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SHORT COMMUNICATION

# Fast Determination of Phenolic Compounds in Brazilian Wines from Vale do São Francisco Region by CE

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**Abstract** Six phenolic compounds were separated and determined by capillary zone electrophoresis in red wine from Brazil's region Vale do São Francisco with total analysis time of 12 min. The limit of detections varied from 1.59 to 2.24 mg $L^{-1}$. The relative standard deviations (for $n = 6$) varied from 0.28 to 3.50 %. The red wine samples analyzed were bought in the local market and the phenolic compound recoveries were in the range of 98–101 %. The concentrations of gallic acid in the samples of wines varied from 16.0 to 42.0 mg $L^{-1}$, caffeic acid (3.16–5.18 mg $L^{-1}$), syringic acid (5.73–13.0 mg $L^{-1}$), kaempferol (2.32–4.33 mg $L^{-1}$), quercetin (1.68–4.03 mg $L^{-1}$), myricetin (7.52–25.1 mg $L^{-1}$). The concentrations found agree with data reported in the literature.

**Keywords** CZE · Phenolic compounds · Wines

## Introduction

Phenolic compounds constitute one of the most important quality parameters of wine, because those compounds have a great impact on the sensorial characteristics, especially colour and flavour. Wine has been the subject of many analytical studies [1–3]. Actually these compounds have been reported to have multiple biological effects such as, anti-inflammatory action, inhibition of platelet aggregation, and antimicrobial activities [4]. A major part of the phenolics in wines may act as antioxidants [5]. Many works have been published dealing with the analysis of red wine polyphenols and the relationship between polyphenol content and antioxidant capacity. The antioxidant properties [6] of red wines have been correlated with their content of flavonols [7, 8], anthocyanins [9, 10], and tannic acid [11], although it is believed that the antioxidant properties of red wines are linked with the total polyphenol concentration [12, 13] rather than with specific compounds. Many methods have been used for analysis of phenolic compounds in wine, with high performance liquid chromatography as the method of choice [14–17]. The HPLC technique has high analysis time, which varies between 30 and 60 min [18].

On the other hand, capillary electrophoresis (CE) [19, 20] is an efficient technique, which spends small sample and electrolyte consumption and rapid analyses, with separation times of few minutes. This last characteristic is the main advantage versus chromatographic methods, which makes CE, micellar electrokinetic chromatography (MEKC) [21], a diode array detector (DAD), electrochemical [22] and fluorimetric detection [23] of great utility in routine analysis, monitoring of processes in a number of industrial fields and in screening methods to give a fast binary response. In addition, CE is relatively well suited to analysis of samples with complex matrices, as it allows in-capillary concentration through electrokinetic stacking. Phenolic compounds have been determined in wine and must using CE [24, 25]. Very often this technique enables the determination of low concentration levels of one analyzed analyte in the presence

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of many other interfering and coeluting components which is possible in combination with an effective sample preparation technique. It is generally known that clean-up and preconcentration of analytes from the biological matrix are the most difficult and time-consuming steps. Traditional separation techniques, include a solvent extraction of the sample [26, 27]. Among the electrophoretic methods, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have been the most used for the analysis of wines and grapes [28–30]. The use of capillary electrophoresis for the determination of flavonols (kaempferol-3-rutinoside, rutin, avicularin, quercitrin, isoquercitrin, isorhamnetin, kaempferol and quercetin) present in fruit juices and wines was explored [31]. Another work studied the effect of organic solvents in the separation of flavonoids in wine by micellar electrokinetic capillary chromatography [32]. Electrophoresis is a separation technique with high efficiency based on differential migration of ionic or ionizable compounds when subjected to an electric field [33]. Thus, the purpose of this work is the determination of polyphenols in wine of Brazil's region Vale do São Francisco by CZE.

## Instrumentation and Capillary Conditioning

Method development and evaluation, as well as sample analyses, were conducted in a capillary electrophoretic system; CE was performed with a "Backman-Coulter" P/ACE MDQ system (Fullerton, CA, USA) equipped with a DAD set at 280 nm for quantification. The temperature was controlled and stabilized at 25 °C using fluorocarbon-based cooling fluid. Samples and standard solutions were injected hydrodynamically (50 mbar for 5 s) and constant voltage of 25 kV was employed. The capillary was a fused-silica (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 50 cm total length, 40.0 cm effective length, 50 um i.d. On each day of analysis, the capillary was conditioning by flushing with 1 mol $L^{-1}$ NaOH for 5 min, followed by purified water (MilliQ System, Millipore, Bedford, MA, USA) for 5 min and the electrolyte solution for 30 min. In between runs, the capillary was flushed with the electrolyte solution (1 min). The electrolytes composed of 10 % MeOH, 20 mM TBS (sodium tetraborate); this solution was prepared daily and filtered through a 0.45-µm membrane. All standards and samples were injected in triplicate.

## Reagents and Solvents

All reagents were of analytical grade and solvents of chromatographic grade, used without previous purification. Methanol was purchased from Tedia Company (Fairfield, OH, USA), hydrochloric acid and sodium tetraborate (TBS) from Merck (Darmstadt, Germany). Water was purified by deionization (18 mΩ) (Milli-Q system, Millipore, Bedford, MA, USA). The standard gallic acid, quercetin, myricetin and caffeic acid were purchased from Aldrich (St. Louis, MO, USA); syringic acid and kaempferol were from Fluka (St. Louis, MO, USA). Stock standard solutions of each of the 6 phenolic compounds were prepared at 1,000 mg $L^{-1}$ in 60:40 v/v water/ethanol. Working solutions were prepared by mixing appropriate volumes of the stock solutions with 60:40 v/v water/ethanol. The structure of the phenolic compounds is illustrated in Fig. 1.

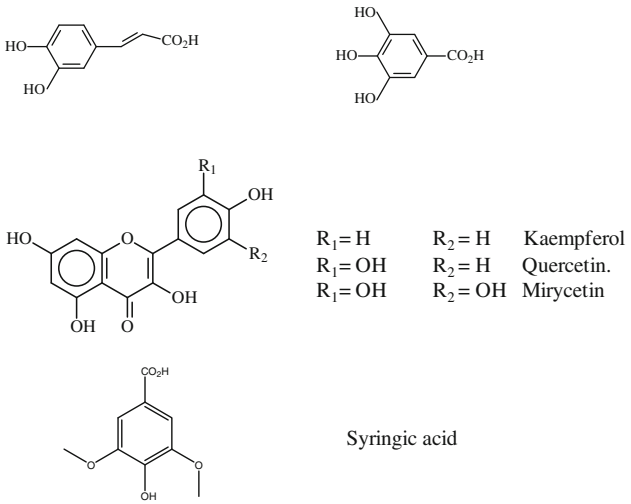


**Fig. 1** Chemical structure of the phenolics

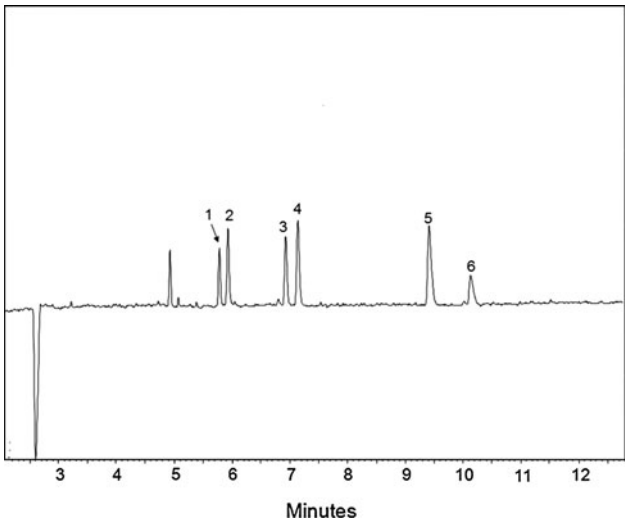


**Fig. 2** Electropherograms of the phenolic compounds in mixture of standards. Buffer, TBS (sodium tetraborate) 20 mmol $L^{-1}$, 10 % (v/v); pH = 9.0; capillary silica capillary uncoated, 50.0 cm × 50 µm I.D.; applied potential, +25 kV; detection, UV at 280 nm peak identification: *1* syringic acid, *2* kaempferol, *3* myricetin, *4* quercetin, *5* caffeic acid, *6* gallic acid

**Table 1** Analytical features of the CE method

| Analyte | Detection limit (μg $mL^{-1}$) | Detection quantification (μg $mL^{-1}$) | Analytical curves | Coefficient of correlation |
|---|---|---|---|---|
| Syringic acid | 0.14 | 0.45 | $y = 447.29x - 2214.9$ | 0.999 |
| Kaempferol | 0.19 | 0.58 | $y = 684.7x - 1324.2$ | 0.999 |
| Myricetin | 0.25 | 0.84 | $y = 398.01x - 1878.8$ | 0.999 |
| Quercetin | 0.31 | 1.03 | $y = 945.13x - 1079.7$ | 0.999 |
| Caffeic acid | 0.33 | 0.96 | $y = 1045.1x - 3116.4$ | 0.999 |
| Gallic acid | 0.22 | 0.72 | $y = 1081.1x - 4881.3$ | 0.998 |

### Samples

Six samples of different types of red wine were purchased from a supermarket in Bahia, Brazil. These wines were chosen to get a good representation of wines produced in the region of Vale do São Francisco-Bahia. The samples of Brazilian wines are novelties in the work, because the region is uncommon for elaboration of wines. These wines are from a tropical region (Shiraz, Cabernet Sauvignon, Cabernet Sauvignon/Shiraz; Shiraz; Ruby Cabernet, Tanat). All wines were stored in the dark at 4 °C until analysis.

### Liquid–Liquid Extraction (LLE)

The contents of two freshly opened bottles were mixed and 1 mL was extracted with ethyl ether at a solvent/wine proportion of 8:5 mL, followed by hydrolysis with 100 μL of hydrochloric acid for 15 min with magnetic stirring. The organic phase was separated from the aqueous phase, dried under nitrogen and dissolved with 2.5 mL of ethanol:water 60:40 v/v. The samples were filtered through a 0.45 μm membrane (Millex LCR PTFE) (Millipore, Sao Paulo, Brazil). The analytes were identified by comparison of the migration times with those of standards and with wine spiked with standards under identical conditions, along with the spectra of the migrated solutes obtained with the PDA detector.

## Results and Discussion

The polyphenols were identified in the electropherogram by comparing both migration time and spectral data obtained from real samples and standards and also with spiked real samples at different concentration levels. Peak areas were used for the quantification of the analytes. The conditions of the separation were optimized and published by Renato et.al. The electropherogram in the best condition is presented in Fig. 2.

### Analytical Features

The analytical curves were produced from results obtained by injecting standard solutions in the range 5–100 μg $mL^{-1}$.

**Table 2** Spike test for phenolic compounds concentrations (μg $mL^{-1}$) in Brazilian wines (Cabernet Sauvignon) obtained by CE

| | Content added (μg $mL^{-1}$) | Content found (μg $mL^{-1}$) | Rec. (%) |
|---|---|---|---|
| Gallic acid | 0.0 | 22.45 | 98.0 |
| | 5.0 | 27.35 | |
| Caffeic acid | 0.0 | 8.04 | 94.0 |
| | 5.0 | | |
| Syringic acid | 0.0 | 8.13 | 98.5 |
| | 2.0 | 10.10 | |
| Quercetin | 0.0 | 3.58 | 96 |
| | 2.0 | 5.50 | |
| Kaempferol | 0.0 | 2.91 | 99.5 |
| | 2.0 | 4.90 | |
| Myricetin | 0.0 | 25.13 | 101 |
| | 5.0 | 30.18 | |

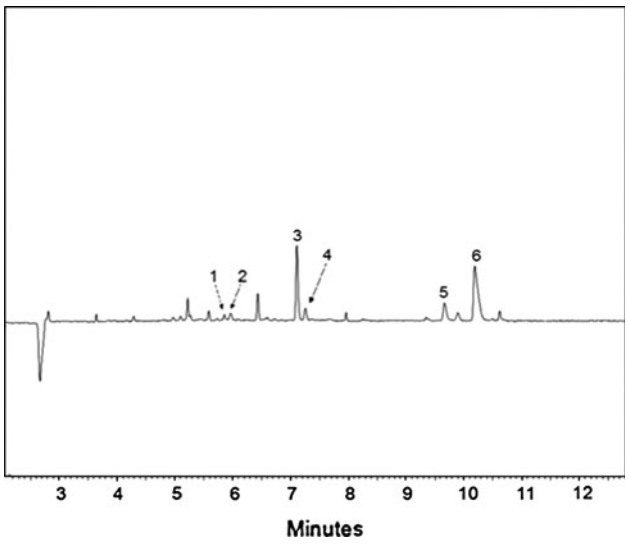


**Fig. 3** Electropherograms of the phenolic compounds in *red* wine samples. Buffer, TBS (sodium tetraborate) 20 mmol $L^{-1}$, 10 % (v/v) MeOH; pH = 9.0; capillary silica capillary uncoated, 50.0 cm × 50 μm I.D.; applied potential, +25 kV; detection, UV at 280 nm peak identification: *1* syringic acid, *2* kaempferol, *3* myricetin, *4* quercetin, *5* caffeic acid, *6* gallic acid

**Table 3** Phenolic compound concentrations (μg $mL^{-1}$) in Brazilian wines obtained by the CE method

| Sample | Syringic | Kaempferol | Myricetin | Quercetin | Caffeic | Gallic |
|---|---|---|---|---|---|---|
| Cabernet Sauvignon/Shiraz | 5.73 ± 0.07 | 4.33 ± 0.21 | 15.34 ± 0.31 | 1.68 ± 0.05 | 5.18 ± 0.30 | 15.77 ± 0.58 |
| Cabernet Sauvignon | 8.13 ± 1.90 | 2.91 ± 0.23 | 25.13 ± 4.82 | 3.58 ± 0.49 | 8.04 ± 0.25 | 22.45 ± 1.00 |
| Ruby Cabernet | 13.05 ± 1.62 | 3.03 ± 0.13 | 7.52 ± 0.26 | 2.97 ± 0.29 | 7.78 ± 0.35 | 22.35 ± 1.07 |
| Tanat | 10.67 ± 1.16 | 3.11 ± 0.21 | 16.31 ± 1.56 | 4.03 ± 0.67 | 13.16 ± 1.09 | 15.47 ± 1.54 |
| Cabernet Sauvignon | 10.43 ± 0.13 | 2.32 ± 0.15 | 23.95 ± 0.43 | 3.76 ± 0.06 | 7.83 ± 0.07 | 41.74 ± 0.98 |
| Shiraz | 11.01 ± 0.42 | 2.48 ± 0.05 | 22.24 ± 1.90 | 3.79 ± 0.31 | 8.49 ± 0.19 | 28.09 ± 1.04 |

The limit of detection (LOD) and limit of quantification (LOQ) were established by analyzing the calibration curves [34].

The corresponding regression equation and other characteristic parameters for the determination of the phenolic compounds are shown in Table 1. The analytical curves exhibit excellent linear behaviour over the concentration range of about three orders of magnitude with the detection limits ranging from 1.59 to 2.24 μg $mL^{-1}$ for all the analytes. The relative standard deviations (RSD) calculated for 20 and 32.5 μg $mL^{-1}$ were 1.05 and 0.74 % for gallic acid, 3.30 and 10.60 μg $mL^{-1}$ were 3.08 and 1.37 % for quercetin, 15.50 and 32.50 μg $mL^{-1}$ were 1.05 and 0.74 % for caffeic acid, 7.50 e 15.30 μg $mL^{-1}$ were 3.0 and 0.8 % for myricetin, 2.30 e 14.20 were 1.01 and 3.50 % for kaempferol, respectively. Absolute recoveries were evaluated to compare the concentrations found in wine sample spiked with known amounts of each polyphenol. The concentrations were obtained using the calibration curve, and the values obtained were about 98 and 101 % of the recuperation (Table 2).

Polyphenols of Brazilian Wines

Figure 3 presents the electropherogram of the phenolic compounds in the red wine samples and Table 3 shows the concentrations of the six phenolic compounds determined in six Brazilian wines: Gallic (16–42 μg $mL^{-1}$), caffeic (3.16–5.18 μg $mL^{-1}$), syringic (5.73–13.05 μg $mL^{-1}$), kaempferol (2.32–4.33 μg $mL^{-1}$), quercetin (1.68–4.03 μg $mL^{-1}$), myricetin (7.52–25.13 μg $mL^{-1}$). In the literature data on phenolic compounds and the ranges are reported: 39–61 μg $mL^{-1}$ gallic acid, 2.2–8.7 μg $mL^{-1}$ caffeic acid [14], syringic (3.62–7.46 μg $mL^{-1}$) [35], kaempferol (0.17–0.54), quercetin (3.19–16.70), myricetin (1.91–11.87 μg $mL^{-1}$) for red wines. In these wines gallic acid is the major component followed by myricetin. The concentration ranges of the phenolic compounds found in the Brazilian wines is comparable with the data obtained in the literature, even though showing small differences. In an another work the reported values of the concentrations for the phenolic compounds found in red wine, determined by GC using matrix solid-phase dispersion extraction, were: (105–33.78 μg $mL^{-1}$), caffeic acid (0.24–4.10 μg $mL^{-1}$), syringic acid (1.21–5.34 μg $mL^{-1}$), kaempferol (0.05–3.04 μg $mL^{-1}$) [36]. The wide variation in the phenolic concentrations obtained in this work can be explained, at least in part, by analytical and natural variability of the data on the levels of these compounds, since phenolic composition presented in the red wine is more complex and the chemical composition of the wine is intimately correlated with the origin of the grapes, soil type, climate and the process of production and conservation during preparation of the wine. The grapes cultivated in the São Francisco region, are new and are still being developed by adaptation processes to the soil and the climate. The wines produced in this region are young; however, they are of good quality.

## Conclusion

A simple, versatile and low-cost CE method, which utilizes methanol, sodium tetraborate and silica capillaries, was applied for rapid and simultaneous determination of polyphenolic compounds in Brazilian wines. The method provided good limits of detection and quantification, as well as linearity, peak area repeatability and good recovery in the concentration levels studied. Applied to the Brazilian wines, good precision was confirmed.

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