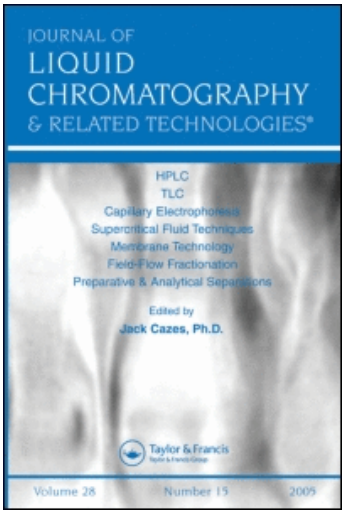


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Improvement of Chiral Discrimination of Acidic Enantiomers on Teicoplanin Stationary Phase by the Use of Chaotropic Effect

J. Flieger[a]
[a] Department of Analytical Chemistry, Medical University of Lublin, Lublin, Poland

To cite this Article Flieger, J.(2009) 'Improvement of Chiral Discrimination of Acidic Enantiomers on Teicoplanin Stationary Phase by the Use of Chaotropic Effect', Journal of Liquid Chromatography & Related Technologies, 32: 7, 948 — 963
To link to this Article: DOI: 10.1080/10826070902787401
URL: http://dx.doi.org/10.1080/10826070902787401

Journal of Liquid Chromatography & Related Technologies®, 32: 948–963, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902787401

Improvement of Chiral Discrimination of Acidic Enantiomers on Teicoplanin Stationary Phase by the Use of Chaotropic Effect

J. Flieger

Department of Analytical Chemistry, Medical University of Lublin, Lublin, Poland

Abstract: This work reveals new advantages of using inorganic salts with chaotropic anions (perchlorate, hexafluorophosphate) as mobile phase additives in enantioseparation of acidic compounds (mandelic acid, tropic acid, phenyllactic acid, 2-(p-methoxyphenoxy)-propionic acid) on teicoplanin-based stationary phase in reversed phase mode. It was observed that the retention factors (k) and the separation factors (α) of acidic analytes increased in the presence of salts in the mobile phase. The standard changes in enthalpy (ΔH^0), entropy (ΔS^0), and the free energy (at 20°C) (ΔG^0) of the binding interactions between enantiomers and the chiral selector in the stationary phase were calculated from the experimental relationships of ln k vs 1/T in the presence of chaotropic salts in eluent systems. The Van't Hoff plots reveal that the interaction mechanism in the presence of chaotropic additives is enthalpy driven. Enthalpy-entropy compensation (EEC) studies revealed mechanistic similarity in retention of the investigated compounds.

Keywords: Chao-tropic effect, Enantiomer separation, Teicoplanin chiral stationary phase, Thermodynamic analysis

Correspondence: J. Flieger, Chair of Chemistry, Department of Analytical Chemistry, Medical University of Lublin, Staszica 6, Lublin, Poland. E-mail: j.flieger@am.lublin.pl

INTRODUCTION

Macrocyclic antibiotics are a recent class of chiral selectors, successfully applied to enantiomeric resolution in liquid chromatography.[1–10] They were introduced by Armstrong in 1994[11–16] as a new chiral selector. Different antibiotics (vancomycin, teicoplanin, thiostrepton, rifamycin B, kanamycin, streptomycin, fradiomycin, and ristocetin A) have been tested as potential chiral selectors, but only a few are commercially available: vancomycin (Chirobiotic V), teicoplanin (Chirobiotic T), teicoplanin aglycone (Chirobiotic TAG), and ristocetin A (Chirobiotic R). Macrocyclic antibiotics are also employed as chiral selectors in other separation techniques like capillary electrophoresis,[17,18] subcritical fluid chromatography,[19] thin layer chromatography.[20] An extensive review devoted to chiral separations using macrocyclic antibiotics was published by T. J. Ward et al.[21]

Teicoplanin-based CSPs belonging to this class of stationary phases possess 20 chiral centres surrounded by four fused macrocyclic rings forming a semi rigid structure. There are many functional groups such as: hydroxylic, aminic, amidic, carboxylic, as well as aromatic moieties and hydrophobic pockets, offering different molecular interactions including: electrostatic interactions, π-π complexation, hydrogen bonding, dipole-dipole, and the weakest ones, of steric and inclusion type. To explain enantioselectivity of these phases, a number of models have been proposed. Most of them are based on the three point interactions theory developed by Dalgliesh in 1952.[22] This theory assumed, that at least three simultaneous interactions (one of them should be stereochemically dependent) between the chiral centre and appropriate enantiomer are *conditio sine qua non* of the chiral recognition process.

Chirobiotic stationary phases have demonstrated enantioselectivity towards different classes of compounds (neutral, basic, acidic), either in normal and reversed phase modes or polar organic, or new polar ionic ones. The polar organic mode is recommended for neutral compounds. In this system, acceptable selectivity could be obtained by the use of pure methanol, ethanol, or acetonitrile. Compounds possessing ionizable groups require an addition of a small amount of base, such as triethylamine and acetic acid, or volatile salt. The most popular additives are triethylamine and acetic acid. Lee et al. tested different ammonium salts such as: formate, acetate, and trifluoroacetate for this purpose.[23] Most of the described enantiomeric separations on the Chirobiotic columns can be accomplished by the use of reversed-phase mode with an aqueous-organic eluent system where selectivity could be controlled by the changes in mobile phase composition: type and concentration of either organic modifier or buffer system. Usually, to enhance the ionization of analytes, the following buffer types are recommended: triethylammonium acetate,

ammonium acetate, ammonium nitrate, and sodium citrate. These systems make it possible to achieve a pH range of 3.5 to 7.0, the safest and the most stable conditions for chirobiotic CSPs. In reversed-phase mode, different interactions may take place: ionic, H-bonds, steric, inclusion, and hydrophobic, but it should be stressed that the primary binding interactions between ionized groups of CSP and appropriate enantiomer appear to occur due to electrostatic attraction forces at high organic modifier content, whereas at low organic solvent concentration hydrophobic interactions are the most important ones.

In the present studies, chosen acidic compounds were enantioseparated on teicoplanin-based CSP using methanol-water as a mobile phase with inorganic salts (hexafluorophosphates, perchlorates) additives as ion-ion interaction reagents. These salts are located at the end of the Hofmeister series. They are known as chaotropic salts or ''order breaking'' (*chaos tropic*), as they can damage the network of the hydrogen bonding between water molecules making hydrophobic interactions stronger. Enrichment of the mobile phase with chaotropic additives causes increasing of salting-in effect, decreasing of surface tension, increasing of ion-pairing process, and decreasing of the ions solvation. So far, the chaotropic effect was beneficial in the chromatography of ionic basic analytes in reversed-phase liquid chromatography.[24–31]

EXPERIMENTAL

Materials and Reagents

Investigated acidic (mandelic acid, phenyllactic acid, tropic acid, 2-p-methoxyphenoxy-propionic acid) compounds were obtained from Sigma (St. Louis. MO, USA). Sodium perchlorate and sodium hexafluorophosphate were obtained from Sigma-Aldrich. HPLC grade methanol (MeOH) was purchased from Merck (Darmstadt, Germany). HPLC water was obtained from Barnstead deionising system (Dubuque, IA, USA). All mobile phases were filtered with Nylon 66 membrane filters (0.45 μm) Whatman (Maidstone, England) by the use of a filtration apparatus.

HPLC Conditions

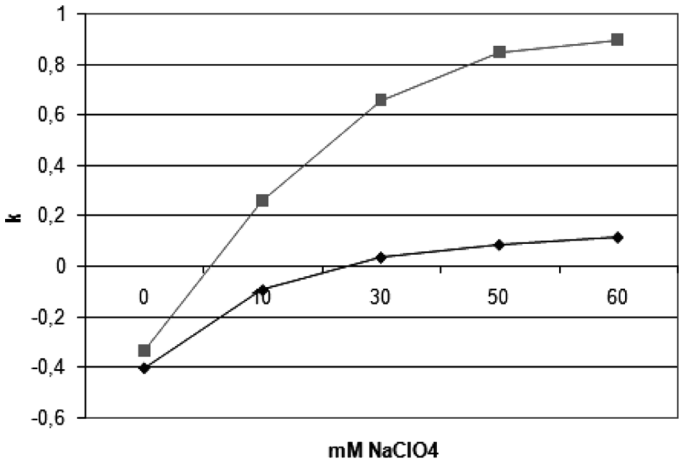
Experiments were performed using a LaChrom HPLC Merck Hitachi (E.Merck, Darmstadt, Germany) model equipped with diode array detector, column oven L-7350, and solvent degasser L-7612. The column (150 mm × 4.6 mm I.D.) was packed with 5 μm Astec Chirobiotic T

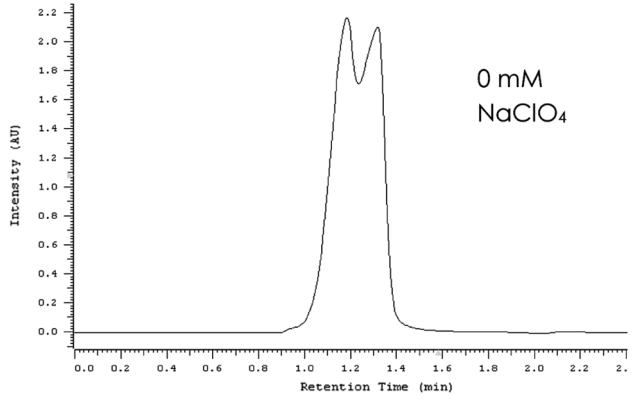
(Sigma-Aldrich), its void volume was determined to be 2.05 mL by injection of blank mobile phase volume at a flow rate of 1 mL/min. Retention data were recorded at a flow rate of 1 mL/min. The column temperature was controlled by a thermostat. Solutions were prepared at 0.1 mg/mL concentrations in methanol. The detection of the drugs was set at 220 nm. Typical injection volumes were 3μL. Mobile phases consisted of 10% aqueous methanol solution and appropriate chaotropic salt additives in concentration ranging from 10 to 60 mM in the whole mobile phase.

RESULTS AND DISCUSSION

Effect of Chaotropic Salt Additives

The conducted analysis pertains to four acidic compounds existing in ionized form in the examined conditions. It is obvious that mobile phase influences not only the dissociation of the analytes (deprotonization of acids) but also the ionizable groups of the chiral selector ($-COO^-$, $-NH_3^+$). When unbuffered MeOH/water is used as the eluent system (the pH is around 7–7.5), the selector is supposed to be net negatively charged and acidic compounds will experience electrostatic repulsion. This may be the reason for the fast elution of the acidic compounds. Upon addition of salt, the ionic strength increases dramatically, which reduces the electrostatic repulsion. This phenomenon is a consequence of electrostatic interactions for which chaotropic ions are the most favorable. So, chaotropic ions: ClO_4^- and PF_6^- can interact with primary amine groups of teicolpanin, thus, facilitating the approach of the acidic analytes to the stationary phase by nonspecific hydrophobic interactions. As a result, both retention factors and separation factors increase with the increasing concentration of these chaotropic salts in the mobile phase (Figures 1–4). Comparison of data obtained for systems modified with $NaClO_4$ and $NaPF_6$ indicates that addition of a greater quantity of more chaotropic salt ($NaPF_6 > NaClO_4$ according to Hofmeister series) to a mobile phase is responsible for higher retention increase of acids (Figure 5). Furthermore, dependency obtained for $NaPF_6$ shows different trends in comparison to the discussed above $NaClO_4$ relationships. Similarly to plots obtained for $NaClO_4$, the retention factor increases with increasing $NaPF_6$ concentration, tending to achieve a limit at higher concentration. It is worthwhile to notice that this limitation could be achieved at lower salt concentration (15 mM), whereas for perchlorates we need fourfold more of added salt to achieve the limiting retention factor. Further increase of salt concentration does not affect retention in the case of addition of $NaClO_4$ as much as for $NaPF_6$, for which the lowering





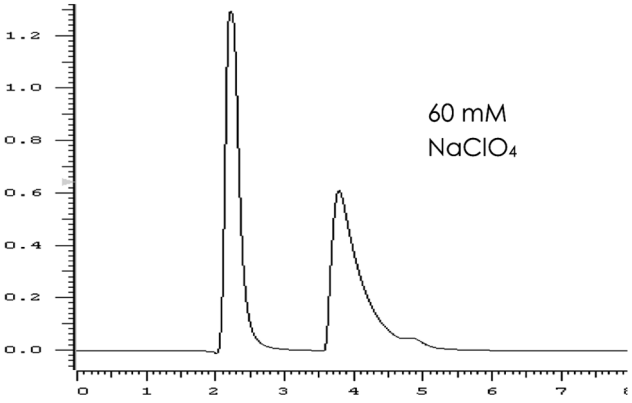


Figure 1. Influence of perchlorate additives on mandelic acid enantiomers resolution.

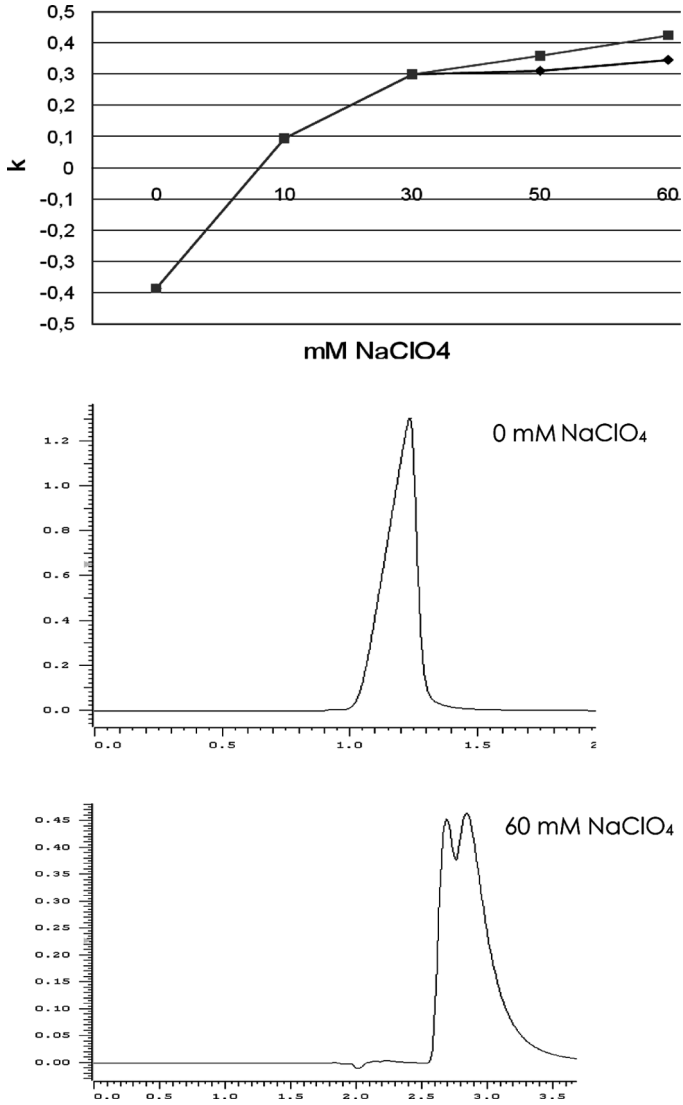


Figure 2. Influence of perchlorate additives on tropic acid enantiomers resolution.

of retention is observed. This lower affinity of anionic analytes to the stationary phase at higher $NaPF_6$ concentration could be explained by excess adsorption of chaotropic anions creating a negatively charged surface, which repulses analytes possessing the same charge.

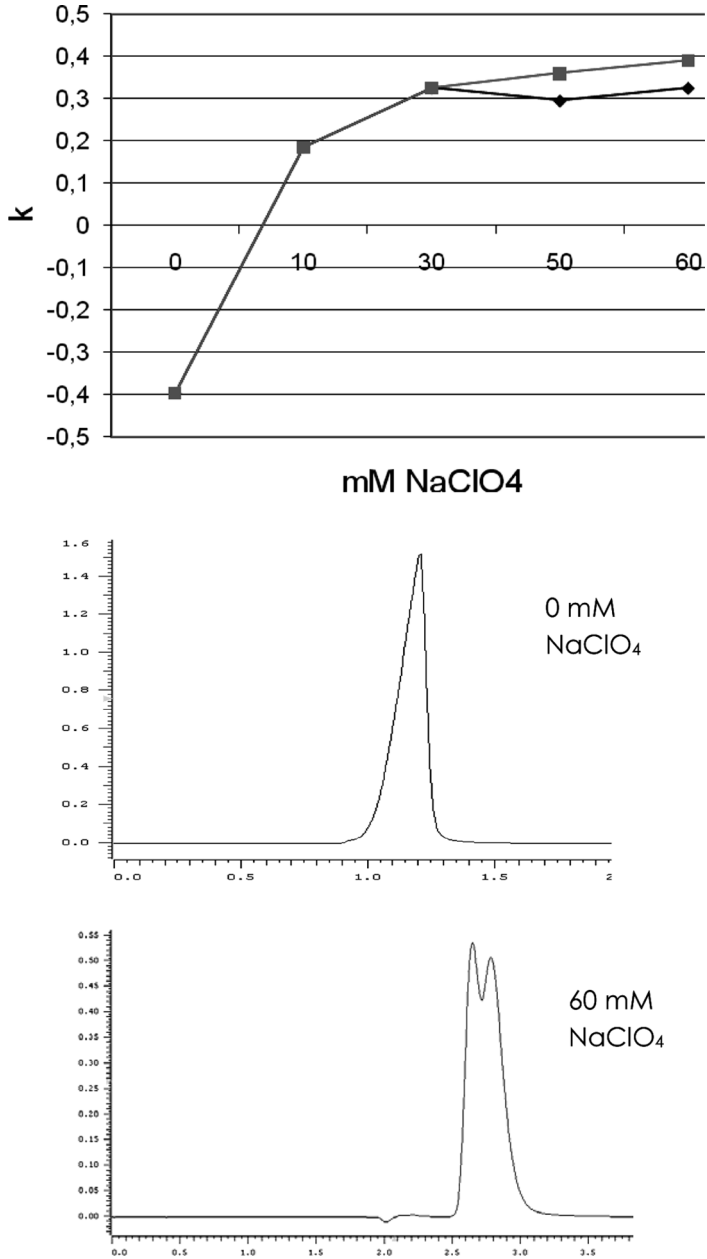


Figure 3. Influence of perchlorate additives on 2-(p-methoxyphenoxy)-propionic acid enantiomers resolution.

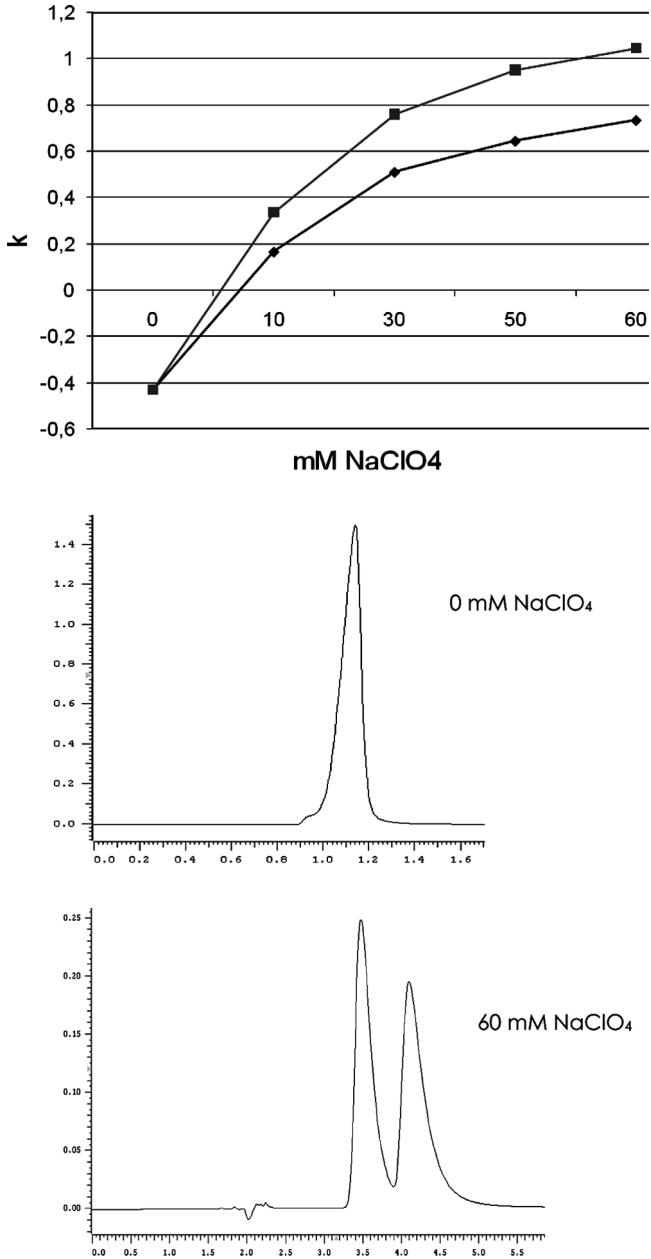


Figure 4. Influence of perchlorate additives on phenyllactic acid enantiomers resolution.

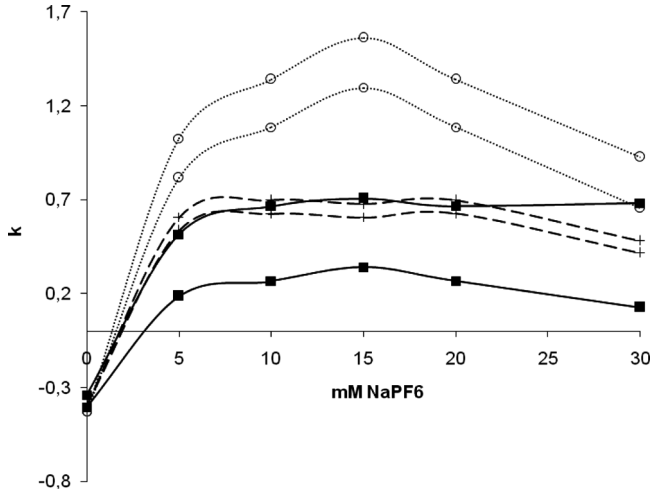


Figure 5. Influence of hexafluorophosphate additives on retention factor of acidic enantiomers: ■ – mandelic acid, + – tropic acid, O – 2-(p-methoxyphenoxy)-propionic acid.

Summarizing, the observed phenomenon of chaotropic salts influence on retention and enantioseparation of acids is a consequence of the overall chaotropic effect, affecting specific and nonspecific interactions occurring in mobile and stationary phases. It is well known that chaotropic ions could be very strong ion-ion interaction reagents and after ion-pairing, could reduce the hydration enhancing the strength of hydrophobic interactions. On the basis of conducted experiments we can expect that the addition of a chaotropic salt to the mobile phase allows an increase of the retention time and improves enantioselectivity for other acids on teicoplanin-based chiral stationary phase in reversed-phase systems.

Thermodynamic Studies

The thermodynamic characteristics of the chromatographic process is described by the following equation:

$$\ln k = -\frac{\Delta H^0}{R}\frac{1}{T} + \frac{\Delta S^0}{R} + \ln \Phi$$

where ΔS^0 and ΔH^0 are standard entropy changes and standard enthalpy changes, respectively, R is gas constant (1.9872 cal $mol^{-1} K^{-1}$), T is

temperature in Kelvin degrees. For a linear plot of ln k versus 1/T, the slope and intercept are respectively: $-\Delta H^0/R$ and $\Delta S^0/R + \ln\Phi$, where Φ is the phase ratio. Determination of Φ is very complex in RP chromatography. Among others, Melander and Horvath,[30] Davydov[31] and Dorsey,[32] proposed different ways of its calculation. The approximate value of Φ for this Chirobiotic column, based on the carbon content equals 0.086. Taking into account that this is the constant value for the individual column, the alternative way to compare entropy variations is calculation of ΔS^{0*} equals $\Delta S^0/R + \ln\Phi$, which can be established directly from the van't Hoff relationship. In this way, all listed entropy variations (ΔS^{0*}) will be biased by $R\ln\Phi$. This bias will be cancelled for $\Delta\Delta S$ entropy changes.[33]

The effect of temperature on the chromatographic retention parameter (k) of investigated acidic enantiomers was determined for two eluent systems containing 5 and 10 mM $NaPF_6$ in 10% MeOH in water, at the temperatures ranging from 5 to 20°C. $NaPF_6$ has a stronger chaotropic effect according to Hofmeister series, causes bigger retention increase, and enables analyzing acidic compounds at a wider range of temperatures. Obtained, results were used to construct van't Hoff plots expressing ln k vs 1/T relationship (Figure 6). The correlations for all the investigated enantiomers were strictly linear with correlation coefficient (R^2) values of 0.98–0.99 indicating no changing retention mechanism in both eluent systems studied. The standard changes in enthalpy (ΔH^0), entropy (ΔS^{0*}), and free energy (at 20°C) (ΔG^0) of the binding interactions between enantiomers and teicoplanin bonded stationary phase, were calculated from the slopes and intercepts of the van't Hoff plots (Table 1). The calculated thermodynamic parameters were all negative. The increase of chaotropic salt concentration, which is responsible for increasing of chiral recognition, caused significant differences, especially in the enthalpy values (ΔH^0), while changes in entropy values were meaningful. The $\Delta\Delta S^0$ and $\Delta\Delta H^0$ values were calculated and indicate that the enantioselectivity of compared eluent systems was predominantly enthalpy driven. Changing of the added salt concentration from 5 mM to 10 mM does not change radically enantioseparation. It is visible in figures and it could be expressed by $\Delta\Delta G^0$ values, which are the following: −2.43, −0.17, −0.55 kJ mol^{-1}, respectively, for mandelic acid, tropic acid, and 2-(p-methoxyphenoxy)-propionic acid in first eluent system and −2.00, −0.17, −0.51 kJ mol^{-1} in the second one. The latter values ($\Delta\Delta G^0$) were in excellent agreement with the $\Delta\Delta G^0$ values derived from the chromatographic separation factor α ($\Delta\Delta G^0 = -RT\ln\alpha$) at 20°C either for system containing 5 mM or 10 mM $NaPF_6$.

Enthalpy-entropy compensation (EEC) studies demonstrating a linear correlation between ΔH^0 and ΔS^{0*}, performed for acidic enantiomers

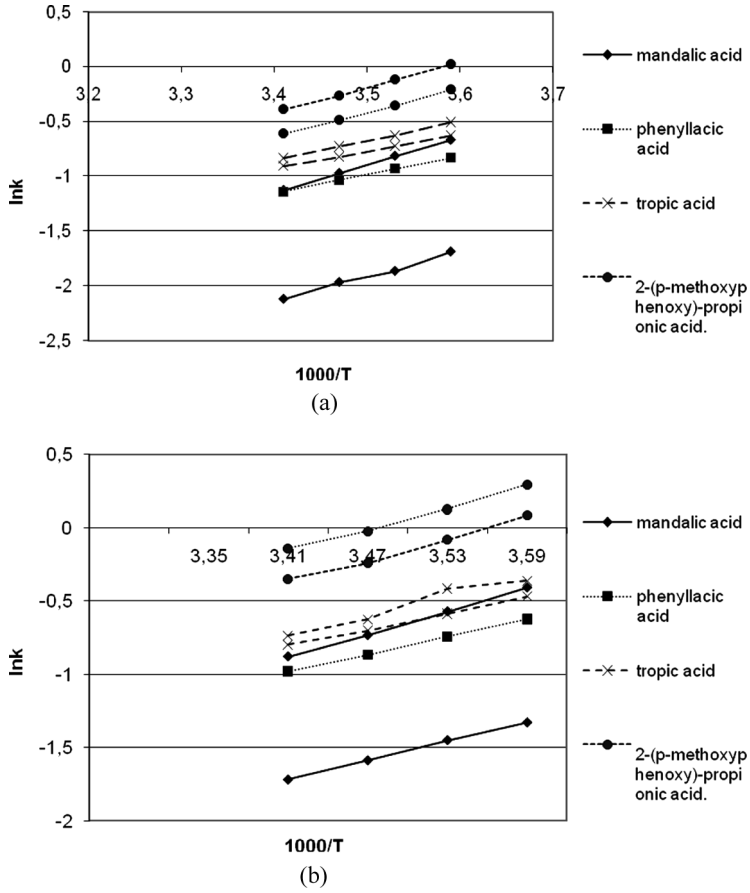


Figure 6. The relationships between chromatographic retention expressed as ln k of investigated acidic enantiomers and temperature expressed as 1/T [1000/K]. (a) eluent system containing 5 mM $NaPF_6$ in 10% MeOH in water; (b) eluent system containing 10 mM $NaPF_6$ in 10% MeOH in water.

were characterized by acceptable linearity (Figure 7). From the slope of these linear plots, the compensation temperature, T_c could be calculated. At this temperature, the enthalpy change is compensated by the entropy change. Some authors suggest that the identical compensation temperatures indicate that the processes occur via the same mechanism.[33,36] However, this common interpretation was definitely rejected by investigations carried out by Carr et al.[37] They showed that only the relative contributions of enthalpy and entropy to the overall free energy are the same. The compensation temperature obtained for acids equals $-30°C$. At this temperature, Gibbs energies of transfer from

Table 1. Thermodynamic parameters obtained with the teicoplanin chiral stationary phase and the two elution modes for acidic enantiomers

Investigated compounds	5 mM $NaPF_6$ in 10% MeOH/water			10 mM $NaPF_6$ in 10% MeOH/water		
	ΔS^{0*} J $mol^{-1}K^{-1}$	ΔH^0 kJ mol^{-1}	ΔG^0 kJ mol^{-1}	ΔS^{0*} J mol^{-1} K^{-1}	ΔH^0 kJ mol^{-1}	ΔG^0 kJ mol^{-1}
Mandelic acid	−83.81 (±2.34)	−19.38 (±0.67)	−5.15 (±0.17)	−75.57 (±0.54)	−17.96 (±0.13)	−4.14 (±0.17)
	−82.10 (±0.41)	−21.31 (±0.13)	−2.72 (±0.08)	−81.94 (±0.71)	−21.86 (±0.21)	−2.14 (±0.08)
Phenyllactic acid	−58.07 (±0.08)	−14.23 (±0.08)	−2.76 (±0.04)	−64.39 (±0.29)	−16.45 (±0.08)	−2.39 (±0.08)
	−58.07 (±0.08)	−14.23 (±0.08)	−2.76 (±0.08)	−64.39 (±0.29)	−16.45 (±0.08)	−2.39 (±0.08)
Tropic acid	−51.62 (±0.62)	−12.90 (±0.17)	−2.18 (±0.04)	−58.70 (±0.92)	−15.24 (±0.25)	−1.93 (±0.21)
	−58.36 (±0.79)	−15.07 (±0.21)	−2.01 (±0.04)	−68.50 (±4.98)	−18.30 (±1.42)	−1.76 (±0.46)
2-(p-Methoxy	−68.62 (±0.92)	−18.63 (±0.25)	−1.47 (±0.04)	−71.68 (±2.34)	−20.10 (±0.67)	−0.84 (±0.13)
phenoxy)-propionic	−68.28 (±0.75)	−19.05 (±0.21)	−0.92 (±0.08)	−69.71 (±2.05)	−20.05 (±0.59)	−0.33 (±0.04)
acid						

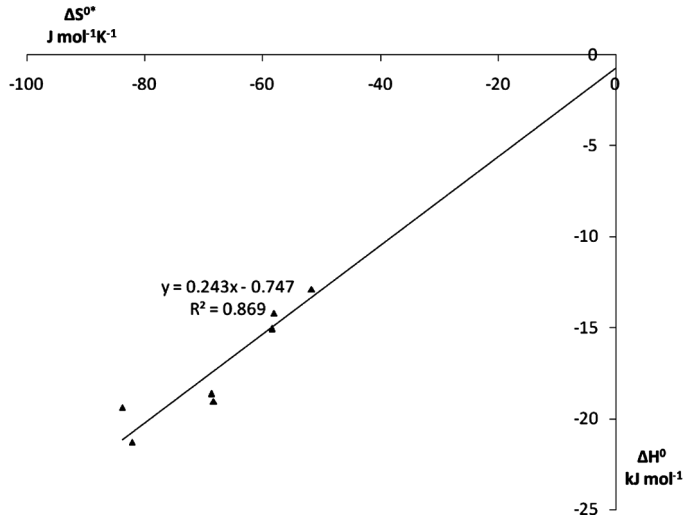


Figure 7. Enthalpy-entropy compensation study on the teicoplanin stationary phase.

mobile to the stationary phase will be the same for each solute from the investigated group.

CONCLUSION

Acidic enantiomers were separated on teicoplanin based stationary phases in reversed phase mode. It was found that the capacity and separation factors were influenced by the chaotropicity and concentration of salt added to the mobile phase. By varying the amount of salt additives, one can affect retention parameters and separation factors. It was found that the increase of chaotropic salt additives causes increase of acidic compounds retention factors, as well as their enantioresolution. On the basis of van't Hoff relationships, it appeared that the separation on teicoplanin-based CSP in reversed-phase mode controlled by a chaotropic effect is enthalpy driven.

REFERENCES

1. Peter, A.; Török, G.; Armstrong, D.W. High-performance liquid chromatographic separationof enantiomers of unusual amino acids on a teicoplanin stationary phase. J. Chromatogr. A. **1998**, *793*, 283–296.
2. Xiao, T.L.; Tesarova, E.; Anderson, J.L.; Egger, M.; Armstrong, D.W. Evaluation and comparison of a methylated teicoplanin aglycone to teicoplanin

aglycone and natural teicoplanin chiral stationary phases. J. Sep. Sci. **2006**, *29*, 429–445.
3. Courderot, C.M.; Perrin, F.; Guillaume, Y.C.; Truong, T.T.; Millet, J.; Thomassin, M.; Chaumont, J.P.; Nicod, L. Anal. Chim. Acta **2002**, *457*, 149–155.
4. Ghassempour, A.; Aboul-Enein, H.Y. Vancomycin degradation products as potential chiral selectors in eantiomeric separation of racemic compounds. J. Chromatogr. A. **2008**, *1191*, 182–187.
5. Clifford, R.M.; Armstrong, D.W.; Berthod, A. Could linear solvation energy relationships give insights into chiral recognition mechanisms? 2. Characterization of macrocyclic glycopeptide stationary phases. J. Chromatogr. A. **2007**, *1166*, 70–78.
6. Lokajova, J.; Tesarova, E.; Armstrong, D.W. Comparative study of three teicoplanin-based chiral stationary phases using the linear free energy relationship model. J. Chromatogr. A. **2005**, *1088*, 57–66.
7. Peter, A.; Török, R.T.; Armstrong, D.W. Direct high-performance liquid chromatographic separation of unusual secondary amino acids and a comparison of the performances of Chirobiotic T and TAG columns. J. Chromatogr. A. **2004**, *1057*, 229–235.
8. Loukili, B.; Dufresne, Ch.; Jourdan, E.; Grosset, C.; Ravel, A.; Villet, A.; Peyrin, E. Study of tryptophan enantiomer binding to a teicoplanin-based stationary phase using the perturbation technique Investigation of the role of sodium perchlorate in solute retention and enantioselectivity. J. Chromatogr. A. **2003**, *986*, 45–53.
9. Peyrin, E.; Ravelet, C.; Nicolle, E.; Villet, A.; Grosset, C.; Ravel, A.; Alary, J. Dansyl amino acid enantiomer separation on a teicoplanin chiral stationary phase: effect of eluent pH. J. Chromatogr. A. **2001**, *923*, 37–43.
10. Jiang, H.; Li, Y.; Pelzer, M.; Cannon, M.J.; Randlett, Ch.; Junega, H.; Jiang, X.; Ji, Q.C. Determination of molindone enantiomers in human plasma by high-performance liquid chromatography–tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases J. Chromatogr. A. **2008**, *1192*, 230–238.
11. Armstrong, D.W.; Rundlett, K.; Chen, J.R. Evaluation of the macrocyclic antibiotic vancomycin as a chiral selector for capillary electrophoresis. Chirality. **1994**, *6*, 496–509.
12. Armstrong, D.W.; Gasper, M.; Rundlett, K. Highly enantioselective capillary electrophoretic separations with dilute solutions of the macrocyclic antibiotic ristocetin A. J. Chromatogr. A. **1995**, *68*, 285–304.
13. Armstrong, D.W.; Liu, Y.; Ekborgott, K.H. A covalently bonded teicoplanin chiral stationary phase for HPLC enantioseparations. Chirality. **1995**, *7*, 474–497.
14. Armstrong, D.W.; Tang, Y.; Cen, S.; Zhou, Y.; Bagwill, C.; Chen, J.R. Macrocyclic antibiotics as a new class of chiral selectors for liquid chromatography. Anal. Chem. **1994**, *66*, 1473–1484.
15. Armstrong, D.W.; Rundlett, K.K.; Reid, G.R. Use of a Macrocyclic Antibiotic, Rifamycin B, and Indirect Detection for the Resolution of Racemic Amino Alcohols by CE. Anal. Chem. **1994**, *66*, 1690–1695.

16. Berthod, A., Liu, Y.; Bagwill, C.; Armstrong, D.W. Facile liquid chromatographic enantioresolution of native amino acids and peptides using a teicoplanin chiral stationary phase .J. Chromatogr. A. **1996**, *731*, 123–137.
17. Lämmerhofer, M. Chiral separations by capillary electromigration techniques in nonaqueous media II. Enantioselective nonaqueous capillary electrochromatography. J. Chromatogr. A. **2005**, *1068*, 31–57.
18. Ward, T.J.; Dann, III, C.; Blaylock, A. Enantiomeric resolution using the macrocyclic antibiotics rifamycin B and rifamycin SV as chiral selectors for capillary electrophoresis. J. Chromatogr. A. **1995**, *715*, 337–344.
19. Medvedovici, A.; Sandra, P.; Toribio, L.; David, F. Chiral packed column subcritical fluid chromatography on polysaccharide and macrocyclic antibiotic chiral stationary phases. J. Chromatogr. A. **1997**, *785*, 159–171.
20. Zarzycki, P.K.; Nowakowska, J.; Chmielewska, A.; Wierzbowska, M.; Lamparczyk, H. Thermodynamic study of the retention behaviour of selected macrocycles using reversed-phase high-performance thin-layer chromatography plates and methanol-water mobile phases. J. Chromatogr. A. **1997**, *787*, 227–233.
21. Ward, T.J.; Farris, III, A.B. Chiral separations using the macrocyclic antibiotics: A review. J. Chromatogr. A. **2001**, *906*, 73–89.
22. Dalgliesh, C.E. The optical resolution of aromatic amino-acids on paper chromatograms. J. Chem. Soc. **1952**, *17*, 3940–3942.
23. Lee, J.T.; Bell, D.S.; Beesley, T.E. HPLC 2008, 32nd International Symposium on High Performance Liquid Phase Separations and Related Techniques, May 10–16, 2008, Baltimore, MD, USA.
24. LoBrutto, R.; Jones, A.; Kazakevich, Y.V.; McNair, H.M. Effect of the eluent pH and acidic modifiers in high-performance liquid chromatography retention of basic analytes. J. Chromatogr. A. **2001**, *913*, 173–187.
25. Pan, L.; LoBrutto, R.; Kazakevich, Y.V.; Thompson, R. Influence of inorganic mobile phase additives on the retention, efficiency and peak symmetry of protonated basic compounds in reversed-phase liquid chromatography. J. Chromatogr. A. **2004**, *1049*, 63–73.
26. LoBrutto, R.; Jones, A.; Kazakevich, Y.V. Effect of counter-anion concentration on retention in high-performance liquid chromatography of protonated basic analytes. J. Chromatogr. A. **2001**, *913*, 189–196.
27. Kazakevich, Y.V.; LoBrutto, R.; Chan, F.; Patel, T. Interpretation of the excess adsorption isotherms of organic eluent components on the surface of reversed-phase adsorbents Effect on the analyte retention. J. Chromatogr. A. **2001**, *913*, 75–87.
28. Flieger, J. The effect of chaotropic mobile phase additives on the separation of selected alkaloids in reversed-phase high-performance liquid chromatography. J. Chromatogr. A. **2006**, *1113*, 37–44.
29. Flieger. J. Effect of mobile phase composition on the retention of selected alkaloids in reversed-phase liquid chromatography with chaotropic salts. J. Chromatogr. A. **2007**, *1175*, 207–216.
30. Melander, W.; Horvath, Cs. in *High-Performance Liquid Chromatography-Advances and Perspectives*, Vol. 2; Horvath, C.S., Ed.; Academic Press: New York, 1980.

31. Davydov, V.Y.; Gonzales, M.E.; Kiselev, A.V.; Lenda, K. Physico-chemical applications of liquid chromatography II. Investigations of the surface properties of chemically modified silica gels and of the adsorption of cardiac glycosides from solutions. Chromatographia. **1981**, *14*, 13–18.
32. Cole, L.A.; Dorsey, J.G. Temperature dependence of retention in reversed-phase liquid chromatography. 1. Stationary-phase considerations. Anal. Chem. **1992**, *64*, 1317–1323.
33. Berthod, A.; He, B.L.; Beesley, T.E. Temperature and enantioseparation by macrocyclic glycopeptide chiral stationary phases. J. Chromatogr. A. **2004**, *1060*, 205–214.
34. Dai, J.;. Carr, P.W. Role of ion pairing in anionic additive effects on the separation of cationic drugs in reversed-phase liquid chromatography. J. Chromatogr. A. **2005**, *107*, 169–184.
35. Dai, J.; Mendonsa, S.D.; Bowser, M.T.; Lucy, C.A.; Carr, P.W. Effect of anionic additive type on ion pair formation constants of basic pharmaceuticals. J. Chromatogr. A. **2005**, *1069*, 225–234.
36. Miyabe, K.; Guiochon, G. Extrathermodynamic Relationships in Reversed-Phase Liquid Chromatography. Anal. Chem. **2002**, *74*, 5754–5765.
37. Ranatunga, R.; Vitha, M.F.; Carr, P.W. Mechanistic implications of the equality of compensation temperatures in chromatography. J. Chromatogr. A. **2002**, *946*, 47–49.

Received September 20, 2008
Accepted November 4, 2008
Manuscript 6417