Fast Separations of Organic Acids in an Orange Juice Sample Using High-Pressure Capillary IC

Terri Christison, Fei Pang, and Linda Lopez
Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words

Food and Beverage, IonSwift MAX-100 column, HPIC, ICS-5000+ system, Organic acids

Goal

Demonstrate fast separations in a juice sample using increased flow rates.

Introduction

Determinations of organic acid profiles in fruit juices are used in the beverage industry to characterize flavor, identifiy spoilage and potential sources of adulteration, and to meet labeling requirements for food products. High-Pressure Capillary Reagent-Free™ (RFIC™) ion chromatography systems are the latest advancement in ion chromatography. Typically with an RFIC system, the system pressure is limited to < 3000 psi because of the limitations of materials in the RFIC accessories. The Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ capillary system can operate at system pressures < 5000 psi. This advance in technology allows increased flow rates with all the same advantages as standard pressure capillary IC, resulting in low consumption of water (30 to 40 mL/day of water) and low waste generation. In capillary IC, the system can remain on without loss in resources, i.e., capillary IC is always on and ready for analysis. These advantages compared to standard or microbore flow rates result in:

- Greater ease-of-use
- Longer eluent generator cartridge life
- Lower cost of ownership

With the increase in mass sensitivity, comparable results are achieved as they are with a standard bore system using a sample injection of only 0.4 µL. In a previous study published in AB 137, inorganic anions and organic acids were determined in two juice samples using standard pressure capillary IC.[2]

In this study, inorganic anions and organic acids from a diluted orange juice sample are separated by anion-exchange chromatography on a capillary size Thermo Scientific™ Dionex™ IonSwift™ MAX-100 monolith IC column and detected by suppressed conductivity detection, using the Thermo Scientific™ Dionex™ ACES™ Anion Capillary Electrolytic Suppressor specifically optimized for capillary IC.

Equipment

Dionex ICS-5000+ HPIC Reagent-Free capillary IC system

- Dionex ICS-5000+ SP Single Pump or DP Dual Pump module with high pressure capillary pumps
- Dionex ICS-5000+ EG Eluent Generator module
- Dionex ICS-5000+ DC Detector/Chromatography module with Thermo Scientific™ Dionex™ IC Cube™ cartridge and high pressure degas cartridge
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data system, version 6.8 and 7.1

Reagents and Standards

18 MΩ-cm degassed deionized water

Thermo Scientific Dionex Combined Seven Anion II Standard (Dionex P/N 057590)

Samples

A commercially-packaged 100% orange sample

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Conditions	
Columns:	Dionex IonSwift MAX 100 guard column, MAX 100, 0.25 × 250 mm
Eluent Source:	Thermo Scientific Dionex EGC KOH capillary cartridge with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column (Capillary)
Gradient:	A: 0.1 mM KOH from -10 to 0.1 min, 0.1–2 mM from 0.1 to 5 min, 2–25 mM from 5 to 20 min, 25–65 mM from 20 to 30 min, 65 mM from 30 to 45.1 min B: Same gradient adjusted for flow rate
Flow Rate:	A: 0.012 mL/min B: 0.024 mL/min
Dionex IC Cube Temp.:	30 °C*
Compartment Temp.:	15 °C
Detection:	Suppressed conductivity, Dionex ACES 300, Thermo Scientific Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode, A: 8 mA B: 18 mA
Background Conductance:	< 1.0 µS-cm conductance
Noise:	< 1.0 nS
System backpressure:	A: ~ 2500 psi B: < 4500 psi

* The Dionex IC Cube heater controls the separation temperature by controlling the column cartridge temperature. The original term of "column temperature" refers to the temperature in the bottom DC compartment which is not used for capillary IC. The part numbers of the consumables for this method are shown in Table 1.

Standard and Sample Preparation

The Dionex Seven Anion II Standard was diluted appropriately for calibration. The orange juice sample was diluted 40-fold with 18 MΩ-cm deionized water, and filtered with a syringe filter (0.2 µm) to minimize any potentially unknown matrix effects, and particles prior to injection.

Tip: It is important to use 18 MΩ-cm resistivity, deionized water for standards, eluent, and autosampler flush solution. It is recommended to degas the deionized water intended for eluent. (An appropriate degassing method is vacuum filtration.) Using deionized water with resistivity less than 18 MΩ-cm can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification. Results can vary and contamination introduced from samples can affect the chromatography.

Instrument Setup and Installation

Tip: To achieve the best chromatography with capillary IC, it is important to minimize void volumes between connections by using factory precision cut tubing, high pressure connectors and fittings (colored blue) and by seating the ferrule minimum of 2 mm above the end of the tubing. These tips are thoroughly discussed in "TN 113: Practical Guidance for Capillary IC".[3] Extra care should be used to prevent introducing air into any of the consumables or tubing by observing a steady liquid flow before installing the next device in a serial fashion. The high pressure Dionex ICS-5000$^+$ HPIC capillary IC system is designed to operate continuously up to 5000 psi which results in very low noise and increased pump stability at high pressures. To setup this application, plumb the consumables and modules of the Dionex ICS-5000$^+$ HPIC system, according to Figure 1.

Table 1. Consumables Table.

Product name	Type, Capillary	Dionex Part Number
Dionex EGC-KOH	Eluent Generator Cartridge	072076
Dionex CR-ATC	Electrolytic trap column	072078
Dionex IonSwift MAX-100	Guard column	074247
Dionex IonSwift MAX-100	Separation column	074246
Dionex CRD 200	Carbonate removal device	072054
Dionex ACES	Suppressor	072052
Dionex HP fittings (blue)	Bolts / Ferrules	074449 / 074373
EG Degas HP cartridge	High pressure degas cartridge, up to 5000 psi	AAA-074459
Dionex AS-AP autosampler vials	Package of 100, polystyrene vials, caps, blue septa	074228

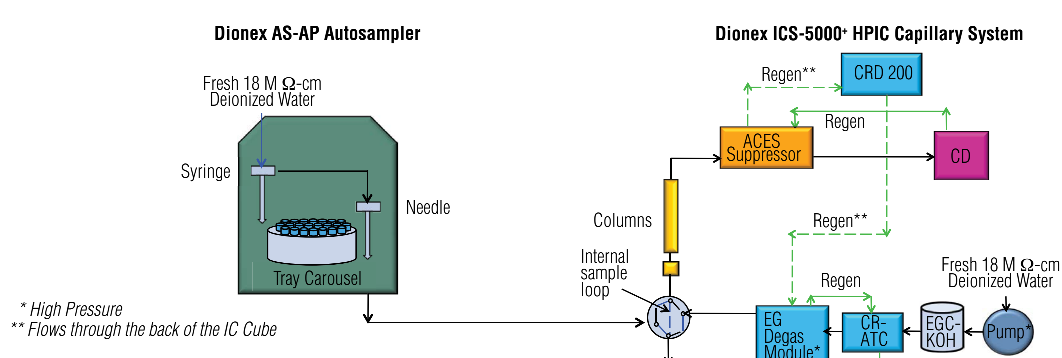

Figure 1. Flow diagram.

Install and hydrate the Dionex EGC-KOH capillary cartridge and Dionex CR-ATC trap. Install the Dionex EG Degas cartridge, Dionex CRD 200 and the Dionex ACES capillary devices into the Dionex IC Cube (Figure 2). Hydrate the devices according to the product manuals and Section 3.18 of the Dionex ICS-5000+ installation manual.[3–7] Install the columns and complete the configuration according to Figure 1. Detailed instructions are described in Technical Note 131, the product manuals, and the instrument installation and operator's manuals.[3–7]

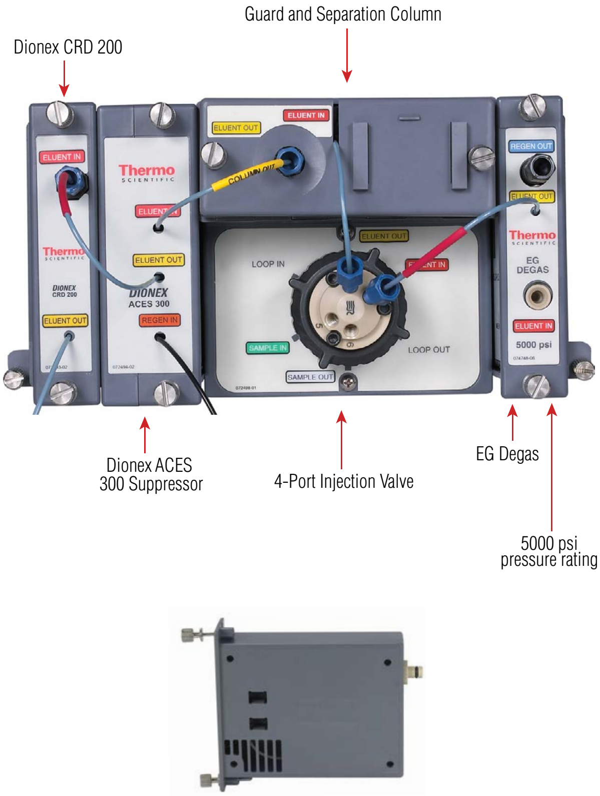

Figure 2. Dionex ICS-5000+ IC Cube.

Results and Discussion

Inorganic anions and organic acid determinations characterize fruit juices to prevent illegal adulteration by a lower cost juice, achieve a desired blend and product, and to determine product degradation from microbial activity. Therefore, to determine these anions in a juice product, the Dionex IonSwift MAX 100 column with a monolith backbone and Thermo Scientific™ Dionex™ IonPac™ AS11 chemistry, was selected for its high resolution anion-exchange chemistry and fast flow rate characteristics. In these experiments, twelve inorganic and organic acid anions were separated at 0.012 and 0.024 mL/min flow rates using an electrolytically-generated gradient. At 0.024 mL/min flow rate conditions, the system backpressure approaches 4200 psi which is well within the within the high pressure tolerances of the new Dionex ICS-5000+ system while reducing the run time from 30 to 15 min. The analytes are detected by suppressed conductivity detection, using the Dionex ACES Anion Capillary Electrolytic Suppressor specifically optimized for capillary IC.

To determine the analyte concentrations, we measured the peak area responses to concentration by calibrating with duplicate injections of the 50-, 100-, and 200-fold diluted Dionex Combined Seven Anion II Standard. Quinate, lactate, formate, malate, maleate, and oxalate were calibrated similarly using duplicate injections of 1, 10, and 50 mg/L standards. Additional lower concentration standards were added to quantify formate and maleate in the sample. Citrate was calibrated using 50, 100, and 150 mg/L concentration standards. A linear regression curve was used for all anions, resulting in linear coefficients of $r^2 > 0.99$.

Figure 3 shows the separation of a 0.4 µL injection of the 1:40 diluted, orange juice sample filtered with a syringe filter to minimize any unknown interferences and to remove particles prior to injection. The twelve ions were sufficiently baseline-resolved for quantification at both flow rates. These chromatograms demonstrate the advantages of using a high-pressure capable system. The system backpressure at 0.024 mL/min results in run times 50% of those at the 0.012 mL/min flow rates. The system pressure of 4200 psi at 0.024 mL/min would exceed the allowable system pressure for an RFIC system. However, this system pressure is well within the tolerances of the Dionex ICS-5000+ capillary HPIC system which can operate at pressures up to 5000 psi. This application shortens the run times 50% from 30 to 15 min by just increasing the flow rate.

	Dionex MAX-100, capillary, 0.25 mm
Eluent Source:	Dionex EGC-KOH capillary cartridge
Gradient:	A: 0.1 mM KOH from -10 to 0.1 min, 0.1–2 mM from 0.1 to 5 min, 2–25 mM from 5 to 20 min, 25–65 mM from 20 to 30 min, 65 mM from 30 to 45.1 min B: Same gradient adjusted for flow rate
Column Temp.:	30 °C
Flow Rate:	A: 12 µL/min; B: 24 µL/min
Inj. Volume:	0.4 µL
Detection:	Suppressed conductivity, Dionex ACES, recycle
Sample Prep.:	1:40 dilution, filter, 0.45 µm

Peaks:

Quinate	2.0 mg/L	8. Maleate	0.1 mg/L
Lactate	3.2	9. Sulfate	2.0
Formate	0.7	10. Oxalate	4.6
Chloride	1.0	11. Phosphate	7.5
Nitrate	--	12. Citrate	125
Glutarate	--		
Malate	25		

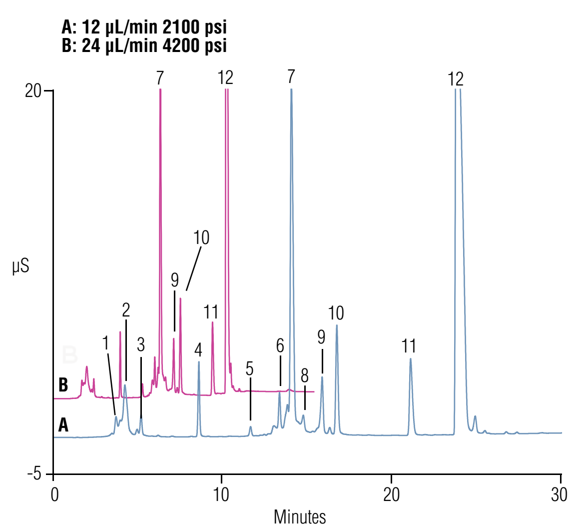

Figure 3. Fast separations of organics acids in a diluted orange juice sample using high-pressure capillary IC.

Conclusion

This application demonstrates how using higher flow rates combined with a high resolution column and a capillary IC system capable of high system pressures can provide comparable separations with 50% shorter run times, therefore increasing sample throughput and saving money and labor.

For additional information on inorganic anion and organic acid determinations in fruit juice samples by high pressure and standard pressure capillary IC, please refer to AB137 and the Food and Beverage section of the Dionex Capillary IC Applications Library website.[2,8] Organic acid determinations in fruit juice samples using standard bore and microbore IC are thoroughly discussed in AN 21, AN 143, and AN 273.[9–11]

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