Journal of Chromatography A, 1217 (2010) 2804–2811

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Improving liquid chromatography–tandem mass spectrometry determinations by modifying noise frequency spectrum between two consecutive wavelet-based low-pass filtering procedures

Hsiao-Ping Chen[a], Hui-Ju Liao[a], Chih-Min Huang[a], Shau-Chun Wang[a,*], Sung-Nien Yu[b]

[a] Department of Chemistry and Biochemistry, National Chung Cheng University, 168 University Road, Min-Hsiung, Chia-Yi 621, Taiwan
[b] Department of Electrical Engineering, National Chung Cheng University, 168 University Road, Min-Hsiung, Chia-Yi 621, Taiwan

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 2 February 2010
Accepted 18 February 2010
Available online 24 February 2010

Keywords:
Wavelet transforms
Liquid chromatography
Mass spectrometry

ABSTRACT

This paper employs one chemometric technique to modify the noise spectrum of liquid chromatography–tandem mass spectrometry (LC–MS/MS) chromatogram between two consecutive wavelet-based low-pass filter procedures to improve the peak signal-to-noise (S/N) ratio enhancement. Although similar techniques of using other sets of low-pass procedures such as matched filters have been published, the procedures developed in this work are able to avoid peak broadening disadvantages inherent in matched filters. In addition, unlike Fourier transform-based low-pass filters, wavelet-based filters efficiently reject noises in the chromatograms directly in the time domain without distorting the original signals. In this work, the low-pass filtering procedures sequentially convolve the original chromatograms against each set of low pass filters to result in approximation coefficients, representing the low-frequency wavelets, of the first five resolution levels. The tedious trials of setting threshold values to properly shrink each wavelet are therefore no longer required. This noise modification technique is to multiply one wavelet-based low-pass filtered LC–MS/MS chromatogram with another artificial chromatogram added with thermal noises prior to the other wavelet-based low-pass filter. Because low-pass filter cannot eliminate frequency components below its cut-off frequency, more efficient peak S/N ratio improvement cannot be accomplished using consecutive low-pass filter procedures to process LC–MS/MS chromatograms. In contrast, when the low-pass filtered LC–MS/MS chromatogram is conditioned with the multiplication alteration prior to the other low-pass filter, much better ratio improvement is achieved. The noise frequency spectrum of low-pass filtered chromatogram, which originally contains frequency components below the filter cut-off frequency, is altered to span a broader range with multiplication operation. When the frequency range of this modified noise spectrum shifts toward the high frequency regimes, the other low-pass filter is able to provide better filtering efficiency to obtain higher peak S/N ratios. Real LC–MS/MS chromatograms, of which typically less than 6-fold peak S/N ratio improvement achieved with two consecutive wavelet-based low-pass filters remains the same S/N ratio improvement using one-step wavelet-based low-pass filter, are improved to accomplish much better ratio enhancement 25-folds to 40-folds typically when the noise frequency spectrum is modified between two low-pass filters. The linear standard curves using the filtered LC–MS/MS signals are validated. The filtered LC–MS/MS signals are also reproducible. The more accurate determinations of very low concentration samples (S/N ratio about 7–9) are obtained using the filtered signals than the determinations using the original signals.

1. Introduction

Spectral analysis of varying types usually requires the measurement of temporal and/or spatial signals in order to generate comprehensible data. These signals, containing time-resolved traces, are often smoothed or filtered prior to the extraction of their characteristic frequency components. In addition, the signal and noise spectra of a trajectory or an image can also be modified in its frequency domain in order to recognize the temporal or spatial features more clearly. Recently, the application of spectral analysis and noise modification procedures have been used in biomedical engineering technologies such as deciphering the electroencephalographic (EEG) morphology for the control of a brain–computer interface and improving the qualities of digital radiographic images [1,2]. Similar approaches have also been utilized in analytical technologies. For instance, noise

* Corresponding author. Tel.: +886 5 2720411.
E-mail address: chescw@ccu.edu.tw (S.-C. Wang).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.02.033

spectrum modification techniques have been used to improve the peak signals of liquid chromatography mass spectrometry [3,4].

There have been previously reported techniques where one match-filtered or second derivative-filtered chromatogram is multiplied with another artificial chromatogram of similar peak features containing only thermal noises. This multiplication procedure can smear the bandwidth-limited noise frequency spectrum of a filtered chromatogram to a wider range. As a result, the noise on the modified chromatogram can be more efficiently removed with the second filtering procedure, resulting in a better signal-to-noise (S/N) ratio. This noise spectrum modification technique has been used to remedy the deficiency of matched filters, allowing the removal of spike-like noise on liquid chromatography–tandem mass spectrometry (LC–MS/MS) signals [3,5].

The problems of fluctuating backgrounds on the mass spectrometry chromatograms can also be solved when similar noise modification techniques are employed between two consecutive second derivative-filtered procedures [4,6]. However, peak broadening is inevitable when chromatographic peaks are processed with matched filters or second derivative filters. Peak broadening, in particular, is even more problematic when the filtering procedures are performed more than once.

Wavelet-based filters have also been used to remove spike-like noises and to suppress the background shifting of chromatograms or spectrum and improve the S/N ratios [7–11]. Unlike the band-pass or low-pass filters using Fourier transform, wavelet-based filters efficiently reject noises in the chromatograms directly in the time domain without distorting the original signals. In addition, the wavelet-based filters can also avoid peak broadening problems. However, these published wavelet methods usually require more sophisticated software procedures and tedious trials in their computational procedures.

The discrete wavelet transformation of a digitized signal is calculated by passing it through a series of filters. The first filter set to process the signal is a pair of quadrature mirror filters, consisting of a low-pass and a high-pass filter. As a result the signal is decomposed into two bands or wavelets. The low frequency and the high frequency bands of this signal are represented by approximation and detail coefficients, respectively. Similar transformations can be calculated again to decompose the low frequency band, consisting of approximation coefficients, into two other bands of high and low frequency, respectively. The original signal is finally decomposed into $m+1$ bands when discrete wavelet transformation is performed m times. Each transformation step results in one more set of approximation and detail coefficients. The number of transformation steps, m, is called the resolution level. Fig. 1 shows the band decomposition procedures using wavelet transform, where $h(n)$ and $g(n)$ are the high and low pass filter respectively [12]. These filters consecutively convolve against the original data points and then transform coefficients for i times. Each convolution step is followed with a down-sampling procedure. The detail and approximation coefficients at the ith resolution level are finally obtained. In the case of processing chromatographic data, the filtered chromatograms of each decomposition step can be reconstructed using the obtained approximation and detail coefficients via up-sampling and low pass filtering procedures.

Properly specifying the weighting factor of each band to shrink the filtered signal can work as a customized band-pass filter. However, the selection of weighting factor for each wavelet requires tedious trials to obtain a suitable band-pass filtered signal. On the other hand, the simple sequential convolutions of the filtered chromatograms using low-pass filters at each resolution level results in filtered signals being obtained without time-consuming weight factor adjustments.

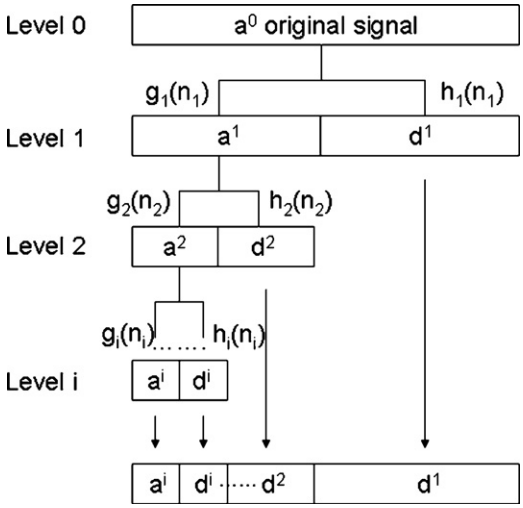

Fig. 1. Schematic illustration of wavelet decomposition procedures to obtain detail and approximation coefficients at each resolution level.

Our previous works have shown that the noise spectrum modification technique employed between two consecutive matched filters can tremendously improve the S/N ratios of LC–MS/MS peaks [3]. In theory, this technique can be implemented between any two consecutive low-pass filters. Hence, in this paper the simple summation of low-frequency bands obtained via wavelet decomposition will be used as low-pass filters. The noise spectrum modification technique is performed between these two wavelet-based low-pass filters. Since wavelet-based low-pass filter does not significantly widen the filtered signal peak, the peak broadening problem encountered in matched filter procedures can be avoided. Also, tedious trials were not required to implement wavelet filters in the signal processing procedures.

As described previously, the noise modification technique involves the multiplication of the filtered chromatogram with the other simulated chromatogram containing a peak ridden with pure random thermal noises. When the chromatogram noises are modified with this multiplication step, the second filtering procedures are able to process the noise-tailored chromatogram. The simulated chromatogram, $sc(t)$, is the summation of a smooth peak, $s(t)$, and noise, $n(t)$. The features of $s(t)$ are estimated with those of a reference chromatographic peak. The features of this reference peak are similar to those of the low-passed filtered chromatogram, $lf(t)$. When the low-pass filtered chromatogram, $lf(t)$, is multiplied by $sc(t)$, the expansion can be expressed using Eq. (1);

$$lf(t) \cdot sc(t) = lf(t) \cdot s(t) + lf(t) \cdot n(t) \quad (1)$$

Since the features of $lf(t)$ are similar to those of $s(t)$, the first term $lf(t)\cdot s(t)$ maintains the signal peak. Therefore, the tailored noise spectrum of the above multiplication product is mainly contributed by the second term $lf(t)\cdot n(t)$.

The multiplication of two time-dependent waveforms is in equivalence to the convolution between the two frequency spectra of these time-dependent waveforms. The Fourier transformation of the multiplication product, $lf(t)\cdot n(t)$, to the frequency domain is proportional to the convolution of two spectra, $LF(\nu)$ and $N(\nu)$, which are the Fourier transformations of $lf(t)$ and $n(t)$, respectively. This is expressed as Eq. (2);

$$\int [lf(t) \cdot n(t)] \exp(-i\omega t) dt = \frac{1}{2\pi} \int LF(v) N(\omega - v) dv \quad (2)$$

The Fourier transformation of a low-pass filtered peak, $LF(\nu)$, only contains low frequency components. $LF(\nu)$ is estimated as an exponential-decay profile as shown in Fig. 2A. The profile of $N(\nu)$,