ELSEVIER

Journal of Chromatography A, 965 (2002) 195–205

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Temperature selectivity in reversed-phase high performance liquid chromatography

John W. Dolan

LC Resources Inc., 3138 NE Rivergate, Building 301C, McMinnville, OR 97128, USA

Abstract

Column temperature plays two important roles in reversed-phase high-performance liquid chromatography (RP-HPLC): control of retention (k) and control of selectivity (α). While changes in retention as a function of temperature are ubiquitous, selectivity changes for any given solute pair are more pronounced for ionized samples and samples with more polar substituents. With many samples, column temperature can be selected in a manner that optimizes resolution. The selectivity effects observed for temperature changes in RP-HPLC generally are complementary to those observed for mobile phase strength changes, so it is often possible to improve resolution by simultaneous optimization of temperature and mobile phase percent organic or gradient steepness. Computer simulation is a powerful tool for such optimization experiments. This paper reviews the influence of temperature on chromatographic selectivity for RP-HPLC.

Keywords: Reviews; Temperature selectivity; Column temperature

Contents

1. Author's note

This paper was prepared for the June 2001 symposium honoring Lloyd Snyder's tenure as an editor of the *Journal of Chromatography*, and in that context concentrates on the contributions of Dr. Snyder and his colleagues to the understanding of temperature as a selectivity variable in reversed-phase high-performance liquid chromatography (RP-HPLC).

References to temperature in HPLC may refer to the temperature of the column packing, the mobile phase, or the column heating device. The temperature inside the column is of primary interest, but is

E-mail address: john.dolan@lcresources.com (J.W. Dolan).

0021-9673/02/$ – see front matter
PII: S0021-9673(01)01321-8

difficult to measure without physically modifying the column. A simpler way to determine the column temperature is to use a flow-through thermocouple to measure the temperature of the mobile phase entering and/or exiting the column. An average of the inlet and outlet mobile phase temperature will give a reasonable value for the average column temperature and information on the temperature gradient along the column. The column oven temperature generally will differ from the temperature inside the column, but as long as the column oven temperature is constant, this measurement will provide a suitable reference value for most applications.

2. Pre-1970

Column temperature has long been recognized as an important parameter in HPLC separation. For example, Giddings [1] described the role of temperature in separation. First, he noted that significant changes in resolution would be expected for changes of approximately 20°C in temperature. Second, Giddings presented the relationship of Eq. (1):

$$\Delta K/K = a/T - b \quad (1)$$

where K is the distribution coefficient (concentration of solute in the stationary phase/concentration in the mobile phase), $\Delta K/K$ is the relative selectivity, T is the temperature, and a and b are constants. He went on to suggest that based on Eq. (1), temperature increases were expected to have a detrimental effect on $\Delta K/K$ in most cases – it was only the exception that increases in temperature would improve the separation. Only many years later was it realized that increases in temperature commonly provide improvements in separation. Finally, Giddings noted the effect of temperature on plate height. Temperature increases will reduce the diffusion coefficient, and for most flow-rate conditions used in routine HPLC, will result in a larger column plate number and narrower peaks. This influence on resolution is expected to be small because resolution is proportional to $N^{0.5}$ (see Eq. (2)).

Snyder [2] also acknowledged the influence of temperature on selectivity in normal-phase chromatography. He stated that lowered temperatures should amplify small differences in K, and thus enhance selectivity. He cautioned that changes in temperature could negatively impact the separation, citing one worker [3] in the mid-1940s who reported retention order reversals with a change in temperature of 75°C. The primary effect of temperature was presumed to be a detrimental change in a carefully engineered separation, so workers were cautioned to carefully control temperature.

As RP-HPLC became widely accepted as an analytical technique, many workers recognized and took advantage of mobile phase and column chemistry changes to manipulate selectivity. However, even in the late 1970s, temperature was acknowledged primarily for its influence on retention, with increased temperatures producing shorter retention times. The observation that in general shorter retention times give poorer resolution argued against the use of elevated temperature as a routine practice. Snyder and Kirkland's popular book, *Introduction to Modern Liquid Chromatography* [4], stated that a change in temperature had little effect on selectivity for normal- and reversed-phase HPLC, but could be a useful tool for ion-exchange and ion-pair.

3. The 1970s

The introduction of two tools, the window diagram and the hand-held programmable calculator, had a strong influence on the rise in understanding of the role of temperature (as well as other variables) in RP-HPLC selectivity. The presentation of the window-diagram concept by Laub and Purnell [5–9] laid the groundwork for later development of the resolution map. A window diagram is a plot of selectivity, α, for every peak pair in a separation as a function of a variable, such as temperature or mobile phase composition. The area under all the overlapping plots is a ''window'' in which all peaks are separated. With the aid of such plots, one can visually determine the conditions that will give the best α-value for the least-separated peak pair.

Window diagrams and resolution maps gave chromatographers the ability to graphically express selectivity or resolution as a function of one or more chromatographic variables. The second contribution was the introduction of the programmable hand-held

electronic calculator, followed by the personal computer. These devices gave the chemists tools which previously had been available only to researchers. The combination of the personal computer and the resolution map gave rise to software that maps resolution as a function of one or more chromatographic parameters. Mapping is the generation of a graphical plot of resolution or selectivity as a function of one or more separation parameters, such as mobile phase percent organic, gradient steepness, or temperature. Snyder and Dolan used these tools to develop DryLab separation simulation software (LC Resources) in the mid-1980s, and several other groups followed with similar programs.

Eq. (2), one form of which is referred to as the Purnell equation, forms the foundation of understanding of the role of temperature in separation and resolution in RP-HPLC:

$$R_s = 1/4 \quad N^{0.5} \quad (\alpha - 1) \quad [k/(1+k)] \qquad \text{(i)} \quad \text{(ii)} \quad \text{(iii)} \tag{2}$$

where R_s is resolution, N is the column plate number, α is the separation factor (k_2/k_1), and k is the retention factor (in this case the average retention factor of two adjacent peaks k_1, and k_2). Although terms (i), (ii), and (iii) are often considered to be independent, there are interactions that can significantly affect resolution. For example, small changes in k often result in changes in α, and thus a larger change in resolution.

Snyder's group first described the utility of temperature in controlling resolution with the aid of the window diagram and hand-held calculator in 1979 [10]. This paper described the relationships that have formed the basis of all the later work on combined temperature–solvent strength selectivity effects. The relationship between temperature and retention for a solute at two different temperatures can be stated as:

$$\log k_T = \log k_R - a(1/T_R - 1/T) \tag{3}$$

where k_R and T_R are the retention factor and (absolute) temperature for a reference condition (e.g., 25°C = 298.3 K) and k_T and T are the retention factor and temperature for a new temperature. As long as a (Eq. (3)) is different for two compounds, α should change with a change in T causing a resolution change as predicted by Eq. (2).

The well-known semi-empirical relationship:

$$\log k = b - c\Phi \tag{4}$$

states that retention, k, is a function of the volume fraction of organic in the mobile phase, Φ (b and c are constants for a particular solute). Eq. (4) can be expressed in a similar manner to Eq. (3):

$$\log k_\Phi = \log k_R - c(\Phi - \Phi_R) \tag{5}$$

where the subscripts refer to reference conditions (R) and some offset condition (Φ). Eqs. (3) and (5) can be combined [10] as:

$$\log k_{\Phi,T} = \log k_\Phi - a(1/T_R - 1/T) \tag{6}$$

where the retention factor $k_{\Phi,T}$ is a function of temperature and mobile phase organic composition.

Eq. (6) allows one to use four well-chosen experiments to determine the required constants. Two experiments are required to determine c (Eq. (5)) and two for a (Eqs. (3) or (6)), then k can be calculated for other values of T and Φ. For example, if the reference conditions were 25°C and 50% organic (B), experiments could be run at 25°C/50% B, 25°C/40% B, 40°C/50% B, and 40°C/40% B. From these data plus Eq. (6), one could determine k, for example, at 30°C and 45% B. The report by Gant et al. [10] showed that Eq. (6) was suitably accurate and provided a program for a then-popular hand-held calculator to facilitate the calculations. It is interesting to note that following this publication, the use of temperature as a selectivity variable in RP-HPLC was largely ignored for another 10 years.

4. Recognition of temperature's importance

After lying fallow for a decade, the use of temperature as a selectivity variable in RP-HPLC was again addressed by Snyder's group. There were likely several factors contributing to this resurgence of interest. The personal computer stimulated the development of resolution mapping software (e.g., Refs. [11–15]) that was becoming widely accepted as a tool to aid HPLC method development. As an extension of the HPLC application of resolution mapping, Snyder's group had developed a similar application for exploiting the influence of tempera-

ture on selectivity in gas chromatography [13]. Finally, with the help of resolution mapping programs, workers were able to take maximum advantage of organic solvent strength and type, as well as mobile phase pH to optimize separations. When the power of these parameters was exhausted, it was natural to more closely examine parameters previously considered to be of minor importance in controlling selectivity, such as temperature.

A multi-parameter scheme for mapping % B, pH, buffer concentration, and temperature was reported by Snyder's group [14,15]. By use of a limited range for each variable, it was possible to ignore interaction effects and optimize the separation for all parameters over a restricted region. Samples included non-ionized substituted benzenes, benzoic acids, steroids, and dialkyl phthalates. In terms of temperature, several important conclusions were drawn from this study. Temperature was determined to be a useful parameter to effect changes in selectivity. This recognition of the combined selectivity power of T and % B (and in later work gradient time, t_G) would gradually change the perception that temperature is only of minor use in controlling selectivity.

The introduction of mapping resolution vs. temperature allowed the chromatographer to evaluate the potential of any set of temperature conditions in providing both acceptable and rugged separations. Ref. [15] illustrated this with separation of substituted benzenes for which conditions could be found that would tolerate a ±5°C variation in temperature while maintaining a satisfactory separation.

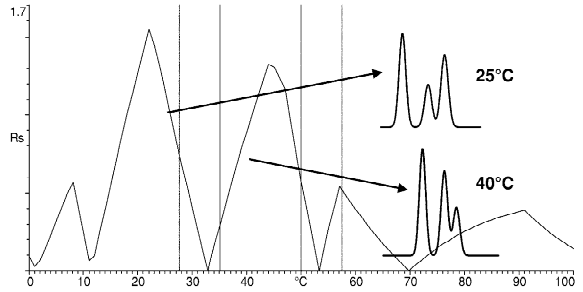

Fig. 2. Resolution map for a mixture of food additives. Insets show peak reversal between 25 and 40°C.

Figs. 1 and 2 illustrate the change that had come about in understanding of the role of temperature in RP-HPLC separation. Each of these figures plots the resolution for the least-resolved band pair (the ''critical pair'') against the temperature. Such plots are derived from Eq. (2), in which k and α can be determined from two experimental runs and Eq. (3). N can be measured experimentally, calculated for isocratic (e.g., Ref. [10]) or gradient (e.g., Ref. [16]) runs, or simply estimated based on ''typical'' column performance (e.g., $N \approx 10\,000$ for a 150 mm, 5 μm particle column).

Fig. 1 shows the common understanding to this point of temperature's role. The primary role in temperature was thought to be its effect on retention (Eq. (3)), with resolution generally increasing with decreasing temperature. Thus, although increases in temperature result in larger column plate numbers and lower mobile phase viscosity (translating into lower column back pressure), an overall loss in

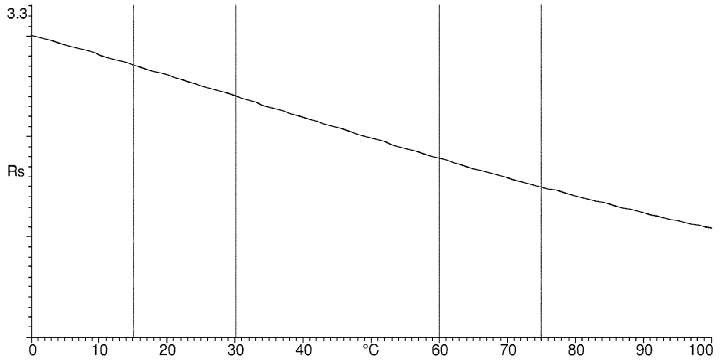

Fig. 1. Resolution map for a mixture of benzoic acids showing decreasing resolution with increasing temperature. Data of Ref. [14].

resolution was expected. This is the case with many samples. Fig. 2 is a resolution map illustrating a separation behavior that is much more common than previously thought. Here temperature plays an important role in resolution. In fact, peak reversals are seen for the three peaks in the insets when temperature is changed from 25 to 40°C. At both temperatures, the critical peak pair is the same (peaks 2 and 3 in the inset), but the retention order has reversed. At about 33°C, peaks 2 and 3 co-elute.

5. Temperature + gradient time

In the mid-1990s, Snyder and colleagues began an extensive series of studies to examine how to take maximum advantage of temperature as a selectivity variable in RP-HPLC. A key study by Hancock and co-workers [17,18] influenced the decision to study temperature selectivity further. This work showed surprisingly large effects of temperature and gradient time on resolution of different peptide and protein samples. Up to this point, when temperature was considered at all, it, along with other variables, was considered in a one-at-a-time optimization strategy. That is, mobile phase organic, pH, and temperature might each be optimized, but usually by optimizing one variable, then the next, and so forth. With such practice came the realization that different parameters could influence a particular separation in different ways.

One summary of the role of different parameters in RP-HPLC separation is given in Table 1 [19]. For example, solvent strength and solvent type are widely recognized as having a powerful influence on the separation and being generally easy to manipulate. Ion-pairing, column type changes, and mobile phase additives are less convenient to change and the resulting methods may not be rugged. Changing mobile phase pH, while a powerful variable for adjusting selectivity, is experimentally inconvenient and can require many experiments to optimize. When the table was prepared, the power of temperature in effecting changes in selectivity was not well appreciated (? in Table 1).

In the series of papers to follow [19–22], the role of temperature was carefully examined for a wide range of samples. It was determined that the selectivity changes brought about by changes in temperature were complementary to those effected by changing solvent strength. That is, the selectivity effects of temperature and solvent strength are more or less orthogonal to each other. So a peak pair that is difficult to separate by optimizing solvent strength

Table 1
Some factors that affect the choice of variables for HPLC method development (++, very favorable; +, favorable; −, unfavorable; − −, very unfavorable)

Variable	Impact on different factors						
	A	B	C	D	E	F	G
1. Solvent strength (% B)	+	++	++	+	+	++	+
2. Temperature	?	++	++	+	+	++	+
3. Solvent type							
ACN	+		+	+	+		+
MeOH	+		+	+			+
THF	++	−	−	−	−	−	
4. pH	++	−	+	− −	−	−	− −
5. Ion-pairing	++	−	− −	− −	−		
6. Column type	+	−	−		+	−	−
7. Column source	−	−	−	− −	+	−	−
8. Mobile phase additives			−	− −	−		
9. Buffer type and concentration	−				−	−	

Reprinted from Ref. [19].
A, Ability to change α; B, experimental convenience, need for operator intervention; C, column equilibration (fast or slow); D, method ruggedness; E, compatible with low-UV detection; F, large or small number of runs required to select optimum value of variable; G, peak tracking problems.

may be separated by temperature optimization. For experimental convenience, the studies concentrated on the combination temperature and gradient elution although isocratic solvent strength changes also are useful in conjunction with temperature. A large number of samples of several sample classes were studied to determine the universal nature of the role of temperature selectivity. Samples included ionizable and non-ionized small molecules, polycyclic aromatic hydrocarbons, fatty acid methyl esters, carotenoids, pharmaceuticals, herbicides, chlorophylls, peptides, and proteins. The conclusions were that gradient steepness and temperature effects on selectivity were about threefold greater for ionized vs. un-ionized samples. This observation seems logical when one considers that temperature and solvent strength changes will affect the pK_a and thus ionization. Changes in sample ionization (as with changes in mobile phase pH) will change selectivity. For all samples, the effect of gradient steepness on selectivity was about 1.5 times as important as the role of temperature. In both cases, there were enough exceptions to the rule that even sample types that are expected to gain little advantage from temperature selectivity (e.g., homologs) should be considered as candidates for temperature optimization. These papers [19–22] demonstrate that method development for most samples can be a straight forward process based on data gathered from four runs (two temperatures and two gradient times using the same % B range) such as in Fig. 3. In order to obtain separation methods that can be transferred from one laboratory to another, it was noted [19] that care needs to be taken to preheat the solvent before it enters the column oven, to ensure that the reported temperature is accurate, and to specify the system dwell volume.

The work reported in Refs. [19–22] showed the utility of using gradient steepness and temperature together, but data manipulations were tedious – based on treatment of each variable with the resolution mapping tools that were used in Figs. 1 and 2. For practical and convenient application of the technique, software was needed to facilitate simultaneous mapping of resolution against temperature and gradient steepness. The popular DryLab software (LC Resources) was expanded to accommodate this need. Snyder and co-workers reported [23,24] the successful application of this mapping software to 14 samples of varying complexity. Additional reports showed the application of the use of temperature and gradient time for optimization of other samples such as achiral isomers [25] and peptide and protein mixtures [26]. The results of this work corroborated the earlier studies, providing convincing evidence that the use of temperature with gradient elution gives a powerful tool to control selectivity for nearly any sample.

Fig. 4 illustrates the power of this three dimensional mapping technique with an example of an easy separation of nine anilines in which the additional dimension of temperature selectivity is helpful, but not essential. Resolution for the critical band pair is plotted on the *x*-axis for gradient time, t_G, and on

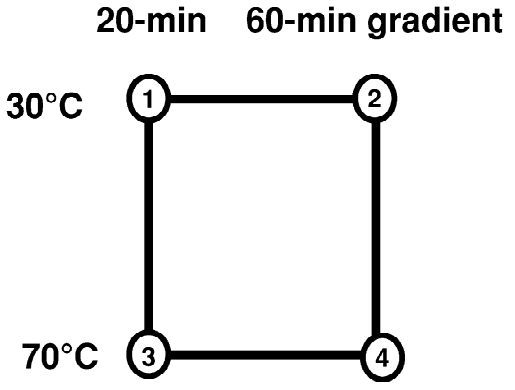

Fig. 3. Experimental design for four input runs to map gradient time (or isocratic % B) and temperature.

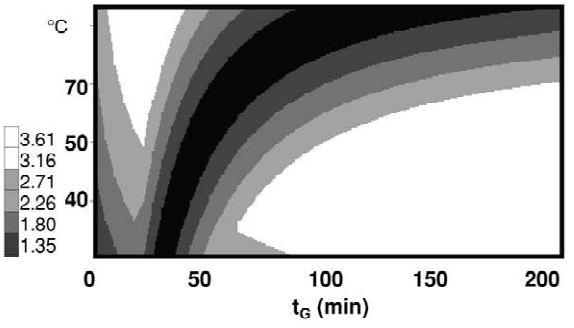

Fig. 4. Temperature vs. gradient time resolution map for nine-component aniline mixture of Ref. [19]. Key at left shows resolution values.

the *y*-axis for temperature (°C). In this sample of substituted anilines, $R_s \geq 2$ is possible at 30°C, but requires gradient times of >50 min. At 40°C, however, $R_s \geq 2$ can be obtained easily with gradients of about 20 min. With column temperatures of 50°C or greater, $R_s > 3$ will be realized. Over-resolving a separation will generally allow the user to shorten the run time even more by changing the column conditions (length, flow-rate, and particle size). The use of the t_G/T map allows one to quickly find combinations of T and t_G that will give satisfactory results. It also allows one to compare the practicality of predicted separations.

Identification of acceptable separation conditions for the substituted aniline sample of Fig. 4 probably could be found without the help of resolution mapping. As might be expected, however, more complex samples can be a challenge to separate without this powerful tool. An example of the power of resolution mapping can be seen in Fig. 5. In this case, the separation of sample of 47 drugs in a toxicology screening panel is attempted. Two observations are immediately apparent upon examining Fig. 5. First, the maximum resolution for this sample, even with the aid of temperature selectivity, is $R_s \approx 0.5$ – hardly enough to recognize that two peaks are present, let alone provide qualitative or quantitative data about the sample composition. Second, there are many combinations of t_G and T in which one or more band pairs overlap completely (black regions with $R_s \leq 0.2$). Although complete resolution of this sample was not possible in a single run, the use of just four experimental runs as in Fig. 3 allow one to determine the best possible conditions for such samples. Fig. 6a shows the chromatogram for the critical peak pairs at the optimum conditions of Fig. 5.

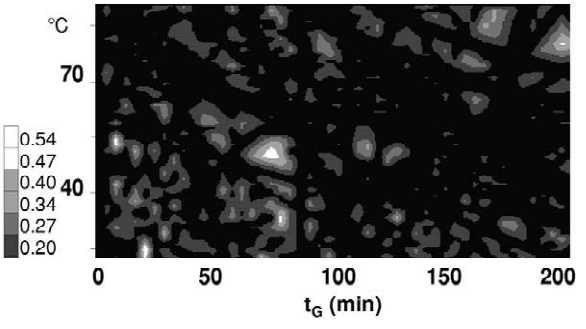

Fig. 5. Resolution map for complex mixture of 47-component toxicology screen sample of Ref. [19].

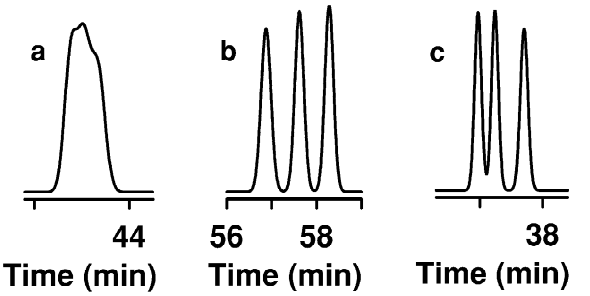

Fig. 6. Chromatograms for three peaks including critical peak pair at optimum conditions of Fig. 5. (a) Separation at optimum of Fig. 5 ($t_G = 64$ min, $T = 47$°C, $R_s = 0.5$); (b) same peaks from Fig. 7 ($t_G = 100$ min, $T = 73$°C, $R_s = 1.7$; (c) same peaks from Fig. 7 ($t_G = 50$ min, $T = 30$°C, $R_s = 1.25$).

Examination of complex samples, such as that of Fig. 5, leads to the question of how many peaks should one be able to resolve by using the combination of gradient time and temperature. A study of this question [27] led to the conclusion that for a sample of 15–20 components, there is at least a 50% probability of achieving $R_s \geq 1$ by varying t_G and T. Baseline resolution ($R_s \geq 1.5$) can be achieved with similar probability for samples with a maximum of 10–15 components. If only enough resolution to distinguish peaks is required ($R_s \geq 0.7$), a maximum of 20–25 peaks can be expected. So it is unrealistic to expect baseline resolution for the 47-component sample of Fig. 5.

Another approach to separation of complex samples, such as that of Fig. 5, is to use more than one run [28], in which a subset of the sample is optimized instead of the separation of all components. For example, if only the cluster of three peaks shown in Fig. 6a is considered, a new resolution map can be generated, as in Fig. 7. Now the resolution map is much simpler, and conditions for baseline resolution can be found, such as those of Fig. 6b ($t_G = 100$ min, $T = 73$°C, $R_s = 1.7$). For this particular application, it is unlikely that many of the original 47 compounds are going to be present in any given sample; as long as each peak can be clearly distinguished, the

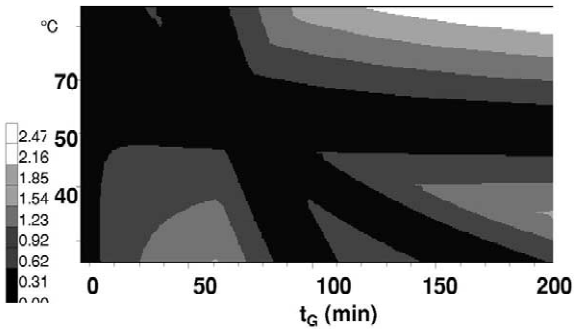

Fig. 7. Resolution map for three peaks of Fig. 6, including critical peak pair from optimum of Fig. 5.

resolution is acceptable. If it is unlikely that more than one of the three components will be present in any given sample, the run of Fig. 6c ($t_G = 50$ min, $T = 30$°C, $R_s = 1.25$) is more acceptable than that of Fig. 6b because the run time is half as long.

With complex samples, such as that of Fig. 5, it is imperative that the predictive accuracy of the model be acceptable for any model-based software to be of practical utility. When the predictive accuracy of t_G vs. T and % B vs. T resolution mapping was addressed [29], it was proposed that complex samples with 15–20 or more peaks could tolerate errors in predictive accuracy of up to 0.2 resolution units and that simpler samples might allow errors of up to 0.4 resolution units. Within these guidelines, it was found that for samples in which gradient time was varied along with temperature, resolution errors were <0.2 units for most cases. The exceptions were samples that contained molecules that differed markedly in shape and samples containing partially ionized acids or bases. As was noted earlier, temperature affects sample ionization characteristics, so the latter conclusion is not surprising. For experiments in which isocratic % B and T are varied, predictions are acceptable for intermediate % B values and a moderate range of temperatures (e.g., 10–20°C). For example, with input runs of 40 and 55% B, predictions between 35 and 60% B are acceptable. For % B<30%, three runs are advised for best results. Finally, predictions of resolution for isocratic % B and temperature which are based on gradient input runs are less accurate than when isocratic runs are used to predict isocratic resolution. Thus, it was shown that resolution mapping based on t_G and T or % B and T inputs is acceptably accurate for most applications. In cases for which errors are observed, a run used to check accuracy can be fed back into the model to refine the predictions using a ''reflection'' technique [30] to correct for the error. This is an easy process with separation simulation software (e.g., DryLab). If an experimental run is made for a predicted condition and found to be in error, the new run can be used to estimate the required adjustment. For example, if the experimental run for 55% B and 35°C did not match the prediction, the software could be used to find predicted conditions that produced a run that matches the experimental one. Perhaps 52% B and 39°C predicted a separation that matched the experimental 55% B/35°C run. One could apply the difference in % B (−3%) and °C (+4°C) in the opposite direction, to obtain adjusted conditions of 58% B and 31°C. This technique proved to be useful in correcting errors in resolution predictions [30].

In an innovative application of resolution mapping of gradient time and temperature, Snyder's group showed [31,32] that resolution maps could be used to identify RP-HPLC columns that can be used as alternatives to the primary selection. This is of interest when an alternative supplier is desired so that method continuity can be maintained in a routine analysis environment. The process is begun by selecting a minimum acceptable resolution value (e.g., $R_s \geq 1.5$). Next, four calibration runs (e.g., as in Fig. 3) are carried out for each column. The resolution maps for each column are compared to determine if a common set of conditions exists for which the minimum resolution criteria are satisfied. In the test set of 11 pharmaceutical compounds from a degradation sample, this technique was used [32] to identify common conditions that generated $R_s > 1.5$ for eight of 10 columns tested (one C_8 and nine C_{18} stationary phases).

6. Control of temperature

The importance of column temperature control is well known (e.g., Refs. [19,33–39]) for its influence on retention, selectivity, system pressure, and col-

umn stability. When temperature is used as a variable to vary selectivity, its control is especially critical. This is especially important when column temperatures are significantly above room temperature (e.g., >40°C). The need to know the true temperature of the column is obvious, especially when a temperature-controlled method must be transferred from one instrument to another. A study of several factors relating to temperature control [40] led to a better understanding of the variables. Column temperature commonly is controlled by ovens using block heaters, circulating heated air, Peltier heaters, or water baths. It was shown that if the conditions were adjusted so that the average column temperature was the same, an identical separation could be obtained using any oven design tested, all other factors being equal. Two key factors were required to obtain separation equivalency. The first is trivial – adjust the temperature controller so that the set-point produces the desired temperature. A second factor is often ignored – ensuring that the solvent at the inlet of the column is at the desired temperature. This can be achieved by using a length of pre-heating tubing, for example 0.5 m of 0.005 in. (0.13 mm) I.D. tubing, upstream from the column. Some commercial ovens incorporate an efficient pre-heater by embedding the column inlet tubing in a heated block. Laboratory-made pre-heaters can be made by coiling the pre-heat tubing and clamping it to the heating source inside the oven.

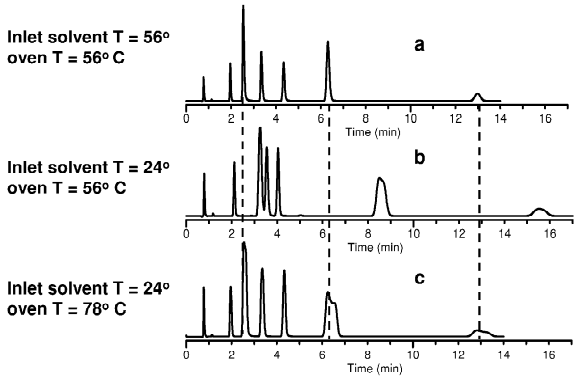

Fig. 8. Effect of temperature equilibration on the chromatogram. (a) Fully equilibrated column temperature; (b) effect of cooler inlet solvent; (c) retention correction by attaining same average temperature as (a). Data from Ref. [40].

The role of temperature control is shown dramatically in Fig. 8 [40]. The chromatogram of Fig. 8a was obtained with a fully equilibrated system in which the inlet solvent and oven temperature were the same. When no pre-heating was used (Fig. 8b), the cooler incoming solvent caused two problems. First, the average temperature of the column was lowered, so retention times increased. Because there was a temperature gradient along the column, peak shape deteriorated. By manipulating the incoming solvent temperature and the column oven setting (Fig. 8c), conditions could be obtained in which the retention times matched those of the fully equilibrated system, although peak shapes were terrible. This suggests that the average temperature of the column controls retention, and that one could empirically adjust the column settings to obtain equal retention times between two otherwise nominally identical systems. The temperature gradient along the column causes poor band shape; this condition can be avoided by pre-heating the solvent. The study concluded [40] that when elevated temperatures were used (e.g., $T > 40$°C), the incoming solvent should be heated to within ±6°C of the column temperature for acceptable performance.

7. Conclusions

Column temperature is now recognized as an important variable in controlling selectivity in RP-HPLC separations. Although generally not providing as much leverage on changes in selectivity as isocratic % B, gradient steepness, solvent type, or pH, temperature can be a strong complimentary variable for any of these parameters. Simply programmed in many HPLC systems, changes in temperature during method development are more convenient than solvent type or pH changes. One recommended approach to method development (e.g., Refs. [34,41,42]) is to use the four-run combination of t_G (or % B) and T as the first step for nearly all methods because of the experimental ease of this approach and its effectiveness for such a broad range of compound types. If these initial experiments plus resolution mapping do not guide the user to an acceptable method, additional parame-

ters can be varied and substituted in the resolution mapping scheme for t_G, % B, or T [41].

8. Nomenclature

a, b, c	Constants
B	Organic solvent component of the mobile phase (% B = 100Φ)
k	Retention factor, $(t_R - t_M)/t_M$
k_T	Value of k for some new temperature
k_Φ	Value of k for a specified value of Φ
$k_{\Phi,T}$	k for a specific value of T and Φ
k_R	Value of k for some reference set of conditions
K	Distribution coefficient (concentration of solute in the stationary phase/concentration in the mobile phase)
$\Delta K/K$	Relative selectivity
N	Column plate number
R_s	Resolution
t_G	Gradient time, in min (t_G = gradient range/gradient steepness)
t_M	Column dead time
t_R	Retention time of a solute
T	Temperature, K; value of T for some new set of conditions
T_R	Value of T for some reference set of conditions selectivity (k_2/k_1)
Φ	Volume fraction of organic in the mobile phase (Φ = %B/100)
Φ_R	Value of Φ for some reference set of conditions

References

[1] J.C. Giddings, in: Dynamics of Chromatography Part I. Principles and Theory, Marcel Dekker, New York, 1965, p. 283.
[2] L.R. Snyder, in: Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968, p. 340.
[3] H.H. Strain, Ind. Eng. Chem. Anal. Ed. 18 (1946) 605.
[4] L.R. Snyder, J.J. Kirkland, in: Introduction to Modern Liquid Chromatography, 2nd ed., Wiley-Interscience, New York, 1979, p. 79.
[5] R.J. Laub, J.H. Purnell, J. Chromatogr. 112 (1975) 71.
[6] R.J. Laub, J.H. Purnell, P.S. Williams, J. Chromatogr. 134 (1977) 249.
[7] R.J. Laub, J.H. Purnell, J. Chromatogr. 155 (1978) 1.
[8] R.J. Laub, J.H. Purnell, J. Chromatogr. 161 (1978) 49.
[9] R.J. Laub, J.H. Purnell, J. Chromatogr. 161 (1978) 59.
[10] J.R. Gant, J.W. Dolan, L.R. Snyder, J. Chromatogr. 185 (1979) 153.
[11] L.R. Snyder, J.W. Dolan, D.C. Lommen, J. Chromatogr. 485 (1989) 65.
[12] J.W. Dolan, D.C. Lommen, L.R. Snyder, J. Chromatogr. 485 (1989) 91.
[13] D.E. Bautz, J.W. Dolan, W.D. Raddatz, L.R. Snyder, Anal. Chem. 62 (1990) 1560.
[14] J.W. Dolan, D.C. Lommen, L.R. Snyder, J. Chromatogr. 535 (1990) 55.
[15] L.R. Snyder, J.W. Dolan, D.C. Lommen, J. Chromatogr. 535 (1990) 75.
[16] L.R. Snyder, M.A. Stadalius, in: Cs. Horvath (Ed.), High-Performance Liquid Chromatography, Advances and Perspectives, Vol. 4, Academic Press, Orlando, FL, 1986, p. 195.
[17] W.S. Hancock, R.C. Chloupek, J.J. Kirkland, L.R. Snyder, J. Chromatogr. A 686 (1994) 31.
[18] R.C. Chloupek, W.S. Hancock, B.A. Marchylo, J.J. Kirkland, B.E. Boyes, L.R. Snyder, J. Chromatogr. A 686 (1994) 45.
[19] P.-L. Zhu, L.R. Snyder, J.W. Dolan, N.M. Djordjevic, D.W. Hill, L.C. Sander, T.J. Waeghe, J. Chromatogr. A 756 (1996) 21.
[20] P.-L. Zhu, L.R. Snyder, J.W. Dolan, J. Chromatogr. A 756 (1996) 41.
[21] P.-L. Zhu, L.R. Snyder, J.W. Dolan, D.W. Hill, L. Van Heukelem, T.J. Waeghe, J. Chromatogr. A 756 (1996) 51.
[22] P.-L. Zhu, L.R. Snyder, J.W. Dolan, N.M. Djordjevic, D.W. Hill, J.-T. Lin, L.C. Sander, L. Van Heukelem, J. Chromatogr. A 756 (1996) 63.
[23] J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, D.C. Locke, D.L. Saunders, L. Van Heukelem, T.J. Waeghe, J. Chromatogr. A 803 (1998) 1.
[24] J.W. Dolan, L.R. Snyder, D.L. Saunders, L. Van Heukelem, J. Chromatogr. A 803 (1998) 33.
[25] L.R. Snyder, J.W. Dolan, J. Chromatogr. A 892 (2000) 107.
[26] J.W. Dolan, L.R. Snyder, LC·GC 17 (4S) (1999) S17.
[27] J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, T.J. Waeghe, J. Chromatogr. A 857 (1999) 1.
[28] J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, T.J. Waeghe, J. Chromatogr. A 857 (1999) 21.
[29] J.W. Dolan, L.R. Snyder, R.G. Wolcott, P. Habner, T. Baczek, R. Kaliszan, L.C. Sander, J. Chromatogr. A 857 (1999) 41.
[30] R.G. Wolcott, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 869 (2000) 3.
[31] J.W. Dolan, L.R. Snyder, T. Blanc, L. Van Heukelem, J. Chromatogr. A 897 (2000) 37.
[32] J.W. Dolan, L.R. Snyder, T. Blanc, J. Chromatogr. A 897 (2000) 51.
[33] J.W. Dolan, L.R. Snyder, in: Troubleshooting LC Systems, Humana Press, Clifton, NJ, 1989, p. 68.
[34] L.R. Snyder, J.J. Kirkland, J.L. Glajch, in: Practical HPLC Method Development, 2nd ed., Wiley, New York, 1997, p. 218.

[35] B. Ooms, LC·GC 14 (1996) 306.
[36] J. Paesen, J. Hoogmartens, LC·GC 10 (1992) 364.
[37] J. Paesen, E. Roets, J. Hoogmartens, Chromatographia 32 (1991) 62.
[38] R.G. Wolcott, J.W. Dolan, LC·GC 16 (1998) 1080.
[39] P.-L. Zhu, J.W. Dolan, LC·GC 14 (1996) 944.
[40] R.G. Wolcott, J.W. Dolan, L.R. Snyder, S.R. Bakalyar, M.A. Arnold, J.A. Nichols, J. Chromatogr. A 869 (2000) 211.
[41] P. Haber, T. Baczek, R. Kaliszan, L.R. Snyder, J.W. Dolan, C.T. Wehr, J. Chromatogr. Sci. 38 (2000) 386.
[42] L.R. Snyder, J.W. Dolan, Chem. Anal. (Warsaw) 43 (1998) 495.