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Determination of organic acids in food samples by capillary electrophoresis

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ABSTRACT

Capillary electrophoresis and a new separation chemistry were investigated for the determination of organic acids in food samples. Features of this approach include minimal sample preparation, excellent specificity, low cost of operation and application to a variety of food matrices. The method is quantitative, with recoveries in the range 97–103%, and linear over more than one order of magnitude, and the precision is better than 2–14% for real samples. The chemistry, with slight modifications, can be applied to other ions in many types of matrices.

INTRODUCTION

Measuring organic acid levels in foods and beverages is important from the standpoint of monitoring the fermentation process, checking product stability, validating the authenticity of juices and concentrates and studying the organoleptic properties of fermented products.

Other methods of analysis can be tedious and cost-ineffective. For example, enzyme assay requires separate kits for each acid, which slows down throughput and increases the cost per test for a multiple acid determination. It had been hoped that simultaneous acid determinations with appropriate instrumentation would be an acceptable solution. However, the current instrumental methods are not without limitations.

For example, high-performance liquid chromatographic (HPLC) separations based on ion-exclusion or ion-suppression columns with UV absorbance (typically at 214 nm) or refractive index detection suffer from carbohydrate and phenolic interferences from the sample. Approaches to minimize this lack of specificity include use of two analytical columns with meticulous data handling [1] or multi-step and multi-device sample preparation [2]. These two solutions add to the cost and tedium of analysis. Another instrumental approach, ion chromatography (IC) with conductivity detection, suffers from inorganic ion interferences, which necessitate the use of a gradient or multiple isocratic separations. Also, organic substances in the matrix need to be removed by sample preparation as a limited column lifetime or complicated chromatogram may result [3]. In addition, with both HPLC and IC, very little can be done to manipulate the selectivity as the analytical columns are used with simple

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buffers or dilute acid only. Therefore, it is difficult to optimize the separation for specific analytes from specific matrices.

The above discussion implies the need for alternative methods for organic acids. Capillary electrophoresis (CE), as a new and powerful separation technique, proved to be a good choice for investigation. Part of the power of CE is its unique selectivity; sample components are separated based on their charge. Therefore, negatively charged species (*e.g.*, organic acids) migrate away from more neutral and positively charged species (*e.g.*, sugars and phenolics). Also, with CE, a large number of theoretical plates is possible for the separation. The separation efficienty, N, is typically in the range 100 000–200 000, compared with 5000–10 000 for HPLC or IC [4].

A new separation chemistry for anion separation by CE has been used for organic acid separations. Known as NICE-Pak™ Chemistry, it separates strongly and weakly charged anions such as inorganics, carboxylic acids and alkylsulfonates.

The NICE-Pak Chemistry includes a fused-silica capillary and an electrolyte which contains an osmotic flow modifier. This modifier serves to direct bulk flow of electrolyte towards the anode for fast detection of the anions. The other chemical component of the electrolyte can vary according to the anionic species and sample matrices of interest. It will, however, contain a UV chromophore which absorbs at 254 nm. This type of detection is indirect, that is, the anions absorb less UV radiation than the electrolyte yielding a negative response in the detector. Positive peaks appear on the electopherogram (comparable to a chromatogram) by reversing the detector signal cables to the data device. Those sample components which absorb more UV light than the electrolyte appear as negative peaks.

Other parameters that can be optimized for the separation include electrolyte pH and voltage applied to the system. A change in pH will affect the mobility of the analytes and therefore their migration. Both efficiency and migration time are affected by a change in applied voltage. Therefore, many variables can be used to enhance the separation of sample components.

Samples are automatically introduced into the capillary hydrostatically, that is, by immersing the injection end of the capillary into the sample and raising both by 10 cm for a user-specified period of time. This results in an injection volume of a few nanoliters. This approach to anion determination is quantitative, with reproducibility, linearity and sensitivity comparable to those of other instrumentation techniques [3].

As described, there is flexibility within NICE-Pak Chemistry, including choice of capillary length, applied voltage, electrolyte pH and UV absorber. The following organic acids separation resulted by optimizing these parameters.

EXPERIMENTAL

Chemicals

All water was provided by a Milli-Q Plus water purification system (Millipore, Bedford, MA, U.S.A.). All standards and phthalic acid, monopotassium salt, were obtained from Sigma (St. Louis, MO, U.S.A.). The OFM™ Anion-BT (Osmotic Flow Modifier Anion-BT) reagent (part of NICE-Pak Chemistry) was provided by Waters Chromatography Division of Millipore (Milford, MA, U.S.A.).

Samples and preparation

Samples were purchased at local grocery and liquor stores. They were diluted to appropriate concentrations with water and, when indicated, mixed with an internal standard, butyric acid (100 μg/ml). The only clean-up necessary was microfiltration through a 0.45-μm Millex HV filter (Millipore) when pulp was present in the sample.

Capillary electrophoresis

The instrument, a Quanta™ 4000 capillary electrophoresis system, was interfaced to an 820 Maxima Workstation via a System Interface Module (all from Waters Chromatography Division). The capillary (100 cm × 75 μm I.D.) was constructed of fused silica. The electrolyte consisted of 0.5 m*M* OFM Anion-BT–5 m*M* potassium phthalate (pH 7.0). The applied voltage was 20 000 V using the negative power supply and the injection time was 45 s.

RESULTS AND DISCUSSION

Separation

The electropherogram of citric, tartaric, malic, succinic, acetic and lactic acid and the internal standard butyric acid is shown in Fig. 1. Other inorganic and organic anions that have been characterized by this method are listed in Table I. The migration times were normalized to that of butyric acid. Also listed are equivalent conductance values, which relate directly to the mobilities of the ions. A general pattern that inversely relates migration time to equivalent conductance is indicated. Therefore, anion migration patterns with CE are reasonably predictable. Also, as the equivalent conductances for malic and succinic acid are identical (58.8), their separation is close. Orotic acid yields a negative peak as it absorbs more UV radiation at 254 nm than does the electrolyte. Fumaric acid does not respond under these specific conditions.

For maximum reproducibility of migration time, the use of an internal standard is necessary. For example, the data in Table II compare migration time reproducibility with a without an internal standard, both on a single day (within-run) and on five

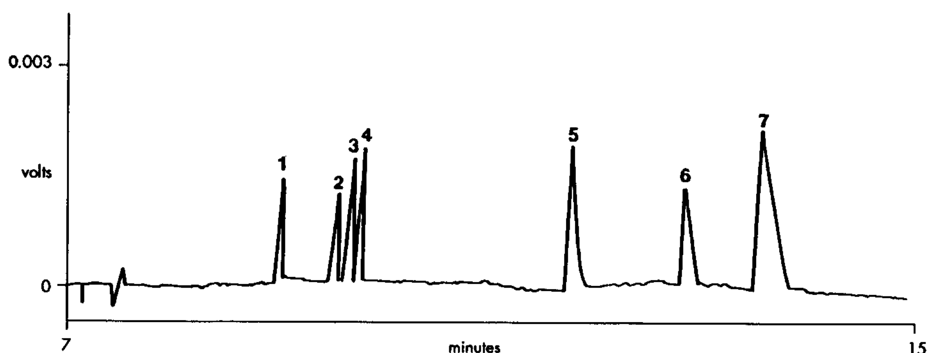

Fig. 1. Electropherogram of a standard mixture. Peaks: 1 = citric acid, monohydrate; 2 = tartaric acid, disodium salt; 3 = malic acid, sodium salt; 4 = succinic acid, calcium salt, monohydrate; 5 = acetic acid; 6 = lactic acid (all 20 μg/ml); 7 = butyric acid (internal standard, 50 μg/ml).

TABLE I

RELATIVE MIGRATION TIMES AND EQUIVALENT CONDUCTANCES

Entries in italics are those from the standard in Fig. 1.

Analyte	Relative migration time[a]	Equivalent conductance[b]
Bromide	0.568	78.1
Chloride	0.577	76.3
Sulfate	0.605	80.0
Nitrite	0.608	71.8
Nitrate	0.617	71.4
Oxalic	0.630	74.1
Sulfite	0.669	79.9
Citric	*0.676*	*70.2*
Tartaric	*0.725*	*59.6*
Fluoride	0.730	55.4
Formic	0.733	54.6
Malic	*0.736*	*58.8*
Succinic	*0.743*	*58.8*
Ketoglutaric	0.743	
Phosphate	0.797	33.0
Carbonate	0.834	69.3
Acetic	*0.868*	*40.9*
Pyruvic	0.877	
Propionic	0.934	35.8
Lactic	*0.950*	*38.8*
Butyric	*1.000*	*32.6*
Orotic[c]	1.023	
Quinic	1.095	
Shikimic	1.095	
Gluconic	1.101	
Fumaric	No response	61.8

[a] Relative to butyric acid.
[b] From ref. 5.
[c] Negative peak.

TABLE II

REPRODUCIBILITY OF MIGRATION TIME WITH AND WITHOUT AN INTERNAL STANDARD (I.S.) AND WITHIN RUN AND BETWEEN RUN (R.S.D., %)

Analyte	Without I.S.	With I.S.
Within-run, n = 10[a]		
Citric acid	0.5	0.3
Succinic acid	0.2	0.3
Lactic acid	0.1	0.1
Between-run, n = 5[b]		
Citric acid	3.1	0.8
Succinic acid	3.2	0.6
Lactic acid	3.6	0.1

[a] Single day, same electrolyte.
[b] Five days, five electrolyte batches.

different days, with five different electrolyte batches (between-run). The relative standard deviation (R.S.D.) is much lower with the internal standard, especially day-to-day. Butyric acid acts as an internal standard as it does not occur naturally in many beverage products.

Linearity of the method was evaluated between 10 and 167 μg/ml with respect to both area and height response. As the correlation coefficient for peak area (0.999) is much better than that for peak height (0.962), the best results are obtained by an area calculation. The detection limit is 1 μg/ml using a signal-to-noise ratio of 3:1.

Sample analysis

Sample preparation, usually a deterrent to the success of an analytical method owing to complexity and cost, appears to be a small issue with this approach. For example, Fig. 2 shows the separation of organic acids in apple juice. The sample is simply diluted with water, internal standard is added and the mixture is injected. Not even filtration is needed. Sugars and phenolics remain in the capillary at the end of the analysis. Programming the autopurge function on the Quanta 4000 cleans the capillary of those compounds prior to the next injection.

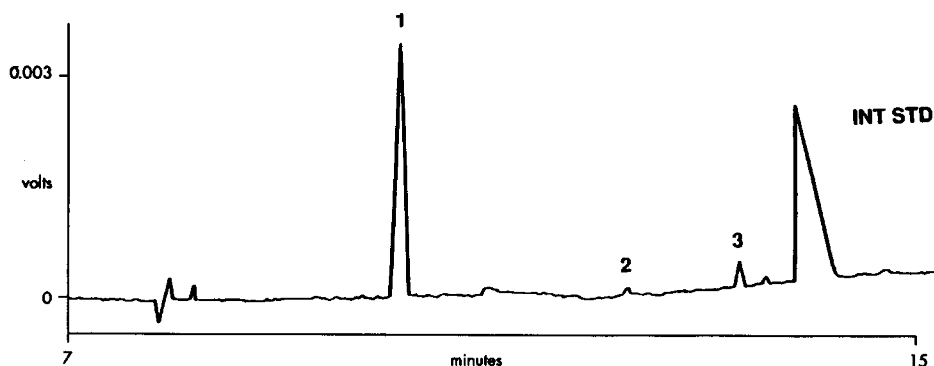

Fig. 2. Electropherogram of apple juice. Sample preparation: dilute and inject. Peaks: 1 = malic acid, 4352 μg/ml; 2 = acetic acid, 48 μg/ml; 3 = lactic acid, 254 μg/ml; INT STD = Internal standard.

Some samples, such as pulp containing large particles. It is then necessary to apply microfiltration to remove such large insolubles from the matrix. This approach works well for orange, grapefruit and tomato juice. The juice is first diluted with water, filtered (0.45-μm Millex HV), then the internal standard is added. Fig. 3 shows an electropherogram of tomato juice, prepared as just described. Chloride in the juice is also identified.

For a few samples, butyric acid is not a good choice of internal standard as it occurs naturally. Fig. 4 shows te separation of acids in soy sauce diluted 1:500 with water. Butyric acid is evident in the electropherogram. Also evident is a huge response for chloride, as would be expected in this sample.

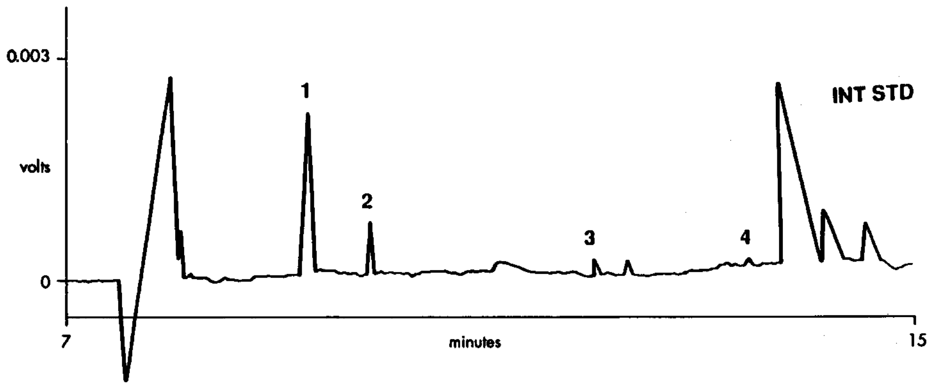

Fig. 3. Electropherogram of tomato juice. Sample preparation: dilute, filter and inject. Peaks: 1 = Citric acid, 3579 μg/ml; 2 = malic acid, 404 μg/ml; 3 = acetic acid, 99 μg/ml; 4 = lactic acid, 69 μg/ml. The large peak on the left is chloride.

Precision and recovery

Results of reproducibility and recovery studies using wine and dark grape juice samples are given in Tables III and IV. Fig. 5 shows an electropherogram for a Chablis wine sample generated after simple dilution. Reproducibility data for ten different analyses of the same wine showed an acceptable R.S.D., especially for tartaric and malic acid (<2%) (Table III). It should be noted that the chosen dilution optimizes the resolution of tartaric, malic and succinic acid and a smaller dilution

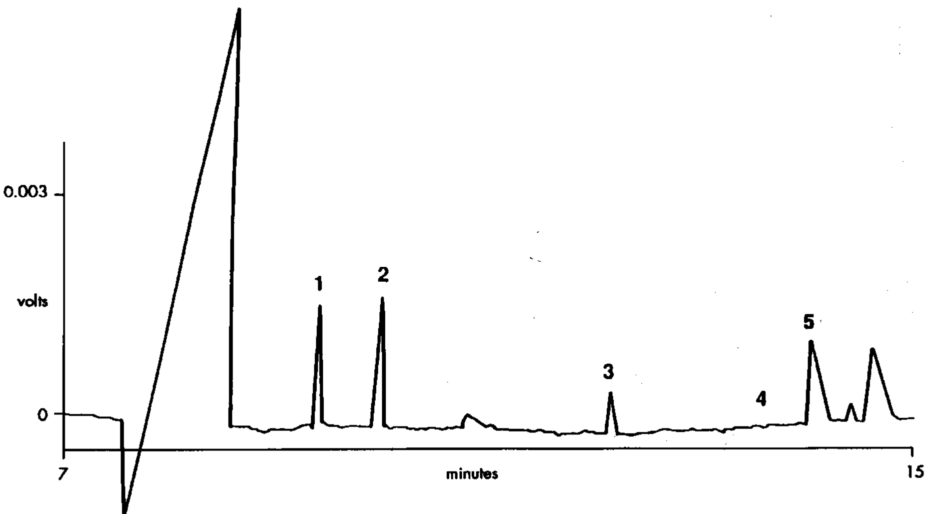

Fig. 4. Electropherogram of soy sauce. Sample preparation: dilute and inject. Peaks: 1 = citric acid; 2 = tartaric acid; 3 = acetic acid 4 = lactic acid; 5 = butyric acid (not added as an internal standard). The large peak on the left is chloride.

TABLE III

RESULTS FOR CHABLIS WINE SAMPLE

Analyte	Concentration (μg/ml)	R.S.D. % ($n = 10$)[a]
Citric acid	127	14.1
Tartaric acid	2645	1.9
Malic acid	3291	1.3
Succinic acid	300	2.7
Acetic acid	260	5.4
Lactic acid	296	9.1

[a] Ten different aliquots of sample.

TABLE IV

RECOVERY OF ACIDS FROM GRAPE JUICE

Analyte	Added (μg/ml)	Found (μg/ml)	Recovery (%)
Tartaric acid	0	4330	–
	1160	5525	103
	2319	6703	102
Malic acid	0	3867	–
	1289	5153	100
	2577	6283	94

would increase the response of the other acids. This, in turn, would improve the reproducibility. The recovery from dark grape juice for tartaric and malic acid at two different levels averaged 103% and 97%, respectively (Table IV).

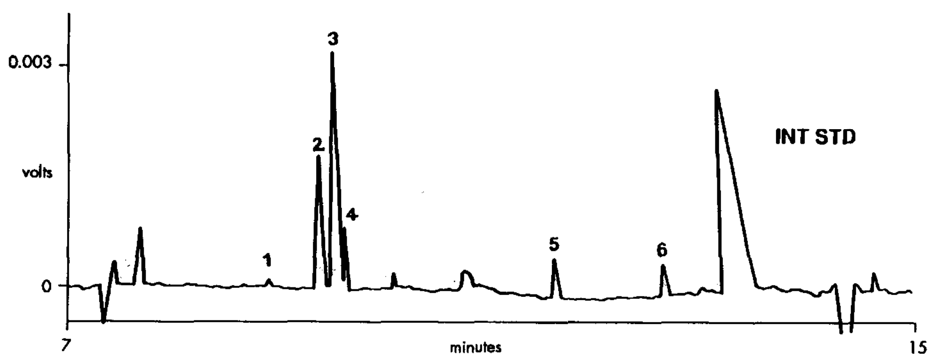

Fig. 5. Electropherogram of Chablis wine. Sample preparation: dilute and inject. Peaks: 1 = citric acid, 127 μg/ml; 2 = tartaric acid, 2645 μg/ml; 3 = malic acid, 3291 μg/ml; 4 = succinic acid, 300 μg/ml; 5 = acetic acid, 260 μg/ml; 6 = lactic acid, 296 μg/ml.

CONCLUSIONS

The determination of organic acids by CE with NICE-Pak Chemistry appears to be a simple alternative to other techniques for many food and beverage matrices. One point not yet stressed is its cost effectiveness. If one compares the cost per test for a six-acid determination in Chablis wine using different techniques, the advantage for CE becomes clear. Enzyme assay, HPLC–IC, and CE were compared with the following results: enzyme assay, US$12.00 (cost of kits divided by number of tests per kit, not including tartaric acid); HPLC–IC, US$ 2.72 (column, filter, mobile phase and vial cost); and CE, US$ 0.39 (capillary, electrolyte and vial cost). On a cost per test basis, CE offers 97% and 86% cost savings over enzyme assay and HPLC–IC, respectively.

Capillary electrophoresis also offers much in terms of automation. Not only are samples introduced into the capillary automatically, if one includes the autopurge function to clean the capillary between injections sample preparation to prevent interferences is also automated.

The flexibility, low cost of operation and automation of CE with the NICE-Pak Chemistry approach should make it an attractive alternative to HPLC–IC for inorganic ion and organic acid determinations.

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