Influence of Integration Domain on Precision of Measurements in Liquid Chromatography

Yuzuru HAYASHI and Rieko MATSUDA

National Institute of Health Sciences, Kami-Yoga, Setagaya, Tokyo 158, Japan

This paper focuses on the relative standard deviation (RSD) of measurements obtained by the integration of noisy data in both Monte Carlo simulation and HPLC experiments. No peak overlap is considered. As the integration domain, T , which is symmetrical around the peak center is increased up to ten times the width, σ (SD), of a Gaussian peak, the integration over the peak has a minimal RSD at $T = (-1.4\sigma, +1.4\sigma)$ in the measurement model where the white noise is the only error source. This optimal integration domain, if described as $T/\sigma = (-1.4, +1.4)$ in the scale of width, is shown to be independent of the entire peak area, width and noise level. Similar results are obtained by the Monte Carlo simulation for skewed Gaussian peaks and by differentiating the RSD of a triangle signal with respect to the integration domain. The probability theory proposed here exactly predicts the RSD observed by the simulation for the integration of the Gaussian and non-Gaussian peaks. Although a minimal RSD is not evident in the HPLC data for some aromatic compounds, an important property of the model integration is retained in a trace analysis: the precision of the integration around the optimal domain (e.g., $\pm 1.5\sigma$) is superior to that over a wide domain (e.g., $\pm 4\sigma$).

Keywords Chromatography, precision, integrator, signal processing, chemometrics

Integration methods have been used commonly in chromatography for quantitative purposes.¹⁻⁵ There have been several publications concerning the availability of the mathematical techniques to many chromatographic situations where peaks are overlapped.¹⁻⁴ The integration of a single peak or completely separated peak is also a usual strategy for quantification.⁵

This paper focuses on the statistical reliability of integrating an isolated peak. No peak overlap is considered. A simple measurement model is adopted in which the white noise is the only error source in the output of chromatography. In this model, the accuracy is perfect or the bias is zero. The only concern of this paper is the precision which is another important criterion when evaluating analytical methods.⁶

First, Gaussian peaks are taken in the measurement model. Needless to say, such symmetrical peaks are idealistic and seldom appear in the real circumstances. Therefore, skewed Gaussian peaks which are known to give even better band fitting in HPLC are also taken.

The optimal integration domain is defined as the time period of the signal processing that provides the minimum RSD (relative standard deviation) of measurements. This paper demonstrates that the optimum is rather narrower (indicated by the shaded region in Fig. 1) than the $\pm 3\sigma$ domain which covers 99.7% of the entire Gaussian peak area around the center.

The confidentiality of the above-mentioned result is verified in this paper by computer simulation, called a Monte Carlo technique, and by probability theory.

These disparate approaches are shown to be in good agreement on the optimization problem of the integration. Additional support is given by differentiating the RSD of a triangle signal with respect to the integration domain. The extremum of the derivative also identifies as the optimum a rather narrower domain than the base of the triangle.

The measurement model based only on white noise is far from the real-situations where other types of noise

Fig. 1 Gaussian signal and integration domain, T .

exist. Therefore, it is essential to compare the results from the simple measurement model and theoretical approach with the real data of HPLC analysis. A narrow integration domain (e.g., $\pm 1.5\sigma$) is also shown to be preferred in the light of the statistical aspects, rather than a wide domain (e.g., $\pm 4\sigma$).

The optimal integrator which always gives the minimal RSD in a variety of real chromatographic situations would be enormously difficult to design. The optimal integrator cannot be found or formulated, until the stochastic properties of most of random noise involved in the chromatographic apparatus is elucidated. The conclusion of this paper is not a proposition of the optimal integrator. This paper, however, will play an important role in giving a clue to a solution of this kind of optimization problem in quantitative analysis.

Precision of Integration in Monte Carlo Simulation

The RSD of the signal integration is obtained by the Monte Carlo simulation as follows. Figure 1 illustrates a discrete Gaussian signal and integration domain, T . Random noise, called white noise, with zero mean and normal distribution of standard deviation, $\tilde{w}$, is computer-generated and the Gaussian peak is overlain at every data point by the white noise. The noisy data are summed or integrated over a time period, T . This sum is recorded on the computer memory as estimate 1. These procedures are repeated n times on the same Gaussian peak, fixed time domain, T , and the same SD of the white noise, but on different noise appearances. The RSD of the integration in the situation is calculated from the n estimates.

Figure 2 shows the dependence of the observed precision on the integration domain. The RSD at $T=0$ means the precision of the peak height measurement.

Fig. 2 Influence of entire peak area, A , on precision. $A=100$ (Δ); 2000 ($\bullet$); 50000 ($\circ$). $T+1$ denotes the number of data points integrated ($\Delta T=1$); $\sigma=10$ data points; $\tilde{w}=1$; $n=10000$.

As the integration domain increases from $T=0$, the observed RSD of the estimates decreases and then increases gently. The first decrease in the RSD occurs in the region of high signal-to-noise ratio (S/N). As long as the signal intensity is much higher than the noise level, $\tilde{w}$, the wider the integration domain, the higher the precision.

The integration of raw data accumulates not only the true signal but also the unnecessary noise. If the signal intensity at the edge of the peak is comparable to or lower than the noise level, the further accumulation of the relatively large noise deteriorates the precision of the integration. Clearly, the integration has an RSD minimum or precision maximum at an integration domain, $\hat{T}$. Such integration is called optimum for a while for convenience, although it is not necessarily the optimum in real HPLC situations as mentioned below.

The influence of the entire peak area, A , on the precision is also demonstrated in Fig. 2. Although the RSD of measurements decreases with increasing the entire peak area, A , the statistical behavior of the data handling method against the integration domain is quite similar for the peaks of different entire areas. Moreover, the optimal integration domains, $\hat{T}$, are constant irrespective of peak area, A ; $\hat{T}=2.8\sigma$ or 28 data points ($\sigma=10$ in Fig. 2).

Figure 3 shows the dependence of the observed RSD values on the peak width, σ . The entire peak area is kept constant in this situation. As the peak width decreases and the peak becomes sharp, the optimal integration domain decreases and the RSD at the optimum also decreases. However, the optimal domain, $\hat{T}$, in the scale of peak width is invariant irrespective of the change in the peak width: $\hat{T}/\sigma$ was always observed to be 2.8.

To recapitulate the above results, the optimal integration domain, $\hat{T}/\sigma$, is determined to be 2.8 and is independent of the entire peak area, A , width and noise

Fig. 3 Influence of peak width, σ , on precision. $\sigma=20$ (Δ); 10 ($\bullet$); 5 ($\circ$). $T+1$ denotes the number of data points integrated ($\Delta T=1$); $A=5000$; $\tilde{w}=1$; $n=10000$.

level. All the results from the simulation in Figs. 2 and 3 coincide with the theoretical lines (solid lines). This error prediction theory is described in the subsequent section.

Probability Theory on Precision of Integration

If an estimate is obtained by integrating the raw data in k data points, then the RSD of the estimates can be described as (see Appendix):

$$\text{RSD}_1^2 = \frac{k\tilde{W}^2}{(F_1 + F_2 + \cdots + F_k)^2} \quad (1)$$

where $F_i (i=1, 2, \cdots, k)$ denotes the signal intensity (or true intensity) of the peak at data point i . The whole area of the Gaussian peak is given as:

$$\lim_{k \rightarrow \infty} F_1 + F_2 + \cdots + F_k = A \quad (2)$$

The integration domain (k data points) contains a part of the whole area. The theoretical lines in Figs. 2 and 3 were obtained from Eq. (1).

The existence of a minimal RSD can easily be imagined from Eq. (1). If a narrow integration domain, k , is taken around the peak center, the increase in the square of the partial peak area, $(F_1 + F_2 + \cdots + F_k)^2$, will exceed the increase in the noise accumulation, $k\tilde{W}^2$. The RSD will decrease with increasing k in this situation. However, if the integration domain reaches the edge of the peak, the increase in the partial area will level off because of the shape of a Gaussian peak; however, the noise accumulation continues constantly. Then, the RSD will increase with k . A minimal RSD should be located somewhere between the extreme domains considered above.

If the integration domain is expressed by the time period, T , then Eq. (1) can be written as:

$$\text{RSD}_1^2 = \frac{(T/\Delta T + 1)\tilde{W}^2}{(F_1 + F_2 + \cdots + F_{T/\Delta T + 1})^2} \quad (3)$$

where ΔT denotes the sampling interval of an A/D converter and $T/\Delta T + 1 = k$.

The signal intensity, y , of a Gaussian peak at time, x , is represented by:

$$y(x) = \frac{A}{(2\pi)^{1/2}\sigma} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \quad (4)$$

The height, h , of the peak maximum ($x=\mu$) is given as:

$$h = \frac{A}{(2\pi)^{1/2}\sigma} \quad (5)$$

Then, the ratio, R , of the signal intensity at the edge of the optimal integration domain to the maximum peak

height, h , is written as:

$$R = y(\mu \pm 1.4\sigma)/h = \exp[-1.4^2/2] = 0.38 \quad (6)$$

R is a constant which is independent of the peak area, width and noise level.

Integration of Triangle Signal

The optimal integration domain has been determined so far by the repeated computer simulations. The primary demand for a theoretical solution of the optimum is to differentiate Eq. (1) or (3). However, the most serious problem is that the integral of a Gaussian peak remaining in the derivative of the equation for RSD is impossible to solve using elementary mathematics. Instead, a triangle signal is examined here for the understanding of the property of the optimal integration.

The signal intensity, y , of a triangle in the first quadrant is described as:

$$y = h - \frac{h}{\sigma} x \quad (7)$$

where h denotes the height of the triangle and 2σ the length of the base (see Fig. 4). Then, the partial area, $f(T)$, under the triangle over the symmetrical integration domain, T , is given as:

$$f(T) = 2 \int_0^{T/2} \left(h - \frac{h}{\sigma} x \right) dx = 2 \left(h \frac{T}{2} - \frac{h}{2\sigma} \left(\frac{T}{2} \right)^2 \right) \quad (8)$$

The RSD of the integration over the domain, T , is written as:

Fig. 4 Triangle signal and integration domain, T .

$$\text{RSD}_1 = \frac{(T/\Delta T + 1)^{1/2} \tilde{W}}{2 \left(h \times \frac{T}{2} - \frac{h}{2\sigma} \left(\frac{T}{2} \right)^2 \right)} \quad (9)$$

The extremum of the RSD is indicated by the derivative:

$$\frac{d\text{RSD}_1}{dT} = 0 \quad (10)$$

Differentiating Eq. (9) and noticing Eq. (10), we can obtain the value of T at the extremum:

$$\frac{2}{3} \left\{ \sigma - \Delta T \pm \sigma \left[\left(\frac{\Delta T}{\sigma} \right)^2 + 4 \frac{\Delta T}{\sigma} + 1 \right]^{1/2} \right\} \quad (11)$$

This equation demonstrates that the optimal integration domain has no connection with the peak height, h , or entire area, σh .

If $\sigma \gg \Delta T$ (i.e., the number of data points is sufficiently large), Eq. (11) takes the approximate form:

$$\hat{T} \approx \frac{4}{3} \sigma \quad (12)$$

The optimal integration domain is clearly narrower than the entire region, 2σ , of the triangle signal. The integration domain in Fig. 4 represents the optimum.

Equation (12) indicates all of the properties of the optimal integration mentioned above. For example, the ratio, R , of the signal intensity at the edge of the optimal domain, $\hat{T}$, to the height, h , is given as:

$$R = \left(h - \frac{h}{\sigma} \left(\frac{2}{3} \sigma \right) \right) / h = 1/3 \quad (13)$$

The optimal integration can be achieved by summing the raw data over the data points at which the intensity of a model peak is more than one third of the maximum peak height. This result bears a close resemblance to that for a Gaussian peak.

Precision of Kalman Filter and Integration

In general, different signal processing methods yield different precision.^{7,8} However, it is important to know the similar behavior of a Kalman filter, one of the least squares, to those of the integration.

As far as the simple measurement model is concerned, the RSD of the Kalman filter is known to be described as:^{7,8}

$$\text{RSD}_L^2 = \frac{\tilde{W}^2}{F_1^2 + F_2^2 + \dots + F_k^2} \quad (14)$$

Unlike the integration, the RSD of the Kalman filter is a monotone decreasing function with the domain, k , (compare Eqs. (1) and (14)) and has no minimum RSD.

The RSD of the filter almost reaches the minimum, even if the filtering region, k , is much narrower than the $\pm 3\sigma$ domain of a Gaussian peak.⁹ In the theory of the Kalman filter, the square of the RSD in Eq. (14) is equal to the error variance, $P_k (= \text{RSD}_L^2)$. In addition, the precision of the Kalman filter is generally higher than that of the integration in the situations treated in this paper: $\text{RSD}_1 \geq \text{RSD}_L$ (see Appendix).

Precision of Integration for Skewed Gaussian Peaks

The asymmetrical shape, F_k , of a skewed Gaussian peak at data point k can be described as:^{10,11}

$$F_k = a_0 \exp \left(-\log 2 \times \left\{ \frac{\log [1 + 2b(k - k_0)/X_{1/2}]}{b} \right\}^2 \right) \quad (15)$$

where a_0 , b , k_0 and $X_{1/2}$ are constants to be determined by the least squares curve fitting of a noisy peak. The skewness of a peak is related to constant b ; the peak maximum is located at k_0 ; $X_{1/2}$ can be connected with the peak broadening. For some antipyretics (acetaminophen, caffeine, salicylamide, etc.), the fitting parameter and goodness of fit are given in ref. 11; the determined parameter b for the skewness ranged from 0.2 to 0.4.

Figure 5 illustrates typical skewed Gaussian peaks with a skewness parameter b of 0.3 (A) and 0.5 (B). Skewed peaks with b of 0.1 or 0.2 appear to be symmetrical upon a quick glance. Peaks with b of 0.7 will be out of question in chromatography because of excessive tailing. The shaded domain gives the minimal RSD of the integration results under the same situations as that for the Monte Carlo simulation in Figs. 1 to 3. The integration domains for the highest precision of Figs. 1, 4 and 5 are quite similar to each other.

Table 1 lists the statistical results from the simulation and theory (Eq. (1)). The heading half and tailing half

Fig. 5 Typical skewed Gaussian signals and optimal integration domains, T . $k_0=0$; $a_0=1000$; $X_{1/2}=50$. A: $b=0.3$; B: $b=0.5$.

Table 1 Maximal RSD (%) obtained at optimal integration domain for skewed Gaussian peaks

<i>b</i>	Data point		RSD, %	
	Head	Tail	Observed	Predicted
0.1	-27(0.4)	31(0.38)	0.171	0.173
0.2	-26(0.4)	33(0.38)	0.171	0.172
0.3	-24(0.4)	36(0.37)	0.171	0.172
0.5	-21(0.4)	42(0.36)	0.174	0.170
0.7	-19(0.4)	49(0.34)	0.160	0.168

The peak maximum is fixed at $k_0=0$; $a_0=1000$; $x_{1/2}=50$. The values in the parentheses denote the ratio of the signal intensity at the edge of the integration domain to that at the peak maximum.

of the skewed Gaussian peaks are separately treated for convenience and different domains for the highest precision are obtained for each half. The combination of the optimal domains of the half parts is defined to be the domain of the highest precision for the entire peak, assuming that there is no probabilistic interference between the halves. The theoretical prediction (Eq. (1))

of the precision of the integration results is also excellent in this situation. Like the Gaussian peaks, the intensity of the skewed Gaussian signals at the edge of the best integration domain ranges from 0.3 to 0.4 of the intensity at the peak maximum. Irrespective of the peak shape (Gaussian or skewed Gaussian), the optimal integration can be achieved over the peak-shaped signals more than the limit ($\approx$ ca. 40% of the maximum peak height).

Precision of Results from Integration and Least Squares in HPLC

The statistical results of the HPLC experiments for aromatic compounds are listed in Table 2. The width, σ , is calculated from the peak area and height of the peak maximum by approximating the real peak to a Gaussian peak (using Eq. (5)). Table 2 demonstrates that the precision from the narrow integration domain is superior to that from the wide integration domain (compare the RSD values of $\pm 1.5\sigma$, 15 points and peak height measurement at the diluted samples (1/500 and 1/200) with those of Area and $\pm 2.5\sigma$). The domain of the

Table 2 Observed RSD (%) of measurements obtained from different signal processing methods in HPLC ($n=8$)

	Conc. ^a	Integration domain				Peak maximum height	Least squares
		Area ^b	$\pm 2.5\sigma$	$\pm 1.5\sigma$	15 points		
Naphthalene ($\sigma=12$ data points)	1/500	8.81	6.63	5.28	4.42	4.33	5.29
	1/200	4.89	3.73	2.66	2.07	2.15	2.78
	1/100	2.36	2.29	2.14	2.02	1.94	2.36
	1/50	0.55	0.51	0.45	0.44	0.44	0.42
	1/20	0.29	0.26	0.27	0.26	0.22	0.35
	1/10	0.20	0.19	0.24	0.26	0.22	0.43
	1	0.17	0.16	0.25	0.33	0.29	0.28
Acenaphthene ($\sigma=14$ data points)	1/500	19.91	13.66	10.82	8.88	8.09	10.95
	1/200	5.36	2.97	2.14	2.01	2.67	2.30
	1/100	4.13	3.59	3.24	2.94	2.48	3.18
	1/50	1.85	1.42	1.16	1.20	1.13	1.19
	1/20	0.85	0.52	0.39	0.32	0.42	0.46
	1/10	0.48	0.36	0.31	0.29	0.35	0.40
	1	0.23	0.20	0.22	0.33	0.53	0.26
Pyrene ($\sigma=17$ data points)	1/500	7.11	6.70	5.83	4.96	4.63	5.99
	1/200	3.64	2.51	1.90	1.52	1.51	2.30
	1/100	3.21	2.81	2.57	2.51	2.38	2.51
	1/50	0.75	0.62	0.58	0.53	0.50	0.78
	1/20	0.45	0.35	0.26	0.24	0.27	0.26
	1/10	0.56	0.23	0.23	0.24	0.28	0.37
	1	0.38	0.23	0.21	0.31	0.25	0.22
Perylene ($\sigma=23$ data points)	1/500	13.69	10.61	8.58	6.96	6.87	8.43
	1/200	6.11	4.50	3.52	2.81	2.75	3.46
	1/100	3.28	3.03	2.82	2.65	2.55	2.80
	1/50	1.51	1.44	1.32	1.26	1.06	1.51
	1/20	0.68	0.49	0.42	0.47	0.39	0.37
	1/10	0.32	0.20	0.17	0.18	0.21	0.27
	1	0.26	0.19	0.15	0.23	0.29	0.26

a. Unit concentration denotes 0.05 mg/ml for naphthalene and acenaphthene, 0.02 mg/ml for pyrene and 0.03 mg/ml for perylene (for detailed experimental conditions, see ref. 8). b. A wide integration domain which is equal to the working region for the least squares curve fitting: $\pm 5\sigma$ for naphthalene; $\pm 4\sigma$ for acenaphthene; $\pm 5\sigma$ for pyrene; $\pm 4\sigma$ for perylene.

15 data points is even narrower than the integration domain for Area. For example, since the width, σ , of the naphthalene peak is 12 points and that of Area is $\pm 5\sigma$, the number of data points involved in the Area domain is 120.

This observation coincides with the conclusion obtained from the above-mentioned measurement model, but is available only for the diluted samples. As for the concentrated samples (1 and 1/10), the precision can be considered almost the same for every analyte within the experimental error. In a macro analysis of HPLC, the predominant error source in the total precision comes from the sample injection, whereas in a trace analysis, random noise observable in chromatograms outweighs the injection volume error.⁸ The precision is almost commensurate for every analyte in the macro analysis, irrespective of the peak shape (area and width), though it greatly depends on the peak shape in the micro analysis.⁸ Therefore, the simple model in which the white noise is the only error source is a better approximation to the uncertainly structure of the micro analysis than to that of the macro analysis.

The precision around the optimal integration domain ($\pm 1.4\sigma$) is still excellent in the HPLC experiments (see Table 2). However, the precision for the three integration domains ($\pm 1.5\sigma$, 15 data points and peak height measurement (one point) does not seem to be distinguished from each other, although it has been shown to be higher than that for the integration over the wide domains. Random noise rather than the white noise would affect the results from the diluted samples in Table 2, making obscure or shifting the integration domain of the highest precision.

In theory, the precision of the integration at the optimal domain (Eq. (1)) is nearly equal to that of the least squares at a wide domain (Eq. (14)). Interestingly, Table 2 shows that the observed RSD of the integration measurements at $\pm 1.5\sigma$ is comparable to that for the least squares for the diluted samples. On the other hand, as for the concentrated samples, the precision seems to be independent of not only the peak shape but also the signal processing.

Discussion

The theory given in this paper has been developed on the simple assumption that the white noise is the only contribution to the imprecision of measurements. However, real chromatograms are contaminated by some kinds of noise other than the white noise. Recently, the real noise in HPLC was proved to be well approximated by the white noise and Brownian motion⁸, and the prediction of the real RSD of measurements based on this approximation is excellent for some aromatic compounds.⁸

If the probability theory presented in this paper is evolved in the light of the Brownian motion as well as white noise, the results will more or less differ from those

obtained here. For example, the optimal integration domain will be narrowed due to the adverse effect of the Brownian motion. This subject is now under investigation by our group.

It is not easy to repeat a measurement many times in chromatography, unlike NMR. The advantage of the uncertainty prediction presented here lies just in this point. The reliability of a measurement can be judged based on the predicted RSD as a criterion, dispensing with the tiresome repetition of measurement. The improved reliability by the repetition can also be evaluated using the same criterion.

Various signal processing methods are feasible for quantification in linear measurement system in which the linearity between the analyte quantity and signal intensity at every data point is satisfied. Not only the entire peak area measurement but also a measurement at a fixed, narrow time domain (e.g., the optimal integration here) is directly proportional to the quantity to be determined. Even a one-point measurement (peak height measurement) is available. However, the estimation of the entire peak area is necessary for the same purposes, especially in a non-linear system. As long as the repeatability of the measurement is ensured, Eq. (1) and the optimal integration are also applicable to this adverse situation, but the quantification based on the narrow integration domain would lead to erroneous determination.

The absolute error is assumed to be constant throughout this paper *i.e.*, SD of the white noise is fixed. This assumption seems to be appropriate in liquid chromatography⁸, but it has yet to be verified by further experiments on photomultiplier tube and photodiode. In the case of constant relative error (RSD of the white noise is constant), the equation for the error prediction (Eq. (1)) also holds good except for a slight modification.

The authors would like to thank Dr. T. Hanai for his useful suggestions.

Appendix

Derivation of Eq. (1)

If the white noise is the only disturbance in the output of chromatography, the area estimate measured in k data points is described as:

$$Y(k) = F_1 + W_1 + F_2 + W_2 + \cdots + F_k + W_k \quad (\text{A1})$$

where $F_i (i=1, 2, \dots, k)$ denotes the signal intensity at data point i and W_i the intensity of the white noise with standard deviation $\tilde{W}_i$. By definition, the mean of the white noise is zero and the mean of the area estimates in k data points is given as:

$$f(k) = F_1 + F_2 + \cdots + F_k \quad (\text{A2})$$

The standard deviation of the white noise is assumed to be the same for every data point ($\tilde{W}_i = \tilde{W}$). Then, the variance of the area estimates takes the form:

$$\text{Var}(f(k)) = k \tilde{W}^2 \quad (\text{A3})$$

The division of Eq. (A3) by the squared average, $f(k)^2$, leads to the relative standard deviation of the area estimates:

$$\text{RSD}_I^2 = \frac{k \tilde{W}^2}{f(k)^2} \quad (\text{A4})$$

This is equal to Eq. (1).

Derivation of inequality, $\text{RSD}_L < \text{RSD}_I$

The denominators of Eqs. (1) and (14) are compared:

$$\begin{aligned} & k(F_1^2 + F_2^2 + \cdots + F_k^2) - (F_1 + F_2 + \cdots + F_k)^2 \\ &= (k-1)(F_1^2 + F_2^2 + \cdots + F_k^2) - 2F_1F_2 - 2F_1F_3 - \cdots - 2F_{k-1}F_k \\ &= (F_1 - F_2)^2 + (F_1 - F_3)^2 + \cdots + (F_{k-1} - F_k)^2 \\ &\geq 0 \end{aligned} \quad (\text{A5})$$

This equality is satisfied, only if the signal is rectangular ($F_1 = F_2 = \cdots = F_k$).

References

1. N. Dyson "Chromatographic Integration Methods", Royal Society of Chemistry, Cambridge, 1990.
2. L. R. Snyder, *J. Chromatogr. Sci.*, **10**, 200 (1972).
3. J. P. Foley *J. Chromatogr.*, **384**, 301 (1987).
4. A. Westerberg, *Anal. Chem.*, **41**, 1770 (1969).
5. A. N. Papas and M. F. Delaney, *Anal. Chem.*, **59**, 54A (1987).
6. R. A. Day, Jr. and A. L. Underwood, "Quantitative Analysis", Prentice Hall, New Jersey, 1991.
7. Y. Hayashi and R. Matsuda, *Chemom. Intell. Lab Syst.*, **18**, 1 (1993).
8. Y. Hayashi and R. Matsuda, *Adv. Chromatogr.*, **34**, 347 (1994).
9. Y. Hayashi, S. Yoshioka and Y. Takeda, *Anal. Chim. Acta*, **212**, 81 (1988).
10. R. D. B. Fraser and E. Suzuki, *Anal. Chem.*, **41**, 37 (1969).
11. Y. Hayashi and R. Matsuda, *Anal. Sci.*, **5**, 459 (1989).

(Received February 10, 1994)

(Accepted May 18, 1994)