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Periodic modulation-based stochastic resonance algorithm applied to quantitative analysis for weak liquid chromatography–mass spectrometry signal of granisetron in plasma

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Abstract

The periodic modulation-based stochastic resonance algorithm (PSRA) was used to amplify and detect the weak liquid chromatography–mass spectrometry (LC–MS) signal of granisetron in plasma. In the algorithm, the stochastic resonance (SR) was achieved by introducing an external periodic force to the nonlinear system. The optimization of parameters was carried out in two steps to give attention to both the signal-to-noise ratio (S/N) and the peak shape of output signal. By applying PSRA with the optimized parameters, the signal-to-noise ratio of LC–MS peak was enhanced significantly and distorted peak shape that often appeared in the traditional stochastic resonance algorithm was corrected by the added periodic force. Using the signals enhanced by PSRA, this method extended the limit of detection (LOD) and limit of quantification (LOQ) of granisetron in plasma from 0.05 and 0.2 ng/mL, respectively, to 0.01 and 0.02 ng/mL, and exhibited good linearity, accuracy and precision, which ensure accurate determination of the target analyte.

Keywords: Stochastic resonance; Periodic modulation; Weak signal; Granisetron; LC–MS

1. Introduction

Stochastic resonance (SR) was first introduced by Benzi et al. to explain the periodicity of Earth's ice ages [1,2]. In recent decades, SR has received increasing interest since weak signals can be amplified significantly in a nonlinear system by the assistance of noise. Typical stochastic resonance phenomena have been found in many simple physical systems [3,4] and complex chemical systems [5,6]. Its application in chromatographic analysis was first found in Pan et al.'s work [7]. And in our previous studies, it was applied successfully to quantitative analysis for weak chromatographic signals of phenazopyridine [8], glyburide [9] and roxithromycin [10] in plasma.

The algorithm based on stochastic resonance (SRA) is an efficient approach to the detection of weak signal buried in noise. However, when a chromatographic peak is extremely weak, the output signal obtained by SRA is usually distorted seriously because of the existence of noise. It was found that the distorted signal could be corrected by introducing a periodic force into the nonlinear system, and the new algorithm was named the periodic modulation-based stochastic resonance algorithm (PSRA) [11]. Previously, PSRA has been successfully employed to improve the Roman signal of CCl_4, but its application to chromatographic analyses has never been reported.

Granisetron is a selective 5-HT3 receptor antagonist particularly effective for the relief of nausea and vomiting induced by cancer therapy [12]. Several methods have been reported for the determination of granisetron in biological fluids [13–15]. In the present work, liquid chromatography coupled with mass spectrometry (LC–MS) was applied to the determination of granisetron in plasma and then the LC–MS signal was processed by PSRA. By applying PSRA, the LC–MS peak was amplified significantly and characterized by good shape, and those peaks buried in noise could be determined accurately. Therefore, only small volumes were needed in the determination of plasma granisetron concentration and this was beneficial for volunteers. The limit of detection (LOD) and limit of quantification (LOQ) given by LC–MS in the current work were originally 0.05 and

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0.2 ng/mL, respectively. With the application of PSRA, the LOD and LOQ were, respectively, improved to 0.01 and 0.02 ng/mL. This method greatly extended the lower limit of quantification without an exhaustive exploration of the extraction methodology or a more advanced detector.

2. Theory and algorithm

The nonlinear Langevin equation has been frequently employed to describe the phenomenon of SR. It has the following formula [16]:

$$\frac{dx}{dt} = -U'(x) + MI(t) + C\xi(t) \quad (1)$$

where the variable x is the output of the nonlinear system, representing the position of signal. $I(t) = S(t) + N(t)$ denotes an input signal embedded in a noisy environment with the signal $S(t)$ and the intrinsic noise $N(t)$ caused by instrument. $\xi(t)$ is external noise added to induce the occurrence of SR. M and C, adjustable parameters, are respectively set to 1 and 0 to avoid the damage of external noise to the characteristic of intrinsic noise and reduce variable factors in SRA [7].

The symmetric double-well potential $U(x)$ used in traditional SRA can be expressed as the following equation with the constants a and b characterizing the system:

$$U(x) = -\frac{a}{2}x^2 + \frac{b}{4}x^4 \quad (2)$$

The profile of the double-well potential is presented in Fig. 1(a). Between the two stable minimum points located at $x = \pm\sqrt{a/b}$, there is a potential barrier with the height given by $\Delta U = a^2/4b$. The weak analytical signal may rest in one potential well initially. As the input signal, noise and nonlinear system match well, namely the stochastic resonance condition is reached, the weak signal can surmount the energy barrier and hop from one potential well to the other with the assistance of noise. As a result, the signal will obtain an increased intensity. However, for the relative low energy, the noise is confined in the original well with the intensity suppressed by the nonlinear system. Therefore, the output signal of the system will obtain a better signal-to-noise ratio (S/N).

When the input signal is extremely weak, the energy of the noise may be too high relative to that of useful signal and it may cause the noise to hop from one potential well to another together with the signal. As a result, the output signal will be distorted. Generally, distorted peaks are not suitable for chromatographic analysis. To correct the distorted peak shape of output signal, in this work, we added a periodic modulation into the nonlinear system [11]. Thus, the potential function can be developed into the following formula by adding such a periodic modulation:

$$U(x,t) = -\frac{a}{2}x^2 + \frac{b}{4}x^4 + x\varepsilon \ \sin(\omega t) \quad (3)$$

In the system described by Eq. (3), the potential barrier will vary with the periodic force (Fig. 1(b)). When the values of ε and ω are satisfactory, the signal is allowed to surmount the potential barrier while the noise is not. Thus, the peak shape of output signal will be improved.

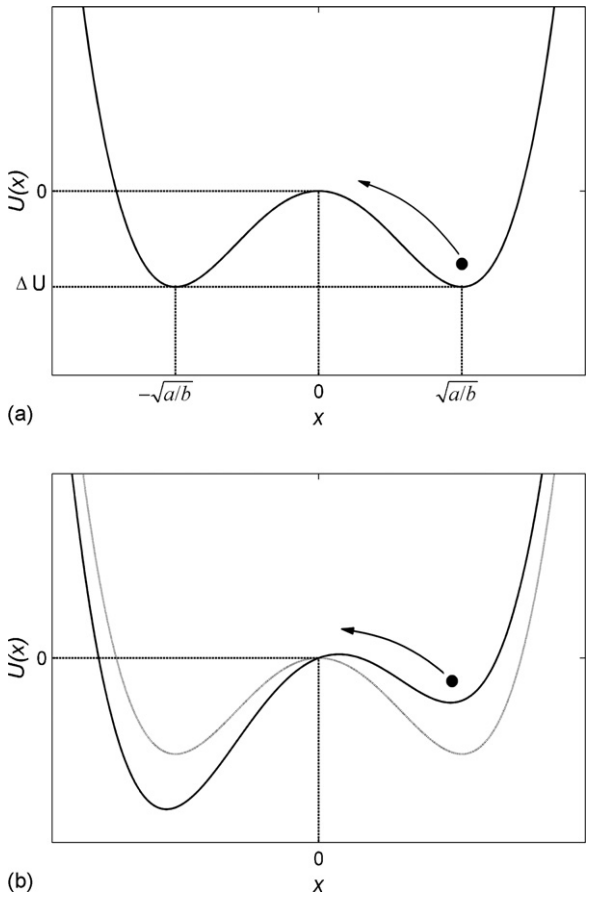

Fig. 1. The profile of double-well potential function. The potential of: (a) traditional SRA; (b) PSRA.

Eqs. (1) and (3) can be solved by a fourth-order Runge-Kutta method [17], which is more accurate than the Euler method used in the previous work [11]. Supposing that there are N sampling points in the input signal, if the nth sample of time t, the input signal $I(t)$ and the output signal $x(t)$ are denoted by t_n, I_n and x_n, respectively, the output signal can be calculated by the following equation:

$$x_{n+1} = x_n + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4),$$
$$n = 0, 1, \ldots, N-1, \quad (4)$$

where k_1, k_2, k_3 and k_4 are given by

$$k_1 = ax_n - bx_n^3 - \varepsilon \ \sin(\omega t_n) + I_n,$$
$$k_2 = a\left(x_n + \frac{k_1}{2}\right) - b\left(x_n + \frac{k_1}{2}\right)^3 - \varepsilon \ \sin(\omega t_{n+1}) + I_{n+1},$$
$$k_3 = a\left(x_n + \frac{k_2}{2}\right) - b\left(x_n + \frac{k_2}{2}\right)^3 - \varepsilon \ \sin(\omega t_{n+1}) + I_{n+1},$$
$$k_4 = a(x_n + k_3) - b(x_n + k_3)^3 - \varepsilon \ \sin(\omega t_{n+2}) + I_{n+2}, \quad (5)$$

The calculation procedure starts with the normalization of $I(t)$ to the interval $[-1, 1]$ and the normalized signal is then operated on by the algorithm to give the output signal. The final results can be obtained by inverse normalization of the output signal.

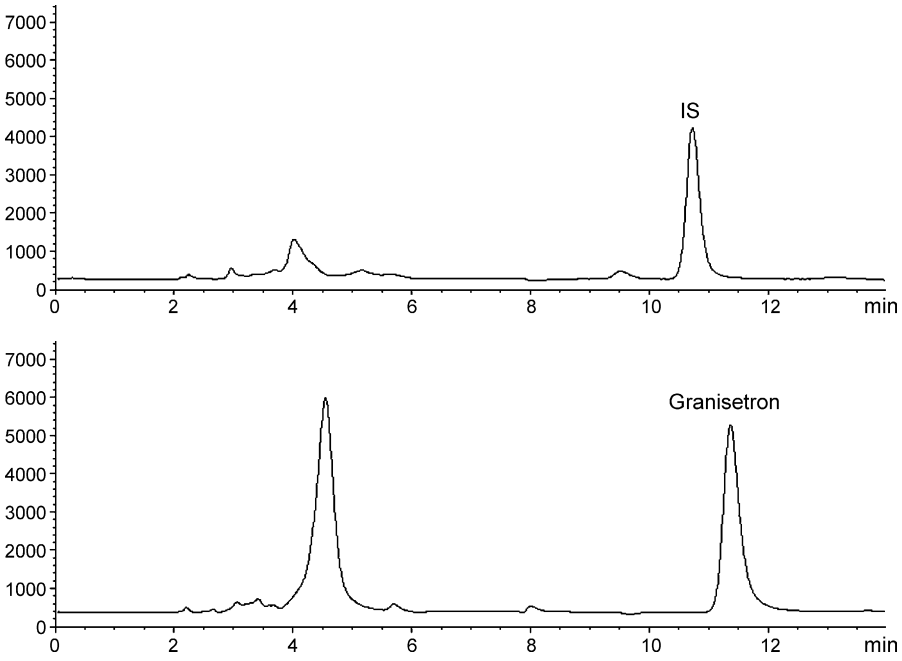

Fig. 2. Typical chromatogram of plasma spiked with IS and granisetron.

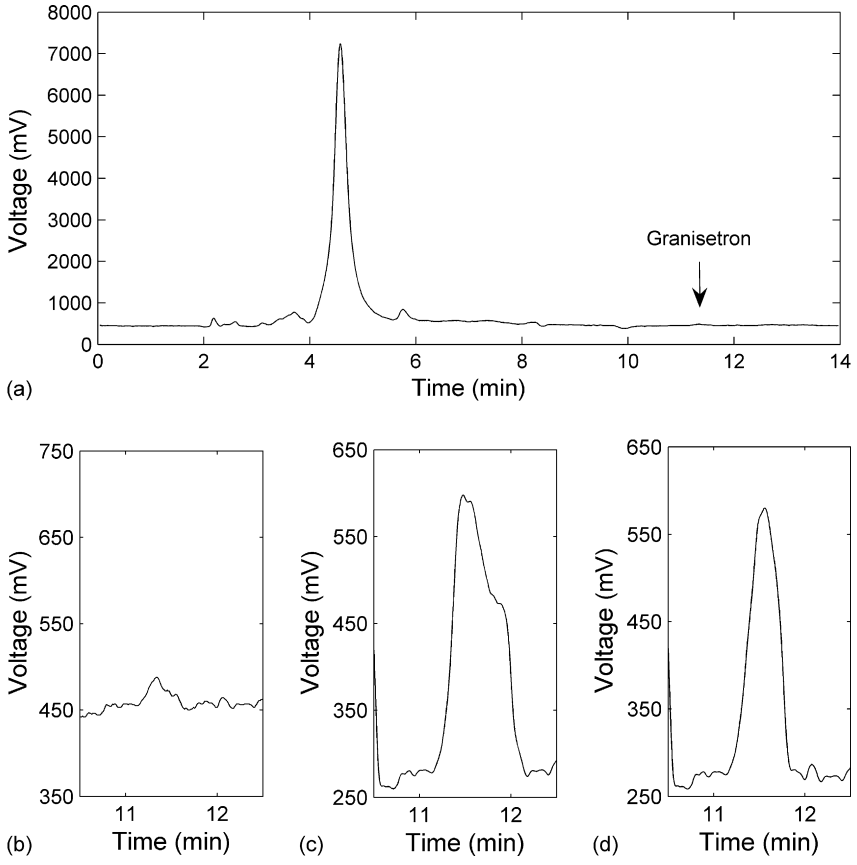

Fig. 3. Chromatogram of plasma spiked with granisetron at 0.02 ng/mL. (a) Full length of the chromatogram. (b) Especially enlarged chromatogram of granisetron. (c) The peak of granisetron obtained by traditional SRA ($a = 0.17$, $b = 0.0045$). (d) The peak of granisetron obtained by PSRA ($\varepsilon = 0.32$, $\omega = 0.31$).

3. Experimental

3.1. Reagents and standards

Granisetron and metoclopramide hydrochloride were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) was purchased from Merck Company (Germany). *n*-Hexane and ammonium acetate were analytical grade and obtained from Nanjing Chemical Reagent Co. (Nanjing, China). Double distilled water was used throughout the study.

3.2. Sample preparation

A 300 μL aliquot of plasma spiked with granisetron was extracted with 2 mL *n*-hexane after addition of 50 μL of metoclopramide hydrochloride solution (internal standard, IS, 0.1 μg/mL) and 0.2 mL of 0.1 M NaOH. Following centrifugation and separation, the organic phase was evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in 100 μL of mobile phase, and a 50 μL aliquot of the supernatant was injected into the LC–MS system.

3.3. LC/MS analysis

All the chromatographic analyses were performed on a HP1100 LC/DAD/MSD system (Hewlett-Packard, USA) equipped with a binary pump and automatic sampler. The separation was carried out on a kromasil CN column (4.6 mm × 250 mm, 5 μm, AKZO NOBEL, Sweden) maintained at 25 °C. The mobile phase consisted of 0.05 M ammonium acetate buffer (pH 6.9)–methanol (15:85, v/v) at a flow-rate of 1.0 mL/min. The MS system was a single quadrupole mass spectrometer equipped with an electrospray interface (ESI) and the parameters were set with a drying gas (N_2) flow of 10.5 L/min, nebulizer pressure of 45 psi, drying gas temperature of 350 °C, capillary voltage of 4 kV and fragmentor voltage of 70 V. Positive ionization with selected-ion monitoring (SIM) mode was used for all analyses. The protonated molecules $(M+H)^+$ were detected at *m/z* 313 for granisetron and *m/z* 300 for metoclopramide hydrochloride (IS).

4. Results and discussion

4.1. The selection of the system parameters

The typical chromatogram of plasma spiked with IS and granisetron is exhibited in Fig. 2. The retention time of granisetron is about 11.5 min. The enhancement of the peaks of interest might be affected by other peaks present in the chromatograms (which will also extract energy from noise), and so if these other peaks are included in the input, the output peaks of interest may not be ideal. Therefore, we pick up the 10.5–12.5 min segment of chromatogram including the peak of interest and noise around as the input of the algorithm for choosing the proper parameters.

It is difficult for traditional SRA to give attention to both the S/N and peak shape of the output signal by adjusting a and b only. According to Eq. (3), there are two additional parameters that are adjustable in PSRA, where ε and ω denote the amplitude and frequency of the added periodic force, respectively. The optimization of parameters a and b was at first carried out with ε and ω set to 0 and with the value of S/N as an evaluating index. When $a = 0.17$ and $b = 0.0045$, the weak chromatographic peak of granisetron at 0.02 ng/mL (see Fig. 3(a and b)) was magnified remarkably with a maximum S/N, but with a badly distorted shape (Fig. 3(c)). To improve the peak symmetry, the parameters ε and ω were investigated subsequently to obtain a best value of the peak height to peak half-width ratio. The values of ε and ω were finally set to 0.32 and 0.31, respectively, to obtain a satisfactory output signal (Fig. 3(d)). According to our trial, the improvement of peak shape is largely dependent on the frequency of introduced force, i.e., the value of ω. When an appropriate ω is selected, the value of ε needs only a slight adjustment to improve the peak shape.

4.2. Analysis of granisetron in human plasma

Although all the samples have different intensities at different concentrations, the same parameters will be used throughout to keep the quantitative relationship of the output signals. A set of working solutions spiked with granisetron at different concentration levels were prepared and assayed, and then the chromatograms were processed by PSRA with the optimized parameters developed above. As described in previous work [8–10], the signal of internal standard was not processed by the algorithm.

The specificity of the assay was checked by analyzing blank plasma samples. No endogenous interferences were encountered either before or after PSRA. Signal-to-noise ratio of 3 and 10 are generally considered as limit of detection and limit of quantification, respectively. The LOD and LOQ of granisetron obtained by LC–MS were originally 0.05 and 0.2 ng/mL, and improved by PSRA to 0.01 and 0.02 ng/mL, respectively.

Table 1
Calibration curve of granisetron by PSRA

	Concentration (ng/mL)						
	0.1	0.2	0.5	1.0	2.0	5.0	10.0
Ratio (*f*)	0.62	1.04	1.57	2.92	5.33	11.08	23.75
Calibration curve	$f = 2.295(\pm 0.049)c + 0.452(\pm 0.213)$; $r = 0.9989$; S.D. = 0.441						

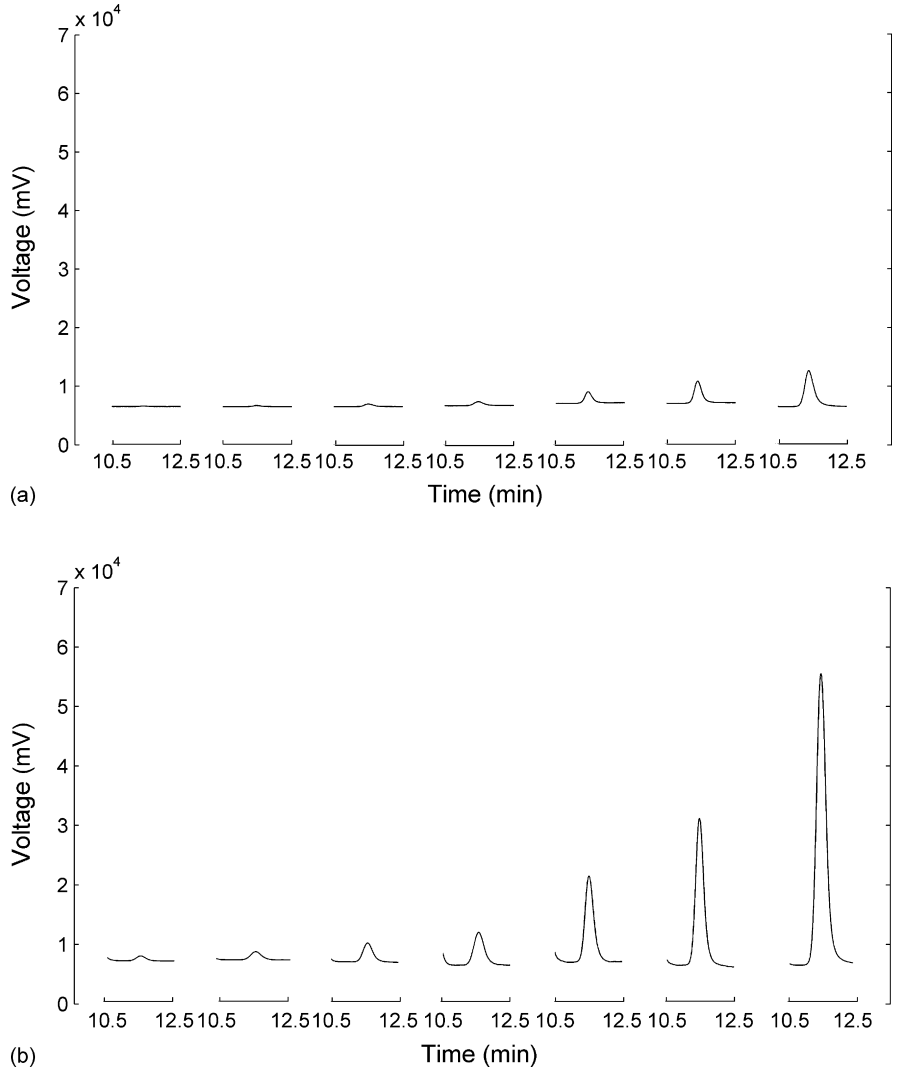

Fig. 4. Chromatograms of calibration samples of granisetron. (a) The original chromatograms of granisetron. (b) The chromatograms of granisetron obtained by PSRA ($a = 0.17$, $b = 0.0045$, $\varepsilon = 0.32$ and $\omega = 0.31$). From left to right, the concentrations of granisetron are 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 ng/mL, respectively.

Calibration standards at seven different concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 ng/mL) were prepared and assayed in duplicated. A good linear relationship was observed between the peak area ratio of granisetron (after PSRA) to IS (original) and the plasma concentration of granisetron (shown in Table 1). In Fig. 4, a comparison of calibration curve samples is made between the original signals and those obtained by PSRA. The result shows satisfactory amplification and good shape of the granisetron peak at each concentration when using PSRA.

The investigation of accuracy and precision was carried out with spiked preparations at concentrations of 0.2, 1.0 and 5.0 ng/mL. Intra-day accuracy and precision were determined by repeated analysis of prepared samples on one day ($n = 6$), and inter-day accuracy and precision were determined by repeated analysis of prepared samples on three different days ($n = 6$ series per day). The intra- and inter-day assays are summarized in Table 2 and the results indicate that the accuracy and precision of the assay are acceptable.

Table 2
Accuracy and precision of granisetron by PSRA

Added concentration (ng/mL)	Intra-day assay			Inter-day assay		
	Measured concentration (mean ± S.D.) (ng/mL)	Accuracy (%)	Precision R.S.D. (%)	Measured concentration (mean ± S.D.)(ng/mL)	Accuracy (%)	Precision R.S.D. (%)
0.2	0.207 ± 0.019	103.5	9.20	0.204 ± 0.024	102.2	11.90
1.0	0.99 ± 0.065	99.4	6.50	1.01 ± 0.071	100.7	7.02
5.0	4.84 ± 0.24	96.8	4.95	4.92 ± 0.29	99.2	5.89

5. Conclusion

To our knowledge, the application of stochastic resonance has never been reported in the LC–MS field. In the present work, the periodic modulation-based stochastic resonance algorithm was applied successfully to the detection of weak chromatographic signals from a LC–MS system. PSRA effectively corrected the distortion of peak shape that usually appeared in the traditional SRA, and provided good linearity as well as satisfactory accuracy and precision. The current work suggests that PSRA should be an effective tool in enhancing the sensitivity of mass spectrometry for the assay of therapeutic compounds and their metabolites.

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