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Prediction of the Elution Profile of Aromatic Compounds in RP-HPLC

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Abstract: An efficient prediction method for the elution profile was used to separate aromatic compounds such as benzene, toluene, chlorobenzene, *o*-xylene, and 1,2-dichlorobenzene by RP-HPLC. The retention factor and bandwidth were predicted under linear-gradient condition with the three retention models. The elution profiles were calculated based on the linear and quadratic equations of retention factor, $\ln k = \ln k_w + S\varphi$, $\ln k = L + M\varphi + N\varphi^2$, $k = A + B/\varphi$, where φ was the vol.% of methanol. The elution profiles were calculated by the Gaussian distribution with obtained retention factor and bandwidth. Two kinds of experiments were performed; one is the isocratic runs to estimate the coefficients of three retention models, and the other is the linear gradient runs that were carried out with same initial mobile phase composition (water/methanol = 96/4, vol.%), two final mobile phase compositions (water/methanol = 24/76 and 40/60, vol.%), and three gradient times (20, 40, and 60 min). The predicted elution profiles by the three retention models and new prediction method have good agreement with experimental data in the employed gradient conditions. The minimum average errors of calculated and experimental results of aromatic compounds were lower than 3.5% by Bi-poly equation.

Keywords: Retention factor, bandwidth, elution profile, aromatic compounds, RP-HPLC

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INTRODUCTION

A widely used parameter in reversed-phase high-performance liquid chromatography (RP-HPLC) is the retention factor (k) of a compound in isocratic elution (1). Many chemical substances are actually complex mixtures of organic chemicals and environmental contamination is almost invariably caused by mixtures of compounds. Recently, several experimental methods were developed for which a fractionation according to hydrophobicity is required for complex mixtures, which contain organic micropollutants of very diverse structures. This fractionation can be used to perform tests for the presence of potentially bioconcentrating compounds in environmental samples or to obtain information on the hydrophobicity distribution profile of complex mixtures of unknown composition (1).

Optimization in RP-HPLC involves the selection of the optimum experimental conditions for adequate separation and acceptable retention time for each individual sample. Nevertheless, obtaining a balance between resolution and analysis time is not always easy in chemical and pharmaceutical laboratories. An efficient optimization method should be employed during the method development process in order to deal with these optimization problems. Computers have been used as an aid in HPLC method development since the late 1970s (2–4). Such software developments have been frequently demonstrated, with an increasing range of applications (5–9). The basic function enables the prediction of sample retention times with given mobile phase composition, as well as column efficiency, resolution, and sample elution profile in specific and optimized operating conditions.

In isocratic elution, the mobile phase composition is unchanged during the separation. The various sample components have a wide range of k values. However, the disadvantages of the isocratic mode are poor resolution of early-eluting bands, broadening of late-eluting bands making detection difficult, tailing peaks, and unnecessarily long separation time. These disadvantages are often overcome by changing the solvent strength during the operation. Gradient elution is usually performed by changing the mobile phase compositions. The changes in the solvent strength can be made stepwise or continuously. Gradient elution offers several advantages: total analysis time can be significantly reduced, overall resolution of a mixture is increased, peak shape is improved (less tailing), and effective sensitivity is increased since there is little variation in peak shape. More importantly, it provides the maximum resolution per unit time. Optimization of gradient elution is very important for analytical HPLC and scale-up column chromatography. The theory of the gradient elution process contains two general problems. The first one is connected with a total theory of solute migration under stepwise gradient conditions. Under the assumption that the relationships between the capacity factor and the composition of the mobile phase are known, this problem was considered in. To calculate the retention of solutes in the gradient program having five steps, Markowski and Golkiewicz

obtained the analytical expressions (10). The second problem is to predict the value of the retention factor for any compositions of the multi-component mobile phase by empirically determined equations. More often the correlations are based on a linear dependence of $\ln k$ via the content of one or more of the components in the mobile phase for binary and ternary mixtures (11).

In isocratic elution, when the adsorption isotherm is assumed as linear, the retention behavior of the solute has a relationship with the composition of the mobile phase, especially the content of organic modifier in RP-HPLC. The migration velocity also can be assumed as constant in the chromatographic column. Several retention models that explained the relationship between the retention factor of the solute and the volumetric fraction of organic modifier in the mobile phase had been proposed. Snyder et al. proposed the empirical linear relationship between the logarithm of retention factor and the volumetric fraction of organic modifier (12). This model is commonly used to predict the retention factor and measure the hydrophobicity of the solute. However, it is hard to estimate the retention factor with this model when a low content of organic modifier is used (13). So, Row et al. proposed as the retention model Langmuir-type relationship between the retention factor and the volumetric fraction of organic modifier (14).

By combining the retention model and migration trace in the chromatographic column, many researchers have tried to predict the retention time in gradient elution liquid chromatography. However, it is difficult to develop the analytical solution of the migration trace. Therefore, empirical or numerical methods to predict retention time in gradient elution have been developed and applied to optimize the mobile phase composition of gradient elution (15–17). In our previous work, we have developed the analytical solution of the migration trace of solutes in the chromatographic column concerning the multi-step gradient elution (18–20). Even though the composition of the mobile phase is changed linearly, the actual mobile phase composition is not exactly changed linearly because of the mixing effect of the system mixer and the dispersion of modifier in the column. However, any gradient elution can be divided into the discrete step gradient elution during the gradient time. In this paper, a new method to predict the retention time and bandwidth in gradient elution is proposed and applied to predict the retention time, retention factor, and the bandwidth of aromatic compounds in linear-gradient elution RP-HPLC. Furthermore, the experimental and calculated elution profiles of the aromatic compounds were compared.

THEORETICAL BACKGROUND

The retention factor, k , can be calculated as $(V_R - V_0)/V_0$ and it is related to the partition coefficient of the solute between the stationary phase and the mobile phase and to the free energy change associated with the

chromatographic process (21). Thus, the solute retention is affected by the thermodynamics of distribution between the two phases. The retention volume in the isocratic mode is expressed by the retention factor as follows:

$$V_{r,n} = V_0(1 + k_n) \quad (1)$$

where $V_{r,n}$, and k_n are the retention volume and retention factor in the n th mobile phase composition, respectively, and V_0 is the dead volume of unretained compound. The prediction of retention time under gradient conditions has been described by assuming that a gradient step is similar to a sequence of short isocratic steps. A modified equation is proposed for predicting the retention volume of step-gradient elution (18):

$$V_{R,g} = V_0(1 + k_2) + (k_1 - k_2) \frac{V_{g,1}}{k_1} \quad (2)$$

where k_1 and k_2 are the retention factors in the first and second mobile phase compositions, respectively, and are obtained by Eq. (2). $V_{g,1}$ is the volume of the first mobile phase in the step-gradient elution passing through an inlet of the chromatographic column until the second mobile phase is introduced to the column inlet. It can be calculated by the summation of the HPLC mixer volume and gradient volume. In the case of the linear-gradient mode, the mobile phase composition changes gradually and continuously. As the linear-gradient mode may be envisaged as the infinite small segments of a step gradient, Eq. (2) is modified and extended to a linear-gradient mode. The retention volume in the linear-gradient mode can be calculated (20):

$$V_{R,g} = V_{r,\infty} + (V_{r,\infty} - V_0) \sum_{i=1}^{\infty} \frac{V_{g,i}(V_{r,i} - V_{r,i+1})}{(V_{r,i} - V_0)^2} \quad (3)$$

The number of theoretical plates, N , is calculated in isocratic mode as:

$$N = 16 \left(\frac{t_R}{w} \right)^2 \quad (4)$$

and it is assumed to be independent of the mobile phase composition throughout this work. It was obtained by the average value from several runs. In gradient mode, the number of theoretical plates was calculated by substituting t_R into t_{Rg} in Eq. (4), w was calculated by substituting t_R into t_{Rg} or $t_{R,n}$ in Eq. (3) according to mobile phase shape and inserting the average value of N . The resolution between components 1 and 2 is given by:

$$R_{12} = \frac{2(t_{R1} - t_{R2})}{w_1 - w_2} \quad (5)$$

The optimum resolution was obtained by calculating the retention time and peak width from Eqs. (2)–(4). According to the plate theory, the chromatographic column is mathematically equivalent to a plate column where the total length is divided into N plates. It is assumed that instantaneous

equilibrium is established for the solute between mobile and stationary phases. A material balance on solute around the plate N leads to the following equation (22):

$$C_N = C_0 \sum_{i=N-r}^{N-1} \frac{(av)^i}{i!} e^{-av} \quad (6)$$

where c_N is the outlet concentration of solute, c_0 the initial concentration, v is the volume of the mobile phase which flows through the column, and a is the following equilibrium constant,

$$a = \frac{1}{v_m + Kv_s} \quad (7)$$

where v_m and v_s are the volume of mobile and stationary phases in a theoretical plate, respectively. Eq. (6) enables the prediction of the concentration elution profile for each component. The equilibrium constant (K) is correlated in terms of partition coefficient as:

$$K = k \left(\frac{\varepsilon}{1 - \varepsilon} \right) \quad (8)$$

where ε is the total porosity of the chromatographic column, and it is assumed to be 0.75.

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 (21). Eq. (9) is the material balance of solutes in the chromatographic column:

$$\frac{\partial c}{\partial t} + \left(\frac{u}{1 + \beta K} \right) \frac{\partial c}{\partial x} = 0 \quad (9a)$$

$$a_s = \frac{u}{1 + \beta K} \quad (9b)$$

where c is the concentration of the solute in the mobile phase, t is the duration time from feed injection, u is the interstitial velocity of the mobile phase, x is the axial distance of 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 composition of the mobile phase is constantly maintained, the denominator in Eq. (9b) is t_R/t_0 and $a_s = L/t_R$. In Eq. (9b) the migration velocity is assumed to be constant under constant composition of the mobile phase.

Retention Models

In this work, the logarithmic retention factor, k , is correlated by the following equations involving the vol.% (φ) of the organic modifier (13):

$$\ln k = \ln k_w + S\varphi \tag{10}$$

$$\ln k = L + M\varphi + N\varphi^2 \tag{11}$$

$$k = A + B/\varphi \tag{12}$$

where k is the retention factor, k_w is the retention factor with pure water as the mobile phase, A , B , L , M , N , $\ln k_w$, and S are empirical constants which should be experimentally determined. Eqs. (10), (11), and (12) were applied to the binary mobile phase in RP-HPLC. In this work, the above three equations are called Snyder, Bi-poly, and Langmuir equations, respectively.

Migration Trace in a Chromatographic Column

Common gradient elution can be estimated by infinitely discrete steps gradient elution. Therefore, we consider step gradient elution to predict the gradient retention time. Figure 1 shows the schematic migration trace of the solute in the chromatographic column with step gradient elution. In single step-gradient elution, it is assumed that the organic modifier is not adsorbed on the stationary phase and the second mobile phase is identically changed

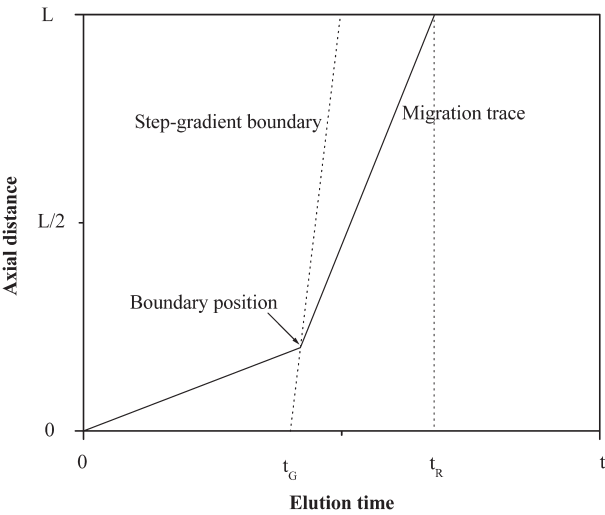


Figure 1. Schematic illustration of the migration trace of solute in the column under step-gradient elution.

through the whole axial position of the column. In other words, the dispersion or mixing effect is negligible when the mobile phase composition is changed. The position of the solute on the boundary between two mobile phases can be obtained by the intersection of two straight lines as shown in Fig. 1:

$$\tau_{b,1} = \frac{a_0}{a_0 - a_S(\varphi_1)} t_{sg1} \quad (13)$$

$$\eta_{b,1} = a_0(\tau_{b,1} - t_{sg1}) = \frac{a_0 a_S(\varphi_1)}{a_0 - a_S(\varphi_1)} t_{sg1} \quad (14)$$

where $\tau_{b,1}$ and $\eta_{b,1}$ are the time and axial distance when the solute is located on the boundary of the 1st step-gradient and t_{sg1} is the time when the 2nd mobile phase flows into the column inlet. From this boundary position, the second migration trace function is derived as

$$l(\varphi_2) = a_S(\varphi_2)t + \theta_2 \quad (15a)$$

$$\theta_2 = \eta_{b,1} - a_S(\varphi_2)\tau_{b,1} = \frac{a_0 t_{sg1}}{a_0 - a_S(\varphi_1)} (a_S(\varphi_1) - a_S(\varphi_2)) \quad (15b)$$

where θ_2 is the intercept of the 2nd 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 next step-gradient. With the same process, the boundary position of the end of $n - 1$ th step-gradient elution and the intercept of migration function in the n th step-gradient elution are obtained as

$$\tau_{b,(n-1)} = \frac{a_0 t_{sg(n-1)} + \theta_{(n-1)}}{a_0 - a_S(\varphi_{(n-1)})} \quad (16)$$

$$\eta_{b,(n-1)} = a_0(\tau_{b,(n-1)} - t_{sg(n-1)}) = \frac{a_0}{a_0 - a_S(\varphi_{(n-1)})} (a_S(\varphi_{(n-1)})t_{sg(n-1)} + \theta_{(n-1)}) \quad (17)$$

$$l(\varphi_{(n)}) = a_S(\varphi_{(n)})t + \theta_{(n)} \quad (18)$$

$$\begin{aligned} \theta_{(n)} = & \frac{a_0 t_{sg(n-1)}}{a_0 - a_S(\varphi_{(n-1)})} (a_S(\varphi_{(n-1)}) - a_S(\varphi_{(n)})) \\ & + \frac{a_0 - a_S(\varphi_{(n)})}{a_0 - a_S(\varphi_{(n-1)})} \theta_{(n-1)} \end{aligned} \quad (19)$$

where a_0 is the migration velocity of unretained solute, the subscript $(n - 1)$ and (n) represent $n - 1$ th and n th step-gradient elution. In 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, because of the dispersion and mixing effect between different compositions of mobile phase. Generally, a packed bed filled with inert material is used to mix two different mobile phases homogeneously. It is assumed that the mobile phase components have no interaction with the stationary phase. The dispersion of mobile phase is negligible, because the mobile phase passes through the chromatographic column rapidly. Therefore, the shapes of the inlet and outlet gradient profiles of the column are assumed to be identical. In step-gradient elution, the actual inlet gradient profile is estimated by the following growth function:

$$\varphi(t) = \varphi_I + \frac{\Delta\varphi}{1 + e^{(-4a_g/\Delta\varphi)(t-t_{sg})}} \quad (20)$$

where a_g is the slope of actual step-gradient profile and φ_I and φ_F are the volumetric fraction of the organic modifier in the initial and the final mobile phase ($\Delta\varphi = \varphi_F - \varphi_I$), respectively. The actual linear gradient profile is obtained by the transformation of rectangular coordinate to the oblique coordinate.

Elution Profile

The Gaussian distribution function is the most widely used function to describe the elution profile in analytical conditions (21). However, it has a symmetric band profile centering on the highest point of the peak. In gradient elution, the front and rear bandwidths are generally not equivalent. Therefore, it is difficult to represent the elution profile of the solute in gradient elution. In order to describe the asymmetric profile, the following modified Gaussian distribution is used:

$$c = \frac{A_0}{w_{1/2}\sqrt{\pi/2}} \exp\left(-2\left(\frac{t-t_R}{w_{1/2} + As(t-t_R)}\right)^2\right) \quad (21)$$

where c is the concentration of analyte at the outlet of column, A_0 is the area of the peak profile, $w_{1/2}$ is the mean value of w_f and w_r , and As is the coefficient for the asymmetry of the peak shape ($As = (w_b - w_f)/(w_b + w_f)$).

EXPERIMENTAL

Reagents and Chemicals

Analytical grade solvents, five standard aromatic compounds, benzene, toluene, chlorobenzene, *o*-xylene, and 1,2-dichlorobenzene were purchased

from Ducksan Pure Chemical (Kyungki-Do, Korea). HPLC grade solvent, methanol was from Ducksan Pure Chemical (Kyungki-Do, Korea). Twice distilled water was filtered by decompressing pump (Division of Millipore, Waters, USA) and filter (FH-0.45 μm).

Sample Preparation

Five 0.025 mg, standard aromatic compounds were dissolved in 1 ml of methanol, and the concentration of the solutions was adjusted to 25 $\mu\text{g/ml}$. The constant 10 μl injection volume of mixtures solution was used throughout.

Apparatus and Method

The HPLC experiments were performed with Shimadzu LC-6AD pump and SIL-10Avp autosampler (Younglin, Korea) and SPD-M10Avp PDA detector (Younglin, Korea) and CTS30 column oven (Younglin, Korea). Sufficient times were allowed for the stabilization of the column and detector signal after each injection, and the solvents in the reservoirs were continuously stripped with helium to degas the mobile phase. The flow rate of the mobile phase was 1 ml/min and was monitored at the fixed wavelength of 270 nm. The column was purchased from RS-tech Co (Daejeon, Korea). The Waters symmetry column size was 0.46×15 cm and packed by C_{18} , 100 Å, 5 μm . All the experimental runs were carried out at 30°C.

To calculate a retention factor, the hold-up time of the used HPLC system should be measured. From the breakthrough curves without column installing, the mixer volume of system was measured as 4.385 ml, and the dead time of the column was measured as 1.434 min with KNO_3 injection. Three replicate injections were made to determine the retention time of each substance, and the average values were used to calculate the retention factors.

RESULTS AND DISCUSSION

The gradient elution is achieved by the programmed separation conditions such as changes of mobile phase composition, column temperature, and flow rate of mobile phase. The mobile phase composition is principally changed during the elution step to control the elution bandwidth and resolutions of solutes. Until now, in linearly programmed gradient elution, the composition of mobile phase is changed linearly during the gradient elution step because it is simple to estimate the retention behavior based on the tendency of isocratic retention and easy to operate the gradient condition of the mobile phase composition.

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 (7). 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. On the contrary, when a low content of organic modifier is used, it is inadequate to assume that the decrement rate of retention factor is constant (19, 20). To determine the coefficients of all three retention models, different range of methanol contents were used. Table 1 shows the retention factors of aromatic compounds in isocratic elution. The elution order of the five aromatic compounds was benzene, toluene, chlorobenzene, *o*-xylene, and 1,2-dichlorobenzene, and it was not changed with mobile phase composition. The retention factor decreased when the methanol contents increased. The empirical coefficients of three retention models were estimated by nonlinear regression method with the experimental retention factors in Table 1. The agreements of calculated and experimental retention factors were higher than that of the other two equations. Table 2 shows the empirical coefficients of three retention models. According to the regression coefficients (R^2) in Table 2, the calculated retention factors of aromatic compounds by Bi-poly equation, Eq. (11), showed close agreement with the experimental data as compared with the other two retention models.

To determine the separation conditions of the five aromatic compounds, several experimental runs were performed in isocratic mode. The required data for the elution profile included the retention factors, factor of front side and back side, column dead volume, column specification (diameter and length), packing diameter, and mobile phase flow rate to predict elution profiles through the chromatographic column. If the retention factor is expressed as a function of mobile phase composition, the elution profile of a solute can be estimated for any change in mobile phase composition by the prediction method.

In Fig. 2, the experimental and calculated retention factors for all the studied compounds by using the Snyder Eq. (10), Bi-poly Eq. (11), and

Table 1. Retention factor (k_R) of aromatic compounds in isocratic elution

MeOH (vol.%)	Benzene	Toluene	Chlorobenzene	<i>o</i> -Xylene	1,2-Dichlorobenzene
36	4.82	11.23	12.71	22.81	29.23
44	3.12	6.59	7.21	12.26	14.43
52	2.00	3.85	3.95	6.54	7.17
60	1.24	2.15	2.16	3.46	3.49
68	0.77	1.24	1.21	1.85	1.79
76	0.50	0.74	0.70	1.03	0.97

Table 2. Parameters of the Snyder, Bi-poly, and Langmuir equations on content of methanol mobile phases

	k_w	s	—	r^2
Eq. (10)				
Benzene	36.50	2.44×10^{-2}	—	0.9997
Toluene	129.28	2.94×10^{-2}	—	0.9998
Chlorobenzene	175.50	3.16×10^{-2}	—	0.9998
<i>o</i> -Xylene	379.82	3.39×10^{-2}	—	0.9999
1,2-Dichlorobenzene	693.88	3.82×10^{-2}	—	0.9999
Eq. (11)				
	L	M	N	r^2
Benzene	1.47	-2.03×10^{-2}	-4.22×10^{-5}	0.9999
Toluene	2.04	-2.61×10^{-2}	-3.56×10^{-5}	0.9999
Chlorobenzene	2.17	-2.84×10^{-2}	-3.54×10^{-5}	0.9999
<i>o</i> -Xylene	2.56	-3.30×10^{-2}	-1.00×10^{-5}	0.9999
1,2-Dichlorobenzene	2.88	-3.99×10^{-2}	1.85×10^{-5}	0.9999
Eq. (12)				
	A	B	—	r^2
Benzene	-3.64	300.30	—	0.9934
Toluene	-9.53	726.30	—	0.9810
Chlorobenzene	-11.17	830.96	—	0.9734
<i>o</i> -Xylene	-20.55	1498.77	—	0.9652
1,2-Dichlorobenzene	-27.30	1932.76	—	0.9476

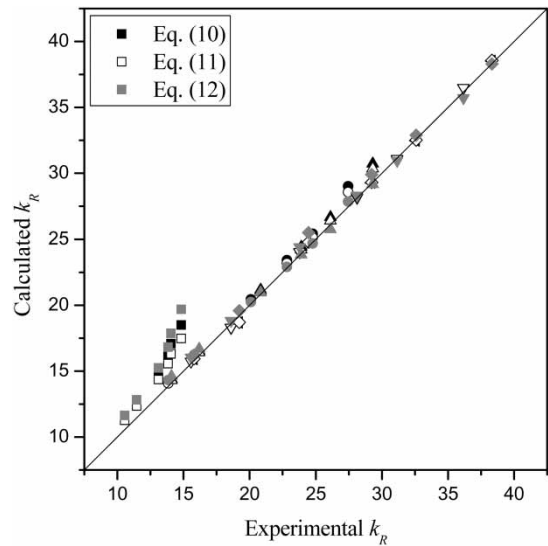


Figure 2. Comparisons of the experimental and calculated retention factors by Snyder, Bi-poly, and Langmuir equations at methanol modifier. Squares, circles, triangles, reversed triangles, and diamonds represent benzene, toluene, chlorobenzene, *o*-xylene, and 1,2-dichlorobenzene, respectively.

Langmuir Eq. (12) equations were compared. These aromatic compounds exhibited good resolutions only in the water-rich mobile phase, but simultaneously, the retention time was very long. Therefore, the separation condition of the five aromatic compounds was determined to be gradient mode.

Six linear gradient runs for methanol was carried out to compare the experimental and calculated retention times in gradient elution. All linear gradient runs were started from 4% methanol content (the minimum content of organic modifier to control) and changed from 0.0 min. The volume of mixer in this HPLC system is 4.385 ml, so the composition of mobile phase is actually changed 4.385 min later. Mixer volume is measured from breakthrough curve of step gradient elution without column installation and sigmoid function is used to estimate actual gradient profile of organic modifier. The actual and estimated gradient profile of organic modifier in step-gradient elution has a good agreement. The obtained actual slope of step-gradient elution is 103.05%/min. The linear gradient profiles are converted from this sigmoid gradient profile by the transformation of rectangular coordinate into oblique coordinate. This gradient profile is not any more linear gradient. Therefore, it is hard to get an analytical solution for the retention time of the gradient elution. A new prediction method that transforms gradient profiles into discrete step-gradient profiles is adopted to predict retention time in sigmoid gradient profile. The discrete time interval is fixed to 0.01 min.

Figure 3 shows the calculated and experimental chromatograms for the five aromatic compounds in the gradient 4–60% methanol in 60 min. The calculated values were obtained by the Gaussian equation by using Eq. (10)–Eq. (12). However, on the gradient condition, the quadratic Eq. (11) gave a better fit with the experimental data. Some of the deviation from the experimental data might be attributed to the curvature of the gradient (gradient dispersion or mixing) at the junction of a segmented gradient. In the experimental conditions, the agreement between the experimental data and the calculated values was relatively good.

Table 3 also shows the average errors of predicted retention factors in achieved linear gradient elution. In all six runs, Bi-poly Eq. (11) has good agreements with experimental data. The average errors were increased with the increasing the gradient time. Average percentage errors of retention times were 2.96% and 3.32% at respectively two different mobile composition conditions. All linear gradient conditions are started from 4% of organic modifier content and this mobile phase composition is maintained at least during 4.385 min. With this result, it is able to conceive Bi-poly retention model, Eq. (11), to precisely extrapolate the retention factor in this content of the organic modifier. However, the errors of predicted retention times of the fastest eluted compound (benzene) are mainly bigger than other compounds. It is supposed that some shocks occurred by demixing of two different mobile phases when the linear gradient is started.

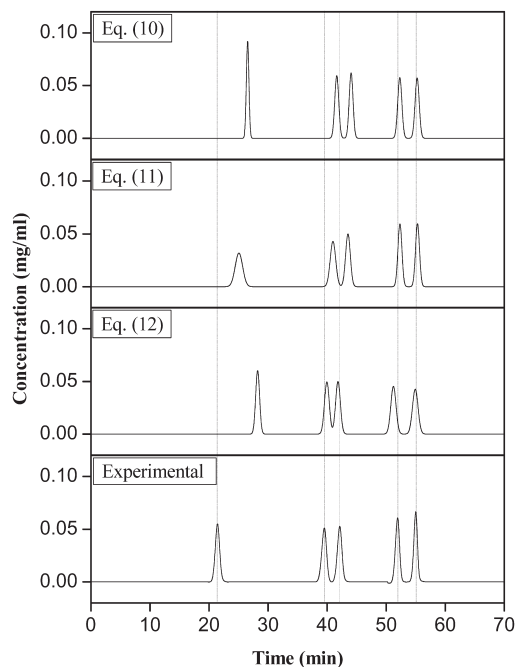


Figure 3. Contrast of experimental and calculated elution profile of aromatic compounds by linear gradient from 4% to 60% methanol in water at 60 min.

Table 3. The average errors of calculated and experimental retention factors for the two gradient conditions

Mobile phase composition	Gradient time (min)	Eq. (10)	Eq. (11)	Eq. (12)
Average error (%); Methanol from 4 to 76%	20	2.88	2.41	4.63
	40	3.80	2.83	4.91
	60	5.17	3.65	5.13
Average errors (%); Methanol from 4 to 60%	20	1.95	1.35	4.19
	40	4.51	3.23	4.92
	60	7.34	5.41	6.50
Average errors (%)		4.60	3.33	5.20

CONCLUSION

A new prediction method for gradient elution can predict the retention times and bandwidth in any combination of gradient conditions, such as multiple

linear or curvilinear gradient conditions by the approximation of discrete step-gradient conditions. Based on the retention theory, elution profiles were predicted by introducing the concept of solute migration in the mobile phase with the linear and quadratic dependency of $\ln k$ in terms of the organic modifier content. The recommended experimental conditions of the mobile phase composition and gradient step were suggested, and the elution profiles calculated by the Bi-poly equation of $\ln k$ showed better coincidence with the experimental data than the linear correlation did. When five aromatic compounds are used as solutes and the organic modifiers (methanol) are individually used as organic modifier, the calculated results obtained by the Bi-poly equation and the prediction method have good agreements with experimental data within lower than 3.5% of the average error in achieved linear gradient runs.

NOMENCLATURE

a_0	the migration velocity of unretained solute, $= u$ [cm/min]
a_g	the slop of actual step-gradient profile [%/min]
a_S	the migration velocity of solute in the column [cm/min]
c	the concentration of solute in the mobile phase of the column [mg/ml]
K	the partition coefficient of solute [–]
k	the retention factor of the solute, $= (t_R - t_0)/t_0$ [–]
k_S	the dwindling rate constant of k_w and S in Eq. (10) [–]
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 solute [cm]
S	the empirical constant in Eq. (10) [–]
t	the duration time from feed injection [min]
t_0	the dead time of the column [min]
t_B	the time of back side band [min]
t_F	the time of front side band [min]
t_{Gi}	the time when i th gradient mobile phase flows into the column inlet [min]
t_R	the retention time of the solute [min]
t_{sgi}	the time when i th step-gradient mobile phase flows into the column inlet [min]
u	the interstitial velocity of the mobile phase in the column, $= L/t_0$ [cm/min]
x	the axial distance of the column from inlet [cm]
$\eta_{b,1}$	the axial distance when solute meets the i th step-gradient mobile phase in the column [cm]

φ	the volumetric fraction of organic modifier in the mobile phase [%]
φ_I	the volumetric fraction of initial organic modifier in step-gradient [%]
φ_F	the volumetric fraction of final organic modifier in step-gradient [%]
θ_i	the intercept of solute migration trace function in i th step-gradient mobile phase [cm]
$\tau_{b,i}$	the time when solute meets the i th step-gradient mobile phase in the column [min]

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