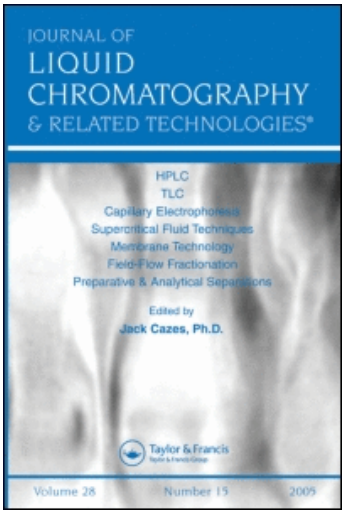

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Prediction of the Gradient Retention Times of Purine Compounds in Reversed Phase High Performance Liquid Chromatography

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Abstract: Various retention models have been developed and adopted to predict the retention behaviors of solutes in high performance liquid chromatography. Although the retention times can be successfully predicted in a gradient elution, it is difficult to predict the fundamental parameters of the eluted peaks, such as the number of theoretical plates, the resolution between neighboring peaks, as well as asymmetry factors. Thus far, the Snyder retention model has widely been used to predict the retention behavior in both isocratic and gradient conditions. However, it has a critical defect in that it cannot closely follow the retention behaviors of solutes when a relatively low content of organic modifier is used in the mobile phase. This is a result of the nonlinear relationship between the logarithm value of the retention factor and the volumetric percentage of organic modifier in the mobile phase. To overcome this shortcoming, a modified retention model was adopted to predict the retention times in several linear gradient conditions. A numerical method that transforms any gradient condition into discrete step gradient conditions was also proposed to predict the retention times in a gradient elution. The model is suitable to apply to nonlinear and multilinear gradient conditions, including actual obtained gradient profiles. Two kinds of organic modifiers, methanol and acetonitrile, were employed, and four purine compounds were used as solutes. The predicted retention times obtained by the modified retention model are in good agreement with experimental data in terms of the achieved gradient conditions.

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Keywords: Gradient elution, HPLC, Migration velocity, Prediction of elution band, Prediction of retention time, Retention model

INTRODUCTION

In reversed phase high performance liquid chromatography (RP-HPLC), a gradient elution is commonly used to enhance the separation and detection capacities.[1] The gradient elution is achieved by programmed separation conditions such as the changes of mobile phase composition, column temperature, or flow rate of the mobile phase. The mobile phase composition is principally changed during the elution step to control the elution times and resolutions of solutes. Thus far, linear programmed gradient elution, which is performed to change the composition of mobile phase linearly during the gradient elution step, has frequently been applied, as using it simplifies the estimation of the retention behavior based on the tendency of isocratic retention. In addition, the gradient condition of the mobile phase composition is easy to operate.

In the analytical HPLC, adsorption behavior can be assumed as a linear isotherm because a small amount and concentration of analytes is injected. The partition coefficient of the solute is changed when the mobile phase composition, particularly the content of the organic modifier in RP-HPLC, is changed. Several retention models that explain the relationship between the retention factor of the solute and the mobile phase composition have been proposed. Snyder et al. proposed an empirical linear relationship between the logarithm of the retention factor and the volumetric fraction of an organic modifier.[2] This model is commonly used to predict the retention times of a solute in isocratic and gradient conditions and also used to measure the octanol/water partition coefficient of the solute.[3,4] However, it is difficult to estimate the retention factor with this model when a small amount of organic modifier is used. To overcome this problem, Row et al. proposed a Langmuir-type retention model.[5,6] It is important to predict the retention factors of solutes because the mobile phase composition is usually changed by the diluted content of organic modifier when gradient elution is used for the analysis. Many researchers have attempted to predict the retention time in gradient elution liquid chromatography. However, it is difficult to develop an analytical solution of the migration trace in a chromatographic column. Therefore, numerical methods to predict the retention time in a gradient elution have been developed and applied to optimize the mobile phase composition of a gradient elution.[7–10] In a previous work by the authors, an analytical solution of the migration trace of solutes in a chromatographic column was developed in relation to an ideal multi-step gradient elution.[11–13] Even if

the composition of the mobile phase is changed linearly, the actual mobile phase composition does not linearly change much owing to the mixing of different composition mobile phases in the mixer. However, any gradient elution can be divided into discrete step gradients during the gradient time.

In order to predict the retention time in a gradient elution, experimental data of isocratic elutions are required to determine the parameters of the retention model. However, it is difficult to obtain experimental data with a low content of organic modifier in the mobile phase because of the low detection of the elution profile resulting from the longer retention time and the broader bandwidth. Therefore, a modified retention model was validated for the extrapolation of a retention factor, and a numerical method to predict the retention time in a gradient elution is proposed and applied to predict the retention time of purine compounds in gradient elution RP-HPLC.

THEORY

Migration Velocity

The migration velocity of the solute is derived from a simple wave equation that is based on a material balance equation concerning only convection.[14] Equation (1a) shows the material balance of solutes in a chromatographic column:

$$\frac{\partial c}{\partial t} + \left(\frac{u}{1+\beta K}\right)\frac{\partial c}{\partial x} = 0 \tag{1a}$$

$$a_S = \frac{u}{1+\beta K} \tag{1b}$$

where c is the concentration of the solute in the mobile phase, t is the duration time from the feed injection, u is the interstitial velocity of the mobile phase, x is the axial distance of the column, a_S is the migration velocity of the solute in the chromatographic column, β is the phase ratio, and K is the partition coefficient of the solute. When the volumetric flow rate is constantly maintained during operation, u is obtained by the column length (L) and the dead time (t_0) of the column ($u = L/t_0$). When the composition of the mobile phase is constantly maintained, the denominator of Equation (1b) corresponds with the retention time (t_R) in an isocratic elution ($a_S = L/L/t_R$). By Equation (1b), it can be assumed that the migration velocity is constant under a constant composition of the mobile phase.

Retention Models

In this study, two retention models are applied to predict the migration velocity of a solute. Equation (2), proposed by Snyder et al., presents the linear relationship of the logarithm of the retention factor and the volumetric fraction of the organic modifier:[2]

$$\ln k = \ln k_w + S\varphi \tag{2}$$

where k is the retention factor, k_w is the retention factor with pure water as the mobile phase, S is the empirical coefficient, and φ is the volumetric fraction of organic modifier. In the linear isotherm, the migration velocity has a relationship with the retention factor as defined in Equation (1b). Therefore, the migration velocity is expressed as:

$$a_S = \frac{L}{t_R} = \frac{L/t_0}{1 + k_w e^{S\varphi}} \tag{3}$$

where L is the length of the column and t_0 is the dead time of the column. Equation (3) is a well known growth function. It has a symmetric shape centering on the inflection point. Row et al. reported that the retention model, Equation (2), is not suitable for application to some mobile phase conditions with a low content of organic modifier.[5,6] With low organic modifier content, the calculated retention factors from Equation (2) have much larger errors compared to conditions with a high content of organic modifier in the reversed phase condition. To reduce these errors, a dwindling rate parameter is considered to regulate two parameters, k_w and S, of Equation (2). The modified retention model of Equation (2), which builds on the three parameter retention models that have been proposed by previous researchers,[7,8] is proposed as follows:

$$\ln k = \frac{\ln k_w - S\varphi}{1 + k_S\varphi} \tag{4}$$

where k_S is the empirical coefficient for the dwindling rate of parameters k_w and S. When the k_S value reaches zero, this model is identical to Equation (2). With Equation (4), it is possible to obtain a curved trend line in the plane of $\ln k$ and φ. The k_w values represent the hydrophobicity of the compounds. However, it is difficult to present the hydrophobicity of compounds only using the k_w value. Numerous studies have been carried out in an effort to determine the relationship between the k_w value and the n-octanol/water partition coefficient.

Migration Trace in a Chromatographic Column

Common gradient elutions can be estimated by infinitely discrete step gradient elution. Therefore, step gradient elution is utilized to predict the gradient elution retention time. Figure 1 shows a schematic migration trace of the solute in a chromatographic column with step gradient elution. In a single step gradient elution, it is assumed that the organic modifier is not adsorbed on the stationary phase and that the second mobile phase changes identically over the entire axial position of the column. In other words, the dispersion or mixing effect is negligible when the composition of the mobile phase changes. In order to obtain the migration trace of a solute in a multi step gradient elution, the nth step-gradient condition was utilized. The coordinate of the solute on the boundary between two mobile phases in a time axial distance plane can be obtained by the following equations:

$$\tau_{b,(n-1)} = \frac{a_0 t_{sg(n-1)} + \theta_{(n-1)}}{a_0 - a_S(\varphi_{(n-1)})} \tag{5}$$

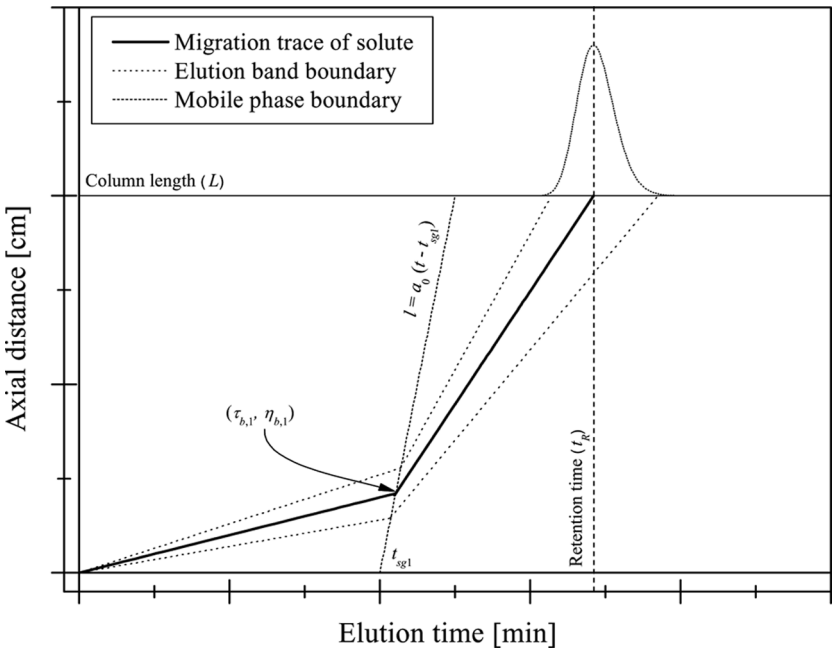

Figure 1. Schematic illustration of the migration trace of the solute band in a chromatographic column under step gradient elution.

$$\eta_{b,(n-1)} = a_0(\tau_{b,(n-1)} - t_{sg(n-1)})$$
$$= \frac{a_0}{a_0 - a_S(\varphi_{(n-1)})}\left(a_S(\varphi_{(n-1)})t_{sg(n-1)} + \theta_{(n-1)}\right) \quad (6)$$

where $\tau_{b,(n-1)}$ and $\eta_{b,(n-1)}$ are the elapsed time from a sample injection and the axial distance of the column from the column inlet when the solute is located on the boundary between $(n-1)$th and the nth mobile-phase composition, and $t_{sg(n-1)}$ is the time when the nth mobile phase reaches the column inlet. From this boundary position $(\tau_{b,(n-1)}, \eta_{b,(n-1)})$, the next migration trace function is derived as:

$$l(\varphi_{(n)}) = a_S(\varphi_{(n)})t + \theta_{(n)} \quad (7)$$

$$\theta_{(n)} = \frac{a_0 t_{sg(n-1)}}{a_0 - a_S(\varphi_{(n-1)})}(a_S(\varphi_{(n-1)}) - a_S(\varphi_{(n)})) + \frac{a_0 - a_S(\varphi_{(n)})}{a_0 - a_S(\varphi_{(n)-1})}\theta_{(n-1)} \quad (8)$$

where $\theta_{(n)}$ is the intercept of the nth migration trace function and $l(\varphi)$ is the migration trace function of the solute. The boundary position is obtained by the previous step gradient elution trace and the boundary of the next step gradient. In a gradient elution, the coordinates of a migration trace can be obtained by the calculation of τ_b and η_b during a given time t.

Gradient Profile

In actual conditions, the mobile phase composition is not linearly changed during linear gradient elution due to the dispersion occurred by the slope of the gradient profile and the mixing of different compositions of mobile phase in the system, such as the mixer, fittings, and tubes. Generally, a packed bed filled with inert material is used to mix two different mobile phases homogenously. It is assumed that the mobile phase components do not interact with the stationary phase. The dispersion of the mobile phase is negligible, as the mobile phase passes through the chromatographic column rapidly. Therefore, the shapes of the inlet and outlet gradient profiles of the column are assumed identical. In a step gradient elution, the actual inlet gradient profile can be estimated by the following cumulative distribution function:[15]

$$\varphi(t) = \varphi_I + \frac{\Delta\varphi}{1 + e^{-\frac{4a_g}{\Delta\varphi}(t - t_{sg})}} \quad (9)$$

where a_g is the slope of the actual step gradient profile and φ_I and φ_F are the volumetric fraction of organic modifier in the initial

and final mobile phase ($\Delta\varphi = \varphi_F - \varphi_I$), respectively. The actual linear gradient profile is obtained by the coordinate transformation as follows:

$$t' = t + \frac{\Delta t'_g}{\Delta\varphi}\left(\varphi - \frac{\Delta\varphi}{2}\right) + t'_{g,S} + \Delta t'_g + t_{Mix} \tag{10a}$$

$$\varphi' = \frac{\Delta\varphi'}{\Delta\varphi}\varphi + \varphi'_S \tag{10b}$$

where t' and φ' are the coordinates of the transformed gradient profile, t and φ are the coordinates of the original gradient profile, $\Delta\varphi$ and $\Delta\varphi$' are the differences of the level of organic modifier in the original and transformed gradient profiles, respectively, $\Delta t'_g$ is the gradient time interval of the transformed gradient profile, $t'_{g,S}$ is the gradient start time of the transformed gradient profile, and t_{Mix} is the dwell time of the system.

EXPERIMENTAL

Four purine compounds (adenine, theobromine, theophylline, and caffeine) were purchased from Sigma (St. Louis, MO, U.S.A.) and dissolved in deionized water to 50 μg/mL. Deionized water and HPLC grade methanol and acetonitrile purchased from Duksan Pure Chemical (Kyunggi-do, Korea) were used as a mobile phase. A Shimadzu HPLC system composed of two LC-6AD pumps, a SIL-10Avp autosampler, a SPD-M10Avp PDA detector, and a Younglin CTS30 column oven were used. A Waters Symmetry C_{18} column (150 × 4.6 mm, 5 μm) was also used.

A mixture solution of four purine compounds mixed with equivalent volumes of pure purine components was injected into the column in a quantity of 8.0 μL and 2.0 μL single solutions of each purine compound. The column temperature was maintained at 30°C and the flow rate of the mobile phase was fixed at 1.0 mL/min. The several isocratic runs were carried out with the contents of methanol and acetonitrile as 10% to 25% and 3% to 24%, respectively. In addition, the intervals of the methanol and acetonitrile contents in isocratic runs were 2.5% and 3.0%, respectively. All gradient runs were carried out by mixing pure water and 30% methanol and acetonitrile in the water. From the breakthrough curves without a column installed, the dwell time of the system was measured as 4.385 min and the dead time of the column was measured as 1.434 min with a KNO_3 injection.

RESULT AND DISCUSSION

It is well known that the retention factor exponentially decreases when the organic modifier content in the mobile phase increases in reversed phase liquid chromatography. However, many researchers have tacitly assumed that the exponential decrement rate of the retention factor is constant. This assumption is suitable when a high content of organic modifier is used as a mobile phase. Contrarily, when a low content of organic modifier is used, it is inadequate to assume that the decrement rate of the retention factor is constant.[9,10] The modified retention model, Equation (4), forms a rational function, and the empirical constant (k_S) in this model determines the nonlinearity between the logarithm of the retention factor and the organic modifier content. To determine the coefficients of two retention models, different ranges of acetonitrile and methanol content were used. The lower bound of acetonitrile (3%) is lower than that of methanol (10%), whereas the upper bound of acetonitrile and methanol are similar at 24% and 25%, respectively. Table 1 shows the empirical coefficients of the two retention models. When acetonitrile is used as an organic modifier, the retention factors of four purine compounds change nonlinearly with variations of the mobile phase composition. Therefore, it is difficult to fit the experimental retention factor to Equation (2) as referred with the regression coefficients. Nonlinear retention behaviors can also be observed with the k_S values of Equation (4); the acetonitrile case is approximately two times higher than the methanol case. Equation (4) precisely follows the experimental retention factors from both the acetonitrile and methanol cases (the regression coefficients of Equation 4) are higher than 0.999).

Table 1. Empirical coefficients of the retention models, Equations (2) and (4)

	Equation (2)			Equation (4)			
Materials	$\ln k_w$	S	$^*R^2$	$\ln k_w$	S	k_S	$^*R^2$
Methanol							
Adenine	1.511	0.0779	0.99292	2.093	0.1101	0.0271	0.99951
Theobromine	2.354	0.1006	0.99151	3.225	0.1363	0.0315	0.99946
Theophylline	2.830	0.1017	0.99185	3.672	0.1225	0.0301	0.99932
Caffeine	3.848	0.1184	0.99123	4.902	0.1265	0.0324	0.99947
Acetonitrile							
Adenine	1.143	0.1199	0.95106	2.076	0.2484	0.0664	0.99845
Theobromine	1.794	0.1477	0.95295	2.916	0.2771	0.0646	0.99977
Theophylline	2.265	0.1586	0.95538	3.419	0.2706	0.0616	0.99983
Caffeine	3.175	0.1717	0.95206	4.504	0.2532	0.0659	0.99991

*: Regression coefficient.

To calculate the retention factor from the retention time in an isocratic elution, the dead time of the column was measured as 1.434 min (void fraction is 0.58). Normally, the total void fraction of commercial C_{18} column ranges from 0.75 to 0.80. Therefore, the measured void fraction appears as the inter-void fraction of the column. However, here it was assumed that the organic modifier is not retained on the stationary phase. Moreover, the time difference between the breakthrough curves obtained with and without a column connection was found to have the same value of the measured dead time of the column. The migration velocity was utilized to predict the retention time and migration trace in the column. As shown in Equation (3), the migration velocity can be obtained by the ratio of the column length and the retention time (L/t_0). Therefore, the apparent dead time was used in this work. Furthermore, the prediction procedure becomes complicated if the dead time and the mobile phase retention time have different values.

In order to validate the extrapolation over the experimental range (for methanol, 10 to 25 vol. % and for acetonitrile, 3 to 24 vol. %), the $\log k_w$ values obtained from Equation (2) and Equation (4) were compared. The $\log k_w$ of the solutes are important values to determine the *n*-octanol/water partition coefficient indirectly. These values must be identical whenever any organic modifier is used. This implies that the ratio of the $\log k_w$ values from the methanol and acetonitrile cases must be 1. Table 2 presents a comparison of the $\log k_w$ values obtained from different organic modifiers (methanol and acetonitrile). The $\log k_w$ values obtained from Equation (4) are much higher compared to those from Equation (2), due to steep changes of the retention factor in below the experimental range of the organic modifier contents. Furthermore, the $\Delta \log k_w$ values of Equation (4) are closer to 1.0 compared to those of

Table 2. Comparisons of $\log k_w$ values obtained from different organic modifiers

	$\log k_w$ from Equation (2)			$\log k_w$ from Equation (4)		
Materials	MeOH	ACN	$\delta \log k_w$	MeOH	ACN	$\delta \log k_w$
Adenine	0.66	0.50	0.758	0.91	0.90	0.989
Theobromine	1.02	0.78	0.765	1.40	1.27	0.907
Theophylline	1.23	0.98	0.797	1.59	1.48	0.931
Caffeine	1.67	1.38	0.826	2.13	1.96	0.920
Average			0.786			0.937
**?			0.032			0.036

qg: Ratio of $\log k_w$ values between acetonitrile and methanol cases.
qq: Standard deviation.

Equation (2) (the average is 0.937 and the standard deviation is 0036). Hence, it can be said that Equation (4) follows the retention behavior more closely than Equation (2) below the experimental range of the organic modifier contents. Thus, Equation (4) can provide precise $\log k_w$ values to determine the *n*-octanol/water partition coefficient, and can extrapolate the retention factor below the experimental range.

Fourteen linear gradient runs, including eight runs for methanol and six runs for acetonitrile as an organic modifier, were carried out to compare the experimental and calculated retention times in a gradient elution. Table 3 shows the gradient conditions of each run. All linear gradient elutions started from a methanol content of 1.5% (the minimum content of organic modifier to control) and changed from 0.0 min. The dwell time of this HPLC system is 4.385 min, and thus, the composition of the mobile phase changes 4.385 min later. The dwell time was measured from the breakthrough curve of a step gradient elution without a column installation, and a sigmoid function was used to estimate the actual gradient profile of the organic modifier. Figure 2 shows good agreement between the actual and estimated

Table 3. Linear-gradient conditions

	Linear-gradient condition for methanol			
Run No.	Initial (min)	MeOH (%)	Final (min)	MeOH (%)
Run 1	0.0	1.5	10.0	10.5
Run 2	0.0	1.5	20.0	10.5
Run 3	0.0	1.5	10.0	16.5
Run 4	0.0	1.5	20.0	16.5
Run 5	0.0	1.5	10.0	22.5
Run 6	0.0	1.5	20.0	22.5
Run 7	0.0	1.5	10.0	28.5
Run 8	0.0	1.5	20.0	28.5
	Linear-gradient condition for acetonitrile			
Run No.	Initial (min)	ACN (%)	Final (min)	ACN (%)
Run 9	0.0	1.5	10.0	9.0
Run 10	0.0	1.5	20.0	9.0
Run 11	0.0	1.5	10.0	15.0
Run 12	0.0	1.5	20.0	15.0
Run 13	0.0	1.5	10.0	24.0
Run 14	0.0	1.5	20.0	24.0

*: Dwell volume of system is 4.385 ml.

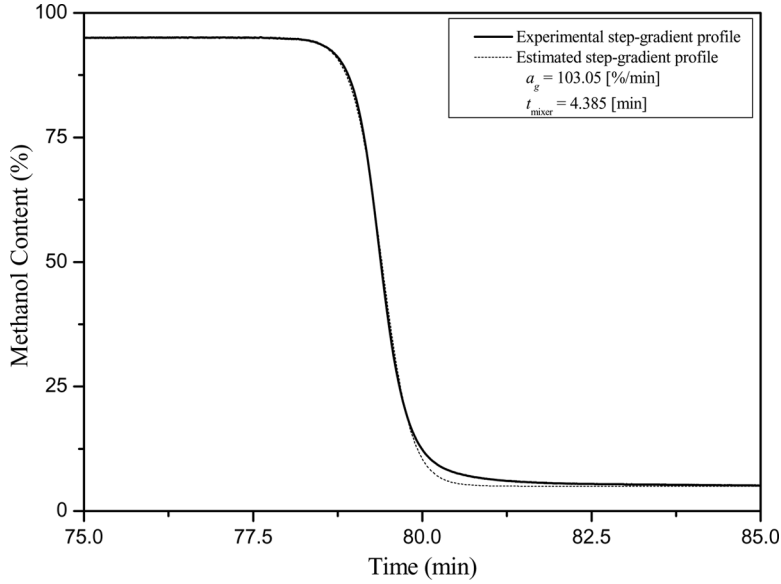

Figure 2. Comparison of the experimental and estimated gradient profiles. The thick solid line is the experimental step gradient profile when the methanol content of the mobile phase changes from 95% to 5% at 75 min, and the thin dotted line is the estimated step gradient profile by Equation (9).

gradient profiles of the organic modifier in a step gradient elution. The obtained actual slope of the step gradient elution was 103.05%/min. Linear gradient profiles were converted from the sigmoid gradient profile via a transformation of rectangular coordinates into oblique coordinates. This gradient profile was then no longer linear gradient. Therefore, it is difficult to obtain an analytical solution for the retention time of a gradient elution. A new prediction method that transforms gradient profiles into discrete step gradient profiles was adopted to predict the retention time in a sigmoid gradient profile. The discrete time interval was fixed to 0.01 min.

Table 4 shows comparisons of the experimental and predicted retention times for Runs 1 and 9 (Table 3). These two gradient runs were carried out under the lowest content of organic modifiers. In particular, Run 1 was carried out with parameters out of the experimental range. The lowest bound of the experimental range is 10%, but the gradient range of Run 1 is 1.5 to 10%. Equation (4) was adopted to predict the retention times, yielding values within 1.85% and 4.36% average error for Runs 1 and 9, respectively. With these results, it is verified that the three

Table 4. Comparisons of experimental and predicted retention times in Run 1 and 9 (Table 3)

Run No.	Retention model	Retention time				Average
		Adenine	Theobromine	Theophylline	Caffeine	
Run 1	*Exp.	10.073	14.981	18.356	30.909	
	Equation (2)	7.097	12.098	15.900	27.636	
	Equation (4)	9.386	14.931	18.300	30.928	
	**Error (2)	29.55	19.25	13.38	10.59	18.19
	**Error(4)	6.82	0.33	0.30	0.06	1.85
Run 9	Exp.	8.171	11.029	13.045	17.259	
	Equation (2)	5.187	7.939	10.521	16.386	
	Equation (4)	7.538	10.540	12.593	16.953	
	**Error (2)	36.52	28.02	19.35	5.06	22.23
	**Error(4)	7.74	4.43	3.47	1.78	4.36

*: Experimental data.
**: $|(t_{R,Exp} - t_{R,Cal})/t_{R,Exp} \times 100|$.

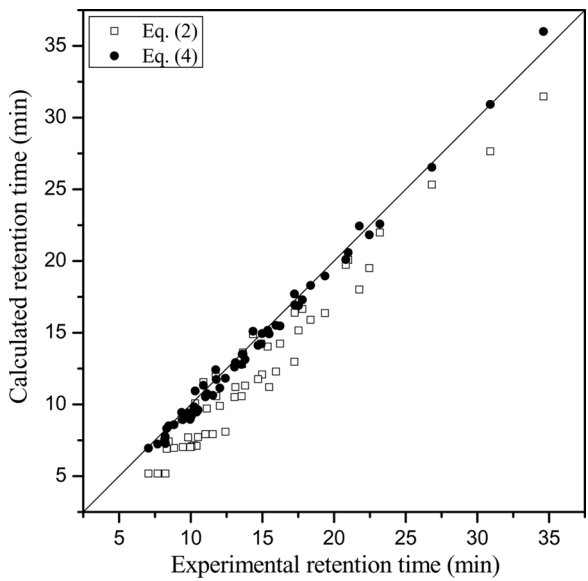

Figure 3. Comparison of the experimental and calculated retention times in linear gradient runs (Table 3). The solid line shows the diagonal, open squares as calculated by Equation (2), and the closed circles are calculated by Equation (4).

parameter retention model, Equation (4), can precisely extrapolate the retention factor below the experimental data to obtain the parameters of the retention model, including $\log k_w$. However, the errors in the predicted retention times of the fastest eluted compound (adenine) are generally larger compared to those of other compounds. In the beginning of the gradient elution, 5% of a premixed solution containing 30% organic modifier was used as the mobile phase. Therefore, it is thought that unexpected behaviors occurred as a result of shock when two different mobile phases are mixed at the start point of the linear gradient condition. Figure 3 shows a comparison between the calculated retention times and the experimental retention times for the achieved gradient conditions. The predicted retention times by Equation (2) are lower than the experimental retention times, as all gradient runs were carried out with a lower organic modifier content than the isocratic elution to obtain the parameters of the two retention models. The average error of the retention time predicted by Equation (4) is nearly four times smaller than that of Equation (2) (the average errors of Equation (2) and (4) are 17.5% and 4.0%, respectively).

CONCLUSIONS

In this work, the three parameter retention model modified from Equation (2), which is a very well known retention model, is proposed to explain the nonlinear relationship between the logarithm of the retention factor and the volumetric fraction of the organic modifier. This retention model is suitable to extrapolate $\log k_w$ values and for a prediction of the retention behaviors below the experimental range. Therefore, when this modified retention model is used, the $\log k_w$ values that are used to determine the *n*-octanol/water partition coefficients can be more precisely estimated compared to the use of Equation (2). The numerical method for the prediction of the retention time in a gradient elution can provide predicted retention times in any combination of gradient conditions, such as multiple linear or curvilinear gradient conditions, by an approximation of the discrete step gradient conditions and a simple coordinate transformation from the actual step gradient profile, as expressed by Equation (9). Four purine compounds were used as solutes and two organic modifiers (methanol and acetonitrile) were individually used as organic modifiers in the experiments to validate the modified retention model. The calculated results obtained by the modified retention model and the numerical prediction method were in good agreement with the experimental data, with a 4.0% average error of the retention time.

NOMENCLATURE

a_0 the migration velocity of unretained solute = u [cm/min]
a_g the slop of the actual step-gradient profile [%/min]
a_S the migration velocity of the solute in the column [cm/min]
c the concentration of the solute in the mobile phase of the column [mg/ml]
K the partition coefficient of the solute [-]
k the retention factor of the solute = (tR-t0)/t0 [-]
k_S the dwindling rate constant of k_w and S in Equation (4) [-]
k_w the retention factor of the solute when pure water is used as the mobile phase [-]
L the length of the column [cm]
$l(\varphi)$ the migration trace function of the solute [cm]
S the empirical constant in Equation (1) [-]
t the elapsed time from the feed injection [min]
t' the elapsed time of the transformed gradient profile [min]
t_0 the dead time of the column [min]
t_{Mix} the dwell time of the system [min]
t_R the retention time of the solute [min]
$t_{sg(i)}$ the time when the ith step-gradient mobile phase reaches the column inlet [min]
$\Delta t'_g$ the gradient time interval of the transformed gradient profile [min]
$t'_{g,S}$ the gradient start time of the transformed gradient profile [min]
x the axial distance of the column from the inlet [cm]
u the interstitial velocity of the mobile phase in the column = L/t_0 [cm/min]
$\eta_{b,(i)}$ the axial distance of the column from the inlet when solute meets the ith step-gradient mobile phase in the column [cm]
φ the volumetric fraction of the organic modifier in the mobile phase [%]
φ' the volumetric fraction of the transformed gradient profile [%]
$\Delta\varphi$ the differences in the organic modifier in the original gradient profiles [%]
$\Delta\varphi$' the differences in the organic modifier in the transformed gradient profiles [%]
φ_I the volumetric fraction of the initial organic modifier in step-gradient [%]
φ_F the volumetric fraction of the final organic modifier in step-gradient [%]
θ_i the intercept of the solute migration trace function in the ith step-gradient mobile phase [cm]

$\tau_{b,(i)}$ the time when the solute meets the ith step-gradient mobile phase in the column [min]

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