Fast Separations of Anions and Organic Acids in a Carbonated Beverage Using High-Pressure Capillary IC

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Key Words
Food and Beverage, Dionex IonSwift MAX-200, phosphate, HPIC, citrate, Dionex ICS-5000+

Goal
Demonstrate fast separations in a carbonated beverage using increased flow rates on a high-pressure capillary IC system.

Introduction
Determinations of organic acids in beverages are important to the beverage industry because these acidulants and flavoring agents maintain beverage freshness, minimize microbiological growth, and add a characteristic flavor. Additionally, analysis is required to meet product labeling requirements in 21 CFR part 101.[1]

High Pressure capillary Reagent-Free™ ion chromatography (RFIC™) systems are the latest advancement in ion chromatography instrumentation. Typically with an RFIC system, the system pressure is limited to < 3000 psi because of the limitations of materials in the RFIC accessories. Now with the upgrade to high pressure, the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ capillary system can operate at system pressures up to 5000 psi. This advance in technology allows 2× to 3× the µL/min flow rates with all the same advantages as standard-pressure capillary IC, resulting in low consumption of water (30 to 40 mL/d of water) and low waste generation. In capillary IC, the system can remain on with minimal consumption of resources, i.e., capillary IC is always on and ready for analysis. These advantages result in:

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

Additionally, with the increase in mass sensitivity, comparable results are achieved using a sample injection of only 0.4 µL as with a 40 µL injection on a standard bore column. In this study, inorganic anions and organic acids from a degassed, diluted carbonated cola beverage were determined using a gradient separation on a capillary-format Thermo Scientific™ Dionex™ IonSwift™ MAX-200 monolith anion-exchange column at increasing flow rates and detection by suppressed conductivity detection with a Thermo Scientific™ Dionex™ ACES™ 300 Anion Capillary Electrolytic Suppressor specifically designed for capillary IC.

Equipment
- High pressure Dionex ICS-5000+ Reagent-Free HPIC 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™ and high-pressure degas cartridge
  - Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software

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Reagents and Standards

- 18 MΩ-cm degassed deionized water
- Thermo Scientific Dionex Combined Seven Anion II Standard (Dionex P/N 057590)

Samples

- A carbonated diet cola beverage

Conditions	
Columns:	Dionex IonSwift MAX 200, 0.25 × 250 mm
Eluent Source:	Thermo Scientific Dionex EGC KOH Eluent Generator Capillary Cartridge with Thermo Scientific Dionex capillary CR-ATC Continuously Regenerated Anion Trap Column
Gradient:	A: 2 mM KOH for 0.1 min, 2–10 mM (0.1–10 min), 10–50 mM (10–15 min) B and C: Same gradient adjusted for flow rate
Flow Rate:	A: 10; B: 20; C: 25 µL/min
IC Cube Temp.: *	30 °C
Compartment Temp.:	15 °C
Inj. Volume:	0.4 µL
Detection:	Suppressed conductivity, Dionex ACES 300 Anion Capillary Electrolytic Suppressor, Thermo Scientific Dionex CRD 200 Carbonate Removal Device (Capillary), recycle mode, A: 8 mA; B: 15 mA; C: 18 mA
Background Conductance:	0.5–0.8 µS conductance
Noise:	< 0.3 nS
System Backpressure:	A: 1900 psi; B: 3700 psi, C: 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.

Table 1. Consumables list.

Product name	Type, Capillary	Dionex Part Number
Dionex EGC-KOH	Eluent Generator cartridge	072076
Dionex CR-ATC	Electrolytic trap column	072078
Dionex IonSwift MAX-200	Separation column	075889
Dionex CRD 200	Carbonate removal device	072054
Dionex ACES	Suppressor	072052
Dionex HP fittings (blue)	Bolts / Ferrules*	074449 / 074373
Dionex AS-AP autosampler vials	Package of 100, polystyrene vials, caps, blue septa	074228

Standard and Sample Preparation

The Dionex Seven Anion II Standard was diluted appropriately for calibration. The diet cola beverage was degassed by vacuum filtration and ultrasonic agitation; diluted 10-fold with 18 MΩ-cm deionized water, and filtered with a syringe filter (0.45 µm) to minimize introduced carbonate and 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 precision cut tubing, high pressure connectors and fittings (colored blue), and by seating the ferrule > 2 mm above the end of the tubing. These tips are thorough discussed in "TN 113: Practical Guidance for Capillary IC".[2] 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 line. 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.

To setup this application, plumb the consumables and modules of the Dionex ICS-5000+ HPIC system, according to Figure 1.

Install and hydrate the Dionex EGC-KOH capillary cartridge and Dionex CR-ATC trap column. Install the Dionex EG Degas cartridge, Dionex CRD 200 cartridge, and the Dionex ACES 300 suppressor 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.[2–6] Install the columns and complete the configuration according to Figure 1. Detailed instructions are described in TN 131, the product manuals, and the instrument installation and operator's manuals.[2–6]

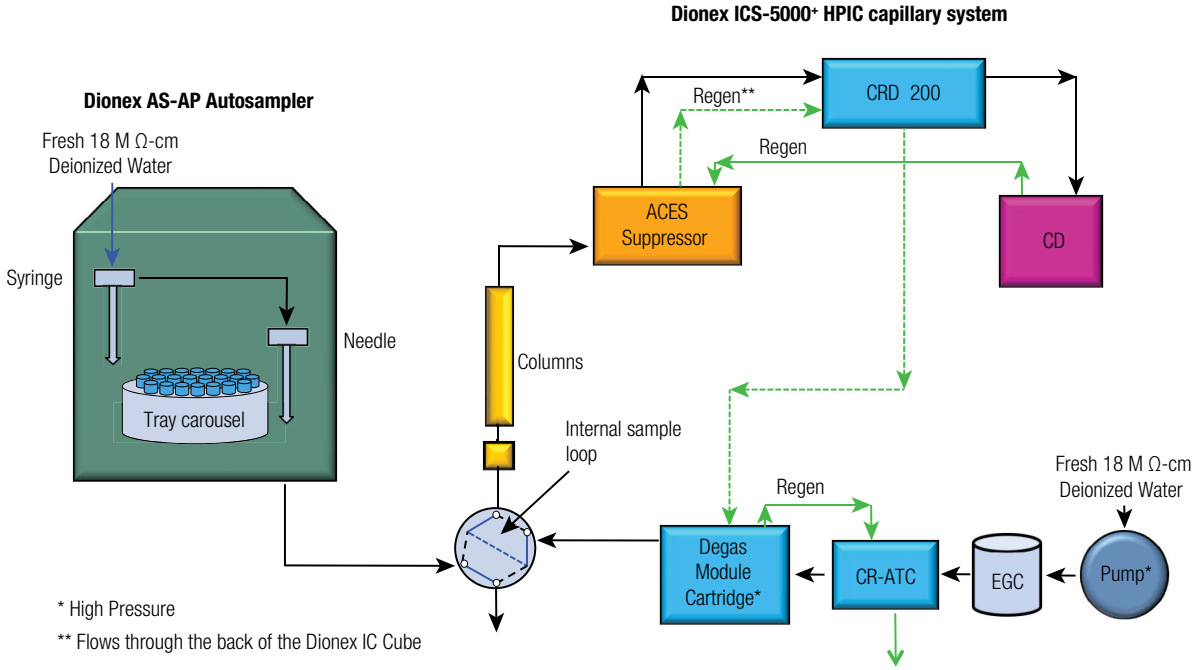

Figure 1. Flow diagram for the Dionex ICS-5000+ HPIC capillary RFIC system.

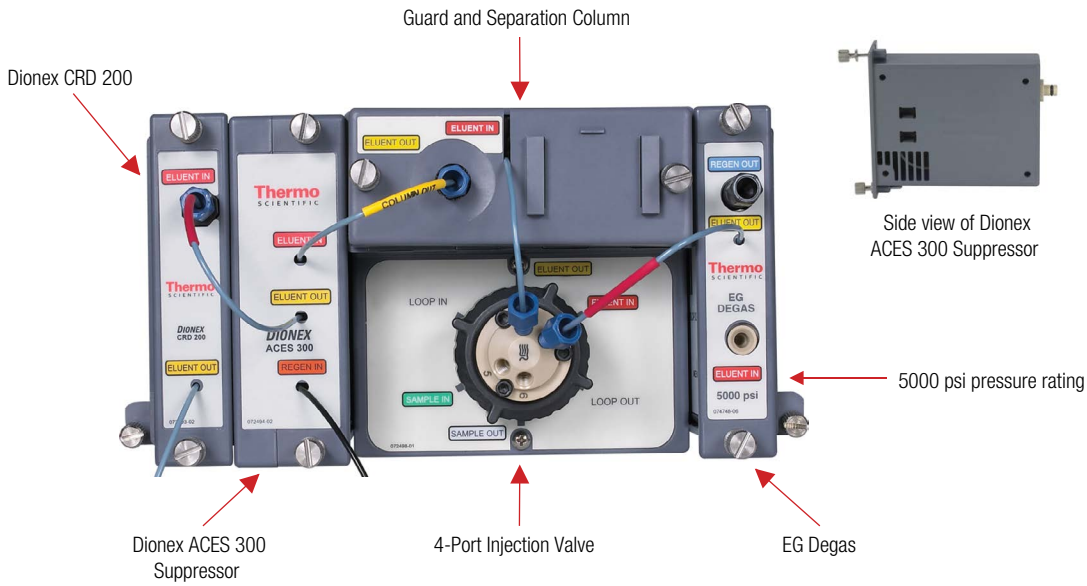

Figure 2. Dionex ICS-5000+ IC Cube.

Results and Discussion

Determinations of anions and acidulants, such as phosphoric acid and citric acid as their anions phosphate and citrate, respectively are important to maintain product quality and flavor when receiving raw ingredients and during the processing of carbonated beverages. The Dionex IonSwift MAX-200 column, with a monolith backbone and the Thermo Scientific™ Dionex™ IonPac™ AS19 anion-exchange chemistry, was selected for this application because of the column's high efficiency and high flow rate characteristics suitable for high pressure cap IC experiments.

To determine the analyte concentrations, the peak area responses as a response to concentration were obtained by calibrating with duplicate injections of the 10-, 50-, and 100-fold diluted Dionex Combined Seven Anion II Standard. Citrate was calibrated similarly using duplicate injections of 1, 10, and 50 mg/L standards. A linear regression curve was used for all anions, resulting in linear coefficients of $r^2 > 0.999$.

Figure 3 shows the gradient separation from 10 to 50 mM KOH of a 0.4 µL injected sample using electrolytically generated KOH eluent. The carbonated diet cola sample was degassed, diluted 10-fold, and filtered to reduce carbonate concentrations, minimize potential interferences, and remove particles prior to injection. The five ions were sufficiently baseline-resolved for quantification at all three flow rates with 60% reduction in run times from 20 to 8 min. However, at these flow rates (20 and 25 µL/min), the system backpressure increases to 3700 and 4500 psi, respectively, which can only be run on a high-pressure IC system, such as the Dionex ICS-5000+ HPIC high-pressure capillary IC.

Column:	Dionex IonSwift MAX-200G, MAX-200, 0.25 mm × 250 mm	
Eluent Source:	Dionex EGC-KOH capillary cartridge	
Gradient:	A: 2 mM KOH for 0.1 min,2–10 mM (0.1–10 min), 10–50 mM (10–15 min) B–C: Same gradient adjusted for flow rate	
Inj. Volume:	0.4 µL	
Column Temp.:	30 °C	
Detection:	Suppressed Conductivity, Dionex ACES 300, Anion Capillary Electrolytic Suppressor, capillary Dionex CRD 200	
Sample Prep.:	Degassed, diluted 10-fold, filtered, 0.45 µm	
		Total
Peaks:	1. Chloride	26.0 mg/L
	2. Nitrate	7.3
	3. Sulfate	50.3
	4. Phosphate	226.0
	5. Citrate	48.1

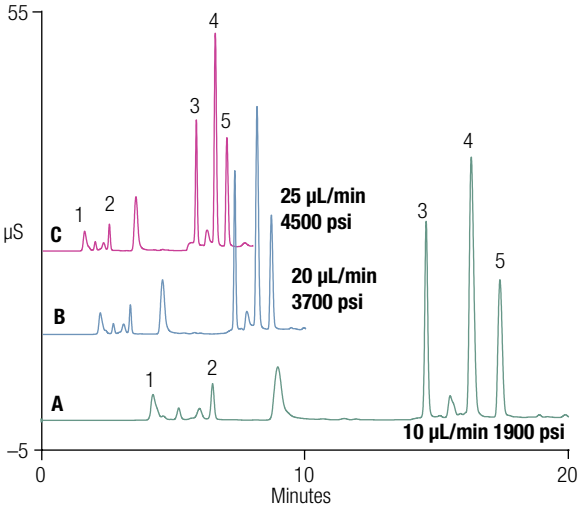

Figure 3: Fast separations of anions in a diet cola beverage by high-pressure capillary IC.

Conclusion

This application demonstrates the power of using higher flow rates on a high-resolution column and a capillary IC system capable of high system pressures, such as the high-pressure Dionex ICS-5000⁺ capillary HPIC system. Sample throughput is increased by just increasing the flow rate, hereby and saving money and labor.

For additional information on inorganic anion and organic acid determinations in beverage samples by high pressure and standard pressure capillary IC, please refer to Application Brief (AB) 137 and Food and Beverage section of Dionex Capillary IC Applications Library website.[7,8] Organic acid determinations in fruit juice samples using standard bore and microbore IC are thoroughly discussed in AN 169 and AU 142.[9,10]

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