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Simple determination of main organic acids in grape juice and wine by using capillary zone electrophoresis with direct UV detection

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Abstract

An accurate, simple and rapid capillary zone electrophoresis (CZE) method with direct UV detection has been set up for the determination of main organic acids in grape juice and wine. The determination of tartaric, malic, and citric acids in grape juices and tartaric, malic, succinic, acetic, lactic and citric acids in wines can be achieved in less than 3 min with only a simple dilution and filtration treatment of the sample. Validation parameters of the method as detection and quantification limits, linearity, precision (intraday and interday analysis) and recovery were also studied in grape juice, white wine, rose wine and red wine, separately. The proposed method decreases the analysis times of the previous reported CZE methods and allows the rapid control of the grape maturity, the winemaking processes and the detection of wine alterations and/or illnesses.

Keywords: Organic acids; Capillary zone electrophoresis; Grape juice; Wine

1. Introduction

The determination of organic acids in grape juices and wines is important because they have influence on the organoleptic properties (flavour, colour, and aroma) and on the stability and microbiological control of the products. Tartaric and malic acids are the predominant organic acids in grape juices and succinic and citric acids are present in minor proportion. In the case of wines, a common differentiation is made between acids which come directly from the grape (tartaric, malic and citric acids) and those that are originated, fundamentally, in the fermentation process (succinic, lactic and acetic acids) (Belitz & Grosch, 1992; Peynaud, 1999).

The evolution of tartaric and malic acids in grapes are useful for checking their processes of maturation (Lamikanra, Inyang, & Leong, 1995). In the case of wines, the analysis of organic acids allows to control the evolution of the acidity during the different steps of the winemaking process (alcoholic fermentation, malolactic fermentation, aging process, etc.). These organic acids also have great importance in the detection of wine alterations and/or illnesses, because they suppose a modification of acids content, as for example, acetic or lactic sharpness.

Nowadays, several methods have been developed for identifying and quantifying these organic acids in grape juices and wines so much individually, as non-enzymatic spectrophotometric and enzymatic methods or as a group of them simultaneously, as chromatographic and electrophoretic methods (Mato, Suárez-Luque, & Huidobro, 2005; Saavedra & Barbas, 2003; Vereda, García de Torres, Rivero, & Cano, 1998). In recent years, chromatographic techniques have been replaced by capillary electrophoresis due to its good resolution, automation, simplicity, high speed, low consumption of chemicals and reduced sample preparation (Heiger, 1992; Thibault & Dovichi, 1998).

Table 1 summarizes the characteristics of some electrophoretic methods published to determine low molecular

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Table 1
Studies of the analysis of organic acids in grape juices and wines by capillary zone electrophoresis (CZE)

Matrix	Organic acids[a]	Electrolyte	Capillary	Injection	Detection	Separation times (min)	Others conditions	References
Grape juice	mal., tart., cit.	180 mM phosphate, 1 mM CTAB, 15% (v/v) methanol (pH 7.2)	Fused silica capillary, 50 cm × 50 μm ID	Hydrodynamic injection – pressure (15 psi s)	Direct UV detection (200 nm)	7	$V = -15$ kV	Vorarat et al. (2002)
Wine	tart., mal., succ., acet., lact.	3 mM phosphate, 0.5 mM MTAB (pH 6.5)	Fused silica capillary, 60 cm × 75 μm ID	Hydrodynamic injection – siphoning (30 s)	Direct UV detection (185 nm)	6	$V = -20$ kV	Castiñeira et al. (2000), Castiñeira et al. (2002)
Wine	acet., cit., fum., lact., mal., oxal., succ., tart.	200 mM phosphate buffer (pH 7.50)	Polyacrylamide coated capillary, 50 cm × 50 μm ID	Hydrodynamic injection – pressure (0.035 mbar, 20 s)	Direct UV detection (200 nm)	13	$V = -14$ kV, $T^a = 20$ °C	Saavedra and Barbas (2003)
Wine	tart., mal.	5 mM phthalate, 0.5 mM TTAB, 50 mM MES (pH 5.2)	Fused silica capillary, 70 cm × 75 μm ID	Electrokinetic injection (−10 kV, 1 s)	Indirect UV detection (205 nm)	5	$V = -30$ kV, $T^a = 20$ °C	Kelly and Nelson, 1993
Wine	tart., mal., lact.	5 mM phthalate, 0.5 mM EOF modifier (pH 5.6)	Fused silica capillary, 100 cm × 75 μm ID	Electrokinetic injection (−10 kV, 5 s)	Indirect UV detection (254 nm)	20	$V = -20$ kV, $T^a = 20$ °C	Levi et al. (1993)
Wine	mal., lact., acet., succ., cit.	5 mM chromate, 0.154% (w/v) PDDPi chromate (pH 8)	Fused silica capillary, 60 cm × 77 μm ID	Hydrodynamic injection – siphoning (15 s)	Indirect UV detection (254 nm)	8	$V = -30$ kV	Stathakis and Cassidy (1995)
Wine	tart., mal., cit., succ., pyr., acet., lact.	5 mM PDC, 0.5 mM CTAB (pH 5.6)	Fused silica capillary, 80.5 cm × 75 μm ID	Hydrodynamic injection – pressure (50 mbar, 2 s)	Indirect UV detection (200 nm)	7	$V = -25$ kV, $T^a = 20$ °C	Soga (1996)
Wine	tart., mal., succ, adip., glut., acet., lact., shyk.	7.5 mM PAB, 10.5 mM BIS-Tris, 0.1 mM TTAB (pH 7.0)	Fused silica capillaries, 48 cm (UV) and 60 cm (cond) × 50 μm ID	Hydrodynamic injection – pressure (25 mbar, 0.2 s)	Indirect UV detection (254 nm) and conductivity	7 min (cond), 8 min (UV)	$V = -30$ kV	Klampfl et al. (1998)
Wine	tart., mal., cit., succ., acet., lact.	7.5 mM PDC, 0.5 mM CTAB, 0.5 mM EDTA (pH 5.6)	Fused silica capillary, 110 cm × 75 μm ID	Electrokinetic injection (−10 kV, 2 s)	Indirect UV detection (210 nm)	18	V = ramp from 0 to −22 kV in 0.5 min, $T^a = 15$ °C	De Villiers et al. (2003)
Wine	tart., mal., succ., cit., acet., lact.	3 mM BTA, 15 mM Tris, 1.5 mM TEPA (pH 8.4)	Fused silica capillary, 65 cm × 50 μm ID	Hydrodynamic injection – siphoning (20 s)	Indirect UV detection (240 nm)	9	$V = -25$ kV	Sing Fung and Man Lau (2003)
Wine	tart., mal., succ., acet., lact.	5 mM PDC, 0.5 mM CTAB (pH 5.6)	Fused silica capillary, 78 cm × 75 μm ID	Hydrodynamic injection – pressure (0.3 psi, 2 s)	Indirect UV detection (200 nm)	7.5	$V = -25$ kV, $T^a = 18$ °C	Esteves et al. (2004)
Wine	tart., mal., succ., acet., lact.	22 mM benzoic acid, 35 % (v/v) methanol (pH 6.10)	Fused silica capillary, 31.2 cm × 75 μm ID dinamically coated by flushing 0.1 % (w/v) HDB	Hydrodynamic injection – pressure (0.5 psi, 5 s)	Indirect UV detection (214 nm)	3.5	$V = -10$ kV, $T^a = 25$ °C	Bianchi et al. (2005)

(*continued on next page*)

Table 1(*continued*)

Matrix	Organic acids[a]	Electrolyte	Capillary	Injection	Detection	Separation times (min)	Others conditions	References
Grape juice, wine	succ., mal.,tart., acet., lact.	10 mM tetraborate, 0.5 mM TTAOH, 10 ppm Ca^{2+} and Mg^{2+} (pH 9.3)	Fused silica capillary, 60 cm × 75 μm ID	Hydrodynamic injection – siphoning (30 s)	Direct UV detection (185 nm)	20	$V = -7$ kV, $T^a = 20$ °C	García Moreno et al. (2001), Moreno et al. (2003)
Grape juice, wine	cit., tart., mal., succ., acet., lact.	5 mM phthalate, 0.5 mM OFM-Anion BT (pH 7.0)	Fused silica capillary, 100 cm × 75 μm ID	Hydrodynamic injection – siphoning (45 s)	Indirect UV detection (254 nm)	15	$V = -20$ kV	Kenney (1991)
Grape juice, wine	tart., mal., succ., cit., acet., lact.	3 mM PMA, 3 mM DETA (pH 7.5)	Fused silica capillary, 44 cm × 75 μm ID	Hydrodynamic injection – pressure (1.5 psi, 2 s)	Indirect UV detection (220 nm)	12	$V = -20$ kV, $T^a = 30$ °C	Arellano et al. (1997), Arellano et al. (1997)
Grape juice, wine	tart., mal., cit., succ., acet., lact.	5 mM PDC, 0.5 mM CTAB (pH 5.6)	Fused silica capillary, 80.5 cm × 75 μm ID	Hydrodynamic injection – pressure (50 mbar, 2 s)	Indirect UV detection (200 nm)	7.5	$V = -25$ kV, $T^a = 18$ °C	Kandl and Kupina (1999)

[a] acet.: acetic acid; adip.: adipic acid; cit.: citric acid; fum.: fumaric acid; glut.: glutaric acid; lact.: lactic acid; mal.: malic acid; oxal.: oxalic acid; pyr.: pyruvic acid; shyk.: shykimic acid; succ.: succinic acid; tart.: tartaric acid.

weight organic acids in grape juice (Vorarat, Aromdee, & Podokmai, 2002), wine samples (Bianchi, Careri, & Corradini, 2005; Castiñeira, Peña, Herrero, & García-Martín, 2000; Castiñeira, Peña, Herrero, & García-Martín, 2002; De Villiers, Lynen, Crouch, & Sandra, 2003; Esteves, Lima, Lima, & Duarte, 2004; Kelly & Nelson, 1993; Klampfl, Katzmayr, & Buchberger, 1998; Levi, Wehr, Talmadge, & Zhu, 1993; Saavedra & Barbas, 2003; Sing Fung & Man Lau, 2003; Soga, 1996; Stathakis & Cassidy, 1995) and both grape juice and wines (Arellano, Andrianary, Dedieu, Couderc, and Puig, 1997; Arellano, Couderc, and Puig, 1997; García Moreno, Jurado Campoy, and García Barroso, 2001; Kandl and Kupina, 1999; Kenney, 1991; Moreno, Jurado, and Barroso, 2003).

Tacking into account the analysis time, the separation time of the methods cited in Table 1 ranged between 3.5 min (Bianchi et al., 2005) and 20 min (García Moreno et al., 2001; Levi et al., 1993; Moreno et al., 2003). Nevertheless, only seven methods have determined the most important acids to control the winemaking process simultaneously, as tartaric, malic, succinic, acetic, lactic and citric acids (Arellano et al., 1997; Arellano et al., 1997; De Villiers et al., 2003; Kandl & Kupina, 1999; Kenney, 1991; Saavedra & Barbas, 2003; Sing Fung & Man Lau, 2003; Soga, 1996). The separation time of these methods ranged between 7 min (Soga, 1996) and 18 min (De Villiers et al., 2003).

Although indirect UV detection was the detection mode most widely used in capillary zone electrophoresis (CZE) for the determination of organic acids in these samples, direct UV detection seems to be more suitable due to the stability of the baseline (Buchberger, Klampfl, Eibensteiner, & Buchgraber, 1997). In the group of direct UV detection, only Saavedra and Barbas (2003) quantified all the acids with importance in winemaking process (tartaric, malic, succinic, acetic, lactic and citric acids). They have used a polyacrylamide coated capillary and phosphate buffer as electrolyte to analyse organic acids in 13 min.

The aim of this paper has been to validate a simple and rapid capillary zone electrophoresis method with direct UV detection for the determination of main low-molecular weight organic acids in grape juices and wines which allows the rapid control of the maturity and the winemaking processes.

2. Materials and methods

2.1. Chemicals

All analytical standard-grade organic acids were obtained from Sigma Chemical Co. (St. Louis, MO, USA) as their sodium or potassium salts. Stock standard solutions were obtained by dissolution of salts of the acids in Milli-Q water (10 g/L) and they were stored at 4 °C for 1 month. The Milli-Q water was purified by passage through a Compact Milli-RO and Milli-Q water system

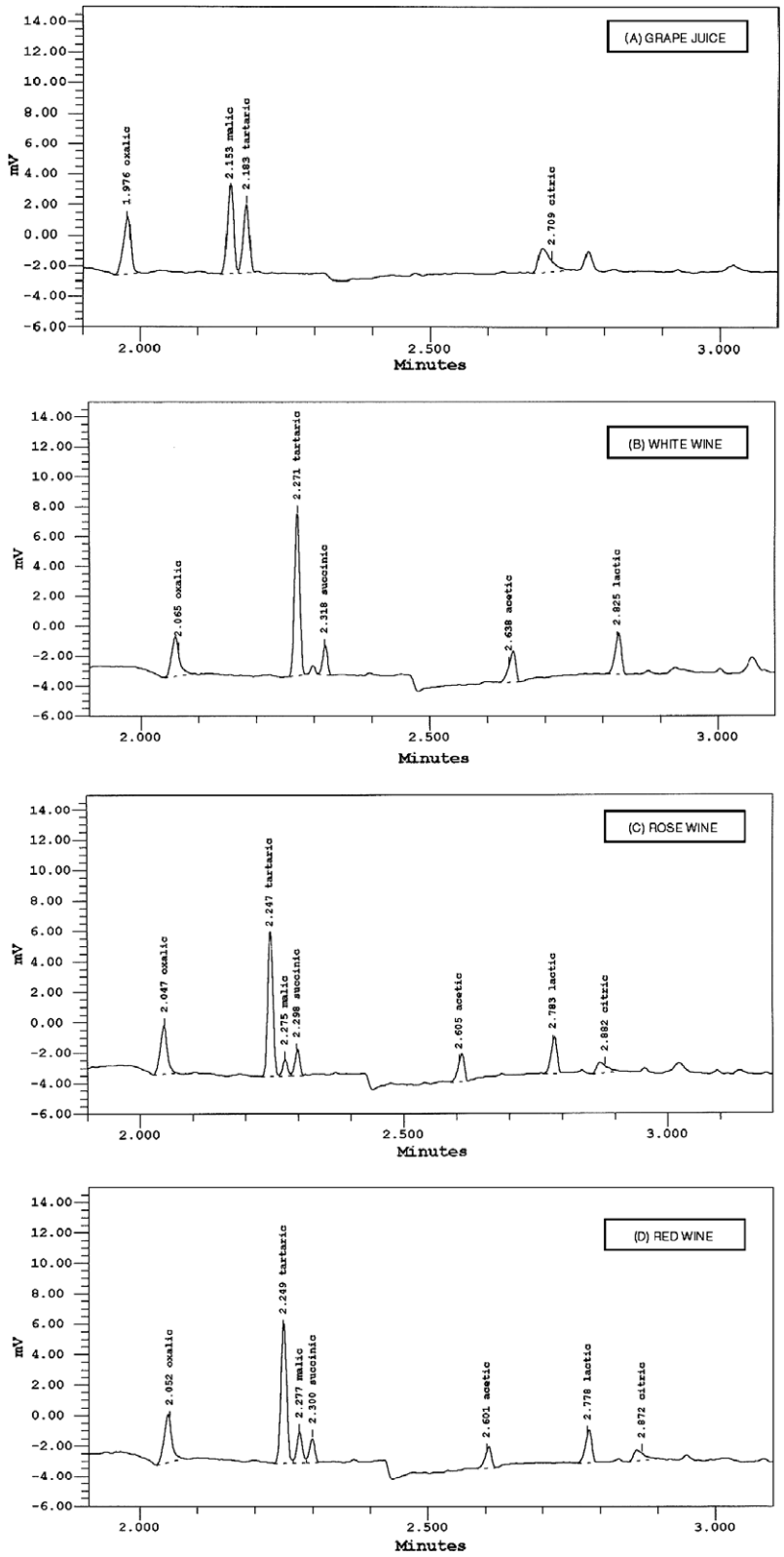

Fig. 1. Electropherogram of organic acids analysed in a grape juice and in three types of wine (white, rose and red wines) by the proposed CZE method (oxalic acid was added as reference acid to calculate the relative migration times of the organic acids). Conditions were phosphate buffer: 7.5 mM NaH_2PO_4 and 2.5 mM Na_2HPO_4, 2.5 mM TTAOH, 0.24 mM Ca^{2+}, pH 6.40, −25 kV, 25 °C, hydrodynamic injection (30 s) and direct UV detection (185 nm).

from Millipore (Milford, MA, USA). Working standard solutions were prepared daily by dilution with Milli-Q water.

Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), di-sodium hydrogen phosphate (Na_2HPO_4), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), hydrochloric

Table 2
A summary of the validation parameters of the CZE methods previously employed by other authors for the quantification of organic acids in grape juice and wine

LOD (mg/L)	LOQ (mg/L)	Intraday analysis (RSD %)	Interday analysis (RSD %)	Recovery (%)	References
Tartaric acid					
0.040	0.132	0.96	–	92.0	Castiñeira et al. (2000), Castiñeira et al. (2002)
–	1.02	0.4–0.8	1.6–5.1	100.3–100.4	Saavedra and Barbas (2003)
1.4	–	2.2	–	–	Soga (1996)
0.277	0.462	1.0	–	–	Klampfl et al. (1998)
25.0	–	1.2	–	–	De Villiers et al. (2003)
0.3	1.5	–	–	–	Sing Fung and Man Lau (2003)
19.92	66.39	–	–	–	Esteves et al. (2004)
1.413	4.709	–	–	97.92–98.40	García Moreno et al. (2001), Moreno et al. (2003)
–	–	1.9	–	103	Kenney (1991)
0.175	0.583	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	1.21–2.98	–	99–101	Kandl and Kupina (1999)
Malic acid					
–	5	2.29	3.07	100.9	Vorarat et al. (2002)
0.037	0.122	0.74	–	90.0	Castiñeira et al. (2000), Castiñeira et al. (2002)
–	0.05	1.0–2.9	5.0–9.3	90.7–100.0	Saavedra and Barbas (2003)
1.2	–	1.5	–	–	Soga (1996)
0.228	0.379	3.5	–	–	Klampfl et al. (1998)
30.6	–	1.5	–	–	De Villiers et al. (2003)
0.27	1.34	–	–	–	Sing Fung and Man Lau (2003)
19.68	65.61	–	–	–	Esteves et al. (2004)
1.583	5.278	–	–	98.82	García Moreno et al. (2001), Moreno et al. (2003)
–	–	1.3	–	97	Kenney (1991)
0.087	0.287	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	0.57–2.95	–	97–103	Kandl and Kupina (1999)
Succinic acid					
0.015	0.050	0.82	–	91.5	Castiñeira et al. (2000), Castiñeira et al. (2002)
–	0.76	0.5–1.0	1.3–4.2	99.8–99.9	Saavedra and Barbas (2003)
1.2	–	1.5	–	–	Soga (1996)
0.228	0.379	1.9	–	–	Klampfl et al. (1998)
37.8	–	5.0	–	–	De Villiers et al. (2003)
0.24	1.18	–	–	–	Sing Fung and Man Lau (2003)
6.13	20.44	–	–	–	Esteves et al. (2004)
0.449	1.495	–	–	98.28	García Moreno et al. (2001), Moreno et al. (2003)
–	–	2.7	–	–	Kenney (1991)
0.040	0.134	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	2.07–3.79	–	101–107	Kandl and Kupina (1999)
Acetic acid					
0.054	0.178	0.40	–	101.2	Castiñeira et al. (2000), Castiñeira et al. (2002)
–	0.53	1.4–1.6	1.9–4.4	100.1–100.3	Saavedra and Barbas (2003)
0.9	–	1.4	–	–	Soga (1996)
0.182	0.304	1.4	–	–	Klampfl et al. (1998)
46.4	–	4.9	–	–	De Villiers et al. (2003)
0.12	0.60	–	–	–	Sing Fung and Man Lau (2003)
1.78	5.94	–	–	–	Esteves et al. (2004)
0.929	3.097	–	–	98.22	García Moreno et al. (2001), Moreno et al. (2003)
–	–	5.4	–	–	Kenney (1991)
0.006	0.020	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	2.54–8.87	–	98–111	Kandl and Kupina (1999)
Lactic acid					
0.032	0.106	0.58	–	102.0	Castiñeira et al. (2000), Castiñeira et al. (2002)
–	0.2	0.5–3.4	3.6–3.9	98.1–100.8	Saavedra and Barbas (2003)
1.2	–	1.8	–	–	Soga (1996)
0.185	0.308	1.1	–	–	Klampfl et al. (1998)
28.5	–	5.1	–	–	De Villiers et al. (2003)
0.27	0.90	–	–	–	Sing Fung and Man Lau (2003)
36.74	122.48	–	–	–	Esteves et al. (2004)
5.550	18.499	–	–	98.24	García Moreno et al. (2001), Moreno et al. (2003)
–	–	9.1	–	–	Kenney (1991)
1.072	3.574	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	2.70–5.00	–	96–103	Kandl and Kupina (1999)

Table 2 (*continued*)

LOD (mg/L)	LOQ (mg/L)	Intraday analysis (RSD %)	Interday analysis (RSD %)	Recovery (%)	References
Citric acid					
–	2.5	1.50	3.26	98.6	Vorarat et al. (2002)
–	0.11	1.2–1.8	2.5–6.3	99.6–100.2	Saavedra and Barbas (2003)
2.2	–	1.0	–	–	Soga (1996)
75.0	–	5.0	–	–	De Villiers et al. (2003)
0.38	1.92	–	–	–	Sing Fung and Man Lau (2003)
1.442	4.805	–	–	–	García Moreno et al. (2001), Moreno et al. (2003)
–	–	14.1	–	–	Kenney (1991)
0.375	1.250	–	–	–	Arellano et al. (1997), Arellano et al. (1997)
1.5	–	1.19–4.99	–	92–101	Kandl and Kupina (1999)

acid fuming (37%) and sodium hydroxide pellets were analytical reagent-grade and supplied by Merck (Darmstadt, Germany). Tetradecyltrimethylammonium hydroxide (TTAOH), commercial name OFM-OH, was supplied by Waters (Milford, MA, USA). The electrolyte was filtered through 0.45 μm nylon membrane filters Phenomenex, AFO-0504 (Phenomenex, CA, USA) and must be prepared fresh daily. The samples were filtered through 0.5 μm PTFE membrane filters (MFS, USA).

2.2. Apparatus

Separation was carried out on a Waters Capillary Ion Analyser (CIA System, 1.3 version) equipped with a negative power supply and a fixed-wavelength UV–vis detector with mercury lamp (Waters Chromatography, Milford, MA, USA). Fused-silica capillaries Waters Accusep Part No. 250-05 with 60 cm in length and 75 μm of internal diameter were used. The distance from the point of injection to the window of on-column detection was 52.5 cm. Electropherograms were collected and plotted by a Millennium 2010 v. 2.15 data acquisition system with specific option CIA for capillary electrophoresis (Waters Chromatography, Milford, MA, USA).

A Crison micropH 2002 pH meter (Crison Instruments S.A., Alella, Barcelona, Spain) and a Selecta Agimatic-S magnetic stirrer (Selecta, Abrera, Barcelona, Spain) were also used.

2.3. Electrophoretic procedures

2.3.1. Capillary column conditioning

Prior to first use, a new capillary was pretreated with the following sequence: Milli-Q water (10 min), 1 M NaOH (10 min), 0.01 M NaOH (10 min), Milli-Q water (30 min) and running electrolyte (90 min). Prior to daily use, the capillary was conditioned with 0.01 M NaOH for 10 min, followed by Milli-Q water for 30 min and carrier electrolyte for 90 min. Before each run, the capillary was flushed with running electrolyte for 2 min. After all analysis of the day, the capillary was also washed with 0.01 M NaOH (10 min) and Milli-Q water (30 min).

2.3.2. Separation conditions

Sample injection was carried out in a hydrodynamic mode by elevating the sample at 10 cm for 30 s. The running voltage was −25 kV at thermostated temperature of 25 °C. The detection mode was UV direct and the wavelength was 185 nm. The electrolyte composition was phosphate as the carrier buffer (7.5 mM NaH_2PO_4 and 2.5 mM Na_2HPO_4), 2.5 mM tetradecyltrimethylammonium hydroxide (TTAOH) as electroosmotic flow modifier and 0.24 mM $CaCl_2$ as selectivity modifier, adjusting the pH at 6.40 constant value. All standards and samples were injected in triplicate.

2.4. Samples

The proposed method was applied to two grape juice samples and to six wine samples (white wines, rose wines and red wines). These samples were purchased at a local supermarket. For grape juice samples, 0.3 mL was dissolved in 100 mL of Milli-Q water and for wine samples 0.5 mL was dissolved in 100 mL of Milli-Q water. All samples are filtered through 0.5 μm PTFE membrane filters and injected directly without any other sample treatment.

3. Results and discussion

When this proposed method was applied to the samples, the identification and quantification of tartaric, malic and citric acids were carried out in grape juices and tartaric, malic, succinic, acetic, citric and lactic acids were achieved in wines (Fig. 1). Fluctuations in absolute migration times of solutes are one of the major reasons for the lack of reproducibility in capillary zone electrophoresis (Yang, Bose, & Hage, 1996). In order to minimise this problem, relative migration times were calculated with regard to oxalic acid which was chosen as reference standard.

In order to compare the validation results of the proposed method with the results obtained by other authors, a summary of the validation parameters of these CZE methods was included in Table 2. In this table, the precision (intraday and interday analysis) and recovery data of these previous works are only included if they were car-

ried out in samples, not only in standards. As you can see, there is a lot of lacks of data because these studies are not as deep as it must be. In this work, a thorough validation (detection and quantification limits, linearity, linear range, precision with intraday and interday analysis and recovery) has been studied in each sample: grape juice; white, rose and red wine.

3.1. Detection and quantification limits

The detection limit (LOD) was calculated as $s_b + 3s$, where s_b is the average signal of 10 blank injections (the absolute value of the area comprised between the migration time of each organic acid ±2%) and s the standard deviation. The quantification limit (LOQ) was calculated as $s_b + 10s$, where s_b is the average signal of 10 blank injections and s the standard deviation (ACS, 1980). LODs were 0.38 mg/L for tartaric acid, 0.05 mg/L for malic and succinic acids, 0.29 mg/L for acetic acid, 0.10 mg/L for lactic acid and 0.23 mg/L for citric acid. LOQs were 1.31 mg/L for tartaric acid, 0.53 mg/L for malic acid, 0.29 mg/L for succinic acid, 0.84 mg/L for acetic acid, 0.65 mg/L for lactic acid and 0.71 mg/L for citric acid. These units are mg of standard/L, so these LODs and LOQs have to multiply by the dilution factor to transform these units in mg of acid/L of sample. It is important to establish this differentiation because in previous works the wide range of LOD and LOQ found may be due to different units.

3.2. Calibration curves

The quantification of organic acids was carried out by using an external standard calibration method. Calibration curves were determined for seven different concentrations of a mixture of organic acid standard solutions. Each calibration point was injected in triplicate. Calibration graphs for each compound were obtained daily by plotting peak area against concentration and applying the least squares method. In CZE peak areas are linearly related to sample concentration over a broader range than peak heights. For this reason peak areas are used as the basis for quantitative analysis (Ross, 1997). All correlation coefficients of the calibration plots are greater than 0.9996. The linear range for grape juice is LOQ until 13 g/L for tartaric, malic and succinic acids and LOQ until 33 g/L for acetic, lactic and citric acids. In the case of wines, this linear range is LOQ until 8 g/L for tartaric, malic and succinic acids and LOQ until 20 g/L for acetic, lactic and citric acids.

3.3. Precision

Repeatability (intraday and interday) was studied to obtain the method precision (Table 3). This precision was carried out on grape juice and white, rose and red wines separately because the composition and the interferences are different in these samples. Intraday analysis was established by injecting the samples five times at the same day. Interday repeatability was determined by analysing each sample on three different days over about one month.

Regarding the precision of absolute and relative migration times of organic acids, RSD % of the intraday analysis were similar to absolute and relative migration times. However, variation of relative migration times (RSD %) were much better than variation of absolute migration times for all organic acids in the interday analysis. Therefore, the use of relative migration times instead of absolute migration times minimise the lack of reproducibility in times of capillary zone electrophoresis. In regard to the precision of concentrations, the relative standard deviation was ⩽3.69% for intraday analysis and ⩽3.98% for interday analysis for all organic acids in all samples (Table 3).

Table 3
Precision (RSD %) of the migration times and the content of analysed organic acids by the proposed method in grape juice and wine samples

Organic acids	Sample											
	Grape juice			White wine			Rose wine			Red wine		
	Migration times		Content	Migration times		Content	Migration times		Content	Migration times		Content
	Absolute	Relative		Absolute	Relative		Absolute	Relative		Absolute	Relative	
Intraday analysis (n = 5)												
Tartaric	0.14	0.07	0.82	0.18	0.11	0.40	0.06	0.11	0.94	0.21	0.05	1.09
Malic	0.14	0.06	1.48	–	–	NQ	0.05	0.08	2.94	0.18	0.09	2.19
Succinic	–	–	ND	0.16	0.13	0.97	0.06	0.10	2.07	0.17	0.09	2.31
Acetic	–	–	ND	0.13	0.19	1.68	0.09	0.19	3.69	0.11	0.10	2.70
Lactic	–	–	ND	0.16	0.21	1.79	0.10	0.17	2.47	0.11	0.13	1.36
Citric	0.06	0.18	2.32	–	–	NQ	0.12	0.18	1.92	0.22	0.25	2.86
Interday analysis (n = 3)												
Tartaric	0.85	0.29	3.70	2.72	0.22	3.53	3.44	0.24	3.04	1.99	0.25	2.44
Malic	0.77	0.18	0.92	–	–	NQ	3.32	0.12	2.34	1.97	0.20	3.98
Succinic	–	–	ND	2.61	0.13	3.70	3.17	0.04	0.33	1.96	0.17	3.83
Acetic	–	–	ND	2.87	0.48	3.24	3.45	0.25	3.56	2.23	0.51	3.42
Lactic	–	–	ND	3.03	0.65	2.88	3.63	0.43	0.29	2.40	0.67	1.34
Citric	1.58	1.02	1.78	–	–	NQ	3.93	0.72	3.02	2.50	0.74	1.15

ND: non detectable; NQ: non quantifiable.

3.4. Recovery

The accuracy of the organic acid analysis was established by using the method of standard additions. Different amounts of each organic acid standards were added to equal volumes of the sample and then diluted to the same volume. As well as precision, the recovery study was carried out on grape juice and white, rose and red wines separately because interferences could be different in these samples. Table 4 summarizes the percentage of recoveries obtained for each organic acid in the analysed samples.

As you can see, the proposed CZE method has similar validation results (Tables 3 and 4) as other CZE methods when validation parameters are compared (Table 2). Furthemore, the proposed method quantifies the most important acids to control the maturity and the winemaking processes (tartaric, malic, succinic, acetic, lactic and citric acids) in less time than previous reported CZE methods. Tacking into account the methods that have determined the most important acids to control the winemaking process simultaneously, as tartaric, malic, succinic, acetic, lactic and citric acids (Table 1), the separation time of these methods ranged between 7 min (Soga, 1996) and 18 min (De Villiers et al., 2003). The analysis time of our method decrease two, four or, even, six times the analysis time of the previous reported CZE methods. Therefore, time and reagent savings have been achieved and a lot of advantages as simplicity, speed and economy.

3.5. Samples

Table 5 summarizes the content of organic acids in the samples analysed by the proposed method. The values are within the range of values previously described in the literature, but obviously depend on the origin, type and ageing of grape juice or wine.

Tartaric acid was the characteristic acid of the grapes and grape products as wines. As you can see in Table 5, this acid represents at least the 50% of the total acid content in wines analysed. Malic acid was found in percentages of more than 30% in grape juices, but these percentages was less than 10% in the majority of wines. Only one wine (white wine 2) has a major content of malic acid because of malolactic fermentation have been not carried out after alcoholic fermentation in this wine. Succinic acid was not detectable in grape juices. When acetic acid is found in quantities greater than 1 g/L and lactic acid greater than various g/L, an alteration of wine could occur. As you can see in Table 5, acetic and lactic acids are found in less than these quantities in all wines. Citric acid is found in low quantities in grapes. This acid disappears due to the malolactic fermentation in wines. However, citric acid is found in samples analysed because, in some countries, the addition of citric acid is allowed to increase the acidity and to complex iron for avoiding the precipitation of Fe^{3+} (Ough & Amerine, 1988; Peynaud, 1999).

Table 4
Recoveries (%) obtained by the method of standard additions for analysed organic acids in grape juice and wine samples

Organic acids	Samples							
	Grape juice		White wine		Rose wine		Red wine	
	Mean ± SD	RSD %	Mean ± SD	RSD %	Mean ± SD	RSD %	Mean ± SD	RSD %
Tartaric	104.3 ± 0.3	0.3	105.8 ± 0.3	0.3	100.7 ± 0.2	0.2	102.5 ± 0.9	0.9
Malic	100.4 ± 1.2	1.2	99.5 ± 4.3	4.3	101.7 ± 1.4	1.4	99.0 ± 4.3	4.3
Succinic	–	–	97.4 ± 0.9	0.9	99.6 ± 1.6	1.6	100.2 ± 1.4	1.4
Acetic	–	–	94.9 ± 3.4	3.6	96.0 ± 1.2	1.3	99.5 ± 0.4	0.4
Lactic	–	–	102.7 ± 1.1	1.1	96.8 ± 1.2	1.2	99.7 ± 2.3	2.3
Citric	99.0 ± 0.1	0.1	95.3 ± 2.1	2.2	92.7 ± 1.4	1.5	99.4 ± 0.4	0.4

Table 5
Organic acid contents (mg/L of sample) of analysed samples by the proposed method

Organic acids	Tartaric	Malic	Succinic	Acetic	Lactic	Citric
Grape juice 1	2302 ± 24	2509 ± 25	ND	ND	ND	1622 ± 16
Grape juice 2	3555 ± 39	2350 ± 22	ND	ND	ND	1710 ± 13
White wine 1	2964 ± 8	NQ	206 ± 2	239 ± 5	783 ± 19	NQ
White wine 2	3221 ± 9	1968 ± 13	612 ± 5	180 ± 4	467 ± 11	459 ± 8
Rose wine 1	2660 ± 27	171 ± 2	241 ± 4	248 ± 3	696 ± 6	414 ± 11
Rose wine 2	3658 ± 37	NQ	183 ± 3	182 ± 2	829 ± 7	862 ± 22
Red wine 1	2492 ± 16	369 ± 5	217 ± 4	248 ± 6	590 ± 4	530 ± 12
Red wine 2	2918 ± 18	410 ± 5	236 ± 5	709 ± 17	1083 ± 8	677 ± 15

Samples are analysed by triplicate (mean ± SD).
ND: non detectable; NQ: non quantifiable.

4. Conclusions

The proposed reliable, simple and rapid CZE method with direct UV detection could be used for routine and automated analysis of the main organic acids in grape juices and wines due to the simple sample pre-treatment, the low cost, the good validation results (LOD, LOQ, linearity, precision and recovery) and the short analysis time which decrease two, four or, even, six times the analysis times of the previous reported CZE methods. Also, with this method could be possible to control the addition of citric acid in grape juices and wines, to monitorize the malolactic fermentation in wine samples by the determination of malic and lactic acids levels and to detect wine changes and/or wine illness related to these organic acids.

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References

ACS Committee on Environmental Improvement (1980). Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Analytical Chemistry, 52*, 2242–2249.

Arellano, M., Andrianary, J., Dedieu, F., Couderc, F., & Puig, P. (1997). Method development and validation for the simultaneous determination of organic and inorganic acids by capillary zone electrophoresis. *Journal of Chromatography A, 765*, 321–328.

Arellano, M., Couderc, F., & Puig, P. H. (1997). Simultaneous separation of organic and inorganic acids by capillary zone electrophoresis. Application to wines and fruit juices. *American Journal of Enology and Viticulture, 48*(4), 408–412.

Belitz, H. D., & Grosch, W. (1992). *Química de los alimentos*. Zaragoza, España: Acribia.

Bianchi, F., Careri, M., & Corradini, C. (2005). Novel approach for the rapid determination of water-soluble organic acids in wine by co-electroosmotic flow capillary zone electrophoresis. *Journal of Separation Science, 28*, 898–904.

Buchberger, W., Klampfl, C. W., Eibensteiner, F., & Buchgraber, K. (1997). Determination of fermenting acids in silage by capillary electrophoresis. *Journal of Chromatography A, 766*, 197–203.

Castiñeira, A., Peña, R. M., Herrero, C., & García-Martín, S. (2000). Simultaneous determination of organic acids in wine samples by capillary electrophoresis and UV detection: optimization with five different background electrolytes. *HRC – Journal of High Resolution Chromatography, 23*(11), 647–652.

Castiñeira, A., Peña, R. M., Herrero, C., & García-Martín, S. (2002). Analysis of organic acids in wine by capillary electrophoresis with direct UV detection. *Journal of Food Composition and Analysis, 15*(3), 319–331.

De Villiers, A., Lynen, F., Crouch, A., & Sandra, P. (2003). A robust capillary electrophoresis method for the determination of organic acids in wines. *European Food Research and Technology, 217*, 535–540.

Esteves, V. I., Lima, S. S. F., Lima, D. L. D., & Duarte, A. C. (2004). Using capillary electrophoresis for the determination of organic acids in Port wine. *Analytica Chimica Acta, 5131*, 163–167.

García Moreno, M. V., Jurado Campoy, C. J., & García Barroso, C. (2001). Method devised for determining low molecular weight organic acids in vinic samples by capillary electrophoresis: validation of the method with real samples. *European Food Research and Technology, 213*, 381–385.

Heiger, D. N. (1992). *High performance capillary electrophoresis: An introduction*. France: Hewlett–Packard Company.

Kandl, T., & Kupina, S. (1999). An improved capillary electrophoresis procedure for the determination of organic acids in grape juice and wine. *American Journal of Enology and Viticulture, 50*(2), 155–161.

Kelly, L., & Nelson, R. J. (1993). Capillary zone electrophoresis of organic acids and anions. *Journal of Liquid Chromatography & Related Technologies, 16*(9–10), 2103–2112.

Kenney, B. F. (1991). Determination of organic acids in food samples by capillary electrophoresis. *Journal of Chromatography A, 546*, 423–430.

Klampfl, C. W., Katzmayr, M. U., & Buchberger, W. (1998). Separation of inorganic and organic anions by capillary zone electrophoresis with simultaneous indirect UV and conductivity detection. *Electrophoresis, 19*, 2459–2464.

Lamikanra, O., Inyang, I. D., & Leong, S. (1995). Distribution and effect of grape maturity on organic acid content of red muscadine grapes. *Journal of Agricultural and Food Chemistry, 43*, 3026–3028.

Levi, V., Wehr, T., Talmadge, K., & Zhu, M. (1993). Analysis of organic acids in wines by capillary electrophoresis and HPLC. *American Laboratory, 25*, 29–32.

Mato, I., Suárez-Luque, S., & Huidobro, J. F. (2005). A review of the analytical methods to determine organic acids in grape juices and wines. *Food Research International, 38*, 1175–1188.

Moreno, M. V. G., Jurado, C. J., & Barroso, C. G. (2003). Determination of organic acids by capillary electrophoresis with simultaneous addition of Ca and Mg as complexing agents. *Chromatographia, 57*(3–4), 185–189.

Ough, C. S., & Amerine, M. A. (1988). *Methods for analysis of must and wines*. New York: John Wiley & Sons Inc.

Peynaud, E. (1999). *Enología práctica. Conocimiento y elaboración del vino*. Madrid, España: Mundi-Prensa.

Ross, G. A. (1997). Precision and quantitation in capillary electrophoresis. In H. Shintani & J. Polonský (Eds.). *Handbook of capillary electrophoresis applications* (pp. 41–70). London: Chapman & Hall.

Saavedra, L., & Barbas, C. (2003). Validated capillary electrophoresis method for small-anions measurement in wines. *Electrophoresis, 24*, 2235–2243.

Sing Fung, Y., & Man Lau, K. (2003). Analysis of organic acids and inorganic anions in beverage drinks by capillary electrophoresis. *Electrophoresis, 24*, 3224–3232.

Soga, T. (1996). *Hewlett–Packard Application Note 12-5963-1138E*.

Stathakis, C., & Cassidy, R. M. (1995). Effect of electrolyte composition in the capillary electrophoretic separation of inorganic/organic anions in the presence of cationic polymers. *Journal of Chromatography A, 699*(1 and 2), 353–361.

Thibault, P., & Dovichi, N. J. (1998). General instrumentation and detection systems including mass spectrometric interfaces. In P. Camilleri (Ed.). *Capillary electrophoresis: Theory and practice* (pp. 23–89). Boca Raton, FL: CRC Press LLC.

Vereda, E., García de Torres, A., Rivero, A., & Cano, J. M. (1998). Determination of organic acids in wines. A review. *Química Analítica, 17*(4), 167–175.

Vorarat, S., Aromdee, C., & Podokmai, Y. (2002). Determination of alpha hydroxy acids in fruits by capillary electrophoresis. *Analytical Science, 18*(8), 893–896.

Yang, J., Bose, S., & Hage, D. S. (1996). Improved reproducibility in capillary electrophoresis through the use of mobility and migration time ratios. *Journal of Chromatography A, 735*, 209–220.