Prediction of Retention Time of Solutes under Linear Gradient Elution Conditions in RP-HPLC

YANG, Geng-Liang*,[a,b](杨更亮) TIAN, Bao-Juan[a,c](田宝娟) CHEN, Yi[b](陈义)
WANG, De-Xian[a](王德先)
[a] College of Chemistry and Environmental Science, Hebei University, Baoding, Hebei 071002, China
[b] Molecular Science Center, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China
[c] Department of Chemistry, Normal College of Baoding, Baoding, Hebei 071051, China

A model for predicting retention time of solutes under linear gradient elution conditions has been established. In this model, the theoretical expressions under different elution modes were derived and tested with the retention behaviors of *p*-hydroxybenzaldehyde, vanillin, biphenyl, phenanthrene in gradient elution. With taking into account the dwell time of the instrumental system, the theoretically predicted retention times agree well with those experimentally determined.

Keywords high performance liquid chromatography, gradient elution, retention time, prediction

Introduction

As an important separation technology, gradient elution is widely used for separation of complex mixtures in RP-HPLC in order to obtain better resolution or shorter elution time. Theoretical treatment of gradient elution processes is a complex problem and it has been given much attention in recent years.[1-12] In a series of papers, Snyder[1,2] derived the equation of retention time based on a theory for the variation of the retention factor k with $\%B$ in RP-LC, which is popularly used for its simple mathematical calculation. But it is only fit for strongly retentive solute. There are as well some other studies done by Heinish,[5,6] Agostino,[7] and Zhang.[9,12] In linear gradient elution, the composition or the strength of the mobile phase in the column changes with time or position and so does the retention factor k. Because of the existence of the dwell time of mobile phase and instrumental system, there is an isocratic elution before gradient run in real procedure of linear gradient elution and the isocratic time is larger than the dwell time of instrumental system t_d. In this paper, a model is developed and theoretical expressions are derived for the retention time of the empirical component. The model is described by three sections: the linear gradient elution; gradient-isocratic elution; isocratic-gradient-isocratic elution. The model has been tested by the retention behavior of *p*-hydroxybenzaldehyde, vanillin, biphenyl, phenanthrene in gradient RP-HPLC.

Theoretical

Retention prediction for ideal linear gradient elution mode

Many different models are available for the prediction of the retention time in gradient elution. These models generally based on the equation (1).[10]

$$u = u_0/(1+k) \tag{1}$$

$$i.e., \quad dx/dt = u_0/(1+k) \tag{2}$$

where u and u_0 indicate respectively the linear velocity of solute and mobile phase in the column, k is the retention factor. In isocratic elution, $\ln k = \ln k_0 - S\varphi$, $i.e.$, $k = k_0 \exp(-S\varphi)$, while in gradient elution, the retention factor is changed with time, if the gradient time of the column entrance is t, the changed mobile phase will take (x/u_0) to arrive at position x,

$$k = k_0 \exp[\beta g(t - x/u_0)] \tag{3}$$

When the gradient elution is a linear process, from equations (1)—(3), the retention time t_R of the eluted component can be expressed as

$$t_R = L/u_0 + (1/\beta g)\cdot\ln[u_0/(u_0 - Lk_0\beta g)] \tag{4}$$

where L is the length of the column, u_0 is the linear velocity of the mobile phase in the column, β is the an empirical constant for a given solute and g is the slope of the gradient.

* E-mail: ygl@mail.hbu.edu.cn
Received November 7, 2002; revised January 21, 2003; accepted March 3, 2003.
Project supported by the National Natural Science Foundation of China (No. 20075005), the Grant from Ministry of Science and Technology and the Natural Science Foundation of Hebei Province (No. 202096).

Retention prediction for isocratic-gradient elution mode

In this mode, gradient elution is carried out after a period of isocratic elution and the change of the solvent strength is represented in Fig. 1. If the isocratic time of pumps is t_2, the retention time t_R of the eluted component can be obtained by the following equation

$$t_R = t_c + (L - x_c)/u_0 + (1/\beta g) \cdot \ln\{u_0/[u_0 - (L - x_c)k_0\beta g]\} \quad (5)$$

where $x_c = u_0(t_d + t_2)/k_0$, is the total isocratic elution distance of solute in column before gradient elution; $t_c = (t_d + t_2)(1 + 1/k_0)$, is the real isocratic elution time of solute in column; t_d is the dwell time of instrumental system.

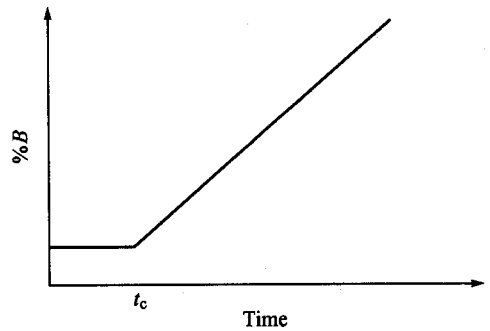

Fig. 1 Representation of isocratic-gradient elution.

Retention prediction for the isocratic-gradient-isocratic elution mode

As shown in Fig. 2, in this mode, gradient elution is performed after a period of isocratic elution, and followed by another isocratic elution. If the gradient time of pumps is t_1 and the isocratic time of pumps of the first step is t_0, the retention time t_R is given by equation (6):

$$t_R = t_c + t_1 + x_g/u_0 + (L - x_c - x_g)/u_0 \quad (6)$$

$$u_d = u_0/[1 + k_0 \exp(\beta g t_1)] \quad (7)$$

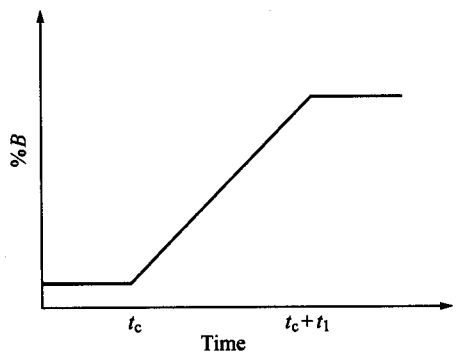

Fig. 2 Representation of isocratic-gradient-isocratic elution.

$$x_g = [u_0 - u_0/\exp(\beta g t_1)]/k_0\beta g \quad (8)$$

where x_g is the distance of solute moved in column during the gradient elution process; u_d is the end velocity of gradient elution; $t_c = (t_0 + t_d)(1 + 1/k_0)$, is the real isocratic retention time of the solute in column, in which the dwell time introduced by instrumental is also included, and $x_c = (t_0 + t_d)u_0/k_0$, is the distance of the solute moved in the column during this time.

Experimental

Reagents and chemicals

All the reagents were of analytical grade. Methanol, *p*-hydroxybenzaldehyde, vanillin, phenanthrene were from Beijing Chemical Reagent Co (Beijing, China), Biphenyl was from Shanghai Reagent Factory (Shanghai, China), and deionized water (Millipore, France) with a resistivity of 18.2 $M\Omega \cdot cm^{-1}$ was used throughout. Methanol and water were used as the mobile phase.

Instruments and apparatus

HPLC were performed with JASCO (Japan) PU-1580 and PU-1586 solvent delivery system, a Rheodyne model 7725i injector, and a Jasco-UV-1570 multi-wavelength detector connected to a computer station and a Jasco-HG-980-30 solvent mixing module. The chromatographic column used was C_{18} column (250 mm × 4.6 mm i.d., 5 μm). The column temperature was controlled at 30 ℃, the detection wavelength was 254 nm, the flow-rate was 1.00 $mL \cdot min^{-1}$ and the injection volume was 5 μL.

Determination of dead time (t_0) and dwell time of instrumental system (t_d)

The dead time of the column was determined with methanol as the unretained marker, which was 1.37 min. The dwell volume V_d from the sample mixer to column head was 1300 μL and the dwell time of instrumental system t_d was 1.30 min ($t_d = V_d/F$) at 1.00 mL/min flow-rate.

Determination of constant β

The retention time of each sample determined at different solvent strength was used to calculate the retention factor k and the value of β was obtained by equation (3). The results are listed in Table 1.

Table 1 β values of four samples

Solute	*p*-Hydroxybenzaldehyde	Vanillin	Biphenyl	Phenanthrene
β	−3.17	−3.64	−6.21	−6.79

Results and discussion

Prediction of retention time under linear gradient elution mode

When $t_2 = 0$, *p*-hydroxybenzaldehyde and vanillin were carried out under the following gradient conditions: initial $\%B$ (methanol, volume factor) = 30%, g = 2% min^{-1} and 4% min^{-1} respectively. Biphenyl and phenanthrene were carried out under: initial $\%B$ = 70%, g = 2% min^{-1} and 1% min^{-1} respectively. t_R was calculated according to equation (5) and the results are listed in Table 2. The average errors and the relative errors of t_R are 0.24 and 2.34% respectively.

When $t_2 \neq 0$, biphenyl, phenanthrene were used in the experiments and the pumps began with $\%B$ (methanol, volume factor) = 70% by isocratic elution for 7 min and then gradient elution with g = 2% min^{-1} and 1% min^{-1} respectively. Predicted retention time t_R was calculated according to equation (5) and the results are listed in Table 3. The average errors and the relative errors of t_R are 0.40 min and 2.55% respectively.

Prediction of retention time under isocratic-gradient-isocratic elution mode

In this mode, biphenyl, phenanthrene were used in the experiments and the pumps began with $\%B$ (methanol, volume factor) = 65% by isocratic elution for 5 min and gradient elution with g = 1% min^{-1} for 15 min and then isocratic elution with 80% methanol until the solutes come out form the column. Predicted retention time t_R was calculated according to equation (6) and the results are listed in Table 4. The average error and the relative error of t_R are 0.68 min and 2.35% respectively.

Conclusion

From the above results, it can be seen that the developed model agrees well with the experiments and presents a model for the quantitative interpretation of the gradient elution process.

Table 2 Experimental and predicted retention times under linear gradient elution ($t_2 = 0$)

Solute	g ($\%B$) (min^{-1})	Exp. t_R (min)	Predicted t_R (min)	Error (min)	Relative error (%)
p-Hydroxybenzaldehyde	2.00	9.35	9.00	-0.35	3.74
	4.00	8.05	8.08	0.03	0.37
Vanillin	2.00	9.79	10.47	0.68	6.94
	4.00	8.62	9.00	0.38	4.41
Biphenyl	1.00	11.01	11.08	0.07	0.64
	2.00	13.06	13.18	0.12	0.92
Phenanthrene	1.00	12.58	12.66	0.07	0.56
	2.00	15.64	15.82	0.18	1.15
Average				0.24	2.34

Table 3 Experimental and predicted retention times under isocratic-gradient elution mode (t_2 = 7 min)

Solute	g ($\%B$) (min^{-1})	Exp. t_R (min)	Predicted t_R (min)	Error (min)	Relative error (%)
Biphenyl	2.00	14.50	15.13	0.63	4.34
	1.00	15.84	16.04	0.20	1.26
Phenanthrene	2.00	16.86	17.60	0.74	4.39
	1.00	19.59	19.63	0.04	0.20
Average				0.40	2.55

Table 4 Experimental and predicted retention times under isocratic-gradient-isocratic elution mode

Solute	g ($\%B$) (min^{-1})	t_1(min)	Exp. t_R (min)	Predicted t_R (min)	Error (min)	Relative error (%)
Biphenyl	1.00	15.00	26.75	27.22	0.47	1.76
Phenanthrene	1.00	15.00	30.58	29.68	0.90	2.94
Average					0.68	2.35

References

1 Dolan, W.; Lommen, D. C.; Snyder, L. R. *J. Chromatogr.* **1989**, *91*, 485.

2 Snyder, L. R.; Dolan, J. W. *J. Chromatogr., A* **1996**, *721*, 3.

3 Jandera, P. *J. Liq. Chromatogr.* **1989**, *12*, 117.

4 Jandera, P. *J. Chromatogr., A* **1998**, *797*, 11.

5 Heinish, S.; Rocca, J. L. *Analyst* **1990**, *18*, 83.

6 Heinish, S.; Rocca, J. L.; Kolosky, M. *Chromatographia* **1990**, *29*, 482.

7 Agostino, G. D.; O'Hare, M. J.; Mitchell, F. *Chromatographia* **1988**, *28*, 343.

8 Schoenmakers, P. J.; Billiet, H. A. H.; Galan, L. D. *J. Chromatogr.* **1981**, *205*, 13.

9 Shan, Y.-C.; Zhao, R.-H.; Zhang, W.-B.; Zhang, Y.-K. *Chin. J. Chromatogr.* **2001**, *19*, 256 (in Chinese).

10 Snyder, L. R.; Kirkland, J. J. *Introduction to Modern Liquid Chromatography*, 2nd Ed., Chemical Industry Press, Beijng, **1988**, p. 24 (in Chinese).

11 Valko, K.; Snyder, L. C.; Glajch, J. L. *J. Chromatogr., A* **1993**, *656*, 501.

12 Li, R.-J.; Zhang, W.-B.; Ni, J.-Y.; Zou, H.-F.; Zhang, Y.-K. *Chem. J. Chin. Univ.* **1998**, *19*, 1556 (in Chinese).

(E0211077 PAN, B. F.; ZHENG, G. C.)