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Determination of Polyphenols in Spanish Wines by Capillary Zone Electrophoresis. Application to Wine Characterization by Using Chemometrics

Helena Franquet-Griell, Antonio Checa, Oscar Núñez,* Javier Saurina, Santiago Hernández-Cassou, and Lluis Puignou

Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain

ABSTRACT: A capillary zone electrophoresis (CZE) method for the simultaneous determination of 20 polyphenols in wine was developed. The separation was performed using fused-silica capillaries of 75 μm i.d. and a 30 mM sodium tretraborate buffer solution at pH 9.2 with 5% isopropanol as a background electrolyte. A capillary voltage of +25 kV with pressure-assisted (3.5 kPa) separation from minute 18 was applied, thus achieving a total analysis time of <25 min. Instrumental quality parameters such as limits of detection (LOD, values between 0.3 and 2.6 mg/L), linearity (r^2 > 0.990), and run-to-run and day-to-day precisions (RSD values lower than 6.5 and 15.7%, respectively) were established. Three different calibration procedures were evaluated for polyphenol quantitation in wines: external calibration using standards prepared in Milli-Q water, standard addition, and pseudomatrix-matched calibration using wine as a matrix. For a 95% confidence level, no statistical differences were observed, in general, between the three calibration methods (p values between 0.11 and 0.84), whereas for some specific polyphenols, such as cinnamic acid, syringic acid, and gallic acid, results were not comparable when external calibration was used. The CZE method using pseudomatrix-matched calibration was then proposed and applied to the analysis of polyphenols in 49 Spanish wines, showing satisfactory results and a wide compositional variation between wines. Electrophoretic profiles and other compositional data (e.g., peak areas of selected peaks) were considered as fingerprints of wines to be used for characterization and classification purposes. The corresponding data were analyzed by principal component analysis (PCA) to extract information on the most significant features contributing to wine discrimination according to their origins. Results showed that a reasonable distribution of wines depending on the elaboration areas was found, tyrosol and gallic, protocatechuic, p-coumaric, and caffeic acids being some representative discriminant compounds.

KEYWORDS: *polyphenols, phenolic acids, wines, capillary zone electrophoresis, PCA*

INTRODUCTION

Moderate consumption of wine has been associated with reduced risks of cardiovascular diseases and cancer, as well as with several beneficial effects on the human immune system and cognitive functions.[1] Health-promoting properties such as antioxidant, antibacterial, anti-inflammatory, antiallergic, and antithrombotic activities have been related with the presence of polyphenols.[2] Other phenolic compounds, such as phenolic acids, catechins, and some flavonoids, play an important role in wine quality, contributing to flavor and color properties, especially in red wines.[3,4] Thus, the determination of polyphenols in wines, using reliable methods, for quality control and assessment of wines because of their effects on health and the taste of these products is considered at the moment a priority.

High-performance liquid chromatography (HPLC) has been the technique of choice for the quantification of phenolic compounds in wine using either UV absorption spectroscopy[5−12] or mass spectrometry (LC-MS).[13,14] Other analytical techniques such as gas chromatography coupled to mass spectrometry,[15] polycyclic sensors,[16,17] or cyclic voltammetry[18] have also been recently reported for the analysis of these compounds.

Lately, the utilization of capillary electrophoresis (CE) has increased as an alternative to LC because of his high efficiency, rapid analysis, and low reagent consumption. The application of CE to the determination of phenolic compounds in beverages[19] and foods,[20,21] including wine, has been reviewed. A specific revision of methods for quantifying resveratrol in wine is also given elsewhere.[22] For instance, capillary zone electrophoresis (CZE) methods using phosphate- or borate-based electrolytes have been described for the quantitative analysis of phenolic acids,[23−28] resveratrol,[26,29] flavonols,[26,30] catechins,[27−30] and different flavonoids.[24,31] Other CE techniques, such as micellar electrokinetic chromatography (MEKC) with sodium dodecyl sulfate (SDS), have also been applied to the determination of phenolic acids[32,33] and flavonoids.[32−34] However, from the point of view of wine analysis, no more than 10 common polyphenols are usually quantified in many of these works. Some of these CE studies focused solely on the determination of the phytoalexin resveratrol.[35−37] Detections often rely on UV spectroscopy using diode array devices, but other techniques such as voltammetry[29] or CE coupled to mass spectrometry (CE-MS)[14] have also been employed.

Obtaining reliable quantitative data for the quantification of polyphenols in wine using capillary electrophoresis is still necessary. For instance, some comparisons between the quantitative performance

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of HPLC and CE methods have been carried out. In some studies, no significant qualitative and quantitative differences in the results were obtained by the two techniques.[28] In other cases, small differences were reported.[30,31] For this reason, different calibration procedures must be evaluated for polyphenol quantitation in wine samples by CE.

The characterization and classification of wines can be tacked from compositional profiles as a source of analytical information. Families of natural wine components such as low molecular weight organic acids, alcohols, esters, polyphenols, amino acids, biogenic amines, and inorganic species have been found to be efficient descriptors of some climatic, agricultural, and enological features. Hence, such compositional data can be treated by chemometric methods such as principal component analysis (PCA), partial least-squares regression (PLS), and discriminant analysis (DA) for classification, quantification, and authentication purposes.[38]

This work was aimed at developing and evaluating a CZE method for the simultaneous determination of 20 polyphenols in wine, without any sample treatment. Quality parameters, such as limits of detection (LODs), limits of quantitation (LOQs), linearity, and run-to-run and day-to-day precisions, were established by using two different CE instruments. Three calibration procedures (external calibration, standard addition, and pseudomatrix-matched calibration) were also evaluated and compared for the analysis of polyphenols in wine samples. The proposed CZE method was applied to the quantification of polyphenols in various Spanish wines. The contents of representative compounds were exploited as potential descriptors of the geographical region of wines. Graphs of the wine distribution obtained by using PCA showed significant clustering as a function of origin.

MATERIALS AND METHODS

Reagents and Solutions. Syringic acid, *p*-coumaric acid, homovanillic acid, protocatechuic acid, resveratrol, fisetin, (−)-epicatechin, quercitrin hydrate, and 4-hydroxybenzoic acid standards of analytical grade were obtained from Sigma-Aldrich (Steinheim, Germany). 2-(4-Hydroxyphenyl)ethanol (tyrosol), *trans*-cinnamic acid, gallic acid, veratric acid, homogentistic acid, caffeic acid, sinapic acid, ferulic acid, vanillin, and (+)-catechin were purchased from Fluka (Steinheim, Germany), and quercetin dihydrate was from Riedel-de Haën (Seelze, Germany).

HPLC gradient grade methanol and isopropanol were obtained from Merck (Darmstadt, Germany), and sodium tetraborate was purchased from Sigma-Aldrich.

Stock standard solutions of all polyphenols (~1000 mg/L) were prepared in methanol. Intermediate working solutions were prepared weekly from these stock standard solutions by appropriate dilution with water. All stock solutions were stored at 4 °C for not more than 1 month. Background electrolyte (BGE) was prepared daily by diluting a 100 mM sodium tetraborate solution and adding the appropriate amount of isopropanol. BGE solutions were filtered through 0.45 μm nylon filters (Whatman, Clifton, NJ, USA).

Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA) and filtered through a 0.22 μm nylon filter integrated into the Milli-Q system.

Apparatus. The experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Fullerton, CA, USA) equipped with a diode array detection system. The electrophoretic separation was carried out using uncoated fused silica capillaries (Beckman) with a total length of 60 cm (effective length 50 cm) × 75 μm i.d. The BGE consisted of 30 mM sodium tetraborate buffer solution (pH 9.2) containing 5% (v/v) isopropanol. Capillary temperature was held at 25 °C. The BGE was filtered through a 0.45 μm membrane filter and degassed by sonication before use. Samples were loaded by pressure-assisted hydrodynamic injection (10 s, 3.5 kPa). The electrophoretic separation of polyphenols was performed by applying a capillary voltage of +25 kV. Pressure-assisted separation (3.5 kPa) from minute 18 was used. Direct UV absorption detection was carried out from 190 to 310 nm (sample quantitation was performed at 280 nm). This CE instrument was controlled using Beckman 32 Karat software, version 5.0. Peak integration was performed valley-to-valley by taking into account the baseline shift showed in the electropherograms.

To study the method performance, a Beckman P/ACE 5500 CE System (Beckman) was also used. With this instrument, a fused silica capillary with a total length of 57 cm (effective length 50 cm) × 75 μm i.d. was used. This CE instrument was controlled using Beckman P/ACE station software, version 1.2. All other acquisition conditions were equal to those of the MDQ CE instrument.

Capillary Conditioning. New capillaries were pretreated with 0.1 M hydrochloric acid for 60 min, with water for 60 min, with 0.1 M sodium hydroxide for 60 min, and finally with water for 60 min. At the beginning of each working session, the capillary was rinsed with sodium hydroxide for 30 min, with water for 30 min, and with the BGE for 60 min. The capillary was rinsed with the BGE for 5 min between runs. At the end of each session, the capillary was stored after rinsing with water.

Data Analysis. MATLAB (version 6.5) was used for calculations. PCA was from the PLS-Toolbox.[39] A detailed description of this method is given elsewhere.[40]

The plot of scores showing the distribution of the samples on the principal components (PCs) may reveal patterns that may be correlated to sample characteristics, in this case sample origin. The study of the distribution of variables (loadings plot) provided information dealing with their correlations and possible relationships with wine properties. Additionally, the simultaneous study of the scores and loadings (biplot) was used to explore the relationships between samples and variables.

Samples. A total of 102 red wines were purchased from a supermarket in Barcelona, Spain. These wines were chosen in two batches: (i) one batch of 49 wines chosen to get a variety of wines produced in several regions of Spain to establish the CZE method and (ii) another batch of 53 wines chosen from three selected Spanish regions (Catalunya, La Rioja, and Castilla-La Mancha) to study wine characterization according to their region of origin. All wines were analyzed from freshly opened bottles; determinations were always done in <48 h to preserve polyphenol content. Samples were directly injected into the CE system after a filtration step using 0.45 μm nylon filters (the first 1−2 mL of filtrate was rejected). No further sample treatment was performed. The analytes were identified by comparison of the migration times with those of aqueous standards as well as those obtained by spiking the wines with standards.

RESULTS AND DISCUSSION

Optimization of the Separation. As was mentioned in the Introduction, most of the works dealing with analysis of polyphenols in wines by CE have been focused on a few compounds (the most abundant ones). However, for wine characterization and better understanding of health-promoting properties, it can be interesting to study the presence of other polyphenols, although they may not occur at relatively high concentrations. For this reason, in this study a CZE method was developed for the simultaneous separation and determination of 20 polyphenols in red wines. Borate-based buffers were chosen as BGE for the electrophoretic separation as they provided pH values around 9.2, making them suitable for the separation of this family of compounds in positive polarity mode. However, the addition of organic solvents is mandatory to improve the electrophoretic separation. In this work, a solution of sodium tetraborate containing isopropanol as organic modifier was selected as BGE separation. The optimization of the percentage of organic solvent and electrolyte concentration

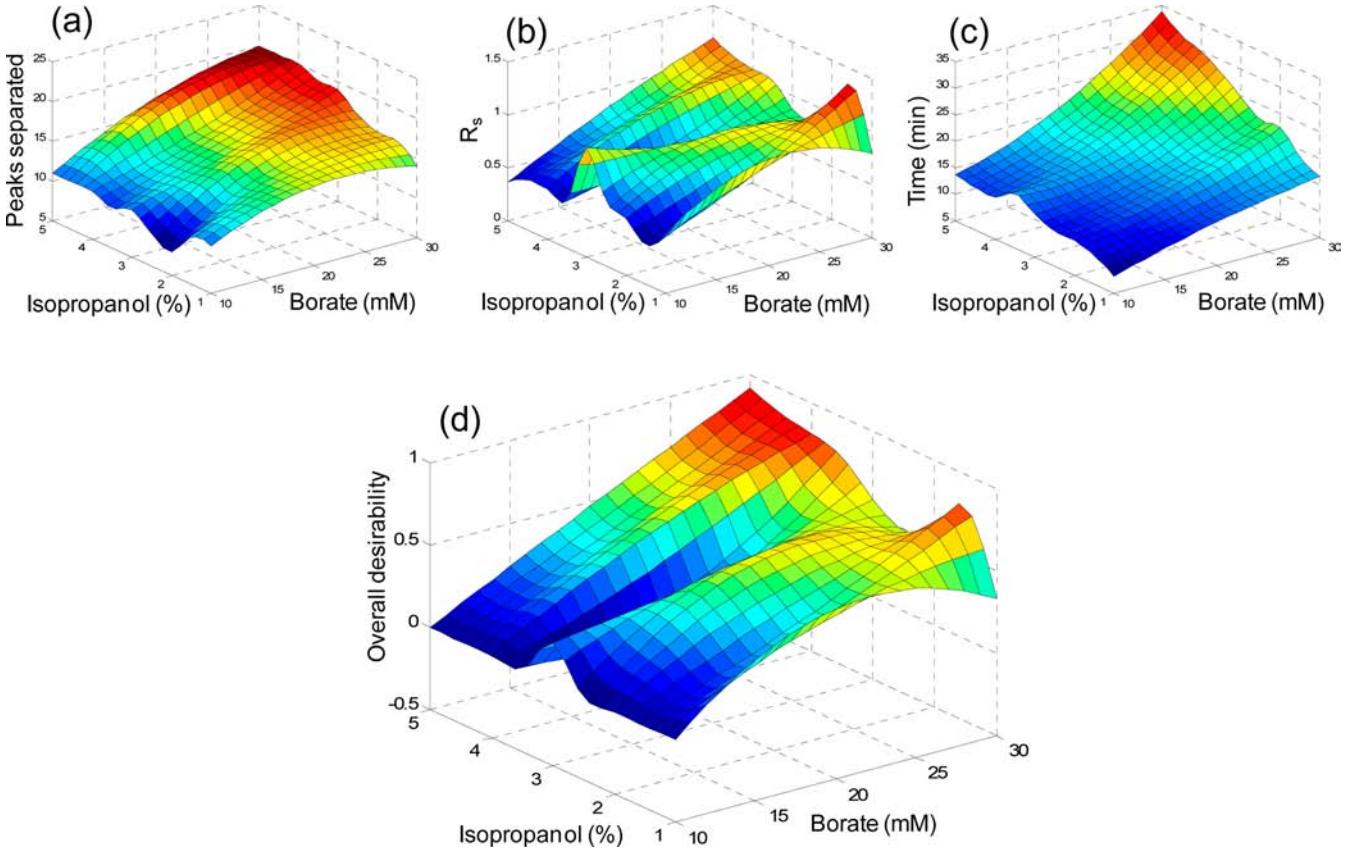

Figure 1. Simultaneous optimization of isopropanol percentage and borate buffer concentration from a 5 × 3 grid design: (a) number of peaks separated; (b) resolution between *p*-coumaric and quercetin peaks; (c) run time; (d) overall desirability.

in the running buffer relied on experimental design. A standard mixture containing the 20 polyphenolic compounds under study was prepared to evaluate the performance of the separation. In this case, a two-factor grid design was defined. Concentrations of isopropanol and borate buffer were assayed at five levels (from 1 to 5%, in steps of 1%) and three levels (10, 20, and 30 mM), respectively. As a result, a total of 5 × 3 experiments were carried out. The criterion for finding the optimal experimental conditions was based on obtaining the best separation, in terms of number of resolved peaks (N_{peaks}) and resolution (R_s), in the minimum run time (t_{run}). Figure 1 shows the response surfaces obtained for each of the objectives considered. In the case of N_{peaks}, the maximum was achieved at 5% isopropanol and 30 mM borate buffer. For R_s of *p*-coumaric and quercetin peaks, two maxima were found, which corresponded to 5% isopropanol and 10 and 30 mM borate. For t_{run}, which was estimated from the migration time of the last peak of the electropherogram (2,3-dihydroxybenzoic acid), the faster runs were obtained at 1% isopropanol and 10 mM borate.

To reach a suitable compromise among these three objectives, a combined desirability response was defined as follows: $D = (d_{peaks} \times d_{res} \times d_{time})^{1/3}$, where d_{peaks}, d_{res} and d_{time} are the normalized (desirability) contributions of N_{peaks}, R_s, and t_{run}, respectively. Experimental values of N_{peaks}, R_s, and t_{run} were used to estimate the corresponding individual desirabilities according to the following transformations: (i) $d_{peaks} = 0$ for $N_{peaks} \leq 10$, $d_{peaks} = 1$ for $N_{peaks} = 20$, and $0 < d_{peaks} < 1$ for $10 < N_{peaks} < 20$; (ii) $d_{res} = 0$ for $R_s \leq 0.7$, $d_{res} = 1$ for $R_s \geq 1.5$, and $0 < d_{res} < 1$ for $0.7 < R_s < 1.5$; (iii) $d_{time} = 0$ for $t_{run} \geq 45$ min, $d_{time} = 1$ for ≤10 min R_s, and $0 < d_{time} < 1$ for $45 < t_{run} < 10$ depicts the overall desirability *D*. The maximum values of this surface were attained at 5% isopropanol and 30 mM borate buffer, so these experimental conditions were selected as optimal. Under these conditions, analytes were separated in about 40 min by applying +25 kV. An increase in capillary voltage was not useful to reduce analysis time because the electrophoretic separation worsened significantly. However, as the last migrating polyphenols 4-hydroxybenzoic acid, caffeic acid, gallic acid, and 3,4-dihydroxybenzoic acid (peaks 17−20, respectively) presented a high separation, an overimposed pressure of 3.5 kPa was applied at minute 18 to reduce the analysis time. Separation was then accomplished in <25 min. Figure 2 shows the electropherogram of a 30 mg/L standard of all polyphenols obtained under optimal conditions: 30 mM tetraborate buffer with 5% isopropanol as BGE, separation at +25 kV, and pressure-assisted separation (3.5 kPa) from minute 18. Although some pairs of compounds were not baseline separated (pairs 3/4, 8/9, and 15/16 with resolutions of 0.7, 0.8, and 1.0, respectively), the separation can be considered to be acceptable as a compromise between resolution and analysis time. Hydrodynamic injection time (2−25 s) was also studied to increase sensitivity. An injection time of 10 s (3.5 kPa) was selected as an optimal compromise between peak signal and resolution.

Instrumental Quality Parameters. Instrumental quality parameters of the proposed CZE method under optimal conditions were evaluated using two CE instruments. Figures of merit are given in Table 1. LODs, based on a signal-to-noise ratio of 3:1, were calculated using standard solutions at low concentration levels (in the range of 0.3−2.6 mg/L). The values obtained are similar to those reported in the literature with CE

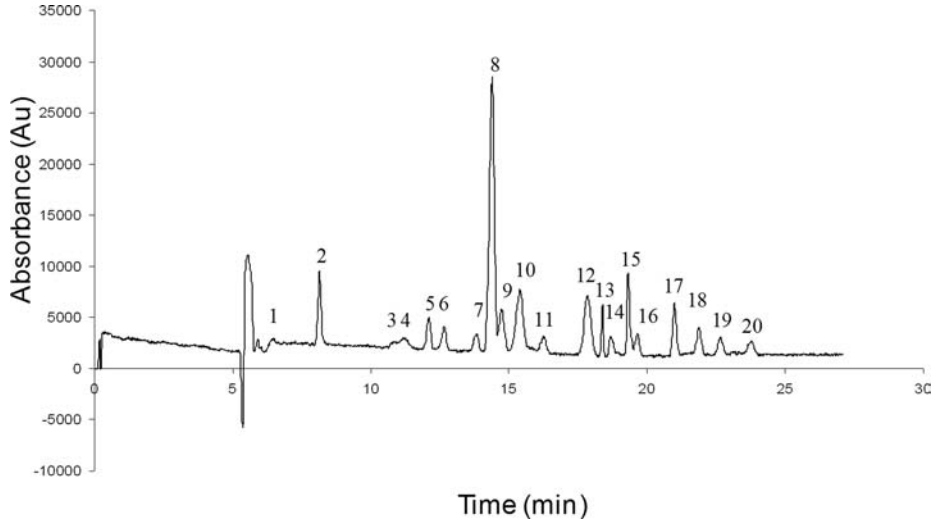

Figure 2. Electrophoretic separation of an aqueous standard mixture of 20 polyphenols. BGE, 30 mM tetraborate buffer with 5% isopropanol; capillary voltage, +25 kV; pressure-assisted separation (3.5 kPa) from minute 18; acquisition wavelength, 280 nm. For peak identification, see Table 1.

Table 1. Instrumental Quality Parameters

						run-to-run precision (% RSD, n = 5)				day-to-day precision (% RSD, n = 3 × 5)			
						migration time		concentration[a]		migration time		concentration[a]	
peak	compound	LOD (mg/L)	LOQ (mg/L)	working range (mg/L)	linearity	MDQ CE	5500 CE	MDQ CE	5500 CE	MDQ CE	5500 CE	MDQ CE	5500 CE
1	2-(4-hydroxyphenyl)ethanol	0.5	1.7	2−200	>0.990	0.1	0.3	2.1	4.5	0.8	1.6	9.5	11.0
2	resveratrol	1.6	5.1	5−200	>0.992	0.3	0.1	0.6	1.7	1.2	1.3	6.7	6.7
3	(−)-epicatechin	2.4	8.0	8−100	>0.990	0.6	0.5	1.2	2.3	0.6	0.8	8.5	9.2
4	(+)-catechin	2.5	8.1	8−100	>0.996	0.3	0.4	1.5	2.8	0.7	1.2	7.8	8.9
5	veratric acid	0.3	1.0	1−100	>0.997	0.2	0.4	2.9	4.2	0.8	1.8	12.3	11.5
6	homovanillic acid	0.3	1.1	1−200	>0.998	0.3	0.2	1.4	2.1	0.6	1.9	11.4	10.7
7	vanillin	0.7	2.4	2−100	>0.999	0.1	0.3	2.3	6.5	1.9	2.2	10.1	10.3
8	*trans*-cinnamic acid	0.4	1.4	1−100	>0.998	0.3	0.4	3.1	2.1	0.7	2.2	15.7	13.7
9	sinapic acid	0.9	3.1	3−100	>0.996	0.2	0.3	2.9	1.5	0.5	2.3	11.6	10.6
10	quercitrin	0.9	2.8	3−100	>0.990	0.3	0.3	2.5	1.3	0.4	2.5	14.6	11.8
11	homogentistic acid	0.9	2.8	3−100	>0.998	0.4	0.4	3.9	2.1	0.7	2.7	13.8	10.5
12	syringic acid	0.6	1.9	2−100	>0.996	0.2	0.6	4.4	2.8	1.5	3.4	11.6	11.3
13	ferulic acid	0.5	1.8	2−100	>0.998	0.2	0.1	3.6	1.8	1.5	2.3	13.3	13.9
14	fisetin	0.7	2.2	2−100	>0.999	0.6	0.1	2.7	5.8	0.9	1.0	14.8	10.9
15	*p*-coumaric acid	0.7	2.3	2−100	>0.999	0.04	0.1	1.6	3.4	1.4	0.8	14.1	12.7
16	quercetin	2.6	8.5	8−100	>0.998	0.2	0.2	1.4	2.4	0.7	1.0	10.2	10.6
17	4-hydroxybenzoic acid	0.4	1.4	1−100	>0.999	0.1	0.1	1.9	2.5	1.3	0.8	9.9	9.8
18	caffeic acid	0.5	1.7	2−100	>0.998	0.2	0.2	2.8	4.7	2.1	0.9	11.7	10.6
19	gallic acid	2.1	6.9	7−250	>0.998	0.2	0.1	2.5	4.0	2.1	0.9	12.7	11.1
20	3,4-dihydroxybenzoic acid	0.6	2.1	2−100	>0.998	0.2	0.2	5.0	4.2	2.2	1.8	10.7	11.6

[a]Concentration = 30 mg/L. Quantitation performed by external calibration.

methods when using UV detection.[26,33] LOQs, based on a signal-to-noise ratio of 10:1, between 1.0 and 8.5 mg/L were obtained. Calibration curves based on peak area at concentrations between 1 and 100 mg/L (higher concentrations for some compounds) were established. Good linearity was observed for all compounds with correlation coefficients (r^2) >0.990.

Run-to-run and day-to-day precisions for compound quantification, at a concentration level of 30 mg/L (using standard solutions), were calculated by external calibration for the two CE instruments (P/ACE MDQ and P/ACE 5500). To obtain the run-to-run precision, five replicate determinations were carried out. Similarly, day-to-day precision was calculated by performing 15 replicate determinations on three nonconsecutive days (five replicates each day). To better validate the proposed method, precision was evaluated using two different CE instruments. The RSDs obtained for run-to-run and day-to-day precisions were similar using both CE instruments (in the ranges of 0.6−6.5 and 6.7−15.7%, respectively). These results showed that the proposed method was satisfactory in terms of precision for the quantitative analysis of polyphenols and phenolic acids. Run-to-run precision was also evaluated using pseudomatrix-matched calibration by performing five replicate determinations of a wine sample matrix spiked at two concentration levels (10 and 30 mg/L). RSD values in the ranges 5.7−11.2 and 3.4−8.9% for concentration levels of 10 and 30 mg/L, respectively, were obtained. Pseudomatrix-matched

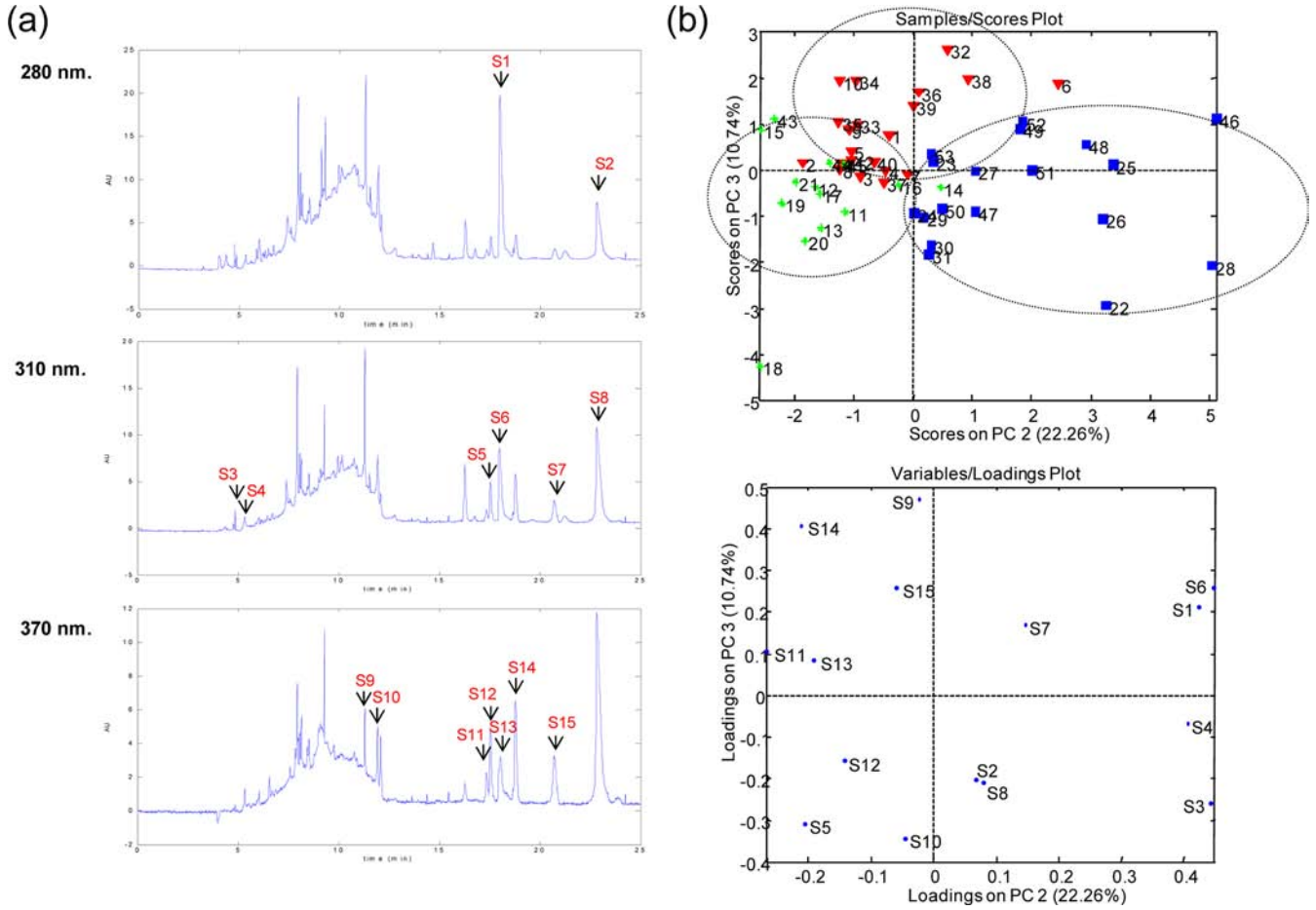

Figure 3. (a) Electropherograms of a wine sample recorded at 280, 310, and 370 nm. (b) PCA results (score and loading plots) using selected peak areas as analytical data.

calibration showed better precision as expected because it allows the correction of the baseline shift observed in the wine electropherograms. Finally, Table 1 also shows that good run-to-run and day-to-day precisions of migration times were also obtained, with RSD values of <3.4%.

Analysis of Polyphenols in Spanish Wines. To evaluate the applicability of the proposed method to the determination of 20 polyphenols and phenolic acids in real samples, 49 commercial Spanish wines were analyzed. No sample treatment was applied, and the wines were only filtered through 0.45 μm nylon membranes before injection. Figure 3a shows, as an example, the electropherogram obtained for the analysis of a wine sample at three different acquisition wavelengths. As can be seen, electrophoretic profiles of standards are much simpler than those of the wines due to the components of the sample matrix. For this reason, prior to analysis of all wine samples, three different quantitation methods were evaluated: (i) external calibration using standards prepared in water, (ii) standard addition, and (iii) pseudomatrix-matched external calibration (using a wine sample as matrix). These three calibration methods were applied to the analysis of five selected wines.

First, wine samples were analyzed using standard addition to establish the concentration of polyphenols in each sample. All of the analyses were performed in triplicate, and the results are given in Table 2. Compound identification was based on the concordance of retention time and UV absorption spectrum with those of the standards. The same samples were then analyzed by external calibration using standards prepared in Milli-Q water and by pseudomatrix-matched calibration. As no wine free of polyphenols can be found, for pseudomatrix-matched calibration two wines with low concentrations of polyphenols were used as sample matrices to prepare all of the other standards to be used in the calibration, and the concentration of each standard was then calculated by taking into account the basal level in the native wine. These analyses were also performed in triplicate with each quantitation method, and the results are also given in Table 2. In all cases pseudomatrix-matched calibration provided results similar to the standard addition calibration. External calibration using standards prepared in Milli-Q water seems to give also results similar to, or slightly different from, those observed with standard addition. Nevertheless, to see if there is any statistical difference between these results, a statistical paired-sample comparison analysis was performed with the results obtained using either external calibration or pseudomatrix-matched calibration procedures with those established by standard addition. For a 95% confidence level, the results achieved with the three calibration procedures were not significantly different, with p values (Table 2) >0.05 (probability at the confidence level) in all cases. However, it must be mentioned that for some compounds (such as *trans*-cinnamic, syringic acid, and gallic acid) in some wines, statistical differences between external calibration and standard addition were observed. As a consequence, the optimized CZE method, using pseudomatrix-matched calibration with standards prepared in wine matrix, can be proposed as an economic and rapid method for the analysis of polyphenols

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Table 2. Comparison of Calibration Procedures for Polyphenol Quantitation in Spanish Wines by the Proposed CZE Method[a]

		wine 1			wine 2			wine 3			wine 4			wine 5		
peak	compound	EC	SA	pMM	EC	SA	pMM	EC	SA	pMM	EC	SA	pMM	EC	SA	pMM
1	2-(4-hydroxyphenyl) ethanol	60.2 ± 5.0	56.3 ± 2.0	58.9 ± 4.0	89.7 ± 6.5	75.9 ± 4.3	80.3 ± 6.1	115.1 ± 14.9	98.71 ± 9.9	109.0 ± 9.9	85.1 ± 6.3	86.3 ± 4.3	84.1 ± 2.9	~LOD	~LOD	~LOD
2	resveratrol	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD
3	(−)-epicatechin	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD
4	(+)-catechin	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD
5	veratric acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	homovanillic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	vanillin	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	nd	nd	nd	~LOD	~LOD	~LOD	nd	nd	nd
8	*trans*-cinnamic acid	5.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	5.1 ± 0.2	1.5 ± 0.3	2.1 ± 0.3	6.5 ± 0.1	2.5 ± 0.2	2.0 ± 0.2	~LOD	~LOD	~LOD	6.1 ± 0.4	2.3 ± 0.5	3.4 ± 0.5
9	sinapic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	quercitrin	20.5 ± 0.4	23.6 ± 1.3	20.4 ± 0.7	12.9 ± 0.1	10.6 ± 0.1	11.9 ± 0.1	34.5 ± 2.5	25.6 ± 0.8	30.2 ± 0.9	21.7 ± 1.7	28.5 ± 5.6	27.0 ± 2.4	15.9 ± 2.0	20.7 ± 3.0	19.0 ± 2.3
11	homogentistic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	syringic acid	7.6 ± 0.8	3.2 ± 1.0	4.0 ± 1.0	~LOD	~LOD	~LOD	4.7 ± 0.4	3.1 ± 0.4	2.2 ± 0.4	12.7 ± 2.0	8.0 ± 4.7	9.1 ± 5.0	19.2 ± 0.9	9.6 ± 1.6	10.2 ± 1.3
13	ferulic acid	~LOD	~LOD	~LOD	16.3 ± 1.9	6.8 ± 0.7	7.5 ± 0.5	nd	nd	nd	nd	nd	nd	~LOD	~LOD	~LOD
14	fisetin	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	*p*-coumaric acid	nd	nd	nd	4.8 ± 0.7	3.3 ± 1.5	6.7 ± 1.5	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	2.5 ± 0.3	5.1 ± 0.8	4.9 ± 0.7
16	quercetin	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	nd	nd	nd	11.8 ± 0.8	5.1 ± 0.5	3.9 ± 0.5
17	4-hydroxybenzoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18	caffeic acid	~LOD	~LOD	~LOD	9.0 ± 1.2	8.0 ± 1.9	8.9 ± 1.7	2.2 ± 0.3	3.8 ± 1.0	3.9 ± 0.9	5.3 ± 0.8	6.6 ± 1.2	6.6 ± 1.1	8.4 ± 0.7	9.1 ± 0.7	12.7 ± 0.5
19	gallic acid	43.1 ± 1.9	82.3 ± 19.5	91.0 ± 4.6	71.0 ± 4.2	51.6 ± 4.5	63.1 ± 5.2	100.7 ± 5.4	87.6 ± 1.2	80.5 ± 1.0	45.5 ± 3.6	77.0 ± 23.7	58.4 ± 4.3	35.2 ± 4.2	43.5 ± 16.7	43.8 ± 5.0
20	3,4-dihydroxybenzoic acid	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD	nd	nd	nd	~LOD	~LOD	~LOD	~LOD	~LOD	~LOD
	p value[b]	0.48		0.46	0.09		0.11	0.12		0.70	0.35		0.38	0.84		0.61

[a]All concentrations are in mg/L. Quantitations performed by triplicate ($n = 3$); results expressed as concentration mean of samples analyzed ± standard deviation. EC, external calibration; SA, standard addition; pMM, pseudomatrix-matched calibration; nd, not detected. [b]For a 95% confidence level.

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Table 3. Polyphenol Concentration Levels in Spanish Wines Obtained by the Proposed CZE Method[a]

peak	compound	wine 6	wine 10	wine 16	wine 18	wine 20	wine 25	wine 36	wine 38	wine 40	wine 45	wine 47	wine 49	concentration range	av ± SD
1	2-(4-hydroxyphenyl) ethanol	99.4 ± 9.7	125.8 ± 17.5	59.7 ± 2.0	63.4 ± 0.8	77.23 ± 2.1	45.8 ± 1.2	61.1 ± 0.5	70.0 ± 2.6	68.1 ± 1.4	54.2 ± 8.0	145.9 ± 4.9	65.2 ± 2.5	0.3–145.9	71.34 ± 22.59
2	resveratrol	9.4 ± 0.6	~LOD	22.5 ± 0.2	~LOD	23.9 ± 0.1	24.1 ± 0.1	25.6 ± 0.05	25.6 ± 0.2	28.0 ± 0.1	21.2 ± 0.2	20.9 ± 0.01	20.7 ± 0.1	0.8–28	18.00 ± 9.78
3	epicatechin	~LOD	~LOD	16.9 ± 1.7	nd	12.9 ± 2.3	13.9 ± 2.0	52.6 ± 17.6	50.3 ± 4.8	15.4 ± 1.2	nd	5.1 ± 0.1	2.2 ± 0.04	1.2–154.1	24.34 ± 31.93
4	catechin	~LOD	~LOD	0.7 ± 0.1	nd	0.8 ± 0.05	0.6 ± 0.05	nd	4.0 ± 0.6	nd	11.2 ± 1.1	nd	1.2 ± 0.5	0.6–86.5	7.99 ± 19.68
5	veratric acid	7.14 ± 0.8	10.7 ± 1.3	35.6 ± 0.1	9.5 ± 0.0	39.9 ± 6.0	31.0 ± 2.9	19.3 ± 1.6	nd	23.4 ± 0.7	2.0 ± 0.05	6.9 ± 0.1	19.6 ± 2.3	2–40.6	17.49 ± 11.26
6	homovanillic acid	nd	nd	nd	nd	nd	21.6 ± 3.3	nd	nd	3.6 ± 0.4	nd	nd	nd	2.24–181	49.17 ± 68.27
7	vanillin	nd	nd	nd	nd	nd	11.0 ± 1.1	16.6 ± 2.1	17.6 ± 0.8	5.8 ± 0.4	nd	6.8 ± 0.8	nd	0.35–21.1	8.39 ± 6.48
8	*trans*-cinnamic acid	nd	2.1 ± 0.1	10.3 ± 0.1	nd	nd	7.7 ± 0.1	12.6 ± 1.0	12.9 ± 0.4	2.1 ± 0.1	4.3 ± 0.4	6.1 ± 0.1	6.2 ± 0.02	0.2–19.7	5.98 ± 4.21
9	sinapic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
10	quercitrin	13.0 ± 2.6	3.6 ± 0.5	7.0 ± 1.4	3.5 ± 0.6	13.8 ± 1.3	12.8 ± 0.1	4.9 ± 0.9	11.9 ± 1.0	24.1 ± 0.7	2.6 ± 0.2	8.3 ± 0.9	7.5 ± 0.3	1.4–31.9	12.90 ± 7.77
11	homogentisic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
12	syringic acid	~LOD	3.9 ± 0.5	5.7 ± 0.2	7.9 ± 0.4	nd	3.4 ± 0.5	6.9 ± 0.3	9.3 ± 0.07	1.7 ± 0.3	3.6 ± 0.4	4.6 ± 0.7	5.3 ± 0.4	0.3–15.5	4.94 ± 3.19
13	ferulic acid	~LOD	~LOD	nd	10.0 ± 0.4	9.9 ± 0.02	9.7 ± 0.05	12.7 ± 0.6	11.6 ± 0.6	7.5 ± 0.2	nd	5.4 ± 0.7	nd	0.25–15.5	8.69 ± 4.04
14	fisetin	6.1 ± 0.7	10.6 ± 1.6	nd	15.0 ± 0.3	nd	nd	18.5 ± 1.3	17.4 ± 0.5	7.5 ± 1.2	11.6 ± 2.0	nd	nd	0.35–18.5	10.40 ± 5.17
15	*p*-coumaric acid	1.35 ± 0.01	7.9 ± 1.2	16.1 ± 0.6	19.0 ± 0.5	11.7 ± 0.5	8.6 ± 0.5	15.0 ± 0.3	14.4 ± 0.02	6.4 ± 0.3	2.3 ± 0.4	9.1 ± 0.6	11.0 ± 0.3	0.35–19	9.37 ± 5.42
16	quercetin	~LOD	~LOD	30.7 ± 0.1	32.6 ± 1.2	8.5 ± 0.06	30.4 ± 1.0	34.2 ± 0.6	33.9 ± 0.02	33.3 ± 0.9	28.6 ± 0.4	1.6 ± 0.2	1.7 ± 0.3	0.3–34.7	18.88 ± 14.36
17	4-hydroxybenzoic acid	~LOD	~LOD	nd	nd	nd	4.9 ± 0.1	nd	8.2 ± 0.2	nd	nd	nd	nd	0.2–13.2	3.92 ± 4.70
18	caffeic acid	1.9 ± 0.2	4.3 ± 0.1	8.3 ± 0.1	11.1 ± 0.3	9.1 ± 0.02	8.4 ± 0.1	13.9 ± 0.3	12.6 ± 0.3	3.9 ± 0.2	4.5 ± 0.3	2.4 ± 0.3	4.3 ± 0.01	0.25–15.6	7.55 ± 3.92
19	gallic acid	57.9 ± 8.1	103.5 ± 3.5	46.7 ± 2.5	59.8 ± 0.9	69.8 ± 2.5	16.3 ± 0.6	35.8 ± 0.3	53.7 ± 2.0	54.6 ± 0.7	111.8 ± 15.7	50.9 ± 7.2	9.1 ± 5.5	9.1–209.2	55.40 ± 30.49
20	3,4-dihydroxybenzoic acid	4.0 ± 0.6	~LOD	9.4 ± 0.9	nd	8.8 ± 0.0	6.7 ± 0.4	14.0 ± 0.3	21.45 ± 0.9	13.1 ± 0.7	2.3 ± 0.3	2.9 ± 0.03	4.6 ± 0.1	0.3–21.45	7.04 ± 5.12

[a]All concentrations are in mg/L. Quantitations performed by triplicate (n = 3); results are expressed as mean of samples analyzed ± standard deviation.

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in wine samples, providing a good idea of polyphenol concentration levels for wine characterization.

Table 3 shows the concentration levels of polyphenols found in 12 of the 49 commercial Spanish wines analyzed, and the concentration range observed for each polyphenol, as well as the average concentration and the standard deviation, are also included. As shown in the table, a wide compositional variation was observed. Five polyphenols were found in all of the analyzed samples: 2-(4-hydroxyphenyl)ethanol, resveratrol, quercitrin, caffeic acid, and gallic acid. Coumaric acid, veratric acid, cinnamic acid, syringic acid, quercetin, and 3,4-dihydroxybenzoic acid were also found in almost all wines analyzed. Gallic acid was usually found at relatively high concentrations, with values ranging from 9 to 209 mg/L. 2-(4-Hydroxyphenyl)ethanol was also found at relatively high concentrations in most of the samples (from 33 to 145 mg/L). The other polyphenols found in the analyzed samples presented, in general, concentration levels ranging from LOD to ~50 mg/L, although in some wines high concentration levels were observed for some specific polyphenols such as homovanillic acid in wines 22 and 23 (155 and 181 mg/L, respectively), epicatechin in wine 49 (154 mg/L), or catechin in wines 22 and 24 (66 and 70 mg/L, respectively). Only 2 of the 20 polyphenols analyzed (sinapic acid and homogentisic acid) were not detected in any sample. It should be pointed out that polyphenol levels found in this work for red wines are, in general, in agreement with those described in the literature for these kind of samples.[25,33] The wide compositional variation and number of polyphenols found in the analyzed wines show that the determination of a high number of polyphenols is necessary for a better wine characterization.

Principal Component Analysis. The developed CZE-UV method was also evaluated to see if either the electrophoretic profile or the polyphenol profile was useful for wine characterization in relation to the region of origin. For this purpose, a batch of 53 Spanish wines from three different regions (Catalunya, La Rioja, and Castilla-La Mancha) was analyzed with the proposed CZE-UV (average concentrations for each polyphenol compound are presented in Table 4), and the results were treated by PCA.

Raw electrophoretic profiles were first evaluated as a source of analytical information for building characterization models. Because electropherograms showed a certain degree of variability in the migration time of components, the extraction of solid conclusions was hindered. This drawback was solved by peak alignment of electropherograms at each recorded wavelength using Correlation Optimized Warping (COW) written for MATLAB. Owing to the complexity of the electrophoretic profiles, COW was inefficient in dealing with peak shifting in the whole time range, so the correction was performed on three different time window subsets as follows: 0−11, 11−19, and 19−25 min. After COW application, electropherograms at each wavelength were reconstituted, and the resulting data sets were analyzed by PCA. Exploratory results showed the predominance of Catalunya and Rioja wines in some parts of the plots of scores, although some of the samples appeared in the wrong positions. With regard to the Castilla-La Mancha region, samples lay in an intermediate zone and mixed with the other classes.

Because the presence of irrelevant data in the set under study may hinder the extraction of reliable conclusion regarding origin, the next step was focused on the selection of discriminat features. In this case, peak areas of the most descriptive peaks were taken as analytical data to be treated by PCA. In particular, the data set consisted of 15 peak areas of known and unknown

Table 4. Polyphenol Concentration Levels in the Three Regions Analyzed[a]

peak	compound	Catalunya	La Rioja	Castilla-La Mancha
1	2-(4-hydroxyphenyl) ethanol	77.7 ± 12.7	62.4 ± 15.7	77.9 ± 14.7
2	resveratrol	22.7 ± 4.4	23.9 ± 1.6	13.1 ± 7.0
3	epicatechin	58.0 ± 22.7	21.3 ± 14.7	nd
4	catechin	5.7 ± 3.9	2.2 ± 2.6	nd
5	veratric acid	9.1 ± 6.4	23.3 ± 11.6	16.8 ± 10.2
6	homovanillic acid	nd	13.4 ± 8.1	nd
7	vanillin	11.1 ± 5.6	11.9 ± 4.9	nd
8	*trans*-cinnamic acid	7.2 ± 3.1	8.1 ± 3.7	4.9 ± 1.2
9	sinapic acid	nd	nd	nd
10	quercitrin	12.7 ± 6.6	12.7 ± 9.1	11.3 ± 7.6
11	homogentisic acid	nd	nd	nd
12	syringic acid	6.0 ± 2.5	5.6 ± 2.0	7.2 ± 5.4
13	ferulic acid	9.2 ± 2.9	11.0 ± 2.6	7.7 ± 5.9
14	fisetin	13.7 ± 3.8	15.2 ± 2.2	7.9 ± 2.0
15	*p*-coumaric acid	7.5 ± 4.5	13.9 ± 3.7	7.5 ± 6.1
16	quercetin	31.2 ± 1.8	31.5 ± 1.6	15.2 ± 12.4
17	4-hydroxybenzoic acid	10.7 ± 3.5	6.3 ± 4.3	nd
18	caffeic acid	7.9 ± 3.0	9.0 ± 3.0	6.2 ± 3.8
19	gallic acid	51.4 ± 26.4	49.4 ± 21.4	42.5 ± 17.1
20	3,4-dihydroxybenzoic acid	3.0 ± 2.0	3.5 ± 2.3	12.6 ± 3.7

[a]All concentrations are in mg/L. Results expressed as mean of samples analyzed ± standard deviation.

compounds extracted as follows: two peaks at 280 nm, six peaks at 310 nm, and seven peaks at 370 nm (see Figure 3a). PCA results showed that PC1 was mainly focused on the description of the peak intensities, and variance dealing with geographical characteristics was not retained. Information of the origin of wines was captured by PC2 and PC3. The scatter plot of scores of PC2 versus PC3 (Figure 3b) suggested that wines from Catalunya were located on the right part, whereas Rioja wines appeared on the top and central-left side. Castilla-La Mancha wines were mainly on the left side, and they seemed to be less distinguishable from the other classes. The distribution of variables with respect to PC2 and PC3 showed that samples with higher contents of compounds S1, S3, S4, and S6 were typical of Catalunya. Species S9, S14, and S15 were quite characteristic of Rioja, and compounds S5, S11, and S12 were more abundant in Castilla-La Mancha wines. Some of these peaks have not been identified yet. For the known components, tyrosol and gallic acid were more characteristic of Catalunya, *p*-coumaric and caffeic acids were encountered at higher levels in Rioja samples, and protocatechuic was more specific of Castilla-La Mancha wines.

The results obtained in this study show that the developed CZE method, using pseudomatrix-matched calibration with standards prepared in wine matrix, can be proposed as a rapid and economical method for the determination of polyphenols in wine samples. The method was applied to analyze these compounds in 49 commercial Spanish wines from different regions. Eighteen of the 20 polyphenols studied were detected and, in most of the samples, quantified, gallic acid and 2-(4-hydroxyphenyl)ethanol being the compounds found at higher concentrations. The peak areas of the most abundant compounds (some of them identified by comparison with standards and some of them unknown) resulted in an excellent source of information to carry out the wine characterization. Results from PCA proved that such compositional data allowed wines to be clustered

according to their origins. Besides, the most discriminant analytes representative of each geographical area were identified.

AUTHOR INFORMATION

Corresponding Author

*Phone: 34-93-403-37-06. Fax: 34-93-402-1233. E-mail: oscar.nunez@ub.edu.

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Notes

The authors declare no competing financial interest.

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