Baseline Noise in High-Performance Liquid Chromatography with Electrochemical Detection

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Abstract. The chromatographic baseline was converted to power spectrum by Fourier transform to show the frequency dependence of noise. The spectral analysis of chromatographic baseline was made under various experimental conditions for high-performance liquid chromatography with electrochemical detection (HPLC-ECD). From the spectral analysis, it was found easily that a system using a small stroke pump and a wall jet type cell with a plastic formed carbon working electrode had a low level of the baseline noise. The optimization of HPLC-ECD system was performed by evaluation of the detection limit based on a chemometric tool, called the Function of Mutual Information (FUMI) theory, which treats the chromatographic baseline fluctuation as a 1/f fluctuation model. We found that the observed relative standard deviation (RSD) of peak area by repetitive measurements \(n = 5\) of chromatograms was parallel to the predicted RSD curve based on the FUMI theory. The result indicates that the FUMI theory can provide a measure for estimating the precise and reliable detection limit from a single measurement of noise and signal in HPLC-ECD. The present spectral analysis of baseline noise and the prediction of RSD based on the FUMI theory are an efficient optimization strategy for determining catechins using HPLC-ECD.

Keywords: HPLC; Electrochemical detection; FUMI theory; Power spectrum; catechins

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INTRODUCTION

High-Performance liquid chromatography with electrochemical detection (HPLC-ECD) is a sensitive and selective method for the determination of redox compounds. However, ECD has sometimes low reproducibility, because current signals and noise are susceptible to the characteristics of interface between an electrode and solution in the electrochemical flow cell. If suitable conditions of HPLC-ECD were selected for keeping stable interface, the above drawback would be overcome. For selecting and describing the conditions of HPLC-ECD, repetitive measurements on the same samples have been required to obtain a relative standard deviation (RSD) of measurements, thus a lot of experimental time and effort are usually required.

In this study, we proved that an optimization strategy for determining catechin by HPLC-ECD using the spectral analysis of chromatographic baseline noise. Moreover, the optimization of HPLC-ECD system was performed by 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 [1,2]. The above methods were an attempt to save considerable amounts of chemicals and experimental time for the optimization of HPLC-ECD systems.

**EXPERIMENTAL SECTION**

The HPLC-ECD system consisted of a pump, an ODS column (250 mm x 4 mm i.d. 5 µm), a mobile phase (acetonitrile: 0.1 mol/L phosphate buffer, pH 2.5, 15:85, v/v), and an electrochemical detector. Jasco PU-880 (a dual series of piston pump, 80 µL/stroke, pump A) or Tosoh DP-8020 (a dual parallel piston pump 6 µL/stroke x 2, pump B) was used. Figure 1 shows the composition of the electrochemical detectors of Jasco EC-840 (abbreviated as detector A, Fig. 1A) and Kotaki EDP-1 (abbreviated as detector B. The detection potential for determining (-)-epicatechin was +600 mV vs. SCE. The analog data of a chromatogram from the electrochemical detector were amplified by an amplifier, converted to digital chromatogram data by an A/D converter, and the digital data were recorded by a personal computer at sampling intervals of 0.2 s/point.

![Electrochemical cells and dimensions of working electrodes for Jasco EC-840 (A) and Kotaki EC-840 (B). WE, working electrode; RE, reference electrode (saturated calomel electrode, SCE); CE, counter electrode (stainless). (A) Glassy carbon (GC) was used as WE. (B) GC or plastic formed carbon (PFC) was used as WE.](image)

**RESULTS AND DISCUSSION**

The baseline noise of the chromatogram in HPLC-ECD was converted to power spectrum by Fourier transform. The power density of the low frequency was larger than that of the high frequency, and it was apparent that the baseline noise contained 1/f noise. These data demonstrate that the reduction of the low frequency noise in 1/f fluctuation improves sensitivity and precision on HPLC-ECD analysis. To trace the source of low frequency noise, and to select a suitable pump, electrochemical flow cell, and working electrode material for the cell, the analysis of power spectra of chromatographic baselines was made.

To show the effect of pulsation on chromatographic baselines in HPLC-ECD, the time series and power spectra of baselines were examined. Fig. 2A illustrates the chromatographic baseline obtained using pump A and detector A at a flow rate of 0.45 mL/min, but it is difficult to recognize the effect of pulsation on the noise by visual
inspection. However, two major bands were clearly spotted in the power spectra in Fig. 2B. At a flow rate of 0.45 mL/min, the rotation speed of pump A (dual piston series type, 80 µL/stroke) was 0.09 Hz, when the flow delivery was ideal. This frequency was identical with the low frequency of the one band in Fig. 2B. The higher frequency of another band was twice the low frequency. Hereinafter, the low and high frequencies are called the fundamental tone and harmonic, respectively, from acoustics terminology. The frequencies of the fundamental tone and harmonic were proportional to the flow rate. Clearly, the cause of the excess fluctuation was the reciprocating motion of the pistons.

**FIGURE 2.** Baseline of chromatogram (A) and power spectrum (B). Baseline was obtained using the HPLC-ECD system composed of pump A (PU-880) and detector A (EC-840) at a flow rate of 0.45 mL/min. The baselines of chromatograms were converted to power spectra by Fourier transform. The arrows indicate the noises derived from the pulsation of the pump.

Fig. 3 shows the power spectra of chromatographic baselines obtained using various pumps and detectors. With the pulse damping system in HPLC-ECD, the bands of pulsation were remarkably decreased (Fig. 3(a); compare Fig. 2A). The power spectrum shown in Fig. 3(b) was obtained using the HPLC system using pump B (dual piston parallel type) and detector A. The power density for pump B (Fig. 3(b)) was considerably smaller than that for pump A (Fig. 2B). Fluctuations in flow rate caused by piston pump mechanics often plague high-sensitive determination using HPLC-ECD. However, the frequencies of the fundamental tone and harmonic for pump B were difficult to recognize these noises at the frequencies on the power spectrum. Pulse damping systems and small stroke pump are useful for decreasing pump pulsation. A spectral analytical comparison was made of the effects of the electrochemical cell structure and working electrode material (GC or PFC) on chromatographic baseline noise. These spectra were obtained using detector B with the GC electrode (Fig. 3(c)) and the PFC working electrode (Fig. 3(d)). In the former case, bands at 0.09 and 0.18 Hz in the power spectrum in Fig. 2B were no longer present in Fig. 3(c). Detector B was a wall jet type cell whose inlet line was perpendicular to the working electrode though the inlet line of detector A and was oblique; the power density for the detector B was considerably less than that of the detector A. The wall jet type cell thus reduces the noise created by pump pulsation. With detector B with the PFC working electrode, power density was found to be very small, as seen in Fig. 3(d). When the wall jet type cell and PFC working electrode were used in HPLC-ECD, the noise level of baseline was very low.

Moreover, the detection limit for determining (-)-epicatechin by HPLC-ECD was obtained by the FUMI theory [1,2]. When the main cause of the uncertainty is the
chromatographic baseline noise, the FUMI theory can predict measurement RSD and detection limits using noise and signal from a single measurement of a chromatogram. In the case of HPLC-ECD, the predicted RSD based on the FUMI theory was parallel to the observed RSD by repetitive measurements (n = 5), indicating the FUMI theory is useful for predicting the measurement precision and detection limit in HPLC-ECD without repetitive measurements of chromatograms. The detection limits at which 33% RSD was given for (-)-epicatechin at various combinations of the pump, the pulse damping system, electrochemical detector, and working electrode are shown in Table 1. Using the FUMI theory, the optimization of HPLC-ECD systems is easily carried out in the shortest possible time.

We conclude that the present power spectral analysis of baseline noise and the detection limit based on the FUMI theory are thus an efficient optimization strategy for determining catechins using HPLC-ECD.

FIGURE 3. Power spectra of baselines of chromatograms using HPLC systems of (A) pump A, detector A and pulse damper system, (B) pump B and detector A, (C) pump A and detector B using GC working electrode, and (D) pump A and detector B using PFC working electrode.

<table>
<thead>
<tr>
<th>Pump</th>
<th>Detector</th>
<th>WE</th>
<th>Detection limit (fmol)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>+ Damper</td>
<td>GC</td>
<td>96.0</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>GC</td>
<td>38.4</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>GC</td>
<td>26.1</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>GC</td>
<td>74.5</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>PFC</td>
<td>25.7</td>
</tr>
</tbody>
</table>

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REFERENCES