

Simplex optimization of liquid chromatography with the function of mutual information as a criterion

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ABSTRACT

This paper demonstrates that the function of mutual information is a valid objective function for the simplex optimization problems in liquid chromatography. The optimum is indicated by the maximum of the function of mutual information over all the examined conditions. When a new peak which is fused with another peak is discovered by an experiment, the function of mutual information increases substantially to make it easy to approach the optimum. The simplex optimization of mobile phase composition (acetonitrile volume fraction) in reversed-phase liquid chromatography for the determination of a mixture of antipyretics (five analytes) is taken as an example.

INTRODUCTION

The simplex optimization has been successfully used, especially in high-performance liquid chromatography [1–7]. Details of the simplex algorithm and concepts can be found elsewhere [8,9].

The most important advantages of the sequential simplex methods are [1,7]: (1) no preconceived model of the retention behavior of solutes is required; (2) the identification or recognition of solutes in individual separations is unnecessary; (3) computational requirements are minimal; (4) any number of independent variables (mobile phase composition X , temperature T , etc.) can be considered.

As pointed out by Berridge [1], a stumbling block of the simplex optimization is the lack of a universally acceptable objective function. Unfortunately, most of the criteria so far proposed for optimization have been acknowledged to suffer to a greater or lesser extent from difficulties in reaching the global optimum or even of reaching the unequivocal definition of the optimum in liquid chromatography [10].

Particularly at the early stage of the simplex optimization, situations can arise where fused peaks

occur in a chromatogram and the number of solutes in a sample is unknown. Without knowledge of the number of peaks to be found, the optimal values of some criteria (TOV and CRF) will produce chromatograms comprising fused or strongly overlapped peaks [10]. A prerequisite for an ideal objective function must be as follows: if a subsequent experiment offers further separation and the proof that a peak previously observed is made up of two components, the criterion should give a much better response for this second experiment than for the previous experiment where the coexistence of the two components was not yet recognizable.

This paper demonstrates that the FUnction of Mutual Information, abbreviated as FUMI [11,12], meets the above-mentioned prerequisite for an ideal criterion.

FUMI can be calculated from the peak parameters (area A_j , width σ_j and retention τ_j) for every individual peak and from the noise level \tilde{a} (see Table I). The optimum is defined as the chromatogram which provides the maximum of FUMI among all the examined conditions. Many variables (mobile phase composition X , column length L , wavelength λ , flow-rate v , the amount of added

TABLE I
FUMI AND ITS RELATED FUNCTIONS

σ_j denotes the width (standard deviation) of Gaussian peak j ; A_j is peak area; \bar{a} is the power spectrum intensity of the filtered white noise [11]; $\kappa_f(j)$ and $\kappa_r(j)$ are the filter-off points [16]; τ_q is the retention time of the last peak q . Φ denotes the total information of a multi (q)-component chromatogram.

$\phi_j = \psi_j - \delta\phi_j$	FUMI for a peak
$\psi_j = \frac{1}{2} \log \left(\frac{A_j^2}{2\pi^{1/2} \sigma_j \bar{a}} \right)$	Intact information
$\delta\phi_j = -\frac{1}{2} \log \left(\frac{\kappa_r(j) - \tau_j}{\pi^{1/2} \sigma_j} + \frac{\kappa_f(j) - \tau_j}{\pi^{1/2} \sigma_j} \right)$	Information loss
$\Phi = \sum_{j=1}^q \phi_j$	FUMI for q peaks

internal standard, etc.) have been simultaneously optimized with FUMI Φ in reversed-phase liquid chromatography [11].

To scientists who are familiar with the traditional optimization methodology, FUMI Φ might seem to be far different from the traditional strategies for separation. On the contrary, the theory of FUMI has been proven to involve as a special case the concepts of commonly used optimization criteria based on the resolution R_s [13]. Only if peak j strongly overlaps with another (e.g., $R_s < 1.5$) is the increase in FUMI ϕ_j for peak j equivalent to the increase in the resolution R_s except for coefficient C (> 0) [13]:

$$\frac{\partial \phi_j}{\partial Z} = C \frac{\partial R_s}{\partial Z} \quad (1)$$

where Z denotes a chromatographic variable (X , L , λ , etc.). However, another equation derived from FUMI holds for sufficiently or unduly separated peaks (e.g., $R_s > 1.5$). In this situation, FUMI involves the sensitivity function s_j proposed by Snyder *et al.* [14] and also corresponds to the R_s minimum method under a special condition [13,15].

THEORY

FUMI ϕ_j for peak j is expressed as the subtraction of the information loss $\delta\phi_j$ due to peak overlap from the intact information ψ_j which is inherent to the

peak shape itself (see Table I). For simplicity, two peaks are considered. The total information Φ for a chromatogram is described as the sum of the individual peak information ϕ_j :

$$\Phi = \phi_j + \phi_{j+1} \quad (2)$$

If the peaks separate sufficiently ($\delta\phi_j = 0$ and $\phi_j \approx \psi_j$) and if they have almost the same information ϕ_j ($\psi_j \approx \psi_{j+1}$; almost the same area A_j and width σ_j), then Φ takes the simple form:

$$\Phi \approx 2\psi_j \quad (3)$$

If the peaks fuse, $\Phi = 0$ (see below).

If the fused peaks are treated as a single peak of doubled area $2A_j$, the apparent total information Φ^* can be calculated as:

$$\Phi^* \approx \psi_j + \log 2 \quad (4)$$

Eqn. 4 can be derived by substituting $2A_j$ into A_j of ψ_j .

Often, in practice [8], $\psi_j \approx 8$. Then, $\Phi \approx 16$ (eqn. 3) and $\Phi^* \approx 8.7$ (eqn. 4) ($\log 2 \approx 0.693$). Note that $\Phi \gg \Phi^*$.

Calculation of FUMI and simplex

The algorithm of FUMI was described in detail previously [11,12]. The arbitrary constant β involved in FUMI was set at 5.25, which means that the optimal peak resolution (R_s) is about 1.5 if the peak areas are the same [11,16].

A peak-search routine was used to detect individual peaks and calculate Φ^* (eqn. 4). Only if R_s exceeds 0.25 can the two peaks be recognized by the routine. The following terminology is used: the peaks (a) fuse, if $R_s < 0.25$; (b) strongly overlap, if $0.25 \leq R_s < 1.5$; (c) separate, if $R_s \geq 1.5$. The peak-search routine recognizes the fused peaks (j and $j + 1$) not as "two" but as "one" peak. In this case, Φ^* is calculated from the apparent peak parameters (e.g., $2A_j$ and σ_j in eqn. 4) of the apparently single peak. The apparent FUMI Φ^* is used as a criterion of the simplex optimization in this paper.

On the other hand, calculation of the exact FUMI Φ requires all the exact peak parameters A_j , σ_j and τ_j of every individual solute to be known before optimization. The fused peaks should be identified as being "two" peaks for the calculation of Φ . If all the values of Φ can be predicted over the

experimental conditions to be examined, then the optimum (the maximum of Φ) can easily be found, as shown by our previous papers [11,12,15,16]. This is called an interpretive method, and the simplex optimization is a sequential method [2].

For analytical purposes (precision Φ or efficiency ϑ of analysis), the optimum can be defined as the chromatogram which provides the maximum of FUMI Φ or the maximal transmission rate ϑ of the information FUMI [11,12]. In this paper, the Φ optimum (the maximal Φ) is focused, because the Φ optimum coincides with the ϑ optimum (the maximum of ϑ), especially in the optimization of mobile phase composition X in reversed-phase liquid chromatography [12].

The super-modified simplex (SMS) was used to determine the optimum of the variable X (mobile phase composition). FUMI ϕ_j represents the data-processing error relative standard deviation (R.S.D.) for peak j , which depends on the peak shape (A_j and σ_j), overlap (τ_{j-1} , τ_j , τ_{j+1} , A_{j-1} , etc.) and noise level \bar{a} . Then, the experiments of SMS were finished when the difference in the R.S.D. values of the updated vertices was less than 0.1%. SMS has been described in detail [9].

The simplex optimization was simulated on a PC9801 T laptop computer (NEC) and the language used was Quick BASIC.

Simplex optimization with FUMI

Fig. 1 shows the model dependence of the apparent total information Φ^* on the volume fraction X of an organic modifier in reversed-phase liquid chromatography. Two peaks are considered in Figs. 1 and 2 for the purpose of demonstration. The elution order is reversed at $X \approx 18\%$. The logarithm of the capacity factor k_j for peak j is assumed to be linearly related to X according to the proposition of Snyder *et al.* [14] (see the legend of Fig. 1). This $\log(k_j) - X$ relationship is illustrated in Fig. 2 of ref. 17. Throughout this paper, peak area A_j is assumed to be invariable in the optimization of X .

The typical chromatograms A–E in Fig. 1 are illustrated in Fig. 2 with the same letters. The values of Φ^* and Φ for chromatograms A–E are listed in Table II. The exact FUMI Φ takes the minimum (zero) in the X range from 16 to 20% (see chromatograms B, C and D and Table II). This means that no precise measurements can be obtained by chroma-

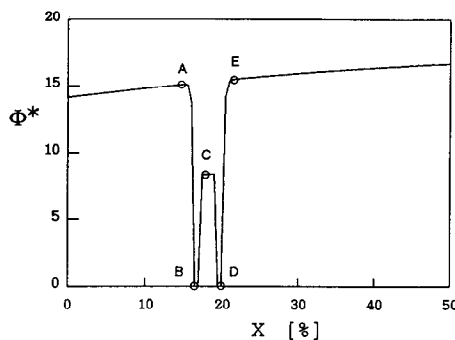


Fig. 1. Dependence of the apparent total information Φ^* on the mobile phase composition X for two peaks. $A_j = 10\,000$; $\bar{a} = 1$; $N = 10\,000$; $\beta = 5.25$; $\tau_0 = 100$ s. The linear $\log(k_j) - X$ relationship is assumed: $\log(k_w) = S_j X$ where S_j denotes the solvent strength for peak j [11]. $S_1 = 3.6$; $\log(k_{w1}) = 1.4$. $S_2 = 2.5$; $\log(k_{w2}) = 1.2$. This linear relationship is illustrated in Fig. 2 of ref. 17.

tophography in the above region because of the strong peak overlap or fusion ($R_s < 1.5$). The success of the chromatographic quantitation is ensured by the large amount of Φ (see chromatograms A and E) which is located in the X range except for around the elution reversal.

The apparent FUMI Φ^* takes a different value

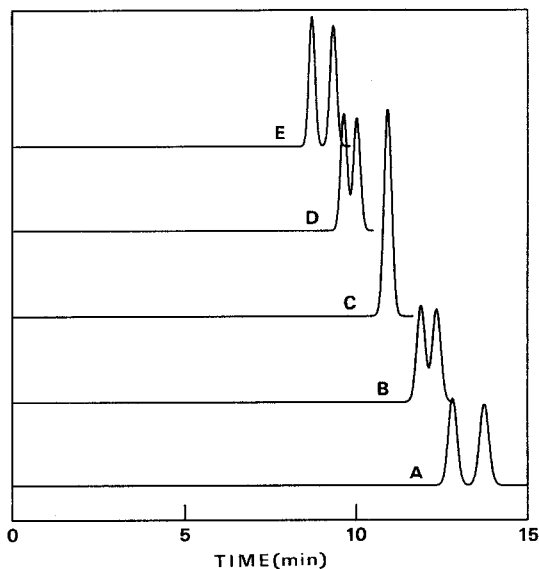


Fig. 2. Chromatograms indicated in Fig. 1. Chromatograms A–E take the information Φ^* indicated with the same letters A–E in Fig. 1. For Φ^* and R_s , see Table II.

TABLE II
 Φ^* AND Φ OF CHROMATOGRAMS A-E IN FIG. 1

Chromatogram (X)	Type of resolution (R_s)	Φ^*	Φ
A (15%)	Separated (1.76)	15.08	15.08
B (16.5%)	Strongly overlapped (0.92)	0	0
C (18.2%)	Fused (0)	8.31	0
D (20%)	Strongly overlapped (0.96)	0	0
E (21.5%)	Separated (1.71)	15.47	15.47

only for the fused peaks ($R_s < 0.25$; see chromatogram C). That is, Φ^* and Φ take the same values as long as the peak-search routine can recognize the individual peaks ($R_s \geq 0.25$). The value of Φ^* for the fused peaks in chromatogram C is much larger than that of Φ (=0) because the peak-search routine cannot recognize the individual peaks and Φ^* is calculated by regarding the almost fused peaks as a large single-component peak (see eqn. 4). This recognition of the apparent FUMI Φ^* is clearly not true. However, Φ^* must be a simple criterion for the simplex optimization in which the number of components in a sample is unknown.

This paper demonstrates the successful application of the apparent FUMI Φ^* to the simplex optimization. Again, we should note that Φ^* gives false values only if peaks fuse ($R_s < 0.25$). Then, Φ^* takes an exact value for the optimum ($\Phi^* = \Phi$).

The false recognition of the fused peaks (C) produces the hillock in the Φ^* - X line around

$X = 18\%$ (see Fig. 1). There is no hillock in the response surface of the exact FUMI Φ . The Φ - X line can easily be imagined from Fig. 1 and Table II and was given previously in Fig. 2 of ref. 17.

The hillock in Fig. 1 would have caused the most serious problem in using FUMI Φ^* as a simplex criterion if the probability that the top of the hillock (chromatogram C) was selected as the optimum by the simplex procedure was high. However, the information Φ^* at the top of the hillock is even lower than Φ^* at the high ridge where the optimum should be located and the above-mentioned probability can be concluded to be negligibly low (see below).

The most favorable property of FUMI for the simplex optimization is that FUMI Φ^* has a tendency to increase greatly when a new peak is discovered by an experiment with superior separation conditions (compare chromatogram C with A or E and their Φ^* values in Table II). Chromatograms A and E take almost the same resolution R_s , but FUMI indicates that chromatogram E is in terms of information theoretically superior to A because of the greater amount of information Φ^* (= Φ) that E provides compared with A. This difference in Φ^* arises from the distinguishable peak shape (mainly σ_j).

Fig. 3 shows the influence of the acetonitrile volume fraction X in water on FUMI for the reversed-phase liquid chromatographic analysis of a mixture of antipyretics [18]. The analytes were acetaminophen (S1), caffeine (S2), salicylamide (S4), guaifenesin (S5, as internal standard) and ethenz-

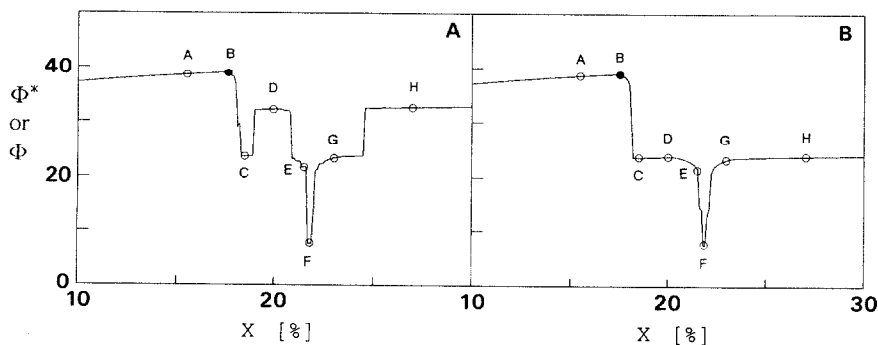


Fig. 3. Influence of the volume fraction X of acetonitrile in water on the apparent FUMI Φ^* (A) and the exact FUMI Φ (B). Analytes: acetaminophen (S1); caffeine (S2); salicylamide (S4); guaifenesin (S5, as internal standard); ethenzamide (S6). ● = The optimum (the maximum of Φ). The capacity factor k_j and peak width σ_j (standard deviation) were approximated by the simple model of Fritz and Scott [18,19]. The $\log(k_j)$ - X profiles (observed) are illustrated in Fig. 1 of ref. 18. The column used was Inertsil ODS (150 \times 4.6 mm I.D.; Gasukuro Kogyo), the temperature was 35°C and the detector was set at 290 nm [18].

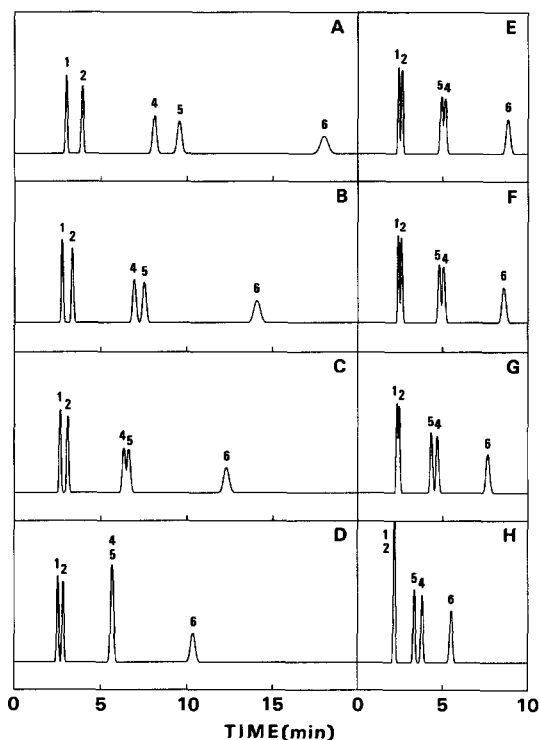


Fig. 4. Chromatograms of different information. Chromatograms A-H of this figure are indicated by the same letters in Fig. 3. X , Φ^* , Φ : (A) 15.5, 38.58, 38.58; (B) 17.4, 38.96, 38.96; (C) 18.5, 23.61, 23.61; (D) 20.0, 32.25, 23.73; (E) 21.5, 21.68, 21.62; (F) 21.8, 7.65, 7.65; (G) 23.0, 23.36, 23.22; (H) 27.0, 32.66, 23.8. Capital letter S is omitted.

amide (S6) (called mixture B in ref. 18). The retention behaviors of these analytes against X are illustrated in Fig. 1 of ref. 18.

The response surface of Fig. 3A denotes the

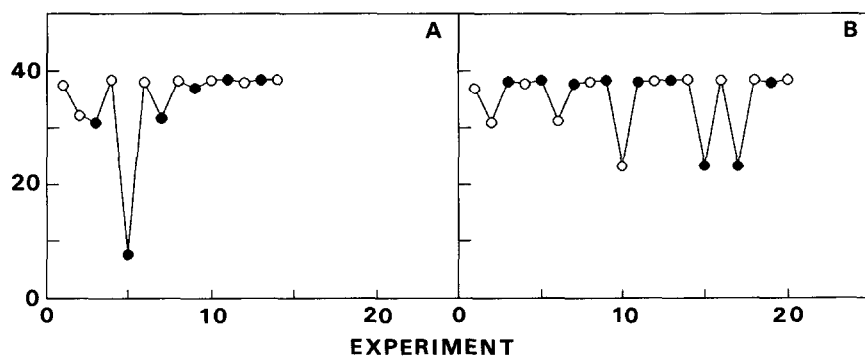


Fig. 5. Progress of the simplex Φ . A = RUN 1; B = RUN 2. The reflection points are denoted with closed circles. FUMI Φ^* was calculated. The detailed values of Φ^* and X are listed in Tables III and IV. Simulation is based on the $\log(k_j)$ - X profiles of Fig. 1 of ref. 18.

apparent total information Φ^* and that of Fig. 3B is the exact information Φ . The hillocks inherent to Φ^* appear around letters D and H. The chromatograms indicated with letters A-H in Fig. 3 are illustrated in Fig. 4 with the same letters.

As X increases from 10 to 17.4% (point B), the information Φ^* increases gently because of the increase in peak sharpness under the conditions of the complete peak separation (compare Fig. 4A and B). The value of X at point B (=17.4%) is the optimum (the maximum of FUMI).

Slightly above the optimum (point B), FUMI decreases abruptly because of the strong overlap of peaks S4 and S5 (Fig. 4C). When these peaks fuse into one and are not recognizable by the peak-searching routine, FUMI Φ^* , for Fig. 4D takes more information than for Fig. 4C, but is still much less than FUMI for the optimum (Fig. 4B).

In Fig. 4E, the elution order of peaks S4 and S5 is reversed. Although these peaks overlap strongly, they can be recognized individually. Peaks S1 and S2 weakly overlap and suffer from a slight information loss.

The strong overlaps of peak pairs of S1 and S2 and of S4 and S5 cause FUMI to decrease substantially (see Fig. 4F). The relaxation of the strong overlap of peaks S4 and S5 increases the information, as shown in Fig. 3G. When peaks S1 and S2 fuse (Fig. 4H), the apparent FUMI Φ^* takes a greater value ($\Phi^* = 32.7$), which is also much less than the maximal information ($\Phi = 39.0$) at point B.

Fig. 5A shows the evolution of FUMI Φ^* during the simulated simplex optimization (RUN 1). When a bad condition is selected, FUMI takes a low value

TABLE III
RESULTS OF THE SIMPLEX OPTIMIZATION WITH
FUMI Φ^* (RUN 1)

Experiment	X	Φ^*	Simplex movement
1	12.0	37.5	Initial
2	28.0	32.2	Initial
3	20.0	31.0	Reflection
4	16.8	38.5	Vertex
5	21.6	7.6	Reflection
6	14.6	38.1	Vertex
7	19.1	31.7	Reflection
8	15.8	38.3	Vertex
9	17.8	37.0	Reflection
10	16.4	38.4	Vertex
11	17.2	38.5	Reflection
12	17.6	38.0	Vertex
13	16.8	38.5	Reflection
14	17.0	38.5	Vertex

and the simplex proceeds further. Table III gives the experimental conditions and value of Φ^* at each experiment of the simplex (RUN 1). The chromatograms corresponding to the experiments can be imagined from the typical chromatograms of Fig. 4 and the information FUMI. The simplex was ter-

TABLE IV
RESULTS OF THE SIMPLEX OPTIMIZATION WITH
FUMI Φ^* (RUN 2)

Experiment	X	Φ^*	Simplex movement
1	10.0	37.0	Initial
2	20.0	31.0	Initial
3	15.0	38.2	Reflection
4	13.2	37.8	Vertex
5	16.4	38.4	Reflection
6	19.6	31.2	Vertex
7	13.2	37.8	Reflection
8	14.8	38.1	Vertex
9	16.4	38.4	Reflection
10	18.0	23.3	Vertex
11	14.8	38.1	Reflection
12	15.6	38.3	Vertex
13	16.4	38.4	Reflection
14	17.2	38.5	Vertex
15	18.8	23.3	Reflection
16	16.5	38.4	Vertex
17	18.0	23.2	Reflection
18	16.8	38.5	Vertex
19	17.6	38.0	Reflection
20	17.1	38.1	Vertex

minated after the large amount of FUMI was obtained.

As long as the initial conditions of X (two points) take a wide range in the simplex algorithm, evolution of FUMI similar to Fig. 5A can be obtained for this problem (see Fig. 5B and Table IV for RUN 2). The range of initial conditions for RUN 2 is narrower than for RUN 1 and the required number of experiments is larger for RUN 2.

Experiments 2, 3 and 7 are located on the hillocks of Φ^* (see Table III). Nevertheless, the simplex with FUMI is not at all thwarted by the hillocks. This holds true even if one of the initial conditions of the simplex falls on the hillock (see experiment 2 in Table IV and Fig. 5B).

DISCUSSION

The simplex with FUMI was terminated not after a good separation was obtained, as is the case for the traditional methods [7], but after a large amount of Shannon information was obtained. FUMI contains the concept of R_s in the information loss $\delta\phi_j$ and the sensitivity function s_j [14] in the intact information ψ_j as demonstrated by previous papers [13,17]. In other words, FUMI depends not only on the peak overlap (described by $\delta\phi_j$) but also on the peak shape itself (described by ψ_j and involving area A_j and width σ_j). Therefore, FUMI defines the optimal chromatogram as comprising the peaks of largest possible A_j and smallest possible σ_j without overlap. Of course, a small noise level \tilde{a} is preferred (see Table I).

Simplex is a hill-climbing method and is susceptible to the well known, important problem of locating the local, rather than the global, optimum [1]. FUMI cannot circumvent this problem, which is inherent to the mathematics of the simplex optimization.

FUMI Φ^* can overcome the problem produced by itself (the hillock in Figs. 1 and 3). On the other hand, the exact FUMI Φ has been proved to be a reliable criterion in the optimization of liquid chromatography [11,12]. As evident from Fig. 3A and B, given knowledge on a number of solutes, FUMI can provide smoother response surfaces (Fig. 3B) and will enable more rapid and less disturbed advance of the simplex optimization than the simplex presented in this paper (Fig. 3A). The

currently available multi-channel detectors will be useful for the disclosure of fused peaks [1]. The most significant advantage of FUMI is that Φ^* increases greatly when a new peak is discovered by chromatographic experiments.

Fig. 4G was proposed to be the optimum in previous papers [16,18]. This optimum is suitable for a data processor of excellent peak-resolving powers, such as the Kalman filter ($\beta = 3.25$ and $R_s \approx 1$ for the optimal peak separation). The optimal chromatogram presented here (Fig. 3B) consists of peaks of R_s greater than 1.5 ($\beta = 5.25$) and precise quantitative data can be collected with a data processor of inferior peak-resolving powers, such as perpendicular dropping.

The simulation in this paper involves a problem which has been considered difficult in chromatographic optimization, *i.e.*, the reversion of elution order. In the X region below 10%, the observation time becomes more than 1 h. Most of the peaks overlap strongly or fuse in the X range of more than 30%. If the initial conditions are X of 0 and 100%, then the result will be the same as the 10–30% of this paper. However, the initial conditions of 20–40% may not reach the global optimum. The simulation (RUN 1 and RUN 2) will suffice for the aim of this paper, which is to demonstrate the applicability of FUMI to the simplex optimization of liquid chromatography.

CONCLUSION

The authors and their colleagues have studied the applicability of FUMI to the total chromatographic optimization (TOCO) of many variables [11] and to

theoretical interpretation, in terms of information, of the analytical roles of chromatographic variables [12,13,17]. This paper has further expanded the dimension of FUMI to the simplex optimization..

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