

Gradient RP-HPLC에서 퓨린 유도체의 용출 곡선 예측

이주원, 노경호*
 인하대학교 화학공학과
 (rowkho@inha.ac.kr*)

Prediction of Elution Profiles of Purine Compounds in Gradient RP-HPLC

Ju Weon Lee, Kyung Ho Row*
 Department of Chemical Engineering, Inha University
 (rowkho@inha.ac.kr*)

Introduction

The partition coefficient of solute is changed when the mobile phase composition, especially the content of organic modifier in RP-HPLC, is changed. Several retention models that explained the relationship between the retention factor of solute and the mobile phase composition had been proposed. Snyder et al. proposed the empirical linear relationship between the logarithm of retention factor and the volumetric fraction of organic modifier [1]. This model is commonly used to predict the retention times of solute in isocratic and gradient condition and also used to measure the octanol/water partition coefficient of solute. However, it is hard to estimate the retention factor with this model when low organic modifier is used. To overcome this controversial point, Row et al. proposed the retention model that is called Langmuir-type retention model [2]. Numerical methods to predict retention time in gradient elution have been developed and applied to optimize the mobile phase composition of gradient elution [3]. In our previous work, we have developed the analytical solution of migration trace of solutes in the chromatographic column concerning the ideal multi-step gradient elution [4]. 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 of different composition mobile phases in the mixer. However, any gradient elution can be divided into the discrete step gradient elution during the gradient time.

To determine the optimum gradient condition of the mobile phase, two important properties of elution profile, such as the retention time and the bandwidth, are considered as the run time and resolutions between neighboring peaks. Therefore, the bandwidth of elution profile must be predictable in gradient condition. van Deemter et al. [5] also proposed that HETP is related on the flow velocity of the mobile phase, which was widely applied in column separation. With the analog that the migration velocity becomes fast with high flow velocity of the mobile phase or high content of organic modifier, it is presupposed that HEPT will relies on the migration velocity of solute.

Theory

The migration velocity of solute is derived from the simple wave equation that is based on the material balance equation concerning only convection. Eq. (1) is the migration velocity derived from simple wave equation of solute in the chromatographic column:

$$a_s = u / (1 + \beta K) \quad (1)$$

where u is the interstitial velocity of the mobile phase, a_s is the migration velocity of solute in the chromatographic column, β is the phase ratio, and K is the partition coefficient of 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 Eq. (1b) is same with the retention time (t_R) in isocratic elution ($a_s = L / t_R$). By the Eq. (1), we can assume that the migration velocity is constant under the constant composition of the mobile phase. In this study two retention models are applied to predict the migration velocity of solute. Eq. (2) proposed by Snyder et al. presents the linear relationship of the

logarithm of retention factor and the volumetric fraction of organic modifier [1]:

$$\ln k = \ln k_w + S \varphi \quad (2)$$

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 linear isotherm, the migration velocity has a relationship with retention factor as following Eq. (1). Eq. (2), is not agreeable to apply to some mobile phase condition with low content of organic modifier [3, 4]. In low content of organic modifier case, calculated retention factors from Eq. (2) have much bigger errors than high content of organic modifier in reversed-phase condition. To reduce these errors, new dwindling rate parameters are considered to regulate two parameters, k_w and S , of Eq. (2), respectively. A modified retention model of Eq. (2) is proposed as follows,

$$\ln k = (\ln k_w + S \varphi) / (1 + k_S \varphi) \quad (3)$$

where k_S is the empirical coefficient for the dwindling rate of parameters k_w and S . When k_S value is reached to zero this model is identically same with Eq. (2). With Eq. (3), we can obtain a curved trend line in the plane of $\ln k$ and φ . Common gradient elution can be estimated by the infinitely discrete step gradient elution. Therefore, we consider the step gradient elution to predict the gradient elution retention time. 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 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 solute on the boundary between two mobile phases can be obtained by following equations:

$$\tau_{b,(n-1)} = \frac{a_0 t_{sg(n-1)} + \theta_{(n-1)}}{a_0 - a_S(\varphi_{(n-1)})} \quad (4a)$$

$$\eta_{b,(n-1)} = a_0 (\tau_{b,(n-1)} - t_{sg(n-1)}) = \frac{a_0}{a_0 - a_S(\varphi_{(n-1)})} (a_S(\varphi_{(n-1)}) t_{sg(n-1)} + \theta_{(n-1)}) \quad (4b)$$

where $\tau_{b,(n-1)}$ and $\eta_{b,(n-1)}$ are the time and axial distance when solute is located on the boundary of $(n-1)^{\text{th}}$ step-gradient and $t_{sg(n-1)}$ is the time when n^{th} mobile phase flows into the column inlet.

In analytical HPLC system, few amount of sample solution is injected to the column. However, the elution profile at column outlet is dispersed because of dispersion. The migration velocity is linearly changed with the flow velocity of the mobile phase. Therefore, van Deemter Eq. is rewritten as follows:

$$H = A + B / a_S + C a_S \quad (5)$$

where H is HETP, A , B , and C are coefficients for eddy diffusion, longitudinal diffusion, and mass transfer resistance, respectively. Because the concentration of solute is very low in the column, the changes of diffusion or dispersion rate is negligible when solute band passes through the column. Therefore, it can be assumed that the band broadening rate is constant under the same mobile phase condition.

In actual condition, 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. Usually a packed bed filled with inert material is used to mix two different mobile phases homogenously. 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 inlet and outlet gradient profiles of column are supposed to be same. In step-gradient elution, actual inlet gradient profile is estimated by the following sigmoidal function:

$$\varphi = \varphi_I + \Delta\varphi / (1 + \exp(4 a_G (t - t_{sg}) / \Delta\varphi)) \quad (6)$$

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

Results and Discussions

Newly proposed retention model, Eq. (3), forms rational function and new empirical constant (k_S) determines the nonlinearity between logarithm of retention factor and organic modifier content. To determine the coefficients of two retention models, different range of acetonitrile and methanol contents were used. Under bound of acetonitrile (3%) is lower than it of methanol (10%), but upper bound of acetonitrile and methanol is almost similar as 24% and 25%, respectively. Table 1 shows the empirical coefficients of two retention models. When acetonitrile is used as organic modifier, the retention factors of four purine compounds are nonlinearly changed with the variation of mobile phase composition. Therefore, it is difficult to fit the experimental retention factor to Eq. (2) as compared with the regression coefficients. And the nonlinearities of retention behaviors also can be observed with the k_S values of Eq. (3); acetonitrile case is approximately two times higher than methanol case.

Table 1 Empirical Coefficients of the retention models, Eq. (2) and (3)

(* : regression coefficient)

Materials	Methanol						
	Eq. (2)			Eq. (3)			
	$\ln k_w$	S	R^2	$\ln k_w$	S	k_S	R^2
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

Materials	Acetonitrile						
	Eq. (2)			Eq. (3)			
	$\ln k_w$	S	R^2	$\ln k_w$	S	k_S	R^2
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

relation between HETP and the migration velocity to predict HETP in different mobile phase composition. Table 2 shows the coefficients of Eq. (5). With this relationship, the band broadening rate can be predictable in certain mobile phase composition. Fig. 2 shows the comparison of experimental HETP to Eq. (9) with obtained coefficients. When acetonitrile is used as organic modifier, HETP is lower than methanol is used. It is empirically appropriate.

Table 2 Coefficients of van Deemter equation in methanol and acetonitrile

	$A (\times 10^{-3})$	$B (\times 10^{-4})$	$C (\times 10^{-4})$	R^2
Methanol	7.45	2.46	2.87	0.99916
Acetonitrile	6.86	2.95	1.36	0.99971

Fourteen linear gradient runs including eight runs for methanol and six runs for acetonitrile as organic modifier were carried out to compare the experimental and calculated retention times in gradient elution. Fig. 2 (a) shows the comparison between calculated retention times and experimental retention times in achieved gradient conditions. The predicted retention times by Eq. (2) are lower than experimental retention times because all gradient runs were carried out from low organic modifier content. Average error of retention time predicted by Eq. (3) is smaller than Eq. (2) about four times (average errors of Eq. (2) and (3) are 17.5% and 4.0%, respectively). Fig. 2 (b) shows the comparison between calculated bandwidths and experimental bandwidths. Elution band becomes spread while

In different flow velocity of mobile phase, the migration velocity of solute linearly changed and HETP of solute is changed by the longitudinal diffusion and finite mass transfer kinetics between mobile phase and stationary phase. In addition, the migration velocity of solute also changed in different mobile phase composition. Therefore, van Deemter equation is derived as

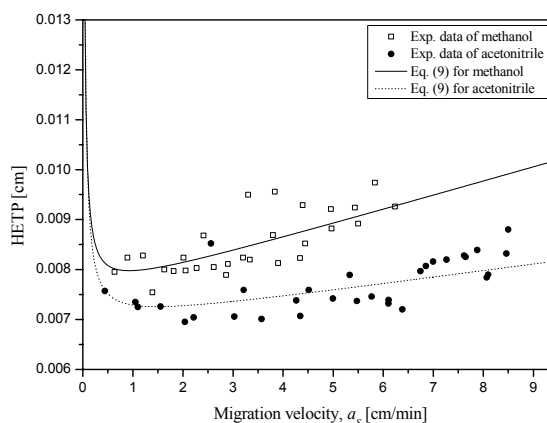


Fig. 1 The changes of HETP with the variation of the migration velocity of solute in isocratic elution.

solute is eluted from the column. So, precise retention model is required to predict bandwidth of elution profile. As shown in Fig. 2 (b), elution bandwidths were well predicted with the adoption of Eq. (3), newly proposed accurate retention model. The average errors of bandwidths are 24.5% and 9.5% with adoption of Eq. (2) and Eq. (3), respectively.

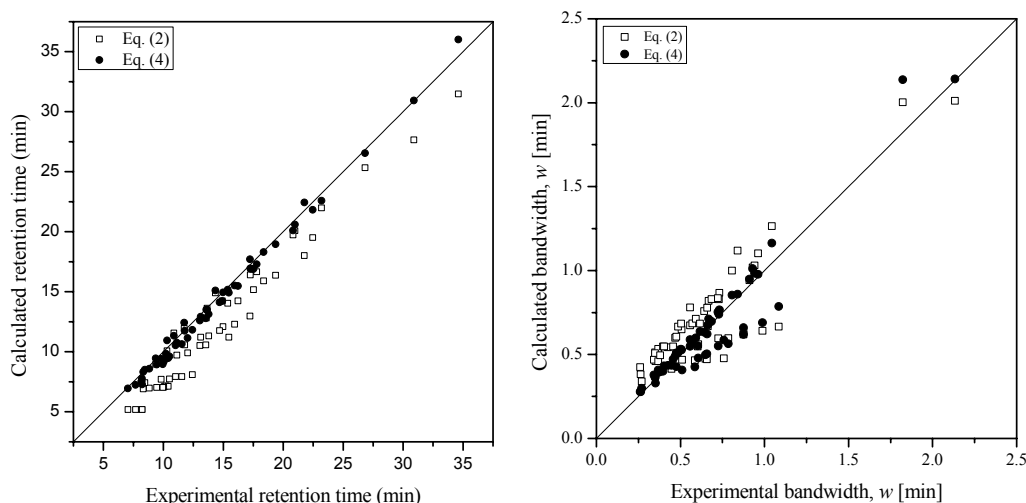


Fig. 4 Comparison of experimental and calculated retention times (a) and bandwidths (b) in linear gradient runs.

Conclusions

In this work, new retention model modified from Eq. (2) is proposed to explain nonlinear relationship between the logarithm of retention factor and the volumetric fraction of organic modifier and new relationship between HETP and mobile phase composition is proposed with the analog of flow velocity of the mobile phase. In the constant flow rate of the mobile phase, migration velocity is changed by only variation of mobile phase composition. The migration velocity of solute can be predictable with the known retention model. Accordingly, the band broadening rate is also predictable in certain mobile phase composition. New prediction method for gradient elution can predict the elution profiles in any combination of gradient conditions, such as multiple linear or curvilinear gradient conditions by the approximation of discrete step-gradient conditions. When four purine compounds are used as solutes and two organic modifiers (methanol and acetonitrile) are individually used as organic modifier, calculated results obtained by newly proposed retention model and prediction method have good agreements with experimental data within 4.0% and 9.5% average errors of retention times and bandwidths, respectively.

Acknowledgement

This study was supported by the ERC for Advanced Bioseparation Technology, KOSEF.

References

- [1] L. R. Snyder and M. A. Quarry, *J. Liquid Chromatogr.*, **10**, 1789 (1987).
- [2] Y. W. Lee, M. S. So, J. W. Lee, S. T. Chung and K. H. Row, *Korean J. of Chem. Eng.*, **13**, 578 (1996).
- [3] P. Nikitas and A. Pappa-Louisi, *J. of Chromatogr. A*, **1068**, 279 (2005).
- [4] K. H. Row and J. W. Lee, *Chromatographia*, **51**, 61 (2000).
- [5] J.J. van Deemter, F.J. Zuiderweg, and A. Klinkenberg, *Chem. Eng. Sci.*, **5**, 271 (1956).