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Journal of Chromatography A, 752 (1996) 101–109

JOURNAL OF CHROMATOGRAPHY A

Enantioseparation of a protease inhibitor, indinavir, by subcritical fluid chromatography

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Received 26 March 1996; revised 21 May 1996; accepted 23 May 1996

Abstract

Indinavir is a protease inhibitor which possesses five chiral centers. Enantioseparation of indinavir and its enantiomer was performed on an amylose type stationary phase, Chiralpak® AD, under normal-phase HPLC and subcritical fluid chromatography conditions. Under the utilized chromatographic conditions, it is believed that the leading interactions are hydrogen bonding between one or both hydroxyl groups of the solute with the carbonyl group of the carbamate. An inclusion mechanism appears to control the chiral recognition. The effect of various modifiers, pressure, and temperature were investigated.

Keywords: Enantiomer separation; Subcritical fluid chromatography; Indinavir

1. Introduction

The necessity to possess and to develop analytical methods that provide accurate quantitation of drug substance enantiomers for the purpose of process control or for pharmacokinetic studies has become a growing priority in the pharmaceutical industry. This growth can be attributed to the increased awareness that the enantiomers of many drug substances possess pharmacological and toxicological differences [1]. Chromatography, by direct or indirect methods, is a common analytical tool for enantiomeric quantitation.

High-performance liquid chromatography has traditionally been the favored choice for achieving an enantioseparation. More recently supercritical fluid chromatography (SFC) and subcritical fluid chromatography (SubFC) have emerged as viable alternatives to HPLC. Super- and sub-critical fluids possess higher diffusion constants than liquids. This higher diffusivity results in higher optimum linear velocities and higher efficiencies per unit time. The overall result of these factors, is generally, higher resolution in shorter times and greater sensitivity as compared to HPLC [2,3].

There are additional advantages gained in using SFC or SubFC over HPLC. Parameters such as pressure, flow, temperature, and composition can be altered with greater ranges and with greater effects on selectivity than with HPLC. One of the more important parameters is the composition of the mobile phase which can be altered through the variation of the concentration and type of modifier. Alcohols are commonly used as modifiers. Acetonitrile and methanol are not generally used in normal-phase LC due to their limited solubility in hexane, but can be used in SFC or SubFC allowing for the use of a wider range in polarity of the modifiers than

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0021-9673/96/$15.00
PII S0021-9673(96)00509-2

with LC. Mourier et al. were the first to demonstrate chiral resolution by SFC and SubFC [4]. Subsequently, a number of chiral separations performed under these conditions have been documented [5–12].

The field of chromatographic enantioseparations has progressed to the extent that there are currently a wide range of chiral stationary phases (CSPs) available to achieve a desired resolution. One of the more popular types of CSPs are the polysaccharide derivatives. Separations were initially performed on these CSPs by Hesse and Hagel who successfully separated Troger's base on microcrystalline cellulose triacetate [13]. Evidence indicated that the primary enantioselective mechanism was through inclusion [13,14]. The dominant enantioselective mechanism for this class of CSPs varies however, and is dependent upon the class of polysaccharide, the morphology of the CSP, the type of derivative, and the mode of preparation [15].

This paper describes a chromatographic enantioseparation using a polysaccharide derivative as the CSP. The specific CSP investigated was amylose with an achiral functionality (carbamate) derivatized onto its hydroxy groups (Chiralpak® AD, Fig. 1). It is one of a number of derivatized cellulose and amylose CSPs that have been commercially developed [16,17] and have been used to perform enantioseparations under HPLC, SFC, and SubFC conditions [7–12,18–22].

With the carbamate derivative of amylose, chiral recognition is achieved through the formation of transient diastereomeric complexes between the analyte and the CSP. The main adsorbing site is believed to be the carbamate group which can interact with the solute through hydrogen bonding and dipole interactions [19,23–26]. These interactions orient the analyte and the CSP within the complex. Additionally, there is the possibility of interactions between the aromatic groups of the stationary phase and the solute. Chiral discrimination is based primarily on the differences of steric fit within the cavities created by the amylose chains [27,28]. Intramolecular hydrogen bonding contributes to the rigid regular structure of the chains which potentially increases enantioselectivity [18,29]. The analyte is eluted usually by the use of alcoholic modifiers. These modifiers compete with the analyte for both chiral and achiral sites. Furthermore, they can alter the steric environment of the CSP by binding at achiral sites near or at the chiral cavities affecting the stability of the transient diastereomers [30].

Enantioseparations under normal-phase HPLC and SubFC conditions using the Chiralpak®) AD column were performed. The compounds used were [1*S*-[1α[α*S**,γ*R**,δ*R**],2α]]-N-(2,3-dihydro-2-hydroxy-1*H* - inden - 1 - yl) - 2 - [[(1,1 - dimethylethyl)amino]-carbonyl] - γ - hydroxy - α(phenylmethyl) - 4 - (3 - pyridinylmethyl) - 1 - piperazinepentanamide sulfate (indinavir sulfate, Fig. 2) and its enantiomer. Indinavir is a member of a class of dipeptides that have been found to be potent inhibitors of the HIV protease [31–33]. The molecule possesses five chiral centers, two hydroxyl groups, amine and amide groups, and three aromatic rings including a pyridine ring. Consequently, there are many potential sites for both chiral and achiral interactions with the derivatized amylose. This multiplicity of possible sites of interaction could result in the occurrence of competitive chiral recognition mechanisms. These mechanisms may not all favor the same enantiomer and as a consequence they can oppose each other. Since the net chromatography is essentially the weighted average of the contributions of all possible interactions [34], resolution will occur only when the contribu-

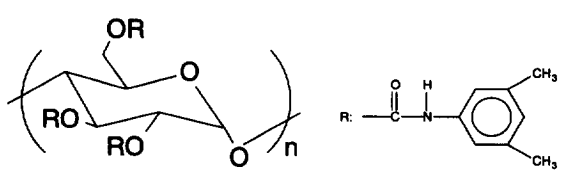

Fig. 1. Structure of Chiralpak® AD.

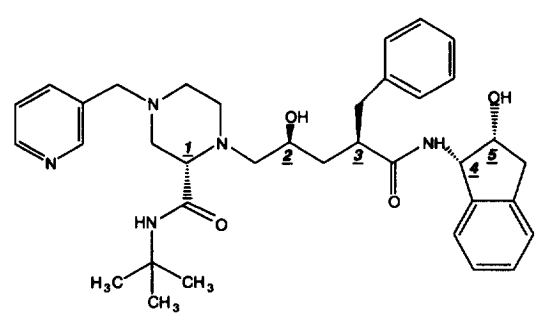

Fig. 2. Structure of indinavir.

tions of the interactions at the five chiral centers predominantly favors one enantiomer and when the achiral interactions have been effectively reduced. Optimization and the effect of various modifiers, pressure, and temperature were investigated along with the effect of inversion at only one or two of the chiral centers on indinavir.

2. Experimental

2.1. Chromatographic equipment

The column used was a Chiralpak® AD 250×4.6 mm (Chiral Technologies, Exton, PA, USA), with a stationary phase of amylose tris (3,5-dimethylphenylcarbamate) coated on 10 μm silica-gel support.

The SubFC system consisted of an HP G1205A SFC pumping module, an HP GC/SFC 7673 injector, an HP SFC 5890 oven, and an HP 1050 detector (Hewlett–Packard, Wilmington, DE, USA). All the data were processed by PE Nelson Access Chrom version 1.7 (PE Nelson, Cupertino, CA, USA). Temperature control below 25°C was performed using liquid nitrogen from a Thermo 30 transfer vessels (Thermolyne, Dubuque, IA, USA).

The HPLC instrumentation included a Spectra-Physics SP 8875 autosampler (TSP, San Jose, CA, USA), a Varian 9010 pump (Varian, San Fernando, CA, USA), and an Applied Biosystems 759A absorbance detector (Applied Biosystems, Foster, CA, USA). The column temperature was controlled by a Beckman 235 column heater (Beckman, Fullerton, CA, USA).

2.2. Chromatographic conditions

For SubFC chromatography, the mobile phase consisted of carbon dioxide modified with the appropriate alcohol at a flow-rate of 2 ml/min unless otherwise specified. Samples were dissolved in methanol and the injection volume was 5 μl. Detection was performed at 210 nm.

For normal-phase HPLC, the mobile phase consisted of hexane with the appropriate alcohol as the polar additive at a flow-rate of 1 ml/min. Samples were dissolved in hexane–isopropanol (50:50) and the injection volume was 10 μl. Detection was performed at 254 nm.

2.3. Chemicals

Indinavir, its enantiomer, and three diastereomers were provided by Process Research, Merck Research Laboratories. SFC grade carbon dioxide was purchased from Air Products (Allentown, PA, USA). Methanol, hexane, *n*-propanol, and isopropanol were all obtained from Fisher Scientific (Pittsburgh, PA, USA). Ethanol (200 proof) was obtained from Quantum Chemical (Newark, NJ, USA).

3. Results and discussion

3.1. Chiral recognition model

When polymers such as amylose tris (3,5-dimethylphenylcarbamate) are used as a CSP, an elucidation of a definitive model for its steric interaction with a solute is difficult. The difficulty arises as a consequence of the unknown nature of its exact steric structure. The general types of interactions which have been identified, however, can be incorporated into a broad model describing the interaction between indinavir and amylose tris (3,5-dimethylphenylcarbamate).

The two hydroxyl groups and the two carbonyl groups of indinavir can undergo hydrogen bonding with the hydrogen from the NH of the carbamate on the CSP. Additionally, the two hydroxyl groups and the two amide groups of indinavir can hydrogen bond with the carbonyl group of the CSP. Along with hydrogen bonding, there is the possibility of interactions between the aromatic portions of indinavir and the CSP. The steric bulk around the chiral centers of indinavir and the geometry of the chiral cavities within the CSP will contribute towards determining which interactions will dominate. Any proposed model must also consider that the various functional groups of indinavir can undergo achiral interactions with the CSP.

3.2. Enantioseparation under normal-phase HPLC conditions

Method development was undertaken using ethanol, *n*-propanol, and isopropanol as the polar modifier. The optimized enantioseparation of indinavir from its enantiomer was performed with a mobile phase of hexane–isopropanol (85:15) (Fig. 3). A baseline separation was not achieved. The separation factor under these conditions was 1.12 but broad peaks resulted in a resolution of only 1.31. The capacity factor of the first eluted enantiomer was 6.9. The lowering of the percentage of isopropanol to decrease competition between the alcohol and the solute for chiral discriminating sites resulted in minimal changes in the separation factor. In fact, the concurrent reduction in competition for achiral sites resulted in much longer retention times, broader peaks and a further diminished resolution.

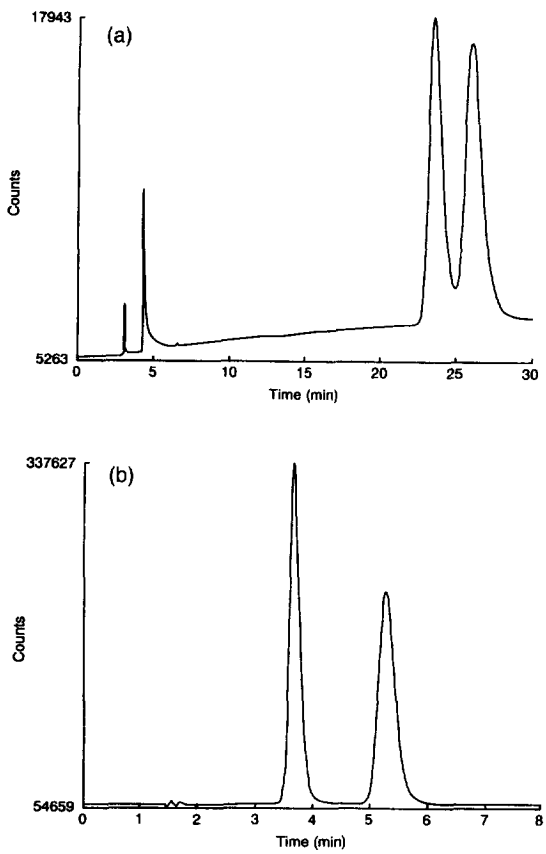

Fig. 3. (a) Normal-phase separation of the two enantiomers. Mobile phase consisted of hexane modified with 15% isopropanol. Other chromatographic conditions are as detailed in the experimental section. (b) Optimized SubFC separation of the two enantiomers. Mobile phase consisted of carbon dioxide modified with 25% methanol. The pressure was 250 bar and temperature was 40°C. Other chromatographic conditions are as detailed in Section 2.

3.3. Effect of modifier under SubFC conditions

Improved resolution and faster analyses, relative to the normal-phase method, is obtained under SubFC conditions dependent upon the modifier (Fig. 3). The aim of adding a modifier to carbon dioxide in SubFC is twofold. The modifier blocks the active sites of the silica through hydrogen bonding and reduces their secondary interactions with the solute [35]. The modifier also changes the solvating power of the carbon dioxide affecting its selectivity.

It has already been indicated that indinavir and its enantiomer has a number of functional groups which can interact with the CSP. In terms of the functional groups' ability to interact through hydrogen bonding, it would be expected that the hydroxyl groups would have the greatest capability [36]. At such high levels of alcohol modifier in the mobile phase the strength of the hydroxyl groups interaction with the CSP will be further accentuated relative to the other groups since it is unlikely that they would be able to compete appreciably with the alcohol modifier for the hydrogen bonding sites on the CSP. It has been already shown that the presence of hydroxyl groups on the solute greatly enhances stereoselectivity during interaction with carbamated amylose [37–39]. Thus it appears most likely that one of the major chiral discriminating interactions would be through one or both of the hydroxyl groups of the solute.

The influence of the nature of the mobile phase modifier, on the separation of indinavir from its enantiomer, was studied at 40°C and 250 bar outlet pressure. For a constant alcohol concentration of 3.7 *M*, from methanol to ethanol to *n*-propanol the capacity factors (k'), of both indinavir and its enantiomer, and the stereoselectivity α decreased (Table 1). The observed decrease of the k' can be attributed in part to the increasing polarity of the mobile phase [36,40]. Interestingly, in going from *n*-propanol to isopropanol an increase in the capacity factors of both enantiomers were observed despite

Table 1
Effect of different organic modifiers at constant molarity (3.7 *M*) on the capacity factors and selectivities for indinavir and its enantiomer

	k_1	k_2	α	Polarity ρ'
Methanol	6.28	11.17	1.78	0.77
Ethanol	3.75	4.80	1.28	0.95
n-Propanol	2.22	2.31	1.04	1.00
Isopropanol	2.90	3.05	1.05	1.09

the fact that the polarity had increased. This observation indicates that steric factors play a significant role in the interaction of the solute with the stationary phase.

The effect of the modifiers were investigated for a fixed polarity in the mobile phase ($\rho'=1.02$). Polarity of the binary mobile phase was calculated as the summation of the product of the polarity of each component times its volume fraction [40]. Liquid carbon dioxide is non-polar with characteristics similar to that of hexane [41–44] and was consequently given a polarity value of 0.1. The results are shown in Table 2. The trend in α was found to be similar to that at fixed molar concentration of the alcoholic modifier. The effect of modifiers concentration on retention and chiral selectivity were investigated in order to further substantiate the above findings. The capacity factors of both enantiomers decreased with increasing concentration of methanol or isopropanol (Fig. 4). The change in k' with modifier concentration was very large and both enantiomers were strongly retained below 15% modifier concentration reflecting the strong interaction that the solute undergoes with the CSP. Conversely, there was a relatively small change in α when using methanol and no change when using isopropanol (Fig. 5). The small change in α with respect to the change in k' suggests that there are many more or stronger achiral interactions than there

Table 2
Effect of different organic modifiers at constant polarity on capacity factors and selectivities for indinavir and its enantiomer

	k_1	k_2	α
Methanol	2.91	5.04	1.73
Ethanol	3.08	3.88	1.26
n-Propanol	2.22	2.31	1.04
Isopropanol	3.78	4.01	1.06

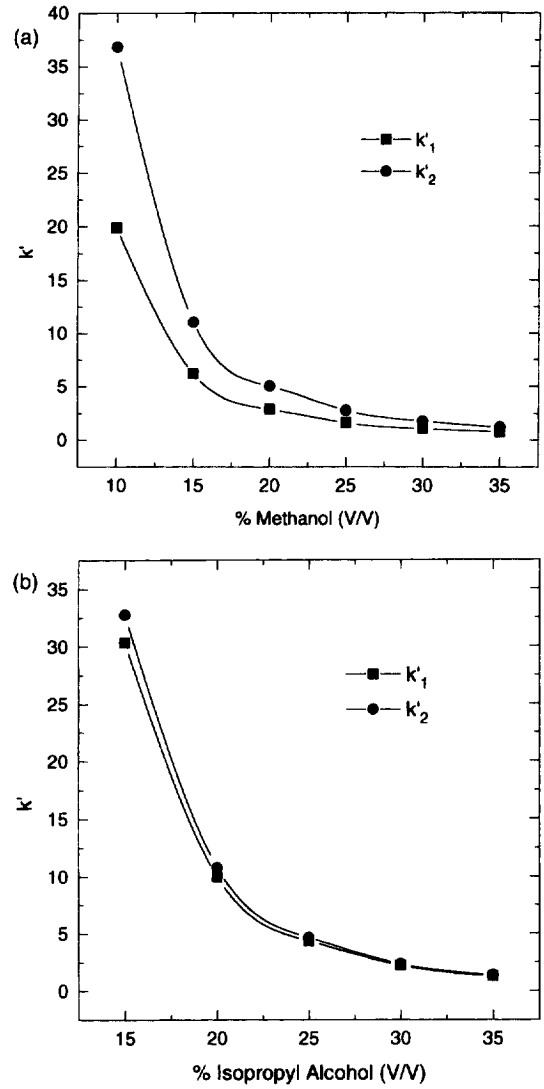

Fig. 4. Effect of the concentration of the alcohol modifier on k'. (a) Methanol, (b) isopropanol. Chromatographic conditions are as detailed in Section 2.

are chiral interactions. The achiral interactions are heavily dependent on the polarity of the mobile phase while the chiral interactions show little or no dependency. These findings indicate that while polarity may have an effect on the overall interaction between the two solutes and the stationary phase, it has little effect on the chiral recognition mechanism.

Investigations into the steric structure of alcoholic mobile phase modifiers on k' and α on Pirkle type

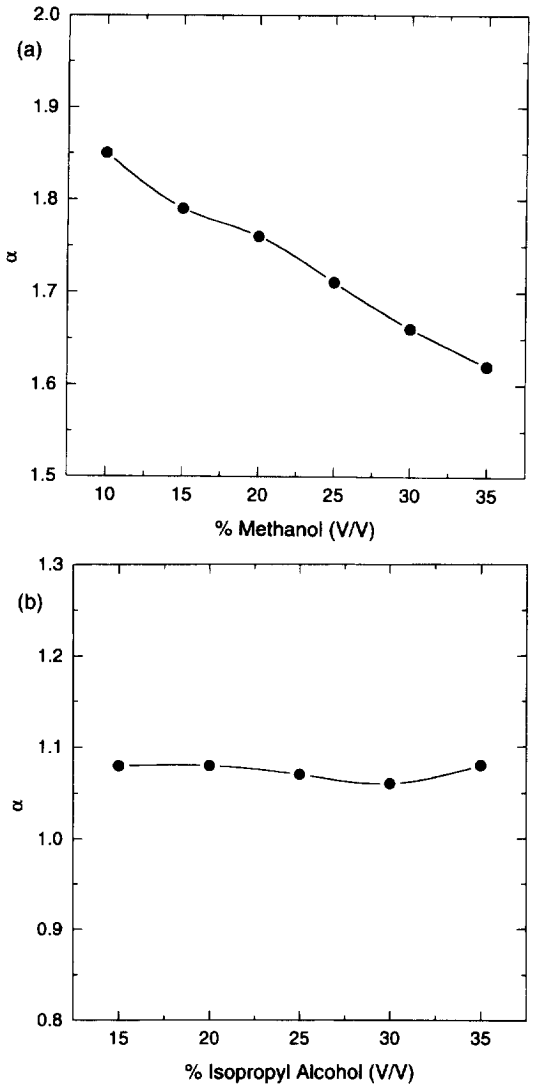

Fig. 5. Effect of the concentration of the alcohol modifier on α. (a) Methanol, (b) isopropanol. Chromatographic conditions are as detailed in Section 2.

CSPs has indicated that α increases with the bulk of the alcohol [45–47]. This behavior was accredited to the increasing bulk around the hydroxyl group of the alcohol. Increasing bulk decreased its ability to displace the enantiomers from the CSP with the extent of this decrease differing for the two enantiomers based on steric factors. However, in the case of the Pirkle type CSP, the interaction of the solute with the CSP takes place at the surface of the CSP while with the carbamated amylose, interactions also takes place in the chiral cavities of the CSP. When the alcoholic mobile phase modifier interacts with the CSP, it not only competes for chiral binding sites, but also alters the steric nature of the chiral cavity by binding to achiral sites at or near the chiral cavity [20]. A bulkier alcohol would have a greater impact on the cavity structure by creating greater steric hinderance for solutes being inserted into the cavity. The observed decrease in stereoselectivity in the case of the carbamated amylose is primarily due to the steric changes of the chiral cavity introduced by the modifier. The above findings are consistent with inclusion being the dominant chiral recognition mechanism with the steric factor introduced by the modifier being a dominant force [14].

3.4. Effect of pressure and temperature under SubFC conditions

The effect of pressure on the enantioseparation under SubFC conditions with 30% methanol and at 40°C were investigated. The pressure was varied from 100 to 300 bar. Negligible changes were noted over this range. The biggest change was in the capacity factor which decreases from 2.70 to 1.53 in going from 100 to 300 bar. The separation factor was constant over this range. The fact that no changes in the separation factor were observed indicates that pressure has no effect on the chiral discrimination and affects only the overall partitioning of both enantiomers between the stationary phase and the mobile phase.

Temperature is an important variable for separation in SFC or sub-FC. For interactions which possess a large enthalpic contribution, it is expected that selectivity will increase with decreasing temperature. The effect of temperature on the enantioseparation under Sub-FC conditions with 30% methanol and under 250 bars of pressure were investigated. The temperature was varied from 0 to 30°C. It was found that the second eluting enantiomer was retained to a greater degree relative to the first eluting enantiomer, indinavir, with decreasing temperature. As a consequence the separation factor increased with decreasing temperature. Plots of the natural log of both capacity factors and the separation factor versus the reciprocal temperature (K^{-1})

were linear with correlation constants greater than 0.994 (Fig. 6) and with ΔH values of −2732.1 and −1779.3 calories/mole for indinavir and its enantiomer respectively. The thermodynamic parameters $\Delta\Delta H$ and $\Delta\Delta S$ for the two enantiomers were found to be −952.8 calories/mol and −2.04 cal/mol· K respectively. While inclusion may be critical for chiral recognition, the large negative value for $\Delta\Delta H$, which reflects the interaction to form the diastereomeric complexes through hydrogen bonding, is also important. This data can be interpreted as the enantiomer which is being included more strongly is also undergoing further interactions through hydrogen bonding within the cavity. The increase in the separation factor with decreasing temperature can in part be attributed to the increased rigidity of the polymeric CSP resulting in a greater discrimination of the steric fit between the two enantiomers.

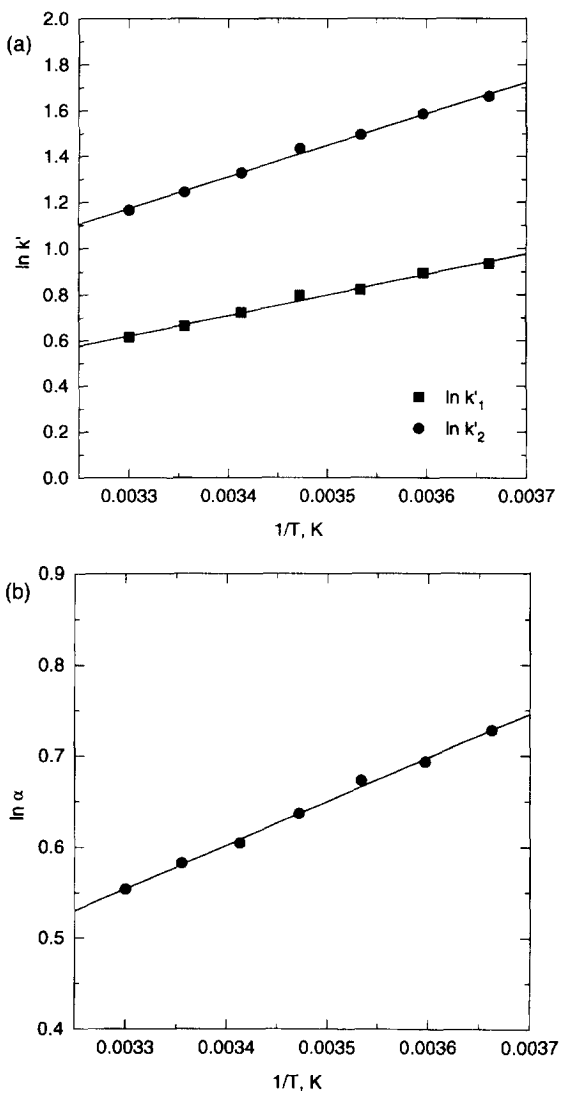

Fig. 6. Effect of temperature on (a) k' and (b) α for the two enantiomers. Chromatographic conditions are as detailed in Section 2.

3.5. Retention characteristics of various epimers of indinavir

Indinavir possesses five chiral centers. The contribution of each individual chiral center to the overall enantioseparation is dependent on the strength of its interaction with the CSP. The overall retention is based on the weighted average of the interactions at these chiral centers and the achiral interactions. The retention characteristics of three additional optical isomers were investigated and compared to indinavir and its enantiomer. The first of the additional optical isomers, 4-*epi* MK0639, possesses an inversion of configuration at the chiral site labeled *2* in Fig. 2. The second optical isomer, bis-*epi* MK0639, possess inversions of configuration at the chiral sites labeled *2* and *3*. The third optical isomer, *epi*-carboxamide MK0639 possesses an inversion of configuration at the chiral site labeled *1*.

The retention of these five isomers were measured at 40°C, 250 Bar, and with 25% methanol modifier. Indinavir and its enantiomer had capacity factors of 1.59 and 2.73 respectively. The capacity factor of 4-*epi* MK0639 was 2.34. The increase in the capacity factor relative to indinavir indicates that the inversion at chiral site *2* leads to a stronger interaction with the CSP. The capacity factor of bis-*epi* MK0639 was 1.56. Since the capacity factor of bis-*epi* MK0639 is similar to that of indinavir it appears that when the hydroxyl group at chiral center *2* and the phenyl group at chiral center *3* are projected in the same plane, interactions are decreased with the CSP. The capacity factor for *epi*-carboxamide MK0639 was 10.0. The tremendous increase in capacity factor indicates that interactions are strongest when the amide at chiral center *1* is not projected into the same plane as the cis amidoindanol portion (constituting chiral centers *4* and *5*) of the

molecule. These findings indicate that, for the given configurations of indinavir and its enantiomer, some of the chiral centers oppose each other in terms of their contribution to the overall interaction and retention on the CSP. In summary it appears, that for indinavir and its enantiomer, interactions between chiral sites *2* and *3* with the CSP are of opposing nature as are the interactions between chiral sites *1* and combined chiral sites *4* and *5* with the CSP which also appear to be stronger in nature.

4. Conclusion

Subcritical fluid chromatography has been utilized for the chiral discrimination of indinavir from its enantiomer on a carbamated amylose stationary phase. The many functional groups of the solute provides for numerous types of interaction with the chiral stationary phase. Under conditions of high alcoholic modifier content, it is expected that the leading interaction is between one or both hydroxyl groups of the solute interacting with the carbonyl group of the carbamate to form a diastereomeric complex with a portion of the solute then being included into the chiral cavity of the stationary phase. Chiral recognition is then dependent upon steric contributions of the alcoholic modifiers.

Acknowledgments

The authors would like to express our gratitude to Dr. Kan Eng for supplying us with the enantiomer and diastereomers of indinavir and to Thomas Loughlin for his cryogenic expertise.

References

[1] I.W. Wainer and D.E. Drayer, (Editors), Drug Stereochemistry, Analytical Methods and Drug Pharmacology, Marcel Dekker, New York, 1988.
[2] D. Gere, Science, 222 (1983) 253.
[3] K. Anton, J. Eppinger, L. Frederiksen, E. Francotte, T. Berger and W. Wilson, J. Chromatogr. A, 666 (1994) 395.
[4] P. Mourier, E. Eliot, M. Caude, R. Rosset and A. Tambute, Anal. Chem., 57 (1985) 2819.
[5] P. Macaudiere, M. Caude, R. Rosset and A. Tambute, J. Chromatogr., 405 (1987) 135.
[6] P. Macaudiere, M. Caude, R. Rosset and A. Tambute, J. Chromatogr. Sci., 27 (1989) 583.
[7] P. Macaudiere, M. Caude, R. Rosset and A. Tambute, J. Chromatogr., 450 (1988) 255.
[8] T. Nitta, Y. Yakushijin, T. Kametani and T. Katayama, Bull. Chem. Soc. Jpn., 63 (1990) 1365.
[9] Y. Kaida and Y. Okamoto, Bull. Chem. Soc. Jpn., 65 (1992) 2286.
[10] W. Wilson, Chirality, 6 (1994) 216.
[11] C., Lee, J. Porziernsky, M. Aubert and A. Krstolovic, J. Chromatogr., 539 (1991) 55.
[12] J. Juvancz, K. Grolimund and E. Francotte, Chirality, 4 (1992) 459.
[13] G. Hesse and and R. Hagel, Chromatographia, 6 (1973) 277.
[14] H. Koller, K. Rimbock and A. Mannschreck, J. Chromatogr., 282 (1983) 89.
[15] A. Ichida and T. Shibata, Cellulose derivatives as chiral stationary phases, in M. Zief and L. Crane (Editors), Chromatographic Chiral Separations, Marcel Dekker, New York, 1988.
[16] A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, Chromatographia, 19 (1984) 280.
[17] Y. Okamoto, M. Kawashima and K. Am, J. Chem. Soc. 106 (1984) 5357.
[18] I. Wainer, R. Stiffin and T. Shibata, J. Chromatogr., 411 (1987) 139.
[19] I. Wainer, and M. Alembik, J. Chromatogr., 358 (1986) 85.
[20] I. Wainer, M. Alembik and E. Smith, J. Chromatogr., 388 (1987) 65.
[21] Y. Okamoto, R. Aburatani and K. Hatada, J. Chromatogr., 389 (1987) 95.
[22] T. Loughlin, R. Thompson, N. Grinberg, G. Bicker and P. Tway, Chirality, 8 (1996) 157.
[23] T. Shibata, I. Okamoto and K. Ishii, J. Liq. Chromatogr., 9 (1986) 313.
[24] Y. Okamoto, M. Kawashima and K. Hatada, J. Chromatogr., 363 (1986) 173.
[25] Y. Okamoto, Y. Kaida, R. Aburatani and K. Hatada, J. Chromatogr., 477 (1989) 367.
[26] O. Azzolina, S. Collina and V. Ghislandi, Il Pharmacao, 48 (1993) 1401.
[27] E, Francotte, M. Romain, D. Lohmann and R. Muller, J. Chromatogr., 347 (1985) 25.
[28] P. Ficarra, R. Ficarra, A. Chimirri, G. Romeo, S. Tommasini, M. Calabro, D. Costantino, A. Montforte and M. Carulli, Chromatographia, 38 (1994) 57.
[29] B. Chankvetadze, E. Yashima and Y. Okamoto, J. Chromatogr., 670 (1994) 39.
[30] M. Gaffney, R. Stiffin and I. Wainer, Chromatographia, 27 (1989) 15.
[31] J.P. Vacca, B.D, Dorsey, W.A. Schlief, R.B. Levin, S.L. McDaniel, P.L. Darke, J. Zugay, J.C. Quintero, O.M. Blahy, E.; Roth, V.V.; Sardana, A.J.; Schlabach, P.I.; Graham, J.H.; Condra, L.; Gotlib, M.K.; Holloway, J.; Lin, I.-W.; Chen, K.; Vastag, D.; Ostovic, P.S.; Anderson, E.A.; Emini and J.R. Huff, Proc. Natl. Acad. Sci. USA, 91 (1994) 4096–4100.

[32] B.D. Dorsey, R.B. Levin, S.L. Vacca, J.P. McDaniel, J.P. Guare, P.L. Darke, J. Zugay, E.A. Emini, W.A. Schlief, J.C. Quintero, J. Lin, I.-W. Chen, M.K. Holloway, P.M. Fiztgerald, M.G. Axel, D. Ostovic, P.S. Anderson and J.R. Huff, J. Medicinal Chem., 37 (1994) 3443.
[33] D. Askin, K. Eng, K. Rossen, R. Purick, K. Wells, R. Volante and P. Reider, Tetrahedron Lett., 35 (1994) 673.
[34] W. Pirkle, and T. Pochapsky, Chem. Rev., 89 (1989) 347.
[35] J., Janssen, P. Schoenmakers and C. Cramers, J. High Resolut. Chromatogr., 12 (1989) 645.
[36] L. Snyder, J. Chromatogr. Sci., 16 (1978) 223.
[37] S. Levin, S. Abu-Lafi, J. Zahalka, and R. Mechoulam, J. Chromatogr., 654 (1993) 53.
[38] S. Abu-Lafi, M. Sterin, S. Levin and R. Mechoulam, J. Chromatogr., 664 (1994) 159.
[39] S. Abu-Lafi, M. Sterin and S. Levin, J. Chromatogr., 679 (1994) 47.
[40] L. Snyder, J. Chromatogr., 92 (1974) 223.
[41] M. Sigman, S. Lindley and J. Leffler, J. Am. Chem. Soc., 107 (1985) 1471.
[42] J. Hyatt, J. Org. Chem., 49 (1984) 5097.
[43] C. Yonker, S. Frye, D. Lalkwarf and R. Smith, J. Phys. Chem., 90 (1986) 3022.
[44] M. Lee and K. Markides, (Editors), Analytical Supercritical Fluid Chromatography and Extraction, Chromatography Conferences Inc., Provo, Utah, 1990.
[45] M. Zief, L. Crane and J. Horvath, J. Liq. Chromatogr., 7 (1984) 709.
[46] P. Pescher, M. Caude, R. Rosset, and A. Tambute, J. Chromatogr., 371 (1986) 159.
[47] P. Macaudiere, A. Tambute, M. Caude, R. Rosset, M. Alembik and I. Wainer, J. Chromatogr., 371 (1986) 177.