

Determination of Organic Acids in Fruit Juices

Swati Gokhale and Jeff Rohrer
Thermo Fisher Scientific, Inc.

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 Thermo Scientific™ Dionex™ IonPac™ AS11-HC column is the ideal column for anion chromatographic determination of organic acids in fruit juices. These organic acids are present in concentrations ranging from less than 1 mg/L to hundreds of milligrams per liter. The Dionex IonPac AS11-HC column 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 Dionex IonPac AS11-HC column consists of 9.0 µm diameter macroporous resin beads, functionalized with quaternary ammonium groups.

The Dionex IonPac AS11-HC column uses hydroxide eluent gradients and can be used with the Thermo Scientific Dionex EG50 eluent generator. Through electrolysis, the Dionex EG50 generator 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 Dionex IonPac AS11-HC column, Dionex 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:

- Thermo Scientific Dionex GP50 Gradient Pump
- Dionex EG50 Eluent Generator
- Thermo Scientific Dionex LC30 Chromatography Oven
- Thermo Scientific Dionex CD25 Conductivity Detector
- Thermo Scientific Dionex AS40 Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, Workstation 6.6
- Thermo Scientific™ Dionex™ ASRS™ ULTRA Anion Self-Regenerating Suppressor

**Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-2100 or the Thermo Scientific Dionex ICS-5000+ system.*

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 Dionex EG50 generator 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 Dionex IonPac ATC Anion Trap column after the proportioning valve. A rise in background during gradient elution is observed if the Dionex IonPac ATC column needs regeneration. For instruction on Dionex IonPac ATC column conditioning and regeneration, see the Dionex EG50 generator manual (P/N 031908). Prior to sample analysis, determine a system blank by analyzing a 10 μ L injection of deionized water using the chromatographic method described below. An equilibrated system has a background conductance of 1–4 μ S 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 H₂O), Type I reagent-grade, 18 M Ω -cm resistance or better was used to feed the Dionex EG50 generator, 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	Trifluoroacetic 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 and diluted 1:10 prior to analysis.

Conditions

Columns:	Dionex IonPac AS11-HC Analytical, 4 mm Dionex IonPac AG11-HC Guard, 4 mm
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:	Dionex EG50 generator
Flow Rate:	1.5 mL/min
Temperature:	30 °C
Detection System:	Suppressed conductivity, Dionex ASRS ULTRA suppressor, 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 Dionex EG40 generator 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 Dionex 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.

Peaks:	1. Quinate	10 mg/L	15. Nitrate	10
	2. Fluoride	3	16. Glutarate	10
	3. Lactate	10	17. Succinate	15
	4. Acetate	10	18. Malate	15
	5. Glycolate	10	19. Malonate/Tartrate	35
	6. Propionate	10	20. Maleate	20
	7. Formate/Butyrate	20	21. Sulfate	15
	8. Pyruvate	10	22. Oxalate	15
	9. Valerate	10	23. Fumarate	15
	10. Galacturonate	10	24. Phosphate	20
	11. Bromate	10	25. Citrate	20
	12. Chloride	5	26. Isocitrate	20
	13. Trifluoroacetate	10	27. <i>Cis</i> -aconitate	20
	14. Bromide	10	28. <i>Trans</i> -aconitate	20

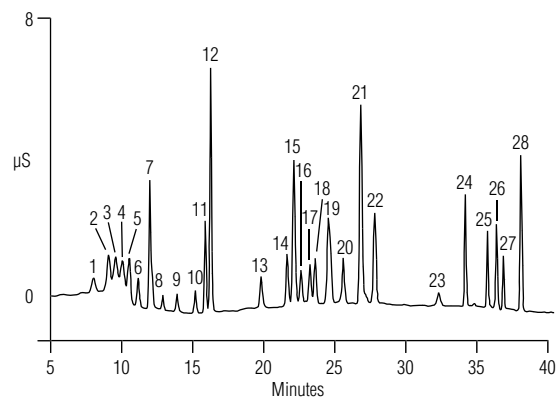


Figure 1. Standard for fruit juice analysis.

Peaks:	1. Quinate	55 ppm	9. Malate	116.0
	2. Fluoride	1.3	10. Malonate/Tartrate	190.0
	3. Lactate	46.2	11. Maleate	4.0
	4. Galacturonate	60	12. Sulfate	22.1
	5. Chloride	1.2	13. Oxalate	19.4
	6. Nitrate	0.6	14. Phosphate	27.0
	7. Glutarate	0.7	15. Citrate	80.0
	8. Succinate	1.2	16. Isocitrate	1.8

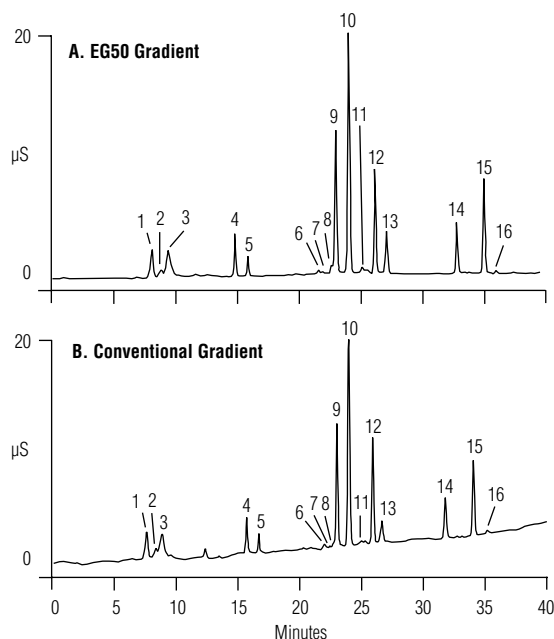


Figure 2. Determination of anions and organic acids in grape juice.

Peaks:	1. Quinate	4.0 ppm	9. Succinate/Malate	161.0
	2. Lactate	9.0	10. Malonate	3.0
	3. Acetate	0.5	11. Sulfate	4.4
	4. Propionate	1.9	12. Oxalate	13.8
	5. Formate	3.0	13. Phosphate	23.0
	6. Chloride	2.0	14. Citrate	400
	7. Nitrate	0.1	15. Isocitrate	4.0
	8. Glutarate	13.0		

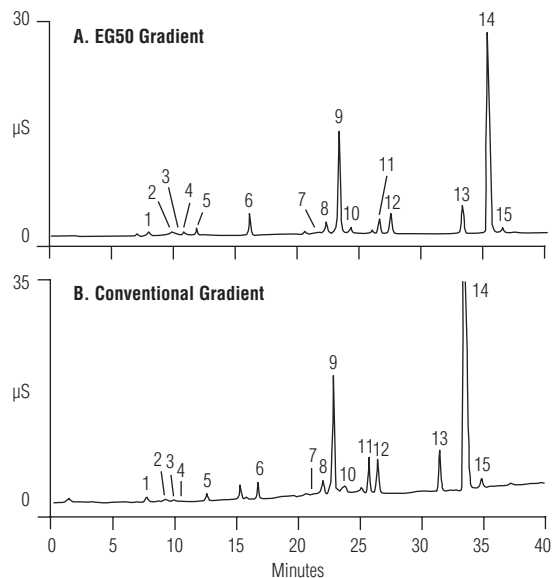


Figure 3. Determination of anions and organic acids in orange juice.

Figures 2 and 3 show analyses of grape and orange juices using this method. These figures also show a comparison of Dionex EG50-generated eluent and conventional eluent preparation. The baselines of the chromatograms that used the Dionex EG50 eluent generator 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 concentration of citric acid (citrate ion).

Peaks:	1. Quinate	34.5 mg/L	10. Malate	250.1
	2. Lactate	14.8	11. Malonate/Tartrate	0.8
	3. Glycolate	0.6	12. Maleate	1.6
	4. Formate	0.5	13. Sulfate	1.7
	5. Pyruvate	1.2	14. Oxalate	0.5
	6. Galacturonate	1.5	15. Phosphate	12.6
	7. Chloride	0.4	16. Citrate	6.6
	8. Nitrate	0.8	17. Isocitrate	0.4
	9. Succinate	9.0	18. <i>Cis</i> -aconitate	0.4

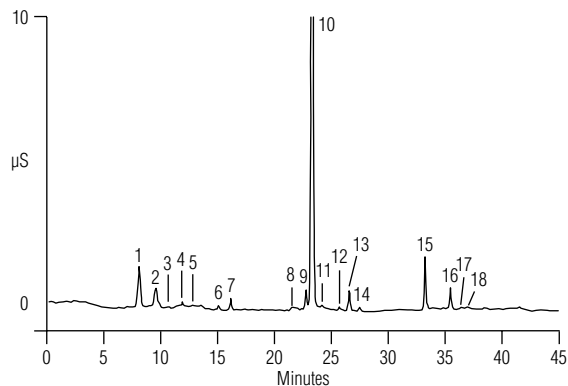


Figure 4. Determination of anions and organic acids in apple juice.

Peaks:	1. Quinate	210 mg/L	11. Succinate	257
	2. Fluoride	<0.1	12. Unknown	-
	3. Lactate/Acetate	10	13. Sulfate	10.3
	4. Glycolate	2.6	14. Oxalate	14.8
	5. Formate	3.7	15. Phosphate	1.8
	6. Pyruvate	2.1	16. Unknown	-
	7. Unknown	-	17. Citrate	163
	8. Galacturonate	16.9	18. Isocitrate	1.0
	9. Chloride	2.3	19. <i>Trans</i> -aconitate	2.7
	10. Nitrate	<0.1	20. Unknown	-

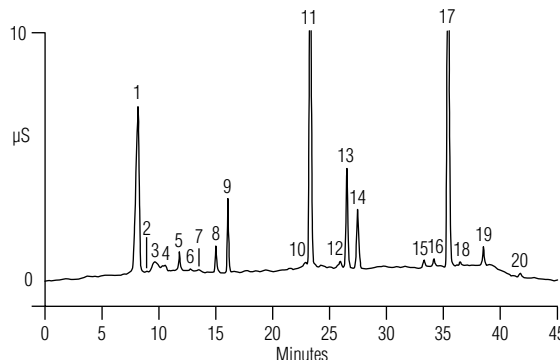


Figure 5. Determination of anions and organic acids in cranberry juice cocktail.

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 Dionex EG50 generator to generate high-purity, carbonate-free eluents to suppress baseline drift and therefore improve retention time and integration reproducibility. The Dionex IonPac AS11-HC column is the ideal column for this method because its high capacity improves separation of a wide range of organic acids.

References

- Henshall, A. *Cereal Foods World* **1998**, *43*(2), 98–103.
- Dong, M. *LC-GC*, **1998**, *16*(12), 1092–1097.
- Masson, P. J. *J. of Chromatogr., A* **2000**, *881*, 387–394.
- Saccani, G. et al. *J. of Chromatogr., A* **1995**, *706*, 395–403.
- Trifiro, A. et al. *J. of Chromatogr., A* **1997**, *770*, 243–252.

www.thermoscientific.com/chromatography

©2014 Thermo Fisher Scientific Inc. All rights reserved. ISO is a trademark of the International Standards Organization. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic)
 400 650 5118

Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591

Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00

Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo
 SCIENTIFIC

A Thermo Fisher Scientific Brand