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Capillary electrophoresis analysis of *trans*- and *cis*-resveratrol, quercetin, catechin and gallic acid in wine

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A study was conducted on the use of micellar electrokinetic capillary chromatography (MEKC) in the analysis of *trans*- and *cis*-resveratrol, quercetin catechin and gallic acid in wine. Standards and wine samples were directly injected into CE after a simple filtration, under a pressure of 50 mbar. Separation of the compounds was done in bare fused-silica capillary-extended light path with 50 μm i.d. An excellent correlation coefficient ($r=0.99998$) was obtained with a linear analytical response from 8 to 1000 ppm of resveratrol, quercetin, catechin and gallic acid in MEKC. The high separation efficiency obtained recommend CE as a good application for *trans*- and *cis*-resveratrol detection in wine. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Resveratrol, which has been found in grape berries (Jeandet *et al.*, 1991), in wines (Goldberg *et al.*, 1995) has prompted intense scientific interest in its anticancer and cardio-protective activity (Frankel *et al.*, 1993). This phytoalexin in *cis* and *trans* isomers were found in wine with gallic acid and other antioxidants such as catechin, quercetin. The latter two, together with resveratrol can protect low-density lipoproteins against oxidation (Frankel *et al.*, 1993). In addition, wine consumption may be beneficial due to the anti-cancer properties of resveratrol and quercetin (Avila *et al.*, 1994).

Separation of resveratrol from other compounds has been carried out by thin layer chromatography (TLC) (Jeandet *et al.*, 1992), quantification by high-performance liquid chromatography (HPLC) (Seamann and Creasy, 1992; Roggero and Archier, 1994; Jeandet *et al.*, 1995), and gas–liquid chromatography (GC) then identity confirmation by GC–MS (Jeandet *et al.*, 1993).

Capillary electrophoresis is, nowadays, on the verge of extensive development in the field of research and analytical application similar to that of HPLC two decades ago. Its greater efficiency compared to HPLC means that CE finds a role in several applications (Wätzig and Dette, 1993; Marina and Torre, 1994) including food analysis (Cancalon, 1995). The aim of the present study was to evaluate the use of this highly efficient instrument with a simple method development to analyse gallic acid, catechin, quercetin and determine the concentration of *cis* and *trans*-resveratrol in wines.

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MATERIALS AND METHODS

Equipment, reagents and samples

Capillary electrophoresis system HP^{3D} CE instrument equipped with a diode array detector, ChemStation data software and 60 cm effective length bare fused silica capillary (50 μm i.d. × 64.5 cm) with extended light path (150 μm i.d. bubble), all from Hewlett-Packard, were used.

Resveratrol, quercetin and gallic acid were obtained from Sigma Chemical Co, St Louis, MO. Wine samples were obtained from Shiraz (Narioopta and Mudgee).

Standards

Stock solution: pure solid resveratrol (Sigma) was dissolved in methanol (HPLC grade) at 20 mg ml^{-1} and stored at −4°C in the dark. This was used to prepare the resveratrol standard dilution at 1000, 500, 250, 100, 50, 10, 5, and 1 μg ml^{-1} in the mobile phase of CE. Similarly, stock solutions in methanol (HPLC grade) of

quercetin, catechin and gallic acid were prepared daily before being diluted at 1000, 500, 250, 100, 50, 10, 5, and 1 $\mu g\,ml^{-1}$ in the mobile phase of CE. These standards were filtered with a 0.22 μm filter (Millex-HV, Millipore) into a microvial with cap (Hewlett-Packard) before being injected into CE.

Samples preparation

Wine samples of 100 μl were diluted in 900 μl of CE mobile phase and then filtered with a 0.22 μm filter into a microvial with cap (Hewlett-Packard) prior to injection into CE.

Initialising and conditioning of CE

A capillary cassette was set at a temperature of 40°C and the capillary was preconditioned between runs by washing with 0.1 M sodium hydroxide and Milli-Q water before flushing with the CE mobile phase. Applied voltage, current and power were set at limits of 20 kV, 50 μA and 4.5 W respectively. The polarity setting was positive. The mobile phase of CE and sample diluent consisted of a mixture of 0.05 M sodium deoxycholate, 0.01 M disodium hydrogen phosphate, and 0.006 M disodium tetraborate at a pH of 9.3 corrected by phosphoric acid. Sample injection was done under a pressure of 50 mbar in 7 s then completed with a 3.7 s of mobile phase injection. The absorbance was recorded at 220 nm wavelength. A standard curve with a correlation coefficient at 0.99998 was developed from 8 to 1000 ppm.

RESULTS AND DISCUSSION

Standards of resveratrol, quercetin, catechin and gallic acid are clearly separated in a single analysis in less than 11 min (Fig. 1). According to the conditions set, no tail was observed.The resolution of resveratrol and gallic acid (1.6–1.6; 1.6–3.1) and their theoretical plate numbers (27 955; 54 697) were automatically calculated by ChemStation software (System HP^{3D} CE, Hewlett-Packard).

Since wine contains a series of compounds and their mobility in the capillary depends on electro–osmotic flow, selection of buffer components depends on charge or pH. Hence, phosphate, borate and citrate, the polybasic buffers at basic pH were tested. The surfactant anionic (SDS) and organic modifiers (7% acetonitrile) were added to help the control the migration direction and separation. A number of assays were done to review the possibility, and quality, of the separation. However, further study will need to study statistically the effect of each component of the buffer on the separation of each compounds in the wine. The mixture of 0.05 M sodium deoxycholate, 0.01 M disodium hydrogen phosphate, and 0.006 M disodium tetraborate pH 9.3 was found, in trials, to maintain the migration direction of *trans*- and

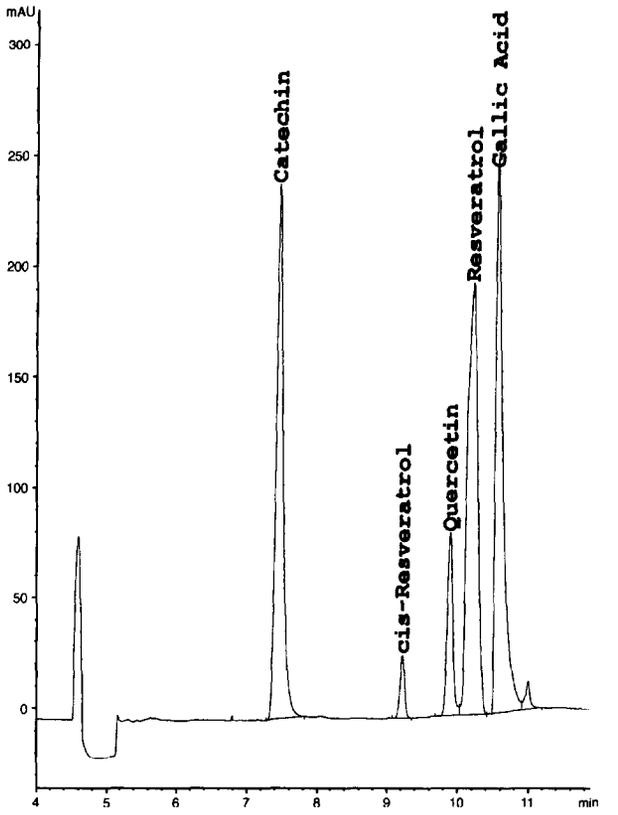

Fig. 1. Electropherogram of the standards of resveratrol, catechin, gallic acid (250 $\mu g\,ml^{-1}$) and Quercetin (100 $\mu g\,ml^{-1}$). Conditions: bare fused silica capillary-extended light path, 50 μm i.d.×64.5 cm, 60 cm effective length; mobile phase composition, 0.05 M sodium deoxycholate, 0.01 M disodium hydrogen phosphate, and 0.006 M disodium tetra borate, pH 9.3; applied voltage 20 kV/50 μA and power 4.5 W; cassette temperature, 40°C and run time 12 min. A peak of noise appeared at approx. 11 min was not observed in the other electropherograms of standards.

cis-resveratrol, quercetin, catechin and gallic acid individually or in a mixture. However, the peaks of gallic acid, resveratrol and quercetin were close and even merged in a temperature higher than 40°C, the conditions that allow for each analysis were subsequently shortened to 10 min. This caused the peaks to become broad. Spectral analysis and library searching in CE sometimes gave a low probability and Joule heating was not suitable at higher temperatures. Reducing the temperature to 30°C induced a better separation and shape. However, the time for each analysis was considerably increased. Adequate separation of the three compounds was observed at 40°C. Higher concentration of the buffer's components was restricted at this temperature.

The standard variation between inter-assay varied between 0.64 and 2.37% dependent upon the amount of resveratrol. The between day varied greatly for compounds other than resveratrol, which may be due to the instability of the compounds themselves. In contrast, the standard variation of resveratrol between days was recorded from 1.4 to 4.9%. The variation was greater in a low concentration of resveratrol. However, the

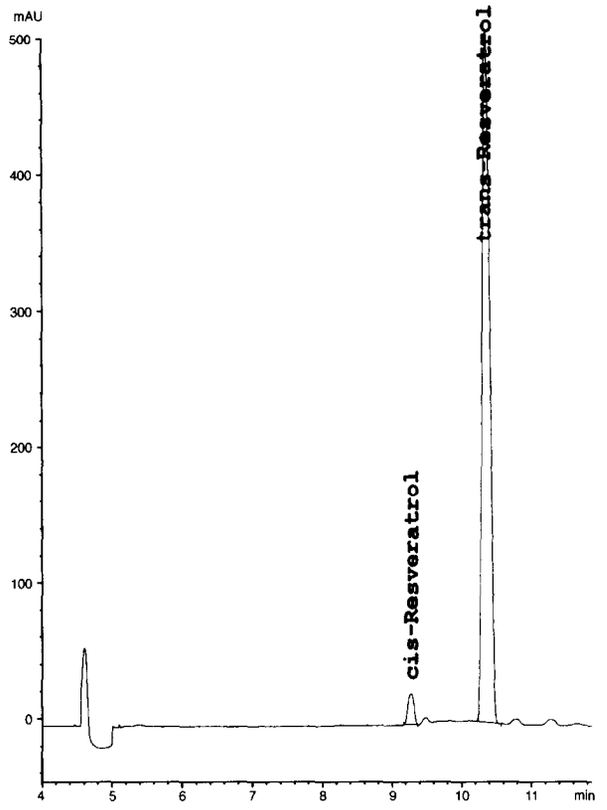

Fig. 2. Electropherogram of the *cis*-resveratrol obtained after 1 h of irradiation at 366 nm on *trans*-resveratrol.

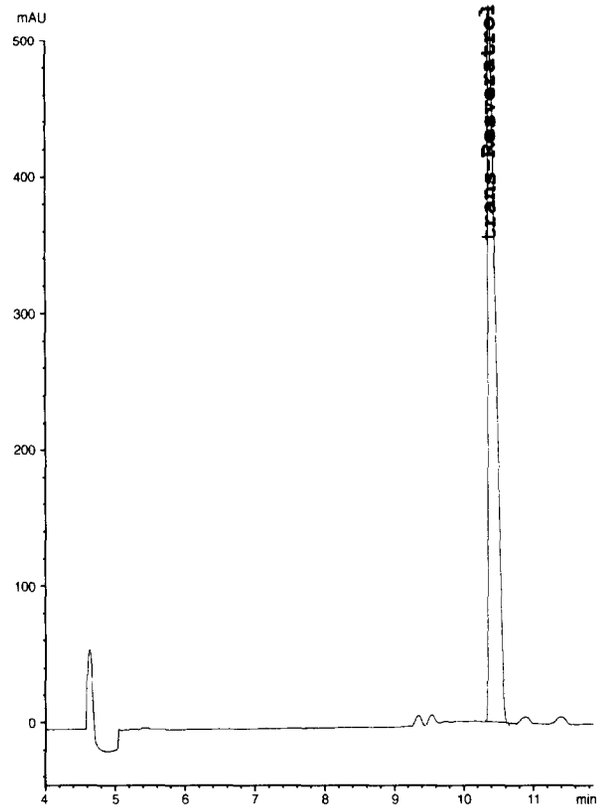

Fig. 3. Electropherogram of the *trans*-resveratrol prior to UV irradiation.

exchange of a new set of mobile phase at every fourth analysis was needed to maintain such sensitivity. A replenishment system needs to be employed with large numbers of samples. An automatic on-line injected system with a 40 samples cartridge was used.

The conversion of *trans*-resveratrol and *cis*-resveratrol was conducted in a manner similar to that previously reported (Jeandet *et al.*, 1993, 1995). However, the condition used to obtain the *trans*-formation of *trans*- to *cis*-resveratrol was to expose *trans*-resveratrol in a solution to UV-visible light at 366 nm wavelength, the source being a UV-visible spectrophotometer (Pharmacia LKB, Biochrom 4060). Thus, several parameters were controlled, such as distance between source of UV and sample, temperature and time of exposure. Figure 2 was the result of *trans*-resveratrol in CE mobile phase after an 1 h exposure to visible 366 nm. Figure 3 shows *trans*-resveratrol prior to UV treatment. The result of the analysis of wine was consistent with the report of Jeandet *et al.* (1995), in that the level of resveratrol was many times higher in concentration in red wine than in white wine. The results of this finding are already published (Skurray and Prasongsidh, 1997).

The similarity in migration time of *trans* and *cis*-resveratrol between red wine samples was found (Figs 4 and 5). The shape of the peaks in Fig. 1 was not identical to that in Figs 4 and 5. The peaks in Fig. 1 were standard peaks in a simple mixture of standard compounds in methanol and CE mobile phase only.

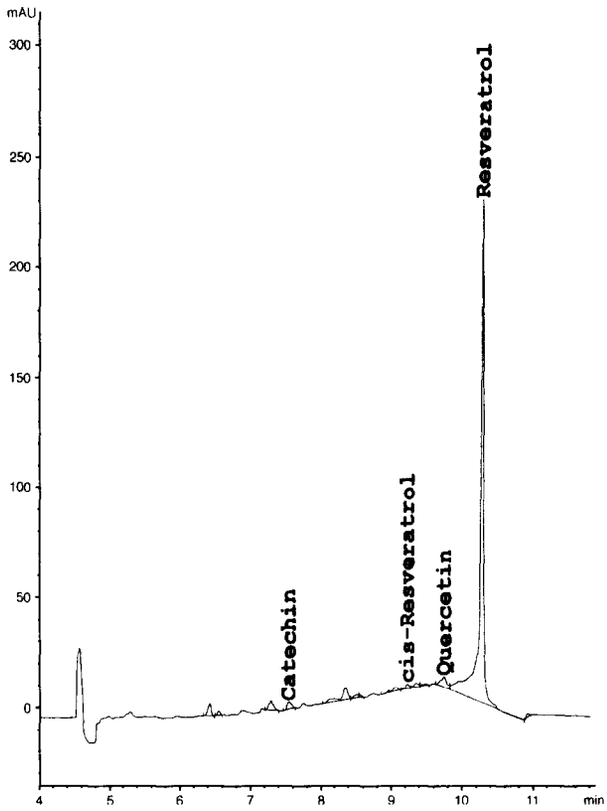

Fig. 4. Electropherogram of the *cis*- and *trans*-resveratrol, catechin and quercetin in a wine sample.

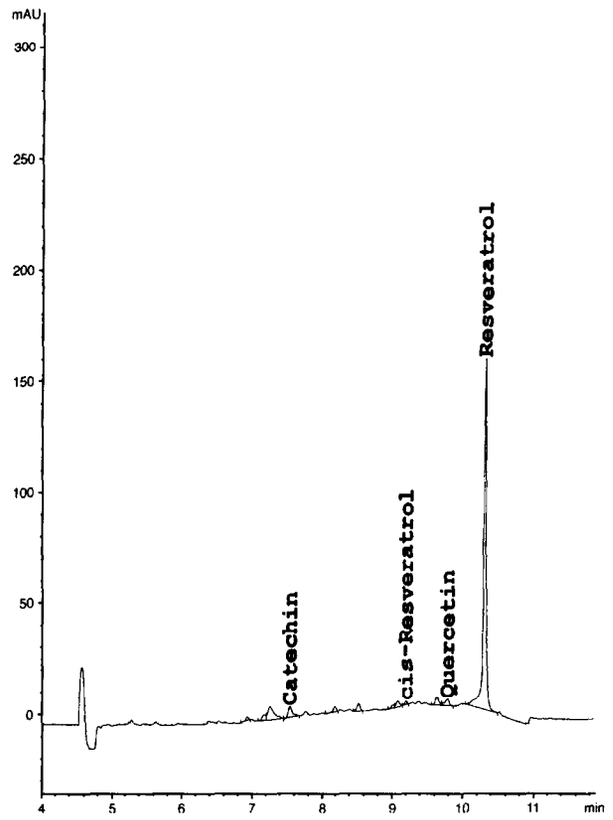

Fig. 5. Electropherogram of the *trans*-resveratrol detected in a different wine sample.

In contrast, the peaks in Figs 4 and 5 were the compounds in wine samples diluted in mobile phase (1:10). The mixture of wine samples and the mobile phase might induce conductivity differences between the buffer and the wine, hence peak shapes distortions occurred. Therefore, the peaks shapes were different. Migration time and peak shape are often insufficient for peak identity.

In this study, spectral analysis was used together with stored spectral libraries. Probability of peak identity in the figures was done by using automated comparison to match the peaks. Therefore, differences in mobility and shape did not prevent peak identification. Catechin and quercetin concentrations were low when compared to *trans*-resveratrol in red wine sample. Methanol was used to assess the repeatability migration time. Direct injection of a wine sample without dilution with CE mobile phase may interfere with the migration time and thus lead to false results. Preparation of a sample by a dilution of 1:10 in mobile phase avoided such problems. However, the mixture containing some wine might become viscous. This was overcome by simple filtration through a 0.22 μm filter.

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