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Reversal of elution order for profen acid enantiomers in normal phase LC on Chiralpak AD

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Abstract

Enantiomeric separations of four 2-substituted propionic acid drugs and two related acids have been studied using normal phase liquid chromatography with amylose (tris 3,5-dimethylphenylcarbamate) coated on silica as support (Chiralpak AD). At standard conditions (i.e. flow-rate, 1.0 ml/min; column temperature, 30 °C) the elution order can be reversed when the polar alcohol modifier in isohexane, 2-propanol, is replaced by methanol/ethanol 2:1. This is the case for ibuprofen with 2.5% (v/v) alcohol and for mandelic acid with 10% (v/v) alcohol using synthetic mixtures with unequal proportions of the respective enantiomer. Thermodynamic studies in the range 10–45 °C on retention and selectivity of ibuprofen and mandelic acid gave both linear and curved plots. These results stress the importance of investigating enantiomer elution order during the development of enantioselective methods when both old and new CSPs are evaluated. One should also keep in mind that reversal can take place for rather common analytes in well established enantioselective chromatographic systems.

Keywords: Normal phase liquid chromatography; NSAIDs; Enantioseparation; Naproxen; Ibuprofen; Flurbiprofen; Ketoprofen; Mandelic acid; 2-Phenyl propionic acid; Amylose (tris 3,5-dimethylphenylcarbamate); Chiralpak AD; Temperature studies

1. Introduction

In a packed column SFC system with Chiralpak AD as CSP, the elution order of some profen acid enantiomers was reversed when the polar alcohol modifier used in the bulk carbon dioxide mobile phase was switched between methanol and 2-propanol at a column temperature of 30 °C [1]. This behaviour has been explained as due to a different swelling or solvation of the stationary phase that result in marked changes in enantioselective recognition and consequently a change in elution order of the enantiomers [2].

During our previous study, one hypothesis was that for some analytes 30 °C was below the T_{iso}, i.e. the column temperature at which both enantiomers are equally retained, while for others 30 °C was above the T_{iso}. Thermodynamic studies do not support this assumption but mostly showed non-linear van't Hoff plots [1].

Generic screening of racemic drugs by sophisticated enantioselective chromatographic systems is becoming increasingly popular as such systems can significantly reduce method development time [3–8]. However, most instrumental set-ups use UV-detection and no information on enantiomer elution order is acquired. Profen drugs are often included in test sets of acidic analytes for such screening protocols [4,5,8]. Phinney and Sander have proposed standard reference materials for chiral stationary phases that contain uneven proportions of the enantiomers in mixtures [9]. For the LC analysis of profen drugs, the reader can use the review by Mullangi et al. as an introduction to the literature [10].

In the present communication, a corresponding normal phase LC system has been investigated using the same column and analytes as in the previous study in which carbon dioxide with an alcohol was used as mobile phase [1]. Two other acids were also included as analytes. The bulk mobile phase was isohexane with methanol, ethanol or 2-propanol as polar modifier. Trifluoroacetic acid was included as buffering agent and to suppress ionization and interaction with silanol groups [11]. Since the miscibility of methanol in isohexane is poor, a fraction of ethanol

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was used as co-modifier in order to maintain a homogenuous organic mobile phase.

2. Experimental

2.1. Instrumentation and chromatographic conditions

An Agilent HPLC 1100 system was available dedicated for normal phase LC use (Waldbronn, Germany). Besides standard parts, the set-up had a degasser and a dry column bath. The UV-signal at 220 nm was collected throughout this study after 5 μl sample injections. The software was ChemStation for LC 3D Rev.A.09.01 combined with ChemStore for security. Basic chromatographic conditions were as follows: a flow-rate of 1.0 ml/min of 10% (v/v) of alcohol in isohexane with 0.1% (v/v) of trifluoroacetic acid at 30 °C.

Thermodynamic studies were performed on ibuprofen and mandelic acids in a temperature range of 10–45 °C. The system was equilibrated for at least 1 h. The chromatographic output k and α was based on average of the last four injections. The polar modifier was 2.5% (v/v) for ibuprofen and 12% (v/v) for mandelic acid. Both mobile phases also contained 0.025% (v/v) of trifluoroacetic acid.

2.2. Chemicals and column

The profen analytes investigated were those presented in a previous work [1]. Furthermore, (D)-(−)- and (L)-(+)-mandelic acid (i.e. (*R*)-α- and (*S*)-α-hydroxyphenylacetic acid) were from Fluka (Buchs, Switzerland). (*S*)-(+)-2-phenyl propionic acid (Norse Laboratories, Santa Barbara, CA, U.S.A.) and (*R*)-(−)-2-phenyl propionic acid (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) were also included. Suitable diluted solutions were prepared in isohexane containing 10% (v/v) 2-propanol with an excess of the (*S*)-enantiomer. Typical concentrations were in the range 20–50 μg/ml. Solvents used were p.a. quality from E. Merck (Darmstadt, Germany). Ethanol 99.5% was from Kemetyl AB, Haninge, Sweden. Trifluoroacetic acid "zur Synthese" was from E. Merck. The Chiralpak AD column (250 mm × 4.6 mm i.d.) was from Daicel Chemical Industries (Tokyo, Japan).

3. Results and discussion

3.1. Alcohol modifiers and reversal of elution order

In the development of enantioselective methods based on separation on CSPs the composition of the mobile phase is often set before the effect of temperature variation is studied in any detail and devlopment work is done using racemic mixtures at room temperature (LC) or near room temperature (SFC). This is especially true when new columns are evaluated and a wide selection of analytes are tested. Recently, we observed reversal of elution order of three well-known profen acids out of four on the CSP Chiralpak AD at 30 °C when the organic modifier of the bulk carbon dioxide was switched from methanol to 2-propanol [1].

Table 1
Selectivity and elution order (α = S/R)

Analyte	Selectivity α		
	MeOH/EtOH	EtOH	2-PrOH
Ibuprofen	1.0	1.0	1.0
Ketoprofen	1.0	1.17	1.20
Flurbiprofen	1.76	1.65	1.50
Naproxen	1.08	1.0	1.09
Mandelic acid	1.11[a]	0.97	0.84[b]
2-Phenyl propionic acid	1.07	1.10	1.19
Ibuprofen[c]	1.06	n.m.	0.94

Conditions: 10% (v/v) alcohol in isohexane 0.1% trifluoroacetic acid, Chiralpak AD column at 30 °C, MeOH/EtOH: two parts methanol and one part ethanol by volume. n.m. = not measured.
[a] D before L.
[b] L before D.
[c] 2.5% alcohol and 0.025% trifluoroacetic acid in isohexane by volume.

Results obtained at normal phase LC conditions and 30 °C are summarized in Table 1. Most of the analytes showed consistent elution order when the polar alcohol modifier was changed. Interestingly enough, the reversal was observed for ibuprofen when the concentration of alcohol modifier was lowered from 10 to 2.5% (v/v). Two representative chromatograms are shown in Fig. 1. The enantioseparation of ibuprofen in normal phase LC has often been difficult to achieve compared to related acids such as flurbiprofen, ketoprofen, naproxen and suprofen [4,5,12].

Two structurally related acids (mandelic and 2-phenyl propionic) acid were also available as individual enantiomers, and mixtures with skewed concentrations of these were prepared and analyzed (Table 1). Mandelic acid showed reversal in elution order (Fig. 2). These results show that carbon dioxide is not critical for the change of elution order to occur.

3.2. Temperature dependence of the retention and enantioselectivity

These studies were performed on ibuprofen and mandelic acid with methanol/ethanol 2:1 and 2-propanol, respectively,

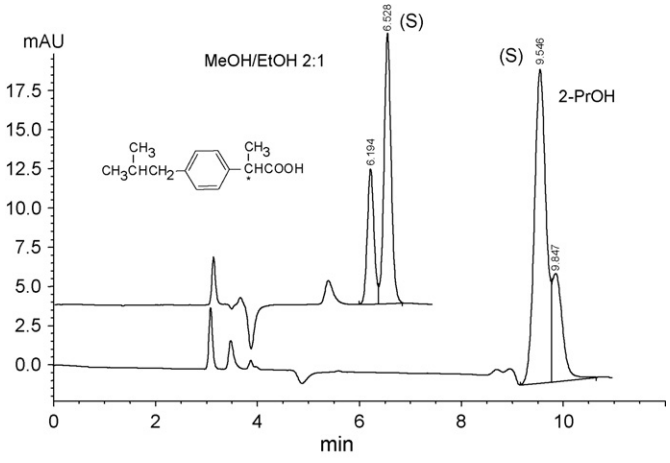

Fig. 1. Influence of alcohol modifier on enantioselectivity and elution order for (S/R: 7:3)-ibuprofen. Conditions: Chiralpak AD 250 mm × 4.6 mm i.d. at 30 °C, 1.0 ml/min of 2.5% (v/v) alcohol and 0.025% trifluoroacetic acid in isohexane. UV-detection at 220 nm.

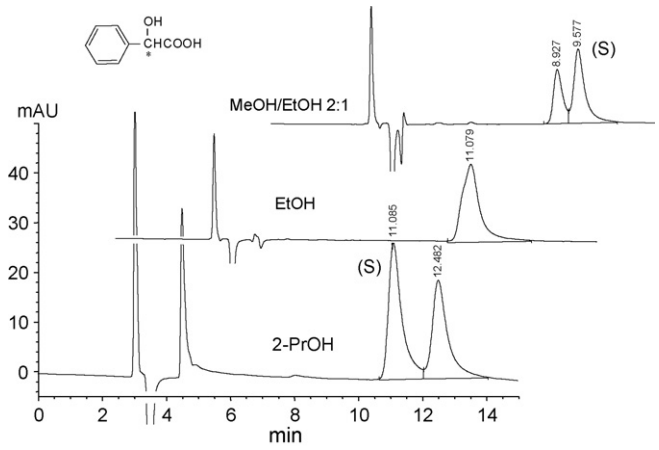

Fig. 2. Influence of alcohol modifier on enantioselectivity and elution order for (S/R: 6:4)-mandelic acid. Conditions: Chiralpak AD 250 mm × 4.6 mm i.d. at 30 °C, 1.0 ml/min of 10% (v/v) alcohol and 0.1% trifluoroacetic acid in isohexane. UV-detection at 220 nm.

as alcohol modifiers. The plots of ln k or ln α versus $1/T$ were generated. In the temperature range studied, 10–45 °C, linear plots were obtained for the retention for ibuprofen with methanol/ethanol (Fig. 3a) and for mandelic acid with 2-propanol (Fig. 4a). On the other hand, the van't Hoff plots for the selectivity α with ibuprofen were always more or less curved, e.g. Fig. 3b (methanol/ethanol). Since the resolution of the ibuprofen enantiomers was rather poor with 2-propanol as modifier (Fig. 1), the measurements were based on analysis of a racemic solution. As a precaution, at each temperature the elution order was checked with the solution containing an excess of the (*S*)-enantiomer.

For mandelic acid with the methanol/ethanol mixture, the corresponding van't Hoff plot was almost flat (not shown), whereas with 2-propanol the curve has a positive slope, as expected, i.e. the selectivity decreased with increasing temperature (Fig. 4b). This was also true for plots on retention; with increasing temperature the retention decreased (Fig. 4a). The very small change in the selectivity between 10 and 45 °C indicates that the site responsible for enantioselective retention is little affected in this temperature range (α from 1.100 to 1.090, average 1.096). On the other hand, the plot for the retention showed a positive slope, though the curves were slightly s-shaped.

From the linear plots, estimated T_{iso} values can be evaluated, but the temperatures needed are outside the chro-

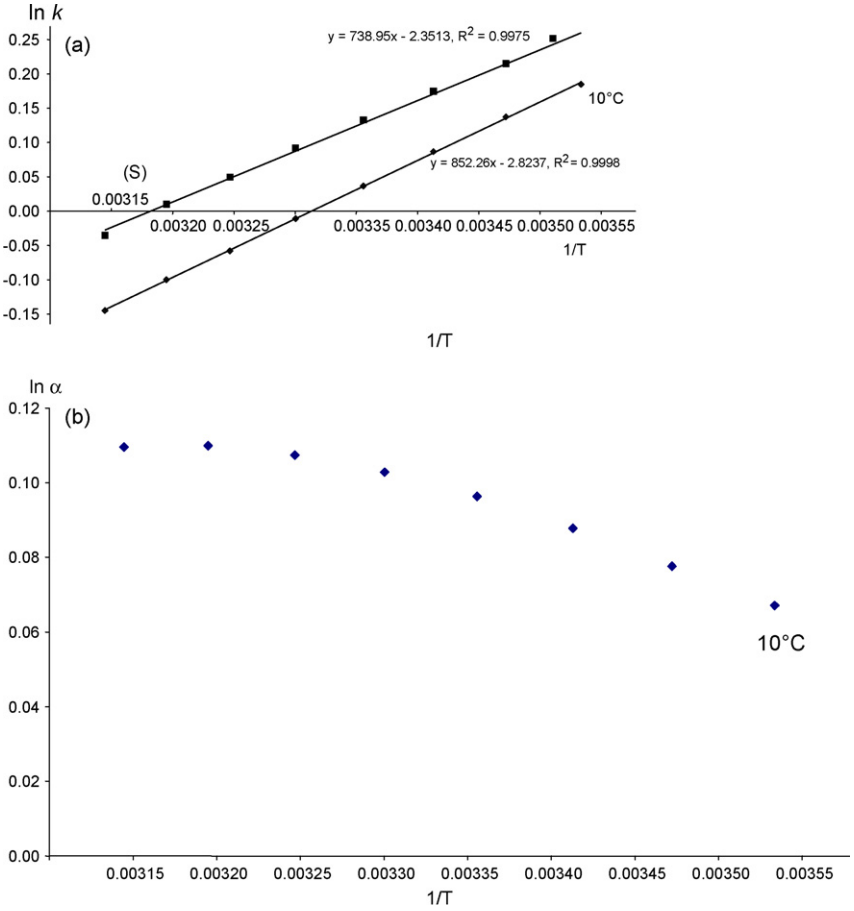

Fig. 3. van't Hoff plots for ibuprofen on Chiralpak AD: (a) retention k and (b) selectivity α. Conditions: flow-rate, 1.0 ml/min of 2.5% (v/v) methanol/ethanol 2:1 (v/v) and 0.025% trifluoroacetic acid in isohexane. Each data point is the average from at least four injections.

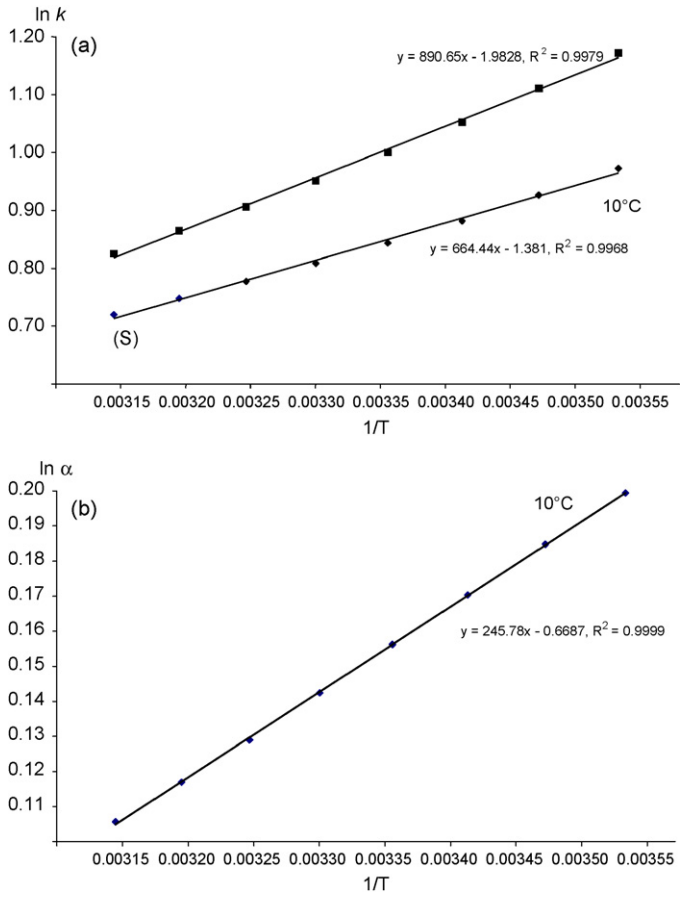

Fig. 4. van't Hoff plots for mandelic acid on Chiralpak AD: (a) retention k and (b) selectivity α. Conditions: flow-rate, 1.0 ml/min of 12% (v/v) 2-propanol and 0.025% trifluoroacetic acid in isohexane. Each data point is the average from at least four injections.

matographic conditions recommended by the manufacturer of this CSP.

4. Conclusions

In this communication, we would like to stress the importance of checking the elution order during optimization of enantioseparations and that wrong conclusions could otherwise be drawn. We have used synthetic enantiomer mixtures with one of the chiral components in excess, a procedure also proposed by others [9]. Other somewhat more elaborate alternatives could be the use of a circular dichroism-detector [13] or a laser polarimeter detector [14]. The apparent reversal of elution order should then be confirmed [15].

Thermodynamic studies on the two analytes, ibuprofen and mandelic acid, showed a reversal in elution order where van't Hoff plots were linear for the retention and for the selectivity of mandelic acid (Figs. 3a and 4a and b) but curved for the selectivity of ibuprofen (Fig. 3b). This means that even if the mechanism of the retention appears to be constant in the temperature range studied, the mechanism of enantioselective retention can change with temperature.

From these observations, and from the curves not presented, one can conclude that the properties of this CSP under certain simple conditions can change in such a way that retention and selectivity behaves in an unpredictable/unexpected way. The curved nature indicate that conformational changes might take place, however, even in the case of linear relationships, the slopes for two enantiomers often differ indicating differences in the Gibbs free energy for interaction with the binding sites. Hence, rather small changes in mobile phase composition or temperature could cancel one or several of the binding sites without any conformational change on the CSP taking place. Such an outcome is not a matter of being above or below the iso-elution temperature T_{iso}.

Even if methanol is not freely soluble in isohexane, this solvent should not be overlooked as polar modifier in normal phase LC. For ibuprofen, near baseline resolution was obtained (R_s 1.1) at 40 °C versus 0.7 at 10 °C. For 2-propanol, it was about 0.6 in the whole temperature range studied. The mandelic acid enantiomers virtually co-eluted using ethanol (Fig. 2) but resulted in a R_s 0.9 and 1.4 with methanol/ethanol and 2-propanol as organic modifier, respectively.

Another conclusion that can be drawn when comparing this LC situation and the situation with SFC conditions [1] is that reversal is more likely to be observed when the selectivity of the system is low.

Since reversal can so easily take place when shifting alcohol modifier, the possibility for this behaviour when other modifiers such as dichloromethane, acetone or 1,4-dioxane are used, should be kept in mind. This is especially important with the availability of more stable columns based on amylose coated with dimethylphenylcarbamate [16]. Reports on elution order reversal are now and then reported in the literature. There is one review on the topic [17] and some more references of interest for Chiralpak AD can be found [1].

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