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Validated capillary electrophoresis method for small-anions measurement in wines

A capillary electrophoresis method has been developed and validated for acetic, citric, fumaric, lactic, malic, oxalic, succinic, and tartaric acids plus the measurement of nitrate and sulfite ions in white and red wines. The separation was carried out in a neutral coated capillary. Separation was performed at −14 kV of applied potential. Temperature was maintained at 20°C. The background electrolyte used was 200 mM phosphate buffer at pH 7.50. Separation was obtained in less than 13 min. Validation parameters obtained for the method permit it to be considered adequate for routine analysis.

Keywords: Capillary electrophoresis / Carboxylic acid / Short-chain organic acid / UV / Wine
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CE and CEC

1 Introduction

The synthesis of organic acids during the physiological development of grapes and the microbial fermentation of their juice are the principal sources of these constituents in wines. Their origins are diverse, the most important being the biosynthesis by the vine, the metabolic pathways related to sugar fermentation, malolactic fermentation and ethanol oxidation. The equilibrium between these acids is probably the most important of the many physical and chemical systems found in wines. The acid composition and pH are of fundamental importance not only to the perception of sourness and related organoleptic properties, but also the microbial stability, the incidence and extent of the malolactic fermentation, the solubility of potassium bitartrate and calcium tartrate, the rates of formation and hydrolysis of esters, the ionization and rate of polymerization of anthocyanin pigments in red wines, and the incidence of protein instabilities in white wines. A considerable volume of literature on the acidic components of grapes and wines has been revised by Fowles [1].

The growing demand of grape concentrates with different purposes involves the need for manufacturing stable products with various acidity levels. The increasing interest of consumers in the so called “organic food” which avoids the use of chemical treatment makes it necessary to determine in grapes when the natural sugars and acids are in perfect balance, and tasting for the moment of adequate maturity for harvesting. The more demanding quality control, the need for origin and variety control in wines as well as the winemaking process investigations make frequent analysis of organic acids necessary. As an example, in certain northerly wine regions, notably Burgundy, malolactic fermentation is actively encouraged, since a dramatic acidity of the wine often results, and certain bouquet and flavor-enhancing by-products are produced [2], this not being the case in other wines. Therefore, a renewed interest in quality control of organic acids in wine products has emphasized the need for more rapid and validated procedures.

Table 1 includes a chronological summary of the most common analytical methods previously employed for short-chain organic acids in wines. Because of the well-known problems related with GC analysis of these compounds GC has been scarcely used [2]. The most common technique has been HPLC both in reverse phase with ionic suppression (working at pH under 2.4) [3–6] or with ion exchange and different detectors: UV-detector [7, 8], conductivity detector [9, 10], refractive index detection [11–13], or even electrochemical detection [14].

The most common drawbacks are the need for some type of sample pretreatment (frequently clean-up with SPE) and the poor resolution and LOD in samples, which makes the measurement of minor compounds difficult. Enzymatic techniques has also been employed, but for specific compounds [15, 16]. Nowadays, capillary electrophoresis is a technique used more and more in analytical laboratories mainly due to its exceptional resolving power, fast separations, many analyses under 10 min, reduced sample preparation compared to many other techniques, and minimum solvent consumption. Moreover, the same technique has proved to be a good choice for enantiomeric resolutions using chiral selectors in the

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Table 1. Summary of analytical methods for short chain organic acids in wines

	Technique	Method	Comments	Ref.
Citric, tartaric, malic, succinic, lactic, fumaric, acetic acids	Isocratic HPLC ion exchange	Aminex HPX(/-H) UV detection at 210 nm	Succinic acid coelutes with shikimic; poor resolution in samples; 12 min	[7]
Tartaric, malic, lactic, acetic, citric, shikimic, fumaric, succinic acids	Isocratic HPLC	Spherisorb ODS-2 column with a Bondapak C_{18}/Corasil-based precolumn UV detection at 210 nm	Working pH 2.47; $<$ 9 min	[3]
Tartaric, malic acetic lactic, succinic, citric acids	Isocratic HPLC	Spherisorb ODS-2 column with a Bondapak C_{18}/Corasil-based precolumn UV detection at 254 nm	Precolumn derivatization needed; computer-assisted optimization software used; $\geq$ 26 min	[4]
Tartaric, malic, citric acids	Gradient ion chromatography	Dionex OmniPac PAX-500 column conductivity detection	Sample filtration and dilution; range tested: 0.25–2.5 g/L; 35 min	[9]
Lactic acid	GC	1.5 m $\times$ 4 mm Porapak Q FID detector	Oxidation with Ce(IV)	[2]
L-Malic, L-lactic acids	FIA	Enzymatic with covalently immobilized L-MDH and L-LDH on-line dialysis; Fluorimetric detection of the common product NADH	Enantiomer-specific reactions; SO_2 can produce interferences	[15]
L-Malic, L-lactic acids	FIA	Enzymatic with nicotinamide adenine dinucleotide coenzyme, catalyzed by the L-malate or L-lactate dehydrogenase enzymes spectrophotometric detection	LOD, 20 mg/L	[16]
Citric, tartaric, malic, succinic, lactic, acetic acids, glucose, fructose, ethanol	Ion-exchange HPLC	Refractive index detection	Statistical methods for optimization; poor resolution for three compounds; 25 min	[11]
Tartaric, malic, lactic acids		Lichrospher Merck CH-18, 10 cm UV detection at 220 nm	SPE Sep-Pak cartridges; range around 1 g/L; 4 min	[5]
Tartaric acid	Rebelein's-based method and HPLC	Polymeric column ION 300 set at 70°C; colorimetric detection at 530 nm	Redisolution of precipitated tartrates needed	[27]
Acetic, lactic, succinic, malic, citric, tartaric acids, sulfate, nitrate, chloride	Ion-exchange HPLC	Shimpak IC-AI column filled with quaternary ammonium polymethacrylate conductimetric detection	LOD, 0.31–4.7 mg/L; filtration and Sep-Pak C_{18} cartridge sample treatment	[10]
Citric, tartaric, malic, succinic, lactic, acetic acids, glycerol, ethanol, glucose, fructose	Isocratic HPLC ion exchange	Two Aminex HPX-87H columns connected in series set at 75°C. Refractive index and UV detection at 214 nm	Sensitivity is rather poor for some analytes; 45 min	[12]
Citric, tartaric, galacturonic, malic, succinic, lactic acids, plus fructose, glycerol	Ion-exclusion HPLC	300 $\times$ 7.8 mm ION-300 column, containing an ion-exchange polymer, 0.0015 N sulfuric acid as mobile phase Refraction index detection	Sample filtration and dilution; LOD, 3–6 mg/L; 15 min; poor resolution in samples	[13]

Table 1. Continued

	Technique	Method	Comments	Ref.
Malic, tartaric acids	Radial compression HPLC	Waters Radial Pack 100 × 8 mm column; UV detection at 230 nm	SPE with Sep Pak cartridges; 7 min	[8]
Chloride, sulfate, tartrate, malate, succinate, citrate, phosphate, acetate, lactate	CE	Inverted polarity; pyromellitic acid as BGE with Tris-base and DETA at pH 7.5. Indirect detection at 220 nm	LOD from 0.017 to 1.072 mg/L; samples have to be diluted 50-fold	[18, 19]
Chloride, sulfate, tartrate, malate, succinate, adipate, glutarate, acetate, lactate, shikimate	CE	Inverted polarity; 4-aminobenzoic acid, Bis-Tris and LiOH to pH 7.0	LOD from 0 054 to 0.51 mg/L; samples have to be diluted 100-fold	[21]
Tartaric, galacturonic, malic, shikimic, lactic, acetic, citric, fumaric, succinic acids	Isocratic HPLC	Kromasil 100 C_{18} column with a Guard-Pack C_{18} precolumn UV detection at 214 nm	Working pH 2.3; no baseline resolution in some cases; > 27 min	[6]
Tartaric, malic, succinic, acetic, lactic acids	CE	Inverted polarity; phosphate buffer pH 6.5 Direct UV detection at 185 nm	Sample filtration and dilution; LOD, 0.02–0.05 mg/mL; different BGE compared; 6 min analysis	[22]
Malic, tartaric acids, both enantiomers of lactic acid	HPLC with columns witching	Two different mobile phases and two pumps needed UV detection at 230 nm	No baseline resolution for lactic acid enantiomers; ≦ 10 min	[17]
Citric, malic tartaric, lactic, formic, acetic acids	Ion-exclusion HPLC	Sulfonated styrene-divinylbenzene (PS-DVB) copolymer resin; electrochemical detection based on PtOH species formation/inhibition	LOD, 0.5–7 μM; poor resolution in samples; 15 min	[14]

DETA, diethylenetriamine; FIA, flow injection analysis; ODS, octadecyl silica

separation buffer which is capable of solving a problem nowadays treated with more complicated approaches [17].

CE has previously been used with indirect UV detection for chloride, sulfate, tartrate, malate, succinate, citrate, phosphate, acetate, and lactate measurement in wine [18, 19]. Although the LOD were reported as ranging from 0.006 to 1.07 mg/L, they were calculated with standards. Real samples had to be diluted 50-fold, and therefore, the LOD for samples ought to be multiplied by this number, which means 0.3–53.5 mg/L. Moreover, organic acids of special interest for quality control, such as fumaric acid, cannot be detected with indirect UV detection. The same group proposed a method for tartaric acid in raw powder to be applied in the solid wine residues obtained as by-products of wine preparation [20]. This method permits to measure just one compound but authors say it is not only very quick (2 min analysis) but also highly reproducible.

On the other hand, the separation of inorganic and organic anions by CE with simultaneous indirect UV detection and conductivity detection was applied to a sample of white wine [21]. Only the combination of both detection techniques allowed the quantification of most of the analytes of interest. Nevertheless, oxalate could not be measured, tartrate, malate and succinate were not baseline-resolved, and fumarate was not included in the method. Moreover, common commercial equipment do not include the conductivity detector, and therefore this method cannot be considered for routine analysis. Tartaric, malic, succinic, acetic, and lactic acids have also been measured in wine by CE [22], but in our opinion

the application could be extended and the LODs for minor acids improved. Thus, the aim of the present work was the validation of a CE method for acetic, citric, fumaric, lactic, malic, oxalic, succinic, and tartaric acids plus the measurement of nitrate and sulfite in red and white wines by CE with UV detection which can be applied very simply in the routine quality control of these beverages.

2 Materials and methods

2.1 Instrumentation

The separation was performed in a CE P/ACE 5500 system (Beckman, Palo Alto, USA) with UV detection at 200 nm. The injection was by pressure (0.035 bar) for 20 s. The separation was carried out in a neutral coated capillary (polyacrylamide), 50 cm long to the detection window and 50 μm internal diameter (Beckman). Separation was performed at −14 kV of applied potential. Temperature was maintained at 20°C. The background electrolyte used was 200 mM phosphate buffer at pH 7.50 and was prepared by adding the necessary amount of NaOH solution to a phosphoric acid solution properly weighted prior to the volume adjustment. The current observed under these conditions was 110 μA. At the beginning of its use, the capillary was conditioned by a pressure flush of 0.1 M HCl (1 min), BGE (10 min) and an electrokinetic flush of electrolyte with 0.5 kV/cm (10 min). In-between runs, the capillary was flushed by pressure with deionized water (2 min) and BGE (2 min). The indirect detection method, developed and validated by The Laboratoire Interregional De La Direction Generale De La Concurrence, De La Consommation Et De La Repression Des Fraudes (Talence, France) employed an uncoated capillary (Beckman) with 50 μm of internal diameter and 57 cm total length. The BGE was pH = 5.6 distributed by Hewlett Packard (Cat. No. 8500-6785; Madrid, Spain). The voltage applied was −18 kV, the temperature was maintained at 30°C and the injection was by pressure for 30 s (0.5 psi). Detection was performed at 214 nm. The current observed under these conditions was 5 μA. At the beginning of its use, the capillary was conditioned by a pressure flush of 0.1 M NaOH solution (5 min), deionized water (5 min) and BGE (10 min) followed by an electrokinetic flush of electrolyte (−18 kV for 10 min). In-between runs, the capillary was just replenished with fresh BGE (2 min, pressure flush).

2.2 Chemicals

L-Lactic acid disodium salt was from Fluka (Buchs, Switzerland), citric acid trisodium salt, tartaric acid disodium salt, fumaric acid disodium salt, succinic acid, phthalic acid, and malic acid were obtained from Sigma (St. Louis, MO, USA), oxalic acid disodium salt, KNO_3 and Na_2SO_3 were obtained from Panreac (Madrid, Spain), acetic acid was from Sharlab (Barcelona, Spain), phosphoric acid (85%) from Merck (Darmstadt, Germany), sodium hydroxide from Panreac.

2.3 Samples

Samples used during the development and validation of the method were obtained from one of the many brands available in the market (Rioja, Spain). Samples were diluted as 1:1 v/v with Milli-Q water quality (Millipore, Madrid, Spain) before its filtration for the measurement. Filtration was made through nylon filters of 0.45 μm pore size. In the indirect method, 1 mL of wine is added with 1 mL of chlorate solution as internal standard and made up to 50 mL with purified water, before its filtration in the same way as described above.

2.4 Validation

Validation experiments were performed separately for white and red wine and, in each case, fumaric acid was validated separately from sulfite. A previous stock solution (stock 0) containing the two minor compounds was prepared with 4.038 mg/mL oxalic acid and 3.822 mg/mL KNO_3 in Milli-Q quality water. The first working stock solution (Stock 1) containing six acids was prepared with 8.98 mg/mL succinic acid, 23.456 mg/mL tartaric acid, 2.72 mg/mL citric acid, 3.172 mg/mL acetic acid, and 1/25 parts of stock 0, which contains oxalic acid and KNO_3, as mentioned before. A second stock solution (Stock 2) was prepared with 22.6 mg/mL lactic acid and 4.26 mg/mL Na_2SO_3. Finally, a third stock solution (stock 3) was prepared with 14.12 mg/mL of malic acid. In the second validation, the stock solution was 0.0614 mg/mL of fumaric acid. In all cases, phthalic acid was employed as internal standard and the stock solution was prepared with 0.09 mg/mL. Linearity of response for standards was tested assaying by triplicate five levels of concentrations in the ranges described in Table 2. These levels were prepared as follows: in 10 mL volumetric flasks we mixed 0.1, 0.25, 0.5, 0.75, or 1 mL of stock 1, plus 0.1 or 0.5 mL of a previously 1/10 diluted stock 2 or 0.1, 0.5 or 1 mL of the undiluted stock 2 and 0.5 or 1 mL of a previously diluted 1/100 stock 3 or 1 mL of a previously 1/10 diluted stock 3, or 0.5 or 1 mL of the undiluted stock 3. Linearity of response for samples was tested in the same way but replacing a half part of the water with wine (red and white separately) and by adding half volume of each stock solution that in samples. Recovery was estimated comparing the values obtained in samples linearity, with those in

Table 2. Main validation parameters of the method with direct UV detection for red wine

			Acetic	Citric	Fumaric	Lactic	Malic	Oxalic	Succinic	Tartaric	Nitrate	Sulfite
	Range (mM)		0.53–5.28	0.11–1.05	0.004–0.04	0.2–20.2	0.05–10.5	0.012–0.12	0.76–7.6	1.02–10.19	0.015–0.15	0.03–3.4
Linearity	Standards	Intercept ± CI	0.03±0.01	−0.002±0.02	−0.01±0.01	0.02±0.02	0.02±0.05	0.001±0.003	0.08±0.04	0.2±0.1	0.12±0.02	0.2±0.4
		Slope ± CI	0.524±0.005	2.35±0.04	12.4±0.4	0.644±0.002	1.107±0.009	3.07±0.04	0.975±0.008	1.38±0.02	8.1±0.2	3.2±0.2
		r	0.9999	0.9999	0.999	0.9999	0.9999	0.999	0.9999	0.999	0.999	0.992
	Samples	Intercept ± CI	0.07±0.02	−0.01±0.02	−0.03±0.01	0.5±0.2	0.04±0.02	0.004±0.003	0.08±0.1	0.5±0.2	0.13±0.02	0.4±0.3
		Slope ± CI	0.51±0.02	2.40±0.05	11.5±0.8	0.59±0.02	1.07±0.01	2.9±0.07	1.0±0.05	1.31±0.03	8.0±0.3	3.1±0.3
		r	0.998	0.999	0.993	0.998	0.999	0.999	0.996	0.999	0.998	0.99
Accuracy	Standards	Recovery (%)	99.4	100.3	102.0	99.2	94.1	100.3	99.4	99.5	100.1	91.9
		RSD (%)	2.48	1.76	6.8	3.9	12.0	3.9	2.3	2.8	3.0	25.5
	Samples	Recovery (%)	100.3	99.6	96.5	100.8	90.7	100.3	99.9	100.4	101.6	100.8
		RSD (%)	1.9	2.2	6.1	2.2	17.1	1.9	4.6	1.2	1.4	6.5
Precision standards	Intra-assay	*n*	10	10	9	10	10	10	10	10	10	10
		Mean (mM)	2.64	0.53	0.019	2.02	1.05	0.06	3.8	5.1	0.076	0.338
		RSD (%)	0.80	0.70	1.24	1.3	0.8	1.0	0.7	0.5	0.9	3.5
	Intermediate	*n*	20	20	19	20	20	20	20	20	20	20
		Mean (mM)	2.64	0.53	0.019	2.02	1.05	0.06	3.8	5.1	0.076	0.338
		RSD (%)	0.96	0.76	1.95	1.4	0.9	1.6	0.8	0.7	1.1	4.2
Precision samples	Intra-assay	*n*	7	7	7	7	7	7	7	7	7	7
		Mean (mM)	2.84	0.14	0.0029	10.45	0.09	0.031	2.35	5.95	0.057	0.014
		RSD (%)	1.4	1.8	2.0	0.5	2.9	3.6	0.5	0.4	7.6	11.9
	Intermediate	*n*	14	14	13	14	14	14	14	14	14	14
		Mean (mM)	2.88	0.14	0.0034	10.80	0.094	0.031	2.37	6.03	0.055	0.015
		RSD (%)	1.9	2.5	13.9	3.6	9.3	3.7	1.3	1.6	9.45	29.7

CI, confidence interval

standards linearity, taking into account the wine concentrations which had been previously measured. Within-day precision was tested to check both the constancy of instrumental response to a given analyte and the concentration and migration time repetitiveness, since the latter is a key parameter for peak assignment. For this purpose, the assay was performed with six solutions of standards and ten of samples on the same day, in the medium concentration of the calibration curve for all the compounds. For intermediate precision, six standards and ten samples were measured on different days.

3 Results and discussion

Our work group has developed several methods for short-chain organic acids measurement in different matrices: urine [23, 24], latex serum [25], orange juice [26]. For these compounds inverted polarity is needed (injection in the negative end) and, therefore, either coated capillaries or a cationic surfactants added to the background BGE have to be employed to avoid the electroosmotic flow. In our experience, coated capillaries present higher reproducibility than uncoated ones, and as the purpose of the present method was routine quality control, polyacrylamide coated capillaries were chosen, to obtain higher precision.

On the other hand, a compromise between ionic strength and current generated was achieved with the concentration of the BGE. Higher concentrations in the BGE permit lower dilution of samples with the corresponding better LODs for the minor compounds, but it also produces higher currents with the well known deleterious effects. Phosphate buffer (200 mM) at pH 7.50 and −14 kV of voltage were the conditions employed. These conditions were in the lineal range of the Ohm's plot and temperature was maintained by the equipment at 20°C without problems.

Figure 1 shows the separation obtained for standards, white and red wines. Peaks were identified by their migration times as compared with those of pure standards and by spiking and they corresponded to acetic, citric, fumaric, lactic, malic, oxalic, succinic, and tartaric acids plus nitrate and sulfite ions. Baseline separation is achieved in around 13 min without any other sample pretreatment than dilution and filtration. Separation was compared with that obtained with the method of indirect detection quoted above which is shown in Fig. 2 for standards, a white and a red wine. Obviously, migration times are shorter with the indirect method, but this method is only intended for tartaric, malic, citric, succinic, lactic, and gluconic acids plus sulfate and phosphate (perchlorate is used as internal standard). In practice, sulfate is many times at the edge of a system peak and cannot be quanti-

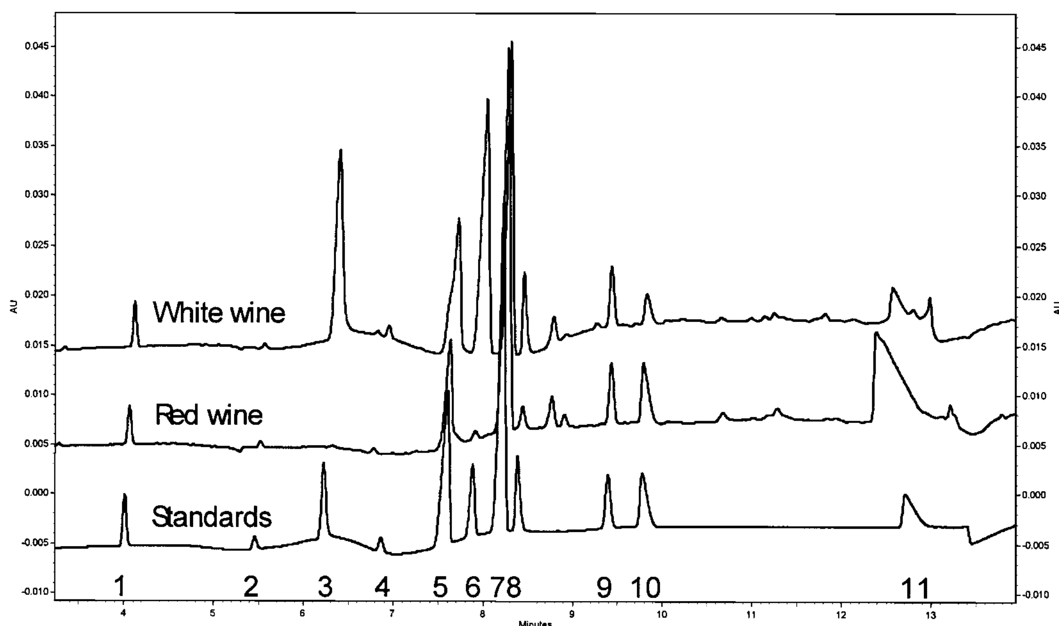

Figure 1. Electropherogram for standards and medium red wine (lower level) and white wine (upper level) with direct detection. Peaks: 1, nitrate; 2, oxalate; 3, sulfite; 4, fumarate; 5, succinate; 6, tartrate; 7, malate; 8, citrate; 9, phthalate (IS); 10, acetate; 11, lactate. Conditions: polyacrylamide-coated capillary, 200 mM phosphate buffer at pH 7.50; −14 kV; direct detection at 200 nm.

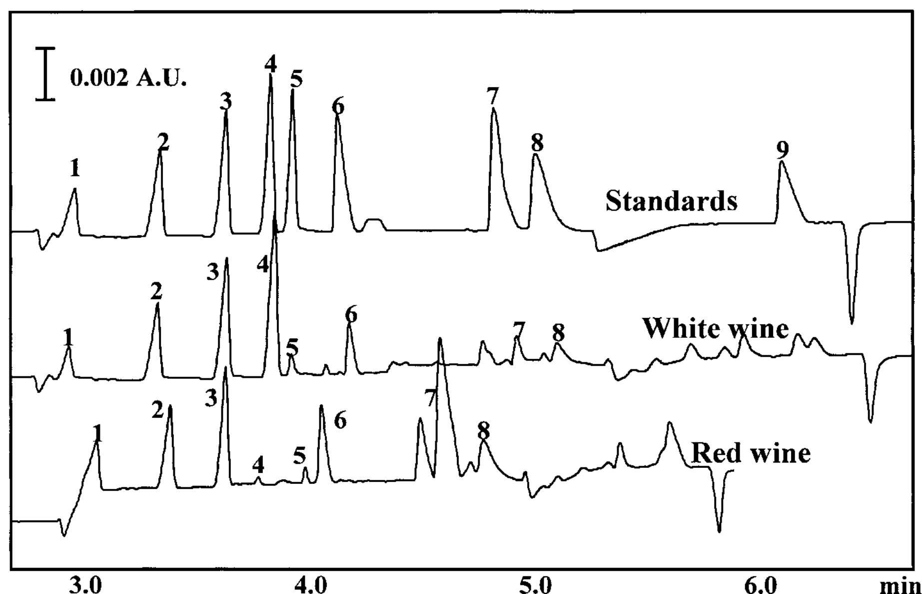

Figure 2. Electropherogram of a sample of white wine with indirect detection. Peaks: 1, sulfate; 2, perchlorate (IS); 3, tartrate; 4, malate; 5, citrate; 6, succinate; 7, lactate; 8, phosphate; 9, gluconate. Conditions: uncoated capillary, UV-absorbing comercial BGE pH 5.6; −18 kV; indirect UV detection at 214 nm.

fied, while phosphate and gluconate are not resolved. Moreover, although indirect detection is said to give lower detection limits than direct detection, samples have to be diluted 50 times to avoid the baseline noise and, finally, as can be seen in Fig. 2, peaks for malic and citric are very small in the samples. Finally, as can be seen in the Fig. 2, there is a between run variation in migration times with this method, and that makes peak identification difficult in many cases.

Small-anions measurement in white and red wines was validated separately because interferences could be different in both types of wine. Moreover, during prevalidation assays it was detected that fumaric acid was not stable in the presence of sulfite. Obviously, when real samples are measured these compounds have got an equilibrium and real concentrations at this time will be measured at one time, but when fumaric acid and sulfite are simultaneously increased through a linearity test, recoveries are lower than expected. For that reason validation for fumaric acid was performed independently. Main validation parameters of the method are summarized in Table 2 for white wine and in Table 3 for red wine.

The linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes in samples within a given range. Linearity has been determined by a series of three replicates of five levels of standards whose concentrations span 10–200% of the expected concentration range. The linear regression equation applied to the results gave an intercept not significantly different from zero in most cases. When a significant non-zero intercept was obtained (acetate, succinate, tartrate, and nitrate), it was mainly due to the good fit of the points to the regression line which made the limits of confidence very narrow and there was no effect on the accuracy of the method. The slopes are different from zero in all cases and correlation coefficients are 0.999 in most cases and over 0.99 in all cases. Samples linearity is also adequate for the same reason, but this experiment was mainly developed to study the recoveries. All the recoveries include 100% and RSDs presented very adequate values with exception of sulfite which gets oxidized during the assay. Intra-assay precision of the method for standards gave RSDs ranging from 0.5% to 1.2% not considering sulfite that was 3.5%. These small RSD values allow us to confirm the better performance of coated capillaries in relation to repetitivity. When method precision for standards was considered on two different days, RSDs ranged from 0.7% to 2.0%, still very low, being 4.2% for sulfite. For red wines, RSDs ranged from 0.4% to 3.3% for short-chain organic acids, being higher for the anions (7.6% for nitrate and 11.9% for sulfite) when intra-assay method precision was tested. On two different days, RSDs in red wine samples ranged from 1.3% to 13.9% for the organic acids. For white wine samples RSDs ranged from 0.8% to 4.7% for short-chain organic acids, meanwhile for the anions it was 7.0% for nitrate and 1.4% for sulfite when intra-assay method precision was tested. The values obtained for RSDs in white wine on two different days ranged from 3.1% to 6.3% for the organic acids. All the values can be considered adequate for the levels of analytes and characteristics of the method.

Table 3. Main validation parameters of the method with direct UV detection for white wine

			Acetic	Citric	Fumaric	Lactic	Malic	Oxalic	Succinic	Tartaric	Nitrate	Sulfite
	Range (mM)		0.53–5.28	0.11–1.05	0.004–0.04	0.2–20.2	0.05–10.5	0.012–0.12	0.76–7.6	1.02–10.19	0.015–0.15	0.03–3.4
Linearity	Standards	Intercept ± CI	0.03±0.01	−0.002±0.02	−0.01±0.01	0.02±0.02	0.02±0.05	0.001±0.003	0.08±0.04	0.2±0.1	0.12±0.02	0.2±0.4
		Slope ± CI	0.524±0.005	2.35±0.04	12.4±0.4	0.644±0.002	1.107±0.009	3.07±0.04	0.975±0.008	1.38±0.02	8.1±0.2	3.2±0.2
		r	0.9999	0.9999	0.999	0.9999	0.9999	0.999	0.9999	0.999	0.999	0.992
	Samples	Intercept ± CI	0.04±0.02	0.02±0.03	−0.011±0.009	0.01±0.06	0.28±0.04	0.001±0.004	0.11±0.06	0.36±0.07	0.13±0.01	0.4±0.3
		Slope ± CI	0.518±0.006	2.30±0.06	11.7±0.6	0.64±0.01	1.032±0.009	2.9±0.1	0.96±0.02	1.33±0.01	8.2±0.2	3.1±0.3
		r	0.999	0.999	0.996	0.999	0.9999	0.997	0.999	0.999	0.999	0.99
Accuracy	Standards	Recovery (%)	99.4	100.3	102.0	99.2	94.1	100.3	99.4	99.5	100.1	91.9
		RSD (%)	2.48	1.76	6.8	3.9	12.0	3.9	2.3	2.8	3.0	25.5
	Samples	Recovery (%)	100.1	100.2	99.7	98.1	100.0	99.5	99.8	100.3	101.3	101.3
		RSD (%)	1.84	1.57	5.8	5.0	0.82	3.4	1.3	0.6	2.1	5.5
Precision standards	Intra-assay	*n*	10	10	9	10	10	10	10	10	10	10
		Mean (mM)	2.64	0.53	0.019	2.02	1.05	0.06	3.8	5.1	0.076	0.338
		RSD (%)	0.80	0.70	1.24	1.3	0.8	1.0	0.7	0.5	0.9	3.5
	Intermediate	*n*	20	20	19	20	20	20	20	20	20	20
		Mean (mM)	2.64	0.53	0.019	2.02	1.05	0.06	3.8	5.1	0.076	0.338
		RSD (%)	0.96	0.76	1.95	1.4	0.9	1.6	0.8	0.7	1.1	4.2
Precision samples	Intra-assay	*n*	6	6	8	7	7	6	7	6	7	6
		Mean (mM)	1.24	0.57	0.0086	1.74	5.4	0.021	3.69	4.89	0.062	1.278
		RSD (%)	1.6	1.2	2.3	3.4	1.0	4.7	1.0	0.8	7.0	1.4
	Intermediate	*n*	13	13	14	13	13	13	13	13	13	13
		Mean (mM)	1.28	0.54	0.0086	1.72	5.7	0.020	3.82	4.65	0.061	1.430
		RSD (%)	4.4	6.3	3.1	3.9	5.0	5.3	4.2	5.1	11.5	10.5

CI, confidence interval

The values obtained for the acids measured in a random bottle of white Rioja Spanish wine were: 0.149 g/L for acetic, 0.294 g/L for citric, 0.003 g/L for fumaric, 0.390 g/L for lactic, 1.448 g/L for malic, 0.006 g/L for oxalic, 0.872 g/L for succinic, and 2.250 g/L for tartaric acids plus 0.008 g/L for nitrate and 0.204 g/L for sulfite ions. In a red Rioja Spanish wine they were 0.341 g/L for acetic, 0.072 g/L for citric, 0.001 g/L for fumaric, 2.342 g/L for lactic, 0.024 g/L for malic, 0.008 g/L for oxalic, 0.555 g/L for succinic, and 2.738 g/L for tartaric acids plus 0.007 g/L for nitrate and 0.002 g/L for sulfite ions. Those values are within the range of values described in literature, but obviously depend on the origin, type and ageing of the wine.

4 Concluding remarks

A CE method has been validated that permits the quantification of acetic, citric, fumaric, lactic, malic, oxalic, succinic, and tartaric acids plus nitrate and sulfite ions in white and red wines in less than 13 min, and without any other sample pretreatment than a dilution with equal volume of water and filtration. This method permits oxalate and fumarate measurement which was not possible with previous CE methods [18, 19, 21]. Fumaric acid can prevent malolactic fermentation, but its addition is forbidden in some countries and therefore, its measurement is of especial interest for quality control. The method has been developed to be applied with common, commercial equipment and the resolution permits an automatic and easy integration of the peaks in real samples and, therefore, it can be applied in routine work. Moreover, the use of coated capillaries provides a higher precision in migration times than that obtained with uncoated capillaries and that permits a higher security in peak identification in samples. Finally, although LODs have not been established, the validation has been extended to a 10% of the expected values for these compounds to ensure that all of them can be measured. In comparison with other well-established methods included in Table 1, such as ion-exchange HPLC, the main advantages are: (i) samples do not need a clean-up prior to the analysis; (ii) analysis time and equilibration times are shorter (higher throughput); (iii) in many cases resolution is higher with CE, mainly in samples. Moreover, the advantages related to the technique, explained in the introduction, are mainly the low consumption of solvents and reagents.

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