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ORIGINAL ARTICLE

Analysis of Organic Acids in Wine by Capillary Electrophoresis with Direct UV Detection

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A capillary electrophoretic method using a phosphate-based carrier electrolyte and direct UV detection at pH 6.5 for the routine determination of five organic acids in wine samples is described. The present method shows advantages over other commonly applied method based on phthalic acid buffer with indirect UV detection for the quantification of organic acids. Factors that affect capillary electrophoretic separation such as concentration and pH of the background electrolyte, concentration of the electroosmotic flow modifier, and methanol addition were considered. Separation and determination of tartaric, malic, acetic, succinic and lactic acids were achieved in approximately 6 min. The method was quantitative, with recoveries in the 98–107% range, and linear over more than one order of magnitude. The precision is better than 0.94–1.06% for migration time and 0.40–0.96% for peak area. The method is sensible, with detection limits between 0.015 and 0.054 mg L^{-1}. The usefulness of the method was successfully demonstrated by the analysis of the considered organic acids in 39 red wines from two different Spanish Certified Brands of Origin (CBO). Determinations were made by direct injection after the appropriate sample dilution and filtration. Distributions of organic acids in Ribeira Sacra wines show a predominance of lactic (3784–452 mg L^{-1}) and tartaric acids (1987–866 mg L^{-1}) followed by succinic (897–398 mg L^{-1}), malic (2844–not detected mg L^{-1}) and acetic (749–116 mg L^{-1}) acids. An analogous distribution was observed in the Bierzo wines: lactic acid (4037–179 mg L^{-1}), tartaric acid (1819–772 mg L^{-1}), succinic acid (646–389 mg L^{-1}), malic acid (1513–not detected mg L^{-1}) and acetic acid (553–214 mg L^{-1}).

Key Words: capillary electrophoresis; direct UV detection; organic acids; wine.

INTRODUCTION

The determination of organic acids in foods and beverages provides relevant information from the standpoint of monitoring the fermentation process, checking

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product stability, validating the authenticity of juices or concentrates and studying the organoleptic properties of fermented products (Klampfl *et al.*, 2000). Generally, organic acids have been determined by a range of chromatographic techniques such as gas chromatography (GC) (Deng, 1997; Haila *et al.*, 1992; Hu *et al.*, 1994), high performance liquid chromatography (HPLC) (Dong, 1998; Vonach *et al.*, 1998; Linget *et al.*, 1998; Escobal *et al.*, 1997; Falque-Lopez and Fernandez-Gomez, 1996) and ion chromatography (IC) (Zhu *et al.*, 1997; Ding *et al.*, 1995; Lourdes *et al.*, 1998). These methods are precise and accurate but there is still a demand for techniques that while offering the same degree of automatization, provide better separation efficiency than those obtained in liquid chromatography and that can avoid time-consuming derivatization procedures often necessary in GC. Taking into account the above considerations, the need for alternative methods of determination for organic acids is clear. In the last few years, capillary electrophoresis (CE) has become a versatile analytical technique employed in the determination of different types of analytes in a great variety of matrices (Sádecká and Polonsky, 2000; Landers, 1997; Khaledi, 1998) due to its adequate analytical characteristics such as low consumption of chemicals, high resolution, high speed, and simplicity. Therefore, CE was applied for the determination of certain organic acids in grapes, wine, solid wine residues and alcoholic beverages (Mallet *et al.*, 1999; Nutku and Erim, 1999; Kandl and Kupina, 1999).

In this paper, a recently developed capillary electrophoretic method using a phosphate-carrier electrolyte and direct UV determination (Castiñeira *et al.*, 2000) is studied comparatively with another CE method (phthalic acid buffer with indirect UV detection) for the analysis of organic acids in wine samples as an alternative technique for the simultaneous, quick and simple analysis of five organic acids having enological interest in wine samples. In order to obtain the best separation and quantification properties, co-electroosmotic conditions such as the pH, the concentration of background electrolytes (BGE), concentration of electroosmotic flow (EOF) modifiers and the addition of organic solvents were evaluated.

MATERIALS AND METHODS

Instrumentation

All experiments were performed using a Waters Quanta 4000 capillary electrophoresis system (Waters Chromatography, Milford, MA) equipped with a UV on-column detector and a negative power supply. Separation was carried out on fused silica capillaries with 60 cm total length $\times$ 75 μm of internal diameter (Composite Metal Services Ltd., U.K.). Data acquisition and processing were carried out by means of the Package Millennium v. 2.15 (Waters Chromatography, Milford, MA) in a Digital Venturis 446 computer. The filtration system used consisted of an All-Glass filter support equipped with 0.45 μm HA membrane filters (Millipore Co., Bedford, MA). The pH measurements were made using a Digilab 517 pH meter (Crison Instrumental S.A., Barcelona, Spain).

Reagents

All chemicals, obtained from different suppliers, were of analytical grade and all of them were used without any purification. Carrier electrolytes and standard solutions were prepared in ultra-pure water (resistivity 18.2 $M\Omega\,cm^{-1}$) provided by a Milli-Q

system (Millipore Co., Bedford, MA), except for the phthalic acid which was dissolved in a 50:50 v/v methanol–ultra-pure water mixture.

Organic Acids

Tartaric acid (dihydrate sodium salt), succinic acid (hexahydrate sodium salt) and acetic acid (potassium salt) were purchased from Avocado (Barcelona, Spain). Malic acid (disodium salt) and lactic acid (sodium salt) were obtained from Aldrich (Madrid, Spain). Standards of the organic acids were prepared daily from a $1\,g\,L^{-1}$ stock solution and diluted to the required concentration before use.

Background electrolytes (BGE) and carrier electrolyte preparation. NaH_2PO_4 was supplied by Panreac (Barcelona, Spain); Na_2HPO_4 by Acros Organics (Geel, Belgium); phthalic acid by Avocado (Barcelona, Spain); myristyltrimethylammonium bromide (MTAB) by Aldrich (Madrid, Spain) and methanol by Merck (Darmstadt, Germany). The carrier electrolytes were prepared daily, filtered through a Millipore 0.45 μm HA membrane filter and degassed in an ultrasonic bath before use. Two carriers were optimized, and their compositions (unless otherwise specified) were as follows.

- Carrier A: 3 mM phosphate with 0.5 mM MTAB as EOF modifier at pH 6.5.
- Carrier B: 7 mM of phthalic acid, 2 mM of MTAB, 5% v/v of methanol at pH 6.1.

Capillary Column Conditioning

In order to obtain a stable baseline, the capillary was conditioned daily by washing it according to the following cycle: 0.1 M NaOH for 5 min followed by ultra-pure water for 10 min and finally, the carrier electrolyte for 10 min.

Separation Conditions

The operation conditions, which were optimized in a previous work in the case of the phosphate (Castiñeira *et al.*, 2000), were different for the two carrier electrolytes studied. In the case of Carrier A (phosphate-based electrolyte), the separation was performed at room temperature, the applied voltage was 20 kV, the current intensity achieved under these conditions was $6.5 \pm 1\,\mu A$. Hydrostatic injection at 10 cm for 30 s was employed and detection was performed by direct UV at 185 nm. The running time for the analysis was 6 min. When Carrier B (phthalic-acid-based electrolyte) was applied, the separation was also performed at room temperature using the same injection mode; in this case, a 15 kV voltage producing a current intensity of $16 \pm 1\,\mu A$ was applied and indirect UV detection at 254 nm was employed.

Wine and Preparation Samples

Thirty-nine commercial red wines were analyzed. Eighteen of these samples came from the Certified Brand of Origin (CBO) *Ribeira Sacra* and the other 21 samples belonged to *Bierzo* CBO. All wines, with guaranteed origin, were made from the traditional grape varieties for these Spanish regions by following the common winemaking practices used in these wine-producing areas. Samples were collected in 750 mL glass bottles and stored at 3–4°C before analysis. The preparation of wine

samples consisted merely of a 1:40 dilution with ultra-pure water to achieve the appropriate concentrations. After filtration through a Millipore 0.45 μm HA membrane filter, the obtained solutions were directly injected into the capillary electrophoresis system.

RESULTS AND DISCUSSION

Background Electrolyte Selection

Two background electrolytes (phosphate and phthalic acid) were compared for the CE separation of the following organic acids with enological interest: tartaric, malic, acetic, succinic and lactic acids. In this step, a test solution containing 10 mg L^{-1} of each organic acid studied in this work was injected into the capillary electrophoresis system for each of the two BGEs considered. The carrier electrolytes were prepared with 10 mM of the BGE and 0.5 mM of MTAB as an EOF modifier at a final pH of 6.5. With these BGE concentrations and under the conditions indicated in the section on *Separation Conditions*, resolution, baseline noise, time analysis and sensitivity were evaluated. Good resolutions were obtained for the organic acids studied using both phosphate and phthalic acids; as can be seen in Figure 1, the

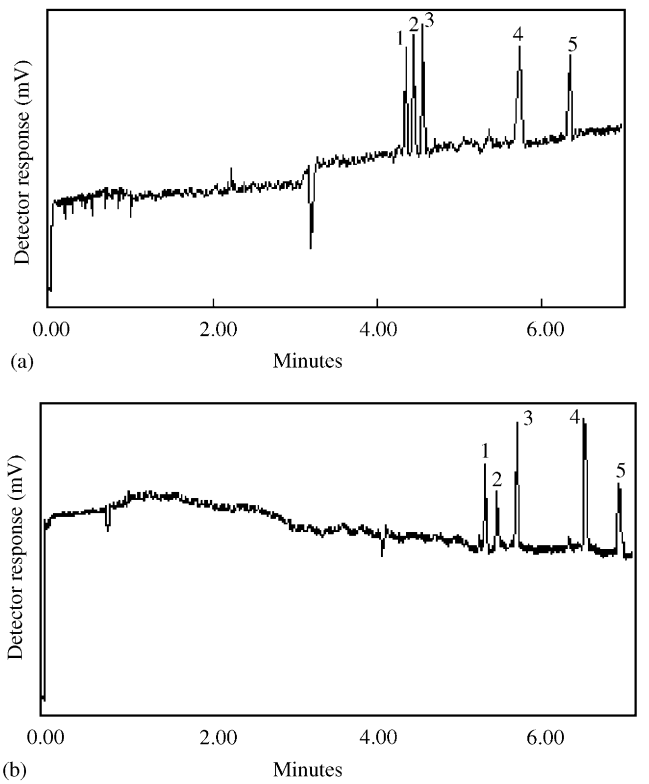

FIGURE 1. Electropherograms of organic acids: (a) phosphate (direct UV detection 185 nm), (b) phthalic acid (indirect UV detection 254 nm). Peak identification: 1 — tartaric acid; 2 — malic acid; 3 — succinic acid; 4 — acetic acid and 5 — lactic acid. Conditions: 10 mM BGE, 0.5 mM MTAB at pH 6.5, hydrostatic injection mode at 10 cm for 30 s.

electropherograms showed well-resolved peaks. Time analysis was slightly shorter for phosphate (6.5 min) than for phthalic acid (7.0 min). At the same level of concentration of organic acids (10 mg L^{-1}), the responses obtained using phosphate were between two- and five-fold higher than those obtained when phthalic acid was used as the BGE.

Influence of pH

The mobility of both BGE and organic acids is pH dependent because their ionizations are controlled by pH of the electrolyte. High changes in mobility are reported in the pH range 2.0–5.5 due to the organic acid ionizations. Taking into account the lower values of the dissociation constants for the studied species (Table 1), an important impact of the pH buffer on the separation of the considered organic acids cannot be expected at pH electrolyte values higher than 5.5. In previous studies concerning the effect of the pH (Soga and Ross, 1997), it was demonstrated that reproducibility in the migration time is not adequate in the region in which the mobility of the solutes is highly affected by the pH. Consequently, the effect of the pH electrolyte on the migration time of the solutes was studied in the 5.5–7.5 range. In these cases, a reduced effect of the electrolyte pH was observed for the Carrier A. At a pH higher than 6.5 the resolution between tartaric, malic, and succinic acid is not optimal. According to this consideration and the fact that 6.5 is the natural pH of carrier A prepared as indicated in the section on Reagents this pH was considered to be optimum. Thus, no acid or base additions were necessary to adjust the pH. In the case of carrier B, no differences in the effective mobility of the solutes were detected in the pH range studied but, at a pH higher than 6.3 the resolution between tartaric, malic, and succinic acids experimented a significant decrease. Further determinations were performed at the optimum pH value of 6.1.

BGE Concentration

The influence of the concentration of both BGEs was studied by varying the concentrations of phosphate and phthalic acid in a range of 3–10 mM and 5–10 mM, respectively. In Figure 2, it can be observed that the detection response increases as the concentration of BGE in the electrolyte decreases. In addition, elevated concentrations of both BGEs in the electrolyte produced increases in migration time for the organic acids. The optimum BGE concentrations were selected by considering which one produces the best relationship sensitivity/time of analysis: 3 mM for phosphate and 7 mM for phthalic acid, respectively.

EOF modifiers

In CE separations of anionic solutes, electroosmotic and electrophoretic movements occur in opposite directions. In several applications, which require the suppression or the reversal of the electroosmotic flow, the use of organic solvents and alkyl ammonium salts as cationic surfactants has been reported (Chen *et al*., 1999). In this work, the addition of myristyltrimethylammonium bromide (MTAB) as the surfactant and methanol as the organic solvent were studied. The effect of the MTAB concentration in the carrier electrolyte on the separation of the organic acids was examined in a concentration range between 0.5 and 2.0 μM for phosphate and in a 0.5 and 3.0 range for phthalic acid. The results of the variation in migration time of solutes for different concentrations of MTAB can be seen in Figure 3. Generally, effective mobility increases as the MTAB concentration increases, but this effect is

TABLE 1

pK_a values for the species studied and analytical figures of merit

Compound	Tartaric acid		Malic acid		Succinic acid		Acetic acid		Lactic acid	
pK_a^1	2.98, 4.34		3.40, 5.11		4.16, 5.16		4.76		3.86	
BGE	Phosphate	Phthalic acid	Phosphate	Phthalic acid	Phosphate	Phthalic acid	Phosphate	Phthalic acid	Phosphate	Phthalic acid
Migration time (min)	3.3	5.7	3.5	5.9	3.7	6.3	4.6	7.2	5.1	7.7
Repeatability migration time ($n=10$) (RSD) (%)	1.01	0.55	1.02	0.64	0.94	1.24	1.05	0.70	1.06	0.67
Repeatability peak area ($n=10$) (RSD) (%)	0.96	3.12	0.74	5.99	0.82	3.43	0.40	2.97	0.58	3.82
Detection limit ($mg\,L^{-1}$)	0.040	1.407	0.037	2.296	0.015	1.197	0.054	1.451	0.032	1.563
Quantitation limit ($mg\,L^{-1}$)	0.132	4.691	0.122	7.349	0.050	3.398	0.178	4.838	0.106	5.209
Linearity (correlation coef.)	0.9996	0.9990	0.9997	0.9985	0.9999	0.9992	0.9993	0.9990	0.9998	0.9990
Linear range ($mg\,L^{-1}$)	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50	Up to 50

[1]Data of pK_a values from Lide and Frederiske (1999).

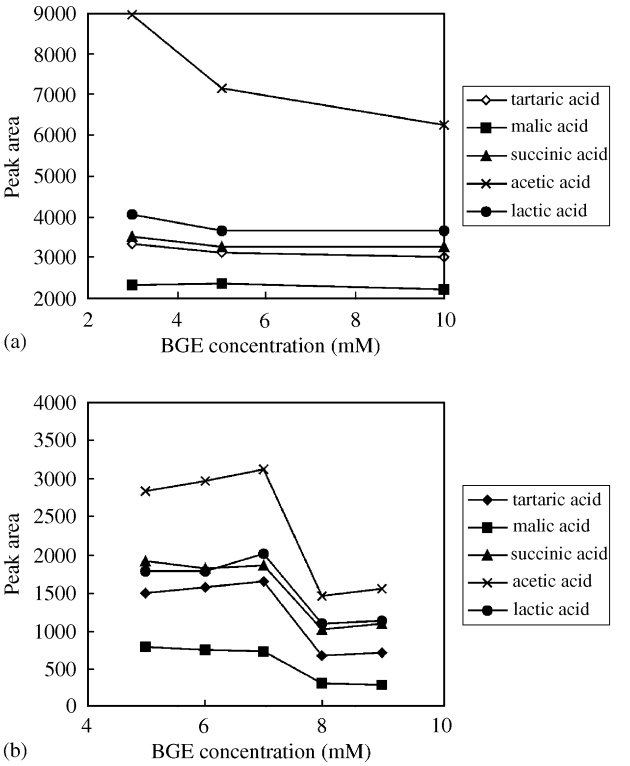

FIGURE 2. Effect of BGE concentration on the detection sensitivity. (a) Phosphate-based electrolyte, (b) phthalic-acid-based electrolyte (each data point is the mean for three determinations).

higher in the phosphate-based electrolyte than in the carrier based on phthalic acid. In the case of phosphate [see Figure 3(a)], high concentrations of MTAB produced higher effective mobilities and, thus, lower migration time. However, a poor resolution between tartaric, malic, and succinic acids and a heavy baseline noise was observed. A MTAB concentration of 0.5 mM in the electrolyte was found to be optimum. In the case of phthalic acid, as can be seen in Figure 3(b), the influence of the MTAB concentration on the effective mobility is lower; the addition of a 2 mM concentration of MTAB to the electrolyte produces a slight diminution of analysis time while the appropriate resolution between the three faster analytes—tartaric, malic, and succinic acids—is not reduced. This concentration of MTAB was considered optimum for carrier B.

In capillary electrophoresis, certain organic solvents are added to the electrolyte in order to improve solutes separation due to the changes produced in the EOF (Soga and Ross, 1997). In this work, the effect of methanol as an organic modifier was studied up to 20% v/v by adding the appropriate quantities to electrolyte. As a general rule (see Figure 4), the effective mobility of the solutes decreased with higher methanol content. Moreover, no improvements in resolution and peak shapes were observed for the phosphate electrolyte; thus, no additions of methanol to the electrolyte were carried out in this case. However, as can be observed in Figure 4(b), for phthalic acid a 5% addition of methanol did not drastically increase the migration time but the resolution between tartaric and malic acid was improved.

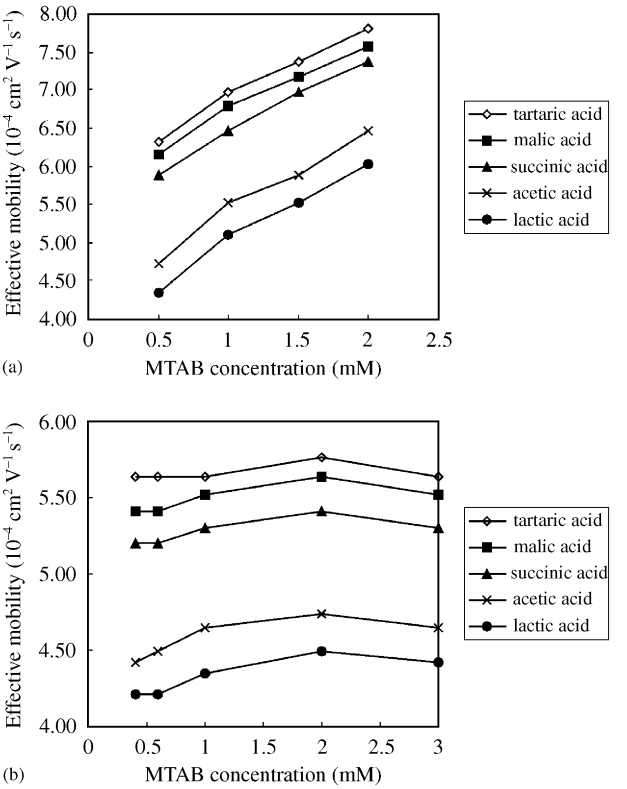

FIGURE 3. Effect of MTAB addition to the electrolyte on the effective mobility of the solutes. (a) Phosphate-based electrolyte, (b) phthalic-acid-based electrolyte (each data point is the mean for three determinations).

Therefore, the addition of methanol as an organic solvent in the indicated concentration was chosen for further measurements.

Sensitivity: Comparison Between Direct and Indirect UV Detection

Working at low UV wavelength, certain anionic species can be detected by direct UV detection. When this approach can be applied it is usually more specific and sensitive than indirect UV detection (Shirao *et al.*, 1994; Turcat *et al.*, 1994; Buchberger and Winna, 1996). Electrolytes based on non-absorbing species such as sulfate or phosphate are used. In order to evaluate the sensitivity of both methods with direct and indirect UV detection, a comparison between phosphate and phthalic acid in the analysis of organic acids was made. With an optimized composition of the carrier electrolytes indicated in the section on Reagents and under the experimental conditions described in the section on *Separation Conditions*, calibration lines for the five organic acids studied were performed by injecting different amounts of each solute up to 50 mg L^{-1}. LOD and LOQ were calculated taking into account a relation signal–noise 3:1 and 10:1, respectively. From the results obtained that are presented in Table 1 it can be concluded that the sensitivity of the analysis carried out using direct detection was from 35- to 80-folds higher than for the indirect procedure. Since the quantification of malic acid in most of the wines analyzed using

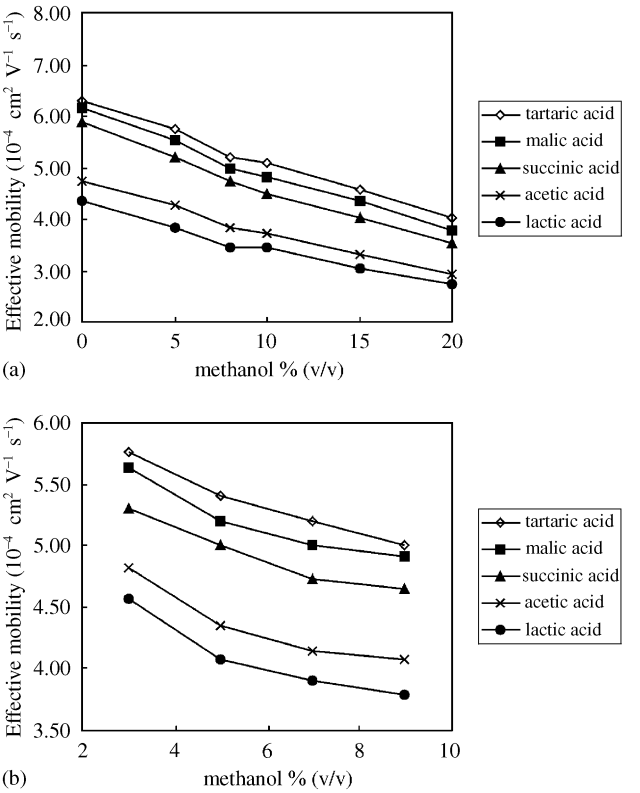

FIGURE 4. Effect of methanol content in the electrolyte on the effective mobility of organic acids. (a) Phosphate-based electrolyte, (b) phthalic-acid-based electrolyte (each data point is the mean for three determinations).

TABLE 2

Recovery results for the five organic acids determined by capillary electrophoresis

Compound	Recovery %	
	Phosphate	Phthalic acid
Tartaric acid	92.0±1	104±2
Malic acid	90.0±0.7	—
Succinic acid	91.5±0.8	98.0±2
Acetic acid	101±0.4	107±2
Lactic acid	102±0.6	100±1

phthalic acid and indirect UV detection was not possible because of their unfavorable detection limit (see Table 1), the direct detection method based on phosphate as a BGE was demonstrated to be more useful and to provide important advantages in sensitivity.

Repeatability, Linearity Range and Recovery

The repeatability of migration time and peak area were evaluated for each organic acid studied (10 mg L^{-1}; $n=10$) and expressed as the relative standard deviation

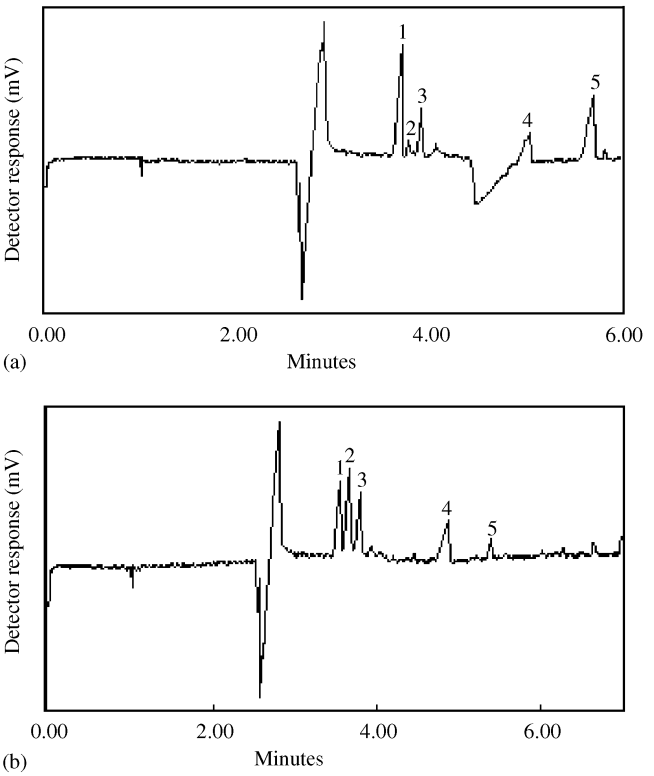

FIGURE 5. Electropherograms obtained for wine samples analysed: (a) Ribeira Sacra CBO wine, (b) Bierzo CBO wine. Peak identification: 1 — tartaric acid; 2 — malic acid; 3 — succinic acid; 4 — acetic acid and 5 — lactic acid. Conditions: 3 mM phosphate, 0.5 mM MTAB at pH 6.5, applied voltage 20 kV, hydrostatic injection for 30 s, direct UV detection at 215 nm.

(RSD) in Table 1. In our results, the phosphate BGE method showed an acceptable peak area RSD in the range comprised between 0.40 and 0.96%. According to their lower sensitivity, poorer results have been achieved for the phthalic acid BGE in terms of repeatability in peak area with an RSD range comprising between 2.97 and 5.99%. Comparable results were obtained for both procedures concerning repeatability in migration time.

The linearity of the response was evaluated by injecting various concentrations of the organic acids studied. The calibration functions obtained by plotting the peak area versus the concentration of the test organic acids were linear in all cases up to 50 mg L^{-1} with correlation coefficients in the range of 0.9985–0.9999.

Recovery was studied using wine samples spiked with different quantities of the organic acids under analysis. The recovery results, shown in Table 2, were satisfactory in both cases, ranging from 90 to 102% for phosphate BGE and from 98 to 107% for phthalic acid BGE.

Analysis of Real Samples

The analysis of organic acids in wine samples is a determination of high interest for the winemaking industry. Grapes present appreciable amounts of various organic

acids. Thus, both grape juice and must can be considered as dilute acid solutions containing mainly tartaric, malic and citric acid. Wine includes the acids from the musts and also those produced during the alcoholic fermentation: acetic, succinic, lactic, and others. Since acids play an important role in the final characteristics of the wine, it is very important to follow the changes in acidity during the different steps of the winemaking process. The acidity level in grapes is one of the criteria, which determines the optimal harvesting time. In addition, controlling the changes in certain fixed acids is also essential in fermentation and during the ageing process. Levels of malic and lactic acids must be monitored in order to ascertain the malolactic fermentation. In wine stabilization, the level of tartrate is a critical control parameter (Ough and Amerine, 1988).

Considering the importance of the determination of organic acids in wine and taking into account the results obtained in the comparative study performed above (in which the phosphate-based method with direct detection was established as most suitable for further determinations), this method was applied for the analysis of organic acids in wine samples under the optimized conditions listed previously. Only a 1:40 dilution and a subsequent filtration of the sample were necessary before direct injection into the capillary electrophoresis system. Two examples of the electropherograms obtained in the analysis of Spanish Certified Brand of Origin red wines are presented in Figure 5. Organic acids were well separated and sharp peaks were observed. The solutes migrated in the order of tartaric, malic, succinic, acetic, and lactic acids.

The results for the determination of the five organic acids studied are presented in Table 3. The levels obtained in the Galician wines studied were comparable to those reported by other authors for wine samples from diverse origins (Ough and Amerine, 1988). All the analyzed wines showed the same qualitative organic acid profile, but some quantitative differences between the two geographical origins and also certain differences between samples in relation to the content of malic and lactic acids were observed. From the results, it can be concluded that the wines from the CBO Ribeira Sacra have a higher content of all organic acids evaluated than those from the Bierzo origin, except for acetic acid which presented a similar content in both CBOs. Malolactic fermentation is a common practice in both of these winemaking regions in order to obtain sweeter sensorial properties due to the natural acidity of the grapes produced there. A low ratio of malic/lactic acid was observed for wines in which malolactic fermentation was performed. Figure 5(a) shows a wine with this characteristic (a great part of malic acid was transformed into lactic acid). On the

TABLE 3

Concentrations of five organic acids in red wines from Spanish CBOs Ribeira Sacra and Bierzo (all results are expressed in mg L^{-1})

		Tartaric acid	Malic acid	Succinic acid	Acetic acid	Lactic acid
Ribeira	Mean	1483	547	556	359	2108
Sacra	Maximum	1987	2844	897	749	3784
CBO	Minimum	866	n.d.	398	116	452
($n=18$)	S.D.	359	789	126	171	907
Bierzo	Mean	1195	311	485	373	1731
CBO	Maximum	1819	1513	646	553	4037
($n=21$)	Minimum	772	n.d.[1]	389	214	179
	S.D.	267	414	71	102	831

[1]n.d.: not detected.

other hand, in Figure 5(b), an electropherogram for a wine without malolactic fermentation is presented. In this case, a high malic/lactic acid ratio is indicative of the absence of this viticulture practice. Of the 39 wines analyzed, malolactic fermentation is not performed in only three samples.

CONCLUSIONS

A simple CE method with UV detection for the determination of organic acids in wine, which can be used as an alternative to other chromatographic techniques, is described. The use of phosphate as a BGE provided a well-resolved and reproducible electrolyte system. The results have shown that the type of BGE and its pH, as well as the EOF modifiers, have significant effects on separation and detection sensitivity. The phosphate-based carrier electrolyte with direct UV detection procedure has been compared advantageously in terms of sensitivity (35–80-folds better) with another CE procedure using phthalic acid and indirect UV detection. The proposed method was successfully applied in the analysis of five organic acids with enological interest. The determination was performed in red wine samples from two Spanish CBOs in less than 6 min with a very simple sample pre-treatment. Thus, the described procedure is able to complement other methods commonly used for the determination of organic acids in wines**.** With one single quick analysis, it provides relevant information for the monitoring of malolactic fermentation as well as other important organic acids.

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REFERENCES

Buchberger, W. and Winna, K. (1996). Optimization of the separation of polycarboxylic acids by capillary zone electrophoresis. *J. Chromatogr. A* **739,** 389–397.

Castiñeira, A., Peña, R. M., Herrero, C., and García-Martín, S. (2000). Simultaneous determination of organic acids in wine samples by capillary electrophoresis and UV detection: optimization with five different background electrolytes. *J. High Resol. Chromatogr.* **23,** 647–652.

Chen, Z., Tang, C., and Yu, J. (1999). Simultaneous determination of inorganic anions and organic acids in environmental samples by capillary zone electrophoresis with indirect UV detection. *J. High Resol. Chromatogr.* **22,** 379–385.

Deng, C. R. (1997). Determination of total organic acids in wine by interfacial derivatization gas chromatographic method. *Sepu* **15,** 505–507.

Ding, M. Y., Suzuki, Y., and Koizumi, H. (1995)**.** Simultaneous determination of organic acids, inorganic anions and cations in beverages by ion chromatography with a mixed-bed stationary phase of anion and cation exchangers. *Analyst* **120,** 1773–1777.

Dong, M. W. (1998). HPLC analysis of organic acids in juice and wine using resin and reversed-phase columns. *LC-GC* **16,** 1092–1097.

Escobal, A., Gonzalez, J., Iriondo, C., and Laborra, C. (1997). Liquid chromatographic determination of organic acids in txakoli from Bizkaia. *Food Chem.* **58,** 381–384.

Falque-Lopez, E. and Fernández-Gómez, E. (1996). Simultaneous determination of the major organic acids, sugars, glycerol, and ethanol by HPLC in grape musts and white wines. *J. Chromatogr. Sci.* **35,** 254–257.

Haila, H., Kumpulainen, J., Hakkinen, U., and Tahvonen, R. (1992). Sugar and organic acid contents of vegetables consumed in Finland during 1988 to 1989. *J. Food Comp. Anal.* **5,** 100–107.

Hu, D. G., Cheng, J. S., and Zhu, Y. (1994). Quantitative determination of free organic acids in Chinese white wine. *Sepu* **12,** 265–267.

Kandl, T. and Kupina, S. (1999). An improved capillary electrophoresis procedure for the determination of organic acids in grape juice and wine. *Am. J. Enol. Vitic.* **50,** 155–161.

Khaledi, M. G. (1998). *High Performance Capillary Electrophoresis, Theory, Techniques and Applications Part* IV, p.637. Wiley, New York.

Klampfl, C. W., Buchberger, W., and Haddad, P. R. (2000). Determination of organic acids in food samples by capillary zone electrophoresis. *J. Chromatogr. A* **881,** 357–364.

Landers, J. P. (1997). *Handbook of Capillary Electrophoresis*, Chap. 6, pp. 156–483. CRC Press, Boca Raton, FL.

Lide, D. R and Frederiske, H. R. (1999). *Handbook of Chemistry and Physics*, 80th edn, Section 8, pp. 46–47. CRC Press, Boca Raton, FL.

Linget, C., Netter, C., Heems, D., and Verette, E. (1998). Online dialysis with HPLC for the automated preparation and analysis of amino acids, sugars and organic acids in grape juice and wines. *Analusis*, **26,** 35–39.

Lourdes, M., Gustavo, A., and Troncoso, A. M. (1998). Ion-exclusion-chromatographic determination of organic acids in vinegars. *J. Chromatogr. A* **822,** 45–51.

Mallet, S., Arellano, M., Boulet, J. C., and Condera, F. (1999). Determination of tartaric acid in solid wine residues by capillary electrophoresis and indirect UV detection. *J. Chromatogr. A* **853,** 211–214.

Nutku, M. S. and Erim, F. B. (1999). Polyethylenimine-coated capillary electrophoresis capillaries for the analysis of organic acids with an application to beverages. *J. Microcolumn Sep.* **11,** 541–543.

Ough, C., and Amerine, M. (1988). *Methods for Analysis of Musts and Wines*, Chap. 2, pp. 50–80. John Wiley, New York.

Sádecká, J. and Polonský, J. (2000) Electrophoretic methods in the analysis of beverages. *J. Chromatogr. A* **880**(1 + 2), 243–279.

Shirao, M., Furuta, R., Suzuki, S., Nakazawa, H., Fujita, S., and Maruyama, T. (1994). Determination of organic acids in urine by capillary zone electrophoresis. *J. Chromatogr. A* **680,** 247–251.

Soga, T. and Ross, G. A. (1997). Simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates by capillary electrophoresis. *J. Chromatogr. A* **767,** 223–230.

Turcat, S., Masclet, P., and Lissolo, T. (1994). Use of capillary electrophoresis to monitor concentrations of organic acids in snow and rainwater. *Sci. Total Environ.* **158,** 21–29.

Vonach, R., Lendl, B., and Kellner, R. (1998). High-performance liquid chromatography with real-time Fourier-transform infrared detection for the determination of carbohydrates, alcohols and organic acids in wines. *J. Chromatogr. A* **824,** 159–167.

Zhu, Y., Zhang, X. D., and Niu, W. J. (1997). Simultaneous determination of carbohydrates and organic acids in beer and wine by ion chromatography. *Mikrochim. Acta* **127,** 189–194.