Application Note 1068

Determination of Organic Acids in Fruit Juices and Wines by High-Pressure IC

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Key Words
Dionex IonPac AS11-HC-4 µm Column, Suppressed Conductivity, Grape Juice, Apple Juice, Pomegranate Juice

Introduction

Organic acids play important roles in juices and wines because of their influence on the organoleptic properties (flavor, color, and aroma) as well as the stability and microbiological control of the products.[1] The total content of organic acids in juices and wines affects the drink's acidity, whereas the levels of a specific organic acid can directly influence the flavor and taste of the drink. Therefore, organic acid profiles are monitored to determine the freshness of certain fruit juices; winemakers also monitor the concentration of various organic acids to ensure the quality of their wines.

The determination of organic acids also plays an important role when testing the authenticity of fruit juices and wines.[2,3] Certain fruit juices, such as those obtained from pomegranate and various types of berries, are popular because of their high levels of antioxidants and the resulting putative health benefits. The high economic value and the large market demand for these juices make them a likely target for adulteration. The most frequent profit-driven adulturation procedures include dilution with water, addition of sugars or pulp wash, and blending with cheaper alternatives. Characterizations of the organic acid content of certain juices are therefore required to verify their authenticity.

Many analytical methods are available to determine organic acids in juices and wines. However, several organic acids have poor UV absorption and therefore lack sufficient sensitivity for detection. In addition, other components commonly present in these types of samples—such as sugars and phenolic compounds—have a much higher UV absorption, which can interfere with the detection of target analytes. In contrast, virtually all carboxylic acids ionize sufficiently; therefore, ion chromatography (IC) with suppressed conductivity detection is the technique of choice to separate a large variety of organic acids with inorganic anions and detect them with high sensitivity while minimizing the sugar interferences.

Goal

To develop a method to determine organic acids in fruit juices and wines using IC with suppressed conductivity detection

Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system, including:
  - SP Single Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment
  - AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer Line, 1.2 mL (P/N 074989)
- Thermo Scientific Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column (P/N 075550)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software version 7.2

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Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- D(+)-Galacturonic Acid Monohydrate, 99% (Fisher Scientific P/N AC22782)
- L(-)-Malic Acid, 99% (Fisher Scientific P/N AC15059)
- L-(+)-Tartaric Acid, Powder, Certified ACS, 99.0% (Fisher Scientific P/N A315)
- Citric Acid Anhydrous, Crystalline, USP (Fisher Scientific P/N A95)
- Methanol (CH_3OH), Certified ACS, ≥99.8% (Fisher Scientific P/N A412)

Consumables

- Vial Kit, 10 mL, Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)
- AirTite All-Plastic Norm-Ject™ Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)
- Thermo Scientific™ Dionex™ OnGuard™ II RP Cartridges, 1 cc (P/N 057083)

Samples

- Apple Juice
- Grape Juice
- White Grape Juice
- Pomegranate Juice
- Pomegranate/Blueberry Juice (Pomegranate 85%, Blueberry 15%)
- Merlot (Red Wine)
- Chardonnay (White Wine)
- White Zinfandel (Rosé Wine)

Conditions

System 1 (9 µm)

Columns:	Thermo Scientific™ Dionex™ IonPac™ AS11-HC Guard, 2 × 50 mm (P/N 052963) Dionex IonPac AS11-HC Analytical, 2 × 250 mm (P/N 052961)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
Eluent A:	DI Water
Eluent B:	CH_3OH

Time (min)	KOH (mM)	Time (min)	B (%)
-2.000	1	-2.000	7
0.000	1	0.000	7
10.070	1	19.000	7
10.071	1	20.000	10
24.000	15	30.000	10
24.010	15	31.000	7
35.000	27	33.000	7
40.000	60	33.010	0
44.000	60	44.000	0
44.010	1	44.010	7
45.000	1	45.000	7

Flow Rate:	0.4 mL/min
Inj. Volume:	2.5 µL
Detection:	Suppressed Conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor™ (2 mm),* 82 mA, external water mode
System Backpressure:	~2100 psi (1 mM KOH/7% CH_3OH), ~2500 psi (60 mM KOH/10% CH_3OH)
Background Conductance:	~0.14–0.64 µS
Noise:	~0.8–1 nS/min, peak-to-peak
Run Time:	47 min

* Equivalent or improved results can be achieved on the Thermo Scientific™ Dionex™ AERS 500 Anion Electrolytically Regenerated Suppressor.

System 2 (4 µm)

Columns:	Dionex IonPac AS11-HC-4 µm Guard, 2 × 50 mm (P/N 078036) Dionex IonPac AS11-HC-4 µm Analytical, 2 × 250 mm (P/N 078035)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
Eluent A:	DI Water
Eluent B:	CH_3OH

Time (min)	KOH (mM)	Time (min)	B (%)
-2.000	1	-2.000	8
0.000	1	0.000	8
10.070	1	19.000	8
10.071	1	20.000	11
24.000	15	30.000	11
24.010	15	31.000	8
35.000	27	33.000	8
40.000	60	33.010	0
44.000	60	44.000	0
44.010	1	44.010	8
45.000	1	45.000	8

Flow Rate:	0.4 mL/min
Inj. Volume:	2.5 µL
Detection:	Suppressed Conductivity, Dionex ASRS 300 Anion Self-Regenerating Suppressor (2 mm),* 82 mA, external water mode
System Backpressure:	~3900 psi (1 mM KOH/8% CH_3OH), ~4800 psi (60 mM KOH/11% CH_3OH)
Background Conductance:	~0.16–0.7- µS
Noise:	~0.6–0.9 nS/min, peak-to-peak
Run Time:	47 min

* Equivalent or improved results can be achieved on the Thermo Scientific™ Dionex™ AERS 500 Anion Electrolytically Regenerated Suppressor.

Preparation of Solutions and Reagents

Stock Solutions of 29 Anions

To prepare 1000 mg/L stock solutions of 29 inorganic and organic acid anions, use the compounds and masses listed in Table 1. To prepare a standard mixture, mix appropriate volumes of the 1000 mg/L stock solutions.

Table 1. Masses of compounds used to prepare 1 L of 1000 mg/L anion stock solutions.

Anion	Compound	Mass (g)
Quinate	Quinic Acid	1.000
Fluoride	Sodium Fluoride	2.210
Lactate	Lactic Acid	1.000
Acetate	Sodium Acetate	1.390
Glycolate	Glycolic Acid	1.000
Propionate	Sodium Propionate	1.315
Formate	Sodium Formate	1.511
Butyrate	Butyric Acid	1.000
Pyruvate	Pyruvic Acid	1.000
Valerate	Valeric Acid	1.000
Galacturonate	Galacturonic Acid	1.098
Bromate	Sodium Bromate	1.179
Chloride	Sodium Chloride	1.648
Bromide	Sodium Bromide	1.288
Nitrate	Sodium Nitrate	1.371
Glutarate	Glutaric Acid	1.000
Succinate	Succinic Acid	1.000
Malate	Malic Acid	1.000
Malonate	Malonic Acid	1.000
Tartrate	Tartaric Acid	1.000
Maleate	Maleic Acid	1.000
Sulfate	Sodium Sulfate	1.479
Fumarate	Fumaric Acid	1.000
Oxalate	Sodium Oxalate	1.522
Phosphate	Potassium Phosphate, Monobasic	1.433
Citrate	Citric Acid	1.000
Isocitrate	DL-Isocitric Acid Trisodium Salt Dihydrate	1.306
cis-Aconitate	*cis*-Aconitic Acid	1.000
trans-Aconitate	*trans*-Aconitic Acid	1.000

Working Standard Solutions

Dilute 1000 mg/L galacturonate stock solution to prepare 2, 5, 10, 20, 50, 100, and 200 mg/L standards. Dilute 1000 mg/L malate stock solution to prepare 2, 5, 10, 20, 50, 100, 200, and 500 mg/L standards. Dilute 1000 mg/L tartrate stock solution to prepare 2, 5, 10, 20, 50, 100, and 200 mg/L standards. Dilute 1000 mg/L citrate stock solution to prepare 1, 2, 5, 10, 20, 50, 100, and 200 mg/L standards.

Sample Preparation

Dilute fruit juice samples 1:20 and filter through a Nalgene syringe filter prior to analysis.

Dilute wine samples 1:20 and filter through a Dionex OnGuard II RP cartridge prior to analysis. Prepare the Dionex OnGuard II RP cartridge before use by flushing it first with 5 mL of methanol and then with 10 mL of DI water with maximum flow rate of 4 mL/min. After filling a 5 mL syringe with sample, push the first 3 mL through the cartridge into a waste container and collect the next 2 mL for injection.

Recovery Study

For fruit juice samples, spike the appropriate amount of stock solutions into the samples during the 1:20 dilution before the filtration described above.

For wine samples, spike the appropriate amount of stock solutions into the samples during the 1:20 dilution. Then filter the spiked samples through a Dionex OnGuard II RP cartridge before injection.

System Preparation and Configuration

Install and configure the Dionex AS-AP Autosampler in Push Mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361) to calibrate the sample transfer line to ensure accurate and precise sample injections.

Prepare the Dionex ASRS 300 Anion Self-Regenerating Suppressor for use by hydrating the internal membrane. Push 3 mL of DI water through the Eluent Out port and 5 mL of DI water through the Regen In port.

Note: Allow the suppressor to sit for 20 min to ensure complete hydration before installing it in the system. Also note that when methanol is added to the eluent stream, the suppressor must be operated in the External Water mode.

Configure the pressurized water reservoirs to supply external water for suppressor regeneration. Use at least two 4 L bottles plumbed in tandem to ensure uninterrupted external water delivery. Fill the reservoirs with DI water and apply 5–15 psi to the reservoir to deliver DI water through the regenerant channel. Ensure that the cap of the reservoir is sealed tightly. For more information on installation and operation of the Dionex ASRS 300 Anion Self-Regenerating Suppressor, consult the product manual (Document No. 031956).

Condition the Dionex EGC 500 KOH cartridge before first use by running 50 mM KOH at 1 mL/min for 45 min. For more information on installation and operation of the Dionex EGC 500 KOH cartridge, consult the product manual (Document No. 065018-04).

Install the Dionex IonPac AG11-HC-4 µm Guard (2 × 50 mm) and the Dionex IonPac AS11-HC-4 µm Analytical (2 × 250 mm) columns in the lower compartment of the DC detector. After connecting the inlet of the column, pump 30 mM KOH through the column with the outlet directed to waste for at least 30 min before connecting the column outlet to the suppressor using 0.005 in. i.d. PEEK tubing. Keep the lengths of the connective tubing to a minimum.

After configuring the system, pump 8% CH_3OH (92% Eluent A, 8% Eluent B) through the Dionex EGC 500 KOH cartridge at 0.4 mL/min, set the KOH concentration at 1 mM, and set the suppressor current at 82 mA. Allow the system to equilibrate for at least 30 min before injection.

Results and Discussion

Summary

In this study, the determination of organic acids in juices and wines was demonstrated using a Dionex ICS-5000⁺ system. The efficient separation was achieved on a Dionex IonPac AS11-HC-4 µm column set, a high-resolution high-capacity anion-exchange product designed to resolve a large number of organic acids and inorganic anions using hydroxide gradient elution. The Dionex EGC 500 KOH eluent generator cartridge produced high-purity KOH, which ensured the excellent reproducibility of the method. A solvent gradient of 8–11% CH_3OH was added to the KOH eluent to improve the resolution of a few close-eluting peaks. The separated analytes were detected using suppressed conductivity detection.

Separation

The performance of the Dionex IonPac AS11-HC (9 µm) and Dionex IonPac AS11-HC-4 µm column sets were compared for separation of a standard mixture containing 29 inorganic and organic acids anions. The chromatographic conditions were individually optimized for the two column sets. Despite a difference in optimal CH_3OH concentration for the two column sets, a similar strategy for the separation was applied to both column sets. A KOH gradient was used to separate anions of different degrees of retention with minimal background shift. The separation was further optimized with CH_3OH, because the solvating power and hydrophobicity of the organic solvent can influence the retention mechanism and improve the resolution of coeluting species.[4,5] However, with the addition of CH_3OH to the eluent stream, the suppressor had to be operated in the External Water mode. The use of CH_3OH caused a small increase in retention time and a certain decrease in peak response.

A low eluent concentration (1 mM KOH) was used to separate the weakly retained anions, such as quinate, fluoride, lactate, acetate, and glycolate. Methanol was added to resolve acetate and glycolate, which would otherwise coelute. The eluent concentration was then gradually increased to elute more strongly retained anions. The percentage of CH_3OH was increased to 11% at 20 min and remained at that level for 10 min, during which three previously coeluting groups of anions resolved, including nitrate, glutarate, succinate, and malate in the first group; malonate and tartrate in the second group; and fumarate and oxalate in the third group. To expedite the elution of late-eluting peaks, including phosphate, citrate, isocitrate, *cis*-aconitate, and *trans*-aconitate, no CH_3OH was used from 33–44 min. The eluent condition was restored to the initial condition at 44 min to re-equilibrate the column prior to the next injection.

As shown in Figure 1, 30 anions were separated on the Dionex IonPac AS11-HC (9 µm) and Dionex IonPac AS11-HC-4 µm column sets with the same elution order, as both are high-capacity anion-exchange products with a similar selectivity and capacity. The Dionex IonPac AS11-HC column set is packed with 9 µm particles, whereas the Dionex IonPac AS11-HC-4 µm column set is packed with 4 µm particles. Because smaller particle sizes yield better overall peak efficiencies, the Dionex IonPac AS11-HC-4 µm column set offers much sharper peaks and thus improved resolution for close-eluting peaks.[6] Significant improvements in resolution were observed among weakly retained monovalent anions, including lactate, acetate, and glycolate, formate and butyrate; as well as more strongly retained divalent anion pairs, such as succinate and malate, malonate and tartrate, and sulfate and fumarate. Therefore, the remainder of this study was conducted using the Dionex IonPac AS11-HC-4 µm column set.

Peaks:								
1. Quinate	5 mg/L	11. Galacturonate	5	21. Tartrate	5
2. Fluoride	1	12. Bromate	10	22. Maleate	10
3. Lactate	5	13. Chloride	2.5	23. Sulfate	5
4. Acetate	5	14. Bromide	5	24. Fumarate	20
5. Glycolate	5	15. Nitrate	5	25. Oxalate	5
6. Propionate	5	16. Glutarate	5	26. Phosphate	10
7. Formate	5	17. Succinate	10	27. Citrate	10
8. Butyrate	5	18. Malate	20	28. Isocitrate	10
9. Pyruvate	5	19. Carbonate	—	29. *cis*-Aconitate	10
10. Valerate	5	20. Malonate	5	30. *trans*-Aconitate	10

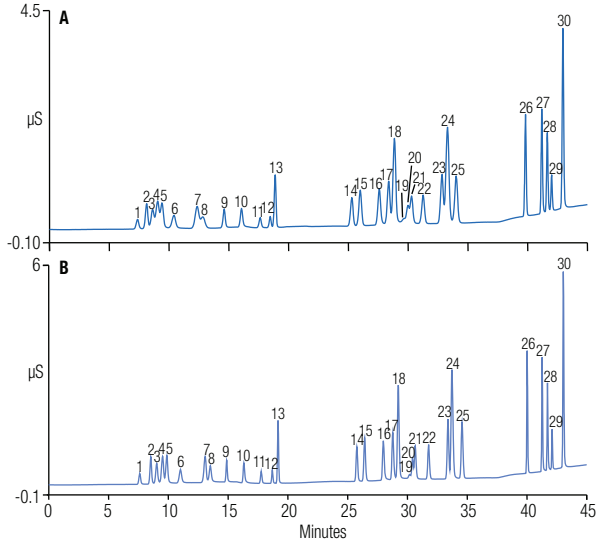

Figure 1. The organic and inorganic anion standard on (A) the Dionex IonPac AS11-HC (complete conditions as shown for System 1 on page 2) and (B) the Dionex IonPac AS11-HC-4 µm columns (complete conditions as shown for System 2 on page 2).

Calibration, Limit of Detection, and Limit of Quantitation

In this study, four representative monovalent, divalent, and trivalent organic acids were selected for the calibration study. Galacturonate, malate, tartrate, and citrate are four of the major organic acids found in fruit juices and wines. Calibration curves with seven concentration levels ranging from 2 mg/L to 200 mg/L were constructed for galacturonate and tartrate. Calibration curves with eight concentration levels ranging from 2 mg/L to 500 mg/L and from 1 mg/L to 200 mg/L were constructed for malate and citrate, respectively.

Due to incomplete dissociation of these weak carboxylic acids at high concentrations, the calibration curves show deviation from linearity in the selected calibration ranges.[7] Therefore, the calibration plots of peak area versus concentration were fit using quadratic regression functions with coefficients of determination (r^2) >0.999. To determine the limit of detection (LOD) and limit of quantification (LOQ), the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1-min segment of the baseline where no peaks elute but close to the peak of interest. The LOD and LOQ were then calculated from the average peak height of five injections of 0.2 mg/L each of the standards. The results of the calibration, LOD, and LOQ are summarized in Table 2.

Table 2. Results of calibration, LOD, and LOQ of galacturonate, malate, tartrate, and citrate.

Analyte	Range (mg/L)	Coefficient of Determination (r^2)[a]	LOD[b] (mg/L)	LOQ[c] (mg/L)
Galacturonate	2–200	0.9999	0.069	0.23
Malate	2–500	0.9997	0.041	0.14
Tartrate	2–200	0.9998	0.053	0.18
Citrate	1–200	0.9997	0.036	0.12

[a] Quadratic fit
[b] LOD = 3 × S/N
[c] LOQ = 10 × S/N

Sample Analysis

A number of fruit juices and different wine samples were studied. The various organic acids were identified by comparing their retention times with those of the standards. The concentrations of all the anions were estimated using the 29-anion standard mixture, except for galacturonate, malate, tartrate, and citrate, which were accurately quantified from their respective calibration curves. As noted in the chromatograms of the selected samples, dissolved CO_2 appeared as the carbonate peak in all samples, but did not interfere with the peaks of interest.

Pomegranate juice is gaining great attention for its perceived health benefits.[8] Because it is a high-value product, there is interest in authenticity testing for pomegranate juice. One of the common adulterants of pomegranate juice is grape juice, which can be added as a sweetener and coloring agent substitute for natural pomegranate color. One distinguishing difference is that tartaric acid is present in large amounts in grape juice but is either absent or present only in small quantities in pomegranate juice.[9,10]

Citric acid is the predominant organic acid found in large quantity in pomegranate juice, as reported in other studies.[11,12] As shown in Figure 2, Chromatogram A, malic acid and citric acid are the main organic acids in the pomegranate juice sample. In comparison, the amount of tartaric acid is very low, indicating that this pomegranate juice is not adulterated with grape juice. Figure 2B shows the anionic profile of a pomegranate/blueberry juice sample. Quinic acid is found in blueberry juices and, as shown in Figure 2, quinate is absent in pomegranate juice but is present in the pomegranate/blueberry juice sample.[13,14]

Peaks:		A	B		A	B
	1. Quinate	— mg/L	6.78	13. Succinate	1.44	2.41
	2. Fluoride	0.137	0.217	14. Malate	40.8	38.2
	3. Lactate	3.40	18.5	15. Carbonate	—	—
	4. Acetate	0.270	0.553	16. Tartrate	0.127	0.109
	5. Glycolate	0.100	0.312	17. Sulfate	4.51	4.43
	6. Formate	0.0546	0.0942	18. Oxalate	2.14	2.31
	7. Butyrate	0.302	0.230	19. Phosphate	14.4	14.1
	8. Pyruvate	0.0521	0.229	20. Citrate	330	265
	9. Valerate	0.0689	0.0671	21. Isocitrate	7.08	6.17
	10. Galacturonate	3.38	15.6	22. *cis*-Aconitate	4.59	1.73
	11. Chloride	22.7	21.1	23. *trans*-Aconitate	0.419	0.367
	12. Glutarate	0.591	0.550			

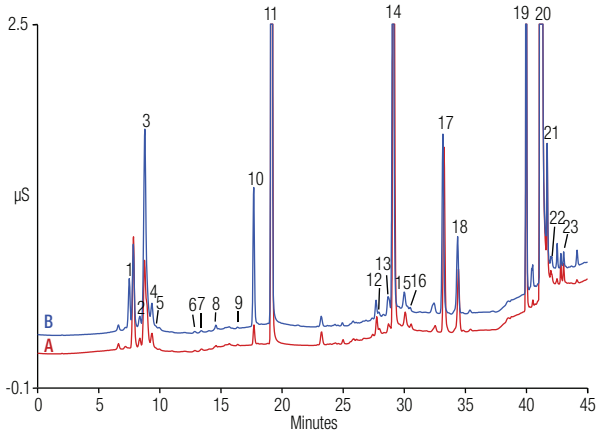

Figure 2. (A) Pomegranate juice and (B) pomegranate/blueberry juice with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

In grape and white grape juices, malic acid, tartaric acid, and citric acid are the major acids (Figures 3 and 4). Among them, tartaric acid is the most abundant acid and its concentration is an important criterion for grape juice and wine stabilization.[15] Compared to white grape juice, grape juice contains a higher content of galacturonic acid, malic acid, and citric acid.

		A		B
Peaks:	1. Fluoride	1.05	mg/L	1.36
	2. Lactate	3.37		3.23
	3. Acetate	0.703		1.21
	4. Glycolate	—		0.376
	5. Formate	0.128		0.510
	6. Butyrate	0.0842		0.295
	7. Pyruvate	0.190		0.0847
	8. Galacturonate	39.5		71.1
	9. Chloride	2.87		2.28
	10. Bromide	0.218		0.125
	11. Nitrate	0.611		1.72
	12. Succinate	1.17		2.17
	13. Malate	86.2		141
	14. Carbonate	—		—
	15. Tartrate	93.5		85.4
	16. Maleate	5.90		—
	17. Sulfate	14.8		10.1
	18. Fumarate	0.345		—
	19. Oxalate	7.59		8.74
	20. Phosphate	20.6		21.4
	21. Citrate	58.4		75.8
	22. Isocitrate	2.32		3.30
	23. *cis*-Aconitate	0.161		0.900
	24. *trans*-Aconitate	0.0995		0.0466

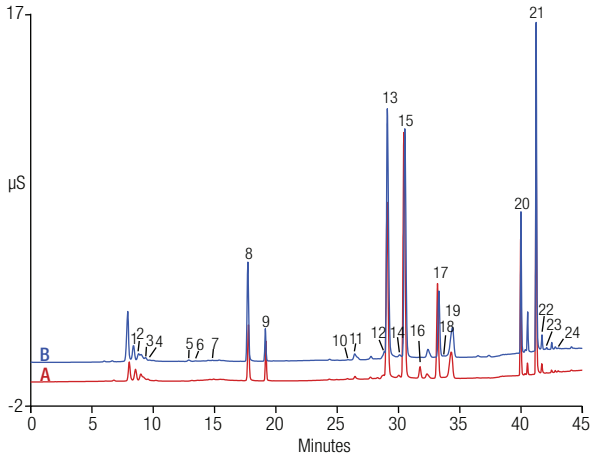

Figure 3. (A) White grape juice and (B) grape juice with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

		A		B
Peaks:	1. Fluoride	1.05	mg/L	1.05
	2. Lactate	3.37		3.37
	3. Acetate	0.703		0.703
	4. Formate	0.128		0.128
	5. Butyrate	0.0842		0.0842
	6. Pyruvate	0.190		0.190
	7. Galacturonate	39.5		87.9
	8. Chloride	2.87		2.87
	9. Bromide	0.218		0.218
	10. Nitrate	0.611		0.611
	11. Succinate	1.17		1.17
	12. Malate	86.2		165
	13. Carbonate	—		—
	14. Tartrate	93.5		186
	15. Maleate	5.90		5.90
	16. Sulfate	14.8		14.8
	17. Fumarate	0.345		0.345
	18. Oxalate	7.59		7.59
	19. Phosphate	20.6		20.6
	20. Citrate	58.4		110
	21. Isocitrate	2.32		2.32
	22. *cis*-Aconitate	0.161		0.161
	23. *trans*-Aconitate	0.0995		0.0995

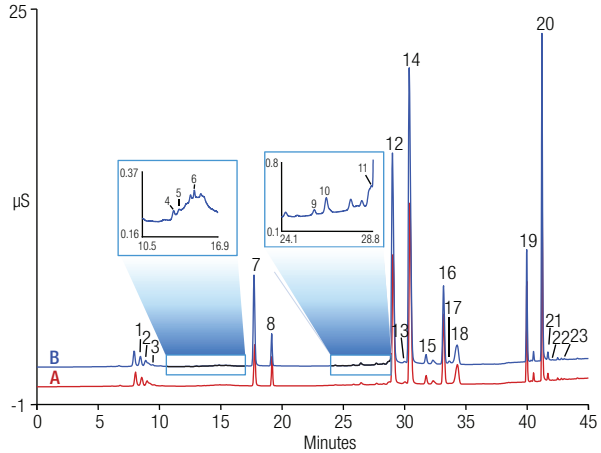

Figure 4. (A) White grape juice and (B) spiked white grape juice with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

In apple juice, quinic, galacturonic, and malic acids are the major acids, whereas tartaric acid is present in a trace amount. Malic acid is the most abundant acid in authentic apple juice, whereas tartaric acid is absent when quinic acid is present in apples, as shown in Figure 5.[16] Galacturonic acid originates from pectin contained in the primary cell walls of terrestrial plants and can be used to indicate the pectin content in fruit samples.[17] Compared to other juices in this study, apple juice contains a low content of citric acid.

		A		B
Peaks:	1. Quinate	17.6	mg/L	17.6
	2. Fluoride	0.214		0.214
	3. Lactate	4.07		4.07
	4. Acetate	0.0515		0.0515
	5. Glycolate	0.258		0.258
	6. Formate	2.08		2.08
	7. Pyruvate	0.496		0.496
	8. Galacturonate	83.5		165
	9. Chloride	2.19		2.19
	10. Succinate	1.59		1.59
	11. Malate	235		461
	12. Carbonate	—		—
	13. Tartrate	0.335		0.335
	14. Sulfate	3.08		3.08
	15. Oxalate	3.16		3.16
	16. Phosphate	8.48		8.48
	17. Citrate	2.11		4.28
	18. Isocitrate	0.628		0.628
	19. *cis*-Aconitate	3.00		3.00
	20. *cis*-Aconitate	0.0241		0.0241

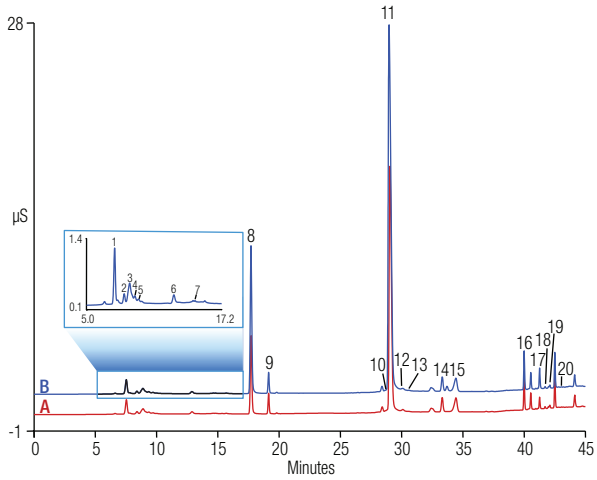

Figure 5. (A) Apple juice and (B) spiked apple juice with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

For wine, a common differentiation is made between acids that originate from the grape (tartaric, malic, and citric acids) and those from the fermentation process (succinic, lactic, and acetic acids).[15,18,19] Two dominant acids in wines are malic and tartaric acids, which are present in large quantities in ripe grapes and virtually determine the acidity of wines. As noted in Figures 6–8, the ratio of tartrate to malate in Merlot wine is significantly higher than in Chardonnay and White Zinfandel wines. In addition, the Merlot wine contains the lowest concentration of citric acid among the three wine samples, because citric acid is usually not added to red wines. Although lactic acid was found in relatively small quantities in the selected juice samples, these wine samples contain much larger concentrations of lactic acid, which originated from the microbial fermentation process.[20] Similarly, these wine samples contain a higher amount of succinic acid when compared to the juice samples, again due to fermentation.

Peaks:		A		B
	1. Quinate	4.31	mg/L	4.31
	2. Fluoride	0.508		0.508
	3. Lactate	79.9		79.9
	4. Acetate	21.3		21.3
	5. Glycolate	2.05		2.05
	6. Galacturonate	59.7		120
	7. Chloride	1.41		1.41
	8. Nitrate	0.578		0.578
	9. Succinate	33.2		33.2
	10. Malate	11.2		43.3
	11. Carbonate	—		—
	12. Tartrate	99.6		193
	13. Maleate	5.31		5.31
	14. Sulfate	20.1		20.1
	15. Oxalate	0.145		0.145
	16. Phosphate	42.6		42.6
	17. Citrate	4.61		8.76
	18. Isocitrate	1.96		1.96
	19. *cis*-Aconitate	0.450		0.450
	20. *cis*-Aconitate	0.0626		0.0626

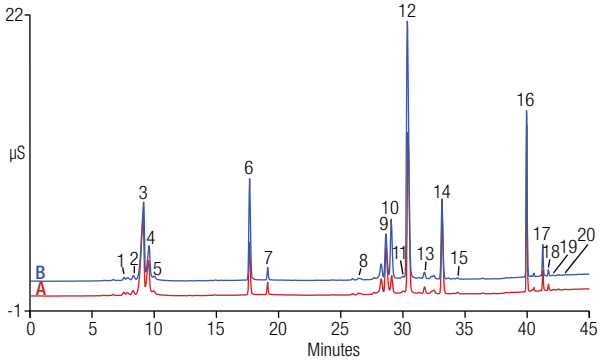

Figure 6. (A) Merlot wine and (B) spiked Merlot wine with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

Peaks:		A		B
	1. Quinate	6.01	mg/L	6.01
	2. Fluoride	1.59		1.59
	3. Lactate	47.0		47.0
	4. Acetate	14.0		14.0
	5. Glycolate	1.97		1.97
	6. Formate	0.149		0.149
	7. Pyruvate	0.908		0.908
	8. Galacturonate	20.7		42.0
	9. Chloride	1.53		1.53
	10. Nitrate	0.555		0.555
	11. Succinate	14.7		14.7
	12. Malate	117		232
	13. Carbonate	—		—
	14. Tartrate	83.7		163
	15. Maleate	12.6		12.6
	16. Sulfate	18.3		18.3
	17. Fumarate	0.0390		0.0390
	18. Oxalate	0.402		0.402
	19. Phosphate	25.5		25.5
	20. Citrate	20.5		41.0
	21. Isocitrate	2.38		2.38
	22. *cis*-Aconitate	0.117		0.117
	23. *trans*-Aconitate	0.0496		0.0496

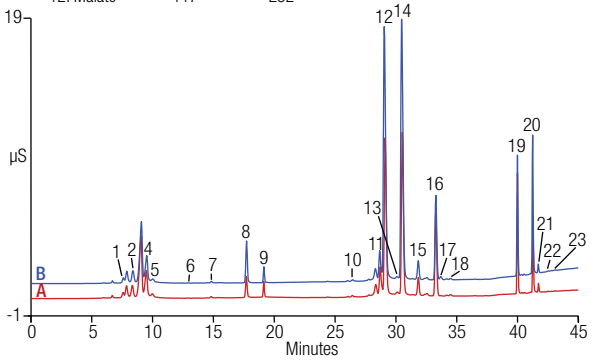

Figure 7. (A) Chardonnay wine and (B) spiked Chardonnay wine with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

Peaks:		A		B
	1. Quinate	3.01	mg/L	3.01
	2. Fluoride	1.12		1.12
	3. Lactate	26.1		26.1
	4. Acetate	8.08		8.08
	5. Glycolate	1.39		1.39
	6. Pyruvate	0.907		0.907
	7. Galacturonate	31.9		63.1
	8. Chloride	2.25		2.25
	9. Nitrate	0.850		0.850
	10. Succinate	13.6		13.6
	11. Malate	175		338
	12. Carbonate	—		—
	13. Tartrate	108		212
	14. Maleate	12.8		12.8
	15. Sulfate	16.2		16.2
	16. Fumarate	0.134		2.20
	17. Oxalate	0.335		0.335
	18. Phosphate	27.5		27.5
	19. Citrate	29.4		59.7
	20. Isocitrate	2.31		2.31
	21. *cis*-Aconitate	0.187		0.187
	22. *trans*-Aconitate	0.0563		0.0563

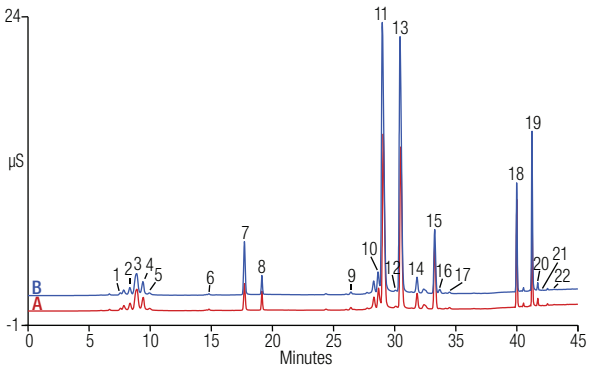

Figure 8. (A) White Zinfandel wine and (B) spiked White Zinfandel wine with a 5% signal offset applied (complete conditions as shown for System 2 on page 2).

Table 3. Recoveries of galacturonate, malate, tartrate, and citrate in selected fruit juices and wines.

Sample	Galacturonate				Malate			
	Found (mg/L)	Spiked (mg/L)	Total (mg/L)	Recovery (%)	Found (mg/L)	Spiked (mg/L)	Total (mg/L)	Recovery (%)
White Grape Juice	39.5	45.0	87.9	108	86.2	85	165	92.4
Apple Juice	83.5	83.0	165	98.2	235	235	461	96.1
Merlot	59.7	60.0	120	100	11.2	30	43.3	107
Chardonnay	20.7	20.0	42	107	117	115	232	100
White Zinfandel	31.9	32.0	63.1	97.6	175	175	338	93.2

Sample	Tartrate				Citrate			
	Found (mg/L)	Spiked (mg/L)	Total (mg/L)	Recovery (%)	Found (mg/L)	Spiked (mg/L)	Total (mg/L)	Recovery (%)
White Grape	93.5	90.0	186	103	58.4	55.0	110	94.1
Apple Juice	<0.2	—	—	—	2.11	2.00	4.28	109
Merlot	99.6	95.0	193	98.4	4.61	4.00	8.76	104
Chardonnary	83.7	80.0	163	99.1	20.5	20.0	41.0	102
White Zinfandel	108	107	212	97.5	29.4	30.0	59.7	101

Note the higher resolving power of the Dionex IonPac AS11-HC-4 µm column set over the Dionex IonPac AS11-HC column set in separating 30 anions in the standard mixture (Figure 1). The superior performance of the Dionex IonPac AS11-HC-4 µm column set is also shown through the comparison of anion separations in fruit juices and wines described in older Dionex Application Notes (ANs), such as AN 143 and AN 273. Considering the slight variations in composition of the fruit juice and wine samples studied in AN 143 and AN 273, the characteristic profiles of the selected samples (such as apple juice, grape juice, red wine, and white wine) show general similarities between the previous studies and this study.

Compared to AN 143, better signal-to-noise (S/N) ratios for various organic acids are observed in the chromatograms of the apple and grape juices presented in this work as a result of higher-efficiency peaks.[21] In AN 273, the anions in wine samples were separated on a Thermo Scientific™ Dionex™ OmniPac™ PAX-100 column with slightly different column selectivity. This column was chosen because poor separations were observed among acetate, shikimate, and lactate and between succinate and malate using the Dionex IonPac AS11-HC column set, even with the aid of organic solvent elution.[22] In this study, acetate, lactate, succinate, and malate peaks are nearly baseline resolved and more anions are observed in the chromatograms here, likely the result of higher peak capacity delivered by the Dionex IonPac AS11-HC-4 µm column set.

Table 4. Precisions of peak area and retention time for galacturonate, malate, tartrate, and citrate.

Analyte	Peak Area RSD	Retention Time RSD
Galacturonate	3.75	0.04
Malate	1.67	0.01
Tartrate	2.69	0.01
Citrate	4.03	0.01

Sample Accuracy and Precision

To validate the determination of galacturonate, malate, tartrate, and citrate in the juices and wines, the selected samples were spiked with known amounts of standards at ~100% of the native concentrations. The recoveries of galacturonate, malate, tartrate, and citrate were in the range of 97.6–107%, 92.4–107%, 97.5–103%, and 94.1–109%, respectively. The results obtained from the recovery study are summarized in Table 3. Figures 4–8 show an overlay of the spiked and unspiked samples of white grape juice, apple juice, Merlot wine, Chardonnay wine, and White Zinfandel wine, respectively.

Precision of the method was evaluated with five injections of a standard mixture containing 0.2 mg/L each of galacturonate, malate, tartrate, and citrate. The retention time RSDs and peak area RSDs of the four analytes are within 4% and 0.04% respectively (Table 4), indicating excellent method precision.

Conclusion

This study presents the characterization of ionic composition profiles in fruit juices and wines and the determination of organic acids in a selection of juice and wine samples. The separation of 30 anions on the Dionex IonPac AS11-HC (9 µm) and the Dionex IonPac AS11-HC-4 µm column sets are compared. The Dionex IonPac AS11-HC-4 µm column set offers superior resolving power for separation of the target anions. The suppressed conductivity detection offers high sensitivity for the anions, including various organic acids—even those present at low concentrations. The specificity and sensitivity of this method allow simple sample treatments without complex procedures such as extraction and/or derivatization. In addition, the recovery study shows good accuracy of the method. The electrolytically generated high-purity KOH and precise delivery of CH_3OH through the proportioning valve ensure good peak area and retention time precisions.

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