General retention parameters of chiral analytes in cyclodextrin gas chromatographic columns

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Abstract

Two thermodynamic parameters – entropy (ΔS) and enthalpy (ΔH) – ideally describe the thermodynamics of how the retention of an analyte in a stationary phase depends on the temperature. The paper examines the conversion of an analyte's entropy and enthalpy into chromatographically more meaningful equivalents: its characteristic temperature and thermal constant. Thermodynamic and characteristic parameters of 29 enantiomer pairs of chiral analytes, analysed with four cyclodextrin stationary phases, were measured, tabulated, and investigated. The distribution of all newly-measured characteristic parameters was found to be similar to the known distribution of these parameters for some 12,000 pairs of analytes, analysed with several stationary phases. This similarity suggests that the peak widths of the investigated analytes in temperature-programmed analyses should be generally the same as the peak widths of other similarly retained analytes. It also suggests that the previously-known optimum general heating rate (about $10\,^{\circ}\mathrm{C}/t_{\mathrm{M}}$, i.e. 10ºC per hold-up time) is also the general optimum for temperature-programmed enantioselective GC analyses with cyclodextrins as stationary phases.

The optimum general heating rate corresponds to the shortest analysis time for a predetermined peak capacity. It can substantially differ from specific optima corresponding to the best separation of particular peak pairs. Theoretical prediction of these specific optima requires more complex non-ideal thermodynamic models, and more accurate measurement of the parameters involved – these topics that are outside the scope of this report.

Keywords: characteristic temperature, characteristic thermal constant, optimal heating rate, enantioselective gas chromatography, cyclodextrin stationary phases

1 Introduction

An *ideal thermodynamic model* of the distribution of an analyte between stationary and mobile phases in a column describes the analyte's *distribution constant* (K_c) as [1]

$$\ln K_c = -\frac{\Delta S}{R} + \frac{\Delta H}{RT} \tag{1}$$

where $R \approx 8.3145$ J/K/mol is the *molar gas constant*, T is the column (absolute) *temperature*, and ΔS and ΔH are the *entropy* and *enthalpy* of the distribution.

The ideal thermodynamic model in Eq. (1) is not sufficiently accurate for prediction of peak retention times and separations, and of other chromatographic details. More accurate (and more complex) models are needed to predict these specifics [2-7]. However, Eq. (1) adequately describes the *general thermodynamic properties*, which are sufficient to evaluate some *general performance characteristics* of isothermal and temperature-programmed analyses, including analysis times, peak widths, and peak capacities [8-10]. In addition, the accuracy requirements for measuring parameters to evaluate general performance characteristics of GC analyses are less stringent than those required for more specific predictions.

This report studies the general retention properties of chiral analytes in chiral recognition, with four cyclodextrin stationary phases coated on open-tubular (*capillary*) columns [11]. Thermodynamic parameters ΔS and ΔH provide sufficient information for these studies, although they present substantial shortcomings. They do not offer a direct and intuitive representation of the analyte's elution parameters, nor of the corresponding peaks' parameters. These properties of an analyte are more directly and intuitively represented by its characteristic temperature (T_{char}) and characteristic thermal constant (θ_{char}) [8, 12]. The former is the temperature at which the analyte retention factor (k) is equal to one; the latter is the negative of the inverse of the slope of the function $\ln k(T)$. Parameters T_{char} and θ_{char} have several useful properties. T_{char} is close to the elution temperature of the analyte in a typical temperature-

programmed analysis. This makes T_{char} a good indicator of the elution order of enantiomer pairs. θ_{char} directly affects the peak widths, and is proportional to the optimal heating rate corresponding to the best trade-off between peak capacity and analysis time in a temperature-programmed GC analysis [9, 10, 12, 13].

In our previous study [14], we found that the heating rates ($R_{T,chiral}$) providing acceptable separation of the target enantiomer pairs in the shortest time were several times lower than general optimal heating rate ($R_{T,Opt}$, 10ºC per hold-up time [9, 13]) corresponding to the shortest analysis time for a given peak capacity. Possible explanations could be: i) the characteristic thermal constants (θ_{char}) of the investigated analytes [14] were proportionally lower compared to previously known θ_{char} of thousands of other analytes [8, 10]; ii) the separation of closely spaced enantiomer pairs substantially depends on the difference in their θ_{char} [8] while the general optimal heating rate only depends on the average value of θ_{char} for all analytes in a given analysis, and not on the difference between θ_{char} of specific analytes [9, 13]. These reasons can be sorted out by measurement of thermodynamic parameters of enantiomer pairs. This study is divided into two stages. This report is the first stage aimed to measure the thermodynamic and characteristic parameters of the target analytes and to find if there is a meaningful difference between the newly found values of θ_{char} and their known counterparts for thousands of other analytes. As shown below, such difference does not exist. This opens the way and provides justification for the next more detailed and more time-consuming measurements targeting evaluation of the differences between θ_{char} of enantiomer pairs. This investigation is under way.

This paper reports the thermodynamic and characteristic parameters of 29 chiral enantiomer pairs, analysed in four cyclodextrin stationary phases, together with the optimal heating rates corresponding to the best trade-off between the peak capacity and analysis time in temperature-programmed chiral analyses [9, 13]. Unless otherwise is explicitly stated, all temperatures (T) in this report are in kelvins.

2 Theory

2.1 Characteristic retention parameters of analytes

It follows from Eq. (1) that the temperature-dependence, $k(T)$, of the *retention factor* (k) of an analyte in a wall-coated open-tubular (*capillary*) column, with *internal diameter* d_c and stationary phase *film thickness* d_f, can be described as [8]

$$\ln k = \ln k(T) = \ln(4\varphi) - \frac{\Delta S}{R} + \frac{\Delta H}{RT} \tag{2}$$

where $R \approx 8.3145$ J/K/mol is the molar gas constant, ΔS and ΔH are the entropy and enthalpy of evaporation of the analyte from the stationary phase, and φ is the *relative film thickness*, defined as [8]

$$\varphi = \frac{V_f}{4V_g} = \frac{1}{4\beta} \tag{3}$$

where V_f, V_g, and β are, respectively, the volume of the stationary phase film, the volume of the gas, and the *phase ratio* in the column. The advantages of φ over β have been discussed elsewhere [8]. Typically, d_f is much smaller than d_c, i.e. $d_f << d_c$; in this case, φ can be approximated as $\varphi \approx d_f / d_c$. This report will assume that

$$\varphi = \frac{d_f}{d_c} \tag{4}$$

The function $k(T)$ in Eq. (2) depends on four parameters: R, φ, ΔS and ΔH. Only the last two (ΔS and ΔH) may differ for different analytes in a single stationary phase; of the two others, R is the universal constant, and φ is a column parameter that is the same for all analytes. The thermodynamic parameters ΔS and ΔH of an analyte provide a straightforward description of the thermodynamics of its interaction with the stationary phase, but do not offer a direct representation of chromatographic parameters of the analyte and of the corresponding peak. Consider, for example, an analyte with $\Delta S = 70$ J/mol/K and $\Delta H = 50$ kJ/mol. What would be its elution temperature in a typical temperature-programmed analysis?

What should be the temperature change in isothermal analysis e. g. in order to double the analytes' retention factor? The answers to these questions could be found from ΔH and ΔS parameters, although not directly. Chromatographically meaningful parameters that directly answer these and similar chromatographic questions can be found from reducing the number of parameters in Eq. (2) from the total of four (R, φ, ΔS and ΔH) to the minimum of two mutually independent parameters. Such modification of Eq. (2) can be expressed as [8-10]:

$$\ln k = \ln k(T) = \frac{T_{\text{char}}}{\theta_{\text{char}}}\left(\frac{T_{\text{char}}}{T} - 1\right) \tag{5}$$

where T_{char} and θ_{char} are, respectively, the *characteristic temperature* and *characteristic thermal constant* [8-10, 12] of retention of a given analyte in a given column. The key properties of these parameters will be examined after describing their relationship to parameters ΔS and ΔH.

The pair ΔS and ΔH can be transformed into the pair T_{char} and $\theta_{\text{char,}}$ and vice versa, through the following equations (Eqs. (6) and (7)) which are obtained by solving together Eqs. (2) and (5) at an arbitrary T and at $T = T_{\text{char}}$ [9, 10]:

$$T_{\text{char}} = \frac{\Delta H}{\Delta S - R\ln(4\varphi)}, \quad \theta_{\text{char}} = \frac{R\,\Delta H}{(\Delta S - R\ln(4\varphi))^2} \tag{6}$$

$$\Delta S = R\left(\frac{T_{\text{char}}}{\theta_{\text{char}}} + \ln(4\varphi)\right), \quad \Delta H = \frac{R T_{\text{char}}^2}{\theta_{\text{char}}} \tag{7}$$

Eq. (6) shows that the characteristic parameters (T_{char} and θ_{char}) depend on the relative film thickness (φ). Assume that the parameters T_{char1} and θ_{char1} corresponding to φ_1 are known, it follows directly from Eqs. (6) and (7) that if parameters T_{char1} and θ_{char1} at φ_1 are known then parameters T_{char2} and θ_{char2} at φ_2 can be found as

$$T_{\text{char2}} = \frac{T_{\text{char1}}^2}{T_{\text{char1}} + \theta_{\text{char1}}\ln\left(\varphi_1/\varphi_2\right)} \tag{8}$$

$$\theta_{\text{char2}} = \frac{T_{\text{char1}}^2 \theta_{\text{char1}}}{\left(T_{\text{char1}} + \theta_{\text{char1}} \ln\left(\varphi_1/\varphi_2\right)\right)^2} \tag{9}$$

2.1.1 *Chromatographic properties of characteristic parameters T_{char} and θ_{char}*

From the mathematical standpoint, the T_{char} of an analyte is the *T*-intercept (Figure 1) of the function $\ln k(T)$ for the analyte: at $T = T_{\text{char}}$, Eq. (5) yields $\ln k = 0$. θ_{char} is the negative (multiplied by -1) of the inverse of the slope of $\ln k(T)$ at its *T*-intercept (Figure 1): differentiation of the right hand side of Eq. (5) yields the following expression for the negative of the inverse of the slope, $\mathrm{d}\ln k(T)/\mathrm{d}T$, of $\ln k(T)$ at $T = T_{\text{char}}$:

$$-\left(\frac{\mathrm{d}\ln k}{\mathrm{d}T}\right)^{-1} = -\left(\frac{T_{\text{char}}}{\theta_{\text{char}}}\frac{\mathrm{d}}{\mathrm{d}T}\left(\frac{T_{\text{char}}}{T} - 1\right)\right)^{-1} = \frac{T^2\theta_{\text{char}}}{T_{\text{char}}^2} = \theta_{\text{char}} \tag{10}$$

The reason why the negative and inversion of the slope of $\ln k(T)$ are adopted in defining θ_{char} stems from the following considerations. An increase in column temperature (*T*) reduces the analyte retention factor (*k*); as a result, the function $\ln k(T)$ has a negative slope. Taking its negative conveniently makes θ_{char} into a positive quantity. Furthermore, the slope of $\ln k(T)$ is measured in inverse temperature units (e.g. 1/K or 1/ºC). Inverting the slope, as in θ_{char}, gives a quantity that is measured in the more convenient units of temperature. This approach has the advantage of expressing both the temperature ranges of heating ramps, and the ranges of characteristic temperatures, in units of θ_{char} [8-10, 12]. Thus, since the typical value of θ_{char} is about 30ºC [8, 10, 15] (see also below), it may be said that a heating ramp from 50ºC to 260ºC covers the temperature range of approximately 7 characteristic temperatures. This and similar assessments have important fundamental implications regarding the peak capacity of temperature-programmed GC analyses [10].

Since θ_{char} is the inversion of the slope of $\ln k(T)$, it may be viewed as its *anti-slope* – a measure of the *insensitivity* of the retention factor (*k*) to changes in column temperature (*T*). The larger is θ_{char}, the

larger must the change in T be to produce the same change in k. The average value of characteristic thermal constants (θ_{char}) is approximately 30ºC, suggesting that a temperature increase of 30° should cause about an e-fold reduction in k ($e \approx 2.72$, the base of natural logarithms); approximately a 20ºC ($\theta_{\text{char}} \ln 2$) temperature increase is required to reduce k by a factor of two; a 1ºC increase in column temperature causes about a 3.3% ($1/\theta_{\text{char}} \approx 0.033/\text{K}$) reduction in k. These theoretical observations [8] are supported by experimental results [16-18].

The characteristic temperature (T_{char}) also has direct chromatographic interpretation: as was already mentioned, at $T = T_{\text{char}}$, Eq. (5) yields $\ln k = 0$, i.e. $k = 1$. In a single-ramp temperature-programmed analysis, all analytes elute with approximately the same retention factor [8, 12], and the elution temperature of each solute is close to its T_{char}. In particular, an analyte eluting in a temperature-programmed analysis at its own characteristic temperature elutes with a retention factor of one. Typically, the *elution temperature* (T_{R}) of an analyte in a temperature-programmed analysis may differ from T_{char}, but will closely approximate to it [8, 9, 12]. The T_{char} of an analyte can therefore be viewed as its approximate elution temperature in a typical temperature-programmed analysis. As a result, from a knowledge of the characteristic temperature (T_{char}) of an analyte, its elution temperature can be estimated and, in consequence, other temperature-dependent parameters. These include the diffusivities of the eluting analytes in temperature-programmed analysis, the optimal carrier gas flow rate, and the viscosity at the time of the analyte elution. [8]. Taking the case, considered above, of an analyte having ΔH=50kJ/mol and $\Delta S = 70$ J/mol/K: these data provide little direct chromatographic information. However, they can be transformed into and θ_{char}. For a column with $\varphi = 0.001$ (Figure 1), T_{char} is 431K (≈158°C) and θ_{char} is 31°C. This indicates that, in a typical temperature-programmed GC analysis, the analyte elution temperature is close to 160ºC, and the column temperature should be reduced by about 21.5ºC ($31 \cdot \ln 2 \approx 21.5$) in order to double the analyte retention factor in isothermal analysis,

An important property of θ_{char} is that the optimal heating rate in temperature-programmed GC is a direct function of parameters θ_{char} for all analytes in the sample [9, 13]. Each analyte in the sample has its own value of T_{char} and θ_{char}. However, the general trend is that the analytes with higher T_{char} values tend also to have higher θ_{char} values [8, 10]. This means that the retention factors of later-eluting analytes tend to be less sensitive to temperature change than are the retention factors of earlier-eluting ones. The characteristic parameters also depend on film thickness, as is shown in Eqs. (8) and (9); this dependency can be described by a simpler approximation, which is sufficiently accurate for the purpose of general evaluation [8]. On the basis of a study carried out on a large number of analytes (more than 12,000 analyte-phase pairs [8, 10]) with about 60 different stationary phases, the general trend of the dependence of θ_{char} on T_{char}, in a column with relative film thickness φ, can be described as (see also Figure 2)

$$\theta_{char} = \left(\frac{T_{char}}{T_{st}} \right)^{0.7} \theta_{char,st}, \quad \theta_{char,st} = (10^3 \varphi)^{0.09} 22^{o}\mathrm{C} \tag{11}$$

where $T_{st} = 273.15\,\mathrm{K}$ (0ºC) is the *standard temperature*. Eq. (11) shows that an increase in the relative film thickness (φ) tends to produce an increase in θ_{char} for a given T_{char}, although the dependence of θ_{char} on φ is rather weak. Thus a 10-fold increase in φ causes about a 23% increase in θ_{char} at a given T_{char}. For each T_{char}, the θ_{char} values of a large majority of the earlier investigated analytes [8, 10] lie within ±10ºC of the values found from Eq. (11).

Other properties of the characteristic parameters T_{char} and $\theta_{char,}$ including additional details of their dependence on film thickness, have been described elsewhere [8, 12].

3 Experimental

3.1 Samples

Pure standards of the analytes investigated were from the collection in the authors' laboratory. All standard compounds were solubilised in cyclohexane at a concentration of 100 ppm mg/L each. Solvents were all HPLC grade from Riedel-de Haen (Seelze, Germany). Table 1 reports the list of the analytes investigated in this study.

[*Please insert Table 1 here*]

3.2 Columns

The analyses were carried out on 25m×0.25mm×0.25μm ($L \times d_c \times d_f$) columns from Mega (Legnano – Italy) coated with the following cyclodextrin derivatives:

$6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$-$3^{I\text{-}VII}$-*O*-acetyl-β-CD (DA)

$6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$-$3^{I\text{-}VII}$-*O*-ethyl-β-CD (DE)

$6^{I\text{-}VII}$-*O*-TBDMS-$2^{I\text{-}VII}$-$3^{I\text{-}VII}$-*O*-methyl-β-CD (DM)

$3^{I\text{-}VII}$-*O*-pentyl-$2^{I\text{-}VII}$-$6^{I\text{-}VII}$-*O*-methyl-β-CD (PEN)

Each cyclodextrin derivative was at a concentration of 30% in PS086 as diluting stationary phase.

3.3 Instruments

Analyses were carried out on a Shimadzu GC 2010 system (Shimadzu, Milan, Italy) provided with an FID; data were processed with Shimadzu GC Solution 2.53SU1 software.

206 *3.4 GC conditions*

207 The retention parameters of all analytes in all columns were found from isothermal analyses at the
208 following temperatures: 50, 75, 100, 125, 150, 175, 200, 210, 220, and 230°C. Carrier gas was helium at
209 1 mL/min flow rate. Injector and detector temperatures were 220°C and 230°C, respectively.

210 **4 Results and discussion**

211 The values of the thermodynamic (ΔS and ΔH) and characteristic (T_{char} and θ_{char}) retention
212 parameters for the analytes listed in Table 1 are summarized in Table 2.

213 [*Please insert Table 2 here*]

214

215 The dimensionless film thickness (φ) in the investigated column was 0.001. Figure 3 shows the
216 distribution maps of the (T_{char}, θ_{char})-points for each stationary phase, and Figure 4 shows the combined
217 distribution map. The least-square fit of the line $AT^{0.7}$ for the combined data can be expressed as

218 $$\theta_{\text{char}} = \theta_{\text{char,st}} \left(\frac{T_{\text{char}}}{T_{\text{st}}} \right)^{0.7}, \quad \theta_{\text{char,st}} = 18^{\text{o}}\text{C} \tag{12}$$

219 The difference between this line and that for the other analytes and stationary phases in Eq. (11) is only
220 in the scale. θ_{char} for the analytes investigated in this report are generally about 18% lower compared to
221 the ones in Eq. (11). As a result, the peak widths in temperature-programmed analyses of chiral analytes
222 investigated here should be practically the same as the peak widths of other analytes under the same
223 conditions.

224 An important property of characteristic thermal constants (θ_{char}) of the analytes in a sample analyzed
225 by a column is that the optimal heating rate ($R_{\text{T,Opt}}$) corresponding to the best separation-time trade-off is
226 a direct function of the distribution (Figure 2 or Figure 4) of quantities θ_{char} [9, 13]. The best separation-
227 time trade-off means here to obtain the shortest analysis time at a given peak capacity or, conversely, the

largest peak capacity at a given analysis time. The numerical value of $R_{T,Opt}$ significantly depends on column dimensions, carrier gas type, flow rate, and other conditions. For example, $R_{T,Opt}$ for a 10m×0.25mm column in GC-MS is much higher than for a 100m×0.25mm column. However, there is a metric that uniquely expresses $R_{T,Opt}$ for different columns under various chromatographic conditions. This is the *normalized heating rate* defined as the product $R_T t_M$ which is measured in units of temperature and describes R_T in terms of the temperature change during the time span equal to t_M [8-10, 13, 19]. The *optimal normalized heating rate* ($R_{T,Opt} t_M$) is proportional to the scale factor ($\theta_{char,st}$) in Eqs. (11) and (12) describing the distribution (Figure 2 or Figure 4) of θ_{char} [9, 12, 13]. $R_{T,Opt} t_M$ also slightly depends on the dimensionless film thickness (φ).

At $\varphi = 0.001$ in Eq. (11), $\theta_{char,st}$ is equal to 22ºC. For this condition, $R_{T,Opt} t_M$ in isobaric analyses should be approximately $10^{\circ}C/t_M$ (10ºC per hold-up time) [9, 13]. Eq. (11) and, therefore, the result of $R_{T,Opt} \approx 10^{\circ}C/t_M$ are valid for all previously evaluated distributions of θ_{char} illustrated in Figure 2. On the other hand, in the θ_{char}-distribution for 29 enantiomer pairs in four cyclodextrin phases evaluated here, $\theta_{char,st} = 18^{\circ}C$ (Eq. (12), Figure 4), i.e. 18% lower than $\theta_{char,st}$ in Eq. (12). This suggests that $R_{T,Opt}$ for the columns and analytes investigated in this report should be about 18% lower than $R_{T,Opt}$ for the majority of other analyses. Thus, the general recommendation, to use $10^{\circ}C/t_M$ in isobaric temperature-programmed analyses [9, 13], should be reduced to $8^{\circ}C/t_M$ for the chiral stationary phases and analytes investigated here. In our view explained below, this difference is insignificant, and may be disregarded in practice. [9, 13].

The following factors should also be considered [9, 13].

- The optimum heating rate depends on the pressure conditions in the column. The optimum is typically close to $10^{\circ}C/t_M$ when gas decompression along the column is strong, and the GC instrument is capable of providing the required pressure. These conditions are typical for all GC-

MS analyses (vacuum at the column outlet), and for analyses of complex mixture requiring relatively long and narrow-bore columns. The 8°C/t_M is optimal for these conditions when cyclodextrin stationary phases are used for GC chiral recognition. Relatively short wide-bore columns with atmospheric pressure at the outlet are typically used to analyse relatively simple mixtures; these analyses do not always require temperature programming, but if they do, the optimum heating rate is about 15°C/t_M. In some relatively rare cases, heating rate optimization can only be achieved at the maximum pressure available from the GC instrument; under these conditions, the optimum heating rate is close to 7.5°C/t_M.

- Theoretical and experimental graphs describing the quantitative dependence of the separation/time trade-off on the heating rate suggest that, in the vicinity of its optimal value, the normalized heating rate causes relatively small changes in analysis time at a given peak capacity. Thus, increasing the normalized heating rate to 50% above its optimum value requires less than 10% increase in analysis time, to maintain a peak capacity constant. An increase of the normalized heating rate to 20% above its optimum value causes a peak capacity reduction that is insignificant in practical terms . This suggests that the difference between 10ºC/t_M and 8ºC/t_M is insignificant in practice.
- Real-world samples contain additional components other than those investigated here. The optimum heating rate to analyse such mixtures on cyclodextrin columns is likely to be somewhere between 10ºC/t_M and 8ºC/t_M.

These observations suggest that 10ºC/t_M is appropriate as a single practical recommendation for a default heating rate in all temperature-programmed GC analyses, including analysis of chiral analytes with cyclodextrins.

In a previous study [14], we found that the heating rates ($R_{T,chiral}$) providing acceptable separation of the target enantiomer pairs in the shortest time were several times lower than $R_{T,Opt}$ of 10ºC/t_M. Since

θ_{char} and $R_{T,Opt}$ for the chiral analytes investigated here are about the same as their counterparts for all other previously evaluated analytes and stationary phase pairs [9, 13], the substantial departure of $R_{T,chiral}$ from $10^{o}C/t_M$ can only be explained by the difference in θ_{char} of the enantiomers [8] rather than by the absolute values of their θ_{char}.

The differences in enantiomer pairs' characteristic parameters and their effect on optimal conditions for the separation of specific pairs will be the subject of a forthcoming publication.

5 Conclusions

The transformation of entropy (ΔS) and enthalpy (ΔH) of distribution of analyte between the stationary and the mobile phases in a wall-coated open tubular column, into the more chromatographically-meaningful characteristic temperature (T_{char}) and characteristic thermal constant (θ_{char}) was examined, together with the inverse transformation of T_{char} and θ_{char} into ΔS and ΔH. The physical meaning of the characteristic parameters (T_{char} and θ_{char}) of analyte retention, and their advantages over their thermodynamic counterparts (ΔS and ΔH), were discussed. All these parameters (ΔS, ΔH, T_{char}, θ_{char}) were measured experimentally and tabulated for 29 chiral pairs of analytes, in four different cyclodextrin stationary phases, for a total of 232 analyte/phase pairs. The distribution maps of (T_{char},θ_{char})-points for these 232 analyte-phase pairs were similar to those for some 12,000 previously investigated analyte-phase pairs for about 60 different stationary phases. This similarity implies that the general optimal heating rate, corresponding to the best trade-off between peak capacity and analysis time, in temperature-programmed chiral analyses with cyclodextrins as stationary phases, is approximately the same as that for conventional GC analyses (about $10^{o}C/t_M$, i.e. $10^{o}C$ per hold-up time). The optimal heating rates, corresponding to the best performance in terms of separation of specific peak pairs, may differ substantially from the general optimum of $10^{o}C/t_M$, and deserve a special study.

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6 List of the acronyms

Symbol	Description
d_c	column internal diameter
d_f	stationary phase film thickness
ΔH	enthalpy of evaporation
k	retention factor
K_c	distribution constant, Eq. (1)
R	molar gas constant, $R \approx 8.3145$ J/K/mol
ΔS	entropy of evaporation
T	temperature
T_{char}	characteristic temperature, Eq. (6)
T_{st}	standard temperature, $T_{st} = 273.15$ K
t	time
t_M	hold-up time
θ_{char}	characteristic thermal constant, Eq. (6)
$\theta_{char,st}$	characteristic thermal constant at $T_{char} = T_{st}$ in Eq. (11)
φ	relative film thickness, Eq. (4)

7 References

[1] J. C. Giddings, Unified Separation Science, Wiley, New York, 1991.

[2] R. C. Castells, E. L. Arancibia, A. M. Nardillo, J. Chromatogr. 504 (1990) 45.

[3] S. Vezzani, P. Moretti, G. Castello, J. Chromatogr. 677 (1994) 331.

[4] F. R. González, J. Chromatogr. A 942 (2002) 211.

[5] K. Aryusuk, K. Krisnangkura, J. Sep. Sci. 26 (2003) 1688.

[6] F. Aldaeus, Y. Thewalim, A. Colmsjö, Anal. Bioanal. Chem. 389 (2007) 941.

[7] B. Karolat, J. Harynuk, J. Chromatogr. A 1217 (2010) 4862.

[8] L. M. Blumberg, Temperature-Programmed Gas Chromatography, Wiley-VCH, Weinheim, 2010.

[9] L. M. Blumberg, in C. F. Poole (Ed.), Gas Chromatography. Elsevier, Amsterdam, 2012, p. 19.

[10] L. M. Blumberg, J. Chromatogr. A 1244 (2012) 148.

[11] V. Schurig, J. Chromatogr. A 906 (2001) 275.

[12] L. M. Blumberg, M. S. Klee, Anal. Chem. 72 (2000) 4080.

[13] L. M. Blumberg, M. S. Klee, J. Microcolumn Sep. 12 (2000) 508.

[14] C. Bicchi, L. M. Blumberg, C. Cagliero, C. Cordero, P. Rubiolo, E. Liberto, J. Chromatogr. A 1217 (2010) 1530.

[15] L. M. Blumberg, M. S. Klee, Anal. Chem. 73 (2001) 684.

[16] J. C. Giddings, J. Chem. Educ. 39 (1962) 569.

[17] E. Grushka, Anal. Chem. 42 (1970) 1142.

[18] A. B. Fialkov, A. Gordin, A. Amirav, J. Chromatogr. A 991 (2003) 217.

[19] L. M. Blumberg, M. S. Klee, Anal. Chem. 70 (1998) 3828.

329 **Table headings**

330 Table 1. Standard racemates of 29 analytes of natural origin, having different chemical structures and
331 volatilities (for a total of 58 analytes) investigated in this study. The analytes were diluted in
332 cyclohexane at a concentration of 100ppm.

333

334 Table 2. Thermodynamic (ΔS, ΔH) and characteristic (T_{char}, θ_{char}) retention parameters of the analytes
335 listed in Table 1.

336

337

Figure Captions

Figure 1. Graph of the function $\ln k(T)$, Eq. (2), (solid line) for $\varphi = 0.001$, $\Delta H = 50\,000$ J/mol and $\Delta S = 70$ J/mol/K. The dashed line is the tangent to $\ln k(T)$ at $\ln k = 0$, and thus at $T = T_{\text{char}}$.

Figure 2. Map of (T_{char}, θ_{char})-points for more than 12,000 solute-liquid combinations involving capillary columns with more than 50 liquid stationary phases. There are more than 12,000 dots on the map. Each dot represents one solute (analyte) in one liquid polymer stationary phase at $\varphi = 0.001$ [8, 10]. The dashed line represents Eq. (11) at $\varphi = 0.001$.

Figure 3. Distribution maps of (T_{char}, θ_{char})-points for the analytes in Table 1; based on the data in Table 2.

Figure 4. Combined distribution map of (T_{char}, θ_{char})-points for the analytes in Table 1 on all four investigated columns; based on the data in Table 2. The solid and dashed lines are the graphs of equations Eq. (12) and Eq. (11) at $\varphi = 0.001$.

Tables

Table 1

#	Analyte	#	Analyte	#	Analyte
1	2-methylbutanol (R)	21	α pinene(S)	41	δ-undecalactone (X)
2	2-methylbutanol (S)	22	α pinene (R)	42	δ-undecalactone (Y)
3	2-octanol (X)	23	limonene (S)	43	δ-dodecalactone (X)
4	2-octanol (Y)	24	limonene (R)	44	δ-dodecalactone (Y)
5	menthol (+)	25	pulegone (R)	45	γ-hexalactone (X)
6	menthol (-)	26	pulegone(S)	46	γ-hexalactone (Y)
7	isobornyl acetate (X)	27	camphor (S)	47	γ-eptalactone (X)
8	isobornyl acetate (Y)	28	camphor (R)	48	γ-eptalactone (Y)
9	linalyl acetate (R)	29	rose oxide (4R4S cis)	49	γ-octalactone (X)
10	linalyl acetate (S)	30	rose oxide (2S4R cis)	50	γ-octalactone (Y)
11	cis 2-methyl-3.-hexenyl butyrate (X)	31	rose oxide (2R4R trans)	51	γ-nonalactone (X)
12	cis 2-methyl-3.-hexenyl butyrate (Y)	32	rose oxide (2R4R trans)	52	γ-nonalactone (Y)
13	hydroxycitronellal (X)	33	δ-hexalactone (X)	53	γ-decalactone (X)
14	hydroxycitronellal (Y)	34	δ-hexalactone (Y)	54	γ-decalactone (Y)
15	chrysanthemic acid (X)	35	δ-octalactone (X)	55	γ-undecalactone (X)
16	chrysanthemic acid (Y)	36	δ-octalactone (Y)	56	γ-undecalactone (Y)
17	2-phenylpropionic acid S)	37	δ-nonalactone (X)	57	γ-dodecalactone (X)
18	2-phenylpropionic acid (R)	38	δ-nonalactone (Y)	58	γ-dodecalactone (Y)
19	2-methylbutyric acid (S)	39	δ-decalactone (X)		
20	2-methylbutyric acid (R)	40	δ-decalactone (Y)		

354 Table 2

#	DA column				DE column				DM column				PEN column			
	ΔS, J/mol/K	ΔH, kJ/mol	T_{char}, ºC	θ_{char}, ºC	ΔS, J/mol/K	ΔH, kJ/mol	T_{char}, ºC	θ_{char}, ºC	ΔS, J/mol/K	ΔH, kJ/mol	T_{char}, ºC	θ_{char}, ºC	ΔS, J/mol/K	ΔH, kJ/mol	T_{char}, ºC	θ_{char}, ºC
1	171.2	65.7	94.5	17.10	181.3	69.6	94.9	16.19	144.8	58.5	110.7	20.94	131.2	51.6	98.6	22.26
2	175.2	67.3	94.7	16.73	185.8	71.2	95.0	15.83	148.3	59.8	110.5	20.46	132.8	52.2	98.5	22.00
3	143.3	60.8	129.6	22.19	169.4	69.6	120.1	18.48	143.2	62.6	142.1	22.89	133.9	58.2	137.8	24.14
4	143.3	60.8	129.6	22.19	171.3	70.3	119.9	18.27	144.4	63.1	141.9	22.71	133.9	58.2	137.8	24.14
5	143.6	64.1	150.4	23.29	161.8	70.7	144.4	20.49	139.7	66.1	175.6	25.32	150.5	68.8	161.7	22.86
6	148.2	66.0	150.4	22.60	163.8	71.5	144.1	20.24	140.4	66.4	175.7	25.21	151.6	69.2	161.7	22.70
7	104.5	48.9	162.9	32.33	116.6	53.3	155.6	28.69	112.4	53.9	176.4	31.15	116.5	54.5	165.8	29.39
8	104.5	48.9	162.9	32.33	118.0	53.8	155.2	28.34	113.8	54.6	176.1	30.75	117.8	55.0	165.6	29.08
9	129.7	57.7	146.8	25.43	132.0	58.8	148.0	25.08	125.8	58.8	168.0	27.50	116.9	53.8	159.1	28.86
10	131.8	58.5	146.1	25.00	135.1	60.0	147.5	24.50	126.4	59.1	167.9	27.36	116.9	53.8	159.1	28.86
11	138.1	60.6	142.4	23.71	141.3	62.3	145.4	23.37	126.8	59.0	166.1	27.17	134.2	60.5	153.3	25.00
12	139.8	61.2	142.0	23.42	143.8	63.3	145.1	22.96	128.4	59.7	165.9	26.84	135.5	61.0	153.1	24.77
13	160.8	75.9	177.2	22.23	161.1	72.5	156.5	21.17	142.9	69.1	186.0	25.36	130.7	62.4	178.3	27.13
14	160.9	76.0	178.0	22.26	162.4	73.0	156.3	21.00	144.0	69.6	185.9	25.17	130.7	62.4	178.3	27.13
15	123.4	58.1	170.7	28.18	153.6	70.8	166.2	22.65	148.9	71.8	185.6	24.36	138.1	66.8	185.2	26.15
16	123.4	58.2	170.8	28.16	162.1	74.6	166.3	21.53	150.1	72.4	185.6	24.18	145.0	70.1	185.8	24.99
17	145.7	70.2	184.9	24.84	167.4	80.1	184.4	21.74	161.9	80.8	203.4	23.37	145.2	73.2	205.8	26.05
18	151.1	72.6	184.6	23.98	171.5	82.0	184.8	21.26	164.1	81.8	203.0	23.05	148.4	74.7	205.7	25.51
19	192.3	80.1	127.7	16.67	183.8	76.0	123.9	17.25	140.3	62.0	146.1	23.56	145.7	62.7	136.0	22.19
20	192.6	80.3	127.8	16.65	185.0	76.5	124.3	17.16	141.4	62.5	146.2	23.40	148.7	64.0	136.5	21.79
21	103.9	40.6	90.8	27.13	146.4	57.3	99.1	20.10	118.9	50.2	123.7	26.09	118.5	48.2	109.1	25.20
22	106.6	41.5	90.4	26.46	147.2	57.7	99.2	19.99	125.3	52.8	123.8	24.83	121.6	49.4	109.4	24.61
23	106.3	44.3	115.9	28.40	130.7	54.7	122.2	23.76	120.3	53.3	143.9	27.11	122.6	52.5	130.0	25.75
24	106.3	44.3	115.9	28.40	135.5	56.7	123.1	23.02	125.0	55.4	144.3	26.17	124.5	53.3	130.4	25.39
25	105.8	49.3	161.4	31.86	132.1	59.8	154.9	25.47	129.5	61.6	175.9	27.22	129.0	60.0	165.9	26.71
26	105.8	49.3	161.4	31.86	134.4	60.8	154.8	25.06	132.7	62.9	175.3	26.57	131.4	61.0	165.6	26.23
27	115.6	51.3	142.9	28.07	126.7	55.1	137.3	25.41	116.6	54.1	162.7	29.17	112.8	51.6	155.1	29.58
28	121.2	53.6	142.8	26.84	130.1	56.5	137.0	24.75	119.0	55.2	162.5	28.60	112.8	51.6	155.1	29.58
29	128.1	54.4	127.7	24.56	135.5	57.7	129.9	23.42	116.9	53.4	155.5	28.63	126.8	55.4	138.7	25.48

30	129.2	54.8	127.5	24.34	136.8	58.2	130.0	23.20	120.4	55.0	156.2	27.88	128.8	56.1	138.5	25.09
31	129.1	55.1	130.1	24.53	136.7	58.5	132.4	23.36	114.6	52.7	158.1	29.35	120.1	53.5	145.8	27.28
32	130.0	55.5	129.9	24.34	138.6	59.3	132.3	23.05	114.6	52.7	158.1	29.35	120.1	53.5	145.8	27.28
33	182.2	85.8	178.9	19.80	162.7	71.1	144.3	20.37	144.1	67.2	169.8	24.28	111.8	51.7	159.4	30.11
34	187.4	88.3	179.6	19.30	164.7	71.9	144.0	20.13	145.8	68.0	170.0	24.02	111.8	51.7	159.4	30.11
35	154.2	73.9	183.7	23.48	138.6	64.4	167.3	25.03	131.4	64.3	189.2	27.65	138.4	65.7	176.9	25.62
36	159.2	76.3	184.3	22.80	141.5	65.7	167.4	24.56	134.3	65.6	189.4	27.09	139.6	66.2	176.7	25.41
37	153.9	75.3	192.9	23.98	137.2	65.3	177.8	25.88	134.5	67.3	200.1	27.69	121.0	60.4	196.3	30.33
38	157.1	76.8	193.0	23.53	138.6	65.9	177.5	25.62	136.4	68.2	200.1	27.32	121.0	60.4	196.3	30.33
39	143.7	72.1	203.7	26.21	142.4	69.3	188.5	25.58	132.3	67.9	211.9	28.81	126.2	64.4	208.0	29.90
40	147.0	73.7	203.7	25.64	144.0	69.9	188.1	25.30	133.9	68.6	211.8	28.49	126.2	64.4	208.0	29.90
41	146.1	74.8	213.4	26.32	147.7	73.2	198.4	25.24	137.0	71.6	222.1	28.48	124.9	65.3	219.6	30.92
42	149.7	76.5	213.1	25.70	149.2	73.9	198.1	24.99	138.8	72.5	221.8	28.11	124.9	65.3	219.6	30.92
43	145.0	75.9	224.4	27.10	135.7	69.9	214.7	28.30	141.9	75.4	231.5	28.06	129.7	69.1	230.2	30.47
44	147.9	77.3	224.2	26.58	136.4	70.2	214.5	28.15	143.7	76.3	231.1	27.71	129.7	69.1	230.2	30.47
45	168.9	79.0	174.2	21.07	138.8	60.5	140.2	23.48	131.5	60.8	163.7	26.10	125.7	56.2	148.4	26.28
46	176.9	82.9	176.4	20.25	145.7	63.6	141.9	22.51	136.5	63.2	165.1	25.28	128.0	57.2	148.6	25.85
47	170.6	80.3	177.5	21.03	140.6	62.9	151.6	23.83	130.1	61.7	174.9	27.06	128.2	59.2	162.8	26.69
48	173.5	81.7	178.0	20.71	147.9	66.2	152.6	22.76	133.9	63.5	175.7	26.36	131.0	60.4	163.0	26.17
49	168.5	80.8	185.4	21.65	141.5	65.1	163.1	24.33	133.5	64.9	186.6	27.10	134.7	63.8	175.1	26.20
50	171.9	82.4	185.8	21.26	148.7	68.3	163.6	23.22	136.9	66.5	187.0	26.48	137.2	64.9	175.1	25.73
51	159.8	78.4	195.1	23.26	133.7	63.5	175.9	26.42	138.3	68.7	197.7	26.82	134.3	65.4	187.7	27.00
52	163.3	80.0	195.2	22.79	139.5	66.1	175.9	25.37	141.9	70.4	197.8	26.20	136.7	66.5	187.5	26.55
53	156.6	78.7	206.5	24.29	131.0	64.3	191.1	27.85	131.6	67.8	213.8	29.07	139.4	69.4	198.9	26.69
54	159.7	80.3	206.6	23.83	134.6	66.0	191.1	27.15	134.7	69.3	213.5	28.43	141.7	70.4	198.6	26.27
55	167.0	84.8	212.4	23.11	136.8	68.4	200.3	27.25	138.4	72.1	220.3	28.09	137.2	70.1	211.0	27.79
56	169.5	86.0	212.6	22.80	140.4	70.1	200.2	26.58	141.0	73.3	220.2	27.59	139.5	71.2	210.5	27.32
57	165.0	85.6	222.7	23.89	134.7	69.0	212.1	28.35	143.3	75.9	229.8	27.70	142.2	74.0	221.0	27.42
58	167.7	86.9	222.6	23.51	137.4	70.3	211.8	27.81	144.8	77.2	229.5	27.21	144.6	75.1	220.3	26.96

Figures

Figure 1:

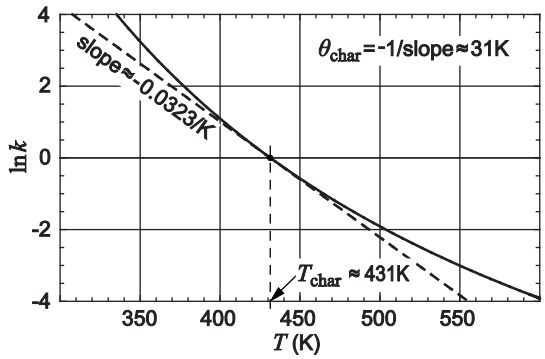

Figure 2:

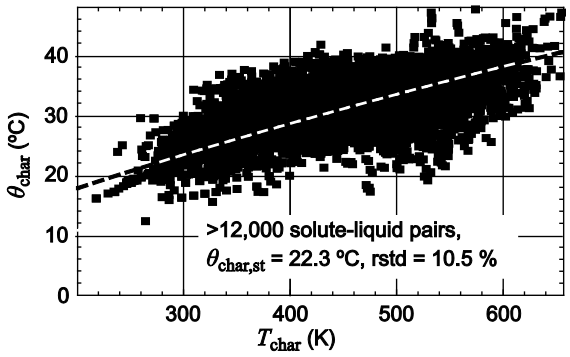

Figure 3

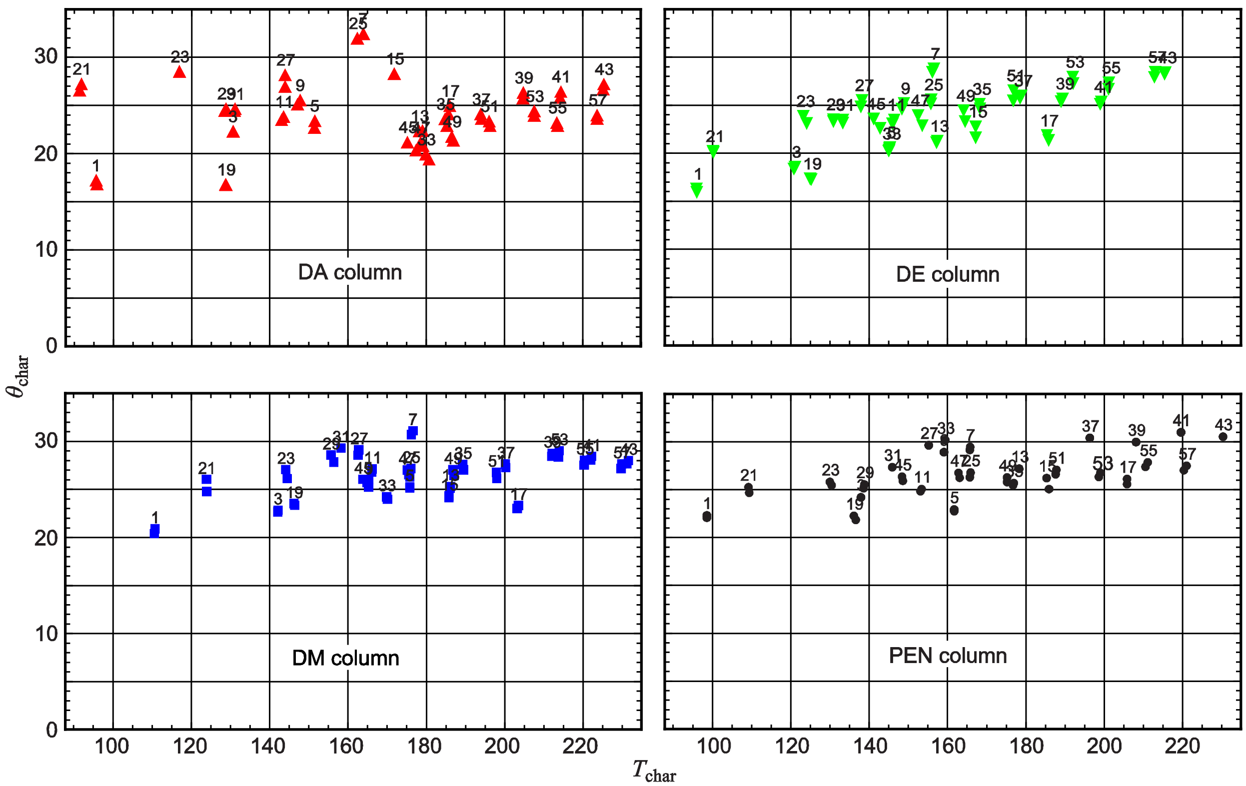

Figure 4