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Direct analysis of *trans*-resveratrol in red wine by high performance liquid chromatography with chemiluminescent detection

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Abstract

A novel high-performance liquid chromatography with chemiluminescence detection has been developed for the determination of *trans*-resveratrol in red wines based on the enhancement by *trans*-resveratrol of the chemiluminescence from luminol–potassium ferricyanide system in alkaline medium. Samples were separated on a C_8 column using isocratic elution of methanol/water (35:65). Quantification of *trans*-resveratrol in Chinese red wines was performed without any sample pretreatment. It allows for the determination of *trans*-resveratrol in the range 0.5–750 μg/L with a detection limit of 0.166 μg/L (S/N = 3). The relative standard deviation (RSD) is 1.16% for 7.5 μg/L *trans*-resveratrol ($n = 11$). *trans*-Resveratrol was detected in Chinese red wines with the recoveries of 92.2–114.7%. Concentrations ranged from 0 to 1.607 mg/L in Chinese red wines.

Keywords: *trans*-Resveratrol; Liquid chromatography; Chemiluminescence; Chinese red wine

1. Introduction

trans-Resveratrol (3,4′,5-trihydroxystilbene) (Fig. 1) is a stilbene produced by plant in response to fungal infection or abiotic stresses such as heavy metal ion or UV light exposure. It occurs in mulberries, peanuts, and grapes as well as in wines. In recent years, it has been discovered that resveratrol has several biological effects, including anticancer activity (Jang et al., 1997), lifespan extension (Howitz et al., 2003), cardioprotection (Hung, Chen, Huang, Lee, & Su, 2000), antioxidant activity (Frankel, Waterhouse, & Kinsella, 1993; Fremont, Belguendou, & Delpal, 1999), inhibition of platelet aggregation (Bertelli et al., 1995) and antiinflammatory activity (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995).

There are increasing interests in resveratrol research owing to its pharmacological activity. A variety of methods have been developed for the quantification of resveratrol in different matrixes such as plant extracts, wine, serum and tissue. High-performance liquid chromatography (HPLC) is the most commonly used procedure with different detection techniques such as UV diode array detection (DAD) (de Lima et al., 1999; Goldberg et al., 1995a; Goldberg et al., 1996; Juan, Lamuela-Raventos, de la Torre-Boronat, & Planas, 1999), electrochemistry (Mcmurtrey, Minn, Pobanz, & Schultz, 1994; Zhu et al., 2000), fluorimetry (Jeandet etal., 1997; Rodriguez-Delgado, Gonzalez, Perez-Trujillo, & Garcia-Montelongo, 2002; Vitrac, Monti, Vercauteren, Deffieux, & Merrillon, 2002) and mass spectrometry (Gamoh & Nakashima, 1999; Wang, Catana, Yang, Roderick, & Van Breemen, 2002). The detections based on electrochemistry, fluorimetry and mass spectrometry can offer higher sensitivity and selectivity than DAD. As an alternative to HPLC based assays, gas chromatography-mass spectrometry (GC-MS) (Goldberg et al., 1994; Luan, Li, & Zhang, 2000; Soleas, Dam, Carey, & Goldberg, 1997a, 1997b) has been used to detect resveratrol. Although GC-MS analysis provides excellent sensitivity

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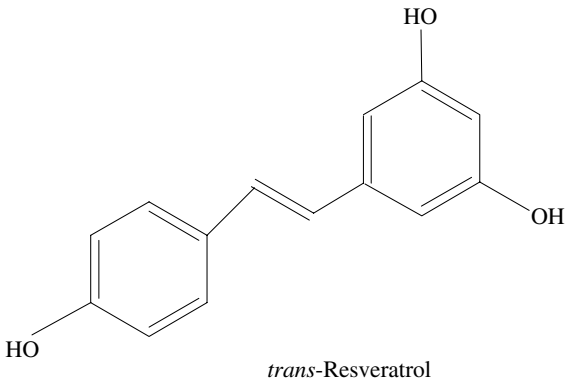

Fig. 1. Chemical structures of *trans*-resveratrol.

and specificity, it usually needs derivatization in order to increase thermal stability and volatility of resveratrol. Furthermore, the high temperature used might cause partial isomerization. Most recently, capillary electrophoresis technique (Arce, Tena, Rios, & Valcarcel, 1998; Brandolini et al., 2002) and laser desorption coupled resonant ionization spectrometry (Orea, Montero, Jimenez, & Urena, 2001) have also been utilized to detect resveratrol.

In recent years, chemiluminescence (CL) has become an attractive detection method for liquid chromatography due to the very low detection limits and wide linear working ranges, which can be obtained with relatively simple instrumentation. However, to our knowledge, there are no reports using chemiluminescence to detect resveratrol in the literatures. In this work, we found that *trans*-resveratrol could enhance strongly the chemiluminescence of luminol–ferricyanide system. A highly sensitive method was developed for the determination of *trans*-resveratrol in red wines by using HPLC coupled with chemiluminescent detection for the first time. Although a number of investigations have focused on the determination of resveratrol in wines from different countries (de Lima et al., 1999; Goldberg et al., 1995b; Romero-Perez, Lamuela-Raventos, Waterhouse, & de la Torre-Boronat, 1996), there is no similar study about the wines produced in China. Accordingly, the *trans*-resveratrol content in 19 Chinese red wines was determined.

2. Materials and methods

2.1. Reagents

Methanol of HPLC gradient grade was purchased from Zhengxing Company (Shanghai, China). Luminol was supplied by Merck (Darmstadt, Germany). $K_3Fe(CN)_6$ was obtained from Wenzhou Chemicals Company (Wenzhou, China). *trans*-Resveratrol was purchased from Oais. Biotech. Inc., (Xi'an, China).

2.2. Standard solutions

A stock solution of *trans*-resveratrol (0.1 mg/mL) was prepared weekly in the mobile phase and stored at 4 °C in the dark. A stock solution of luminol (1×10^{-2} mol/L) was prepared in 0.1 mol/L NaOH without further purification. A stock solution of $K_3Fe(CN)_6$ (1.0×10^{-2} mol/L) was prepared by dissolving 0.3293 g $K_3Fe(CN)_6$ in 100 mL redistilled water.

2.3. Apparatus

The schematic diagram shown in Fig. 2 illustrated HPLC-CL detection system used in our experiments. The HPLC system was Agilent 1100 series (Agilent Technologies, USA), including a binary pump, a thermostat column compartment, a diode array detector (DAD) and a manual sample injection valve with a 100 μL loop. Chromatographic separation was carried out using a Zorbax Eclipse XDB-C_8 (150×4.6 mm i.d., 5 μm) column and the column temperature was set at 25 °C. Data collection and analysis were performed using Agilent Chemstation (version A.08.03). The CL detection was conducted on a IFFM-D flow injection chemiluminescence (FIA-CL) system consisted of a model peristaltic pump, a mixing tee and a model IFFM-D CL detector equipped with a glass coil (used as reaction coil and detection cell) and a photomultiplier. The data from the CL detector was acquired by Agilent Interface 35900E and processed by Agilent Chemstation (version A.08.03). The connection between DAD and the mixing tee was carried out via a PEEK tube (600×0.25 mm i.d., Agilent technologies).

2.4. Sample preparation

Commercial Chinese red wines (Table 1) were analyzed from the following varieties: Greatwall, Dynasty, Suntime, Aili, Changyu, Baiyanghe, Gujing, Huatai, Xuelanshan, Malina. All wines were stored in the dark at 4 °C and analyzed immediately after bottle opening. Wine samples were filtered through 0.22 μm membrane filter (Xinya, Shanghai, China). The filtrated solutions were injected directly for the DAD detection. A 0.05 mL aliquot of the filtered wine samples was diluted to 5.0 mL with mobile phase and injected directly into HPLC without further treatment for the CL detection.

2.5. HPLC-CL analysis

The HPLC separation was carried out by isocratic elution with a mobile phase of methanol/water (35:65) at a flow rate of 1.0 mL/min. Detection by diode array was performed at 306 nm. The UV spectra were recorded between 250 and 400 nm for the identification of *trans*-resveratrol in wine samples and for the test of peak

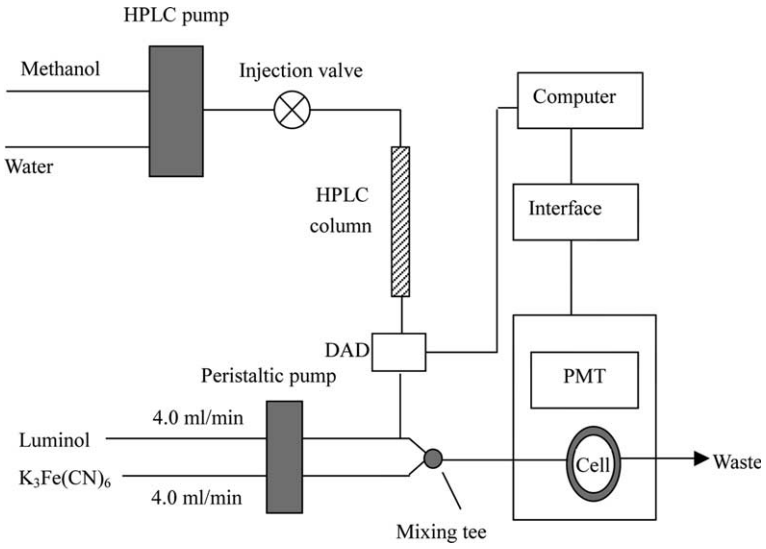

Fig. 2. Schematic diagram of HPLC-CL system.

purity. The column effluent from DAD was first mixed with luminol solution at a mixing tee via a PEEK tube, then combined with $K_3Fe(CN)_6$ solution. The CL reagents were pumped into the flow cell at 4.0 mL/min, respectively. The light emission was monitored by the photomultipler tube. For both detection, each sample was injected in triplicate and the height of peak was used for quantitative determination.

3. Results and discussion

3.1. CL enhancement of trans-resveratrol

In basic media, luminol can react with ferricyanide to produce CL. *trans*-Resveratrol was found to enhance the CL intensity. Fig. 3(A) showed the kinetic characteristics of the enhanced CL in a flow injection system. After the injection of *trans*-resveratrol, the CL intensity of luminol–ferricyanide system increased quickly. The maximum response was obtained at 4 s after the mixing of the reagents and the emission ended at 22 s, which was important for design of the connecting tube of the CL detector so that maximum of the CL kinetic curves could pass through the detector window. For *cis*-resveratrol, the enhancing signal was much lower than that of *trans*-resveratrol. Therefore, the present work is focused on *trans*-resveratrol.

It is well known that 3-aminophthalate (3-APA) is the luminophor of luminol CL system, the CL spectra in the presence and absence of *trans*-resveratrol shown in Fig. 3(B) demonstrated that the luminophor of luminol–ferricyanide–*trans*-resveratrol was still 3-APA. Diaz and Garcia (1994) observed the enhancement by protocatechuic acid and caffeic acid of luminol–potassium ferricyanide CL. They proposed that the enhancers reacted with potassium ferricyanide to produce enhancer radical anion, followed by the reaction with O_2 to generate $O_2^{\cdot -}$, accelerating the CL reaction. We believed that the CL enhancement of *trans*-resveratrol might follow similar pathways.

3.2. Optimization of chromatographic separation

The mobile phase for the chromatographic separation of *trans*-resveratrol, also compatible with the CL reaction, was investigated. Gradient elution and acidic mobile phase were applied in most methods reported to separate *trans*-resveratrol in wines (Goldberg et al., 1996; Rodriguez-Delgado et al., 2002; Vitrac et al., 2002; Wang et al., 2002). However, these separation conditions were not compatible with the CL detection. The gradient elution led to CL baseline drift that made quantification imprecisely. The acids in mobile phase would react with Na_2CO_3–$NaHCO_3$ buffer solution to generate bubble, which would result in lower sensitivity and worse reproducibility of the CL detection. The mobile phase containing methanol and water was found to be suitable for the HPLC-CL detection. Moreover, *trans*-resveratrol could not be separated from other compounds with the methanol concentration higher than 35%, whereas, lower concentrations led to longer analytical time. Therefore, the mobile phase of 35% methanol was chosen for the separation.

3.3. Optimization of the CL reaction conditions

The factors influencing the CL reaction such as the pH of luminol solution, the concentrations of luminol and potassium ferricyanide, and flow rate of solutions were studied in order to obtain maximal enhanced CL intensity.

Table 1
Concentration[a] of *trans*-resveratrol in Chinese red wines

Appellation	Variety	Area	HPLC-DAD[b] (mg/L)				HPLC-CL (mg/L)			
			Original[c]	Added	Found[c]	Recovery (%)	Original[c]	Added	Found[c]	Recovery (%)
Dried	Greatwall	Hebei	1.528 ± 0.031	0.500	2.015 ± 0.086	97.4	1.607 ± 0.019	1.000	2.591 ± 0.051	98.4
Dried	Dynasty	Tianjin	1.180 ± 0.031	0.500	1.657 ± 0.034	95.2	0.792 ± 0.004	1.000	1.844 ± 0.032	105.2
Suntime	Suntime	Xinjiang	0.164 ± 0.004	0.500	0.651 ± 0.071	97.4	0.160 ± 0.004	1.000	1.243 ± 0.019	108.3
Rose	Aili	Xinjiang	ND	0.500	0.519 ± 0.007	103.8	0.075 ± 0.002	1.000	0.997 ± 0.021	92.2
Full juice	Changyu	Yantai	0.329 ± 0.004	0.500	0.805 ± 0.048	95.2	0.283 ± 0.007	1.000	1.355 ± 0.023	107.2
Chefoo	Changyu	Yantai	0.737 ± 0.004	0.500	1.257 ± 0.055	104.0	0.756 ± 0.009	1.000	1.839 ± 0.027	108.3
Natural	Changyu	Yantai	0.447 ± 0.007	0.500	0.912 ± 0.028	93.0	0.415 ± 0.010	1.000	1.370 ± 0.010	95.5
Yuanjiang	Baiyanghe	Yantai	0.203 ± 0.002	0.500	0.659 ± 0.025	91.2	0.213 ± 0.005	1.000	1.201 ± 0.021	98.8
Love	Baiyanghe	Yantai	0.241 ± 0.003	0.500	0.728 ± 0.012	97.4	0.256 ± 0.007	1.000	1.242 ± 0.026	98.6
Yantai	Baiyanghe	Yantai	0.466 ± 0.013	0.500	1.016 ± 0.045	110.0	0.474 ± 0.008	1.000	1.507 ± 0.015	103.3
Wild	Baiyanghe	Yantai	ND	0.500	0.535 ± 0.013	107.0	0.072 ± 0.002	1.000	1.148 ± 0.023	107.6
Full juice	Gujing	Anhui	0.434 ± 0.001	0.500	0.940 ± 0.034	101.2	0.452 ± 0.010	1.000	1.569 ± 0.030	111.7
Gudaohong	Gujing	Anhui	0.349 ± 0.009	0.500	0.819 ± 0.021	94.0	0.367 ± 0.008	1.000	1.388 ± 0.035	102.1
Nature	Gujing	Anhui	0.202 ± 0.004	0.500	0.731 ± 0.021	105.8	0.197 ± 0.003	1.000	1.286 ± 0.024	108.9
Shuangxi	Gujing	Anhui	0.138 ± 0.002	0.500	0.635 ± 0.018	99.4	0.123 ± 0.003	1.000	1.092 ± 0.016	109.2
Shan	Gujing	Anhui	ND	0.500	0.473 ± 0.008	94.6	ND	1.000	1.055 ± 0.005	105.5
Nature	Huatai	Jilin	ND	0.500	0.544 ± 0.012	108.8	ND	1.000	0.982 ± 0.012	98.2
Full juice	Xuelanshan	Jilin	ND	0.500	0.538 ± 0.009	107.6	ND	1.000	1.107 ± 0.029	110.7
Shuangkou	Malina	Qingdao	ND	0.500	0.489 ± 0.010	97.8	ND	1.000	1.147 ± 0.007	114.7
Average			0.338		0.497	99.4	0.329		1.044	104.4

ND, not detected.
[a] Original concentration.
[b] Wavelength = 306 nm.
[c] Mean value ± SD ($n = 3$).

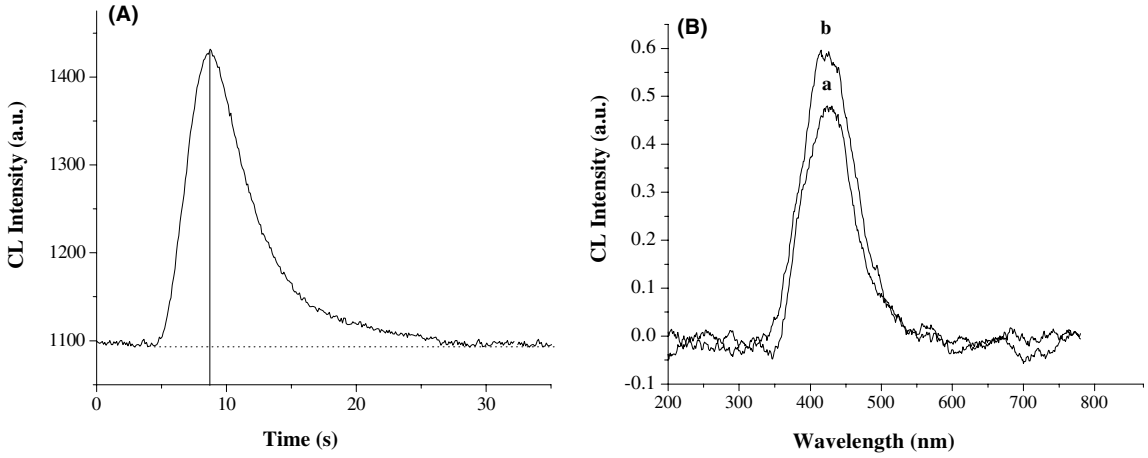

Fig. 3. Kinetic curves and CL spectra of *trans*-resveratrol-enhanced luminol–potassium ferricyanide CL system. (A) Kinetic curves of the CL system. Reaction condition: luminol, 1×10^{-4} mol/L; potassium ferricyanide, 1.0×10^{-4} mol/L. Flow rate: 4.0 mL/min, *trans*-resveratrol, 10 μg/L. (B) CL spectra of the CL system. Reaction condition: luminol, 1×10^{-3} mol/L; potassium ferricyanide, 5×10^{-4} mol/L. *trans*-resveratrol: 100 ng/mL. (a) luminol–potassium ferricyanide, (b) luminol–potassium ferricyanide–*trans*-resveratrol.

It is well known that the CL of luminol–ferricyanide system can only be produced in alkaline media. Na_2CO_3–$NaHCO_3$ and NaOH media were studied and the CL intensity was stronger in Na_2CO_3–$NaHCO_3$ medium than that in NaOH medium. Thus, Na_2CO_3–$NaHCO_3$ was chosen in this research. The effect of the pH on the CL intensity was studied ranging from 9.0 to 11.0 and the maximum enhanced CL intensity could be reached at pH = 10.0. Thus, a Na_2CO_3–$NaHCO_3$ buffer solution with pH 10.0 value was considered to be optimal.

The effect of the concentration of luminol solution was evaluated from 1×10^{-5}–1×10^{-3} mol/L. Increasing the luminol concentration (1×10^{-5}–110^{-4} mol/L) resulted in an increase in CL intensity and the luminol concentrations higher than 1×10^{-4} mol/L caused a decrease in CL intensity. 1×10^{-4} mol/L of luminol was selected as optimum.

The effect of potassium ferricyanide concentration on the enhanced CL intensity was investigated in the range 1×10^{-5}–1×10^{-3} mol/L. With the increase of concentration of potassium ferricyanide in the range 1×10^{-5}–1×10^{-4} mol/L, the enhanced CL intensity increased sharply and then decreased dramatically beyond 1×10^{-4} mol/L. 1.0×10^{-4} mol/L of potassium ferricyanide was selected as optimum for the maximum CL intensity.

The effect of flow rate (2.6–5.0 mL/min) on CL reaction was investigated. The CL intensity increased with the increase of the flow rate in the range 2.6–4.0 mL/min, but the flow rate higher than 4.0 mL/min caused lower CL intensity, higher pressure in the connection and excessive consumption of reagents. Therefore, 4.0 mL/min of flow rate was chosen in the work.

Under the optimal conditions described above, the chromatograms of *trans*-resveratrol in standard solution using DAD and CL detection were shown in Fig. 4(A) and (C), respectively. The retention time of *trans*-resveratrol was less than 12 min.

3.4. Method validation

Linearity, detection limit, precision, selectivity, and recovery were established to evaluate the method performance.

The calibration curves established for wines spiked with seven concentrations of *trans*-resveratrol varying 75–10,000 μg/L for DAD detection and 0.5–750 μg/L of *trans*-resveratrol for CL detection, respectively. Good linearity was obtained over more than 2 orders of magnitude for both detections. The regression equations of DAD and CL detection were $\mathrm{Log}H = -2.695 + 1.192\,\mathrm{Log}C$ ($r = 0.9997$) and $\mathrm{Log}H = 2.412 + 0.798\,\mathrm{Log}C$ ($r = 0.9978$), respectively. The limits of detections (LODs) (S/N = 3) were 25 and 0.166 μg/L for DAD (306 nm) and CL detection, respectively. The LOD of CL detection was 1–2 orders of magnitude lower than those methods in literatures (Arce et al., 1998; Gamoh & Nakashima, 1999; Goldberg et al., 1996; Soleas et al., 1997b; Vitrac et al., 2002; Zhu et al., 2000) and present HPLC-DAD. The detail data were listed in Table 2.

The precisions were determined by performing 11 replicate analysis of the center point of the calibration curves for both detections. The relative standard deviations (RSD) for the determination of 750 μg/L *trans*-resveratrol with DAD and for the determination of 7.5 μg/L *trans*-resveratrol with CL detection were 1.45% and 1.16%, respectively.

The identification of *trans*-resveratrol in red wines was carried out by retention time and UV spectra. The chromatogram at 306 nm (Fig. 4(B)) indicated that the *trans*-resveratrol was well resolved from interference

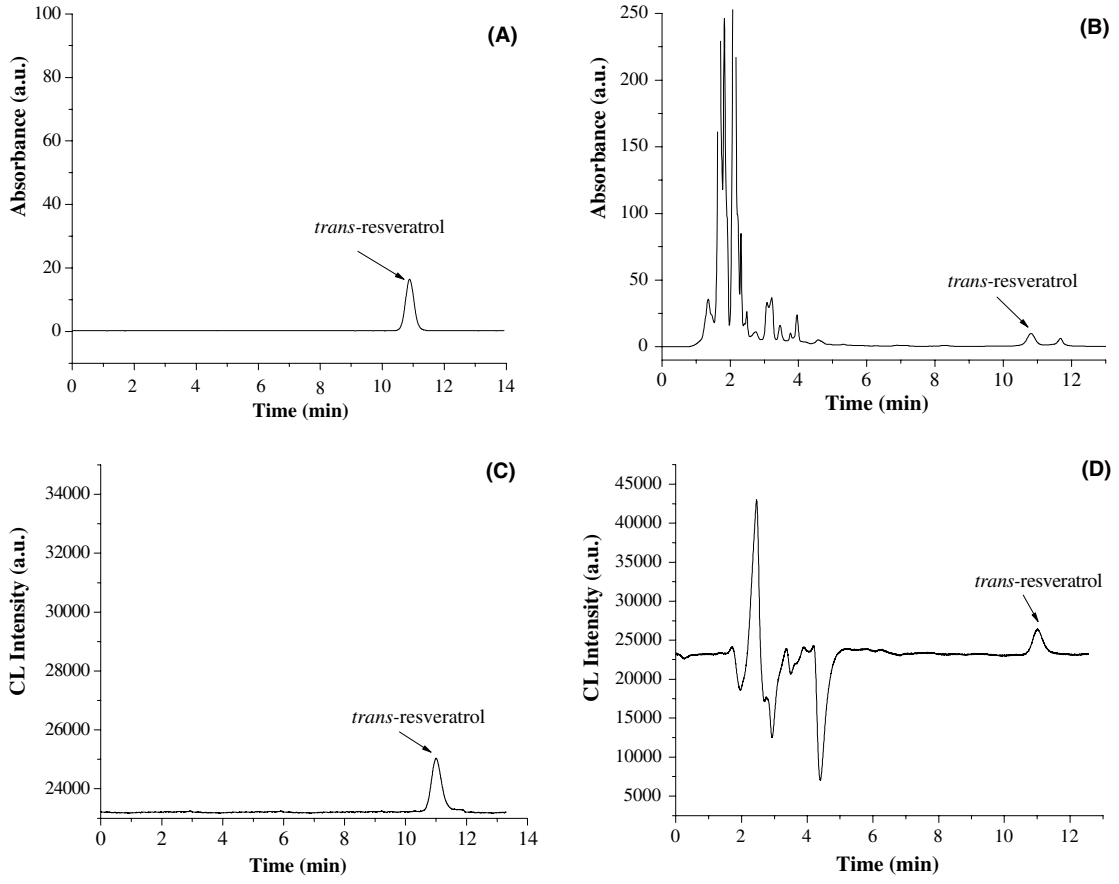

Fig. 4. Chromatograms of *trans*-resveratrol with DAD at 306 nm and CL detection. (A) Chromatogram of standard solution by DAD detection. *trans*-Resveratrol, 1000 μg/L. (B) Chromatogram of Dynasty dried red wine by DAD detection. (C) Chromatogram of standard solution by CL detection. *Trans*-resveratrol: 10 μg/L. (D) Chromatogram of Dynasty dried red wine (diluted 100 times) by CL detection.

Table 2
Detection of *trans*-resveratrol with different methods

Method	Linear range (μg/L)	Detection limit (μg/L)	References
This HPLC-DAD method	75–10,000	25	
This HPLC-CL method	0.5–750	0.166	
HPLC-DAD	400–8500	30	Goldberg et al. (1996)
HPLC-electrochemistry	5–1000	2	Zhu et al. (2000)
HPLC-fluorimetry	500–10,000	10	Vitrac et al. (2002)
HPLC-MS	40–8000	20	Gamoh and Nakashima (1999)
GC-MS	84–33,400	84	Soleas et al. (1997a)
CE-DAD	500–100,000	50	Arce et al. (1998)

peaks. For CL detection, there was not interference by matrix (Fig. 4(D)). The comparison between the UV spectra of standard *trans*-resveratrol and the corresponding peak showed the match factor 999.475.

The accuracy of the method was tested by determining the recovery of *trans*-resveratrol spiked to the wine samples. As shown in Table 1, the recoveries of wines varied from 91.2 to 110.0 for DAD detection and from 92.2 to 114.7 for CL detection, respectively, and the relative standard deviations of each wine were less than 3% for both detections. Thus, the recoveries for both methods in Table 1 were acceptable. Since the recoveries fluctuated around 100%, the error of the determination arose from random error and systematic error.

Comparison between the two methods was carried out with a paired *t*-test and *p* was 0.44.

3.5. Wine sample analyses

Nineteen different Chinese red wines were analyzed for the concentration of *trans*-resveratrol by DAD and CL detection. The results were summarized in Table 1.

Concentrations of *trans*-resveratrol varied from 0 to 1.528 mg/L for DAD detection with a mean value of 0.338 mg/L. As for CL detection, concentrations of *trans*-resveratrol were in the range 0–1.607 mg/L with a mean value of 0.329 mg/L. From data in Table 1, the results from the CL detection were comparable with those from DAD. Furthermore, lower concentrations of *trans*-resveratrol in wines could be detected not by DAD but by the CL detector.

The mean concentration found in these red wines was higher than 0.135 mg/L in Spain (Romero-Perez et al., 1996), 0.132 mg/L in California (Lamuela-Raventos & Waterhouse, 1993), 0.157 mg/L in Japan (Okuda & Yokotsuka, 1993), but lower than 0.77 mg/L in Canada (Soleas et al., 1995), 0.998 mg/L in California (Juan et al., 1999), 1.00 mg/L in Portugal (de Lima et al., 1999), 1.21 mg/L in Chile/Argentina (Goldberg et al., 1995b) and 2.46 mg/L in California (Mcmurtrey et al., 1994). These differences could be attributed to environmental conditions, such as humidity and fungal disease, which are factors influencing the production of *trans*-resveratrol by grapevines (Goldberg et al., 1995b). However, Soleas et al. (1997c) stated that the difference between the statistical parameters, sample pretreatment (direct injection or not) and the chromatographic method (GC or HPLC) might cause different result. For example, wines from California presented mean values varying from 0.132 to 2.46 mg/L.

4. Conclusion

A novel HPLC-CL method for the quantitative analysis of *trans*-resveratrol has been developed, validated and applied to the analysis of red wines. The method is sensitive, selective, simple, rapid and reliable. It allows us for the determination of *trans*-resveratrol in wines by direct injection without sample pretreatment. Additionally, the levels of *trans*-resveratrol of Chinese red wines from different varieties were examined for the first time. Furthermore, this assay is a promising tool for the analysis of biological fluids and further work is under investigation including the analysis of other piceid components.

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References

Arce, L., Tena, M. T., Rios, A., & Valcarcel, M. (1998). Determination of *trans*-resveratrol and other polyphenols in wines by a continuous flow sample clean-up system followed by capillary electrophoresis separation. *Analytica Chimica Acta, 359*, 27–38.

Bertelli, A. A. E., Giovannini, L., Gianness, D., Migliori, M., Bernini, W., Fregoni, M., & Bertelli, A. (1995). Antiplatelet activity of synthetic and natural resveratrol in red wine. *International Journal of Tissue Reactions, 17*, 1–3.

Brandolini, V., Maietti, A., Tedeschi, P., Durini, E., Vertuani, S., & Manfredini, S. (2002). Capillary electrophoresis determination, synthesis, and stability of resveratrol and related 3-O-â-D-glucopyranoisides. *Journal of Agriculture and Food Chemistry, 50*, 7407–7411.

de Lima, M. T. R., Waffo-Teguo, P., Teissedre, P. L., Pujolas, A., Vercauteren, J., Cabanis, J. C., & Merillon, J. M. (1999). Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid, and *cis*- and *trans*-resveratrol) in Portuguese wines. *Journal of Agriculture and Food Chemistry, 47*, 2666–2670.

Diaz, A. N., & Garcia, J. A. G. (1994). Nonlinear Multicomponent kinetic analysis for the simultaneous stopped-flow determination of chemiluminescence enhancers. *Analytical Chemistry, 66*, 988–993.

Frankel, E. N., Waterhouse, A. L., & Kinsella, J. E. (1993). Inhibition of human LDL oxidation by resveratrol. *Lancet, 341*, 1103–1104.

Fremont, L., Belguendou, L., & Delpal, S. (1999). Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sciences, 64*, 2511–2521.

Gamoh, K., & Nakashima, K. (1999). Liquid chromatography mass spectrometric determination of *trans*-resveratrol in wine using a tandem solid-phase extraction method. *Rapid Communications in Mass Spectrometry, 13*, 1112–1115.

Goldberg, D. M., Ng, E., Karumanchiri, A., Yan, J., Diamandis, E. P., & Soleas, G. J. (1995a). Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography. *Journal of Chromatography A, 708*, 89–98.

Goldberg, D. M., Tsang, E., Karumanchiri, A., Diamandis, E. P., Soleas, G., & Ng, E. (1996). Method to assay the concentrations of phenolic constituents of biological interest in wines. *Analytical Chemistry, 68*, 1688–1694.

Goldberg, D. M., Yan, J., Ng, E., Diamandis, E. P., Karumanchiri, A., Soleas, G., & Waterhouse, A. L. (1994). Direct injection gas chromatographic mass spectrometric assay for *trans*-resveratrol. *Analytical Chemistry, 66*, 3959–3963.

Goldberg, D. M., Yan, J., Ng, E., Diamands, E. P., Soleas, G. J., & Waterhouse, A. L. (1995b). A global survey of *trans*-resveratrol concerntrations in commercial wines. *American Journal of Enology Viticulture, 46*, 159–165.

Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L. L., Scherer, B., & Sinclair, D. A. (2003). Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature, 425*, 191–196.

Hung, L. M., Chen, J. K., Huang, S. S., Lee, R. S., & Su, M. J. (2000). Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovascular Research, 47*, 549–555.

Jang, M. S., Cai, E. N., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., & Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science, 275*, 218–220.

Jeandet, P., Breuil, A. C., Adriun, M., Weston, L. A., Debord, S., Meunier, P., Maume, G., & Bessis, R. (1997). HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. *Analytical Chemistry, 69*, 5172–5177.

Juan, M. E., Lamuela-Raventos, R. M., de la Torre-Boronat, M. C., & Planas, J. M. (1999). Determination of *trans*-resveratrol in plasma by HPLC. *Analytical Chemistry, 71*, 747–750.

Lamuela-Raventos, R. M., & Waterhouse, A. L. (1993). Occurrence of resveratrol in selected California wines by a new HPLC method. *Journal of Agriculture and Food Chemistry, 41*, 521–523.

Luan, T. G., Li, G. K., & Zhang, Z. X. (2000). Gas-phase postderivatization following solid-phase microextraction for the rapid determination of *trans*-resveratrol in wine by gas chromatography-mass spectrometry. *Analytica Chimica Acta, 424*, 19–25.

Mcmurtrey, K. D., Minn, J., Pobanz, K., & Schultz, T. P. (1994). Analysis of wines for resveratrol using direct injection high-performance liquid chromatography with electrochemical detection. *Journal of Agriculture and Food Chemistry, 42*, 2077–2080.

Okuda, T., & Yokotsuka, K. (1993). *Trans*-resveratrol concentration in berry skins and wines from grapes grown in Japan. *American Journal of Enology Viticulture, 47*(1), 93–99.

Orea, J. M., Montero, C., Jimenez, J. B., & Urena, A. G. (2001). Analysis of *trans*-resveratrol by laser desorption coupled with resonant ionization spectrometry. Application to *trans*-resveratrol content in vine leaves and grape skin. *Analytical Chemistry, 73*, 5921–5927.

Pace-Asciak, C. R., Hahn, S. E., Diamandis, E. P., Soleas, G., & Goldberg, D. M. (1995). The red wine phenolics *trans*-resveratrol and quercetin block human platelet-aggregation and eicosanoid synthesis – implications for protection against coronary heart-disease. *Clinica Chimica Acta, 235*, 207–219.

Rodriguez-Delgado, M. A., Gonzalez, G., Perez-Trujillo, J. P., & Garcia-Montelongo, F. J. (2002). *Trans*-resveratrol in wines from the Canary Islands (Spain). An analysis by high-performance liquid chromatography. *Food Chemistry, 76*, 371–375.

Romero-Perez, A. I., Lamuela-Raventos, R. M., Waterhouse, A. L., & de la Torre-Boronat, M.C. (1996). Levels of *cis* and *trans*-resveratrol and their glucosides in white and rose *vitis vinifera* wines from Spain. *Journal of Agriculture and Food Chemistry, 44*, 2124–2128.

Soleas, G. J., Dam, J., Carey, M., & Goldberg, D. M. (1997a). Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. *Journal of Agriculture and Food Chemistry, 45*, 3871–3880.

Soleas, G. J., Diamandis, E. P., Karumanchiri, A., & Goldberg, D. M. (1997b). A multiresidue derivatization gas chromatographic assay for fifteen phenolic constituents with mass selective detection. *Analytical Chemistry, 69*, 4405–4409.

Soleas, G. J., Goldberg, D. M., Ng, E., Karumanchiri, A., Tsang, E., & Diamandis, E. P. (1997c). Comparative evaluation of four methods for assay of *cis*- and *trans*-resveratrol. *American Journal of Enology Viticulture, 48*, 169–176.

Soleas, G., Goldberg, D. M., Diamandis, E. P., Karumanchiri, A., Yan, J., & Ng, E. (1995). A derivatized gas chromatographic-mass spectrometric method for the analysis of both isomers of resveratrol in juice and wines. *American Journal of Enology Viticulture, 46*, 346–352.

Vitrac, X., Monti, J. P., Vercauteren, J., Deffieux, G. J., & Merrillon, M. (2002). Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. *Analytica Chimica Acta, 458*, 103–110.

Wang, Y., Catana, F., Yang, Y. N., Roderick, R., & Van Breemen, R. B. (2002). An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. *Journal of Agriculture and Food Chemistry, 50*, 431–435.

Zhu, Y. X., Huang, T. H., Gregor, M., Long, H., Kissinger, C. B., & Kissinger, P. T. (2000). Liquid chromatography with electrochemical detection for the determination of *trans*-resveratrol in rat blood utilizing an automated blood sampling device. *Journal of Chromatography B, 740*, 129–133.