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Theory and Application of the Two-Mode Gradient Elution in Liquid Chromatography Involving Simultaneous Changes in Temperature and Mobile-Phase Composition

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The theory of the dual-mode gradient elution in liquid chromatography involving any type of simultaneous changes in column temperature and mobile-phase composition is developed following Drake's approach. The theory was tested in the retention prediction of six alkylbenzenes in aqueous eluting systems modified by acetonitrile. Significant delay phenomena, i.e., a lag between the programmed gradient temperature and the temperature in the oven, and a lag between the oven temperature and the effective temperature the analyte feels in the column, were detected. When these phenomena were taken into account, the retention prediction obtained for all solutes under all dual-mode gradient conditions was excellent. The average percentage error between experimental and predicted retention times is below to 2%.

In gradient elution liquid chromatography (LC), the elution conditions are changed according to a preset program.[1–4] The most often used programming technique in LC is solvent gradient elution, where the composition of the mobile phase changes during the run by mixing two or more components. Other useful modes for the separation of nonionized solutes are the flow rate and the column temperature. Although modern gradient instruments offer the simultaneous coupling of solvent and flow or temperature programming, the majority of the work on gradient elution is carried out using single gradients, simply programmed changes in organic modifier content. Possible reasons are the lack of a theory for prediction of the solute elution times under multimode gradient conditions and the higher number of experimental variables that should be taken into account. Moreover, effective temperature gradients presume certain instrumental specifications, like mobile-phase preheating, fast-temperature change column ovens, columns stable at high temperatures, and preferably columns with small diameter or capillary columns.[5]
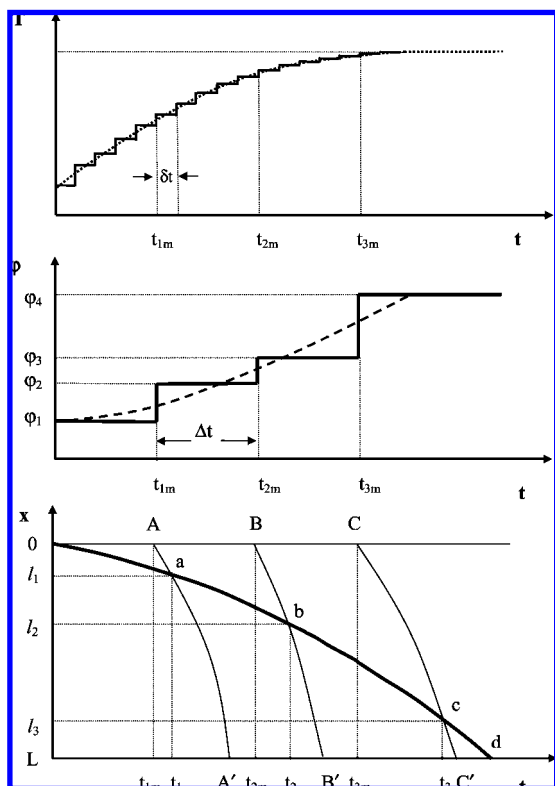
Due to the lack of an appropriate theory, the majority of reports concerning "dual-mode gradient"[6–22] are either empirical or pseudomultimode type in the following sense. For example, temperature gradients are applied under different isocratic conditions or solvent gradients under different isothermal conditions.

In two recent papers,[23,24] we have developed the theory of the dual-mode gradient elution in liquid chromatography involving any type of simultaneous changes in flow rate and mobile-phase composition based on Drake's approach.[25] Here, we extend this treatment to dual gradients composed of any type of solvent composition and temperature variations.

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Figure 1. Schematic representation of the movement of the fronts of the mobile phase when the φ vs t profile (broken line) at the inlet of the chromatographic column is approximated for simplicity by four steps under variable temperature and the corresponding path of an analyte inside the column shown by the thick line $0abcd$.

THEORETICAL SECTION

Basic Expressions for the Solute Retention. In previous studies,[23,24,26] we found that Drake's approach[25] is very effective for developing the fundamental equations of gradient elution. According to this approach, each gradient is approximated by a stepwise curve composed of a large number of very small time steps. In this respect, we consider that the curve of φ versus t is approximated by a stepwise curve of very small time steps, Δt, and similarly, the T versus t curve is approximated by a stepwise curve composed of infinitesimally small time steps, δt, where $\delta t \ll \Delta t$. Thus, the steps of the φ versus t curve occur at $t_{1m} = \Delta t = m\delta t$, $t_{2m} = 2\Delta t = 2m\delta t$, $t_{3m} = 3\Delta t = 3m\delta t$, ..., $t_{nm} = n\Delta t = nm\delta t$, where $m = \Delta t/\delta t$. The solute meets these steps at $t_1 = n_1\delta t$, $t_2 = n_2\delta t$, ..., $t_n = n_n\delta t$, where $n_1, n_2, ..., n_n$ are integers that should be determined.

Figure 1 depicts schematically the approximation of a φ versus t profile (broken line) by only four steps in φ, for the shake of simplicity, with time steps Δt and a T versus t profile (dotted line) approximated by a stepwise curve of time steps $\delta t = \Delta t/5$, i.e., $m = 5$. Due to the variations in the temperature, the fronts of the mobile phase may move with variable velocity and for this reason their representation in the plot of x versus t, where x is the distance from the beginning of the chromatographic column, is not linear. The fronts of the mobile phase are indicated by the curved lines AA′, BB′, and CC′, whereas the analyte follows inside the column the path $0abcd$ indicated by the thick line. In this figure a, b, and c are the points where the analyte meets the fronts of the mobile

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phase. In the simplified example of Figure 1, the steps of the φ versus t curve occur at $t_{1m} = 5\delta t$, $t_{2m} = 10\delta t$, $t_{3m} = 15\delta t$ and $t_1 = n_1\delta t = 6\delta t$, $t_2 = n_2\delta t = 12\delta t$, and $t_3 = n_3\delta t = 20\delta t$.

In order to determine the unknown integers $n_1, n_2, ..., n_n$ we may work as follows. Consider the second step with $\varphi = \varphi_2$ of the φ versus t curve in the simplified example of Figure 1. The beginning of this step moves along the curved line AA′ and its end follows the line BB′. The end of the step meets the analyte at $t_2 = n_2\delta t$ at the point b, whose distance from the inlet of the column is l_2. From Figure 1, we note that the end of the second step travels this distance, i.e., the distance Bb along the curve BB′, for a time period $t_2 - t_{2m}$. In the example of Figure 1 from t_{2m} to t_2, there are two time steps δt. In the first of these steps, the front of the mobile phase is moving with constant velocity equal to $L/t_{o,\varphi_2 T_{11}}$ and in the second with velocity equal to $L/t_{o,\varphi_2 T_{12}}$, where L is the column length and $t_{o,\varphi_2 T_j}$ is the column holdup time at $\varphi = \varphi_2$ and $T = T_j$, T_j being the jth step in the T versus t plot, since the column holdup time might depend on φ and T. Therefore, the distance l_2 may be calculated from

$$\frac{l_2}{L} = \frac{\delta t}{t_{o,\phi_2 T_{11}}} + \frac{\delta t}{t_{o,\phi_2 T_{12}}} = \sum_{i=2\cdot5+1}^{n_2} \frac{\delta t}{t_{o,\phi_2 T_i}} = \sum_{i=2\cdot m+1}^{n_2} \frac{\delta t}{t_{o,\phi T}} \quad (1)$$

where subscripts 2 and i in the last term of eq 1 have been eliminated for simplicity.

In addition, in the second step the analyte is under the influence of $\varphi = \varphi_2$ for time $t_2 - t_1 = (n_2 - n_1)\delta t$, and at each time step δt of this interval it moves with a constant velocity $v_a = L/t_{R,\varphi_2 T_j}$, where $t_{R,\varphi_2 T_j}$ is the solute retention time when $\varphi = \varphi_2$ and $T = T_j$ ($j = 7, 8, ..., 12$ or $j = n_1 + 1, ..., n_2$) are constant. Therefore, the distance traveled by the analyte during the time period $t_2 - t_1$ is equal to $l_2 - l_1$ given by

$$\frac{l_2 - l_1}{L} = \frac{\delta t}{t_{R,\phi_2 T_7}} + \frac{\delta t}{t_{R,\phi_2 T_8}} + \cdots + \frac{\delta t}{t_{R,\phi_2 T_{12}}} = \sum_{i=n_1+1}^{n_2} \frac{\delta t}{t_{R,\phi T}} \quad (2)$$

The above two equations are readily extended to the pth step of the φ versus t curve. We have

$$\frac{l_p}{L} = \sum_{i=pm+1}^{n_p} \frac{\delta t}{t_{o,\phi T}} \quad (3)$$

and

$$\frac{l_p - l_{p-1}}{L} = \sum_{i=n_{p-1}+1}^{n_p} \frac{\delta t}{t_{R,\phi T}} \quad (4)$$

If we combine these equations, we obtain the recursive relationship

$$\sum_{i=pm+1}^{n_p} \frac{1}{t_{o,\phi T}} = \sum_{i=n_{p-1}+1}^{n_p} \frac{1}{t_{R,\phi T}} + \frac{l_{p-1}}{L\delta t} \quad (5)$$

where $l_0 = 0$ and $n_0 = 0$. After rearrangement, eq 5 is simplified to

$$\sum_{i=n_{p-1}+1}^{n_p}\left(\frac{1}{t_{o,\phi T}}-\frac{1}{t_{R,\phi T}}\right)=\sum_{i=(p-1)m+1}^{pm}\frac{1}{t_{o,\phi T}} \tag{6}$$

which may be rewritten as

$$\sum_{i=n_{p-1}+1}^{n_p}\frac{k_{\phi T}}{t_{o,\phi T}(1+k_{\phi T})}=\sum_{i=(p-1)m+1}^{pm}\frac{1}{t_{o,\phi T}} \tag{7}$$

where the solute retention capacity $k_{\phi T}$ at each time interval δt is a function of φ and T defined from

$$k_{\phi T}=\frac{t_{R,\phi T}}{t_{o,\phi T}}-1 \tag{8}$$

Equation 7 allows for the determination of n_p and through eqs 3 and 4 for the calculation of the distance l_p. Moreover eq 7 may take the following approximate expression

$$\sum_{i=n_{p-1}+1}^{n_p}\frac{k_{\phi T}}{1+k_{\phi T}}=m,\ p=1,2,3,4, \tag{9}$$

which arises if we consider a mean value of t_o taking into account that it depends slightly on φ and T.

The Fundamental Equation. From eq 4 we obtain that the analyte is eluted when

$$\sum\frac{l_p-l_{p-1}}{L}=\sum_{p=1}^{n}\sum_{i=n_{p-1}+1}^{n_p}\frac{\delta t}{t_{R,\phi T}}\geq 1 \tag{10}$$

where n is the least number of terms of the sum that makes the above inequality valid. From this equation we obtain directly the fundamental equations of single gradient when either φ or T is constant.

(a) If T = constant, eq 10 yields

$$\sum_{p=1}^{n}\frac{(n_p-n_{p-1})\delta t}{t_o(1+k_p)}\geq 1 \tag{11}$$

which in the limit $\delta t\to 0$ results in the fundamental equation of gradient elution with changes in the mobile-phase composition only[26]

$$\int_0^{t_R}\frac{dt_c}{t_0(1+k_\phi)}=\int_0^{t_R-t_0}\frac{dt}{t_0 k_\phi}=1 \tag{12}$$

(b) If φ is constant, eq 10 is transformed to

$$\sum_{i=1}^{n_n}\frac{\delta t}{t_{o,i}(1+k_i)}\geq 1 \tag{13}$$

which exhibits the following integral expression[2,3,27]

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$$\int_0^{t_R}\frac{dt}{t_o(1+k)}=1 \tag{14}$$

Equation 10 may also take an integral expression as follows. If we consider that the quantity $\delta t_c=t_p-t_{p-1}$ is, in fact, an infinitesimal small quantity and make use of the first mean value theorem of integrals, we have

$$\sum_{i=n_{p-1}+1}^{n_p}\frac{\delta t}{t_{R,\phi T}}=\int_{t_{p-1}}^{t_p}\frac{dt}{t_{R,\phi T}(t)}=(t_p-t_{p-1})\frac{1}{t_{R,pT}}=\frac{\delta t_c}{t_o(1+k_p)} \tag{15}$$

Then eq 10 may be readily expressed as

$$\int_0^{t_R}\frac{dt_c}{t_o(1+k)}=1 \tag{16}$$

which is the fundamental equation of the dual gradient elution involving simultaneous changes in temperature and mobile-phase composition.

Note that the time parameter in eq 16 is t_c. The physical meaning of this parameter is discussed in ref 26. If an infinitesimal step lasts in the mixer Δt, the same step is transformed to Δt^* through eq 3 at the inlet of the chromatographic column, and finally, the analyte feels this step inside the column for a time period equal to δt_c. This means the initial φ versus t profile formed in the mixer is transformed to a new φ versus t^* profile at the beginning of the column, whereas inside the column the analyte is subject to a φ versus t_c profile.

It is evident that the application of eq 16 for the calculation of t_R requires the expression of t_c in terms of t or t^*. As in ref 26, this can be done numerically by integration of the δt_c versus Δt dependence determined via eq 9. However, a more direct calculation of t_R can be performed by means of eqs 3 and 9. The analyte is eluted when

$$l_{p-1}/L<1\ \text{and}\ l_p/L\geq 1 \tag{17}$$

Therefore, the retention time may be calculated from

$$t_R=n_p\delta t \tag{18}$$

with an accuracy of the order of Δt.

Hysteresis Phenomena. When a T-gradient is formed in the oven, the effective gradient the analyte experiences is different due to hysteresis phenomena. There are two hysteresis phenomena: There is a lag between the programmed T-gradient and the temperature in the oven, i.e., a program delay, and a lag between the oven temperature, actual temperature inside the oven, and the effective temperature the analyte feels in the column. The first hysteresis phenomenon can be treated by recording the actual temperature and use it in place of the programmed temperature. In what concerns the hysteresis between actual (T_a) and effective (T) temperature, we may assume that it is described by Newton's law

$$\frac{dT}{dt}=-h(T-T_a) \tag{19}$$
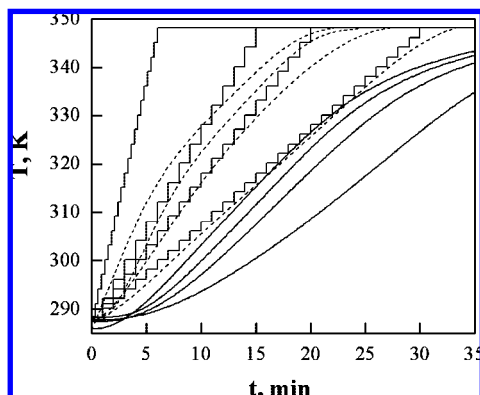
Figure 2. Four stepwise programmed gradients of temperature (−), the corresponding actual gradients (- - -) inside the oven, i.e., the oven temperature profiles, and the effective gradients (−) that the analyte experiences.

Table 1. Experimental Retention Times t_R (in min) of the Solutes under Dual-Mode Gradient Conditions

T-gradient	gT_1	gT_2	gT_3	gT_4
φ_{min}	0.4	0.4	0.4	0.4
φ_{max}	0.7	0.7	0.7	0.7
t, min	20	30	40	50
solute				
B	8.51	9.39	9.66	10.06
T	11.47	13.18	13.98	14.99
EB	14.47	17.19	18.73	20.63
iPB	17.06	20.71	22.99	25.83
PB	17.83	21.75	24.26	27.39
tBB	19.12	23.55	26.47	30.07

Table 2. Experimental Retention Times t_R (in min) of the Solutes Obtained under Isocratic and Temperature Gradient Conditions

T-gradient	φ	Solute					
		B	T	EB	iPB	PB	tBB
gT_1	0.50	6.363	9.545	14.107	19.577	21.523	25.284
gT_4	0.45	8.357	13.493	21.262	30.625	33.786	40.133

where h is an hysteresis constant characteristic of the chromatographic system. The T_a gradient can always be expressed as a power series of t

$$T_a = a_0 + a_1 t + \cdots + a_n t^n \quad (20)$$

Then eq 19 yields

$$T = \left(a_0 + a_1 t + a_2 t^2 + \cdots + a_n t^n\right) - \frac{1}{h}\left(a_1 + 2a_2 t + \cdots + n a_n t^{n-1}\right) + \frac{1}{h^2}\left(2a_2 + \cdots + n(n-1)a_n t^{n-2}\right) + \cdots \left(-\frac{1}{h}\right)^n$$
$$(n! \, a_n) + c e^{-ht} \quad (21)$$

where c is an integration constant. It is calculated from the initial condition $T(t=0) = T_a(t=0)$.

EXPERIMENTAL SECTION

The chromatographic system as well as the test mixtures of solutes used in this study is the same as that in ref 28. Thus, the liquid chromatography system consisted of a Shimadzu LC-20AD pump, a model 7125 syringe loading sample injector fitted with a 20 μL loop, a Zorbax SB-C18 column (3.5 μm, 150 × 4.6 mm) thermostated by a CTO-10AS Shimadzu column oven and a Shimadzu UV−visible spectrophotometric detector (model SPD-10A) working at 254 nm. The oven temperature was measured electronically during each chromatogram and a homemade mobile-phase preheating arrangement was used to achieve a better control of the column temperature. Note also that, according to the manufacturer, the column is stable at temperatures <90 °C. The solutes were a mixture of six nonpolar solutes: benzene (B), toluene (T), ethylbenzene (EB), isopropylbenzene (iPB), propylbenzene (PB), and *tert*-butylbenzene (tBB). Alkylbenzene sample solutions were prepared in neat acetonitrile at a concentration of 4.7 mM of each compound. The flow rate was 1.0 mL/min. The holdup time was estimated to be $t_0 = 1.37$ min, and the dwell time, t_D, was 1.1 min. Mobile phases were binary mixtures of acetonitrile−water.

In order to test the theory, we used four T-gradients each one coupled with one φ-gradient. The initial temperature in all T-gradients was 15 °C, the final was 75 °C, and the gradient time, i.e., the time between the initial and the final temperature, was 6 (gT_1), 15 (gT_2), 20 (gT_3), and 30 (gT_4) min. Note that, due to oven limitation, the programmed T-gradient profile was stepwise. The steps we used were 0.3 min for gT_1 and 1 min for the other gradients, gT_2−gT_4; see Figure 2. For the φ-gradients the acetonitrile content in the mobile phase was varied from $\varphi = 0.4$ to 0.7 at $t = 20$, 30, 40, and 50 min. The retention data of the solutes under the dual-mode gradients applied are given in Table 1. The combined changes of both T and φ alter the efficiency of the peaks, i.e., the ratio t_R/w_b, where w_b is the peak width at base. Thus, under isocratic/isothermal conditions, the average peak efficiency ranges from 42 to 44 when we pass from benzene to *tert*-butylbenzene at $\varphi = 0.4$ and from 34 to 51 at $\varphi = 0.7$. Under the combined T and φ conditions of the present work the peak efficiency ranges from 66 to 134.

The application of the theory to predict retention times requires the evaluation of the hysteresis constant h. For this reason, we have recorded two isocratic chromatograms under gT_1 and gT_4 gradient conditions. The retention data of these chromatograms are listed in Table 2.

The algorithms used for the determination of the effective temperature as well as for the retention prediction of solutes, according to the theory presented above, were homemade written in C++. The exe files of the basic programs are free available upon request from the authors.

RESULTS AND DISCUSSION

As pointed out in the Experimental Section, due to a limitation of the oven we used, the programmed T-gradient profiles were stepwise. The actual temperature, i.e., the real oven temperature, does not exhibit steps. Depending on the slope of the programmed gradient, a significant or insignificant lag between the oven temperature and the programmed gradient may occur. The real oven temperature is shown by the broken lines in Figure 2. These data were fitted to a sixth-order polynomial, and the coefficients of these polynomials are given in Table 3.

The actual temperature inside the oven is not expected to be identical to the effective temperature that the analyte feels inside

Table 3. Coefficients of the Actual (T_a) and the Effective (T) Temperature Gradients Inside the Oven[a]

gradient	a_0	a_1	$a_2 \times 10$	$a_3 \times 10^2$	$a_4 \times 10^3$	$a_5 \times 10^5$	$a_6 \times 10^7$	c
gT$_1$	285.8300	5.0601	4.2729	−12.1245	10.259	−37.900	51.60	−4697.5595
gT$_2$	288.3573	−2.3391	21.2149	−29.0660	18.707	−57.984	69.19	−7953.2481
gT$_3$	287.2482	0.9741	5.1990	−5.2962	2.577	−6.298	5.99	−994.4793
gT$_4$	287.6700	0.5493	2.9710	−2.6900	1.197	−2.520	1.95	−437.1989

[a] Calculated from eqs 20 and 21, Respectively, Using $h = 0.12$ min^{-1}.

Table 4. Coefficients of Eq 24 Obtained after Fitting to Isocratic/Isothermal Data Presented in Table 7 of ref 28

solute	c_1	c_2	c_3	c_4	c_5	c_6
B	−2.943	2592.57	0.077	2010.918	−0.343	307.213
T	−4.033	3491.376	−1.1	2841.285	−0.434	389.068
EB	−7.112	5035.241	−4.007	4253.887	−0.859	559.574
iPB	−10.536	6694.943	−7.36	5822.678	−1.128	679.404
PB	−11.408	7094.553	−8.158	6172.672	−1.18	701.224
tBB	−13.575	8108.077	−10.345	7163.753	−1.331	763.902

the column. To calculate this temperature by means of eq 21 we need to know the delay constant h and the integration constant c. The determination of h was based on the experimental data of Table 2, which were analyzed by means of eq 13 since φ is constant. This equation shows that under constant φ the retention time of an analyte, t_R, may be calculated from

$$t_R = n\delta t \quad (22)$$

where n is the least number of terms of the sum that makes the following inequality valid

$$\frac{\delta t}{t_0(1+k_{T_1})} + \frac{\delta t}{t_0(1+k_{T_2})} + \cdots + \frac{\delta t}{t_0(1+k_{T_n})} \geq 1 \quad (23)$$

Using $\delta t = 0.1$ min and a grid search for h, we found that the value of h that yields the minimum deviations between experimental and calculated t_R values is 0.12; that is, in our system we have $h = 0.12 \pm 0.02$ min^{-1}. Now the integration constant c of eq 21 is calculated from the initial condition $T(t{=}0) = T_a(t{=}0)$. The values of c obtained are also shown in Table 3.

The curves of the effective temperature the analyte feels inside the column calculated from eq 21 using data of Table 3 are shown in Figure 2. We observe that there is a significant lag between actual and effective temperature, which is independent of the extent of the lag between programmed and actual temperature inside the oven. The extent of this lag is significant, probably due

Table 5. Predicted Retention Data Obtained under Dual-Mode Gradient Conditions and Percentage Error between Experimental and Predicted Data When the Delay between Actual and Effective Temperature Is Taken into Account

T-gradient	gT$_1$		gT$_2$		gT$_3$		gT$_4$	
φ_{min}	0.4		0.4		0.4		0.4	
φ_{max}	0.7		0.7		0.7		0.7	
t, min	20		30		40		50	
solute	t_R (calc)	% error	t_R(calc)	% error	t_R(calc)	% error	t_R(calc)	% error
B	8.594	1.0	9.071	3.4	9.591	0.7	9.890	1.7
T	11.473	0.0	12.886	2.2	13.772	1.5	14.678	2.1
EB	14.586	0.8	16.883	1.8	18.571	0.8	20.380	1.2
iPB	17.183	0.7	20.376	1.6	22.977	0.1	25.570	1.0
PB	17.985	0.9	21.483	1.2	24.277	0.1	27.171	0.8
tBB	19.288	0.9	23.283	1.1	26.582	0.4	29.978	0.3
av % error		0.7		1.9		0.6		1.2

Table 6. Predicted Retention Data Obtained under Dual-Mode Gradient Conditions and Percentage Error between Experimental and Predicted Data When the Delay between Actual and Effective Temperature Is Ignored

T-gradient	gT$_1$		gT$_2$		gT$_3$		gT$_4$	
φ_{min}	0.4		0.4		0.4		0.4	
φ_{max}	0.7		0.7		0.7		0.7	
t, min	20		30		40		50	
solute	t_R(calc)	% error	t_R(calc)	% error	t_R(calc)	% error	t_R(calc)	% error
B	7.899	7.2	8.588	8.5	8.983	7.0	9.489	5.7
T	10.585	7.7	11.876	9.9	12.887	7.8	13.871	7.5
EB	13.481	6.8	15.572	9.4	17.279	7.7	19.281	6.5
iPB	15.970	6.4	18.978	8.4	21.374	7.0	24.280	6.0
PB	16.787	5.8	19.984	8.1	22.575	7.0	25.672	6.3
tBB	18.085	5.4	21.785	7.5	24.883	6.0	28.377	5.6
av % error		6.6		8.6		7.1		6.3

to the fact that we have used a column with diameter 4.6 mm, i.e., a rather large diameter. The use of small-diameter columns or, preferably, capillary columns is expected to reduce the lag between the oven temperature and the effective temperature. Moreover, another phenomenon similar to the ones above, a temperature gradient from the column walls to the column center may take place.[29] However, as we shall show below, the treatment presented in this work takes into account effectively the lag between actual (oven) and effective temperature, whereas it minimizes any effect from a possible radial gradient.

For the application of eqs 3, 9, 17, and 18 to predict t_R values under dual gradient conditions, we used $\Delta t = 0.1$ min and $\delta t = 0.000\,05$ min ($m = \Delta t/\delta t = 5000$) since, according to the theory, $\delta t \ll \Delta t$. Values of m higher than 5000 give practically converged results. At this point we should clarify the following. The programmed temperature gradients were composed of 0.3- or 1-min step changes. However, these steps are totally irrelevant to the δt time steps, which are the steps of the stepwise profile that approximates the actual (oven) T versus t profile (dotted lines in Figure 2).

The calculations for t_R need an analytical expression for $k_{\varphi T}$. In the present study, we adopted the following relationship.[28]

$$\ln k_{\phi T} = c_1 + \frac{c_2}{T} - \frac{\left(c_3 + \dfrac{c_4}{T}\right)\left[\exp\left(c_5 + \dfrac{c_6}{T}\right)\right]\phi}{1 + \left[\exp\left(c_5 + \dfrac{c_6}{T}\right) - 1\right]\phi} \quad (24)$$

where $c_1, c_2, \ldots, c_6$ are adjustable parameters. Equation 24 is an extension of the following equation[30–32]

$$\ln k_\phi = a + \frac{b\beta\phi}{1 + (\beta - 1)\phi} \quad (25)$$

if we assume that constants a and b depend linearly upon $1/T$, i.e., $a = c_1 + c_2/T$, $b = c_3 + c_4/T$, and a similar expression is valid for the equilibrium constant β of the retention process

$$\ln\beta = c_5 + \frac{c_6}{T} \quad (26)$$

For the determination of the adjustable parameters $c_1, c_2, \ldots, c_6$ of eq 24, we used the RND_LM algorithm described in ref 33 and based on the isocratic/isothermal data presented in ref 28. Their values are listed in Table 4.

Tables 5 and 6 summarize the comparison between calculated and experimental retention times obtained under dual-mode gradients examined when we take into account the delay

between actual and effective temperature (Table 5) and when we ignore it (Table 6). It is seen that the agreement is excellent in all-different types of dual-mode gradient programs tested provided that all hysteresis phenomena are properly embodied into the treatment. In this case, the average percentage error between experimental and predicted retention times is less than 2%. This small error is an indirect indication that the approach we used to take into account the hysteresis between the actual and the effective temperature (a) is very successfully applicable to columns with rather large diameters (5 mm) and (b) eliminates or minimizes effects from radial temperature gradients if such gradients do exist. In contrast, if we ignore the hysteresis between the actual and the effective temperature the prediction error may reach 10%.

To sum up, this paper continues our attempts to the development of a mathematical treatment describing the peak position within a chromatogram when two variables vary simultaneously with time. Up to now, this task has been accompliced for simultaneous flow rate and φ changes as well as T and φ changes. The fundamental equations of both these two dual gradient elution modes are identical to each other as well as to the single-mode gradient provided that the proper time variable is used. In the near future, we are going to extend these models in order to describe any multimode gradient elution in liquid chromatography.

GLOSSARY

L	column length
T	temperature
T_a	actual temperature inside the oven
a, b	coefficients of eq 25, which are functions of temperature
c	integration constant in eq 21
h	hysteresis constant characteristic of the chromatographic system
k	retention factor of the sample solute
k_p	retention factor of the sample solute during the pth step of the approximated stepwise φ vs t curve
k_φ	retention factor of the sample solute at constant φ value
k_T	retention factor of the sample solute at constant T value
$k_{\phi T}$	the solute retention factor at constant φ, T values
l_p	distance traveled by the mobile phase during the pth step of the approximated stepwise φ vs t curve until it meets the solute
m	integer equal to $\Delta t/\delta t$
n_i	integer numbers equal to $t_i/\delta t$
t	time variable
t^*	time variable that expresses the transformation of the initial φ vs t profile formed in the mixer to a new φ vs t^* profile at the beginning of the column
t_C	time variable that expresses the transformation of the initial φ vs t profile formed in the mixer to a new φ vs t_C profile that feels the analyte inside the column
t_D	dwell time
t_R	solute retention time
t_0	column holdup time
$t_{jm} = j\Delta t$ $= jm\delta t$	steps of the approximate stepwise φ vs t profile occuring at $t_{1m}, t_{2m}, t_{3m}, \ldots$

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$t_i = n_i \delta t$ — solute meets the steps of the φ vs t stepwise profile at $t_1, t_2, t_3, \ldots$

$t_{o,\varphi T}$ — column holdup time, which might depend on φ and T

$t_{R,\varphi T}$ — solute retention time when φ and T are constant

w_b — peak width at base

Δt — time steps of the stepwise φ vs t curve that approximates the original φ vs t curve formed in the mixer

β — equilibrium constant of the retention process

δl_p — distance traveled by the mobile phase at each δt time step from t_{pm} to t_p

δt — time steps of the stepwise T vs t curve that approximates the T vs t profile in the oven. It holds $\delta t \ll \Delta t$

δt_c — $= t_p - t_{p-1}$

φ — volume fraction of the organic modifier in the mobile phase