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Theoretical repeatability assessment without repetitive measurements in gradient high-performance liquid chromatography

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ABSTRACT

This paper puts forward a time and material-saving method for evaluating the repeatability of area measurements in gradient HPLC with UV detection (HPLC-UV), based on the function of mutual information (FUMI) theory which can theoretically provide the measurement standard deviation (SD) and detection limits through the stochastic properties of baseline noise with no recourse to repetitive measurements of real samples. The chromatographic determination of terbinafine hydrochloride and enalapril maleate is taken as an example. The best choice of the number of noise data points, inevitable for the theoretical evaluation, is shown to be 512 data points (10.24 s at 50 point/s sampling rate of an A/D converter). Coupled with the relative SD (RSD) of sample injection variability in the instrument used, the theoretical evaluation is proved to give identical values of area measurement RSDs to those estimated by the usual repetitive method ($n = 6$) over a wide concentration range of the analytes within the 95% confidence intervals of the latter RSD. The FUMI theory is not a statistical one, but the "statistical" reliability of its SD estimates ($n = 1$) is observed to be as high as that attained by thirty-one measurements of the same samples ($n = 31$).

1. Introduction

In a quantitative analysis using an HPLC method, repeatability estimation for chromatographic peak area and/or height is of great importance to obtain reliable analytical results and/or data. The repeatability, which is statistically expressed as standard deviation (SD) and relative SD (RSD) of chromatographic peak area and/or height, can be obtained from repetitive measurements using the same samples [1–3]. Thus, vast amounts of experimental time, effort, and chemicals are wasted on obtaining a reliable measurement RSD by repetitive measurements.

In the ISO 11843 part 7 (ISO 11843-7:2012) [4], two theories have been introduced for estimating detection limits in instrumental analyses such as chromatography and spectroscopy. To determine detection limits, measurement SD and RSD were obtained based on the stochastic aspects of instrumental noise and signal data involved in a chromatogram and spectrum without repetitive measurements of real samples. This paper adopts one of two theories, called Function of Mutual Information (FUMI) theory that can perform a simulation of the time variation in instrumental noise by a mixture of well-defined stochastic processes of white noise and Markov process [5]. The power spectra of white noise and the Markov process are shown as a horizontal line and a Lorentzian curve, respectively [6]. Because the chromatographic baseline noise in most of the HPLC systems exhibited flicker noise (or $1/f$ noise) [7–10], it corresponded to white noise and Markov process [5,11]. Thus, the chromatographic baseline noise in HPLC was approximated by a mixed stochastic processes of white noise and Markov process, and their stochastic parameters were determined from the power spectrum analysis of chromatographic baseline noise. Recently, repeatability estimations based on the FUMI theory, which provided measurement RSDs stochastically [12], were proposed to avoid repetitive measurements for obtaining measurement RSD statistically. Repeatability estimations based on the FUMI theory have been utilized to determine the detection limits and measurement precision in isocratic HPLC with UV detection (HPLC-UV), electrochemical detection (HPLC-ECD), refractive index detection (HPLC-RI), and mass spectrometric detection (LC–MS) [12–14]. Thus, the statistical properties of chromatographic baseline noise without drift in isocratic HPLC-UV,

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HPLC-ECD, HPLC-RI, and LC–MS have been well investigated to be evaluated by the FUMI theory in our previous reports [12–14].

In a gradient HPLC, chromatographic baseline drift on a chromatogram is usually observed by the change of the mobile phase composition [15,16]. However, it has not been clarified whether chromatographic baseline drift in gradient HPLC can also be treated in the similar way to when repeatability in isocratic HPLC methods is estimated. Because HPLC methods using gradient elution are widely used for the analysis of medicine and pharmaceutical preparations in various country's pharmacopoeia, repeatability estimation is desirable in gradient HPLC.

In this study, we demonstrate whether chromatographic baselines which are contaminated by drifts resulting from gradient elution in HPLC can be handled by the FUMI theory which has been proved to be successful for repeatability evaluation in case of "flat" baselines in a variety of isocratic HPLC systems. This evaluation is experimentally tried in the gradient HPLC-UV for the analysis of terbinafine hydrochloride and enalapril maleate.

2. Theory

In the FUMI theory, the time variation in the chromatographic baseline noise is approximated by a mixed random process of white noise and Markov process [5,11]. The white noise is a stochastic process with a horizontal power spectrum, whereas the Markov process has a monotonously decreasing power spectrum such as a Lorentzian curve [6]. The SD of white noise ($\tilde{w}$), SD of the Markov process ($\tilde{m}$), and the retention parameter (ρ) of the Markov process are utilized as noise parameters, and the three noise parameters are determined in the frequency space. The digital chromatographic baseline noise data of 2 to the nth points are transformed into the real power spectrum by the Fourier transform, and then the three noise parameters, $\tilde{w}$, $\tilde{m}$, and ρ, are determined by the least squares fitting of the theoretical power spectrum to a real power spectrum. The following equation, $P(f)$, is the theoretical power spectrum of the mixed stochastic processes of white noise and the Markov process [5,11]:

$$P(f) = \frac{\tilde{m}^2}{1-\rho^2} \times \frac{2\alpha}{\alpha^2 + 4\pi f} + \tilde{w}^2 \quad (1)$$

where f is frequency, $\alpha = (1\text{-}\rho)/\Delta t$, and Δt is the sampling rate.

The three noise parameters are used for the estimation of measurement SD caused by the chromatographic baseline noise (S_B) in the integration domain (k_e data points) [17]:

$$S_B{}^2 = k_e\tilde{w}^2 + \frac{\tilde{m}^2}{(1-\rho)^2}\left(k_e - 2\rho \times \frac{1-\rho^{k_e}}{1-\rho} + \rho^2 \times \frac{1-\rho^{2k_e}}{1-\rho^2}\right) + \beta^2\tilde{w}^2 + \tilde{m}^2\left[\beta^2\frac{1-\rho^{2k_e}}{1-\rho^2} - 2\beta \times \sum_{i=1}^{k_e}\left(\rho^{k_e-i} \times \frac{1-\rho^{k_e+1-i}}{1-\rho}\right)\right] \quad (2)$$

where $\beta = (k_e + 1)/2$. The first term of Eq. (2) denotes the $\tilde{w}$ in k_e data points; the second term, $\tilde{m}$ in k_e data points; the third term, the effect of the white noise in the oblique zero line; the fourth term, the effect of the Markov process in the oblique zero line. The third and fourth terms are added to take into consideration the baseline drift. In the case of isocratic HPLC, the third and fourth terms should be neglected (=0) for the horizontal zero line because chromatographic baseline drift is not included [12].

Thus, a measurement RSD of the chromatographic signal of an analyte, which has a peak area (A) is shown as follows [18–20]:

$$\mathrm{RSD}^2 = \frac{S_B^2}{A^2} + I^2 \quad (3)$$

where I is the RSD of the volume error in the auto sample injector.

The reproducibility of a chromatographic peak area which results from the variability of injected sample volumes into the apparatus is also of importance in theory and practice. The area reproducibility can be expressed as an RSD of injected volumes, which can be considered invariant irrespective of samples in a chromatography system, except for viscous solutions. Therefore, a constant RSD, not constant SD, is used as an indicator for the injection volume error. In Eq. (3), the injection volume error is described as an independent term, I, because this error and the noise of the instrumental output are probabilistically independent of each other. In the FUMI theory, the RSD of injection volume variability (=I in Eq. (3)) can be substituted for by a value cited in a specification sheet of auto and/or manual sample injectors. At high concentrations, a chromatographic peak area of analyte, A, becomes so large that the volume error of the injector is the most predominant factor in the precision. At low concentrations, the contributions of the Markov process and white noise (Eq. (2)) are much higher than the injection volume error.

On the whole, baseline drifts, often appearing in gradient HPLC as shown in Fig. 1A, are not indeterminate or a periodic function, rather systematic and monotonous. Therefore, the Fourier transform of such drifts over a limited time region has a tendency to produce unfavorable, extra frequencies in the frequency space, probably leading to an erroneous interpretation of random processes included in them. To circumvent this problem, the computer software of this study comprises the least squares fitting algorithm of a linear function to eliminate, though not completely, the systematic drifts from innate background noises in the analytical instrument used. Furthermore in the theoretical approach, dc components in the baseline are neglected during the analysis in the frequency space.

3. Experimental

3.1. Chemicals and reagents

All chemicals and solvents were of analytical-reagent grade. Terbinafine hydrochloride (>98%), methanol (>99.7%), triethylamine (>99%), and acetic acid (>99.7%) were obtained from Wako Pure Chemical Industries, Co. Ltd. (Osaka, Japan). Enalapril maleate (Japanese Pharmacopoeia (JP) reference standard, >99%) was obtained from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ). Acetonitrile (>99.9%) was obtained from Honeywell (Muskegon, MI). Distilled deionized water (resistivity 18 MΩ cm) was prepared using a Barnstead NANO pure ultra-pure water system (Barnstead Co. Ltd., Boston, MA, USA).

Terbinafine hydrochloride was dissolved in a water-acetonitrile (50:50, v/v) mixture and was used as a test solution. Enalapril maleate was dissolved in a 50 mM phosphate buffer (pH 2.5)-acetonitrile (19:1, v/v) mixture and was used as a test solution.

3.2. Gradient HPLC-UV system

The gradient HPLC-UV system consisted of a pump (L-2130, Hitachi, Tokyo), an auto sample injector (L-2200, Hitachi), a column oven (CTO-10ASvp, Shimadzu, Kyoto, Japan), and a UV detector (L-2400, Hitachi). Chromatographic analog data from the UV detector were converted to digital data by an A/D converter (SS420X, Scientific Software, Inc., California, USA), and the digital data were recorded by a personal computer at a sampling rate of 50 point/s.

3.3. Chromatographic conditions for the analysis of terbinafine hydrochloride

The chromatographic conditions in the present gradient HPLC-UV system were set according to the purity test for terbinafine