

Capillary electrophoretic separation of phenolic acids

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Abstract

Phenolic acids such as syringic, *p*-coumaric, vanillic, caffeic, 3,4-dihydroxybenzoic and gallic acid, which are present in wines and other alcoholic drinks, were determined by capillary electrophoresis using an uncoated fused-silica capillary. The optimum conditions for their separation were investigated. Examples of electropherograms of phenolic acids contained in some Italian wines are reported.

1. Introduction

Phenolic acids such as syringic, vanillic, caffeic, *p*-coumaric, gallic and 3,4-dihydroxybenzoic acid are present in wines and other alcoholic drinks [1–3]. Considering the importance of their determination, we studied the possibility of the application of capillary electrophoresis. Many papers have been published on the electrophoretic determination of organic acids [4–8]. The determination of organic aliphatic acids in wine by HPLC and capillary electrophoresis has been reported [9]. Oxalic, citric, tartaric, malic, succinic, formic, fumaric and acetic acid have been determined by indirect detection. The HPLC analysis of wine has been described [10–12]. This paper describes the determination of phenolic acids in wine by capillary electrophoresis using an uncoated fused-silica capillary.

2. Experimental

2.1. Instrumentation

Electrophoresis was carried out using a Spectrophoresis 1000 instrument (Spectra Physics, Palo Alto, CA, USA) with rapid scan spectrophotometric detection. An uncoated fused-silica capillary (SGE, Melbourne, Australia) of I.D. 50 μm and total length 43 cm was used. The distance between the anode end of the capillary, where the sample was injected, and the detector was 36 cm. Sampling was carried out using the hydrodynamic method with times ranging between 2 and 5 s. The volumes of sample solution injected were about 4.5 and 10.0 nl, respectively. The operating temperature was 25°C.

2.2. Reagents

All the reagents used were of high purity. Monobasic phosphate, dibasic phosphate, phos-

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phoric acid and sodium carbonate and hydrogencarbonate were supplied by Carlo Erba (Milan, Italy). Phenolic acid standards were supplied by Fluka (Buchs, Switzerland). The solvents used were methanol and diethyl ether, supplied by Carlo Erba.

2.3. Solutions

Solutions of the acid standards studied were prepared by dissolving the pure compounds in water–methanol (1:1) at concentrations of 0.5 mg/ml each. The following buffers were used as background electrolyte (BGE): phosphate (pH 7.2), hydrogencarbonate (pH 8.3) and carbonate–hydrogencarbonate (pH 9.2 and 10.1). All solutions were filtered before use. Benzyl alcohol was used as a marker of electroosmotic flow.

2.4. Operating conditions

Before carrying out analysis, the capillary was washed for 2 min with 0.1 M NaOH solution and then for 2 min with distilled water, and finally conditioned for 4 min with the buffer solution which was to be used for the analysis. Electroosmotic flow was then checked by injecting benzyl alcohol. The reproducibility in terms of migration times of the neutral marker was 0.6% (run-to-run) and 3.0% (day-to-day). After ca. 2 weeks the capillary became less active, as detected by a lower electroosmotic flow. Washing with NaOH solution was insufficient to restore the initial activity. Washing with 1 M HCl restored the initial conditions of the electroosmotic flow and migration times. In order to optimize the conditions for the separation of the phenolic acids studied, several tests were carried out by appropriately varying the pH, ionic strength (concentration of BGE) and applied potential. Table 1 summarizes the parameters concerning the experimental conditions of the tests performed.

For the analysis of real wine samples, it was necessary to extract the phenolic acids from the material, as follows. The wine was evaporated under vacuum to eliminate the ethanol. After addition of buffer of pH 8.2 to 2 ml of evapo-

Table 1
Experimental parameters

pH	V (kV)	C (mM)
7.2	10	50
		100
	15	50
8.3	10	100
		20
		50
	15	80
		20
		50
9.1	20	80
		20
		50
	10	80
		100
		15
10.1	15	80
		100
		50
	20	50
		80
		50

rated wine, the sample was extracted with diethyl ether (the ether extract was discarded). After acidification with HCl to pH 1, the wine was extracted three times with diethyl ether. The ether extract was evaporated under nitrogen and the residue was dissolved in 50 μ l of water–methanol (1:1). Electrophoretic analysis was carried out on this sample. Extraction with diethyl ether at pH 8 allowed the separation of the neutral and basic substances present in the undissociated form. The ionized phenolic substances remained in the aqueous phase. Later, after acidification, the phenolic substances were in undissociated form and were thus extractable with diethyl ether.

3. Results and discussion

Phenolic acids are weak carboxylic acids. They are partly dissociated and hence present in

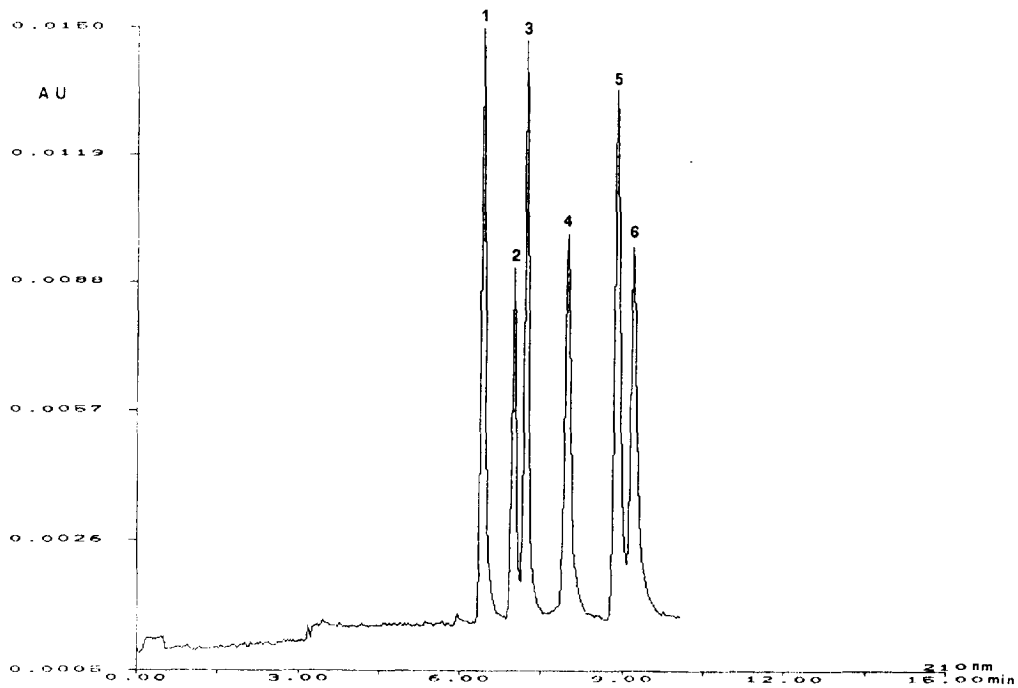


Fig. 1. Electropherogram of standard acid mixture obtained under optimum conditions: pH = 8.3; $V = 15$ kV; BGE concentration = 50 mM hydrogencarbonate buffer; current = 25 μ A. Peaks: 1 = syringic acid; 2 = *p*-coumaric acid; 3 = vanillic acid; 4 = caffeic acid; 5 = 3,4-dihydroxybenzoic acid; 6 = gallic acid.

anionic form. In an electric field they migrate towards the anode. By carrying out electrophoretic analysis in an uncoated fused-silica capillary, there is an electroosmotic flow. Under

these conditions the anions move towards the cathode and it is then possible to detect them. Other experimental parameters being equal, the higher the electroosmotic flow the higher is the

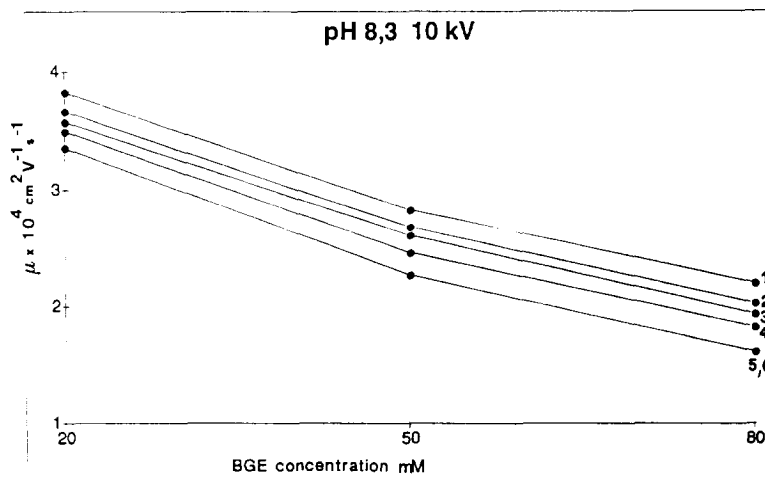


Fig. 2. Apparent mobility of phenolic acids. Conditions: pH = 8.3; $V = 10$ kV.

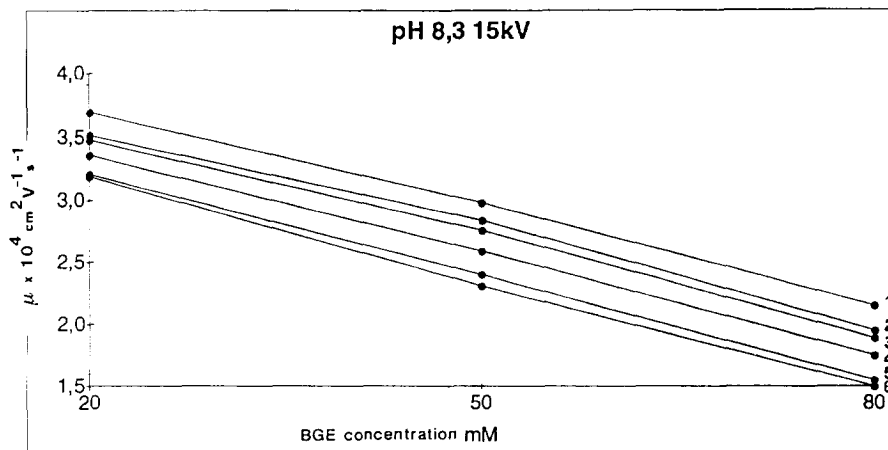


Fig. 3. Apparent mobility of phenolic acids. Conditions: pH = 8.3; $V = 15$ kV.

anion migration velocity [13,14]. Separation tests on phenolic acids carried out under different conditions (see Table 1) demonstrated that the optimum conditions were pH = 8.3 (hydrogen-carbonate buffer), BGE concentration = 50 mM and applied potential = 15 kV. Only these conditions allowed an electropherogram with six distinct peaks (Fig. 1). The results obtained using a longer capillary (76 cm total length) did not significantly change the separation. Figs. 2–4) show the variation of the apparent mobility of

the phenolic acids with BGE concentration. As can be seen, the apparent μ decreases as the BGE concentration rises. This is probably due to the greater screening of the capillary walls by the BGE and, as a result, a decrease in electroosmotic flow. Fig. 5 shows the variation of the apparent mobility with pH. The experimental data show that the apparent mobility decreases as the pH rises. Since the electroosmotic flow was not significantly influenced by the hydrogen ion concentration in the pH range explored (7–

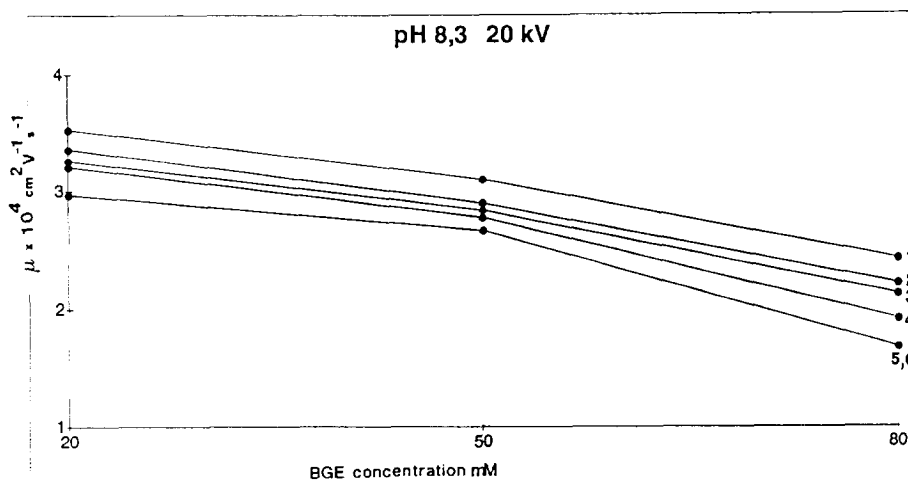


Fig. 4. Apparent mobility of phenolic acids. Conditions: pH = 8.3; $V = 20$ kV.

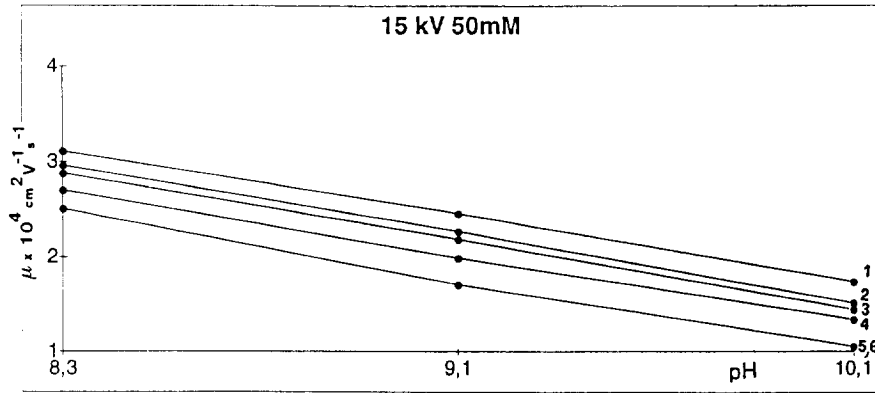


Fig. 5. Apparent mobility of phenolic acids. Conditions: BGE = 50 mM; V = 15 kV.

10), the decrease in the apparent mobility of the acids was probably due to the greater dissociation of the molecules studied (with the resulting “braking” effect of anions with a greater negative charge).

In order to check the reproducibility of the experimental data, the relative standard deviation (R.S.D.) was calculated for the migration times of the acids in the mixture. This value (run-to-run) was always less than 1%. Peak-area integration was carried out on the electropherograms relative to the phenolic acid standard mixture. The peak-area reproducibility (run-to-run) was R.S.D. 1.0–2.2%. Two electrophero-

grams relative to the phenolic acid standard mixture. The peak-area reproducibility (run-to-run) was R.S.D. 1.0–2.2%. Two electrophero-

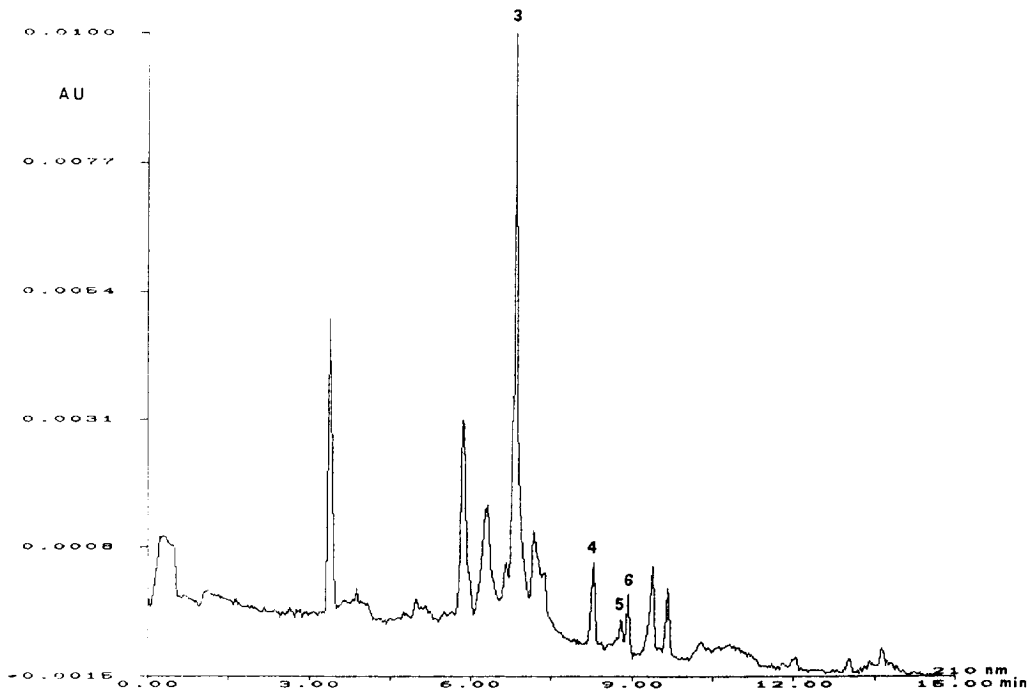


Fig. 6. Electropherogram of sample extracted from Corvo wine. Conditions as in Fig. 1.

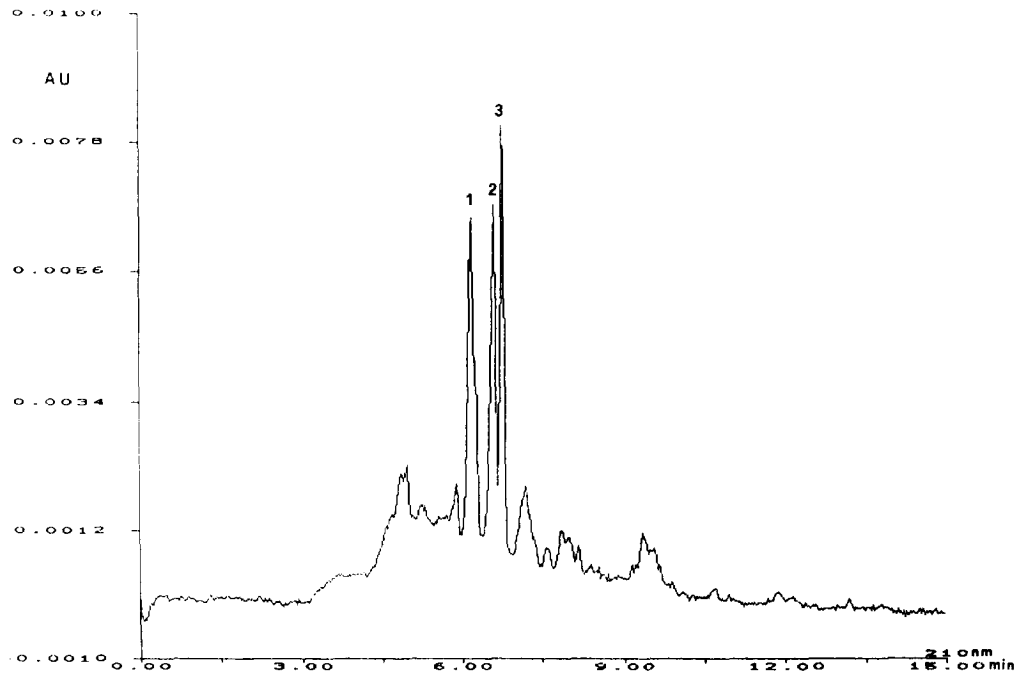


Fig. 7. Electropherogram of sample extracted from Chianti wine. Conditions as in Fig. 1.

grams of samples extracted from Italian red wines are shown: Corvo (Fig. 6) and Chianti (Fig. 7).

References

- [1] L.W. Wulf and C.W. Nagel, *J. Chromatogr.*, 116 (1976) 271–279.
- [2] C.S. Ough and M.A. Amerine, *Methods for Analysis of Musts and Wine*, Wiley, New York, 1988, pp. 196–200.
- [3] A.A. Qureshi, N. Prentic and W.C. Burger, *J. Chromatogr.*, 170 (1979) 343–353.
- [4] W.R. Jones and P. Jandik, *J. Chromatogr.*, 608 (1992) 385–393.
- [5] S.C. Smith and M.G. Khaledi, *J. Chromatogr.*, 632 (1993) 177–184.
- [6] J. Karovicova, J. Polonsky, M. Drdak, P. Simko and V. Vollek, *J. Chromatogr.*, 638 (1993) 241–246.
- [7] S.C. Smith and M.G. Khaledi, *Anal. Chem.*, 65 (1993) 193–198.
- [8] Y. Liu and S.J. Sheu, *J. Chromatogr., A* 663 (1994) 239–243.
- [9] V. Levi, T. Wehr, K. Talmadge and M. Zhu, *Int. Chromatogr. Lab.*, 15, September (1993) 4.
- [10] K.V. Castlelee, H. Geiger and C.F. Van Sumere, *J. Chromatogr.*, 258 (1983) 114–124.
- [11] C.G. Barroso, R.C. Torrijos and J.A.P. Bustamante, *Chromatographia*, 17 (1983) 249–252.
- [12] D.A. Roston, P.T. Kissinger, *Anal. Chem.*, 53 (1981) 1695–1698.
- [13] T. Tsuda, K. Nomura and G. Nagakawa, *J. Chromatogr.*, 264 (1983) 385–392.
- [14] J. Kohr and H. Engelhardt, *J. Microcol. Sep.*, 3 (1991) 491–495.