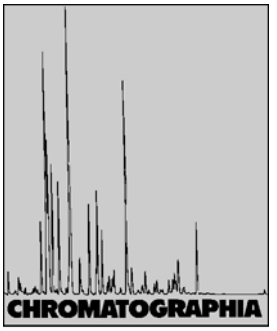
Comparative Analysis of the Organic Acid Content of Vinegar by Capillary Electrophoresis and Ion-Exclusion Chromatography with Conductimetric Detection



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Key Words

Capillary electrophoresis
Ion-exclusion chromatography
Organic acid
Vinegar

Summary

Ion-exclusion chromatography (IEC) and capillary electrophoresis (CE) have been compared for determination of organic acids in samples of Sherry wine vinegar. The accuracy of each technique was evaluated by use of the standard addition method. There were no differences between the techniques at a significance level of 5%, except for determination of malic acid by CE. Both analytical methods were used to analyse sixteen samples of Sherry wine vinegar supplied by different producers. The regression coefficients (r^2) for analysis by IEC and CE exceeded 0.94 for all acids. Results from both methods were in good agreement and the methods are sufficiently selective and sensitive to be applied directly to sherry wine vinegars.

Introduction

In recent years vinegar has ceased to be regarded as a food product of secondary importance in the Jerez-Xérès-Sherry, Manzanilla de Sanlúcar, and Vinagre de Jerez Denomination of Origin (D.O.) region (SW Spain). It is now a highly valued, high-quality product on a par with the wines and brandies typical of the region. Vinegars are produced from a variety of different raw materials (white and red wine, cider, malted barley, honey, pure alcohol, etc.) and by different methods. In our D.O. Sherry vinegars are produced from Sherry wines by following traditional methods of acetification [1]. For this reason, the producers have recently been seeking techniques for objectively determining appropriate properties for characterizing a special vinegar such as Sherry wine vinegar.

Organic acids are compounds of interest for characterizing all products derived from grapes. They are present in a wide variety of products for human consumption – foods, drink, drugs, etc. – that are, consequently, of analytical interest. The level and nature of the organic acids present in a particular vinegar can provide information both about its origin and about the techniques of processing and ageing to which it has been subjected.

The organic acids in vinegars comprise volatile (acetic, propionic, etc.) and non-volatile (tartaric, citric, malic, succinic, etc.) compounds. The acid that identifies the product as a vinegar is acetic acid, the amount of which can vary depending on the carbohydrate substrate used; acetic acid is the only organic acid present in vinegars derived from pure alcohol or from cereals [2].

The type and content of the non-volatile organic acids seem to depend on the type of vinegar analysed [3]. In cider vinegars malic acid is the most abundant organic acid whereas in malt vinegars lactic and malic acids are the most abundant. Wine vinegars are characterized by their tartaric acid content, but contain relatively little malic acid, the amount of which depends on the origin of the wine and on the enological techniques to which it has been subjected. Because this acid is converted into lactic acid during the malolactic fermentation, the ratio of the amounts of these two acids in the vinegar can be indicative of the extent to which this key fermentative process had developed in the particular base wine. The lactic acid content can, in turn, be reduced during the acetic fermentation. The amounts of citric and succinic acids, formed during the alcoholic fermentation, can sometimes be reduced by the presence of microorganisms which can transform these compounds into acetic acid [4].

In a study of the effect of the procedure used for ageing Morales et al. [5] found clear differences between the amounts of organic acids and aromatic compounds in Sherry wine vinegars aged traditionally in barrels of American oak and those produced in a laboratory fermenter. Because

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of their unusual and specific production process (acetification and subsequent ageing in wooden casks that have previously held wine), Sherry wine vinegars are different in composition from vinegars produced by other methods – the composition of Sherry wine vinegars is similar to that of the more oxidized Sherry wines (olorosos and amontillados) and brandies typical of the region.

Traditional methods used for the determination of organic acids include the techniques of liquid and gas chromatography [6]. HPLC analysis of these acids in wines is usually performed by reversed-phase chromatography [6–10], ion-exchange chromatography [11], or ion-exclusion chromatography [5, 12, 13], usually with refractive index or UV detection.

Analytical methods based on capillary electrophoresis (CE) have recently been widely studied and developed. CE is a method of great potential for the high-resolution separation of a variety of substances. The detection and determination of organic acids by CE has been performed for many raw materials, e.g. sugars [14], margarines [15], vegetable products [16, 17], fruit juices [15, 18, 19], beer [20, 21], wine [22], orange juice [18], brandy [23], and cigarette smoke [24].

The aim of the work described in this paper was to perform a comparative study of the determination of organic acids in Sherry wine vinegar by both CE and ion-exclusion chromatography with conductimetric detection. Both methods have previously been used to determine the organic acids in Brandies de Jerez [13, 23] and, because of the similarity between these products and Sherry wine vinegar, it was decided to use them for the comparative study of this type of sample, the high acetic acid content of which might lead to interference.

The method of ion-exclusion chromatography optimized by Guillen et al. [13] for analysis of organic acids in Brandy de Jerez is based on chromatographic separation on an ion-exclusion column with a dilute solution of trifluoracetic acid as mobile phase, and conductimetric detection. To increase the sensitivity before detection, a buffer of pH 6.5 is added to the mobile phase to ensure ionization of the analytes.

García Moreno et al. [23] have proposed a method for CE determination of organic acids in Brandies de Jerez by use of an electrolyte consisting of sodium tetraborate buffer at pH 9.3, TTAOH as OFM (organic flow modifier), and $CaCl_2$ and $MgCl_2$ as complexing agents.

Neither method requires any sample preparation and, after their validation, they have been applied to a variety of Sherry wine vinegars.

Experimental

Chemicals

Citric and lactic acids were supplied by Fluka (Buchs, Switzerland), malic and succinic acids by Sigma (St Louis, MO, USA), and acetic and tartaric acids by Merck (Darmstadt, Germany). The water used was purified by means of a Milli-Q system (Millipore, Bedford, MA, USA). Solvents used as mobile phases for ion-exclusion chromatography (trifluoroacetic acid, bis-[2-hydroxyethyl]imino-tris-[hydroxymethyl]methane, and EDTA) were purchased from Merck. All solvents were filtered through 0.45-μm membranes. The calcium chloride and magnesium chloride used for capillary electrophoresis ($MgCl_2.6H_2O$ and $CaCl_2.2H_2O$) were purchased from Merck (Darmstadt, Germany) and the sodium tetraborate ($Na_2B_4O_7.10H_2O$) from Panreac (Barcelona, Spain). The electroosmotic flow modifier TTAOH (tetradecyltrimethylammonium hydroxide) was from Waters (Milford, MA, USA).

Instrumentation

For both methods Millennium 2100 software was used for control and data acquisition.

Ion-exclusion chromatography was performed with two model 2150 pumps and a model 2155 column oven from LKB (Pharmacia, Sweden), a Conductomonitor model III conductivity detector from Milton Roy (LDC, Florida, USA), and a model 717 automatic injector from Waters.

Capillary electrophoresis was performed with a Waters Capillary Ion Analyzer, equipped with a UV-visible detector operated at 185 nm. Compounds were separated in conventional 60 cm (53 cm effective length) × 75 μm i. d. fused-silica capillaries.

Procedures

Ion-Exclusion Chromatography

The chromatographic separation was performed on a 300 mm × 4.6 mm i. d. ION-300 ion-exclusion column (Interaction Chromatography, S. José, CA, USA). The oven temperature was set at 60 °C. The sample volume injected, after filtration through a 0.45-μm filter, was 40 μL. The mobile phase was a 2.5 mM solution of trifluoroacetic acid (TFA; 0.4 mL min^{-1}). To increase the detection sensitivity a solution consisting of 2.5 mM TFA, 20 mM bis-[2-hydroxyethyl]imino-tris-[hydroxymethyl]methane (bis-tris buffer), and 100 mM EDTA was added at the column outlet, at a flow rate of 0.4 mL min^{-1}.

Capillary Electrophoresis

Before each injection the capillary was washed first with a solution of sodium hydroxide (0.01 M, 1 min), then with a solution of sodium tetraborate (10 mM, pH 9.3, 1 min) [25], and finally with the electrolyte (3 min).

Samples were introduced hydrostatically into the capillary (height 10 cm); the injection time was 30 s. The voltage applied was 7 kV, using a negative feed source. UV detection was performed at 185 nm; the temperature was 20 °C. The electrolyte was tetraborate buffer (10 mM, pH 9.3) containing electroosmotic flow modifier (TTAOH; 0.5 mM), and Ca^{2+} and Mg^{2+} (10 p.p.m. of each, as the chlorides) as complexing agents. The prepared electrolyte was filtered before use.

Standards and Samples

Standards

Ion-exclusion chromatography: The main stock solution of each acid was prepared in Milli-Q water. Working solutions of mixtures of acids for calibration were prepared with concentrations ranging from 1 to 0.02 g L^{-1} for citric, succinic, and malic acids, 2 to 0.03 g L^{-1} for tartaric acid, 2.25 to 0.04 g L^{-1} for lactic acid, and 40 to 1 g L^{-1} for acetic acid.

Capillary electrophoresis: Individual standard solutions of each acid were prepared in Milli-Q water. Working solutions of mixtures of all the acids were prepared for use as standard solutions. These were prepared with concentrations ranging from 0.032 to 0.001 1 g L^{-1} for citric and

Table I. Characteristics of the calibration curves obtained for ion-exclusion chromatography.

Compound	Linear range ($g\,L^{-1}$)	Regression coefficient	Linearity (LOL, %)	Slope ± S.D. ($\mu VsL\,g^{-1}$)	Intercept ± S.D. (μVs)
Citric acid	1.01–0.0200	0.9990	99.12	6473.82 ± 56.77	56.53 ± 22.01
Tartaric acid	2.01–0.0331	0.9997	99.51	277095.16 ± 1358.13	4249.16 ± 1025.18
Malic acid	1.02–0.0166	0.9999	99.70	284748.85 ± 850.22	– 94.53 ± 324.00
Succinic acid	0.99–0.0166	0.9998	99.65	320405.60 ± 1109.30	– 1081.96 ± 417.09
Lactic acid	2.25–0.0402	0.9998	99.65	133238.59 ± 470.04	–3163.291 ± 449.28
Acetic acid	10–1.33	0.9995	99.16	247309.16 ± 2090.95	44085.78 ± 14063.51

Table II. Characteristics of the calibration curves obtained for capillary electrophoresis.

Compound	Linear range ($mg\,L^{-1}$)	Regression coefficient	Linearity (LOL, %)	Slope ± S.D. ($\mu VsL\,mg^{-1}\cdot min^{-1}$)	Intercept ± S.D. ($\mu Vs\,min^{-1}$)
Citric acid	4.0–32.0	0.9989	98.71	124.18 ± 1.61	126.00 ± 31.59
Tartaric acid	5.0–39.0	0.9994	99.05	142.82 ± 1.35	191.06 ± 32.79
Malic acid	1.0–32.0	0.9990	98.01	203.52 ± 2.08	–35.80 ± 36.33
Succinic acid	1.0–8.0	0.9985	98.47	244.14 ± 3.73	76.17 ± 18.35
Lactic acid	1.0–50.0	0.9989	97.97	69.62 ± 1.14	197.79 ± 34.75
Acetic acid	527.2–1318.0	0.9978	96.14	173.98 ± 6.71	–2371.52 ± 6176

malic acids, 0.008 to 0.001 g L^{-1} for succinic acid, 0.400 to 0.005 g L^{-1} for tartaric acid, 0.050 to 0.001 g L^{-1} for lactic acid, and 1.300 0.500 g L^{-1} for acetic acid. All solutions were filtered before injection.

Samples

Ion-exclusion chromatography: After filtration (0.45-µm membrane) and dilution (1:20 with Milli-Q water, for determination of tartaric and acetic acids) Sherry wine vinegar samples were injected into the system.

Capillary electrophoresis: All the vinegar samples analysed in this study were highly saline, making difficult their direct determination by capillary electrophoresis. Desalination of the samples by dilution with water is easy to perform, does not require excessive manipulation, and does not saturate the detector with high concentrations of some of the target organic acids in some of the samples studied. For this reason, before CE analysis vinegar samples were diluted fiftyfold for citric acid determination and one-hundredfold for determination of the other organic acids.

Results

Performance Characteristics

Calibration, Linearity (LOL)

Seven levels of concentration, covering the ranges expected for these organic acids in vinegars, were tested in triplicate. Online linearity (LOL) was determined by use of the equation:

$$LOL\,(\%) = 100 - RDS(b)$$

where $RSD(b)$ is the relative standard deviation of the slope, b (expressed as a percentage).

Ion-exclusion chromatography: The peak area obtained by use of direct electric conductivity detection was studied for each organic acid except citric acid, for which peak height was used. Excellent linearity was always obtained, with LOL values >99% (Table I). For acetic acid concentrations higher than 10 g L^{-1} there was no linear relationship between conductimetric signal and concentration. For this reason, and to determine the tartaric acid content, vinegar samples were diluted 1:20 with Milli-Q water.

Capillary electrophoresis: The normalized peak area (quotient between the peak area and the retention time) was used for quantification [26]. Although linearity in CE was lightly less than in ion-exclusion chromatography LOL values were always >96% (Table II).

Table III. Performance characteristics for ion-exclusion chromatography (IEC) and capillary electrophoresis (CE).

Compound	Analytical sensitivity		Detection limit (LOD, $g\,L^{-1}$)		Quantitation limit (LOQ, $g\cdot L^{-1}$)		Recovery (%)	
	IEC	CE	IEC	CE	IEC	CE	IEC	CE
Citric acid	0.0155	0.0005	0.0462	0.0015	0.1532	0.0050	105.77	102.92
Tartaric acid	0.0152	0.0005	0.0453	0.0013	0.1501	0.0044	97.20	107.96
Malic acid	0.0047	0.0005	0.0140	0.0014	0.0464	0.0048	103.90	40.03
Succinic acid	0.0053	0.0004	0.0161	0.0013	0.0522	0.0042	112.60	104.36
Lactic acid	0.0139	0.0011	0.0413	0.0033	0.1371	0.0106	94.07	101.32
Acetic acid	0.0996	0.0247	0.2881	0.0641	0.9601	0.2136	95.31	98.25

Detection and Quantitation Limits, Recovery, and Analytical Sensitivity

Detection and quantitation limits and analytical sensitivity (Table III) were calculated from the calibration curves constructed for each acid, by means of the Alamin Computer Program [27]. Analytical sensitivity and detection and quantitation limits for CE were lower than for ion-exclusion chromatography.

To check the accuracy of both methods the technique of standard additions was used. A sample of representative vinegar was taken as the matrix and known quantities of each acid were added at five levels, in triplicate. The slopes of the lines thus obtained for each of the acids and methods were compared with the corresponding slopes obtained from calibration with standards (t criterion).

Table III gives recovery data for each acid added and for each analytical method; the values were determined from the slope of a plot of concentration found against the concentration expected.

Table IV. Results from study of the repeatability of ion-exclusion chromatography (IEC) and capillary electrophoresis (CE).

Compound	Intra-day repeatability *RSD* (%, $n = 5$)		Inter-day repeatability *RSD* (%, $n = 15$)		Mean concentration ($g \cdot L^{-1}$)	
	IEC	CE	IEC	CE	IEC	CE
Citric acid	1.96	5.24	4.12	7.99	0.4506	0.5442
Tartaric acid	2.51	8.15	4.87	9.22	2.1157	2.0835
Malic acid	1.47	6.57	5.14	7.76	0.2890	0.2320
Succinic acid	1.65	7.36	6.99	7.71	0.3232	0.3423
Lactic acid	6.36	3.74	9.92	11.89	0.7858	0.8572
Acetic acid	4.12	1.57	7.48	6.06	75.57	76.33

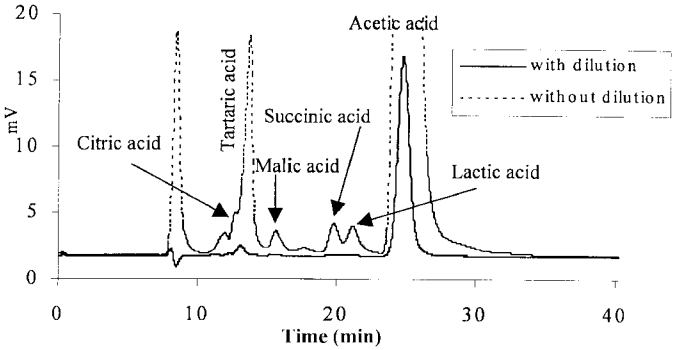

Figure 1. Chromatograms obtained from a Sherry wine vinegar with and without dilution.

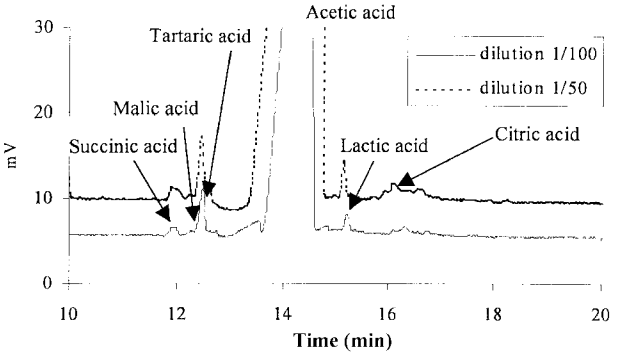

Figure 2. Electropherograms obtained from a Sherry wine vinegar after hundredfold and fiftyfold dilution.

Intra- and Inter-Day Repeatability

To evaluate the intra-day repeatability five samples of the same vinegar were injected, directly and diluted, into the two systems, on the same day. For inter-day repeatability, fifteen samples, directly and diluted, were injected on three consecutive days. Daily relative standards deviations (*RSD*) (intra-day repeatability, Table IV) were relatively low (1.47–6.36% for ion-exclusion chromatography and 1.57–8.15% for CE). *RSD* values for inter-day precision were slightly higher than for intra-day precision (4.12–9.92% for ion-exclusion chromatography and 6.06–11.89% for CE), as might be expected.

Determination of Organic Acids in Vinegars. Comparison of the Two Analytical Methods

Both analytical methods were used to analyse sixteen Sherry vinegar samples supplied by different producers. The chromatogram and the corresponding electropherogram obtained from a Sherry wine vinegar are shown in Figures 1 and 2. It is apparent that the order of elution was different for each technique. Separation of organic acids by ion-exclusion chromatography depends mainly on differences between their pK_a when a strongly acid ion-exchange resin in its acidic form is used as stationary phase. Weak acids behave as non-electrolytes at low pH and are retarded by the resin whereas the stronger acids are eluted first. In CE the relationship between load and mass of the complexes formed with the added ions (Ca^{2+} and Mg^{2+}) determines the order of separation of these acids.

The results obtained for these vinegars are shown in Table V. Malic acid was determined by ion-exclusion chromatography because of its relatively low recovery when determined by CE. As expected, levels of acetic acid are very high. Tartaric acid is also present in substantial amounts in all wine vinegars. Succinic and lactic acids are present in smaller amounts and citric acid is not present in all vinegars. One sample (sample 10) contained an abnormally high level of citric acid, possibly because of adulteration of this vinegar.

Discussion

There was no difference between the accuracy of the methods at a significance level of 5% except for CE determination of malic acid, for which the recovery was only 40.03% (Table III). This might be because of the use of insufficient quantities of complexing agents in this technique and the high concentration of acetic acid in the samples.

Relative standard deviations for the six organic acids were acceptable for both methods. The highest *RSD* values were obtained for ion chromatography of tartaric and acetic acids (Table IV). Note that the concentrations of these acids were calculated after dilution.

Although the values obtained for these organic acids by ion-exclusion chromatography were similar to those obtained from capillary electrophoresis (Table V), values obtained by ion-exclusion chromatography for succinic, tartaric, and acetic acid were usually higher than values obtained from analysis by CE. This is probably because the quantities of complexing agents added were insufficient for these types of sample, which contained relatively high concentrations of acetic acid. Despite this, the regression coefficients (r^2) for analysis by ion-exclusion chromatography and capillary electrophoresis always exceeded 0.94 (Table V); this indicates that results from the methods are in good agreement.

Conclusions

Both methods used for determination of organic acids were sufficiently selective

Table V. Comparison of ion-exclusion chromatography (IEC) and capillary electrophoresis (CE) for the determination of organic acids in Sherry wine vinegars.

Sample	Citric acid ($g L^{-1}$)		Tartaric acid ($g L^{-1}$)		Succinic acid ($g L^{-1}$)		Lactic acid ($g L^{-1}$)		Acetic acid ($g L^{-1}$)		Malic acid ($g L^{-1}$)
	IEC	CE	IEC	CE	IEC	CE	IEC	CE	IEC	CE	IEC
1	0.543	0.665	3.667	3.138	0.832	0.657	0.367	0.496	114.58	108.06	0.638
2	n.d.	0.033	1.971	1.873	0.313	0.271	0.232	0.235	119.82	113.64	0.152
3	0.228	0.221	1.555	1.502	0.468	0.371	0.269	0.293	82.82	81.73	0.059
4	0.480	0.405	1.730	1.596	0.410	0.387	0.422	0.397	81.74	75.90	0.198
5	0.496	0.412	1.911	1.884	0.372	0.301	0.841	0.750	73.12	71.13	0.339
6	0.451	0.298	1.805	1.679	0.262	0.250	0.710	0.662	91.44	89.96	0.237
7	0.241	0.312	1.638	1.627	0.280	0.236	0.179	0.238	95.87	95.56	0.499
8	0.137	0.165	1.192	1.425	0.271	0.230	0.269	0.287	95.35	92.02	0.162
9	0.332	0.564	2.039	2.017	0.440	0.332	0.459	0.495	113.16	109.70	0.362
10	4.095	4.652	2.013	1.879	0.098	0.078	0.175	0.130	56.46	54.62	0.153
11	0.169	0.229	2.976	2.699	0.369	0.270	0.216	0.266	79.42	75.94	0.191
12	n.d.	0.020	0.146	0.205	0.303	0.277	0.499	0.487	54.00	52.78	0.015
13	0.148	0.282	1.005	1.074	0.472	0.361	0.210	0.273	101.25	104.30	0.133
14	0.349	0.290	1.886	1.786	0.522	0.456	0.626	0.563	96.34	96.78	0.201
15	0.281	0.118	1.094	1.154	0.385	0.341	0.416	0.395	88.28	92.79	0.178
16	n.d.	n.d.	0.770	0.876	0.315	0.277	0.705	0.694	99.49	98.38	0.115
y = IEC x = CE	$y = 1.1342x + 0.0235$ $r^2 = 0.9897$		$y = 0.8174x + 0.2523$ $r^2 = 0.9850$		$y = 0.7667x + 0.0255$ $r^2 = 0.9625$		$y = 0.8416x + 0.0693$ $r^2 = 0.9423$		$y = 0.9642x + 1.3587$ $r^2 = 0.9741$		

and sensitive for direct application to Sherry wine vinegars. Recovery was poor only for determination of malic acid by CE. Both techniques enabled rapid analysis (30 min for ion-exclusion chromatography and 20 min for CE) and results were in good agreement. Either method could, therefore, be used for routine determination of these acids in vinegars, samples with a high acetic acid content.

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