Capillary Electrophoretic Determination of Resveratrol in Wines†

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A rapid and sensitive capillary electrophoretic method for analysis of resveratrol in wine was established. The protocol consists of sample preparation using a C-18 solid-phase extraction cartridge. Baseline separation of *trans*- and *cis*-resveratrol from other compounds in wine was achieved in ~8 min using a micellar mode. The limits of detection for *trans*- and *cis*-resveratrol were 0.1 and 0.15 μmol/L, respectively. Recovery rates for *trans*-resveratrol using the protocol described ranged from 94.6 ± 8.5 to 101.9 ± 7.2%. These procedures were used to analyze the *trans*- and *cis*-resveratrol levels in 26 wines. It was found that the concentration of *trans*-resveratrol ranged from 0.987 to 25.4 μmol/L, whereas the concentration of *cis*-resveratrol was much lower.

Keywords: *Resveratrol; wine; capillary electrophoresis*

INTRODUCTION

The antioxidant resveratrol (3,5,4*N*-trihydroxystilbene) exists as both *trans* and *cis* isomers. *trans*-Resveratrol is found in a number of plants, including grapes, whereas the *cis* form is absent. However, both isomers are found in red wines. Resveratrol has become of interest because of its suggested role as a factor associated with lowering the risk of coronary heart disease (CHD) (Trela and Waterhouse, 1996; Sato et al., 1997). CHD has been associated with a high intake of saturated fat in the diet, but an exception to this rule seems to exist in France (Renaud and deLorgeril, 1992). It is thought that the consumption of red wines (i.e., resveratrol) might provide protection against CHD in this population. Similarly, the oriental medicine, Kojo Kon, purportedly provides protection against arteriosclerosis. This preparation has also been found to contain resveratrol (Goldberg et al., 1995a). Resveratrol has also been associated with anticarcinogenic activity. Resveratrol (both *cis* and *trans*) showed an ability to inhibit protein kinase activity, a factor related to cancer (Jayatilake et al., 1993).

Thus, the significance of this compound has spawned numerous methods for its analysis. These include gas chromatography/mass spectrometry (GC/MS) (Siemann et al., 1992; Goldberg et al., 1994, 1995a; Soleas et al., 1995; Lamikanra et al., 1996) and high-performance liquid chromatography (HPLC) (Lamuela-Raventos et al., 1993; McMurtrey et al., 1994; Pezet et al., 1994; Goldberg et al., 1995b, 1996; Jeandet et al., 1995, 1997). Among these methods, either liquid−liquid extraction (Siemann et al., 1992; Lamuela-Raventos et al., 1993) or solid-phase extraction (Soleas et al., 1995) was used for sample pretreatment. Alternatively, direct injection of wine samples was reported by Goldberg et al. (1994, 1995b).

For most GC methods, derivatization of resveratrol with bis(trimethylsilyl)trifluoroacetamide was utilized to enhance resveratrol volatility (Goldberg et al., 1994, 1995a). Good peak resolution was achieved for both resveratrol isomers in the above procedures with detection limits in the low micromolar range.

The use of other dection methods in HPLC such as fluorometric (Pezet et al., 1994) or electrochemical detection (McMurtrey et al., 1994) results in substantially enhanced sensitivity when compared with ultraviolet (UV) detection. However, a problem of intermethod bias has been noted with these methods. Soleas et al. (1997) reported a 10-fold variation in resveratrol concentration determined by different methods for the same generic wines.

Our laboratory has recently reported the use of capillary electrophoresis (CE) as an alternative method for the analysis of resveratrol in wine (Chu et al., 1998). CE has unique potential advantages including high separation efficiency and speed (Vesterberg, 1989; Zeece, 1992; Cancalon, 1995). Our previous method utilized direct injection to analyze *trans*-resveratrol in wine samples. However, we report here several improvements that result in a lower limit of detection, shorter separation time, and resolution of both *trans*- and *cis*-resveratrol.

MATERIALS AND METHODS

Chemicals and Materials. *trans*-Resveratrol was purchased from Sigma Chemical Co. The mixture of *cis*- and *trans*-resveratrol was produced by UV irradiation of *trans*-resveratrol in acetonitrile solution. A 2 min exposure to UV irradiation of 20 μM *trans*-resveratrol in acetonitrile was used to generate the *cis*-resveratrol standard. Sodium dodecyl sulfate (SDS), boric acid, dibasic sodium phosphate, and ethyl acetate were also obtained from Sigma Chemical Co. Ethyl alcohol was supplied by Fisher Scientific, and acetonitrile was from EM Science. Solid-phase extraction (SPE) cartridges were Supelco Supelclean LC-18 SPE tubes (3 mL). The 26 red wines sampled were obtained from a local liquor vendor.

Instrumentation. The instrument used for analysis was a Beckman P/ACE system 5510 equipped with a diode array detector. Data collection and analysis were performed by using P/ACE system Gold software. The SPE was performed using

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a Supelco Visiprep extraction box. The samples were dried under vacuum with the Labconco Centrivap cold trap and concentrator.

SPE. The SPE cartridges were preconditioned with 3 mL of absolute ethanol and followed with 1 mL of 10% ethanol. Two milliliters of the red wine sample was then loaded and allowed to flow through the C-18 bed in 5−7 min. After the wine had passed through, the cartridge was washed with 2 mL of 1 mM HCl, and excess liquid was removed by application of a vacuum (−15 to −20 in Hg) for 2 min. Finally, the bound materials were eluted from the cartridge with 1 mL of ethyl acetate. The ethyl acetate eluate was placed in a Labconco Centrivap concentrator chamber and dried (∼30 min). Dried samples were stored in a −20 °C freezer until CE analysis. Just prior to CE analysis, the samples were reconstituted in 40 μL of CE run buffer. The SPE cartridge used above was washed with 3 mL of ethanol and can be reused ∼20 times.

CE Analysis. The analysis was performed with CE run buffer of 75 mM SDS, 30 mM boric acid, 30 mM dibasic sodium phosphate, and 15% acetonitrile, pH 9.2. The buffer was filtered through a 0.45 μm microfilter before use. The separation was conducted with a fused silica capillary (50 μm i.d., 37 cm total length and 30 cm to detectable aperture) at 25 kV and 20 °C. The capillary was rinsed with CE run buffer for 2 min before separation. The sample was induced into the capillary with low pressure (0.5 psi) for 3 s. Resveratrol isoforms were detected at 314 nm, and a UV scan from 200 to 400 nm was also recorded. Resveratrol isoforms in red wine samples were identified by comparison of peaks' migration times and UV spectrum patterns with the standards. The coinjection of resveratrol standards with wine samples was also conducted. The quantification of *trans*- and *cis*-resveratrol was performed from peak areas with an external standard curve. A standard curve, which covered the concentrations of *trans*-resveratrol in red wine samples to be determined, was prepared daily.

RESULTS AND DISCUSSION

CE Separations. Several variations of the micellar separation conditions were tried to optimize the resolution of *trans*- and *cis*-resveratrol in wine samples. These variations included the composition and pH of the run buffer and voltage. We found that *trans*- and *cis*-resveratrol are more readily separated with the run buffers containing boric acid and dibasic sodium phosphate together with acetonitrile (compare Figure 1a−d). Baseline separation of *trans*- and *cis*-resveratrol from coextracted matrix compounds was achieved with CE run buffer consisting of 75 mM SDS, 30 mM boric acid, 30 mM dibasic sodium phosphate, and 15% acetonitrile, pH 9.2 (Figure 1d). Finally, conducting separations at 25 kV resulted in high separation efficiency, greater sensitivity, and shorter analysis time. Under these CE conditions, a run can be completed in 10 min, which includes a 2 min prerinse of the capillary.

Linearity and Limit of Detection. The standard curves were generated using *trans*-resveratrol standard solutions. Standards ranging from 0.2 to 200 μM were analyzed with CE, and linear regressions of peak areas versus concentrations or peak heights versus concentrations were calculated. A good linear relationship between peak area and concentration of *trans*-resveratrol ranging from 0.2 to 100 μM was found with a linear correlation of $R^2 = 0.99993$. Poor linearity was obtained if peak height was used as a parameter ($R^2 = 0.93947$). Therefore, the peak area was always used for quantification in this study. The limits of detection were 0.1 and 0.15 μM for *trans*- and *cis*-resveratrol, respectively, which were calculated at a peak height of 5 times the baseline.

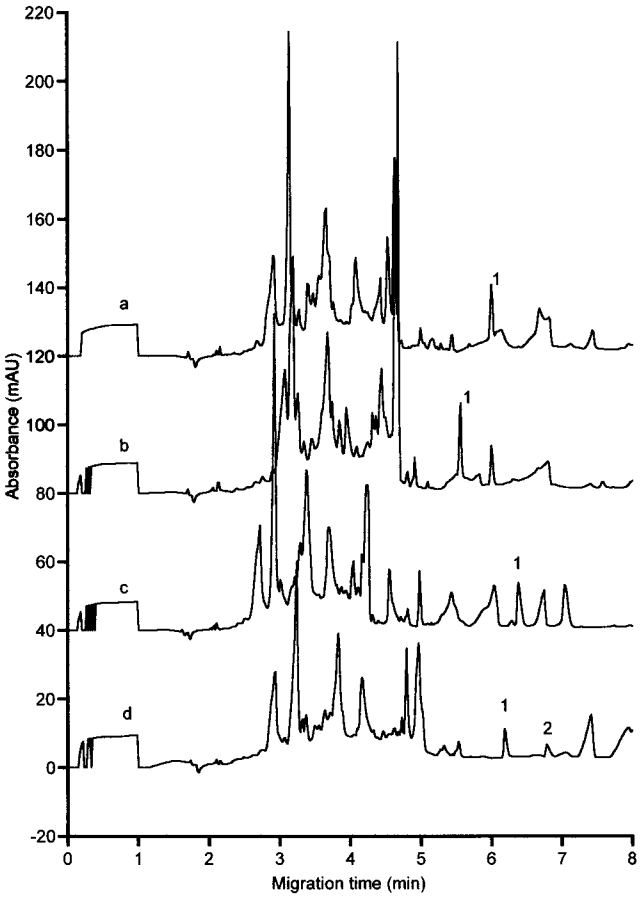

Figure 1. Optimization of red wine electrophoretic separations. Electrophoretic separations of red wine were performed with a Beckman P/ACE 5510 equipped with a diode array detector. The capillary was a fused silica capillary (50 μm i.d., 37 cm total length and 30 cm to detectable aperture). Voltage was at 25 kV, and temperature was kept constant at 20 °C. Samples were injected by application of low pressure (0.5 psi) for 3 s. Detectable wavelengths were from 200 to 400 nm. Electropherograms a−d represent separations in which the buffer was varied: (a) 75 mM SDS, 25 mM boric acid, 25 mM dibasic phosphate, 12.5% acetonitrile, pH 9.2; (b) 75 mM SDS, 25 mM boric acid, 25 mM dibasic phosphate, 15% acetonitrile, pH 9.2; (c) 75 mM SDS, 30 mM boric acid, 30 mM dibasic phosphate, 12.5% acetonitrile, pH 9.2; (d) 75 mM SDS, 30 mM boric acid, 30 mM dibasic phosphate, 15% acetonitrile, pH 9.2. Peaks 1 and 2 correspond to *trans*- and *cis*-resveratrol, respectively.

Table 1. Recovery Rates of *trans*-Resveratrol in Spiked Wine

concn (mM)	recovery (%) ± SD	concn (mM)	recovery (%) ± SD
0.5	96.03 ± 8.6	10	99.41 ± 5.1
2.0	101.9 ± 7.2	25	94.58 ± 8.5

Recovery Rates. The *trans*-resveratrol recovery tests were conducted using spiked red wine with four different concentration levels of *trans*-resveratrol standard solution. The triplicate spiked samples and three blank wine samples were used for each concentration level of *trans*-resveratrol. After SPE extraction and CE analysis, the recovery of *trans*-resveratrol was calculated by deduction of the concentration of *trans*-resveratrol in blank wine and comparing it to the concentration in spiked samples. Excellent recovery rates were achieved, which on average ranged from 94.6 ± 8.5 to 101.9 ± 7.2% (Table 1).

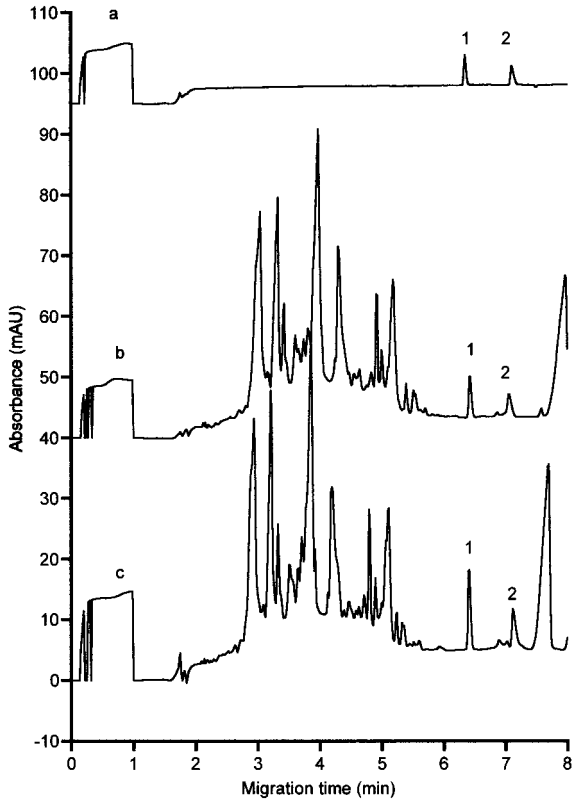

Figure 2. Identification of *trans*- and *cis*-resveratrol in red wine (co-injection of standards). Separation conditions were the same as in Figure 1d. Peaks 1 and 2 in these separations correspond to *trans*- and *cis*-resveratrol, respectively. Electropherograms show separation of *trans*- and *cis*-resveratrol standards (a), 1996 Pinot Noir (Saintsbury, California) (b), and 1996 Pinot Noir co-injected with *trans*- and *cis*-resveratrol standard.

Determination of *trans*- and *cis*-Resveratrol in Red Wine. The wine samples selected for this study were from three states in the United States and seven other countries. Each wine sample was opened just prior to analysis. The triplicate SPE extractions were conducted for each wine, and three CE runs were performed for each SPE extraction. The SPE extraction and CE separation conditions shown in Figure 1d were found to be well suited for analyses of all 25 other wines. The peaks of resveratrol were identified by migration times and also by co-injection with the standards. Figure 2 shows the electropherograms of *trans*- and *cis*-resveratrol standards (Figure 2a), wine extract (Figure 2b), and co-injection of wine extract with *trans*- and *cis*-resveratrol standards (Figure 2c). It can be seen from parts a and b of Figure 2 that *trans*- and *cis*-resveratrol in the wine sample have the same migration times as the standards. Similarly, co-injection of this wine extract with standard *trans*- and *cis*-resveratrol resulted in increases of both presumptive *trans*- and *cis*-resveratrol peaks (Figure 2c). To further confirm the identification of these peaks, the UV adsorption spectra were also examined. The UV spectra of these two compounds in the wine separation matched very well with *trans*- and *cis*-resveratrol standard (Figure 3a,b). From the above results, we concluded that these two peaks identified in wine were *trans*- and *cis*-resveratrol, respectively.

The peak purity checks were performed at the inflections and apex of each peak, and the three spectra were drawn and compared in a normalized and overlaid mode. The results showed that there were no impurities found in either *trans*- or *cis*-resveratrol peaks in all 26 wines using these CE separation conditions.

The maximum UV absorbances for *trans*- *and cis*-resveratrol were found to be 314 and 278 nm, respectively (Figure 3a,b). Both forms had similar absorbance profiles around these wavelengths, and therefore the determination of both *trans*- and *cis*-resveratrol was performed at 314 nm in this study. The concentrations and standard deviations of *trans*- and *cis*-resveratrol were determined as described under Materials and Methods and are listed in Table 2. The concentration of *trans*-resveratrol ranged from 0.987 to 25.49 μmol/L in the 26 wines, whereas the concentration of *cis*-resveratrol is much lower. For most wines, the content of *cis*-resveratrol is about one-third that of *trans*-resveratrol. However, *cis*-resveratrol was not detected in some wines. In our SPE extraction and CE conditions, we found that there was no *trans*- to *cis*-resveratrol conversion. Therefore, *cis*-resveratrol detected in this study was in the wine.

At the beginning of wine fermentation, the concentration of *trans*-resveratrol is very low and *cis*-resveratrol is absent (Mattivi et al., 1995; Vrhosek et al., 1997). *trans*- and *cis*-resveratrol are gradually released into wine when their glucosides are hydrolyzed during fermentation (Vrhosek et al., 1997). In addition to the type of grape used and the region of cultivation, different yeast strains can also significantly affect the resveratrol content in wine (Vrhosek et al., 1997).

In general, we found that Pinot Noir and Merlot wines had the highest levels of resveratrol concentrations (Table 2), which is consistent with previous studies (Goldberg et al., 1995c; Sato et al., 1997). Californian Cabernet Sauvignons had extremely low *trans*-resveratrol contents. Regionally, Pinot Noir wine from Oregon had the highest resveratrol concentration (*trans*-resveratrol = 25.49 μmol/L). French wines also had very high resveratrol concentrations (*trans*-resveratrol = 6.5−12.7 μmol/L; total resveratrol = 8.8−15.0 μmol/L), whereas Californian wines had the lowest resveratrol concentrations (*trans*-resveratrol = 1.0−10.2 μmol/L; total resveratrol = 1.0−14.4 μmol/L).

We have previously reported the use of CE for the separation of *trans*-resveratrol in wine (Chu et al., 1998). This method used direct injection of the wine sample and was capable of detecting 2−3 μM levels of *trans*-resveratrol. However, the method did not detect *cis*-resveratrol in the wines analyzed, and separation times were ∼15 min under the conditions employed.

We have reported here a significantly improved (compared to our previous work) protocol for the analysis of *cis*- and *trans*-resveratrol in wine. The new procedure has a 10-fold lower limit of detection and a significantly shorter separation time. The protocol was found to work well when tested with a variety of wines. The levels of resveratrol determined by our protocol in these samples were similar to those reported using other methodologies (Table 2). Thus, this work illustrates the potential application of CE for the analysis of resveratrol in wine. CE may also be useful for the determination of other antioxidant components found in wine. For example, CE methods for identification quercetin and catechin in wine have been reported (Prasongsidh and Skurray 1998). Similarly, we have developed a CE method that separates quercetin, catechin, epicatechin, kaempferol, myricetin, and resveratrol in the same run (unpublished results). Finally, conditions used in sepa-

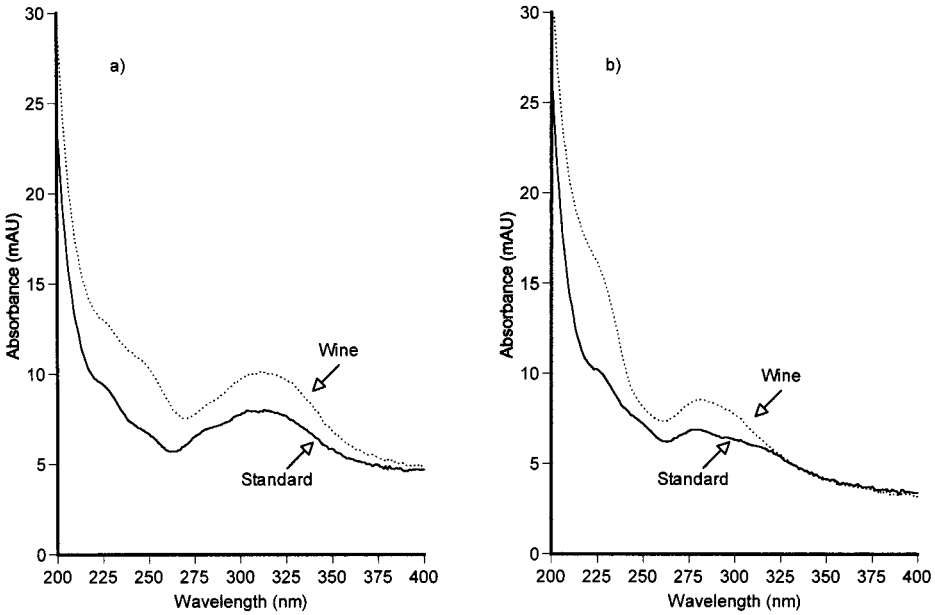

Figure 3. Identification of *trans*- and *cis*-resveratrol in red wine separations (absorption spectra). The absorption spectra between 200 and 400 nm of peaks 1 and 2 from a wine separation (Figure 2) are shown. The spectra are similar to those for purified *trans*- and *cis*-resveratrol standards and further support the identification of the peaks corresponding to the isoforms.

Table 2. Resveratrol Concentration in Red Wine

variety or name	maker	vintage	*trans*-[a]	*cis*-[a]	total
California					
Cabernet	J. Lohr-Cypress	1994	2.41 ± 0.16	ND	2.41
Zinfandel	Karly-Pokerville	1996	3.26 ± 0.08	ND	3.26
Cabernet Sauvignon	Sutter Home	1995	1.73 ± 0.09	ND	1.73
Special Reserve Red	Mountain View	none	10.16 ± 0.57	4.29 ± 0.13	14.45
Cabernet Sauvignon	Hawk Crest	1995	1.90 ± 0.29	0.65 ± 0.01	2.56
Merlot	Saintsbury	1996	1.90 ± 0.13	0.68 ± 0.10	2.58
Pinot Noir	Parducci	1996	7.93 ± 0.26	2.44 ± 0.07	10.37
Cabernet Sauvignon	Frey Mendocino	1995	0.99 ± 0.10	ND	0.99
Oregon					
Pinot Noir	Bethel Heights	1996	25.49 ± 2.34	ND	25.49
Washington					
Merlot	Paul Thomas	1995	11.78 ± 0.38	3.34 ± 0.07	15.12
France					
Cotes-Du-Rhone	George Duboeuf	1993	7.62 ± 0.62	1.18 ± 0.07	8.79
Beaujolais Villages	George Duboeuf	1996	6.52 ± 0.16	2.98 ± 0.11	9.50
Bordeaux	Chauteau Larose	1994	7.60 ± 0.31	1.66 ± 0.07	9.26
Bordeaux	Christian Moueix	1995	12.71 ± 0.89	2.37 ± 0.15	15.08
Chile					
Merlot	Sunrise-Concha Toro	1997	5.80 ± 0.29	2.52 ± 0.05	8.32
Cabernet Sauvignon	Castillero del Diablo	1996	4.02 ± 0.16	1.19 ± 0.06	5.21
Spain					
Tinto Reserva Pendes	Mont Marcal	1989	5.66 ± 0.15	0.69 ± 0.02	6.35
Red Navarra	Guelbenzu	1995	10.10 ± 0.27	1.47 ± 0.123	11.57
Australia					
Shiraz	Rosemount Estate	1997	6.78 ± 0.29	2.46 ± 0.08	9.24
Cabernet Sauvignon	Rosemount Estate	1995	6.40 ± 0.29	1.42 ± 0.07	7.82
Argentina					
Cabernet Sauvignon	Santa Julia	1995	5.11 ± 0.37	ND	5.11
Cabernet Sauvignon	Santa Julia	1995	6.78 ± 0.30	ND	6.78
Italy					
Vino Nobile	Montepalciano	1991	2.88 ± 0.20	ND	2.88
Chianti Classico	Castello D'alboa	1995	4.99 ± 0.23	0.83 ± 0.03	5.82
Valpolicella Classico	Zenato	1994	5.06 ± 0.33	0.75 ± 0.03	5.82
Portugal					
Porto	Warre's	none	2.26 ± 0.10	0.70 ± 0.02	2.95

[a] Values for *trans*- and *cis*-resveratrol represent micromolar concentrations ± SD of the mean of three determinations. ND = not detected.

rations presented here will contribute to the development of chip-based CE analysis. The rapidly developing chip-based separation technology (Effenhauser et al., 1997) will provide significant advantages over other analytical methods by facilitating high throughput and sensitive analysis of antioxidants and other wine analytes.

Conclusions. A sensitive, fast, and reliable CE procedure for analysis of both *trans*- and *cis*-resveratrol in wines was developed in this study. Excellent recovery with SPE and baseline CE separation was achieved. Runs were completed in ~8 min with the limits of detection for *trans*- and *cis*-resveratrol at 0.1 and 0.15 μmol/L, respectively. The analyses of 26 samples dem-

onstrated that this method can be used for the determination of *trans*- and *cis*-resveratrol in wine.

As a note of added proof for the CE approach to the analysis of resveratrol in wine, a similar procedure has been reported by Nevado et al. (1999). They employed SPE of wine samples and determined *cis*- and *trans*-resveratrol at the low micromolar level. This paper was published during the time this manuscript was in review.

ABBREVIATIONS USED

CE, capillary electrophoresis; GC, gas chromatography; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; SPE, solid-phase extraction.

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