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Computer aided optimization of multilinear gradient elution in liquid chromatography

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ABSTRACT

Analytical expressions for retention time and peak compression factor are deduced by assuming quadratic solvent strength model and multilinear gradient elution. Based on these expressions, a program for the optimization of multilinear gradient profile is written with Visual Basic for Applications in Excel using genetic algorithm. The program is applied to search for a gradient profile for the separation of twelve compounds that are degraded from lignin. It is shown that the predicted and experimental chromatograms are well consistent. A better separation of the compounds is achieved under an S-shaped multilinear gradient profile than that obtained under linear gradient profile.

1. Introduction

Gradient elution is a powerful separation technique in liquid chromatography (LC) [1-4]. It is generally implemented in practice by changing mobile phase composition (φ, denoting volume fraction of strong solvent) with time (t). Compared with another technique isocratic elution, gradient elution can shorten analysis time and narrow peak width. It, however, also makes the separation mechanisms more complicated. One feature of gradient elution is that retention factor (k) will vary with φ. Linear solvent strength model (LSSM), which is also the basis of the well-known Drylab software of chromatography [5-8], has been used to account for such variation [5,9,10],

$$\ln k = \ln k_0 - S\phi \tag{1}$$

where k_0 is retention factor of analyte in 100% of the starting (weak) solvent, and S is solvent strength parameter. In practice, the ln$k$ vs. φ plots are generally found to be curved [11-13]. Quadratic solvent strength model (QSSM) has been used to account for such curvature [14-17],

$$\ln k = \ln k_0 - S_1\phi + S_2\phi^2 \tag{2}$$

where S_1 and S_2 are solvent strength parameters. Due to the curvature in the ln$k$ vs. φ plot, Schellinger et al have pointed out that Drylab can be used to provide an estimate of resolution and optimum conditions but does not seem to provide accurate chromatograms [8].

Another feature of gradient elution is peak compression that does not exist in isocratic elution. It is resulted from stronger elution strength of mobile phase at the rear side of the band than at the front side. The well-known plate height equation that has been proposed by van Deemter is derived from isocratic elution [18]. The effects of peak compression are not included in it. As pointed out by Guiochon, peak compression has not gained as much attention as it deserves [19,20]. Concerning LSSM and linear gradient elution, Poppe et al have derived a well-known expression for peak compression factor (G) under linear gradient elution [21]. However, it has been reported that the experimental values of peak width are generally larger than the values predicted by theory [22,23]. Neue et al have shown that it is necessary to take into account the curvature in the ln$k$ vs. φ plot to understand the concept of peak compression correctly [24].

Multilinear gradient elution that consists of two or more linear gradient segments has been widely used in practice [25,26]. The multilinear gradient profile will be more flexible than linear gradient profile, which can be obtained by assembling the linear gradient segments in any form. Multilinear gradient elution provides more options for LC separation. However, the combination of linear gradient segments will produce a great number of gradient profiles, and it is challenging for analysts to find out one from them to obtain a satisfactory separation. In the search of the gradi-

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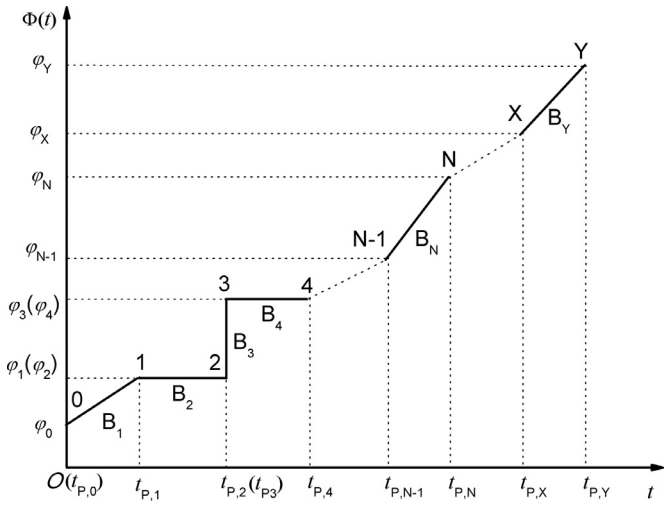

Fig. 1. Illustration of multilinear gradient profile.

ent profile, it is important to ensure the accuracy in the prediction of retention time (t_R) and peak width (W) under different gradient conditions. Due to the curvature in the ln$k$ vs. φ plot, it is still difficult to obtain accurate prediction of the values, especially in the prediction of W.

In this work, we present analytical expressions for t_R and W which are deduced by assuming QSSM and multilinear gradient elution. Compared with numerical methods such as the finite difference or the finite element methods, analytical expressions have the advantages of higher precision and less time for calculation. Especially in the prediction of W, numerical methods will inevitably introduce numerical diffusion due to discretization [27,28]. This will lead to artificial peak broadening; i.e., the predicted values of W will be always larger than the actual ones. For modern columns that have high column efficiency, the effects of numerical diffusion on peak width will be more significant. In contrast, analytical expressions can avoid numerical diffusion and thus have more accuracy. The analytical expressions obtained in this work are incorporated in a program that is written with Visual Basic for Applications (VBA) in Microsoft Excel using genetic algorithm (GA). This program is testified by applying it to search for a gradient profile for the separation of twelve compounds that may be degraded from lignin.

2. Theoretical

2.1. Expressions for retention time under QSSM and multilinear gradient elution

A typical example of multilinear gradient profile is shown in Fig. 1. In this figure, $\Phi(t)$ accounts for the time program of mobile phase composition which is input into the delivery system of chromatograph. Under gradient elution, the general expression for retention time t_R is [29,30]

$$t_0 = \frac{t_D}{k_{\phi_0}} + \int_0^{t_R - t_0 - t_D} \frac{1}{k_{\Phi(t)}} dt \tag{3}$$

where t_0 is dead time, t_D is dwelling time of the system that accounts for the time needed for a certain change in the mixer to reach the beginning of the column. Under multilinear gradient elution, the expression for t_R can be rewritten as

$$t_0 = \frac{t_D}{k_{\phi_0}} + \sum_{i=1}^{N-1} \int_{t_{P,i-1}}^{t_{P,i}} \frac{1}{k_{\Phi(t)}} dt + \int_{t_{P,N-1}}^{t_R - t_0 - t_D} \frac{1}{k_{\Phi(t)}} dt \tag{4}$$

where $t_{P,i}$ is time coordinate of the ith turning point on the gradient profile ($t_{P,0}$ is equal to zero), $k_{\varphi 0}$ and $k_{\Phi(t)}$ are retention factors of analyte at initial mobile phase composition (φ_0) and at mobile phase composition of $\Phi(t)$, respectively, and N is the index of the last gradient segment within which the analyte is eluted from the column (i.e. t_R-t_0-$t_D \in (t_{P,N-1}, t_{P,N}]$).

The calculation of t_R appearing in Eq. (4) in the case of general solvent strength model is discussed in detail in Section 1 of the Supplementary Material (SM). Here we present analytical expressions that are obtained in the case of QSSM. In order to solve Eq. (4), it is firstly necessary to figure out N. The axial distance Z_i of analyte to column inlet at the end of the ith gradient segment can be calculated by

$$Z_i = Z_0 + \sum_{k=1}^{i} (Z_k - Z_{k-1}) = \frac{u t_D}{k_{\phi 0}} + \sum_{k=1}^{i} (Z_k - Z_{k-1}) \tag{5}$$

where $Z_0 (= u t_D / k_{\varphi 0})$ is the position at which the mobile phase generated in the mixer catches up with the analyte [31]. When the slope of gradient segment B_i=0 (e.g. B_2 in Fig. 1), we have

$$Z_i - Z_{i-1} = \frac{u(t_{P,i} - t_{P,i-1})}{k_{\phi,i}} \quad \text{for} B_i = 0 \tag{6}$$

where $k_{\varphi,i}$ is retention factor of analyte at the mobile phase composition of φ_i. According to QSSM, the value of $k_{\varphi,i}$ can be calculated by

$$k_{\phi,i} = k_0 \exp(-S_1 \phi_i + S_2 \phi_i^2) \tag{7}$$

When the gradient segment is perpendicular to the t axis, i.e., B_i=∞ (e.g. B_3 in Fig. 1), the analyte will not move within this segment and thus we have

$$Z_i - Z_{i-1} = 0 \quad \text{for} B_i = \infty \tag{8}$$

When $B_i \neq 0$ (e.g. B_1 in Fig. 1), we have

$$Z_i - Z_{i-1} = \frac{\sqrt{\pi} u}{2 B_i k_0 \sqrt{S_2}} \exp\left(\frac{S_1^2}{4 S_2}\right) \mathrm{erf}\left(\frac{2 S_2 \phi_{i-1} - S_1}{2\sqrt{S_2}}, \frac{2 S_2 \phi_i - S_1}{2\sqrt{S_2}}\right) \quad \text{for } B_i \neq 0 \tag{9}$$

where erf(a,b) is general error function, $\mathrm{erf}(a,b) = \frac{2}{\sqrt{\pi}} \int_a^b \exp(-t^2) dt$, which has been built in Excel. By substituting the values of Z_i-Z_{i-1} into Eq. (1), we can calculate the value of Z_i. For the Nth segment within which the solute is eluted from the column, there will be

$$Z_{N-1} < L \leq Z_N \tag{10}$$

The above criterion can be used to figure out N. The retention time t_R can be calculated as follows. When B_N=0, we have

$$t_R = t_0 + t_D + t_{P,N-1} + k_{\phi,N}(L - Z_{N-1})/u \quad \text{for } B_N = 0 \tag{11}$$

When $B_N \neq 0$, we have

$$t_R = t_0 + t_D + t_{P,N-1} + (\phi_R - \phi_{N-1})/B_N \quad \text{for } B_N \neq 0 \tag{12}$$

where φ_R, which denotes eluted mobile phase composition at which the analyte is eluted from the column, is calculated by

$$\phi_R = \frac{1}{\sqrt{S_2}}\mathrm{erf}^{-1}\left[\mathrm{erf}\left(\frac{2S_2\phi_{N-1}-S_1}{2\sqrt{S_2}}\right)+\frac{2B_N k_0\sqrt{S_2}(t_0-Z_{N-1}/u)}{\sqrt{\pi}}\exp\left(-\frac{S_1^2}{4S_2}\right)\right] + \frac{S_1}{2S_2} \quad (13)$$

In Eq. (12), erf(x) is error function, and $\mathrm{erf}^{-1}(y)$ is inverse error function that can be calculated by using NORM.S.INV function in Excel; i.e., $\mathrm{erf}^{-1}(y)=(1/2)^{0.5}$NORM.S.INV($y$/2+0.5).

2.2. Expressions for peak compression factor under QSSM and multilinear gradient elution

It is assumed that the column efficiency varies little with mobile phase composition. In this case, the general expression for peak compression factor G is [31,32]

$$G^2 = \frac{k_{\phi R}^2}{t_0(1+k_{\phi R})^2}\left\{\frac{t_D(1+k_{\phi 0})^2}{k_{\phi 0}^3}+\int_0^{t_R-t_0-t_D}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt\right\} \quad (14)$$

Under multilinear gradient elution, the expression for G can be rewritten as

$$G^2 = \frac{k_{\phi R}^2}{t_0(1+k_{\phi R})^2} \times\left\{\frac{t_D(1+k_{\phi 0})^2}{k_{\phi 0}^3}+\sum_{i=1}^{N-1}\int_{t_{P,i-1}}^{t_{P,i}}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt+\int_{t_{P,N-1}}^{t_R-t_0-t_D}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt\right\} \quad (15)$$

where $k_{\varphi R}$ is retention factor of analyte at eluted mobile phase composition. When B_i=0, we have

$$\int_{t_{P,i-1}}^{t_{P,i}}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt = \frac{(t_{P,i}-t_{P,i-1})(1+k_{\phi,i})^2}{k_{\phi,i}^3} \quad \text{for } B_i=0 \quad (16)$$

$$\int_{t_{P,N-1}}^{t_R-t_0-t_D}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt = \frac{(t_R-t_0-t_D-t_{P,N-1})(1+k_{\phi,N})^2}{k_{\phi,N}^3} \quad \text{for } B_N=0 \quad (17)$$

When B_i≠0, according to QSSM, we have

$$\int_{t_{P,i-1}}^{t_{P,i}}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt=\frac{\sqrt{\pi/S_2}}{2B_ik_0}\exp\left(\frac{S_1^2}{4S_2}\right)\cdot\left\{\frac{1}{\sqrt{3}k_0^2}\exp\left(\frac{S_1^2}{2S_2}\right)\mathrm{erf}\left[\sqrt{3}F(\phi_{i-1}),\sqrt{3}F(\phi_i)\right] + \frac{\sqrt{2}}{k_0}\exp\left(\frac{S_1^2}{4S_2}\right)\mathrm{erf}\left[\sqrt{2}F(\phi_{i-1}),\sqrt{2}F(\phi_i)\right]+\mathrm{erf}[F(\phi_{i-1}),F(\phi_i)]\right\} \quad \text{for } B_i\neq 0 \quad (18)$$

$$\int_{t_{P,N-1}}^{t_R-t_0-t_D}\frac{\left[1+k_{\Phi(t)}\right]^2}{k_{\Phi(t)}^3}dt = \frac{\sqrt{\pi/S_2}}{2B_Nk_0}\exp\left(\frac{S_1^2}{4S_2}\right)\cdot\left\{\frac{1}{\sqrt{3}k_0^2}\exp\left(\frac{S_1^2}{2S_2}\right)\mathrm{erf}\left[\sqrt{3}F(\phi_{N-1}),\sqrt{3}F(\phi_R)\right] + \frac{\sqrt{2}}{k_0}\exp\left(\frac{S_1^2}{4S_2}\right)\mathrm{erf}\left[\sqrt{2}F(\phi_{N-1}),\sqrt{2}F(\phi_R)\right] + \mathrm{erf}[F(\phi_{N-1}),F(\phi_R)]\right\} \quad \text{for } B_N\neq 0 \quad (19)$$

where φ_R is calculated from Eq. (13), and the function $F(x)$ is defined by

$$F(x) = \frac{2S_2x-S_1}{2\sqrt{S_2}} \quad (20)$$

Detailed mathematical derivations of Eqs. (18)-(20) are presented in Section 2 of the SM. When the value of G is obtained, the peak width W can be calculated by [24,31]

$$W = 4Gt_0(1+k_{\phi R})/\sqrt{N_c} \quad (21)$$

where N_c is the number of theoretical plates of the column.

3. Experimental

Twelve compounds that may be degraded from lignin are chosen as analytes. These compounds include ferulic acid (FEA), caffeic acid (CAA), syringic acid (SYA), *p*-hydroxybenzoic acid (HYA), vanillic acid (VAA), *p*-coumaric acid (COA), sinapic acid (SIA), *p*-hydroxybenzaldehyde (HA), vanillin (VL), syringylaldehyde (SA), acetovanillone (AV) and acetosyringone (AS). Chromatographic conditions are the same as those reported previously [31]. Briefly, a C18 column was chosen as stationary phase which dimension was 150 mm × 4.6 mm (i.d.). The particle size of the packing was 5 μm diameter. The mobile phase was a gradient mixture of water containing 1% acetic acid in volume fraction (solvent A) and methanol containing 1% acetic acid in volume fraction (solvent B). The flow rate and detector wavelength were set at 0.8 mL/min and 280 nm, respectively. The column temperature was kept at 35°C. The values of t_0 and t_D were measured as 2.155 and 3.715 min, respectively.

4. Results and discussion

4.1. Optimization of multilinear gradient profile using genetic algorithm

GA is a global optimizing method which imitates biological evolution [33,34]. Generally, it involves iterations that include: (1) creation of population with a number of chromosomes; (2) reproduction of child chromosomes by means of crossover and mutation; (3) evaluation of each chromosome according to cost function (CF); and (4) Selection of new chromosomes according to the values of CF to replace the old population. In this work, GA is adopted for the optimization of multilinear gradient profile. The chromosome is constructed by the coordinates of turning points on the multilinear gradient profile. For example, if there are five turning points on the gradient profile, the chromosome is coded as

$$\{(0,\ \varphi_0),\ (t_{P,1},\ \varphi_1),\ (t_{P,2},\ \varphi_2),\ (t_{P,3},\ \varphi_3),\ (t_{P,4},\ \varphi_4),\ (t_{P,5},\ \varphi_5)\}$$

It can be seen that one chromosome corresponds to one multilinear gradient profile. For one chromosome, the retention time and peak width for each analyte can be calculated by using the analytical expressions that are presented in Section 2.1 and 2.2. Then, the resolution R between adjacent peaks is calculated by

$$R = 2(t_{R,2}-t_{R,1})/(W_1+W_2) \quad (22)$$

and the CF in this work is defined by

$$\mathrm{CF} = \mathrm{Round}(R_{\min},2)\times 10^5 + \mathrm{Round}(t_S-t_{R,L},1)\times 10 \quad (23)$$

where $R_{\min}$ is the minimum value of R, t_S is specified value of analysis time, $t_{R,L}$ is retention time of the last eluted analyte, and Round(*value*, *n*) is rounding operation of *value* with *n* decimal places retained. By using Eq. (23), the first three digits in the cost value indicate the resolution between analytes. The last three digits indicate the analysis speed. It is also specified that the first or the last three digits are equal to 999 when R>9.99 or t_S-$t_{R,L}$>99.9, respectively. When t_S-$t_{R,L}$<0, the cost value is returned to zero. It can be seen that the larger value of CF the better performance of the chromosome.

A program based on GA for the optimization of multilinear gradient profile is written in house with VBA in Excel. This file is named as *MultiGradient.xls* and attached to the SM. Detailed instructions to it are presented in Section 3 of the SM. It should also be noted that there are differences between the approach presented in this work and that proposed by Nikitas et al in which GA is adopted [35]. Firstly, in the Nikitas' approach, the retention time is calculated by subdividing the ln$k$ vs. φ curve into small portions so that each portion can be treated as linear. This approach

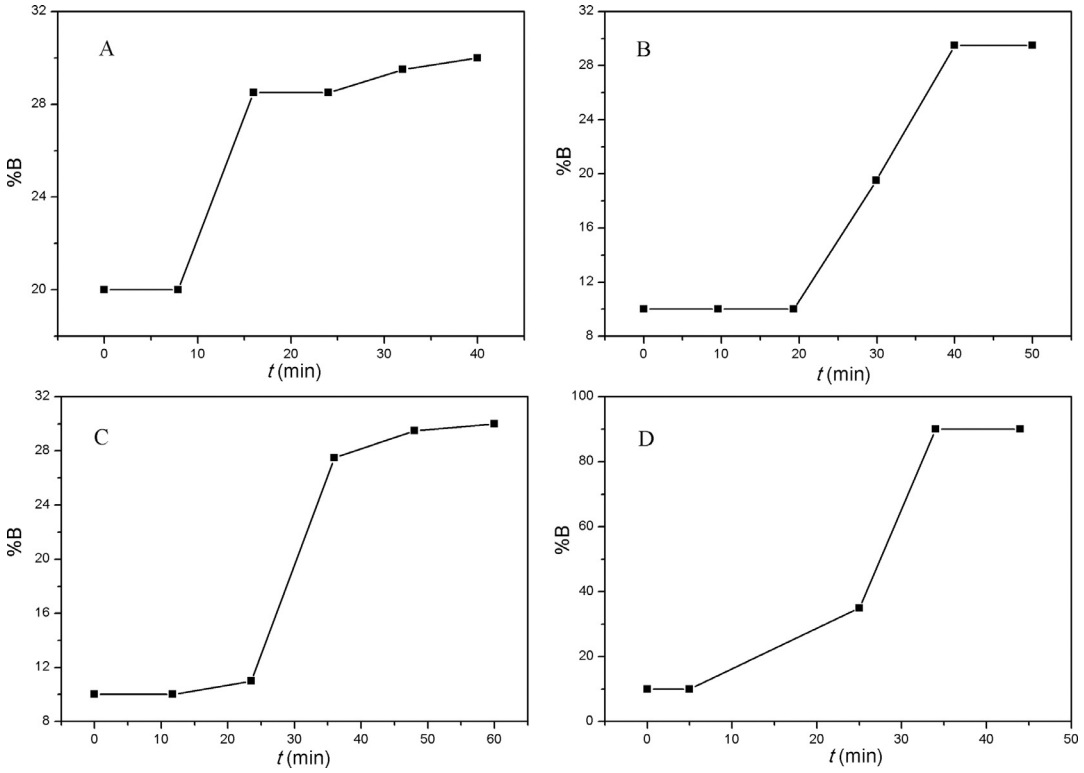

Fig. 2. Multilinear gradient profile: A, B and C, obtained from the program by setting t_S=40, 50 and 60 min, respectively; D, same as adopted in Ref. [36].

is equivalent to the finite element method that belongs to the numerical techniques. Due to the error introduced by discretization, the precision of the Nikitas' approach will be less than that of analytical expressions. In addition, in the Nikitas' approach, it needs to subdivide the gradient profile into small portions so that the intervals of φ of these portions should be in agreement with those of linear portions on the ln$k$ vs. φ curve. This will complicate mathematical treatments somewhat and increase the time for calculation. By using analytical expressions presented in Section 2.1, such subdivision of the gradient profile is not needed. Secondly, in the Nikitas' approach, the values of peak width under multilinear gradient elution are not calculated. The difference of retention time between adjacent peaks is adopted in the CF [35]. In practice, the resolution R has generally been used to evaluate the performance of chromatographic separations. In this work, an approach to predict peak widths under multilinear gradient elution is proposed, and this makes it possible to calculate the values of R.

4.2. Comparison between predicted and experimental chromatograms

The program in *MultiGradient.xls* attached to the SM is applied to search for a gradient profile for the separation of twelve compounds that are degraded from lignin. The parameters used in the GA are presented in the worksheet *OptGradient*. The regression coefficients for QSSM are the same as those reported in Ref. [31]. The optimization can be implemented by running the macro *OptFradientwithN* in *MultiGradient.xls*. In this work, the number of segments in multilinear gradient profile was set to be five. The analysis time (t_S) for the separation was set to be 40, 50 and 60 min, respectively. The gradient profiles obtained by the program with different t_S are shown in Fig. 2A-C. Fig. 2D is the multilinear gradient profile that has been used in our previous work [36]. The gradient conditions are also presented in Table S1 in Section 4 of the SM.

Fig. 3 shows a comparison between predicted and experimental chromatograms in which t_S=60 min. The comparisons for t_S=40 and 50 min are shown in Figs. S4 and S5 in Section 4 of the SM.

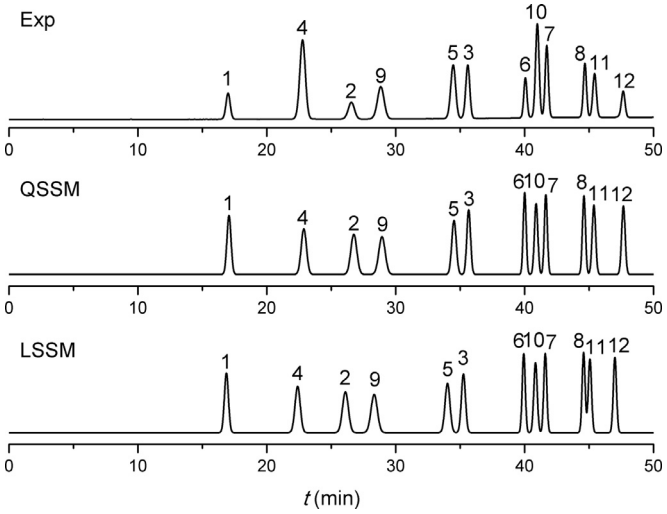

Fig. 3. Comparison between predicted and experimental chromatograms: Exp, experimental chromatogram that is obtained by using the gradient profile shown in Fig. 2C; QSSM, predicted by QSSM; LSSM, predicted by LSSM. The number above each peak: 1, HYA; 2, VAA; 3, SYA; 4, HA; 5, VL; 6, SA; 7, AV; 8, AS; 9, CAA; 10, COA; 11, FEA; 12, SIA. The peak area for each analyte in predicted chromatograms is set to be one.

It is found that when t_S=40 min some compounds cannot be well resolved under multilinear gradient elution. When t_S is increased to 50 or 60 min, the separation is improved by changing the shape of gradient profile. In addition, it should be noted that the separation shown in Fig. 3 is better than that obtained under linear gradient elution, of which the gradient time is also 60 min and the chromatogram corresponding to it is shown in Fig. 5 in Ref. [31]. Under linear gradient elution, the peaks of SYA (3) and VL (5) are poorly resolved, and the peaks of AV (7) and COA (10) also overlap partly. By applying multiliear gradient elution, the separation can

Table 1
Comparison between predicted and experimental values of retention time (t_R) and peak width (W) under multilinear gradient profiles that are shown in Fig. 2.

Gradient	Analyte	t_R					W				
		Exp (min)	QSSM (min)	RE (%)	LSSM (min)	RE(%)	Exp (min)	QSSM (min)	RE (%)	LSSM (min)	RE (%)
A	HYA	9.843	9.762	-0.82	9.863	0.20	0.387	0.375	-3.09	0.378	-2.20
	HA	12.313	12.231	-0.67	12.479	1.35	0.472	0.460	-2.61	0.468	-0.78
	VAA	12.587	12.499	-0.70	12.814	1.80	0.460	0.469	1.90	0.480	4.28
	CAA	12.985	12.886	-0.76	13.187	1.56	0.511	0.482	-5.71	0.493	-3.66
	SYA	15.145	15.078	-0.44	15.645	3.30	0.533	0.508	-4.68	0.500	-6.24
	VL	16.048	15.960	-0.55	16.435	2.41	0.536	0.510	-4.78	0.506	-5.60
	SA	18.608	18.507	-0.54	19.118	2.74	0.495	0.472	-4.66	0.457	-7.77
	AV	20.805	20.688	-0.56	21.138	1.60	0.491	0.476	-3.12	0.456	-7.08
	COA	21.109	20.967	-0.67	21.186	0.36	0.488	0.489	0.27	0.479	-1.75
	AS	22.656	22.539	-0.52	22.989	1.47	0.465	0.453	-2.55	0.441	-5.12
	FEA	24.073	23.967	-0.44	23.788	-1.18	0.531	0.525	-1.10	0.488	-8.10
	SIA	25.353	25.300	-0.21	24.860	-1.94	0.549	0.541	-1.45	0.486	-11.6
B	HYA	17.059	17.062	0.02	16.862	-1.15	0.638	0.629	-1.35	0.622	-2.46
	HA	22.938	22.956	0.08	22.446	-2.14	0.870	0.840	-3.42	0.822	-5.52
	VAA	26.903	26.981	0.29	26.310	-2.20	0.902	0.872	-3.32	0.904	0.25
	CAA	28.925	28.911	-0.05	28.379	-1.89	0.825	0.800	-3.05	0.836	1.31
	VL	33.789	33.783	-0.02	33.342	-1.32	0.734	0.687	-6.41	0.735	0.07
	SYA	34.84	34.861	0.06	34.493	-1.00	0.630	0.604	-4.16	0.651	3.36
	SA	39.767	39.704	-0.16	39.678	-0.22	0.551	0.517	-6.23	0.540	-1.95
	COA	40.843	40.728	-0.28	40.706	-0.34	0.622	0.597	-4.00	0.607	-2.49
	AV	41.687	41.607	-0.19	41.627	-0.14	0.565	0.535	-5.22	0.546	-3.38
	AS	44.993	44.909	-0.19	44.989	-0.01	0.470	0.453	-3.65	0.438	-6.83
	FEA	45.684	45.601	-0.18	45.405	-0.61	0.510	0.495	-3.02	0.464	-9.00
	SIA	47.723	47.693	-0.06	47.150	-1.20	0.499	0.489	-1.98	0.427	-14.4
C	HYA	16.991	17.062	0.42	16.862	-0.76	0.640	0.629	-1.66	0.622	-2.77
	HA	22.769	22.867	0.43	22.386	-1.68	0.848	0.814	-4.04	0.801	-5.50
	VAA	26.553	26.744	0.72	26.093	-1.73	0.941	0.923	-1.92	0.910	-3.32
	CAA	28.832	28.929	0.34	28.323	-1.77	1.008	0.983	-2.52	0.972	-3.57
	VL	34.452	34.514	0.18	34.009	-1.29	0.751	0.688	-8.35	0.753	0.25
	SYA	35.583	35.652	0.19	35.244	-0.95	0.615	0.574	-6.61	0.632	2.84
	SA	40.055	40.000	-0.14	39.928	-0.32	0.483	0.453	-6.30	0.469	-2.82
	COA	40.981	40.886	-0.23	40.838	-0.35	0.550	0.524	-4.75	0.530	-3.69
	AV	41.712	41.641	-0.17	41.596	-0.28	0.492	0.465	-5.43	0.468	-4.89
	AS	44.671	44.597	-0.17	44.570	-0.23	0.486	0.471	-3.16	0.462	-4.84
	FEA	45.422	45.368	-0.12	45.059	-0.80	0.551	0.536	-2.70	0.504	-8.45
	SIA	47.639	47.655	0.03	46.996	-1.35	0.561	0.541	-3.48	0.493	-12.1
D	HYA	15.867	15.905	0.24	15.828	-0.25	0.420	0.434	3.33	0.440	4.83
	HA	19.082	19.087	0.03	19.042	-0.21	0.425	0.435	2.24	0.445	4.81
	VAA	20.3	20.256	-0.22	20.257	-0.21	0.390	0.401	2.73	0.410	5.20
	CAA	20.846	20.860	0.07	20.881	0.17	0.387	0.388	0.33	0.395	2.18
	SYA	23.143	23.031	-0.48	23.144	0.00	0.351	0.362	3.06	0.364	3.77
	VL	23.315	23.227	-0.38	23.312	-0.01	0.393	0.400	1.72	0.403	2.42
	SA	25.743	25.607	-0.53	25.725	-0.07	0.357	0.362	1.54	0.352	-1.46
	COA	27.197	27.133	-0.24	27.147	-0.18	0.391	0.403	3.14	0.394	0.88
	AV	27.309	27.161	-0.54	27.195	-0.42	0.372	0.385	3.48	0.367	-1.27
	AS	28.842	28.674	-0.58	28.582	-0.90	0.342	0.356	4.11	0.325	-4.93
	FEA	29.638	29.593	-0.15	29.138	-1.69	0.363	0.378	4.26	0.341	-6.07
	SIA	30.45	30.420	-0.10	29.632	-2.69	0.336	0.351	4.61	0.302	-10.3
		Average		-0.19		-0.34			-1.95		-3.03
		SD		0.34		1.32			3.38		4.70

Symbols: Exp, experimental values; QSSM, predicted values by using QSSM; LSSM, predicted values by using LSSM; RE, relative error between predicted (pre) and experimental (exp) values, RE= (pre- exp)/exp × 100%.

be improved. This result shows the flexibility of multiliear gradient profile. The program presented in this work can be used for the optimization of its shape.

In Section 2.1 and 2.2, we present analytical expressions for t_R and W which are based on QSSM. The expressions based on LSSM and for multilinear gradient profile are also presented in Section 1 and 2 of the SM. Under LSSM, the values of t_R and W can be calculated by using the same methods as under QSSM, except that Eqs. (9), (12), (18) and (19) are replaced by Eqs. (S26), (S29), (S41) and (S42) in the SM, respectively. The chromatograms predicted by LSSM are also shown in Fig. 3 and Figs. S4-S6 in Section 4 of the SM. The comparisons between the predicted and experimental values of t_R and W are presented in Table 1. It is found that the chromatograms predicted by QSSM are closer to experimental chromatograms than predicted by LSSM. For example, for the last two eluted analytes (i.e. FEA and SIA), the retention times predicted by LSSM are generally smaller than the experimental values. The differences in peak width between experimental values and those predicted by LSSM are also more significant than the differences which are obtained by QSSM. These differences may be ascribed to the curvature in the ln$k$ vs. φ plot. It is shown that the application of QSSM will help to improve the prediction of the chromatograms. This will have little effect on the speed of calculation, because the error functions appearing in the analytical expressions presented in Section 2.1 and 2.2 have been built in common software such as Excel.

5. Conclusions

In this work, we present analytical expressions for retention time and peak compression factor by assuming QSSM and multilinear gradient elution. These expressions will help to improve the prediction of the chromatograms and reduce the time for computation. Based on the expressions, a program is written for the optimization of multilinear gradient profile in which GA is adopted. The predicted chromatograms are found to be well consistent with the experimental ones. The program is testified by applying it to search for a gradient profile for the separation of twelve compounds that are degraded from lignin. It is shown that an S shaped multilinear gradient profile can provide a satisfactory separation. In order to validate the program, it still needs more experiments with different analytes. Such studies are under way in our laboratory and will be reported in future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Weiqiang Hao: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Bo Li:** Investigation, Data curation. **Yuying Deng:** Visualization, Validation. **Qiang Chen:** Project administration, Resources. **Lijuan Liu:** Investigation. **Qiaoyin Shen:** Investigation.

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Supplementary materials

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