CASE 6

Evaluation of Component Separation Using a Dynamic Operating Window

Abstract: Liquid chromatographic (LC) analysis is an effective instrument for use in pharmaceutical analysis and pharmaceutical science. Various methods have been utilized in attempts to optimize LC analytical conditions. To obtain a high degree of separation, optimum conditions were determined by use of the dynamic SN ratio as an index for evaluation of the separation process. An L_{18} orthogonal array was used in this experiment. It was demonstrated that the SN ratio was an appropriate scale to indicate the separation of some peaks in an LC. Also, appropriate types of SN ratios to show the degree of separation in an LC are discussed.

1. Introduction

In a high-performance liquid chromatograph (HPLC), after moving components placed on liquid, called a *mobile phase*, we separated each component by taking advantage of its degree of adsorption for a solid phase, called a *carrier*, in the column. A flow rate indicates the mobile phase. The result of separation was recorded as a peak by sensing an optical deflection or a difference in the wavelength of a desorbed component in the liquid using a detector. Although components were separated according to a degree of adsorption, in actuality this distance is determined by the type and constitution of the mobile phase (primarily, organic solvent), pressure, temperature, and type of column (i.e., the solid phase's capability of adsorption and separation inside the column). Therefore, using parameter design we selected levels and types of parameters leading to an appropriate degree of separation from various variables.

As shown in Figure 1, in separating two peaks we used the separability, R_s, as a degree of separation of two components, which is defined as

$$R_S = \frac{2(t_{R_2} - t_{R_1})}{W_1 + W_2} \tag{1}$$

The larger R_s becomes, the better the degree of separation of two components. However, since there are numerous factors related to the separation of components' peaks, it really is difficult to conduct a simultaneous multiple-factor evaluation regarding a relationship between the separability and influential factors. In our study we attempted to evaluate the relationship quantitatively using the SN ratio in parameter design for the separation of components.

The HPLC method detects the peak value of a component. In this case, since the desorbing time of a component was unknown, we determiend that a reciprocal of a flow rate M in separation, $1/M$, was proportional to a desorbing time Y (time when a peak shows up), and clarified that the dynamic operating window method for components M_1^*, M_2^*, and M_3^* can be utilized with the SN ratio based on a desorbing time Y as an output. Yet we have come to the conclusion that it is more natural to choose a flow rate M per se in response to the function of the HPLC instead of selecting reciprocal $1/M$ as a
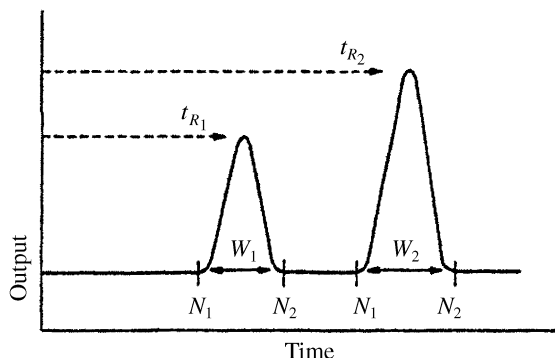
Figure 1
Correspondence of evaluation scale and separability

Figure 2
Chromatography and conceptual diagram of separation

signal factor, because we considered it rational to separate adsorbed components by flowing energy.

Then, setting a reciprocal of a desorbing time Y, $1/Y$, to y, we obtained

$$y = \beta M \qquad (2)$$

Based on this equation, our objective was to compute a variation of differences in proportional terms between components' M* values, denoted by $S_{M^*\beta}$. As a component of a signal in the dynamic operating window method, we studied the following SN ratio η^*:

$$\eta^* = \frac{(\sigma_{M^*\beta})^2}{\sigma_N^2} \qquad (3)$$

2. Quantification of Component Separation

The basic idea of the dynamic operating window method for component separation in a chemical reaction is shown in Figure 2. However, if we consider the principle of chromatography in the HPLC process, we let liquid flow in a column, and by doing so, desorb components in the column and flush the desorbed components using pressure. It is more reasonable to regard this phenomenon as a physical thrust of desorbed components by energy of liquid motion rather than as a chemical reaction. In other words, a flow rate is equivalent to energy that thrusts components by raising the flow rate of liquid,
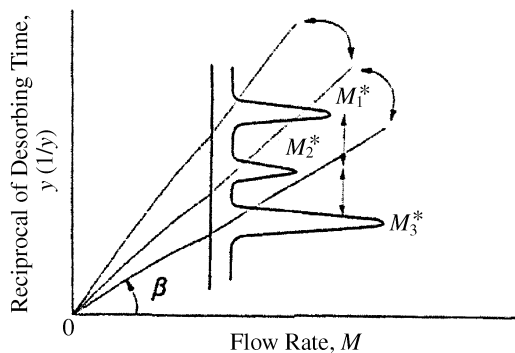
thereby applying pressure. Since this thrust energy removes components, a component with weak adsorption is pushed off earlier than one with strong adsorption.

We adopted an extended version of a basic dynamic operating window method rather than a reaction rate for time in a chemical reaction. That is, we focused our analysis on improving the detectability of a difference of components in the case of multiple operating windows. As a noise factor, we selected rising and falling times before and after a peak in the amount of desorbed components, N_1 and N_2 (refer to Figure 1). We consider the following flow rates:

Signal:	M_1	M_2	M_3
Flow Rate (mL/min):	5.0	4.2	3.3

Using the data in the second column of the L_{18} orthogonal array shown in Table 1, we calculated the SN ratio following the procedure outlined below. The original value was a peak distance from the origin (mm), and 80 mm was equivalent to 1 minute. Therefore, we divided the original value by 80 into a desorbing time in minutes and took its reciprocal:

$$S_T = \left(\frac{1}{64^2} + \frac{1}{76^2} + \cdots + \frac{1}{145^2} \right) 80^2 = 12.8243 \quad (4)$$

$$r = 5.0^2 + 4.2^2 + 3.3^2 = 53.53 \qquad (5)$$

Table 1
Results of experiment 2 (mm)

Component		Flow Rate			Linear Equation
		M_1	M_2	M_3	
M_1^*	N_1	64	76	96	L_1
	N_2	73	85	107	L_2
M_2^*	N_1	84	98	124	L_3
	N_2	96	110	138	L_4
M_3^*	N_1	89	106	132	L_5
	N_2	93	116	145	L_6

$$L_1 = \left[(5.0) \left(\frac{1}{64} \right) + (4.2) \left(\frac{1}{76} \right) + (3.3) \left(\frac{1}{96} \right) \right] 80$$

$$= 13.4211 \tag{6}$$

By doing the same calculations, we obtain

$$L_2 = 11.8997$$

$$L_3 = 10.3195$$

$$L_4 = 9.1343$$

$$L_5 = 9.6642$$

$$L_6 = 9.0183$$

$$S_\beta = \frac{(L_1 + \cdots + L_6)^2}{(3)(2r)} = 12.5375 \tag{7}$$

$$S_{M*\beta} = \frac{(L_1 + L_2)^2 + (L_3 + L_4)^2 + (L_5 + L_6)^2}{2r} S_\beta$$

$$= 0.2462 \tag{8}$$

$$S_{N\beta} = \frac{(L_1 + L_3 + L_5)^2 + (L_2 + L_4 + L_6)^2}{3r} - S_\beta$$

$$= 0.0350 \tag{9}$$

$$S_e = S_T - S_\beta - S_{N\beta} = 0.0056 \tag{10}$$

$$V_e = \frac{S_e}{14} = 0.0004 \tag{11}$$

$$V_N = \frac{S_{N\beta} + S_e}{14 + 1} = 0.0027 \tag{12}$$

$$\eta^* = 10 \log \frac{(1/2r)(S_{M*\beta} - 2V_e)}{V_N} = -0.71 \text{ dB} \tag{13}$$

$$S = 10 \log \frac{1}{(3)(2r)} (S_\beta - V_e) = -14.09 \text{ dB} \tag{14}$$

$$S^* = 10 \log \frac{1}{2r} (S_{M*\beta} - 2V_e) = -26.40 \text{ dB} \tag{15}$$

The SN ratio η^* represented the variability in the operating window's width between peaks M_1^*, M_2^*, and M_3^*. In other words, it indicated the stability of the differences among β_1, β_2, and β_3, each of which was the slope of each of M_1^*, M_2^*, and M_3^*, and $S_{M*\beta}$ is the signal's effect. Considering the separability for each of M_1^*, M_2^*, and M_3^*, the square root of an SN ratio represents the degree of separation. Therefore, improvement in the SN ratio leads to increased separability. Taking into account the definition of an SN ratio, an SN ratio is a scale not only for the degree of improvement in separability but also for a ratio of the variance between components to the variance within components. On the other hand, sensitivity S was used to accelerate the desorbing time. In addition, S^* was used to magnify an operating window's width, that is, to judge whether or not the gap among β values for M_1^*, M_2^*, and M_3^* is large. Separability was adjusted by tuning each parameter's sensitivity.

As illustrated in Table 2, we selected factors that were assumed to affect component separation and allocated them to an L_{18} orthogonal array. The levels of the control factors were selected according to the preliminary study of our current analytical con-

Table 2
Control factors and levels

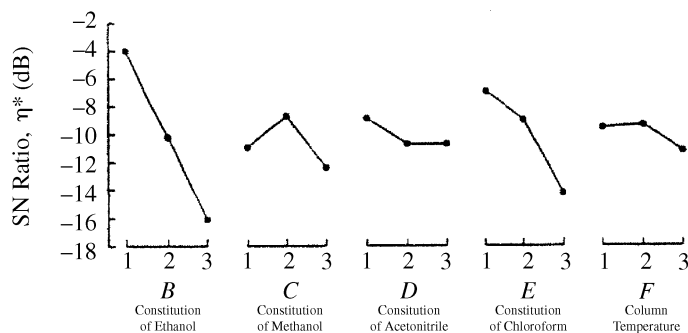| Control Factor		Level		
		1	2	3
A:	error			
B:	constitution of ethanol (%)	Low	Mid	High
C:	constitution of methanol (%)	Low	Mid	High
D:	constitution of acetonitrile (%)	Low	0	High
E:	constitution of chloroform (%)	Low	0	High
F:	temperature of column (°C)	Low	Mid	High
G:	error			
H:	error			

Figure 3
Factor effect plot of SN ratio η^*

ditions. The components chosen in this research were three types of components included in nature resources. The second component was not easily separated from the third. In addition, because of its high constitution, the third tended to overlap with the others in terms of the peak. Therefore, it was difficult to gain high separability.

3. Optimal Configuration and Confirmatory Experiment

On the basis of the idea outlined above, we designed experiments on component separation and conducted them and a subsequent analysis.

Figures 3 to 5 show the response graphs; using them, we selected levels with large decibel values of SN ratio η^* and sensitivities S and S^* to estimate the

optimal configuration. Primarily, we found that the factors largely affecting the SN ratio η^* were factors B, C, D, and E, whereas factor F had little influence on the SN ratio. Secondarily, looking at the sensitivities S and S^*, we noticed that they both became high at high SN ratio η^* levels. Then we determined the combination $B_1C_1D_1E_2F_1$ by choosing a level with a high decibel value as an optimal level.

Although essentially, the second level should be selected for factor C, we picked up the first level for efficiency because there was little influence of the SN ratio. The reason for selecting the second level for factor E in most cases is that in a product development process, technical issues are prioritized. On the other hand, as the worst configuration, we selected $B_3C_3D_3E_3F_3$ to estimate the SN ratio η^* and sensitivities S and S^*.

We conducted a confirmatory experiment and compared the results with those under the worst
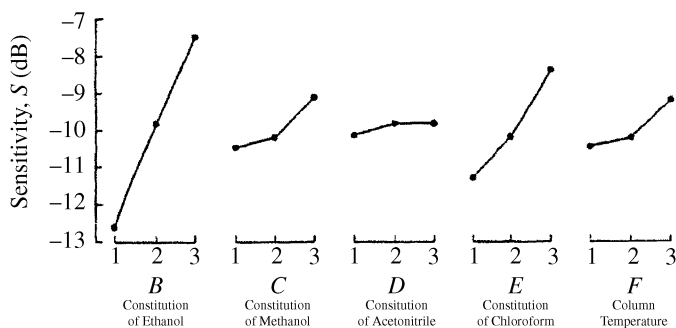

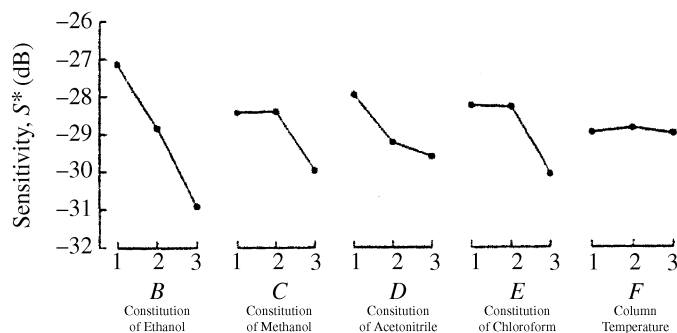**Figure 4**
Factor effect plot of sensitivity S

Figure 5
Factor effect plot of sensitivity S

configuration. As a result of the confirmatory experiment, we attained improvement in separation of peaks of M_1^*, M_2^*, and M_3^* (Table 3). Although separation was difficult in M_1^*, M_2^*, and M_3^*, by magnifying the operating window of three types of peaks and making an adjustment, we achieved sufficient separation. The remaining problem was poor reproducibility in gain. The response graphs explicitly revealed numerous interactions. Yet since reproducibility in the absolute value of the SN ratio under the worst configuration was poorer than that under the optimal configuration, we supposed that the worst configuration might have extremely poor separability. Despite inconsistent reproducibility in gain, by taking advantage of the adjustment factor (flow rate), our significant achievement was to reduce the analysis time from 10 to 20 minutes in the conventional process to only 1 minute.

The problem with our experimentation was that since the noise factors rely on the haphazard external disturbance of the variability in desorbing speed or desorbing time at the peak, the effects of the noise factor became less visible. The reason that the reproducibility in gain was poor was considered to be because the noise factor selected for our study was insufficient. Although recent analytical apparatuses have high accuracy and reproducibility, there remain some technical issues regarding inconsistent reproducibility in gain defined by quality engineering.

Although HPCL is well established as a means of evaluation, still needed is a comprehensive evaluation method to select an optimal configuration among various factors used for HPLC. In our traditional technique, there was no way other than use of a one-by-one method, based on our experience.

Table 3
Results of confirmatory experiment (dB)

Characteristic	Condition	Configuration		Gain
		Optimal	Current	
SN ratio η^*	Estimation	23.98	1.23	22.74
	Confirmation	28.96	−11.99	40.95
Sensitivity S	Estimation	0.43	−9.86	10.29
	Confirmation	−2.78	−8.20	5.42
Sensitivty S	Estimation	−11.82	−39.17	27.35
	Confirmation	−7.60	−50.19	42.59

Especially for the issues of stability and reproducibility in measurement, the only solution at present is to rely on a precision test. Additionally, for evaluation of separation, despite several proposed scales, comprehensive judgment has been limited. We see as our significant accomplishment the discovery that we can achieve not only a comprehensive judgment on separability but can also solve problems such as reproducibility and robustness by use of an SN-ratio-based evaluation method.

Reference

Kouya Yano, Hironori Kitzazki, and Yoshinobu Nakai, 1995. Evaluation of liquid chromatography analysis using quality engineering. *Quality Engineering*, Vol. 3, No. 4, pp. 41–48.

This case study is contributed by Kouya Yano.