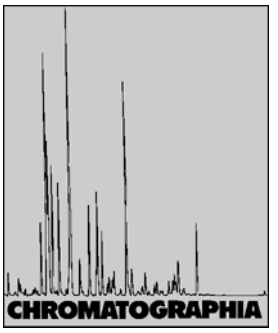
Determination of Organic Acids by Capillary Electrophoresis with Simultaneous Addition of Ca and Mg as Complexing Agents



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Key Words

Capillary electrophoresis
Complexing agents in electrolyte
Organic acid
Wine samples

Summary

A capillary electrophoretic method, with divalent cations as complexing agents in the electrolyte, has been developed for separation and determination of the low molecular weight organic acids most commonly found in wine, viz. formic, fumaric, succinic, oxalic, malic, tartaric, acetic, lactic, and citric acids. The separation conditions optimized were electrolyte concentration, organic flow modifier concentration, type and concentration of complexing agents in the electrolyte, and injection time. The best resolution of some of the acids studied was achieved by use of an electrolyte containing tetraborate buffer (10 mM) at pH 9.3, an organic flow modifier (tetradecyltrimethylammonium hydroxide), and Ca^{2+} (10 ppm) and Mg^{2+} (10 ppm) as complexing agents. Other conditions used in the method were hydrostatic injection (10 cm height for 30 s), detection at 185 nm, and temperature 20 °C. For all the acids studied detector response was linear for the concentration ranges considered. The repeatability of each point on the calibration plot for standards (n = 4) was generally better than 1%. The method was applied to samples of must, wine, brandy, and vinegar from the Jerez region.

Introduction

Organic acids are important constituents of grape-derived products (must, wine, brandy, or vinegar). They are not quantitatively significant, because they occur in small concentrations only; it is, instead, qualitative aspects that make them important, because of their substantial effect on such important properties as the products' organoleptic characteristics (colour, aroma, flavour, and taste) and their stability, or the control of microbiological quality [1, 2]. This makes their quantitative determination very important. These acids can be determined individually, either enzymatically or spectroscopically, after they have been separated from other components [3, 4].

Traditionally, gas chromatography (GC) has been used for the analysis of carboxylic acids [3]. This technique has a disadvantage: The organic acids cannot be determined directly, because they require derivatization to make then volatile. Another much-used technique for the analysis of this type of compound is high-performance liquid chromatography (HPLC) [1, 2, 5–11]; because of the complexity of samples of enological origin, however, pretreatment of the samples is necessary. Activated charcoal, ionic exchange resins, or Sep-Pak C_{18} cartridges are some of the materials used for this pretreatment.

Capillary zone electrophoresis (CZE) is currently acquiring considerable importance as a technique for separation of ionic species of low molecular weight. Methods based on CE have also been used for analysis of a wide variety of organic acids of low molecular weight [10–27] in different matrices, e.g. blood [12], urine [25], other biological fluids, industrial samples [10], the complex fluids of organic plants [11], environmental samples such as air [13], and cigarette smoke [10].

CZE has also been used for analysis of these compounds in food [27] and drinks. Analysis of organic acids is important in quality control in the refining of sugars [14] and quantification of sugars in vegetable products [15]. These acids are also determined in beer [16, 17], in fruit juices [18–20], and wine [20–23].

Most of these papers report the analysis of carboxylic acids with indirect UV detection [11–17, 19–24], or conductivity detection [16, 17, 21]; few papers describe the analysis of these acids by use of direct detection [18, 25, 26] and only the method of Saavedra et al. [18] has been used with food samples. The aim of this work was optimization of a CE method with direct UV detection for separation and determi-

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nation of the principal organic acids contained in samples of grape-derived products (must, wine, vinegar, and brandy).

Experimental

Chemicals

All the reagents used in this study were of analytical quality or better. Citric, lactic, formic, and oxalic acids were supplied by Fluka (Buchs, Switzerland), fumaric, malic, and succinic acids by Sigma (St Louis, MO, USA), acetic and tartaric acids, calcium chloride ($CaCl_2.2H_2O$) and magnesium chloride ($MgCl_2.6H_2O$) by Merck (Darmstadt, Germany), sodium tetraborate ($Na_2B_4O_7.10H_2O$) by Panreac (Barcelona, Spain), and the organic flow modifier tetradecyltrimethylammonium hydroxide (TTAOH) by Waters (Milford, MA, USA).

Instrumentation

All separations were performed with a Waters (Milford, MA, USA) capillary ion analyser equipped with a UV-visible detector, operated at 185 nm, with Millennium 2100 software for control and data acquisition. The separations were performed in a fused-silica capillary of 75 µm internal diameter and 60 cm total length (53 cm effective length to the detector). Because the wall of the capillary is covered with ionizable silanol groups, it is necessary to prepare the capillary before each analysis to ensure the surface is completely and uniformly charged. For this purpose first a solution of 10 mM sodium hydroxide (1 min), then de-ionized water (1 min), and finally the electrolyte (3 min) were passed through the capillary immediately before injection of the samples. The potential applied was 7 kV, by use of a negative feed source.

The samples were injected hydrodynamically. In CE the peaks do not all pass the detector at the same speed and, therefore, the more slowly-moving peaks spend more time in the detector, giving rise to larger peak areas. To obtain peak areas independent of time it is usual to divide these areas by the migration time; the areas thus calculated are designated normalized areas [28]. Normalized areas have been used in this study.

Standards

Individual standard solutions (1000 ppm) were prepared from each acid. Immediately before performing analyses working solutions of a mixture of acids were prepared as stock solutions. Other solutions were prepared by dilution of these stock solutions. Before injection the samples were filtered through 0.45 µm Millipore filters. All solutions were prepared in Milli-Q quality water (Millipore, Bedford, MA, USA).

Samples

The method was applied to samples of grape-derived products (must, wine, brandies and vinegar) from the Jerez region. All real samples were diluted with Milli-Q water before filtration and injection. Must samples were diluted 1:100 (1 mL must in 100 mL total solution) before injection. Wine samples were diluted and injected twice, the first time in 1:50 ratio (1 mL wine in 50 mL total solution) for determination of acids found in relatively large concentrations (tartaric and succinic acids) and a second time in 1:10 ratio (1 mL wine in 10 mL total solution) for determination of acids found in lower concentrations (acetic and lactic acids). Brandy samples were diluted 2:5 (2 mL wine in 5 mL total solution) before injection. Vinegar samples were diluted 1:100 for determination of acids found in relatively large concentrations (acetic, tartaric, malic, and lactic acids) and diluted 1:50 for determination of acids found in lower concentrations (principally citric acid).

Results and Discussion

Choice of Electrolyte

The literature reports the use of different types of electrolyte for the analysis of these acids with both inverse and direct detection, including phthalate [11, 14], *p*-hydroxybenzoate [19, 24], and borate buffer [25, 26]. Initial tests conducted on the basis of methods found in the literature identified borate buffer as the carrier electrolyte giving the best results.

Concentration of EOF Modifier

To achieve better analysis of negative species by capillary electrophoresis it is necessary to apply a negative voltage, so that the ions migrate together with the electrosmotic flow (EOF) towards the detector, situated at the anodic end of the capillary. To do this, species that invert the direction of electrosmotic flow (i.e. modifiers of the electrosmotic flow) must be used. Of the many organic species used as modifiers [19] the surfactant TTAOH was the flow modifier affording the best resolution and baseline stability in previous work. To determine the optimum concentration of flow modifier different electrolytes were prepared containing from 0.5 to 5.0 mM OFM. These were tested with a control sample of acids. No significant improvement in the resolution of the peaks was observed when the concentration of the flow modifier was increased. Consequently a flow modifier concentration of 0.5 mM was selected as optimum.

Concentration of the Carrier Electrolyte

The concentration of the electrolyte can have a considerable effect on baseline noise, sensitivity, and the linear dynamic range. Different electrolytes containing between 2.5 and 10 mM sodium tetraborate were tested to find the optimum concentration of carrier electrolyte. On the basis of peak resolution we observed that the best results were obtained for a borate concentration of 10 mM.

Complexing Agents

In initial experiments it was observed that under the conditions used similar ionic mobilities were obtained for tartaric and malic acids, regarded as the most important acids in the samples studied. On the basis of results reported in the literature [14, 24, 26] it was decided to add complexing agents to modify the mobility of these ions. Tests were conducted with the divalent cations calcium and magnesium (as their chlorides) added to the carrier electrolyte, both individually and together, as complexing agents of the carboxylic acids; 5 ppm or 10 ppm of Ca^{2+} or Mg^{2+} or 5 ppm or 10 ppm of both Ca^{2+} and Mg^{2+} were added to the electrolyte. The results in Figure 1 show that when the concentration of Ca was increased, the resolution of the peaks also increased. When Ca^{2+} was added, however, citric acid was not detected. For this reason we used Mg^{2+} as

the complexing agent. Study of the results obtained by addition of different amounts of Mg^{2+} to the electrolyte, as complexing agent, showed that good peak resolution was obtained irrespective of concentration.

Finally, the effect of adding both Ca and Mg, i.e. in combination, to the electrolyte was investigated. Two tests were performed. In the first the concentrations of the two cations in the electrolyte were 5 ppm and in the second the concentrations were 10 ppm. As is apparent from Figure 1C the best result it is obtained in the second test, i.e. when the electrolyte contained 10 ppm of both cations. Although the separation could be better in the electropherogram obtained, there are no overlapping peaks and all are resolved.

Injection Time

In this analytical technique short injection times lead to larger percentage standard deviations of peak areas. On studying the mean percentage standard deviations for the normalized areas of the acids analysed, for four different injection times (10, 20, 30, and 40 s), it was found that injection for 30 s resulted in the lowest value (4.92%). The dependence on injection time of the normalized peak areas for the acids studied is depicted in Figure 2. It is apparent that injection for 10 and 20 s gives very similar values whereas after injection for 20, 30, and 40 s there is a direct correlation between normalized area and injection time. This indicates that injection times of 10 or 20 s are not significant in terms of the quantity of sample injected, in contrast with times of 30 or 40 s, which are significant. For both reasons it was decided to inject samples for 30 s.

Performance of the Method

The linearity of the method was confirmed by analysis of standard solutions of samples containing a mixture of the acids at concentrations similar to those expected in grape-derived samples. Table I shows the linear regression equations and other characteristic data for determination of the analytes studied. Each plot was been constructed with five points, with four replicate analyses per point. The corrected area values were used to perform linear regression by the minimum least squares method. The values obtained for the correlation coefficients show the method is linear for the acids in the ranges of concentrations studied. In general the results are fairly satisfactory; the lowest correlation coefficient was that for lactic acid (0.989) and the highest that for formic acid (0.9998).

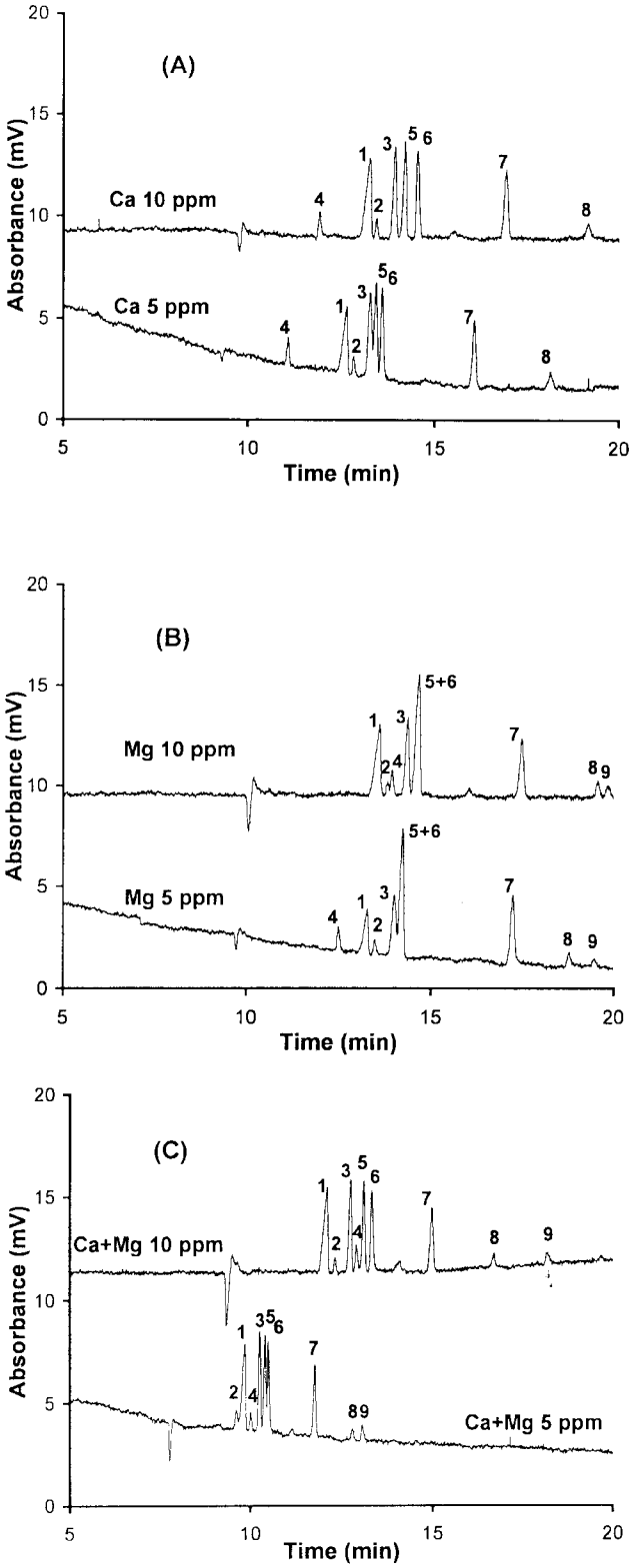

Figure 1. Effect of addition of divalent cations to the background electrolyte. (**A**) addition of Ca^{2+}; (**B**) addition of Mg^{2+}; (**C**) addition of Ca^{2+} and Mg^{2+}. Other electrolyte conditions: 10 mM $Na_2B_4O_7$ and 0.5 mM TTAOH (pH 9.3). Electrophoretic conditions: separation potential −7 kV; capillary temperature 20 °C; UV detection at 185 nm. Peaks: **1** = formic acid, **2** = fumaric acid, **3** = succinic acid, **4** = oxalic acid, **5** = malic acid, **6** = tartaric acid, **7** = acetic acid, **8** = lactic acid, **9** = citric acid.

Table I. Summary of calibration and precision data for the optimized method.

Analyte	Range	$y = a + bx$ ($P = 0.02\%$; $n = 20$)	r	*Lin*	*AS*	$S_{y/x}$	*LOD*	*LOQ*
Formic acid	5–40	$a = 64.01 \pm 27.00$, $b = 166.14 \pm 2.10$	0.9998	99.685	0.221	36.67	0.662	2.207
Fumaric acid	0.5–4	$a = 88.37 \pm 14.33$, $b = 239.14 \pm 5.84$	0.9982	98.837	0.080	19.18	0.241	0.802
Oxalic acid	1–8	$a = 121.47 \pm 63.30$, $b = 239.67 \pm 12.87$	0.9910	97.443	0.359	85.96	1.076	3.586
Succinic acid	1–8	$a = 88.30 \pm 27.90$, $b = 240.07 \pm 6.06$	0.9977	98.803	0.150	36.04	0.449	1.495
Malic acid	4–32	$a = 32.61 \pm 76.11$, $b = 200.68 \pm 3.74$	0.9990	99.112	0.528	105.92	1.583	5.278
Tartaric acid	5–40	$a = 202.93 \pm 50.21$, $b = 141.97 \pm 2.13$	0.9993	99.286	0.471	66.85	1.413	4.709
Acetic acid	3–24	$a = 228.06 \pm 33.77$, $b = 148.07 \pm 2.29$	0.9992	99.186	0.310	45.85	0.929	3.097
Lactic acid	5–40	$a = 237.87 \pm 167.66$, $b = 120.28 \pm 7.15$	0.9890	97.171	1.850	222.51	5.550	18.499
Citric acid	4–32	$a = 115.68 \pm 44.06$, $b = 124.51 \pm 2.24$	0.9990	99.144	0.481	59.83	1.442	4.805

Range is the concentration range in ppm; a is the intercept, b the slope, r the correlation coefficient, *Lin* the linearity, *AS* the analytical sensitivity, $S_{y/x}$ the standard deviation of the residuals, *LOD* the limit of detection, and *LOQ* the limit of quantification.

Table II. Concentration of organic acids in different vinic samples.

Organic acid	Concentration (ppm) ($P = 0.02\%$; $n = 5$)			
	Must	Wine	Brandy	Vinegar
Formic acid	n.d.	n.d.	47.8 ± 0.7	n.d.
Fumaric acid	n.d.	n.d.	2.2 ± 0.3	n.d.
Succinic acid	n.d.	420.7 ± 11.05	5.9 ± 0.5	342.3 ± 17.9
Oxalic acid	n.d.	n.d.	10.6 ± 1.1	n.d.
Malic acid	1868.21 ± 63.0	n.d.	15.7 ± 1.7	232.0 ± 70.8
Tartaric acid	578.34 ± 61.8	2074.4 ± 33.9	66.5 ± 1.4	2083.5 ± 56.4
Acetic acid	n.d.	73.6 ± 22.3	347.4 ± 6.1	76330.0 ± 144.2
Lactic acid	n.d.	n.q.	44.7 ± 5.5	857.2 ± 234.9
Citric acid	n.d.	n.d.	17.2 ± 1.5	544.2 ± 29.3

n.d. = not detected; n.q. = not quantified.

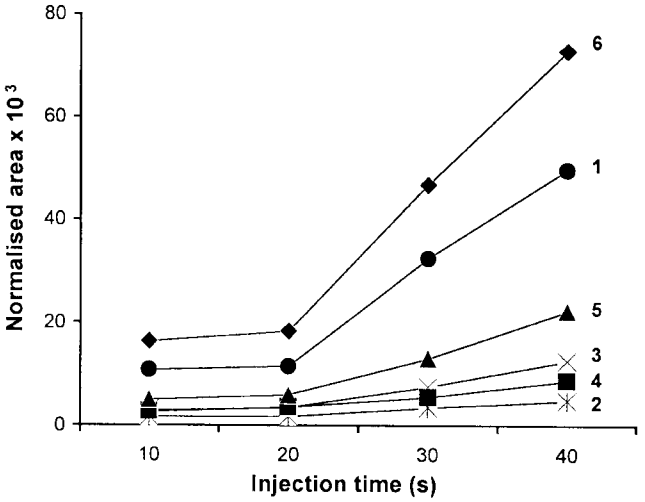

Figure 2. Dependence of normalized peak areas on injection time. Peak identification as for Figure 1.

The repeatability data obtained for each point on the plot obtained for the standards, with four replicate analyses per point, were usually lower than 1%. Only exceptionally are some values above 5%, e.g. that obtained for 2 ppm oxalic acid (6.61%).

The limit of detection (*LOD*) was calculated as three times the standard deviation of the residual divided by the slope, and the limit of quantification (*LOQ*) as ten times the standard deviation of residual divided by the slope. *LOD* values obtained are usually lower than the minimum values used to obtain the calibration plots.

The analytical sensitivity (*AS*) was calculated as the standard deviation of residual divided by the slope. The linearity (*Lin*) of the plots [29] was calculated by use of the equation:

$$Lin\,(\%) = 100 - RSD_b$$

where the relative standard deviation of the slope (RSD_b) is expressed as a percentage.

Analysis of Real Samples

In two previous papers we have reported the practical application of the method, and its validation for samples of different grape-derived products, e.g. must, wine, brandy [30], and vinegar [31] from the Jerez region. We validated the method for these real samples by comparing the slopes obtained by use of standards and those obtained by the method of standard additions. With one exception the method was valid for all the acids studied, and satisfactory regression values were obtained by use of the technique for analysis of the samples studied. The one exception was acetic acid, for which a matrix effect was observed, depending on the type of electrolyte used; this could be associated with the alcohol content of the sample injected.

The concentrations of the different organic acids of interest found in the samples analysed are given in Table II. It is apparent that the concentrations of the different acids vary significantly from one sample to another.

Conclusion

The proposed method enables easy determination of the organic acids most commonly found in samples of vinic origin. It was necessary to add two cations (calcium and magnesium) to the electrolyte to form complexes with the acids in question.

The reproducibility and selectivity of the method, and the limits of detection, show that the method usually gives satisfactory results. The technique also uses small amounts of inexpensive reagents.

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