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# Temperature dependence of chiral discrimination in supercritical fluid chromatography and high-performance liquid chromatography

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**Abstract**

Chiral separations of related compounds within a series of potassium channel activator (KCA) analogues were compared by supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC) at several temperatures between 0° and 52°C. Mobile phases as close as possible in performance were selected for the two modes of analysis, and the same chiral stationary phase (CSP) was used, namely, cellulose tris(3,5-dimethylphenyl-carbamate) (Chiralcel-OD). Two of the compounds, which differed only by replacement of a benzoyl group by a *n*-pentanoyl group, showed quite strikingly different temperature dependencies. These indicate that one compound is above, and the other below, its isoenantioselective temperature ($T_{iso}$), at which separation of enantiomers is not possible. The thermodynamic parameters for these chiral discriminations support the conclusion that, in spite of their very similar structures, quite different chiral recognition factors operate for these two racemic mixtures.

## 1. Introduction

Chiral separations are increasingly important in the development and production control of pharmaceutical compounds, but the way in which temperature should be controlled when developing a new chiral analysis procedure is not well understood, although it is well established that temperature effects are important in chiral selectivity. Recently, several authors [1–4] have reported ways in which temperature variations may alter chiral selectivities. We therefore embarked on a comparison of the role of temperature variation on chiral selectivity in supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC), to determine whether any distinctive differences between the role of temperature in these two modes of chiral analysis might emerge.

The compounds selected for this study are a set of analogues of Cromakalim™ (**1**), a SmithKline Beecham potassium channel activator [5]. For the purposes of this report, only two members of the series are considered, namely compounds **2** and **3**, in which the ex-ring amide function changes from a benzamide in (**2**) to a *n*-pentanoylamide in (**3**): all other structural features remain the same.

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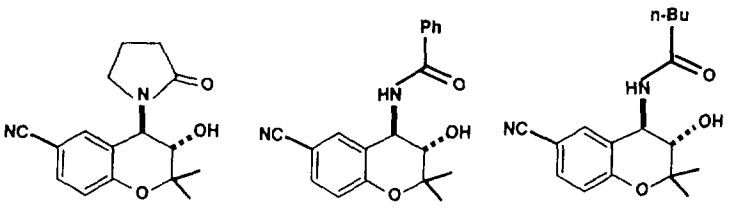


## 2. Equipment

For SFC, a Gilson packed-column system was used, comprising a Model SFC2 Cooler, Model 305 carbon dioxide pump, Model 306 organic modifier pump, a Model 811B Dynamic Mixer, and a Model 805S Manometric Module and a Tescom Series 26-1700 back-pressure regulator to provide outlet pressure restriction. Injection was via a manually operated 20 $\mu$l Model 7125 Rheodyne loop injector, and detection was achieved using an Applied Biosystems Model 757 SFC UV detector. The 25 cm × 4 mm I.D. stainless-steel column contained Diacel Chiralcel-OD stationary phase on a silica support, and was housed in a modified Pye Unicam Model 740592 GC oven. Pure dry liquid carbon dioxide was supplied via a dip-tube from a pressure regulated cylinder.

For HPLC experiments below 32°C, a Waters Model 6000A pump, Rheodyne 7125 injector and Perkin-Elmer Model LC75 UV absorption detector was used. For HPLC experiments above ambient temperature, an integrated Perkin-Elmer Model 4000 HPLC system fitted with a photodiode array UV detector was used. HPLC grade solvents (Rathburn) were used throughout the work, and the 25 cm × 4 mm I.D. stainless-steel column was identical to that used for SFC, containing the same chiral stationary phase (CSP) (Chiralcel-OD).

## 3. Experimental procedure

The HPLC conditions for each analyte were chosen initially, working at ambient temperature and using *n*-hexane containing propan-2-ol (IPA). The amount of IPA was determined such that the mean $k'$ of the two enantiomers was between 3.0 and 3.5; it was then kept constant for a given analyte as the temperature was varied. This procedure ensured that although the % IPA differed for the different analytes, their $k'$ values remained very close.

For SFC, since supercritical carbon dioxide resembles *n*-hexane in solvent strength, an appropriate amount of IPA modifier was then sought, starting at 15% and successively reducing it by 1%, until the observed $k'$ values were closely similar to those obtained in HPLC. Since $t_m$ in SFC is less than in HPLC for a comparable flow-rate, the retention times we obtained by SFC are lower than those for HPLC by a factor of ca. 2 (this, one of the main advantages of SFC, has been well documented [6]). The outlet pressure was set at 200 atm (ca. 20 MPa) and the temperature of the SFC column was then varied between 22 and 52°C, without other changes of operating conditions. Subcritical conditions apply below 31°C, but it is not likely that there is any discontinuity of behaviour below the critical value [4].

## 4. Discussion of results

As can be seen from the chromatograms in Figs. 1 and 2, the HPLC analyses at a series of temperatures in the range 0–42°C show that compounds **2** and **3** behave quite differently. In the case of compound **2**, the selectivity deteriorates somewhat as the temperature is decreased, but this process is accompanied by such a large degree of peak-broadening that baseline resolution is lost below ambient temperature. In con-

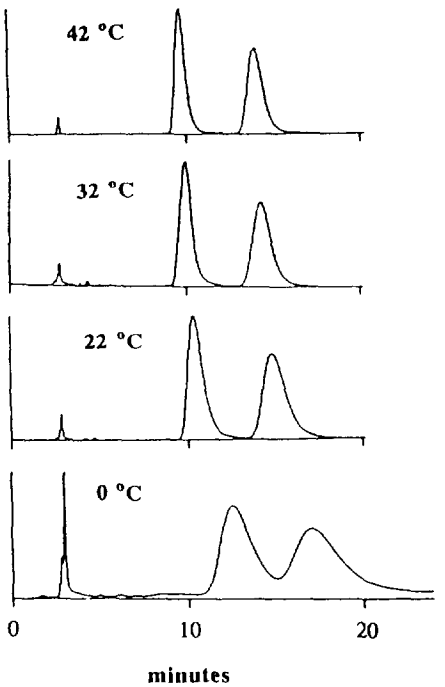


Fig. 1. Chromatograms showing temperature dependence of chiral separation on Chiralcel-OD of compound **2** in HPLC. Mobile phase: *n*-hexane containing 10% IPA.

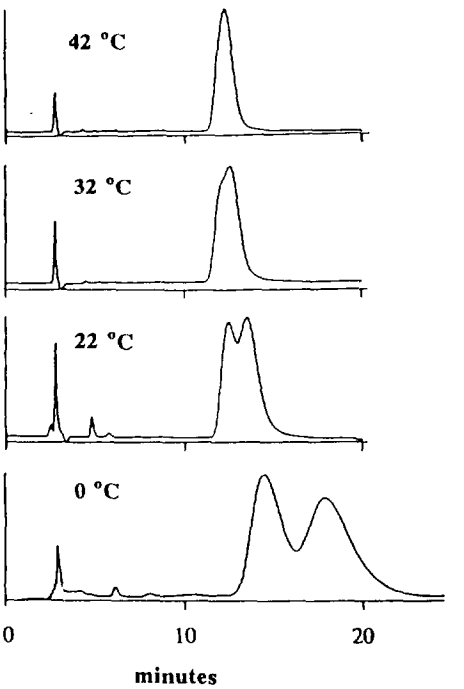


Fig. 2. Chromatograms showing temperature dependence of chiral separation of compound **3** on Chiralcel-OD in HPLC. Mobile phase: *n*-hexane containing 5% IPA.

trast, compound **3** is completely unresolved at the highest temperature used, but approaches a separation factor of 50% at the lowest temperature (0°C). Plots of $\ln(k')$ for both enantiomers of these two compounds (Figs. 3 and 4) explain these observations by showing that compound **2** is well above, whereas compound **3** is at or just below, the temperature at which the two lines intersect.

The temperature at which the enantiomers' $\ln(k')$ vs. $1/T$ lines cross corresponds to the temperature at which the selectivity ($\alpha$) is 1 (i.e. when $k'_R = k'_S$). This temperature is termed the isoenantioselective temperature ($T_{iso}$), a parameter of chiral separations well known in gas chromatography as a result of the work of Schurig and co-workers [7–9], but less well documented in HPLC or SFC. Using the $\ln(k')$ vs. $1/T$ plots, or the corresponding graph of $\ln(\alpha)$ vs. $1/T$, we were able to measure, albeit without great precision, not only the isoenantioselective temperatures of compounds **2** and **3** in HPLC on Chiralcel-OD, but also the partial molar excess thermodynamic parameters $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$, and hence could determine at any temperature the value of $\Delta\Delta G^\circ$, using the relationships shown below.

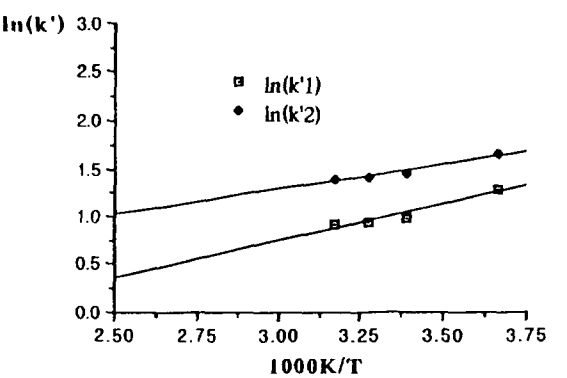


Fig. 3. Plot of $\ln(k')$ vs. $1/T$ for compound **2** in HPLC on Chiralcel-OD.

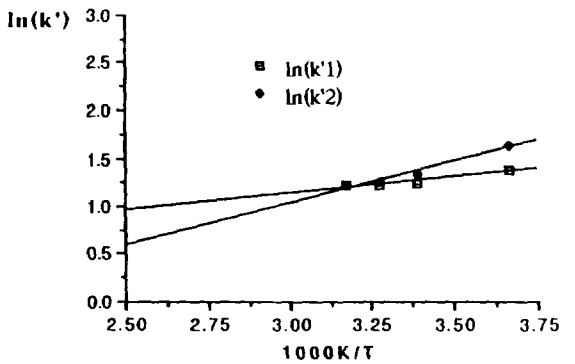


Fig. 4. Plot of $\ln(k')$ vs. $1/T$ for compound **3** in HPLC on Chiralcel-OD.

Since

$$\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ \text{ and } \Delta G^\circ = -RT\ln(K)$$

$$\ln(k')_R = -\Delta H^\circ_R/RT + \Delta S^\circ_R/\mathrm{R} + \ln(\beta) \quad (1)$$

where

$\beta$ = phase ratio $= V_s/V_m$ and $k' = \beta K$

$$\ln(k')_S = -\Delta H^\circ_S/RT + \Delta S^\circ_S/R + \ln(\beta) \quad (2)$$

whence

$$\ln(\alpha) = -\Delta\Delta H^\circ/RT + \Delta\Delta S^\circ/R \quad (3)$$

where

$$\Delta\Delta H^\circ = \Delta H^\circ_R - \Delta H^\circ_S$$

and

$$\Delta\Delta S^\circ = \Delta S^\circ_R - \Delta S^\circ_S$$

The resulting values are shown in Table 1, along with the corresponding values of $\Delta\Delta G^\circ$ at the lowest and highest temperatures used (0 and 42°C). Note that the change of sign of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ between compound **2** and compound **3** can be ignored, since it arises because the value for the more retained isomer is generally given preference over the less retained isomer when calculating selectivity $\alpha$. Since compound **2** is above, and compound **3** below, its isoenantioselective temperature, and there has necessarily been an invertion of elution order as the $T_{iso}$ temperature is passed, this change of sign can be ignored.

The most striking feature of this data is the very large difference (127°C) between their respective $T_{iso}$ values. This temperature, at which $\Delta\Delta H^\circ = T\Delta\Delta S^\circ$, is reached more quickly, on heating from absolute zero, for compound **2** than for compound **3**, because its $\Delta\Delta H^\circ$ is much smaller (1929 J mol$^{-1}$) than for compound **3** (4265 J mol$^{-1}$); their $\Delta\Delta S^\circ$ values (10.3 and 13.6 J mol$^{-1}$ K$^{-1}$) are not so different. Enthalpy changes in this context arise mainly due to heats of adsorption during retention, and as a consequence of partial bonding to the selector, since for two enantiomers solvation enthalpies must be identical. They determine the slopes of the enantiomers' $\ln(k')$ vs. $1/T$ graphs. When two enantiomers reveal large $\Delta\Delta H^\circ$ values (which may be considered as very temperature dependent differences in their $\Delta H^\circ$ values), one possible conclusion is that hydrogen bonding, a very temperature dependent phenomenon, is involved in their chiral discrimination. In the case of compound **3**, which has an ex-ring alkylamide function, this is understandable, since chiral discrimination must reflect to some extent the degree to which the enantiomers form or disrupt hydrogen bonds while retained in the CSP. In the case of compound **2**, chiral recognition may depend critically upon the additional arene com-

Table 1
Thermodynamic parameters for compounds **2** and **3** on Chiralcel-OD in HPLC

| Compound | $\Delta\Delta H^\circ$ (J mol$^{-1}$) | $\Delta\Delta S^\circ$ (J mol$^{-1}$ K$^{-1}$) | $\Delta\Delta G^\circ_0$ (J mol$^{-1}$) | $\Delta\Delta G^\circ_{42}$ (J mol$^{-1}$) | $T_{iso}$ (°C) |
|---|---|---|---|---|---|
| **2** | 1929 | 10.3 | −883 | −1316 | −86 |
| **3** | −4265 | −13.6 | −552 | 19 | 41 |

ponent, and H-bonding may be less important than $\pi-\pi$ interactions which are, evidently, less temperature dependent.

An important objective of this research was the comparison of chiral SFC separations of the same analytes on the same CSP, to see if the factors in their chiral recognition were affected by the change of solvent system and phase. As the chromatograms (Figs. 5 and 6), ln($k'$) vs. $1/T$ graphs (Figs. 7 and 8) and tabulated data (Table 2) clearly show, the results are strikingly similar. Compound **2** is again better resolved at higher temperature, and compound **3** at lower temperature, because compound **2** is above, and compound **3** at or below, its $T_{iso}$ value. Both compounds are, as expected, eluted more rapidly in SFC under comparable conditions with comparable $k'$ values. This arises, as previously stated, because of the lower value of $t_m$ in SFC, and is a major advantage SFC provides for chiral analyses, which often require long retentions in HPLC.

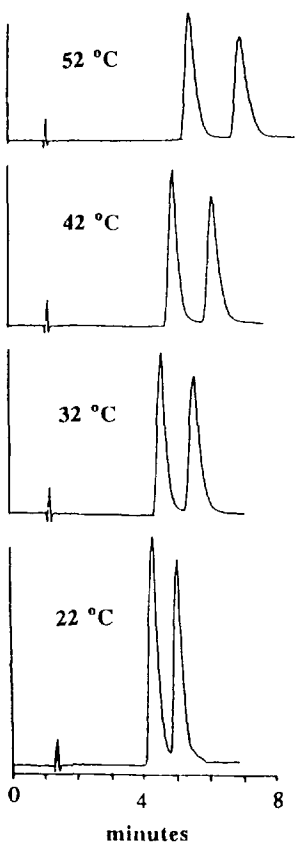


Fig. 5. Chromatograms showing temperature dependence of chiral separation on Chiralcel-OD of compound **2** in SFC. Mobile phase: carbon dioxide containing 12% IPA.

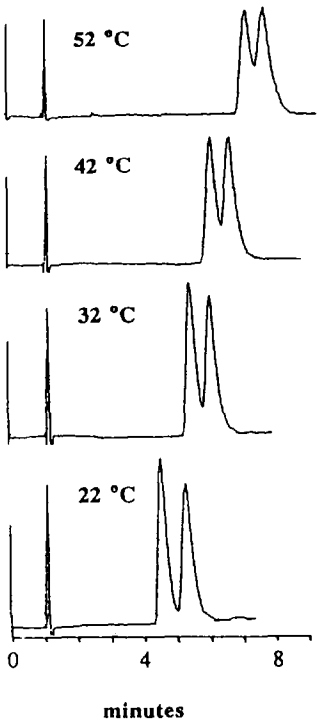


Fig. 6. Chromatograms showing temperature dependence of chiral separation of compound **3** on Chiralcel-OD in SFC. Mobile phase: carbon dioxide containing 7% IPA.

The effect of temperature on retention in SFC differs from HPLC: at constant column outlet

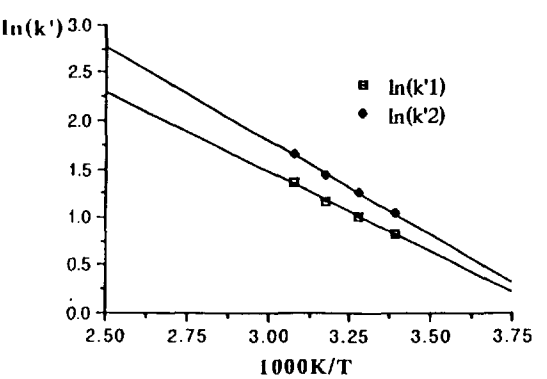


Fig. 7. Plot of ln($k'$) vs. $1/T$ for compound **2** in SFC on Chiralcel-OD.

Table 2
Thermodynamic parameters for compounds **2** and **3** on Chiralcel-OD in SFC

| Compound | $\Delta\Delta H^\circ$ (J mol$^{-1}$) | $\Delta\Delta S^\circ$ (J mol$^{-1}$ K$^{-1}$) | $\Delta\Delta G^\circ_0$ (J mol$^{-1}$) | $\Delta\Delta G^\circ_{42}$ (J mol$^{-1}$) | $T_{iso}$ (°C) |
|---|---|---|---|---|---|
| **2** | 2102 | 9.0 | −544 | −813 | −39 |
| **3** | −2654 | −7.5 | −444 | −220 | 81 |

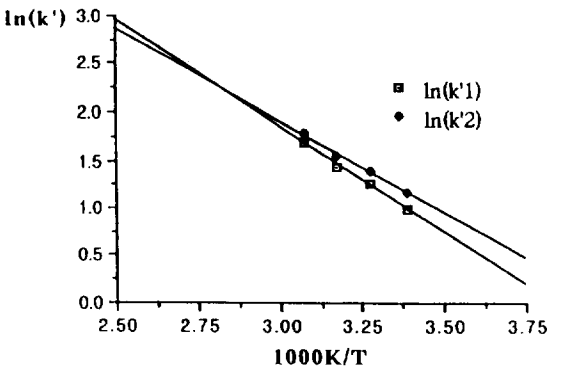


Fig. 8. Plot of $\ln(k')$ vs. $1/T$ for compound **3** in SFC on Chiralcel-OD.

pressure in SFC, increasing temperature makes for a less dense, lower eluent strength, mobile phase. The normal pattern of behaviour in SFC is, therefore, that retention increases initially on heating, but eventually reaches a maximum and then decreases at substantially higher temperatures. In our experiments, at just above ambient temperature, the graphs of $\ln(k')$ vs. $1/T$ have opposite signs of slopes to those observed in HPLC, and do not reach a maximum. The behaviour is similar to HPLC, however, in that there is still a difference of 120°C in their isoenantioselective temperatures, and compound **2** still has the lower value; $\pi-\pi$ interactions are still apparently more important than H-bonding, since the discrimination is less temperature dependent and $\Delta\Delta H^\circ$ is much smaller for compound **2**.

In practical terms, since compound **2** is always above its $T_{iso}$ and compound **3** always below its $T_{iso}$, in our experiments using either HPLC or SFC, the correct use of temperature in the optimization of their chiral resolution is completely different. One should heat the column to improve the enantioselectivity in the case of compound **2** and any racemic mixture which, at the expense of H-bonding factors, involve more $\pi-\pi$ interactions. By contrast, one should cool the column to improve the resolution of the enantiomers of compounds such as **3**, which are, we presume, more dependent upon H-bonding.

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