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Separation of polyphenols in Canary Islands wine by capillary zone electrophoresis without preconcentration ☆

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Abstract

A method for separation and determination of polyphenols in wine by capillary zone electrophoresis (CZE) without any preconcentration step is described. The sensitivity and limits of detection for gentisic and *p*-coumaric acid are better than those previously published. The effect of a possible C_{18} solid-phase extraction prior to the CZE analysis was examined. The developed optimized method (without any extraction step) was applied to the analysis of wines from Tenerife, Canary Islands.

Keywords: Wine; Food analysis; Polyphenols

1. Introduction

In most of the published methods for the determination of trace concentrations of polyphenols in water or wines by HPLC or CE [1–7], typically a sample preconcentration is performed. Such steps, however, consist of several repeated operations (evaporation under vacuum, liquid–liquid extraction, re-extraction, etc.) which are time-consuming and often skill-demanding. With such treatments, the quality of Canary Islands wines has been estimated using various methods [8,9]. On the other hand, Goldberg et al. [10] have shown an HPLC determination of several polyphenolic compounds in wines where the sample was directly injected into the chromatographic column without any pre-treatment. The authors determined concentrations of resveratrol (1–10 mg/l), quercetin (10–20 mg/l), rutin (about 10 mg/l), catechin (30–300 mg/l) and epicatechin (50–100 mg/l).

Wine analysis applications of CE as an alternative to HPLC or other separation methods had been limited to a few papers [7,11,12]. Recently, Arce et al. [1] published a method for the determination of polyphenols in wines by capillary zone electrophoresis (CZE) with on-line flow injection enrichment by solid-phase extraction (SPE) on a C_{18}-cartridge. The authors showed that the recovery for wine analysis is quantitative for *trans*-resveratrol, (−)-

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epicatechin, (+)-catechin, gentisic acid, quercetin, *p*-coumaric acid, salicylic acid and caffeic acid. However, since wine is an ethanolic mixture of dozens of compounds, recovery after such a simple SPE step *cannot* principally be quantitative for all the compounds, i.e., there may be a loss of some analytes. Therefore, direct analysis of samples is often desirable and applicable if it includes a separation technique that is efficient and selective for the analyte.

The aim of this work was to study the separation conditions for the determination of polyphenols in wines by CZE suggested by Ref. [1] and to apply the improved procedure without the SPE step to wine samples from the Canary Islands.

2. Experimental

2.1. Chemicals

(−)-Epicatechin, (+)-catechin, *p*-coumaric acid, gentisic acid, *trans*-resveratrol, caffeic acid and sorbic acid were purchased from Sigma (St. Louis, MO, USA). Ascorbic acid, lactic acid, gallic acid and sodium tetraborate were obtained from Lachema (Brno, Czech Republic). Quartz bidistilled water from a quartz apparatus (Hanau, Germany) was always used. Methanol and ethanol of analytical-grade were obtained from Lachema (Brno, Czech Republic). Sodium tetraborate and tris(hydroxymethyl)aminomethane (Tris) were obtained from Lachema. Mesityloxide from Sigma was used for the electroosmotic flow (EOF) measurements.

2.2. Preparation of solutions

Electrolytes were made from a weighted amount of solid compound dissolved in water. If necessary, the pH of all the electrolytes (except sodium tetraborate) was adjusted with 1 *M* hydrochloric acid to 9.5. Several background electrolytes (BGEs) were tested: a mixture of 25 m*M* Tris and 2-*N*-morpholineethanesulphuric acid (Tris–MES), a mixture of 25 m*M* Tris and 25 m*M* boric acid (Tris–borate) and 25 m*M* sodium tetraborate.

Stock solutions of standards were prepared by dissolving a weighted amount of the standard in ethanol. For experiments with a mixture of standards, the stock solutions were dissolved to the final concentration by water so that 10% (v/v) ethanolic solution of the standards was made.

2.3. Apparatus for CZE

A Spectraphoresis 2000 sytem from Thermo Separation Products, (TSP, San Jose, CA, USA) equipped with fused-silica capillary (Composite Metal Services, The Chase, Hallow, UK), 43 cm (effective length 36.5 cm)×75 μm I.D., was used. The apparatus was thermostated by a Peltier unit and equipped with a fast scanning detector (ranges: 190–350 and 350–600 nm). The apparatus was connected to a PC, data acquisition and electropherograms evaluation were done with PC1000 software of TSP.

After each run the capillary was washed with 0.1 *M* LiOH, then water and BGE. Lithium hydroxide was used rather than sodium hydroxide because it attacks the inner capillary surface less [13].

2.4. EOF measurement

Electroosmotic flow was measured using mesityloxide. For the optimized conditions, the value obtained was $+6.6\cdot10^{-8}$ $m^2/V\,s$. Since our analytes were polyphenols (and weak carboxylic acids) at pH 9.5, the separation process was a cathodically driven electrophoresis of anions.

2.5. Equipment for solid-phase extraction

Columns Silica CART filled with 500 mg of a sorbent Separon SGX RPS 60 μm from Tessek (Prague, Czech Republic) (C_{18} was the stationary phase) were used. Liquids were delivered on the

Table 1
Summary of the optimized conditions

BGE	25 m*M* sodium tetraborate
pH	9.5 (not adjusted)
Voltage	+20 kV
Detection wavelength	305 nm
Injection	hydrodynamic, 5 s (vacuum=7.5 kPa)
Sample preparation	dilution five-fold with water
Temperature	25°C

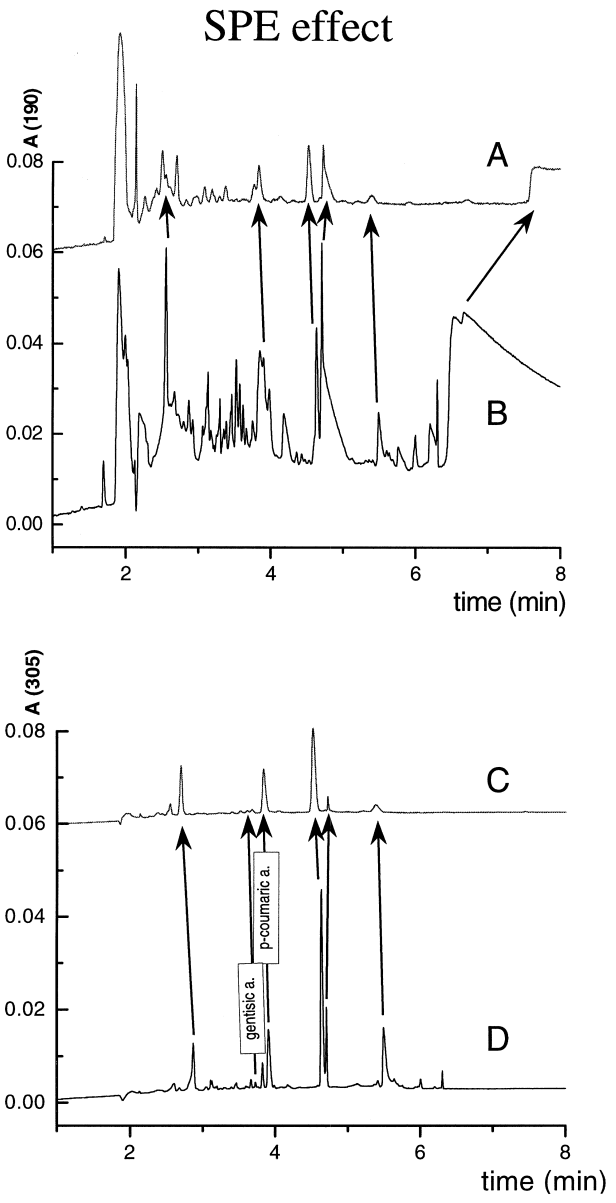

Fig. 1. Effect of solid-phase extraction. The upper pair of electropherograms demonstrates the effect of SPE on white wine monitored at 190 nm: electropherogram A corresponds to sample with SPE, electropherogram B without SPE. Similarly, the lower pair of electropherograms shows the effect of SPE monitored at 305 nm: electropherogram C corresponds to sample with SPE, electropherogram D without SPE. Arrows relate some of the peaks, some of them substantially decreased. The wine was B4 (see Table 3). Experimental conditions: BGE 25 m*M* sodium tetraborate (pH 9.5), voltage +20 kV, injection hydrodynamic 5 s (vacuum, 7.5 kPa), sample was diluted five times with water, temperature 25°C.

column by a peristaltic pump PCR01 from Labeco (Prague, Czech Republic) at a constant flow-rate of 1.0 ml/min.

3. Results and discussion

We studied the basic factors affecting the sepa-

ration in CZE in detail, i.e., the sort of BGE, detection wavelength, separation voltage, sample concentration and injection time. The length of capillary was kept constant. In finding the optimum conditions, the effect of the SPE preconcentration step suggested by Arce et al. [1] was tested.

3.1. CZE separation

As the optimum, 25 m*M* sodium tetraborate was selected, which was also used in Arce et al. [1]. The pH of the samples was in the range 3.0–3.9 and had a negligible effect on the migration times.

Arce et al. [1] used the wavelengths of 280 or 306 nm. We used the wavelength of 305 nm, which is selective for polyphenolic compounds. Standard deviation of signal-to-noise at 305 nm was $s_n = 0.013$ mAU (time constant was 0.5 s). For some experiments with sorbic acid, a wavelength of 260 nm was also used. For comparison, some experiments were also monitored at 190 nm.

Five different separation voltages of +8, +10, +15, +20 and +25 kV (anode at sample side), respectively, were tested and the corresponding number of theoretical plates were calculated for peaks in the range of the product voltage×time of 80–100 kV min.

Sorbic acid was selected for plotting the height equivalent to a theortical plate (HETP) vs. separation voltage. According to the maximum of the graph, the voltage of +20 kV was selected as the optimum, which corresponds to the total run time of 8 min when all the polyphenolic compounds of interest pass through the detection window.

The amount of the sample injected (the product of injection time×concentration) was optimized in order to get an acceptable S/N ratio and so as not to deteriorate the resolution. To guarantee not dis-

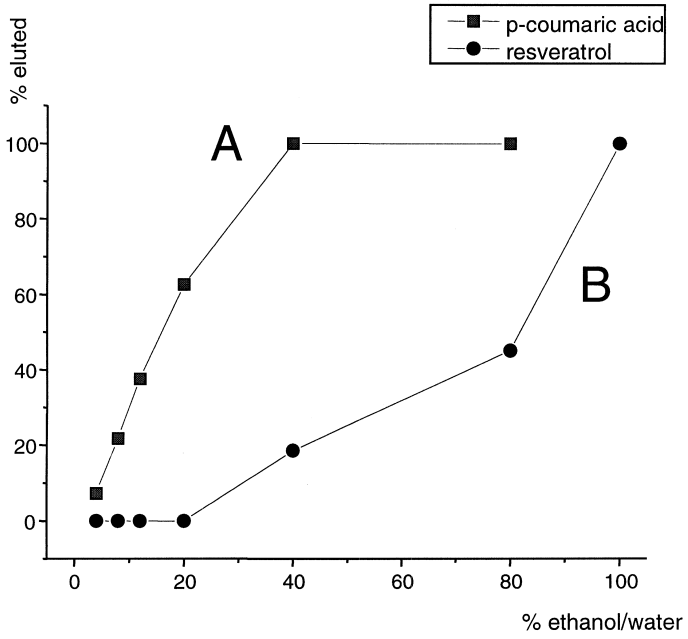

Fig. 2. Recovery after elution by ethanol–water mixture. The graph shows the amount of *trans*-resveratrol and *p*-coumaric acid, respectively, eluted from the SPE column after 5 ml of wine passed the column. The elution was carried out discontinuously by doses of 2 ml an ethanol–water mixture of increasing ethanol concentration. The lower line B shows that *trans*-resveratrol starts to be released from the column at ethanol concentration of 20% which excludes errors in quantative determination. On the other hand, *p*-coumaric acid (graph A) is partially eluted from the column already at ethanol concentration of 4% which illustrates the risk of analyte loss during the SPE procedure.

Table 2
Parameters of the calibration of gentisic and *p*-coumaric acid[a]

Compound	Calibration curve: area(μAU s)= a+conc(mg/l)·b	s_a[b]	s_b	r[c]	RSD[d] (%)	Min. linearity range: area/height (mg/l)	LOD[e] (mg/l)	
							This work	Ref. [1]
Gentisic acid	Area=74.2 + 571.4·conc	90	8	0.9988	3.2	0–20/0–20	0.05	0.27
p-Coumaric acid	Area=−7571.4+ 4467.4·conc	362	28	0.9997	3.1	0–20/0–20	0.01	0.14

[a] The calibration was done with solutions of standards in 10% (v/v) ethanol. Experimental conditions: 25 m*M* sodium tetraborate as the BGE, pH 9.5 (not adjusted), separation voltage=+20 kV, detection wavelength=305 nm, injection hydrodynamic (5 s, vacuum=7.5 kPa), temperature=25°C.

[b] s_a and s_b are the standard deviation of regression parameters a (intercept) and b (slope).

[c] Correlation factor.

[d] LOD was calculated as a concentration read from calibration curve in peak height units corresponding to the signal of $3 \cdot s_n$ (3×standard deviation of blank).

[e] RSD was calculated from three parallel determinations.

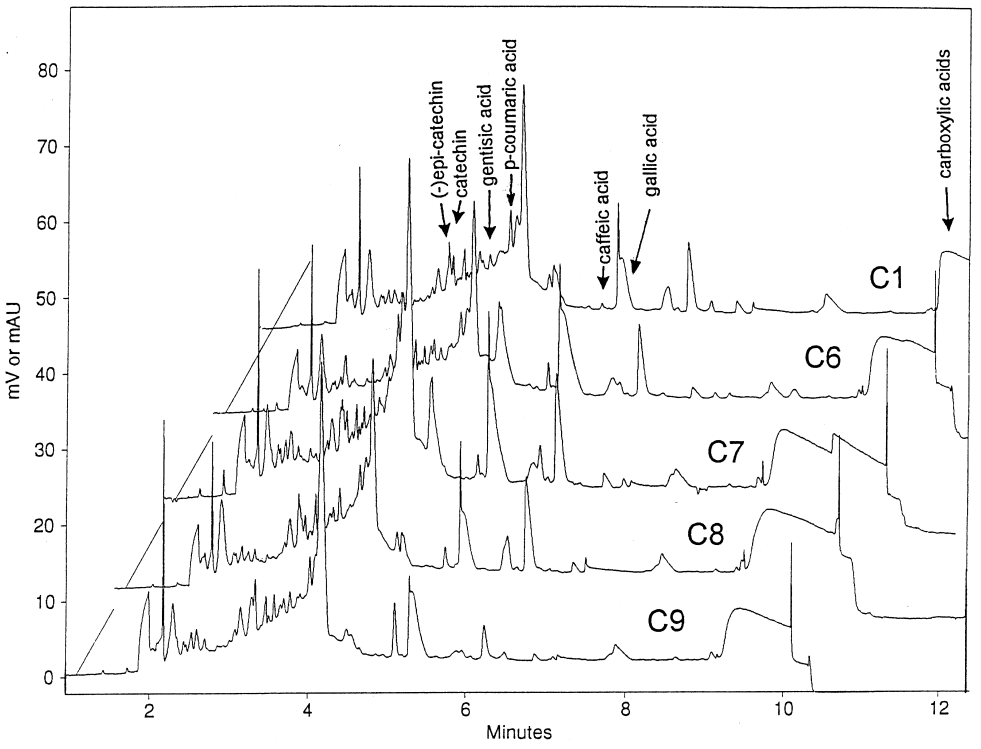

Fig. 3. Electropherograms of red wine samples. Electropherograms are indicated by the codes C1–C9 according to Table 3. Experimental conditions: 25 m*M* sodium tetraborate as the BGE, pH 9.5 (not adjusted), separation voltage +20 kV, detection wavelength 190 nm, injection hydrodynamic (5 s, vacuum=7.5 kPa), sample dilution five times by water, temperature 25°C. The typical intensive fronting peak (elution time about 4 min) is seen.

criminating the introduction of sample constituents, the hydrodynamic mode of injection was selected (vacuum of 7.5 kPa) rather than the electrokinetic one. Due to the fact that injection times of about 1 s exhibit lower repeatability, the injection time of 5 s was selected which implied a five-fold dilution of the sample. A summary of these optimized conditions is given in Table 1.

3.2. The effect of solid-phase extraction

3.2.1. Procedure

The SPE column was conditioned by 3 ml of methanol followed by 10 ml of water, and then 5 ml of wine was introduced on the column and eluted by 2 ml of methanol. To make a reasonable comparison of samples with and without SPE, the methanolic eluate (2 ml) obtained after SPE was diluted to 5 ml by water. The pH of the wine samples varied in a short range between 3.0 and 3.9 and had no principal effect on the procedure.

In Fig. 1 it can been seen that after the SPE treatment (electropherograms A and C), both the heights and areas of the peaks decreased in comparison to the samples without SPE (electropherograms B and D), illustrating a removal of several compounds by which the peak pattern is simplified. As a result, the migration times of the extracts are shifted also. The removal of the compounds would have a positive impact on the analysis if the analytes are not removed, i.e., if they are selectively adsorbed on the SPE column. However, the natural content of ethanol in wines is about 12.5% (v/v). This means that wine itself does exhibit a non-negligible elution strength for C_{18} columns. A graph in Fig. 2 shows

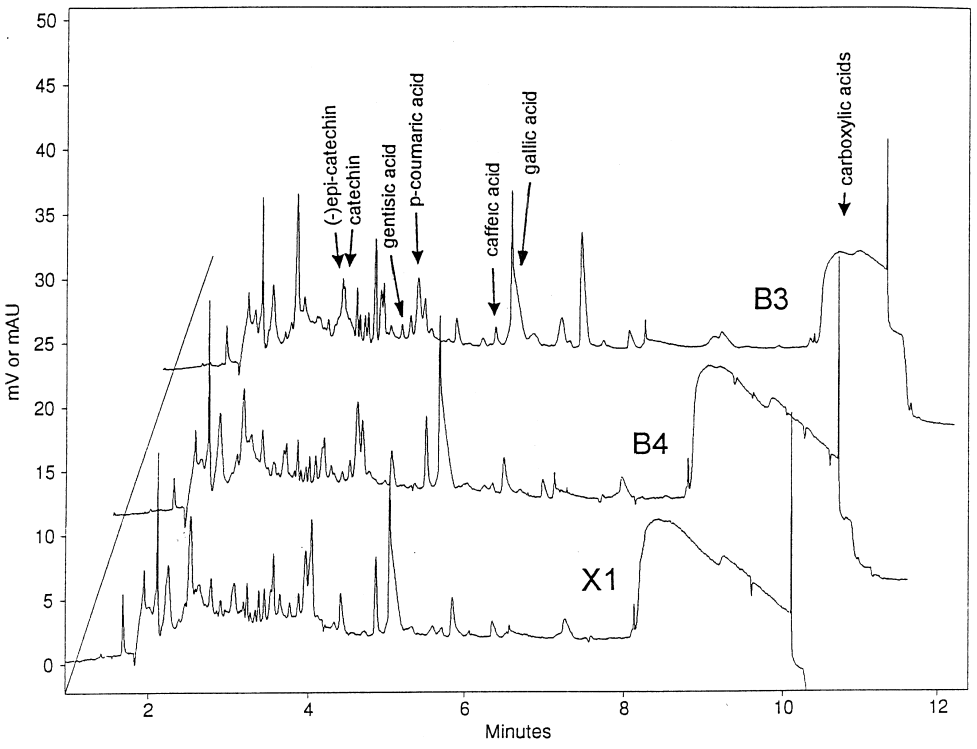

Fig. 4. Electropherograms of white wine and rosé samples. Electropherograms are indicated by the codes B3, B4 and X1 according to Table 3. Experimental conditions: 25 m*M* sodium tetraborate as the BGE, pH 9.5 (not adjusted), separation voltage +20 kV, detection wavelength 190 nm, injection hydrodynamic (5 s, vacuum), sample dilution five times by water, temperature 25°C. The broad tailing peak at 9–10 min corresponds to carboxylic acids naturally present in wines.

the amount of two polyphenolic compounds (*trans*-resveratrol and *p*-coumaric acid) eluted from the SPE column after 5 ml of wine was passed on the column. The elution was carried out discontinuously by doses of 2 ml of an ethanol–water mixture of increasing ethanol concentration. Although these conditions do not exactly simulate the reality of SPE, where the ethanolic solution always contains a mixture of naturally presented compounds already, the figure clearly shows the possibility of analyte loss due to SPE applied to non-diluted wines. Another relevant fact supporting the use of direct analysis of wines is that a SPE step must decrease the precision of any analysis.

These facts show that direct analysis is preferable if a separation method selective enough is used. Since wine is a mixture of many compounds, selectivity of determination by CZE can be reached by the use of 305 nm as the detector wavelength rather than 190 nm (Fig. 1). The wavelength of 260 nm is selective and useful for direct determination of sorbic acid. On the other hand, the number of peaks obtained at 190 nm is suitable for fingerprints even if some of them do not represent resolved compounds.

Table 2 summarizes the parameters obtained from calibration of direct analysis of two polyphenols – gentisic and *p*-coumaric acid. The calibration was done with solutions of standards in 10% (v/v)

Table 3
Determination of gentisic and *p*-coumaric acids in wines[a]

	Code	Wine, year, producer and denomination of origin	Gentisic acid (mg/l)	*p*-Coumaric acid (mg/l)
White wines	B3	Viña Donia, 1996, S.A.T. Tajinaste, Tenerife, Ycoden Daute-Isora, Spain	0.08	3.81
	B4	Viñátigo, 1997, Bodegas Viñátigo, C.B. Tenerife, Ycoden-Daute-Isora, Spain	0.08	4.72
Rosé	X1	Viñátigo Rosado, 1997, Bodegas Viñátigo C.B., Tenerife, Ycoden-Daute-Isora, Spain	0.42	3.46
Red wines	C1	Viña el Valle, 1996, J.C. González, Tenerife, Valle de la Orotava, Spain	0.39	1.41
	C6	La isleta, 1996, A. González Cruz, Bodega la Isleta, Taroconte Acentejo, Spain	0.27	0.82
	C7	Monje, 1997, Bodgas Monje, El Sauza, Tenerife, Taroconte Acentejo, Spain	0.86	2.27
	C8	El lomo, 1997, Afecan L.A., Taroconte Acentejo, Spain	1.26	1.36
	C9	Brumas de Ayosa, 1996, SAT Güímar Arafo, Valle de Güímar, Spain	0.65	0.58

[a] Experimental conditions: 25 m*M* sodium tetraborate as a BGE, pH 9.5, separation voltage +20 kV, wavelength 305 nm, injection hydrodynamic (5 s, vacuum 7.5 kPa), sample dilution 5 times by water, temperature 25°C.

ethanol. For a comparison, the last column shows limits of detection (LODs) published in Ref. [1].

3.3. Fingerprints of wine samples

In Figs. 3 and 4 fingerprints of wine samples are displayed. The identification of some of the peaks was done by spiking.

A typical characteristic of red wines can be observed – an intensive peak elution time of about 4 min. The pattern of the electropherogram at 190 nm of rosé wine resembles that of white wine though the

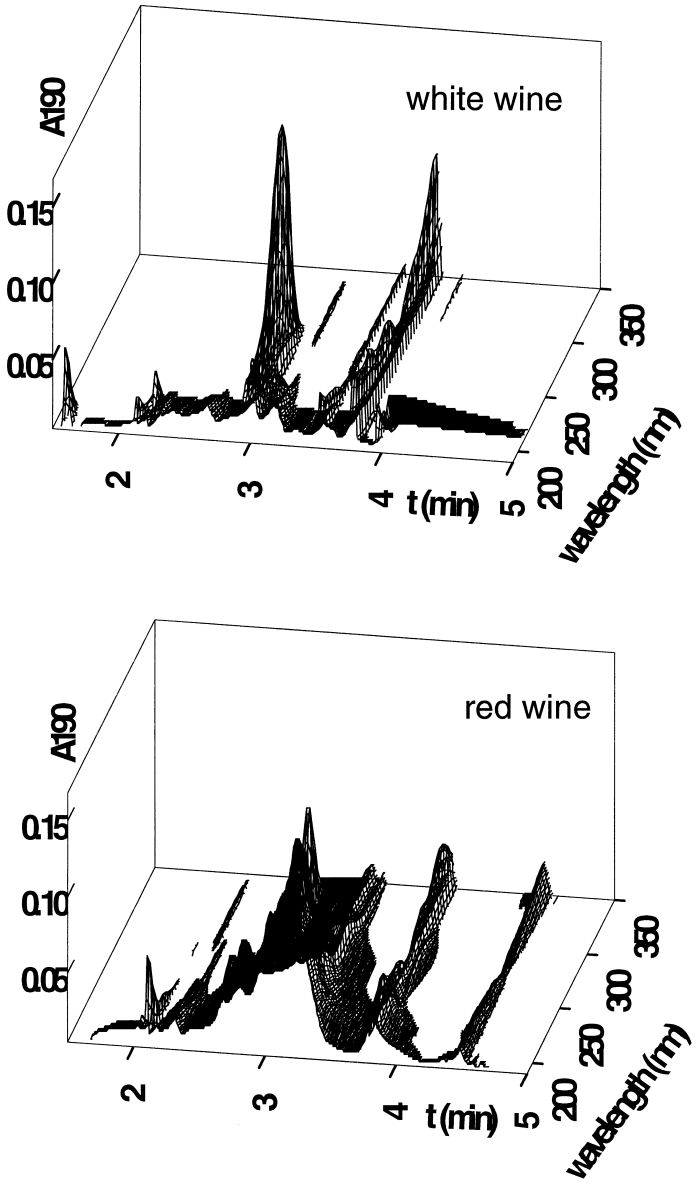

Fig. 5. Three-dimensional-electropherograms of white (B3) and red (C1) wines. To make the graph more illustrative, signals below 0.005 AU were suppressed. Experimental conditions: 25 m*M* sodium tetraborate as the BGE, pH 9.5 (not adjusted), separation voltage +20 kV, detection wavelength 190 nm, injection hydrodynamic (20 s, vacuum=7.5 kPa), sample dilution five times by water, temperature 25°C.

higher content of gentisic acid (Table 3) is similar to that of red wines (red wines have a higher content of gentisic acid than white wines). Red wines exhibit a lower content of *p*-coumaric acid than white wines. The differences between red and white wines are better visible from the three-dimensional-electropherograms in Fig. 5. The pattern of a white wine exhibits two dominant peaks (with maxima at ca. 3 min/300 nm and 3.8 min/350 nm, respectively) while a red wine pattern shows several less intensive peaks (with maxima at ca. 2.8 min/300 nm, 3.0 min/290 nm, 3.7 min/350 nm and 4.5 min/350 nm). We must point out that not all the signals represent individual or identified compounds, which, however, does not exclude the use of the characteristic patterns for the purposes of qualitative comparison.

4. Conclusions

For the determination of compounds that cannot be quantitatively (without a loss) preconcentrated by SPE, the advantages of direct analysis of wine by capillary electrophoresis was found.

The optimized procedure of direct analysis was applied to wine samples from different denominations of origin localized in Tenerife, Canary Islands, producing a more precise, faster and technically simpler analysis, and therefore resulting in a higher throughput of the method. Moreover, the limits of detection were better than those obtained by other methods previously published.

Electropherograms at 190 nm, or the three dimensional-electropherograms exhibit characteristics of wine and may be used as fingerprints.

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