DIONEX

Application Note 143

Determination of Organic Acids in Fruit Juices

INTRODUCTION

Organic acids are important in characterizing the flavor of fruit juices. Their presence and concentration determine tartness and other flavor attributes. In some cases, it is necessary to determine organic acids to assess whether an expensive juice has been illegally adulterated with a cheaper juice. Because organic acid profiles are distinct to each type of fruit juice,[1] evidence of tampering can be evaluated by comparing the known juice fingerprint to that of the suspected adulterated juice. Organic acid profiles can also determine juice freshness or spoilage.[2] Masson used ion chromatography to determine the organic acids in grape juice.[3]

The IonPac® AS11-HC is the ideal column for anion chromatographic determination of organic acids in fruit juices. These organic acids are present in concentrations ranging from less that 1 mg/L to hundreds of milligrams per liter. The AS11-HC is a high-capacity anion-exchange column that is solvent-compatible, allowing for the addition of organic solvents to enhance performance. The column's high capacity yields an improved separation of lactate, acetate, and formate. The column packing of the AS11-HC consists of 9.0-µm diameter macroporous resin beads, functionalized with quaternary ammonium groups.

The AS11-HC uses hydroxide eluent gradients and can be used with the EG50 Eluent Generator. Through electrolysis, the EG50 produces high-purity, carbonate-free potassium hydroxide (KOH) eluent. This on-line generation of KOH eliminates carbonate contamination and therefore increases baseline stability and chromatographic reproducibility, making peak integration more accurate. This application note shows how organic acids can be determined in fruit juices at low to high mg/L concentrations using a simple dilution, an IonPac AS11-HC, EG50-generated eluent gradients, and suppressed conductivity detection. The fruit juices analyzed in this application note are orange, grape, apple, and cranberry.

EQUIPMENT

Dionex DX-600 IC system consisting of:

- GP50 Gradient Pump
- EG50 Eluent Generator
- LC30 Chromatography Oven
- CD25 Conductivity Detector
- AS40 Autosampler
- Chromeleon® Chromatography Workstation
- ASRS® ULTRA Anion Self-Regenerating Suppressor

SYSTEM SETUP

To ensure a quiet baseline, the following steps must be taken during the system setup. Add 1000 psi of backpressure to the degas module on the eluent generator. (See the EG50 manual, P/N 031908, for details on adding backpressure to the degas module.) The final system backpressure should be approximately 2900 psi, but should not exceed 3000 psi. Install a conditioned ATC after the proportioning valve. A rise in background during gradient elution is observed if the ATC needs regeneration. For instruction on ATC conditioning and regeneration, see the EG50 manual (P/N 031908). Prior to sample analysis, determine a system blank by analyzing a 10-µL injection of deionized water using the chromatographic method described below. An equilibrated system has a background conductance of 1–4 µS with the peak-to-peak noise typically 10–20 nS, and no peaks eluting with the same retention time as an analyte of interest.

PREPARATION OF STANDARDS

One thousand mg/L standards of 30 organic acids and inorganic anions were prepared using the compounds and masses listed in Table 1. The mixed standard whose separation is shown in Figure 1 was prepared by mixing appropriate volumes of the 1000 mg/L standards. To determine method linearity for organic acids, representative monovalent, divalent, and trivalent organic acids were analyzed. These organic acids were quinate, tartrate, and citrate, respectively. A 10,000 ppm solution of quinate was diluted to prepare the following standards: 1, 2, 5, 10, and 20 mg/L. A 1000 ppm solution of tartrate was diluted to prepare 50, 75, 100, 150, and 200 mg/L standards. To prepare citrate standards of 75, 100, 200, 300, and 400 mg/L, a 10,000 ppm solution was used. Deionized water (DI H_2O), Type I reagent-grade, 18 MΩ-cm resistance or better was used to feed the EG50, prepare all standards, and dilute samples.

Table 1. Amounts of Compounds Used to Prepare 1 L of 1000 mg/L Anion Standards

Anion	Compound	Mass (g)
Quinate	Quinic acid	1.000
Fluoride	Sodium fluoride	2.210
Lactate	Lithium lactate	1.067
Acetate	Sodium acetate, trihydrate	2.305
Glycolate	Glycolic acid	1.000
Propionate	Sodium propionate	1.315
Formate	Sodium formate	1.511
Butyrate	Sodium butyrate	1.250
Pyruvate	Pyruvic acid	1.000
Valerate	Valeric acid	1.000
Galacturonate	D-Galacturonic acid, monohydrate	1.000
Bromate	Sodium bromate	1.179
Chloride	Sodium chloride	1.648
Trifluoroacetate	Trifluoroacetatic acid	1.000
Bromide	Sodium bromide	1.288
Nitrate	Sodium nitrate	1.371
Glutarate	Glutaric acid	1.000
Succinate	Sodium succinate	1.396
Malate	L-Malic acid	1.000
Malonate	Malonic acid	1.000
Tartrate	Sodium tartrate	1.311
Maleate	Maleic acid	1.000
Sulfate	Sodium sulfate	1.479
Oxalate	Sodium oxalate	1.522
Fumarate	Fumaric acid	1.000
Phosphate	Potassium phosphate, monobasic	1.433
Citrate	Citric acid	1.000
Isocitrate	Isocitric acid trisodium dihydrate	1.306
Cis-aconitate	*Cis*-aconitic acid	1.000
Trans-aconitate	*Trans*-aconitic acid	1.000

SAMPLES

Samples were filtered (0.45 µM filter IC Acrodisk®, Gelman P/N 4483; or Anotop™ IC, Whitman P/N 68099232) and diluted 1:10 prior to analysis.

CONDITIONS

Columns:	IonPac AS11-HC Analytical, 4 mm (P/N 052960)
	IonPac AG11-HC Guard, 4 mm (P/N 052962)
Eluent:	Potassium hydroxide gradient:
	1 mM from 0–8 min
	1 mM to 30 mM, 8–28 min
	30 mM to 60 mM, 28–38 min
	Methanol: 10%, 0–38 min
Eluent Source:	EG50
Flow Rate:	1.5 mL/min
Temperature:	30 °C
Detection System:	Suppressed conductivity, ASRS ULTRA 4 mm, AutoSuppression®, external water mode (10 mL/min)
Backpressure:	2900 psi
Background Conductance:	1–4 µS
Degas Setting:	30 s every 2 min
Injection Volume:	10 µL

RESULTS AND DISCUSSION

Figure 1 shows the separation of 30 organic acids and inorganic anions in a single injection of a fruit juice standard. Of these 30 analytes, only the two pairs (formate/butyrate and tartrate/malonate) are not resolved. This separation demonstrates that monovalent (e.g., quinate), divalent (e.g., tartrate), and trivalent (e.g., citrate) organic acids can be separated with this method. The stable baseline is due to the use of the EG40 for KOH eluent generation. The responses of quinate, tartrate, and citrate were measured over the concentration ranges described in the "Preparation of Standards" section, and good linearity was obtained (r^2 = 0.999, 0.993, and 0.998, respectively) for each class of organic acid (mono-, di-, and trivalent). Using an IonPac AS11 column, Masson also found good linearity of organic acid standards ($r^2 = (0.990)^3$. These studies show that this method can be used to measure a wide range of organic acids in fruit juices with a single sample injection.

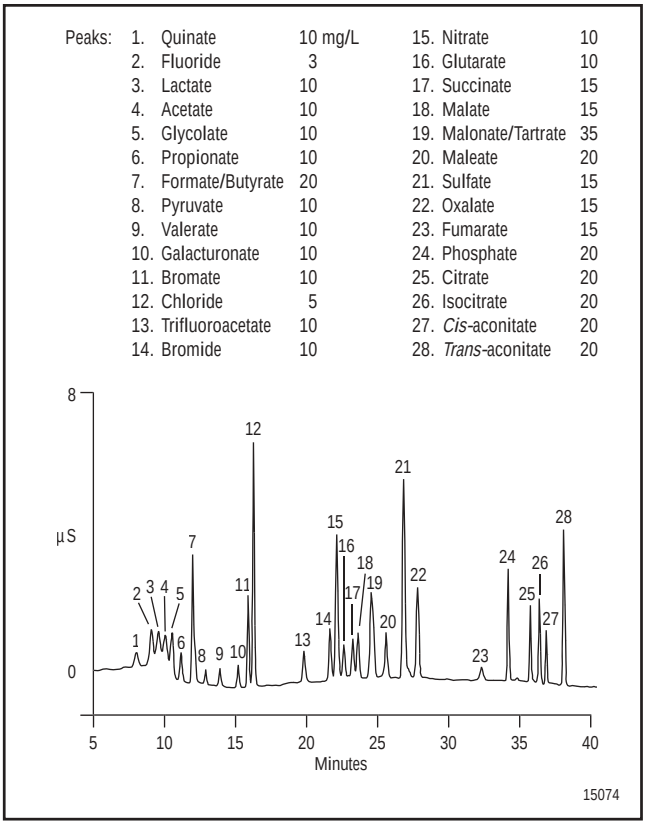

Figure 1. *Standard for fruit juice analysis.*

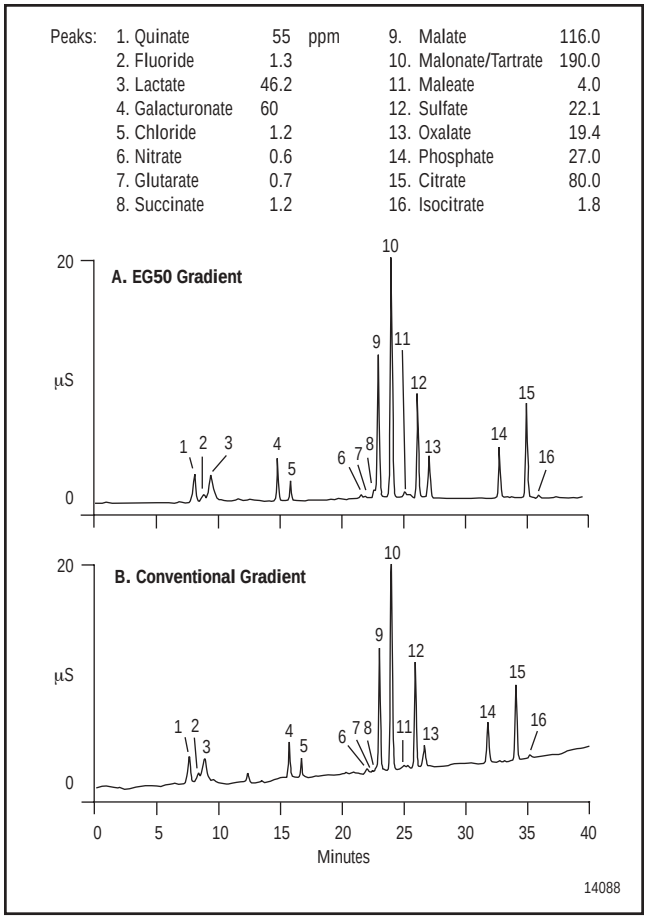

***Figure 2**. Determination of anions and organic acids in grape juice.*

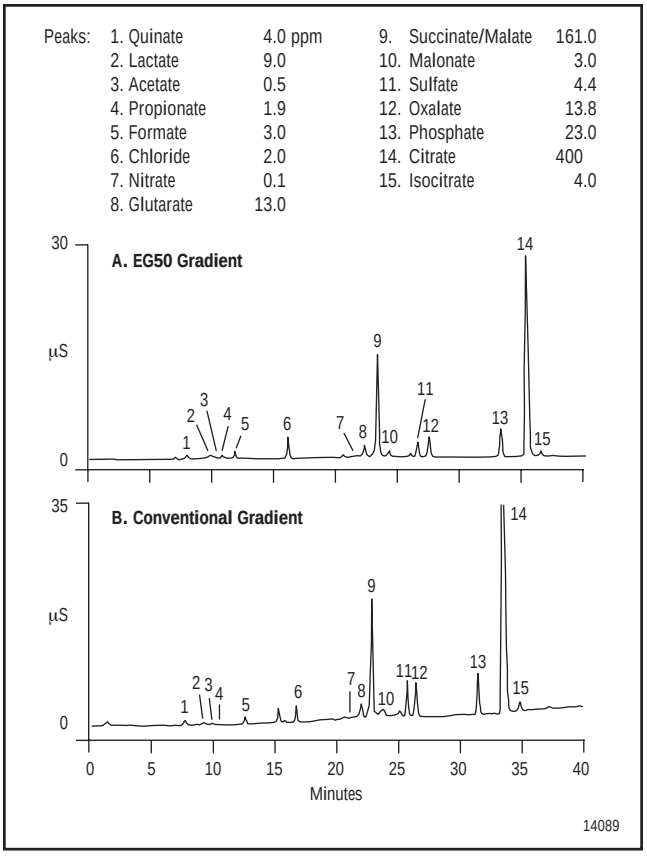

***Figure 3**. Determination of anions and organic acids in orange juice.*

Figures 2 and 3 show analyses of grape and orange juices using this method. These figures also show a comparison of EG50-generated eluent and conventional eluent preparation. The baselines of the chromatograms that used the EG50 are noticeably flatter and more stable than those from the chromatograms that used manually prepared eluents. Figure 2 shows that the major organic acids in grape juice are malate, tartrate, and citrate, as has been reported by others.[2–4] Figure 3 shows that orange juice has, as expected, a high concentrate of citric acid (citrate ion).

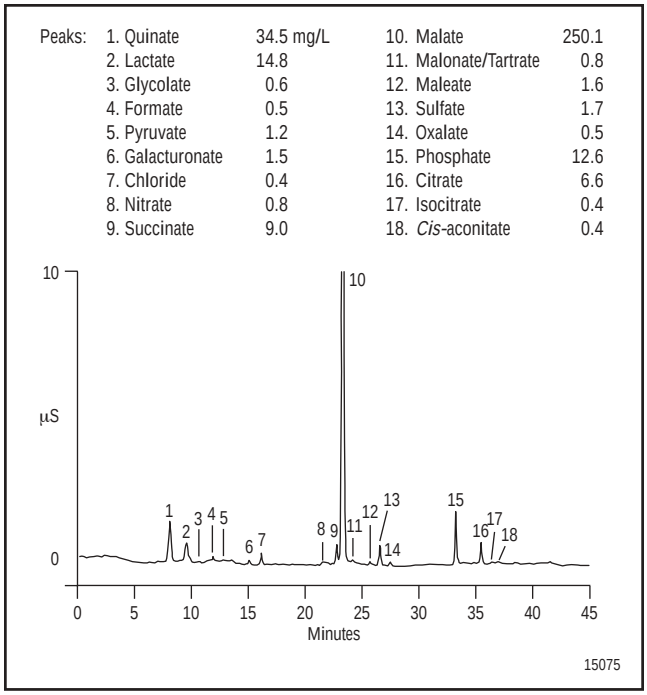

***Figure 4**. Determination of anions and organic acids in apple juice.*

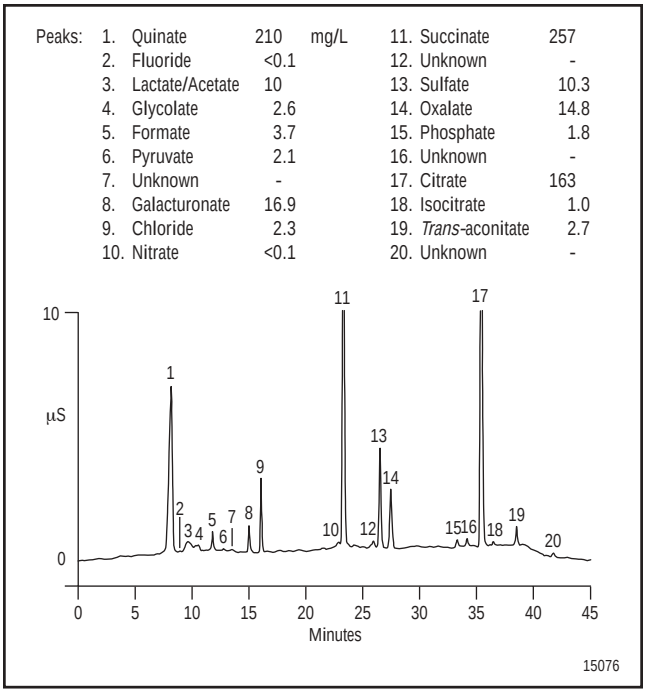

***Figure 5**. Determination of anions and organic acids in cranberry juice cocktail.*

Figures 4 and 5 show organic acid determination in apple juice and cranberry juice cocktail, respectively. Notice that both lactic and acetic acids are low in both figures. Elevated levels of lactic and acetic acids may be caused by microbiological spoilage,[4,5] so it is important to monitor the concentrations of these organic acids as a measure of product quality. Malate is the major constituent of apple juice. In cranberry juice cocktail, there are high concentrations of succinate and quinate, which provide a tart taste. Oxalate and citrate are also present at high levels. Note the presence of galacturonate in cranberry juice cocktail and grape juice. The presence of galacturonate can be attributed to the degradation of pectins in the skins of fruit.[4] Freshly squeezed juices generally show lower levels of galacturonate. Note the lack of galacturonate in the orange juice.

SUMMARY

The method described in this application note can be used to determine organic acids in fruit juices. This method uses the EG50 to generate high-purity, carbonate-free eluents to suppress baseline drift and therefore improve retention time and integration reproducibility. The IonPac AS11-HC is the ideal column for this method because its high capacity improves separation of a wide range of organic acids.

REFERENCES

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2. Dong, M. *LC-GC*, **1998**, *16(12)*, 1092–1097.
3. Masson, P. *J. of Chromatogr., A* **2000**, *881*, 387–394.
4. Saccani, G. et al. *J. of Chromatogr., A* **1995**, *706*, 395–403.
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