitude of the resolution on the AD phase was similar to that on the AD-H phase in the whole temperature range. The resolution difference in the heating/cooling cycle was not as big as that of apparent selectivity factors because of the efficiency loss of the *S* enantiomer at the high temperatures (Fig. 7C).

For DHP ester, the resolution curves of the AD-H phase during heating and cooling processes show the similar curve-shape (Fig. 8C). They increased with the increase in the temperature at first. Both reached their optima at 15 °C. Then, both decreased with the further increase in the temperature (Fig. 8C). The resolution curves of DHP ester on the AD phase (Fig. 8D) show the same trend as the apparent selectivity factors (Fig. 5) as previously reported [15]. This indicates that the apparent selectivity factors, not the efficiency, were the main contributor to the resolution on the AD phase. The overall resolution of DHP ester on the AD-H phase was much higher than that on the AD

phase in the whole temperature range. This agreed with the application notes from the vendor.

4. Conclusion

The comparison study of the AD-H and AD phases using DHP acid and methyl ester as the probe compounds showed that the differences between the two phases were not only in their thermodynamic behaviors (such as retention and selectivity factors, resolution) but also in their kinetic behaviors in the thermally-induced conformational transition. Overall, the AD-H phase did not show any better selectivity of DHP acid enantiomers than that of the AD phase while the AD-H phase showed better selectivity and resolution of DHP ester than those of the AD phase.

Acknowledgements

The authors wish to thank Dr. Daniel R. Sidler for providing the DHP acid and methyl ester racemates, and the single enantiomers. The authors also wish to thank Drs. Bing Mao, T. O'Brien and Tao Wang for their helpful discussions.

References

[1] T.E. Beesley, R.P.W. Scott, in: R.P.W. Scott, C.F. Simpson, E.D. Katz (Eds.), Chiral Chromatography, Wiley, Chichester, England, 1998.
[2] G. Subrananian (Ed.), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, New York, 1994.
[3] S. Allenmark. Chromatographic Enantioseparation: Methods and Applications, Ellis Horwood, Chichester, 1991 (Chapter 7).
[4] A.M. Krstulovic (Ed.), Chiral Separations by HPLC: Applications to Pharmaceutical Compounds, Ellis Horwood, Chichester, 1989.
[5] G. Gubitz, M.G. Schmid, Biopharm. Drug Dispos. 22 (2001) 291.
[6] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[7] V.A. Davankow, J. Chromatogr. A 666 (1994) 55.
[8] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[9] E. Yashima, Y. Okamoto, Bull. Chem. Soc. Jpn. 68 (1995) 3289.
[10] E. Yashima, C. Yamamoto, Y. Okamoto, Synlett (1998) 344.
[11] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[12] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[13] Chiral Technologies, Inc., Application Notes, production information brochure provided with columns.
[14] Chiral Technologies, Inc., Use of Polar Mobile Phase Solvents, production information brochure provided with columns.
[15] F. Wang, T. O'Brien, T. Dowling, G. Bicker, J. Wyvratt, J. Chromatogr. A 958 (2002) 69.
[16] F. Wang, R. Wenslow Jr., T. Dowling, K. Mueller, I. Santos, J. Wyvratt, Anal. Chem. 75 (2003) 5877.
[17] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730.