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Stochastic resonance is applied to quantitative analysis for weak chromatographic signal of roxithromycin in beagle dog plasma

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Abstract

Based on the theory of stochastic resonance, the signal to noise ratio (SNR) of HPLC/UV chromatographic signal of roxithromycin is enhanced by cooperation of signal, noise and nonlinear system. A simple new method for the determination of low concentration of roxithromycin in beagle dog plasma is presented. Using signal enhancement by stochastic resonance, this method extends the limit of quantitation from the reported 0.5 to 0.1 μg/ml. During validation of the new method, HPLC/MS was used as a comparison technique. The results indicate that the recovery and low concentrations of roxithromycin in beagle dog plasma were equivalent between the two methods ($P > 0.05$). Stochastic resonance may be a promising tool for improving detection limits in trace analysis.

Keywords: Stochastic resonance; Roxithromycin; Weak signal; HPLC/UV; HPLC/MS

1. Introduction

Stochastic resonance generally occurs in bistable dynamical systems attacked by a weak signal corrupted by noise through the nonlinear internal dynamics of the system, the small signal is amplified by the presence of noise [1]. It has been shown in numerous research works that the strength of signals will increase and that of noise will decrease by the cooperation of proper noise, weak signals and nonlinear system with a threshold [2–4]. Since then SR has attracted considerable interest [5–7], e.g. Wang has applied SR to analyze the weak laser-Raman spectrum of CCl4 [8]. Pan et al. have improved the detectability of analytes in gas chromatography [9].

Roxithromycin is a semisynthetic macrolide antibiotic derived from erythromycin. It is rapidly absorbed with long elimination half time and gives plasma levels that are higher than those of erythromycin [10]. Several chromatographic methods have been reported for determination of roxithromycin in biological fluids. Electrochemical detection was employed in most of them. In comparison with other macrolides, roxithromycin has somewhat higher absorbance at lower wavelengths. Macek et al. have established a method with spectrophotometric detection [11]. With a limit of quantitation of 0.5 μg/ml, it is difficult to determine low concentration with UV detection in plasma.

The goal of this work is to apply stochastic resonance algorithms (SRA) to satisfy the requirement of quantitative analysis of weak chromatographic signals. The method was used to determine the roxithromycin in beagle dog plasma with limit of quantitation of 0.1 μg/ml, and a limit of detection of 0.05 μg/ml. This method greatly extended the low limit of quantitation compared with the published work. A HPLC/MS method, whose limit of detection was as low as 0.05 μg/ml, was also developed to determine the concentrations of roxithromycin in plasma in order to compare with the UV. The result showed that there was no significant difference between HPLC/UV with SRA and HPLC/MS analyses.

2. Theory and algorithm

The Langevin equation has been frequently employed in order to describe the phenomenon of SR [8,9]:

$$\dot{x} = -U'(x) + MI(t) + \xi(t) \quad (1)$$

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where $I(t) = S(t) + N(t)$ denotes an input signal embedded in a noisy environment with the signal $S(t)$ and the intrinsic noise $N(t)$; x is the displacement of particle; $\xi(t)$ is external noise added to induce SR, M is an adjustable parameter. $U(x) = -(1/2)ax^2 + (1/4)bx^4$ is a simple double-well potential with the constants a and b characterizing the system. The minima are located at $x = \pm\sqrt{a/b}$, where $x_{\min} = (a/b)^{1/2}$. These are separated by a potential barrier with the height given by $\Delta U = a^2/4b$. The barrier top is located at $x = 0$. When the input signal, noise and nonlinear system cooperate well, the potential barrier will lower and the signal which rest at one of the two minima of the potential may surmount the energy barrier hopping from one potential well to another. Thus, the intensity of signals will increase and that of the noise will reduce. The output signal of the system will be obtained with a better SNR compared to the input [1–3,5,6,12]. Supposing the input signal is a sinusoid, $I(t) = A\sin(\varpi_0 t)$, and D is the noise intensity, where A is the intensity of input signal and ϖ_0 is frequency of the input signal. Based on the adiabatic approximate theory [13], the SNR can be described as:

$$\mathrm{SNR} = \frac{\sqrt{2}\mu^2 A^2 \mathrm{e}^{-\mu^2/4D}}{4D^2} = \sqrt{2}\Delta U\left(\frac{A}{D}\right)^2 \mathrm{e}^{-\Delta U/D} \quad (2)$$

The equation shows that the SNR of the output signal is decided by the potential barrier ΔU and the noise intensity D. The SNR of the Eq. (2) has a maximum at $D_{\max} = \Delta U/2$. Based on the theory, SR can be observed by adding external noise, adjusting the input signal intensity, and modulating the nonlinear system simultaneously. Previous literatures [14] clearly showed that it was impossible to do quantitative determination by adding external noise. Because noise in analytical signals is often assumed to be Gaussian white noise, but added external noise, which is usually simulated is somewhat colored. The addition of external noise will damage the characteristic of the intrinsic noise and result in the serious distortion of the obtained signals. It is impossible to do quantitative determination under this condition. In order to do quantitative determination, only the parameters a and b of the system are modulated to match the input signal including real signal and intrinsic noise to achieve SR.

Eq. (1) is solved by a fourth-order Runge–Kutta method [9,15,16] and algorithm can be described as follows:

$$x_{n+1} = x_n + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4), \quad n = 0, 1, \cdots, N-1$$
$$k_1 = ax_n - bx_n^3 + u_n$$
$$k_2 = a(x_n + k_1/2) - b(x_n + k_1/2)^3 + u_n$$
$$k_3 = a(x_n + k_2/2) - b(x_n + k_2/2)^3 + u_{n+1}$$
$$k_4 = a(x_n + k_3/2) - b(x_n + k_3/2)^3 + u_{n+1}$$

where $u_n = I_n + \xi_n$.

Because there were other peaks in the signal of plasma sample, which would also absorb energy from noise in SNR, the magnification of roxithromycin might be affected and the output might be not ideal. Thus, it is not possible to process the whole chromatogram. A section of chromatograph signal included weak signal of roxithromycin and noise around was first chosen. In this work, the retention time of roxithromycin is 10.24 min, so we choose a section of signals during the period of 9.8–11.0 min to perform stochastic resonance. The input signal just over the range of time from 9.8 to 11.0 min is first prepared by normalizing in the range [−1, 1]. Therefore, all samples in analyzed series will have the same intensity in the nonlinear system. Then the output signal can be obtained by solving Langevin equation. The final results can be obtained by inverse normalization of the output signals. The algorithm was implemented in matlab6.5 (Math-Works, Natick, MA, USA) by authors and the calculations were carried out on a computer (Pentium®4 Cpu 2.00 GHZ, Memory 512 M).

3. Experimental

3.1. Materials

Roxithromycin (98.0% purity) and clarithromycin (internal standard, 98.0% purity) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) and acetonitrile (HPLC-grade) were purchased from Merk Company (America). Ammonium acetate, ether and sodium carbonate (analytical grade) were from Nanjing Chemical Co. (Nanjing, China). Heparinized blank (drug free) beagle dog plasma was provided by the Experimental Animal Center of China Pharmaceutical University. Distilled water was used throughout the study.

3.2. HPLC/UV system

The LC-10AT vp HPLC (SHIMADZU) system was used with SPD-10A vp UV–vis detector. The N2000 chromatography data system (Zhejiang University Star Instrument Technology Co, Ltd.) was used with sampling frequency of 10 Hz. The samples were separated on a Hanbang C18 column (250 mm × 4.6 mm i.d., 5 μm, Jiangsu Hanbon Science & Technology Co, Ltd., China) column. The mobile phase was a mixture of methanol–10 mmol/l ammonium acetate buffer (68:32, v/v) adjusted to pH 4.5 at a flow-rate of 1 ml/min. The detector was set at 215 nm.

The plasma was prepared by a liquid–liquid extraction method for spectrophotometric detection. 0.5 ml of plasma and 50 μl clarithromycin acetonitrile solution (internal standard, 200 μg/ml) were added to the centrifuge tube, and mixed. Then 50 μl saturated Na_2CO_3 solution was added and vortex mixed. Five milliliters of ether serving as extraction solvent was added and the tubes were vortex mixed for 3 min. About 4 ml of the organic phase was drawn out to another centrifuge tube after centrifugation at 2130 × g for 10 min and was evaporated to dryness under nitrogen atmosphere in the water bath at 45 °C. Before HPLC analysis, the residues were reconstituted by 50 μl of mobile phase. The reconstituted plasma extract was centrifuged at 16,880 × g for 10 min and 20 μl of supernatant fluid was injected into the HPLC for analysis.

3.3. HPLC/MS system

An Agilent series 1100 HPLC with a mass detector, including a binary pump (Model G1312A), a vacuum degasser (Model G1322A), an autosampler (Model G1313A) and a column oven (Model G1316A). The chromatographic conditions were a Hanbang C18 column (250 mm × 4.6 mm i.d., 5 μm) column and a mobile phase (75:25, v/v) of methanol:water (10 mmol/l ammonium acetate buffer) at a flow-rate of 1 ml/min. The mass detector was set at SIM mode. The monitored ions were 837.5 ($M + H^+$) for roxithromycin and 748.5 ($M + H^+$) for the internal standard (clarithromycin).

Because of high sensitivity of mass detector, the plasma sample was injected directly for determination after being deproteinized. 0.2 ml volume of plasma was transferred to a 1.5 ml centrifuge tube, mixed with 50 μl of internal standard solution (5 μg/ml), followed by the addition of 0.8 ml acetonitrile. The sample was vortexed for 60 s and centrifuged at 16,880 × *g* for 10 min and 20 μl supernatant fluid was injected into HPLC for analysis.

4. Results and discussion

4.1. Effect of the system parameters a and b on the performance of SRA

The parameters *a* and *b* in Eq. (1) not only define the height of the potential barrier, but also affect the profile of the potential well. When the input signal was fixed, the parameters *a* and *b* effected the quality of final output signal directly. Therefore, it is necessary to optimize the parameters *a* and *b* in order to get a good output result. The optimization method in the references reported was to fix one parameter among *a* and *b*, then to adjust another to optimize peaks with different height [9]. In such way, some information obtained from the adjusting on parameters of the target signal may be lost. This article presented a new method to optimize *a* and *b* synchronously for the first time with the ratio between peak height to peak half-width as evaluating indicator. A greater value of the ratio of peak height to half-width indicates a better output signal. Generally, our experience showed that *a* and *b* should be small to get a satisfactory result, so we optimized *a* and *b* in the range from 0 to 0.01 firstly with the results showed in Fig. 1. It is obvious that parameter *b* has a much influence on the ratio than *a* and the result is better when *b* is near to 0. Then, *a* and *b* were varied in the range from 10^{-6} to 10^{-4} and from Fig. 2, we can see that the ratio of peak height to peak half-width becomes larger with increasing *b* until it has reached the maximum when *a* equals to 9.9×10^{-5} and *b* equals to 8.0×10^{-6}. After that, the ratio was reduced rapidly with the increase of *b*. Thus, with $a = 9.9 \times 10^{-5}$ and $b = 8.0 \times 10^{-6}$, the signal get the best output result.

4.2. Analysis of the roxithromycin and the recovery of the two methods

As shown in Fig. 3, roxithromycin and internal standard were separated well from the biological background under the described HPLC/UV system, and the retention time were 10.24 and 8.89 min, respectively. There were no endogenous plasma components interfering with them both before and after SRA. When the concentration of blood sample was lower than 0.5 μg/ml, the peak of roxithromycin was too weak to perform quantitative analysis. So stochastic resonance algorithm was used to extend the instrumental linear range. Fig. 4A was chromatogram of roxithromycin of 0.05 μg/ml, Fig. 4B was the especially enlarged chromatogram of roxithromycin and Fig. 4C was the chromatogram of roxithromycin which was obtained after SRA with the parameters $a = 9.9 \times 10^{-5}$, $b = 8.0 \times 10^{-6}$, and Fig. 4D was especially enlarged chromatogram of Fig. 4C. It does seem clear that there is an increase in S/N between Fig. 4A and C. The peak area ratio *f*, which was the ratio of the peak area of this sample after SRA to the origin peak area of the internal standard, was applied to make the regression analysis. Peak area ratios were plotted against roxithromycin concentrations and standard curves were in the form of $f = A + Bx$. To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on separate 5 days. Effective concentrations in plasma samples were 0.1, 0.2, 0.5, 1, 2, 5 and 10 μg/ml for roxithromycin. Visual inspection of the plotted duplicate cali-

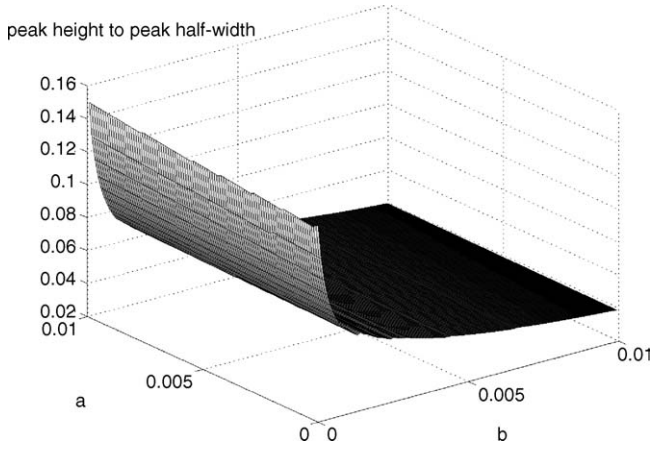

Fig. 1. Peak height to half-width to parameters *a*, *b* (0–0.01).

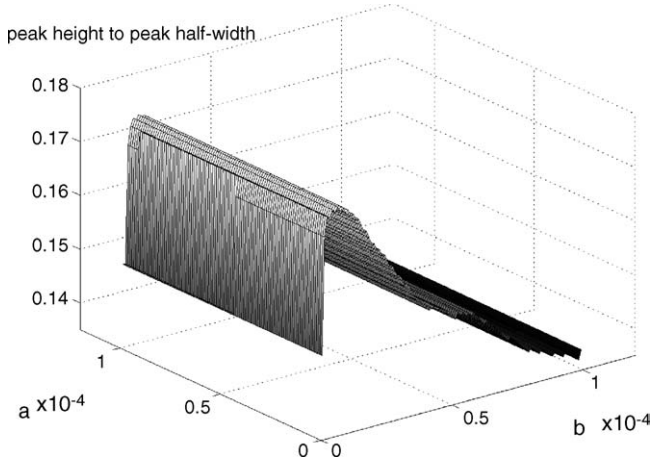

Fig. 2. Peak height to half-width to parameters *a*, *b* (10^{-6} to 10^{-4}).

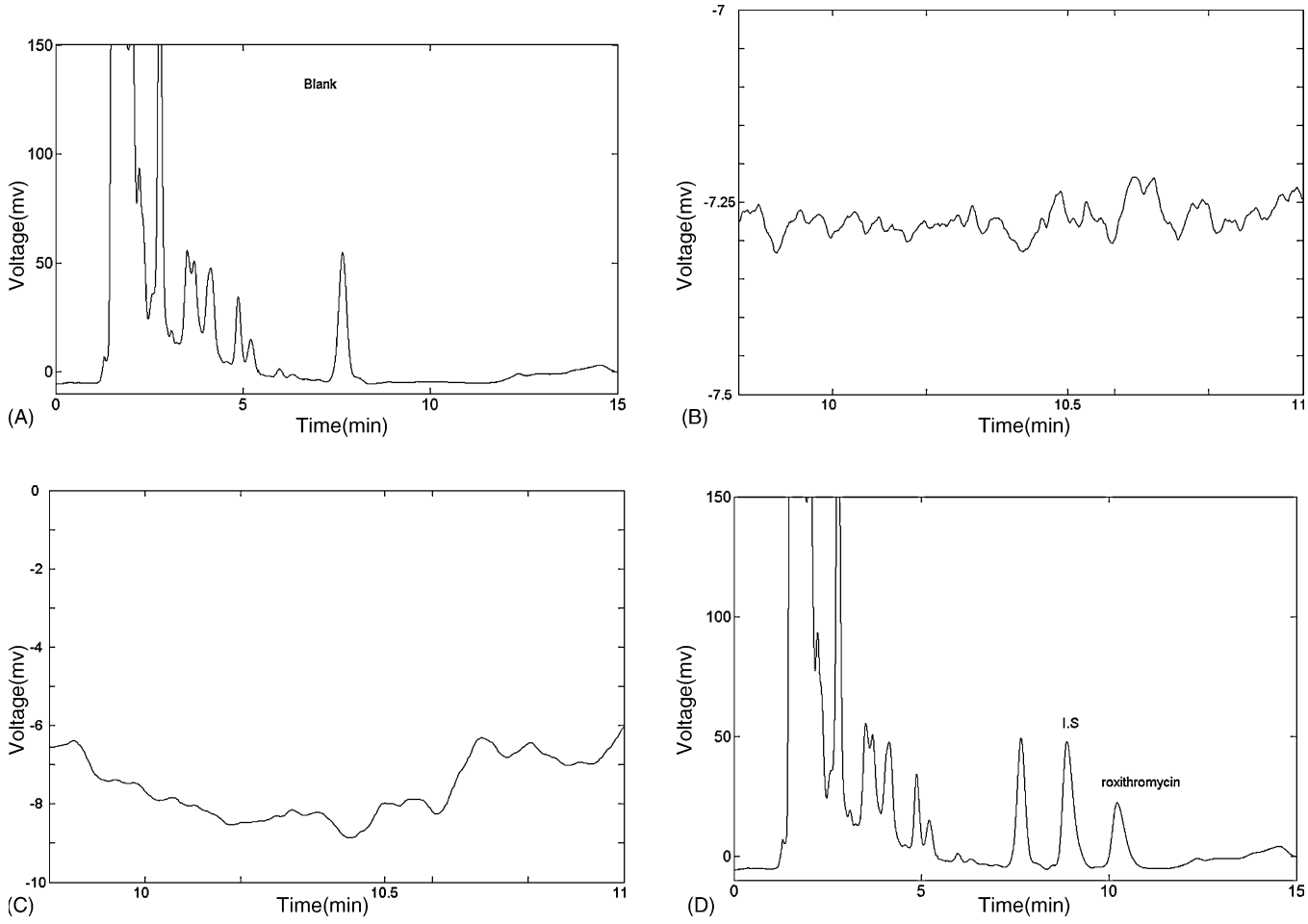

Fig. 3. (A) Chromatogram of a blank plasma sample; (B) especially enlarged chromatogram of a blank plasma sample; (C) especially enlarged chromatogram of a blank plasma sample by SRA with $a = 9.9 \times 10^{-5}$ and $b = 8.0 \times 10^{-6}$; (D) a blank plasma sample spiked with roxithromycin at 1.5 μg/ml and I.S obtained by HPLC/UV.

bration curves and correlation coefficients = 0.9994 confirmed that the calibration curves were linear over the concentration ranges 0.1 to 10 μg/ml for the analyte. Typical standard curve was $f = 5.265 \times c - 0.2738$, where f represents the ratios of roxithromycin peak area to that of I.S. and c represents the plasma concentrations of roxithromycin. The corresponding confidence intervals ($\alpha = 0.05$) was intercept: −1.629 to 0.8834 and slope: 5.088–5.671.

Fig. 5 was a typical chromatogram of roxithromycin and internal standard analyzed by HPLC/MS. The approximate retention times of roxithromycin and internal standard were 4.678 and 4.260 min, respectively. The calibration curve also showed good linearity over the concentration range of 0.1–10 μg/ml in plasma. The assay methods were validated in terms of recovery. It was evaluated by processing three different concentration levels of the samples (each level analyzed five times). The data are given in Table 1, and the results showed that the two methods had no significant difference.

4.3. Comparisons between HPLC/UV with SRA and HPLC/MS

The blood samples were determined at concentrations at 0.1, 0.2 and 0.4 ng ml^{-1} by different methods—HPLC/UV with SRA and HPLC/MS (each level analyzed five times). Table 2 showed that there was no significant difference.

Table 1
Recovery

Content (μg/ml)	HPLC/UV/SRA ($n = 5$)		HPLC/MS ($n = 5$)	
	Recovery (%)	(R.S.D.%)	Recovery (%)	(R.S.D.%)
0.1	98.62	9.84	102.4	7.53
1	96.53	5.26	99.37	4.86
10	102.3	3.64	101.5	3.21

Table 2
Comparisons between HPLC/UV with SRA and HPLC/MS

Content (μg/ml)	HPLC/UV/SRA ($n = 5$)		HPLC/MS ($n = 5$)	
	Content (ng/ml)	(R.S.D.%)	Content (ng/ml)	(R.S.D.%)
0.1	0.1024	12.48	0.1040	10.16
0.2	0.2024	8.42	0.2098	7.13
0.4	0.3889	7.82	0.4037	6.18

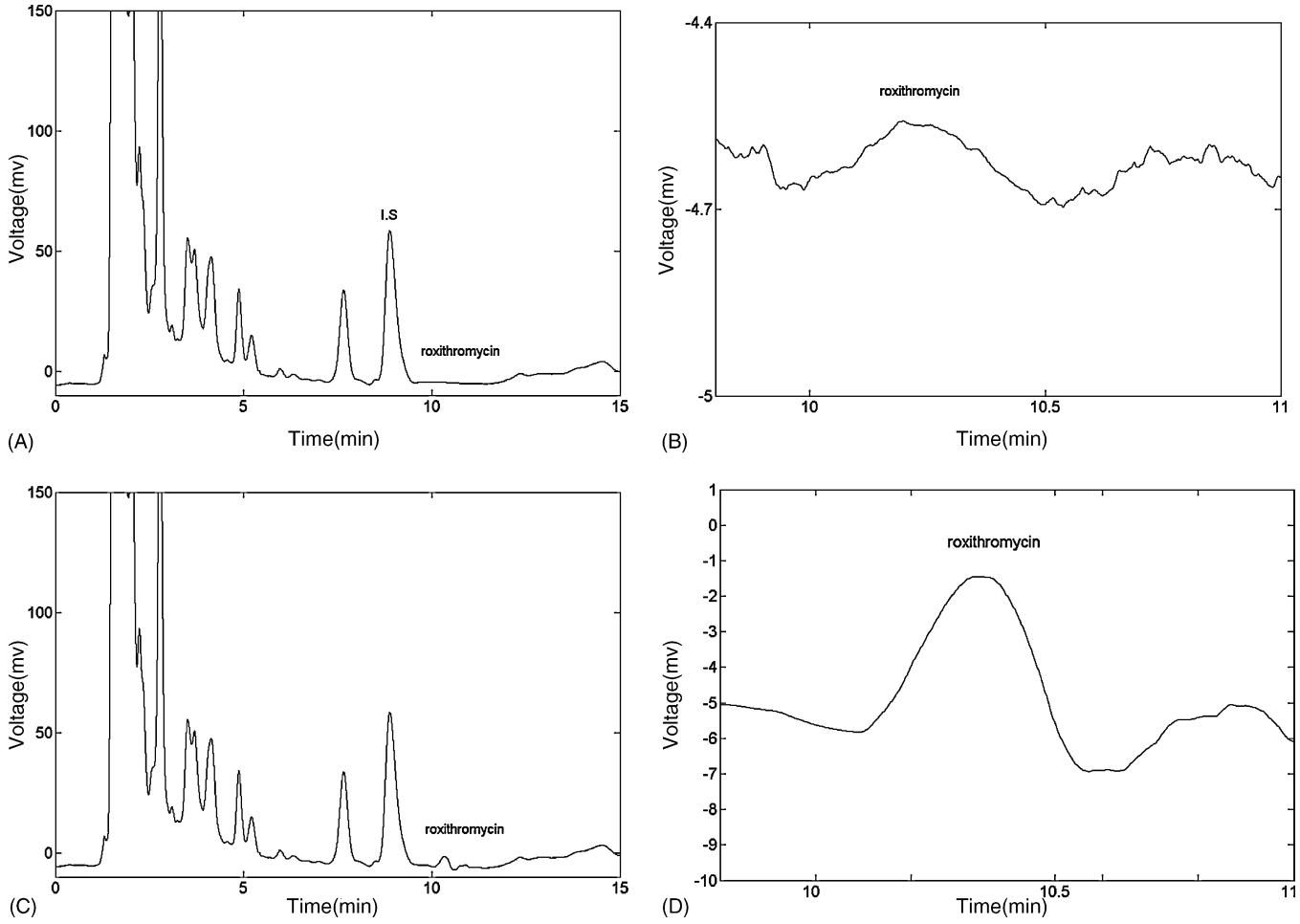

Fig. 4. (A) Chromatogram of the internal standard and roxithromycin at 0.05 μg/ml; (B) especially enlarged chromatogram of roxithromycin at 0.05 μg/ml; (C) chromatogram of roxithromycin at 0.05 μg /ml by SRA with $a = 9.9 \times 10^{-5}$ and $b = 8.0 \times 10^{-6}$; (D) especially enlarged chromatogram of roxithromycin at 0.05 μg/ml by SRA with $a = 9.9 \times 10^{-5}$ and $b = 8.0 \times 10^{-6}$.

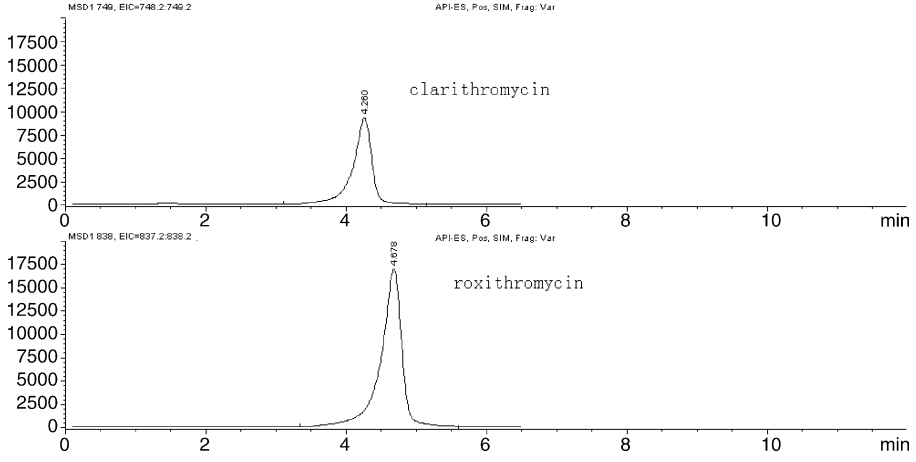

Fig. 5. Chromatogram of roxithromycin at 6.0 μg/ml and the internal standard in plasma obtained by HPLC/MS.

5. Conclusion

The accurate quantitative analysis of roxithromycin in plasma showed that SR could not only improve the quantification limit of instruments but also kept good quantitative linearity between concentration and peak strength. This method gives analysts a completely different way to improve the instrumental detectability. The new method is applied to trace analysis in a biological sample. It can be expected that the proposed SRA may be a promising chemometrics method for trace analysis.

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References

[1] R. Zhu, Q.S. Li, Phys. Lett. A 292 (2001) 58.
[2] F. Chapeau-Blondeau, X. Godivier, Phys. Rev. E 55 (1997) 1478.
[3] L. Gammaitoni, P. Hanggi, P. Jung, F. Marchesoni, Rev. Mod. Phys. 70 (1998) 223.
[4] S. Mitaim, B. Kosko, Proc. IEEE 86 (1998) 2152.
[5] P. Jung, P. Hanggi, Phys. Rev. A 44 (1991) 8032.
[6] P. Jung, P. Hanggi, Europhys. Lett. 8 (1989) 505.
[7] G. Hu, Phys. Rev. A 46 (1992) 3250.
[8] L.Y. Wang, Chem. J. Chin. Univ. 21 (2000) 53.
[9] Z.X. Pan, W.M. Guo, et al., Chemom. Intell. Lab. Syst. 66 (2003) 41.
[10] A. Markham, D. Faulds, Drugs 48 (1994) 297.
[11] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. B 723 (1999) 233.
[12] L.Y. Wang, C.S. Yin, Q. Ren, Chin. J. Anal. Chem. 27 (1999) 1391.
[13] L. Gammaitoni, P. Hanggi, P. Jung, et al., Rev. Mod. Phys. 70 (1998) 223.
[14] H.Y. Song, X.G. Shao, Q.D. Su, Fresenius J. Anal. Chem. 370 (2001) 1087.
[15] B. McNamara, K. Wiesenfeld, Phys. Rev. A 39 (1989) 4854.
[16] A.S. Asdi, A.H. Tewfik, IEEE Trans. Signal Proc. 2 (1995) 1332.