Chemometric Evaluation of Repeatability Using the Autocorrelation Method in High-Performance Liquid Chromatography with Ultraviolet Detection

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The mixed random processes of the first order autoregressive process (AR(1)) and white noise have been proved to provide a good approximation of baseline noise in a variety of analytical instruments, and may therefore be useful for estimating precision profiles. This study aims to examine a recently proposed autocorrelation method for estimating three noise parameters involved in the mixed processes (two for AR(1) and one for white noise) of HPLC, which can then be used to calculate the precision profile. This chemometric method was applied to repeatability evaluations of estriol determination using HPLC with UV detection (HPLC-UV). The relative standard deviations (RSDs) of peak area measurements for 5.0 mg/L estriol were observed to be 1.42% for the autocorrelation method and 1.63% for actual repeated measurements of real samples (n = 6). The theoretical RSDs of the autocorrelation method fell within the 95% confidence intervals of the repeated measurements. It is found that the noise parameters are obtained from real chromatographic baseline via the autocorrelation method. Moreover, the instrumental detection limit of estriol based on ISO 11843 was obtained from the precision profile (plot of RSD of measurements against concentration). This is the first paper to describe the autocorrelation method as a practically useful technique for evaluating the precision profile of HPLC-UV analyses without recourse to the repeated measurements of real samples.

Key words autocorrelation method; baseline noise; FUMI theory; repeatability; stochastic process

Introduction

Repeatability assessments are integral to reliable quantitative analyses using HPLC. The repeatability of HPLC analyses is generally expressed as the relative standard deviation (RSD) of peak area and/or peak height measurements.1–5 As a single chromatogram normally requires more than 10 min to generate, statistical repeatability assessments based on multiple measurements involve substantial quantities of chemical samples and experimental time.

To reduce this experimental burden, various theoretical approaches have been developed to enable the mathematical interpretation of noise that ubiquitously exists in analytical equipment regardless of whether a sample is being analyzed.6–13 The principal advantages of these chemometric strategies include reducing the need for materials, time, energy, and human effort that would otherwise be required to perform repeated measurements. As an example, the function of mutual information (FUMI) theory, the practical applicability of which has been proved experimentally in a variety of settings, has been adopted as an international standard (ISO 11843–7).15 The FUMI theory approximates the baseline noise with the mixed random processes of the first order autoregressive process (designated AR(1)) and white noise, the parameterization of which is attained by the least-squares fitting to noise power spectra.13,14 Recently, Hayashi and Zhang proposed another method that utilized the autocorrelation method instead of the power spectrum.15 The most prominent merit of their method is the simplicity of the algorithm used to elicit the information needed for repeatability assessments of baseline noise. In the previous study,16 simulated time series of white noise and AR(1) mixtures were only examined, and not real chromatographic baselines in an HPLC. Real chromatographic baselines are generally constituted by various noises such as fluctuation, drift, and spike noise, etc. in addition to white noise and AR(1). Thus, using real chromatographic baselines in HPLC, we must confirm that the autocorrelation method is acceptable to perform the repeatability assessments in HPLC with UV detection (HPLC-UV) system.

This study aims to demonstrate experimentally the practical usefulness of the autocorrelation method as a means for estimating the detection limit, precision profile and noise characteristics in an HPLC-UV. To verify whether the chemometric evaluation of repeatability in HPLC-UV is suitable in this study, a reliable HPLC-UV method for determining estriol and baicalin in the Japanese Pharmacopoeia 17th edition was applied as an example.

Experimental

Preparation of a Simulated Time Series Normal random numbers to prepare \( \omega(t) \) with \( \sigma^2_w = 1 \) and \( m(t) \) with \( \sigma^2_m = 1 \) were generated in Microsoft Excel (Microsoft Corp., Washington, U.S.A.). A value of \( \phi \) was arbitrarily set at 0.98 for our analysis. The simulated time series were analyzed using MATLAB (MathWorks Inc., Massachusetts, U.S.A.).

Preparation of Real Baseline Noise and Chromatograms The HPLC-UV system (Hitachi, Tokyo, Japan) consists of a pump with a degasser (Model 5110), an auto sample injector (Model 5210), a column oven with a Peltier cooling module (Model 5310), and a UV detector (Model 5410). Real baseline noise produced in an HPLC-UV system during the determi-
Optimization of the Autocorrelation Method to Estimate Noise Parameter $\phi$ Using Simulated Data  The theoretical estimation of the dependence parameter of the AR(1) process $\phi$ (as described in Eq. S9) was first proposed in a previous study,\textsuperscript{16} but has yet to be examined with respect to the optimal value of $J$. The noise data were transformed into the autocorrelation function described in Eq. S7, which in turn was used to estimate $\phi$ in Eq. S9. Table 1 lists the estimated values of $\phi$ and their standard deviations. The accuracy and precision of these statistics were calculated using 1000 simulated repetitions of noise data ($n = 2000$). The estimates of $\phi$ deviated gradually from the true value ($= 0.98$) with increases in $J$; in addition, the standard deviations were observed to increase slightly. This suggests that the value of $J$ should be kept to a minimum in practice.

Table 1. Dependence of the Observed $\phi$ ($= 0.98$) on $J$ Calculated Using Eq. S9

| $J$ | Observed $\phi$    |
|-----|--------------------|
| 1   | 0.9773 (0.0055)    |
| 2   | 0.9772 (0.0052)    |
| 3   | 0.9772 (0.0052)    |
| 4   | 0.9772 (0.0052)    |
| 5   | 0.9772 (0.0053)    |
| 6   | 0.9772 (0.0053)    |
| 7   | 0.9772 (0.0053)    |
| 8   | 0.9772 (0.0054)    |
| 9   | 0.9772 (0.0054)    |
| 10  | 0.9771 (0.0055)    |
| 11  | 0.9771 (0.0055)    |
| 12  | 0.9771 (0.0056)    |
| 13  | 0.9770 (0.0057)    |
| 14  | 0.9770 (0.0057)    |
| 15  | 0.9770 (0.0058)    |

Values in parentheses indicate the corresponding standard deviations. $\sigma^2_w = 1$.

Results and Discussion
The theoretical evaluation of repeatability, $\sigma_y$, requires the application of Eqs. S9–S11 to the sample autocorrelation function given in Eq. S7. The arbitrary parameters included in Eqs. S7–S11 are the number of baseline noise data points ($n$) and the repetition number ($J$) in Eq. S9 for estimating the dependence parameter ($\phi$) of the AR(1) process. The following sections describe the examination of the optimal values of $J$ and the lower limit of $n$ using simulated and real HPLC data, respectively.

Optimization of the Autocorrelation Method to Estimate Noise Parameter $\phi$ Using Simulated Data  The theoretical estimation of the dependence parameter of the AR(1) process $\phi$ (as described in Eq. S9) was first proposed in a previous study,\textsuperscript{16} but has yet to be examined with respect to the optimal value of $J$. The noise data were transformed into the autocorrelation function described in Eq. S7, which in turn was used to estimate $\phi$ in Eq. S9. Table 1 lists the estimated values of $\phi$ and their standard deviations. The accuracy and precision of these statistics were calculated using 1000 simulated repetitions of noise data ($n = 2000$). The estimates of $\phi$ deviated gradually from the true value ($= 0.98$) with increases in $J$; in addition, the standard deviations were observed to increase slightly. This suggests that the value of $J$ should be kept to a minimum in practice.

Estimation of Precision Profiles Obtained via Autocorrelation Method  Figure 1A shows a graph of typical baseline noise in the HPLC-UV analysis of estriol. This baseline noise was regarded to be $Y(t)$ in Eq. S1, and the variance of the measurement error (Eq. S4) was determined using the autocorrelation method described in Eqs. S9–S11. Figure 1B shows a chromatogram of estriol in a standard solution. The estriol peak was observed at approximately 600 s under the chromatographic conditions described in caption of Fig. 1. The width of the signal region $k$ in Eqs. S2 and S4 was determined from the actual width of the estriol peak in Fig. 1B ($k = 300$ data points).

Figure S1 demonstrates the dependence of the RSD of the $\sigma_y$ values estimated by the autocorrelation method on the values of $J$ over a range of data points ($n = 1500, 2000, 3000, 4000,$ and $5000$) used for the fundamental calculations in Eqs. S7 and S8. The RSD of the estimated $\sigma_y$ values did not exceed 3.0% over the entire range of $J$ with 3000, 4000, and 5000 data points. However, a smaller number of data points ($n = 1500$ and 2000) failed to produce satisfactory levels of repeatability. If $n = 3000$ (Fig. 1A), the estimation of HPLC-UV precision can be accomplished in as little as 10 min (3000 data points).
points = 600 s × 5 data points/s). Although the repetition numbers in Fig. S1 are much smaller (10 repetitions for each $J$ and $n$) than in the simulation shown in Table 1 (1000 repetitions), the repeatability of the error variance $\sigma_j$ in the analysis of real data demonstrated a similar trend toward $J$ as observed in the simulation. For an adequate amount of data sets, $J = 1$ was optimal in the simulation (Table 1), whereas $J$ was fixed at 7 for the practical experimental situations (Fig. 1) as a compromise due to the scarcity of data sets in practice.

Figure 2 shows the precision profiles as indicated by the relationships between the RSDs of peak area measurements and the concentration of estriol. Two types of measurement RSDs are shown: $\sigma_j$ determined using repeated measurements ($n = 6$) at each concentration (open circles) and using the autocorrelation method (solid line). As an example, the RSDs of peak area measurements for 5.0 mg/L estriol were observed to be 1.63% for actual repeated measurements of real samples ($n = 6$) and 1.42% for the autocorrelation method. The 95% confidence intervals of the statistical RSDs were calculated based on the chi-square distribution using $\chi^2_{0.025}$ (5) and $\chi^2_{0.975}$ (5), and the confidence intervals were calculated from those of the sample SD under the assumption that the scattering of the mean was negligibly small compared to that of the SD. Thus, statistical SDs ($n = 6$) have 95% confidence intervals ranging between ±60% around the population SD. In Fig. 2, the error bars around the open circles denote the 95% confidence intervals of the statistical RSDs ($n = 6$) at each concentration (0.5, 1.0, 2.5, and 5.0 mg/L). The theoretical RSDs (solid line) fall within the 95% confidence intervals of the statistical RSDs, i.e., the theoretical RSDs fall within acceptable error ranges. For parameterization of baseline as shown in Fig. 1A, $\sigma_w^2$, $\sigma_r^2$, and $\phi$ were determined to be $6.59 \times 10^{-3}$, $3.82 \times 10^{-3}$, and 0.974, respectively. Thus, it is found that noise parameters are obtained via the autocorrelation method from the baseline, which consists of the intensities of white noise larger than that of AR(1) ($\sigma_w^2 > \sigma_r^2$). In Fig. 2, the measurement time to obtain statistical RSDs at 4 concentrations was 384 min (16 min × 6 time repetitive measurements × four concentration), while the measurement time to obtain theoretical RSD via the autocorrelation method was 32 min (16 min for the monitoring baseline noise and 16 min for the chromatographic measurement). Here, the instrumental detection limit (IDL) is defined as IDL = 3.3 $s/a$ where $s$ means the SD of measurement ($=\sigma_j$) and $a$ implies the slope of calibration line. Thus, the IDL is the...
concentration at which the RSD of measurements, \( (s/a)/IDL \), shows 30\% \((=1/3.3)\). The IDL \((3.3s/a)\) of estriol was 0.29 mg/L by the precision profile in Fig. 2. Usually an IDL in chromatographic methods is obtained by the means of a signal-to-noise ratio \((s/N = 3)\), and thus the IDL \((s/N = 3)\) of estriol was 0.31 mg/L. Thus, the IDLs of estriol obtained from both the autocorrelation method and \(S/N\) were apparently the same.

The same analysis was performed to assess repeatability in HPLC-UV for determining baicalin. Figures 3A and 3B show a graph of typical baseline noise in HPLC-UV analysis of baicalin and a chromatogram of baicalin in standard solution, respectively. As shown in Fig. 4, the theoretical RSDs (solid line) also fall within the 95\% confidence intervals of the statistical RSDs at each concentration \((0.016, 0.04, 0.08, (solid line)\) also fall within the 95\% confidence intervals of the latter). In the previous study using \((s/N = 3)\), and thus the IDL \((s/N = 3)\) of estriol was 0.31 mg/L. Thus, the IDLs of estriol obtained from both the autocorrelation method and \(S/N\) were apparently the same.

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Thus, these results in Figs. 2 and 4 support the conclusion that the autocorrelation method is applicable for estimating measurement RSDs over a wide range of sample concentrations in HPLC analyses. In other words, the RSDs of peak areas can be predicted at arbitrary concentrations of analytes by Eq. S5. These findings indicate that the autocorrelation method is a practical useful technique for evaluating the precision profile in Fig. 2. Usually an IDL in chromatographic methods is obtained by the means of a signal-to-noise ratio \((S/N)\) via the autocorrelation method from the real baseline, which consists of the intensities of white noise smaller than that of AR(1) \((\sigma^2_\omega < \sigma^2_\epsilon)\). And, the IDLs of baicalin obtained from the autocorrelation method and \(S/N = 3\) were 5.0 \(\mu\)g/L and 5.3 \(\mu\)g/L, respectively.

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### Conclusion

This paper demonstrates the practical applicability of the autocorrelation method in evaluating the precision profile of HPLC-UV analysis. The results of the autocorrelation method were in concordance with the repeated measurements of real samples \((i.e., \text{the theoretical RSDs fell within the 95\% confidence intervals of the latter})\). In the previous study using simulated time series, the autocorrelation method is only demonstrated using the random processes made up of the noises of the same strength \((i.e., \sigma^2_\omega = 1 \text{ and } \sigma^2_\epsilon = 1)\). While real chromatographic baselines, which consist of the intensities of white noise larger than that of AR(1) \((\sigma^2_\omega > \sigma^2_\epsilon)\) and the other way around \((\sigma^2_\omega < \sigma^2_\epsilon)\), were use in the present study. We show another fact that the autocorrelation method is applicable to obtain noise parameters from the two kinds of real baseline behaviors \((\sigma^2_\omega > \sigma^2_\epsilon \text{ and } \sigma^2_\omega < \sigma^2_\epsilon)\) in HPLC-UV. Moreover, the IDL based on the ISO 11843 was obtained from the precision profile of analyte, and this value was reasonable in the comparison with the IDL \((S/N = 3)\). The IDL in the HPLC-UV system is obtained from a real chromatographic baseline, not simulated time series as used in the previous study. We show a fact that the precision profile obtained via the autocorrelation method is applied to estimate an IDL based on ISO 11843.

The autocorrelation method represents a useful technique for evaluating repeatability in HPLC analyses, and may have applications in examining the allowable limits of system repeatability in LC as described in the United States, European, and Japanese pharmacopoeias.

### Conflict of Interest

The authors declare no conflict of interest.

### Supplementary Materials

The online version of this article contains supplementary materials.

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