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# Determination of *trans*-Resveratrol in wines, herbs and health food by capillary electrophoresis with electrochemical detection

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**Abstract**

A method based on capillary electrophoresis with electrochemical detection (CE–ED) was developed for the determination of *trans*-Resveratrol in wines, Chinese medicinal herb *Polygonum cuspidatum* Sied. et Zucc., and Zijin capsule. The effects of some important factors such as injection time, and applied potential to working electrode were investigated. Operated in a wall-jet configuration, a 300 μm diameter carbon-disk electrode was used as the working electrode, which exhibits good response at +0.85 V (vs. SCE) for *trans*-Resveratrol. Linearity was obtained in the concentration range from $1.0\times10^{-4}$ to $5.0\times10^{-7}$ g/ml. The detection limit (S/N = 3) was $5.96\times10^{-8}$ g/ml. This proposed method has been successfully applied to analyze several actual samples with satisfactory assay results. 

*Keywords:* Capillary electrophoresis; Electrochemical detection; *trans*-Resveratrol; Wine; *Polygonum cuspidatum* Sied. et Zucc.; Zijin capsule

## 1. Introduction

It is well known that the phenolic compound *trans*-Resveratrol (*trans*-3,5,4′-trihydroxystilbene; Fig. 1) is a phytoalexin, a class of antibiotic compounds (Celotti, Ferrarini, Zironi, & Conte, 1996). Over the past decade, resveratrol has gained much attention, as it was proven to have cancer chemopreventive activity in assays representing three major stages of carcinogenesis (Jang et al., 1997). This compound was found in some grapes and many other plants such as eucalyptus, spruce, and lily. A number of investigations on the determination of resveratrol in grape-derived products such as wine have been conducted. However, a Chinese medicinal herb known as *polygonum cuspidatum* Sied. et Zucc. has not drawn much attentions from analytical chemists, despite the fact that it contains hundreds of times more resveratrol than wines. *Polygonum cuspidtum* sieb. et Zucc, widely distributed in China, belongs to the family Polygonaceae. It is also called Huzhang, Zi Jinlong, Banzhang, etc. in China and has long been used for treatment of scalds and burns. Recently, a health food Zijin capsule with trade name of Viogaron appeared on the Chinese market, its primary ingredient is *trans*-Resveratrol. Therefore, the analysis of *trans*-Resveratrol in wines, herb and health food is of primary importance.

For the analysis of resveratrol in wines, there have been several analytical methods including high performance liquid chromatography (HPLC; Adrian, Jeandet, Breuil, Levite, Debord, & Bessis, 2000; Domínguez, Guillén, & Barroso, 2001; Dourtoglou, Makris, Bois-Dounas, & Zonas, 1999; Martinez-Ortega, Garcia-Parrilla, & Troncoso, 2000), gas chromatography (Jeandet, Bessis, Maume, & Sbaghi, 1993), and simple oscillographic voltammetry (Zhang, Zhang, Zheng, & Gao, 2001). Capillary zone electrophoresis (CZE) with UV detection (Berzas Nevado, Contento Salcedo & Castañeda Peñalvo, 1999; Gu, Chu, O'Dwyer, & Zeece, 2000) has also been employed for this purpose. For the analysis of resveratrol in Chinese medicinal herb *Polygonum cuspidatum* Sied. et Zucc., only a few reports can be found, including the HPLC approach (Zhu, 2001) and the high-speed counter-current chromatography method (Chen, Yang, Zhang, Han, & Chen, 2000).

HPLC, regarded as a prime separation method, has some apparent shortcomings in the analysis of traditional Chinese medicines, including long analysis time, low resolution and short lifetime of columns owing to numerous co-existent interfering compounds, some of which can be absorbed strongly by column packing, resulting in fast column degradation. Capillary electrophoresis (CE) is becoming increasingly recognized as an

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important analytical separation technique for its speed, efficiency, reproducibility, ultra-small sample volume, and little consumption of solvent. In addition, with electrochemical detection (ED), CE–ED offers high sensitivity and good selectivity for electroactive analytes.

In this work, an alternative method for *trans*-Resveratrol determination in some real samples (wines, medicinal herb and health food) by using CE–ED approach is described, which has been proven to be simple and convenient, as well as sensitive and selective.

## 2. Experimental

### 2.1. Apparatus

The laboratory-built CE–ED system has been constructed and described previously. (Ye, Jin, Zhao, & Fang, 1998). A ±30 kV high-voltage d.c. power supply (Shanghai Institute of Nuclear Research, China) provided separation voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and outlet end of capillary was maintained at ground. The separation was proceeded in a 65 cm length of 25-μm i.d. and 360-μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA). In order to prevent operator from the high voltage and assure the safety of the CE–ED system, the entire capillary, the buffer reservoir for CE, and all electrodes were enclosed in a Plexiglas box with a safety switch wired to turn off the power supply whenever the box was opened (Ye & Baldwin, 1993, 1994).

A carbon-disk electrode with 300-μm diameter was employed as the working electrode as described previously (Fang, Ye, & Fang, 1996). Before the use, the surface of the carbon-disk electrode was polished with emery sandpaper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel Corp. (Stratford, CT, USA) Model 14901 micropositioner. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode and a SCE reference electrode was used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN, USA). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument factory, China). Samples were all injected electrokinetically, applying 10 kV for 6 s.

### 2.2. Reagents

*trans*-Resveratrol was purchased from Sigma (St. Louis, MO, USA). Stock solution of *trans*-Resveratrol ($2.00\times10^{-4}$ g/ml) was prepared in anhydrous ethanol (A.R. grade), stored in the dark and below 0 °C, and was diluted to the desired concentrations with the running buffer (100 mmol/l $H_3BO_3$–$Na_2B_4O_7$ buffer, pH = 9.24), in this solution carbon working electrode shows excellent response to phenolic compounds (Cao, Zhang, Fang, & Ye, 2001). Before use, all solutions were filtered through 0.22-μm nylon filters.

### 2.3. Sample preparation

Five wine samples were Huaxia Great Wall dry red wine, Merlot France dry red wine, Zhangyu dry red

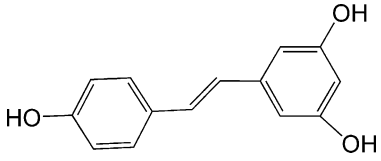


Fig. 1. Structure of *trans*-Resveratrol.

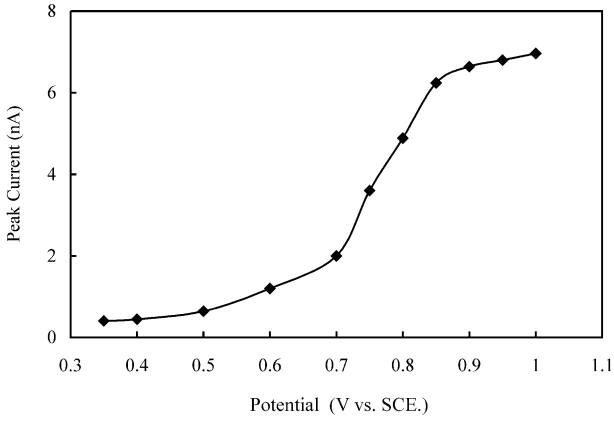


Fig. 2. Hydrodynamic voltammogram (HDV) of *trans*-Resveratrol. Fused-silica capillary: 25 μm i.d.×65 cm; working electrode: 300-μm diameter copper disk electrode; electrophoresis medium: 100 mmol/l borate buffer (pH = 9.24); separation voltage: 10 kV; injection: 10 kV/6s; *trans*-Resveratrol concentration: $2\times10^{-5}$ g/ml.

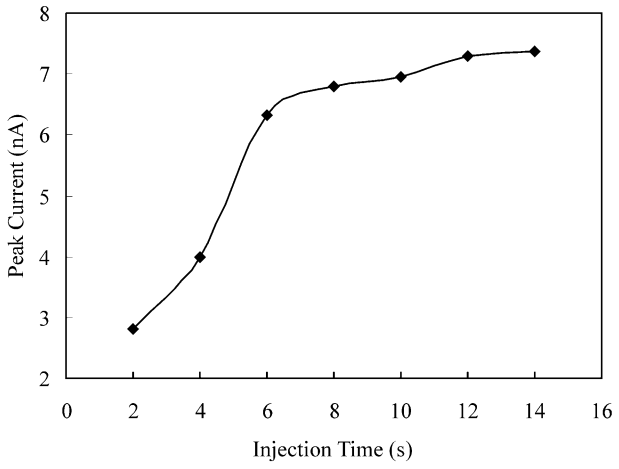


Fig. 3. Effect of the injection time on *trans*-Resveratrol peak current. Working electrode potential is 0.85 V (vs. SCE). Other conditions are the same as in Fig. 2.

wine, Dynasty dry red wine, and Dynasty medium dry white wine, respectively. All the wine samples were directly filtered through 0.22-μm nylon filters, 1.5 ml filtrate was then diluted with running buffer to 2 ml in volume. Chinese medicinal herb *Polygonum cuspidatum* Sied. et Zucc.was purchased from a drug store in Shanghai (China). Zijin capsules (Trade name: Viograron) were obtained from Xi'an Tiancheng Drugs & Bio-Engineering Co., Ltd. (Xi'an, China). One gram of dried *Polygonum cuspidatum* Sied. et Zucc. and 3.3 grams of Zijin capsules (10 capsules) were ground into powder and accurately weighed. Each weighed sample (0.1715 g *Polygonum cuspidatum* Sied. et Zucc. powder, 0.0175 g Zijin capsule powder) was extracted with 25 ml anhydrous ethanol (A.R. grade) and the running buffer (1:1) for 40 minutes in an ultrasonic bath. Then each of the samples was filtered through filter paper first, then through a 0.22-μm syringe filter, and made up to 10 ml in volume. Sample solution was stored in the dark.

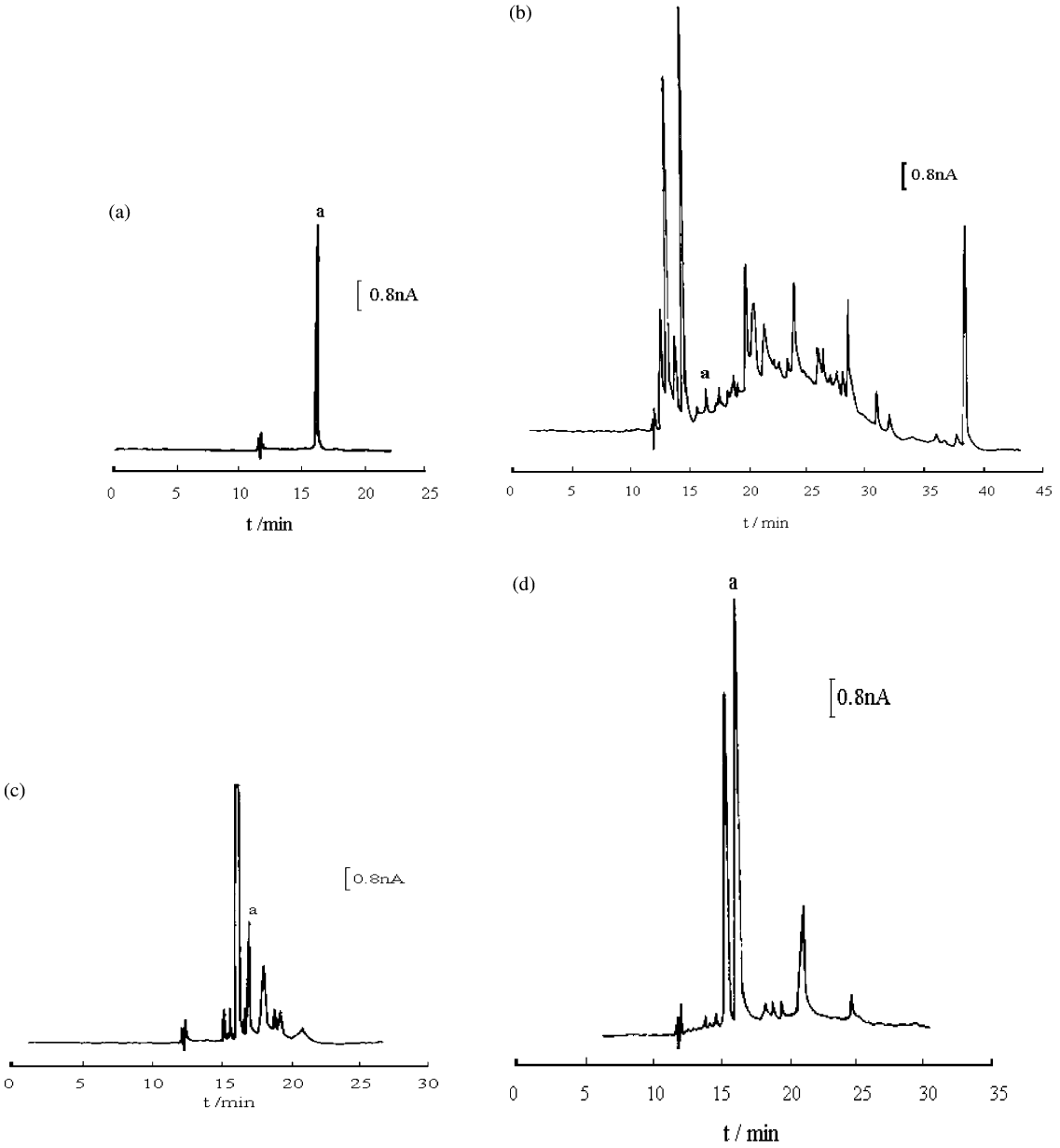


Fig. 4. The typical electropherogram of (A) *trans*-Resveratrol standard solution ($2\times10^{-5}$ g/ml); (B) red wine (Huaxia Great Wall) sample; (C) *Polygonum cuspidatum* Sied. et Zucc. sample solution ($6.86\times10^{-3}$ g/ml); and (D) Zijin capsule sample solution ($3.50\times10^{-4}$ g/ml). Experimental conditions are the same as in Fig. 3. *peak a*: *trans*-Resveratrol.

## 3. Results and discussion

### 3.1. Hydrodynamic voltammogram (HDV)

In amperometric detection the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Therefore, the effect of working electrode potential on the peak current (calculated by measuring the peak height) of the analyte was investigated to obtain optimum detection. Fig. 2 illustrates the hydrodynamic voltammogram of *trans*-Resveratrol. When the applied potential reaches 0.6 V (vs. SCE), the peak current increases rapidly. However, when the potential exceeds 0.85–0.90V (vs. SCE), the current levels off. Although applied potential greater than +0.90V (vs. SCE) results in larger peak current, solvent oxidation becomes pronounced, both the baseline noise and the background current increase, resulting in an unstable baseline, which is a disadvantage for sensitive and stable detection. Therefore the applied potential to the working electrode was maintained at +0.85 V (vs. SCE) where the background current is not too high and the S/N ratio is the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

### 3.2. Effect of injection time

Injection time determining the amount of sampling affects both the peak current and peak shape. The effect of injection time on CE separation was investigated by changing the sampling time from 2 to 14 s at injection voltage of 10 kV. As shown in Fig. 3, from 2 to 6 s, peak current increases with increasing sampling time. But when the injection time is longer than 6 s, the peak current does not increase obviously, moreover, the peak broadening becomes more severe. In this experiment, injection time of 6 s at 10 kV is selected. At the optimum conditions, the typical electropherogram for *trans*-Resveratrol standard is shown in Fig. 4, and the *trans*-Resveratrol peak appears at 16.8 min.

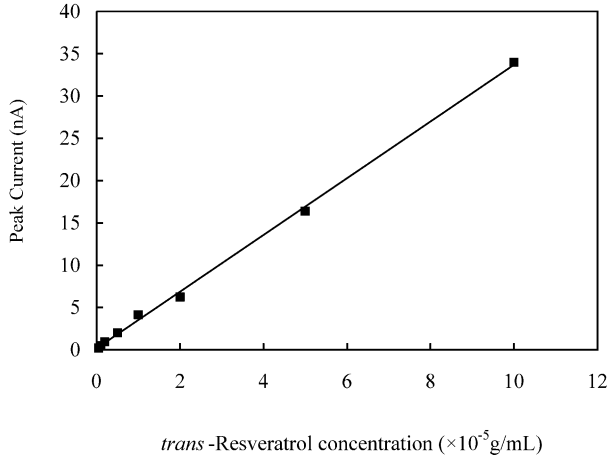


Fig. 5. *trans*-Resveratrol calibration curve ($5\times10^{-7}$–$1\times10^{-4}$ g/ml). Experimental conditions are the same as in Fig. 3. The regression equation is $y = 3.3536\times10^{5}x + 0.154$ [the $x$ value is the concentration of analyte (g/ml), the $y$ value is the peak current (nA)].

### 3.3. Reproducibility, linearity, detection limit of the analyte

The reproducibility of the peak current was estimated by making repetitive injections of the *trans*-Resveratrol standard ($2.0\times10^{-5}$ g/ml) into the system under the selected optimum conditions. The relative standard derivation (RSD) was found to be 2.07% for peak current, and 1.71% for migration time ($n=7$).

To determine the linearity of *trans*-Resveratrol, a series of standard solutions with the concentration range of $1.0\times10^{-7}$–$1.5\times10^{-4}$ g/ml was tested to determine the linearity of this method. Shown in Fig. 5, the calibration curve exhibits excellent linear behavior over the concentration range from $5.0\times10^{-7}$ to $1.0\times10^{-4}$ g/ml with the correlation coefficient of 0.9993. The detection limit is $5.96\times10^{-8}$ g/ml, which is evaluated on the basis a signal-to-noise ratio of 3.

### 3.4. Sample analysis

#### 3.4.1. Analysis of wine samples

Under optimum conditions, the determination of *trans*-Resveratrol in four red wines and one white wine samples was carried out according to the procedures described earlier. Typical electrophorogram of wine

Table 1
Results of *trans*-Resveratrol determination in wine samples

| Samples | *trans*-Resveratrol found (mg/l) | RSD (%; $n=3$) |
|---|---|---|
| Huaxia Great Wall dry red wine | 2.621 | 2.84 |
| Merlot France dry red wine | 3.156 | 2.44 |
| Zhangyu dry red wine | 1.084 | 2.71 |
| Dynasty dry red wine | 1.985 | 3.53 |
| Dynasty medium dry white wine | N.F.[a] | |

[a] N.F. refers to not found.

Table 2
Results of *trans*-Resveratrol determination in herb and capsule samples ($n=3$)

| Samples | *trans*-Resveratrol found (mg/g) | RSD (%) |
|---|---|---|
| *Polygonum cuspidatum* Sied. et Zucc. | 1.556 | 1.24 |
| Zijin capsule | 75.93[a] | 1.35 |

[a] Manufacturer claimed content is 76.8 mg/g (Zhang et al., 2001).

Table 3
Results of recovery experiments for real samples ($n = 3$)

| Samples | *trans*-Resveratrol original amount | *trans*-Resveratrol added amount | *trans*-Resveratrol found | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Sample 1[a] | 1.311 (mg/l) | 0.500 (mg/l) | 1.799 (mg/l) | 97.60 | 1.24 |
| Sample 2[b] | 1.556 (mg/g) | 0.729 (mg/g) | 0.729 (mg/g) | 97.53 | 2.34 |
| Sample 3[c] | 75.93 (mg/g) | 28.57 (mg/g) | 28.57 (mg/g) | 98.63 | 1.35 |

[a] Huaxia Great Wall dry red wine.
[b] *Polygonum cuspidatum* Sied. et Zucc.
[c] Zijin capsule.

sample is shown in Fig. 4B. By comparing its migration time with that of Fig. 4A, and by standard-addition method, the active ingredient *trans*-Resveratrol (*peak a*) in wine samples can be identified and quantified. The assay results are listed in Table 1.

### 3.4.2. Analysis of herb and capsule samples

*trans*-Resveratrol in both chinese medicinal herb *Polygonum cuspidatum* Sied. et Zucc. and the commercial health food Zijin capsules were determined by CE-ED under the optimum conditions. Typical electropherograms of *Polygonum cuspidatum* Sied. et Zucc., and Zijin capsules are shown in Fig. 4C and D, respectively. Based on the migration time and peak height of *peak a* (Fig. 4A), *trans*-Resveratrol contents in these samples can be determined. The assay results are listed in Table 2.

### 3.4.3. Recovery

The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. The recovery experiments of *trans*-Resveratrol in Huaxia Great Wall dry red wine sample, *Polygonum cuspidatum* Sied. et Zucc. sample, and Zijin capsule sample under the optimum condition were determined by standard-addition method, and the results are listed in Table 3.

## 4. Conclusion

In this work, a useful quantitation method is described for the analyses of both wines and traditional Chinese medicine by CE–ED. It could be concluded that CE–ED could be an alternative to traditional methods for the determination of *trans*-Resveratrol in real samples. The earlier assay results indicate that this method is accurate, sensitive and reproducible.

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