



Prediction of measurement precision of apparatus using a chemometric tool in electrochemical detection of high-performance liquid chromatography

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Abstract

The relative standard deviation (RSD) of measurements in high-performance liquid chromatography with electrochemical detection (HPLC–ECD) was predicted by a chemometric tool based on the $1/f$ fluctuation model which is made up of white noise and a Markov process, called the Function of Mutual Information (FUMI) theory. FUMI theory can provide a precise and reliable detection limit from a single measurement of noise and signal in HPLC–ECD. To obtain RSD ($n=5$) for determination of (–)-epicatechin at five concentrations required 12.5 h, while the predicted RSD by FUMI theory required only 0.5 h (one measurement). Moreover, to trace the source of instrumental noise, power spectra of chromatographic baseline were used. Selection of a suitable apparatus in HPLC–ECD system, acquisition of RSD, and detection limits for determination of catechins by HPLC–ECD were simply and easily made by this chemometric tool within a very short time. The use of the FUMI theory for the prediction of measuring precision was more efficient and the optimization was less time-consuming to be suited for determination.

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1. Introduction

For the development and/or the optimization of experimental conditions of high-performance liquid chromatography (HPLC), the precision and detection limit of the instrumental analysis is of great importance [1]. The precision, which is expressed in a statistical term as standard deviation (SD) or relative standard deviation (RSD), can usually be obtained

by repeated experiments on the same samples [2], thus a lot of time and effort are usually required. In spectrometry and chromatography, much effort has been made to predict the uncertainty or precision of measurement in instrumental analyses [3–8]. Recently, Hayashi and Matsuda proposed a chemometric tool based on the Function of Mutual Information (FUMI) theory to improve the uncertainty prediction in instrumental analysis. Two types of noises, shot and flicker, have been cited for instrumental analysis [9]. The time variation in instrumental noise was simulated by the mixture of well-defined random processes, called white noise and the Markov pro-

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cess. The power spectrum of the white noise was indicated as a horizontal line and that of the Markov process a Lorentzian curve. It has been noted that white noise and Markov process correspond to flicker noise (or $1/f$ noise) [10]. Thus, baseline drift in HPLC was approximated by a mixed random process of white noise and a Markov process. The stochastic parameters of the random process were determined from the power spectral density of the baseline drift. FUMI theory was verified for HPLC with UV detection [11], with diode-array detection [12], with refractive index detection [13], atomic absorption spectrometry [10], and capillary electrophoresis [14] to save chemicals and experimental time. FUMI theory makes it possible to predict measurement RSD, to calculate detection limit from a single measurement of noise and signal on a chromatogram, and to select the best analytical condition. Therefore, the tool is expected to be useful for RSD prediction of measurements in HPLC with electrochemical detection (HPLC–ECD).

HPLC–ECD is a sensitive and selective method for the determination of redox compounds. The determination of redox compounds such as catechins by HPLC–ECD using a microbore column [15] should be more sensitive than HPLC–UV, HPLC with a fluorescence detector, or HPLC with a chemiluminescence detector. But, ECD may have low reproducibility, because of the effect of the interface between an electrode and a solvent in the flow cell. If a suitable condition of HPLC–ECD were to be present, the above drawback would be overcome. HPLC–ECD is flow-rate sensitive caused by piston pump mechanics. Therefore, many researchers often use a pulse dampening system to exclude pump pulsation [16]. For the best condition of HPLC–ECD to be present, reparative measurements have been required [17]. If RSD and detection limit are predicted by FUMI theory from a single measurement of noise and signal on a chromatogram, the improvement of the HPLC–ECD system and experimental conditions will easily be made to yield experimental results with more sensitivity and precision.

In this study, we first attempted to prove that the chemometric tool based on FUMI theory was useful for predicting the measurement uncertainty in HPLC–ECD. Secondly, using the tool, we were better able to select a suitable pump, electrochemical

detector, and working electrode to determine (–)-epicatechin in HPLC–ECD.

2. Experimental

2.1. Reagents

(–)-Epicatechin was purchased from Kurita Industrial Co. (Tokyo, Japan). Phosphoric acid, sodium dihydrogenphosphate, and acetonitrile (HPLC grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Apparatus

The present HPLC–ECD system consisted of a dual series of piston pumps, Jasco PU-880 (80 μ l/stroke, abbreviated as pump A) or dual parallel piston pumps, Tosoh DP-8020 (6 μ l/stroke \times 2, abbreviated as pump B), a 7125 sample injector fitted with a 5- μ l injection loop (Reodyne, Cotati, CA, USA), a LiChrospher 100 RP-18 ODS column (250 \times 4 mm I.D. 5 μ m, Cica-Merck, Japan), electrochemical detectors, Jasco EC-840 (abbreviated as detector A) or Kotaki EDP-1 (abbreviated as detector B), and a mobile phase (acetonitrile: 0.1 mol/l phosphate buffer, pH 2.5, 15:85, v/v). The electrochemical cell of detector A was made from a glassy carbon (GC) working electrode, saturated calomel reference electrode (SCE), and a stainless steel counter electrode (Fig. 1A). That of detector B, was made from a GC or plastic formed carbon (PFC) working electrode, an SCE, and a stainless steel counter electrode (Fig. 1B). Between the pump and the sample injector, a pulse damping system (HPD-1, GL Sciences, Tokyo, Japan) was connected. The mobile phase was made to flow at 0.36, 0.45 and 0.54 ml/min through the pump operation. The detection potential for determining (–)-epicatechin was set at +600 mV vs. SCE.

The analog data of a chromatogram from the electrochemical detector was amplified by an amplifier (L1201, Yokogawa, Tokyo, Japan), converted to digital chromatogram data by an A/D converter (NR-110, Keyence, Tokyo, Japan), and the digital data were recorded by a personal computer at

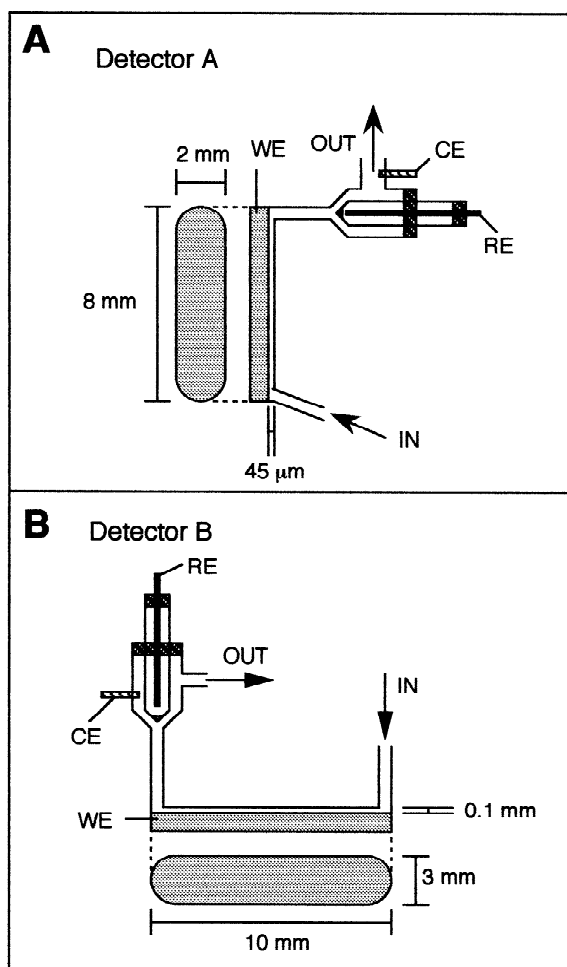


Fig. 1. Electrochemical cells and dimensions of GC or PFC working electrode for Jasco EC-840 (Detector A) and Kotaki EDP-1 (Detector B). WE, working electrode; RE, reference electrode; CE, counter electrode; IN, inlet of mobile phase; OUT, outlet of mobile phase.

sampling intervals of 0.2 s/point. Low-pass filter of the amplifier cut-off 10 Hz frequency was used.

3. Theory

The time variation in the baseline of a chromatogram is described as the mixed random processes of the white noise and a Markov process. The white noise is a time-independent process with one parameter, but the Markov process has a time-correlation

(also called the auto-correlation) with two parameters [18]. Digital data of 1024 data point (0.2 s/point) transferred from the analogue signals of baseline of chromatogram were converted to power spectra by Fourier transform for determining the three noise parameters, the SD, \tilde{w} , of the white noise and the SD, \tilde{m} , and retention parameter, ρ , of the Markov process. The noise parameters can be determined by the least squares fitting of the theoretical power spectrum (see Fig. 2) and are in turn used for the prediction of measurement SD in the time space. To determine SD of the baseline, we used a power spectrum from 0.01 to 2.5 Hz, because low pass filter was used at 10 Hz. The theoretical power spectrum ($P(f)$) in accordance with FUMI has been expressed as follows:

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

where \tilde{w} is SD of the white noise, \tilde{m} , SD of the Markov process, ρ , retention parameter of the Markov process, f , frequency, $\alpha, \alpha = (1 - \rho)/\Delta t$ and Δt , sampling intervals.

The power spectrum derived from noise in frequency space is utilized for the parameterization of \tilde{w} , \tilde{m} and ρ . Coupled with the parameters with the information on the signal shape over the integration

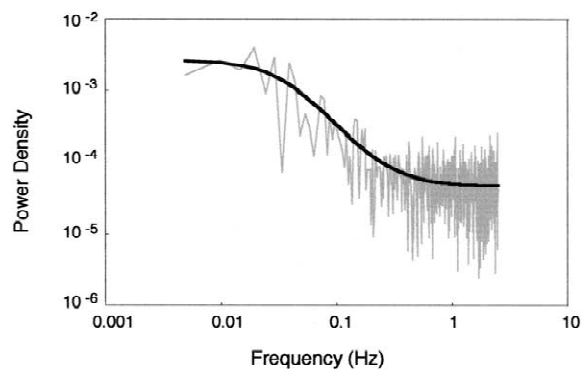


Fig. 2. Power spectra of baseline of a chromatogram obtained using HPLC–ECD system composed of pump B (DP-8020) and detector A (EC-840). The smooth solid line designates the best fit of theoretical line (white noise + Markov process) to the observed power spectrum (zig-zag line). HPLC conditions: mobile phase, acetonitrile: 0.1 mol/l phosphate buffer (pH 2.5), (15:85, v/v); column, LiChrospher 100 RP-18 ODS column (250×4 mm I.D., 5 μm); flow-rate, 0.45 ml/min; applied potential, +600 mV vs. SCE.

domain, the theory can provide the measurement RSD.

$$\text{RSD}^2 = \frac{k_f \tilde{w}^2}{A^2} + \frac{\tilde{m}^2}{(1-\rho)^2 A^2} \left(k_f - 2\rho \frac{1-\rho^{kf}}{1-\rho} + \rho^2 \frac{1-\rho^{2kf}}{1-\rho^2} \right) + I^2 \quad (2)$$

where k_f , integration domain, A , area of signal parameter, and I , independent error.

At higher concentrations, peak area A is large, the independent error is the most predominant factor on the precision, and the terms for the white noise and Markov process are negligibly small. At lower concentrations, the contributions of random process are much higher than independent error.

4. Results and discussion

4.1. Determination of theoretical RSD based on the FUMI theory

The most prominent advantage of the theory is that repeated measurements are dispensed with. The power spectrum of a baseline on a chromatogram by an HPLC system composed of pump B is shown in Fig. 2. The zig-zag line refers to the observed power spectrum, and the smooth one is the best fit of the baseline model based on the FUMI theory. This result indicates that FUMI theory makes it possible to predict precision in HPLC–ECD without repeated measurements.

In order to verify the usefulness of the FUMI theory for measurements in HPLC–ECD, we compared the theoretical predicted RSD with an experimentally observed RSD. A determination of (–)-epicatechin was taken as an example. Chromatograms for 0.5 pmol (–)-epicatechin were obtained five times. This measurement required 2.5 h as shown in Fig. 3. Injection noise was observed at 5 min, and (–)-epicatechin peak was observed at 20 min. The experimentally observed RSD for 0.5 pmol (–)-epicatechin was calculated using peak heights on the chromatograms to be 2.14% ($n=5$). The observed RSD at 0.15, 0.25, 0.5, 1.5 and 2.5

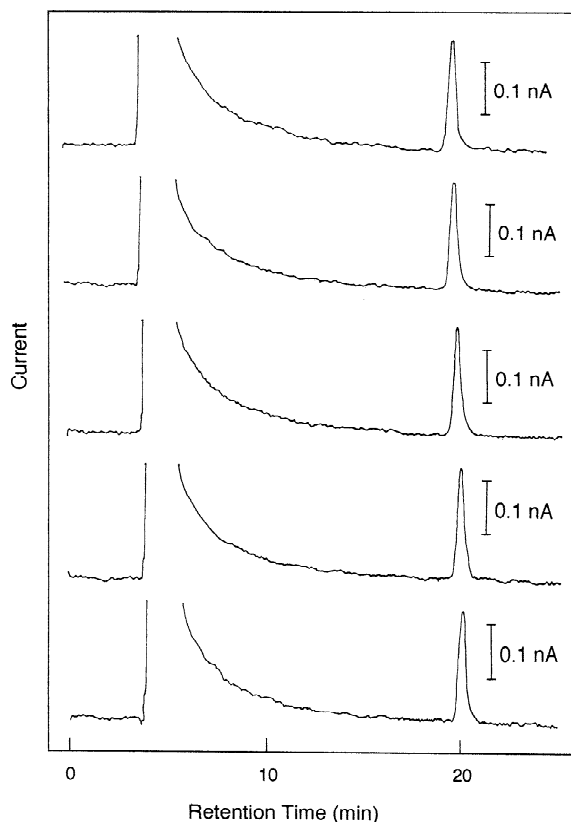


Fig. 3. Chromatograms of each 0.5 pmol (–)-epicatechin using pump B (DP-8020) and detector A (EC-840) in HPLC system. HPLC conditions are the same as those in Fig. 2.

pmol (–)-epicatechin was obtained using data from each five times repeated measurement.

In Fig. 4, the observed RSD values are plotted against the amount of (–)-epicatechin by HPLC–ECD using pump A (curve (a)) or pump B (curve (b)) and detector A. As shown in Fig. 4, the predicted RSD (solid line) over a wide concentration range was calculated from only one measurement of a chromatogram for 0.5 pmol (–)-epicatechin. It is clear that HPLC–ECD using pump B and detector A gave a smaller RSD than that using pump A and detector A.

The observed RSD was parallel to the curve of the predicted RSD. The error bar shows 95% of the confidence intervals of the observed RSD ($n=5$). The confidence interval of the RSD by five repeated measurements is $\pm 70\%$ of the SD error, which was

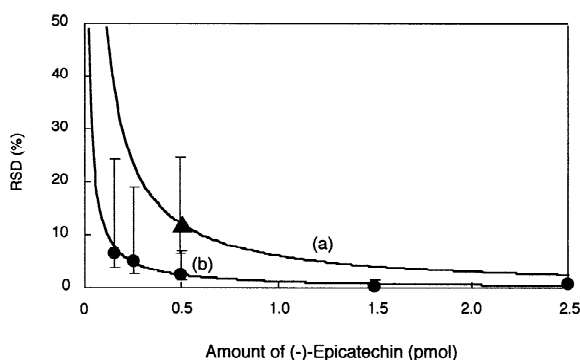


Fig. 4. Precision plots for determination of (-)-epicatechin using HPLC-ECD. Observed RSD were indicated (\blacktriangle) using pump A (PU-880), detector A (EC-840) and (\bullet) using pump B (DP-8020) and detector A in HPLC system. The error bar shows 95% of the confidence intervals. The repetition number of measurements (n) is 5 at each concentration. HPLC conditions are the same as those in Fig. 2. Solid lines mean predicted RSD using chromatograms for 0.5 pmol (-)-epicatechin obtained with (a) pump A, detector A and with (b) pump B and detector A and the FUMI theory.

proved by Chi-Square distribution. Because the amounts of the injected (-)-epicatechin were less than 2.5 pmol, the independent error could be much lower than the contributions of the random processes. The predicted RSD by FUMI theory fit in the range of 95% of the confidence interval indicating that the predicted RSD was parallel to the observed RSD. It is thus shown that the FUMI theory is useful for predicting the measurement precision in HPLC-ECD without repeated measurement. While experimental time to obtain observed RSD at five concentrations was 12.5 h (0.5 h \times five time repeated measurement \times five concentrations), the predicted RSD required only 0.5 h. That is, both chemicals and experimental time were saved by this chemometric tool based on the FUMI theory.

4.2. Baseline noise in HPLC-ECD

As shown in Fig. 2, the power density of the low frequency was larger than that of the high frequency, and it was apparent that the baseline noise of the chromatogram in HPLC-ECD contained $1/f$ noise. This data demonstrates that reduction of low frequency noise in $1/f$ fluctuation improves sensitivity and precision on HPLC-ECD analysis. To trace the source of low frequency noise, the chromatographic

baseline was converted to power spectrum by Fourier transform. To select a suitable pump, electrochemical cell, and working electrode material for HPLC-ECD, the analysis of power spectra of chromatographic baseline was made.

To show the dependence of pulsation on flow-rate in HPLC-ECD, characteristics of baselines of chromatograms at various flow rates using power spectra were examined. Fig. 5A illustrates the baselines of chromatograms obtained using pump A and detector A. The baseline noise looks different at each flow-rate, but it is difficult to recognize the effect of flow-rate on the noise by visual inspection. Power spectra of baselines were obtained using the baselines of chromatograms for 3 min, and shown in Fig. 5B. The power spectra provided the feature of frequencies and amplitude of the fluctuation, depending on flow-rate. It was clear that two major bands were spotted in the power spectra in Fig. 5B. At a flow-rate of 0.45 ml/min (Fig. 5B(b)), the rotation speed of pump A (dual piston series type, 80 μ l/stroke) was 0.09 Hz, when the flow delivery was ideal. This frequency was the low frequency at 0.45 ml/min in Fig. 5B. The higher frequency observed was almost twice the low frequency. Hereinafter, the

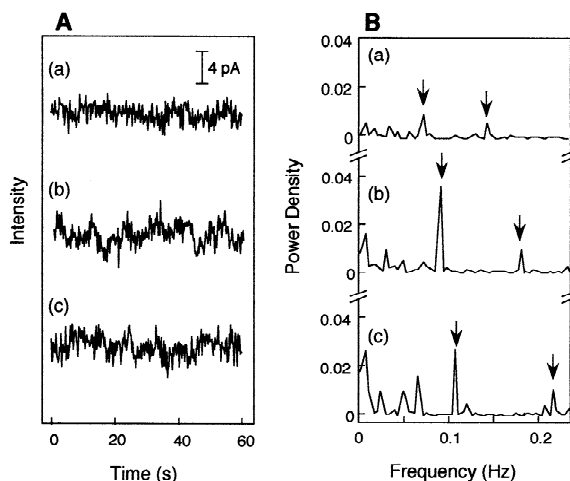


Fig. 5. Baselines of chromatogram (A) and power spectra (B). Baseline of chromatograms were obtained using HPLC-ECD system composed of pump A (PU-880) and detector A (EC-840) at flow-rates of (a) 0.36, (b) 0.45, and (c) 0.54 ml/min. The baselines of chromatograms were converted to power spectra by Fourier transform. The arrows indicate the noises derived from pulsation of pump.

low and high frequencies are called the fundamental tone and harmonic, respectively, from acoustics terminology. The frequencies of the fundamental tone and harmonic were positively proportional to the flow-rate. At 0.36 ml/min, the frequencies were almost 0.8 times lower and at 0.54 ml/min, the frequencies were almost 1.2 times higher than that of the flow-rate at 0.45 ml/min. This rule also held true for the harmonic. Clearly, the case of the excess fluctuation was the reciprocating motion of the pistons.

Fig. 6 shows the power spectra of chromatographic baseline obtained using various pump and detectors. The bands appearing around 0.05 Hz in Fig. 6 were the $1/f$ components. With the pulse damping system in HPLC–ECD, the bands of pulsation were remarkably decreased (Fig. 6(a)). Moreover, the pump noise was regarded as being a medium term noise [19,20]. This type of noise has already been discussed by Scott [21].

The power spectrum shown in Fig. 6(b) was obtained using HPLC system using pump B (dual piston parallel type) and detector A. The power density for pump B (Fig. 6(b)) was considerably smaller than that for pump A (Fig. 5B). At a flow-rate of 0.45 ml/min and 6 μ l/stroke, the frequencies of the fundamental tone and harmonic were expected to be 0.63 and 1.3 Hz, respectively, for pump B. But, it is difficult to recognize these noises at the frequencies on the power spectrum, as fluctuations in flow-rate caused by piston pump mechanics often plague high-sensitive determination using HPLC–ECD. Pulse damping systems and small stroke pump are useful for decreasing pump pulsation.

To select the electrochemical cell and working electrode materials most suitable for improving sensitivity, baseline of chromatograms obtained using each cell were examined for 3 min. Comparison was made of effects of the electrochemical cell structure and working electrode material (GC or PFC) on chromatographic baseline noise based on power spectra. These spectra were obtained using detector B with the GC electrode (Fig. 6(c)) and the PFC working electrode (Fig. 6(d)). In the former case, bands at 0.09 and 0.18 Hz in the power spectrum in Fig. 5B(b) were no longer present in Fig. 6(c). Detector B was a wall-jet type cell [22] whose inlet line was perpendicular to the working

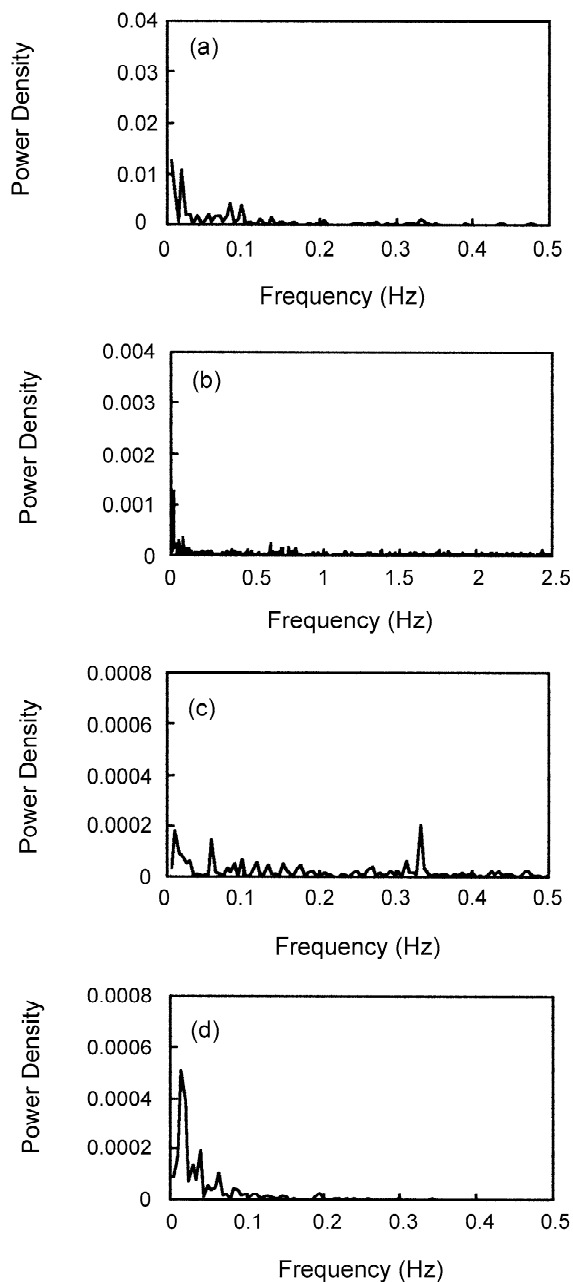


Fig. 6. Power spectra of baselines of chromatograms using HPLC systems of (a) pump A (PU-880), detector A (EC-840) and pulse damper system, (b) pump B (DP-8020) and detector A, (c) pump A and detector B using GC working electrode, and (d) pump A and detector B using PFC working electrode.

electrode though the inlet line of detector A and was oblique; the power density of the former was considerably less than that of the latter. The wall-jet type cell thus resists noise created by pump pulsation. With detector B and the PFC working electrode in HPLC, power density was found to be very small, as seen in Fig. 6(d). When the wall-jet type cell and PFC working electrode were used in HPLC–ECD, noise level of baseline was very low. The power spectra of chromatographic baseline for only 3 min would also be useful for daily checks of the performance of an HPLC–ECD apparatus.

4.3. Detection limits of (–)-epicatechin obtained using various HPLC–ECD systems

When σ is defined as blank SD of chromatogram baseline, detection limit is the defined concentration or amount at which peak height is 3σ . Thus, detection limit can also be expressed as a concentration or amount of 33% RSD. FUMI theory therefore can predict detection limit, too. The confidence interval of the detection limit by FUMI theory is $\pm 20\%$ which is equal to the SD error from 40 repeat measurements [23]. Thus, the reliable detection limit (3σ) would be calculated from SD obtained using the FUMI theory and a chromatogram, where σ is means SD of the false height of the baseline of a chromatogram.

Detection limits of (–)-epicatechin determination obtained at various combinations of the pump, the pulse damping system, electrochemical detector, and working electrode are shown in Table 1. When pulsation was removed by the pulse damping system, the detection limit was remarkably improved. The detection limit for the system with pump B compared favorably with the system with pump A and the

pulse damping system. In the absence of pulsation with the detector B and GC working electrode, detection limit noted to improve. For the PFC working electrode whose surface was electrochemically highly active [24,25], good sensitivity was noted. The present method is thus shown to make possible prediction of the detection limits of a detector with only one measurement of chromatogram.

5. Conclusion

It was reported that the FUMI theory was useful for predicting the measurement precision compared with other mathematical and physical approaches, and was an efficient method for predicting the measurement precision of a slow analysis such as liquid chromatography [10]. The method has been applied to liquid chromatography and capillary electrophoresis equipped with ultraviolet absorption spectrometers [11]. The present paper is the first attempt to extend its applicability to HPLC–ECD. The FUMI theory makes it possible to predict RSD and calculate a detection limit in the HPLC–ECD from only one chromatogram.

We found that ECD was susceptible to the pulsation of pumps. Weber used a sigmoidal filter function based on Fourier transform to minimize flow-associated noise in HPLC–ECD [26]. But, it could not be applied to all chromatographic peaks, because a narrow peak would disappear through the sigmoidal filter function. In this study, in order to investigate the effect of pulsation, we have used the power spectra of baseline on chromatogram for only 3 min. It was clear that the selection of a pump was done within a short time. In the case of the wall-jet type cell, the effects caused by pump pulsation lessened. We have used the electrochemical cell in HPLC–ECD, so as to the best select an electrochemical detector and electrode material in the shortest possible time.

A detection limit derived from the signal–noise ratio is usually less reliable compared to those based on repeated measurements of chromatogram [27]. Using FUMI theory, however, reliable values could be obtained from only one chromatogram with no requirement for repetitive chromatographic measure-

Table 1
Detection limit of (–)-epicatechin determination, calculated by FUMI theory

Pump	Detector	Working electrode	Detection limit (fmol)
A	A	GC	96.0
A	+ Damper	GC	38.4
B	A	GC	26.1
A	B	GC	74.5
A	B	PFC	25.7

ments. In short, amounts of chemicals and experimental time were remarkably saved by the method, and thus the present method is useful in the fabrication of an HPLC–ECD system.

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